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Sunlight, Vitamin D and Skin Cancer

Third Edition

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Preface

The powerful rays of the sun represent a *conditio sine qua non* for life on earth in its present form and a major driver for human evolution. However, solar radiation exerts both positive and negative effects on human health. As a result of this dilemma, there is an ongoing controversy and intense discussion in scientific communities and the general population to answer this fundamental question of environmental medicine: how much sun is good for human health? The first two editions of "*Sunlight, Vitamin D and Skin Cancer,*" designed and organized to be up-to-date reviews, were widely recognized benchmarks on the subject when published in 2008 and 2014, respectively. This new and extended volume continues to include extensive, in-depth chapters covering the most important aspects of the ongoing debate on how much sun is good/optimal for human health and how to balance between positive and negative effects of solar and artificial UV radiation. As a result of a mountain of new information about the health benefits caused by the UV-induced cutaneous synthesis of vitamin D, this book has been expanded substantially to include many new topics. It is generally accepted that UV exposure represents the most important risk factor for the development of non-melanoma skin cancer. Additionally, assessment of sun exposure parameters has consistently shown an association between the development of malignant melanoma and short-term intense UV exposure, particularly burns acquired in childhood. As a consequence, protection of the skin from UV radiation is an integral part of skin cancer prevention campaigns. However, more chronic less-intense UV exposure has not been found to be a risk factor for melanoma and in fact has been found in some studies to be protective. Moreover, besides many other photoproducts, 90% of all requisite vitamin D is formed within the skin through the action of the sun – a serious problem, for a connection between vitamin D deficiency and many severe diseases, including various types of cancer (e.g., colon, prostate, and breast cancer), has been demonstrated in a large number of studies. Hence, the association between vitamin D deficiency and various diseases, including internal malignancies, has opened a debate among dermatologists and other clinicians on how to balance between positive and negative effects of solar and artificial UV exposure. The goal of this volume is to provide a comprehensive highly readable, updated, and extended overview on our present knowledge of positive and negative effects of UV exposure, with a focus on vitamin D and skin cancer. Topics are discussed in depth by leading

researchers and clinicians ranging from the newest findings in endocrinology (including the relevance of non-classical vitamin D metabolites), epidemiology, histology, photobiology, immunology, cytogenetics, and molecular pathology to new concepts for disease prevention and treatment. Experts in the field as well as health-care professionals not intimately involved in these specialized areas have provided the most significant and timely information related to these topics. It is the aim of this third edition to summarize essential up-to-date information for every clinician or scientist interested in how to balance between positive and negative effects of UV exposure to minimize the risks that are associated with insufficient (e.g., developing vitamin D deficiency) and excessive (e.g., skin cancer) exposure. Again, all the chapters are written by authors who are experts in their respective research areas, and I am grateful for their willingness to contribute to this book. I am convinced that this edition will be as successful as the previous ones. I would also like to express my thanks to Larissa Albright, Anthony Dunlap, Murugesan Tamilselvan, and all the other members of the Springer Nature staff for their expertise, diligence, and patience in helping me complete this work.

Enjoy the reading!

Homburg/Saar, Germany   Jörg Reichrath

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Part I

Introduction

Lessons Learned from Paleolithic Models and Evolution for Human Health: A Snap Shot on Beneficial Effects and Risks of Solar Radiation

Jörg Reichrath

Abstract

How to deal with the powerful rays of the sun represents a fundamental question of environmental medicine, affecting skin cancer prevention campaigns and many other aspects of public health. However, when preparing recommendations for sunlight exposure, physicians, scientists, and other health authorities are in a dilemma, because solar radiation exerts both positive and negative effects on human health. While positive effects are at least in part mediated via the UV(Ultraviolet)-B-induced cutaneous synthesis of vitamin D, negative effects include the UV-mediated photocarcinogenesis of skin cancer. During the last century, interest in the positive effects of the sun on our health increased dramatically after the introduction of the so-called vitamin D/cancer hypothesis. In the late 1930s, Peller and Stephenson reported higher rates of skin cancer but lower rates of other cancers among the US Navy personnel. Several years later, Apperly reported an association between latitude and cancer mortality rate in North America. He argued that the "relative immunity to cancer is a direct effect of sunlight". Although the hypothesis that sun exposure may be beneficial against cancer had been proposed early, these observations supporting the hypothesis were ignored for nearly 40 years, until a clear mechanism was proposed. In the 1980s, Garland and Garland published a pilot study focusing on colon cancer and suggested that the possible benefits of sun exposure could be attributed to vitamin D. Later, the proposed protective role of vitamin D was extended to many other types of cancer. Subsequent laboratory investigations supported potential anticarcinogenic effects of vitamin D compounds. We know today that many, but not all, of the positive effects of the sun on human health are mediated by the UV-induced cutaneous synthesis of vitamin D and other photoproducts. However, because of the abovementioned dilemma, there is an ongoing controversial discussion in scientific communities and in the general population that how much sunlight is optimal for human health. This chapter summarizes the content of the third edition of "*Sunlight, Vitamin D and Skin Cancer,*" a book specifically designed and organized to be an up-to-date review covering the most important aspects of the ongoing debate on how much sun is good for human health and how to balance between the positive and negative effects of solar and artificial UV-radiation, including lessons learned from Paleolithic models and evolution.

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When preparing recommendations for sunlight
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tion exerts both positive and negative effects on
human health (rev. in [11]). While positive effects
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introduction of the so-called vitamin D/cancer
hypothesis (rev. in [12]). Although the hypothesis
that sun exposure may be beneficial against can-
cer had been proposed early in the last century,
these observations were ignored for nearly
40 years, until a convincing mechanism was pro-
posed (rev. in [12]). In the late 1930s, Peller and
Stephenson reported higher rates of skin cancer
(i.e., eight times higher) but lower rates of other
cancers among the US Navy personnel ([16]; rev.
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quently conferred immunity against other cancers
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cer mortality rate in North America ([1]; rev. in
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latitude regions had higher rates of total cancer
mortality when compared to those in low-latitude
regions. He argued that the "relative immunity to
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sun exposure could be attributed to vitamin D
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made from exposure to solar Ultraviolet-B
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protective role of vitamin D was later extended
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effects of the sun on human health are mediated
by the UV-induced cutaneous synthesis of vita-
min D and other photoproducts (rev. in [11]).
However, because the powerful rays of the sun
may also exert negative effects on human health,
we are in a dilemma, and there is an ongoing
controversial discussion in scientific communities
and in the general population that how much
sunlight is good for human health. When the
first edition of "*Sunlight, Vitamin D and Skin
Cancer,*" designed and organized to be an up-
to-date review, was published in 2008, it was
the benchmark on the subject. This new and
extended volume continues to include extensive,
in-depth chapters covering the most important
aspects of the ongoing debate on how much sun
is good for human health and how to balance
between the positive and negative effects of
solar and artificial UV radiation. As a result of a
mountain of new information about the health
benefits of the UV-induced cutaneous synthesis
of vitamin D, including lessons learned from
Paleolithic models (rev. in [22]) and evolution
[11], this book has been expanded substantially
to include many new topics.

The first section of this book focusses on pho-
tobiology and the positive biological effects of
vitamin D compounds. In Chap. 2 entitled "Sun-
light, UV-Radiation, Vitamin D and Skin Cancer:
How Much Sunlight Do We Need?" Michael
F. Holick gives an excellent overview on the
multiple biological effects of the sunshine vita-
min, which is vitamin D [11]. He points out that
during exposure to sunlight, the ultraviolet B
photons enter the skin and photolyze
7-dehydrocholesterol to previtamin D3, which in
turn is isomerized by the body's temperature to
vitamin D3. He further explains that most humans
depend on sun for their vitamin D requirement
and that skin pigment, sunscreen use, aging, time
of day, season, and latitude dramatically affect
previtamin D3 synthesis. Michael Holick reports

that vitamin D deficiency was thought to have been conquered, but it is now recognized that more than 50% of the world's population is at risk for vitamin D deficiency [11]. This deficiency is in part due to the inadequate fortification of foods with vitamin D and the misconception that a healthy diet contains an adequate amount of vitamin D [11]. Vitamin D deficiency causes growth retardation and rickets in children and will precipitate and exacerbate osteopenia, osteoporosis and increase risk of fracture in adults. Holick further explains that the vitamin D deficiency pandemic has been associated with other serious consequences, including increased risk of common cancers, autoimmune diseases, infectious diseases and cardiovascular disease [11]. He concludes that there needs to be a renewed appreciation for the beneficial effect of moderate sensible sunlight for providing all humans with their vitamin D requirement for health [11].

In the Chap. 3 entitled "Vitamin D Status and Cancer Incidence and Mortality," Hanseul Kim and Edward Giovannucci explain that there have been numerous efforts in studying the relationship between sun exposure and cancer incidence and mortality [12]. The authors point out that in the late 1930s, Peller and Stephenson reported higher rates of skin cancer (i.e., eight times higher) but lower rates of other cancers among the US Navy personnel ([16]; rev. in [12]). Peller and Stephenson suggested that sun exposure induced skin cancer, which consequently conferred immunity against other cancers ([16]; rev. in [12]). After several years, Apperly reported an association between latitude and cancer mortality rate in North America ([1]; rev. in [12]). He observed that individuals in high-latitude regions had higher rates of total cancer mortality when compared to those in low-latitude regions. He argued that the "relative immunity to cancer is a direct effect of sunlight" ([1]; rev. in [12]). Although the hypothesis that sun exposure may be beneficial against cancer had been proposed early, these observations supporting the hypothesis were ignored for nearly 40 years, until a clear mechanism was proposed. In the 1980s, Garland and Garland suggested that the

possible benefits of sun exposure could be attributed to vitamin D ([7]; rev. in [12]). They hypothesized that vitamin D was protective against colon cancer, based on the premise that most vitamin D in humans is made from exposure to solar Ultraviolet-B (UV-B) radiation ([7]; rev. in [12]). While this study focused on colon cancer, the proposed protective role of vitamin D was later extended to cancers in the breast, ovary, prostate, and other multiple sites. Subsequent laboratory studies supported potential anti-carcinogenic properties of vitamin D including increased differentiation and apoptosis and inhibited proliferation, invasiveness, angiogenesis, and metastatic potential. This chapter provides a review and synthesis of up-to-date epidemiologic evidence on the association between vitamin D and the incidence and mortality for various cancers. After Garland and Garland's initial hypothesis, numerous epidemiologic studies have supported the protective role of vitamin D (or sun exposure) on different cancer sites. In this chapter, Hanseul Kim and Edward Giovannucci first discuss epidemiologic studies that assess the association between serum vitamin D levels and cancer incidence and mortality and then discuss vitamin D intake studies, including evidence from the most recent randomized controlled trial (RCT) data. Hanseul Kim and Edward Giovannucci, the authors, consider three endpoints: cancer incidence (newly onset cases diagnosed during the study period in an initially cancer-free population), cancer mortality (fatal cases occurring during the study period in an initially cancer-free population), and cancer survival (survival or mortality from cancer among individuals already diagnosed with cancer). They demonstrate that, over the last several decades, vitamin D has received substantial study in relation to the common cancers, and less so for the rarer malignancies (rev. in [12]). For cancer incidence, based on observational studies, a consistent inverse association has only been observed for colorectal cancer. RCTs also do not support a general effect of vitamin D on cancer incidence. Although these RCTs potentially provide more evidence for a causal association, there exist important limitations. Trials with longer duration

are warranted for studies on cancer incidence because of potential long durations required to observe an effect. For example, epidemiologic evidence suggests that at least 10 years are needed for any influence of calcium or vitamin D to show on colorectal cancer occurrence. Since most cancers generally arise through a multi-stage process that lasts for a long period of time, studies with relatively short duration would not capture the benefit of vitamin D on cancer risk, if there is any. In addition, in trials, it is difficult to choose a single "proper" or "effective" dosage that a susceptible population could benefit from. Therefore, although RCTs are generally considered as a gold standard, their results should still be interpreted with caution for issues mentioned above and other issues such as noncompliance. In contrast to the studies on cancer incidence, both RCTs and many, though not all observational, studies suggest that vitamin D may play a role in cancer mortality or survival. Approximately 15% reduction in total cancer mortality was observed in those who were randomized to receive vitamin D supplement over placebo, and the VITAL study suggested that this effect size could increase over duration of vitamin D use (rev. in [12]). Most of the follow-up time in the studies was less than 5 years. In VITAL, after excluding the first 2 years, the risk reduction was 25%. Benefits were seen even at fairly high doses of 2000 IU/ day and when levels of >100 nmol/L were attained (rev. in [12]). While the reason for the divergent findings for the incidence and mortality of total cancer is not apparent, plausible mechanisms exist for vitamin D operating at the multiple stages of carcinogenesis (rev. in [12]). The authors explain that vitamin D may decrease tumor invasiveness and propensity to metastasize, which may occur at the later stages of carcinogenesis. In the RCTs, which showed benefits on mortality, vitamin D administration generally started before cancer diagnosis, likely during the later stages of carcinogenesis and continued during and after the diagnosis. Thus, the potential benefit for vitamin D status on cancer mortality could operate during all stages of carcinogenesis and tumor progression, from prediagnostic stages until late-stage tumor progression (e.g., invasion

and metastatic seeding, during the treatment phase possibly by complementing or enhancing effects of therapies (rev. in [12]). Kim and Giovannucci explain that it is unclear if similar benefits could be attained by beginning vitamin D treatment at the time of diagnosis because some of the effects of vitamin D could be during the metastatic seeding phase during the prediagnostic period. Almost ten million cancer deaths were estimated to have occurred in 2018 worldwide (rev. in [12]). With the increasing population size and ageing, cancer incidence and mortality is likely to increase over time. The authors conclude that results from their meta-analysis support that achieving circulating levels of 25(OH)D, around 54–135 nmol/L, may contribute to reducing cancer mortality. Although the optimal 25 (OH)D level for prevention is not established, it is likely to be higher than 50 nmol/L, and currently, a substantial portion of the world's population is even below this threshold. The Endocrine Society recommends at least 1,500–2000 IU/day intake of vitamin D to maintain the level of 25 (OH)D above 75 nmol/L (rev. in [12]). The authors conclude that further studies are needed to confirm these findings, establish the optimal dose and timing of vitamin D intake for prevention, find which cancer types are affected, and determine the underlying mechanisms of action (rev. in [12]).

In the Chap. 4 entitled "Vitamin D Receptors Polymorphisms and Cancer," Patrizia Gnagnarella, Sara Gandini, and coworkers point out that increasing scientific evidence supports the link between vitamin D and cancer risk [9]. The active metabolite 1,25(OH)2D exerts its activity by binding to the vitamin D receptor (VDR), an intracellular receptor that mediates transcriptional activation and repression of target genes. The binding of 1,25(OH)2D to VDR is able to regulate hundreds of different genes. VDR is active in virtually all tissues, including: colon, breast, lung, ovary, bone, kidney, parathyroid gland, pancreatic b-cells, monocytes, T lymphocytes, melanocytes keratinocyte, and also in cancer cells. The relevance of *VDR* gene restriction fragment length polymorphisms for various types of cancer has been investigated by

a great number of studies. Patrizia Gnagnarella, Sara Gandini and coworkers have carried out a systematic review of the literature to analyze the relevance of more VDR polymorphisms (*Fok1*, *Bsm1*, *Taq1*, *Apa1*, and *Cdx2*) for individual malignancies considering ethnicity as a key factor for heterogeneity [9]. Until December 2018, they identified 177 independent studies with data to calculate risk estimate for breast, prostate, colorectal, skin (melanoma and nonmelanoma skin cancer), lung, ovarian, kidney, bladder, gallbladder, esophageal, thyroid, head and neck, liver, and oral squamous cell carcinoma; non-Hodgkin lymphoma; multiple myeloma; sarcoma [9]. Significant associations with VDR polymorphisms have been reported for prostate (*Fok1, Bsm1, Taq1, Apa1, Cdx2*), breast (*Fok1, Bsm1, Apa1, CdX2*), colorectal (*Fok1, Bsm1, Taq1, Apa1*), and skin cancer (*Fok1, Bsm1, Taq1*). Very few studies reported risk estimates for the other cancer sites. Conflicting data have been reported for most malignancies, and at present, it is still not possible to make any definitive statements about the importance of the *VDR* genotype for cancer risk. It seems probable that other factors such as ethnicity, phenotype, 25(OH)D plasma levels, and UV radiation exposure play a role as confounding factors and introduce heterogeneity. The authors conclude, there is some indication that *VDR* polymorphisms may modulate the risk of some cancer sites, and in future studies *VDR* genetic variation should be integrated also with the assessment of vitamin D status and stratified by ethnicity [9].

In Chap. 5 entitled "On the Relationship Between Sun Exposure and All-Cause Mortality," Pelle G Lindqvist makes a short update on the knowledge regarding sun exposure and all-cause mortality [14]. He points out that data support the hypothesis that low sun exposure habits are a major risk factor for all-cause mortality [14]. Low sun exposure is related to an increased risk of death due to cardiovascular disease (CVD) and noncancer/non-CVD and a minor reduction in risk of cancer [14]. Active sun exposure habits have a dual effect; it increases the incidence of skin cancer but also improve the prognosis in terms of all-cause mortality. The author concludes that in a low solar intensity region, both risk and benefits of sun exposure should be carefully assessed in order to obtain balanced recommendations. In 2011, a 30% lower rate of all-cause mortality was reported among those who took a sunbathing vacation at least once a year over the course of three decades (rev. in [14]). A 15-year prospective follow-up of the Melanoma in Southern Sweden (MISS) cohort of women demonstrated a significant dose-dependent decrease in all-cause mortality with increasing sun exposure habits and the mortality rate was doubled (2.0, 95% CI 1.6 – 2.5) among those avoiding sun exposure compared to the highest sun exposure group (rev. in [14]). The population attributable risk (PAR) for mortality for the group avoiding sun exposure was estimated to be 3%. In a 20-year follow-up of the same cohort, analyzed in a competing risk scenario, it was shown that the shorter life expectancy of women who avoided sun exposure was mainly due to a dose-dependent significantly increased risk of cardiovascular disease (CVD) and noncancer/non-CVD deaths, compared to the moderate and high sun exposure groups (rev. in [14]). While the risk of dying in the CVD and noncancer/non-CVD groups decreased with increasing sun exposure, the relative contribution of death due to cancer increased as a result of extended life expectancy (rev. in [14]). Thus, the overall *prevalence* of death due to cancer increased, but not the age-adjusted risk. In an analysis stratified for smoking, there was a similar risk of death among nonsmokers avoiding sun exposure as for smokers in the highest sun exposure groups. Pelle G Lindqvist interpreted this as that sun exposure avoidance is a risk factor for all-cause death of the same magnitude as smoking. He concludes that the increased mortality rate among those who avoid sun exposure is mainly due to an increased risk of death from CVD and noncancer/non-CVD. He hopes our findings add to a more balanced and adequate view regarding the effects of sun exposure on our health.

The following section of this book focusses on the role of solar radiation as a major environmental risk factor for the photocarcinogenesis of skin cancer. In Chap. 6 entitled "Epidemiology of Skin

Cancer and UV Radiation – Update 2019," Ulrike Leiter, Ulrike Keim, and Claus Garbe explain that melanoma and keratinocyte skin cancer (KSC) are the most common types of cancer in white-skinned populations [13]. Both tumor entities showed increasing incidence rates worldwide, but stable or decreasing mortality rates. Rising incidence rates of cutaneous melanoma (CM) and KSC are largely attributed to increasing exposure to ultraviolet (UV) radiation, the main causal risk factor for skin cancer. Incidence rates of KSC, comprising of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are much higher than that of melanoma. BCC development is mainly the cause of an intensive UV exposure in childhood and adolescence, while SCC development is related to chronic, cumulative UV exposure over decades. Although mortality is relatively low, KSC is an increasing problem for health care services, causing significant morbidity. Cutaneous melanoma is rapidly increasing in white populations, with an estimated annual increase of around 3–7% over the past decades. The authors further explain that in contrast to SCC, melanoma risk is associated with intermittent and chronic exposure to sunlight [13]. The frequency of its occurrence is closely associated with the constitutive color of the skin and the geographical zone. Changes in outdoor activities and exposure to sunlight during the past 70 years are an important factor for the increasing incidence of melanoma. Mortality rates of melanoma show stabilization in the United States, Australia, and in European countries. In the United States, even dropping numbers of death cases were recently reported, probably reflecting efficacy of the new systemic treatments. Among the younger cohorts in some populations (e.g., Australia and New Zealand,), stabilizing or declining incidence rates of CM are observed, potentially caused by primary prevention campaigns aimed at reducing UV exposure [13]. The authors further explain that in contrast, incidence rates of CM are still rising in most European countries and in the United States and that ongoing trends toward thinner melanoma are largely ascribed to earlier detection [13].

In the next paper entitled "Solar UV Exposure and Mortality from Skin Tumors: An Update," Marianne Berwick and Amy Garcia explain that solar UV exposure is critical and complex in the etiology and prognosis of skin cancer, particularly cutaneous malignant melanoma [2]. Sun exposure and one of its "derivatives," vitamin D, have been implicated in protection against mortality from melanoma. The authors conclude that the relationships are inconsistent and that, at this time, it is not possible to make clear recommendations for or against sun exposure in relationship to melanoma prognosis [2]. However, this relationship deserves continued exploration.

In Chap. 8 [5] entitled "Solarium Use and Risk for Malignant Melanoma: Many Open Questions, Not the Time to Close the Debate," Barbara Burgard and Jörg Reichrath shed further light on the ongoing debate whether sunbed use may increase melanoma risk, critically assessing the scientific literature that is at present available, focusing on a meta-analysis that these authors have published previously. Their literature search identified several meta-analyses that report a weak association for ever-exposure to UV radiation from a solarium with melanoma risk. However the quality of studies included in these meta-analyses, the resulting evidence levels and grades of recommendation were very low due to the lack of interventional trials and because of severe limitations of many of the observational studies. The results of cohort and case–control studies published until today do not prove causality, not even by the Hill criteria. The overall quality of these observational studies and the resulting evidence levels are low due to severe limitations (including unobserved or unrecorded confounding), which leads to bias. It must be recognized that, in the majority of studies, published to date, many of the confounding factors, including sun exposure, sunburns, and skin type, have not been adequately and systematically recorded and adjusted for. We conclude that the many limitations of the individual studies and the resulting low levels of evidence and grades of recommendation do at present not allow postulation of a causal relationship between solarium use and melanoma risk. At present, there

is no convincing evidence that moderate/responsible solarium use increases melanoma risk.

The next section of this book covers various aspects of the molecular biology and photocarcinogenesis of skin cancer. In the next contribution entitled "Molecular Biology of Basal and Squamous Cell Carcinomas," Lars Boeckmann, Christine Martens, and Steffen Emmert explain that the prevalent keratinocyte-derived neoplasms of the skin are basal cell carcinoma and squamous cell carcinoma [4]. The authors point out that both so-called nonmelanoma skin cancers comprise the most common cancers in humans by far and that common risk factors for both tumor entities include sun exposure, DNA repair deficiencies leading to chromosomal instability, or immunosuppression [4]. Yet, the fundamental differences in the development of the two different entities have been and are currently unveiled. The constitutive activation of the sonic hedgehog signaling pathway by acquired mutations in the PTCH and SMO genes appears to represent the early basal cell carcinoma developmental determinant. Although other signaling pathways are also affected, small hedgehog inhibitory molecules evolve as the most promising basal cell carcinoma treatment options systemically as well as topically in current clinical trials. For squamous cell carcinoma development mutations in the p53 gene, especially UV-induced mutations have been identified as early events. Yet, other signaling pathways including epidermal growth factor receptor, RAS, Fyn, or p16INK4a signaling may play significant roles in squamous cell carcinoma development. The authors conclude that improved understanding of the molecular events leading to different tumor entities by the de-differentiation of the same cell type have begun to pave the way for modulating new molecular targets therapeutically with small molecules [4].

In Chap. 10 entitled "Human Papillomaviruses and Skin Cancer," Sigrun Smola explains that human papillomaviruses (HPVs) infect squamous epithelia and can induce hyperproliferative lesions [21]. More than 220 different HPV types have been characterized and classified into five different genera. While mucosal high-risk HPVs have a well-established causal role in anogenital carcinogenesis, the biology of cutaneous HPVs is less understood. From patients with epidermodysplasia verruciformis (EV), a rare genetic disorder, and animal models, evidence accumulated suggests that cutaneous PVs of genus β synergize with ultraviolet (UV) radiation in the development of cutaneous squamous cell carcinoma (cSCC). In 2009, the International Agency for Research on Cancer (IARC) classified the genus β-HPV types 5 and 8 as "possible carcinogenic" biological agents (group 2B) in EV. As Sigrun Smola further explains, epidemiological and biological studies indicate that genus β-PV infection may also play a role in UV-mediated skin carcinogenesis in non-EV patients [21]. However, they rather act at the early stages of carcinogenesis and become dispensable for the maintenance of the malignant phenotype, compatible with a "hit and run" mechanism. In summary, Sigrun Smola gives in this chapter an excellent overview on genus β-PV infections and discusses the similarities and differences between cutaneous and genus α mucosal high-risk HPV in epithelial carcinogenesis.

In Chap. 11 entitled "The Immune System and Pathogenesis of Melanoma and Nonmelanoma Skin Cancer," Kory P Schrom, InYoung Kim, and Elma D. Baron explain that tumor development is the result of genetic derangement and the inability to prevent unfettered proliferation [19]. The authors further point out that genetic derangements leading to tumorigenesis are variable, but the immune system plays a critical role in tumor development, prevention, and production. In this chapter, Kory P Schrom, InYoung Kim, and Elma D. Baron discuss the importance of the immune system as it relates to the development of skin cancer—both melanoma and non-melanoma skin cancers (NMSC). As the authors explain, the human immune system functions not only to protect us from pathogens but also to prevent tumor development and eradicate malignant cells. A complex interplay between the immune system, tumor cells, and molecular mediators dictates whether or not the immune system will be successful at this task. Kory P Schrom, InYoung Kim, and Elma

D. Baron explain that at this time, research has not uncovered a single sentinel event that leads to tumor evasion of the immune system and its subsequent proliferation, spread, and ultimately death of the host. Our current understanding of immunosuppression by UVR and cancer development in organ transplant recipients (OTRs) has allowed us to harness the immune system via employing immunotherapies to treat skin malignancies. The authors conclude that continued scientific research to expand our understanding of the immune system, its role in carcinogenesis and skin-cancer-related mutations, will continue to impact their approach and improve management of patients afflicted by cutaneous malignancies.

The following four chapters focus on the relevance of the vitamin D endocrine system for pathogenesis and progression of skin cancer. In Chap. 12 entitled "Protection from Ultraviolet Damage and Photocarcinogenesis by Vitamin D Compounds," Warusavithana Gunawardena Manori De Silva, Rebecca S. Mason, and coworkers explain that exposure of skin cells to UV radiation results in DNA damage, which if inadequately repaired, may cause mutations [6]. UV-induced DNA damage and reactive oxygen and nitrogen species also cause local and systemic suppression of the adaptive immune system. Together these changes underpin the development of skin tumors. The hormone derived from vitamin D, calcitriol (1,25-dihydroxyvitamin D_3), and other related compounds, working via the vitamin D receptor and at least in part through endoplasmic reticulum protein 57 (ERp57), reduce cyclobutane pyrimidine dimers and oxidative DNA damage in keratinocytes and other skin cell types after UV. Calcitriol and related compounds enhance DNA repair in keratinocytes, in part through decreased reactive oxygen species, increased p53 expression and/or activation, increased repair proteins, and in part through increased energy availability in the cell when calcitriol is present after UV exposure. There is mitochondrial damage in keratinocytes after UV. In the presence of calcitriol, but not vehicle, glycolysis is increased after UV, along with increased energy conserving

autophagy and changes consistent with enhanced mitophagy. Reduced DNA damage and reduced ROS/RNS should help reduce UV-induced immune suppression. Reduced UV-immune suppression is observed after topical treatment with calcitriol and related compounds in hairless mice. The authors conclude that these protective effects of calcitriol and related compounds presumably contribute to the observed reduction in skin tumor formation in mice after chronic exposure to UV, followed by topical postirradiation treatment with calcitriol and some, though not all, related compounds.

In Chap. 13 entitled "The Role of Classical and Novel Forms of Vitamin D in the Pathogenesis and Progression of Nonmelanoma Skin Cancers," Andrzej T. Slominski and coworkers explain that nonmelanoma skin cancers, including basal and squamous cell carcinomas (SCC and BCC), represent a significant clinical problem due to their relatively high incidence, imposing an economic burden to healthcare systems around the world [20]. It is accepted that ultraviolet radiation (UVR: $\lambda = 290 - 400$ nm) plays a crucial role in the initiation and promotion of BCC and SCC, with UVB ($\lambda = 290 - 320$ nm) having a central role in this process. On the other hand, UVB is required for vitamin D3 (D3) production in the skin, which supplies >90% of the body's requirement for this prohormone. Prolonged exposure to UVB can also generate tachysterol and lumisterol. Vitamin D3 and its canonical (1,25(OH)2D3) and noncanonical (CYP11A1-intitated) D3-hydroxyderivatives show photoprotective functions in the skin. These include regulation of keratinocytes proliferation and differentiation, induction of anti-oxidative responses, inhibition of DNA damage and induction of DNA repair mechanisms, and anti-inflammatory activities. The authors further explain that studies in animals have demonstrated that D3-hydroxyderivatives can attenuate UVB or chemically induced epidermal cancerogenesis and inhibit growth of SCC and BCC. Genomic and nongenomic mechanisms of action have been suggested. In addition, vitamin D3 itself inhibits hedgehog signaling pathways, which have been implicated in many cancers. Silencing of the

vitamin D receptor leads to increased propensity to develop UVB or chemically induced epidermal cancers. Other targets for vitamin D compounds include 1,25D3-MARRS, retinoic orphan receptors α and γ, arylhydrocarbon receptor, and Wnt signaling. Most recently, photoprotective effects of lumisterol hydroxyderivatives have been identified. Clinical trials demonstrated a beneficial role of vitamin D compounds in the treatment of actinic keratosis. The authors conclude that, in summary, recent advances in vitamin D biology and pharmacology open new exciting opportunities in chemoprevention and treatment of skin cancers [20].

In Chap. 14 entitled "The Vitamin D Receptor as Tumor Suppressor in Skin," Daniel D. Bikle explains that cutaneous malignancies, including melanomas and keratinocyte carcinomas (KC), are the most common types of cancer, occurring at a rate of over 1 million per year in the United States [3]. KCs, which include both basal cell carcinomas and squamous cell carcinomas, are substantially more common than melanomas and form the subject of this chapter. Ultraviolet radiation (UVR), both UVB and UVA, as occurs with sunlight exposure, is generally regarded as causal for these malignancies, but UVB is also required for vitamin D synthesis in the skin. Keratinocytes are the major cell in the epidermis. Daniel Bikle further explains that these cells not only produce vitamin D but contain the enzymatic machinery to metabolize vitamin D to its active metabolite, $1,25(OH)_2D$, and express the receptor for this metabolite, the vitamin D receptor (VDR). This allows the cell to respond to the $1,25(OH)_2D$ that it produces. Based on data reported in the literature, Daniel D. Bikle concludes that vitamin D signaling in the skin suppresses UVR-induced epidermal tumor formation. In this chapter, Daniel D. Bikle focusses on four mechanisms by which vitamin D signaling suppresses tumor formation. They are inhibition of proliferation/stimulation of differentiation with discussion of the roles of hedgehog, wnt/b-catenin, and hyaluronan/CD44 pathways in mediating vitamin D regulation of proliferation/differentiation, regulation of the balance between oncogenic and tumor suppressor long noncoding RNAs, immune regulation, and promotion of DNA damage repair (DDR).

In Chap. 15 entitled "Cancer Prevention in Skin and Other Tissues via Cross-Talk Between Vitamin D- and p53-SIGNALING," Jörg Reichrath and coworkers explain that vitamin D- and p53-signaling pathways have a significant impact on spontaneous or carcinogen-induced malignant transformation of cells [17]. The vitamin D receptor (VDR) and the p53/p63/p73 proteins (the p53 family hereafter) all function typically as receptors/sensors-that-turn-into-transcriptional-regulators-upon-stimulus, with the main difference being that the nuclear VDR is transcriptionally activated after binding its naturally occurring ligand 1,25-dihydroxyvitamin D with high affinity while the p53 clan, mostly in the nucleoplasm, responds to a large and still growing number of alterations in cell homeostasis, commonly referred to as stress. These authors point out that an increasing body of evidence now convincingly demonstrates a cross-talk between vitamin D- and p53 signaling that occurs at different levels, has genome-wide implications, and should be of high importance for many malignancies, including nonmelanoma skin cancer. One interaction involves the ability of p53 to regulate skin pigmentation. It has been shown that p53 upregulates skin pigmentation via POMC derivatives, including alpha-MSH and ACTH. Increased pigmentation protects the skin against UV-induced DNA damage and skin carcinogenesis but, on the other hand, reduces cutaneous synthesis of vitamin D. A second level of interaction may be through the ability of 1,25-dihydroxyvitamin D to increase the survival of skin cells after UV irradiation. UV irradiation-surviving cells show significant reductions in thymine dimers in the presence of 1,25-dihydroxyvitamin D that are associated with increased nuclear p53 protein expression and significantly reduced NO products. A third level of interaction is documented by the ability of vitamin D compounds to regulate the expression of the murine double minute (MDM2) gene in dependence of the presence of wild-type p53. MDM2 has a well-established role as a key negative regulator of p53 activity. Finally, p53 and its

family members have been implicated in the direct regulation of the VDR. In their overview, Reichrath et al. summarize some of the implications of the cross-talk between vitamin D- and p53- signalling for carcinogenesis in the skin and other tissues, focusing on a genome-wide perspective [17].

In Chap. 16 entitled "Sunlight, Vitamin D and Xeroderma Pigmentosum," Marie Christine Martens, Steffen Emmert, and Lars Boeckmann explain that sunlight, in particular UV-B radiation, is an important factor for endogenous vitamin D production as 80–90% of the required vitamin D needs to be photosynthesized in the skin [15]. The active form of vitamin D, vitamin D_3 or calcitriol, binds to the ligand-activated transcription factor vitamin D receptor (VDR) for genomic and nongenomic effects. Recently, calcitriol and analogs have been shown to have anti-proliferative effects in the mouse and human BCC and SCC cell lines in vitro. As UV radiation plays a critical role in the photosynthesis of vitamin D, stringent sun protection, as recommended for xeroderma pigmentosum (XP) patients, may impact their vitamin D levels. XP is a rare autosomal-recessive disorder with a worldwide prevalence of 1 in 1,000,000. XP can be divided into seven different complementation groups: XP-A to XP-G. The complementation groups correspond with the underlying gene defect. Defects in these genes lead to a defective nucleotide excision repair (NER), which is necessary to remove UV-induced DNA damage such as the UV photoproducts cyclobutane pyrimidine dimers (CPD) and 6-pyrimidine-4-pyrimidone dimers (6-4 PP). Additionally, a variant form with a mutation in the translational polymerase η gene (*PolH*), also called XP variant (XPV), exists. Patients with XPV show a defect in translesional synthesis. Due to their inability to repair UV-induced lesions, XP patients exhibit an increased risk for UV-induced nonmelanoma skin cancer (NMSC), such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) as well as melanoma. The authors conclude that although no curative therapy for XP exists today, numerous options for the treatment and prophylaxis of skin cancer have become available.

In Chap. 17 entitled "Update: Solar UV-Radiation, Vitamin D and Skin Cancer Surveillance in Organ Transplant Recipients (OTRs)," Roman Saternus, Thomas Vogt, and Jörg Reichrath explain that although great progress has been achieved during the last decades, the clinical management of organ transplant recipients (OTRs) remains a challenge [18]. OTRs need in general lifelong immunosuppressive therapy, which is associated with an increased risk to develop skin cancer, and with an unfavorable clinical outcome of these malignancies. Skin cancer prevention measures, including regular full-body examinations, are therefore necessary in OTRs to detect and treat suspicious lesions at an early stage. The frequency of aftercare depends on the individual risk factors of the patient. Patients should apply consistent sun protection with sunscreens and clothing, as well as a monthly self-examination. On the other hand, the need of UVR avoidance increases the risk of vitamin D deficiency, which itself is associated with an increased risk for many diseases, including malignancies. OTRs should therefore be monitored for 25(OH)D status and/or should take vitamin D supplements. The authors conclude that it has to be emphasized that an interdisciplinary approach, coordinated by the transplant center, which includes regular skin examinations by a dermatologist, is needed to ensure the best care for the OTRs.

The next section focusses on the risks and benefits of sunscreens. In Chap. 18 entitled "Sunscreens in the United States: Current Status and Future Outlook," Katherine S. Glaser* and Kenneth J. Tomecki explain that incidence rates of nonmelanoma skin cancer and melanoma have been on the rise in the United States for the past 25 years [8]. UV radiation (UVR) exposure remains the most preventable environmental risk factor for these cancers. Aside from sun avoidance, sunscreens continue to provide the best alternative protection. UVR directly damages DNA and causes indirect cellular damage through the creation of reactive oxygen species, the sum of which leads to cutaneous immunosuppression

and a tumorigenic milieu. The current generation of sunscreens protects from UVR through two main mechanisms: absorption and deflection. In the United States, the Food and Drug Association (FDA) regulates sunscreen products, which are considered over-the-counter drugs. With the release of new FDA testing and labeling requirements in 2011 and the enactment of the Sunscreen Innovation Act in 2014, sunscreen manufacturers are now required to evaluate their products not only on the sun protection factor (SPF) but also on broad spectrum UVA protection. The *American Academy of Dermatology Association* and the *American Academy of Pediatrics* have provided specific recommendations for proper sun protection and sunscreen usage with the continual goal of increasing public awareness and compliance with appropriate sun protective measures. Antioxidants, photolyases, and plant polyphenols remain an interesting avenue of research as additives to sunscreens or stand-alone topical or oral products that appear to modulate the immunosuppressive effects of UVR on the skin. The authors conclude that additionally, although UVR induces endogenous cutaneous production of vitamin D, its damaging effects overshadow this positive benefit, especially in light of the ease of achieving recommended amounts of vitamin D through diet and supplementation.

In Chap. 19 entitled "A Handful of Sunscreen for Whole Body Application," Ida M. Heerfordt, Peter A. Philipsen, and Hans Christian Wulf explain that the rule of thumb: "Fill up a handful of sunscreen and spread it all over your body" has been used in several sun-safety campaigns [10]. The intention was to increase the applied sunscreen to obtain a quantity of 2 mg/cm^2 to all accessible skin. The present study is the first to investigate how this advice works in practice, evaluated by the quantity of sunscreen applied and the amount of covered skin. Methods: seventeen volunteers wearing swimwear were asked to: "Fill up a handful and spread it all over your body". Before and after sunscreen application, the volunteers were photographed in black light.

As sunscreen absorbs black light, the darkness of the skin increases with increasing amounts of applied sunscreen, making it possible to identify skin left without coverage. The sunscreen container was weighed before and after to quantify the amount of sunscreen applied. Results: A median of 21% of the accessible skin was left completely without coverage. The 79% covered area was covered with a median of 1.12 mg/cm^2, not the expected 2 mg/cm^2. The authors conclude that in practice, the advice: "Fill up a handful of sunscreen and spread it all over your body" led to a better, but still modest, protection, compared to the intended effect.

In Chap. 20 entitled "Ultraviolet Exposure Scenarios: Balancing Risks of Erythema and Cutaneous Vitamin D Synthesis," Ann R. Webb and Ola Engelsen explain that exposure to sunlight is a major source of vitamin D for most people [23]. Yet public health advice has focused overwhelmingly on avoiding exposure of unprotected skin because of the risks of erythema and skin cancer. Given that there are also health risks associated with low vitamin D status, they explore the possibilities of achieving a range of targets associated with vitamin D and the accompanying erythema risk. They have calculated the exposure required to gain a number of proposed oral-equivalent doses of vitamin D, as functions of latitude, season, skin type, and skin area exposed, together with the associated risk of erythema, expressed in minimum erythema doses. The model results show that a recommended daily intake of 400 IU is readily achievable through casual sun exposure in the midday lunch hour, with no risk of erythema, for all latitudes some of the year, and for all the year at some (low) latitudes. Ann R. Webb and Ola Engelsen also show that such daily, sub-erythemal doses at lunchtime during the summer months is sufficient to avoid winter-time vitamin D deficiency for the UK all-weather climate, provided that lower arms and legs are exposed in the warmer months. At the higher proposed vitamin D dose of 1000 IU, lunchtime sun exposure is still a viable route to the vitamin,

but requires the commitment to expose greater areas of skin, and is effective for a shorter period of the year. The highest vitamin D requirement considered was 4000 IU per day. For much of the globe, and much of the year, this is not achievable in a lunchtime hour, and where it is possible large areas of skin must be exposed to prevent erythema. When the only variable considered was skin type, latitudinal and seasonal limits on adequate vitamin D production were more restrictive for skin type 5 than skin type 2.

In Chap. 21, the last contribution entitled "The Paleolithic Nutrition Model in Relation to Ultraviolet Light and Vitamin D," Reinhold Vieth explains that recent years have seen multiple debates as to what dietary policy should target in terms of circulating levels of 25-hydroxyvitamin D (25(OH)D [22]. He explains that dietary guidelines follow risk-benefit profiles. Reinhold Vieth further points out that the starting point for nutrition policy makers is intakes and levels of nutrient that are typical of people who are regarded as generally healthy. In essence, the perspective is to assume that status that is endemic is the starting point, and that any nutrition or policy requires evidence to motivate any change from that starting point. The purpose of Reinhold Vieth's excellent article is to present a more biologically based perspective. As he points out, Paleolithic nutrition has focused on foods consumed, but the Paleolithic model extends beyond diet to incorporate environment, which is equally relevant to health policies in the context of sunlight exposure and vitamin D nutrition. Biologically based thinking starts from the basic premise, that disease risk may have an evolutionary underpinning and that modern human cultures and environments are probably not substitute for what is natural or optimal. Reinhold Vieth further points out that natural selection is a process that optimizes the matching of the genome for fitness to reproduce. But the environmental stresses due to latitude, clothing, and sun avoidance relate to many aspects of human health, disease and mortality. The traditional perspective of policy makers has been to adhere to extant norms, unless the evidence is overwhelming, that more sun or more vitamin D intake produces a benefit. The alternative perspective merits attention, namely, one should consider that what is optimal for human health should start from the original environment and culture of the first humans. Reinhold Vieth concludes that the sun exposure experienced by the original humans should be regarded optimal and that it is reasonable to reverse the traditional approach of policy groups to ask at what point human health suffers from diminishing exposure to sunshine and vitamin D. He further asks why there are no double-blind placebo-controlled trials of such environmental deprivation. There is no level 1 medical evidence supporting any degree of deprivation.

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UV-Induced Cutaneous Synthesis of Vitamin D and the Physiologic Consequences (I)

Sunlight, UV Radiation, Vitamin D, and Skin Cancer: How Much Sunlight Do We Need?

Michael F. Holick

Abstract

Vitamin D is the sunshine vitamin for good reason. During exposure to sunlight, the ultraviolet B photons enter the skin and photolyze 7-dehydrocholesterol to previtamin D_3 which in turn is isomerized by the body's temperature to vitamin D_3. Most humans have depended on sun for their vitamin D requirement. Skin pigment, sunscreen use, aging, time of day, season, and latitude dramatically affect previtamin D_3 synthesis. Vitamin D deficiency was thought to have been conquered, but it is now recognized that more than 50% of the world's population is at risk for vitamin D deficiency. This deficiency is in part due to the inadequate fortification of foods with vitamin D and the misconception that a healthy diet contains an adequate amount of vitamin D. Vitamin D deficiency causes growth retardation and rickets in children and will precipitate and exacerbate osteopenia, osteoporosis and increase risk of fracture in adults. The vitamin D deficiency pandemic has other serious consequences including increased risk of common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease. There needs to be a renewed appreciation of the beneficial effect of moderate sensible sunlight for providing all humans with their vitamin D requirement for health.

Prehistorical Historic Perspective

The major source of vitamin D for most land vertebrates, including humans, comes from exposure to sunlight. From a prehistoric perspective, some of the earliest unicellular organisms that evolved in the oceans including phytoplankton produced vitamin D when exposed to sunlight [1, 2]. Vertebrates that evolved in the ocean took advantage of their high calcium environment and used it effectively for developing a mineralized endoskeleton. When vertebrates ventured onto land, they needed to adapt to the calcium poor environment by increasing their efficiency for intestinal absorption of dietary

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calcium. They took with them the ability to photosynthesize vitamin D_3 in their skin which became essential for enhancing intestinal calcium absorption and maintaining serum calcium levels in most land vertebrates including homosapiens [1, 2].

In the mid-1600s, Whistler and Glissen reported that children living in industrialized cities in Great Britain had short statute and deformities of their skeleton especially their lower legs [3]. This scourge of the industrialization of Europe and North America persisted for more than 250 years. Even though Sniadecki [4] suggested in 1822 that the most likely reason for why his young patients who lived in Warsaw had a high incidence of rickets while the children whom he cared for living in the countryside did not was due to lack of sun exposure. It would take 100 years to appreciate this insightful observation. Palm in 1889 [5] also recognized that "sunbathing" was important for preventing rickets based on reports from his colleagues who saw children living in the most squalid conditions in India and Asia who were not afflicted with rickets whereas it was epidemic in the industrialized cities in Great Britain. By the turn of the twentieth century, upwards of 90% of children living in Leyden, The Netherlands, and in Boston and New York City were afflicted with this bone deforming disease and suffered its long-term consequences. In 1903, Finsen received the Nobel Prize for his insightful observations that exposure to sunlight cured a variety of diseases including lupus vulgaris (skin infected with tuberculosis) [6]. Finally, in 1919, Huldschinski [7] reported that exposure of children to radiation with a mercury arc lamp was an effective means of treating rickets. This quickly followed by the observation of Hess and Unger [8] that exposure of children to sunlight on the roof of a New York City Hospital was an effective means of treating rickets.

The recognition that exposure of both people and animals to ultraviolet radiation was effective in preventing and treating rickets prompted Hess and Weinstock [9] and Steenbock and Black [10]

to irradiate with ultraviolet radiation a wide variety of substances including lettuce, grasses and corn, olive and cotton seed oils. Before the irradiation, none of the substances had antirachitic activity, but after the irradiation, they were effective in preventing rickets in rodents. It was also known at that time that cod liver oil was an effective method for preventing and treating rickets, and it was Park [11] who demonstrated that rachitic rats could be cured of their bone disease by either cod liver oil or by ultraviolet irradiation suggesting that the two were related. Steenbock [12] appreciated the practical benefit of these observations when he reported that the irradiation of cow's milk imparted antirachitic activity, and, thus, would be an ideal way of preventing rickets in children.

By the early 1930s, it was appreciated throughout Europe and in the northeastern United States that exposing children to sensible and adequate sunlight without causing sunburn was an effective method of preventing rickets in children. The United States set up an agency in the US Government that promoted sensible sun exposure to parents as a means of preventing their children from developing rickets [3, 13].

Photoproduction of Vitamin D_3

When the skin is exposed to sunlight, the ultraviolet B radiation (UVB) that is able to penetrate through the ozone layer with energies 290–315 nm (Fig. 2.1) is absorbed by 7-dehydrocholesterol in the epidermis and dermis [2, 14, 15]. This absorption causes the double bonds to be excited causing the B-ring to open making the rigid steroid structure into a more flexible molecule known as previtamin D_3 (Fig. 2.2). Previtamin D_3 exists into conformations. It is the thermodynamically less favorable cis, cis form that converts to vitamin D_3. Thus, when previtamin D_3 was made in an isotropic organic solution such as hexane or ethanol, it would take several days for it to convert to vitamin D_3 at 37 ° C. To enhance the thermal-

Fig. 2.1 Action spectrum of 7-dehydrocholesterol to previtamin D_3 conversion in human skin. (Holick copyright 2007 with permission)
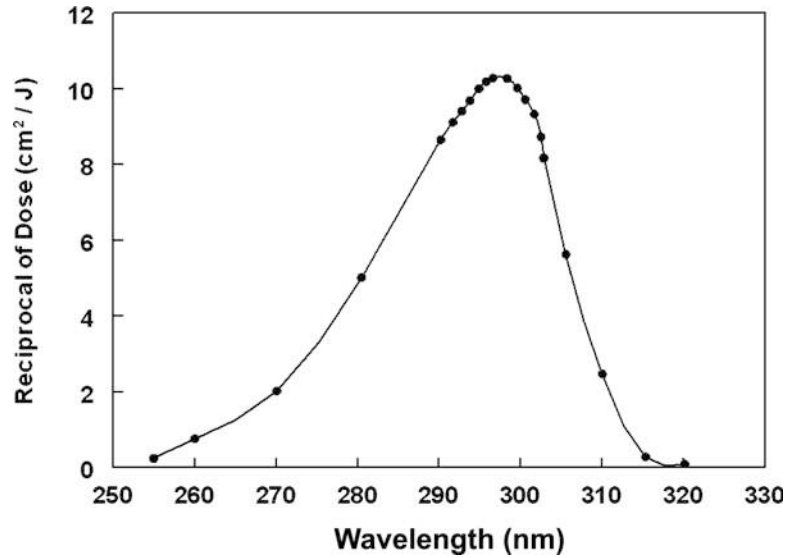
induced isomerization of previtamin D_3 to vitamin D_3, 7-dehydrocholesterol is incorporated within the fatty acid hydrocarbon side chain and polar head group of the triglycerides in the plasma membrane. When exposed to sunlight, 7-dehydrocholesterol is efficiently converted to the cis, cis conformer which rapidly isomerizes to vitamin D_3 (Fig. 2.2). Vitamin D_3 is ejected out of the plasma membrane into the extracellular space where it enters the dermal capillary bed bound to the vitamin D binding protein [16].

There has been a lot of debate as to whether dietary vitamin D_3 is equivalent to vitamin D_3 made in the skin. Although both have the same biologic activity once they are metabolized, the half-life of vitamin D_3 produced in the skin is prolonged in the circulation in part because 100% is bound to the vitamin D binding protein whereas when vitamin D_3 is ingested, only about 60% is bound to the vitamin D binding protein, and 40% is rapidly cleared in the lipoprotein bound fraction [17]. Other explanations include the additional time it takes for previtamin D_3 to isomerize to vitamin D_3 and the slow gradual diffusion of the vitamin D_3 from the epidermis into the dermal capillary bed.

Factors Controlling Cutaneous Vitamin D Synthesis

Melanin evolved as a sunscreen that absorbed UVB and ultraviolet A (390–400 nm) radiation protecting the UV absorbing macromolecules including DNA, RNA, and proteins from the damaging effects from excessive exposure to UVR. However, as people migrated north and south of the equator, they needed to quickly mutate their skin pigment gene in order to have the ability to make enough vitamin D to sustain their calcium and bone metabolism [18]. This is supported by the observation that Neanderthals had a mutation of their melanocyte-stimulating hormone receptor resulting in them being red-headed and having Celtic-like fair skin which would have facilitated the production of vitamin D3 when they migrated into Europe [19].

Melanin is so efficient in absorbing UVB radiation that it markedly reduces the cutaneous photosynthesis of vitamin D_3. The dark melanin pigment of Africans and African Americans with skin types 5 and 6 (never burns, always tans) is so efficient in absorbing UVB radiation that it reduces the capacity of the skin to produce
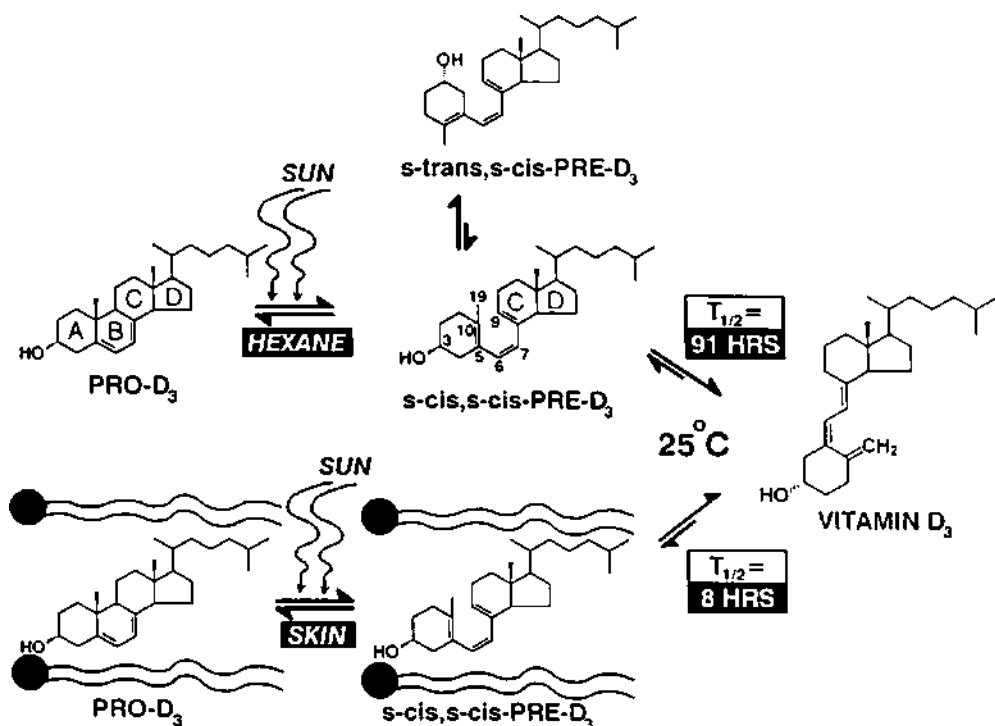
Fig. 2.2 Photolysis of provitamin D_3 (pro-D_3; 7-dehydrocholesterol) into previtamin D_3 (pre-D_3) and its thermal isomerization to vitamin D_3 in hexane and in lizard skin. In hexane is pro-D_3 photolyzed to *s-cis,s-cis*-pre-D_3. Once formed, this energetically unstable conformation undergoes a conformational change to the *s-trans*, *s-cis*-pre-D_3. Only the *s-cis,s-cis*-pre-D_3 can undergo thermal isomerization to vitamin D_3. The *s-cis,s-cis* conformer of pre-D_3 is stabilized in the phospholipid bilayer by hydrophilic interactions between the 3β-hydroxyl group and the polar head of the lipids, as well as by the van der Waals interactions between the steroid ring and side-chain structure and the hydrophobic tail of the lipids. These interactions significantly decrease the conversion of the *s-cis,s-cis* conformer to the *s-trans,s-cis* conformer, thereby facilitating the thermal isomerization of *s-cis,s-cis*-pre-D_3 to vitamin D_3. (Holick copyright 2013 with permission)

previtamin D_3 by 95–99% when compared to a Caucasian with skin type 2 (always burns, sometimes tans) [20].

The application of a sunscreen with a sun protection factor of 30 absorbs approximately 97.5% of UVB radiation, and, thus, reduces the skin's capacity to produce previtamin D_3 by 97.5% [21]. The angle at which the sun's rays hit the earth's surface has a dramatic effect on the cutaneous production of previtamin D_3. As the angle of the sun becomes more oblique to the earth's surface, the UVB photons have to travel a longer path through ozone which efficiently absorbs them. Thus, season, latitude, time of day as well as weather conditions dramatically affect the cutaneous production of previtamin D_3 [22]

(Fig. 2.3). Living above and below approximately 35° latitude, children and adults are able to produce an adequate amount of vitamin D_3 in their skin during the spring, summer, and fall. However, essentially all of the UVB photons are absorbed during the winter months, thus, either completely eliminating or markedly reducing the capacity of the skin to produce vitamin D_3. This is the explanation for why there is a seasonal variation in circulating levels of 25-hydroxyvitamin D_3 [25(OH)D] which is considered to be the major circulating form of vitamin D [23–25] (Fig. 2.4). Similarly, early in the morning and late in the afternoon, the sun's rays are more oblique, and as a result, most of if not all of the UVB photons are absorbed by the ozone layer.
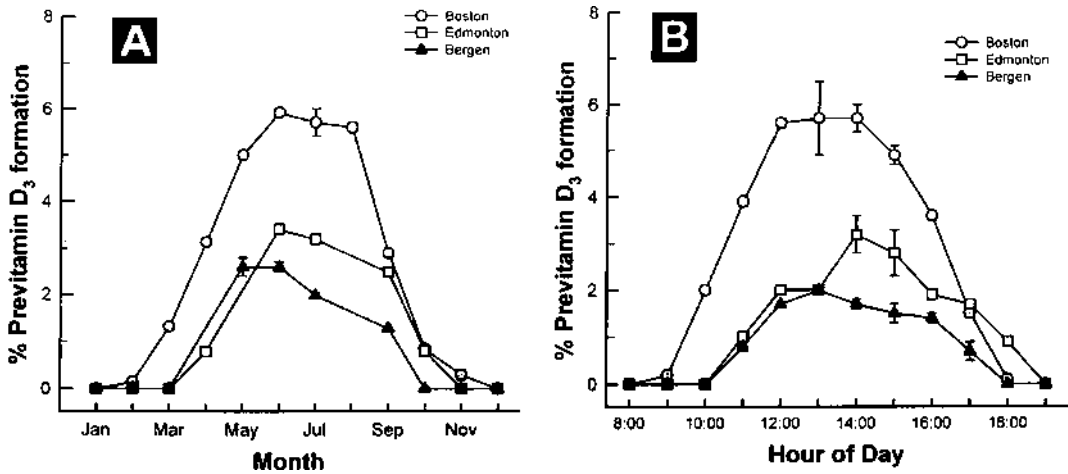
Fig. 2.3 Influence of season, time of day in July, and latitude on the synthesis of previtamin D_3 in Boston (42°N) -○-, Edmonton (52°N) -▯-, Bergen (60°) -▲-. The hour is the end of the 1 h exposure time in July. (Holick copyright 2007 with permission)

Thus, even in the summer in the early morning and late afternoon, little, if any, vitamin D_3 is produced in the skin (Fig. 2.3).

Sources and Metabolism of Vitamin D

The major source of vitamin D (D represents D_2 or D_3) for most humans is exposure to sunlight. Very few foods naturally contain vitamin D. These include oily fish such as salmon, cod liver oil which contains vitamin D_3 and sun-dried mushrooms which contains vitamin D_2 [25]. Although it was thought that vitamin D_3 was 2–3 times more effective in raising blood levels of 25(OH)D compared to the same dose of vitamin D_2, a recent study found that physiologic doses of vitamin D_2 are equally as effective as vitamin D_3 not only in maintaining circulating levels of 25(OH)D but also circulating levels of the active form 1,25-dihydroxyvitamin D [1,25 (OH) $_2$D] [26]. Some foods are fortified with vitamin D including milk and some juice products in the United States and Canada, and some breads, margarines, and cereals in the United States, Canada, and Europe. Sweden and Finland fortify milk with vitamin D_3 and India now

permits the fortification of milk and cooking oil with vitamin D_2 [26]. Typically there is 100 IU (10 micrograms) of vitamin D in a serving such as 8 ounces of milk or orange juice [25].

Once vitamin D is made in the skin or ingested from the diet, it must be metabolized in the liver to 25(OH)D [24, 25, 28] (Fig. 2.5). The metabolite is biologically inactive, however, it is the major circulating form of vitamin D that is used by physicians to determine a patient's vitamin D status. 25(OH)D undergoes an obligate hydroxylation by the 25-hydroxyvitamin D-1-α-hydroxylase (CYP27B1; 1-OHase) in the kidneys to form the biologically active form 1,25(OH)$_2$D. 1,25(OH)$_2$D, a steroid-like hormone, interacts with its nuclear vitamin D receptor (VDR) in target tissues including the small intestine, osteoblasts in bone, and in the renal tubular cells in the kidneys. 1,25(OH)$_2$D is responsible for the maintenance of calcium and phosphate homeostasis and bone health by increasing the efficiency of intestinal calcium and phosphate absorption, stimulating osteoblast function and increase bone calcium resorption. It also enhances the tubular resorption of calcium in the kidneys [24, 25, 28] (Fig. 2.5).
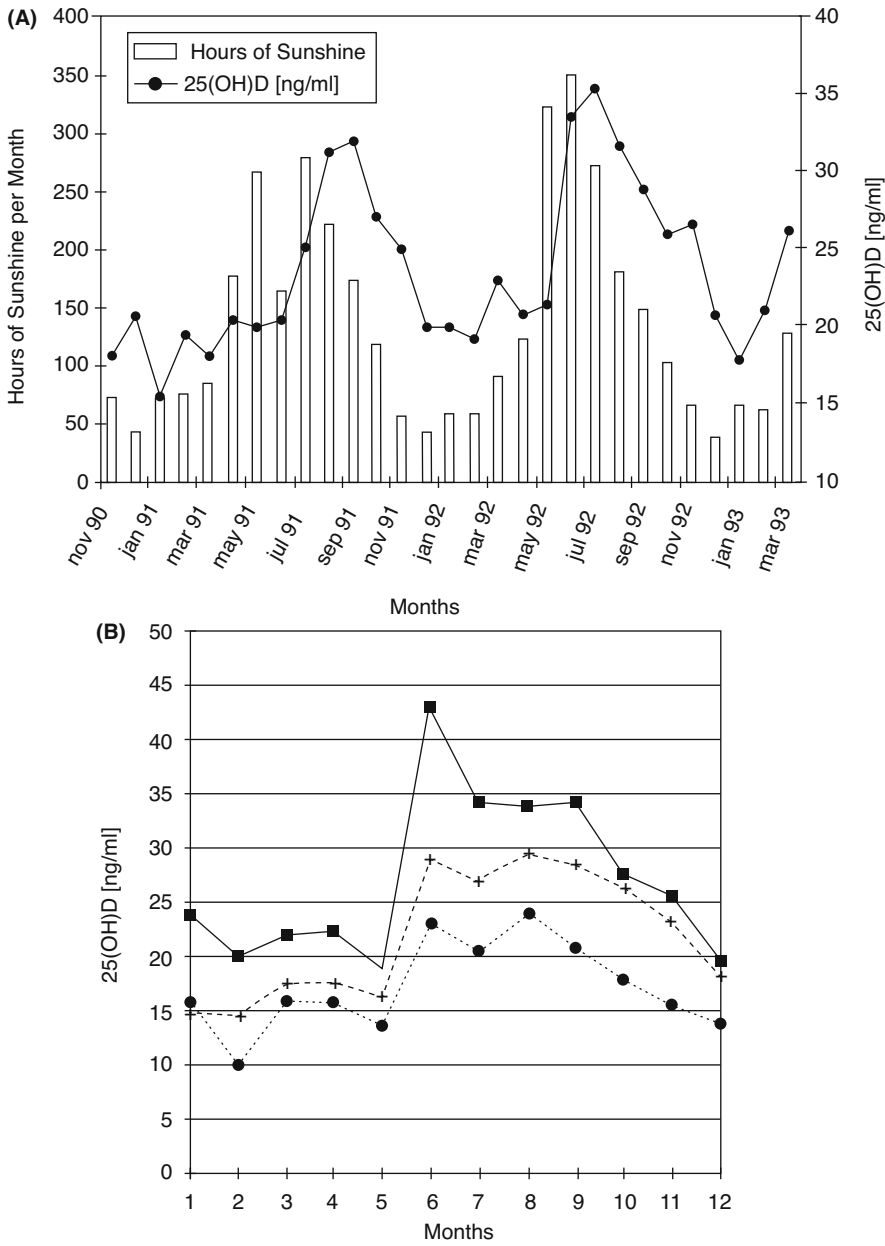
Fig. 2.4 (**a**) Relationship between hours of sunshine and serum 25(OH)D. ■ Hours of sunshine; ● 25(OH)D (ng/ml). (**b**) Seasonal fluctuation of serum 25(OH)D according to frequency of sun exposure. ■ Regular sun exposure; ◆ Occasional sun exposure; ● Avoiding direct sun exposure. (Holick copyright 2013)

$1,25(OH)_2D$ is such a potent regulator of calcium metabolism that in order to control its own actions, it induces its own destruction by enhancing the expression of the 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) [24, 25, 28]. CYP24A1 causes oxidation on carbons 24 and 23 leading to the formation of a C23 acid known as calcitroic acid. This water-soluble inactive metabolite is excreted in the bile (Fig. 2.5).

Role of Vitamin D in the Prevention of Chronic Diseases

Most tissues and cells in the body including brain, skin, breast, prostate, colon, and activated T and B lymphocytes possess a VDR [24, 25, 28–31]. It is now recognized that $1,25(OH)_2D$ is one of the most potent hormones for regulating cell growth and maturation. It is estimated that more than 2000 genes are either directly or indirectly influenced by $1,25(OH)_2D$ [30–32].

There have been numerous studies that have implicated living at higher latitudes and being at increased risk of vitamin D deficiency with many serious and chronic and deadly diseases including cancers of the colon, prostate and breast, autoimmune diseases including multiple sclerosis, type I diabetes and rheumatoid arthritis, infectious diseases including tuberculosis and influenza and hypertension and heart disease [24, 25, 28–50].

What has been perplexing is the fact that exposure to sunlight results in an increase of circulating levels of $25(OH)D$ but not $1,25(OH)_2D$. The reason is that parathyroid hormone, calcium and phosphorus and fiberblast growth factor 23 tightly control the production of $1,25(OH)_2D$ in the kidneys [25, 28] (Fig. 2.5). Since $25(OH)D$ is incapable of altering vitamin D responsive gene expression at physiologic concentrations, there needed to be another explanation for the sunlight-vitamin D health connection.

It has been recognized for more than 30 years that activated macrophages, placenta, and skin expressed the 1-OHase [24, 25, 51–59]. In the late 1990s, there were numerous reports of various cell culture systems that expressed the 1-OHase that were capable of converting $25(OH)D_3$ to $1,25(OH)_2D_3$ including colon, prostate, breast, and lung cell cultures [53–57]. It was also observed that normal prostate cells obtained from prostate biopsies and both normal and colon cancer cells obtained at the time of surgery expressed the 1-OHase and had the capacity to make $1,25(OH)_2D$ [54]. These observations have led to the hypothesis that by raising blood levels of $25(OH)D$, there is enough substrate for many tissues and cells in the body that express the 1-OHase to produce locally $1,25(OH)_2D$. It is believed that the local production of $1,25(OH)_2D$ is important for regulating cell growth and maturation, and, thus, is able to prevent cells from becoming malignant. $1,25(OH)_2D_3$ accomplishes this by either restoring the cell to its normal proliferative state or by inducing its death by apoptosis. If the cell becomes malignant, an additional strategy for $1,25(OH)_2D$ is to inhibit angiogenesis to the malignant cells [58].

$1,25(OH)_2D$ locally produced by macrophages is important for innate immunity in humans. $1,25(OH)_2D$ enhances the production of the bacteriocidal protein cathelicidin which was shown to be ineffective in killing effective agents including Microbacterium tuberculosis [48]. $1,25(OH)_2D$ is also an effective immunomodulator which may be the explanation for why the local production of $1,25(OH)_2D$ by activated macrophages that is released locally and paracrine fashion to modulate lymphocyte activity [25] may be important for reducing the risk of developing multiple sclerosis, rheumatoid arthritis, and Crohn's disease (Fig. 2.5) [25, 28]. In addition, $1,25(OH)_2D$ enhances the production of insulin, and, thus, may play an important role in type II diabetes [59] and metabolic syndrome [60] and inhibits the production of renin [61] which is important for blood pressure regulation.

Vitamin D Deficiency Pandemic

It is estimated that one billion people worldwide are at risk of vitamin D deficiency [25]. Upwards of 30–50% of both children and adults in the United States, Europe, South America, Middle East, and Far East are at risk [24–28, 62–77]. The major cause for this pandemic is the lack of appreciation of the beneficial effect of sunlight in producing vitamin D [24, 28]. In the sunniest areas of the world, vitamin D deficiency is common because of lack of adequate sun exposure [27, 73–75].

It has been previously thought that the adequate intake for vitamin D to satisfy the body's
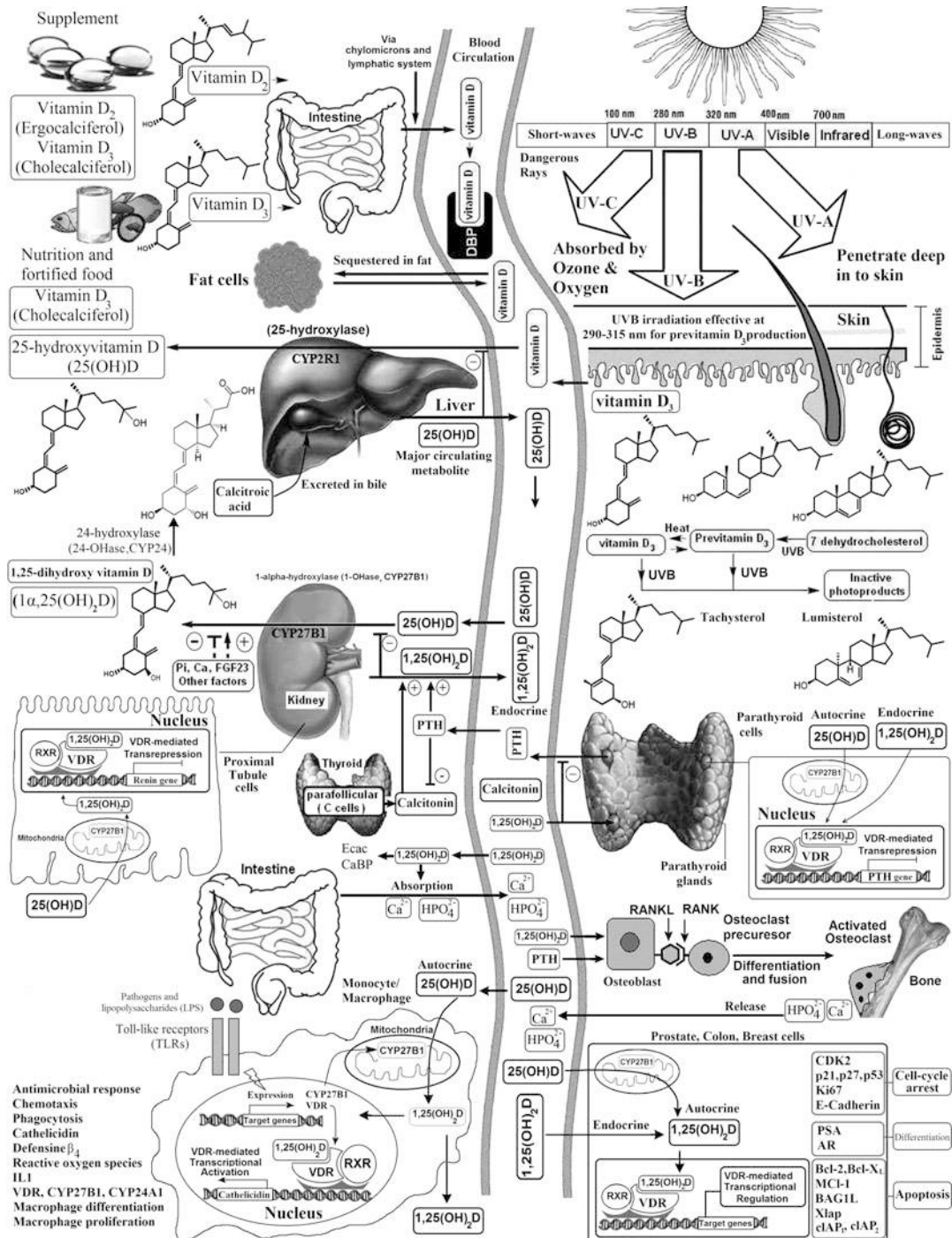
Fig. 2.5 Schematic representation of the synthesis and metabolism of vitamin D for skeletal and non-skeletal function. During exposure to sunlight, 7-dehydrocholesterol in the skin is converted to previtamin D_3. Previtamin D_3 immediately converts by a heat-dependent process to vitamin D_3. Excessive exposure to sunlight degrades previtamin D_3 and vitamin D3 into inactive photoproducts. Vitamin D_2 and vitamin D_3 from dietary sources are incorporated into chylomicrons, transported by the lymphatic system into the venous circulation. Vitamin D (D represents D_2 or D_3) made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D-binding protein(DBP), which transports it to the liver, where vitamin D is converted by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D [25(OH)D]. This is the major circulating form of vitamin D that is used by

requirement was 200 IU for all children and adults up to the age of 50 years, 400 IU for adults 51–70 years, and 600 IU of vitamin D for adults over the age of 70 [78]. In 2010 the Institute of Medicine (IOM; National Academy of Medicine) recommended that infants, children, adults up to the age of 70, and adults over the age of 70 required 400, 600, 600, and 800 IUs of vitamin D daily respectively [79]. After a careful review of the literature the committee for the Endocrine Society's Practice Guidelines on Vitamin D recommended that to treat and prevent vitamin D deficiency infants should receive 400–1000 IUs daily, children 1 year and older 600–1000 IUs daily, and adults 1500–2000 IUs

daily. For obese adults the recommendation was to increase intake by two to threefold because vitamin D is fat soluble and is diluted in the body fat and less bioavailable [67]. The IOM defined vitamin D deficiency, insufficiency and sufficiency with the measurement of serum 25 (OH)D of <12 ng/mL, 12–19 ng/mL, and 20 and greater ng/mL respectively [79]. The Endocrine Society recommended that vitamin D deficiency, insufficiency, and sufficiency for maximum bone health should relate to blood levels of 25(OH)D of >20 ng/mL, 21–29 ng/mL, and 30–100 ng/mL respectively. In addition, The Endocrine Society considered the UL (upper level causing no harm) for vitamin D for infants, children, and adults to

←

Fig. 2.5 (continued) clinicians to measure vitamin D status (although most reference laboratories report the normal range to be 20–100 ng/ml, the preferred healthful range is 30–60 ng/ml). It is biologically inactive and must be converted in the kidneys by the 25-hydroxyvitamin D-1a-hydroxylase (1-OHase) to its biologically active form 1,25-dihydroxyvitamin D [1,25(OH)$_2$D]. 1,25(OH)$_2$D$_3$ is then taken up by target cells and targeted to intracellular D-binding proteins (IDBP) to mitochondrial 24-hydroxylase or to the vitamin D receptor (VDR). The 1,25(OH)$_2$D$_3$-VDR complex heterodimerizes with the retinoic acid receptor (RXR) and binds to specific sequences in the promoter regions of the target gene. The DNA bound heterodimer attracts components of the RNA polymerase II complex and nuclear transcription regulators. Serum phosphorus, calcium fibroblast growth factors (FGF-23), and other factors can either increase or decrease the renal production of 1,25(OH)2D. 1,25(OH)$_2$D feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone (PTH) in the parathyroid glands. 1,25(OH)$_2$D increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize 1,25(OH)$_2$D to the water-soluble, biologically inactive calcitroic acid, which is excreted in the bile. 1,25(OH)$_2$D enhances intestinal calcium absorption in the small intestine by stimulating the expression of the epithelial calcium channel (ECaC) and the calbindin 9 K (calcium-binding protein, CaBP). 1,25(OH)$_2$D is recognized by its receptor in osteoblasts, causing an increase in the expression of the receptor activator of the NF-kB ligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL, which induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. Adequate calcium and phosphorus levels promote the mineralization of the skeleton. Autocrine metabolism of 25(OH)D; when a macrophage or monocyte is stimulated through its toll-like receptor 2/1 (TLR2/1) by an infectious agent such as Mycobacterium tuberculosis or its lipopolysaccharide, the signal upregulates the expression of VDR and 1-OHase. A 25(OH)D level of 30 ng/ml or higher provides adequate substrate for 1-OHase to convert 25(OH)D to 1,25(OH)$_2$D in mitochondria. 1,25(OH)$_2$D travels to the nucleus, where it increases the expression of cathelicidin, a peptide capable of promoting innate immunity and inducing the destruction of infectious agents such as M. tuberculosis. It is also likely that the 1,25(OH)$_2$D produced in monocytes or macrophages is released to act locally on activated T lymphocytes, which regulate cytokine synthesis, and activated B lymphocytes, which regulate immunoglobulin synthesis. When the 25(OH)D level is approximately 30 ng/ml, the risk of many common cancers is reduced. It is believed that the local production of 1,25(OH)$_2$D in the breast, colon, prostate, and other tissues regulates a variety of genes that control proliferation, including p21 and p27, as well as genes that inhibit angiogenesis and induce differentiation and apoptosis. Once 1,25(OH)$_2$D completes the task of maintaining normal cellular proliferation and differentiation, it induces expression of the enzyme 24-OHase, which enhances the catabolism of 1,25(OH)$_2$D to the biologically inert calcitroic acid. Thus, locally produced (autocrine) 1,25(OH)$_2$D does not enter the circulation and has no influence on calcium metabolism. The parathyroid glands have 1-OHase activity, and the local production of 1,25(OH)2D inhibits the expression and synthesis of parathyroid hormone. The 1,25(OH)$_2$D produced in the kidney enters the circulation and can downregulate rennin production in the kidney and stimulate insulin secretion in the beta islet cells of the pancreas. (Holick copyright 2013 with permission)

be 1000, 2000, and 10,000 IUs daily [67]. There has been concern about vitamin D toxicity which can cause hypercalcemia and hyperphosphatemia resulting in cardiovascular calcification and nephrocalcinosis. Vitamin D toxicity is one of the rarest medical conditions and is caused by intentional or accidental ingestion of huge amounts of vitamin D for a significant period of time, i.e., several 100,000 IUs daily for more than 6 months [25, 67, 80]. It is now recognized by many professional medical and nutrition organizations that a 25(OH)D should be at least 30 ng/mL not only for maximum bone health but also to provide the full benefits of vitamin D for overall health and welfare [24, 25, 27, 28, 67].

When considering how much vitamin D we all require it is worthwhile to consider what our hunter-gatherer forefathers were obtaining from daily sun exposure. To get some insight as to what their blood levels likely were, a study in adults was conducted in Maasai herders and Hadzabe bands who lived 2–4° South of the equator in Tanzania and who were outdoors exposed to equatorial sunlight every day. The overall mean concentration of 25(OH)D was 46 ng/mL [81]. Another study determined the amount of daily vitamin D intake required to maintain adequate vitamin D levels in human breast milk to satisfy the infant's requirement. It is well established that human breast milk contains very little if any vitamin D. From an evolutionary perspective this makes little sense. When lactating women received 6000 IUs of vitamin D daily they were able to add enough vitamin D in their milk to satisfy their infant's requirement [82, 83]. This suggests that the hunter-gatherer lactating women exposed to sunlight on a daily basis were making several thousand IUs of vitamin D a day; enough to satisfy their infant's requirement. It is known that once the serum 25(OH)D level reaches 20 ng/mL it takes approximately 100 IUs of vitamin D daily to raise the blood level by approximately 1 ng/mL [25, 67]. When healthy adults in Boston who had a mean 25(OH)D level of 22 ng/mL ingested 1000 IUs of vitamin D daily for 2 months a majority of them were unable to reach a blood level of at least 30 ng/mL [26]. To achieve a

blood level of the Maasai and Hadzabe adults of 40–50 ng/mL would require adults to ingest approximately 3000–5000 IUs daily. A study of Canadian adults taking varying doses of vitamin D reported that those who were taking approximately 3000–5000 IUs daily were able to achieve blood levels of 25(OH)D in the range of 40–50 ng/mL. They also reported that adults with a BMI >30, they required 2.5 times more vitamin D to achieve the same blood levels as normal-weight adults. Furthermore, they found that adults taking between 10,000 and 20,000 IUs daily for more than 1 year demonstrated no toxicity [84].

Therefore to achieve a blood level of 25(OH)D of at least 30 ng/mL would require a normal weight adult to ingest at least 1500–2000 IUs daily. To achieve what is considered to be the preferred blood level of 40–60 ng/mL, as recommended by the Endocrine Society, would require ingesting 3000–5000 IUs daily. I recommend to my patients that to guarantee vitamin D sufficiency infants, especially breast-fed infants, should receive at least 400 IUs daily and preferably 1000 IUs daily. Children up to the age of 13 should receive at least 600 IUs daily and preferably 1000 IUs daily. Teenagers should be treated as adults. They should receive at least 1500–2000 IUs daily and up to 5000 IUs daily is reasonable and safe to maintain blood levels of 25(OH)D in the preferred range of 40–60 ng/mL.

The consequences of vitamin D deficiency are often silent, but insidious in nature and have been reviewed extensively [3, 25, 28, 31, 85]. For children, it may prevent them from attaining their peak height and bone mineral density [3, 86]. Adults are at increased risk of developing osteopenia, osteoporosis and increased risk of fracture [25, 28, 86]. In addition, vitamin D deficiency increases the risk of a wide variety of chronic diseases including autoimmune diseases, type 1 diabetes, rheumatoid arthritis, Crohn's disease and multiple sclerosis, cardiovascular disease, neurocognitive dysfunction and Alzheimer's disease, type 2 diabetes, and several deadly cancers [25, 28, 31, 85] (Fig. 2.6).
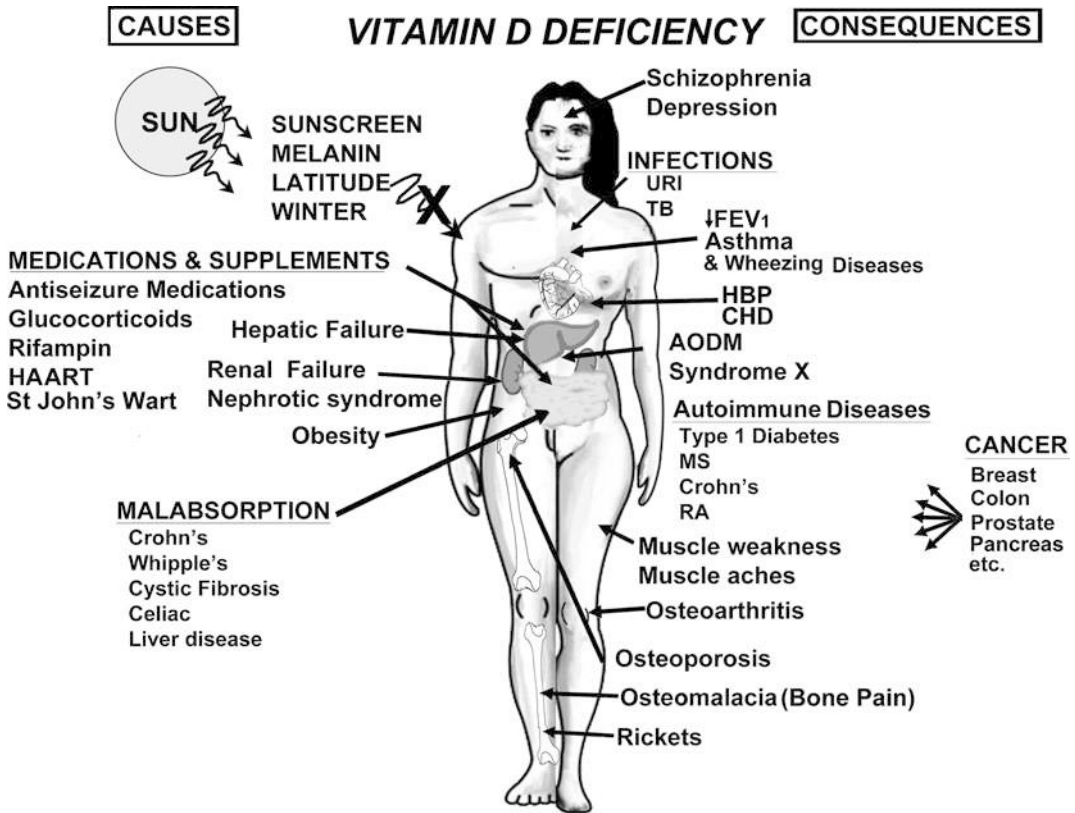
Fig. 2.6 A schematic representation of the major causes for vitamin d deficiency and potential health consequences. (Holick copyright 2007 with permission)

Sunlight, Vitamin D, and the Skin Cancer Conundrum

Humans evolved in sunlight and their skin pigment gene has evolved in order to protect the skin from the damaging effects from excessive exposure to sunlight but permitting enough UVB radiation to enter the skin to produce an adequate amount of vitamin D to sustain health. The pigment gene has rapidly mutated to decrease skin pigmentation [18, 19] in order to permit humans to survive in environments where there is markedly reduced UVB irradiation, and, thus, vitamin D_3 synthesis.

The skin has a large capacity to make vitamin D_3 [24]. When young- and middle-aged adults were exposed one time to one minimal erythemal dose of ultraviolet B radiation, the circulating levels of vitamin D that were observed 24 h

after the exposure were similar to adults who ingested between 10,000 and 25,000 IU of vitamin D_2 [87] (Fig. 2.7). Thus, only minimum suberythemal exposure to sunlight is often adequate to satisfy the body's vitamin D requirement [83, 88].

It is well documented that excessive exposure to sunlight will increase the risk of nonmelanoma skin cancers [89]. However, it is also known that occupational sun exposure decreases the risk of the most deadly form of skin cancer, melanoma [90, 91].

People of color who live near the equator and are exposed to sunlight on a daily basis sustain blood levels of 25(OH)D of 40–60 ng/mL [81]. Their skin was designed to produce an adequate amount of vitamin D and the melanin pigmentation prevents the damaging effects minimizing the risk of nonmelanoma skin cancer.

SERUM CONCENTRATION OF VITAMIN D FROM
ORAL VITAMIN D₂ OR UV EXPOSURE
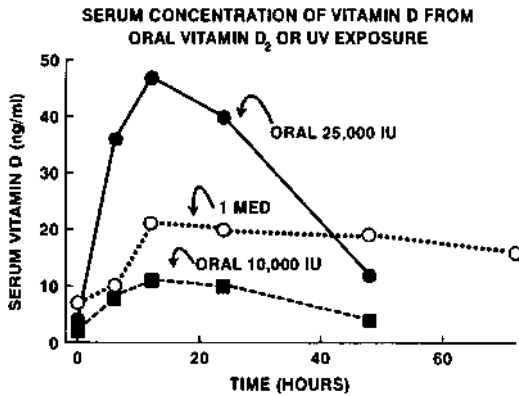
Fig. 2.7 Comparison of serum vitamin D_3 levels after a whole-body (in a bathing suit; trunks for men, bikini for women) exposure to 1 MED (minimal erythemal dose) of simulated sunlight compared with a single oral dose of either 10,000 or 25,000 IU of vitamin D_2. (Holick copyright 2013)

As skin pigment devolved in order to permit humans to produce an adequate amount of vitamin D_3, the skin was perfectly designed to take advantage of the beneficial effect of sun exposure. However, the loss of skin pigment permitted UVB-sensitive macromolecules, including DNA, to absorb the solar UVB radiation that penetrated the epidermis. This absorption caused thymidine dimerization and other alterations in the DNA structure, increasing the risk for the development of nonmelanoma skin cancer [92, 93]. The Surgeon General's report from the United States and many dermatology societies have promoted abstinence from any direct sun exposure, which is thought to be a major contributor for the worldwide vitamin D deficiency epidemic [94].

In support of this recommendation, Peterson et al. [95]. reported that Danish adults exposed to high-intensity sunlight during a vacation in the Marriott Islands had significant and concerning cutaneous DNA damage as measured by increased urinary cyclobutane pyrimidine dimers (CPD), a surrogate for DNA damage. They also reported improvement in vitamin D status and concluded that the detrimental DNA damaging effect of the sun exposure far outweighed the benefits of improvement in the vitamin D status of their subjects. This study however was subject

to criticism because Danes with skin types 1 and 2 were not designed to be exposed to high-intensity sunlight for an average of 38 h over 6 days in an environment that was much farther South from where their ancestors evolved. A study by Felton et al. [96] provided a more realistic insight regarding sun exposure and its beneficial and negative health consequences. They exposed healthy adults with little skin pigmentation (skin type II) to low-level simulated United Kingdom June midday sunlight (equivalent to 13–17 min 6 times weekly) and evaluated its effect on vitamin D status and outcome measures related to cutaneous DNA damage. They observed a significant 49% increase in circulating levels of 25(OH)D at the end of the 6-week study. A histologic evaluation of the skin biopsies revealed after the first week of exposure a significant increase in CPD-positive nuclei in keratinocytes compared to the photoprotective skin of the same volunteer. However, remarkably 1 day after the last exposure of the 6-week study, the authors observed significant clearing of the CPD-positive nuclei that corresponded to undetectable levels of CPD in the urine and no change or accumulation in another marker for DNA damage from baseline, i.e., urinary 8-oxo-2′-deoxyguasine (8-oxo-dG), a measure of oxidatively damaged DNA. These results suggested that the skin adapted to the sun exposure and did not demonstrate accumulating DNA damage but did demonstrate that there was likely continued vitamin D_3 synthesis. They also conducted a study in skin type V adults and as expected found minimum histologic evidence for DNA damage and no significant increase in serum 25(OH)D levels. This again demonstrated how the evolution of skin pigmentation evolved for taking advantage of the beneficial effect of sun exposure while minimizing damaging consequences. This suggests that you can have your cake and eat it too when it comes to the utilization of sensible sun exposure to improve a person's vitamin D status [92]. A study in adults who frequent a tanning bed at least once a week at the end of the winter had robust levels of 25(OH)D of approximately 40–50 ng/mL which was comparable to people of color being exposed to sunlight
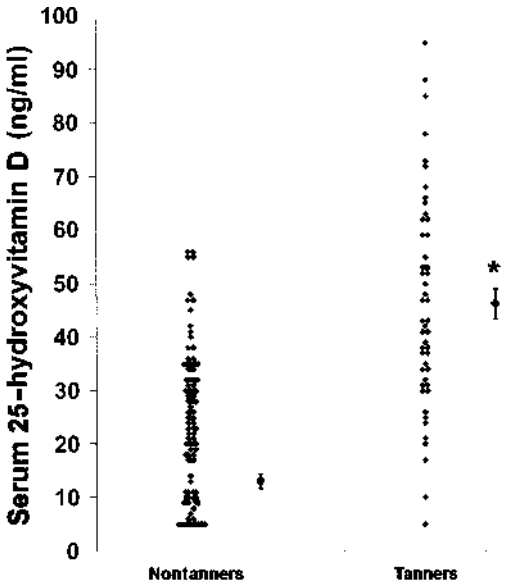
Fig. 2.8 Mean (±SEM) serum 25-hydroxyvitamin D concentrations in tanners and nontanners. Single points for each category are means ±SEMS. *Significantly different from nontanners, P < 0.001. (Holick copyright 2013)

on almost a daily basis living near the equator [81, 97, 98] (Fig. 2.8).

Aging will dramatically affect the amount of 7-dehydrocholesterol in human skin [99]. As a result, a 70-year-old has about 25% of the capacity to produce vitamin D_3 in their skin compared to a young adult. However, because the skin has such a large capacity to produce vitamin D_3, elders exposed to either sunlight [24, 100], a tanning bed [89, 98] or other UVB emitting devices [100] are able to raise their blood levels of 25(OH)D often above 30 ng/mL.

How long should a person be exposed to sunlight to satisfy their vitamin D requirement? It depends on time of day, season of year, latitude, altitude, weather conditions, and the person's degree of skin pigmentation. Typically for a Caucasian's skin type II living at approximately 42° N in June at noon-time, exposure of arms and legs and abdomen and back when appropriate (and always protecting the face since it is the most sun exposed and sun damaged and only represents about 2–4% of the body surface) to suberythemal sunlight (equivalent to approximately 0.75 MED) on a clear day between the hours of 10 and 3 pm for approximately 10–30 min, two to three times a week is often adequate to satisfy the body's vitamin D requirement. I recently helped develop the free app dminder.info that will provide guidance for sensible sun exposure anywhere on this planet for all skin types. It also provides a recommendation when to stop exposure to direct sunlight and to use sun protection to reduced risk for sun burning. After the sensible sun exposure, the application of a sunscreen with an SPF of at least 30 is then recommended if the person stays outside for a longer period of time in order to prevent sun burning and the damaging effects due to excessive exposure to sunlight.

Conclusion

Humans have always depended on sun for their vitamin D requirement. It is curious that the same UVB radiation that is so beneficial for making vitamin D_3 is also the major cause of non-melanoma skin cancer. It is excessive exposure to sunlight and the number of sunburns that is responsible for the alarming increase in non-melanoma skin cancer [90]. The fact that most melanomas occur on the least sun-exposed areas at least raises the question of whether moderate sun exposure is at all related to an increased risk of this deadly disease. Two reports suggest that moderate sun exposure decreases the risk [90, 91]. It is also worth noting that children and young adults who had moderate sun exposure had a decreased mortality if they developed melanoma [101] and a 40% reduced risk of developing non-Hodgkin's lymphoma [102]. It has also been suggested that improvement in vitamin D status may reduce the risk of developing melanoma and decreasing its malignant activity [103].

It is unfortunate that the sun has been demonized for more than 50 years by those who have been poorly informed or lack knowledge about the beneficial effect of sunlight [104] that our forefathers had appreciated more than 1000 years ago when many cultures including

the Egyptian's worshiped the sun for its life-giving properties [24, 94].

There are several new developments with the important health implications in the photobiology of vitamin D that will need further investigation. Slominski et al. [105] have observed the production of novel vitamin D compounds that have a shortened side chain that have little calcemic activity and potent antiproliferative properties. LED technology has made a major advancement by developing LEDs that can emit ultraviolet C, UVB, and UVA radiation. LEDs can be tuned to emit peak wavelengths with minimum bandwidth. This remarkable advancement in LED technology has resulted in the development of LEDs that emit germicidal UV radiation that is effective for water purification and sterilization of surgical suites and home appliances. We tuned LEDs in the region of the UVB spectrum that maximizes the photoproduction of previtamin D [106]. These LEDs demonstrated that peak wavelengths of 293 and 295 nm radiation were not only very effective in producing previtamin D in human skin but were also approximately 300% more efficient compared to sunlight. This suggests that exposure to LEDs emitting UVB radiation for producing previtamin D improves the risk-benefit ratio by approximately 300%. These LEDs can be developed for naturally producing vitamin D in the skin. This is of particular importance for patients who are unable to absorb vitamin D from diet or supplements because of some type of fat malabsorption syndrome.

Sunscreen technology has been developed whereby the ingredients have been altered in a manner that permits the sunscreen to let an additional small amount of vitamin D producing UVB radiation to pass through it to enhance the production of vitamin D in the skin. This was accomplished without altering its sun protection factor [107].

Finally, it should also be realized that there are a wide variety of additional photochemical and biologic processes that occur in the skin during sun exposure [94, 108]. These include among others an increased production of beta-endorphin, nitric oxide, and carbon monoxide that are related to improvement in feeling of well-being,

reduction in blood pressure. In addition, exposure to ultraviolet radiation increased expression of the clock, proopiomelanocortin, aryl hydrocarbon receptor, and nitric oxide synthetase genes [94, 108]. Therefore, sensible sun exposure not only can provide the all-important vitamin D but has demonstrable many other health benefits.

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Part III

Solar Radiation, Vitamin D and Human Health

Vitamin D Status and Cancer Incidence, Survival, and Mortality

3

Hanseul Kim and Edward Giovannucci

Abstract

Over the last several decades, extensive research on vitamin D and its role on cancer incidence, cancer survival (survival or mortality from cancer among individuals diagnosed with cancer), and cancer mortality (fatal cases occurring during the study period in an initially cancer-free population) has been conducted. A variety of study designs were implemented to explore vitamin D status, assessed by measuring sun exposure, vitamin D intake, and circulating 25-hydroxyvitamin D (25(OH)D) concentration. Although not many randomized controlled trials have examined the relationship between vitamin D and cancer incidence, observational studies have consistently shown a protective association between vitamin D and cancer incidence, especially for colorectal cancer. In addition, randomized controlled trials and most observational studies suggested that vitamin D plays a role in reducing cancer mortality. The potential benefit of vitamin D on cancer mortality may operate during the pre-diagnostic stages by affecting late-stage tumor progression and metastatic seeding, during the treatment phase by complementing or enhancing effects of therapies, or during the post-diagnostic stages. However, further studies are needed to confirm these conclusions, establish the optimal dosage and timing of vitamin D intakes for the most benefit, find which cancer types are affected, and understand the underlying mechanisms.

Introduction

There have been numerous efforts in studying the relationship between sun exposure and cancer incidence, survival, and mortality. In the late 1930s, Peller and Stephenson reported higher rates of skin cancer (i.e., 8 times higher) but lower rates of other cancers among the US Navy personnel [1]. Peller and Stephenson suggested that sun exposure induced skin cancer, which consequently conferred immunity against other

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cancers. After several years, Apperly reported an association between latitude and cancer mortality rate in North America [2]. Here, he observed that individuals in high-latitude regions had higher rates of total cancer mortality when compared to those in low-latitude regions. He argued that the "relative immunity to cancer is a direct effect of sunlight [2]." Although the hypothesis that sun exposure may be beneficial against cancer had been proposed early, these observations supporting the hypothesis were ignored for nearly 40 years until a clear mechanism was proposed.

In the 1980s, Garland and Garland suggested that the possible benefits of sun exposure could be attributed to vitamin D [3]. They hypothesized that vitamin D was protective against colon cancer, based on the premise that most vitamin D in humans is made from exposure to solar ultraviolet-B (UV-B) radiation. While this study focused on colon cancer, the proposed protective role of vitamin D was later extended to cancers in breast [4], ovary [5], prostate [6, 7], and other multiple sites [8]. Subsequent laboratory studies supported potential anti-carcinogenic properties of vitamin D, including increased differentiation and apoptosis and inhibited proliferation, invasiveness, angiogenesis, and metastatic potential [9].

This chapter provides a review and synthesis of up-to-date epidemiologic evidence on the association between vitamin D and incidence, survival, and mortality for various cancers. After Garland and Garland's initial hypothesis, numerous epidemiologic studies have supported the protective role of vitamin D (or sun exposure) on different cancer sites. In this chapter, we first discuss epidemiologic studies that assessed the association between serum vitamin D levels and cancer incidence, survival, and mortality and then discuss vitamin D intake studies, including evidence from recent randomized controlled trial (RCT) data. We consider three endpoints: cancer incidence (newly onset cases diagnosed during the study period in an initially cancer-free population), cancer mortality (fatal cases occurring during the study period in an initially cancer-free population), and cancer survival (survival or

mortality from cancer among individuals already diagnosed with cancer).

25-hydroxyvitamin D, Cancer Incidence, Survival, and Mortality

Many initial studies on this topic were ecological studies that examined population cancer incidence or mortality rates in relation to latitudes or regions that differ in UV-B radiation exposure [3–8]. These studies, in general, found that populations residing in regions of higher solar UV-B exposure generally had lower incidence and mortality rates of cancer. Similar findings were reported in Australia, China, France, Japan, and Spain, and at least 15 types of cancer, especially colorectal cancer, were shown to correlate with low sun exposure [10, 11]. An important limitation of ecological studies is that factors that are correlated with latitude or UV-B exposure may be the causal factors (confounders) rather than the UV-B exposure itself. However, the inverse association between regional solar UV-B exposure and cancers was not only observed in the United States but also in other regions such as Japan [12], China [13], and Spain [14]. The unlikelihood that potential confounders have similar relationships with solar UV-B exposure in all these different regions supports the hypothesis that the inverse association between UV-B exposure and cancers is causal.

While ecological studies examine exposure and outcome at the population level, case-control and cohort studies ("analytic epidemiologic studies") assess hypotheses at the individual level. Since more detailed information on covariates can be obtained in analytic studies, confounding is often better controlled for in case-control and cohort studies than ecological studies. In the recent 20 years, there have been numerous epidemiological studies (primarily cohort studies) assessing circulating 25-hydroxyvitamin D (25 (OH)D) levels in relation to cancer risks. Since serum- or plasma-based studies provide the most definite evidence for the role of vitamin D in observational studies, we mainly review evidence from such study designs to assess relationships

between vitamin D and colorectal cancer, breast cancer, prostate cancer, and other cancers. Studies that measured 25(OH)D levels for individuals who were already diagnosed with cancer should be interpreted with caution because of the potential for reverse causation, which is, the cancer may lead to low levels of 25(OH)D rather than vice versa. For example, the cancer may cause pathophysiologic changes that lower 25(OH)D levels or lead to behaviors due to illness that reduce sun exposure.

Colorectal Cancer

Colorectal cancer has been studied the earliest and the most in relation to vitamin D, specifically vitamin D deficiency. In general, studies have consistently shown that low levels of 25(OH)D were associated with higher risks of colorectal cancer or adenoma.

Cancer Incidence

Studies that supported an inverse association between vitamin D and incidence of colorectal cancer were from various populations including the Nurses' Health Study (NHS) [15], the Health Professionals Follow-up Study [16], the Women's Health Initiative [17], the Japan Public Health Center-based Prospective Study [18], the European Prospective Investigation into Cancer and Nutrition Study (EPIC) [19], and the Multi-ethnic Cohort Study [20]. These are among the largest prospective cohort studies of cancer.

Meta-analyses also found evidence favoring a protective association. In a meta-analysis published in 2007, Gorham et al. reported that serum 25(OH)D levels of ≥33 ng/mL were associated with a 50% lower risk of colorectal cancer compared to that of relatively low values of ≤12 ng/mL[21]. From this evidence, the authors suggested that daily intake of 1000–2000 IU/day of vitamin D would reduce colorectal cancer incidence. In support of this, another meta-analysis published in 2011 showed that based on 2630 cases, the summary relative risk for a 10 ng/mL increase in serum 25(OH)D was 0.85 (95% confidence interval [CI]: 0.79 to

0.91) [22]. In a recent large pooling project of 17 cohorts with 5706 colorectal cancer cases and 7107 controls, deficient 25(OH)D levels of <30 nmol/L were associated with 31% higher colorectal cancer risk, compared to 25(OH)D levels of 50 to <62.5 nmol/L (95% CI: 1.05 to 1.62) [23]. Intriguingly, this study reported that the inverse association persisted up until 100 nmol/L; at 25(OH)D levels of ≥100 nmol/L, the risk did not decline further and was not statistically significant. The "effective" dosage of vitamin D that the authors suggested on reducing colorectal cancer risk was higher than doses conventionally recommended (optimal concentrations: 75–100 nmol/L[23]). Based on multiple studies and meta-analyses, it is very clear that there is an inverse association between circulating 25(OH)D levels and colorectal cancer risk. Individuals in the highest quartile of 25(OH)D level had approximately half the risk of colorectal cancer incidence compared to those in the lowest quartile. Statistical adjustment for potential confounding factors generally did not affect the estimates for 25(OH)D and cancer.

Cancer Survival

Previous studies have consistently found that higher circulating 25(OH)D levels were associated with better colorectal cancer survival and prognosis. In a prospective study of 1598 patients with stage I to III colorectal cancer, higher plasma 25(OH)D was significantly associated with better colorectal cancer survival [24]. To be specific, compared to patients in the lowest tertile of 25(OH)D, those in the highest tertile had a hazard ratio of 0.68 (95% CI: 0.50 to 0.90) for colorectal cancer-specific deaths (i.e., higher postoperative 25(OH)D levels were related to better survival). In this study, blood samples were collected postoperatively, and the median time to blood sampling was 105 days after the treatment of colorectal cancer. Since factors like acute illness, surgery, or postoperative recovery could affect vitamin D levels, the authors in this study created a variable describing time from definitive treatment to blood sampling. Furthermore, systematic reviews and meta-analyses supported the benefits of higher circulating

25(OH)D in prognosis and survival among colorectal cancer patients [25–27]. For colorectal cancer-specific deaths, the pooled hazard ratio for the highest versus the lowest category of circulating 25(OH)D levels was 0.65 (95% CI: 0.49 to 0.86) [27]. For overall, not cancer-specific deaths of colorectal cancer patients, the corresponding pooled hazard ratio ranged from 0.55 (95% CI: 0.33 to 0.91) [26] to 0.71 (95% CI: 0.55 to 0.91) [27]. It seems clear from the evidence that there is a strong inverse association between circulating 25(OH)D levels and colorectal cancer deaths among patients (i.e., higher circulating levels associated with better colorectal cancer survival). An important thing to note from these meta-analyses is that included studies had different times of blood collection. For example, one of the studies (included in the meta-analysis) showed that higher pre-diagnostic 25(OH)D levels were significantly associated with better survival among colorectal cancer patients (hazard ratio comparing highest quintile versus lowest quintile for cancer-specific deaths, 0.69; 95% CI: 0.50 to 0.93) [28]. However, the authors from this study warranted further studies investigating the potential effects of vitamin D levels before, at, and after colorectal cancer diagnosis and/or treatment.

Prostate Cancer

Along with colorectal cancer, prostate cancer appears to be a well-studied cancer through case-control and cohort studies. However, unlike colorectal cancer studies that showed a clear inverse association, prostate cancer incidence data have been equivocal.

Cancer Incidence

Although some studies [29–34] suggested a weak inverse association between circulating 25(OH)D levels and risk of prostate cancer, most studies [35–38] reported no association between vitamin D and prostate cancer risk. In particular, two studies [39, 40] that were conducted in Nordic countries (where 25(OH)D levels tend to be low due to high-latitude and low UV-B exposure)

supported an inverse association. Even these studies remained inconclusive as one [40] of them noted a U-shaped risk of prostate cancer (i.e., an increased risk was observed not only when 25(OH)D level decreased from the reference but also when it increased from the reference). Recent large studies also did not find an association between 25(OH)D levels and prostate cancer risk. For instance, Ahn et al. found no statistically significant association between season-standardized serum 25(OH)D level and prostate cancer risk in a large prospective study [41]. Similarly, in a nested case-control study within the EPIC cohort (652 cases matched to 752 controls), the authors found no statistically significant association between 25(OH)D levels and prostate cancer risk (odds ratio for the highest versus the lowest quintile: 1.28; P for trend: 0.188).

Meta-analyses and Mendelian randomization study also showed mixed results regarding the association between 25(OH)D levels and prostate cancer incidence. A meta-analysis published in 2011 showed that based on 3956 cases, the summary relative risk for a 10 ng/mL increase in serum 25(OH)D was 0.99 (95% CI: 0.95 to 1.03) [22]. Such null association was confirmed in another meta-analysis [42]. A recent Mendelian randomization study also supported this and observed that there was no evidence of a causal association (odds ratio per 25 nmol/L increase: 1.00; 95% CI: 0.93 to 1.07) [43]. Mendelian randomization studies are those that utilize genetic variation in genes of known function (in this case, variation in 25(OH)D levels) to examine the presumed causal effect of exposure on disease. However, with a meta-analysis published in 2014 even suggesting a positive association between 25(OH)D level and prostate cancer risk [44], evidence on prostate cancer remains equivocal. Such discrepancies on the results of prostate cancer studies could potentially be attributed to differences in disease aggressiveness, which is critical to account for in prostate cancer epidemiology [45]. For example, in a recent study that aggregated 19 prospective studies (13,462 incident prostate cancer cases and 20,261 controls), a positive association between serum vitamin D

concentrations and total prostate cancer risk (odds ratio for highest versus lowest quintile: 1.22; 95% CI: 1.13 to 1.31) varied by disease aggressiveness [46]. Specifically, higher 25(OH)D levels were associated with increased risk of non-aggressive disease (odds ratio per 80 percentile increase: 1.24; 95% CI: 1.13 to 1.36) but not aggressive disease (odds ratio: 0.95; 95% CI: 0.78 to 1.15; aggressive disease defined as stage 4, metastases, or prostate cancer deaths). Therefore, although there were some studies suggesting a weak inverse association, studies on circulating 25 (OH)D levels and prostate cancer incidence have been inconclusive.

Cancer Survival and Mortality

The literature on prostate cancer survival and mortality in relation to vitamin D has also been inconsistent. Among studies that assessed post-diagnostic circulating 25(OH)D levels and pros-tate cancer deaths in patients, one found a significant protective association (relative risk: 0.16 for high levels of serum 25(OH)D versus low serum levels; 95% CI: 0.05 to 0.43 for cause-specific deaths) [47], but the others found no association [48, 49]. However, we noted that one of the studies that found no association had a short median follow-up (31 months) and only included men with stage IV prostate cancer [49]. Advanced cancers (e.g., stage IV prostate cancer) may be less influenced by vitamin D status and modifiable lifestyle factors in general. Not only post-diagnostic but also pre-diagnostic circulating 25(OH)D studies showed inconsistent results. One study found that higher pre-diagnostic plasma 25(OH)D was associated with improved prostate cancer prognosis [50]. To be specific, prostate cancer patients in the lowest 25(OH)D quartile were more likely to die from their cancer compared to those in the highest quartile (hazard ratio: 1.59; 95% CI: 1.06 to 2.39). In support of this, two survival analyses concluded that higher levels of pre-diagnostic serum 25(OH)D (e.g., above 85 nmol/L[51]) could improve survival in prostate cancer patients [51, 52]. On the other hand, results from a large cohort consortium (518 fatal prostate cancer cases and 2986 controls) showed that there was no statistically

significant relationship between pre-diagnostic circulating 25(OH)D and fatal prostate cancer (odds ratio for extreme quartiles: 0.86; 95% CI: 0.65 to 1.14) [53]. Although it is suggestive that higher levels of serum 25(OH)Ds are associated with better prostate cancer prognosis and survival, further research is warranted.

Breast Cancer

Breast cancer is one of the cancers that has been studied much in relation to vitamin D. However, the results have been inconsistent, and in general, have not been supportive of an association.

Cancer Incidence

The evidence for breast cancer has been mixed. In a nested case-control study within the NHS cohort (701 breast cancer cases and 724 controls), women in the highest quintile of 25(OH)D had a relative risk of 0.73 (95% CI: 0.49 to 1.07), compared to those in the lowest quintile [54]. Although still statistically insignificant, the association was stronger for women who were 60 years old or older (relative risk: 0.57; 95% CI: 0.31 to 1.04). This result suggested that vita-min D could be an important factor, particularly for postmenopausal breast cancer. Interestingly, a recent study observed an inverse association between total baseline 25(OH)D and breast can-cer risk (odds ratio: 0.87 per 10 ng/mL increase; 95% CI: 0.78 to 0.98) [55]. Here, the association remained similar when the analyses were restricted to postmenopausal women. However, this inverse association changed to a significantly positive association when the authors assessed second blood draw measures during follow-up and subsequent breast cancer risk (odds ratio: 1.17 per 10 ng/mL; 95% CI: 1.08 to 1.26). This finding, therefore, suggested that discrepant results among studies on vitamin D and breast cancer incidence may be due to temporal trends in vitamin D and potential reverse causation.

Findings from meta-analyses and Mendelian randomization study were mostly null. A meta-analysis by Gandini et al. reported a null associa-tion between 25(OH)D levels and breast cancer

risk among 5 prospective studies (summary relative risk: 0.97 for a 10 ng/mL increase; 95% CI: 0.92 to 1.03) [22]. In support of this, a more recent meta-analysis published in 2014 observed no statistically significant association between blood 25(OH)D levels and breast cancer incidence among 30 prospective studies (pooled relative risk: 0.92; 95% CI: 0.83 to 1.02) [56]. A recent Mendelian randomization study also suggested a null association, and that there was no evidence of a causal association (odds ratio per 25 nmol/L increase: 1.02; 95% CI: 0.97 to 1.08) [43]. Therefore, based on the studies of breast cancer risk in relation to circulating 25(OH)D levels, no clear association was found in general.

Cancer Survival

Many systematic reviews and meta-analyses supported that higher circulating 25(OH)D levels were associated with better breast cancer prognosis and survival [25–27, 57–59]. For example, a meta-analysis published in 2014 reported that low levels of 25(OH)D were significantly associated with higher risks of overall and breast cancer-specific deaths among breast cancer patients (hazard ratio for the highest versus the lowest tertile: 1.52, 95% CI: 1.22 to 1.88 and hazard ratio: 1.74, 95% CI: 1.23 to 2.40, respectively) [58]. Since studies with longer times from diagnosis to blood collection tend to report no association [57], the protective association seemed to be stronger for studies in which blood samples were drawn close to diagnosis. This may be because serum 25(OH)D concentrations could change from therapy or lifestyle modifications after the diagnosis or due to disease worsening [60]. For instance, the association of serum 25(OH)D levels and mortality was statistically significant only for patients whose blood samples were collected prior to chemotherapy [61].

Although there were many studies in support of an association, some studies on breast cancer treatment trials showed no association between 25(OH)D levels and breast cancer prognosis [62–64]. Since these were treatment trials, all these studies measured post-diagnostic 25(OH)D levels after they recruited the cases. One of the studies mentioned that they collected all the blood

samples before treatment [63]. One explanation for the differences in the results between observational studies conducted in general cohorts and those in the context of treatment trials could be that trials had stricter inclusion criteria, which led the study population to be more homogeneous. Alternatively, it might be due to a potential that adjuvant therapies negated the adverse effect of low 25(OH)D levels. In addition, it should be noted that information on vitamin D supplementation was not available.

Other Cancer Types

Unlike colorectal, prostate, and breast cancers, other cancers have not been examined much in relation to vitamin D. Furthermore, some cancers are too rare to study in individual cohorts.

Cancer Incidence

There have been some studies that examined 25(OH)D levels and risks of cancers in various sites including skin, lung, and pancreas. In a recent study based on 217,244 individuals, there were significant positive associations between 25(OH)D levels and skin (both non-melanoma and melanoma), prostate, and hematological cancers but a significant inverse association for lung cancer [65]. One nested case-control study of blood 25(OH)D levels and pancreatic cancer risk was based on the cohort of male Finnish smokers (200 incident exocrine pancreatic cancer cases matched to 400 controls) [66]. In this study, higher vitamin D concentrations were associated with almost a threefold increased risk of pancreatic cancer, and the association remained significant even after excluding cases early in follow-up. However, since pancreatic cancer is rare, studying it in individual cohorts could result in relatively less statistical power. Therefore, the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (VDPP) was formed to address the role of circulating 25(OH)D in less common cancers [67]. The VDPP, a consortium of ten prospective cohort studies from the United States, Finland, and China, was used to examine the associations between 25(OH)D levels and the

risks of endometrial, kidney, ovarian, pancreatic, and upper gastrointestinal tract cancers and non-Hodgkin lymphoma. The total numbers of cases for each of the malignancies were 830 for endometrial cancer, 775 for kidney cancer, 516 for ovarian cancer, 952 for pancreatic cancer, 1065 for upper gastrointestinal cancers, and 1353 for non-Hodgkin lymphoma. In general, the results from the VDPP showed that there were no statistically significant associations between circulating 25(OH)D levels and risks of cancers mentioned above, except for increased pancreatic cancer risk at high levels ($\geq$ 100 nmol/L) of 25 (OH)D [68–73]. However, such a potential positive association between vitamin D and pancreatic cancer incidence has not yet been entirely confirmed. For example, in a pooled analysis of nested case-control studies from 5 cohorts (451 cases and 1167 controls), higher circulating 25(OH)D levels were associated with a lower risk of pancreatic cancer, suggesting an inverse, not a positive, association [74].

Although there was no overall association between 25(OH)D levels and upper gastrointestinal and ovarian cancers in the VDPP, subgroup analyses and results from other studies deserve attention. For instance, there were racial differences in the association between 25(OH)D levels and gastrointestinal cancers. Among Asians, not Whites, lower concentrations of 25 (OH)D (< 25 nmol/L) were associated with a lower risk of upper gastrointestinal cancers (odds ratio: 0.53; P for trend: 0.003) [69]. However, such positive association could possibly be attributed to reverse causation because one of the Asian cohorts (Shanghai Men's Health Study) had a short follow-up time of 1.7 years. Besides, undiagnosed cancers at baseline blood draw could have affected the 25(OH)D level. In the subgroup analysis by smoking status, concentrations of <25 nmol/L were associated with a decreased risk of upper gastrointestinal cancers among never smokers. Regarding ovarian cancer, a nested case-control study within the Finnish Maternity Cohort observed that having sufficient (> 75 nmol/L) serum 25(OH)D levels compared to insufficient serum 25(OH)D was associated with a decreased risk (odds ratio:

0.32; P-value: 0.03), suggesting an inverse association [75].

Cancer Survival and Mortality

Studies on overall cancer survival and mortality have generally found better prognosis and lower mortality for those with higher 25(OH)D levels. In a meta-analysis of 12 cohort studies, lower 25(OH)D levels were associated with more cancer deaths (pooled relative risk comparing bottom versus top thirds: 1.14; 95% CI: 1.01 to 1.29) [76]. This part of the meta-analysis assessed cancer mortality rather than cancer survival because eligible observational cohort studies included healthy participants at baseline. Similar findings were also reported for cancer survival among patients. A recent study with 4616 cancer cases (2884 died of their cancer during 28 years of follow-up) found that higher 25(OH)D levels were associated with better overall cancer survival (hazard ratio for the highest versus the lowest quintile: 0.76; 95% CI: 0.67 to 0.85) [77]. Here, cancer cases were drawn from the previous nested case-control studies of circulating 25(OH)D levels and cancer risk within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Fasting blood samples were collected at baseline (pre-diagnostic) and stored until analysis. This study also found that significant inverse associations were present for kidney cancer deaths among kidney cancer patients (hazard ratio: 0.59; 95% CI: 0.35 to 0.98) and melanoma deaths among melanoma patients (hazard ratio: 0.39; 95% CI: 0.20 to 0.78), but a significant positive association for lung cancer deaths among lung cancer patients (hazard ratio: 1.28; 95% CI: 1.02 to 1.61).

Studies on vitamin D in relation to lung cancer, lymphoma, melanoma, and pancreatic cancer prognoses, individually, are worthy of notice. For lung cancer, two studies in Norway (which collected serum samples within 90 days of cancer diagnosis) [78] and the United States (which collected samples at the time of diagnosis) [79] observed better survival for patients with higher circulating serum levels of 25(OH)D. However, this was not supported in a small Chinese study with 87 cases [80]. Besides, two studies on

advanced non-small-cell lung cancer did not find any significant association between post-diagnostic serum 25(OH)D levels and cancer survival [81, 82]. In a study of 500 Finnish men, pre-diagnostic serum 25(OH)D levels (median time from blood collection to diagnosis was 10 years) were also not significantly associated with lung cancer survival (hazard ratio comparing the highest to the lowest quartile: 1.18; 95% CI: 0.89 to 1.56) [83]. This study found suggestive associations between higher serum 25(OH)D and better survival from adenocarcinoma (hazard ratio: 0.64; 95% CI: 0.17 to 2.45) and small cell carcinoma (hazard ratio: 0.55; 95% CI: 0.21 to 1.45). However, these estimates were based on a relatively small number of cases and were not statistically significant. A similar null result was observed for lung cancer mortality as well as survival. In a study that analyzed 258 cases of lung cancer deaths, the authors found that there was no association between serum 25(OH)D levels and overall lung cancer mortality. They observed that among nonsmokers, $\geq$ 44 nmol/L versus <44 mol/L of serum 25(OH)D was associated with a decreased risk of lung cancer mortality (hazard ratio: 0.53; 95% CI: 0.31 to 0.92) [84]. Although there were many studies reporting null associations, there were some studies suggesting that higher circulating vitamin D levels could be associated with better lung cancer survival.

Although not many, some studies examined lymphoma, melanoma, and pancreatic cancer prognoses with respect to 25(OH)D levels. In the meta-analysis that showed significant inverse associations between 25(OH)D levels and colorectal and breast cancer deaths, higher 25(OH)D levels measured at or near the time of diagnosis were associated with better lymphoma outcomes (pooled hazard ratio for the highest versus the lowest quartile: 0.48; 95% CI: 0.36 to 0.64) [26]. Other studies also showed that higher 25 (OH)D levels collected at or near diagnosis were associated with favorable prognosis in melanoma [85–87]. For pancreatic cancer, a study of 256 cases showed that baseline 25(OH)D levels were not associated with progression free or overall survival [88]. However, the authors of this

study noted that baseline 25(OH)D levels in cancer patients might represent inadequate nutrition or limited outdoor activity due to the burden of cancer, instead of true steady state [89]. Also, since the median overall survival was very short (less than 6 months) and most of the cases had deficient (< 20 ng/mL; 44.5% of the cases) or insufficient (< 30 ng/mL; 22.5% of the cases) levels of vitamin D, it might have been hard to find an association. To sum up, 25(OH)D levels seem to be inversely associated with cancer deaths in general.

Vitamin D Intake Trials

As RCTs are considered to be a gold standard for epidemiologic evidence (i.e., a causal association), we discuss the results on trials of vitamin D intake and cancer incidence and mortality in this section. We are able to draw a causal inference in a well-designed RCT as issues on confounding will ideally be removed with effective randomization.

Randomized Controlled Trials (RCTs) of Vitamin D and Cancer Incidence

There are not many RCTs that have examined the relationship between vitamin D intake and cancer incidence. Since studies, in general, suffer from a lack of statistical power when examining specific cancers, some trials assessed the role of vitamin D supplements on total cancer incidence. In a meta-analysis summarizing these trials, the authors reported that vitamin D supplementation had no effect on total cancer incidence (summary relative risk: 1.00; 95% CI: 0.94 to 1.06; 4 RCTs with 4333 combined cases) [90]. However, they noted that this summary measure was based on relatively short duration (2–7 years of duration) and a limited dosage (400 to 1100 IU per day). A recent large randomized trial in the United States called the Vitamin D and Omega-3 Trial (VITAL) also found that vitamin D supplementation was not associated with a lower risk of invasive cancer [91]. VITAL was a randomized controlled study

of vitamin D at a dose of 2000 IU per day and omega-3 fatty acids at 1 g per day on cancer and cardiovascular disease among US men ($\geq$ 50 years old) and women ($\geq$ 55 years old). Among the total of 25,871 participants that were followed for a median of 5.3 years, 1617 were diagnosed with cancer (793 in the vitamin D group and 824 in the placebo group). The hazard ratio of the vitamin D group to the placebo group was 0.96, with a 95% CI of 0.88 to 1.06 (P = 0.47). In the VITAL study, supplementation of vitamin D also did not reduce the occurrences of colorectal, breast, and prostate cancers.

The results from VITAL were included in a new meta-analysis of cancer incidence [92]. This updated meta-analysis comprised 10 trials (6547 cases; 3–10 years of follow-up; 54–135 nmol/L of attained levels of circulating 25(OH)D in the intervention group). The summary RR was 0.98 (95% CI: 0.93 to 1.03; P = 0.42). The results remained null across subgroups tested, including even when attained 25(OH)D levels exceeded 100 nmol/L (RR: 0.95; 95% CI: 0.83 to 1.09; P = 0.48).

RCTs of Vitamin D and Cancer Mortality

Unlike the results on cancer incidence, results on cancer mortality tend to show an inverse association. In the meta-analysis mentioned above, the authors found that vitamin D supplementations significantly reduced total cancer mortality (summary relative risk: 0.88; 95% CI: 0.78 to 0.98; three RCTs with combined 1190 cases) [90]. This meta-analysis included RCTs on cancer mortality, not survival. Although only marginally significant, VITAL results also showed a protective association between vitamin D supplementations and cancer mortality (hazard ratio: 0.83; 95% CI: 0.67 to 1.02; 341 cancer deaths, with 154 in the vitamin D group and 187 in the placebo group) [91]. This association became stronger and significant in the analysis that excluded the first 2 years of follow-up, a pre-specified analysis (hazard ratio: 0.75; 95% CI: 0.59 to 0.96). It is common to exclude early years of follow-up in analyzing trials on diet and cancer because the effects of

nutritional factors become clear only after a certain period of time, especially for slow-growing diseases like cancer. In an updated meta-analysis [92], five trials were included to study total cancer mortality. These studies entailed 1591 deaths over 3–10 years of follow-up. The summary RR for vitamin D compared to placebo was 0.87 (95% CI: 0.79 to 0.96; P = 0.005). This result was largely attributable to interventions with daily dosing, rather than infrequent bolus dosing. No statistically significant heterogeneity was observed by attained levels of circulating 25 (OH)D above or below 100 nmol/L.

Conclusions

Over the last several decades, vitamin D has received substantial interest in relation to the common cancers and less so for the rarer malignancies. For cancer incidence, a consistent inverse association has only been observed for colorectal cancer in observational studies. RCTs also have not supported a general effect of vitamin D on cancer incidence. Although these RCTs potentially provide more evidence for a causal association, there exist some important limitations. Trials with extended duration are warranted for studies on cancer incidence because long durations are often required to observe an effect. For example, epidemiologic evidence suggests that at least 10 years are needed for any influence of calcium or vitamin D to show on colorectal cancer occurrence [93]. Since most cancers generally arise through a multi-stage process that lasts for a long period of time, studies with relatively short duration may not capture the benefit of vitamin D on cancer risk, if there is any. In addition, in trials, it is difficult to choose a single "proper" or "effective" dosage that a susceptible population could benefit from. Therefore, although RCTs are generally considered as a gold standard, their results should still be interpreted with caution for issues mentioned above and other issues such as noncompliance.

In contrast to the studies on cancer incidence, both RCTs and many though not all observational studies suggest that vitamin D may play a role in

cancer mortality or survival. Approximately a 15% reduction in total cancer mortality was observed in those who were randomized to receive vitamin D supplements over placebo, and the VITAL study suggested that this effect size could increase over the duration of vitamin D use. Most of the follow-up time in the studies was less than 5 years. In VITAL, after excluding the first 2 years, the risk reduction was 25%. Benefits were seen even at fairly high doses of 2000 IU/day and when levels of >100 nmol/L were attained. While the reason for the divergent findings for incidence and mortality of total cancer is not apparent, plausible mechanisms exist for vitamin D operating at multiple stages of carcinogenesis. Vitamin D may decrease tumor invasiveness and propensity to metastasize, which may occur at the late stages of carcinogenesis. In the RCTs, which showed benefits on mortality, vitamin D administration generally started before cancer diagnosis, likely during the late stages of carcinogenesis and continued during and after diagnosis. Thus, the potential benefit for vitamin D status on cancer mortality could operate during the pre-diagnostic stages by affecting late-stage tumor progression (e.g., invasion) and metastatic seeding, during the treatment phase possibly by complementing or enhancing effects of therapies, or during the post-diagnostic stages. It is unclear if similar benefits could be conferred by beginning vitamin D treatment at the time of diagnosis because some of the effects of vitamin D could be occurring during the metastatic seeding phase in the pre-diagnostic period.

Almost 10 million cancer deaths were projected to occur in 2018 worldwide [94]. With increasing population size and aging, cancer incidence and mortality is likely to increase over time. The results from meta-analyses support that achieving circulating levels of 25(OH)D around 54–135 nmol/L may contribute to reducing cancer mortality. Although the optimal 25 (OH)D level for prevention is not established, it is likely to be higher than 50 nmol/L, and currently, a substantial portion of the world's population is below even this threshold. The Endocrine Society recommends at least 1500–2000 IU/day intake of vitamin D to maintain the levels of 25

(OH)D above 75 nmol/L [95]. Further studies are needed to confirm our conclusions, establish the optimal dose and timing of vitamin D intakes for prevention, find which cancer types are affected, and determine the underlying mechanisms of action.

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Vitamin D Receptor Polymorphisms and Cancer

4

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Abstract

Increasing scientific evidence supports the link between vitamin D and cancer risk. The active metabolite 1,25(OH)2D exerts its activity by binding to the vitamin D receptor (VDR), an intracellular receptor that mediates transcriptional activation and repression of target genes. The binding of 1,25(OH)2D to VDR is able to regulate hundreds of different genes. VDR is active in virtually all tissues including the colon, breast, lung, ovary, bone, kidney, parathyroid gland, pancreatic b-cells, monocytes, T lymphocytes, melanocytes, keratinocytes, and also cancer cells.

The relevance of *VDR* gene restriction fragment length polymorphisms for various types of cancer has been investigated by a great number of studies.

We have carried out a systematic review of the literature to analyze the relevance of more VDR polymorphisms (*Fok1*, *Bsm1*, *Taq1*, *Apa1*, and *Cdx2*) for individual malignancies considering ethnicity as a key factor for heterogeneity.

Up to December 2018, we identified 176 independent studies with data to assess the risk of breast, prostate, colorectal, skin (melanoma and non-melanoma skin cancer), lung, ovarian, kidney, bladder, gallbladder, esophageal, thyroid, head and neck, liver and pancreatic cancer, oral squamous cell carcinoma, non-Hodgkin lymphoma, multiple myeloma and sarcoma.

Significant associations with VDR polymorphisms have been reported for prostate (*Fok1*, *Bsm1*, *Taq1*, *Apa1*, *Cdx2*), breast (*Fok1*, *Bsm1*, *Taq1*, *Apa1*, *CdX2*), colorectal (*Fok1*, *Bsm1*, *Taq1*, *Apa1*), and skin cancer (*Fok1*, *Bsm1*, *Taq1*). Very few studies reported risk estimates for the other cancer sites.

Conflicting data have been reported for most malignancies, and at present, it is still not possible to make any definitive statements about the importance of the *VDR* genotype for cancer risk. It seems probable that other factors such as ethnicity, phenotype, 25(OH)D plasma levels, and UV radiation exposure play a role as confounding factors and introduce heterogeneity.

To conclude, there is some indication that *VDR* polymorphisms may modulate the risk of some cancer sites and in future studies *VDR* genetic variation should be integrated also with assessment of vitamin D status and stratified by ethnicity.

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Abbreviations

BCC	Basal cell carcinoma
BMI	Body mass index
CI	Confidence interval
CRC	Colorectal cancer
EAC	Esophageal adenocarcinoma
HCC	Hepatocellular carcinoma
HNC	Head and neck cancer
H-W	Hardy-Weinberg
MM	Malignant melanoma
NHL	Non-Hodgkin lymphoma
NMSC	Non-melanoma skin cancer
NPC	Nasopharyngeal carcinoma
OR	Odds ratio
OSCC	Oral squamous cell carcinoma
PCa	Prostate cancer
RCC	Renal cell carcinoma
RFLP	Restriction fragment length polymorphism
RR	Relative risk
SCC	Squamous cell carcinoma
SNP	Single nucleotide polymorphism
SRR	Summary relative risk
TC	Thyroid carcinoma
UV	Ultraviolet
VDR	Vitamin D receptor
vs	Versus

Introduction

Most vitamin D is derived from the action of sunlight on the skin, and this source accounts for about 80% of the total vitamin D [84]. Exogenous vitamin D comes from dietary intake through the consumption of foods that are naturally rich in or fortified with it or through supplementation [202]. The overall vitamin D reservoir is the sum of cutaneous and nutritional vitamin D.

Pre-vitamin D undergoes two hydroxylations to become biologically active [46]. First, vitamin D3 from the skin and vitamins D2 and D3 from the diet are metabolized in the liver to 25-hydroxyvitamin D (25[OH]D), which is the main circulating vitamin D metabolite measured to define the patient's vitamin D status. The conversion to its biologically active form, 1,25-hydroxyvitamin D (1,25[OH]D), is under tight hormonal control in the kidneys by the parathyroid hormone, in keeping with its important role in calcium homeostasis.

The vitamin D status varies greatly with season (the highest levels are observed in late summer and autumn) and with body mass index (BMI) (greater BMI is associated with lower 25 (OH)D).

In addition to the pivotal role of vitamin D in the maintenance of musculoskeletal health, it has also been shown to play an important role in other metabolic pathways, such as those involved in the immune response and cancer. It is emerging as a critical regulator of pathogenic processes such as pigmental disorders; cardiovascular, renal, infectious, and autoimmune diseases; as well as several types of cancers [55, 83, 147, 164–167, 184, 195].

The active metabolite 1,25(OH)2D [84] seems to play an important role in the development of cancers by regulating the expression of tumor-related genes and mediating inhibition of cell growth, adhesion, migration, and angiogenesis in vitro and in vivo [32, 41, 53, 58, 61, 80, 150, 156, 229]. It exerts its activity by binding to the vitamin D receptor (VDR), an intracellular receptor and member of the nuclear receptor family

(locus on chromosome 12q12–14) that mediates transcriptional activation and repression of target genes. The VDR controls gene expression through so-called vitamin D response elements on the DNA. The binding of 1,25(OH)2D to VDR is able to regulate hundreds of different genes [23]. VDR is active in virtually all tissues including the colon, breast, lung, ovary, bone, kidney, parathyroid gland, pancreatic b-cells, monocytes, T lymphocytes, melanocytes, keratinocytes, and also cancer cells.

Several meta-analyses of observational studies showed a reduced risk for some cancer sites associated with high vitamin D status. A meta-analysis published by Gandini et al. [64] showed a significant inverse relationship between high level of 25(OH)D levels and the risk of colorectal cancer (CRC): a SRR of 0.85 (95%CI: 0.79–0.91) for 10 ng/ml increase in serum 25-hydroxyvitamin D. This inverse association was further confirmed by another meta-analysis [207] that presents a SRR of 0.96 (95%CI: 0.94–0.97) for 100 IU/L increase of 25(OH)D.

High serum 25(OH)D levels were found to significantly decrease the risk of bladder cancer (SRR: 0.75; 95%CI: 0.65–0.87) in a meta-analysis published by Berlin [19].

A meta-analysis on lung cancer risk showed that vitamin D intake and serum 25(OH)D levels each correlated inversely with lung cancer risk [OR = 0.72 (95%CI: 0.61–0.85) and OR = 0.89 (95%CI: 0.83–0.97)]. Interestingly non-smokers had higher vitamin D levels, which correlated negatively with lung cancer risk (OR = 0.76, 95%CI: 0.65–0.88) [125]. Similar results were found for breast cancer: 25(OH)D deficiency is significantly associated with increased risk (OR = 1.91, 95%CI: 1.51–2.41), and supplemental vitamin D (OR = 0.97, 95%CI: 0.95–1.00, $P = 0.026$) was inversely associated with breast cancer risk [87].

A meta-analysis of randomized clinical trials showed that vitamin D supplementations seem to have little effect on total cancer incidence, but a significant reduction in total cancer mortality (400–833 IU per day, summary RR = 0.88, 95%CI = 0.78–0.98, $I2 = 0\%$, 3 RCTs with combined 1190 deaths) [110].

Genetic variations of VDR may phenotypically appear as interindividual rate-limiting variations of vitamin D synthesis in the skin, hydroxylation in the liver and kidney, and transportation, metabolism, and degradation that could influence individual vitamin D status. Given that single nucleotide polymorphisms (SNPs) in the VDR gene could potentially influence the binding of 1,25(OH)2D, the transcriptional activity of the receptor, and its binding to vitamin D response elements and provided the antiproliferative effects of vitamin D, VDR polymorphisms have been hypothesized to be associated with cancer risk.

The most frequently studied single nucleotide VDR polymorphisms in association with cancer risk are the restriction fragment length polymorphisms *Fok1* (rs2228570) and *Bsm1* (rs1544410) [171, 212]. More recently, other SNPs have been investigated: *Taq1* (rs731236), *Apa1* (rs7975232), and *Cdx2* (rs11568820) [185].

Materials and Methods

We performed systematic literature search of published studies evaluating the association between *VDR* gene restriction fragment length polymorphisms (RFLPs) *Fok1*, *Bsm1*, *Taq1*, *Apa1*, and *Cdx2* and 19 types of cancer, including breast (female and male), prostate, skin (melanoma and non-melanoma skin cancer), colon, ovarian, kidney, bladder, brain, esophageal, gallbladder, gastric, liver, head and neck, lung, multiple myeloma, non-Hodgkin lymphoma, pancreas, sarcoma, and thyroid and mixed cancer sites. Estimates of risk are also available for several ethnic groups (Caucasians, Asian, African, African-American, Hispanic, and others).

Data Extraction and Data Analysis

Data have been extracted retrieving the following information from each publication: authors, journal and year of publication, country of origin, ethnic group of study population, number of cases and controls for each *VDR* genotype and

by variants status and adjustments used for risk estimates.

We considered eligible for the present analysis all independent papers from genotype-based epidemiological studies reporting frequency of *VDR* polymorphisms, for cancers and controls, or estimates of the association between the two *VDR* polymorphisms and cancer, with a corresponding measure of uncertainty (i.e., 95% confidence interval (CI), standard error, variance, or P-value of the significance of the estimate).

When available, we extracted fully adjusted relative risk (RR) estimates separately for heterozygous and minor allele homozygous subjects compared to wild-type subjects. When adjusted estimates were not available, we retrieved the frequencies of *VDR* genotypes in cases and controls and calculated the corresponding study-specific crude odds ratio (OR), with 95%CI for cancer risk, by cancer site. Since the reference group for each polymorphism varied among the studies, we considered the homozygous genotype of the more prevalent allele as reference genotype in our analyses. Articles were reviewed and data were extracted and crosschecked independently by two investigators. Any disagreement was resolved by consensus among the two.

We presented forest plots of risk estimates by cancer sites and ethnic groups.

When zero subjects with homozygous variants were present among controls, we imputed 0.5 in order to be able to calculate the risk estimate.

Exclusion Criteria

– Studies not independent from a study already included, because based on the same population or have in common a subgroup of population. The one with the greater sample size is preferred.
– Studies evaluating the risk of colorectal adenoma and benign prostatic hyperplasia.
– Studies that included as control group not healthy subjects (e.g., benign prostatic hyperplasia).
– Studies with too sparse data that included as control group benign prostatic hyperplasia.

– Studies with zero subjects in the wild-type category among cases or controls.
– Studies that presented no risk estimates for homozygous and heterozygous variants vs wild-type and no crude data to calculate them.
– Studies that presented risk estimates only for additive model.
– Studies for which the Minor Allele Frequency (MAF) was strongly different from HapMap MAF for the corresponding ethnic group.

Fok1 and Cancer

It has been hypothesized that a less active VDR could be associated with either an increased susceptibility to cancer risk or a more aggressive disease. The *Fok1* restriction fragment length polymorphism, located in the coding region of the *VDR* gene, results in the production of a VDR protein that is three amino acids longer. Although no significant differences in ligand affinity, DNA binding, or transactivation activity are found between these two VDR forms when studied independently [70], in transient transfection assays with a vitamin D-responsive reporter gene, the shorter VDR variant displays higher potency than the longer one [216].

Breast Cancer

Fok1 is the most frequently analyzed *VDR* polymorphism, and numerous studies examined its association with breast cancer risk. Between 1999 and 2018, 25 studies have been published, and they are summarized in Table 4.1 and Figs. 4.1 and 4.2. Most of them were carried out in the USA and Canada (n. 8 studies 32%) and in European countries (n. 7 studies 28%). Twelve studies (48%) were case-control studies with population controls, and 15 (60%) analyzed a Caucasian population.

We also included a study investigating the association between VDR gene polymorphism with male breast cancer risk in a Turkish population [111].

Table 4.1 Descriptive characteristics of studies grouped by cancer sites

Cancer site	Author, PY [REF]	Country	Ethnicity	Source of controls	Apa1	Fok1	Bsm1	Cdx2	Taq1
Bladder	Mittal, 2007 [140]	India	Other	Population		x			x
Bladder	Ben Fradj, 2016 [17]	Tunisia	African	Population		x			
Brain	Anic, 2012 [8]	USA	Caucasian	Population		x	x	x	x
Brain	Toptaş, 2013 [205]	Turkey	Other	Hospital		x			
Brain (pediatric)	Yilmaz, 2017 [225]	Turkey	Other	Hospital		x	x		x
Breast	Ruggiero, 1998 [180]	Italy	Caucasian	Hospital			x		
Breast	Curran, 1999 [44]	Australia	Caucasian	Hospital	x	x	x		x
Breast	Dunning, 1999 [51]	UK	Caucasian	Hospital					x
Breast	Hou, 2002 [88]	Taiwan	Asian	Hospital	x		x		x
Breast	Buyru, 2003 [26]	Istanbul	Other	Hospital			x		x
Breast	Guy, 2003 [75]	UK	Caucasian	Population		x	x		
Breast	Hefler, 2004 [82]	Germany	Caucasian	Hospital			x		
Breast	Sillanpaa, 2004 [190]	Finland	Caucasian	Hospital	x		x		x
Breast	VandeVord, 2006 [211]	USA	Mixed	Hospital					
Breast	John, 2007 [104]	USA	A-A, Caucasian, Hispanic	Population		x	x		x
Breast	Trabert, 2007 [208]	USA	A-A	Population			x		
Breast	Abbas, 2008 [1]	Germany	Caucasian	Population		x		x	x
Breast	Barroso, 2008 [15]	Spain	Caucasian	Population		x			x
Breast	Gapska, 2009 [66]	Poland	Caucasian	Population		x	x		
Breast	Sinotte, 2008 [191]	Canada	Caucasian	Hospital		x	x		
Breast	Chakraborty, 2009 [29]	India	Other	Hospital	x				x
Breast	McKay, 2009 [133]	USA	A-A, Asian, Caucasian, Hispanic, other	Population		x	x		
Breast	Anderson, 2011 [6]	Canada	Caucasian	Population	x	x	x	x	x
Breast	Dalessandri, 2012 [45]	USA	Caucasian	Population	x				
Breast	Engel, 2012 [57]	USA	Caucasian	Population	x	x			x
Breast	Huang, 2012 [90]	China	Asian	Hospital	x		x		x
Breast	Rollison, 2012 [178]	USA	Caucasian, Hispanic	Population		x	x		x
Breast	Yao, 2012 [223]	USA	A-A, Caucasian	Population				x	
Breast	Akilzhanova, 2013 [3]	Kazakhstan	Other	Nr		x	x		
Breast	Fuhrman, 2013 [63]	USA	Caucasian	Population		x	x		
Breast	Mishra, 2013 [139]	USA	A-A, Hispanic	Hospital	x	x	x		x
Breast	Shahbazi, 2013 [188]	Iran	Other	Hospital		x	x		

(continued)

Table 4.1 (continued)

Cancer site	Author, PY [REF]	Country	Ethnicity	Source of controls	ApaI	FokI	BsmI	Cdx2	TaqI
Breast	Abd-Elsalam, 2015 [2]	Egypt	Other	Hospital	x	x	x		x
Breast	Clendenen, 2015 [40]	Sweden	Caucasian	Population		x	x	x	
Breast	Guo, 2015 [74]	China	Asian	Hospital	x		x		x
Breast	Iqbal, 2015 [100]	Pakistan	Other	Hospital				x	
Breast	Nemenqani, 2015 [149]	Saudi Arabia	Asian	Hospital		x			x
Breast	Reimers, 2015 [177]	USA	Caucasian	Population	x		x		x
Breast	Deschasaux, 2016 [49]	France	Caucasian	Population		x	x		
Breast	Amadori, 2017 [5]	Italy/Tanzania	Caucasian, African	Hospital		x		x	x
Breast	Atoum, 2017 [11]	Jordan	Other	Population			x		
Breast	Elzehery, 2017 [56]	Egypt	Other	Hospital			x		
Breast	Haikal, 2017 [77]	Egypt	Other	Hospital			x		
Breast	Talaneh, 2017 [201]	Iran	Other	Hospital		x	x		
Breast	Shahabi, 2018 [187]	Iran	Other	Hospital		x	x		
Breast	Shaker, 2018 [189]	Egypt	African	Hospital		x	x		
Breast	Rashid, 2015 [174]	Pakistan	Other	Hospital		x	x		
Breast (male)	Kizildag, 2011 [111]	Turkey	Other	Hospital	x	x			x
CRC	Speer, 2001 [197]	Hungary	Caucasian	Hospital			x		
CRC	Wong, 2003 [217]	Singapore	Asian	Population		x			
CRC (rectal)	Murtaugh, 2006 [146]	USA	Caucasian	Population		x			
CRC	Park, 2006 [160]	Korea	Asian	Population	x	x	x		x
CRC	Flugge, 2007 [60]	Russia	Caucasian	Hospital	x	x	x	x	x
CRC	Kadiyska, 2007 [106]	Bulgaria	Caucasian	Hospital			x		

Cancer	Study	Country	Ethnicity	Source						
CRC	Slattery, 2007 and Slattery 2009 [192, 193]	USA	Caucasian	Population			x	x	x	x
CRC	Yaylim-Eraltan, 2007 [224]	Turkey	Other	Hospital		x		x		x
CRC	Grunhage, 2008 [71]	Germany	Caucasian	Hospital		x		x		
CRC	Li, 2009 [119]	China	Asian	Population Hospital		x		x		
CRC	Ochs-Balcom, 2008 [154]	USA	Caucasian	Population		x			x	x
CRC	Parisi, 2008 [159]	Spain	Caucasian	Population		x	x	x		
CRC	Theodoratou, 2008 [203]	UK	Caucasian	Population	x	x	x	x	x	
CRC	Wang, 2008 [214]	China	Asian	Hospital		x		x		
CRC	Jenab, 2009 [101]	Europe	Caucasian	Population		x		x		
CRC	Mahmoudi, 2010 and 2011 [130, 131]	Iran	Other	Hospital	x	x		x		x
CRC	Hughes, 2011 [95]	Czech republic	Caucasian	Hospital	x	x		x		x
CRC	Bentley, 2012 [18]	New Zealand	Caucasian	Population		x	x		x	x
CRC	Gunduz, 2012 [73]	Turkey	Other	Hospital				x		x
CRC	Rasool, 2013 and 2014 [175, 176]	India	Other	Hospital	x	x		x		
CRC	Atoum, 2014 [12]	Jordan	Other	Population						x
CRC	Laczmanska, 2014 [114]	Poland	Caucasian	Hospital	x	x		x		x
CRC	Sarkissyan, 2014 [183]	USA	Mixed	Hospital	x	x		x		x
CRC	Takeshige, 2015 [200]	Japan	Asian	Population	x	x		x		x
CRC	Alkhayal, 2016 [4]	Saudi Arabia	Other	Hospital	x	x		x		x
CRC	Cho, 2018 [36]	Korea	Asian	Hospital		x				
CRC	Moossavi, 2018 [142]	Iran	Other	Hospital		x				x
CRC	Vidigal, 2017 [212]	Brazil	Mixed	Hospital	x	x		x		
Esophageal	Chang, 2012 [30]	Ireland	Caucasian	Population	x	x		x		x
Esophageal	Gu, 2014 [72]	China	Asian	Hospital		x			x	
Gallbladder	Li, 2014 [120]	China	Asian	Hospital	x	x		x		x
Gastric	Cong, 2015 [42]	China	Asian	Hospital		x				
Gastric	Yin, 2017 [226]	China	Asian	Hospital		x				
Head and neck	Liu, 2005 [124]	USA	Caucasian	Population		x				x
Head and neck (Oral)	Bektas-Kayhan, 2010 [16]	Turkey	Other	Hospital		x				x
Head and neck (nasopharyngeal)	Huang, 2011 [93]	China	Asian	Hospital	x	x		x		

(continued)

Table 4.1 (continued)

Cancer site	Author, PY	Country	Ethnicity	Source of controls	ApaI	FokI	BsmI	Cdx2	TaqI
Head and neck	Zeljic, 2012 [230]	Serbia	Caucasian	Population	x	x	x		x
Kidney	Obara, 2007 [153]	Japan	Asian	Population	x		x		x
Kidney	Karami, 2008 [109]	Europe	Caucasian	Hospital		x	x		x
Kidney	Arjumand, 2012 [10]	India	Other	Hospital		x	x		
Kidney	Southard, 2012 [196]	Finland	Caucasian	Hospital		x			
Kidney	Yang, 2016 [222]	China	Asian	Hospital	x	x	x	x	x
Liver	Falleti, 2010 [59]	Italy	Caucasian	Hospital	x	x	x		x
Liver	Hung, 2014 [96]	Taiwan	Asian	Hospital	x		x		x
Liver	Peng, 2014 [162]	China	Asian	Hospital		x			
Lung (SCLC)	Dogan, 2009 [50]	Turkey	Other	Hospital			x		x
Lung	Kaabachi, 2014 [105]	Tunisia	African	Hospital	x	x	x		x
Lung (NSCLC)	Wu, 2016 [218]	China	Asian	Hospital	x	x	x		x
Lung	Gromowski, 2017 [69]	Poland	Caucasian	Hospital	x	x	x	x	x
Multiple myeloma	Shafia, 2013 [186]	India	Other	Hospital	x	x	x		
Multiple myeloma	Chen, 2017 [34]	China	Asian	Hospital	x	x			
Non-Hodgkin lymphoma	Purdue, 2007 [170]	Australia	Caucasian	Population		x	x		
Non-Hodgkin lymphoma	Purdue, 2007 [169]	USA	Caucasian	Population					x
Non-Hodgkin lymphoma	Smedby, 2011 [194]	Sweden	Caucasian	Population			x		x
Ovary	Lurie, 2007 [127]	USA	Asian, Caucasian	Population	x		x	x	x
Ovary	Clendenen, 2008 [39]	USA + Sweden	Caucasian	Population	x	x	x		x
Ovary	Tworoger, 2009 [209]	USA	Caucasian	Population		x	x	x	
Ovary	Lurie, 2011 [128]	USA + Europe	Caucasian	Population		x			
Ovary	Grant, 2013 [68]	USA	A-A, Caucasian	Population		x	x		x
Ovary	Mohapatra, 2013 [141]	India	Other	Population		x			
Ovary	Mostowska, 2016 [144]	Poland	Caucasian	Hospital		x	x		
Pancreas	Li, 2015 [121]	China	Asian	Hospital		x	x		
Prostate	Ingles, 1998 [98]	USA	A-A	Population					
Prostate	Ma, 1998 [129]	USA	Caucasian	Population					
Prostate	Correa-Cerro, 1999 [43]	France	Caucasian	Population		x			x
Prostate	Blazer, 2000 [22]	USA	A-A, Caucasian	Population					x
Prostate	Habuchi, 2000 [76]	Japan	Asian	Hospital	x		x		x
Prostate	Chokkalingam, 2001 [37]	China	Asian	Population		x	x		

Prostate	Medeiros, 2002 [134]	Portugal	Caucasian	Hospital					x
Prostate	Liu, 2003 [123]	China	Asian	Hospital			x		
Prostate	Nam, 2003 [148]	Canada	A-A, Asian, Caucasian	Hospital			x		
Prostate	Suzuki, 2003 [198]	Japan	Asian	Hospital	x		x		x
Prostate	Huang, 2004 [91]	Taiwan	Asian	Hospital	x		x		x
Prostate	Maistro, 2004 [132]	Brazil	Mixed	Hospital	x		x		x
Prostate	Oakley-Girvan, 2004 [152]	USA	A-A, Caucasian	Population	x	x	x		x
Prostate	Yang, 2004 [221]	China	Asian	Hospital		x			
Prostate	Hayes, 2005 [81]	Australia	Caucasian	Population		x	x	x	
Prostate	John, 2005 [103]	USA	Caucasian	Population					x
Prostate	Mishra, 2005 [138]	India	Other	Hospital		x			x
Prostate	Andersson, 2006 [7]	Sweden	Caucasian	Population					x
Prostate	Chaimuangraj, 2006 [28]	Thailand	Asian	Hospital			x		x
Prostate	Cicek, 2006 [38]	USA	Caucasian	Population	x	x	x	x	x
Prostate	Huang, 2006 [92]	Taiwan	Asian	Hospital		x	x		
Prostate	Holick, 2007 [85]	USA	Caucasian	Population		x	x		x
Prostate	Li, 2007 [117]	USA	Caucasian	Population		x	x		
Prostate	Mikhak, 2007 [137]	USA	Caucasian	Population		x	x	x	
Prostate	Onen, 2008 [155]	Turkey	Other	Hospital	x		x		x
Prostate	Torkko, 2008 [206]	USA	Caucasian, Hispanic	Population		x	x	x	
Prostate	Bai, 2009 [14]	China	Asian	Population	x	x	x		x
Prostate	Holt, 2009 [86]	USA	A-A, Caucasian	Population		x	x		x
Prostate	Szendroi, 2011 [199]	Hungary	Caucasian	Hospital			x		
Prostate	Rowland, 2013 [179]	USA	A-A, Caucasian	Population		x		x	
Prostate	Hu, 2014 [89]	China	Asian	Hospital					x
Prostate	Yousaf, 2014 [227]	Pakistan	Other	Hospital	x	x			x
Prostate	Atoum, 2015 [13]	Jordan	Other	Population	x	x			
Prostate	Gilbert, 2015 [67]	UK	Caucasian	Hospital	x	x	x	x	
Prostate	Jingwi, 2015 [102]	USA	A-A	Hospital	x		x		
Prostate	Deschasaux, 2016 [48]	France	Caucasian	Population		x	x	x	
Prostate	Nunes, 2016 [151]	Brazil	Mixed	Hospital		x	x		

(continued)

Table 4.1 (continued)

Cancer site	Author, PY	Country	Ethnicity	Source of controls	ApaI	FokI	BsmI	Cdx2	TaqI
Prostate	El Ezzi, 2017 [54]	Lebanon	Caucasian	Hospital	x	x	x		x
Prostate	Kambale, 2017 [107]	India	Other	Hospital	x	x			x
Prostate	Braczkowski, 2018 [24]	Poland	Caucasian	Hospital		x	x		x
Sarcoma sarcoma)	Ruza, 2003 [181]	Spain	Caucasian	Population	x	x			x
Skin (melanoma)	Hutchinson, 2000	UK	Caucasian	Hospital					x
Skin (melanoma, BCC, SCC)	Han, 2007 [79]	USA	Caucasian	Population				x	
Skin (melanoma)	Santonocito, 2007 [182]	ITALIA	Caucasian	Population		x	x		
Skin (melanoma)	Gapska, 2009 ([65], [66])	Poland	Caucasian	Population		x	x		x
Skin (melanoma)	Li, 2008 [117]	USA	Caucasian	Hospital		x	x		
Skin (melanoma)	Randerson-Moor, 2009 [173]	UK	Caucasian	Population	x	x	x	x	x
Skin (BCC)	Lesiak, 2011 [116]	Poland	Caucasian	Hospital	x	x	x		x
Skin (BCC, SCC)	Köstner, 2012 [113]	Germany	Caucasian	Hospital	x				x
Skin (melanoma)	Pena-Chilet, 2013 [161]	Spain	Caucasian	Hospital		x			x
Skin (melanoma)	Zeljic, 2014 [231]	Serbia	Caucasian	Hospital	x	x			x
Skin (NMSC)	Burns, 2017 [25]	USA	Caucasian	Hospital	x		x		x
Skin (melanoma)	Cauci, 2017 [27]	Italy	Caucasian	Hospital		x	x		
Solid pediatric tumor	Bienertova-Vasku, 2016 [21]	Czech Republic	Caucasian	Population		x	x	x	x
Tobacco-related	Deschasaux, 2015 [47]	France	Caucasian	Population		x	x	x	
Thyroid (follicular, papillary)	Penna-Martinez, 2009 [163]	Germany	Caucasian	Population	x	x	x		x
Thyroid (papillary)	Beysel, 2018 [20]	Turkey	Other	Hospital	x	x	x		x

A-A: African-American

(a)

FokI (ff vs FF)

Author	Odds Ratio [95% CI]
Breast	
Curran, 1999 (Caucasian)	0.99 [0.45, 2.18]
Guy, 2003 (Caucasian)	0.80 [0.50, 1.28]
John, 2007 (African-American)	0.97 [0.46, 2.05]
John, 2007 (Caucasian)	0.80 [0.46, 1.40]
John, 2007 (Hispanic)	1.45 [0.83, 2.53]
Abbas, 2008 (Caucasian)	0.97 [0.79, 1.19]
Barroso, 2008 (Caucasian)	1.65 [1.02, 2.65]
Gapska, 2008 (Caucasian)	1.41 [1.16, 1.71]
Sinotte, 2008 (Caucasian)	1.33 [1.03, 1.73]
McKay, 2009 (African-American)	0.65 [0.32, 1.30]
McKay, 2009 (Asian)	1.63 [1.07, 2.49]
McKay, 2009 (Caucasian)	1.15 [1.02, 1.28]
McKay, 2009 (Hispanic)	1.34 [0.87, 2.07]
McKay, 2009 (Other-Hawaiian)	1.02 [0.46, 2.27]
Anderson, 2011 (Caucasian)	0.71 [0.57, 0.88]
Kizildag, 2011 (male) (Other)	3.00 [0.87, 10.30]
Engel, 2012 (Caucasian)	1.20 [0.70, 1.90]
Rollison, 2012 (Caucasian)	1.04 [0.81, 1.33]
Rollison, 2012 (Hispanic)	0.91 [0.66, 1.27]
Fuhrman, 2013 (Caucasian)	0.83 [0.59, 1.17]
Mishra, 2013 (African-American)	2.90 [0.30, 26.30]
Mishra, 2013 (Hispanic)	1.00 [0.50, 2.20]
Shahbaz, 2013 (Other)	0.83 [0.36, 1.92]
Akilzhanova, 2014 (Other)	1.71 [1.21, 2.43]
Abd-Elsalam, 2015 (African)	1.43 [0.71, 2.88]
Clendenen, 2015 (Caucasian)	1.05 [0.88, 1.24]
Nemenani, 2015 (Asian)	2.60 [1.11, 6.19]
Rashid, 2015 (Other)	0.89 [0.52, 1.52]
Deschasaux, 2016 (Caucasian)	1.08 [0.67, 1.74]
Amadori, 2017 (African)	35.00 [2.73, 449.12]
Amadori, 2017 (Caucasian)	1.03 [0.28, 3.77]
Talaneh, 2017 (Other)	0.20 [0.06, 0.74]
Shahabi, 2018 (Other)	1.19 [0.58, 2.45]
Shaker, 2018 (African)	3.28 [1.34, 8.02]

0 1 2 3 4 5

(b)

FokI (ff vs FF)

Author	Odds Ratio [95% CI]
Prostate	
Correa-Cerro, 1999 (Caucasian)	0.84 [0.31, 2.28]
Chokkalingam, 2001 (Asian)	1.13 [0.67, 1.91]
Oakley-Girvan, 2004 (African-American)	0.70 [0.18, 2.72]
Oakley-Girvan, 2004 (Caucasian)	0.83 [0.44, 1.55]
Yang, 2004 (Asian)	0.62 [0.26, 1.48]
Hayes, 2005 (Caucasian)	0.99 [0.72, 1.35]
Mishra, 2005 (Other)	0.25 [0.09, 0.72]
Cicek, 2006 (Caucasian)	1.04 [0.71, 1.52]
Huang, 2006 (Asian)	0.79 [0.55, 1.14]
Holick, 2007 (Caucasian)	1.22 [0.87, 1.73]
Li, 2007 (Caucasian)	1.09 [0.86, 1.40]
Mikhak, 2007 (Caucasian)	1.03 [0.72, 1.46]
Rukin, 2007 (Caucasian)	1.11 [0.71, 1.72]
Torkko, 2008 (Caucasian)	1.25 [0.84, 1.87]
Bai, 2009 (Asian)	0.81 [0.41, 1.61]
Holt, 2009 (African-American)	0.30 [0.05, 1.71]
Holt, 2009 (Caucasian)	1.07 [0.78, 1.48]
Torkko, 2008 (Hispanic)	0.92 [0.51, 1.66]
Rowland, 2013 (African-American)	1.14 [0.51, 2.51]
Rowland, 2013 (Caucasian)	1.32 [0.99, 1.76]
Yousaf, 2014 (Other)	0.37 [0.08, 1.74]
Atoum, 2015 (Other)	1.23 [0.40, 3.78]
Gilbert, 2015 (Caucasian)	0.81 [0.61, 1.09]
Deschasaux, 2016 (Caucasian)	1.06 [0.46, 2.48]
Nunes, 2016 (Mixed)	1.67 [0.75, 3.71]
Kambale, 2017 (Other)	1.31 [0.32, 5.45]
Braczkowski, 2018 (Caucasian)	1.51 [0.59, 3.91]

0 0.5 1   2   3   4   5

Fig. 4.1 Forest plot for the association between *Fok1 ff* and *FF* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the pancreas, skin, and thyroid, sarcoma, pediatric solid tumors, and tobacco-related cancers; (**e**) cancers of the kidney, liver, lung, and ovary, multiple myeloma, and non-Hodgkin lymphoma; (**f**) cancers of the bladder, brain, esophagus, gallbladder, and head and neck and gastric cancer

(c)

FokI (ff vs FF)

Author	Odds Ratio [95% CI]

CRC

Wong, 2003 (Asian) — 1.84 [1.15, 2.94]
Murtaugh, (rectal) 2006 (Caucasian) — 1.08 [0.80, 1.46]
Park, 2006 (Asian) — 0.35 [0.19, 0.65]
Flugge, 2007 (Caucasian) — 1.32 [0.79, 2.20]
Yaylim-Eraltan, 2007 (Other) — 4.71 [0.48, 46.23]
Grunhage, 2008 (Caucasian) — 1.12 [0.43, 2.97]
Ochs-Balcom, 2008 (Caucasian) — 1.87 [1.03, 3.38]
Theodoratou, 2008 (Caucasian) — 1.02 [0.87, 1.19]
Wang, 2008 (Asian) — 2.51 [1.21, 5.18]
Jenab, 2009 (Caucasian) — 1.03 [0.79, 1.34]
Li, 2009 (Asian) — 0.89 [0.53, 1.47]
Slattery, 2009 colon (Caucasian) — 0.73 [0.59, 0.90]
Mahmoudi, 2011 (Other) — 0.85 [0.66, 1.59]
Bentley, 2012 (Caucasian) — 1.07 [0.59, 1.94]
Rasool, 2013 (Other) — 1.30 [0.86, 1.65]
Laczmanska, 2014 (Caucasian) — 1.32 [0.71, 2.46]
Sarkissyan, 2014 (Mixed) — 0.34 [0.12, 0.93]
Takeshige, 2015 (Asian) — 0.83 [0.59, 1.17]
Alkhayal, 2016 (Asian) — 1.38 [0.41, 4.59]
Cho, 2018 (Asian) — 0.92 [0.68, 1.24]
Moossavi, 2018 (Other) — 4.84 [0.99, 23.62]

0   0.5   1   2   3   4   5

(d)

FokI (ff vs FF)

Author	Odds Ratio [95% CI]

Pancreas

Li, 2015 (Asian) — 2.98 [1.84, 4.81]

Sarcoma

Ruza, 2003 (Ewing Sarcoma) (Caucasian) — 1.18 [0.47, 2.93]
Ruza, 2003 (Osteosarcoma) (Caucasian) — 0.52 [0.19, 1.44]

Skin

Han, 2007 (BCC) (Caucasian) — 1.50 [0.98, 2.30]
Han, 2007 (Caucasian) — 1.40 [0.86, 2.27]
Han, 2007 (SCC) (Caucasian) — 1.41 [0.92, 2.17]
Santonocito, 2007 (Caucasian) — 0.81 [0.31, 2.09]
Li, 2008 (Caucasian) — 1.08 [0.78, 1.49]
Gapska, 2009 (Caucasian) — 0.99 [0.80, 1.30]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian) — 1.03 [0.73, 1.45]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian) — 1.88 [1.25, 2.82]
Lesiak, 2011 (BCC) (Caucasian) — 7.85 [3.88, 15.86]
Pena-chilet, 2013 (Caucasian) — 0.96 [0.61, 1.52]
Zeljic, 2014 (Caucasian) — 0.11 [0.04, 0.29]
Cauci, 2017 (Caucasian) — 0.93 [0.41, 2.14]

Thyroid

Penna-Martinez, 2009 (Follicular) (Caucasian) — 3.75 [1.45, 9.70]
Penna-Martinez, 2009 (Papillary) (Caucasian) — 0.66 [0.31, 1.42]
Beysel, 2018 (Other) — 2.44 [1.29, 4.62]

Solid tumor pediatric

Bienertová-Vašků, 2016 (Caucasian) — 1.73 [0.92, 3.28]

Tobacco-related cancers

Deschasaux, 2015 (Caucasian) — 1.87 [1.08, 3.23]

0   0.5   1   2   3   4   5

Fig. 4.1 (continued)

(e)

FokI (ff vs FF)

Author		Odds Ratio [95% CI]
Kidney		
Karami, 2008 (Caucasian)		0.91 [0.69, 1.19]
Arjumand, 2012 (Other)		0.85 [0.50, 1.46]
Southard, 2012 (Caucasian)		0.81 [0.45, 1.46]
Yang, 2016 (Asian)		1.05 [0.65, 1.70]
Liver		
Falleti, 2010 (Caucasian)		0.85 [0.34, 2.15]
Peng, 2014 (Asian)		2.15 [1.13, 4.08]
Lung		
Kaabachi, 2014 (African)		0.32 [0.15, 0.66]
Wu, 2016 (Asian)		1.15 [0.84, 1.37]
Gromowski, 2017 (Caucasian)		1.03 [0.79, 1.34]
Multiple Myeloma		
Shafia, 2013 (Other)		5.33 [2.33, 12.10]
Chen, 2017 (Asian)		2.62 [1.66, 4.14]
Non-Hodgkin's lymphoma		
Purdue, 2007 (Caucasian)		1.13 [0.77, 1.66]
Ovary		
Clendenen, 2008 (Caucasian)		1.23 [0.61, 2.51]
Tworoger, 2009 (Caucasian)		1.26 [1.01, 1.57]
Lurie, 2011 (Caucasian)		1.16 [0.97, 1.39]
Grant, 2013 (African-American)		2.06 [0.43, 9.75]
Grant, 2013 (Caucasian)		0.95 [0.63, 1.43]
Mohapatra, 2013 (Other)		3.50 [1.02, 12.06]
Mostowka, 2016 (Caucasian)		1.55 [1.00, 2.40]

0   0.5   1   2   3   4   5

(f)

FokI (ff vs FF)

Author		Odds Ratio [95% CI]
Bladder		
Mittal, 2007 (Other)		0.49 [0.19, 1.25]
Ben Fradj, 2016 (African)		2.67 [1.24, 5.74]
Brain		
Anic, 2012 (Caucasian)		0.89 [0.62, 1.27]
Toptaş, 2013 (Other)		0.43 [0.20, 0.93]
Yilmaz, 2018 (pediatric) (Other)		0.47 [0.01, 15.03]
Esophageal		
Chang, 2012 (Caucasian)		1.12 [0.50, 2.50]
Gu, 2014 (Asian)		1.01 [0.73, 1.40]
Gallbladder		
Li, 2014 (Asian)		0.29 [0.19, 0.46]
Gastric		
Cong, 2015 (Asian)		1.90 [1.07, 3.34]
Yin, 2017 (Asian)		0.97 [0.65, 1.45]
Head and neck		
Liu, 2005 (Caucasian)		0.64 [0.47, 0.87]
Huang, 2011 (Asian)		1.00 [0.56, 1.79]
Zeljic, 2012 (Caucasian)		0.89 [0.36, 2.20]

0   0.5   1   2   3   4   5

Fig. 4.1 (continued)

(a)

FokI (Ff vs FF)

Author		Odds Ratio [95% CI]
Breast		
Curran, 1999 (Caucasian)		1.08 [0.60, 1.92]
Guy, 2003 (Caucasian)		0.96 [0.69, 1.33]
John, 2007 (African-American)		1.29 [0.84, 1.96]
John, 2007 (Caucasian)		0.83 [0.56, 1.24]
John, 2007 (Hispanic)		0.84 [0.56, 1.27]
Abbas, 2008 (Caucasian)		0.89 [0.77, 1.03]
Barroso, 2008 (Caucasian)		1.26 [0.89, 1.66]
Gapska, 2008 (Caucasian)		1.20 [1.02, 1.41]
Sinotte, 2008 (Caucasian)		1.15 [0.95, 1.40]
McKay, 2009 (African-American)		1.13 [0.83, 1.56]
McKay, 2009 (Asian)		1.06 [0.78, 1.45]
McKay, 2009 (Caucasian)		1.05 [0.97, 1.14]
McKay, 2009 (Hispanic)		1.05 [0.75, 1.48]
McKay, 2009 (Other-Hawaiian)		1.02 [0.61, 1.71]
Anderson, 2011 (Caucasian)		1.01 [0.86, 1.17]
Kizildag, 2011 (male) (Other)		0.70 [0.25, 1.95]
Engel, 2012 (Caucasian)		1.30 [0.90, 1.70]
Rollison, 2012 (Caucasian)		0.93 [0.79, 1.11]
Rollison, 2012 (Hispanic)		0.93 [0.72, 1.20]
Fuhrman, 2013 (Caucasian)		0.85 [0.67, 1.09]
Mishra, 2013 (African-American)		2.20 [0.95, 5.10]
Mishra, 2013 (Hispanic)		1.00 [0.50, 1.80]
Shahbazi, 2013 (Other)		1.09 [0.67, 1.77]
Abd-Elsalam, 2015 (African)		1.07 [0.59, 1.94]
Clendenen, 2015 (Caucasian)		0.99 [0.87, 1.13]
Nemenqani, 2015 (Asian)		1.60 [0.88, 3.22]
Rashid, 2015 (Other)		1.06 [0.84, 1.34]
Deschasaux, 2016 (Caucasian)		0.99 [0.69, 1.44]
Amadori, 2017 (African)		6.67 [1.54, 28.84]
Amadori, 2017 (Caucasian)		1.38 [0.54, 3.54]
Talaneh, 2017 (Other)		0.50 [0.08, 3.06]
Shahabi, 2018 (Other)		1.06 [0.50, 2.20]
Shaker, 2018 (African)		1.02 [0.41, 2.54]

0   0.5   1   2   3   4   5

(b)

FokI (Ff vs FF)

Author		Odds Ratio [95% CI]
Prostate		
Correa-Cerro, 1999 (Caucasian)		1.05 [0.59, 1.87]
Chokkalingam, 2001 (Asian)		1.06 [0.69, 1.63]
Oakley-Girvan, 2004 (African-American)		0.52 [0.29, 0.94]
Oakley-Girvan, 2004 (Caucasian)		1.25 [0.81, 1.91]
Yang, 2004 (Asian)		0.72 [0.36, 1.45]
Hayes, 2005 (Caucasian)		0.97 [0.78, 1.20]
Mishra, 2005 (Other)		0.52 [0.31, 0.86]
Cicek, 2006 (Caucasian)		1.07 [0.81, 1.42]
Huang, 2006 (Asian)		0.89 [0.65, 1.21]
Holick, 2007 (Caucasian)		1.09 [0.84, 1.41]
Li, 2007 (Caucasian)		1.08 [0.91, 1.30]
Mikhak, 2007 (Caucasian)		1.25 [0.97, 1.62]
Rukin, 2007 (Caucasian)		1.13 [0.83, 1.54]
Torkko, 2008 (Caucasian)		1.09 [0.82, 1.43]
Bai, 2009 (Asian)		1.32 [0.73, 2.38]
Holt, 2009 (African-American)		1.20 [0.64, 2.25]
Holt, 2009 (Caucasian)		0.96 [0.76, 1.20]
Torkko, 2008 (Hispanic)		1.13 [0.71, 1.80]
Rowland, 2013 (African-American)		1.02 [0.72, 1.43]
Rowland, 2013 (Caucasian)		1.06 [0.87, 1.30]
Yousaf, 2014 (Other)		2.44 [0.05, 124.96]
Atoum, 2015 (Other)		0.85 [0.47, 1.53]
Gilbert, 2015 (Caucasian)		1.10 [0.90, 1.34]
Deschasaux, 2016 (Caucasian)		1.86 [0.98, 3.52]
Nunes, 2016 (Mixed)		1.19 [0.74, 1.93]
Kambale, 2017 (Other)		2.13 [1.23, 3.69]
Braczkowski, 2018 (Caucasian)		1.13 [0.52, 2.42]

0   0.5   1   2   3   4   5

Fig. 4.2 Forest plot for the association between *Fok1 Ff* and *FF* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the pancreas, skin, and thyroid, sarcoma, pediatric solid tumors, and tobacco-related cancers; (**e**) cancers of the kidney, liver, lung, and ovary, multiple myeloma, and non-Hodgkin lymphoma; (**f**) cancers of the bladder, brain, esophagus, gallbladder, and head and neck and gastric cancer

(c)

FokI (Ff vs FF)

Author		Odds Ratio [95% CI]
CRC		
Wong, 2003 (Asian)		1.51 [1.00, 2.29]
Murtaugh, (rectal) 2006 (Caucasian)		1.09 [0.89, 1.34]
Park, 2006 (Asian)		0.57 [0.39, 0.84]
Flugge, 2007 (Caucasian)		1.33 [0.90, 1.97]
Yaylim-Eraltan, 2007 (Other)		0.11 [0.03, 0.44]
Grunhage, 2008 (Caucasian)		1.09 [0.66, 1.79]
Ochs-Balcom, 2008 (Caucasian)		1.00 [0.65, 1.54]
Theodoratou, 2008 (Caucasian)		1.12 [1.00, 1.25]
Wang, 2008 (Asian)		1.25 [0.66, 2.39]
Jenab, 2009 (Caucasian)		0.95 [0.78, 1.15]
Li, 2009 (Asian)		0.99 [0.63, 1.58]
Slattery, 2009 colon (Caucasian)		0.84 [0.73, 0.97]
Mahmoudi, 2011 (Other)		1.09 [0.83, 1.43]
Bentley, 2012 (Caucasian)		1.52 [0.98, 2.35]
Rasool, 2013 (Other)		0.84 [0.60, 1.18]
Laczmanska, 2014 (Caucasian)		1.19 [0.74, 1.93]
Sarkissyan, 2014 (Mixed)		0.66 [0.38, 1.14]
Takeshige, 2015 (Asian)		0.88 [0.70, 1.10]
Alkhayal, 2016 (Asian)		0.90 [0.50, 1.63]
Cho, 2018 (Asian)		0.83 [0.66, 1.04]
Moossavi, 2018 (Other)		1.58 [0.83, 2.99]

0   0.5   1   2   3   4   5

(d)

FokI (Ff vs FF)

Author		Odds Ratio [95% CI]
Pancreas		
Li, 2015 (Asian)		2.03 [1.37, 3.01]
Sarcoma		
Ruza, 2003 (Ewing Sarcoma) (Caucasian)		1.22 [0.60, 2.49]
Ruza, 2003 (Osteosarcoma) (Caucasian)		1.50 [0.80, 2.79]
Skin		
Han, 2007 (BCC) (Caucasian)		1.02 [0.74, 1.41]
Han, 2007 (Caucasian)		0.96 [0.67, 1.37]
Han, 2007 (SCC) (Caucasian)		0.91 [0.66, 1.24]
Santonocito, 2007 (Caucasian)		0.78 [0.41, 1.47]
Li, 2008 (Caucasian)		1.30 [1.05, 1.60]
Gapska, 2009 (Caucasian)		0.91 [0.70, 1.10]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)		1.17 [0.91, 1.51]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)		1.28 [0.93, 1.75]
Lesiak, 2011 (BCC) (Caucasian)		4.87 [2.74, 8.65]
Pena-chilet, 2013 (Caucasian)		1.12 [0.82, 1.51]
Zeljic, 2014 (Caucasian)		0.32 [0.16, 0.64]
Cauci, 2017 (Caucasian)		1.38 [0.80, 2.37]
Thyroid		
Penna-Martinez, 2009 (Follicular) (Caucasian)		1.80 [0.81, 3.98]
Penna-Martinez, 2009 (Papillary) (Caucasian)		1.03 [0.67, 1.57]
Beysel, 2018 (Other)		1.71 [1.15, 2.76]
Solid tumor pediatric		
Bienertová-Vašků, 2016 (Caucasian)		1.44 [0.77, 2.67]
Tobacco-related cancers		
Deschasaux, 2015 (Caucasian)		1.42 [0.96, 2.10]

0   0.5   1   2   3   4   5

Fig. 4.2 (continued)

(e)

FokI (Ff vs FF)

Author		Odds Ratio [95% CI]
Kidney		
Karami, 2008 (Caucasian)		0.90 [0.73, 1.11]
Arjumand, 2012 (Other)		0.99 [0.56, 1.74]
Southard, 2012 (Caucasian)		0.81 [0.53, 1.24]
Yang, 2016 (Asian)		1.15 [0.78, 1.71]
Liver		
Falleti, 2010 (Caucasian)		0.95 [0.54, 1.67]
Peng, 2014 (Asian)		1.73 [0.98, 3.04]
Lung		
Kaabachi, 2014 (African)		0.58 [0.40, 0.84]
Wu, 2016 (Asian)		0.97 [0.73, 1.41]
Gromowski, 2017 (Caucasian)		0.94 [0.76, 1.17]
Multiple Myeloma		
Shafia, 2013 (Other)		1.58 [0.74, 3.38]
Chen, 2017 (Asian)		1.66 [1.30, 2.10]
Non-Hodgkin's lymphoma		
Purdue, 2007 (Caucasian)		1.00 [0.77, 1.31]
Ovary		
Clendenen, 2008 (Caucasian)		1.10 [0.67, 1.81]
Tworoger, 2009 (Caucasian)		1.13 [0.96, 1.33]
Lurie, 2011 (Caucasian)		1.13 [1.00, 1.29]
Grant, 2013 (African-American)		1.12 [0.52, 2.43]
Grant, 2013 (Caucasian)		0.93 [0.70, 1.23]
Mohapatra, 2013 (Other)		1.00 [0.36, 2.81]
Mostowka, 2016 (Caucasian)		1.06 [0.74, 1.52]

```
0   0.5  1    2    3    4    5
```

(f)

FokI (Ff vs FF)

Author		Odds Ratio [95% CI]
Bladder		
Mittal, 2007 (Other)		0.60 [0.39, 0.91]
Ben Fradj, 2016 (African)		1.33 [0.88, 2.01]
Brain		
Anic, 2012 (Caucasian)		0.75 [0.59, 0.97]
Toptaş, 2013 (Other)		0.78 [0.45, 1.35]
Yilmaz, 2018 (pediatric) (Other)		0.63 [0.24, 1.65]
Esophageal		
Chang, 2012 (Caucasian)		0.99 [0.58, 1.70]
Gu, 2014 (Asian)		0.91 [0.70, 1.18]
Gallbladder		
Li, 2014 (Asian)		0.58 [0.41, 0.83]
Gastric		
Cong, 2015 (Asian)		1.52 [0.95, 2.45]
Yin, 2017 (Asian)		0.89 [0.65, 1.23]
Head and neck		
Liu, 2005 (Caucasian)		0.85 [0.68, 1.06]
Huang, 2011 (Asian)		1.11 [0.68, 1.83]
Zeljic, 2012 (Caucasian)		1.38 [0.58, 3.26]

```
0   0.5  1    2    3    4    5
```

Fig. 4.2 (continued)

Only two studies [6, 201] found the *Fok1 ff* genotype significantly inversely associated with breast cancer risk (OR = 0.71; 95%CI: 0.57–0.88 and OR = 0.20; 95%CI: 0.06–0.74, respectively). It is unclear the reason for this association. Anderson analyzed a big sample size and the estimates were adjusted for age. However this study presents a significant departure from Hardy-Weinberg equilibrium for this polymorphism. In the most recent study conducted in Iran [201], the authors suggested that the observed discrepancies could be attributed to the small number of participants, races, and risk factors not considered in the analysis (analyses not adjusted).

On the other hand, eight studies reported a significant positive association [3, 5, 15, 65, 133, 149, 189, 191] between *Fok1 ff* genotype and breast cancer risk. The biggest study, Gapska [65], found a 41% significant increased risk (OR = 1.41; 95%CI: 1.16–1.71) in a Caucasian population (Poland) including 1736 cases and 1484 controls. Another big study was performed in Canada: Sinotte [191] found a significant higher breast cancer risk (OR = 1.33, 95% CI = 1.03–1.73), and this association was also observed for women without family history of breast cancer in first-degree relatives.

McKay [133] found a significant increase in breast cancer risk associated with the *ff* genotype in Japanese (OR = 1.63; 95%CI: 1.07–2.49) and Caucasian (OR = 1.15; 95%CI: 1.02-1.28) women in a multiethnic cohort study conducted in USA.

The remaining studies found no significant association (Figs. 4.1 and 4.2).

Analyzing *Fok1 Ff* genotype, only two studies found a significant increased risk of breast cancer: Gapska [65] (OR = 1.20; 95%CI: 1.02–1.41) and Amadori [5] (OR = 6.67; 95%CI: 1.54–28.84).

Amadori conducted the study on a mix population (indigenous black Tanzanian and Caucasian Italian population), but they found an effect only in the African population, and this result could also be given by the relatively limited sample size (18 cases and 50 controls). Finally, Gapska identified an association between the

Fok1 polymorphisms and early-onset breast cancer risk in a Polish population [65].

In conclusion, the weight of the evidence tends to indicate an association of breast cancer with *Fok1 ff* genotype. The most recent meta-analysis published by Iqbal [99] showed that the *Fok1-f* allele was associated with breast cancer risk with a recessive model, but the SOR was not statistically significant (*Fok1 ff* + F*f* vs *FF*; SOR = 0.25, 95%CI: 0.896–1.759). The authors explained the discrepancies may be due in part to variation in linkage disequilibrium between these functional and marker alleles. Further studies are necessary to clarify these observations.

Prostate Cancer

Twenty-three eligible studies have been published between 1999 and 2018 (Table 4.1, Figs. 4.1 and 4.2) analyzing the association between *Fok1* polymorphism of the *VDR* gene and prostate cancer risk. They are carried out mainly in the USA (eight studies), and the remaining 15 in China (three), France (two), India (two), the UK, Australia, Poland, Brazil, Jordan, Pakistan, Lebanon, and Taiwan. Fourteen (61%) were case-control studies with population controls, and 14 (61%) analyzed a Caucasian population.

There was no strong evidence of altered risk of developing prostate cancer analyzing the *ff* genotype. Two big studies with more than 1000 cases [117, 179] were from the USA. The *Fok1* was not directly associated with prostate cancer risk. An increased risk was associated with the less functional *Fok1 ff* genotype only in the presence of low 25(OH)D status [117] and only among Caucasian and for advanced disease [179].

Two studies carried out in India [107, 138] found an association with prostate cancer risk. Mishra found the Fok1 *ff* and *Ff* genotype significantly inversely associated with prostate cancer risk (OR = 0.25; 95%CI: 0.09–0.72 and OR = 0.52; 95%CI: 0.31–0.86, respectively). This was the first report from an Indian population, suggesting that the *f* allele could be protective in nature and hence less aggressive

[219]. The most recent study [107] found an opposite effect only for the *Ff* genotype (OR = 2.13; 95%CI: 1.23–3.69). On the contrary, *Ff* genotype was associated with a reduced risk of prostate cancer in the study by Oakley-Girvan in the USA; however, this association was found only in the group of African-Americans [152].

The other studies failed in finding any significant association. Two recent meta-analyses have been published on prostate cancer [108, 136]. In the overall analysis, both studies found that *Fok1* polymorphism was not significantly associated with the susceptibility to prostate cancer, but they found a significant association in the subgroup analysis for Caucasians and in the subgroup of population-based controls.

The most recent study, published in Poland [24], indicated a lack of relationship between prostate cancer and the *Fok1 VDR* gene polymorphisms, but these results could also be given by the relatively limited sample size.

In conclusion, there is no evidence of an association between the *VDR* gene *Fok1* polymorphism and prostate cancer risk, and further studies are necessary to clarify possible interactions with other factors.

Colorectal Cancer

Twenty-one studies presented data on *Fok1* and colorectal cancer (CRC). Six studies were performed in Asian countries (two in China, two in Korea, one in Singapore, and one in Japan), nine studies analyzed a Caucasian population, and the reaming six studies were performed on other populations [4, 131, 142, 175, 183, 224] (Table 4.1, Figs. 4.1 and 4.2).

One big study conducted in the USA suggested a significant 27% decreased risk of CRC for *ff* genotype [193]: OR = 0.73 (95%CI: 0.59, 0.90). Accordingly, there are two other small studies published by Sarkissyan [183] and Park [160] suggesting a protective role of 65–66% for CRC, respectively.

Contrasting results were published by three studies [154, 214, 217] reporting a significant increased risk for CRC for *ff* variant versus *FF*.

Two studies were performed in Singapore and China [214, 217]: OR = 1.84 (95%CI: 1.15, 2.94) and OR = 2.51 (95%CI: 1.21, 5.18), respectively. The third study was published in the USA by Ochs-Balcom [154], OR = 1.87 (95%CI: 1.03, 3.38), but the sample size is limited (250 cases and 246 controls). All the others suggest no effect or increased risk.

For heterozygous genotype, three estimates indicated a significant protective effect for *Ff* vs *FF* ranging from 89% (OR = 0.11: 95%CI: 0.03, 0.44) in the very small study from Turkey [224] to 43% in a study from Korea [160] (OR = 0.57: 95%CI: 0.39, 0.84). The big study conducted in the USA [193] suggested a more modest significant decreased risk of 16% [193] OR = 0.84 (95%CI: 0.73, 0.97). Only two studies indicated an increased risk for the heterozygous genotype, *Ff* vs *FF*: a very big study conducted in the UK [203] suggested a borderline significant increased risk: OR = 1.12 (95%CI: 1.00, 1.25). They also found a statistically significant interaction of *Fok1* with vitamin D and calcium dietary intake. Individuals homozygous for the variant and who had a high dietary intake of vitamin D or calcium had a higher risk compared with those homozygous for the wild-type with a high dietary intake of vitamin D and calcium. The other study published by Wong [217] indicated an increased risk of 51% in an Asian population (Singapore).

The most recent meta-analyses published in 2018 included 29 studies finding a borderline significant association comparing *F* allele versus *f* in a mixed model (OR = 1.029, 95%CI: 0.999, 1.059) considering this polymorphism a risk factor for CRC.

Skin Cancer

Nine studies were found on *Fok1* and skin cancer, and all were performed on Caucasian populations (Table 4.1, Figs. 4.1 and 4.2). Three estimates were reported for basal cell carcinoma or squamous cell carcinoma [79, 116] and eight estimates on melanoma. Seven studies were from Europe and two from the USA (Table 4.1, Figs. 4.1 and 4.2).

Three studies indicate positive associations between *ff* and *FF* for skin cancer (Fig. 4.1). Only one study reported an 88% significant increased risk for melanoma in a second Leeds case-control study [173], and one study published in Serbia [231] reported a contrasting result. They found a significant protective effect for the *ff* and for *Ff* variants vs *FF* (OR = 0.11; 95%CI: 0.04, 0.29 and OR = 0.32; 95%CI: 0.16, 0.64, respectively), but these results may be due to the small sample size of the study [231].

A study performed in a Polish population presented a significant and very-high-risk estimate for *ff* variant (OR = 7.85; 95%CI: 3.88, 15.86) but also for *Ff* vs FF (OR = 4.87; 95% CI: 2.74, 8.65) for basal cell carcinoma [116], but the sample size of the study is quite small (100 cases), and there is evidence of significant departure from Hardy-Weinberg equilibrium.

The most recent meta-analysis published in 2015 [115] revealed no association between melanoma and the *Fok1 F* allele in all study subjects (OR = 1.016, 95%CI = 0.869–1.189, p = 0.839). They also did not find association with melanoma susceptibility also comparing recessive and dominant models versus homozygote genotype [115], while the previous meta-analysis published by Zhao [232] found that *Fok1* polymorphism was associated with an overall significant increased risk of skin cancer (*Ff* vs *FF*: OR = 1.20, 95% CI = 1.01–1.44; *ff* vs *FF*: OR = 1.41, 95%CI =1.08–1.84; *Ff* + *ff* vs *FF*: OR = 1.26, 95%CI =1.04–1.53) including melanoma and non-melanoma skin cancer.

Ovarian Cancer

Six studies evaluated the association with ovarian cancer (Table 4.1, Figs. 4.1 and 4.2). Three studies reported positive risk estimates for *FF* genotype. A pooled analysis [209] of the New England Case-Control study and a nested case-control study of three prospective cohort studies (the Nurses' Health Study, NHSII, and the Women's Health Study) observed a significant positive association between the number of *Fok1 f* alleles and ovarian cancer risks (p-trend = 0.03). The

odds ratio for the *ff* versus *FF* genotype was 1.26 (95%CI: 1.01, 1.57).

Two other small studies found positive association between *ff* and *FF* genotype. Mohapatra [141] and Mostowska [144] found an increased risk. The Indian study [141] showed that the *ff* genotype was associated with a threefold increase in ovarian cancer risk, and the authors also found that vitamin D deficiency and *VDR* gene *Fok1* polymorphism acted non-synergistically (p value <0.4).

Only one study, Lurie [128], found a borderline increased risk for the *Ff* heterozygous genotype (OR = 1.13; 95%CI: 1.00, 1.29) in a pooled analysis of five population-based case-control studies within the Ovarian Cancer Association Consortium.

Two recent meta-analyses were published in 2018 analyzing the effect of the *Fok1* polymorphism on ovarian cancer susceptibility [31, 122]. Li suggested that the recessive model of the *Fok1* polymorphism (*ff* vs *Ff/FF*; OR = 1.15, 95%CI: 1.05–1.18; p = 0.000, I^2 = 67.9%) in Caucasian population (*ff* vs *Ff/FF*; OR = 1.12, 95%CI: 1.05–1.19; p = 0.000, I^2 = 73.2%) predicted the risk of ovarian cancer [122]. The other meta-analysis showed a fixed-effect odds ratio of 1.14 (95%CI 1.05–1.23) under a dominant model. They found also that the fixed-effect odds ratios were 1.12 (95%CI 1.03–1.21) and 1.49 (95%CI 1.06–2.09) in Caucasian and Asian populations, respectively.

Other Cancer Sites

Thirty-two publications for the other cancer sites are available for 15 cancer sites (Table 4.1, Figs. 4.1 and 4.2), and 2 studies were performed on different sites, Deschasaux [47] on tobacco-related cancers (2015) and Bienertová-Vašků [21] on pediatric solid tumors (2016).

Ten studies presented a significant increased risk for *ff* genotype and gastric [42], multiple myeloma [34, 186], liver [162], thyroid [20, 163], bladder [17], pancreas [121], and brain [205] cancers. The increased risk range from an 87% [47] on different cancer sites to a

fivefold increased risk reported by Shafia [186] in a small study (75 cases and 150 controls) conducted in India. Chen [34], Li [121], and Beysel [20] reported a statistical increased risk also for the *Ff* genotype versus *FF* and multiple myeloma, pancreas, and thyroid cancers, respectively, not found by the other studies.

Only three studies found a significant protective effect for *ff* and *Ff* genotype for gallbladder cancer for head and neck cancer, for brain cancer and for lung cancer (Figs. 4.1 and 4.2). Kabaki et al. reported a protective role also for the *Ff* variant vs *FF*. The other reports did not show significant associations.

Bsm1 and Cancer

Bsm1 is located at the 3′ end of the VDR gene. Since it is intronic, it apparently does not alter the amino acid sequence of the translated VDR protein [143]; however, in Caucasians, it is in strong linkage disequilibrium with the *poly(A)* microsatellite located in the 3′ untranslated region which appears to influence *VDR* messenger RNA stability and *VDR* translational activity [210] and thus affect local VDR protein levels. Some degree of coupling with *poly(A)* microsatellite was observed even in non-Caucasian populations, but the strength of the linkage disequilibrium varied by ethnicity [97]. A study of 599 healthy men reported that those with the *bb* genotype at the *Bsm1* locus had, on average, 2.3 pg/mL lower levels of $1,25(OH)_2D_3$ compared with *BB* carriers [129], supporting the hypothesis that *Bsm1* polymorphism may be a mediator for the cellular effects of vitamin D.

Breast Cancer

With respect to breast cancer risk, 28 studies were published in 10 years, from 1998 to 2018: 9 from the USA or Canada, 9 from Asia, 6 from Europe, 4 from Egypt, 1 from Kazakhstan, and 1 from Turkey (Table 4.1; Figs. 4.3 and 4.4). Seventeen studies (61%) were hospital-based, nine (32%) were population-based, and two (7%) included

mixed controls (both hospital and healthy subjects), and one was not reported (Table 4.1).

The biggest study is reported by McKay et al. in 2009 [133] and pooled data from six smaller cohort studies carried out in the USA and Europe, including 6473 cases and 8397 controls. The authors indicated no significant association overall between *Bsm1* polymorphism and breast cancer; however, they found a significant 58% risk reduction in the Asian subgroup (Japanese American) (OR = 0.60; 95%CI: 0.42–0.85) for heterozygous *Bb* genotype versus *bb*. In the same study in a subgroup analysis, they found a statistically significant lower risk of advanced breast cancer (OR = 0.74; 95%CI: 0.60–0.92) in women of all races with the *Bsm1 BB* genotype.

Summary estimates obtained in our previous meta-analysis [172] suggested no association between breast cancer and *Bsm1* polymorphism, with very similar estimates for heterozygous and *BB* homozygous subjects: summary odds ratio were indeed SOR = 0.99 (95%CI: 0.93–1.05) and SOR = 0.98 (95%CI: 0.91–1.05) for *Bb* and *BB* genotypes, respectively, compared with *bb* genotype.

After that meta-analysis, 12 new studies were published, most of all in Egypt (four) and Medium-Oriental areas (four from Kazakhstan, Pakistan, and Iran), with contrasting results. One small study conducted in Egypt [2] on 130 cases and 100 controls reported a protective effect of the *B* allele on breast cancer risk, with a significant risk reduction of 44% and 60%, respectively, for *Bb* and *BB* genotypes versus *bb*. Similar results for *BB* genotype were found in further studies conducted in Kazakhstan [3], Pakistan [174], and Iran [187], where a 32%, 33%, and 47% significant reduction of breast cancer risk were found, respectively. On the other side, other two small Egyptian studies [56, 189] found a risk effect of *B* allele on breast cancer, with ORs = 9.71 (95%CI: 2.61–36.11) and OR = 2.51 (95%CI: 1.32–4.77) for *Bb* genotype, respectively, but only Shaker found a significant increased risk for *BB* genotype OR = 2.38 (95% CI: 1.23–4.57). In the same direction, one study on Caucasian subjects from the USA [177] found a borderline significant increase of breast cancer

(a)

BsmI (BB vs bb)

Author		Odds Ratio [95% CI]
Breast		
Ruggiero, 1998 (Caucasian)		1.18 [0.42, 3.33]
Hou, 2002 (Asian)		11.33 [0.37, 346.24]
Buyru, 2003 (Other)		0.76 [0.25, 2.29]
Guy, 2003 (Caucasian)		0.53 [0.34, 0.84]
Hefler, 2004 (Caucasian)		1.12 [0.80, 1.56]
VandeVord, 2006 (Mixed)		0.81 [0.47, 1.38]
Trabert, 2007 (African-American)		1.25 [0.83, 1.88]
Trabert, 2007 (Caucasian)		0.85 [0.68, 1.06]
Gapska, 2008 (Caucasian)		0.97 [0.77, 1.22]
Sinotte, 2008 (Caucasian)		0.81 [0.62, 1.06]
McKay, 2009 (African-American)		0.91 [0.53, 1.57]
McKay, 2009 (Asian)		0.52 [0.12, 2.26]
McKay, 2009 (Caucasian)		1.28 [0.86, 1.91]
McKay, 2009 (Hispanic)		1.32 [0.70, 2.48]
McKay, 2009 (Other)		1.05 [0.31, 3.54]
McKay, 2009 (EPIC) (Caucasian)		0.93 [0.77, 1.13]
McKay, 2009 (MEC) (Caucasian)		0.85 [0.56, 1.30]
McKay, 2009 (NHS) (Caucasian)		0.91 [0.72, 1.15]
McKay, 2009 (PLCO) (Caucasian)		1.21 [0.94, 1.56]
McKay, 2009 (WHS) (Caucasian)		0.94 [0.67, 1.31]
Anderson, 2011 (Caucasian)		1.02 [0.83, 1.25]
Huang, 2012 (Asian)		4.73 [0.16, 141.80]
Rollison, 2012 (Caucasian)		1.05 [0.83, 1.33]
Rollison, 2012 (Hispanic)		1.24 [0.83, 1.86]
Akilzhanova, 2013 (Other)		0.68 [0.49, 0.95]
Fuhrman, 2013 (Caucasian)		0.69 [0.49, 0.97]
Mishra, 2013 (African-American)		0.58 [0.21, 1.64]
Mishra, 2013 (Hispanic)		1.44 [0.63, 3.31]
Shahbazi, 2013 (Other)		0.42 [0.21, 0.85]
Abd-Elsalam, 2015 (Other)		0.40 [0.18, 0.90]
Cleneden, 2015 (Caucasian)		1.03 [0.86, 1.22]
Guo, 2015 (Asian)		1.54 [0.10, 24.79]
Reimers, 2015 (Caucasian)		1.35 [1.00, 1.82]
Deschasaux, 2016 (Caucasian)		0.67 [0.41, 1.09]
Rashid, 2016 (Other)		0.67 [0.50, 0.90]
Elzehery, 2017 (Other)		3.40 [0.77, 14.93]
Haikal, 2017 (Other)		0.88 [0.26, 2.97]
Talaneh, 2017 (Other)		8.49 [1.06, 68.21]
Shahabi, 2018 (Other)		0.53 [0.32, 0.86]
Shaker, 2018 (Other)		2.38 [1.23, 4.57]

0   0.5   1   2   3   4   5

(b)

BsmI (BB vs bb)

Author		Odds Ratio [95% CI]
Prostate		
Ingles, 1998 (African-American)		0.91 [0.45, 1.84]
Habuchi, 2000 (Asian)		0.60 [0.19, 1.89]
Chokkalingam, 2001 (Asian)		1.06 [0.30, 3.67]
Liu, 2003 (Asian)		1.05 [0.02, 53.59]
Nam, 2003 (African-American)		1.25 [0.33, 4.69]
Nam, 2003 (Asian)		1.09 [0.11, 11.15]
Nam, 2003 (Caucasian)		0.88 [0.62, 1.24]
Suzuki, 2003 (Asian)		4.29 [0.84, 22.02]
Huang, 2004 (Asian)		0.40 [0.08, 2.00]
Oakley-Girvan, 2004 (African-American)		0.98 [0.34, 2.50]
Oakley-Girvan, 2004 (Caucasian)		0.93 [0.49, 1.80]
Hayes, 2005 (Caucasian)		0.87 [0.65, 1.17]
Chaimuangraj, 2006 (Asian)		0.29 [0.06, 1.54]
Cicek, 2006 (Caucasian)		0.72 [0.41, 1.28]
Holick, 2007 (Caucasian)		0.87 [0.61, 1.25]
Li, 2007 (Caucasian)		1.05 [0.83, 1.33]
Mikhak, 2007 (Caucasian)		1.18 [0.84, 1.66]
Onen, 2008 (Other)		0.78 [0.35, 1.74]
Bai, 2009 (Asian)		0.47 [0.02, 14.26]
Holt, 2009 (African-American)		3.92 [1.40, 10.95]
Holt, 2009 (Caucasian)		0.98 [0.71, 1.35]
Szendroi, 2011 (Caucasian)		3.71 [1.84, 7.51]
Gilbert, 2015 (Caucasian)		1.02 [0.77, 1.35]
Deschasaux, 2016 (Caucasian)		0.81 [0.34, 1.92]
Nunes, 2016 (Other)		0.65 [0.31, 1.34]
El Ezzi, 2017 (Caucasian)		1.36 [0.50, 3.73]
Braczkowski, 2018 (Caucasian)		0.94 [0.31, 2.81]

0   0.5   1   2   3   4   5

Fig. 4.3 Forest plot for the association between *Bsm1 BB* and *bb* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the pancreas, skin, and thyroid, pediatric solid tumors, and tobacco-related cancers; (**e**) cancers of the lung and ovary, multiple myeloma, and non-Hodgkin lymphoma; (**f**) cancers of the brain, esophagus, gallbladder, head and neck, kidney, and liver

(c)

BsmI (BB vs bb)

Author		Odds Ratio [95% CI]
CRC		
Speer, 2001 (Caucasian)		1.04 [0.44, 2.49]
Park, 2006 (Asian)		0.87 [0.03, 26.07]
Flugge, 2007 (Caucasian)		1.10 [0.65, 1.88]
Kadiyska, 2007 (Caucasian)		0.55 [0.25, 1.24]
Slattery, (colon) 2007 (Caucasian)		0.88 [0.72, 1.08]
Slattery, (rectal) 2007 (Caucasian)		0.94 [0.70, 1.25]
Li, 2008 (Asian)		0.44 [0.21, 0.92]
Parisi, 2008 (Caucasian)		0.89 [0.44, 1.80]
Theodoratou, 2008 (Caucasian)		0.93 [0.79, 1.08]
Jenab, 2009 (Caucasian)		0.76 [0.59, 0.98]
Hughes, 2011 (Caucasian)		0.93 [0.66, 1.30]
Mahmoudi, 2011 (Other)		0.97 [0.65, 1.44]
Gunduz, 2012 (Other)		0.54 [0.12, 2.52]
Laczmanska, 2014 (Caucasian)		0.53 [0.26, 1.06]
Rasool, 2014 (Other)		0.37 [0.21, 0.67]
Sarkyssyan, 2014 (Other)		1.20 [0.52, 2.79]
Takeshige, 2015 (Asian)		0.72 [0.28, 1.86]
Alkhayal, 2016 (Other)		0.49 [0.22, 1.08]
Vidigal, 2017 (Other)		0.89 [0.48, 1.62]

0   0.5   1   2   3   4   5

(d)

BsmI (BB vs bb)

Author		Odds Ratio [95% CI]
Pancreas		
Li, 2015 (Asian)		1.68 [1.06, 2.65]
Skin		
Santocito, 2000 (Caucasian)		0.29 [0.12, 0.71]
Han, 2007 (Caucasian)		0.89 [0.54, 1.48]
Han, 2007 (BCC) (Caucasian)		1.01 [0.66, 1.56]
Han, 2007 (SCC) (Caucasian)		1.51 [1.00, 2.28]
Li, 2008 (Caucasian)		0.78 [0.59, 1.05]
Gapska, 2009 (Caucasian)		0.92 [0.67, 1.27]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)		1.00 [0.71, 1.41]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)		0.69 [0.45, 1.06]
Lesiak, 2011 (BCC) (Caucasian)		0.65 [0.35, 1.22]
Burns, 2017 (Caucasian)		0.74 [0.31, 1.79]
Cauci, 2017 (Caucasian)		1.23 [0.60, 2.52]
Thyroid		
Penna-Martinez, 2009 (Foolicular)(Caucasian)		0.35 [0.11, 1.12]
Penna-Martinez, 2009 (Papillary) (Caucasian)		0.84 [0.46, 1.56]
Beysel, 2018 (Other)		0.67 [0.33, 1.36]
Solid tumor pediatric		
Bienertova-Vasku, 2016 (Caucasian)		1.27 [0.72, 2.21]
Tobacco-related cancers		
Deschasaux, 2015 (Caucasian)		0.73 [0.42, 1.27]

0   0.5   1   2   3   4   5

Fig. 4.3 (continued)

(e)

BsmI (BB vs bb)

Author		Odds Ratio [95% CI]
Lung		
Dogan, 2009 (Other)		0.63 [0.31, 1.28]
Kaabachi, 2014 (Other)		0.89 [0.48, 1.62]
Wu, 2016 (Asian)		0.78 [0.68, 0.96]
Gromowski, 2017 (Caucasian)		0.88 [0.64, 1.19]
Multiple Myeloma		
Shafia, 2013 (Other)		0.46 [0.23, 0.91]
Non-Hodgkin's lymphoma		
Purdue, 2007 (Caucasian)		0.94 [0.68, 1.31]
Purdue, 2007 (Mixed)		1.14 [0.80, 1.63]
Smedby, 2010 (Caucasian)		1.11 [0.93, 1.34]
Ovary		
Lurie, 2007 (Asian)		0.90 [0.20, 4.00]
Lurie, 2007 (Caucasian)		1.30 [0.50, 3.30]
Cleneden, 2008 (Caucasian)		1.08 [0.54, 2.17]
Tworoger, 2009 (Caucasian)		0.95 [0.76, 1.18]
Grant, 2013 (African-American)		1.68 [0.26, 10.64]
Grant, 2013 (Caucasian)		1.01 [0.68, 1.50]
Mostowska, 2016 (Caucasian)		1.62 [0.96, 2.73]

0   0.5   1   2   3   4   5

(f)

BsmI (BB vs bb)

Author		Odds Ratio [95% CI]
Brain		
Anic, 2012 (Caucasian)		0.77 [0.53, 1.12]
Yilmaz, 2017 (pediatric) (Other)		1.10 [0.30, 4.04]
Esophageal		
Chang, 2012 (Caucasian)		1.59 [0.75, 3.40]
Gallbladder		
Li, 2014 (Asian)		1.21 [0.75, 1.95]
Head and neck		
Huang, 2011 (Asian)		0.33 [0.03, 3.22]
Zeljic, 2012 (Caucasian)		0.25 [0.01, 5.11]
Kidney		
Obara, 2007 (Asian)		0.53 [0.02, 15.95]
Karami, 2008 (Caucasian)		0.85 [0.61, 1.18]
Arjumand, 2012 (Other)		0.38 [0.22, 0.66]
Yang, 2016 (Asian)		1.04 [0.02, 52.57]
Liver		
Falleti, 2010 (Caucasian)		0.58 [0.26, 1.32]
Hung, 2014 (Asian)		1.05 [0.02, 53.37]

0   0.5   1   2   3   4   5

Fig. 4.3 (continued)

(a)

BsmI (Bb vs bb)

Author		Odds Ratio [95% CI]
Breast		
Ruggiero, 1998 (Caucasian)		0.57 [0.25, 1.28]
Hou, 2002 (Asian)		2.12 [0.76, 5.91]
Buyru, 2003 (Other)		0.93 [0.50, 1.72]
Guy, 2003 (Caucasian)		0.65 [0.48, 0.87]
Hefler, 2004 (Caucasian)		0.98 [0.77, 1.25]
VandeVord, 2006 (Mixed)		1.19 [0.78, 1.84]
Trabert, 2007 (African-American)		0.97 [0.73, 1.28]
Trabert, 2007 (Caucasian)		0.92 [0.75, 1.12]
Gapska, 2008 (Caucasian)		0.97 [0.83, 1.13]
Sinotte, 2008 (Caucasian)		0.98 [0.81, 1.18]
McKay, 2009 (African-American)		1.10 [0.80, 1.51]
McKay, 2009 (Asian)		0.60 [0.42, 0.85]
McKay, 2009 (Caucasian)		1.22 [0.90, 1.66]
McKay, 2009 (Hispanic)		0.91 [0.66, 1.26]
McKay, 2009 (Other)		1.23 [0.74, 2.04]
McKay, 2009 (EPIC) (Caucasian)		1.02 [0.89, 1.17]
McKay, 2009 (MEC) (Caucasian)		1.02 [0.74, 1.41]
McKay, 2009 (NHS) (Caucasian)		1.01 [0.85, 1.20]
McKay, 2009 (PLCO) (Caucasian)		0.87 [0.72, 1.05]
McKay, 2009 (WHS) (Caucasian)		1.01 [0.78, 1.31]
Anderson, 2011 (Caucasian)		1.09 [0.93, 1.27]
Huang, 2012 (Asian)		1.58 [0.89, 2.80]
Rollison, 2012 (Caucasian)		1.11 [0.93, 1.33]
Rollison, 2012 (Hispanic)		1.13 [0.89, 1.43]
Fuhrman, 2013 (Caucasian)		0.81 [0.63, 1.04]
Mishra, 2013 (African-American)		0.66 [0.36, 1.24]
Mishra, 2013 (Hispanic)		1.18 [0.75, 1.86]
Shahbazi, 2013 (Other)		0.95 [0.57, 1.59]
Abd-Elsalam, 2015 (Other)		0.56 [0.31, 1.00]
Cleneden, 2015 (Caucasian)		1.06 [0.93, 1.21]
Guo, 2015 (Asian)		1.42 [0.86, 2.33]
Reimers, 2015 (Caucasian)		1.11 [0.84, 1.46]
Deschasaux, 2016 (Caucasian)		0.85 [0.59, 1.83]
Rashid, 2016 (Other)		0.84 [0.65, 1.09]
Elzehery, 2017 (Other)		9.71 [2.61, 36.11]
Haikal, 2017 (Other)		0.62 [0.20, 1.95]
Talaneh, 2017 (Other)		1.27 [0.49, 3.32]
Shahabi, 2018 (Other)		1.19 [0.71, 2.00]
Shaker, 2018 (Other)		2.51 [1.32, 4.77]

0   0.5 1   2   3   4   5

(b)

BsmI (Bb vs bb)

Author		Odds Ratio [95% CI]
Prostate		
Ingles, 1998 (African-American)		0.94 [0.59, 1.50]
Habuchi, 2000 (Asian)		0.26 [0.16, 0.43]
Chokkalingam, 2001 (Asian)		1.01 [0.54, 1.90]
Liu, 2003 (Asian)		1.40 [0.47, 4.20]
Nam, 2003 (African-American)		0.74 [0.30, 1.81]
Nam, 2003 (Asian)		1.64 [0.35, 7.60]
Nam, 2003 (Caucasian)		0.87 [0.64, 1.19]
Suzuki, 2003 (Asian)		1.22 [0.59, 2.52]
Huang, 2004 (Asian)		0.50 [0.24, 1.05]
Oakley-Girvan, 2004 (African-American)		0.90 [0.51, 1.60]
Oakley-Girvan, 2004 (Caucasian)		1.30 [0.78, 2.10]
Hayes, 2005 (Caucasian)		0.84 [0.67, 1.05]
Chaimuangraj, 2006 (Asian)		0.95 [0.28, 3.18]
Cicek, 2006 (Caucasian)		0.75 [0.51, 1.11]
Holick, 2007 (Caucasian)		0.77 [0.59, 0.99]
Li, 2007 (Caucasian)		0.98 [0.82, 1.17]
Mikhak, 2007 (Caucasian)		0.94 [0.72, 1.23]
Onen, 2008 (Other)		0.69 [0.42, 1.14]
Bai, 2009 (Asian)		0.36 [0.15, 0.85]
Holt, 2009 (African-American)		3.36 [1.19, 9.46]
Holt, 2009 (Caucasian)		1.09 [0.87, 1.38]
Szendroi, 2011 (Caucasian)		2.94 [1.71, 5.06]
Gilbert, 2015 (Caucasian)		1.08 [0.88, 1.32]
Deschasaux, 2016 (Caucasian)		1.13 [0.57, 2.22]
Nunes, 2016 (Other)		1.16 [0.71, 1.90]
El Ezzi, 2017 (Caucasian)		1.40 [0.66, 2.97]
Braczkowski, 2018 (Caucasian)		0.88 [0.44, 1.77]

0   0.5 1   2   3   4   5

Fig. 4.4 Forest plot for the association between *Bsm1 Bb* and *bb* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the pancreas, skin, and thyroid, pediatric solid tumors, and tobacco-related cancers; (**e**) cancers of the lung and ovary, multiple myeloma, and non-Hodgkin lymphoma; (**f**) cancers of the brain, esophagus, gallbladder, head and neck, kidney, and liver

(c)

BsmI (Bb vs bb)

Author	Odds Ratio [95% CI]
CRC	
Speer, 2001 (Caucasian)	0.65 [0.32, 1.36]
Park, 2006 (Asian)	1.36 [0.78, 2.37]
Flugge, 2007 (Caucasian)	0.95 [0.65, 1.38]
Kadiyska, 2007 (Caucasian)	0.55 [0.30, 1.01]
Slattery, (colon) 2007 (Caucasian)	1.01 [0.87, 1.17]
Slattery, (rectal) 2007 (Caucasian)	0.88 [0.72, 1.08]
Li, 2008 (Asian)	0.55 [0.34, 0.87]
Parisi, 2008 (Caucasian)	1.01 [0.61, 1.67]
Theodoratou, 2008 (Caucasian)	0.88 [0.79, 0.99]
Jenab, 2009 (Caucasian)	1.06 [0.87, 1.28]
Hughes, 2011 (Caucasian)	1.02 [0.80, 1.30]
Mahmoudi, 2011 (Other)	1.11 [0.83, 1.49]
Gunduz, 2012 (Other)	0.28 [0.09, 0.88]
Laczmanska, 2014 (Caucasian)	0.38 [0.17, 0.83]
Rasool, 2014 (Other)	0.15 [0.09, 0.26]
Sarkyssyan, 2014 (Other)	0.97 [0.58, 1.63]
Takeshige, 2015 (Asian)	0.98 [0.75, 1.29]
Alkhayal, 2016 (Other)	0.81 [0.44, 1.50]
Vidigal, 2017 (Other)	1.00 [0.66, 1.52]

(d)

BsmI (Bb vs bb)

Author	Odds Ratio [95% CI]
Pancreas	
Li, 2015 (Asian)	1.09 [0.71, 1.66]
Skin	
Santocito, 2000 (Caucasian)	0.74 [0.40, 1.40]
Han, 2007 (Caucasian)	0.85 [0.60, 1.22]
Han, 2007 (BCC) (Caucasian)	0.90 [0.65, 1.23]
Han, 2007 (SCC) (Caucasian)	1.05 [0.76, 1.45]
Li, 2008 (Caucasian)	0.75 [0.60, 0.93]
Gapska, 2009 (Caucasian)	0.91 [0.73, 1.13]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)	0.93 [0.72, 1.20]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)	0.81 [0.59, 1.11]
Lesiak, 2011 (BCC) (Caucasian)	0.76 [0.45, 1.29]
Burns, 2017 (Caucasian)	1.32 [0.60, 2.88]
Cauci, 2017 (Caucasian)	1.45 [0.78, 2.71]
Thyroid	
Penna-Martinez, 2009 (Foolicular)(Caucasian)	0.71 [0.35, 1.47]
Penna-Martinez, 2009 (Papillary) (Caucasian)	0.95 [0.59, 1.54]
Beysel, 2018 (Other)	0.84 [0.44, 1.64]
Solid tumor pediatric	
Bienertova-Vasku, 2016 (Caucasian)	0.67 [0.42, 1.05]
Tobacco-related cancers	
Deschasaux, 2015 (Caucasian)	1.15 [0.77, 1.70]

Fig. 4.4 (continued)

(e)

(f)

Fig. 4.4 (continued)

risk for *BB* compared to *bb* genotype. Other studies did not report significant association between breast cancer risk and *Bsm1* polymorphism (Figs. 4.3 and 4.4).

In conclusion, while on average it seems that no association existed between breast cancer risk and *Bsm1* polymorphism, recent studies provided very heterogeneous estimates even in populations of similar ethnic group, and this may be warranted to be investigated in future studies and possibly in subgroup analyses.

Prostate Cancer

With respect to prostate cancer, 23 studies published between 1988 and 2018 were found: 8 from the USA or Canada, 7 from Asia, 4 from Europe, and 1 each from Australia, Brazil, Lebanon, and Turkey (Table 4.1; Figs. 4.3 and 4.4). Fourteen studies (52%) were hospital-based; others are population-based (Table 4.1).

In a previous meta-analysis published in 2014 [172], SORs were 0.86 (95%CI: 0.69–1.08) and 0.95 (95%CI: 0.85–1.07) for *Bb* and *BB* genotypes, respectively, compared with *bb* genotype.

Generally, the results of studies on *Bsm1* and prostate cancer are controversial. Heterozygous *Bb* risk estimates give us an indication of protective effect compared to *bb* in earlier studies, while in recent ones, the ORs were usually above 1.00 [48, 54, 67, 151], although results were not significant. The biggest study (the Physicians' Health Study, with 1066 cases) [117] indicated no significant association.

Hayes [81], Cicek [38], Holick [84], Onen [155], Deschasaux [48], Nunes [151], and Braczkowski [24] showed a non-significant decreased risk of *BB* for developing prostate cancer as compared to *bb* genotypes. Only two studyies conducted in Hungary [199] and USA [86] suggested a significant almost 3-fold increased risk of prostate cancer for both *BB* and *Bb* vs wild-type *bb* genotype.

In conclusion, the studies included in this review seem to be not able to demonstrate a strong association between the VDR gene Bsm1 polymorphism and prostate cancer risk.

Colorectal Cancer

Eighteen studies presented data on *Bsm1* and colorectal cancer. Eight were from Europe, six from Asia, and two from the USA, one was from Turkey, and one was from Brazil (Table 3; Figs. 4.3 and 4.4). The two biggest studies with more than 1000 subjects were conducted in the USA [192] and Europe [101]. Twelve studies (67%) were hospital-based, while the remaining were population-based.

Our previous meta-analysis [172] suggested a significant risk reduction of colorectal cancer for carriers of *BB* genotype compared to carriers of *bb* genotype (SOR = 0.89; 95%CI: 0.80–0.98), with no evidence of between-study heterogeneity ($I^2 = 0\%$). This result was mainly due to the big study conducted in Europe, a nested case-control study within the European Prospective Investigation into Cancer and Nutrition [101], which indeed suggested a reduction of 24% for colon cancer risk for carriers of *BB* genotype.

Five studies on different ethnic groups were published after the above-cited meta-analysis, and all of them confirmed a trend of colorectal cancer reduction for *BB* genotype carriers, with significant results obtained in the study by Rasool et al. [176]: OR = 0.37 (95%CI: 0.21–0.67).

A trend of colorectal cancer risk reduction was also suggested for *Bb* genotype compared to *bb* genotype carriers. SOR (95%CI) from the published meta-analysis [172] was 0.88 (95%CI: 0.77–1.01); in line with this result, the five recently published studies reported inverse association with colorectal cancer risk, with significant risk reduction of 62% and 85%, respectively, observed in the study by Laczmanska et al. [114] and Rasool et al. [176].

In conclusion, publications up to now are suggestive of a protective effect of the *Bsm1* both *BB* and *B*b variant allele on colorectal cancer risk.

Skin Cancer

In eight studies, seven estimates were retrieved on *Bsm1* and melanoma and four estimates on non-melanoma skin cancer (NMSC). Five studies were from Europe and 4 from the USA (Table 4.1; Figs. 4.3 and 4.4). Half of the studies were hospital-based and half population-based (table).

Our previous meta-analysis [172] reported a significant protective effect of *Bb* genotype (SOR = 0.86; 95%CI: 0.76–0.98) and borderline protective effect of *BB* genotype (SOR = 0.87; 95%CI: 0.70–1.08) compared to *bb* genotype on overall skin cancer risk.

Only one study [78] reported data for squamous cell carcinoma (SCC), and the *BB* genotype was significantly associated with increased cancer risk (OR = 1.51, 95%CI: 1.00–2.28).

Recently, 2 small studies were published: 1 from Italy [27] included 120 melanoma cases and the second one from the USA [25] 97 NMSC cases. No association was found between *Bsm1* polymorphism and skin cancer, with ORs surprisingly above 1.00 for carriers of *B* allele.

The relative low number of studies on the association between melanoma and NMSC with *Bsm1* polymorphism makes it difficult to reach a firm conclusion, although most of the published studies and our previous meta-analysis seem to suggest a protective effect of the *B* allele on skin cancer risk.

Ovarian Cancer

Five studies evaluated the association with ovarian cancer: three were from the USA, one was mixed (the USA and Sweden), and one was from Poland (Table 4.1; Figs. 4.3 and 4.4). All except one were population-based studies.

Our previous meta-analysis [172] found no association of *Bsm1* polymorphism with ovarian cancer for carriers of neither one (SOR = 1.15;

95%CI: 0.96–1.37) or two (SOR = 1.01; 95%CI: 0.79–1.29) *B* alleles. In a pooled analysis [209] of 3 cohort studies and 1 nested case-control study summarizing 1473 ovarian cases, *Bsm1* was not found significantly associated with ovarian cancer risk.

A recently published update of a previously published study in Poland [144] suggested a significant 56% higher risk of ovarian cancer for carriers of *Bb* compared to *bb* genotype.

In summary, although no significant association was found for *Bsm1* and ovarian cancer, it seems that, contrary to other cancer sites, *B* allele confers a possible higher risk of cancer compared to *b* allele.

Renal Cancer

Four studies were published on the association between *Bsm1* polymorphism and renal cancer. Two were from Asia, one was from Eastern Europe, and one was from India (Table 4.1). Two (50%) were hospital-based and two (50%) population-based studies.

Due to the low number of studies and investigated cases, no significant association was suggested, although a trend toward a protective effect of the *B* allele was apparent, especially for Asian studies [135, 157], in line with results for other cancer sites.

Lung Cancer

Four studies investigated *Bsm1* polymorphism in relation to lung cancer. They were conducted in different countries, China, Turkey, Poland, and Tunisia, and they all were hospital-based studies (Table 4.1).

Three of the four studies were published after our meta-analysis published in 2014 [172]. Almost all the risk estimates were under 1.00, with significant lung cancer risk reduction suggested for carriers of the *Bb* genotype in one study [50], SOR = 0.55 (95%CI: 0.33–0.92), and for carriers of the *BB* genotype in another study [218]: SOR = 0.78 (95%CI: 0.68–0.96).

Other Cancer Sites

Other cancer sites (Table 4.1; Figs. 4.3 and 4.4) were rarely investigated: non-Hodgkin lymphoma (three studies), brain cancer (two studies), hepatocellular carcinoma (two studies), thyroid carcinoma (two studies), multiple myeloma, esophageal adenocarcinoma, gallbladder cancer, nasopharyngeal carcinoma, oral squamous cell carcinoma, pediatric solid tumors, and tobacco-related cancers (Table 4.1).

As for *Bb* genotype, a 52% and 31% borderline significant higher risk of Gallbladder cancer, Non-Hodgkin lymphoma and liver cancer were found, respectively, in studies conducted in China [119], Australia [170] and Italy [59]. Otherwise for *BB* genotype, a 54% significant lower risk of multiple myeloma was found in a study conducted in India [186], while a 68% higher risk of pancreatic cancer was suggested by a Chinese study [121].

No other significant association was found for other cancer sites.

Taq1 and Cancer

The *Taq1* polymorphism is a synonymous SNP, near the 3′ terminus of the *VDR* gene, and does not determine any structural modification of the receptor. *Taq1* is in linkage disequilibrium with two other common *VDR* SNPs, *Bsm1* and *Apa1*, both located in the 3′-UTR region of the gene, thus outside the coding regions. The 3′UTR is known to be involved in regulation of gene expression, possibly through the control of mRNA stability, thus affecting gene transcription, translation, or RNA processing [52, 216]. Thus *Taq1* may act as an indirect marker [112] through its association with other variants (*Bsm1* and *Apa1*).

Breast Cancer

All but two [65, 177] of nineteen studies reported in the literature between 1999 and 2017 confirmed no significant association for *Taq1* polymorphism and breast cancer risk, including also a study investigating the association between VDR gene polymorphism and male breast cancer risk in a Turkish population [111] (Table 2; Figs. 4.3a and 4.8a). Three estimates were reported for Asians, two for African-Americans, twelve in Caucasian and 7 in other ethnicity groups. Among these studies, several were big population-based case-control studies (Abbas [1] with 1408 cases and Anderson [6] with 1546 cases). Overall, estimates are very heterogeneous, some showed a generally not significant trend to increased risk, while others [2, 15, 26, 44, 74] showed a trend to a risk reduction in homozygote and heterozygote subjects versus wild type. Only two studies, Reimers [177] and Gapska [65] presented significant increase risk for *tt* vs *TT* (OR=1.32 95%CI: 1.01, 1.73 and 1.29 95%CI: 1.03- 1.63, respectively).

The most recent meta-analyses reported no significant association [126].

Prostate Cancer

Some studies, reported more estimates for different. Twenty-three studies have been published in 20 years (1998–2018) reporting on *Taq1* SNP and prostate cancer risk (Table 4.1; Figs. 4.5 and 4.6). We obtained 12 estimates for Caucasian, 4 for African-American, 6 for Asian, and 6 for other ethnicity groups. The prevalence of the *t* allele was in average 30% but 17% in control subjects.

In a meta-analysis, the role of *Taq1* polymorphism in prostate cancer was investigated in 17 studies [185]. Overall more than 8800 subjects were included, and no significant association between the *Taq1* polymorphism and prostate cancer risk was observed.

A trend for a protective role for the *Taq1* polymorphism was observed for both homozygous and heterozygous genotype (*tt* and *Tt* vs *TT*, respectively) showing SORs lower than 1.00, although a statistical significance was not reached (SOR = 0.94; 95%CI: 0.78–1.12 and SOR = 0.95; 95%CI: 0.80–1.12 for *tt* and *Tt* vs *TT*, respectively).

(a)

TaqI (TT vs tt)

Author		Odds Ratio [95% CI]
Breast		
Curran, 1999 (Caucasian)		0.47 [0.21, 1.07]
Dunning, 1999 (Combined cohorts) (Caucasian)		0.97 [0.71, 1.32]
Hou, 2002 (Asian)		5.31 [0.10, 273.06]
Buyru, 2003 (Other)		0.41 [0.08, 2.23]
Sillanpää, 2004 (Caucasian)		1.34 [0.83, 2.17]
John, 2007 (African-American)		0.93 [0.52, 1.67]
John, 2007 (Caucasian)		1.59 [0.95, 2.67]
John, 2007 (Hispanic)		0.96 [0.58, 1.58]
Abbas, 2008 (Caucasian)		1.11 [0.91, 1.36]
Barroso, 2008 (Caucasian)		0.72 [0.48, 1.09]
Gapska, 2008 (Caucasian)		1.29 [1.03, 1.63]
Chakraborty, 2009 (Other)		1.09 [0.35, 3.37]
Anderson, 2011 (Caucasian)		1.04 [0.89, 1.22]
Kizildag, 2011 (male) (Other)		1.06 [0.11, 10.27]
Engel, 2012 (Caucasian)		1.10 [0.70, 1.60]
Huang, 2012 (Asian)		1.10 [0.04, 33.00]
Mishra, 2013 (African-American)		1.10 [0.30, 4.40]
Mishra, 2013 (Hispanic)		1.40 [0.50, 4.00]
Abd-Elsalam, 2015 (Other)		0.62 [0.30, 1.28]
Guo, 2015 (Asian)		0.76 [0.44, 1.13]
Nemenqani, 2015 (Asian)		1.50 [0.69, 3.30]
Reimers, 2015 (Caucasian)		1.32 [1.01, 1.73]
Atoum, 2017 (Other)		1.68 [0.65, 4.31]

0   0.5   1   2   3   4   5

(b)

TaqI (TT vs tt)

| Author		Odds Ratio [95% CI]
Prostate		
Ma, 1998 (Caucasian)		0.92 [0.61, 1.38]
Correa-Cerro, 1999 (Caucasian)		1.15 [0.48, 2.74]
Blazer, 2000 (African-American)		1.00 [0.07, 13.64]
Blazer, 2000 (Caucasian)		0.96 [0.42, 2.19]
Habuchi, 2000 (Asian)		0.55 [0.08, 3.93]
Medeiros, 2002 (Caucasian)		0.65 [0.34, 1.25]
Suzuki, 2003 (Asian)		1.41 [0.19, 10.27]
Huang, 2004 (Asian)		1.23 [0.02, 62.17]
Maistro, 2004 (Mixed)		1.58 [0.82, 3.07]
Oakley-Girvan, 2004 (African-American)		1.23 [0.50, 2.98]
Oakley-Girvan, 2004 (Caucasian)		1.04 [0.58, 1.86]
Andersson, 2006 (Caucasian)		1.02 [0.53, 1.94]
Chaimuangraj, 2006 (Asian)		0.48 [0.02, 14.99]
Cicek, 2006 (Caucasian)		0.89 [0.50, 1.61]
Holick, 2007 (Caucasian)		0.91 [0.64, 1.29]
Onen, 2008 (Other)		0.55 [0.26, 1.15]
Bai, 2009 (Asian)		1.08 [0.02, 54.91]
Holt, 2009 (African-American)		0.79 [0.28, 2.24]
Holt, 2009 (Caucasian)		1.06 [0.77, 1.46]
Rowland, 2013 (African-American)		0.97 [0.73, 1.28]
Rowland, 2013 (Caucasian)		0.71 [0.38, 1.32]
Hu, 2014 (Asian)		4.56 [0.41, 50.92]
Yousaf, 2014 (Other)		0.35 [0.11, 1.09]
Gilbert, 2015 (Caucasian)		1.09 [0.82, 1.44]
Jingwi, 2015 (African-American)		0.49 [0.26, 0.95]
Nunes, 2016 (Other)		0.51 [0.23, 1.17]
El Ezzi, 2017 (Other)		1.19 [0.38, 3.72]
Kambale, 2017 (Other)		1.57 [0.31, 8.01]
Braczkowski, 2018 (Caucasian)		0.90 [0.29, 2.78]

0   0.5   1   2   3   4   5

Fig. 4.5 Forest plot for the association between *Taq1 TT* and *tt* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the skin and thyroid, sarcoma, and pediatric solid tumors; (**e**) cancer of the kidney, liver, lung, non-hodggkin's lymphoma, ovary; (**f**) cancers of the bladder, brain, esophagus, gallbladder, head and neck cancer

A statistically not significant trend for an inverse association for the *tt* genotype was observed in several studies. One case-control study in African-Americans reported a statistically significant reduction in risk for *tt* carriers versus *TT* [102] (OR = 0.49 95%CI: 0.26, 0.95).

(c)

TaqI (TT vs tt)

Author		Odds Ratio [95% CI]
CRC		
Park, 2006 (Asian)		1.74 [0.03, 88.00]
Flugge, 2007 (Caucasian)		1.05 [0.62, 1.77]
Yaylim-Eraltan, 2007 (Other)		2.12 [0.61, 7.41]
Ochs-Balcom, 2008 (Caucasian)		1.66 [0.92, 2.97]
Mahmoudi, 2010 (Other)		1.08 [0.54, 2.19]
Hughes, 2011 (Caucasian)		1.07 [0.76, 1.51]
Bentley, 2012 (Caucasian)		1.06 [0.59, 1.92]
Gunduz, 2012 (Other)		4.89 [1.15, 20.79]
Atoum, 2014 (Other)		1.08 [0.46, 2.56]
Laczmanska, 2014 (Caucasian)		0.48 [0.26, 0.88]
Sarkissyan, 2014 (Mixed)		0.87 [0.33, 2.28]
Takeshige, 2015 (Asian)		1.15 [0.39, 3.40]
Alkhayal, 2016 (Other)		0.90 [0.40, 2.01]
Moossavi, 2018 (Other)		0.72 [0.18, 2.81]

```
0  0.5  1    2    3    4    5
```

(d)

TaqI (TT vs tt)

Author		Odds Ratio [95% CI]
Sarcoma		
Ruza, 2003 (Ewing Sarcoma) (Caucasian)		1.31 [0.55, 3.11]
Ruza, 2003 (Osteosarcoma) (Caucasian)		0.70 [0.30, 1.60]
Skin		
Gapska, 2009 (Caucasian)		1.08 [0.80, 1.50]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)		1.07 [0.76, 1.51]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)		0.73 [0.48, 1.13]
Lesiak, 2011 (BCC) (Caucasian)		2.58 [1.35, 4.95]
Köstner, 2012 (BCC) (Caucasian)		1.19 [0.39, 3.66]
Köstner, 2012 (SCC) (Caucasian)		1.66 [0.59, 4.70]
Pena-Chilet, 2013 (Caucasian)		0.85 [0.54, 1.34]
Zeljic, 2014 (Caucasian)		3.56 [1.50, 8.43]
Burns, 2017 (Caucasian)		1.25 [0.51, 3.06]
Thyroid		
Penna-Martinez, 2009 (Follicular) (Caucasian)		0.45 [0.15, 1.38]
Penna-Martinez, 2009 (Papillary) (Caucasian)		1.01 [0.56, 1.83]
Beysel, 2018 (Other)		1.41 [0.67, 2.97]
Solid tumor pediatric		
Bienertová-Vašků, 2016 (Caucasian)		1.24 [0.70, 2.17]

```
0  0.5  1    2    3    4    5
```

Fig. 4.5 (continued)

Similarly the analysis for heterozygous (*Tt* versus *TT*) showed a trend toward a protective effect of this SNP, with three studies that reached a statistical significance reduction of risk: Correa-Cerro [43] (OR = 0.50 95%CI: 0.27, 0.92), Holick [85] (OR = 0.73 95%CI: 0.56, 0.95), and Kambale [107] (OR = 0.29 95%CI: 0.16, 0.50).

The *t* allele was also found to be protective in a meta-analysis published in 2014 [220]. A more recent meta-analysis confirmed this association particularly in Asian populations and suggested that PCa patients carrying the *t* allele or *tt* genotype were less likely to progress to advanced stage [35].

(e)

TaqI (TT vs tt)

Author	Odds Ratio [95% CI]
Kidney	
Karami, 2008 (Caucasian)	0.89 [0.66, 1.20]
Yang, 2016 (Asian)	1.04 [0.02, 52.72]
Liver	
Falleti, 2010 (Caucasian)	0.49 [0.21, 1.15]
Hung, 2014 (Asian)	1.00 [0.02, 50.98]
Lung	
Dogan, 2009 (Other)	0.45 [0.21, 0.95]
Kaabachi, 2014 (African)	0.97 [0.56, 1.69]
Wu, 2016 (Asian)	0.84 [0.57, 0.95]
Gromowski, 2017 (NSCLC) (Caucasian)	0.86 [0.63, 1.17]
Non-Hodgkin's lymphoma	
Purdue, 2007 (Caucasian)	0.89 [0.64, 1.24]
Purdue, 2007 (Mixed)	1.05 [0.73, 1.53]
Smedby, 2010 (Caucasian)	1.01 [0.85, 1.21]
Ovary	
Lurie, 2007 (Asian)	1.70 [0.40, 7.90]
Lurie, 2007 (Caucasian)	1.80 [0.70, 4.40]
Clendenen, 2008 (Caucasian)	0.98 [0.50, 1.94]
Grant, 2013 (African-American)	1.34 [0.36, 5.04]
Grant, 2013 (Caucasian)	1.00 [0.67, 1.49]

0   0.5   1   2   3   4   5

(f)

TaqI (TT vs tt)

Author	Odds Ratio [95% CI]
Bladder	
Mittal, 2007 (Other)	0.64 [0.30, 1.35]
Brain	
Anic, 2012 (Caucasian)	0.72 [0.49, 1.05]
Toptaş, 2013 (Other)	0.82 [0.33, 2.06]
Yilmaz, 2017 (pediatric) (Other)	0.66 [0.18, 2.49]
Esophageal	
Chang, 2011 (Caucasian)	1.70 [0.79, 3.65]
Gallbladder	
Li, 2014 (Asian)	0.65 [0.41, 1.03]
Head and neck	
Liu, 2005 (Caucasian)	0.72 [0.53, 0.98]
Bektas-Kayhan, 2010 (Other)	0.54 [0.18, 1.61]
Zeljic, 2012 (Caucasian)	1.69 [0.62, 4.63]

0   0.5   1   2   3   4   5

Fig. 4.5 (continued)

Colorectal Cancer

Colorectal cancer risk and *Taq1* were analyzed in 14 studies published between 2006 and 2018 (Table 4.1; Figs. 4.5 and 4.6). A consistent detrimental trend was observed among all the studies for the *tt* compared to the *TT*, less evident in the heterozygous condition.

In a recent meta-analysis [185], eight studies with data on CRC and *Taq1* were included. The

(a)

TaqI (Tt vs tt)

Author		Odds Ratio [95% CI]
Breast		
Curran, 1999 (Caucasian)		0.61 [0.35, 1.08]
Dunning, 1999 (Combined cohorts) (Caucasian)		1.04 [0.84, 1.30]
Hou, 2002 (Asian)		2.04 [0.68, 6.17]
Buyru, 2003 (Other)		0.34 [0.10, 1.10]
Sillanpää, 2004 (Caucasian)		0.92 [0.71, 1.20]
John, 2007 (African-American)		0.81 [0.59, 1.12]
John, 2007 (Caucasian)		1.02 [0.71, 1.47]
John, 2007 (Hispanic)		0.77 [0.57, 1.05]
Abbas, 2008 (Caucasian)		1.08 [0.93, 1.26]
Barroso, 2008 (Caucasian)		0.82 [0.59, 1.13]
Gapska, 2008 (Caucasian)		1.02 [0.88, 1.19]
Chakraborty, 2009 (Other)		1.15 [0.72, 1.85]
Anderson, 2011 (Caucasian)		1.01 [0.82, 1.24]
Kizildag, 2011 (male) (Other)		1.26 [0.51, 3.11]
Engel, 2012 (Caucasian)		1.10 [0.80, 1.60]
Huang, 2012 (Asian)		1.12 [0.57, 2.17]
Mishra, 2013 (African-American)		0.60 [0.30, 1.40]
Mishra, 2013 (Hispanic)		0.90 [0.50, 1.60]
Abd-Elsalam, 2015 (Other)		0.69 [0.37, 1.28]
Guo, 2015 (Asian)		1.11 [0.60, 2.01]
Nemenqani, 2015 (Asian)		0.95 [0.50, 1.79]
Reimers, 2015 (Caucasian)		1.06 [0.82, 1.38]
Atoum, 2017 (Other)		0.94 [0.54, 1.65]

0 0.5 1 2 3 4 5

(b)

TaqI (Tt vs tt)

Author		Odds Ratio [95% CI]
Prostate		
Ma, 1998 (Caucasian)		0.95 [0.71, 1.26]
Correa-Cerro, 1999 (Caucasian)		0.50 [0.27, 0.92]
Blazer, 2000 (African-American)		4.50 [0.49, 41.25]
Blazer, 2000 (Caucasian)		1.40 [0.74, 2.65]
Habuchi, 2000 (Asian)		0.80 [0.47, 1.35]
Medeiros, 2002 (Caucasian)		1.39 [0.88, 2.20]
Suzuki, 2003 (Asian)		1.41 [0.70, 2.84]
Huang, 2004 (Asian)		0.66 [0.33, 1.31]
Maistro, 2004 (Mixed)		1.58 [1.01, 2.47]
Oakley-Girvan, 2004 (African-American)		0.89 [0.52, 1.53]
Oakley-Girvan, 2004 (Caucasian)		1.31 [0.84, 2.04]
Andersson, 2006 (Caucasian)		1.04 [0.64, 1.71]
Chaimuangraj, 2006 (Asian)		0.72 [0.21, 2.41]
Cicek, 2006 (Caucasian)		0.72 [0.49, 1.06]
Holick, 2007 (Caucasian)		0.73 [0.56, 0.95]
Onen, 2008 (Other)		0.69 [0.42, 1.13]
Bai, 2009 (Asian)		1.20 [0.47, 3.06]
Holt, 2009 (African-American)		0.83 [0.43, 1.60]
Holt, 2009 (Caucasian)		1.15 [0.91, 1.45]
Rowland, 2013 (African-American)		1.00 [0.82, 1.22]
Rowland, 2013 (Caucasian)		1.07 [0.76, 1.52]
Hu, 2014 (Asian)		1.04 [0.47, 2.27]
Yousaf, 2014 (Other)		3.33 [1.33, 8.31]
Gilbert, 2015 (Caucasian)		1.06 [0.86, 1.29]
Jingwi, 2015 (African-American)		0.68 [0.45, 1.01]
Nunes, 2016 (Other)		0.98 [0.60, 1.58]
El Ezzi, 2017 (Other)		1.32 [0.63, 2.75]
Kambale, 2017 (Other)		0.29 [0.11, 0.76]
Braczkowski, 2018 (Caucasian)		1.10 [0.55, 2.19]

0 0.5 1 2 3 4 5

Fig. 4.6 Forest plot for the association between *Taq1 Tt* and *tt* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the sarcoma, skin, thyroid and pediatric solid tumors; (**e**) cancers of the kidney, liver, lung, non-Hodgkin lymphom and ovary; (**f**) cancers of the bladder, brain, esophagus, gallbladder, head and neck cancer

Taq1 tt genotype showed an increased risk for CRC (SOR = 1.43, 95%CI: 1.30–1.58), but the data lost significance in Caucasians (SOR = 1.21, 95%CI: 0.89–1.64).

The study by Gunduz [73, 204] showed the greatest increase in risk, almost fivefold, for colorectal cancer in subjects with *Taq1 tt* (OR = 4.90; 95%CI: 1.15, 20.79). The *t* allele frequency was highly different between cases and controls in that

(c)   **TaqI (Tt vs tt)**

Author		Odds Ratio [95% CI]

CRC

Park, 2006 (Asian)		1.47 [0.81, 2.68]
Flugge, 2007 (Caucasian)		0.96 [0.66, 1.40]
Yaylim-Eraltan, 2007 (Other)		0.56 [0.18, 1.73]
Ochs-Balcom, 2008 (Caucasian)		1.10 [0.71, 1.69]
Mahmoudi, 2010 (Other)		0.59 [0.37, 0.97]
Hughes, 2011 (Caucasian)		1.07 [0.76, 1.50]
Bentley, 2012 (Caucasian)		1.17 [0.75, 1.84]
Gunduz, 2012 (Other)		1.55 [0.61, 3.95]
Atoum, 2014 (Other)		1.49 [0.81, 2.76]
Laczmanska, 2014 (Caucasian)		0.57 [0.35, 0.92]
Sarkissyan, 2014 (Mixed)		1.21 [0.68, 2.16]
Takeshige, 2015 (Asian)		0.91 [0.70, 1.19]
Alkhayal, 2016 (Other)		0.79 [0.43, 1.44]
Moossavi, 2018 (Other)		0.79 [0.44, 1.41]

0   0.5   1   2   3   4   5

(d)   **TaqI (Tt vs tt)**

Author		Odds Ratio [95% CI]

Sarcoma

| Ruza, 2003 (Ewing Sarcoma) (Caucasian) | | 1.07 [0.52, 2.20] |
| Ruza, 2003 (Osteosarcoma) (Caucasian) | | 0.79 [0.42, 1.47] |

Skin

Gapska, 2009 (Caucasian)		0.96 [0.80, 1.20]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)		0.97 [0.75, 1.26]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)		0.96 [0.70, 1.31]
Lesiak, 2011 (BCC) (Caucasian)		2.46 [1.43, 4.20]
Köstner, 2012 (BCC) (Caucasian)		1.61 [0.75, 3.48]
Köstner, 2012 (SCC) (Caucasian)		1.28 [0.60, 2.70]
Pena-Chilet, 2013 (Caucasian)		1.03 [0.75, 1.41]
Zeljic, 2014 (Caucasian)		2.32 [1.22, 4.43]
Burns, 2017 (Caucasian)		1.61 [0.82, 3.14]

Thyroid

Penna-Martinez, 2009 (Follicular) (Caucasian)		0.73 [0.36, 1.46]
Penna-Martinez, 2009 (Papillary) (Caucasian)		1.13 [0.72, 1.76]
Beysel, 2018 (Other)		1.09 [0.69, 1.72]

Solid tumor pediatric

| Bienertová-Vaškù, 2016 (Caucasian) | | 0.69 [0.44, 1.09] |

0   0.5   1   2   3   4   5

Fig. 4.6 (continued)

study (44% in cases and 27% in controls), and this might in part explain the different results in that study. In the heterozygote subjects, the risk was increased by 55% (OR = 1.55 95%CI: 0.61, 3.95) in the study published by Gunduz, but it did not reach a statistical significance for any of the other studies. The only study the presented significant inverse associations, for both tt and *Tt*, compared to the *TT* group, was the one carried out in Poland [114].

(e)

TaqI (Tt vs tt)

Author		Odds Ratio [95% CI]
Kidney		
Obara, 2007 (Asian)		0.90 [0.52, 1.56]
Karami, 2008 (Caucasian)		1.04 [0.85, 1.27]
Yang, 2016 (Asian)		1.42 [0.86, 2.35]
Liver		
Falleti, 2010 (Caucasian)		0.59 [0.33, 1.07]
Hung, 2014 (Asian)		0.43 [0.16, 1.17]
Lung		
Dogan, 2009 (Other)		0.54 [0.33, 0.90]
Kaabachi, 2014 (African)		0.88 [0.60, 1.28]
Wu, 2016 (Asian)		0.77 [0.59, 0.99]
Gromowski, 2017 (NSCLC) (Caucasian)		1.02 [0.83, 1.24]
Non-Hodgkin's lymphoma		
Purdue, 2007 (Caucasian)		0.98 [0.77, 1.26]
Purdue, 2007 (Mixed)		1.37 [1.04, 1.79]
Smedby, 2010 (Caucasian)		0.93 [0.81, 1.07]
Ovary		
Lurie, 2007 (Asian)		1.10 [0.60, 2.20]
Lurie, 2007 (Caucasian)		1.10 [0.50, 2.10]
Clendenen, 2008 (Caucasian)		1.21 [0.71, 2.06]
Grant, 2013 (African-American)		0.78 [0.37, 1.63]
Grant, 2013 (Caucasian)		1.28 [0.96, 1.70]

0   0.5   1   2   3   4   5

(f)

TaqI (Tt vs tt)

Author		Odds Ratio [95% CI]
Bladder		
Mittal, 2007 (Other)		0.88 [0.57, 1.35]
Brain		
Anic, 2012 (Caucasian)		0.90 [0.69, 1.16]
Topta?, 2013 (Other)		0.97 [0.55, 1.71]
Yilmaz, 2017 (pediatric) (Other)		0.93 [0.31, 2.74]
Esophageal		
Chang, 2011 (Caucasian)		0.98 [0.56, 1.70]
Gallbladder		
Li, 2014 (Asian)		0.98 [0.69, 1.39]
Head and neck		
Liu, 2005 (Caucasian)		0.97 [0.77, 1.22]
Bektas-Kayhan, 2010 (Other)		1.67 [0.81, 3.46]
Zeljic, 2012 (Caucasian)		1.75 [0.87, 3.52]

0   0.5   1   2   3   4   5

Fig. 4.6 (continued)

Skin Cancer

Seven studies have been conducted to investigate the role of VDR *Taq1* SNP and melanoma between 2009 and 2017 (Table 4.1; Figs. 4.5 and 4.6). The majority of the papers, including melanoma and non-melanoma skin cancer, showed a non-significant increased risk for *tt* vs *TT*.

Only two studies found significant associations. Lesiak [116] presented estimates for BCC and found that subjects having *Taq1 tt* genotypes were associated with an increased risk for developing BCC more than twice and half compared to the *TT* genotype (OR = 2.59; 95% CI: 1.35, 4.95).

Zeljic et al. [231] presented results of a study carried out in Serbia and found a significant increased risk of melanoma for *tt* vs *TT* OR = 3.56 (95%CI: 1.50, 8.43) and for *Tt* vs *TT* OR = 2.32 (95%CI: 1.22, 4.43).

However, the meta-analysis by Serrano [185] did not show a significant association between *Taq1* VDR polymorphisms and skin cancer risk (SOR = 1.01; 95%CI: 0.71–1.45 and SOR = 1.0; 95%CI: 0.82–1.45 for tt and Tt vs TT, respectively). After that publication, only one study, carried out in the USA [25], has been published indicating a non-significant increased risk.

Lung Cancer

Four studies published estimates on *Taq1* and lung cancer. They were carried in Turkey, Tunis, Poland, and China. The Turkish [50] and Chinese studies presented significantly inverse association for both *tt* and *Tt* versus *TT*. Furthermore Dogan [50] observed that *tt* homozygous men among the patients who smoked were less likely to develop lung cancer compared to *TT* (for smokers: OR = 0.25, 95%CI: 0.09–0.75, P = 0.012).

In a recent meta-analysis, the *tt* genotype was found inversely associated with lung cancer risk

compared with the *Taq1 Tt + TT* genotype (OR = 0.70, 95%CI = 0.55–0.90) [228].

Other Cancers

Several other cancers were investigated, such as bone cancer, brain cancer, esophageal adenocarcinoma, hepatocellular carcinoma, head and neck cancer, non-Hodgkin lymphoma, oral SCC, renal cell carcinoma, and thyroid carcinoma, with a total of 30 studies included. None of these studies reached significant associations of *Taq1* polymorphism and cancer risk (Table 4.1; Figs. 4.5 and 4.6).

The meta-analysis by Serrano [185] found a borderline significant risk reduction for "other cancer" for the heterozygous *tt* genotype compared with *TT* genotype (SOR = 0.88, 95%CI: 0.78–1.00).

Apa1 and Cancer

Apa1 polymorphism is located near the 3′ UTR of VDR gene similar to *Taq1* and *Bsm1* and does not alter the protein's amino acid sequence. The functional significance of the VDR *Apa1* polymorphism remains unknown.

Breast Cancer

Fourteen epidemiologic studies, counting for more than 5000 subjects, have investigated the association between *Apa1* and breast cancer risk (including also male breast cancer) (Table 4.1). Some studies suggested an increased risk of breast cancer and others a reduction (Table 4.1; Figs. 4.7 and 4.8).

A statistically significant increased risk was found by Curran et al. [44] carried out in Australia with 2.5-fold risk increment for aa compared to *AA* (OR = 2.53; 95%CI 1.20, 5.39), and similar results were achieved in one study [2]. Four studies presented significant decreased

risk of breast cancer for the *Aa* versus *AA* from 30% to 72% [74, 88, 90, 190]. Only one study conducted in the USA found a statistical increased risk for the *Aa* versus *AA* genotype [45]. Other studies reported that the *Apa1* polymorphism was not associated with breast cancer risk.

Prostate Cancer

With respect to *Apa1* polymorphism, 15 studies have been published between 2000 and 2017, and 4 studies found statistically significant association with prostate cancer risk (Table 4.1; Figs. 4.7 and 4.8). Two studies [91, 107] conducted in India and Taiwan suggested a significant decreased risk of prostate cancer for both *aa* and *Aa* vs wild-type *AA* genotype, whereas one study [155] conducted in Turkey presented an increased risk for both. Contrasting results were published by Yousaf [227] conducted in Pakistan with a significant decreased risk for *aa* vs *AA* and a significant decreased risk for *Aa* vs *AA*.

In the most recent meta-analysis [215] including 6427 cases and 6039 controls from 16 case-control studies, Wang suggested that these polymorphisms did not increase the risk of prostate risk in genetic models, which was consistent with our previous meta-analysis [185].

Colorectal Cancer

Eleven studies including subjects from different ethnicities, conducted from 2006 to 2017, have focused on the association between *Apa1* and colorectal cancer with no consistent results (Table 4.1; Figs. 4.7 and 4.8). In six studies, *Apa1* variant was not associated with risk of colorectal cancer. Four studies in different ethnicities suggested that the *Apa1 aa/Aa* polymorphism genotypes may increase [130, 212] or decrease [114, 160, 176] the risk respect to AA genotype. The recent meta-analysis by Pan et al. did not found any association with cancer risk [158].

Skin Cancer

Only a few epidemiological studies have addressed the relationship between *Apa1* polymorphism and risk of melanoma and NMSC [25, 113, 116, 173, 231]. All these studies were conducted in Caucasian populations (Table 4.1; Figs. 4.7 and 4.8). A recent meta-analysis from Von Schuckmann et al. [213] showed a decreased basal cell carcinoma risk in *Apa1* recessive genotype *AA* (SOR = 0.74; 95%CI: 0.56–0.098).

Other Cancers

Nineteen studies were included, investigating different cancer sites, such as esophageal adenocarcinoma, gallbladder cancer, hepatocellular carcinoma, lung cancer, multiple myeloma, oral cancer, ovary cancer, renal cell carcinoma, and thyroid carcinoma.

Regarding lung cancer risk, *Apa1* was investigated in 2 meta-analyses [33, 62] including 5 studies involving 602 patients and 662 healthy controls (examining Asian and Turkish population). No statistically significant association with lung cancer risk was found. Kaabachi et al. [105] found an increased significant association for *aa* and *Aa* versus *AA* genotypes (OR = 2.64; 95%CI: 1.37–5.07 and OR = 2.30; 95%CI: 1.22–4.35, respectively). But in the most recent Polish case-control study [69], *Apa1* was not related to lung cancer risk.

Three studies evaluated the association between *Apa1* and ovarian cancer with null results [39, 67, 128] in Caucasians and a significant increase risk in African American [67] for *aa* vs *AA* genotype. Other studies included esophageal adenocarcinoma, hepatocellular carcinoma, oral squamous cell carcinoma, thyroid cancer, and sarcoma. Interesting results were reported by Zeljic et al. [230] for oral squamous cell carcinoma (110 cases and 122 controls): they found a significant increased risk for *Apa1 aa* vs *AA* (OR = 2.06, 95%CI: 1.04, 4.09) and for *Aa* vs *AA* (OR = 2.44, 95%CI: 1.31, 4.54). Penna-

(a)


Apal (aa vs AA)
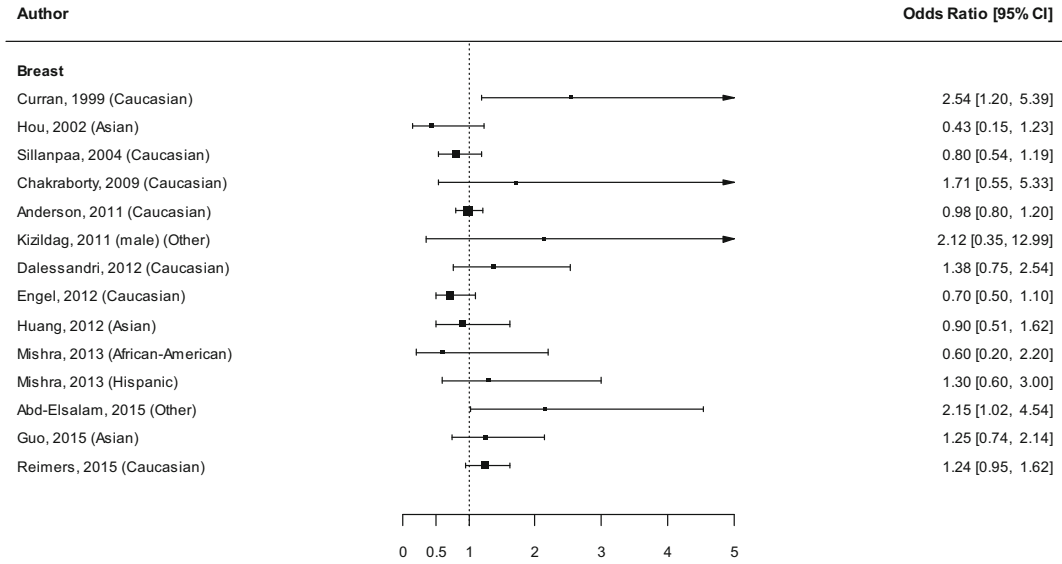
(b)

Apal (aa vs AA)

Fig. 4.7 Forest plot for the association between *Apa1 aa* and *AA* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the skin and thyroid, sarcoma, and pediatric solid tumors; (**e**) cancers of the lung and ovary and multiple myeloma; (**f**) cancers of the esophagus, gallbladder, head and neck, kidney, and liver

(c)

ApaI (aa vs AA)

Author	Odds Ratio [95% CI]

CRC

Park, 2006 (Asian)	0.45 [0.23, 0.89]
Flugge, 2007 (Caucasian)	0.93 [0.57, 1.53]
Theodoratou, 2008 (Caucasian)	1.14 [0.96, 1.37]
Mahmoudi, 2010 (Other)	2.32 [1.19, 4.54]
Hughes, 2011 (Caucasian)	1.19 [0.87, 1.61]
Lacmanska, 2014 (Caucasian)	0.35 [0.19, 0.64]
Rasool, 2014 (Other)	0.50 [0.21, 1.16]
Sarkyssyan, 2014 (Other)	1.07 [0.46, 2.50]
Takeshige, 2015 (Asian)	1.17 [0.81, 1.69]
Alkhayal, 2016 (Other)	1.09 [0.57, 2.09]
Vidigal, 2017 (Mixed)	1.30 [0.68, 2.51]

0   0.5   1   2   3   4   5

(d)

ApaI (aa vs AA)

Author	Odds Ratio [95% CI]

Sarcoma

Ruza, 2003 (Ewing Sarcoma) (Caucasian)	0.81 [0.32, 2.06]
Ruza, 2003 (Osteosarcoma) (Caucasian)	1.61 [0.74, 3.52]

Skin

Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)	1.02 [0.74, 1.41]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)	1.46 [0.97, 2.19]
Lesiak, 2011 (BCC) (Caucasian)	0.71 [0.38, 1.32]
Köstner, 2012 (BCC) (Caucasian)	1.00 [0.37, 2.69]
Köstner, 2012 (SCC) (Caucasian)	0.79 [0.30, 2.06]
Zeljic, 2014 (Caucasian)	0.68 [0.35, 1.35]
Burns, 2017 (Caucasian)	1.52 [0.66, 3.50]

Thyroid

Penna-Martinez, 2009 (Follicular) (Caucasian)	3.82 [1.27, 11.49]
Penna-Martinez, 2009 (Papillary) (Caucasian)	0.95 [0.55, 1.66]
Beysel, 2018 (Other)	0.61 [0.29, 1.25]

0   0.5   1   2   3   4   5

Fig. 4.7 (continued)

(e)

Apal (aa vs AA)

Author		Odds Ratio [95% CI]
Lung		
Dogan, 2009 (Other)		1.74 [0.88, 3.43]
Kaabachi, 2014 (African)		0.45 [0.25, 0.81]
Wu, 2016 (Asian)		0.92 [0.64, 1.34]
Gromowski, 2017 (Caucasian)		1.05 [0.80, 1.38]
Multiple Myeloma		
Shafia, 2013 (Other)		2.48 [1.09, 5.62]
Chen, 2017 (Asian)		0.58 [0.38, 0.87]
Ovary		
Lurie, 2007 (Asian)		0.89 [0.34, 2.30]
Lurie, 2007 (Caucasian)		0.52 [0.22, 1.23]
Clendenen, 2008 (Caucasian)		0.67 [0.35, 1.26]
Grant, 2013 (African-American)		6.38 [1.78, 22.89]
Grant, 2013 (Caucasian)		0.95 [0.65, 1.38]

0   0.5   1   2   3   4   5

(f)

Apal (aa vs AA)

Author		Odds Ratio [95% CI]
Esophageal		
Chang, 2012 (Caucasian)		0.81 [0.37, 1.77]
Gallbladder		
Li, 2013 (Asian)		1.09 [0.72, 1.65]
Head and neck		
Zeljic, 2012 (Caucasian)		2.06 [1.04, 4.09]
Kidney		
Obara, 2007 (Asian)		0.42 [0.19, 0.94]
Yang, 2016 (Asian)		0.39 [0.21, 0.73]
Liver		
Falleti, 2010 (Caucasian)		1.34 [0.60, 2.99]
Hung, 2014 (Asian)		1.97 [0.45, 8.62]

0   0.5   1   2   3   4   5

Fig. 4.7 (continued)

(a)

ApaI (Aa vs AA)

Author	Odds Ratio [95% CI]
Breast	
Curran, 1999 (Caucasian)	1.54 [0.85, 2.80]
Hou, 2002 (Asian)	0.28 [0.09, 0.87]
Sillanpaa, 2004 (Caucasian)	0.70 [0.51, 0.95]
Chakraborty, 2009 (Caucasian)	1.14 [0.71, 1.82]
Anderson, 2011 (Caucasian)	1.00 [0.86, 1.18]
Kizildag, 2011 (male) (Other)	1.14 [0.46, 2.85]
Dalessandri, 2012 (Caucasian)	1.64 [1.01, 2.67]
Engel, 2012 (Caucasian)	0.90 [0.60, 1.20]
Huang, 2012 (Asian)	0.42 [0.27, 0.67]
Mishra, 2013 (African-American)	0.80 [0.30, 1.80]
Mishra, 2013 (Hispanic)	1.30 [0.70, 2.40]
Abd-Elsalam, 2015 (Other)	1.44 [0.77, 2.69]
Guo, 2015 (Asian)	0.52 [0.29, 0.93]
Reimers, 2015 (Caucasian)	1.17 [0.94, 1.45]

0   1.25   2.5   3.75   5

(b)

ApaI (Aa vs AA)

Author	Odds Ratio [95% CI]
Prostate	
Habuchi, 2000 (Asian)	0.53 [0.25, 1.14]
Suzuki, 2003 (Asian)	1.32 [0.60, 2.91]
Huang, 2004 (Asian)	0.41 [0.22, 0.75]
Maistro, 2004 (Mixed)	1.11 [0.70, 1.75]
Oakley-Girvan, 2004 (African-American)	1.39 [0.79, 2.43]
Oakley-Girvan, 2004 (Caucasian)	1.17 [0.74, 1.85]
Chaimuagraj, 2006 (Asian)	0.64 [0.14, 2.87]
Cicek, 2006 (Caucasian)	0.98 [0.73, 1.33]
Onen, 2008 (Other)	1.88 [1.10, 3.20]
Bai, 2009 (Asian)	0.83 [0.31, 2.18]
Yousaf, 2014 (Caucasian)	4.58 [1.26, 16.65]
Gilbert, 2015 (Caucasian)	1.13 [0.91, 1.41]
Jingwi, 2015 (African-American)	0.97 [0.65, 1.45]
Nunes, 2016 (Mixed)	0.69 [0.41, 1.15]
El Ezzi, 2017 (Other)	0.59 [0.29, 1.22]
Kambale, 2017 (Other)	0.14 [0.06, 0.33]

0   1.25   2.5   3.75   5

Fig. 4.8 Forest plot for the association between *Apa1 Aa* and *AA* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the skin and thyroid, sarcoma, and pediatric solid tumors; (**e**) cancers of the lung and ovary and multiple myeloma; (**f**) cancers of the esophagus, gallbladder, head and neck, kidney, and liver

(c)

Apal (Aa vs AA)

Author	Odds Ratio [95% CI]

CRC

Park, 2006 (Asian) — 0.61 [0.30, 1.24]

Flugge, 2007 (Caucasian) — 1.03 [0.68, 1.56]

Theodoratou, 2008 (Caucasian) — 1.07 [0.93, 1.24]

Mahmoudi, 2010 (Other) — 1.46 [0.89, 2.39]

Hughes, 2011 (Caucasian) — 0.97 [0.74, 1.28]

Lacmanska, 2014 (Caucasian) — 0.30 [0.17, 0.52]

Rasool, 2014 (Other) — 0.36 [0.15, 0.85]

Sarkyssyan, 2014 (Other) — 0.84 [0.48, 1.48]

Takeshige, 2015 (Asian) — 1.03 [0.71, 1.49]

Alkhayal, 2016 (Other) — 1.42 [0.71, 2.84]

Vidigal, 2017 (Mixed) — 2.17 [1.17, 4.01]

0   1.25   2.5   3.75   5

(d)

Apal (Aa vs AA)

Author	Odds Ratio [95% CI]

Sarcoma

Ruza, 2003 (Ewing Sarcoma) (Caucasian) — 1.09 [0.53, 2.23]

Ruza, 2003 (Osteosarcoma) (Caucasian) — 1.16 [0.59, 2.28]

Skin

Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian) — 1.17 [0.89, 1.53]

Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian) — 1.17 [0.84, 1.62]

Lesiak, 2011 (BCC) (Caucasian) — 1.51 [0.84, 2.74]

Köstner, 2012 (BCC) (Caucasian) — 1.56 [0.66, 3.67]

Köstner, 2012 (SCC) (Caucasian) — 1.14 [0.50, 2.61]

Zeljic, 2014 (Caucasian) — 0.95 [0.53, 1.68]

Burns, 2017 (Caucasian) — 0.79 [0.37, 1.73]

Thyroid

Penna-Martinez, 2009 (Follicular) (Caucasian) — 3.79 [1.40, 10.26]

Penna-Martinez, 2009 (Papillary) (Caucasian) — 0.82 [0.52, 1.30]

Beysel, 2018 (Other) — 1.00 [0.62, 1.61]

0   1.25   2.5   3.75   5

Fig. 4.8 (continued)

(e)

ApaI (Aa vs AA)

Author	Odds Ratio [95% CI]

Lung
Dogan, 2009 (Other)   1.11 [0.66, 1.86]
Kaabachi, 2014 (African)   0.87 [0.60, 1.26]
Wu, 2016 (Asian)   0.84 [0.59, 1.19]
Gromowski, 2017 (Caucasian)   0.87 [0.68, 1.11]

Multiple Myeloma
Shafia, 2013 (Other)   1.54 [0.79, 3.01]
Chen, 2017 (Asian)   0.69 [0.45, 1.05]

Ovary
Lurie, 2007 (Asian)   1.04 [0.41, 2.67]
Lurie, 2007 (Caucasian)   0.79 [0.41, 1.55]
Clendenen, 2008 (Caucasian)   1.00 [0.58, 1.73]
Grant, 2013 (African-American)   1.40 [0.65, 3.01]
Grant, 2013 (Caucasian)   1.10 [0.81, 1.50]

0   0.5   1   2   3   4   5

(f)

ApaI (Aa vs AA)

Author	Odds Ratio [95% CI]

Esophageal
Chang, 2012 (Caucasian)   0.79 [0.43, 1.43]

Gallbladder
Li, 2013 (Asian)   0.80 [0.55, 1.15]

Head and neck
Zeljic, 2012 (Caucasian)   2.44 [1.31, 4.54]

Kidney
Obara, 2007 (Asian)   0.35 [0.16, 0.78]
Yang, 2016 (Asian)   0.58 [0.32, 1.08]

Liver
Falleti, 2010 (Caucasian)   0.88 [0.48, 1.60]
Hung, 2014 (Asian)   1.00 [0.22, 4.56]

0   0.5   1   2   3   4   5

Fig. 4.8 (continued)

Martinez [163], analyzing the thyroid follicular and papillary carcinoma, showed no correlation between *Apa1* and cancer risk for the papillary carcinoma, whereas an almost fourfold increased risk was observed for the follicular type for *aa* vs *AA* (OR = 3.82, 95%CI: 1.27, 11.49) and for *Aa* vs *AA* (OR = 3.79, 95%CI: 1.40, 10.26). A subsequent study in a Turkish [20] population did not reveal any effect of *Apa1* on papillary carcinoma.

For renal cell carcinoma, we found two papers, both showing a risk reduction for *aa* and Aa vs *AA*. Other studies reported not association for the *Apa1* polymorphism and cancer risk.

Cdx2 and Cancer

Cdx2, located in the 5′ region of the VDR, has been suggested to modulate promoter activity [9].

Since 2005 28 studies included *Cdx2*, 7 in prostate cancer, 6 in breast cancer, 5 in colorectal and 2 in skin cancer, and 9 in other cancer sites. The studies were conducted mainly in North America and Europe, three were in China, one was in Pakistan, and one was in New Zealand (Table 4.1; Figs. 4.9 and 4.10).

Data were summarized in a previous meta-analysis published by Serrano et al. in 2016 [185] that estimated a modest but significant increased risk for all cancer sites: SOR = 1.12 (95%CI: 1.00–1.25) for *gg* versus the *GG* genotype and 1.03 (95%CI: 0.96–1.10) for *Gg* versus the *GG* genotype (Table 4.1; Figs. 4.9 and 4.10).

Breast Cancer

For breast cancer Anderson [6] had the largest series with 1546 cases and 1627 controls. Subjects with gg have a significant 50% increased risk for breast cancer (OR = 1.49, 95%CI: 1.05–2.11), but in the same cohort, the *Gg* is suggested to have a protective effect with a 17% significant risk reduction (OR = 0.83, 95%CI:

0.72–0.97). Pooling estimates, Serrano found a non-significant increased risk for carriers of *gg* genotype (Summary OR = 1.22, 95%CI: 0.70–2.12) and a non-significant reduction in breast cancer for carriers of heterozygous *Gg* genotype (summary OR = 0.97, 95%CI: 0.70–1.36) [185]. In a meta-analysis published by Zhou et al., *Cdx2* might be associated with the risk of breast cancer in African-Americans [233], consistent with the data reported by Huang et al. [94].

The three more recent studies (Clendenen et al. [40] carried out in Sweden, Iqbal et al. [100] carried out in Pakistan, Amadori et al. [5] that presented data for *Cdx2* only for Italian subjects) do not suggest any significant associations.

Prostate Cancer

Five of the seven studies [38, 86, 103, 137, 206] were included in a recent meta-analysis [185]. *Cdx2* was found to be not associated with prostate cancer: SOR was 1.09 (95%CI: 0.73–1.64) and 1.01 (95%CI: 0.83–1.22) for *gg* and *Gg* versus the *GG* genotype, respectively. Results of the two recent studies (Gilbert et al. [67] that presented the results of the ProtecT studies carried out in the UK and Deschasaux et al. [48] that presented the results of the SU. VI. MAX nested case-control study carried out in France) also do not support an association.

Colorectal Cancer

In all studies but one [18] for CRC published since 2007, a consistent trend toward an increased risk is observed for subjects carrying gg genotype [60, 154, 193, 203]; however, the SORs obtained in the meta-analysis by Serrano et al. [185] do not confirm a significant increased risk: 1.24 (95%CI: 0.94–1.63) and 1.09 (95%CI: 0.96–1.24) for *gg* and *Gg* versus the *GG* genotype, respectively.

(a)

Cdx2 (gg vs GG)

Author	Odds Ratio [95% CI]

Breast

Abbas, 2008 (Caucasian)	0.94 [0.66, 1.33]
Anderson, 2011 (Caucasian)	1.49 [1.05, 2.11]
Yao, 2012 (African-American)	1.94 [1.01, 3.74]
Yao, 2012 (Caucasian)	0.83 [0.39, 1.75]
Clendenen, 2015 (Caucasian)	0.77 [0.54, 1.09]
Iqbal, 2015 (Other)	0.55 [0.21, 1.44]
Amadori, 2017 (Caucasian)	1.30 [0.29, 5.85]

0   0.5   1   2   3   4   5

(b)

Cdx2 (gg vs GG)

Author	Odds Ratio [95% CI]

Prostate

John, 2005 (Caucasian)	0.90 [0.48, 1.68]
Cicek, 2006 (Caucasian)	0.63 [0.30, 1.31]
Mikhak, 2007 (Caucasian)	0.91 [0.56, 1.49]
Torkko, 2008 (Caucasian)	2.09 [1.13, 3.85]
Torkko, 2008 (Hispanic)	0.49 [0.18, 1.37]
Rowland, 2013 (African-American)	1.80 [0.98, 3.33]
Rowland, 2013 (Caucasian)	0.98 [0.64, 1.48]
Gilbert, 2015 (Caucasian)	1.31 [0.86, 2.00]
Deschasaux, 2016 (Caucasian)	1.31 [0.45, 3.78]

0   0.5   1   2   3   4   5

Fig. 4.9 Forest plot for the association between *Cdx2 gg* and *GG* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the skin, pediatric solid tumors, and tobacco-related cancers; (**e**) cancers of the brain, esophagus, kidney, lung, and ovary

(c)

Cdx2 (gg vs GG)

Author	Odds Ratio [95% CI]

CRC

Flugge, 2007 (Caucasian)	1.00 [0.53, 1.87]
Ochs-Balcom, 2008 (Caucasian)	2.27 [0.95, 5.41]
Theodoratou, 2008 (Caucasian)	1.15 [0.85, 1.57]
Slattery, 2009 (Caucasian)	1.29 [0.96, 1.72]
Bentley, 2012 (Caucasian)	0.80 [0.27, 2.37]

0   0.5   1   2   3   4   5

(d)

Cdx2 (gg vs GG)

Author	Odds Ratio [95% CI]

Skin

Han, 2007 (Caucasian)	0.58 [0.21, 1.58]
Han (BCC), 2007 (Caucasian)	0.94 [0.45, 1.99]
Han (SCC), 2007 (Caucasian)	1.35 [0.67, 2.71]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)	1.20 [0.69, 2.08]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)	0.99 [0.54, 1.84]

Solid tumor pediatric

Bienertová-Vašků, 2016 (Caucasian)	1.18 [0.66, 2.10]

Tobacco-related cancers

Deschasaux, 2015 (Caucasian)	0.46 [0.16, 1.28]

0   0.5   1   2   3   4   5

Fig. 4.9 (continued)

(e)

Cdx2 (gg vs GG)

Author		Odds Ratio [95% CI]
Brain		
Anic, 2012 (Caucasian)		0.68 [0.36, 1.28]
Esophageal		
Gu, 2014 (Asian)		1.01 [0.73, 1.40]
Kidney		
Yang, 2016 (Asian)		1.10 [0.68, 1.78]
Lung		
Wu, 2016 (Asian)		0.96 [0.79, 1.29]
Gromowski, 2017 (Caucasian)		0.27 [0.08, 0.99]
Ovary		
Lurie, 2007 (Asian)		0.90 [0.40, 1.80]
Lurie, 2007 (Caucasian)		1.50 [0.40, 5.20]
Tworoger, 2009 (Caucasian)		1.00 [0.71, 1.42]

0   0.5   1   2   3   4   5

Fig. 4.9 (continued)

Other Cancers

Several studies presented risk estimates for other cancer sites: skin cancer [79, 173], ovarian cancer [127, 209], brain cancer [8] and esophageal cancer [72], renal cell cancer [222], lung cancer [69, 218], all solid pediatric tumor together [21], and all tobacco-related cancers [47]. None of them found significant association except for Gromowski et al. [69] who observed a significant inverse association with lung cancer of *gg* genotype vs *GG* (SOR = 0.27, 95%CI: 0.08–0.99) (Table 4.1; Figs. 4.9 and 4.10).

Conclusions and Discussion

Over the last 30 years, an increasing number of studies have examined the association of VDR polymorphisms and cancer. We performed a comprehensive review of the literature on the *VDR Fok1*, *Bsm1*, *Taq1*, *Apa1*, and *Cdx2* polymorphisms and cancer risk. We identified 176 independent studies published up to 2018 with data to calculate cancer risk estimates for 19 cancer sites. The four most studied cancer types were prostate, breast, colorectal, and skin cancer.

We found some significant associations with VDR polymorphisms for all genotypes with prostate, breast, and colon-rectum cancer, even if the associations are sometime heterogeneous. VDR *Fok1* polymorphisms might modulate the risk of cancer of breast and possibly affect cancer risk at any site. *Bsm1 B* allele was suggested to reduce cancer risk at most sites, especially colon-rectal and skin. Some opposite effect of *B* allele was suggested for ovarian and bladder cancer and for non-Hodgkin lymphoma, which could be spurious results due to the small number of studies and included subjects. For some cancer sites, especially breast cancer, opposite risk estimates were obtained in some studies, possibly suggesting different effect of *B* allele in sub-populations and/or interaction with other genetic and host factors. This would be warranted to be further investigated in future studies.

For skin cancer significant associations with VDR polymorphisms have been reported for *Fok1*, *Bsm1*, and *Taq1*.

No significant association has been reported for esophageal cancer, non-Hodgkin lymphoma,

(a)

Cdx2 (Gg vs GG)

Author		Odds Ratio [95% CI]
Breast		
Abbas, 2008 (Caucasian)		1.08 [0.94, 1.25]
Anderson, 2011 (Caucasian)		0.83 [0.72, 0.97]
Yao, 2012 (African-American)		1.55 [0.79, 3.03]
Yao, 2012 (Caucasian)		0.99 [0.72, 1.36]
Clendenen, 2015 (Caucasian)		1.15 [0.93, 1.41]
Iqbal, 2015 (Other)		0.52 [0.30, 0.88]
Amadori, 2017 (Caucasian)		0.71 [0.28, 1.83]

0   0.5   1   2   3   4   5

(b)

Cdx2 (Gg vs GG)

Author		Odds Ratio [95% CI]
Prostate		
John, 2005 (Caucasian)		0.86 [0.64, 1.15]
Cicek, 2006 (Caucasian)		0.92 [0.63, 1.34]
Mikhak, 2007 (Caucasian)		1.16 [0.90, 1.48]
Torkko, 2008 (Caucasian)		1.01 [0.76, 1.35]
Torkko, 2008 (Hispanic)		0.83 [0.53, 1.32]
Rowland, 2013 (African-American)		1.55 [0.82, 2.92]
Rowland, 2013 (Caucasian)		0.89 [0.58, 1.36]
Gilbert, 2015 (Caucasian)		0.93 [0.76, 1.13]
Deschasaux, 2016 (Caucasian)		0.71 [0.43, 1.17]

0   0.5   1   2   3   4   5

Fig. 4.10 Forest plot for the association between *Cdx2 Gg* and *GG* genotype for (**a**) breast cancer; (**b**) prostate cancer; (**c**) colorectal cancer; (**d**) cancers of the skin, pediatric solid tumors, and tobacco-related cancers; (**e**) cancers of the brain, esophagus, kidney, lung, and ovary

(c)

Cdx2 (Gg vs GG)

Author	Odds Ratio [95% CI]

CRC

Flugge, 2007 (Caucasian)	1.00 [0.69, 1.44]
Ochs-Balcom, 2008 (Caucasian)	1.07 [0.70, 1.63]
Theodoratou, 2008 (Caucasian)	1.13 [0.99, 1.29]
Slattery, 2009 (Caucasian)	1.07 [0.93, 1.24]
Bentley, 2012 (Caucasian)	0.85 [0.28, 2.57]

0   0.5   1   2   3   4   5

(d)

Cdx2 (Gg vs GG)

Author	Odds Ratio [95% CI]

Skin

Han, 2007 (Caucasian)	1.11 [0.78, 1.59]
Han (BCC), 2007 (Caucasian)	0.89 [0.65, 1.22]
Han (SCC), 2007 (Caucasian)	1.00 [0.73, 1.37]
Randerson-Moor, 2009 (Study: Leeds CCS1) (Caucasian)	0.93 [0.73, 1.20]
Randerson-Moor, 2009 (Study: Leeds CCS2) (Caucasian)	0.90 [0.66, 1.23]

Solid tumor pediatric

Bienertová-Vašků, 2016 (Caucasian)	0.99 [0.58, 1.70]

Tobacco-related cancers

Deschasaux, 2015 (Caucasian)	1.04 [0.72, 1.50]

0   0.5   1   2   3   4   5

Fig. 4.10 (continued)

(e)

Cdx2 (Gg vs GG)

Author		Odds Ratio [95% CI]
Brain		
Anic, 2012 (Caucasian)		1.12 [0.87, 1.45]
Esophageal		
Gu, 2014 (Asian)		1.18 [0.91, 1.52]
Kidney		
Yang, 2016 (Asian)		1.10 [0.70, 1.72]
Lung		
Wu, 2016 (Asian)		1.22 [0.85, 1.47]
Gromowski, 2017 (Caucasian)		0.83 [0.66, 1.04]
Ovary		
Lurie, 2007 (Asian)		0.50 [0.30, 0.90]
Lurie, 2007 (Caucasian)		1.00 [0.60, 1.80]
Tworoger, 2009 (Caucasian)		1.19 [1.02, 1.39]

0   0.5   1   2   3   4   5

Fig. 4.10 (continued)

sarcoma, pediatric solid tumor, and tobacco-related cancers.

In a previous meta-analysis [171], we found that VDR Fok1 and *Bsm1* polymorphisms might modulate the risk of cancer of the breast, skin, and prostate and possibly affect cancer risk at any site in Caucasians. We found a significant 30% increase in skin cancer risk and 14% increase in breast cancer risk with *Fok1 ff* compared to *FF* genotype. We found a significant 17% reduction in prostate cancer risk with *Bsm1 Bb* compared to *bb* genotype (SOR; 95%CI: 0.83; 0.69–0.99). In Caucasian populations, both *Bb* and *BB* carriers had a significant reduced risk of cancer at any site.

The more recent meta-analysis published by Xu et al. [220] indicated that *b* allele of *Bsm1* polymorphism was a risk factor for cancer susceptibility. Moreover, *f* allele of *Fok1* polymorphism was a risk factor for ovarian and skin cancer and a protective factor for glioma. Furthermore, *t* allele of *Taq1* polymorphism was found to be positively associated with oral, breast, and basal cell cancer and inversely with prostate cancer. Finally, *a* allele of *Apa1* polymorphism was a risk factor for basal cell cancer in Asian population.

In 2015, another meta-analysis evaluated the associations between VDR gene polymorphisms (*Cdx-2*, *Fok1*, *Bsm1*, *Apa1*, and *Taq1*) and female reproductive cancers (breast, ovarian, cervical, endometrial, uterine, and vaginal cancers) [145]. Up to April 2014, the authors evaluated the risks for reproductive cancers under the heterozygous, homozygous, dominant, and recessive models with fixed or random effects models. They indicated that the *Fok1* polymorphism was related to increased risks for breast and ovarian cancers, whereas the *Bsm1* polymorphism was associated with a decreased risk for developing these cancers.

A meta-analysis published by Serrano et al. in 2016 [185] assessed the association of *Taq1*, *Apa1*, and *Cdx2* SNPs with the risk of cancer and estimated a modest but significant increased risk for any cancer site for *Cdx2*: summary OR = 1.12 (95%CI: 1.00–1.25) for *gg* versus the *GG* genotype and 1.03 (95%CI: 0.96–1.10) for *Gg* versus the *GG* genotype.

Two meta-analyses were recently published [35, 228]. Yu found the *tt* genotype of *Taq1* inversely associated with lung cancer risk compared with the *Taq1 Tt* + *TT* genotype

(OR = 0.70, 95%CI = 0.55–0.90) [228], while Chen [35] confirmed the association in particular in Asian population and suggested that PCa patients carrying the *t* allele or *tt* genotype were less likely to progress to advanced stage.

There are several potential explanations for contrasting results and inconsistencies in findings for these common SNPs. Design issue or small sample size may limit the generalizability of the results. It is well established that *VDR* genotypes vary widely by ethnicity and it is needed to evaluate these associations among ethnic subgroups to evaluate differences in allele frequency [168, 207]. We considered the deviation from H-W disequilibrium in controls as an indication that the alleles remain constant and are not segregating independently. There are several reasons for heterogeneity, including non-random matching (which encompasses admixture), biased selection of subjects from the population, genotyping error, population stratification, and adjustment for confounders. Sun exposure and dietary consumption are potential modification of the genotype-cancer associations.

To conclude, there is some indication that *VDR* polymorphisms may modulate the risk of some cancer sites and in future studies *VDR* genetic variation should be integrated also with prediagnostic biomarkers of vitamin D status.

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On the Relationship Between Sun Exposure and All-Cause Mortality

5

Pelle G. Lindqvist

Abstract

Increasing sun exposure is related to lower prevalence of death in cardiovascular disease (CVD), type 2 diabetes, and other noncancer non-CVD. In this chapter we aim to make a short update on the knowledge regarding sun exposure and all-cause mortality. Data support the hypothesis that low sun exposure habits are a major risk factor for all-cause mortality. Low sun exposure is related to an increased risk of death due to CVD and noncancer/non-CVD, and a minor reduction in risk of cancer. Active sun exposure habits have a dual effect; it increases the incidence of skin cancer, but also improves the prognosis in terms of all-cause mortality. In a low solar intensity region, we should carefully assess both risk and benefits of sun exposure in order to obtain balanced recommendations.

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In this chapter we aim to make a short update on the knowledge regarding sun exposure and all-cause mortality. Data support the hypothesis that low sun exposure habits is a major risk factor for all-cause mortality. Low sun exposure is related to an increased risk of death due to cardiovascular disease (CVD) and noncancer/non-CVD and a minor reduction in risk of cancer. Active sun exposure habits have a dual effect; it increases the incidence of skin cancer but also improves the prognosis in terms of all-cause mortality. In a low solar intensity region, we should carefully assess both risk and benefits of sun exposure in order to obtain balanced recommendations.

In 2011, a 30% lower rate of all-cause mortality was reported among those who took a sunbathing vacation at least once a year over the course of three decades [1]. A 15-year prospective follow-up of the melanoma in Southern Sweden (MISS) cohort of women demonstrated a significant dose-dependent decrease in all-cause mortality with increasing sun exposure habits [2] (Fig. 5.1), and the mortality rate was doubled (2.0, 95% CI 1.6–2.5) among those avoiding sun exposure compared to the highest sun exposure group (Fig. 5.2). The population attributable risk (PAR) for mortality for the group avoiding sun exposure was estimated to be 3%. In a 20-year follow-up of the same cohort, analyzed in a competing risk scenario, it was shown that the shorter life expectancy of women who avoided sun exposure was mainly due to a dose-dependent

Fig. 5.1 The melanoma in Southern Sweden (MISS) cohort included 1000 women from each age from 25 to 64 years, without cancer from the population registry 1990, and 29,518 women entered the study. Adjusted all-cause survival plot of all 29,518 women in the MISS cohort. Significance of difference $P < 0.001$ among all three sun exposure groups. (Used with permission from Wiley [2])
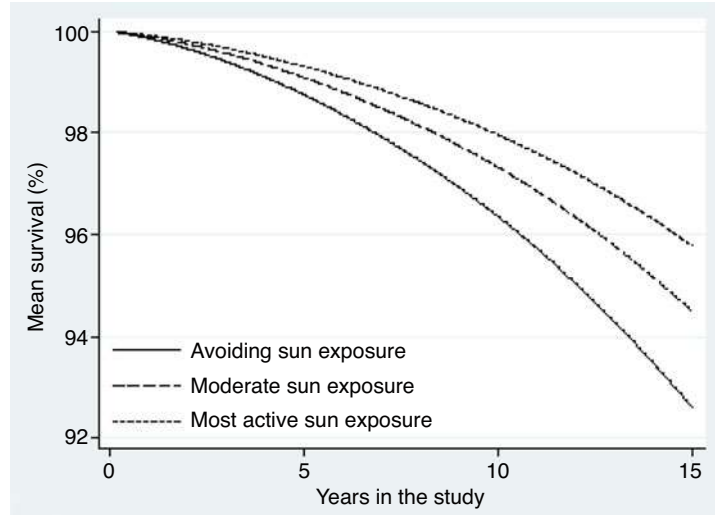
Fig. 5.2 Mortality rate by sun exposure with attained age as time variable. As compared to the highest sun exposure group, the mortality rate was twofold higher (2.0, 95% CI 1.6–2.5) among avoiders of sun exposure and increased by 40% (1.4, 95% CI 1.1–1.7) in those with moderate exposure. (Used with permission from Wiley [2])
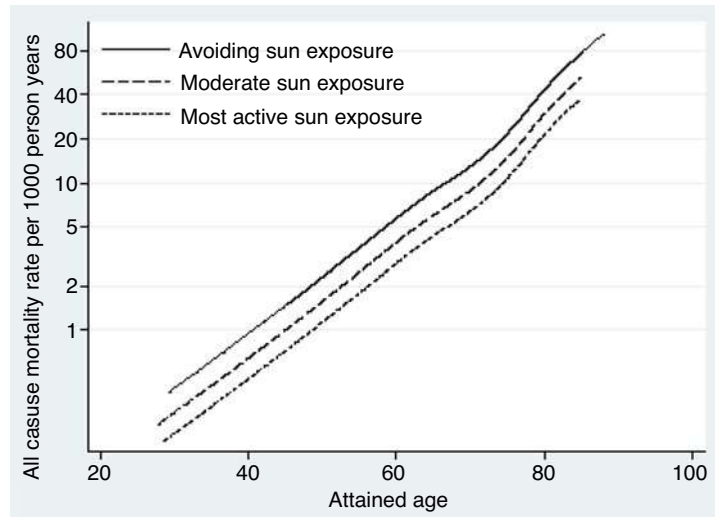
significantly increased risk of cardiovascular disease (CVD) and noncancer/non-CVD deaths, compared to the moderate and high sun exposure groups (Fig. 5.3, top) [3]. While the risk of dying in the CVD and noncancer/non-CVD groups decreased with increasing sun exposure, the relative contribution of death due to cancer increased as a result of extended life expectancy (Fig. 5.3, bottom) [3]. Thus, the overall *prevalence* of death

due to cancer increased, but not the age-adjusted risk. In an analysis stratified for smoking, there was a similar risk of death among nonsmokers avoiding sun exposure as for smokers in the highest sun exposure groups (Fig. 5.4) [3]. We interpreted this that sun exposure avoidance is a risk factor for all-cause death of the same magnitude as smoking.
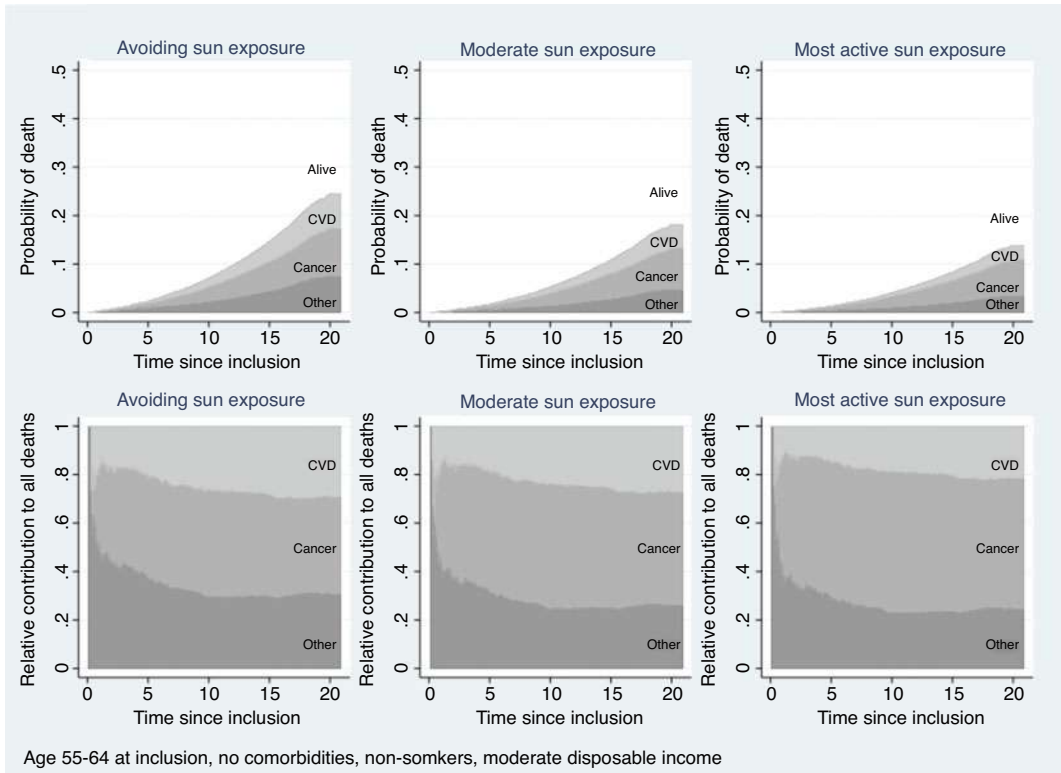
Fig. 5.3 Probability of death by sun exposure habits in a competing risk scenario. Upper three graphs show death categorized into CVD, cancer, and other (noncancer/non-CVD) according to time in years since study inclusion. Bottom three graphs show relative contribution to death is sun exposure habits. As compared to highest sun exposure group, the subdistribution hazard ratios (sHRs) of CVD mortality among sun exposure avoidance and moderate exposure were sHR = 2.3 95% CI 1.8–3.1, and 1.5 95% CI 1.2–1.8, respectively. The corresponding sHRs for noncancer-non-CVD death were 2.1, 95% CI 1.7–2.8, and 1.57, 95% CI 1.3–1.9 and for cancer 1.4, 95% CI 1.04–1.6, and 1.1, 95% CI 0.9–1.4, respectively. (Used with permission from Wiley [3])

Skin Cancer and All-Cause Mortality

Sunlight exposure and fair skin are major determinants of both skin cancer and vitamin D production. Due to similar etiology and prognosis, basal cell carcinoma and squamous cell carcinoma are often grouped as non-melanoma skin cancer (NMSC). NMSC is mainly related to cumulative UV radiation and has a good prognosis in terms of all-cause mortality. Cutaneous malignant melanoma (MM) is the skin malignancy mainly related to increased mortality and is related to (episodic) overexposure to UV radiation and genetic causes [4]. There is a relationship between high sun exposure and MM incidence but an inverse relationship to prognosis [2]. Thus, high UV exposure increases the incidence, while low sun exposure habits/vitamin D levels have been linked to thicker, more aggressive melanomas with shorter survival times [2, 5–7]. The incidence of MM has shown the greatest increase of all cancers during the last 30 years. The disease is reported to be fatal in approximately 20% of patients. In line with this, out of those contracting MM in the MISS cohort, 35% of women with low sun exposure and 10% of those with the greatest sun exposure habits died during the follow-up period [3]. Further, when grouping women based on skin cancer status (no skin cancer, NMSC, or MM) and sun exposure habits (low sun exposure, moderate exposure, or highest exposure), in all three skin cancer groups there was an inverse
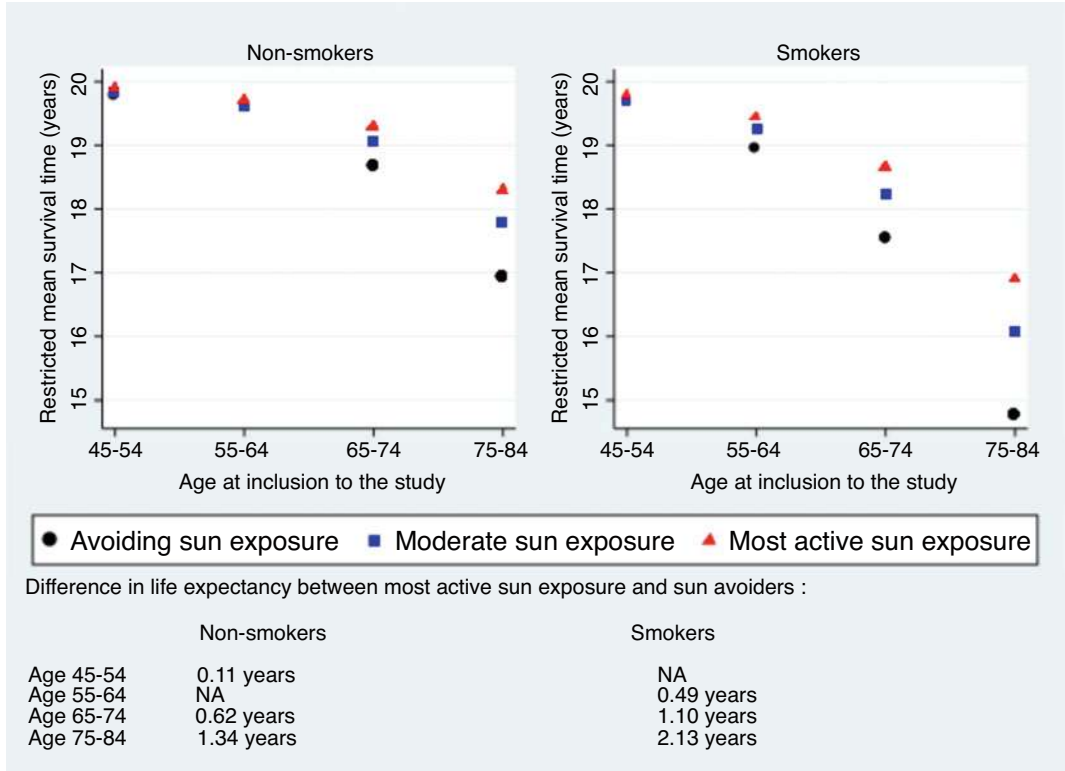
Fig. 5.4 Mean survival by age groups and sun exposure habits, stratified by smoking status, and calculations of mean difference in life expectancy by age groups among smokers and nonsmokers using restricted mean survival, i.e., the area under the curve between two time points based on flexible parametric survival analysis. (Used with permission from Wiley [3])

relationship between sun exposure and all-cause mortality (Fig. 5.5) [3]. In agreement with our findings, US Navy personnel have a higher risk of skin cancer and a reduced risk of other internal cancers, resulting in a 26% reduced all-cause mortality rate [8].

Plausible Explanations for the Inverse Relation Between Sun Exposure and CVD or Noncancer/Non-CVD Mortality

Both coronary heart disease (CHD) and cerebrovascular disease show an increased risk during winter/spring compared to summer in countries at higher latitudes [9–11]. There are several noncancer/non-CVD conditions that increase the

risk of all-cause mortality. In the UK, the risk of autoimmune diseases has been found to be significantly influenced by the season of birth, suggesting the presence of seasonal risk factors such as UVB exposure [12]. Multiple sclerosis (MS) is an immunopathological autoimmune condition with a positive association with both latitude and seasonal differences [13]. The risk of MS is reported to increase approximately threefold among those with low sun exposure habits during their childhood and youth [14]. The incidence of childhood type 1 diabetes mellitus (T1DM) has been shown to have latitude-dependent occurrence with the nadir close to the equator [15]. In a Danish study, mothers exposed to more sunshine during the third trimester had male offspring with a lower risk of developing T1DM before the age of

Odds of all-cause mortality of
Skin cancer (SC) groups by sun exposure groups

We show in all three SC groups a dose dependent worse prognosis with decreasing sun exposure habits.
* The upper visker was cut out in the figure.
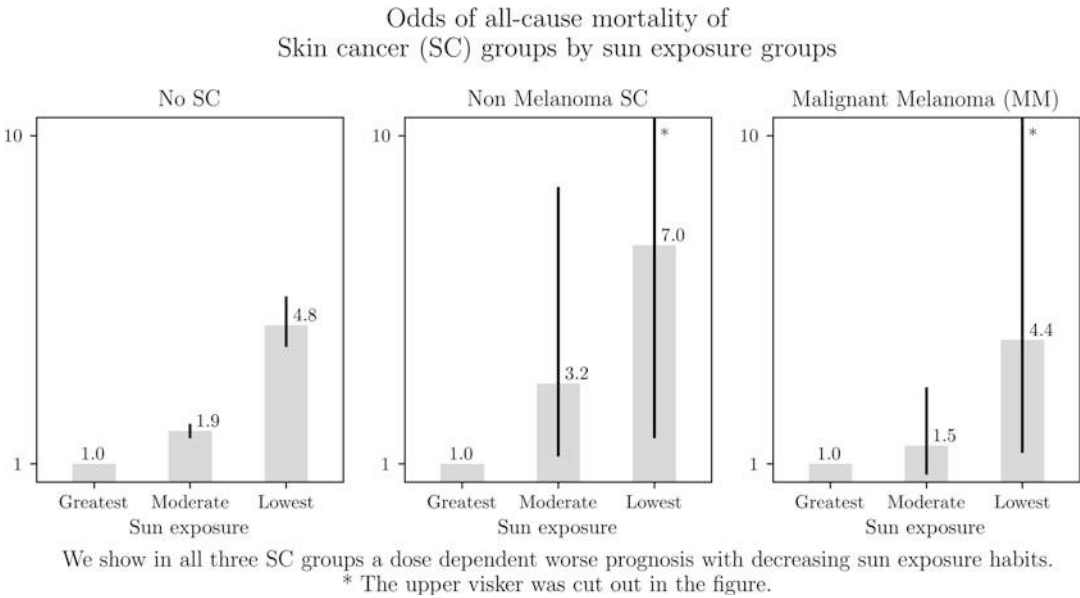
Fig. 5.5 Odds of all-cause mortality of skin cancer (sc) groups by sun exposure groups

15 compared to those who had less sun exposure [16]. Finnish newborns supplemented with vitamin D had an 80% reduced risk of contracting childhood or adolescent T1DM [17]. In the MISS cohort, we showed that there was a dose-dependent reduced risk of incidental type 2 DM (T2DM) with increasing sun exposure and that the risk reduction was accentuated among non-overweight women and independent of physical exercise [18].

Since 1.25 vitamin D induces antimicrobial peptide production, such as cathelicidin and β-defensin, much research has focused on the role of vitamin D in respiratory tract infections [19]. For example, two RCTs with vitamin D supplementation showed reduced antibiotic consumption in patients with primary immune deficiency (60% reduction) and > 70 years of age (50% reduction) compared to the placebo group [20, 21]. These observations could be explained by a direct effect of vitamin D. Vitamin D can modulate IL-8 response to infection through the action of IL-10-producing regulatory lymphocytes IL-1 [22, 23].

Solar UVA radiation causes decreased blood pressure and cardiovascular morbidity. This might be due to an increase in skin-derived nitric oxide (NO) bioactivity and to the mobilization of NO stores [24, 25]. Both high chronic and acute stress levels may activate coagulation and thereby increase the risk of CVD [26, 27]. Sun exposure attenuate stress levels by induced β-endorphin synthesis and, thus, have a cardioprotective effect [28]. The endorphins also induce mood enhancement and feelings of relaxation and socialization [29, 30]. An inborn awarding system to UVB exposure may be interpreted as an evolutionary mechanism indicating that sun exposure is important for our health. Atherosclerosis is a chronic inflammatory condition with cardiovascular dysfunction leading to increased risk of myocardial infarction, stroke, and thromboembolism. In atherosclerosis angiotensin II, levels are increased and NO levels decreased, which might be normalized by sun exposure. Depletion of sun exposure or low levels of vitamin D alone can probably not induce and propagate autoimmune diseases but could facilitate a progression of

cascade events, initiated by virus or other exogenous factors, toward a manifest disease.

There seem to be several plausible mechanisms explaining the inverse relationship between sun exposure and both CVD and noncancer/non-CVD death.

Strengths and Limitations of the Inverse Relation Between Sun Exposure and All-Cause Death

Since the results of an inverse relation between sun exposure and all-cause mortality is observational, it is hypothesis-generating and not necessarily causal.

Serum levels of vitamin D are lower in many diseases. Measurement of circulating vitamin D levels may only provide a surrogate measure of sun exposure [31]. The major shortcoming is, however, that we still cannot exclude the possibility that a bias exists between a healthy lifestyle and high sun exposure habits for which we might not control [18]. Bias due to possible reversed causation has to be taken into consideration. Compared to women with low sun exposure, women in the highest sun exposure group might be better educated, have higher income, smoke less, exercised more, have a better diet, and have had fewer diseases at the inception of the study. In the study we only included women without a diagnosis of cancer, and we adjusted for comorbidity in our analysis. In addition, while omitting the first 10 years in the analysis, the HR for all-cause death were similar [2]. Further, we adjusted for family income, educational level, smoking habits, and marital status in the survival analysis [3].

The findings that there was a dose dependency in sun exposure to inversed risk of all-cause mortality and the magnitude of the differences indicate a causal relationship and not only an association.

Public Health Implications

The MISS cohort is comprised of Swedish-born women before 1966, i.e., before widespread immigration took place, and consists almost entirely of Caucasian women. If avoidance of sun exposure is a major risk factor for all-cause mortality, the problem may even be more serious among women who traditionally cover their skin or who are more densely pigmented. For example, black women in the USA were reported to have a 26% excess all-cause mortality, as compared to Caucasians [32].

Different health issues stand in opposition to each other regarding UV exposure, and a careful weighing of both hazards and benefits is required to get a balanced view. As compared to Northern Australia with strong UV radiation (UV index ≥ 6) during most days of the year, Sweden has low UV intensity (UV index <3) 8–9 months of the year, increasing to strong UV radiation only a few days per year. However, even if there are less than 5 days a year with strong sun, there is a recommendation to stay out of the sun between 1100 and 1400 [33]. Although the use of sun blockers has a very minor position in our present sun protection guidelines, the general perception is "as long as they use sun blockers they can be out for long." This has, however, never been showed. Thus, a plausible explanation for the increasing MM incidence in Sweden is that the old recommendations to rely on sunscreens use result in UV overexposure, explaining the double risk of MM among sunscreen users in Sweden [34, 35]. More importantly, strong recommendations to avoid sun exposure seem to increase the risk of CVD and noncancer-non-CVD morbidity and excess death in our population.

We conclude that the increased mortality rate among those who avoid sun exposure is mainly due to an increased risk of death from CVD and noncancer/non-CVD. We hope our findings add to a more balanced and adequate view regarding the effects of sun exposure on our health.

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Epidemiology of Skin Cancer: Update 2019

6

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Abstract

Melanoma and keratinocyte skin cancer (KSC) are the most common types of cancer in White-skinned populations. Both tumor entities showed increasing incidence rates worldwide but stable or decreasing mortality rates. Rising incidence rates of cutaneous melanoma (CM) and KSC are largely attributed to increasing exposure to ultraviolet (UV) radiation, the main causal risk factor for skin cancer.

Incidence rates of KSC, comprising of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are much higher than that of melanoma. BCC development is mainly the cause of an intensive UV exposure in childhood and adolescence, while SCC development is related to chronic, cumulative UV exposure over decades. Although mortality is relatively low, KSC is an increasing problem for health care services causing significant morbidity.

Cutaneous melanoma is rapidly increasing in White populations, with an estimated annual increase of around 3–7% over the past decades. In contrast to SCC, melanoma risk is associated with intermittent and chronic exposure to sunlight. The frequency of its occurrence is closely associated with the constitutive color of the skin and the geographical zone. Changes in outdoor activities and exposure to sunlight during the past 70 years are an important factor for the increasing incidence of melanoma. Mortality rates of melanoma show stabilization in the USA, Australia, and in European countries. In the USA even dropping numbers of death cases were recently reported, probably reflecting efficacy of the new systemic treatments.

Among younger cohorts in some populations (e.g., Australia and New Zealand,), stabilizing or declining incidence rates of CM are observed, potentially caused by primary prevention campaigns aimed at reducing UV exposure. In contrast, incidence rates of CM are still rising in most European countries and in the USA. Ongoing trends towards thinner melanoma are largely ascribed to earlier detection.

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Introduction

Skin cancers are currently the most frequent solid cancers in White populations, while they are rare in African and Asian populations because these populations have effective pigment protection. The main forms are melanoma, originating from pigment cells of the skin, and basal cell carcinoma and squamous cell carcinoma, originating from keratinocytes. All skin cancers in White populations are caused in 90–95% by UV radiation and are therefore considered to be predominantly caused by population attributable factors [1]. This means that skin cancers could be avoided as far as possible by changing the behavior and avoiding UV exposure.

The important role of UV radiation in the development of skin cancer is also reflected in the mutation patterns of these tumors. The investigation of tumor mutational burden in 27 different tumors showed the following picture: the lowest tumor mutational burden was found in hematological and pediatric tumors, and the highest tumor mutational burden was found in lung cancer and melanoma [2]. These are characteristically caused by exogenous carcinogens such as cigarette smoke and UV radiation. The mutation pattern in melanoma with C-T transitions in about 90% of all mutations is also characteristic for UV-induced mutations. Further studies on skin tumors showed that the mutation load in squamous cell carcinoma of the skin is even significantly higher than in melanomas. In squamous cell carcinoma, 61 mutations/Mb were found, in melanoma just 13/Mb [3].

Incidence and mortality of melanoma are well documented in many registers worldwide. Observed cases are recorded in cancer registries, and estimates based on these data are made for new cancer cases or deaths. The estimates are made in advance and are therefore more up-to-date than the cases observed. The American Cancer Society's Department of Epidemiology and Surveillance Research has been making such case number estimates since 1970 and has reported reliable estimates on melanoma since 1975. The data for the USA are summarized in Table 6.1 for the period 1975–2019 [4–16]. During this period, the number of melanoma cases increased more than tenfold and deaths doubled between 1975 and 2016. While the number of new cases continued to increase and even doubled between 2000 and 2019, the number of deaths showed an interesting development: from 2016 to 2019, there was a significant decrease in the number of deaths. In 2019, 2900 fewer patients died from melanoma than in 2016. Most likely because of the efficacy of the new targeted therapies and the new immunotherapies, probably a certain percentage of patients with metastatic melanoma are currently being cured.

The incidence of keratinocyte skin cancer is much higher than that of melanoma, but keratinocyte skin cancer has a very low mortality rate. This is the reason why keratinocyte is hardly recorded by cancer registries worldwide. In the USA, there is no cancer registry data on keratinocyte skin cancer. In order to collect data on keratinocyte skin cancer, evaluations of health insurance data were carried out. It turned out that 2–3,000,000 procedures for the treatment of keratinocyte were billed annually [17]. In Germany, keratinocyte skin cancer is recorded by several cancer registries in different federal states. Here it was shown that the incidence of keratinocyte skin cancer is about ten times higher than that of melanoma. For 2010, 25 cases of melanoma and 250 cases of keratinocyte skin cancer per 100,000 inhabitants per year were registered in Germany [18, 19]. About 80% of keratinocyte skin cancers are basal cell carcinomas, and about 20% are squamous cell carcinomas.

The purpose of this review is to provide an overview of the data available worldwide on the epidemiology of melanoma and skin cancer. The causal role of UV exposure in the development of melanomas and skin cancer will be addressed in particular. Trends of increases in incidence and mortality are analyzed. Particular attention will be paid to detecting the onset of plateau formation or even a decrease in incidence.

Table 6.1 Annual estimates of new melanoma cases and deaths by the American Cancer Society's Department of Epidemiology and Surveillance Research for the USA from 1975 to 2019

Year	Estimated new cancer cases	Estimated cancer deaths
1975	9000	5000
1980	14,100	4600
1985	22,000	5500
1990	27,600	6300
1995	34,100	7200
2000	47,700	7700
2005	59,580	7770
2010	68,130	8700
2015	73,870	9940
2016	76,380	10,130
2017	87,110	9730
2018	91,270	9320
2019	96,480	7230

Keratinocyte Skin Cancer

Incidence of Keratinocyte Skin Cancer

Keratinocyte skin cancer (KSC) is by far the most frequent cancer in White populations, and numerous studies have shown that incidence rates of KSC are increasing worldwide [20–25]. KSC generally occurs in persons older than 50 years, and in this age group, its incidence is increasing rapidly. In the USA the estimated case numbers of KSC is more than 1000.000 per year of which roughly 20–30% are SCC and 70–80% are BCC [26]. In the White population in the USA, Canada, and Australia, a mean annual increase of KSC of 3–8% was observed since 1960 [20, 26–28]. Few studies found nearly 50-fold differences in the incidence of basal cell carcinoma (BCC) and 100-fold differences in squamous cell carcinoma (SCC) between Caucasian populations in Northern Europe and Australia [20, 29]. Within Australia there is a marked North to South gradient with the most extreme incidence rates of KSC recorded in Queensland [30]. Age-standardized incidence rates (ASIR) of KSC, reported by a 3-year study (2011–2014), were 3105/100,000/year for men and 2296/100,000/year for women [30]. This study also showed that within the 3-year study period, 47% of the patients suffered from multiple KSC which strongly correlated with higher ages. Age-specific

incidence increased from 26/100,000/year among 20–24-year-old people and reached rates of more than 6000/100,000/year among those aged 80–84.

In Germany, the age-standardized incidence rate of KSC was reported to be 113.2/100,000/year in men and 85.1/100,000/year in women in 2014 (European Standard Population ESP) [31]. Between 2007 and 2014, the estimated annual percentage change (EAPC) of the age-standardized incidence rate of keratinocyte skin cancer was 3.6% among men and 5.2% among women [32]. The KSC incidence rates in Germany corresponded well with data from Denmark (126.5/100,000/year for males and 124,8/100,000/year for females in 2012) (Fig. 6.1e) [33].

Increasing incidence rates of BCC and SCC have been reported in several European countries. A study from the Scottish cancer registry over a period of 12 years revealed an annual increase of 1.4–3.5% [25]. The Danish cancer registry also evaluated the incidence rates of BCC and SCC over a period of 30 years, and the incidence rates have risen between 3.1 and 4.6% per year [24]. Finally, a German study, including data from 11 cancer registries over a period of 13 years, reported an annual increase of 3.3–11.6% for BCC and SCC [23]. In the German Federal State of Saarland between 1970 and 2016, the KSC age-standardized incidence rates
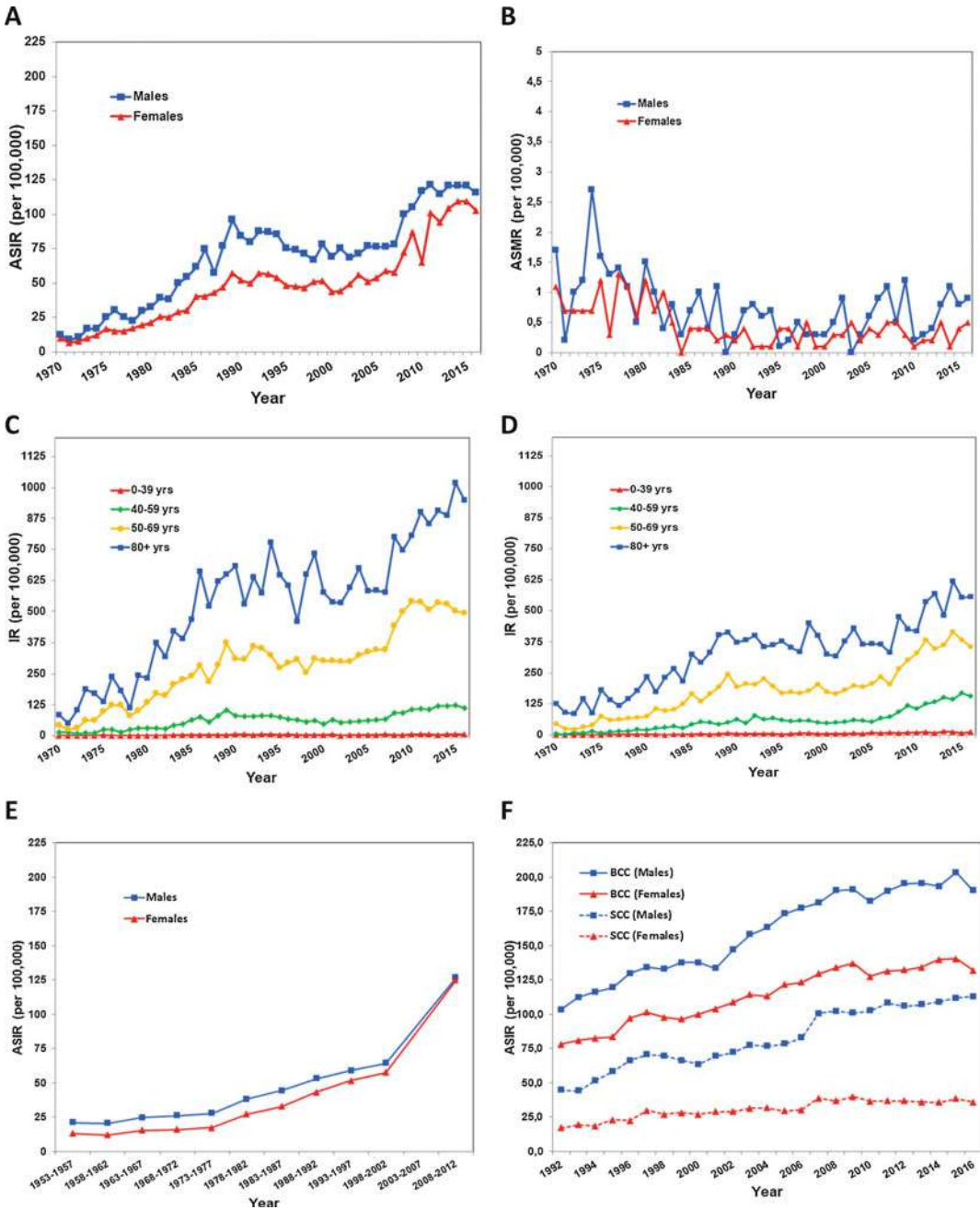
Fig. 6.1 Incidence and mortality rates of keratinocyte skin cancer in Germany (Saarland), Scotland, and Denmark. (**a**) Age-standardized (European Standard Population) incidence rates (ASIR) of keratinocyte skin cancer in Saarland 1970–2016. (**b**) Age-standardized (European Standard Population) mortality rates (ASMR) of keratinocyte skin cancer in Saarland 1970–2016. (**c**) Age-specific incidence rates (IR) of keratinocyte skin cancer in Saarland 1970–2016 (males). (**d**) Age-specific incidence rates (IR) of keratinocyte skin cancer in Saarland 1970–2016 (females). (**e**) Age-standardized (World Standard Population) incidence rates (ASIR) of keratinocyte skin cancer in Denmark 1953–2012. (**f**) Age-standardized (European Standard Population) incidence rates (ASIR) of keratinocyte skin cancer in Scotland 1992–2016, separately for SCC and BCC

increased from 12.0 to 115.6/100,000/year in males and from 9.7 to 102.7/100,000/year in females (Fig. 6.1a) [34]. Age-specific incidence rates continuously increased between 1970 and 2016. Throughout the entire period, the highest incidence rates were observed in persons 80 years and older. In this age group, incidence rates increased from 85.3 in 1970 to 950.1/100,000/year in 2016 in males and from 126.8 to 554.5/100,000/year in females. In the same period, considerably lower incidence rates were observed in the youngest age group (40 years and younger). Between 1970 and 2016, the incidence rates increased for both sexes from less than 0.01 to 6.4/100,000/year in males and to 11.1/100,000/year in females (Fig. 6.1c, d).

These incidence rates may be underestimated as only the first keratinocyte tumor is registered in many registries. To overcome this problem, a cohort study from the UK, assessing the first BCC and SCC per patient per annum for the period 2013–2015, identified 51% additional tumors, leading to threefold higher incidence rates [35]. In this time period, the mean annual percentage increase was 5% for both BCC and SCC.

Basal Cell Carcinoma

Basal cell carcinoma is worldwide the most frequent cancer in fair-skinned people and occurs more frequently than SCC. It is more commonly found in men than in women. In a cohort study from the UK, BCC incidence in 2013–2015 was 352/100,000/year for men and 219/100,000/year for women [35] (Table 6.2), which is clearly higher than the incidence rate found in Germany. Here, an incidence rate for BCC was reported with 113.8/100,000/year in men and 102.5/100,000/year in women [21], similar to rates found in Scotland 2016 (190.1/100,000/year for men and 132/100,000/year for women) [36] (Table 6.2, Fig. 6.1f). According to estimates from the Robert Koch Institute, in 2014 about 43,863 men and 44,257 women were diagnosed with BCC for the first time [37]. Compared to Northern European countries as Scotland or

Germany, incidence rates were found to be three- to tenfold higher in the USA and 10- to 20-fold higher in Australia. In Australia highest yearly age-standardized incidence rates were found dependent on the latitude, in Queensland for BCC with 1538/100,000/year for men and 1191/100,000/year for women [30] (Table 6.2).

Squamous Cell Carcinoma

Squamous cell carcinoma is mostly associated with an older age (mean age 70 years at diagnosis), especially in males, who are about twice frequently affected. About 80% of cases occur in people aged 60 years and above [28, 38]. Highest incidence rates were found in Australia. In Queensland, incidence rates in 2011–2014 accounted for 573/100,000/year in men and for 371/100,000/year in women [30] (Table 6.2). In the USA, incidence rates were lower, 207.5/100,000/year for men and 128.8/100,000/year for women [26] (Table 6.2). In the UK the estimated annual percentage change was 6% in the 3-year period from 2013 to 2015. Incidence rates for cutaneous SCC were 111/100,000/year in men and 42/100,000/year in women [35] (Table 6.2). According to estimates from the Robert Koch Institute, in 2014 about 29,300 men and 20,100 women in Germany were diagnosed with SCC for the first time [37]. The incidence of SCC in Germany has increased fourfold in the last 30 years [21, 23].

Decrease of Mortality in Keratinocyte Skin Cancer

Compared to the incidence, the mortality of KSC is quite low. The age-adjusted US mortality rate for KSC from 1969 to 2000 was 0.69/100,000/year; the rate among men was twice higher than among women. Overall, SCC and BCC death rates have declined [39]. According to the Rhode Island study, decreasing SCC mortality rates for men and women have been observed when comparing two time periods (1979–1987 and 1988–2000) [40, 41]. Also, the BCC

Table 6.2 Incidence rate of basal cell carcinoma and squamous cell carcinoma in Europe (Germany/Federal State of Schleswig-Holstein [21], Scotland [36], UK [35]), the USA (Minnesota) [26], and Australia (Queensland) [30]

	Incidence rates per 100,000 inhabitants and year	
	Basal cell carcinoma	Squamous cell carcinoma
Germany 2008–2010		
Men	113.8	30.0
Women	102.5	15.6
Scotland 2016		
Men	190.1	113.0
Women	132.0	35.8
US Minnesota 2000–2010		
Men	360	207.5
Women	292.9	128.8
Australia Queensland 2011–2014		
Men	1538	573
Women	1191	371
UK 2013–2015[a]		
Men	352	111
Women	219	42

Incidence rates per 100,000 inhabitants and year, age-standardized for the European Standard Population 1976, US Standard Population 2000 and for the Australian Standard Population

[a]Calculated for the first BCC and the first SCC per patient per year

mortality rate for the current period was estimated at 0.05 compared with 0.10 for the earlier period. In Europe, similarly, a decrease of mortality rates was found [42]. In the Netherlands, SCC mortality rates decreased by −1.9% (95% CI: −3.1% to −0.7%) from 1989 to 2008 annually [43]. A meta-analysis from Wehner et al. [44] compared rates from four countries, Germany [45], Denmark [46], the USA [47], and the Netherlands [43]. For BCC all studies showed similar outcomes with a standard mortality rate reaching from 0.87 to 0.97. For SCC the rates were higher, reaching 1.17 in Germany, 1.3 in Denmark, 1.25 in the USA, and 1.27 in the Netherlands. Therefore, patients with SCC had a 25% increased risk of all-cause mortality compared to the general population. Mortality rates from 1970 to 2016 in western Germany (the Federal State of Saarland) revealed a continuous decrease since the 1970s. In men, the age-standardized mortality rate (European Standard Population) decreased from 1.7/100,000/year in 1970 to 0.9/100,000/year in 2016, and in women this rate decreased from 1.1/100,000/year to 0.5/100.000/year for the same period [34] (Fig. 6.1b).

Clinical Epidemiology of KSC

Keratinocyte skin cancers constitute more than one-third of all cancers in the USA, and the standardized ratio of BCC to SCC is roughly 4:1.2 [48]. Recent studies however reported a more balanced overall BCC/SCC incidence ratio of 1.4:1, which equalized as age increases, reaching 1.1:1 in age groups older than 60 years [49, 50].

KSC generally occurs in persons older than 50 years, and in this age group, its incidence is increasing rapidly, patients with SCC were generally older at the time of diagnosis [28, 51, 52]. The anatomic pattern of increase in BCC and SCC incidence was consistent with an effect of higher sunlight exposure. Over 80% of KSC occur on sun-exposed body sites. For KSC the highest body site-specific incidence rates were found for lip, orbit, nasolabial and ear, nose, cheek, and the dorsum of the hands [53]. In 2008 Brantsch et al. [54] showed that tumor thickness is an independent prognostic factor in SCC. Key prognostic factors for metastasis were increased tumor thickness (hazard ratio HR 4.79), immunosuppression (HR 4.32), localization at the

ear (HR 3.61), and increased horizontal size (HR 2.22). The risk of local recurrence depended on increased tumor thickness (HR 6.03) and desmoplasia (HR 16.11) [54].

Sun Exposure and Keratinocyte Skin Cancer

Sun exposure has since long been regarded to be the major environmental risk factor for nonmelanoma skin cancer [55, 56]. Lifelong cumulative sun exposure has been postulated to be a causal factor for SCC [55], while mixed effects of intermittent and cumulative sun exposure have been discussed as being causal for BCC [56]. A dose-response curve for sun exposure and BCC could be reported by several authors [56].

There is strong evidence to suggest that the role of UV radiation in the development of skin cancer is multifold: [1] it causes mutations in cellular DNA that might ultimately lead to unrestrained growth and tumor formation, (and [2]) it induces a state of relative cutaneous immunesuppression that might prevent tumor rejection and [3] might allow the persistent infection with human papilloma viruses (HPV) as shown in immunosuppression patients [57]. Most UV-induced damage to the cellular DNA is repaired; however, mutations may occur as a result of base mispairing of the cellular DNA. The genes involved in the repair process are also potential UV targets. p53 is a nucleoprotein encoded by a tumor suppressor gene. Mutations of the tumor suppressor gene p53 are implicated in the genesis of a wide variety of human neoplasia including KSC [58]. These mutations were reported to be present in 50% to 90% of SCC [58] and approximately 55% of BCC including very small lesions [59]. A second tumor suppressor gene, the gene for the patched (PTCH) protein in the epidermal growth-stimulating Hedgehog pathway, the human gene homolog of the Drosophila segment polarity gene patched, has also been shown to be mutated in more than 90% of sporadic BCC, in patients with Gorlin-Goltz syndrome, and with xeroderma pigmentosum [60–62]. Furthermore, it has been reported that the observed point mutations both in the PTCH and the p53 genes were predominantly UV-specific transitions [61, 63]. These results provide the first genetic evidence that UV radiation is the principal causal factor for KSC. So far, mutations in the PTCH gene seem to be specific for BCC transformation, apart from SCC in patients with a history of multiple BCC [63].

Recently, some studies report on occupational risk factors for the development of KSC. Occupational exposure to tar, mineral oils, and infrared radiation has also been identified as causative agents for KSC. Now, there is consistent epidemiological evidence for a positive association between occupational UV light exposure and an increased risk of SCC and BCC [64, 65]. In Germany, KSC has been defined as an industrial disease in outdoor workers [66, 67].

A systematic review and meta-analysis published in 2011 demonstrated that working people with many years of outdoor employment have a significantly higher risk of SCC compared to people who work indoors [64]. In addition, the causal relationship between UV radiation and the development of cutaneous SCC carcinoma and actinic keratoses is established from a pathophysiological, experimental, and epidemiological point of view.

Melanoma

Increase of Melanoma Incidence in White Populations

The incidence of cutaneous melanoma (CM) has steadily increased over the past 70 years [28, 68–73]. Steep increases were mainly reported from industrial countries with Caucasian populations (Northern America [74–76], Northern Europe [77, 78], and Australia and New Zealand [79–81]), whereas in populations with greater pigmentation (Asia and Africa), melanoma incidence has remained largely unchanged [69, 82]. A variety of behavioral changes in lifestyle (i.e., increased outdoor recreational activities, desire to tan, more frequent holidays spent in tropical climates), associated with increasing exposure to

UV radiation, have largely contributed to the observed increase in melanoma incidence in the past [68, 69, 83, 84]. The highest incidence rates were reported from Australia and New Zealand. In Australia, the age-standardized incidence rates (WHO standard population, Segi) in 2014 were 41/100,000/year for men and 29.4/100,000/year for women [85].

In the USA, the age-standardized incidence rates (US Standard Population, 2000) increased between 1975 and 2015 from 9.4 to 39.3/100,000/year for men and from 8.2 to 27.2/100,000/year for women (Fig. 6.2a) [86].

Incidence rates within Europe show great variation [69, 72, 87]. The highest incidence rates have been reported from North and West Europe, where age-standardized melanoma incidence rates (European Standard Population, 1976) for 2018 ranged between 23 and 25/100,000/year for both sexes. The lowest incidence rates in Europe were found in the Mediterranean and Eastern countries (7–12/100,000/year), which are less than half of that of Western and Northern Europe [82, 88].

In all European countries, incidence rates of CM have steadily increased since the 1950s. During the period 1990–2007, incidence rates have risen by an average of +3.8% p.a. for women and by +4.2% for men [73]. The strongest increases were observed in Northern Europe, followed by Western and later also in Eastern and Southern Europe [69].

Long-term incidence trends are reported from the Scandinavian countries, where first cancer registration had already begun in the 1940s [89, 90]. The Danish Cancer Registry recorded melanoma patients from 1943 to 2015. Age-standardized incidence rates (European Standard Population, 1976) increased from 0.9/100,000/year for men and 0.8/100,000/year for women in 1943 to 29.4/100,000/year for men and 36.8/100,000/year for women in 2015 (Fig. 6.2c) [91].

In Germany, melanoma incidence data since the 1970s are recorded in the Federal State of Saarland. For men, age-standardized incidence rates (European Standard Population) increased from 2.3/100,000/year in 1970 to 12.0/100,000/

year in 2016 and in women from 2.4/100,000/year to 11.4/100,000/year, respectively (Fig. 6.2e). While incidence rates of melanoma continue to rise in most European countries (i.e., in Southern and Eastern Europe), particularly in higher age groups, there have been recent reports from several Northern and Western European countries, Australia, New Zealand, Canada, and the USA of declining incidence rates among younger birth cohorts [69, 79, 81].

Stabilization of Mortality Rates

Mortality from CM has been increasing until the late 1980s in young- and middle-aged populations from most European countries [70, 83, 92] as well as from North America, Australia, and New Zealand [5, 74, 79, 93]. Mortality rates peaked in 1988–1990. Thereafter, mortality trends developed differently. Mortality rates were still rising in several European countries (e.g., Southeastern Europe), particularly for middle-aged and old patients, whereas trends of stabilization or decline were visible among younger cohorts [18, 83, 94–98]. The favorable mortality trends are largely the result of changing patterns of sunshine exposure and sunburn in younger generations as well as to a better and earlier diagnosis of CM [18, 79, 92, 95, 99, 100]. Additionally, a trend towards thinner and less invasive melanomas in both Central Europe and Queensland was observed in the last three decades [101–103].

Age-standardized mortality rates are available for the USA from 1975 onwards. Between 1975 and 2015, the age-standardized mortality rate for men increased from 2.9/100,000/year to 4.1/100,000/year and remained largely the same for women (1.7–1.8/100,000/year) (Fig. 6.2b). Mortality rates have been recorded in Denmark from 1950 to 2015. During this period, mortality rates among men increased from 1.2/100,000/year to 4.0/100,000/year with peaks of up to 4.7/100,000/year in-between. For women, the age-standardized mortality rate increased from 0.9/100,000/year to 2.3/100,000/year with peaks around the 1990s from 3.5/100,000/year
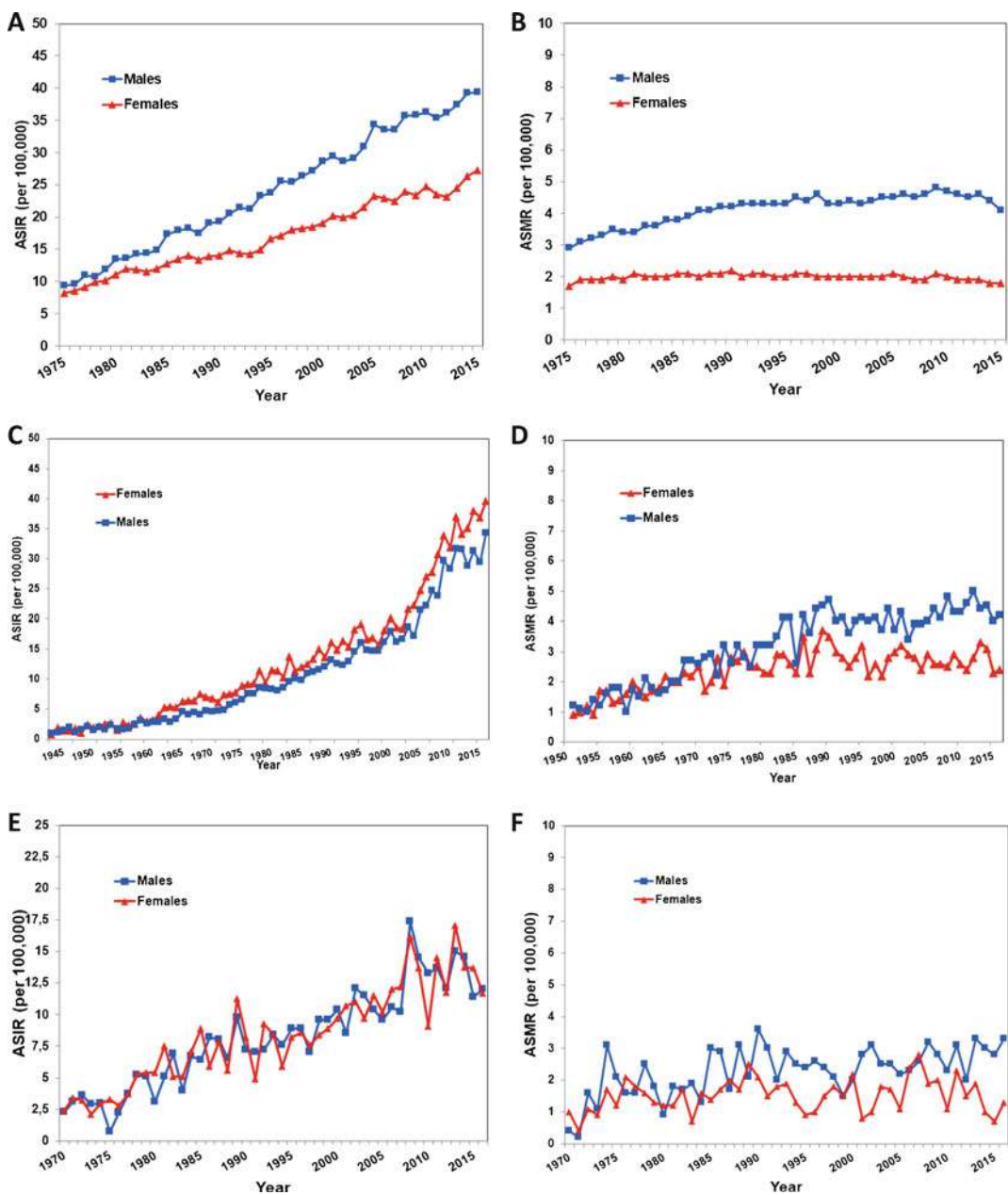
Fig. 6.2 Incidence and mortality rates of melanoma in the USA (SEER 9) and in Denmark. (**a**) Age-standardized (US Standard Population, 2000) incidence rates of melanoma in the USA 1975–2015. (**b**) Age-standardized (US Standard Population, 2000) mortality rates of melanoma in the USA 1975–2015. (**c**) Age-standardized (European Standard Population, 1976) incidence rates of melanoma in Denmark 1943–2015. (**d**) Age-standardized (European Standard Population, 1976) mortality rates of melanoma in Denmark 1943–2015. (**e**) Age-standardized (European Standard Population, 1976) incidence rates of melanoma in Germany (Saarland) 1970–2016. (**f**) Age-standardized (European Standard Population, 1976) mortality rates of melanoma in Germany (Saarland) 1970–2016

(Fig. 6.2d). In the German Federal State of Saarland, age-standardized mortality rates for men rose from 0.4/100,000/year in 1970 to 3.3/100,000/year in 2016, with peaks around 1990 of 3.6/100,000/year, while mortality rates for women only slightly increased from 1.0/100,000/year to 1.3/100,000/year, with peaks of 2.5/100,000/year in 1989 and of 2.8/100,000/year in 2007 (Fig. 6.2f).

Clinical Epidemiology

Incidence trends of melanoma including clinical and histopathological characteristics are based on data from the Central Malignant Melanoma Registry (CMMR). The CMMR is the largest clinical-based melanoma registry worldwide, which was founded in 1983 by the German Dermatological Society [104, 105].

Over the last four decades, the CMMR developed into a large multicenter project, recording data retro- and prospectively from patients diagnosed with CM in more than 70 dermatological centers in Germany (including data from the former Federal Republic of Germany and the former German Democratic Republic), Austria, and the Switzerland. Between 1983 and 2018, a total of 130,600 cases with CM were registered.

Compared to the 1970s where almost 2/3 of CM patients were women (63.5%), equalization in both sexes (51% women and 49% men) was visible in the 1990s in Germany.

In most countries, incidence rates of CM are similar in men and women. Exceptions, with a higher incidence in men, are observed from several high-risk countries (e.g., Australia, New Zealand, and the US Whites) [99, 106]. Higher rates among women are found in countries with lower CM incidence (e.g., Great Britain) [69, 107].

Anatomic Site
The anatomic site varies according to gender. In men most of the tumors are localized on the trunk, and in women the preferred site is lower extremities (Table 6.3). In men 52% of CM are localized at the trunk, thereof 37% at the back, followed by the lower leg (17%). In women 37% of CM are localized at the lower extremity, with 18% at the lower leg, followed by the trunk (27%). CM localized at the head and neck region are nearly equivalent in both sexes [104, 108].

The site-specific incidence of melanoma varies according to age. The incidence of melanoma localized on the trunk and on the lower extremity decreases in higher ages, whereas a significant increase of melanoma localized in head and neck areas was found in older patients [109, 110]. Nearly 80% of melanoma in age groups of 80 and more years were found in head and neck areas. Melanomas developing at different body sites are associated with distinct patterns of sun exposure. Melanomas of the head and neck are associated with chronic patterns of sun exposure, whereas trunk melanomas are associated with intermittent patterns of sun exposure, supporting the hypothesis that melanomas may arise through divergent causal pathways [110–112].

Histological Subtype
The most frequent histological subtype is superficial spreading melanoma which covers nearly 50% of all CM followed by nodular melanoma (16% of all CM), lentigo maligna melanoma (10% of CM), and acrolentiginous melanoma (4% of CM).

Different age distributions are found for the respective histological subtypes. The peak for superficial spreading melanomas is found in patients of 55 to 59 years, for nodular and acrolentiginous melanomas in patients of 65 to 69 years, and in lentigo maligna melanoma in patients of 70 to 74 years.

Tumor Thickness
The tumor thickness is the most important prognostic factor in primary melanoma [101, 113]. In Germany there is an ongoing trend towards thinner melanoma since the 1980s with stabilization from the mid-1990s onwards [114]. For men, the median tumor thickness decreased from 1.61 mm in 1982 to 0.91 mm in 2018 and in women from 1.44 mm to 0.90 mm, respectively (Fig. 6.3).

Table 6.3 Anatomic sites of CM in the CMMR, according to gender. The median age is given at the time point of diagnosis

Anatomic site	Men		Women	
	%	Median age	%	Median age
Face	8.7%	68	9.7%	71
Scalp	7.0%	67	2.6%	64
Neck	2.7%	60	1.8%	57
Anterior trunk	15.3%	58	8.0%	47
Posterior trunk	36.5%	59	18.6%	51
Genital region	0.2%	63	0.5%	64
Upper extremity	11.6%	61	19.4%	60
Lower extremity	16.5%	56	37.0%	54
Others	1.6%	63	2.4%	66
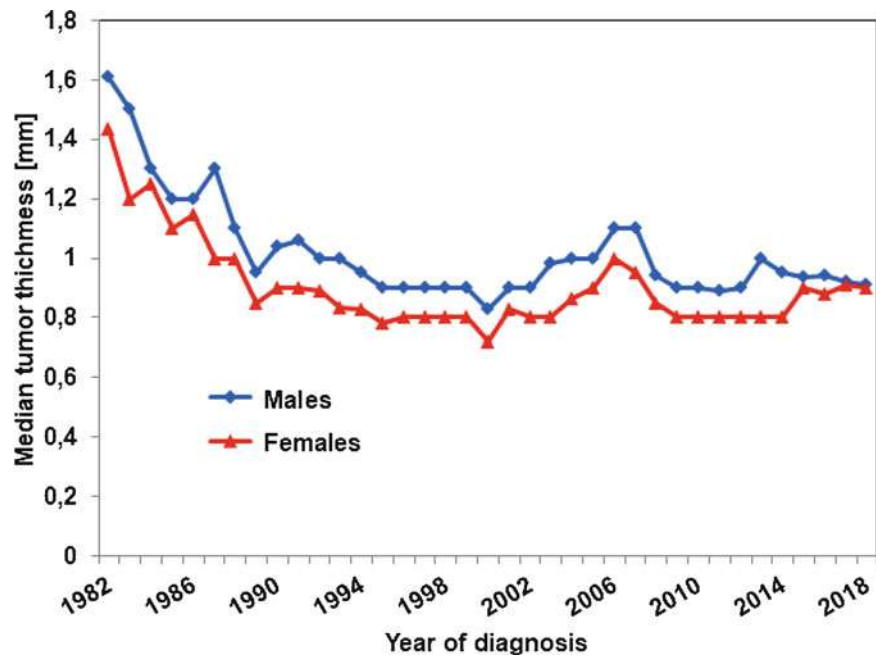
Fig. 6.3 Median tumor thickness of melanoma, recorded in the CMMR (1982–2018), by sex

The tumor thickness at the time point of primary diagnosis is also age dependent. Generally there is a significant decrease of melanoma with a tumor thickness of 1.0 mm or less in higher ages and is less than 50% at the age of 70. In contrast the proportion of thick melanoma increases significantly and reaches 22% at the age of 80 years in both genders.

An analysis of the prognosis of 19,693 patients with primary CM considering tumor thickness was performed based on data recorded by the CMMR since 2000.

In patients with a tumor thickness of 0.8 mm or less, 10-year melanoma-specific survival rates were 97%; for those with a tumor thickness between 0.8 and 1.0 mm, 10-year survival was 90% and decreased to 87% in patients with a tumor thickness of >1.0 to 2.0 mm and to 76% in patients with a tumor thickness of >2.0–4,0 mm. Ten-year survival rates were lowest (58%) in patients with a tumor thickness of more than 4 mm (Fig. 6.4).
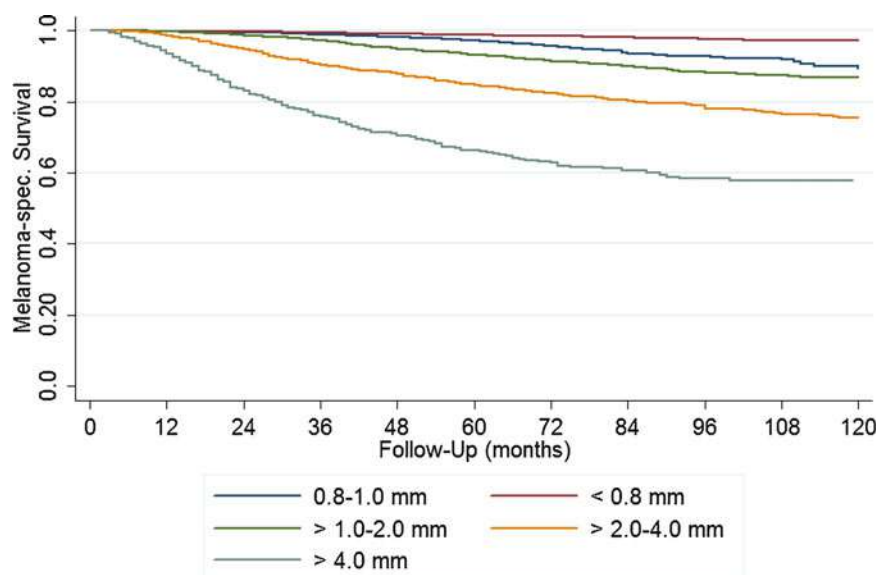
Fig. 6.4 Melanoma-specific survival, according to tumor thickness groups. (AJCC 2017)

Sun Exposure and Melanoma

Population Attributable Fraction: UV Radiation and Melanoma

A series of epidemiological and biological studies have provided sufficient evidence for the causal role of UV exposure in melanoma development [115, 116].

The population attributable fraction (PAF) quantifies the proportion and the numbers of melanoma cases that can be attributed to exposure to UV radiation and that could potentially be avoided by complete elimination of sun exposure. It is helpful in prioritizing melanoma control strategies and for the evaluation of the potential impact of interventions seeking to reduce exposure to UV.

The population attributable fraction is estimated by comparing the observed incidence rates in an "exposed" population with those of a "minimal-exposed" or "low-incidence" reference population (as approximation of an "unexposed" population). The differences in incidence rates are then attributed to corresponding differences in exposure to UV between reference and study population [1, 117].

Population Attributable Fraction: Global Estimates

The proportion of melanoma cases caused by UV exposure varies greatly across different regions, ranging from less than 1% to ≥95%, with the lowest and highest PAF observed in East Asia and Oceania [118, 119]. Most recent estimates for 2012 revealed that around 168,000 cases of melanoma were attributed to excess exposure to UV radiation, representing 75.7% of all melanoma cases worldwide. The burden was higher in men (81.3% attributable cases) than in women (69.4% attributable cases). The vast majority (around 89%, 149,340 of 168,000 cases) of UV-attributable melanoma cases occurred in countries with a very high Human Development Index (HDI), where 86.6% of all melanoma cases (91% among men and 81.4% among women) were due to high UV exposure. This was most pronounced in Australia and New Zealand, where 97.4% of all melanomas in men and 93.4% in women, respectively, were attributable to UV radiation [120]. Similarly high values were estimated for the White US population, with a PAF ranging between 85 and 92% in females and between 94 and 96% in males [1, 121]. Within Europe, the proportion of melanomas attributed to

excess sun exposure shows a great variation. The highest values for the PAF were reported from Northern (90–95%) and Western Europe (86%); lower PAFs were estimated for Eastern (68%) and Southern European countries (78%) [117, 118, 122, 123].

Conclusion

Melanoma and keratinocyte skin cancer (KSC) are now the most common types of cancer in White populations. Both tumor entities show an increasing incidence rate worldwide. The rising incidence rates are predominantly caused by increased exposure to UV radiation. An intensive UV exposure in childhood and adolescence was causative for the development of basal cell carcinoma (BCC), whereas for the etiology of SCC a chronic UV exposure in the earlier decades was accused. Melanoma risk seems to be associated with intermittent and also chronic UV exposure. Although a stabilization of CM incidence rates are observed in younger cohorts in Australia and New Zealand, the impact of primary prevention measures on incidence rates of melanoma is unlikely to be seen in the near future, and rather increasing incidence rates to 40–50/100,000/year should be expected in Europe and in the USA in the next decades.

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Part IV

Epidemiology of Skin Cancer

Solar UV Exposure and Mortality from Skin Tumors: An Update

7

Marianne Berwick and Amy Garcia

Abstract

Solar UV exposure is critical and complex in the etiology and prognosis of skin cancer, particularly cutaneous malignant melanoma. Sun exposure and one of its "derivatives," vitamin D, have been implicated in protection against mortality from melanoma. However, the relationships are inconsistent. At this time, it is not possible to make clear recommendations for or against sun exposure in relationship to melanoma prognosis. However, this relationship deserves continued exploration.

Keywords

Cutaneous melanoma · Nonmelanoma skin cancer · Mortality · UV exposure · Vitamin D

Introduction

Skin Cancer

The three major skin cancers – cutaneous malignant melanoma (CMM), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) – are considered "sun-related" cancers, as detailed by Gordon [18]. Although CMM is considered to be the most lethal of the three major types of skin cancer, SCC can also be quite lethal. A major problem in the assessment of the impact of BCC and SCC is the fact that most, though not all, national tumor registries do not track these skin tumors due to their large numbers and supposed low impact. BCC and SCC are commonly referred to as "nonmelanoma skin cancer" or NMSC. NMSC is the most frequently diagnosed cancer in North America and in Australia and New Zealand. Globocan [7] estimated that there were 1,042,056 new cases of NMSC worldwide in 2018, with 65,155 deaths, or approximately 6% of deaths, attributable to NMSC (mostly SCC). These data are similar to those reported from Spain [37] where overall survival with SCC is 90.1% and with BCC 99.8%. Norway is one of the few countries keeping records for SCC. Robsahm et al. [36] reported that SCC incidence was increasing but that SCC mortality was stable. One group with a high overall mortality of all skin cancer is organ transplant patients [30]. In the United States, skin cancer-specific mortality among organ transplant patients was 35.27 per 100,000 person years. Of the skin cancers in these patients, mortality was greatest for CMM (11.48 per 100,000), followed by SCC (4.94 per 100,000) and others such as Merkel cell carcinoma [17].

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Incidence and Mortality of CMM

Worldwide estimate for the number of CMM diagnosed in 2018 was 287,723, with 60,712 deaths, approximately 21% [7], 3.5 times the rate as NMSC. CMM incidence has increased in developed countries, in places such as Australia, the United States, and Europe. Currently in most developed countries, CMM incidence has generally increased among those with thinner lesions. In the United States, female mortality with CMM has not increased and hovers at 1.9 per 100,000, whereas mortality among males with CMM has continued to increase slightly over time, from 2.88 per 100,000 in 1975 to 4.44 per 100,000, based on 2011–2015 deaths (Fig. 7.1, [28]). The reasons for the differences in male and female rates have been controversial. Some consider behavior to be the determining factor, and others focus on genetic and hormonal factors.

One suggestion to explain the fact that incidence is increasing far faster than mortality is "over-diagnosis," a tendency to diagnose more early lesions than previously [31]. Of course, it is probably best to find lesions early, but it may be that lesions that would not have been called melanoma in the past are now being called melanoma [12]. Over-diagnosis occurs when there is an increase in incidence but little or no corresponding increase in mortality [44]. Over-diagnosis is difficult to prove, but curves for CMM incidence and mortality fit that description. The result is that there is potentially over-diagnosis among the very thin lesions in particular.

Sun Exposure

Behavior and type of sun exposure critically interact in the development and progression of melanoma. Sun exposure is generally divided into three categories: chronic, intermittent, and total.

Chronic Sun Exposure. Chronic sun exposure is defined as a constant or consistently high level of sun exposure. The chronic sun exposure pathway in sun-sensitive people is particularly damaging, although sun-sensitive individuals usually develop melanoma somewhat later in life. This type of exposure has often been measured as occupational exposure.

Intermittent Sun Exposure. Intermittent sun exposure is the type of exposure that indoor workers generally get as they are indoors and relatively sun protected during the week and then outdoors on the weekend. Often this type of exposure is associated with sunbathing, water sports and vacations in sunny places, or simply being outdoors on the weekend.

Timing of Sun Exposure. Many studies indicate that early life sun exposure is critical to the development of melanoma later in life. Our unpublished data, based on 3578 individuals, shows that individuals with high levels of early life sun exposure also have high levels of sun exposure throughout their lifetime, so it is somewhat difficult to differentiate the effects of early life sun exposure from that over the lifetime. It is logical that children might have a stronger association with melanoma risk with excessive sun exposure as their bodies are experiencing rapid cell growth and thus likely to multiply the effects of sun damage more than adults; however, this thesis has not been rigorously tested.

Sun Exposure Measurement. Sun exposure is generally measured by (a) ground-level meter readings, (b) satellite measurement, (c) self-reported outdoor activities, (d) wearable monitors for sun exposure, and (e) a combination of self-reported outdoor activities and satellite measures of ambient ultraviolet radiation (UVR). None of these measures is totally accurate for obtaining an individual's actual sun exposure; each has drawbacks. Ground-level meter readings are geographically distant, so that an individual relatively far from one does not have an accurate measure. Satellite measures are somewhat better but suffer from over-generalization, such that they measure the exposure at a specific spot but do not take into account individual behaviors indoors and outdoors. Self-reported outdoor activities rely on an individual's memory and thus vary considerably over time. Wearable monitors are excellent but can only cover a short time period. Combinations

SEER Observed Incidence, SEER Delay Adjusted Incidence and US Death Rates[a]
Melanoma of the Skin, White, by Sex
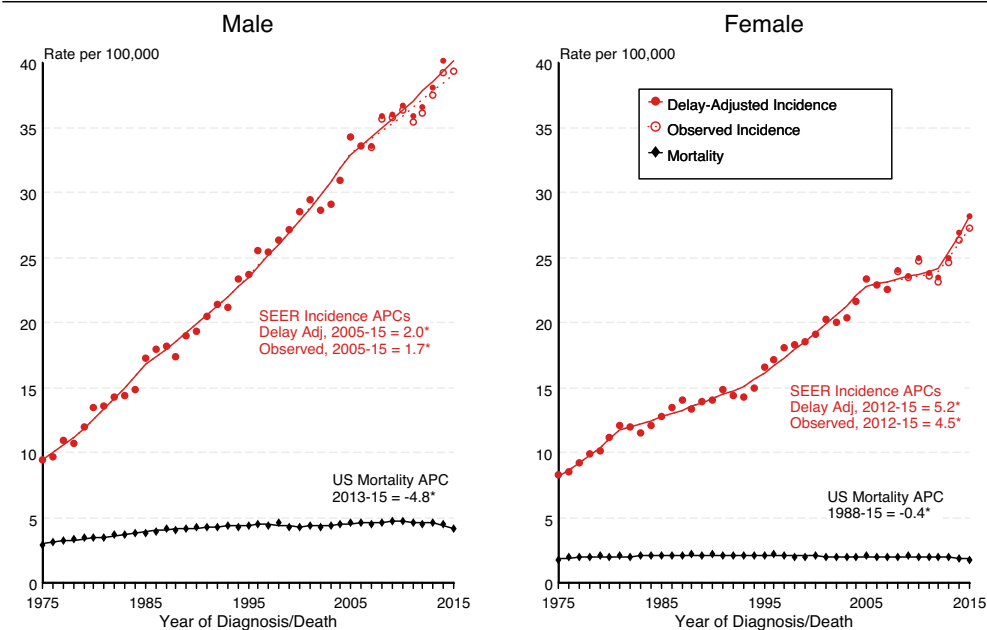
Source: SEER 9 areas and US Mortality Files (National Center for Health Statistics, CDC).
[a]   Rates are age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1103).
      Regression lines and APCs are calculated using the Joinpoint Regression Program Version 4.6, February 2018, National Cancer Institute.
      The APC is the Annual Percent Change for the regression line segments. The APC shown on the graph is for the most recent trend.
*   The APC is significantly different from zero (p < 0.05).

Fig. 7.1 Incidence and mortality for melanoma among different racial/ethnic groups [28]

of various measures suffer from the problems of each measure; however, they are more likely to get at real exposure.

Anatomic Site

The appearance of melanomas on any particular anatomic site is confounded by multiple factors [11]. For example, as more melanomas appear on the legs in females than in males, and as females often wear skirts, many have assumed that they have more exposure, albeit limited, on the legs. However, there is some evidence to suggest that anatomic site may be due to a sex-linked genetic factor or hormonal differences.

Age and Sex Differences. Males are more likely to develop melanoma on the trunk and later in life, while females are more likely to develop melanoma on the legs and earlier in life

[2]; however, females also develop melanoma on the trunk and males on the legs, so this relationship is not rigid.

Mutation Status. There appears to be agreement that *BRAF*-mutated tumors tend to appear in younger individuals on the trunk, whereas *NRAS*-mutated tumors tend to segregate among older individuals on the trunk and upper extremities [40]. As it is quite difficult to sequence primary tumors, there has been little data to date; however, technology is improving and it is likely that more information will be forthcoming.

Survival Difference. Melanomas of the scalp and neck have poorer survival as has been shown many times (e.g., [20]). Those melanomas on the trunk and arms have the next poorer survival, while those on the legs have relatively good survival.

UV and DNA Damage

It has been repeatedly shown that melanomas have more "UV signature mutations" (i.e., C > T or CC > TT transitions) and more total mutations than other cancers in a comparison of the tumor mutations among multiple cancer types from the Cancer Genome Atlas (TCGA) [21]. Strangely, the more mutations, the less "lethal" the melanoma. Perhaps that is due to the development of neo-antigens as the mutations increase. Thus, the tumor is being recognized and combatted by the body's immune system.

Relationships of Sun Exposure and Melanoma Mortality

Studies have found reduced mortality of melanoma located on anatomic areas with increased sun exposure (Table 7.1). Some newer studies demonstrate that melanoma tumors on sun-protected sites have poor survival [19, 35]. However, it is unclear whether that is due to an inability to see those tumors at an early stage or that is due to a different etiology for tumors with poor survival. In addition, different authors describe "sun protected" differently, with some using mucosal and acral lentiginous melanomas as "sun protected" [34] and others using body sites usually covered by clothing, such as the chest, back of the neck, shoulders, and thighs, as "sun protected" or "highly intermittent" [19].

Other studies have evaluated associations of sun exposure and melanoma prognostic factors [16], who found that higher levels of individually reported sun exposure were inversely associated with Breslow thickness and ulceration. In several studies overall survival was associated with reported sun exposure ([6, 23, 46] (supplementary data)). However, in Italy, Fortes et al. [14] found no association between reported sun exposure or estimated UVB levels and melanoma survival, and Lin et al. [22] estimated continuous UVR exposure and found a small association with increased melanoma mortality.

Epidemiologic studies have multiple issues that preclude definitive answers to the role of sun exposure and melanoma survival. However, those studies, such as those by Pozniak et al. [35] and Trucco et al. [42], which evaluate both biological and genetic associations may give insights to this association. Trucco found that patients with UV "signature" mutations had longer disease-free survival and better overall survival independent of stage at diagnosis. This finding was replicated in the TCGA data. Trucco then evaluated the role of UV signature mutations in a BRAF^{V600E} mouse model. While UVA and UVB accelerated melanomagenesis and increased tumor burden, the mice with UV signature mutations had significantly longer survival. Studies such as these provide strong evidence that UV exposure is in fact associated with better survival. These findings need to be evaluated in the context of UV exposure which is also causative for melanoma. Further evaluation is critical to understanding these complex relationships.

Vitamin D and Melanoma Mortality

Numerous case-control, cohort, and ecological studies have shown an inverse relationship between serum vitamin D levels and melanoma mortality (Table 7.1). Timerman et al. [41] found that initial vitamin D deficiency and inadequate repletion confer a worse prognosis. Fang et al. [13] demonstrated that lower vitamin D levels at diagnosis were associated with poorer survival, after adjusting for CRP level. In a small study in Australia, Wyatt et al. [45] reported a strong association between low levels of serum vitamin D and increased Breslow thickness, the major prognostic factor for melanoma, as did Gambichler et al. [15] in Germany. In a large cohort in Leeds, Newton-Bishop et al. [27] demonstrated that lower vitamin D levels at diagnosis were associated with poorer survival. Bade

Table 7.1 Studies addressing sun exposure or vitamin D and melanoma mortality

Author, year	Country of population	Time period	Number followed	Number of deaths or hazard ratio	Comments
Sun exposure and melanoma mortality					
Trucco, 2019	United Kingdom and France	2012–2014	126 primary melanomas, 112 mice and TCGA sun-exposed (n = 372) and non-sun-exposed sites (n = 47)	TCGA: HR = 0.43 (95% CI 025, 0.73) for signature 7; HR 0.53 (0.36, 0.80) for 10 gene set; UK-Fr: HR 0.29 (0.20, 0.84)	Developed a 10-gene recurrent mutation set from the "signature 7" (UV-related) mutation set
Poźniak, 2019	United Kingdom	2001–2003	703	HR 1.64 [95% CI, 1.11–2.41]	Melanoma on sun-protected body sites was associated with worse melanoma-specific survival
Gordon, 2017	Sweden	1976–2003	5973	HR 1.3 [95% CI, 1.1–1.5]	Melanoma on high and moderate UVR-exposed anatomic areas (face, hands, lateral arms, lower legs, dorsum of the feet) was associated with a more favorable prognosis
Gandini, 2016	Italy	2010–2013	2738	Ulcerated melanoma in patients with vacation sun exposure: OR 0.76 [95% CI, 0.61–0.93]	Holidays with sun exposure prior to diagnosis and the number of weeks of vacation with sun exposure were statistically significantly inversely related to Breslow thickness and ulceration
Fortes, 2016	Italy	2001–2003	972	HR 1.02 [95% CI, 0.43–2.41]	No protective effect for UVB or individual sun exposure variables on melanoma mortality.
Berwick, 2014	Australia, Canada, Italy, and the United States	1998–2003	3578	HR 0.27 [95% CI, 0.09–0.85]	This study found only weak evidence that high levels of sun exposure before melanoma diagnosis increased survival. Of the recent sun exposure variables, only one or more sunburns in a year in the decade prior to diagnosis was significantly associated with reduced risk of death from melanoma

Table 7.1 (continued)

Author, year	Country of population	Time period	Number followed	Number of deaths or hazard ratio	Comments
Lindqvist, 2014	Sweden	1990–1992	29,518	MR 2.0 [95% CI, 1.6–2.5]	Data was obtained from the melanoma in southern Sweden cohort. The all-cause mortality rate was twofold higher in those that avoided sun exposure
Lin, 2013	United States	1995–1996	346,615 (417 melanoma)	HR 1.13 [95% CI, 1.02–1.25]	Continuous UVR exposure was associated with increased melanoma mortality
Serum levels of vitamin D and melanoma mortality					
Timerman, 2017	United States	2007–2013	252	HR 1.93 [95% CI, 1.15–3.22]	Retrospective study found 25(OH)D deficiency on presentation and insufficient repletion were associated with worse prognosis in patients with metastatic melanoma
Fang, 2016	United States	1997–2009	3189	HR for overall survival 1.03 per unit decrease of vitamin D [95% CI, 1.01–1.04]	Lower vitamin D levels in melanoma patients were associated with worse outcomes when adjusted for CRP level
Saiag, 2015	France	2003–2008	1171	HR 0.90 [95% CI, 0.82–0.99] for each 20 nmol/L increase in vitamin D levels without adjusting for Breslow thickness	Vitamin D3 levels during follow-up is an independent prognostic marker, but not its level at diagnosis
Wyatt, 2015	Australia	2010–2011	100	OR 3.82 [95% CI, 1.03–14.14]	<50 nmol/L levels of 25(OH)D levels were associated with a nearly fourfold increased risk of greater Breslow thickness
Bade, 2014	Germany	2000–2004	324	None	Primary tumors in patients with low serum levels of 25(OH)D concentrations had significantly higher Breslow thickness. Patients with lower serum vitamin D concentrations had decreased survival

Table 7.1 (continued)

Author, year	Country of population	Time period	Number followed	Number of deaths or hazard ratio	Comments
Newton-Bishop, 2015	United Kingdom	2001–2013	2182	HR 1.79 [95% CI, 1.15–2.78]	Lower vitamin D levels at melanoma diagnosis were associated with thicker primary tumors and worse survival. Vitamin D levels <20 nmol/L were significantly associated with increased risk of melanoma-related death
Gambichler, 2013	Germany	2009–2012	764	Regression coefficient − 1.45 (Breslow) -0.79 (AJCC stage)	Case series that found that lower vitamin D levels were significantly associated with higher Breslow tumor thickness and higher American Joint Committee on Cancer 2002 melanoma stage
Ogbah, 2013	Spain	2004–2008	81	OR 1.1 [95% CI, 0.78–1.54] p = 0.583	Case series that did not find a significant association between vitamin D levels and Breslow thickness
Vitamin D SNPs and melanoma mortality					
Orlow, 2018	Australia, Canada, Italy, and the United States	1998–2003	3336	Each VDR haplotype had variable HRs. The most significant had a HR of 0.67 [95% CI, 0.52–0.88]	Measured sun exposure around the time of diagnosis modifies survival in melanoma patients
Sikora, 2018	Poland		243	None	No correlation was found between VDR genotype and Breslow thickness
Vasilovici, 2018	International	Reviewed studies published from 2000 to 2018	17 studies reviewed	None	Systematic review which concluded that the vitamin D receptor gene is implicated in the pathogenesis and progression of melanoma
Luo, 2017	International		2578	Each SNP had variable HRs. After correction, none reached FDR cut-off of 0.05	No improvement in melanoma prognosis by including vitamin D pathway SNPs into known major prognostic measures (i.e., Breslow thickness, ulceration, etc.)

Table 7.1 (continued)

Author, year	Country of population	Time period	Number followed	Number of deaths or hazard ratio	Comments
Morgese, 2017	Italy	2012–2016	88	Recessive homozygous PIK3CA rs2699887 SNPs showed worse overall survival than dominant or heterozygous genotypes. HR 0.28 [95% CI, 0.02–3.61]	Significant correlation between certain VDR SNPs and longer progression-free survival and disease control rate during treatment with anti-BRAF in patients with melanoma
Orlow, 2016	Australia, Canada, Italy, and the United States	1998–2003	3566	Each VDR haplotype had variable HRs. The most significant had a HR of 1.22 [95% CI, 1.02–1.45)	Researchers found several SNPs mostly located in the coding region for the VDR gene that were associated with melanoma-specific survival. The SNPs were not associated with Breslow thickness, mitosis, or ulceration
Davies, 2014	United Kingdom	2001–2013	3.137	HR 1.22 [95% CI, 1.04–1.43]	Statistically significant increased risk from melanoma death with SNPs associated with lower vitamin D levels
Brozyna, 2014	Poland	2003–2009	69	None	VDR expression was inversely correlated with melanoma progression
Vitamin D supplementation and cancer mortality					
Manson, 2019	United States	2011–2017	25,871	HR 0.83 [95% CI, 0.67–1.02], for death from cancer of any type	Randomized controlled trial of vitamin D administration for the prevention of cancer and cardiovascular disease. Supplementation with vitamin D did not result in lower incidence of invasive cancer

et al. [4] found similar results in Germany. However, in a small study, Ogbah et al. [29] saw no association between vitamin D levels and Breslow thickness. Saiag et al. [38] measured vitamin D levels in patients through follow-up and concluded that it was the change in serum vitamin D levels that was important rather than only vitamin D levels at diagnosis.

Vitamin D Receptors and Melanoma Mortality

Research interest has grown in vitamin D receptor characteristics and melanoma survival (Table 7.1). Brozyna et al. [8] in a small study of 69 found that high VDR expression is associated with reduced melanoma mortality. In

a large international study, Orlow et al. [32] found that VDR SNPs were associated with melanoma survival, and then in 2018 [33], they reported an *interaction* of VDR SNPs with sun exposure that reduced survival in melanoma. However, Luo et al. [24] reported that the addition of VDR genotype to host and clinical factors had no influence on prognosis of melanoma. Sikora et al. [39] in a much smaller study found no association with VDR genotypes and Breslow thickness at diagnosis. This finding is in contrast to a systematic review by Vasilovici et al. [43] demonstrating that the VDR gene is important in progression of melanoma as did Morgese et al. [26]. Underlining this conclusion is the study by Davies et al. [10] demonstrating a small but statistically significantly increased risk for melanoma-specific death related to SNPs associated with lower vitamin D levels.

Vitamin D Supplements and Melanoma Mortality

Despite these promising results, the role of vitamin D in clinical treatment is still not clear. Clinical trials exploring the effects of vitamin D supplementation have been largely inconclusive. In a large randomized controlled trial, Manson et al. [25] concluded that supplemental vitamin D does not lower the incidence of invasive cancer or other chronic diseases. In a meta-analysis, Caini et al. [9] found that vitamin D taken from diet or supplements was not protective against the development of skin cancer. They propose that the active form of vitamin D produced in the skin may serve a different function from the systemic form. Indirect proof for this theory comes from different regulatory mechanisms for vitamin D activated in the kidney and that activated in the skin. While supplementation may not confer protection, serum vitamin D levels at diagnosis have been inversely associated with cutaneous melanoma Breslow thickness and survival, so it is critical to understand the factors leading to this

inverse association. Such factors may be solar UV, or some other factors less obvious.

Conclusion

Findings are inconsistent regarding the role of sun exposure and melanoma mortality. The populations studied are not necessarily representative of the general population, even though some are quite large. At this point it is difficult to separate the role of sun exposure from several other factors: site of sun exposure, serum vitamin D levels, and the genetic signature for sun exposure. For example, many studies demonstrate that melanoma tumors on sun-protected sites have poor survival. However, it is unclear whether that is due to an inability to see those tumors at an early stage or that is due to a different etiology for tumors with poor survival. As pointed out throughout this volume, high or adequate serum vitamin D levels are important and have been associated with better melanoma survival. However, as Autier et al. [3] has pointed out, "This possibility is supported by the observation that of all vitamins and anti-oxidative compounds found in the serum, the 25(OH)D concentration is probably the most sensitive to changes in health status." Thus, perhaps this is a function of what epidemiologists call "reverse causation," so that healthy serum vitamin D levels are a result not a cause of good health. Manson's [25] groundbreaking randomized controlled study demonstrates clearly that vitamin D supplementation will not improve risk for disease. Trucco et al. [42] recently demonstrated in humans that a subset of "signature 7" [1] genes were strongly associated with longer disease-free survival and better overall survival in melanoma. They verified this finding in a melanoma mouse model. Pozniak et al. [35] reporting on gene expression data in 703 patients from a population-based cohort of 2184 patients in Leeds, UK, focused on immune subgroups and the role of tobacco smoking among patients. However, they also controlled

for site of melanoma – sun-exposed vs. non-sun-exposed – which was strongly associated with survival: those with melanomas in non-sun-exposed sites had an increased hazard ratio for melanoma-specific survival (HR 1.64, 95% CI 1.71–2.41). The hazard ratio was increased among each immune subgroup, but only significant overall and among the low immune subgroup, where it was equal in strength to AJCC stage.

Finally, Bataille [5] has called into question the emphasis on sun exposure in melanoma incidence, suggesting more complex etiologies: (1) melanocyte differentiation in embryogenesis may be important for initiation and progression; (2) reduced senescence and increased longevity; (3) body weight and energy expenditure; as well as (4) new gene discoveries. These areas may also be relevant for melanoma survival and should continue to be investigated along with any new leads.

There are multiple strands of evidence supporting different areas of investigation for melanoma mortality. Sun exposure is particularly difficult to measure over a lifetime and after diagnosis of melanoma. Indirect clues lie with histopathologic variables such as solar elastosis, individual reporting of sun exposure over the lifetime, integration of individual reports of sun exposure with ultraviolet radiation flux, serum vitamin D levels, mutations indicating UV exposure, host characteristics such as age and sex, and each of these as interacting with specific host genetic factors. What is most critical is that investigators continue to look for a variety of causes for mortality from melanoma and the natural history of melanoma leading to melanoma-specific mortality. Only by casting a wide net and continuing to investigate hypotheses – new and old – will we be able to improve survival from melanoma.

While sun exposure appears to play a role in lower melanoma mortality, it is unclear how. It is becoming more evident that oral vitamin D supplementation will not improve survival, even as it may increase serum vitamin D levels. At this time, there is no clear recommendation for the role of sun exposure in melanoma mortality. Caution is always the best route.

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Solarium Use and Risk for Malignant Melanoma: Many Open Questions, Not the Time to Close the Debate

8

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Abstract

To shed further light on the ongoing debate whether sunbed use may increase melanoma risk, we have critically assessed the scientific literature that is at present available, focussing on a meta-analysis that we published recently. Our literature search identified several meta-analyses that report a weak association for ever-exposure to UV radiation from a solarium with melanoma risk. However, the quality of studies included in these meta-analyses and the resulting evidence levels and grades of recommendation were very low due to the lack of interventional trials and because of severe limitations of many of the observational studies. The results of cohort and case–control studies published until today do not prove causality, not even by the Hill criteria. The overall quality of these observational studies and the resulting evidence levels are low due to severe limitations (including unobserved or unrecorded confounding), which leads to bias. It must be recognized that in the majority of studies, published to date, many of the confounding factors, including sun exposure, sunburns and skin type, have not been adequately and systematically recorded and adjusted for. We conclude that the many limitations of the individual studies and the resulting low levels of evidence and grades of recommendation do at present not allow postulation of a causal relationship between solarium use and melanoma risk. At present, there is no convincing evidence that moderate/responsible solarium use increases melanoma risk.

Introduction

For the development of life on earth and for human evolution, sunlight represents an important pre-requisite [1, 2]. Of particular importance

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is the ultraviolet (UV) range (UV-C: 200–280 nm; UV-B: 280–315 nm; UV-A: 315–400 nm) of solar radiation, because exposure to solar or artificial UV exerts both positive and negative effects on human health [1–59]. While some of the beneficial UV effects are due to the UV-B-mediated cutaneous synthesis of vitamin D [1–5], hazardous effects include the initiation and promotion of skin photocarcinogenesis [5]. The relevance of the UV-B spectrum in promoting non-melanoma skin cancer (most importantly basal and squamous cell carcinomas; risk factor: high cumulative UV exposure, via, e.g. induction of DNA mutations) and melanoma (risk factor: high intermittent UV exposure, e.g. UV burns, most importantly in childhood) is generally accepted. Besides other effects that include immunomodulation, UV-B radiation is thought to lead in target cells to direct DNA damage through cyclobutane pyrimidine dimer formation and also the production of DNA damaging photoproducts. Recent animal and laboratory studies have indicated a possible additional contribution of the UV-A spectrum to skin photocarcinogenesis [5, 81]. It was demonstrated that UV-A radiation produces reactive oxygen species, thereby causing indirect DNA damage [5, 81]. Malignant melanoma, which develops from uncontrolled proliferation of pigment-producing cells (melanocytes), represents the most aggressive form of skin cancer. Cutaneous melanoma is the 12th most common cancer worldwide, with an estimated age-standardized incidence rate of 3.0 per 100,000 [7]. In contrast to melanoma death rates, which had more than doubled in light-skinned populations between 1955 and 1985, melanoma mortality rates were decreasing from 1985 to 1990 in Australia, the United States and in many European countries [7].

While the most common form of melanoma is cutaneous, it can also arise in the uveal tract, mucosal surfaces and leptomeninges. At present, the pathogenesis of malignant melanoma is far from being completely understood. It has been reported that genetic, epigenetic and environmental factors are of importance for the development of malignant melanoma. One factor that is always mentioned as an important contributor to the development of malignant melanoma is the exposure to ultraviolet radiation (UV-light) either from the sun or from artificial sources, such as tanning devices (e.g. named sunbed, sunlamp, tanning bed, solarium). Since the 1980s, solarium use has become common in Western and Northern Europe, Canada and the United States [8]. Since 2000, it has become common even in sun-rich countries such as Australia [8]. Modern tanning devices produce mostly UV-A radiation; less than 5% of the radiation that they emit is UV-B radiation; UV-C radiation is not being produced [8, 9].

In 2009 and 2012, the International Agency for Research on Cancer (IARC) classified UV-emitting tanning devices as the highest category of carcinogen (Group 1) [6, 9.83]. Based on this evaluation, the World Health Organization recommended to avoid UV-emitting tanning devices.

This decision was based on several observational studies, either cohort or case–control studies, which had been systematically reviewed in subsequent meta-analyses [17–59].

However, the above-mentioned studies had not only been criticized for limitations, unbalanced view and errors, but also because of the study design, the results can show associations but not prove causality [6, 64–68]. Randomized interventional clinical trials had not been performed.

In this chapter, we summarize our present scientific knowledge between solarium use and melanoma risk, focussing on a recent meta-analysis that we published in 2018 [6].

The Association Between Solarium Use and Melanoma Risk: Major Findings Focussing on a Recent Meta-analysis

Study Characteristics of a Recent Meta-analysis (Table 8.1)

Regarding the association between ever-exposure to UV radiation from a solarium and melanoma risk. Most studies included in a recent meta-analysis were conducted in Europe (64.5%), followed by North America (29.0%) and Australia (6.5%). Samples were mainly recruited before 2000 (80.0%), and differed in age and gender distributions. Overall, the included studies comprised 11,706 malignant melanoma cases and 93,236 controls.

Table 8.1 Characteristics of studies ($n = 31$) included in a recent meta-analysis (Ref [19–49]; modified from Ref [6])

Study (reference number)	Design	Recruitment period	Matching	Gender (f/m %)	Age (range in years)	Ethnicity	Place of recruitment
Adam et al. [19]	CC	1971–1976	FM	100%/0%	15–49	Caucasian	GBR
Autier et al. [20]	CC	1991–n/s	FM	n/s	20–n/s	Caucasian	GER, FRA, BEL
Bataille et al. [21]	CC	1989–1993	FM	60.3%/39.7%	16–75	n/s	GBR
Bataille et al. [22]	CC	1998–2001	FM	64.5%/35.5%	18–49	Caucasian	BEL, NLD, FRA, SWE, GBR
Chen et al. [23]	CC	1987–1989	FM	n/s	n/s	Caucasian	USA
Clough-Gorr et al. [24]	CC	1995–1998	FM	48.1%/51.9%	20–69	n/s	USA
Cust et al. [25]	CC	2000–2002	FM	60.1%/39.9%	18–39	Caucasian	AUS
Dunn-Lane et al. [26]	CC	1985–1986	FM	71.0%/29.0%	15–82	n/s	IRL
Elliott et al. [27]	CC	2000–2005	FM	59.6%/40.4%	17–76	n/s	GBR
Elwood et al. [28]	CC	1981–1984	IM	70.0%/30.0%	18–82	n/s	GBR
Farley et al. [29]	CC	2001–2013	NM	56.5%/43.5%	18–50	n/s	USA
Fears et al. [30]	CC	1991–1992	FM	n/s	20–79	Caucasian	USA
Garbe et al. [31]	CC	1983–1990	NM	n/s	n/s	n/s	AUT, GER, CHE
Han et al. [32]	NCC	1989–2000	IM	100%/0%	43–68	Caucasian	USA
Holly et al. [33]	CC	1981–1986	FM	100%/0%	25–59	Caucasian	USA
Holman et al. [34]	CC	1980–1981	FM	n/s	n/s	n/s	AUS
Kaskel et al. [35]	CC	1997–1999	NM	50.5%/49.5%	19–90	Caucasian	GER
Landi et al. [36]	CC	1994–1999	FM	51.4%/48.6%	17–77	Caucasian	ITA
Lazovich et al. [37]	CC	2004–2007	FM	59.7%/40.3%	25–59	Caucasian	USA
MacKie et al. [38]	CC	1987	IM	64.6%/35.4%	11–n/s	n/s	GBR
Naldi et al. [39]	CC	1992–1995	NM	n/s	n/s	n/s	ITA
Nielsen et al. [40]	CO	1990–1992	n/a	100%/0%	25–64	n/s	SWE
Østerlind et al. [41]	CC	1982–1985	FM	n/s	20–79	n/s	DNK
Swerdlow et al. [42]	CC	1979–1984	FM	n/s	15–84	n/s	GBR
Ting et al. [43]	CC	n/s	n/s	61.2%/38.8%	n/s	Caucasian	USA
Veierød et al. [44]	CO	1991–1992	n/a	100%/0%	30–50	n/s	NOR, SWE
Walter et al. [45]	CC	1984–1986	FM	53.0%/47.0%	20–69	n/s	CAN
Westerdahl et al. [46]	CC	1988–1990	IM	51.4%/48.6%	15–75	n/s	SWE
Westerdahl et al. [47]	CC	1995–1997	IM	n/s	16–80	n/s	SWE

(continued)

Table 8.1 (continued)

Study (reference number)	Design	Recruitment period	Matching	Gender (f/m %)	Age (range in years)	Ethnicity	Place of recruitment
Wolf et al. [48]	CC	1993–1994	NM	57.6%/42.4%	15–83	n/s	AUT
Zanetti et al. [49]	CC	1984–1986	NM	54.5%/45.5%	17–92	n/s	ITA

Rounding errors may occur in data table. Gender proportions are approximated for total sample sizes, and may differ from original data

AUS Australia, *AUT* Austria, *BEL* Belgium, *CAN* Canada, *CC* case–control study, *CHE* Switzerland, *CO* cohort study, *DNK* Denmark, *f* female, *FM* frequency matching, *FRA* France, *GER* Germany, *GBR* United Kingdom of Great Britain and Northern Ireland, *HRV* Croatia, *IM* individual matching, *IRL* Ireland, *ITA* Italy, *m* male, *n/a* not applicable, *NCC* nested case–control study, *NLD* The Netherlands, *NM* no matching, *NOR* Norway, *n/s* not stated, *SWE* Sweden, *USA* United States of America

Assessment of Study Quality, Level of Evidence and Grade of Recommendation

The overall quality of studies included in this meta-analysis and the resulting evidence levels were low due to the lack of interventional trials and severe limitations (including unobserved or unrecorded confounding) of many of the observational studies, which might cause a high risk of bias [6]. It has to be emphasized that the results of these cohort and case–control studies published to date represent associations and do not prove causality. Remarkably, in all the studies included in this meta-analysis, risk of bias resulted most likely in an overestimation of melanoma risk. Scores on the modified Newcastle-Ottawa Quality Assessment Scale were on average low, as 67.7% of the 31 included cohort and case–control studies scored less than four stars [6]. Assessing all individual studies according to the recommendations of the Oxford Centre for Evidence-Based Medicine, the association of ever-exposure, first exposure at younger age and high/low exposure to UV radiation from a solarium with melanoma risk was defined as level four of evidence (poor quality cohort and case-control studies) and grade C of recommendation [6]. Only a minority of studies included in this meta-analysis reported risk estimates (odds ratios, ORs) that were adjusted for the same confounding factors (Table 8.2). As many as 35.5% ($n = 11$) of all the included studies did not account for a single confounder. The remaining studies ($n = 20$) adjusted mainly for age ($n = 15$), sex ($n = 11$) and skin colour ($n = 11$). Fewer studies adjusted for hair colour ($n = 10$), sun exposure ($n = 8$), sunburns ($n = 8$), family history of melanoma ($n = 7$), naevi ($n = 7$), freckles ($n = 5$) and education ($n = 5$). Moreover, individual confounders were assessed across the included studies differently, and were only partly comparable. Overall, a relatively high heterogeneity across the included studies (e.g. ever-exposure: $I^2 = 76.89\%$) was observed, and therefore the authors performed a random-effects meta-analysis.

Association Between Ever-Exposure to UV Radiation from a Solarium and Melanoma Risk

The summary risk estimate of this random-effects meta-analysis showed for all studies (cohort and case–control studies combined) a statistically significant weak association for ever-exposure to UV radiation from a solarium with melanoma risk compared with non-exposure (relative risk [RR] = 1.19; 95% CI = 1.05-1.34; Q [30] = 114.33; $p < 0.001$; $I^2 = 74.55\%$) (Tables 8.2 and 8.3). Exclusion of the study by Nielsen et al. [40], which reported an HR instead of an OR, altered results only slightly (Table 8.3; OR = 1.19; 95% CI = 1.04-1.35; Q [29] = 114.33; $p < 0.001$; $I^2 = 75.98\%$). The funnel plot did not show evidence of publication bias (Egger's test; $p=0.169$).

Table 8.2 Risk estimates for case–control and cohort studies ($n = 31$) included in a recent meta-analysis (Ref [19–49]; modified from Ref [6])

Study	Sample size (n)		Ever exposure vs non-exposure				
	Cases	Controls	Cases	Controls	Crude OR (95% CI)	Adjusted OR/HR (95% CI)	Adjustment
Adam et al. [19]	111	342	9/102	11/331	2.66 (1.07–6.59)[a]	n/s	n/a
Autier et al. [20]	420	447	110/310	120/327	0.97 (0.72–1.31)[b]	n/s	n/a
Bataille et al. [21]	413	416	95/314	106/306	0.87 (0.64–1.20)[a]	1.19 (0.84–1.68)	a,p
Bataille et al. [22]	597	622	315/282	354/268	0.85 (0.67–1.06)[b]	0.90 (0.71–1.14)	a,p,q
Chen et al. [23]	624	512	141/483	95/417	1.28 (0.96–1.71)[a]	1.13 (0.82–1.54)	a,p,q,s
Clough-Gorr et al. [24]	423	678	267/156	460/218	0.81 (0.63–1.05)[a]	1.22 (0.83–1.80)	a,e,f,g,p,s,t
Cust et al. [25]	604	479	137/467	84/395	1.38 (1.02–1.87)[a]	1.41 (1.01–1.96)	a,c,e,l,p,q,s,t
Dunn-Lane et al. [26]	100	100	17/83	15/85	1.16 (0.54–2.48)[a]	n/s	n/a
Elliott et al. [27]	959	513	441/414	225/258	1.22 (0.98–1.53)[a]	1.06 (0.83–1.36)	a,c,e,p,s,t
Elwood et al. [28]	83	83	15/68	12/71	1.31 (0.57–2.99)[a]	n/s	n/a
Farley et al. [29]	265	195	140/125	70/125	2.00 (1.37–2.92)[a]	n/s	n/a
Fears et al. [30]	718	945	188/530	282/662	0.83 (0.67–1.03)[a]	n/s	n/a
Garbe et al. [31]	856	705	66/790	50/655	1.09 (0.75–1.60)[a]	1.5 (0.9–2.4)	a,g,k,l,q
Han et al. [32]	200	804	42/140	87/625	2.16 (1.43–3.25)[a]	2.06 (1.30–3.26)	a,e,o,s,r,v
Holly et al. [33]	452	930	n/s	n/s	0.94 (0.74–1.20)[b]	n/s	n/a
Holman et al. [34]	511	511	n/s	n/s	1.1 (0.6–1.8)[b]	n/s	n/a
Kaskel et al. [35]	291	329	6/285	21/308	0.31 (0.12–0.78)[a]	n/s	n/a
Landi et al. [36]	183	179	32/150	38/141	0.79 (0.47–1.34)[a]	1.3 (0.7–2.4)	a,d,m,n,p,q
Lazovich et al. [37]	1167	1101	734/433	563/538	1.62 (1.37–1.92)[a]	1.74 (1.42–2.14)	a,c,d,e,f,g,h,j,p,q,r,s,u
MacKie et al. [38]	280	280	33/247	8/272	4.54 (2.06–10.02)[a]	1.22 (0.54–2.73)	f,k,o,q,r
Naldi et al. [39]	542	538	30/512	36/502	0.82 (0.50–1.35)[a]	0.78 (0.45–1.37)	a,c,d,f,g,i,k,p,q,r,x
Nielsen et al. [40]	206	29,314	n/s	n/s	n/s	1.17 (0.79–1.72)	b,e,f,g,k,r,u,w,x
Østerlind et al. [41]	474	926	66/408	167/759	0.74 (0.54–1.00)[a]	n/s	n/a
Swerdlow et al. [42]	180	197	38/142	10/110	2.94 (1.40–6.17)[a]	2.94 (1.4–6.4)	d,g,k,q,s

Table 8.2 (continued)

| Study | Sample size (n) | | Ever exposure vs non-exposure | | | | |
	Cases	Controls	Cases	Controls	Crude OR (95% CI)	Adjusted OR/HR (95% CI)	Adjustment
Ting et al. [43]	79	1439	34/45	453/986	1.64 (1.04–2.60)[a]	n/s	n/a
Veierød et al. [44]	412	105,954	178/137	40873/37854	1.20 (0.96–1.50)[a]	1.31 (1.03–1.66)	a,g,o,q,s
Walter et al. [45]	583	608	152/431	109/498	1.61 (1.22–2.13)[a]	1.54 (1.16–2.05)	a,p,t
Westerdahl et al. [46]	400	640	115/282	155/479	1.26 (0.95–1.67)[a]	1.3 (0.9–1.8)	e,g,k,r,x
Westerdahl et al. [47]	571	913	250/319	372/538	1.13 (0.92–1.40)[a]	1.2 (0.9–1.6)	g,k,q,r
Wolf et al. [48]	193	319	11/181	16/300	1.14 (0.52–2.51)[a]	1.34 (0.58–3.07)	a,p
Zanetti et al. [49]	208	416	15/193	21/395	1.46 (0.74–2.90)[a]	0.9 (0.4–2.0)	a,c,g,r,t

n/a Not applicable, *n/s* not stated, *a* age, *b* blisters, *c* education, *d* eye color, *e* family history of melanoma, *f* freckles, *g* hair color, *h* income, *i* marital status, *j* moles, *k* naevi, *l* place of recruitment, *m* presence of DN, *n* propensity to tan, *o* region of residence, *p* sex, *q* skin colour, *r* sunburns, *s* sun exposure, *t* sun sensitivity, *u* sunscreen use, *v* susceptibility, *w* ulcers, *x* vacations
[a]Calculated from contingency table
[b]Obtained from publication. Rounding error may occur in data table. Number of cases and controls from risk estimations may differ from total sample sizes due to missing data. Adjusted risk estimates (with max. number of confounders) were obtained from original articles

Sensitivity Analyses Yielded Results Inconsistent with Main Finding

In this meta-analysis [6], subgroup analyses did not show statistically significant associations when separating for geographic region (studies performed in Europe, Table 8.3; OR = 1.10; 95% CI 0.95-1.27; Q [18] = 49.39; $p < 0.001$; $I^2 = 60.15\%$), risk of bias (studies with low risk of bias, Table 8.4; OR = 1.15; 95% CI = 0.94-1.41; Q [10] = 29.63; $p = 0.001$; $I^2 = 66.30\%$) and trends over time (studies conducted after 1990, Table 8.4; OR = 1.09; 95% CI = 0.93−1.29; Q [15] = 72.97; $p < 0.001$; $I^2 = 79.51\%$). According to the Oxford Centre for (64) Evidence-Based Medicine, for the outcome 'ever-exposure to UV radiation from a solarium', an evidence level of 3a− (systematic review of poor quality cohort and case–control studies) and a grade D of recommendation were determined in this meta-analysis [6].

Association of First Exposure to UV Radiation from a Solarium at Young Age with Melanoma Risk

Thirteen studies included in this meta-analysis [6] investigated a possible association between age at first use of a solarium and melanoma risk. However, only four studies reported a risk estimate for the same age threshold (<25 years). For consistency, a meta-analysis was solely performed with these four studies. The summary risk estimate indicated a statistically significant moderate association between first exposure to UV radiation from a solarium before age 25 years and melanoma risk (Table 8.3; OR = 1.59; 95% CI = 1.38-1.83; Q [3] = 1.06; $p = 0.787$; $I^2 = 0.00\%$). According to the Oxford Centre for Evidence-Based Medicine, [64] for the outcome first 'exposure to UV radiation from a solarium at young age,' an evidence level of 3a− (systematic review of poor quality cohort and case–control studies) and a grade D of recommendation were determined in this meta-analysis [6].

Table 8.3 Summary risk estimates from random-effects meta-analyses reported recently (Ref [19–49] modified from Ref [6])

	No. of studies	No. of participants	No. of cases	Crude OR (95%CI)	I^2	Adjusted OR (95%CI)	I^2
	Ever exposure vs non-exposure						
Overall	30	104,942	11,706	1.19 (1.04–1.35)	75.98%	1.21 (1.08–1.36)	62.47%
Study design							
Case–control studies	29	25,900	11,391	1.19 (1.04–1.36)	76.84%	1.21 (1.07–1.36)	63.25%
Geographic region							
America	9	10,229	4041	1.32 (1.05–1.66)	84.24%	1.35 (1.10–1.67)	76.71%
Australia	2	1083	604	1.30 (1.00–1.69)	0.00%	1.31 (0.98–1.74)	0.00%
Europe	19	93,630	7061	1.10 (0.95–1.27)	60.15%	1.11 (0.98–1.25)	34.60%
Recruitment period							
≤1990	13	8621	3896	1.33 (1.07–1.66)	69.35%	1.21 (1.01–1.45)	49.20%
≥1991	16	94,803	7731	1.09 (0.93–1.29)	79.51%	1.19 (1.02–1.38)	69.60%
1991–1999	11	88,435	4243	0.98 (0.82–1.17)	66.67%	1.11 (0.94–1.31)	51.41%
≥2000	5	6368	3488	1.34 (1.05–1.71)	79.95%	1.34 (1.03–1.74)	78.83%
Risk of bias							
Low (MNOS A4)	11	85,219	2385	1.15 (0.94–1.41)	66.30%	1.19 (0.98–1.43)	51.76%
High (MNOS B4)	19	19,723	9321	1.21 (1.02–1.43)	79.66%	1.22 (1.06–1.41)	66.09%
	High exposure vs. non-exposure						
Overall	7	7691	3944	1.43 (1.17–1.74)	60.87%	1.39 (1.08–1.80)	67.45%
	Low exposure vs. non-exposure						
Overall	7	6995	3451	1.13 (0.93–1.38)	58.51%	1.13 (0.92–1.39)	56.49%
	First exposure at young age vs. non-exposure						
Overall	4	4602	2537	1.59 (1.38–1.83)	0.00%	1.52 (1.23–1.89)	38.06%

Rounding errors may occur in data table. Total numbers of participants and cases are based on crude risk estimations and may differ for adjusted risk estimations. Summary-adjusted risk estimates are based on estimates adjusted for the maximum number of covariates (crude risk estimates were used for studies without adjustment). The study of Ting et al. [43] was excluded from subgroup analyses regarding the year of recruitment due to missing information. High and low exposure to UV radiation from a solarium were defined as >10 and ≤10 sessions in lifetime, respectively. First exposure to UV radiation from a solarium at young age refers to exposure before age 25 years

Association of High/Low Exposure to UV Radiation from a Solarium with Melanoma Risk

Several studies (n = 15) included in this meta-analysis [6] determined possible dose–response relationships between exposure to UV radiation from a solarium and melanoma risk. Seven out of these studies used a consistent definition (>10 sessions in lifetime) and were thus appropriate for meta-analysis. The pooled result of this analysis indicated a statistically significant moderate

association for high exposure to UV radiation from a solarium with melanoma risk (Table 8.4; OR = 1.43; 95% CI = 1.17-1.74; Q [6] = 19.32; p = 0.004; I^2 = 60.87%). However, most of the pooled studies (85.7%) had a high risk of bias. A meta-analysis with the same seven studies was performed for low exposure to UV radiation from a solarium (defined as ≤10 sessions in lifetime) and did not show a statistically significant association (Table 8.3; OR = 1.13; 95% CI = 0.93-1.38; Q [6] = 17.06; p = 0.009; I^2 = 58.51%). According to the Oxford Centre for Evidence-Based Medicine [64], for the outcome 'high and low exposure to UV radiation from a solarium and melanoma risk', an evidence level of 3a− (systematic review of poor quality cohort and case–control studies) and a grade D of recommendation were determined in this meta-analysis [6].

Discussion

Several meta-analyses and reviews have investigated the relevance of solarium use as a potential melanoma risk factor. However, many of them have been criticized for limitations, unbalanced view and errors [6, 11, 17, 65, 66]. As Colantonio et al. point out, a comparison of five previously published systematic reviews on this topic reveals an alarming tendency for copying data without referencing the original article, and obviously without checking for errors [11]. For example, the widely recognized report that the IARC Working Group published in 2007 [10] was criticized for numerous errors in content and typography (e.g. giving wrong numbers for controls in studies published in 1989 by MacKie et al. [38] [180 instead of 280] and in 1981 by Adam et al. [19] [207 instead of 507]), which are also found in two subsequent reviews [11]. Moreover, the numbers of participants from several included studies published in the IARC review could not be derived by us and others [6, 11] from the original articles. A recent meta-analysis investigated the quality of individual studies using a modified Newcastle-Ottawa quality assessment scale and a generally accepted

grading system for recommendations in evidence-based medicine [6, 60–64]. The overall evidence level and quality of studies included in this meta-analysis [19–50] were very low due to the lack of interventional trials and because of severe limitations of many of the observational studies. In this meta-analysis of all cohort and case–control studies identified by a literature search, a weak association for ever-exposure to UV radiation from a solarium with melanoma risk was found. The meta-analysis of Boniol et al. [8], which included 27 studies, reported in 2012 an overall relative risk of 1.20 (95% CI = 1.08-1.34) for the association of ever-exposure to UV radiation from a solarium with melanoma risk (heterogeneity: I^2 = 56%). Boniol et al. [8] also estimated that 3,438 (5.4%) of 63,942 new cases of cutaneous malignant melanoma diagnosed each year in the 15 countries that were members of the European Community and the three countries that were part of the European Free Trade Association were related to solarium use [8]. In another investigation, Wehner et al. estimated the population proportional attributable risk of 2.6–9.4% for melanoma, corresponding to more than 10,000 melanoma cases [12] each year attributable to sunbed use in the United States, Europe and Australia. Colantonio et al. reported in their meta-analysis of 31 studies (which included data of 14,956 melanoma cases and 233,106 controls) a summary OR of 1.16 (95% CI = 1.05-1.28) for the association of ever-use of a solarium with melanoma risk [11]. While the overall OR of the studies of Burgard et al. [6] (OR = 1.19; 95% CI = 1.04-1.35, p = 0.009, Ref), Boniol et al. [8] and Colantonio et al. [11] are comparable, the authors disagree in their conclusions. In the view of Burgard et al. (Ref [6]), Boniol et al. [8] and Colantonio et al. [11] did not adequately consider the many limitations of the individual studies and the resulting low levels of evidence and grades of recommendation that do not allow postulation of a causal relationship between sunbed use and melanoma risk. Moreover, the attempts of Boniol et al. [8] and others [12] to attribute melanoma cases to solarium use have been criticized as being speculative and scientifically not sufficiently supported [6].

The meta-analysis of Burgard et al. [6] indicated a moderate association of first exposure at younger age and high exposure to UV radiation from a sunbed with melanoma risk. However, it has to be noted that these results should be interpreted with caution. It was reported that all cohort and case–control studies included in this meta-analysis [19–50] are likely to have overestimated the association of sunbed use with melanoma risk in the general population because of many independent reasons, including (i) selection bias (exclusion of individuals with a likely relatively high UV-exposure in the past [e.g. history of any kind of skin cancer or dermatological conditions] in controls, but not in cases), (ii) information bias (e.g. recall bias, the inclusion of non-sunbed exposure to artificial UV, e.g. phototherapy), (iii) difficulties in appropriately considering or adjusting for other confounding factors (e.g. solar UV or lifestyle, including smoking) and (iv) the restriction of the analysis to a subgroup of the general population, which may have an increased risk for melanoma (e.g. women).

Like others [65], the study of Burgard et al. [6] could not confirm the emphasis of the IARC report [10] and of the report by Boniol et al. [8] on an increased melanoma risk with first use of indoor tanning in younger age. It should be mentioned that both the IARC report [10] and the report by Boniol et al. [8] have to be criticized for defining first use in younger age as first use before the age of 36 years, but included studies that consider first use prior to ages 25–30 years [rev. in 6]. Moreover, some studies restricted their investigation to melanoma cases diagnosed before the age of 36 years [rev. in 6]. However, this could have resulted in the exclusion of older cases and controls that may have been exposed at a younger age, as outlined previously [6].

The obvious difficulties in most studies in considering or adjusting for important confounders have to be emphasized. Interestingly, subgroup analyses for studies performed in Europe, studies with low risk of bias and studies with recruitment in 1991–1999 showed in a recent analysis [6] no association of melanoma risk with solarium use.

Concerning the finding by Burgard et al. [6] of no significant statistical association between ever-exposure to UV radiation from a sunbed and melanoma risk in studies performed in Europe (in contrast to studies performed in the United States), it is of particular relevance that (1) the role of solar UV exposure represents a major confounding factor which is difficult to control or to adjust for and which may well, at least in part, explain latitude-dependent variations in melanoma risk, and (2) other region-specific factors, which include technical differences in solarium devices, must also be taken into account. Since 2008, sunbed devices in Europe and Oceania (Australia and New Zealand) are restricted in intensity to an ultraviolet index of 12 and 36 (which was 60 before 2002), respectively. In contrast, the intensity of a sunbed in the United States is unlimited (however, often a 'maximum recommended exposure time' is given).

Because many factors that may influence the association of sunbed use and melanoma risk, including legal regulations, solarium technology and epidemiology of solarium use, which are subject to frequent change, it is of particular interest to evaluate trends over time. Another interesting observation of sensitivity and subgroup analyses performed by Burgard et al. [6] was the finding that recruitment period had a strong impact on the association of melanoma risk with solarium use. For recruitment before 1991, a higher OR was found as compared with recruitment from 1991 to 1999 or since 2000. It can be speculated that this observation is due to changes in operation and technical modifications of UV-emitting devices (approximately two decades ago, the sunbed industry started to produce devices with higher pressure bulbs emitting larger doses of long-wave UV A). It has to be noted that the results of the meta-analysis by Burgard et al. [6] and previous published studies most likely overestimate the association of melanoma risk with current sunbed use as many countries have recently imposed strict regulations on solarium use that, besides other effects, should reduce first use at younger age and high use of a sunbed. However, the questions whether stricter regulations of recent years and technical progress

have further improved the safety of solarium use are difficult to answer because in many studies, sunbed use is not clearly restricted to distinct time periods of interest.

It has to be emphasized that interventional trials on this topic are lacking and that the results of the cohort and case–control studies published to date represent associations that do not prove causality. Moreover, both the resulting level of evidence and grade of recommendation of studies investigating the association of melanoma risk with sunbed use are weak. Applying recommendations of the Oxford Centre for Evidence-Based Medicine [64], for all outcomes analysed in a recent meta-analysis, a resulting level 3a− of evidence (poor quality cohort and case-control studies) and grade D of recommendation were determined. The poor quality of the cohort and case-control studies included in this meta-analysis is due to severe limitations, which include difficulties in appropriately considering and controlling for known confounders (e.g. exposure to solar UV or artificial UV for medical purposes; lifestyle, including smoking).

It must be recognized that in the majority of studies, published to date, many of the confounding factors, including sun exposure, sunburns and skin type, have not been adequately and systematically recorded and adjusted for [rev. in 6, 65]. As pointed out in a previous meta-analysis [6], only a minority of the studies published so far reported odds ratios (ORs) adjusted for the same confounding factors. As many as 35.5% ($n =$11) of all ($n = 31$) studies included in this meta-analysis [3] did not account for a single confounder. The remaining studies ($n = 20$) adjusted mainly for age ($n = 15$), sex ($n = 11$) and skin colour ($n = 11$). Fewer studies adjusted for hair colour ($n = 10$), sun exposure ($n = 8$), sunburns ($n = 8$), family history of melanoma ($n = 7$), naevi ($n = 7$), freckles ($n = 5$) and education ($n = 5$). Moreover, individual confounders were assessed differently across the studies included in this meta-analysis [6] and were only partly comparable.

In this context, it must be emphasized that risk estimates (e.g. odds ratios, OR) as given in the meta-analyses published until today, including,

Burgard et al. [6], Boniol et al. [8] and Colantonio et al. [11], could well be affected by the issues of lack of standardization in terms of confounding factors for sunbed studies [6] and could well be obtained through the scenario indicated before [6]: moderate sunbed use has no effect on melanoma risk, but an 'unhealthy lifestyle' (e.g. extensive sunbathing, alcohol, smoking) resulted in an inflated OR=1.2 in association with sunbed use (it has been reported previously that 'sun worshippers' and individuals with an 'unhealthy lifestyle' go more frequently to tanning salons).

It has to be noted that the gap between solarium studies and earlier studies on the risk of sun exposures remains remarkable: confounding caused by exposure to the sun – the major UV source – is often neglected or corrected inadequately and most often not accompanied by a proper analysis of covariance (collinearity or other) to eliminate a possible dominance of sun over sunbed exposure due to an a priori highly plausible strong correlation between sunbed use and sunbathing – OR = 2–7 for sunbathing among sunbed users versus non-users [6]. As pointed out in a recent French study [rev. in 6], solaria were estimated to have only a minor contribution to melanoma incidence (1.5% in men and 4.6% in women) compared to the sun (83%), i.e. not likely to be a major driver of the increases in melanoma incidence, and, moreover, authors noted that it remains difficult to disentangle risk from sunbeds from that of the sun (anecdotal attribution of melanoma to solarium use is often offset by excessive sunbathing, as exemplified by Australian publicity campaigns for the regulation of sunbeds [rev. in 6]). But most importantly, earlier studies clearly identified number of sunburns as a good proxy of 'at risk' sun exposure in relation to melanoma risk [6]. Virtually, all studies on solarium and melanoma fail to use this proper proxy of effective UV dosimetry, with notable exception of two studies, which confirmed a strong relationship between UV burns and risk of melanoma [rev. in 6]. This would imply that UV burns specifically increase the risk, where genuine sunburns are far more common than UV burns from sunbeds. The

confounding effects of sun exposure and sunburns may also be one of the reasons why melanoma risk in relation to solarium use varies so strongly between studies, as meta-analyses of European studies show no net significant melanoma risk associated with sunbed use in contrast to American or Australian studies [rev. in 6].

We reiterate that unequivocal proof of an appreciable causal relationship between moderate solarium use and melanoma risk could be provided by randomized controlled trials, but these are lacking [rev. in 6, 65, 66] for various reasons: (a) it is unfeasible (takes too long, too costly and too demanding on compliance); and (b) it would now be considered unethical by many. It must be emphasized that it is a fundamental principle of evidence-based medicine that the level of evidence is not influenced by the reasons why the evidence is lacking [6, 64]. The overall quality of these observational studies and the resulting evidence levels are low due to severe limitations (including unobserved or unrecorded confounding), which leads to bias [rev. in 6]. The results of cohort and case–control studies published until today do not prove causality [rev. in 6]; not even the criteria defined by Hill in 1965 [67] (or as modified by Weed [68]) are fulfilled for the inference that moderate sunbed use per se increases melanoma risk. At least the criteria 'Consistency' ('Consistent findings observed by different persons in different places with different samples strengthens the likelihood of an effect'.), "Specificity" ('Causation is likely if there is a very specific population at a specific site and disease with no other likely explanation'.), "Plausibility" ('A plausible mechanism between cause and effect is helpful in determining causality'.), "Coherence" ('Coherence between epidemiological and laboratory findings increases the likelihood of a causal effect'.) and "Experiment" ('Experimental evidence is helpful in determining causality'.) are not fulfilled for the relationship between moderate sunbed use and melanoma risk, and therefore Hill's criteria do not support causality. The criteria "Consistency" and "Specificity" are not fulfilled for many reasons, including the obvious difficulties of confounding factors. Interestingly, in a recent

meta-analysis [6], subgroup analyses for studies performed in Europe, studies with low risk of bias and studies with recruitment between 1991 and 1999 did not show an association between melanoma risk and solarium use ('ever' vs 'never'). The lack of association in this subgroup analysis is very unlikely to be caused by a lack of power, e.g. because the number of participants in studies performed in Europe is much greater compared to studies from America. The lack of association in studies performed in Europe (in contrast to studies performed in the United States) may be due to several factors which are of particular relevance. Firstly, as outlined above, the role of solar UV exposure represents a major confounding factor which is difficult to document or to adjust for and which may well, in part, explain why latitude-dependent variations in melanoma risk in association with sunbed arise (e.g. due to shifts in effects from sunburns). On the other hand, other region-specific factors, which include technical differences in solarium devices, must also be taken into account as well as skin type, which is also an important confounding factor.

It has to be noted that there is a large body of evidence from epidemiological and animal studies that demonstrates no increase in melanoma risk following chronic (moderate) UV exposure [6, 88–94]. Many studies show that suberythemal chronic exposure to the sun may even be protective and that outdoor workers have a reduced risk of melanoma [6, 92]. It should also be noted that driver mutations in the B-rapidly accelerated fibrosarcoma (*B-RAF*) gene nor in other important drivers of melanomagenesis carry the specific UV signature (mutations in *B-RAF* are similar to those found in *GNA11* and *GNAQ* driver genes in uveal melanomas from UV-protected parts of the inner eye [65, 95]). Initiating melanoma by UV exposure in mice without predisposition by an activated oncogene proved to be very difficult (few exceptions, e.g. by neonatal exposure of *Ink4a-Arf-/-XPC-/-* mice [65, 96] and incidentally successful, 3/20, with repeated sunburn exposures [65, 97]).

Many open questions remain to be answered, including the following: (1) What is the relevance of confounding factors, including solar UV, UV

burns, skin type and age, for the association of solarium use and melanoma risk? (2) Does chronic exposure to moderate (sub-erythemal) UV doses have a preventive effect on melanoma risk? (3) If moderate solarium use does, in contrast to UV-burns, not increase melanoma risk, what means 'moderate'? (What is the dose-response curve? What is the impact of the flux of UV radiation, single and total UV dose as well as duration and frequency of solarium use?) (4) What is the relevance of the wavelength (UV-A vs UV-B) for the association of solarium use with melanoma risk? (5) How to analyze the combined effect of all (beneficial, e.g. cutaneous vitamin D synthesis, and adverse) health effects of moderate solarium use? Considering the fact that most melanomas do not occur in predominantly sun-exposed skin areas and that UV burns in childhood are an important risk factor for melanoma, we need to have a better understanding separate from the risk factors mentioned above of the (at least, in part, likely immunological) mechanisms responsible for inducing melanocytes to become malignant.

We conclude that both the level of evidence and grade of recommendation of studies published previously investigating the association of melanoma risk with solarium use are weak and that our present scientific knowledge does not support the hypothesis of an increased melanoma risk due to solarium use and questions studies that try to attribute melanoma cases to indoor tanning.

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Further Readings

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Molecular Biology of Basal and Squamous Cell Carcinomas

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Lars Boeckmann, Marie Christine Martens, and Steffen Emmert

Abstract

The prevalent keratinocyte-derived neoplasms of the skin are basal cell carcinoma and squamous cell carcinoma. Both so-called non-melanoma skin cancers comprise the most common cancers in humans by far. Common risk factors for both tumor entities include sun exposure, DNA repair deficiencies leading to chromosomal instability, or immunosuppression. Yet, fundamental differences in the development of the two different entities have been and are currently unveiled. The constitutive activation of the sonic hedgehog signaling pathway by acquired mutations in the *PTCH* and *SMO* genes appears to represent the early basal cell carcinoma developmental determinant. Although other signaling pathways are also affected, small hedgehog inhibitory molecules evolve as the most promising basal cell carcinoma treatment options systemically as well as topically in current clinical trials. For squamous cell carcinoma development, mutations in the *p53* gene, especially UV-induced mutations, have been identified as early events. Yet, other signaling pathways including epidermal growth factor receptor, RAS, Fyn, or p16INK4a signaling may play significant roles in squamous cell carcinoma development. The improved understanding of the molecular events leading to different tumor entities by de-differentiation of the same cell type has begun to pave the way for modulating new molecular targets therapeutically with small molecules.

Keywords

Basal cell carcinoma · Squamous cell carcinoma · UV-induced skin cancer · Molecular biology · Sonic hedgehog · PTCH · SMO · GLI · p53 · EGFR · RAS · Fyn · p16INK4a · Micro RNA · Genetic model diseases · Nevoid basal cell carcinoma syndrome (Gorlin syndrome) · Xeroderma pigmentosum · Nucleotide excision repair · Organ transplant recipients · Immunosuppression · Human papilloma virus

Introduction

The skin is composed of three layers: the epidermis, the dermis or cutis, and the subcutis. The subcutis is composed of fat tissue. The dermis contains collagen and elastic fibers produced by fibroblasts, nerve cells, blood and lymph vessels, and several types of immune cells. Skin adnexal structures like sweat glands and hair follicles with sebaceous glands originate from the epidermis and extend into the deeper layers. The epidermis, separated from the dermis by the basal

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membrane, is devoid of vessels. The prevalent resident cell types within the epidermis are keratinocytes but also melanocytes, Langerhans cells, and Merkel cells. The keratinocytes undergo a structured differentiation process from the basal layer of the epidermis toward the corneal layer which lasts about 30 days until they desquamate in the form of corneocytes. Malignant transformation of keratinocytes results in two major different and distinct tumor entities, basal and squamous cell carcinoma [39, 60].

Basal Cell Carcinoma: Epidemiology and Clinical Forms

Basal cell carcinoma, also termed basalioma, basal cell epithelioma, or, when ulcerated, ulcus rodens or ulcus terebrans, was first described by Arthur Jacob in 1827 [93] as a malignant, locally invasive, and destructive cancer. This local growth behavior results in a rather benign course of the disease, with metastases being largely absent [40]. Therefore, basal cell carcinomas are sometimes considered semi-malignant despite the true malignant cell transformation with invasive and destructive growth. The name basal cell carcinoma is retained by the WHO classification since 1974 [191], reflecting the sometimes aggressive growth with extensive tissue destruction and metastasis to lymph nodes and inner organs [123, 129, 189]. Basal cell carcinomas are the most common human invasively growing cancers by far. The total number of persons in the United States treated for non-melanoma skin cancer in 2012 has been estimated at 3.3 million [164]. About 80% of these are basal cell carcinomas [3]. In Germany, the incidence of basal cell carcinoma is reported as about 100 per 100,000 inhabitants [65, 162]. The mean age of patients affected with basal cell carcinomas is currently 60 years with a tendency toward a younger age for first tumor manifestation. A considerable increase in the incidence of BCC has been observed in young women, presumably due to an increased use of tanning beds and a closer attention to their appearance. Men are more frequently affected from basal cell carcinomas than women.

On the good side, mortality rates are very low [34, 57, 68].

Basal cell carcinomas are subdivided according to their clinical appearance [161, 204]. The nodular type (Fig. 9.1a) accounts for 60% of all basal cell carcinomas and is characterized by a pearly skin nodule and telangiectasias. Histologically, the basaloid cells are arranged in palisades at the tumor periphery surrounded by a strong stroma clearly separated from the tumor. The tumor cells show prominent chromatin-rich nuclei with frequent mitoses [104]. The (multicentric) superficial growth form (Fig. 9.1b) accounts for 25% of all basal cell carcinomas and appears as an indurated, erythematous, eczematous plaque. Histologically, there are multiple tumor foci within the plaque that extend from the epidermis to the upper dermis. The most problematic basal cell carcinoma growth pattern is the sclerodermiform or morphea-like type (Fig. 9.1c) due to ill-defined tumor margins. This grown pattern is characterized by strings of tumor cells invading the surrounding tissue and accounts for approximately 2% of all basal cell carcinomas [94, 178]. In addition, several rare forms of basal cell carcinoma growth forms exist that can be discerned based on histological criteria and include basosquamous tumors, pigmented basal cell carcinoma, metatypic basal cell carcinoma, ulcus rodens or ulcus terebrans, fibroepithelioma of Pinkus, or collision tumors.

Squamous Cell Carcinoma: Epidemiology and Clinical Forms

The second most common human cancer following basal cell carcinoma is cutaneous squamous cell carcinoma with approximately 250,000 new cases in the United States per year. In Europe the incidence is estimated as 20–30 per 100,000 inhabitants. Squamous cell cancer is a tumor of the elderly with an increasing risk with older age. The mean age of first occurrence is 70 years with a preponderance of men. Still, the mortality rate of squamous cell cancers of the skin is low. Only about 5% of locally advanced tumors do
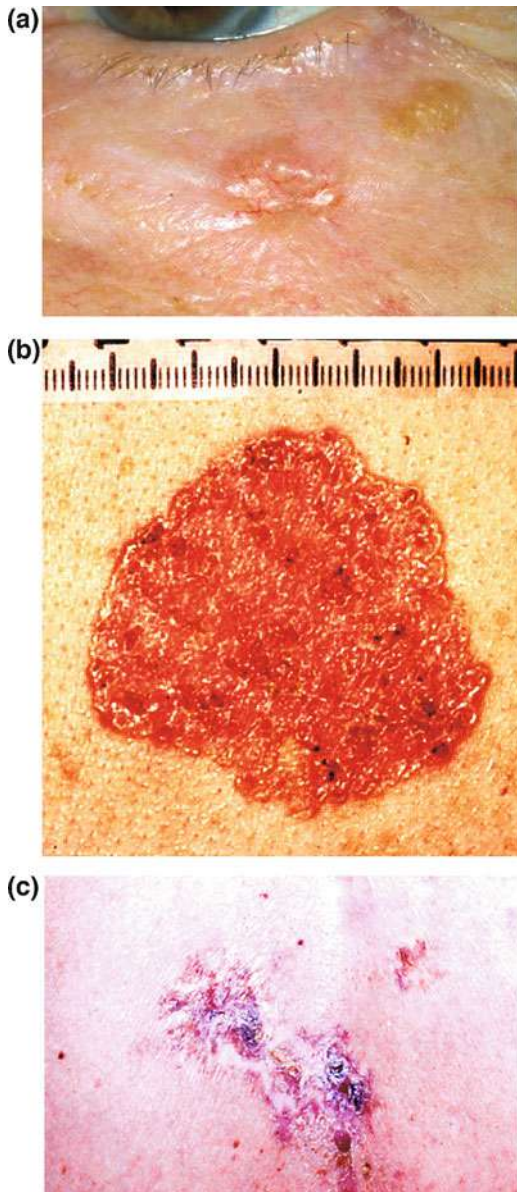
Fig. 9.1 Basal cell carcinomas. (**a**) Nodular type; (**b**) (multicentric) superficial type; (**c**) sclerodermiform (morphea-like) type

metastasize primarily to the lymph nodes, and the 5 years survival rate in this case is 25–50% [65]. Cutaneous squamous cell cancer manifests as a spectrum of progressively advancing malignancies ranging from actinic keratoses as precursor lesions to in situ squamous cell carcinoma (Bowen's disease) followed by invasive and finally metastatic squamous cell cancer [73]. Actinic keratoses present as reddish, hyperkeratotic circumscribed plaques on the skin (Fig. 9.2a), whereas squamous cell carcinomas represent skin-colored non-healing progressively growing papules or plaques with or without central ulceration (Fig. 9.2b). Histologically, tumor keratinocytes exhibit prominent atypical hyperchromatic nuclei. In actinic keratoses there is disrupted epidermal differentiation resulting in disorganized growth in the upper epidermal layers with a thickened corneal layer and corneocytes with retained nuclei (parahyperkeratosis). The degree of the epidermal dysplasia can be graded histologically following the KIN (keratinocytic intraepidermal neoplasia) nomenclature. KIN I represents atypical keratinocytes confined to the lower third of the epidermis. In KIN II atypical keratinocytes can be found in the lower two-thirds of the epidermis, and in KIN III there are atypical keratinocytes throughout the entire epidermis which represents Bowen's disease [27]. It has been shown that the degree of dysplasia is inversely correlated with the potential of a spontaneous regression of actinic keratoses [2]. During 1 year approximately 26% of all actinic keratoses will spontaneously regress [137]. In several publications the progression rate of actinic keratoses into squamous cell carcinomas is estimated between 0.025% and 16% for a single lesion per year [67, 137]. As a patient usually harbors more than one actinic keratosis, the annual risk – depending on the number of actinic keratoses present – can be estimated at 0.15% to 80% [67, 136, 143, 170]. A patient with more than 20 actinic keratoses shows a 20% probability to develop an invasive squamous cell carcinoma within 1 year [29, 113]. This range of uncertainty whether a keratotic lesion may develop into an invasive tumor or not reflects our still limited knowledge about the molecular mechanisms of squamous cell carcinoma progression.
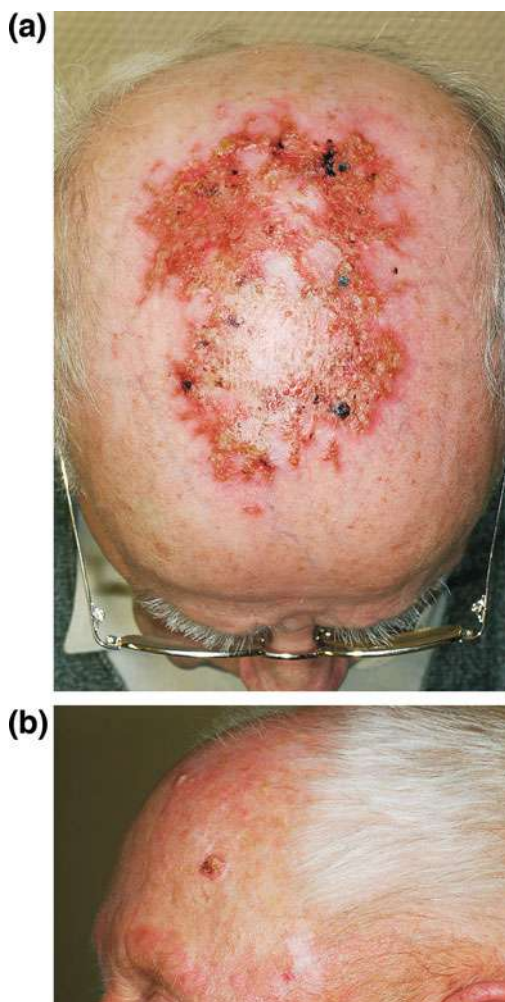
Fig. 9.2 (**a**) Actinic keratoses; (**b**) invasive cutaneous squamous cell carcinoma

Risk Indicators

Over the last decades, several risk indicators for non-melanoma skin cancer development including environmental as well as individual-dependent factors have been well established; however, others like viral co-carcinogens are still under investigation.

Fair-skinned, blue-eyed, and red- or blond-haired individuals who always burn and hardly tan after sun exposure carry an increased risk for non-melanoma skin cancers [63]. In addition, older age and a positive family history are individual skin cancer risk indicators [12, 142, 174]. Further, basal and squamous cell cancers predominately develop in sun-exposed skin areas. About 90% of these cancers are located on the head, neck, or forearms [179]. Extensive and chronic UV exposure especially before puberty remains the most important risk indicator for non-melanoma skin cancer [28, 72]. The role of sun-induced DNA damage as the initiator of those cancers is also vividly demonstrated by genetic syndromes with increased sun sensitivity and defective DNA repair.

The solar spectrum contains infrared (>800 nm) and visible light (400–800 nm), UVA-light (320–400 nm), as well as a portion of UVB-light (290–320 nm). Although the composition of the solar spectrum contains only 5% UVB-light, UVB is considered the most important skin cancer initiator followed by UVA-light [88, 124]. Via direct energy transfer, UVB directly induces DNA photoproducts, the so-called pyrimidine dimers including cyclobutane pyrimidine dimers (CPD) and pyrimidine-6,4-pyrimidone dimers (6–4PP). As the dimer induction is a result of a direct energy transfer, the number of dimers is directly proportional to the amount of UV exposure at a rate of CPD:6–4PP formation of 2:1 [48, 49, 192]. Today, it is widely accepted that UVA can also induce CPDs at the same amount of oxidative DNA damage which appears as the driving force of UVA-induced skin carcinogenesis [168, 169]. Whenever the photoproducts are not repaired properly, typical "UV fingerprint" mutations result. These include C to T base exchanges or CC to TT tandem mutations [20, 47, 48].

DNA photoproducts are almost exclusively repaired by the nucleotide excision repair pathway. This is a multistep process in which all seven currently known xeroderma pigmentosum genes are essentially involved [50, 82]. Briefly, the photoproducts are detected by the XPC and XPE (DNA damage-binding protein 2 – DDB2) proteins, demarcated by the XPB and XPD helicases, and verified by the XPA protein. Then, the XPG and XPF endonucleases incise the strand on both sides of the photoproduct.

The lesion containing oligonucleotide is removed and the gap filled by polymerases and ligases using the opposite strand as a template [46, 193]. If one of the XP proteins does not function properly, the whole repair cascade fails and results in the autosomal recessively inherited syndrome xeroderma pigmentosum (XP). XP patients show severe sun burning after minimal sun exposure, freckling in sun exposed skin, and skin cancer proneness for all types of UV-induced skin cancers, especially non-melanoma skin cancer [19, 47, 112]. Interestingly, the distribution of cutaneous basal and squamous cell carcinomas in XP patients does not differ from the skin cancer distribution in the normal Caucasian population [15]. However, the mean age of first skin cancer development in XP patients is 8 years indicating that the nucleotide excision repair pathway protects our cells over five to six decades from malignant transformation [15, 19]. The tumor cells of XP patients exhibit similar UV-type mutations, however, at a higher level in key regulatory genes compared to tumor cells from normal individuals [32, 33]. Cutaneous melanomas of XP patients showed UV-type mutations in the *PTEN* gene [207], and basal cell carcinomas of XP patients exhibited *PTCH* (73–88%), *SMO* (30%), and *SHH* (18%) mutations [32]. Besides XP there are several other family cancer syndromes that pose a genetic predisposition to non-melanoma skin cancer. These include Bloom syndrome (*BLM*), Cowden syndrome (*PTEN*), Fanconi anemia (*FANCA-N*), Li-Fraumeni syndrome (*TP53*), Rothmund-Thomson syndrome (*RECQL4*), Werner syndrome (*WRN*), dyskeratosis congenita (telomere maintenance), Kindler syndrome (*FERMT1*), Muir-Torre syndrome (mismatch repair), and patients with recessive dystrophic epidermolysis bullosa (*COL7A1*) [78].

Other non-melanoma skin cancer risk indicators include exposure to chemical carcinogens like arsenic, coal tar products, psoralen, as well as ionizing radiation and smoking [18, 26, 38, 62, 81, 103, 106]. The benzo-a-pyrene products induced by tobacco smoke are also a target of the nucleotide excision repair pathway [47]. Viruses such as the human papilloma virus (HPV) may also be a cofactor in basal and squamous cell cancer development. HPV has been associated strongly with malignant progression of warts to cutaneous squamous cell carcinoma and with epidermodysplasia verruciformis [64]. HPV has also been associated with the formation of basal cell cancer [10, 209]. This is especially important for organ transplant recipients who receive continuous immunosuppressive medication [115, 120]. In those patients squamous cell cancer is the most common skin cancer that occurs at a rate that is increased by 100- to 200-fold in comparison with immunocompetent controls. As one would expect, the risk for basal cell carcinoma development is also increased [11, 83, 115]. Cyclosporin A is associated with the highest skin cancer risk clinically due to its inhibition of nucleotide excision repair [114, 194], counteraction of p53-dependent cellular senescence [214], and tumor-promoting effects in SCID mice [84].

For both, the development of basal cell carcinoma and the development of actinic keratoses and their progression into invasive squamous cell carcinomas, the multistep carcinogenesis development model is helpful for our understanding of the molecular mechanisms involved [50, 55, 193]. Mutations accumulate in our cells over the course of our lifetime. A mutation in an essential tumor suppressor gene may lead to increased genetic instability increasing the cell's proneness for further mutations leading to the oncogenic transformation of the cell and ultimately to clinically apparent tumor formation. As few as two specific mutational changes in essential proto-oncogenes may be sufficient to drive skin carcinogenesis [30, 118].

Molecular Biology of Basal Cell Carcinomas

A major breakthrough in the understanding of early molecular events resulting in basal cell carcinoma development came from studying genetic diseases with increased basal cell carcinoma risk (reviewed in [149]). Such syndromes include – besides xeroderma pigmentosum – Bazex-

Dupré-Christol syndrome [202], Rombo syndrome [140], and cartilage-hair hypoplasia [135]. These syndromes are indicative for the involvement of DNA repair and telomere maintenance in basal cell carcinoma development. However, the most "specific" basal cell carcinoma risk (compared to other skin or internal cancers) was described as early as 1894 by Jarisch and White [95, 210] and in extensive detail by Gorlin and Goltz in the 1960s [70, 86] in patients who developed numerous basal cell carcinomas already at a very young age but also other tumors like rhabdomyosarcomas and medulloblastomas. In addition, patients who suffer from this basal cell nevus syndrome (BCNS) exhibit palmar pits (small punctate hyperkeratoses), bone cysts, and calcification of the falx cerebri [13]. The incidence rate of the autosomal dominantly inherited BCNS is about 1:19,000 inhabitants [54]. The underlying genetic cause of BCNS was just identified in 1996 by Hahn et al. [75] who showed that mutations in the *Patched 1* gene (*PTCH*, human ortholog of the drosophila segment polarity gene patched) and abnormal activation of the sonic hedgehog signaling pathway are causative and a prerequisite for basal cell carcinoma development [53, 61, 99]. The patients carry a germline mutation in one allele, and somatic mutations lead to functional loss of the second allele.

Sonic hedgehog (*SHH*), Indian hedgehog (*IHH*), and desert hedgehog (*DHH*) genes constitute the mammalian hedgehog family. SHH signaling is involved in early embryologic development, formation of the neural tube, musculoskeletal system, hematopoietic cells, teeth, and skin. In the skin the SHH pathway controls stem cells and hair follicle development [92]. In adult tissue *SHH* is largely turned off [5]. *SHH* itself and the proteins PTCH1 (patched), SMO (smoothened), and GLI constitute the key components of the signaling pathway [92, 147, 195] (Fig. 9.3). PTCH1 represses SMO, a transmembrane signaling protein. After binding of SHH to PTCH1, SMO repression is relieved and allows phosphorylation of GLI proteins. Here, cofactors such as Fused or Suppressor of Fused (Su(Fu)), KIF7, and others such as Rab23 or protein kinase A (PKA) may be involved

[37, 195]. There are three GLI genes in vertebrates. GLI1 predominantly acts as a transcriptional activator, whereas GLI2 and GLI3 can act as both activators and repressors [134, 165]. The activated GLI proteins translocate into the nucleus to serve as transcription activators. Via GLI the activated hedgehog (Hh) pathway targets Wnt signaling, β-catenin (CTNNB1), platelet-derived growth factors receptor α (PDGFRA), forkhead box (FOX) genes, cyclins (CCND), and PTCH1 itself (self-regulatory loop) via HHIP (hedgehog interacting protein) [92, 195] (Fig. 9.3). The crosstalk with Wnt signaling was demonstrated by the notion that basal cell carcinomas have increased levels of β-catenin [1, 44]. In turn, overexpression of a potent Wnt antagonist, Dkk1, resulted in a reduced tumor growth in mouse models indicating the importance of Wnt signaling in tumor formation [217]. The use of mouse models for studying the roles of Hh and related pathways is certainly very helpful in order to decipher molecular biologics behind basal cell carcinoma development in humans. Toftgard and colleagues summarized and updated the studies in mice [101].

Also in human sporadic basal cell carcinoma development, the role of constitutively activated hedgehog signaling as the driving force is now well established. About 90% of basal cell carcinomas carry molecular alterations in components of the Hh pathway [4, 53, 61, 96, 110, 215, 221]. It was found that 6–20% of sporadic basal cell carcinomas show activating missense mutations in *SMO* [14, 158, 215] and 11–75% show inactivating somatic mutations in *PTCH1* [150]. Of these mutations close to 50% appear to be UV-induced (CC to TT UV fingerprint mutations) [61]. There is also a *PTCH2* gene with 57% similarity to *PTCH 1* which also serves as a receptor [220] and carries mutations in sporadic basal cell carcinomas [182]. In about half of all sporadic basal cell carcinomas, mutations in the *TP53* gene have been detected [7, 35, 150], and an increased expression of (mutated) p53 protein was found immunohistochemically in basal cell carcinomas. About 66% of the *TP53* mutations occurred at nine mutational hotspots
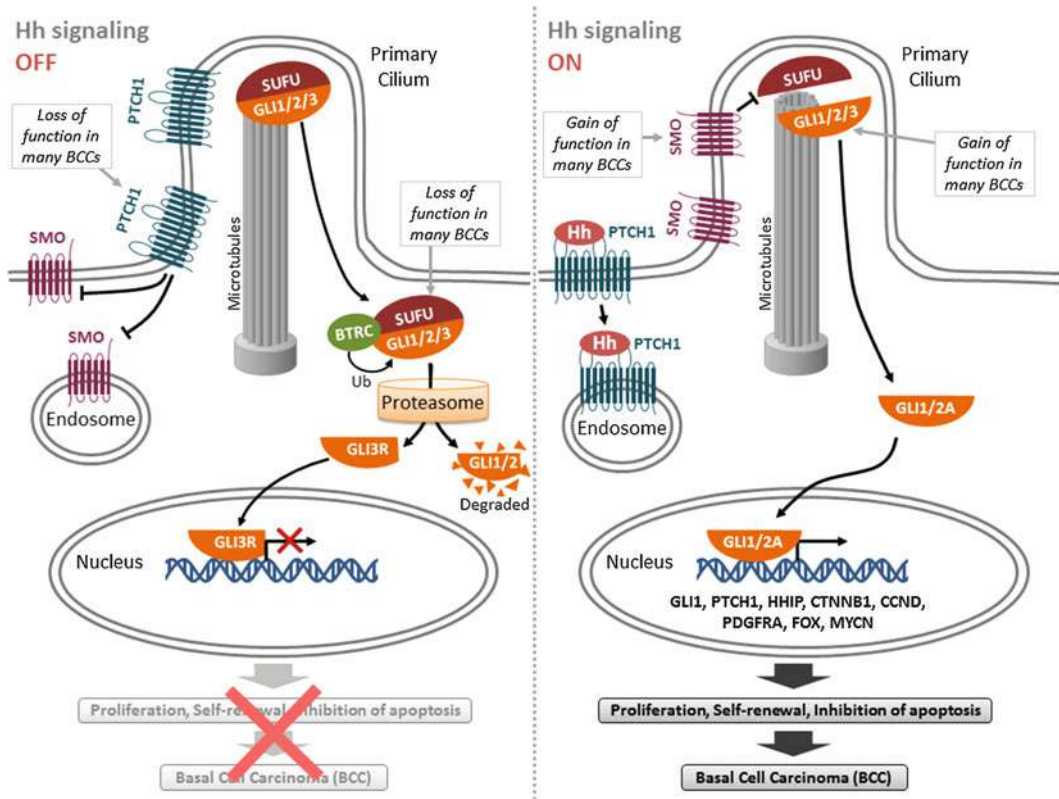
Fig. 9.3 A diagram of the vertebrate sonic hedgehog signaling pathway. The hedgehog (Hh) pathway consists of ligands (sonic hedgehog, Indian hedgehog, and desert hedgehog), receptors (patched-1 [PTCH1] and patched-2), signaling transducers and signaling intermediates (smoothened [SMO], BTRC and SUFU), and transcription factors (GLI1, GLI2, and GLI3). Target genes of this pathway include PTCH1, GLI1, and hedgehog-interacting protein (HHIP), which maintain the hedgehog signaling at an appropriate level in a given cell. The panel on the left shows the inactivated state of the Hh pathway, and the panel on the right shows the activated state

[224] and were UV fingerprint mutations, i.e., C (C) to T(T) conversions. A lower level of *TP53* mutations was identified in basal cell carcinomas from sunscreen users compared to non-sunscreen users [167]. In addition, patients suffering from Li-Fraumeni syndrome (loss of p53) do not exhibit basal cell cancer proneness, indicating that *TP53* mutations may be secondary events in basal cell carcinoma formation. On the other hand, loss of p53 increased the activity of the SHH pathway by increasing SMO expression in a mouse model rendering the mouse interfollicular keratinocytes receptive for basal cell carcinoma induction via SHH [205].

Beyond members of the Hh signaling pathway and the tumor suppressor *TP53*, further tumor suppressor genes and proto-oncogenes have been implicated in the pathogenesis of basal cell carcinomas. While earlier studies [152, 203] reported *RAS* gene mutations in 4 out of 30 (13%) and 5 out of 16 (31%) basal cell carcinomas, respectively, a later study with 293 basal cell carcinomas identified *NRAS*, *KRAS*, or *HRAS* mutations in only 2% of the tumors [14]. Besides mutations in *PTCH1* (73%), *SMO* (20%), and *TP53* (61%), this later study identified further driver mutations in other cancer-related genes in 85% of basal cell carcinomas. Among others, these include genes involved in the Hippo-YAP signaling pathway [14]. The Hippo-YAP pathway is a key regulator of organ size and tissue homeostasis. It is involved in restraining cell proliferation and promoting apoptosis [219]. MST1/2, the human

orthologs of Hippo (Hpo, *Drosophila melanogaster*), phosphorylate and activate LATS1/2, which then phosphorylate and activate the major effector of the Hippo-YAP pathway YAP/TAZ. This prevents the transcription co-activators YAP/TAZ from translocating into the nucleus and from inducing transcription [219]. Through genetic profiling of basal cell carcinomas, recurrent mutations in the Hippo-YAP pathway genes *LATS1* (16%), *LATS2* (12%), and *PTPN14* (23%) have been identified [14]. Other genes with mutations significantly associated with basal cell carcinoma tumorigenesis include MYCN (30%), PPP6C (15%), STK19 (10%), RB1 (8%), FBXW7 (5%), and ERBB2 (4%) [14]. Furthermore, somatic mutations have been identified in regulatory regions such as promoters of the genes *TERT* and *DPH3* [36, 71, 154, 175].

Molecular Biology of Squamous Cell Carcinomas

Compared with other solid tumors, the average burden of mutations in squamous cell carcinomas is very high. This makes the determination of driver genes and their distinction from passenger mutations challenging [78]. The *TP53* gene locus appears to play a significant role in the pathogenesis of squamous cell carcinoma. Roles of the phosphoprotein p53 include cell cycle control, DNA repair, induction of apoptosis, or senescence [85, 102, 180]. In normal skin, wild-type p53 protein is not detectable but appears within 2 h after UV irradiation, peaks at 24 h after irradiation, and again disappears at 36 h after irradiation [76]. Squamous cell carcinomas and actinic keratoses as precursors exhibit *p53* mutations with the typical UV-signature (CC-TT) in 60–90% of all cases. These mutations seem to be an early event in the carcinogenesis. Even in clinically normal appearing UV-exposed skin, *TP53* mutations were found in 74% of the cases compared to a much lower rate of 5% in sun-shielded skin [66, 146]. The transition from the earliest stages of squamous cell carcinoma development (KIN I – KIN III) into invasive cancers may be paralleled by loss of heterozygosity (LOH) of *TP53* [8]. All these findings point toward a driving role of *TP53* loss-of-function in squamous cell carcinoma development. In contrast, internal malignancies appear to develop *TP53* mutations at later stages after formation of an invasive tumor [198]. Also $Trp53^{-/-}$ mouse models ($Trp53$ = ortholog of human *TP53*) which exhibited an increased propensity for developing actinic keratoses-like lesions and squamous cell carcinomas helped to confirm the role of *TP53* in UV-induced skin cancer development [98]. Of note, patients suffering from Li-Fraumeni syndrome harboring a germline *TP53* mutation seem not to have an increased squamous cell carcinoma risk [197]. Also a retrospective study including 250 patients did not find statistically significant differences between the skin from patients with solitary and multiple skin carcinomas. This study rather highlighted an increased frequency of p53 patches with age [119]. This indicates that other pathways may also have implications in cutaneous squamous cell carcinoma development. In accordance, a number of other chromosomes have been shown to carry LOH in squamous cell carcinomas at 3p, 5p, 9p, 9q, 13q, 17p, and 17q [17, 155–157, 208]. Telomeres play an essential role in preserving chromosomal integrity. Due to proliferation-dependent telomere erosion, telomeres may lose their protective function and become prone to chromosomal fusions, anaphase bridge formation, and chromosomal breakage [117, 125]. Two classes of genetically distinct squamous cell carcinomas have been described based on their specific telomere profiles: one class with short/intermediate homogeneous telomeres and the other with longer/heterogeneous telomeres. This suggests two different mechanisms of tumor initiation – one dependent and one independent of telomere erosion [125].

Besides *TP53* mutations also a high frequency of mutations in genes of the Notch family (*NOTCH1*, *NOTCH2*, *NOTCH3*) has been identified. The frequency varies from 22%, 75%, 84%, and up to 86% depending on the study [23, 184, 185, 206]. These mutations tend to be

loss-of-function mutations, indicating a tumor suppressor role of NOTCH [183].

Targeted sequencing of TGF-β receptor genes *TGFBR1* and *TGFBR2* in 91 human primary squamous cell carcinoma samples and 21 human squamous cell carcinomas cell lines revealed mutations in 43% of primary samples [23]. TGF-β signaling has paradoxical roles in suppressing and promoting squamous cell carcinoma [213]. Furthermore, TGF-β promotes heterogeneity and drug resistance in squamous cell carcinoma [148].

UV-induced mutations, amplifications, and activating mutations, in the *RAS* oncogenes, have also been identified, however, at lower frequencies compared to *TP53* mutations in squamous cell carcinomas [17, 197]. Of the three *RAS* genes, Harvey rat sarcoma virus oncogene (*HRAS*) was found to be predominately mutated. About 21% of all squamous cell carcinomas harbor activating *RAS* mutations (9% *HRAS*, 7% *NRAS*, 5% *KRAS*) [9]. Further studies identified *HRAS* mutations in 12–38% of cases [23, 42, 184]. RAS is an upstream activator of the Raf/Mek/Erk kinase pathway, and aberrant activation promotes mitogenesis, drug resistance, angiogenesis, and resistance to apoptosis [90, 105]. However, RAS activation appears not sufficient for squamous cell carcinoma development [160] but needs to be coupled with other aberrant pathways such as the INK4A/Rb (see below) or the NF-κB pathway [30].

In addition, the Fyn Src-family tyrosine kinase may serve as another important effector of oncogenic *HRAS* signaling. Aberrant activation of the epidermal growth factor receptor (EGFR) and Fyn can be found in human squamous cell carcinomas and downregulate *TP53* [111, 223]. *HRAS* upregulates Fyn expression, and Fyn is required for tumor cell mobility and invasion [216]. These data indicate an interesting crosstalk of RAS and EGFR/Fyn which could serve as a target for small therapeutic molecules (kinase inhibitors). In accordance with that, a K14-Fyn Y528F mouse model spontaneously developed actinic keratoses- and squamous cell carcinoma-like lesions [223].

Targeted sequencing of 100 matched squamous cell carcinoma-normal pairs revealed mutations at an UV-signature hotspot in the gene *KNSTRN* in 19% of squamous cell carcinomas. The mutation was also detected in 13% of actinic keratosis but not in normal skin, suggesting that it occurs early in tumorigenesis [122]. *KNSTRN* encodes for the kinetochore protein kinastrin, and a mutation in this gene disrupts chromatid cohesion and correlates with aneuploidy in primary squamous cell carcinomas [122].

Another important pathway especially in the progression of actinic keratosis to invasive squamous cell cancer appears to be *INK4A/Rb* (Fig. 9.4). *CDKN2A*, first identified as a melanoma susceptibility gene located on the short arm of chromosome 9 (9p21), encodes two unrelated proteins: p16INK4a and p14ARF which are strong tumor suppressors involved in cell cycle regulation. The CDK4/CDK6-mediated phosphorylation of the retinoblastoma (Rb) protein that suppresses cell cycle progression from G1 to S phase is inhibited by p16INK4a. p14ARF inhibits oncogenic transformation by stabilizing p53 levels. p16INK4a was absent in invasive squamous cell carcinomas but still present in actinic keratoses [8]. LOH or epigenetic changes like promoter methylation may be involved in this process [21]. Transcriptome profiling of 30 squamous cell carcinomas and 10 actinic keratoses revealed key differences between the two [116]. Based on these findings and others, the PI3K/AKT/mTOR pathway appears to be the most altered mitogenic pathway in squamous cell carcinomas [90, 116, 133, 151]. *EGFR* overexpression is frequently observed especially in advanced squamous cell carcinomas [58, 201]. This results in increased proliferation, migration, cell survival, resistance to apoptosis, and altered differentiation by affecting downstream pathways such as PI3K/AKT/mTOR, PI3K/JAK/STAT, RAS/RAF/MEK/ERK/MAPK, PLCγ/PKC, and NF-κB [111, 163, 201].

A recent analysis of whole-exome data from 40 primary squamous cell carcinomas revealed 22 significantly mutated genes [91]. These include the genes with previously described

Fig. 9.4 A diagram of the *INK4/ARF* locus, the gene products, and their functions. The p16INK4a and p14ARF proteins are encoded by the *INK4/ARF* locus on human chromosome 9. As a CDK inhibitor, the p16INK4a protein inhibits the Rb functions via inactivation of the CDK4/6-cyclin D complex. The p14ARF protein, on the other hand, inhibits p53 functions
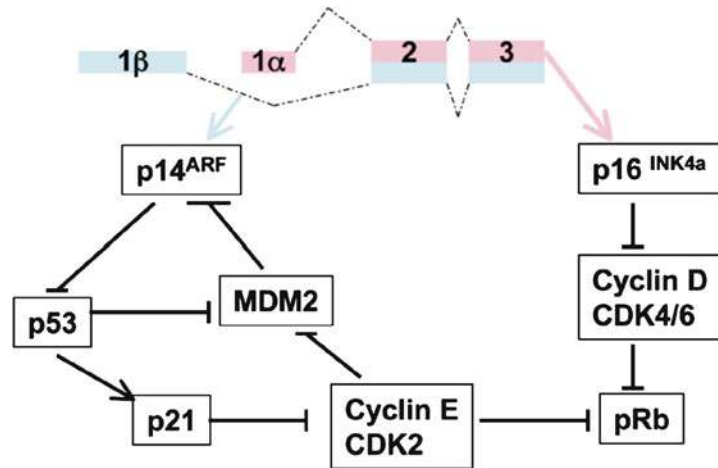
genetic alterations in human malignancies (*NOTCH1*, *TP53*, *NOTCH2*, *CDKN2A*, *MAP3K9*, *VPS41*, *SF3B1*, *PTEN*, *HRAS*, and *WHSC1*) and other genes with a so far unknown significance in tumorigenesis (*FLNB*, *GLIS3*, *CACNA1C*, *HERC6*, *TRAPPC9*, *MAPK1P1L*, *GRHL2*, *CLCN3*, *TMEM51*, *ATP1A1*, *LCLAT1*, and *CRY1*): the frequency and statistical significance are comparable with those observed in other sequencing studies [127, 151].

Although, as described above, mutations in many genes have been associated with squamous cell carcinoma, high levels of somatic mutations have also been identified by ultra-deep genome sequencing of normal sun-exposed eyelid skin from four individuals [138]. The burden of somatic mutations was similar to that observed in many cancers and showed characteristic UV-signatures. Remarkably high levels of mutations were identified in key genes including *TP53* and *NOTCH1–3* with *NOTCH1* as the most frequently mutated gene in the cohort [138].

Specific non-coding RNA transcripts, especially short approximately 22 nucleotide micro RNAs, have also been associated with the development of cutaneous squamous cell carcinoma. Non-coding RNAs are able to regulate important pathways like p53 [87] as well as stem cell maintenance pathways in the epidermis [222]. It was found that the micro RNA profile is altered in squamous cell carcinomas with elevated levels

of miR21 [43]. Others also found that miR21 suppresses tumor suppressors in epithelial cancers [132, 144]. Also miR203, an antagonist of p63 responsible for epidermal stem cell proliferation, was found downregulated in squamous cell cancers [43].

Emerging Treatment Options

The current treatments of actinic keratoses, invasive squamous cell carcinomas, and basal cell carcinomas share considerable similarities; however, some differences based on the increasing knowledge about the underlying molecular mechanisms begin to diverse our options for treatment. Generally, for invasive basal and squamous cell carcinomas, micrographically controlled surgical excision is still the gold standard to ensure elimination of all tumor cells. However, for the actinic keratoses and Bowen's disease as well as for superficially growing basal cell carcinomas, several topical treatment options exist or are being developed. For advanced and metastatic carcinomas, the use of targeted therapies and immune checkpoint inhibition provides innovative treatment options.

The chemotherapeutic 5-fluorouracil (5-FU) has been assessed extensively for the topical treatment of non-melanoma skin cancer. 5-FU is a pyrimidine analog on and by its incorporation

inhibits both RNA and DNA synthesis [130]. This mechanism of action targets the more rapidly dividing malignant keratinocytes [24, 188]. Usually, topical formulations of 5-FU have to be applied twice a week for 2–4 weeks [74]. However, side effects like severe inflammation, ulceration, and sometimes scarring were described which lower the quality of life of the patients and constitute major drawbacks of this therapy. Topical 5-FU may be combined with oral retinoids (isotretinoin) to accelerate the therapeutic effect as retinoids lead to epidermal normo-proliferation as well as normo-differentiation [45, 171]. Other chemotherapeutic molecules that have been used for the treatment of advanced or metastatic squamous cell carcinomas include cisplatin, carboplatin, bleomycin, methotrexate, adriamycin, taxanes, gemcitabine, and ifosfamide [159, 186, 196].

Imiquimod, an immune response modifier, is another widely used topical treatment option. The mode of action comprises the induction of an inflammatory skin reaction triggered by the activation of the innate immune system by activating toll-like receptors (TLR) 7 and 8. Drawbacks of this 5% imiquimod-containing immune therapy are the considerably long treatment time during which the patients carry an inflamed skin (e.g., for actinic keratoses for 4 weeks; application 3 times per week), individually severe inflammation with ulceration, and systemically present immune reactions including flu-like symptoms [74, 173]. Therefore, other formulations of imiquimod with lower concentrations (3.75% and 2.5%) have entered the US (2011) and European market (2012). These are applied on the basis of a 2-week daily on-off regimen and allow for larger treatment areas >25 cm^2.

Topical diclofenac in a 3% formulation with hyaluronic acid is also well established in the treatment of actinic keratoses. The gel formulation has to be applied twice daily for 90 days [153]. The rather long treatment cycle and the slightly lower patient clearance rates in comparison to the other topical formulations are drawbacks. The mode of action depends on the blocking of cyclooxygenases and prostaglandin E2 synthesis by diclofenac, which exerts pro-apoptotic effects in de-differentiated keratinocytes and also anti-angiogenic effects [56].

In photodynamic therapy, delta-aminolaevulinic acid is applied to the lesion-containing skin and irradiated by visible light after 3 h. Different formulations or modifications are on the market. As delta-aminolaevulinic acid predominately penetrates into dysplastic keratinocytes, the resulting phototoxic reaction eliminates selectively de-differentiated keratinocytes. It has been shown that apoptosis is involved in this reaction in squamous cell carcinoma as well as in basal cell carcinoma treatment [97, 100, 145].

With Ingenol mebutate yet another topical agent was licensed to treat actinic keratoses. Ingenol mebutate is a component of the plant *Euphorbia peplus* (also known as radium weed) and induces necrosis in the epidermal cells followed by a profound neutrophilic inflammatory response [166]. Ingenol mebutate is formulated in a gel with a 0.015% (face and scalp) or 0.05% (trunk and extremities) concentration, and the main advantages are that it has to be applied just once daily on two consecutive days and that the inflammatory skin response is mainly resolved after 8–16 days [121].

The understanding of the role of hedgehog signaling in basal cell carcinoma has opened new and somehow unexpected specific treatment options, as the inhibition of the aberrant activation of the hedgehog pathway may result in considerable tumor shrinkage, if not complete clearance indicating an important role of hedgehog not only in the development but also maintenance of basal cell carcinoma cells [92, 101]. Cyclopamine was the first SMO inhibitor discovered for the treatment of basal cell carcinomas given orally in mice [5]. GLI and HIP expression were reduced, and murine basal cell carcinomas were reduced by 90%. Also topical application of cyclopamine was successful in reducing basal cell carcinomas as shown in a small human trial from Turkey [187]. In four patients with basal cell nevus syndrome, the application of cyclopamine 4 times per day resulted already after 2 days in a visible reduction

of tumor size. This prompted the search for new compounds which inhibit SMO or the hedgehog pathway. Several of those are investigated in clinical trials [101]. Promising results were obtained with the orally administered SMO inhibitor GDC-0449 (vismodegib) in phase I and phase II trials [101]. Although no resistance to SMO inhibitors is reported so far, the oral administration harbors some noteworthy side effects including fatigue, nausea, muscle spasms, loss of taste, hyponatremia, and loss of hair [131, 177]. Such drawbacks may be overcome by the use of topically applied hedgehog inhibitors. Indeed, in a study with basal cell nevus patients, a 4 weeks application (twice daily) of a topical formulation of the hedgehog inhibitor LDE225 (sonidegib) resulted in basal cell carcinoma regression without side effects [181]. With longer follow-up, sonidegib demonstrated sustained tumor responses in patients with advanced basal cell carcinomas in a phase II, randomized, double-blind study [41]. However, it has been shown that resistance to hedgehog inhibitors can occur, if SMO is mutated at the inhibitor binding site [218] or if downstream genes like GLI are amplified [22]. Long-term assessment of safety and efficacy of vismodegib in patients with advanced basal cell carcinomas demonstrated durability of response, efficacy across patient subgroups, and manageable long-term safety [176]. But it also revealed response rates of only about 60%. This may be due to the high mutational burden affecting other signaling pathways of these tumors. GLI antagonists acting downstream of SMO were found to be effective in suppressing the hedgehog pathway. Those antagonists include an inhibitor of atypical protein kinase C-iota/lambda (aPKC-ι/λ) that phosphorylates GLI1 and arsenic trioxide that inhibits GLI2 [6, 147].

Alternatives to overcome resistance due to mutations at the inhibitor binding site of SMO would consist in the use of SMO inhibitors that bind at different sites such as itraconazole [109]. The anti-basal cell carcinoma activity of itraconazole in humans has been confirmed in an exploratory phase II trial [107]. Another substance that has been widely used in medical

treatment is vitamin D. Calcipotriol has been used for years in the topical treatment for psoriasis. However, it has been shown that it can also inhibit SMO and it activates the vitamin D receptor [199, 200]. Activation of the vitamin D/vitamin D receptor system induces cell differentiation, and inactivating polymorphisms in genes involved in this system may predispose to carcinogenesis [172]. Thus, vitamin D treatment would target basal cell carcinomas via two different effectors. As mere hedgehog inhibition results in tumor shrinkage, but residual tumor cells may persist rendering this treatment non-curative [89, 181], the second effector of vitamin D treatment may help to fully clear basal cell carcinomas.

For a more specific therapeutic approach to treat advanced cutaneous squamous cell carcinomas, targeting of the EGFR signaling has been established. The monoclonal antibody cetuximab inhibits EGFR signaling resulting in DNA repair inhibition, apoptosis, cell growth inhibition, and immune stimulation [69, 190, 212]. In a phase II study, cetuximab achieved 69% disease control rate as a first-line treatment in patients with unresectable squamous cell carcinomas [139]. Another monoclonal antibody inhibitor of EGFR, panitumumab, demonstrated response as a single-agent in locally advanced squamous cell carcinomas in a phase II study [59]. Further antibodies that inhibit EGFR include matuzumab, nimotuzumab, and zalutumumab. Typical side effects of EGFR inhibition include acneiform skin rashes (45–100%), hair loss (20%), facial hair growth (20%), and paronychia (inflammation of nails; 15%) [51]. Besides monoclonal antibodies, small molecule tyrosine kinase inhibitors can be used to target EGFR. These molecules block the activity of the tyrosine kinase ATP binding site and thereby inactivate downstream pathways. Several of these EGFR tyrosine kinase inhibitors, including gefitinib [126], erlotinib [52, 80], lapatinib [77], and dacomitinib [108], have been deployed in clinical trials for the treatment of advanced squamous cell carcinomas showing some evidence of clinical response.

The novel and highly effective treatment of metastasized melanomas with PD-1 antibodies

(immune checkpoint inhibition) also leads to regression of basal cell carcinomas, which has been shown through the example of xeroderma pigmentosum patients [79]. Clinical trials for the treatment of unresectable and metastasized basal cell carcinomas with PD-1 antibodies are currently being conducted (cemiplimab; REGN2810; NCT-03132636). The use of immune checkpoint inhibitors is also considered for the treatment of squamous cell carcinomas. Nivolumab, pembrolizumab, and cemiplimab represent PD-1 inhibitors, and at least partial responses to these antibodies have been described for several cases [16, 25, 128, 141, 211]. Durable responses have also been described for the CTLA4 inhibitor ipilimumab [31].

Conclusion

Major advances have been made during the last few years in our understanding of the biological processes in the development of basal and squamous cell carcinoma. Activation of the sonic hedgehog pathway is the most important driving force in basal cell carcinoma development. This knowledge has already been transferred into the clinic, as effective inhibition of hedgehog signaling using small molecules showed promising results in clinical trials, such as shrinking extensively progressed or metastasized basal cell carcinomas. While UV-induced mutations and functional loss of p53 are early events in squamous cell carcinoma development, activation of tumor suppressor genes is not possible using targeted therapies. However, through further understanding the multiple genetic alterations at the molecular levels, it will be possible to devise strategies to treat or prevent squamous cell carcinomas. The use of immune checkpoint inhibitors also provides new and innovative treatment options. Most new therapeutic developments may work by topical application avoiding many unexpected physiological and pathological side effects from systemic drug delivery.

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Photocarcinogenesis of Skin Cancer

Human Papillomaviruses and Skin Cancer

Sigrun Smola

Abstract

Human papillomaviruses (HPVs) infect squamous epithelia and can induce hyperproliferative lesions. More than 220 different HPV types have been characterized and classified into five different genera. While mucosal high-risk HPVs have a well-established causal role in anogenital carcinogenesis, the biology of cutaneous HPVs is less well understood.

From patients with the rare genetic disorder epidermodysplasia verruciformis (EV) and animal models, evidence is accumulating that cutaneous PV of genus β synergize with ultraviolet (UV) radiation in the development of cutaneous squamous cell carcinoma (cSCC). In 2009, the International Agency for Research on Cancer (IARC) classified the genus β-HPV types 5 and 8 as "possible carcinogenic" biological agents (group 2B) in EV disease. Epidemiological and biological studies indicate that genus β-PV infection may also play a role in UV-mediated skin carcinogenesis in non-EV patients. However, they rather act at early stages of carcinogenesis and become dispensable for the maintenance of the malignant phenotype, compatible with a "hit-and-run" mechanism.

This chapter will give an overview on genus β-PV infections and discuss similarities and differences of cutaneous and genus α mucosal high-risk HPV in epithelial carcinogenesis.

Introduction: Human Papillomaviruses and Cancer

Human papillomaviruses (HPVs) are double-stranded DNA viruses that infect epithelial cells of skin or mucosa in a species-specific manner and cause hyperproliferative lesions. More than 220 HPV types are classified into five genera on a genetic basis [1] with differences in biology and pathogenicity. Depending on the oncogenic potential of particular HPV types and body-specific sites of infection, lesions induced by HPVs range from benign warts to invasive carcinoma. The genus α mucosal high-risk HPV types has a well-established causal role in anogenital carcinogenesis. In particular, HPV16 and 18 are involved in about 70% of all cervical cancers. In

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2008 the discovery of this important link between viral infection and malignant disease was recognized with the Nobel Prize in Medicine to Prof. Harald zur Hausen. Benign anogenital condylomas are predominantly caused by the genus α-HPV types 6 and 11. Prophylactic vaccines against these most prominent high- and low-risk mucosal HPV types have been developed to prevent infection as well as HPV-induced diseases [2]. Recent epidemiologic studies have demonstrated the high efficacy of HPV vaccination on mucosal HPV-associated disease burden in countries with vaccination programs [3].

A link between HPV infection and skin cancer was first demonstrated in patients suffering from epidermodysplasia verruciformis (EV), a rare inherited disease. EV patients display a particular susceptibility to productive and persistent infection with cutaneous genus β-PV. As a consequence, they have a high risk to develop keratinocyte carcinomas at sun-exposed sites [4]. Two genus β-PV types, HPV5 and HPV8, were classified as "possibly carcinogenic" in patients with EV [5]. Studies in EV patients and animal models have provided evidence for the cocarcinogenic potential of HPV8 together with UV irradiation, and epidemiological studies suggest an association between β-HPV infection and keratinocyte carcinoma development also in the general human population. However, their "commensalic nature" and the fact that they are apparently dispensable for the maintenance of the malignant phenotype in skin cancer raise difficulties to proof this hypothesis [6, 7].

While the mucosal high-risk HPVs and their involvement of the microenvironment in carcinogenesis have been extensively studied [8], the biology of cutaneous genus β-PV is less well understood.

In order to gain more information of the mechanistic role of HPV in skin carcinogenesis, the International Agency for Research on Cancer (IARC) working group expressed the need for further research on potentially oncogenic cutaneous HPV types [5].

This chapter will give an overview on infections with genus β-PV and their roles in skin carcinogenesis in EV patients, in murine models, and in studies in vitro.

Human Papillomaviruses in Epidermodysplasia Verruciformis Patients

Epidermodysplasia verruciformis is a rare autosomal recessive genodermatosis first described by Lewandowsky and Lutz in 1922 [9–11]. EV-specific symptoms start early in life with the development of disseminated persisting flat warts or macular, red or brownish plaques, occasionally with a scaly surface. EV patients are at high risk to develop precancerous lesions and invasive cancer, particularly at sun-exposed areas of the skin [12]. Understanding fundamental mechanisms of β-PV during infection and skin carcinogenesis in EV patients may provide a better understanding of their potential impact on skin cancer in the general population.

Histology of EV Lesions and β-PV Genotypes

Histological analysis of EV lesions reveals large clear dysplastic cells with a granular cytoplasm and vacuolated nuclei found in clusters within the upper layers of the epidermis. This characteristic cytopathic effect is indicative of viral infection. Auto- and heteroinoculation experiments proved the infectious nature of the lesions [13]. Viral particles were then demonstrated in benign lesions by electron microscopy studies [14, 15]. Specific viral genomes were detected and later on characterized as human genus β-PV. In benign lesion of EV patients, the HPV types 5, 8, 9, 12, 14, 15, 17, and 19–25 were detected most frequently. Most cutaneous squamous cell carcinomas (SCC) in EV patients were shown to be associated with infection by the HPV types 5 and 8 pointing to a prominent role of these β-PV types in carcinogenic progression [16]. HPV types 5 and 8 were thus classified as "possibly carcinogenic" in patients with EV [5]. In contrast to earlier in situ hybridization data, highly sensitive techniques have allowed

the detection of viral genomes also in malignant lesions. β-PV positive nuclei were found heterogeneously distributed in the tumor tissues [17]. Also viral gene expression may still be detectable in atypical cells of SCC, albeit at a lower level than in the benign lesions [18]. It can be concluded that viral gene expression is strongest during early stages of skin carcinogenesis. However, in EV skin HPV may persist in the epithelium throughout the process of carcinogenesis.

Genus β-PV Epidemiology

Family transmission studies have shown that genus β-PV populate the skin of healthy individuals already very early in life. The majority of HPV types found in children were also detected in one or both parents indicating intrafamilial transmission [19, 20]. Due to their ubiquity and diversity, a "commensalic nature" of these viruses has been suggested [21]. Thus, β-PVs are found not only in SCC and actinic keratosis of non-EV patients but also in clinically normal skin and plucked hairs [16, 21, 22]. In the various studies, detection rates strongly depended on the methods applied.

Long-term immunosuppressed patients are at particular risk to develop keratinocyte carcinoma [23]. Of note, in plucked hairs from this patient group, HPV was detected more frequently and with increased probability of high viral loads [22, 24]. From this, it was suggested that higher viral loads may contribute to the risk of skin cancer development. Moreover, several recent (sero)epidemiological studies point to an association between genus β-PV infection, UV susceptibility, and skin cancer in organ transplant recipients as well as in the general population [25–28].

Quantification of viral loads in skin lesions in the general population revealed that precancerous lesions contain higher HPV copy numbers than keratinocyte carcinomas [29]. In addition, transcriptome analysis indicated that HPV is no more actively transcribed in non-EV SCC [30]. This suggested that genus β-PV might play an early role during the initiation phase of skin carcinogenesis rather than a role in sustaining the carcinogenic process at later stages of the disease. In fact, animal models using natural infection, conditional transgenic mice, and human explant cultures [31–33] support this hypothesis, compatible with a "hit-and-run" mechanism [34].

The β-PV Life Cycle

HPV infect keratinocytes of the basal layer, and it is assumed that they also reside within the hair follicle compartment comprising epidermal stem cells. As with other HPVs, the life cycle of genus β-PV is tightly linked to the differentiation program of the stratifying epithelium. To indicate the sequence of viral gene expression during the HPV life cycle, viral gene products have been classified as early (E) and late proteins (L). β-PVs encode E1, 2, 4, 6, 7 proteins but lack an E5 ORF. In benign EV lesions, the viral genome is actively transcribed in a differentiation-dependent manner, as demonstrated for HPV5 [35].

E1 and E2 transcription start in the basal cells and particularly E2 expression increases with differentiation in the middle layers of the epithelium. Both proteins play an important role in viral transcription and replication. The nuclear transcription factor E2 interacts with a variety of cellular factors involved in transcriptional regulation [36] and influences cellular gene transcription in favor of the viral life cycle [37]. β4-integrin is the first cellular gene shown to be transcriptionally regulated through specific E2-binding sites. It anchors basal keratinocytes to the basement membrane. Loss of β4-integrin leads to the detachment of keratinocytes from the underlying structures. HPV8 E2 was shown to downregulate β4-integrin transcription in human keratinocytes by displacing the cellular transcriptional activator AP-1 from its promoter [37, 38]. In vivo, β4-integrin downregulation in keratinocytes may initiate their egress from the basal to suprabasal layers. Thus, it is assumed that E2 expression pushes the virally infected cells into the transit-amplifying compartment, a prerequisite for differentiation [37].

In suprabasal cells cellular transcription factors of the CCAAT/enhancer-binding protein (C/EBP) family are expressed in a coordinated manner. HPV8 E2 is able to bind to these proteins and to enhance their transcriptional activity. This ensures the expression of a distinct profile of cellular differentiation-dependent genes in the middle layers of the epidermis [39, 40]. Notably, E2 exploits the same pathway to induce the differentiation-associated S100 proteins A8 and A9. Once they are released, S100 A8/A9 can recruit myeloid cells to the lesion contributing to an inflammatory microenvironment, which may support the viral life cycle and potentially also carcinogenesis [40]. In the nucleus, β-PV E2 proteins bind to pericentromeric regions of cellular DNA and tether viral DNA to host mitotic chromosomes [41]. Interactions of E2 with structural maintenance of chromosome 5 (SMC5) and SMC6 help to maintain viral episomal DNA [42]. E1 together with E2 initiates a DNA damage response [43]. As a consequence, DNA damage and repair proteins are recruited to viral replication foci, which may support vegetative viral DNA replication [44]. This may preferentially occur in suprabasal cells, where cellular DNA replication is normally shut down.

In benign HPV5-positive EV lesions E6 and E7 transcripts are abundantly detected. E7 expression is highest in the terminally differentiated epidermal layers [35]. Functions and roles of these putative oncoproteins in cutaneous HPV infection and skin carcinogenesis have been investigated in vivo as well as in vitro. In view of the viral life cycle, it is assumed that early proteins of cutaneous HPV ensure a cellular environment that allows viral DNA replication in differentiated layers [45].

Replication of the dsDNA genome of β-PV and viral transcription are controlled by the noncoding control region (NCR). This region is located between the 3′ end of the late gene region and the 5′ end of the early gene region. The NCR of EV-associated HPVs differs from that of other HPVs. It is characterized by its small size of about 400 bp. E2 and cellular transcription factors bind to the NCR and regulate its activity. UV irradiation, the major skin carcinogen, activates the NCR of several β-PV [46–48]. Of note, it was shown that UV light induces and activates nuclear expression of the cellular interferon regulatory factor-7 (IRF-7) [49]. IRF-7 then directly binds to the HPV8 NCR and transmits the UV-signal [48]. IRF-7 itself is induced by type I IFN and enhances IFN-α and IFN-β gene expression [50]. Thus, HPV8 utilizes a central part of the natural antiviral IFN pathway for its own gene expression. In contrast, IRF-3, another related interferon regulatory factor, strongly suppresses the HPV8 NCR. IRF-3-mediated suppression prevails over IRF-7-mediated activation of HPV8 transcription. Similarly, suppression is observed in keratinocytes treated with the potent IRF-3 activators, poly(I:C) or RNA bearing 5` phosphates [48]. Thus, local application of IRF-3-activating compounds might be a novel therapeutic concept against cutaneous β-PV infection particularly for EV patients [7].

The Genetic Defect in EV Patients

An important susceptibility locus of EV patients has been mapped to chromosome 17q25 comprising two adjacent genes EVER1/TMC6 and EVER2/TMC8 [51–53]. EVER genes are expressed in keratinocytes and leukocytes. EV patients are not generally prone to infection and EVER2 deficiency is associated only with mild changes in T lymphocytes [54]. Therefore, it is assumed that the EVER proteins function mainly as keratinocyte-intrinsic restriction factors for β-PV [55]. The transmembrane channel-like proteins are located in the endoplasmic reticulum [56], where they form a complex with one of the zinc transporters ZnT-1. However, it was controversially discussed whether EVER proteins regulate zinc homeostasis [57, 58].

Recently, a third EV susceptibility gene encoding the pleiotropic factor calcium- and integrin-binding protein 1 (CIB1) [59] has been identified [58]. In normal cells, CIB1 forms a complex with EVER1 and EVER2, while in EVER1- or EVER2-mutated keratinocytes, CIB1 protein levels are low. The E5 protein encoded by the α-HPV16 and the γ-HPV4 E8

protein were shown to interact with CIB1, and it is hypothesized that they interfere with CIB1-dependent restriction. β-PVs, however, are lacking an E5 ORF, and therefore CIB1 may specifically restrict β-PVs [58]. Conversely, keratinocytes with reduced levels of CIB1 or CIB1-specific defects may efficiently support β-PV replication.

EV-Like Disease

Common Gamma-c or Jak3 Deficiency
In 50% of patients with severe combined immune deficiency (SCID) due to gamma-c cytokine receptor subunit (gamma-c) or Jak3 mutations, EV-like pathologies ("atypical EV") can occur as a late-onset disease after successful hematopoietic stem cell transplantation [60]. They are either as a consequence of a natural killer (NK) cell or a keratinocyte-intrinsic defect.

Immunosuppression in Organ Transplant Recipients (OTRs), Inherited T-Cell Defects, and HIV
Molecular or seroepidemiological studies of OTRs who receive immunosuppressive treatments point to a crucial role of adaptive T-cell immunity for the control of β-PV infection and disease [27]. OTRs display infections with multiple β-PVs, higher viral loads than in the general population, and a more than 100-fold increased incidence of cSCCs [6, 61]. Although no overt EV-like disease is observed [55], β-PVs actively replicate in actinic keratosis and epithelium adjacent to cSCCs of these patients [62]. In addition, low penetrance of EV-like disease and infections with other pathogens are observed in patients with inherited primary T-cell deficiencies (summarized in [55]).

EV-like disease has also been described in HIV-positive individuals. Worsening of symptoms in these patients has been repeatedly observed during immune reconstitution associated with an inflammatory syndrome [63–65]. The relationship between the immune reconstitution syndrome and EV-like disease is, however, not yet fully understood.

Local Immune Control and Immune Escape in EV Patients

Although EV patients are able to mount a pronounced humoral response directed against the L1 major capsid protein [66], genus β-PV persists in the skin of EV patients for long periods of time. An important question is how these viruses, once expressed, manage to escape cutaneous immune control. It is assumed that cellular immunity against the virally infected cells is not efficiently elicited.

In this regard, a striking observation was the dramatic reduction of Langerhans cell numbers (Langerin-positive cells) in lesional areas of EV epidermis where viral replication and gene expression occurs [67]. This finding confirmed previous reports demonstrating the virtual absence of MHC class II or CD1a-positive cells in EV lesions [68, 69]. Skin immunity critically depends on the activity of Langerhans cells, specialized antigen-presenting cells residing in the epidermis. They locally take up antigen and migrate to local lymph nodes. In a homeostatic situation, they may dampen immune responses to self-antigens. However, depending on the microenvironmental stimuli, they will be able to cross-present soluble and cell-associated antigen from neighboring keratinocytes to CD8+ effector cells [70]. Thus, Langerhans cells are key regulators of immune responses in the skin.

Upon UV-light exposure, Langerhans cells leave the skin, which is known as a part of UV-mediated immunosuppression [71]. Under normal conditions the epidermis will then be repopulated again with Langerhans precursor cells migrating along a chemotactic gradient toward the chemokine CCL20 [72–74]. CCL20 was found to be expressed in the most differentiated layers of human epidermis. Of note, lesional areas of EV epidermis devoid of Langerhans cells express only low or no CCL20 protein [67]. Chromatin immunoprecipitation of the CCL20 promoter and functional studies identified the differentiation-associated transcription factor C/EBPβ as a novel critical regulator of CCL20 gene expression in normal human

keratinocytes. In situ studies demonstrated that the expression patterns of CCL20 and nuclear C/EBPβ converge spatially in the most differentiated layers of human epidermis. Of note, the E7 oncoprotein of HPV8 was shown to co-localize and interact with C/EBPβ in the nucleus. The interaction site could be mapped to a FQELL motif within the putative C-terminal zinc-finger loop. Furthermore, it was demonstrated that the interaction between the viral and the cellular factor has important functional consequences. E7 interferes with the binding of C/EBPβ to the CCL20 promoter in vivo and specifically suppresses CCL20 gene expression. In fact, keratinocytes expressing the HPV8 E7 protein produce only very low amounts of the chemokine CCL20 and display strongly reduced chemotactic activity toward Langerhans cells [67]. As a consequence, EV lesions may not be properly repopulated with Langerhans cells after UV exposure resulting in impaired antigen presentation.

Thus, once expressed at sufficiently high levels, HPV8 is able to disrupt the epithelial immune barrier allowing viral persistence.

UV Light and β-PV as Cocarcinogens in EV Patients

Ultraviolet (UV) radiation and β-PVs cooperate as cocarcinogens in EV patients. Recently, investigations of EV lesions have shed light into the molecular mechanism underlying this multistep process, i.e., β-PV-mediated (1) expansion of the epithelial progenitor cell compartment, (2) enhancement of UV-mediated DNA damage, and (3) of chronic inflammation.

β-PV-Mediated Expansion of the Epithelial Progenitor Cell Compartment in EV Lesions

A seminal observation in skin lesions of EV patients was the HPV8-mediated expansion of the ΔNp63-positive stem cell compartment via suppression of the stemness-repressing microRNA-203 [75]. This was particularly interesting, since this compartment displays an

enhanced susceptibility to carcinogenic progression [76]. As the underlying mechanism, the cellular differentiation-regulating transcription factor C/EBPα was identified as a novel regulator of microRNA-203. C/EBPα is strongly downregulated by the major β-HPV oncoprotein E6 and, like miR-203, potently suppressed in EV lesions [75]. In addition, β-HPV E6 also binds to Mastermind-like protein 1 (MAML1), thereby interfering Notch, another important regulator of keratinocyte differentiation [77, 78]. Notably, C/EBPα is not only a key regulator of epidermal differentiation, but it also suppresses UV-induced skin carcinogenesis in mice [79, 80]. Thus, this novel β-HPV E6-driven C/EBPα/microRNA-20/ ΔNp63 profoundly disturbs epidermal homeostasis in EV patients and expands the stem cell compartment, a critical step paving the way for UV-mediated skin carcinogenesis.

UV-Induced p53 Mutations in EV Lesions

UVB displays significant mutagenic activity [81]. An important target gene of UV-induced mutagenesis is the tumor suppressor protein p53 [82, 83]. Upon genotoxic stress wild-type p53 activates cell cycle checkpoints in normal keratinocytes. This leads to growth arrest, which allows DNA repair or initiates the execution of programmed cell death [84]. UV-induced pyrimidine-pyrimidone photoproducts and unrepaired cyclobutane pyrimidine dimers may result in C to T or CC to TT mutations in the p53 gene [85, 86]. p53 mutations may arise causing the inactivation of p53 functions. As a consequence, this eventually results in genomic instability, a major step in carcinogenesis.

In a retrospective study of two EV patients during an 8-year period, p53 mutations were detected in five (62.5%) SCC, two actinic keratoses, and one benign lesion. These comprised UV-signature mutations as well as mutations that might correspond to DNA replication errors. It was speculated that unrepaired DNA lesions caused by other exogenous or endogenous mutagens such as reactive oxygen species might also play a role [87]. β-PV E6 interferes with the DNA damage response and UV-induced apoptosis in vitro [88, 89],

potentially allowing the accumulation of UV-mediated DNA mutations (summarized in [90]). Obviously, p53 mutations are common in HPV-associated skin cancer in EV patients. This is in strong contrast to cervical carcinogenesis, where p53 mutations are rarely detected. A major oncogenic activity of mucosal high-risk genus α-PV involves proteolytic degradation of p53 by the E6 protein forming a complex with the ubiquitin ligase E6-AP [91, 92]. Most β-PV E6 proteins, however, do not bind p53 or lead to p53 degradation [45, 93, 94]. This indicates that oncogenic mechanisms of human genus β-PV are distinct from those of mucosal high-risk genus α-PV. In genus β-PV-initiated carcinogenesis, rather the increased burden of critical mutations, which also affect p53, may substantially contribute to disease progression at later stages.

β-HPV-Mediated Amplification of Inflammation in EV Lesions

While mucosal HPVs suppress inflammatory cytokines and chemokines (summarized in [8, 95]) HPV8-positive skin of EV patients is infiltrated with myeloid cells, starting in the stroma of productive lesions. In the epithelium of EV lesions, S100A8/A9 proteins are tremendously upregulated in cells showing virus-induced cytopathic effects [40]. These differentiation-associated S100A8/A9 proteins form a calprotectin complex. Once released, calprotectin serves as a potent neutrophil chemoattractant [96]. Notably, the β-PV-encoded transcription factor E2 exploits the same C/EBPβ-dependent mechanism to upregulate S100A8/A9 [40] as previously shown for the premature enhancement of differentiation [39]. Also other neutrophil-attracting chemokines including interleukin-8 (IL-8), ENA-78, and NAP-2 are produced by keratinocytes co-expressing HPV8 E2 and C/EBPβ, which may further increase neutrophil infiltration [40].

The ability of β-PV E2 to promote differentiation thus appears to be intimately linked to the induction of inflammation [37–40], and the resulting inflammatory microenvironment may pave the way for tumorigenesis as observed in HPV8 E2 transgenic mice [97].

Functional Studies of Cutaneous PV in Animal Models

Transgenic Mouse Models

The oncogenic potential of β-PV has been explored in transgenic mouse models. Mice expressing the complete early region of HPV8 under the keratin-14 promoter, which directs transgene expression to the basal compartment, spontaneously developed skin tumors. In 6% of the mice, SCC arose without any need for physical or chemical carcinogens [98]. Of note, it was shown that the cellular signal transducer and activator of transcription 3 (STAT3) plays an important role in HPV8-induced skin tumor formation [99]. STAT3 is also activated in the epithelium and inflammatory infiltrate in preneoplastic lesions of the cervix uteri [100, 101]. Thus, STAT3 activation plays a major role in HPV-induced tumorigenesis.

Expression of the HPV8 E6 protein under the keratin-14 promoter generated essentially the same phenotype as seen in mice transgenic for the complete early region of HPV8 [102]. From these experiments it has been deduced that E6 is the major oncoprotein of HPV8 sufficient to induce skin cancer. Application of UV light or skin wounding strongly accelerated and enhanced tumor formation [102].

Under both conditions, UV exposure or wounding, tumors displayed a strong inflammatory infiltrate. Chronic inflammation has an important role in neoplastic progression [103]. This notion is compatible with the observation of EV-like disease during the immune reconstitution phase in HIV patients. Of note, chronic inflammatory infiltrates were also observed in lesional skin from EV patients, and a link between β-HPV E2, keratinocyte differentiation, and inflammation has recently been identified [40]. In vitro experiments have demonstrated that high E2 expression not only initiates premature differentiation of

keratinocytes [37–39], it also upregulates the differentiation-associated S100A8/A9 proteins and thereby leads to the recruitment of myeloid inflammatory cells [40]. Accordingly, in mice expressing the HPV8 E2 protein under the keratin-14 promoter, the epidermis was virtually thin predisposing to ulcerations, similar to a "chronic, non-healing wound" [104]. Lesions in these mice showed chronic inflammation, in 6% severe dysplasia, and some even progressed to skin cancer [97]. Thus, evidence is increasing that the β-PV E2 protein contributes to a chronic protumorigenic inflammatory response observed in vivo.

Expression of HPV38 E6 and E7 under the control of the keratin-14 promoter did not result in spontaneous tumor formation, but precancerous lesions and SCC developed after chronic UV irradiation [105]. Using a heterologous keratin-10 promoter directing HPV38 or HPV20 E6 and E7 transgene expression to the suprabasal compartment did not lead to spontaneous tumor formation, either [106, 107]. Comparison of the different models demonstrated that the oncogenic potency of genus β-PV is highest, if their major oncogene E6 is expressed in the basal layer of the epidermis. Chronic UVB irradiation of HPV20 transgenic mice increased papilloma formation and led to the rare occurrence of SCCs [107]. These animal models clearly demonstrated the oncogenic potential of genus β-PV in vivo when continuously expressed under the keratin-14 promoter. They also underscore a synergism between genus β-PV and UV light as well as the importance of inflammatory responses in β-PV-mediated skin tumor induction.

Evidence for a "Hit-and-Run" Mechanism

Using a novel mastomys coucha model with natural PV infection [108], conditional transgenic mice, as well as human explant cultures, the question has been investigated whether or not cutaneous PV are necessary throughout carcinogenesis [31–33]. Together, all these studies provided evidence that β-PVs have an early role in skin

carcinogenesis, and at later stages, they become dispensable for the maintenance of the malignant phenotype, compatible with a "hit-and-run" mechanism [34].

Molecular and Functional Studies of Human Genus β-Papillomaviruses In Vitro

Comparative analyses demonstrated that several genus β-PV have transforming potential in vitro. For this, an oncogene (activated EJ-ras) cooperation assay in rodent cells was used [109, 110]. A subset of genus β-PVs was shown to extend the life span of primary human keratinocytes. For E6/E7 oncogenes of HPV49, HPV38, and HPV8, although weaker, immortalization of keratinocytes was demonstrated [111–113]. Thus, β-PV oncoproteins clearly have the potential to transform their natural host cells. However, the molecular mechanisms by which genus β-PV oncoproteins support the oncogenic process in skin can strongly differ from α-PVs. This part describes the major functional differences and similarities between mucosal genus α-PV and cutaneous β-PV.

The β-PV E6 Oncoprotein

There is evidence that β-PV E6 proteins have a profound impact on the regulation of epithelial homeostasis, UV-induced DNA damage responses and cell death in keratinocytes.

HPV oncoproteins lack enzymatic activity. Recent studies have unraveled important pathways targeted by genus β-PV E6 proteins. Thus, HPV8 E6 has been shown to transcriptionally suppress C/EBPα [75], a potent inducer of keratinocyte differentiation and suppressor of UV-induced carcinogenesis [79, 80]. C/EBPα was identified as a novel suppressor of this microRNA controlling the stemness factor ΔNp63 [75]. It directly binds to the microRNA-203 gene, and, via the novel C/EBPα/microRNA-20/ΔNp63 pathway, HPV8 E6 potently alters keratinocyte homeostasis. This leads to the

expansion of the ΔNp63-expressing epithelial progenitor compartment keratinocytes, which is highly susceptible to carcinogenic progression [76].

In addition, β-PV E6 proteins specifically bind to the Mastermind-like coactivator MAML1. As a consequence, this leads to suppression of Notch signaling [77, 114–116]. Notch is a key regulator of keratinocyte differentiation. Of note, MAML1 binding was highly specific for the cutaneous E6 proteins and was not observed for eight different genus α-PV E6 proteins. The latter E6 proteins neither interact with MAML1 nor with Notch1, Notch2, or RBPJ, a Notch-regulated transcription factor [45]. In mice, Notch also suppresses skin tumor formation [117]. Thus, interference of β-PV E6 with both, the C/EBPα/microRNA-20/ΔNp63 and the MAML1/Notch pathways, may contribute to tumorigenesis.

A key mechanism of the high-risk mucosal genus α-PV E6 oncoproteins is seen in its interaction with the tumor suppressor protein p53 as well as the ubiquitin ligase E6-AP, which targets p53 to proteasomal degradation [118–120]. In strong contrast, most genus β-PV E6 including HPV8 E6 do not bind to p53. Exceptions from this rule are HPV49 E6, as well as E6 proteins from two further β-PV, HPV38 and 92, which are able to interact with p53 [45, 93, 94, 113]. A comprehensive E6 interaction analysis, however, demonstrated that the p53 protein was rather stabilized by a posttranslational mechanism in keratinocytes expressing HPV38 or 92 E6 proteins. A similar effect was observed by HPV17a E6, a "p53 nonbinder" [45]. The functional significance of these findings and their consequences still has to be elucidated.

As outlined above, mutations of p53 are frequently found during skin carcinogenesis in the general population as well as in HPV-associated skin cancer in EV patients, which is a profound difference to cervical carcinogenesis. Moreover, several other ways might exist how β-PV E6 proteins interfere with p53 function. For example, HPV77 E6 selectively inhibits p53-dependent transcription of proapoptotic genes following UVB irradiation in cell lines [121]. HPV23 E6 was shown to prevent p53 phosphorylation through an interaction with the homeodomain-interacting protein kinase 2 [122]. In case of HPV38, the E6 protein was shown to affect p53 signaling indirectly, by inducing the expression of the deltaN isoform of p73 [123].

β-PV E6 proteins can extend the life span of human keratinocytes, and this was strongest for HPV38 and 8 [124, 125]. Particularly in cells expressing the latter E6 proteins, activation of telomerase was observed, and this occurred in an E6-AP-dependent manner [124], although no stable physical interaction of E6-AP and β-PV E6 proteins was observed in a different study [45]. Another important feature of different β-PV E6 proteins is seen in their ability to abrogate UV-mediated apoptosis. In vitro studies suggested that p53 degradation was not required, and inhibition of apoptosis was also observed in p53 null cells. One mechanism how β-PV E6 proteins exert their antiapoptotic activity is the proteolytic degradation of the proapoptotic molecule Bak [88]. This observation was later on confirmed for HPV5, 8, 20, 22, 38, 76, 92, and 96 in normal human keratinocytes [89].

β-PV E6 was shown to have variety of novel interaction partners including proteins containing PDZ motifs as well as proteins of the Ccr4-Not complex. Moreover, HPV5, 8, 20, and 25 E6 proteins specifically bind the acetyltransferases and transcriptional coactivators p300/CBP [45, 126, 127]. Several studies indicate that p300 binding by β-PV E6 affects important downstream signaling events most relevant for tumorigenesis, such as C/EBPα suppression [75], acetylation of p53, and p53-dependent transcription [128]. Thus, in HPV-associated skin carcinogenesis, p53 might either be mutated or inhibited at a functional level by β-PV E6 proteins.

P300 binding of E6 also contributes to suppression of keratinocyte differentiation and expression of the kinase ATR (ataxia telangiectasia, mutated and Rad3-related), a key regulator of the checkpoint pathway in the DNA damage response [127, 129]. Reduced ATR levels in β-PV HPV5 or 8 E6 expressing keratinocytes can increase the occurrence of UVB-induced double-stranded DNA breaks and thymine dimer

persistence [129] summarized in [90]. These data confirmed previous observations demonstrating a compromised repair of UV-induced thymine dimers in cell lines expressing β-PV E6 proteins [121]. The in vitro observations are also in line with the in vivo finding that HPV8 and 38 oncoproteins can significantly promote UV-induced tumorigenesis in transgenic mice [102, 105]. The fact that E6 enhances UV-induced mutagenesis may explain the accumulation of DNA mutations found in EV lesions including those within the p53 gene [87].

Thus, genus β-PV E6 proteins engage various strategies to promote tumorigenesis. At later stages of carcinogenesis, when E6 expression has promoted UV-induced genomic DNA alterations, p53 may itself be mutated and thereby inactivated. From this stage on, cellular mechanisms driving progression to cancer may dominate, and further persistence of the virus and maintenance of viral oncogene expression may become dispensable.

The β-PV E7 Protein

A key function of the mucosal high-risk E7 protein is seen in binding to and degradation of the retinoblastoma tumor suppressor protein pRb. The G_1-S phase checkpoint is bypassed, and cell cycle regulation is disrupted. This allows viral DNA replication in differentiating keratinocytes and contributes to the oncogenic activity. A recent systematic interaction analysis confirmed previous studies showing that genus α- and β-PV E7 proteins from different HPV species share the ability to bind to pRb as well as CUL3, a cullin-RING E3 ubiquitin ligase [130]. Most cutaneous E7 proteins bind pRb with lower affinities; however, HPV5 and 38 E7 were also shown to destabilize pRb [111, 112, 131].

β-PV E7 proteins may promote epithelial proliferation by further paracrine mechanisms altering the response to the local microenvironment. It has been demonstrated that the antiproliferative cytokine TGF-β is strongly upregulated in keratinocyte-fibroblast cocultures [132]. Keratinocytes expressing the E7 protein, however, showed strongly reduced responsiveness to TGF-β signaling. This was explained by their binding to Smad factors mediating the intracellular TGF-β signal. Again, this was a common feature of mucosal and cutaneous high- and low-risk HPV types [133, 134].

Another feature shared by the β-PV HPV8 and mucosal high-risk HPV16 but not the cutaneous low-risk HPV1 E7 protein is induction of the membrane-bound matrix metalloproteinase MT-1 MMP at mRNA and protein levels [135, 136]. There is a long list of MT-1 MMP substrates including MT-1 MMP itself, plasminogen, chemokines, cytokines, and growth factors promoting keratinocyte proliferation and angiogenesis (for review see [137]).

In addition, the genus β-HPV8 E7 protein may alter the microenvironment in a completely different manner. By binding to the transcription factor C/EBPβ in the granular layer, it specifically suppresses CCL20 expression and impairs Langerhans cell recruitment. This provides an explanation for the deficiency of Langerhans cells in EV lesions [67]. Thus, β-PV E7 proteins apparently do not directly promote carcinogenesis in vivo. However, it has been convincingly demonstrated that they can affect virus-host interactions critical for evading host immune defense and providing a microenvironment that is conducive for skin carcinogenesis.

Conclusions

Evidence is accumulating that cutaneous genus β-PVs are important cocarcinogens in UV-induced skin carcinogenesis. However, underlying mechanisms differ significantly from the carcinogenic process driven by high-risk mucosal genus α-PVs.

In the general population, β-PVs are found in the commensal skin flora. Their expression is tightly controlled by host restriction factors and extrinsic immunity. Patients with disturbed control mechanisms, i.e., mutations in restriction factors or impaired immune control, however, show higher disease penetrance, i.e., EV or

EV-like symptoms or development of skin cancer.

Once expressed, β-PV undergoes a life cycle that is highly adapted to the skin, UV exposure, UV damage, and an inflammatory host microenvironment. They expand the cutaneous epithelial progenitor cell compartment, which is highly susceptible to carcinogenic progression, disturb cutaneous immune homeostasis, fuel tumor-promoting inflammation, and lower the threshold to UV-induced DNA damage while promoting the life span of their host cells through preventing UV-induced apoptosis. This may lead to an enhanced accumulation of genomic mutations in infected cells. In vivo animal studies and ex vivo human studies imply that β-PV can act as powerful cocarcinogens at early stages of skin carcinogenesis. It is reasonable to assume that once genetic alterations, such as p53 mutations, have become established, the continuous presence of the virus may be dispensable for the maintenance of malignancy, compatible with a "hit-and-run" mechanism.

For the development of novel therapeutic strategies specifically interfering with β-PV at early stages of carcinogenesis, more research is needed to better understand the cross talk with their host keratinocytes and the local microenvironment.

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The Immune System and Pathogenesis of Melanoma and Non-melanoma Skin Cancer

11

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Abstract

Tumor development is the result of genetic derangement and the inability to prevent unfettered proliferation. Genetic derangements leading to tumorigenesis are variable, but the immune system plays a critical role in tumor development, prevention, and production. In this chapter, we will discuss the importance of the immune system as it relates to the development of skin cancer—both melanoma and non-melanoma skin cancers (NMSC).

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Brief Introduction to Skin Immunology

As part of the innate immune system, the skin functions primarily as a physical barrier to external pathogens [1]. If this barrier is broken, then a rapid, nonspecific innate immune response is initiated by cells residing in the skin. Subsequently, a slower yet more specific adaptive immune response will develop for definitive pathogen clearance. These immune responses include several immune cell types, divided into at least two different groups: skin-resident cells and recruited cells [1].

Within these two groups, the cells are further characterized as either innate or adaptive. Innate immune cells mount a non-antigen-specific response, whereas the adaptive immune cells mount an antigen-specific attack. The adaptive immune cells including B cells and T cells undergo genetic rearrangements to increase their antigen specificity, and this genetic rearrangement allows them to become long-lived and capable of a rapid, specific response when re-exposed to the same antigen (i.e., memory response). The skin-resident innate immune cells include Langerhans cells, macrophages, dermal dendritic cells, and mast cells. Adaptive, skin-resident immune cells include T cells (memory, natural killer, γδ). Recruited innate immune cells include granulocytes (e.g., neutrophils, eosinophils), natural killer (NK) cells, and monocytes. The recruited, adaptive immune cells include T cells and B cells [1]. In addition to fighting off

pathogens, the immune system is critical in the detection and destruction of tumor cells.

The adaptive immune response is the primary mediator of tumor control and consists of two arms, cellular (i.e., T cells) and humoral (i.e., B cells) but primarily relies on the cellular arm [2]. To initiate an adaptive immune response, antigens must be presented to T cells on human leukocyte antigen (HLA) molecules. HLA molecules are either class I or class II with class I presenting intracellular antigens and class II presenting extracellular antigens following phagocytosis. A cell specifically designed for antigen presentation is an antigen-presenting cell (APC) which can present via HLA I and HLA II. Any nucleated cell, however, can present an antigen to a T cell, but they primarily present via HLA I molecules [1, 2]. In the context of tumor surveillance and removal, cytotoxic T (CD8$^+$) cells will be presented to by APCs via HLA I. A co-stimulatory molecule, however, is required to optimize the response of the cytotoxic T cell, and these co-stimulatory molecules include CD28 on the T cells and B7.1 and 7.2 on APCs [2]. Antigen presentation and co-stimulatory interaction eventually lead to T-cell activation and clonal expansion specific to the presented antigen.

Helper T (CD4$^+$) cells are also involved and are engaged by their reaction with APCs through HLA II molecules. Once activated, helper T cells secrete cytokines that support both B cell and cytotoxic T-cell activities. Their activity also results in the development of memory T cells and regulatory T cells (CD4$^+$, CD25$^+$, FoxP3). Regulatory T cells are important to prevent unwanted collateral tissue injury that may occur if the immune system is not kept in check. It is through these processes of presentation and engagement of co-stimulatory molecules that the skin immune cells are able to effectively detect and address malignant transformation such as melanoma and non-melanoma skin cancers [2].

Immune System Failures and Cancer Development

The skin is an immunologic barrier containing many cell types found in secondary lymphoid organs [3]. The primary mediators of immunologic surveillance in the skin are dendritic antigen-presenting cells (APC) – CD1a$^+$ Langerhans cells in the epidermis and several other dendritic cells in the dermis [4]. These APCs travel to skin-draining lymph nodes, present to T cells, and induce a primary immune response [3]. To create an effective response against tumors, APCs must be able to stimulate an effective Th1-mediated immune response [5]. When immunosuppression occurs, APC function is impaired through local and systemic cytokine-mediated pathways.

The most common cause underlying Langerhans cells and APC impairment is ultraviolet radiation (UVR), which modifies the cell function and depletes the cell population [5, 6]. Ultraviolet B (UVB) in vitro has been shown to disrupt the ability of Langerhans cells to produce Th1-type cytokines while sparing production of the Th2 type [5]. UVR exposure leads to reduced number of APCs migrating into the skin-draining lymph nodes as well as their function [6]. The tumors themselves (e.g., melanoma) can also inhibit the differentiation of Langerhans cell precursors [7].

In addition to Langerhans cells, macrophages may also be impaired by UVR exposure. Macrophages that infiltrate the epidermis after UVR produce a significant amount of interleukin-10 (IL-10), an immunosuppressive cytokine, and produce a tumor-favorable state [8]. Macrophages can be divided into M1 and M2 macrophages. M1 macrophages produce nitric oxide synthase, IL-12, and tumor necrosis factor alpha (TNFα), whereas M2 produce IL-10, transforming growth factor (TGF-β), and prostaglandin E2 [5, 7, 9]. Of these two subsets, M2 macrophages are responsible for tumor

development through stimulation of angiogenesis and tissue remodeling. When a Th2-mediated response is present, NMSC will progress given low levels of interferon γ and high levels of IL-4 favoring M2 macrophage development [7, 9]. M1 macrophages are still present during a Th2-mediated response; however, their presence amplifies the Th2-mediated response by recruiting more M2 macrophages and further stimulating tumor growth [7]. These types of macrophages are supported by dermal neutrophils that secrete IL-4 and IL-10 after UVR exposure [5]. CD4$^+$ T cells will also downregulate interferon γ and IL-2 while increasing production of IL-4 and IL-5 further favoring tumor development.

An effective Th1-mediated immune response is required for tumor killing. However, tumors have the ability to evade detection by T cells. First, tumors downregulate MHC class I molecules which prevent recognition by the T cells [5]. Second, some tumors lack expression of CD28, which is necessary as a co-stimulatory molecule for T-cell activation [5].

Tumors themselves will potentiate their continued growth by suppressing Th1 cytokines while supporting the production of Th2 cytokines (e.g., IL-4, IL-5, IL-10) [10]. These responses subsequently stimulate a humoral immune response which is less effective against tumors [10, 11]. Another major mechanism of skin cancer development is prevention of apoptosis. There are two main ways tumors accomplish this: (1) prevention of CD8$^+$ T cell maturation and (2) derangement of the Fas-Fas ligand (FasL) interaction [5, 12, 13]. With regard to the Fas-FasL interaction, tumor cells become resistant to Fas/FasL killing by hijacking the interaction and promoting depletion of T-cell populations. Tumors downregulate their own Fas and upregulate their FasL, and T cells increase their expression of Fas. With the upregulation of Fas on activated T cells, the increased presentation of FasL on tumors stimulates T-cell apoptosis [5, 11].

Moreover, a regulatory system exists that may hinder clearing the non-melanoma skin cancers (NMSC) including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). For example, 50% of T cells in SCC are FOXp3$^+$ T regulatory cells which favor immunosuppression, with less number of CD8$^+$ effector T cells present. Myeloid dendritic cells are also unable to stimulate T-cell proliferation [14]. Cellular infiltrates in BCC show increased numbers of immature CD11c$^+$ myeloid dendritic cells which may create an overall immunosuppressed state since immature dendritic cells are believed to induce T-cell anergy [10, 14, 15].

In attempt to further characterize the role the immune system plays in cutaneous carcinogenesis, the remainder of this chapter will discuss skin cancer development in the setting of immunosuppression induced by ultraviolet radiation (UVR) and immunosuppressive therapies in organ transplant recipients (OTR). Additionally, we will also discuss the recent rise of immunotherapies in the treatment of malignant melanoma.

UV Radiation-Induced Immunosuppression and Skin Cancer

UVR-Induced Mutagenesis

The development of NMSC due to ultraviolet radiation (UVR) and its cumulative risk with increasing dose is well documented [16]. UVR also leads to malignant melanoma development [17, 18]. Melanoma genetic mutations reflect the level of UV exposure experienced by the afflicted anatomic location, where low UV exposure areas have lower rates of BRAF mutations and higher KIT mutations [18–20]. Skin sites with intermittent UV exposure (e.g., trunk) have higher BRAF and NRAS mutations and less KIT mutations [18, 20–22]. Areas of chronic UV exposure, such as the head and neck, exhibit their own distinct set of mutations [21]. Intermittent intense sun exposure is important for tumorigenesis in BCC and MM, whereas low level but chronic UVR exposure has been more important in SCC development [23].

UVR causes direct and indirect DNA damage and is a key mutagen in NMSC development [24]. UVR induces highly mutagenic cyclobutane

pyrimidine dimers (CPDs) between thymine (T) and cytosine (C) residues and pyrimidine photoproducts that promote C-T transitions [16, 25, 26]. These abnormalities are normally corrected by DNA repair enzymes, and if the damage exceeds repair capacity, the abnormal cells undergo apoptosis. When both of these methods fail, tumor development through uncontrolled cell proliferation is possible [16]. Murine studies have also demonstrated global DNA hypomethylation in keratinocytes after chronic UVR exposure, which may be related to inactivation of the p53 tumor suppressor gene and may contribute to malignant transformation especially given findings showing frequent inactivation of p53 in SCC [12, 23, 25].

UVR-Induced Immunosuppression

As UVR induces DNA damage, it concurrently promotes immunosuppression which further favors carcinogenesis. Murine research has shown resolution of UVR-induced skin cancers after transplantation into syngeneic mice with competent immune systems and growth when the mice are immunosuppressed [26–28].

UVR-induced skin cancers are highly antigenic, and research has demonstrated the transference of UVR-induced immunosuppression by transplanting antigen-specific CD4$^+$CD8$^-$ suppressor T cells into syngeneic mice [27]. These findings demonstrate that UVR suppresses cell-mediated immunity, and the immunosuppression is antigen specific [29, 30]. UVR also suppresses activation of T cells in skin-draining lymph nodes and impacts differentiation into memory T cells capable of producing interferon-γ [29]. UVR has also been implicated in interfering with antigen presentation leading to immune tolerance to sun-damaged cells, and the level of immunosuppression may be dose dependent [24, 30, 31].

Included in these APCs are Langerhans dendritic cells. UVR exposure destroys the network needed to travel to the local skin-draining lymph node [14, 27, 30]. When mice receive Langerhans cells exposed to UVR, there is long-lasting tolerance to specific tumor antigens, leading to

immunosuppression [27]. Langerhans cells normally present to both Th1 and Th2 T cell populations; however, UV-irradiated Langerhans cells present antigens to Th2 cells, which are not effective at combating tumor, while failing to stimulate Th1 cells which are important in combating tumor cells [27, 32]. Development of Th1 cell tolerance was also noted after antigen presentation by the UV-irradiated cells; restimulation by normal APCs was not possible [27].

UVR exposure leads to increased numbers of regulatory T cells and less effector T cells present in the skin. These regulatory T cells are specific to antigens encountered after UVR, and there is a shift toward T-cell-mediated immunosuppression [33]. The phenotype for these regulatory T cells is CD4$^+$, CD25$^+$, FOXp3$^+$, and CTLA-4$^+$, and these cells are cytotoxic for APCs, release IL-10, and suppress proliferation of other effector T cells that stimulate the immune system [26, 27, 29, 30, 33]. These regulatory T cells also express the dectin-2 receptor whose ligand, dectin-2, is expressed on Langerhans cells, and their association results in decreased Langerhans cell activity (i.e., immunosuppression). When a synthetic dectin-2 ligand was injected into mice, it bound to the receptor on regulatory T cells and suppressed immune suppression and tolerance [27].

Regulatory B cells are also activated after UVR exposure and may inhibit APCs as well. Activated regulatory B cells found in skin-draining lymph nodes after UVR exposure secrete IL-10, have B220 upregulation, and increased MHC class II expression [34].

There are skin chromophores (i.e., UV-absorbing molecules) that change conformation after UVR exposure and initiate immunosuppression. Major chromophores responsible for immunosuppression are located in the superficial and epidermal layers of the skin [26, 27, 29, 34]. One of these chromophores, trans-urocanic acid (trans-UCA), is a by-product of histamine deamination and is found in the stratum corneum [28, 32]. Trans-UCA is converted to cis-UCA after UVR exposure and induces immunosuppression, as evidenced by inhibition of APC

function [26–28]. This effect may be blocked by injection of a serotonin receptor antagonist [32].

UVR also affects monocytes, macrophages, and mast cells and renders them immunosuppressive [34]. After UVR exposure, mast cells secrete IL-10, and CD11b^{+} macrophages are specifically recruited to the skin resulting in defective antigen presentation and migration of APCs to the lymph nodes [28, 33]. Complement activation products such as C3 have also been shown to form after UVR, and the subsequent binding to its receptor, CD11b, on monocytes results in increased IL-10 and decreased IL-12 secretion [31, 34]. IL-12 and IL-23 production is also reduced by UVR, which are integral in T-cell activation for DNA repair [29, 35].

UVR and Melanomagenesis

UVR creates an immunologically appropriate microenvironment that specifically allows for melanoma development [36, 37]. UVR reduces Langerhans cell counts resulting in decreased antigen presentation and promotes an immunosuppressive type 2 cytokine response [38]. UVR-related mutations of the mitogen-associated protein kinase (MAPK) pathway like the V600E BRAF mutation also create an immunosuppressive microenvironment. Inhibitors of this pathway have been developed to block this response [38–45]. Prior to treatment with a BRAF inhibitor, there is a low-density of CD8^{+} T cells and granzyme B^{+} lymphocytes. After treatment with a BRAF inhibitor, the density of CD8^{+} T cells and granzyme B^{+} lymphocytes increases [45]. In murine models, melanoma expansion and metastases have been shown to be dependent on activated neutrophils recruited by high mobility group box 1 (HMGB1) secreted from UV-damaged epidermal keratinocytes and driven by toll-like receptor 4 [46]. Neutrophils stimulate angiogenesis and support melanoma migration, and this correlates with phenomenon previously described in human melanoma [46, 47].

Organ Transplantation, Immunosuppressive Therapies, and Skin Cancer

Immunosuppressive therapies promote cutaneous carcinogenesis, and the most robust data for this phenomenon come from organ transplant recipients (OTR), in which the most common malignancy is skin cancer (40%) [48–53]. Compared to the general population, the incidences of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are increased by 65-fold and tenfold, respectively, and the incidence of skin cancer continues to rise years after transplantation [49, 52–54]. Skin cancer incidence correlates with the degree of immunosuppression wherein patients with NMSCs have a lower total CD4^{+} T cell count, and rates of initial and subsequent skin cancers decrease with cessation or reduction of immunosuppression [12, 24, 49–51, 53, 55–59].

With respect to malignant melanoma (MM), the risk of immunosuppression in OTRs is less clear. Various studies have demonstrated at least a $\geq$ 3.4-fold increase in incidence; however, the largest study (n = 5356) evaluating incidence in OTRs showed no increased incidence [56, 60–63]. To further complicate the matter, melanoma is the most common tumor type (28%) inadvertently transplanted in OTRs [62, 64, 65]. A diagnosis of malignant melanoma may occur 6 months to 16 years after transplantation and is associated with a <5% overall 5-year survival rate [66–69]. Fortunately, studies in renal transplant recipients have shown that the host immune system can maintain dormancy of transplanted melanomas and destroy it if it is immunogenic and immunocompetence has been restored [50, 66].

Of several therapies used for immunosuppression in OTRs, calcineurin inhibitors (CNI) are the most strongly linked to skin cancer development. CNIs inhibit Langerhans cells and dermal dendritic cells as well as T-cell signaling and proliferation [70–76]. In psoriasis patients exposed to UVA radiation, the risk of SCC is increased with the use of cyclosporine, and skin cancer has been shown to develop in a dose-dependent manner in

Table 11.1 Immunosuppressive therapies for prevention of organ transplant rejection

Immunosuppressive therapy	Mechanism of action
Cyclosporine [55, 77, 78]	Inhibits calcineurin by complexing with an intracellular protein cyclophilin and subsequently prevents transcription of interleukin-2 and activation of T cells
Tacrolimus [80–82]	Inhibits calcineurin by complexing with an intracellular protein FKBP-12 and subsequently prevents transcription of interleukin-2 and activation of T cells
Azathioprine [78, 83, 84]	Metabolites inhibit purine synthesis and are also incorporated into DNA halting its replication and leading to decreased T-cell development and results in immunosuppression
mTOR inhibitors (e.g., everolimus; sirolimus) [89–99]	Complexes with an intracellular protein to inhibit the protein kinase mTOR which inhibits T-cell proliferation by preventing progression from the G1 phase to S phase in the cell cycle

renal transplant recipients taking cyclosporine [57, 77, 78]. Some studies have implicated ATF3 induction due to CNI use and a bypassing of senescence allowing for tumor development [76, 79]. Tacrolimus, while also linked to an increased risk of skin cancer, shows a lower risk than cyclosporine particularly earlier on posttransplantation [80–82].

Azathioprine is associated with increased skin cancer risk as well, especially SCC. The relationship with SCC is possibly due to azathioprine's ability to increase photosensitivity and UVA-mediated mutagenesis through incorporation of the 6-thioguanine metabolite into DNA [78, 83, 84]. In renal transplant recipients, keratinocytes have been shown to have an increased number of mutant p53 tumor suppressors which corresponds to decreased DNA repair activity and an increased risk of carcinogenesis [85]. Some data suggest a dose-dependent risk of azathioprine for SCC development, which may be even higher than cyclosporine [86].

Mammalian target of rapamycin (mTOR) inhibitors are used for immunosuppression in OTRs because they inhibit T-cell proliferation in response to interleukin-2 [87, 88]. However, they also have direct antitumor properties (i.e., temsirolimus, everolimus) [89]. mTOR is a protein kinase that regulates protein translation, cell survival, cell growth, cell proliferation, cell motility, and secretion of angiogenic factors [90–99]. It is through inhibition of these activities that mTOR suppresses carcinogenesis and subsequent metastasis. The use of mTOR inhibitors, sirolimus and everolimus, as alternatives to

cyclosporine or as maintenance therapy in renal transplant recipients is associated with less skin cancer development and even regression of skin cancers which were present prior to mTOR inhibitor initiation [100–106]. Table 11.1 summarizes the mechanism of action of the abovementioned immunosuppressive therapies.

Alternative forms of immunosuppressive therapies (i.e., biologics) do exist; however, they are used primarily for autoimmune diseases like psoriasis rather than prevention of transplant rejection. Of these biologics, inhibitors of TNFα, IL-17, IL-12/IL-23, and IL-23 are most important to dermatologists. Inhibitors of TNFα have been associated with rapid NMSC development and even with a resurgence of latent metastatic melanoma [107–115]. It has been postulated that this association is related to cancer immunosurveillance disruption and a favoring of a Th2 immune profile. Meta-analyses of randomized controlled trials of TNFα inhibitor use in psoriasis on the other hand have shown that there is no increased risk of malignancy, but this may not hold true in other patient populations [116].

Inhibitors of IL-12/IL-23 or IL-23 alone may also increase skin cancer risk in the setting of UVR given the association of these interleukins and their roles in reducing UVR-induced DNA damage [117]. IL-12 and IL-23 share the same subunit, p40, but IL-12 only contains p35, and IL-23 only contains p19 [117–119]. In mice that lack p40, there is an increased risk of developing skin cancer upon chronic UVR exposure; however, this may be more related to the lack of IL-23 than IL-12. When mice lacking either p35 or p19

were exposed to chronic UVR, only the mice lacking p19 had a significantly increased risk of developing a tumor [117, 120]. These findings suggest that providers treating with biologics that target the p40 and p19 subunits may need to increase their screening for skin cancers, especially in those patients who have been chronically exposed to UVR.

IL-17 inhibitors such as secukinumab and ixekizumab, to date, have not shown a statistically significant increased risk of skin cancers [121–123]. In fact, research into IL-17 and its connection to cutaneous malignancy may suggest a possible protective effect. SCC and BCC tumor infiltrates have high infiltrates of Th17 lymphocytes, and proliferation of these tumor types is supported in vitro and in vivo by IL-17. IL-17 promotes upregulation of IL-6, IL-8, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which are needed for tumor progression, and it enhances human NMSC growth in mice injected with CAL27 [124]. When IL-17 knockout mice are exposed to the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) followed by 12-O-tetradecanoylphorbol-13-acetate (TPA), tumorigenesis was reduced [125]. These findings offer a possible therapeutic benefit to patients especially those historically treated with PUVA wherein NMSC incidence rates are increased [126–128]. However, further research is required to make concrete recommendations for clinicians.

Immunotherapy and Melanoma

Melanoma is a highly immunogenic tumor, likely due to its high rate of carcinogen-induced (e.g., UVR) point mutations [129]. This high frequency lends itself to development of many novel antigens that can be presented to T cells and elicit an immune response [130]. In an attempt to harness this immunogenicity, treatments such as intratumoral injections of bacteria or viruses and inactivated tumor vaccines have been developed and used [131]. When these approaches result in a response, generally ≤10% of the time, the response tends to be durable [132].

Cytokine therapy with IL-2 historically was one of the first attempts at immunotherapy in patients with melanoma. IL-2 is primarily secreted by antigen-stimulated CD4$^+$ T cells. It stimulates cell growth and cell differentiation of CD8$^+$ T cells and is responsible for maintenance of regulatory CD4$^+$ T cells [133]. It was generally used in otherwise healthy patients, and the median overall survival was 9 months [134]. There was modest clinical benefit and significant toxicity [135]. Since then, new immunotherapies have been developed that are directed at cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1), T-cell stimulation, and use of a modified oncolytic herpes virus—talimogene laherparepvec (T-VEC) [136].

Recent advances in immunomodulatory cancer therapy have aimed at stimulating the antitumor response by preventing the inhibition of activation (i.e., anergy) [137]. One inhibitor of T-cell activation is CTLA-4, which is a cell surface molecule homologous to the positive co-stimulatory molecule, CD28. CTLA-4 competes with CD28 for binding to B7.1 and B7.2 on APCs. If CTLA-4 is blocked, then T cells are allowed to proliferate and maintain their activity [131]. This mechanism is exploited by the monoclonal antibody, ipilimumab, which has been shown to improve overall survival in patients with metastatic melanoma, and the response appears durable after 2–3 years [138–140]. Unfortunately, the use of ipilimumab has been shown to result in a significant risk of auto-inflammatory effects including dermatitis, diarrhea, colitis, hepatitis, uveitis, and hypophysitis [138, 139].

The immune system has also been harnessed for the treatment of melanoma through prevention of immune cell apoptosis. The programmed death 1 (PD-1) receptor negatively regulates T cells and is expressed in response to chronic antigen exposure. Its ligand, programmed death ligand 1 (PDL-1), is expressed directly by tumor cells, which makes PD-1/PD-L1 blockade a more specific and appropriate target for antitumor therapy [141]. Nivolumab is one such monoclonal antibody that blocks PD-1 and has been associated

with significant and durable responses in patients with metastatic melanoma; however, a life-threatening pneumonitis may develop [142–144]. Adverse effects, however, were lower in frequency and seriousness compared to ipilimumab [143–146]. Pembrolizumab is an alternative PD-1 inhibitor used in melanoma as well and is similarly effective [147]. Blockade of PD-L1 has also been attempted using a monoclonal antibody (BMS-936559) that had less side effects than nivolumab but has not been shown to be as objectively efficacious [148]. A synergistic effect has been demonstrated when PD-1 and CTLA-4 inhibitors are used together [149–152].

The goal of these two previous types of immunotherapy is intratumoral infiltration by activated T cells that are cytotoxic to the tumor. When melanomas demonstrate a robust infiltration of lymphocytes histologically, there is an associated reduced risk of metastasis [136]. Newer immunotherapeutic approaches have attempted to directly generate this phenomenon via adoptive cell transfer (ACT), wherein large quantities of activated T cells are generated ex vivo and then infused back into the patient. This process begins when T cells are harvested from the tumor environment. They are harvested from the tumor because they are antigen specific but have been suppressed functionally by the tumor environment. Once the T cells are removed from the tumor, they can be expanded, activated, and reinfused. Prior to reinfusion, the tumor environment is prepped by depleting endogenous lymphocytes with chemotherapy and creating an environment conducive to their success and proliferation [153]. Another method of ACT is one where activated T cells are expanded from peripheral blood after repetitive antigen exposure ex vivo [154].

Another recently approved immunotherapy is T-VEC which has been approved in patients with advanced stage, unresectable melanoma [136]. T-VEC is a genetically modified herpes virus that is injected into the melanoma and is engineered to selectively replicate in tumor cells and produce granulocyte-macrophage colony-stimulating factor (GM-CSF) [155]. The virus itself induces an immune response, and the GM-CSF enhances antigen presentation to local macrophages [156]. The virus and tumor cells are then better recognized by T cells that can stimulate an immune response against the virus and the tumor. When injected into a melanoma, a response is seen in the tumor itself as well as adjacent tumors and distant metastases [155, 157]. T-VEC injection has been shown to have a more durable response than GM-CSF injection alone which suggests the importance of both the presence of the virus and expression of GM-CSF. Toxicities seen with T-VEC have been modest. Adverse events experienced by subjects include inflammation at the injection site and short-lived fevers and chills. Autoimmune toxicities similar to those of CTLA-4 and PD-1 inhibitors have not been seen [157]. Currently, studies investigating combination therapy with T-VEC and checkpoint inhibitors like ipilimumab and pembrolizumab are underway [158, 159]. Evidence shows that the efficacy of checkpoint inhibitors is limited to melanomas with a high baseline infiltrate of $CD8^+$ T cells; therefore, T-VEC may be used to increase the level of $CD8^+$ T cell infiltrates prior to or in tandem with checkpoint inhibitor treatment [160, 161]. Table 11.2 summarizes the current immunotherapies discussed above and their mechanisms of action.

Conclusion

The human immune system functions not only to protect us from pathogens but also to prevent tumor development and eradication of malignant cells. A complex interplay between the immune system, tumor cells, and molecular mediators dictates whether or not the immune system will be successful at this task. At this time, research has not uncovered a single sentinel event that leads to tumor evasion of the immune system and its subsequent proliferation, spread, and ultimately death of the host. Our current understanding of immunosuppression by UVR and cancer development in OTRs has allowed us to harness the immune system via employing immunotherapies to treat skin malignancies. Continued scientific research to expand our

Table 11.2 Immunotherapies used for the treatment of malignant melanoma

Immunotherapies	Mechanism of action
Interleukin-2 [133]	Stimulates cell growth and cell differentiation of CD8$^+$ T cell and maintains regulatory T cells
Ipilimumab [138–140]	Monoclonal antibody that binds CTLA-4 and results in T-cell proliferation and maintains anticancer activity
Nivolumab [142–144]; Pembrolizumab [147]	Monoclonal antibodies that bind PD-1 and prevent tumor-induced apoptosis of T cells
BMS-936559 [148]	Monoclonal antibody that binds PD-L1 and prevents tumor-induced apoptosis of T cells
Adoptive cell transfer [153]	Autologous, antigen-specific T cells from the tumor microenvironment are removed, activated, and expanded ex vivo, and reinfused
Talimogene laherparepvec (T-VEC) [155–157]	Genetically modified herpes virus injected into the tumor and results in a viral-induced immune response as well as increased GM-CSF secretion that results in an increased macrophage response

understanding of the immune system, its role in carcinogenesis and skin cancer-related mutations, will continue to impact our approach and improve management of patients afflicted by cutaneous malignancies.

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Protection from Ultraviolet Damage and Photocarcinogenesis by Vitamin D Compounds

12

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Abstract

Exposure of skin cells to UV radiation results in DNA damage, which if inadequately repaired, may cause mutations. UV-induced DNA damage and reactive oxygen and nitrogen species also cause local and systemic suppression of the adaptive immune system. Together, these changes underpin the development of skin tumours. The hormone derived from vitamin D, calcitriol (1,25-dihydroxyvitamin D_3) and other related compounds, working via the vitamin D receptor and at least in part through endoplasmic reticulum protein 57 (ERp57), reduce cyclobutane pyrimidine dimers and oxidative DNA damage in keratinocytes and other skin cell types after UV. Calcitriol and related compounds enhance DNA repair in keratinocytes, in part through decreased reactive oxygen species, increased p53 expression and/or activation, increased repair proteins and

increased energy availability in the cell when calcitriol is present after UV exposure. There is mitochondrial damage in keratinocytes after UV. In the presence of calcitriol, but not vehicle, glycolysis is increased after UV, along with increased energy-conserving autophagy and changes consistent with enhanced mitophagy. Reduced DNA damage and reduced ROS/RNS should help reduce UV-induced immune suppression. Reduced UV immune suppression is observed after topical treatment with calcitriol and related compounds in hairless mice. These protective effects of calcitriol and related compounds presumably contribute to the observed reduction in skin tumour formation in mice after chronic exposure to UV followed by topical post-irradiation treatment with calcitriol and some, though not all, related compounds.

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Introduction

Sun exposure produces both benefits and harms. The damaging effects of UV were reported as early as 1894 [323], and UV damage is a major cause of skin cancer.

Several studies have shown that 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] provides protection against UV-induced DNA damage as well as reduces photocarcinogenesis. The functions of 1,25(OH)$_2$D$_3$ and related compounds in reducing UV-induced DNA damage, including oxidative stress damage and immune suppression, as well as UV-induced skin tumours are discussed below.

Direct DNA Damage by UVB

Although UV radiation is essential for vitamin D biosynthesis, amongst other functions, it is mutagenic and carcinogenic [108]. UV exposure triggers DNA damage. If this damage is not properly repaired, it may lead to mutations, mitochondrial damage, inflammatory cascade, immunosuppression, protein and lipid oxidation and photo-aging [10, 28, 31, 191, 271]. There are protective mechanisms in the skin to reduce UV damage, most of which are induced by UV exposure. These include increased production of melanin that is transferred to keratinocytes to form a shield over the nuclear DNA of the keratinocyte and increased thickness of the stratum corneum to reduce UV transmission through the skin [33, 213].

DNA bases directly absorb photons within the UVB range (290–320 nm). Upon UV absorbance, adjacent pyrimidine bases of the same DNA strand form dimeric photolesions, as reported by Beukers and Berends in 1960 [23, 283]. The most prevalent UV-induced lesions are the *cis-syn* cyclobutane pyrimidine dimers (CPDs), mostly formed between the 5 and 6 bonds of adjacent thymine and cytosine pyrimidines (Fig. 12.1-a). The most common form of CPD are thymine dimers (T-T), which are present in numbers proportional to total CPD of all types [69]. Although T-T are not in theory mutagenic due to

incorporation of the 'A' residue and subsequent restoration of the initial A-T sequence by DNA polymerase, they have been shown to cause mutations in practice [129, 222]. Thymine-cytosine dimers and cytosine-cytosine dimers are highly mutagenic [54, 129].

Such solar signature mutations are found in the p53 genes of many UV-induced cancer cells [54, 186]. The main function of p53 is to induce cell cycle arrest, facilitate DNA repair processes and induce apoptosis for cells that are beyond repair [265]. Mutated p53 gene loses its capacity to induce apoptosis in damaged cells [127, 244, 349]. As a result, these cells may replicate their damaged DNA and progress to cancer cells [4, 31, 129].

The second most common UV-induced photolesions are 6–4 photoproducts (6–4PPs) (Fig. 12.1-a), initially reported by Johns et al. in 1964 [138, 206, 247]. The 6–4PPs have a stable bond between positions 4 and 6 of adjacent pyrimidines, and further UV irradiation around 313 nm isomerizes this to form a Dewar product [46]. The presence of CPDs in the skin after UV exposure is five- to tenfold higher than 6–4PPs [73, 85, 129, 208]. CPDs are more obscured in the nucleosomes causing less helix distortion, while 6–4PPs cause more distortion in the DNA strands [3, 210, 274, 312]. As a result, in humans, 6–4PPs are easily recognized by DNA damage recognition proteins, less mutagenic and more effectively repaired than CPDs [32, 207–209].

Other minor direct UV lesions are pyrimidine mono adducts and purine dimers. Unlike CPDs, these lesions are less likely to contribute to mutagenesis and carcinogenesis [312]. Recent studies have shown that UVA radiation induces CPD production but not 6–4PPs, indicating that both UVB and UVA radiation may contribute to direct DNA damage [214].

Indirect DNA Damage by Oxidative Stress

Indirect DNA damage or oxidative damage to purine bases contributes to mutagenesis, cancer, aging and other pathological conditions [8, 9,
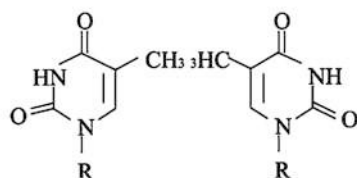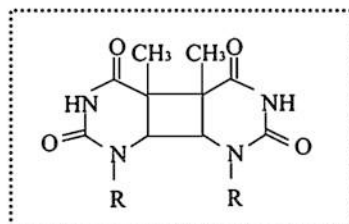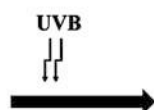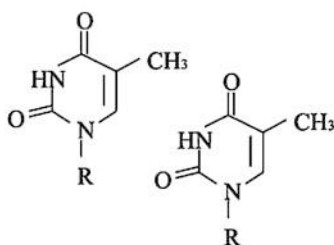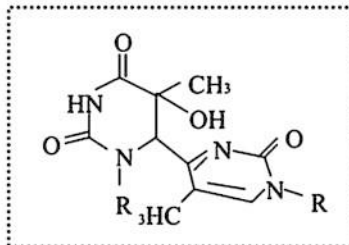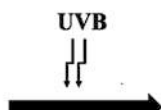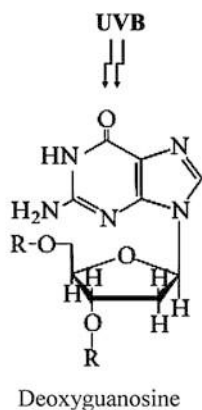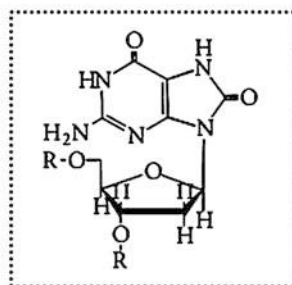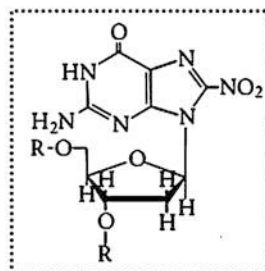
Fig. 12.1 UV-induced DNA damage. (**a**) Under UV radiation, cyclobutane pyrimidine dimers (CPD) or 6–4 photoproducts (6-4PP) form between adjacent pyrimidine bases [129]. (**b**) Formation of 8-oxoguanine and 8-nitroguanine under UV radiation [218]

109]. The main endogenous agents are free radicals such as reactive oxygen species (ROS) [87, 158] and reactive nitrogen species (RNS) [197] (Fig. 12.1-b).

ROS are ubiquitous oxidizing molecules produced by either endogenous metabolic reactions, such as cellular respiration (electron transport chain), or external factors, such as UV exposure or carcinogenic chemicals. Under normal metabolic conditions, 5% of the oxygen consumed in mitochondria is converted to ROS [28, 262]. Usually, the level of free radicals is controlled by innate defence mechanisms such as intrinsic antioxidant enzyme systems [215, 271, 326] and scavenger molecules [320–322]. Exposure to UV radiation of at least one minimal erythemal dose (MED), the amount of UV that produces faint redness of the skin, increases ROS production and depletes antioxidants, resulting in an increased ROS-to-antioxidant ratio, considered to be the main contributor to the formation of photolesions [161, 251, 271, 327]. Types of ROS include singlet oxygen which is highly reactive and toxic [87], hydroxyl radical, hydrogen peroxide and superoxide anion [67, 89]. These free radicals mainly target guanine in the DNA, and this oxidation reaction produces the main photolesion, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoguanine/8-OHdG) [5, 40, 102, 143, 144]. 8-OHdG is used as a biomarker for DNA damage by oxidative stress. Increased levels were observed in 50–90% of cancer cells [129, 337]. Under normal conditions, tissues produce around 10^3 8-OHdG lesions per cell/per day, but 8-OHdG lesions in cancer tissues are around 10^5 per cell/per day [171, 327]. Oxidative stress may cause non-canonical base pairing and incorrect pairing by DNA polymerase, leading to mutations [266, 270]. ROS affect different areas of the cell, including gene mutations [114], DNA strand breakage [176], shortening of telomeres [288], mitochondrial damage [28], structural damage (organelle membranes) and functional protein damage, all of which may contribute to carcinogenesis [18, 31, 75, 239].

Under normal physiological conditions, nitric oxide (NO) is a ubiquitous molecule that regulates physiological functions in the cardiovascular and nervous systems and has been reported to reduce blood pressure on release from the skin after UV [338]. Rapid reaction of NO with free radicals such as superoxide anion produces RNS [20, 98, 246]. Several types of RNS have been identified; these include peroxynitrite, higher oxides of nitrogen, S-nitrosothiols, nitroxyl and nitrosonium cation [62, 226, 253]. RNS cause nitrosative stress, which is closely related to oxidative stress. Oxidative reaction of peroxynitrite with guanine bases forms the main photolesion, 8-nitroguanine (8-NG) [37, 158, 189, 197] or 8-OHdG [197]. While the presence of higher concentrations of ROS induces oxidative stress, ROS have also been shown to react with nitric oxide to induce nitrosative stress [173]. Therefore, RNS contribute indirectly to DNA damage and directly to structural and functional modifications of proteins. Peroxynitrite and nitrogen oxide mainly cause DNA damage by base modification and DNA stand breaks including double-strand breaks [172]. NO affects proteins by nitrosylation of tyrosine and cysteine amino acids which have been shown to inactivate enzymes [188], such as specific nitrosylation of DNA repair pathway proteins, which reduces the removal of photolesions and increases the probability of photocarcinogenesis [18, 134, 229, 341].

Photoimmunosuppression

UV-induced immune suppression increases susceptibility to skin cancer [150, 154]. DNA damage and subsequent mutations produce neoplastic cells, but these are removed by the normal immune system unless there is concomitant immune suppression [56, 77, 103, 346]. Solar radiation consists of around 94% UVA and 6% UVB [63]. UVB-induced immunosuppression (peak immunosuppressive effectiveness at 300 nm) has a linear dose response at shorter

UVB wavelengths [196], while UVA-induced immunosuppression (peak immunosuppressive effectiveness at 370 nm) has a Gaussian-shaped dose response [38, 52]. In mice, the role of UVA in immune responses, where UVA may be protective [257], may be different from those seen in studies in human subjects, where UVA contributes to immune suppression [104]. UVA-induced immunosuppression is threefold higher than UVB-induced immunosuppression at sun exposures similar to those obtained during normal daily activities in summer in human subjects [52, 104].

Immunosuppression is mediated by several mechanisms in the skin including CPDs [13, 154, 157], ROS [125], skin immune cells [74] and urocanic acid [55, 233]. UV radiation triggers local changes in skin immune cells, including Langerhans cells (LCs), by causing cellular damage [1, 329], apoptosis (high UV dose) [74, 220] or alterations in their migration to lymph nodes (low UV dose) [53].

One or a combination of these factors may affect cellular immune function in antigen presentation [1, 219, 273]. ROS have been also shown to induce immune suppression by reducing antigen presentation in UV-irradiated XS52 cell lines (derived from epidermis of newborn BALB/c mice) [39]. UV-induced CPDs are found in epidermal keratinocytes, LCs and dendritic cells in lymph nodes drained from irradiated areas of female albino HRA/Skh hr/hr mice [300, 329]. These CPDs induce keratinocytes to secrete immunosuppressive cytokine interleukin-10 (IL-10) [140, 231, 263] and transforming growth factor-β to induce systemic immunosuppression [186, 235, 300]. Studies have also shown that the UV-induced sunburn reaction releases IL-6 in human primary keratinocytes, which has an immunosuppressive effect [57, 248]. The contact hypersensitivity (CHS) response in IL-6$^{-/-}$ or IL-6$^{+/+}$ mice showed increased IL-6 and IL-10 expression in IL-6$^{+/+}$ mice after UV radiation, but no increase in IL-10 was observed in IL-6$^{-/-}$

mice [232]. In addition, UVB results in increased secretion of IL-1, tumour necrosis factor-α (TNF-α) [153], death receptor Fas [120], platelet-activating factor [225], prostaglandin (COX-2) [225] and IL-4 [287] that all contribute to immune suppression.

Another molecule involved in immunosuppression is urocanic acid (UCA), a metabolic product of the amino acid histidine, found in the outer layer of the epidermis [104, 146]. Under normal conditions, UCA molecules exist in the *trans*-conformation [233]. UV radiation converts them to *cis*-UCA [55], which directly affects the activity of LCs by suppressing contract hypersensitivity (CHS) and inducing secretion of IL-10, leading to LC-dependent immunosuppression [31, 200, 235, 264].

DNA Damage Response in Eukaryotes

Following the detection of erroneous DNA, cell cycle arrest is initiated to interrupt or delay replication, thereby allowing DNA repair to occur. If the damaged DNA can be repaired, DNA repair pathways are initiated, but if the DNA or organelle damage is severe, cells undergo apoptosis [126, 166, 242]. The wide variety of mechanisms are collectively known as DNA damage responses (DDR), which include increased p53 protein, DNA damage check points, DNA repair and apoptosis [270] (Fig. 12.2).

The cellular repair mechanisms in vertebrates are highly complex and sensitive involving many regulatory proteins. Mammalian cells have several DNA repair mechanisms which include nucleotide excision repair (NER) [261, 284], base excision repair (BER) [167, 169, 266], mismatch repair (MMR) [211, 331, 339], DNA double-strand break (DSB) repair [139, 259, 305], post-replication repair (PRR) [145] and cross-link repair [16, 48, 344]. Defects in one or more of these mechanisms are detrimental for genetic stability [270].
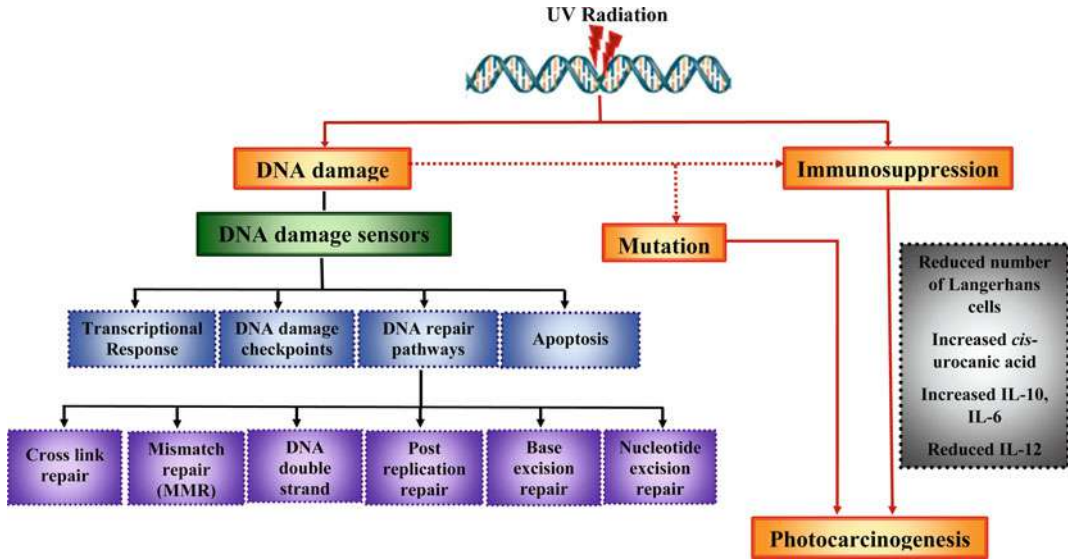
Fig. 12.2 UV radiation leads to DNA damage, DNA damage response and cellular repair mechanisms. Unrepaired damaged DNA and immunosuppression may lead to photocarcinogenesis. (Edited from [270])

DNA Repair Pathways

Nucleotide Excision Repair (NER) Pathways

Bulky photolesions such as CPDs and 6–4PPs are removed by nucleotide excision repair pathway (NER), which is a conserved multistep process involving around 30 proteins [2, 59, 107, 187, 216, 344].

This process involves the following steps: initial DNA damage recognition, unwinding of the DNA strand around the damaged lesion, incision and removal of the damaged DNA and replacement with newly synthesized DNA [350]. Two sub-pathways of NER have been identified: transcription-coupled nucleotide excision repair (TC-NER) [30, 203] and global genomic nucleotide excision repair (GG-NER) [202, 203].

The main difference between TC-NER and GG-NER lies in the initial damage recognition step. Damage recognition proteins are highly specific for each sub-pathway and have the ability to identify the small areas of damaged DNA from the vast amount of undamaged DNA. The initial damage sensor for TC-NER is RNA polymerase II, which recognizes lesions while routinely performing DNA transcription [68]. Cockayne

syndrome protein A (CSA) and B (CSB) are also required for damage recognition and progression in TC-NER [84, 97, 224]. GG-NER repairs photolesions from any position of the genome using several damage recognition proteins as initial damage sensors [350]. Many damage recognition proteins have high affinity for the damaged DNA and work collectively in the initial stages of the pathway.

Although the damage recognition is different for TC-NER and GG-NER, the NER repair pathways converge at Xeroderma Pigmentosum (XP) complementation group A (XPA) protein, and repair from there is similar for both, as discussed below [187]. Two key damage recognition proteins for GG-NER are XP complementation group C (XPC) protein and UV-DNA damage binding (DDB) protein [303, 304]. UV-DDB is involved in the initial damage recognition stage of the GG-NER pathway. Human UV-DDB protein consists of two subunits: DDB1 (XPE binding factor) and GG-NER specific-DDB2. DDB1 (*p127*) gene encodes a 127 kDa protein subunit, and DDB2 (*p48*) gene encodes a 48 kDa protein subunit. Following UV radiation, the two subunits form a heterodimer [148, 230, 303] which then binds

directly to the DNA strand of the UV damaged lesion [44]. The efficiency of NER may depend on the expression levels of cellular DDB2 [324]. Both XPC and DDB2 have higher affinity to damaged DNA than undamaged DNA [17, 44, 254, 302, 342], and UV-DDB has 100- to 1000-fold higher affinity for UV-damaged lesions than XPC [91, 221].

Within the UV-DDB heterodimer, the specific function of DDB1 is unclear. Current research indicates that DDB1 functions as an adaptor molecule between Cullin 4A (Cul4A) and CULA-associated factors (DCAFs) in the process of ubiquitination [12, 130, 304]. In unirradiated cells, DDB1 is mainly located in the cytoplasm, but after UV exposure, it dimerizes with DDB2 to form UV-DDB [97]. UV-DDB forms a super complex with Cullin 4A, Roc1 and COP9 signalosome and functions as an E3 ligase complex [6]. The UV-DDB-E3 ligase complex is inactive in unirradiated cells [6, 97]. After UV exposure, the UV-DDB-E3 ligase complex translocates to the nucleus, binds sites of DNA damage sites and activates ubiquitin ligase in the complex to facilitate the ubiquitination of several proteins including DDB2 itself, XPC and histones [97, 141, 303]. Auto-ubiquitination and proteasomal degradation of DDB2 abolishes the damage recognition capacity of UV-DDB [76, 97, 195, 303]. Under certain conditions, accessibility of damage recognition proteins to the damaged sites are hampered by nucleosomes [183, 201]. Ubiquitination of the above-mentioned molecules may cause conformational changes in the nucleosome, allowing better access for XPC and other repair proteins to the damaged sites [221, 274, 334].

This conservative and highly specific pathway ensures that the UV damaged photolesions are removed from the genome. Otherwise, if the damage is extensive, cells undergo apoptosis [219]. Any deficiencies or mutations in damage recognition proteins in humans give rise to autosomal recessive diseases such as xeroderma pigmentosum, related to defective GG-NER [328], or Cockayne syndrome, related to defective TC-NER [217]. Individuals with these conditions have high sensitivity to sunlight and generally show a higher susceptibility to skin cancer after UV exposure [83].

The enzyme poly (ADP-ribose) polymerase-1 (PARP-1) is also involved in the initial stages of DNA lesion recognition [70]. This enzyme is activated when DNA strand breaks occur under UVB or UVC irradiation [330]. Studies have shown that both inhibition of PARP-1 in human fibroblasts and depletion of PARP-1 in mouse epidermis reduced DNA repair at least in some models, though other PARP proteins may substitute for PARP-1 in some cases [194, 250, 313, 332]. Further, topical application of the PARP-1 inhibitor, 3-Aminobenzamide, on a UV-exposed hairless mouse was shown to increase carcinogenesis [72].

Antioxidant Defences

As mentioned earlier, innate protective mechanisms include antioxidants and scavenger molecule systems. Antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase are reduced 5 days after UV exposure, indicating the negative effect of UV on the regulatory antioxidant system [161]. Another scavenger molecule affected by UV is metallothionein (MT). MT is an intracellular cysteine-rich, metal-binding protein found in many organisms [117, 185]. It has a vital function in metal homeostasis [285] and has a free radical scavenger [309]. UV-irradiated, MT-null mice exhibited higher numbers of apoptotic sunburn cells than wild-type mice indicating the necessity of MT for defence against UV-induced damage [105].

Base Excision Repair (BER)

DNA damage caused by oxidative stress [143, 170], alkylation damage [275] and deamination [147, 240] is repaired by the highly conserved base excision repair (BER) pathway [5].

The BER process is more rapid than NER and involves four repair proteins [156, 168]. Both initial damage recognition and removal of the damaged bases are carried out by a DNA glycosylase. Following the initial damage recognition, a DNA glycosylase catalyzes the cleavage of the N-glycosidic bond between the base and

2'deoxyribose sugar molecule to remove the damaged base [155, 160]. The removal of the damaged base from the strand forms an abasic site known as an apurinic/apyrimidinic (AP) site [167, 255]. The DNA backbone of the abasic site is incised by a DNA AP endonuclease to form 5′ single-strand nick at the AP site [131]. The AP nuclease removes the damaged base at the nick to form a gap in the DNA strand. DNA polymerase β fills the gap with a complementary DNA base [252, 297]. Finally, DNA ligase completes the repair by sealing the nick of the DNA helix [236, 266, 267, 347].

BER pathway is either long patch (2–6 nucleotides gap filling) [88] or short patch (one nucleotide gap filling) [156]. The initial steps are common but differ at the AP site. The resynthesis step involves DNA polymerase β in the short-patch BER [236], while it involves PCNA or polδ/ε in the long-patch BER [88]. The main ROS product, 8-OHdG, is removed by 8-oxoguanine-DNA glycosylase 1 enzyme (OGG1) [81, 152, 325]. This enzyme is more abundant in the outer epidermis layers than in the basal layers [136], likely due to the fact that the outer epidermal layers are naturally exposed to a higher amount and intensity of UV radiation than the basal layers. However, this distribution of OGG1 may reduce the oxidative damage repair in the replicating basal layers of the skin [136] and thus make these cells more susceptible to oxidative stress.

Compounds that Reduce Photolesions in the Skin

Reduction of Photo Lesions

Several studies have shown that vitamin D and related metabolites reduced UV-induced CPDs [58, 64, 66, 94, 311, 343]. The reduction of UV-induced DNA damaged by these compounds has been reported following immunohisto-chemistry, using antibody detection of the lesions, and image analysis [58, 162, 282, 343]. Confirmation of these reductions in both CPD and 8-OHdG with vitamin D compounds

has been made using the Comet assay, which uses endonucleases to identify and cut DNA at sites of specific lesions, followed by gel electro-phoresis [49, 94, 137]. These reductions in UV-induced CPDs by $1,25(OH)_2D_3$ were shown to result from increased DNA repair [269]. The studies showing reductions in UV-induced DNA damage have been carried out on several cell types such as human keratinocytes [58, 162, 282], melanocytes [64, 343] and fibroblasts [65, 343] and in Skh/hr1 mice [64, 66, 100, 151], human skin ex vivo [299] and human volunteers [51]. These studies reported that low concentrations of $1,25(OH)_2D_3$ reduced CPDs, 8-OHdG and 8-NG, even when added only after UV exposure.

Mechanisms of Action

In relation to mechanisms, $1,25(OH)_2D_3$ was shown to increase the expression of the radical scavenger metallothionein in UV-irradiated mouse skin, which may be a major contributor to decreased oxidative DNA damage [142, 162]. In keratinocytes and melanocytes, UV radiation was shown to increase ROS, in part via mitochondrial damage [10, 269] and RNS [62, 61, 268]. After post-irradiation treat-ment of keratinocytes with $1,25(OH)_2D_3$, there was a significant reduction in ROS levels as early as 15 minutes [269] and NO products as early as 30 minutes [66, 100, 282, 298]. The Nrf2-Keap1 antioxidant pathway is activated by $1,25(OH)_2D_3$ under some conditions, and that may also contribute to reduced oxidative products [22, 223] (Fig. 12.3).

Several lines of evidence show that NO and other RNS have inhibitory effects on NER repair proteins, which may result in the disruption of CPD repair [18, 110]. NO and other RNS products may also react with BER repair proteins such as OGG1 enzyme, by covalent NO nitrosylation of cysteine thiols [134, 135, 306, 340, 341]. 1,25-dihydroxyvitamin D_3-dependent reductions in ROS [269] and RNS would reduce direct 8-OHdG formation, while reductions in ROS and RNS would lead to less inhibition of DNA repair after UV [18, 110]. DNA repair after
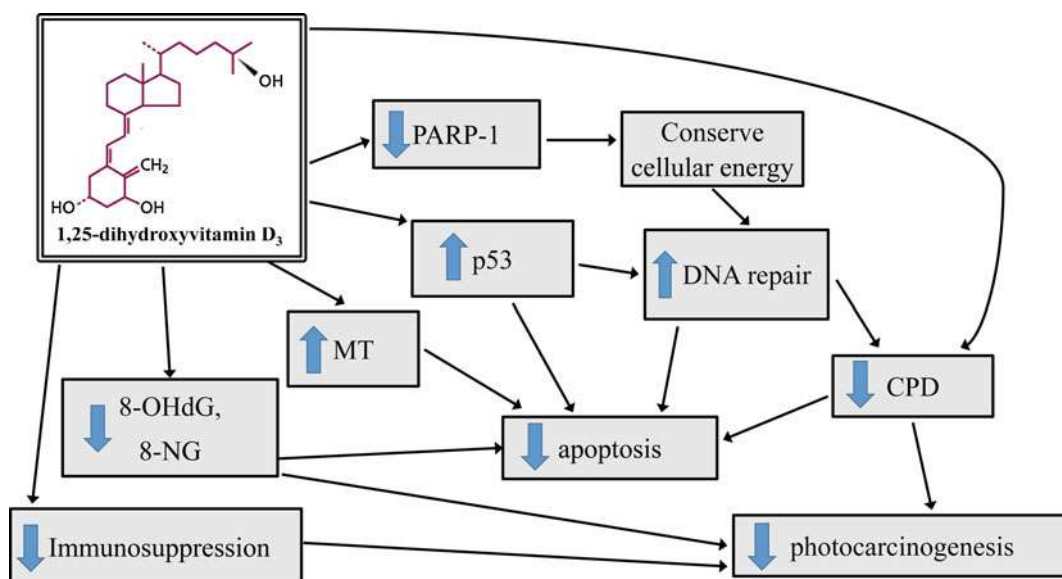
Fig. 12.3 Mechanisms of photoprotection by 1,25 (OH)$_2$D$_3$. Following exposure to ultraviolet radiation, 1,25(OH)$_2$D$_3$ reduces the levels of UV-induced DNA damage in the form of CPDs [64, 65, 299], 8-OHdG and 8-NG [94, 142, 299]. Moreover, 1,25(OH)$_2$D$_3$ increases p53 levels [64, 100, 162, 281], which may induce apoptosis of cells with irreparable DNA damage or facilitate DNA repair. It has also been demonstrated that 1,25 (OH)$_2$D$_3$ can increase DNA repair by increasing levels of NER proteins including XPC and DDB2 [198, 299, 335] and by reducing PARP-1 activation [11, 177] which facilitates DNA repair by conserving cellular energy. Levels of metallothionein (MT) are also increased by 1,25(OH)$_2$D$_3$ [142], leading to a reduction in UV-induced apoptosis. These photoprotective effects of 1,25(OH)$_2$D$_3$, together with its ability to reduce UV-induced immunosuppression [64, 66], lead to a reduction in photocarcinogenesis [66, 151]

UV in human keratinocytes is increased in the presence of 1,25-dihydroxyvitamin D$_3$ [269] and in the skin of mice that have vitamin D receptor, which is necessary for endogenous 1,25-dihydroxyvitamin D$_3$ action [281], compared with mice with vitamin D receptor knockout [71].

DNA repair requires energy [187, 190]. The primary energy production pathway in the cell, oxidative phosphorylation and total cellular energy levels are reduced after UV, probably due in part to mitochondrial damage [28, 29, 133, 245] and also due to utilization of cellular NAD$^+$ by PARP-1 [93, 290]. Further, it is reasonable to propose that the increased PARP-1 activity reported in keratinocytes exposed to UV [245] causes some degree of inhibition of glycolysis through inhibition of hexokinase further depleting cellular energy levels [11, 82]. PARP-1 activity is increased via direct interaction with phosphorylated ERK [47], and Mabley et al.

reported that in immortalized keratinocytes (HaCaT cells), 1,25(OH)$_2$D$_3$ inhibited PARP-1 activity [177]. Whether this occurs in normal keratinocytes is unclear, but since 1,25-dihydroxyvitamin D$_3$ suppresses phosphorylation of ERK after UV [269], it is reasonable to propose that 1,25-dihydroxyvitamin D$_3$ suppresses PARP activity in primary keratinocytes. Any reduction of PARP-1 by 1,25(OH)$_2$D$_3$ may help to increase energy for DNA repair due to less consumption of cellular NAD+. Indeed, DNA repair, as measured by unscheduled DNA synthesis, and glycolysis are significantly increased in response to 1,25-dihydroxyvitamin D$_3$ in primary keratinocytes following UV irradiation [269] (Fig. 12.3).

p53 plays a major role in DNA repair [3, 80, 238, 280, 333, 348], and it is possible that previously reported increases in p53 expression after UV in the presence of 1,25(OH)$_2$D$_3$ may contribute to reduced DNA damage [64, 100,

282]. Nuclear localization of p53 is increased in UV-irradiated keratinocytes and further increased when UV-irradiated cells were treated with $1,25(OH)_2D_3$ [64, 100, 282].

The significance of DNA repair proteins in reducing DNA damage is clearly shown in patients with xeroderma pigmentosum, who harbour mutations in at least one of the key enzymes involved in NER and have a much higher susceptibility to skin cancer [43, 302]. Increased efficiency of the NER pathway can also be attained via upregulated expression of DNA damage repair proteins. Increased XPC and DDB2 expression in nonirradiated human keratinocytes treated with $1,25(OH)_2D_3$ was demonstrated using array techniques [212]. Reduction of UV-induced CPD and upregulation of XPC and DDB2 were reported in UV-irradiated human skin explants topically treated with vitamin D metabolites [299] (Fig. 12.3).

Reduced Immunosuppression

The effects of $1,25(OH)_2D_3$ on immune responses are controversial and appear to depend on the dose used and the model tested [175, 205]. In humans, some research suggests that $1,25(OH)_2D_3$ induces immunosuppression [51]. Other data indicate that $1,25(OH)_2D_3$ reduces UV-induced immunosuppression in Skh/hr1 and other mice [64, 66].

The vitamin D receptor, necessary for $1,25(OH)_2D_3$ actions and the CYP27B1 enzyme necessary for local $1,25(OH)_2D_3$ production are found not only in epidermal keratinocytes [45] but also in Langerhans cells (LC)s and monocytes [24], which may indicate that $1,25(OH)_2D_3$ has a biological effect on these cells [243]. The vitamin D hormone, $1,25(OH)_2D_3$, has been reported to reduce LC numbers [99] and LC chemotaxis, as well as alter the proliferation of antigen-specific T cells [91] and affect the induction of regulatory T cells [95]. Both $1,25(OH)_2D_3$ and a synthetic analogue calcipotriol exhibited immune-

inhibitory properties in a mixed epidermal cell-lymphocyte reaction [15]. Several studies have reported that $1,25(OH)_2D_3$ downregulates various co-stimulatory molecules responsible for LC maturation and reduces antigen presentation, such as increased IL-10 secretion [231, 263] and inhibited IL-12 production [164]. The hormone can also upregulate or downregulate transcription of various genes in CD4+ cells [179].

Topical application of $1,25(OH)_2D_3$ on mouse skin induced immunosuppression similar to UV exposure [95]. In mice, the effects of topical $1,25(OH)_2D_3$ may depend on dose, with higher concentrations (approx. 1.3ug/kg/day) reported to decrease primary contact hypersensitivity responses (CHS) [99], while lower doses (approx. 0.03ug/kg/day) had no effect, and even lower doses (approx. 0.2 ng/kg/day) enhanced CHS [307]. The analogue calcipotriol has also been reported to have varying effects on induction or elicitation of immune responses in mice, depending on dose [92].

On the other hand, topical application of $1,25(OH)_2D_3$ on immunocompetent female Skh/hr1 mice resulted in a reduction in systemic immunosuppression demonstrated by contact hypersensitivity (CHS) reaction to oxazolone 2 weeks after acute UV irradiation [65, 66], along with reduced IL-6 expression [193], which in turn reduces IL-10 [57, 232]. It has also been shown that $1,25(OH)_2D_3$ reduces the immunosuppressive effect of cultured keratinocytes by reducing UVB-induced IL-6 expression in a dose-dependent manner, though the mechanism was not clear [57]. As mentioned before, CPDs induce secretion of immunosuppressive agents such as IL-10 [140, 231, 263]. Therefore, it is reasonable to presume that reduced CPDs in the presence of $1,25(OH)_2D_3$ may lead to reduced secretion of immunosuppressive agents, hence, reduced immunosuppression [64–66, 154]. $1,25(OH)_2D_3$ also increases metallothionein expression (a scavenger molecule), which is responsible for the reduction of UV-induced ROS [142] and RNS [94, 100]. This may also contribute to the reduc-

tion of DNA damage and production of immuno-suppressant molecules [57, 58, 142, 346].

In humans, topical application of the 1,25 $(OH)_2D_3$ analogue calcipotriene applied twice daily for 1 week suppressed local primary CHS responses to dinitrochlorobenzene to an extent comparable to that caused by a single suberythemal UV exposure [106]. Damian et al. (2009) also reported immune suppression in a recall delayed-type hypersensitivity response at topical doses of $1,25(OH)_2D_3$ of approx. 17 ng/kg but saw neither enhancement nor suppression of this response at approx. 9 ng/kg.

There are reported sex differences between males and females, with UV-induced immune responses being more suppressed in male, compared with female, mice or humans [258]. A study with vitamin D deficient or sufficient BALB/c male and female mice showed that vitamin D status and sex influence contact hypersensitivity reactions [182]. The hypothesis has even been proposed that $1,25(OH)_2D_3$ may contribute to UV-induced immune suppression [235].

Reduced Photocarcinogenesis

UV-induced DNA damage and immunosuppression [13, 78, 345] are considered the main contributing factors to photocarcinogenesis [301]. A study carried out on Skh/hr1 mice has shown that those treated with $1,25(OH)_2D_3$ immediately after UV have lower tumour incidence and higher photoprotection [66]. Another study carried out on Skh/hr1 mice for a longer UV exposure duration and using a higher concentration of $1,25(OH)_2D_3$ treatment showed less tumours, smaller tumour size and regression in tumour size in the $1,25(OH)_2D_3$ treated group towards the end of the study [151]. These studies have verified that $1,25(OH)_2D_3$ delays the progression of benign skin tumours (papilloma) to malignant tumours, delaying the progression to photocarcinogenesis.

Vitamin D Signalling Pathways

Classic/Genomic Pathway

The classic or genomic $1,25(OH)_2D_3$ pathway is mediated through the vitamin D receptor (VDR) [34, 111, 204, 234]. The VDR is located in both cell membranes (mVDR) and nucleus (nVDR), but the majority are localized in the cell nucleus [79]. The expression of VDR in skin keratinocytes depends on factors such as UVB exposure [50], presence of calcium [180], interferon γ [279], flavonoids [277], cell density and adherence [275]. In the event of cell growth arrest or differentiation, expression of VDR is reduced [278].

$1,25(OH)_2D_3$ acts as a ligand for VDR, and its binding to the receptor initiates the genomic effects in the cell (Fig. 12.4). The ligand-bound VDR changes its natural conformation and dimerizes with retinoic acid X receptor (RXR) and several other cofactors, forming a complex that acts as a transcription factor [41, 165]. The VDR-RXR complex binds to promotor regions known as vitamin D response elements (VDRE). The 5′ of the VDRE sequence binds to the RXR, and VDR binds to the 3′ end of the sequence. This process activates or represses transcription of numerous target genes for cellular responses. The responses to $1,25(OH)_2D_3$, mediated through the genomic pathway, may take hours to elicit a response [60, 112].

Nonclassic/Non-genomic Pathway

The non-genomic $1,25(OH)_2D_3$ pathway appears to be mediated through plasma membrane-initiated mechanisms (Fig. 12.4). The non-genomic pathway initiates at the membrane-bound receptor known as 1,25-dihydroxyvitamin D-MARRS (membrane-associated, rapid response steroid-binding), which consists of endoplasmic reticulum protein57 (ERp57) [128, 227, 228] in combination with caveolae-associated mVDR [35, 128]. The majority of ERp57 is located in the lumen of the endoplasmic
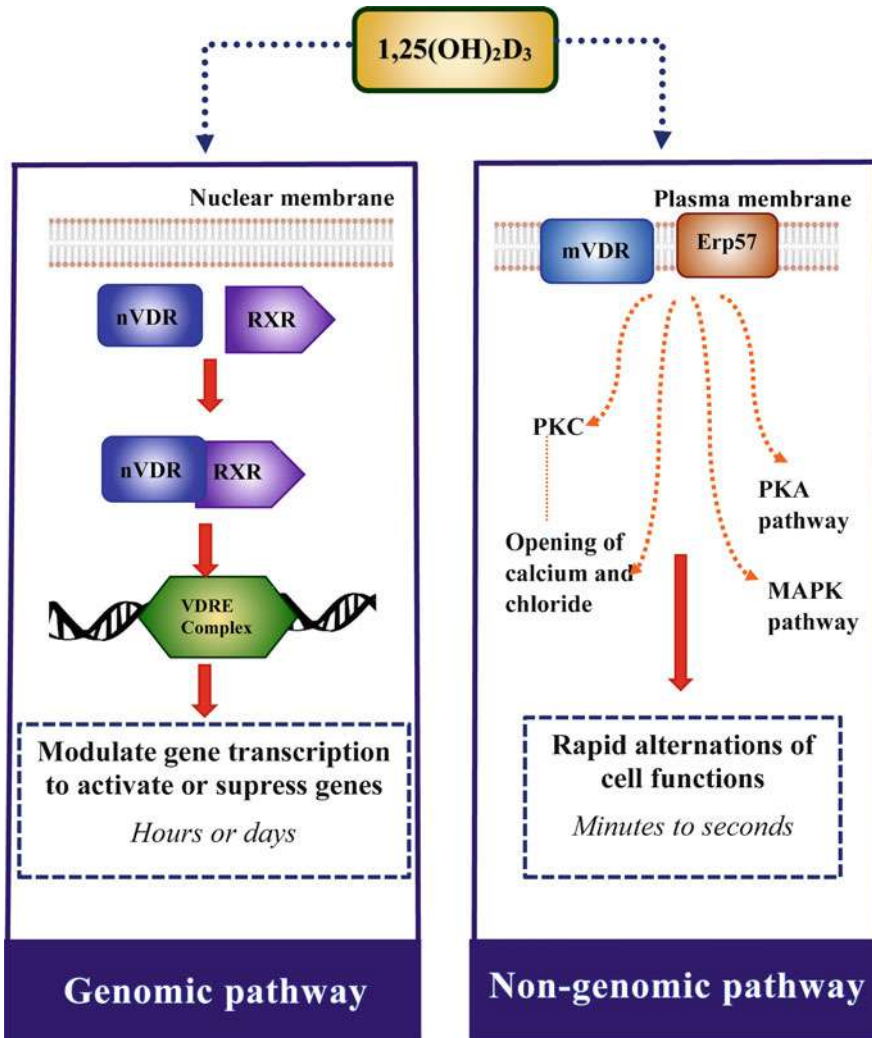
Fig. 12.4 $1,25(OH)_2D_3$ mediated genomic and non-genomic pathway. (Edited from [199])

reticulum, one third is in the cytoplasm, and lower concentrations are expressed in nuclear matrix compartments [7, 317, 318]. ERp57 is a member of the protein disulphide isomerase (PDI) family and is a glycoprotein-specific thiol-reductase with several domains [86]. ERp57 is found in osteoblasts, osteoclasts, other calcium regulating cells and non-calcium regulating cells such as the liver, placenta, lung and other tissues [149]. The binding of $1,25(OH)_2D_3$ to ERp57 or mVDR may initiate several signalling pathways. $1,25(OH)_2D_3$ triggers rapid opening of Ca^{+2} channels to increase the intracellular Ca^{+2} concentrations,

hence activation of protein kinase C (PKC) [184]. The subsequent increase of cellular Ca^{+2} concentrations further activates PKC through positive feedback loop and mitogen-activated protein kinase (MAPK) and cAMP-PKA pathway [21, 184]. MAPK and PKA increase the transcriptional activity of VDR. Recently, it has been shown that $1,25(OH)_2D_3$ modulates a range of phosphoproteins following UV irradiation in primary human keratinocytes collectively resulting in growth arrest, autophagy and mitophagy – all of which are energy-conserving processes for the cell [269].

The initiation of non-genomic pathways may also interconnect with genomic pathways to increase the efficiency of cellular $1,25(OH)_2D_3$ functions [25, 60, 112, 119, 249, 260]. Unlike the classic/genomic pathway, the effects of $1,25(OH)_2D_3$ through the nonclassic/non-genomic pathway tend to be more rapid and are often observed within seconds to minutes [112, 269, 281, 317].

Vitamin D Production and Metabolism

Vitamin D is a fat-soluble secosteroid that humans obtain endogenously upon UVB exposure and exogenously through food or vitamin D supplements. Biosynthesis of the hormonal form of active vitamin D, $1,25(OH)_2D_3$, is a multistep process involving several organs (Fig. 12.5). The active hormone is also biosynthesized locally in skin keratinocytes. The skin contains 25-hydroxylase enzyme (CYP2R1 and CYP27A1) necessary to covert vitamin D_3 into 25-hydroxyvitamin$_3$ and CYP27B1 to convert this to $1,25(OH)_2D_3$ [26, 27, 163] (Fig. 12.5).

On average, the 7-dehydroxycholesterol content in the human skin is 2352 ± 320 ng/cm^2, and previtamin D_3 produced in the skin after UV exposure accounts for around 35% conversion [121, 237]. This depends on several factors such as the individual level of skin pigmentation, duration of UVB exposure, latitude, time of the day and season of the year [123, 124]. Biosynthesis of previtamin D_3 in fair skin peaks after 15 minutes of UVB exposure, while more pigmented skin requires a longer duration to reach the same peak previtamin D_3 concentration [121, 122, 174]. At body temperature, previtamin D_3 thermo-isomerizes into vitamin D_3. Continuous sun exposure does not increase the overall previtamin D_3 concentration, as previtamin D_3 photoisomerizes to produce what were believed to be biologically inactive products known as 'overirradiation products' (Fig. 12.5), preventing vitamin D intoxication. Overirradiation products of vitamin D were initially reported by Windus and Linsert [36, 115, 116] and also by Webster and Bourdillon in 1928 [336]. Overirradiation products include lumisterol, tachysterol and other compounds [96, 113, 115, 132]. The most abundant overirradiation products are lumisterol$_3$ (29%) (L3) and tachysterol$_3$ (T3) (7%) [237]. Other overirradiation products include suprasterols, toxisterol and other compounds [14, 96, 113, 115, 132, 256, 272]. T3 production peaks after 1 hour in over irradiated skin samples, while L3 synthesis increased steadily with continuing UVB exposure for 8 hours [96, 124, 178]. Further, T3 photoisomerizes to L3 during prolonged UV radiation causing L3 to be the most abundant overirradiation product found in the skin [178] (Fig. 12.5). L3 was assumed to be a biologically inactive compound, but recent studies have shown that L3 derivatives have substantial biological activity, though not much in relation to calcium or phosphate homeostasis [42, 297, 316].

Cytochrome P450 side-chain cleavage (P450scc) enzyme (CYP11A1) is a mitochondrial enzyme [241] found in the vertebrate adrenal cortex [192], ovaries, placenta [19, 314], lungs, bones, skin (both epidermis and dermis) and cultured human keratinocytes [292]. CYP11A1 expression increases in mixed cultures of keratinocytes and melanocytes under UVB and UVC radiation, but is not affected by UVA radiation [291, 294, 316]. The main substrate for the CYP11A1 enzyme is cholesterol, and it cleaves the cholesterol side chain to produce pregnenolone [159, 286, 289, 293, 314]. But this enzyme also metabolizes substrates with similar steroid ring structures, such as vitamin D_3, vitamin D_2 [315], 7DHC [101], T3 and L3 [296, 314, 316]. CYP11A1 expressed in the skin catalyzes the production of two main compounds, 20-hydroxyvitamin D_3 [20(OH)D$_3$] and 20,22-dihhydroxyvitamin D_3, from vitamin D_3 [101]. Recent studies have shown that 20(OH) D$_3$ has photoprotective properties against UV-induced DNA damage in in vitro [295] and in vivo [311]. Enzymatic actions of CYP11A1 on L3 result in the production of three main derivatives: 24-hydroxy-L$_3$ [24(OH)L$_3$], 22-hydroxy-L$_3$ [22(OH)L$_3$] and 20,22-dihydroxy-L$_3$ [20,22(OH)L$_3$] [316]. There is now good evidence that both the CYP11A1
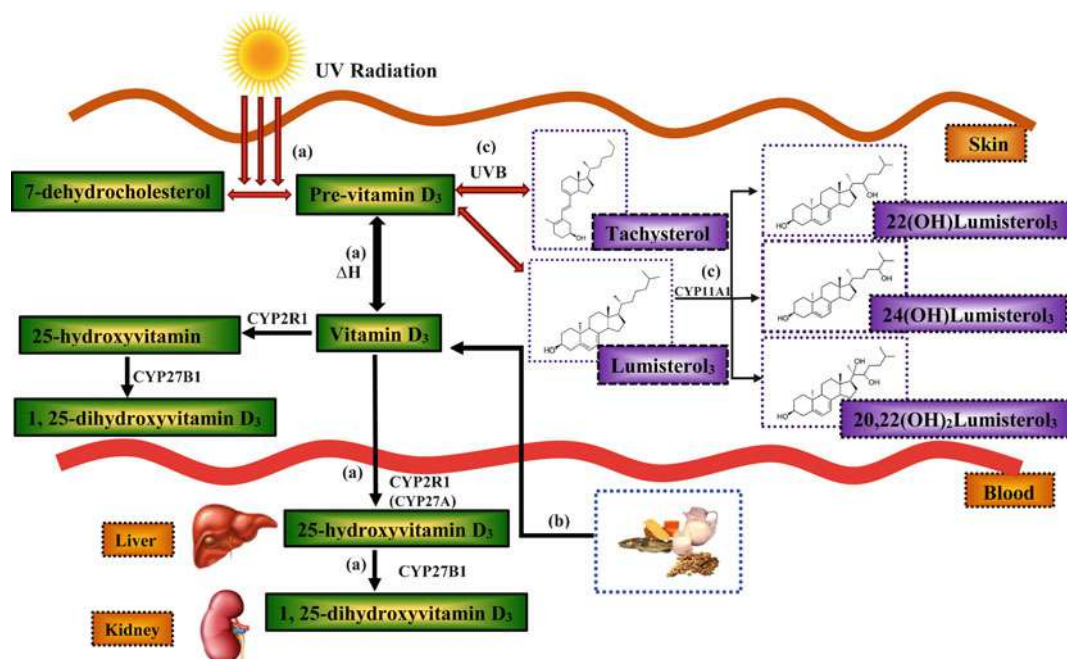
Fig. 12.5 Biosynthesis of vitamin D and overirradiation products upon UV radiation. (a) Vitamin D synthesis pathway initiated in the skin upon UVB exposure then involving the liver and kidney and in the skin to produce biologically active vitamin D hormone, $1,25(OH)_2D_3$, modified from [124]. (b) Exogenous vitamin D acquired through food and supplements, modified from [118]. (c) Further UVB exposure photoisomerizes previtamin D_3 to produce relatively inactive 'overirradiation products' mainly lumisterol$_3$ (L3) and tachysterol$_3$ (T3). CYP11A1 enzyme in skin catalyzes the production of three main derivatives from L3: 22 (OH)Lumisterol$_3$, 24(OH)Lumisterol$_3$ and 20,22 (OH)$_2$Lumisterol$_3$. (Modified from [316])

derivatives of vitamin D and the hydroxyl-lumisterol derivatives exhibit many of the actions of 1,25-dihydroxyvitamin D_3. In cultured human keratinocytes, they reduced UV-induced CPD and 6–4 photoproducts [42]. These test compounds also reduced oxidant levels, at least in part by increased expression of Nrf2 target genes such as glutathione reductase, heme oxygenase-1, superoxide dismutase 1 and 2, catalase and Mn-superoxide dismutase [42]. In addition, these compounds increased the appearance of phosphorylated p53-Ser[15] in the nucleus of irradiated keratinocytes, similar to 1,25-dihydroxyvitamin D_3 [42].

It has been reported that VDR null mice are more susceptible to UV-induced DNA damage and skin tumours [71]. It has been stated that mice which lack the 1α-hydroxylase enzyme, which produces $1,25(OH)_2D_3$, are not more susceptible to UV-induced DNA damage or photocarcinogenesis [308]. One potential explanation is that CYP11A1-derived metabolites of vitamin D or lumisterol contribute to photoprotection but that all these metabolites require the presence of the vitamin D receptor. This remains to be determined experimentally.

Limitations of $1,25(OH)_2D_3$ as a Photoprotective Agent

Despite the outlined evidence for the effectiveness of $1,25(OH)_2D_3$ in reducing UV-induced DNA damage, adverse effects of this compound limit its use for sun protection commercially. The most common adverse effects of $1,25(OH)_2D_3$ are hypercalciuria and hypercalcemia [181]. In addition, the high cost and the biological instability of this compound make $1,25(OH)_2D_3$ an unsuitable additive for sun protection. Alternative vitamin

D-like non-calcemic analogues may be useful to prevent UV-induced damage and provide photoprotection [66]. Mouse studies have shown that 20-hydroxyvitamin D_3 [310] and $1,25$ $(OH)_2$lumisterol$_3$ provide protection against UV-induced DNA damage and potentially photocarcinogenesis [66].

Conclusion

Current research indicates that $1,25(OH)_2D_3$ and vitamin D-related compounds exhibit photoprotective properties against UV-induced damage. The contribution of overirradiation products to photoprotection against DNA damage or skin cancer is yet to be investigated. If this proves to be the case, the incorporation of these compounds in sunscreens may increase the effectiveness of sun protection by reducing DNA damage, oxidative damage and immunosuppression while increasing DNA repair, ultimately leading to decreased carcinogenesis.

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Part VI

**The Relevance of the Vitamin D Endocrine System
for Skin Cancer**

The Role of Classical and Novel Forms of Vitamin D in the Pathogenesis and Progression of Nonmelanoma Skin Cancers

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Abstract

Nonmelanoma skin cancers including basal and squamous cell carcinomas (SCC and BCC) represent a significant clinical problem due to their relatively high incidence, imposing an economic burden to healthcare systems around the world. It is accepted that ultraviolet radiation (UVR: $\lambda = 290$–400 nm) plays a crucial role in the initiation and promotion of BCC and SCC with UVB ($\lambda = 290$–320 nm) having a central role in this process. On the other hand, UVB is required for vitamin D3 (D3) production in the skin, which supplies >90% of the body's requirement for this prohormone. Prolonged exposure to UVB can also generate tachysterol and lumisterol. Vitamin D3 itself and its

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canonical (1,25(OH)$_2$D3) and noncanonical (CYP11A1-intitated) D3 hydroxyderivatives show photoprotective functions in the skin. These include regulation of keratinocyte proliferation and differentiation, induction of antioxidative responses, inhibition of DNA damage and induction of DNA repair mechanisms, and anti-inflammatory activities. Studies in animals have demonstrated that D3 hydroxyderivatives can attenuate UVB or chemically induced epidermal cancerogenesis and inhibit growth of SCC and BCC. Genomic and non-genomic mechanisms of action have been suggested. In addition, vitamin D3 itself inhibits hedgehog signaling pathways which have been implicated in many cancers. Silencing of the vitamin D receptor leads to increased propensity to develop UVB or chemically induced epidermal cancers. Other targets for vitamin D compounds include 1,25D3-MARRS, retinoic orphan receptors α and γ, aryl hydrocarbon receptor, and Wnt signaling. Most recently, photoprotective effects of lumisterol hydroxyderivatives have been identified. Clinical trials demonstrated a beneficial role of vitamin D compounds in the treatment of actinic keratosis. In summary, recent advances in vitamin D biology and pharmacology open new exciting opportunities in chemoprevention and treatment of skin cancers.

Keywords

Squamous cell carcinoma · Basal cell carcinoma · Vitamin D · Ultraviolet radiation · VDR · RORα · RORγ

Introduction to the Ultraviolet Spectrum of Solar Radiation

Ultraviolet radiation (UVR: $\lambda = 290$–400 nm), depending on its wavelength (UVB: $\lambda = 290$–320 nm; UVA: $\lambda = 320$–400 nm), penetrates into different layers of the skin, with UVB being predominantly absorbed by the epidermis and reaching the upper portion of the papillary dermis, while UVA penetrates deep into the reticular dermis [69, 135, 164, 210, 238, 246]. UVR affects the integrity of DNA, RNA, and proteins and cell and tissue homeostasis, induces mutations, and changes the expression of a plethora of genes including oncogenes and tumor suppressor genes [29, 51, 132, 210, 241, 242]. It can also modify the expression and activity of growth factors, cytokines, neurohormones, neuropeptides, and their receptors and have local and systemic immunosuppressive [2, 30, 32, 62, 82, 105, 106, 126, 144, 156, 172, 174, 177–181, 193, 196, 210] as well as pro-pigmentary effects [148, 176, 182].

Excessive exposure to UVR results in skin aging, precancerous states such as solar/actinic keratosis (SA), and finally skin cancers including squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and melanoma (Fig. 13.1). Therefore, UVR (UVB and UVA) is defined as a major environmental stressor and full carcinogen responsible for the development and progression of BCC, SCC, and melanoma [11, 51, 100, 200].

UVB, while representing only ~5% of UVR spectrum, exhibits a high efficiency for inducing biological effects in the skin through its interaction with cutaneous chromophores. It causes direct damage to DNA (a chromophore for UVB) by inducing covalent bond formation between adjacent pyrimidines, which leads to the production of mutagenic photoproducts such as cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidine adducts [29, 121, 241, 242]. To a lesser degree, its mechanism of action is linked to production of reactive oxygen species (ROS). UVB is an important etiological factor of BCC and SCC [121, 200, 241, 242]. UVB fingerprint mutations in p53 and CDKN2A genes have been identified in BCC and SCC [83]. UVB is more efficient in inducing SCC and BCC than UVA [52, 141] with some exceptions [151–153, 159]. The damaging effect of UVA, which is approximately 1,000 less efficient than UVB due to the limited number of target chromophores, is predominantly secondary to the action of ROS [24, 71, 245] or production of nitric oxide (NO) and nitroxyl (HNO) [1, 170, 210].
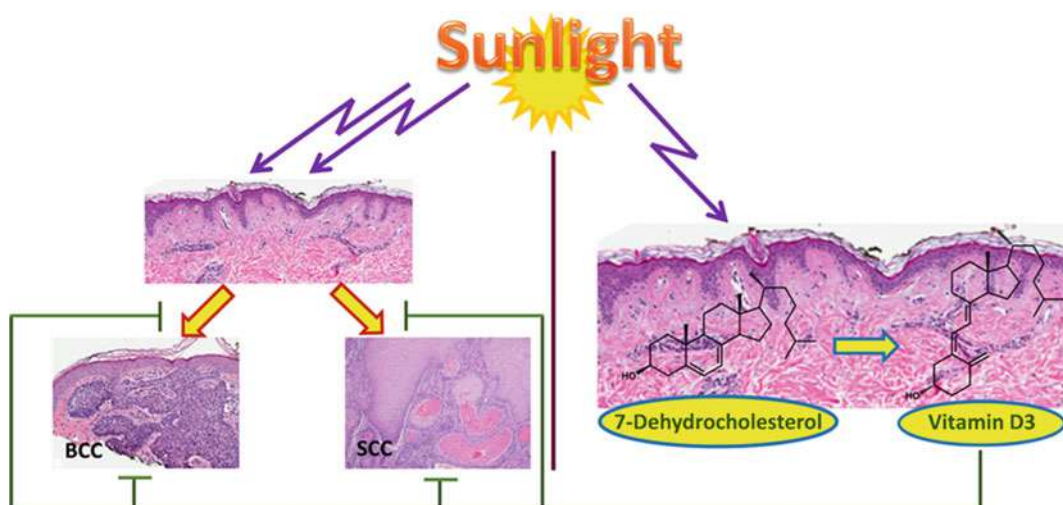
Fig. 13.1 Ultraviolet B as the double-edge sword in skin health
UVB not only induces skin cancers but also is necessary for phototransformation of 7DHC (7-dehydrocholesterol)

to vitamin D3. *BCC* basal cell carcinoma, *SCC* invasive squamous cell carcinoma. (Reprinted from [208] with permission from Elsevier)

Vitamin D in the Skin

Vitamin D and Related Compounds in a Nutshell

UVB is also required for vitamin D3 formation in the skin which usually supplies >95% of the body's requirement for this prohormone [18, 84, 85] (Fig. 13.1). The transformation of 7-dehydrocholesterol (7DHC) to vitamin D3 (D3) after absorption of UVB energy represents the most fundamental reaction in photobiology [84, 87]. The initial photoproduct, previtamin D3, undergoes thermal isomerization to vitamin D3 in the skin. With sustained UVB, previtamin D3 can undergo further photoisomerization to lumisterol (L3) and tachysterol (T3) [84]. These reactions are reversible and are dependent on the temperature and UVB dose.

Vitamin D3 is a prohormone that is activated by sequential hydroxylations in positions C25 and C1α, both at the systemic (liver and kidney) and local (skin) levels, to produce $1,25(OH)_2D3$ [13, 84, 85]. The first reaction is catalyzed by CYP2R1 or CYP27A1, while the C1α hydroxylation is catalyzed by CYP27B1 [15, 16, 84, 85]. Dietary vitamin D2 is activated to 1,25

$(OH)_2D2$ by CYP2R1 and CYP27B1, and inactivated by CYP24A1, by similar pathways [15, 16, 228].

Vitamin D can also be activated by CYP11A1, the first enzyme in the steroid biosynthesis pathway [78, 184, 185]. The major products of CYP11A1 action on vitamin D3 are $20(OH)D3$ and $20,23(OH)_2D3$ [192, 224]. Other products of CYP11A1 action on vitamin D3 are $22(OH)D3$, $20,22(OH)_2D3$, $17,20(OH)_2D3$, and $17,20,23$ $(OH)_3D3$ [184, 224, 225]. The CYP11A1-derived metabolites can be further hydroxylated by CYP27A1, CYP27B1, CYP2R1, and/or CYP3A4 producing many more metabolites including $1,20(OH)_2D3$, $1,20,23(OH)_3D3$, $20,24$ $(OH)_2D3$, $20,25(OH)_2D3$, and $20,26(OH)_2D3$ [213, 215, 217, 218, 223, 228]. Most of these metabolites have been detected in the human skin and/or serum indicating that the pathways occur in vivo (Fig. 13.2), and most have been tested in cultured cells and found to display biological activity, including inhibition of skin cell proliferation [192, 202–204, 228]. CYP11A1 can also act on vitamin D2 producing $20(OH)D2$, which displays activities similar to $20(OH)D3$, plus a number of other metabolites, including $17,20(OH)_2D2$ [140, 185,
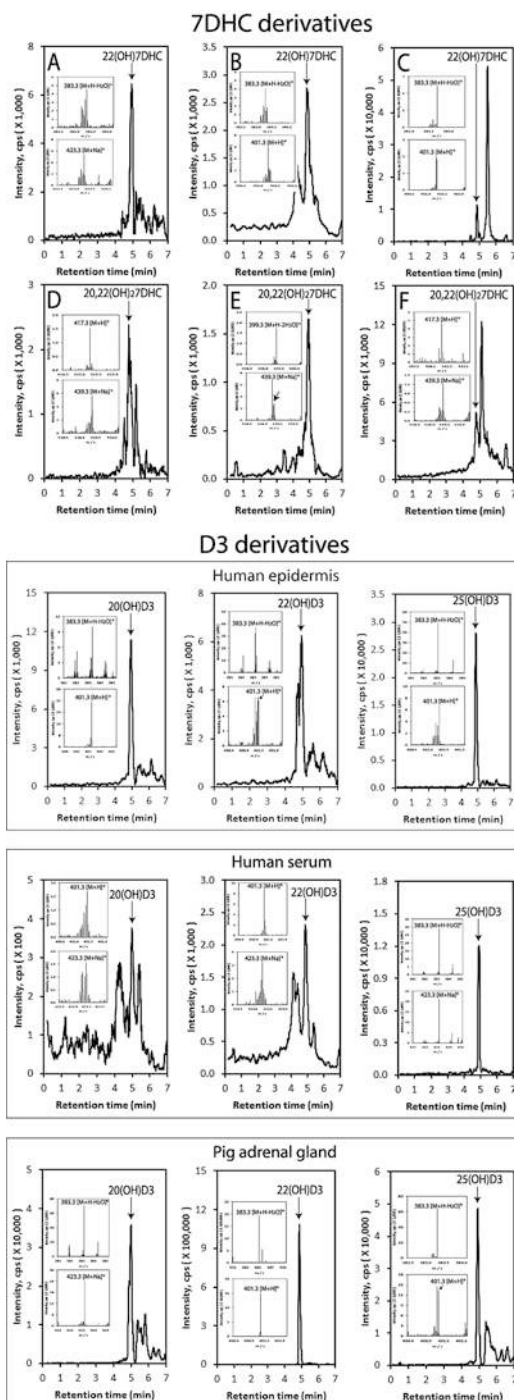
Fig. 13.2 Detection of CYP11A1-derived 7DHC and D3 hdroxyderivatives in the human epidermis and serum LC-MS spectra were measured on fractions with retention times corresponding to either 22(OH)7DHC or 20,22 (OH)$_2$7DHC or 20(OH)D3, 22(OH)D3, or 25(OH)D3 that were pre-purified on a Waters C18 column (250 × 4.6 mm, 5 μm particle size) with a gradient of acetonitrile in water as described in [202]. Arrows indicate the retention times of the corresponding standards. Inserts show the mass spectra corresponding to the retention time of detected compound. In the outer panel, extracted ion chromatograms are shown for human epidermis (**a** and **d**), serum (**b** and **e**), and the pig adrenal (**c** and **f**). The work is reprinted from [202] under the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/) with small modifications
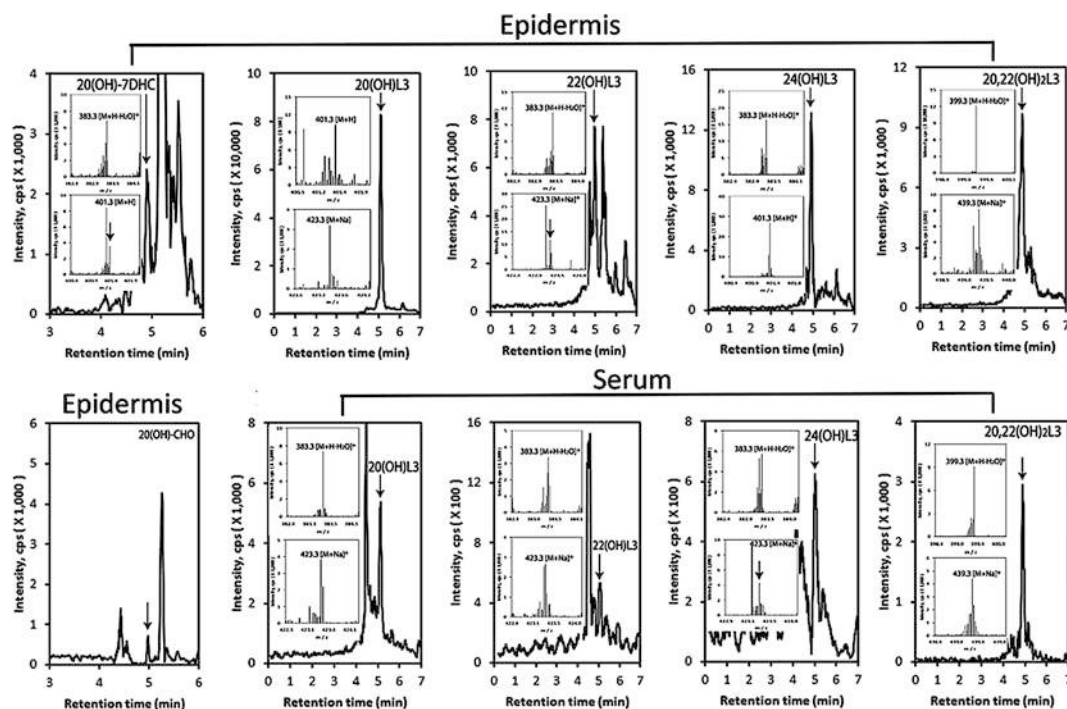
Fig. 13.3 Detection of novel lumisterol hdroxyderivatives in the human epidermis and serum
LC-MS spectra were measured on fractions with retention times corresponding to either of the hydroxyderivatives listed that were pre-purified on a Waters C18 column as described in [202]. Arrows indicate the retention times of the corresponding standards. Inserts show the mass spectra corresponding to the retention time of the detected compound. The work is reprinted from [202] under the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/) with small modifications

188, 198, 228]. 20(OH)D2 can also be metabolized further by CYP27B1.

Lumisterol (L3), the major 7DHC photoproduct found in the skin following prolonged UVB radiation [86], can be metabolized by both CYP11A1 and CYP27A1 [206, 226, 227]. CYP11A1 produces primarily 22(OH)L3, 24(OH)L3, and 20,22(OH)$_2$L3, with only minor production of pregnalumisterol which contains a cleaved side chain [226]. Lumisterol and its hydroxyderivatives have been detected in the skin and serum, illustrating that this pathway occurs in vivo (Fig. 13.3). The presence of relatively high concentrations of L3 in the serum indicates that it can leave the site of its production in the skin and potentially be delivered to tissues, such as the adrenal cortex, which expresses a high level of CYP11A1, for further metabolism [206]. The major products of CYP11A1 action on L3 are biologically active, with some, but not all activities, being similar to those of 1,25 (OH)$_2$D3 (see below) [41, 206]. More recently, we reported that lumisterol is an excellent substrate for CYP27A1, which converts it to 25(OH) L3 and both C25 epimers of 27(OH)L3, which in initial testing are able to inhibit melanoma cell proliferation [227].

Finally, tissues expressing CYP11A1 are able to transform 7DHC to 22(OH)7DHC, 20,22 (OH)$_2$7DHC, and finally to 7-dehydropregnenolone (7DHP) [183, 186, 191]. The latter can be further hydroxylated or converted to dehydroprogesterone by steroidogenic enzymes [191]. 20(OH)7DHC has been identified in human epidermis [206], while 22 (OH)7DHC, 20,22(OH)$_2$7DHC, and 7DHP were detected in human epidermis and serum (Fig. 13.2) [202]. 7DHP and its metabolites can be transformed by UVB to the corresponding secosteroids, as predicted [183] and as has been
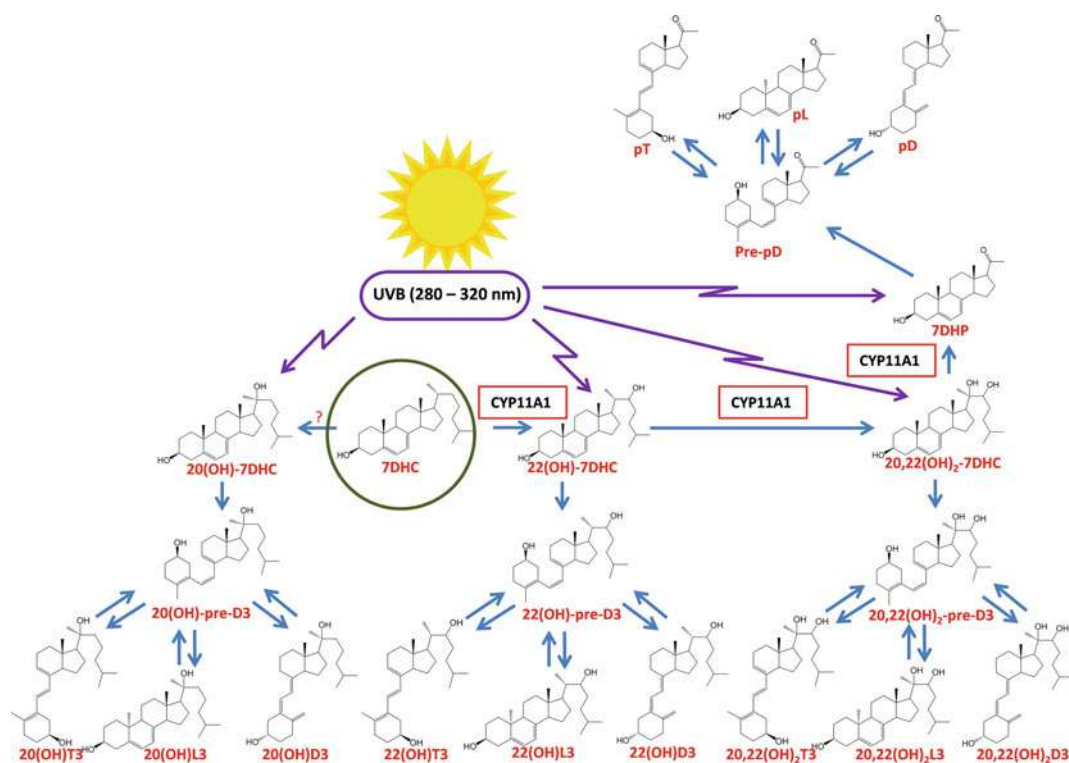
Fig. 13.4 UVB-induced phototransformation of 7DHC, its hydroxyderivatives, and 7DHP to the corresponding secosteroidal, lumisterol, and tachysterol compounds Shown is the metabolism of 7DHC by CYP11A1, the skin, and the subsequent transformations to the corresponding photoproducts after exposure to UVB. (?) – the enzyme transforming 7DHC to 20(OH)7DHC remains to be

identified, since none of the products of 7DHC hydroxylation by CYP11A1 has its retention time. Because of the similarity of 20(OH)7DHC and 20-hydroxycholesterol, it is likely to be the same enzyme that transforms cholesterol into 20-hydroxychaolesterol, which is also detectable in the epidermis. (Reprinted from [208] with permission from Elsevier)

experimentally substantiated [251–253] (Fig. 13.4). In addition, 20(OH)7DHC, 22(OH) 7DHC, and 20,22(OH)₂7DHC can be converted to the corresponding vitamin D3, lumisterol and tachysterol hydroxyderivatives, after absorption of UVB energy by the B-ring (Fig. 13.4).

Phenotypic Effects of Active Forms of Vitamin D: An Overview

$1,25(OH)_2D3$, in addition to regulating calcium homeostasis, has important pleiotropic activities that include stimulation of differentiation and inhibition of proliferation of different cell types, anti-cancerogenic effects, stimulation of innate immunity, and inhibition of adaptive immunity

and inflammation [13, 15, 28, 46–48, 55, 65, 73–75, 84, 85, 149, 240]. In the skin, vitamin D3 plays a significant role in the formation of the epidermal barrier and adnexal structures, including hair follicles, and has a wide variety of ameliorating effects in skin cancer and proliferative and inflammatory cutaneous diseases [12, 14, 23, 63, 84, 85, 94, 143, 157, 158]. These properties of $1,25(OH)_2D3$ have been extensively reviewed as listed above and, therefore, will not be detailed.

Similar effects are exerted by CYP11A1-derived hydroxyderivatives of vitamin D3, including mono, dihydroxy, and trihydroxy forms with or without the hydroxyl group at position C1α (reviewed in [197, 205, 207, 208]). Specifically, they exert antiproliferative,

pro-differentiation, and anti-inflammatory effects in cultured cells that are comparable or stronger than those of 1,25(OH)$_2$D3 [41, 95, 96, 112, 114–116, 119, 123, 189, 190, 194, 195, 207, 225, 248]. In addition, they exhibit antifibrotic activities both in vitro [189, 194, 195] and in vivo [194]. They also display anti-melanoma and antitumor properties that are cell type-dependent [44, 97, 173, 187, 188, 190, 195, 207, 234, 235, 237]. Moreover, similar to 1,25 (OH)$_2$D3, they can stimulate different elements of the cutaneous hypothalamus-pituitary-adrenal axis in human keratinocytes including CRH, urocortins, and POMC, together with their corresponding receptors CRHR1, CRHR2, MC1, MC2, MC3, and MC4 [238]. The newly identified hydroxyderivatives of lumisterol also show antiproliferative and pro-differentiation properties in human normal and malignant epidermal keratinocytes [41, 206]. Finally, vitamin D-, lumisterol-, and tachysterol-like compounds with a short or absent side chain also show antiproliferative and antitumor properties [102, 145, 186, 187, 195, 235, 252, 253]. Importantly, 20(OH)D3 and 20,23(OH)$_2$D3 are non-calcemic, while 1,20(OH)$_2$D3 show low-calcemic activity [44, 187, 194, 234].

Receptors for Vitamin D in the Skin

Vitamin D Receptor (VDR)

An Overview The main phenotypic activities of canonical hydroxyderivatives of vitamin D are mediated through their interaction with the ligand-binding domain of the nuclear receptor, vitamin D receptor (VDR, NR1I1) [22, 28, 39, 46, 81, 130, 131, 142]. This interaction promotes heterodimerization of the VDR with the retinoid X receptor (RXR) and its translocation to the nucleus where it interacts with VDR-responsive elements (VDRE) to regulate the transcription of target genes (transactivation or repression). VDR is expressed in all tissues, including the skin [22, 28, 39, 157], and is reported to regulate approximately 3% of the mammalian genome. The human epidermis is rather unique in this

context in that it is both the source of vitamin D3 and a target tissue. The CYP11A1-derived secosteroids with a full-length side chain can bind to the VDR and act via a VDRE-dependent mechanism, with compounds containing a hydroxyl group at C1α exhibiting a higher affinity than those without it [102, 118, 119, 188, 207]. Most importantly, the crystal structures of 20(OH)D3, 1,20(OH)$_2$D3, and 1,25(OH)$_2$D3 bound to the genomic LBD of the VDR were obtained [118, 119] which illustrated similarities and differences between these compounds in their interaction with the VDR receptor (Fig. 13.5), as reported in [119].

VDR transcriptional activity is dependent on the availability of VDR agonists and antagonists and their effect on receptor conformation (allostery [160], the recruitment of different cofactors [18, 22], and chromatin accessibility [39, 136, 142]). Moreover, VDR activity can be influenced by single-nucleotide polymorphisms (SNPs) [254]. This plays, for example, a role in the etiology of nonmelanoma skin cancers (NMSC) and melanoma [38, 54, 104, 111, 117]. Interestingly, CYP11A1-derived D3 hydroxyderivatives without a hydroxyl group at C1α display a subset of the activities possessed by 1,25(OH)$_2$D3 (see above) and lack calcemic activity, acting as biased agonists on the VDR [197, 207].

In addition to genomic (G), VDRE-mediated regulation of gene expression, the VDR can also induce rapid responses via a non-genomic, membrane-associated mechanism that involves an alternative ligand-binding site (A-pocket) [81, 130, 131]. The list of ligands interacting with the A-pocket of VDR includes 25(OH)D3, 1,25(OH)$_2$D3, 1,25(OH)$_2$L3 [57, 130], and some CYP11A1-derived hydroxylumisterol derivatives [206], but not CYP11A1-derived vitamin D3 hydroxyderivatives [207]. An additional cell membrane-linked mechanism of action includes the interaction between VDR and caveolin-associated signal transducers [249].

Finally, different alternatively spliced forms of VDR have been described [5, 64, 68, 212]. It has been suggested that they can have different
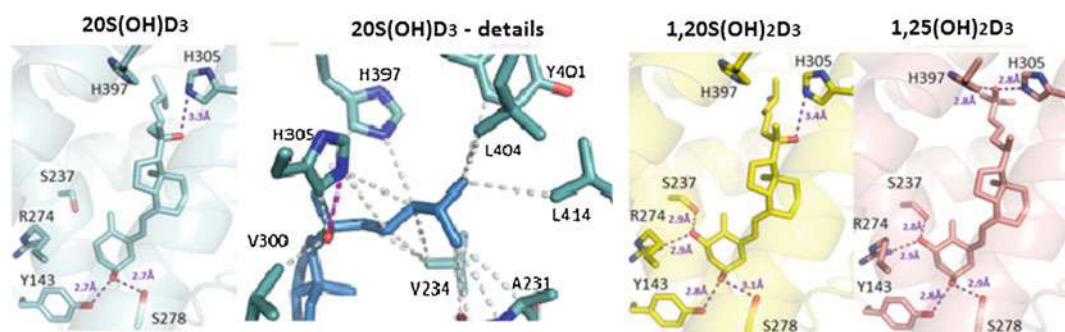
Fig. 13.5 Crystal structures of 20(OH)D3, 1,20(OH)$_2$D3, and 1,25(OH)$_2$D3 in complexes with the VDR ligand-binding domain

The crystal structures of 20*S*(OH)D3, in complex with the *Danio Rerio* VDR (zVDR) LBD, were determined and compared to those of 1,20(OH)$_2$D3 and 1,25(OH)$_2$D3 VDR complexes as described previously [119]. The complexes with 20(OH)D$_3$ (PDB ID 5OW9), 1,20 (OH)$_2$D3 (PDB ID 5MX7), and 1,25(OH)$_2$D3 (PBD ID 2HC4) are shown in cyan, yellow, and salmon, respectively. Hydrogen bonds between the ligands and LBD are represented by purple dashed lines. Details of the interactions mediated by the side chains of 20(OH)D3 are in the second image from the left. Hydrophobic interactions are indicated by gray dashed lines, and hydrogen bonds are depicted as pink dashed lines. Only residues within 4 Å of the ligand are shown by stick representation. The residue numbers correspond to human VDR. The detailed description and analysis are in [119]. (The work is reprinted from [119] under the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/) with small modifications)

transcriptional activity and promote VDR-ligand-independent functions [5]. Most recently, alternatively spliced forms have been detected in human melanoma cells [236]. Using the same methodology and the same primers with sequencing of the resulting cDNA fragments [49, 236], we identified VDR isoforms a, b, c, 1a, 1d, and 1f, similar to those described previously [49, 236], in normal adult and neonatal human epidermal keratinocytes and the skin fragments from white and black subjects. The immediate challenges in this area are to determine whether alternatively spliced VDR isoforms exhibit distinct functions in skin cells and regulate the expression of different genes and whether the alternative splicing is regulated by endogenous or environmental factors, as has been shown for other receptors such as CRH-R1 [146, 147, 196, 250]. In addition, it further needs to be determined whether these isoforms display different affinities for different vitamin D3 hydroxyderivatives and exhibit differences in their interaction with RXR, cofactors, and DNA or to understand mechanisms by which they regulate VDR-ligand-independent functions.

VDR in the Skin VDR is expressed in all skin cell types [17]. However, its level of expression can change depending on the specific pathology, as documented in VDR knockout mice. For example, VDR$^{-/-}$ mice show significant defects in cutaneous structures, alopecia [46, 143], and have significantly increased propensity to develop epidermal skin cancer [21, 23, 216]. The later indicates that VDR functions as a tumor suppressor [19, 20].

With respect to melanomagenesis, significant changes in the level of VDR expression were observed during progression of melanocytic tumors, with reduced nuclear and cytoplasmic VDR levels correlating with tumor progression and Clark levels, with highest VDR levels in normal skin and common melanocytic nevi, and with lowest VDR levels in advanced and metastatic melanomas [33, 35]. Low or lack of VDR expression also positively correlated with poor prognostic markers of melanoma and poorer outcome of the diseases as measured by shortening of the survival and disease-free times [33, 35]. The combined analysis of CYP27B1 and VDR showed an even stronger correlation

with disease progression, with the lowest levels of expression in highly advanced melanomas and metastases [34]. Interestingly, an inverse correlation between VDR and nuclear expression of HIF-1α was found with the highest HIF-1α expression observed in pT3-pT4 VDR-negative melanomas [37]. Also, nuclear VDR expression was significantly lower than in normal uveal cells including melanocytes [125]. Finally, VDR single-nucleotide gene polymorphisms are associated with a higher probability of developing melanoma and a poorer disease outcome (reviewed in [205]).

NMSC studies performed in animal models have convincingly demonstrated a role for VDR in photoprotection and prevention or attenuation of skin cancer development [12, 21–23, 40, 57, 92, 94, 233]. The latter involves inhibition of the hedgehog and Wnt signaling pathways and induction of keratinocyte differentiation [3, 79, 120, 216, 230]. Inhibition of the hedgehog pathway has also been implicated in the attenuation of other tumors, including rhabdomyosarcoma [231] and renal carcinomas [60]. The inhibition of hedgehog signaling by vitamin D compounds might be mediated by VDR-dependent and VDR-independent mechanisms [214].

Although VDR polymorphisms have been linked to various malignancies, including cutaneous melanomas [205, 208], studies on the relationship between VDR polymorphisms and the risk of developing NMSC (*Apa*I, *Bsm*I, and *Taq*I) [54, 80, 111] were not fully conclusive with some, but limited, evidence indicating a relationship between VDR SNPs and NMSCs. In a German population, a correlation between the combined ApaI/TaqI/BglI AaTtBb genotypes of VDR with BCC risk was observed (aaTTBB VDR genotype was found only in controls). The aaTTbb VDR genotype was much more frequent in BCCs and SCCs that in the control population. Also, a higher frequency of the BB VDR genotype on sun-exposed versus nonexposed areas both in BCCs and SCCs was identified. In addition, Apa1 and Taq1 genotypes were associated with BCCs, but not with SCC photocarcinogenesis [104]. In a Polish population, the TT genotype of FokI VDR polymorphism was correlated with greater than tenfold higher risk of BCC development [111]. Burns et al. found that the BsmI b or TaqI t genotypes of VDR were more frequent in NMSC patients, suggesting that individuals with these genotypes are more likely to develop skin cancer [38]. A very recent nested case control study and meta-analysis showed that patients with rs2228570, rs927650, and rs1544410 recessive genotypes were characterized by a lower risk of SCC development, while rs7975232 and rs739837 recessive genotypes were related to decreased BCC risk [107]. Another study identified two new SNPs in VDR binding sites (rs16917546 and rs79824801) associated with BCC risk. This study also confirmed the association of the rs3769823 SNP in the VDR binding site with increased BCC risk [117], while a study performed on a population in the mid-south of the USA (96 cases vs. 100 controls) showed that subjects with *Bsm*I SNP had two times higher probability of developing NMSC in comparison to controls [38]. Thus, VDR polymorphisms should be considered as factors related to NMCS risk; however, additional studies are needed with larger population cohorts.

The vitamin D system has been analyzed in cell cultures and clinical samples of NMSCs. Reichrath's group found a significant increase in nuclear VDR expression (as detected with immunohistochemistry) in SCC samples compared to normal skin, however no correlation with histological type, grading or markers for proliferation, differentiation, or apoptosis, and increased expression of *VDR*, *CYP27A1*, *CYP27B1* and *CYP24A1* in SCC was observed [155]. Reichrath et al. [154] and Mitschelle et al. [129] also analyzed the expression of VDR in BCCs and found a pattern similar to SCC, with significantly elevated nuclear expression of VDR in BCCs in comparison to normal skin, adjacent epidermis, and unaffected epidermis. VDR expression was moderate or strong, and the strongest VDR expression was found in peripheral *palisade cells.* VDR expression was not correlated with a particular histological type of BCC. Similar to SCCs, the expression of *VDR*, *CYP27B1*, and *CYP24A1*, but not of *CYP27A1*,
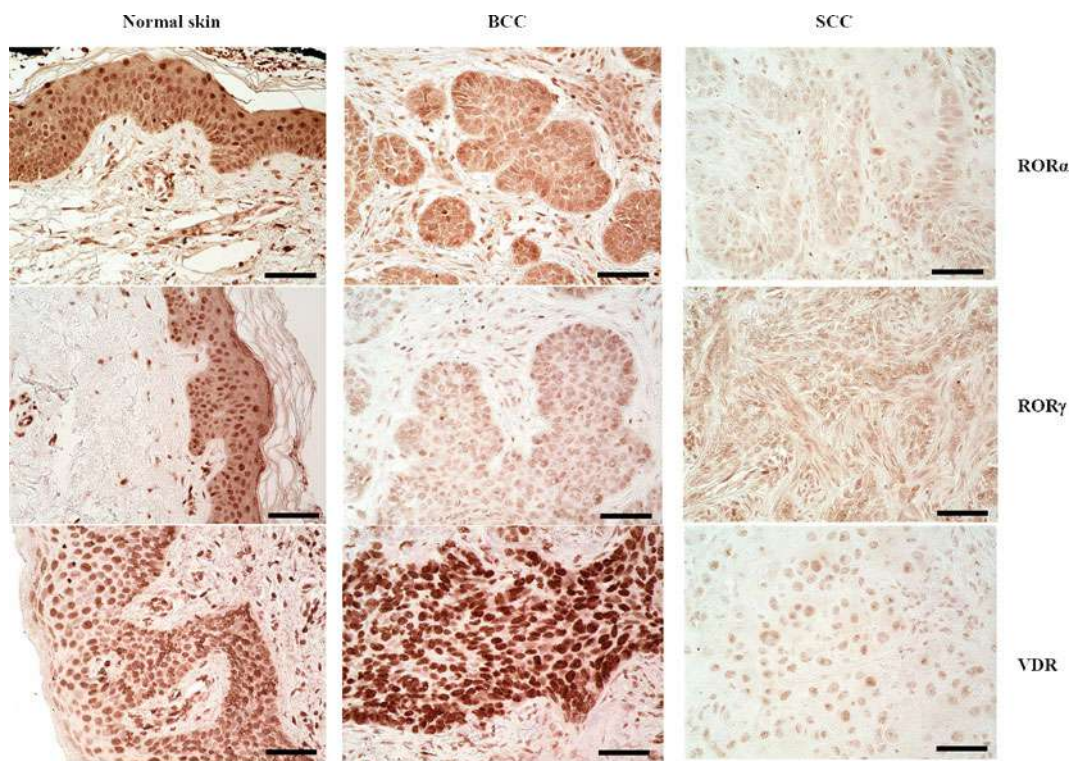
Fig. 13.6 Immunohistochemical detection of RORα (upper), RORγ (middle), and VDR (lower) in normal skin (left panel), BCC (middle), and SCC (right). Scale bar: 50 μm. Archival formalin-fixed paraffin-embedded sections, after heat-induced antigen retrieval in Tris-based antigen unmasking solution (Vector Laboratories, Inc., Burlingame, CA) and endogenous peroxidase blocking, were incubated over night at 4 °C with primary antibodies (rabbit anti-RORα (provided by Dr. Anton M. Jetten), 1:400; rabbit anti-RORγ (provided by Dr. Anton M. Jetten), 1:50; rat anti-VDR (Abcam,

MA1-710; Thermo Fisher Scientific, Waltham, MA)). Next, sections were incubated with secondary antibodies conjugated with HRP (anti-rabbit ImmPRESS antibody (ready to use, Vector Laboratories, Inc., Burlingame, CA) for RORα and RORγ; anti-rat antibody (1:200, Abcam, Cambridge, UK) for VDR), followed by peroxidase substrate ImmPACT NovaRED (Vector Laboratories Inc., Burlingame, CA, USA) application and mounting with permanent mounting media and glass coverslip (Thermo Fisher Scientific, Waltham, MA)

was increased in comparison to normal skin [129]. We also detected the VDR in human biopsies of BCC and SCC (Fig. 13.6). These studies show that both receptors for active forms of vitamin D and enzymes activating or inactivating vitamin D are expressed in NMSC, providing a rationale for targeting vitamin D signaling in the therapy of NMSC.

Other Receptors for Vitamin D: An Overview

Other receptor candidates for $1,25(OH)_2D3$ include the 1,25D3-membrane-associated, rapid-response steroid-binding protein (1,25D3-

MARRS), which is also known as ERp57/GRp58 and also serves as a protein disulfide isomerase A3 (PDIA3) that acts as a chaperone protein [101, 137] and has additional unexpected functions [138, 219]. According to some reports, it functions as a membrane-bound receptor for active forms of D3 and is involved in the regulation of some of its phenotypic functions [101, 137]. Other studies have shown interactions between plasma membrane 1,25D3-MARRS, VDR, and calveolin-1 via a non-genomic signal transduction pathway initiated by $1,25(OH)_2D3$ [43, 169]. Our molecular modeling predicts that

the CYP11A1-derived secosteroids are unlikely to interact with 1,25D3-MARRS [207].

Retinoic acid-related orphan receptors (ROR) α and γ, members of the nuclear receptor superfamily, provide an alternative mechanism by which vitamin D3 and its derivatives can regulate biological functions and gene expression and affect pathology [99, 199, 207]. CYP11A1-derived hydroxyderivatives of D3 can act as inverse agonists on RORα and RORγ. Similarly, hydroxyderivatives of lumisterol can function as RORα and RORγ inverse agonists [206]. Molecular modeling where these vitamin D3 metabolites exhibit high docking scores predicts that they interact strongly with the ligand-binding pocket of RORα/RORγ [208]. These receptors are expressed in normal and pathological skin [36, 199], including BCC and SCC (Fig. 13.6). Their expression inversely correlates with human melanoma progression, and higher expression in the nucleus correlates with significantly longer overall and disease-free survival times [36]. Interestingly, RORα and RORγ expression positively correlates with HIF-1 expression in cutaneous melanomas [37]. In uveal melanoma, expression of RORs was lower than in normal uveal cells [125]. This suggests that RORs may play an important role in melanomagenesis, melanoma progression, and host responses against the tumor [205, 208]. RORγ is essential for the generation of T-helper 17 (Th17) cells and production of the pro-inflammatory cytokine interleukin 17 (IL-17) which plays a critical role in various autoimmune diseases, including psoriasis, and also has antitumor as well as pro-tumor effects in melanoma [42, 99, 211]. Thus, these hydroxyderivatives could potentially inhibit inflammation and tumor progression in the skin through an RORγ-mediated mechanism.

Most surprising was a recent discovery showing that hydroxyderivatives of vitamin D3, including 20(OH)3, 20,23(OH)$_2$D3, 17,20,23 (OH)$_3$D3, and classical 1,25(OH)$_2$D3, can act on the aryl hydrocarbon receptor (AhR) in a manner dependent on the positions of hydroxyl groups on the structure [209]. This discovery is consistent with the promiscuous nature of AhR and its activity [134]. It opens up an exciting opportunity to study the regulation of the skin phenotype by different vitamin D3 hydroxyderivatives acting via AhR signaling, taking into consideration its complex role in skin physiology and pathology [27, 67, 93, 133] (Fig. 13.7).

Thus, different forms of vitamin D3, in addition to acting via the genomic canonical pathway of VDR, can potentially act via noncanonical pathways, including those involving the nuclear receptors, RORs and AhR. While the classical 1,25(OH)$_2$D3 can exert non-genomic activities through action via the non-genomic binding site of VDR or via 1,25D3-MARRS, similar functions for CYP11A1-derived secosteroids are less likely [207] and remain to be established experimentally. The receptors for pregnacalciferol derivatives [195] remain to be identified.

In summary, vitamin D hydroxyderivatives exhibit different affinities for multiple receptor targets and through their modulation of these distinct receptor signaling pathways regulate different physiological functions and influence pathologies in different ways.

Nonmelanoma Skin Cancers

Human Skin Cancer: An Overview

NMSCs, encompassing SCC and BCC, are the most common malignancies in humans. The cost of their treatment is an enormous economic burden to the healthcare system of the USA and to healthcare systems worldwide [113]. The role that UV radiation plays in the pathogenesis was first proposed in the late nineteenth century by Unna, who made the important observation that sailors, who had chronic exposure to sunlight, had a disproportionate increase in the incidence of skin cancer [25]. In fact, over 80% of NMSCs occur in sun-exposed skin sites, i.e., head and neck and back of the hands [11, 51, 100, 162, 200]. Studies in experimental animal models have demonstrated that wavelengths within the UVB range are primarily responsible for these malignancies [25, 66]. Immunocompromised patients, including solid organ transplant
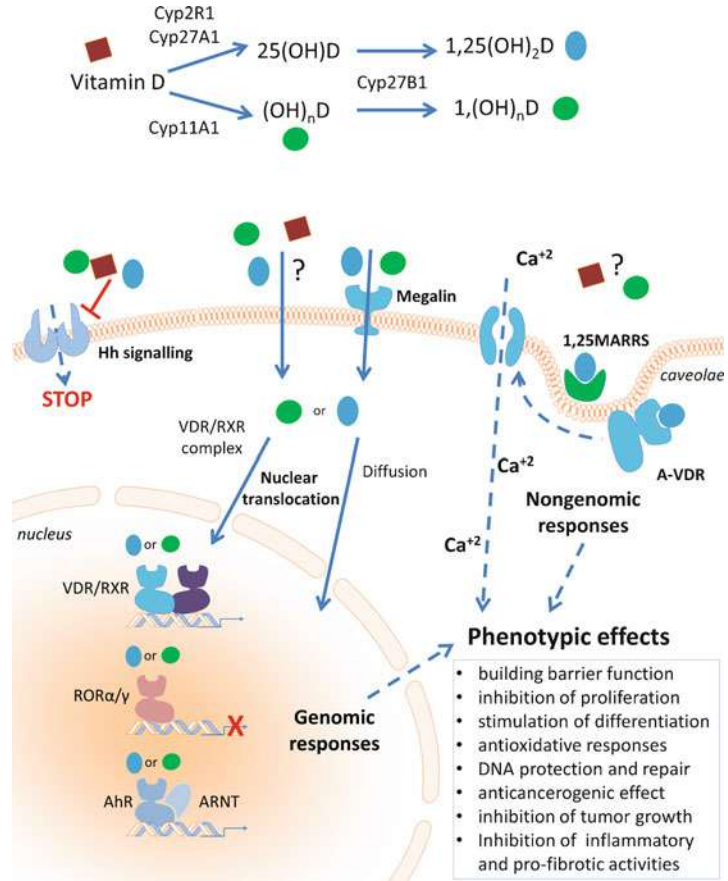
recipients who require drugs that suppress immunological function in order to prevent rejection of their transplanted organ, are at greatly increased risk of developing nonmelanoma skin cancers, particularly cutaneous squamous cell carcinomas [6]. Tumors in this population behave more aggressively and are more likely to metastasize [6]. Military personnel also have an increased risk of NMSCs [7]. They are exposed to high doses of UVR during deployment to locations with high solar radiation including the desert and high-altitude environments. This often happens in situations in which adequate attention to photoprotective measures is unavoidable. It should be noted that there was an unusually high incidence of NMSCs in World War II

veterans who served in the Pacific and elsewhere in the tropics. Currently, the incidence of skin cancer in the military is greater than in the general population.

Although there has been an intensive effort by healthcare institutions around the world to take preventative measures against excessive sun exposure, the incidence of these malignancies continues to rise [161]. Therefore, there is an urgent need to establish proper measures to stimulate photoprotective or reparative mechanisms in the skin of civilian and military personnel against UVR-induced damage. These measures need to be taken at as early an age as possible for young and older individuals alike, since skin cancers often develop after a long latency period.

Therapy of NMSC

The mortality for most NMSCs is low. However, they, and the treatment required for their removal, can be disfiguring with significant morbidity. Given the frequency with which they occur, the management of NMSC is a tremendous economic burden [113]. In the USA alone, the estimated cost for the treatment of actinically damaged skin is $1.68 billion [113].

Guidelines and appropriate use criteria for the management of both basal cell carcinomas and squamous cell carcinomas have been created by the American Academy of Dermatology and the National Comprehensive Cancer Network [4, 9, 10, 103, 244]. In most instances, the treatment of nonmelanoma skin cancers is surgical. This includes electrodessication and curettage, excision with appropriate tumor-free margins, and Mohs micrographic surgery. Electrodessication and curettage is used primarily for lower risk skin cancers, chiefly on the trunk and extremities. The procedure involves scraping away malignant tissue with a curette followed by electrodessication of the treatment area; the procedure is repeated up to three times. The cure rate has been reported to be up to 95% for low-risk lesions but is considerably lower for higher-risk tumors [9, 45, 108]. Standard excision followed by histological evaluation of margins is another option. Recurrence or metastasis rates of less than 6% can be achieved for primary tumors; cure rates for recurrent lesions, however, are substantially lower [163]. Subclinical involvement for cutaneous squamous cell carcinomas is present in up to 15% of primary tumors and up to 50% of recurrent squamous cell carcinomas [8, 110]. For this reason, Mohs micrographic surgery is the treatment of choice for most high-risk nonmelanoma skin cancers. Mohs micrographic surgery is an outpatient surgical procedure in which the tumor is debulked. Then a thin layer of underlying tissue is removed and examined histologically by frozen section to determine if it is free of tumor. If not, then further surgical layers are removed until there is no microscopic evidence of tumor. Surgery is performed all in one session with the patient remaining in the clinic while tissue sections are evaluated. Mohs micrographic surgery minimizes the amount of normal tissue that must be taken and provides microscopic verification that the tumor has been completely removed. Retrospective studies have found a 5-year cure rate of 97% for primary tumors and 90% for recurrences [229]. This is compared with 92% for primary tumors and 77% for recurrences with other procedures.

Radiotherapy, especially for low-risk tumors, is employed in some situations based on patient preference or other factors [9, 10]. It is contraindicated in patients with certain genodermatoses such as basal cell nevus syndrome and in individuals less than 60 years of age because of the potential for long-term consequences. Five-year cure rates of 93% for primary tumors and 90% for recurrent tumors have been accomplished with radiotherapy [9].

Topical imiquimod has received regulatory approval for treatment of superficial BCCs and premalignant actinic keratoses [10, 70, 139, 150, 166, 171]. It has also been used off-label for nodular BCCs [77, 239]. Imiquimod stimulates innate and acquired immunity by binding to the TLR7 and, as a consequence, stimulates dendritic cells and augments production of interferon-gamma, TNF-alpha, and other pro-inflammatory cytokines [77]. Recent studies have shown that it also has actions independent of TLR7 stimulation [232]. The end result is an antitumor immune response capable of eradicating BCCs. Treatment requires daily application of imiquimod for 6 (superficial BCC) to 12 (nodular BCC) weeks. Five-year response rates with imiquimod are significantly less than with surgical excision [239].

While metastasis of BCC is very rare, it can occur. Furthermore, neglected BCCs can enlarge to the point at which sufficient destruction of cutaneous and even non-cutaneous tissue occurs, making it impossible to remove the lesions surgically. The sonic hedgehog pathway plays an essential role in BCC pathogenesis. Two oral sonic hedgehog inhibitors, vismodegib and sonidegib, are commercially available, and both cause BCC regression [61, 122, 128, 168]. They are employed for the treatment of locally

advanced and metastatic BCC. These agents have many adverse effects including hair loss, muscle spasms, weight loss, and dysgeusia, which reduce patient compliance. Moreover, BCCs can develop resistance to these medications, and discontinuation often results in BCC regrowth. Thus, these medications are not used for routine BCCs.

Other treatment options for nonmelanoma skin cancers include cryotherapy, PDT, 5-FU, and intralesional methotrexate but are only utilized in special circumstances [9, 10, 103, 244].

Vitamin D in Chemoprevention of NMSC

Photoprotective Activity of Active Forms of Vitamin D3

A significant number of studies have shown protective effect of different vitamin D analogs against UVR in human skin cells and hairless mice [53, 56, 58, 59, 72, 76, 109, 127, 220, 243]. Specifically, 1,25(OH)$_2$D3 and 1,25(OH)$_2$L3 reduced UV-induced DNA damage including formation of CPD and reduced production of pro-inflammatory cytokines in human keratinocytes in culture and in mouse and human skin [127]. The photoprotective effects of these compounds were also connected with increased expression of P53 in the nucleus and a decrease in the number of apoptotic sunburn cells and attenuation of UVB-induced immunosuppression [57]. The authors suggested non-genomic actions of 1,25(OH)$_2$D3 and 1,25(OH)$_2$L3 [57]. Similarly, topical application of CYP11A1-derived 20(OH)D at 23 or 46pmol/cm^2 protected mouse skin against UVB-induced DNA damage at comparable level to that of 1,25(OH)$_2$D3 [221]. It also reduced the sunburn edema and protected against UVR-induced immunosuppression in a similar manner to 1,25(OH)$_2$D3. Thus, these in vivo photoprotective effects were independent of C1α-hydroxylation [221]. The same group demonstrated that in addition to 1,25(OH)$_2$D3, low-calcemic analogs of D3 reduced UV-induced CPDs in both skin fibroblasts and keratinocytes and their cell death

after UV exposure [58]. They were equally effective as 125(OH)$_2$D3 in increasing levels of p53 in cultured human keratinocytes. In a hairless mouse line, these compounds reduced UV immunosuppression. However, the low-calcemic analog was not as effective as 1,25(OH)$_2$D3 in reducing tumorigenesis [58]. Most recently, an interesting mechanism of action for 1,25(OH)$_2$D3 in UVB-irradiated keratinocytes was demonstrated. Specifically, it enhanced glycolysis along with energy-conserving processes such as autophagy and mitophagy, resulting in increased repair of CPDs and decreased oxidative DNA damage [165]. Finally, high doses of vitamin D3 given orally shortly after exposure to UVB could reverse the induced skin damage with attenuation of the inflammation and induction of barrier repair mechanisms [167].

Our studies on photoprotective functions of 20(OH)D3 and 20,23(OH)$_2$D3 in cultured human epidermal keratinocytes, melanocytes, and HaCaT keratinocytes have shown that they can attenuate ROS, H$_2$O$_2$, and NO production induced by UVB to a similar level to that for 1,25(OH)$_2$D3, with 25(OH)D3 and 20(OH)7DHC having lower efficiency [201]. The photoprotection was accompanied by increased expression of genes involved in defense against oxidative stress. Furthermore, these compounds reduced the UVB-induced CPDs and DNA fragmentation in the comet assay and enhanced expression of p53 phosphorylated at Ser-15, but not at Ser-46 [201]. The most recent tests on an extended list of CYP11A1-derived vitamin D3 and lumisterol hydroxymetabolites (1,25(OH)$_2$D3, 20(OH)D3, 1,20(OH)$_2$D3, 20,23(OH)$_2$D3, 1,20,23(OH)$_3$D3, 20(OH)L3, 22(OH)L3, 20,22(OH)$_2$L3, and 24(OH)L3), and lumisterol itself, have shown that they can protect human epidermal keratinocytes against UVB [41]. Treatment of cells with the D3 or lumisterol derivatives showed a dose-dependent reduction in UVB-induced oxidant formation, protection against DNA damage, and/or induction of DNA repair by enhancing the repair of 6-4PP and attenuating CPD levels and the tail moment of comets. They also stimulated the expression of antioxidant response genes downstream of Nrf-2

(GR, HO-1, CAT, SOD1, and SOD2) and expression at the protein level of HO-1, CAT, and MnSOD [41]. With respect to their mechanism of action, these compounds increased the phosphorylation of p53 at Ser-15 with stimulation of p53 and Nrf2 translocation into the nucleus. We have also shown that not only pre-treatment but also posttreatment of keratinocytes with D_3 and lumisterol derivatives can reverse UVB-induced keratinocyte damage [41] which is similar to other natural products [98, 175]. Thus, CYP11A1-derived D3 or lumisterol derivatives, and to some degree lumisterol itself, act as photoprotectors with their mechanism of action involving stimulation of the Nrf2-dependent and p53 responses, as well as stimulation of the DNA repair system.

Chemoprevention Against UVR and Chemically Induced NMSC in Animal Models

As discussed in subheading "Vitamin D Receptor (VDR)", the chemopreventive and potentially therapeutic roles of D3 hydroxyderivatives in NMSC are indicated by experiments with VDR$^{-/-}$ and RXR$^{-/-}$ (partner for VDR) mice on cutaneous carcinogenesis [12, 21–23, 40, 57, 92, 94, 233]. For example, Dixon et al. [57] have shown that 1,25 (OH)$_2$D3 and 1,25(OH)$_2$L3 inhibited UVB-induced development of papillomas and squamous cell carcinomas in immunocompetent mice (Skh:hr1). They suggested a non-genomic mechanism of action, at least in part [57]. Studies on low-calcemic analog, 1α-hydroxymethyl-16-ene-24,24-difluoro-25-hydroxy-26,27-bis-homovitamin D3, have shown that while it protected against UVB-induced damage, it was not as effective as 1,25(OH)$_2$D3 in reducing tumor formation and progression [58].

Others using 1,25(OH)$_2$D3 have shown that it inhibits proliferation and growth of BCC of Ptch mutant mice in vivo and of established murine BCC lines in vitro [230]. Two mechanisms of action have been shown, e.g., the activation of the VDR and induction of keratinocyte differentiation and inhibition of Hh signaling at the level of

Smo in a VDR-independent manner [230]. The 1,25(OH)$_2$D3 effects on BCC growth were stronger than those of the cyclopamine (Hh inhibitor), indicating that its dual action makes 1,25 (OH)$_2$D3 an excellent therapeutic for BCC and other tumors in which Hh signaling is disrupted [230]. Of great interest was the study showing that unmodified D3 inhibited Hh signaling and growth of murine BCCs both in vitro and in vivo [214]. D3 blocked both proliferation and Hh signaling to similar degree as cyclopamine. 7DHC, 25(OH)D3, and 1,25(OH)$_2$D3 were less effective in these actions. The D3 effect appeared to be independent of the VDR [214]. An important study led by Epstein on UVB-induced BCC carcinogenesis in Ptch1(+/−) mice showed that inhibition of UVB-induced production of D3 in the skin accelerated BCC carcinogenesis [124]. Furthermore, topical application of the D3 prohormone inhibited UVB-induced BCC tumorigenesis, while orally delivered D3 had no protective effect [124]. The authors concluded that UVB-induced production of D3 in keratinocytes significantly restrains murine BCC tumorigenesis and that UVB has anti-BCC carcinogenic effects through induction of D3 formation [124].

Studies on the chemically induced development and progression of SCC in mice showed that calcipotriol (analog of 1,25(OH)$_2$D3) inhibited the cancerogenesis and growth of tumors [50]. The mechanism of anti-cancerogenic action included induction of thymic stromal lymphopoietin [50].

Vitamin D in Chemoprevention or Adjuvant Therapy in NMSC in Humans

Currently, a few clinical trials have investigated the effects of vitamin D on NMSCs. The synergistic effects of calcipotriol and 5-FU treatment in optimally activating a CD4+ T cell-mediated immunity against actinic keratoses in randomized, double-blind clinical trial involving 131 participants were reported [50]. Another human trial has shown that calcipotriol combined with methyl aminolaevulinate photodynamic

therapy (MAL-PDT) was more efficacious than MAL-PDT alone and well tolerated [222]. The already completed Dutch phase II clinical trial (ClinicalTrials.gov Identifier: NCT01358045, start date November 2011, completed date May 2013) ([31], https://clinicaltrials.gov/ct2/show/NCT01358045?term=vitamin+d&cond=BCC&rank=3) was a randomized trial on the treatment of primary, histologically confirmed BCC (nodular of superficial subtype) with topical application of vitamin D3, diclofenac, or a combination of both twice daily under occlusion on BCC lesion. After 8 weeks, tumors were excised, and proliferation (Ki-67) and antiapoptotic (Bcl-2) markers were examined, and no effect of calcitriol alone was found. Combination therapy resulted in decreasing Ki-67 level in superficial BCC subtype, while diclofenac application was related to a significantly reduced expression of both Ki-67 and B-cl2 in superficial BCC. Another two clinical trials are related to BCC in basal cell nevus syndrome (BCNS) treatment with photodynamic therapy (PDT) and vitamin D as neoadjuvant. The first one is a clinical, double-blinded, randomized trial (ClinicalTrials.gov Identifier: NCT03467789, start date October 2018) (https://clinicaltrials.gov/ct2/show/NCT03467789?term=vitamin+d&cond=BCC&rank=2) on the vitamin D effect (10,000 IU/day) prior to the first or second PDT visit (treatment for 14 days when patients are deficient for 25-hydroxy-D3 serum levels or 5 days when 25-hydroxy-D3 levels are normal, and to maintain vitamin D3 level patients are supplemented with 2000 IU/day or 1000 IU/day for adults and children, respectively). The tumor clearance measured as change in lesion diameter per month is the primary outcome of this trial. The second one is randomized Phase 1 clinical trial (ClinicalTrials.gov Identifier: NCT03483441, start date March 2018, (https://clinicaltrials.gov/ct2/show/study/NCT03483441?term=vitamin+d&cond=BCC&rank=1), with a similar study design. Patients will take 10,000 units of cholecalciferol for several days prior to PDT, and differences in tumor BCC tumor diameter between treatments will be measured. The recruitment to these clinical trials has been opened; however, no results are

available yet. There is also a completed early Phase 1, double-blinded clinical trial on actinic keratosis, a precursor of SCC, treated with calcipotriol plus 5-fluorouracil (5-FU) in patients with multiple actinic keratoses (ClinicalTrials.gov Identifier: NCT02019355, start date October 2013, completed date March 2015, ([50], https://clinicaltrials.gov/ct2/show/NCT02019355)). A significantly reduced number of actinic keratosis was found in patients treated for 4 days with calcipotriol plus 5-FU when compared to only 5-FU treated patients. Currently, there is no open clinical trial on SCC treatment with vitamin D. Thus, vitamin D could enhance NMSC treatment; however, additional clinical trials are needed to fully justify its use and to select the most optimal vitamin D derivative for treatment of keratinocyte-derived cancers.

Perspective and Conclusions

The pleiotropic activities of D3 that are in addition to the regulation of body calcium homeostasis and include radioprotective and anticarcinogenic activities are consistent with the actions of multiple vitamin D derivatives produced in the human body and multiple target receptors in addition to the VDR. In vivo and in vitro studies reviewed above clearly document an important if not crucial role for different vitamin D compounds and the VDR, not only in photoprotection but also in the prevention or attenuation of NMSCs. With respect to cutaneous carcinogenesis, a key question is which chemical configurations of vitamin D compounds are the most efficacious with relatively minimal site effects and what is their mechanism of action, e.g., genomic or no-genomic. For genomic activities, new receptor candidates in addition to the VDR are emerging such as RORα and RORγ and AhR, which may be targeted in addition to the targeting of the Hh signaling pathway. Finally, different routes of delivery with preferred topical application have to be considered that require proper formulation.

Due to toxic (calcemic) effects, the therapeutic use of $1,25(OH)_2D3$ at pharmacological doses or

chronic oral use of D3 has its limitations. The discovery of an alternative pathway of D3 activation initiated by CYP11A1, producing at least 15 metabolites (OH)$_n$D3) with a full-length side chain and potentially several others with a short or absent side chain, opens new possibilities for treatment, since they have antiproliferative, pro-differentiation, anti-inflammatory photoprotective effects on normal and malignant epidermal cells. Many of them are non-calcemic and non-toxic at suprapharmacological doses. Furthermore, with the contribution of UVB acting on Δ7-steroids or sterols produced in the skin, the corresponding lumisterol and tachysterol compounds can be produced with photoprotective properties. Thus, novel secosteroids, lumisterol, and/or tachysterol compounds are excellent candidates to serve as radioprotectors and chemopreventive agents for skin cancers. They potentially can induce the repair of damaged DNA and/or attenuate or reverse UVR-induced skin aging [26].

In summary, recent advances in vitamin D, lumisterol and 7DHC biochemistry, skin biology, and pharmacology are opening up new exciting opportunities in skin healthcare and treatment of different cutaneous pathologies.

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The Vitamin D Receptor as Tumor Suppressor in Skin

14

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Abstract

Cutaneous malignancies including melanomas and keratinocyte carcinomas (KC) are the most common types of cancer, occurring at a rate of over one million per year in the United States. KC, which include both basal cell carcinomas and squamous cell carcinomas, are substantially more common than melanomas and form the subject of this chapter. Ultraviolet radiation (UVR), both UVB and UVA, as occurs with sunlight exposure is generally regarded as causal for these malignancies, but UVB is also required for vitamin D synthesis in the skin. Keratinocytes are the major cell in the epidermis. These cells not only produce vitamin D but contain the enzymatic machinery to metabolize vitamin D to its active metabolite, $1,25(OH)_2D$, and express the receptor for this metabolite, the vitamin D receptor (VDR). This allows the cell to respond to the $1,25(OH)_2D$ that it produces. Based on our own data and that reported in the literature, we conclude that vitamin D signaling in the skin suppresses UVR-induced epidermal tumor formation. In this chapter we focus on four mechanisms by which vitamin D signaling suppresses tumor formation. They are inhibition of proliferation/stimulation of differentiation with discussion of the roles of hedgehog, Wnt/β-catenin, and hyaluronan/CD44 pathways in mediating vitamin D regulation of proliferation/differentiation, regulation of the balance between oncogenic and tumor suppressor long noncoding RNAs, immune regulation, and promotion of DNA damage repair (DDR).

Introduction

Over one million skin cancers occur annually in the United States, 80% of which are basal cell carcinomas (BCC), 16% squamous cell carcinomas (SCC), and 4% melanomas, making skin cancer by far the most common cancer afflicting humankind [1]. Ultraviolet radiation

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(UVR) from the sun is the major etiologic agent for these cancers. The highest energy UVR, UVC (below 280 nm), does not penetrate the atmosphere. Of the solar radiation that does reach the earth, 95% is UVA and 5% UVB. UVB (280–320 nm), although it does not penetrate past the epidermis, is absorbed by DNA in the epidermal cells creating characteristic mutations identified as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP), which if not repaired result in C to T or CC to TT mutations, the UVB "signature" lesion [2, 3]. UV wavelengths between 320 and 400 nm (UVA) are capable of penetrating into the dermis and do their DNA damage (e.g., 8 hydroxy 2′ deoxyguanosine and peroxynitrite production) primarily by oxidative processes [4], although at high enough dose levels UVA can produce CPDs [5]. On the other hand, UVB is required to convert 7-dehydrocholesterol in the skin to previtamin D_3, which then isomerizes to vitamin D_3. Moreover, the skin is capable of converting the vitamin D produced to its active metabolite $1,25(OH)_2D$ [6], and this conversion is potentiated by UVR at least in part by cytokines such as TNF-α [7] which are increased by UVR in the epidermis [8]. Both melanocytes [9] and keratinocytes [10] express the vitamin D receptor (VDR) and respond to $1,25(OH)_2D$ with reduced proliferation and increased differentiation [11, 12]. Sun avoidance may reduce one's risk of developing skin cancer, but this practice could result in suboptimal levels of vitamin D in the body. Vitamin D supplementation can compensate, but the skin remains the major source of vitamin D availability for most of the world's population. Moreover, low-dose UVR may be protective against skin cancer via the vitamin D signaling mechanisms that will be reviewed in this article, and some epidemiologic evidence is consistent with a potential benefit of low-dose UVR. In a recent report, an international panel of experts in endocrinology, dermatology, photobiology, epidemiology, and anthropology [13, 14] concluded that sunscreens could be protective against the harmful effects of solar radiation while still enabling vitamin D production. In this chapter, after a review of vitamin D metabolism and VDR function, I will examine potential mechanisms that have been proposed for vitamin D-induced antitumor mechanisms in general and then focus on those mechanisms that have been shown to be operative in the epidermis.

Vitamin D Metabolism

Vitamin D_3 is produced from 7-dehydrocholesterol (7-DHC). UVB breaks the B ring of the 7-DHC to produce previtamin D_3, which subsequently undergoes a temperature-dependent rearrangement of the triene structure to form vitamin D_3, lumisterol, and tachysterol. This process is relatively rapid and reaches a maximum within hours [13, 15, 16], although both the amount of epidermal pigmentation and the intensity of exposure influence the time required. With continued UV exposure the biologically inactive lumisterol and tachysterol accumulate eliminating the risk of excessive production of vitamin D. Sunlight exposure increases melanin production, which can absorb UVB, and so provides another mechanism by which excess vitamin D_3 production can be prevented. The intensity of UVR is dependent on latitude and season. In Edmonton, Canada (52°N), very little vitamin D_3 is produced in exposed skin from mid-October to mid-April, while in San Juan (18°N) the skin is able to produce vitamin D_3 all year long [17]. Clothing and sunscreen effectively prevent vitamin D_3 production in the covered areas. Vitamin D_3 produced in the skin can be carried to the liver and other tissues for further metabolism to 25-hydroxyvitamin D (25OHD) and then to the kidney to produce $1,25(OH)_2D$ by the enzyme CYP27B1. The major 25-hydroxylase in the liver is CYP2R1, but the main 25-hydroxylase in keratinocytes appears to be CYP27A1 [18]. However, CYP2R1 has been found in dermal fibroblasts [19]. The expression of CYP27A1 like that of CYP27B1 is mitochondrial. Its expression is increased by vitamin D and UVB irradiation [18], but otherwise its regulation is unclear. The microsomal CYP2R1 is a more

specific 25-hydroxylase, but as of yet its expression in keratinocytes has not been reported.

The production of $1,25(OH)_2D$ in the skin is under quite different regulation compared to its production by the kidney, although the same enzyme, CYP27B1, is involved. In the kidney parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and $1,25(OH)_2D$ itself are the principal hormonal regulators: PTH stimulates, whereas FGF23 and $1,25(OH)_2D$ inhibit $1,25(OH)_2D$ production. Keratinocytes respond to PTH with increased $1,25(OH)_2D$ production, but these cells do not have the classic PTH receptor and do not respond to cyclic AMP [6] unlike the kidney. The effect of FGF23 on keratinocyte CYP27B1 expression or function has not been reported. Furthermore, unlike the kidney, $1,25(OH)_2D$ does not directly affect CYP27B1 expression in keratinocytes. Rather, $1,25(OH)_2D$ regulates its own levels in the keratinocyte by inducing CYP24A1, the catabolic enzyme for $1,25(OH)_2D_3$ [20]. In the keratinocyte the major regulators of $1,25(OH)_2D$ production are cytokines such as tumor necrosis factor-α (TNF) [7] and interferon-γ (IFN) [21]. These cytokines are activated in the skin by UVB, which of course also increases the substrate via increased vitamin D production. The differences in regulation of CYP27B1 in kidney vs nonrenal cells such as macrophages and most likely keratinocytes have recently been shown to involve different regions of the CYP27B1 promoter that are accessible to regulatory factors in a tissue-specific manner [22].

Vitamin D Receptor: Mechanism of Action

The VDR is a member of the nuclear hormone receptor superfamily [23]. These members are characterized by a highly conserved DNA-binding domain characterized by two zinc fingers and a structurally conserved ligand-binding domain that has at its C-terminal end the AF2 domain to which coactivator complexes bind [24]. The ligand-binding domain also serves as the region to which VDR binds to its transcriptional partners, RXR being the major one. In general ligand (i.e., $1,25(OH)_2D$) binding is required for the VDR/RXR heterodimer to form and bind to those regions on the DNA called vitamin D response elements (VDRE). Ligand binding also alters the structure of the VDR with major movement of helix 12 (C-terminus) into position to enclose the ligand while exposing sites on the VDR in helices 3, 5, and 12 to which coactivators bind. These coactivators can in turn recruit chromatin-modifying enzymes such as histone acetyl transferases (SRC, CBP/p300, pCAF) and DNA demethylases or proteins that bridge the gap between the VDRE and the transcription machinery (Mediator complex) including TATA-associated factors, TFIIb, and RNA polymerases (primarily RNA pol II). In the absence of ligand binding, sites for corepressors are exposed. These corepressors recruit another set of chromatin-modifying enzymes such as histone deacetylases and DNA methyl transferases [25]. The most common VDRE is comprised of two head-to-tail half sites of hexanucleotides separated by three nucleotides, referred to as DR3 VDREs. The sequence of these DR3 half sites is heterogeneous, with a consensus approximated by RGKTSA where R = A or G, K = G or T, and S=C or G. Moreover, many VDREs are not DR3s, although DR3s tend to have the highest affinity for VDR/RXR heterodimers.

The VDREs can be quite distant from the transcription start site of the gene being regulated, occurring in introns, between genes, and in either a 5′ or 3′ relationship to the coding region [26]. Moreover, putative VDREs as demonstrated by techniques such as ChIP-seq, in which binding sites to the genome by VDR are identified using a combination of chromatin immunoprecipitation of the VDR to DNA followed by high-throughput sequencing of those binding regions, number in the thousands, with a substantial degree of cell/tissue specificity [27]. Most genes have several VDREs. In a review of two such ChIP-seq studies, Carlberg et al. [28] noted that the study in a lymphoblastoid line identified 2776 VDREs for 232 genes, whereas the study in THP-1 monocytes identified 1820 VDREs for 638 genes. In the latter study 408 of the genes

were upregulated and 230 downregulated. Most of the VDREs for the upregulated genes were within 400kbp of the transcription start site; this was less true for the downregulated genes. Only 93 of the upregulated genes had VDREs within 30kbp of the transcription start site. Moreover, only 31.7% of the VDREs were DR3s. These two studies had only 18% overlap of the VDREs identified but differed not only in cell line but dose and time after 1,25(OH)$_2$D was administered before the cells were analyzed. Earlier microarray studies had likewise demonstrated the many genes regulated by 1,25(OH)$_2$D and the surprising lack of consensus from one study to the next perhaps due to tissue specificity and/or differences in dose and time of 1,25(OH)$_2$D exposure. These studies demonstrate the diversity of vitamin D-regulated genes and diversity in type and location of VDREs. Such studies have revolutionized our concepts of the scope and means of vitamin D signaling and reveal many potential mechanisms by which vitamin D signaling can regulate cancer formation. In this regard an unbiased systems biology approach mapping genetic loci underlying susceptibility to skin cancer puts the VDR in the center of a complex set of networks linking regulation of barrier function, inflammation, and tumor formation [28].

The VDR is essential for most actions of 1,25(OH)$_2$D and its analogs. Tumors that are unresponsive to vitamin D have either lost their ability to produce 1,25(OH)$_2$D (i.e., decreased CYP27B1) [29, 30], increased their metabolism of 1,25(OH)$_2$D via upregulation of CYP24A1 [31], lost VDR transcriptional activity through posttranslational alterations in RXR [32], or decreased their VDR expression. The latter may be secondary to increased activity in tumors of inhibitors of VDR expression such as SNAIL [33] and SLUG [34], increased methylation of the VDR promoter [35], or increased expression of miRNA125b, an inhibitor of VDR expression [36]. In melanoma cell lines, the

administration of 5-aza cytidine (to inhibit DNA methyltransferase) and trichostatin (to inhibit HDAC activity) could restore responsiveness to 1,25(OH)$_2$D by increasing VDR levels and reducing miR125b expression [37]. P53 cooperates with VDR in its antitumor functions [38]. The nuclear levels of p53 are increased by 1,25(OH)$_2$D by nongenomic mechanisms [39]. Nevertheless p53 mutations in tumors can also contribute to resistance to vitamin D in tumors. The E3 ubiquitin ligase and transcriptional regulator murine double minute (MDM2) is also regulated by 1,25(OH)$_2$D [40], and unlike p53 it inhibits VDR levels and transcriptional activity [41] as well as that of p53.

However, not all actions of 1,25(OH)$_2$D involve the VDR, and not all actions of VDR are genomic. Nongenomic actions of 1,25(OH)$_2$D are rapid and include a number of signaling pathways such as the opening of chloride and calcium channels, activation of mitogen-activated protein kinases, protein kinase C, phosphatidylinositol 3-kinase, and phospholipase C (review in [42]. As will be noted subsequently, these nongenomic actions contribute to the protective effect of topical administration of 1,25(OH)$_2$D and its nongenomic analogs on UVB-induced CPD formation in the skin [43]. These rapid effects of 1,25(OH)$_2$D are mediated by either or both VDR acting in the membrane and an unrelated receptor variably known as membrane-associated rapid response steroid-binding protein (MARRS), ERp57, GRp58, ERp60, or protein disulfide isomerase family A, member 3 (PDIA3). In a study with fibroblasts from patients with variable mutations in the VDR, Sequeira et al. [39] demonstrated that both receptors including VDR lacking the DNA-binding domain mediated the protective effect of 1,25(OH)$_2$D on UVB-induced CPD formation. That said, most attention to VDR as a tumor suppressor has focused on its genomic actions.

Mechanisms of Tumor Suppression by Vitamin D: General

The demonstration that many genes and pathways are influenced by vitamin D signaling has suggested a large number of potential means by which vitamin D signaling can control tumor growth (recent reviews in [44, 45]).

Cell Cycle Regulation

Regulation by $1,25(OH)_2D$ of the cell cycle in a number of cells, normal and malignant, has been demonstrated. This results from an upregulation of cell cycle inhibitors such as $p21^{cip}$ and $p27^{kip}$ (cyclin-dependent kinase inhibitors) [46] and retinoblastoma-like protein 2 and retinoblastoma-binding protein 6 [47] and decreased expression of cyclins [48] and cyclin-dependent kinases [49]. In addition $1,25(OH)_2D$ increases the interaction of FoxO proteins (tumor suppressors controlling proliferation [50]) with VDR and FoxO regulators Sirt1 and protein phosphatase 1 that maintain FoxO in the nucleus by blocking MAPK phosphorylation [51]. Increased c-MYC expression and activity are frequently found in cancer [52]. c-MYC induces the expression of a number of cell cycle regulatory genes such as cyclin D2 and cdk4. $1,25(OH)_2D$ inhibits the expression of c-MYC [53], and c-MYC expression is increased in the skin and gut of VDR null mice [54].

Growth Factors

$1,25(OH)_2D$ and its analogs regulate a number of growth factor pathways. Insulin-like growth factor (IGF)-stimulated proliferation of the breast and prostate cells is reduced by $1,25(OH)_2D$ via its induction of IGF-I-binding protein 3 [55, 56]. TGFβ2 exerts antiproliferative actions in epithelial cells. $1,25(OH)_2D$ and its analogs increase the expression of TGFβ2 and TGFβ

receptors in breast and prostate cancer cells [47, 49, 57] while suppressing the expression of the latent TGFβ-binding protein [49, 58]. GDF15 (growth differentiation factor 15) is a member of the TGFβ superfamily and like TGFβ is antiproliferative in prostate cancer cells. Its expression is increased by $1,25(OH)_2D$ [59, 60]. Bone morphogenic proteins (BMPs) are also members of the TGFβ superfamily that have been found to be dysregulated in certain cancers [61]. The expression of several BMPs is regulated by $1,25(OH)_2D$ and its analogs in a number of malignant cell lines [48, 49, 62]. Wnt/β-catenin signaling will be dealt with in depth when we focus on vitamin D-regulated pathways in the skin, but this pathway has been extensively studied in the colon based on the frequency of mutations in the adenomatous polyposis coli (APC) gene in colon cancer [63]. In the canonical pathway of Wnt/β-catenin signaling, the APC complex that would otherwise bind and phosphorylate β-catenin, targeting it for proteasomal degradation, is inactivated, allowing β-catenin to move to the nucleus where it binds to LEF/TCF leading to transcription of genes involved with proliferation. $1,25(OH)_2D$/VDR binds to β-catenin, preventing its movement into the nucleus and/or binding to LEF/TCF [64, 65]. Moreover, by increasing the levels of E-cadherin, which binds β-catenin in the plasma membrane, $1,25(OH)_2D$ can further reduce the translocation of β-catenin into the nucleus [64, 65]. Furthermore, $1,25(OH)_2D$ can suppress Wnt signaling by stimulating the expression of the Wnt antagonist DKK-1 [66]. Cystatin D, an inhibitor of several cysteine proteases of the cathepsin family that appear to be involved in Wnt signaling, has likewise been shown to be a target gene of $1,25(OH)_2D$ [67]. The induction of cystatin D and other $1,25(OH)_2D$ target genes such as E-cadherin appears to involve a nongenomic action requiring calcium activation of RhoA-ROCK-p38MAPK-MSK in colon cancer cells [68]. We have shown that this pathway requires the $1,25(OH)_2D$-induced calcium receptor in

keratinocytes [69]. These and other studies point to the interaction between calcium and vitamin D signaling in the regulation of tumor formation [70], an interaction that to date has received little attention, but which is supported by our observations that mice lacking both VDR and the calcium-sensing receptor (CaSR) develop KC spontaneously [71, 72].

Apoptosis

In addition to inhibiting proliferation, $1,25(OH)_2D$ promotes apoptosis in a number of malignant cell lines in part by downregulation of anti-apoptotic genes Bcl-2 and Bcl-X_L [73, 74] and upregulation of the proapoptotic gene GOS2 (G_0G_1 switch gene 2) [48, 75]. Transcripts of other pro-apoptotic genes increased by $1,25(OH)_2D$ include death-associated protein-3, caspase 8 apoptosis-related cysteine peptidase, and fas-associated death domain-like apoptosis regulator as well as a number of caspases [47]. Telomerase is a mechanism that enables cancer cells to escape apoptosis. $1,25(OH)_2D$ suppresses telomerase expression by inducing miRNA498, a transcript in the complementary strand of CTC-360P6 [76]. Of interest is this miRNA has its own VDRE [76]. On the other hand, a mutant form of p53 has been shown to reverse the anti-apoptotic effect of $1,25(OH)_2D$ [77].

Oxidative Stress

As noted previously in the discussion of UVA-induced effects on the epidermis, oxidative stress can lead to oxidative DNA damage, marked by 8 hydroxy $2'$-deoxyguanosine and reactive nitrogen species. In VDR knockout mice, 8 hydroxy $2'$-deoxyguanosine levels are increased in the colon [78] and reduced by vitamin D supplementation in humans [79]. $1,25(OH)_2D$ induces several antioxidant enzymes in cancer cells including thioredoxin reductase 1 [47, 49], superoxide dismutase [49, 59], and glucose-6

phosphate dehydrogenase [58]. The induction of genes associated with DNA repair will be discussed at greater length when we focus on UVB damage to the epidermis, but the induction by $1,25(OH)_2D$ of GADD45α (growth arrest and DNA-damage inducible α), p53, RAD23B, PCNA, and DAP-1α may all contribute to this aspect of tumor suppression by $1,25(OH)_2D/$ VDR [47, 75, 80].

Prostaglandins

Prostaglandins have been shown to stimulate cancer cell growth [81]. $1,25(OH)_2D$ blocks prostaglandin signaling by inhibiting COX2 expression and that of prostaglandin receptors while increasing the expression of hydroxyprostaglandin dehydrogenase 15-NAD, the prostaglandin-inactivating enzyme [60, 82].

Angiogenesis

Growing tumors require a blood supply. $1,25(OH)_2D$ inhibits angiogenesis by blocking the expression and function of VEGF (vascular endothelial growth factor) and other proangiogenic factors [83–85]. Mice lacking VDR had larger and more vascular tumors when implanted with prostate cells from TRAMP mice [86].

Immune System

The immune system plays an important protective role in cancer protection [87] as evidenced by the increased numbers of malignancies in immunosuppressed hosts including SCCs in immunosuppressed renal transplant patients [88]. UVB results in immunosuppression [89], which can either be ameliorated [43] or enhanced [90] by $1,25(OH)_2D$ as will be dealt with in more depth when we focus on the skin, as it has not received much study in the context of tumor protection in general by $1,25(OH)_2D$.

Mechanisms of Tumor Suppression by 1,25(OH)$_2$D/VDR in the Epidermis

The potential for vitamin D signaling as protection against epidermal tumor formation was demonstrated when Zinser et al. [91] observed that 85% of the VDR null mice but none of the controls developed skin tumors within 2 months following 7,12 dimethylbenzanthracene (DMBA) administration. These were primarily papillomas. These results have been confirmed using topical administration of DMBA/TPA [92]. However, although only papillomas were seen in the VDR null mice, RXRα null mice developed both BCC and SCC [92]. Subsequently, Ellison et al. [93] and our own group [94] demonstrated that VDR null mice were also more susceptible to tumor formation following UVB and many of the tumors were SCC and BCC. Moreover, as mentioned previously, deletion of both VDR and CaSR resulted in spontaneous tumor formation albeit in 1-year-old mice [72]. The appearance of BCC in these studies is surprising since the typical malignancy induced in mouse skin by UVR, ionizing radiation, or chemical carcinogens is SCC not BCC [95]. Given that BCC generally result from increased hedgehog (Hh) signaling [96], and that lack of VDR results in BCC when β-catenin signaling is increased [97], we became interested in the relationship between vitamin D, Hh, and β-catenin signaling in tumor suppression. Along with these studies, we noted that UVR increased the breakdown of hyaluronan to shorter forms that are known to promote inflammation and tumor progression stimulating our examination of this pathway [98]. We also discovered that VDR regulated the expression of long noncoding RNAs (lncRNAs) such that in VDR null mouse epidermis the balance between oncogenic and tumor suppressor lncRNAs was shifted to oncogenic species [99]. The lack of a normal innate immune response in CYP27B1 null mice to wounding [100] or infection [101] and the increased numbers of SCC in immunocompromised patients [88] suggested that disruption of the immune system might contribute to the increased susceptibility to tumor formation when vitamin D signaling was impaired. Moreover, we [94] noted a reduction in clearance in CPDs following UVB exposure of the skin of VDR null mice, suggesting that disruption of DNA damage repair was playing a role in tumor susceptibility in these mice. In what follows I will examine potential mechanisms and pathways within those mechanisms for their contribution to the role of VDR as a tumor suppressor including regulation of proliferation and differentiation with particular attention to the hedgehog (Hh), Wnt/β-catenin, hyaluronan/CD44 pathways, long noncoding RNAs, immunoregulation, and DNA damage repair (Fig. 14.1).

Vitamin D Regulation of Epidermal Proliferation and Differentiation

The epidermis is composed of four layers of keratinocytes at different stages of differentiation (reviewed in [11]). The basal layer (stratum basale, SB) rests on the basal lamina separating the dermis and epidermis. Within this layer are the stem cells. These cells proliferate, providing the cells for the upper differentiating layers. The basal cells are characterized by keratins K5 and K14 as well as the stem cell marker K15 and integrin α6β4. These cells have the highest expression of CYP27B1 and VDR in the epidermis. As the cells migrate upward from this basal layer into the spinous layer (stratum spinosum, SS), they initiate the production of the keratins K1 and K10, the keratins characteristic of the more differentiated layers of the epidermis. Cornified envelope precursors such as involucrin also appear in the spinous layer as does the enzyme transglutaminase K, responsible for the ε-(γ-glutamyl)lysine cross-linking of these substrates into the insoluble cornified envelope. Migrating further into the granular layer (stratum granulosum {SG}), lying above the spinous layer, the cells acquire the electron-dense keratohyalin granules containing profilaggrin and loricrin that give the SG its name. Loricrin is a major component of the cornified envelope. Filaggrin serves to bundle the keratin filaments, but also when proteolyzed is thought to contribute to the
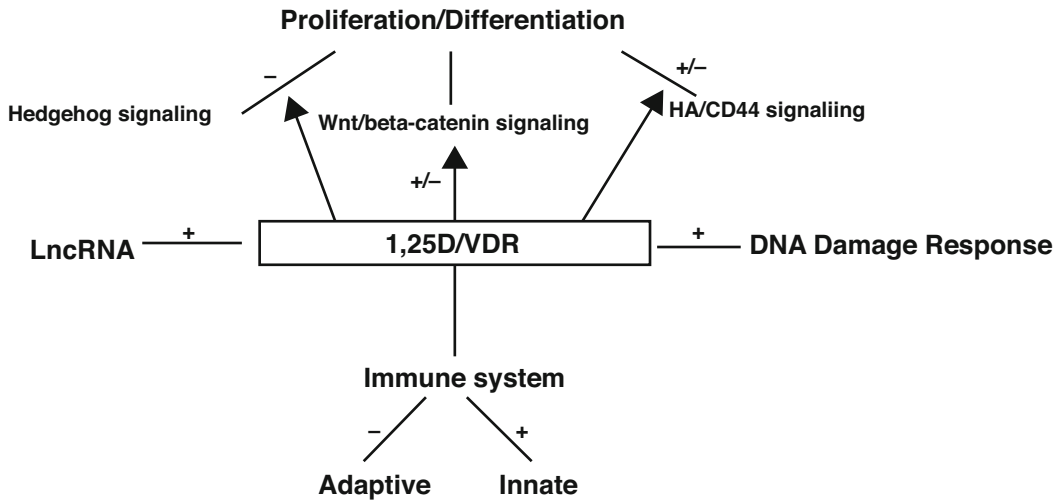
Fig. 14.1 Multiple mechanisms by which 1,25(OH)2D/ VDR suppresses tumor formation. Vitamin D signaling has the potential to suppress tumor formation by affecting a number of pathways. As depicted in this figure and discussed in the text, there are four major mechanisms: regulation of proliferation and differentiation via three pathways: hedgehog signaling, Wnt/b-catenin signaling, and HA/CD44 signaling; regulation of long noncoding RNAs by increasing the balance of tumor suppressors to oncogenic lncRNAs; immunity by suppressing adaptive and stimulating innate immunity; and promoting DNA damage response

hydration of the outer layers. The granular layer also contains lamellar bodies – lipid, enzyme, and antimicrobial peptide-filled structures that fuse with the plasma membrane, divesting their contents into the extracellular space between the SG and stratum corneum (SC). The secreted enzymes process the lipids that contribute to the permeability barrier of the epidermis in conjunction with the keratin bundles and cornified envelope. The antimicrobial peptides provide a barrier against infectious organisms in the SC.

$1,25(OH)_2D$ increases essentially every step of this differentiation process [102–107] while inhibiting proliferation at least at concentrations above 1 nM. These actions complement those of calcium [69]; the response to which is enhanced by $1,25(OH)_2D$ via its induction of the CaSR [108, 109] and the phospholipase C enzymes [110–112] that regulate intracellular calcium and other signaling molecules critical for the differentiation process. The antiproliferative effects are accompanied by a reduction in the expression of c-myc [113] and cyclin D1 [114] and an increase in the cell cycle inhibitors $p21^{cip}$ and $p27^{kip}$. In addition, $1,25(OH)_2D$ and its receptor regulate the processing of the long-chain glycosyl-ceramides that are critical for permeability barrier formation [115] and induce the receptors, toll-like receptor 2 (TLR2) and its coreceptor CD14, that initiate the innate immune response in skin [100]. Activation of these receptors leads to the induction of CYP27B1 (the enzyme that produces $1,25(OH)_2D$), which in turn induces cathelicidin resulting in the killing of invasive organisms [100, 116]. Deletion of either VDR [117, 118] or CYP27B1 [119] results in defects in the differentiation process leading to an abnormal barrier and increased proliferation of the epidermis with a defective innate immune response [100]. Three pathways that appear to be important in vitamin D signaling in the epidermis with respect to proliferation and differentiation that we believe underlie the predisposition of the VDR null mouse to tumor formation are the Hh, Wnt/β-catenin, and hyaluronan/CD44 pathways, any and all of which could be influenced by the changes in long noncoding RNAs altered in their expression by the VDR.

The Hedgehog (Hh) Pathway

In the skin sonic hedgehog (Shh) is the ligand for patched (Ptch) 1, a 12-transmembrane domain protein that in the absence of Shh inhibits the function of another membrane protein smoothened (Smo). Smo in turn maintains a family of transcription factors, Gli1 and Gli2 in particular, in the cytoplasm bound to suppressor of fused (Sufu) [120, 121]. When Shh binds to Ptch 1, the inhibition of Smo is relaxed and Gli1 and 2 are released from Sufu and move into the nucleus where they initiate transcription of a number of factors including each other as well as Ptch 1, the antiapoptotic factor bcl2, cyclins D1 and D2, E2F1, and cdc45 (all of which promote proliferation), while suppressing genes associated with keratinocyte differentiation such as K1, K10, involucrin, loricrin, and the VDR [122–126].

The appearance of BCC is characteristic of tumors formed when Hh signaling is disrupted [127], although activation of Hh signaling also predisposes to UVR-induced SCC formation [128]. VDR null animals overexpress elements of the Hh signaling pathway in their epidermis and the epidermal portion (utricles) of the hair follicles [94]. Moreover, $1,25(OH)_2D$ suppresses the expression of all elements of the Hh pathway in a dose-dependent fashion that requires the VDR [94, 129] and reduces tumor growth in *Ptch* 1 null mice. The promoters of Shh and Gli1 have binding sites for VDR [130] suggesting that the effects of $1,25(OH)_2D$ on these genes are direct. However, vitamin D has also been shown to bind to and inhibit the actions of smoothened (Smo) directly without seeming to require further metabolism to $1,25(OH)_2D$ [131, 132].

The Wnt/β-Catenin Pathway

Wnt signaling via activation of β-catenin has a complex role in VDR function as discussed briefly earlier. In the canonical pathway, the receptor for Wnt ligands is a family of seven-transmembrane Frizzled receptors and an LRP5 or LRP6 coreceptor. When Wnt binds to this complex, disheveled (Dvl) is phosphorylated resulting in disruption of the axin/APC complex and inhibition of glycogen synthase kinase 3β ((GSK-3β)). In the basal state, GSK-3β phosphorylates the serine(s) within exon 3 of β-catenin resulting in its degradation by the E3 ubiquitin ligase. Wnt signaling, by blocking this phosphorylation, increases the availability of β-catenin in the nucleus, where it binds to transcription factors of the T-cell factor (TCF) and lymphoid enhancer factor (LEF) families to promote expression of genes such as cyclin D1 and c-myc [133] important for proliferation. β-catenin also forms part of the adherens junction complex with E-cadherin where it may play an important role in keratinocyte differentiation [134]. Tyrosine phosphorylation of E-cadherin, as occurs after calcium administration to keratinocytes, promotes the binding of β-catenin and other catenins to the adherens junction complex [134, 135] making it less available for transcriptional activity. $1,25(OH)_2D$ increases E-cadherin expression and its membrane localization [136]. Overexpression and/or activating mutations in the β-catenin pathway lead to skin tumors, in this case pilomatricomas or trichofolliculomas (hair follicle tumors) [137–139]. As noted earlier VDR binds to β-catenin and reduces the transcriptional activity of β-catenin in a $1,25(OH)_2D$-dependent fashion [64]. On the other hand binding of β-catenin to VDR in its AF-2 domain enhances the $1,25(OH)_2D$-dependent transcriptional activity of VDR [65]. Palmer et al. [97] evaluated the interaction between VDR and β-catenin in transcriptional regulation in keratinocytes and identified putative response elements for VDR and β-catenin/LEF in a number of genes. These interactions were either positive or negative, depending on the gene being evaluated. The hypothesis put forward is that genes in which the interaction was positive (i.e., stimulated transcription) benefited from β-catenin acting as a coactivator for VDR on VDREs, whereas in situations where the interaction was negative (i.e., suppression of transcription), VDR prevented β-catenin from binding to TCF/LEF required for transcription in those genes. We [114] have found in keratinocytes that

knockdown of VDR reduces E-cadherin expression and formation of the β-catenin/E-cadherin membrane complex resulting in increased β-catenin transcriptional activity, whereas 1,25(OH)$_2$D administration has the opposite effect. This was associated with increased (with VDR knockdown) or decreased (with 1,25(OH)$_2$D administration) keratinocyte proliferation and cyclin D1 expression. On the other hand Cianferotti et al. [140] found a reduction in proliferation of keratinocytes in the dermal portion of the hair follicle (below the bulge) in VDR null mice, and no stimulation of proliferation when β-catenin was overexpressed in these cells in contrast to the stimulation of proliferation in control animals. Moreover, when we examined mice lacking VDR specifically in their keratinocytes, we observed a reduction in both epidermal and hair follicle stem cells and a reduction in β-catenin signaling opposite to our original observations in keratinocyte cultures [141]. Thus VDR/β-catenin interactions can be positive or negative, depending on the gene/cell/function being evaluated and the cellular context, but disruption of this pathway does appear to affect stem cell numbers and their differentiation.

The β-catenin and Shh pathways interact [55] [97]. Both are required for normal hair follicle development and cycling. Putative β-catenin/LEF response elements have been found in a number of Hh pathway genes [97]. Conditional deletion of β-catenin eliminates Shh expression from the hair follicle [142] and tongue [62], whereas Shh inhibits β-catenin transcriptional activity [143]. However, the degree to which β-catenin/Shh interactions occur in the formation of skin cancer has not been carefully examined.

The Hyaluronan/CD44 Pathway

Hyaluronan (HA), a major glycosaminoglycan within the extracellular matrix, binds to CD44 [144], a functionally important membrane receptor found in most cells including keratinocytes. CD44 is encoded by 19 exons of which 12 can be alternatively spliced, generally involving exons 6–14 to form variants (v1–10)

[145]. Differentiated keratinocytes express epican, CD44v3–10 (i.e., CD44 containing exons 9–14 in addition to exons 1–5, 15–17,19), whereas undifferentiated keratinocytes, mouse skin after chronic UVR, and SCCs express a variety of shorter CD44s with variable numbers of exons between the first 5 and last 5 [146]. These different isoforms of CD44 appear to signal differently [146]. HA is synthesized by different HA synthases, and its size is further modified by hyaluronidases. UVR alters both HA synthesis and its degradation [147]. Large HA predominates in normal mouse skin, whereas small HA predominates in cancer tissue [148]. Large HA promotes transcriptional activation and differentiation, whereas small HA induces the expression of proinflammatory cytokines/chemokines as well as cell proliferation and migration [149]. We have proposed that large HA/CD44 epican promotes keratinocyte differentiation and DNA repair through Rac/PKNγ and p38MAPK signaling, whereas small HA/CD44 variant promotes proliferation and inflammation through RhoA/ROK-dependent NFκB/Stat-3 signaling [98]. 1,25(OH)$_2$D blocks small HA/CD44-mediated RhoA/ROK activation and NFkB-p65 signaling as well as inflammatory gene expression and proliferation in transformed keratinocytes and SCC [98].

Long Noncoding RNAs (LncRNAs)

Only about 2% of the genome is actively transcribed and translated into proteins, while a much larger percentage of the genome is actively transcribed without protein coding potential [150]. These noncoding transcripts can be broadly categorized into short and long noncoding RNAs. The arbitrary size delineation is at 200 bases in length: small noncoding RNAs are less than 200 bases, whereas lncRNAs are endogenous cellular RNAs larger than 200 bases and can even be greater than 100 kb in length [151]. LncRNAs account for 80% of the transcriptome [150]; they are spliced and contain polyadenylation signals, much like messenger RNAs [152]. LncRNAs are expressed across all

mammalian genomes and have emerged as master regulators of embryonic pluripotency, differentiation, and body axis patterning, promoting developmental transitions [152, 153] and regulating histone modifications hence influencing the epigenetic programs of the transcriptome [154]. A number of these lncRNAs when aberrantly expressed are associated with cancers. We explored the potential role of lncRNAs in VDR protection against skin tumor formation by profiling 90 well-annotated mouse lncRNAs from mouse keratinocytes cultured in vitro and mouse epidermis from epidermal-specific *VDR* null mice and their normal littermates [99, 155]. We found that several well-known oncogenes, including *H19, HOTTIP*, and *Nespas*, are significantly increased, whereas tumor suppressor lncRNAs (*Kcnq1ot1, lincRNA-p21*) were attenuated in VDR-deleted keratinocytes. A similar pattern of lncRNA-expression profiling was observed in the epidermis of epidermal-specific VDR null mice vs. control littermates. In addition to the altered lncRNAs (*H19, HOTTIP, Nespas, Kcnq1ot1, lincRNA-p21)* in VDR-deleted cultured keratinocytes*, there was an increase in other oncogenes (*mHOTAIR, Malat1*, *and SRA*) and a decrease in other tumor suppressors (*Foxn2-as, Gtl2-as, H19-as*) in VDR null mouse epidermis. This study reveals a novel mechanism for protection by VDR against skin cancer formation by maintaining the balance of oncogenic to tumor-suppressing lncRNAs, although the relevant pathways involved require further investigation.

Vitamin D Regulation of Immune Function in the Skin

VDR and CYP27B1 are found in professional immune cells, namely, dendritic cells, macrophages, and lymphocytes [156, 157], responsible for both innate and adaptive immune responses as well as in epithelial cells expressing the components of the innate immune response. $1,25(OH)_2D$ regulates the proliferation and function [158] of these cells. Although it is not clear the extent to which dysregulated immune function contributes to cancer development in the skin, a link between inflammation and cancer susceptibility in the skin involving VDR has been established [28].

Adaptive Immunity

The adaptive immune response involves the ability of T and B lymphocytes to produce cytokines and immunoglobulins, respectively, in response to antigens presented to them by cells such as macrophages and dendritic cells. $1,25(OH)_2D$ suppresses the adaptive immune response by inhibiting proliferation, immunoglobulin production, and differentiation of B-cell precursors into plasma cells [157]. $1,25(OH)_2D$ inhibits T cell proliferation [159] and the differentiation of CD4 cells into Th1 cells capable of producing IFN-γ and IL-2 and activating macrophages [160] and Th17 cells capable of producing IL17 and IL22 [161, 162]. On the other hand, $1,25(OH)_2D$ stimulates IL-4, IL-5, and IL10 production [163] by increasing CD4 cell differentiation into Th2 and regulatory T cells (Treg) [164]. The IL-10 so produced is one means by which Treg block Th1 and Th17 development. Part of these effects is mediated by the negative impact of $1,25(OH)_2D$ on the maturation and antigen presenting capability of dendritic cells [165]. It is unclear if this suppression of the adaptive immune system alters tumor surveillance in the skin.

Innate Immunity

The innate immune response involves the activation of toll-like receptors (TLRs) [166] that serve as transmembrane pathogen-recognition receptors detecting specific membrane patterns (PAMP) shed by a wide variety of infectious agents [167]. Activation of TLRs leads to the induction of antimicrobial peptides and reactive oxygen species, which kill the organism. Cathelicidin is the best studied of these antimicrobial peptides. The expression of cathelicidin is induced by $1,25(OH)_2D$ in both myeloid and epithelial cells [168, 169], cells that also express

CYP27B1 and so are capable of producing 1,25 (OH)$_2$D needed for this induction. Stimulation of TLR2 in macrophages [170] or keratinocytes [100] results in increased CYP27B1 expression, which in the presence of adequate substrate (25OHD) induces cathelicidin expression. Lack of substrate (25OHD), VDR, or CYP27B1 blunts the ability of these cells to respond with respect to cathelicidin production [100, 169, 170].

The major cells involved in adaptive immunity in the skin include the Langerhans cells, dendritic cells, and T cells. The Langerhans cells are dendritic-like cells within the epidermis that when activated by invading organisms migrate to the lymph nodes serving the skin where they present the antigens to the T cells, initiating the adaptive immune response [171]. Keratinocytes, on the other hand, are equipped with toll-like receptors that enable them to respond to invading organisms with elaboration of antimicrobial peptides such as cathelicidin [116]. However, cathelicidin also induces an inflammatory response [172]. UVB leads to a reduction in Langerhans cells and blunts their antigen presenting activity [173–175] but stimulates the innate immune function of keratinocytes perhaps as a consequence of UVB-induced vitamin D/1,25(OH)$_2$D production in the skin [176, 177].

The potential role of altered skin immunity by UVB with respect to skin carcinogenesis was suggested by Kripke and Fisher [178]. They found that skin tumors originally induced in mice by chronic UVR would grow when transplanted into mice that had been UV irradiated but not when transplanted into control mice. The role of 1,25(OH)$_2$D production in UVR immunosuppression is not clear. Topical application of high concentrations of 1,25(OH)$_2$D protected against UVR induced suppression of contact hypersensitivity in the mouse [179], but a study in humans by the same group showed suppression of delayed hypersensitivity (Mantoux test) by topical 1,25(OH)$_2$D [180]. These data are limited but raise some concern about the balance between innate and adaptive immunity in tumor surveillance and how that balance is affected by vitamin D.

Vitamin D Regulation of the DNA Damage Response

DNA damage response (DDR) is the means by which UVR- and chemical-induced DNA damage is prevented from producing fixed DNA mutations [181]. DDR involves a cascade of damage recognition, repair, and signal transduction that coordinates the response of the cell to DNA damage. DDR activates checkpoints that delay the cell cycle, provides time for repair, and directs damaged cells into senescent or apoptotic pathways. DDR involves a number of components and is well orchestrated, tightly controlled, and highly accurate in normal primary cells such that the spontaneous mutation rate is very low, and changes in copy number are negligible [182–184]. As noted earlier UVB causes CPD and 6-4PP formation, which are bulky adducts that block the movement of replicative DNA polymerase, a high-fidelity enzyme, with a shift to translesion synthesis by lower-fidelity DNA polymerases [185]. Moreover, CPDs if they occur in promoter regions can block the binding of transcription factors [186]. With malignant transformation DDR becomes less controlled, and mutation rates and copy number abnormalities increase by orders of magnitude [182, 183, 187, 188]. Nucleotide excision repair (NER) is the principal means by which UVR damage is repaired, enabling repair before DNA replication begins. This is important as NER plays a major role in reducing the amount of damage that becomes fixed as mutations during replication [189–191]. During NER, the DNA damage is recognized by XPC acting in a complex with hRAD23B supported in some cases by the DNA damage-binding protein DDB1 and 2 [192, 193], the DNA is unwound around the lesion, and 30 base pair portions of DNA containing the lesion are excised by endonucleases such as XPF and XPG followed by fill in with DNA polymerases such as Pol δ,ε,κ.

The NER process has two main branches involving different mechanisms for the initial recognition of DNA damage [194]:

transcription-coupled repair (TCR) during which DNA polymerases stop replication at the site of the lesion until it is repaired [195–199] and global genomic repair (GGR), during which non-transcribed regions of the genome are repaired [200]. Keratinocytes in the epidermis of mice lacking VDR are deficient in DDR as demonstrated by a reduced rate of clearing CPDs and 6-4PPs following UVB [201]. Moreover, $1,25(OH)_2D$ increases CPD clearance in VDR intact mice [202, 203]. These actions have been demonstrated with $1,25(OH)_2D$ analogs that are not thought to have genomic activity [202]. However, at least part of this enhancement of CPD clearance is due to the upregulation of two genes important for DDR: XPC (xeroderma pigmentosum complementation group C) and DDB2 (damage-specific DNA-binding protein 2 also known as XPE) [201, 204]. Furthermore, $1,25(OH)_2D$ has been shown to increase the levels of p53, which could enhance apoptosis in those cells bearing excess DNA damage [203], and reduce UVR-induced oxidative stress contributing to the DNA damage [203]. As such these actions of vitamin D signaling on DDR contribute to the reduced susceptibility of normal skin to UVB-induced tumor formation.

Summary

The VDR is present in nearly every cell in the body. Moreover, the enzyme, CYP27B1, required for the production of the VDR ligand, $1,25(OH)_2D$, is likewise widely distributed. Because of its abundance of 7-DHC, the epidermis is unique in its capability to produce vitamin D, metabolize it to $1,25(OH)_2D$, and respond to $1,25(OH)_2D$ in a number of ways. Recent data from RNA-seq and ChIP-seq studies have demonstrated hundreds, perhaps thousands, of genes regulated by $1,25(OH)_2D$/VDR via VDREs which are located throughout the gene. The selection of the genes regulated by $1,25(OH)_2D$/VDR at any one time is cell specific and most likely dose and time specific with respect to exposure to $1,25(OH)_2D$. As a result of these studies, numerous pathways have been

discovered by which $1,25(OH)_2D$/VDR may prevent cancer. In the skin UVB is critical for vitamin D production, but UVB is also the major cause of skin cancer. This chapter examines the question of whether the beneficial effects of UVB on vitamin D production can counter the harmful effects on carcinogenesis. Epidemiologic data suggest that there may be a threshold below which UVR is not carcinogenic, a threshold that would suffice for adequate vitamin D production. Conceivably, vitamin D production at such levels of UVB exposure might even be protective. Four mechanisms for such protection were examined. The first mechanism focuses on the role of vitamin D signaling in keratinocyte proliferation and differentiation. Three pathways affecting proliferation and differentiation, namely, the hedgehog, Wnt/β-catenin, and hyaluronan/CD44 pathways, were evaluated. Mice lacking the VDR have increased expression of the hedgehog pathway, a key pathway involved in BCC formation. The role and regulation of the Wnt/β-catenin pathway in tumor formation is less clear as it is reduced in stem cells of the hair follicle and epidermis in VDR null mice in vivo but increased in keratinocytes lacking VDR when evaluated in vitro. Hair follicle tumors occur when Wnt/β-catenin signaling is excessive, but only in mice lacking VDR are malignant tumors (BCC) found. VDR/$1,25(OH)_2D$ inhibits expression of the components of the Hh pathway, but the interaction of VDR with β-catenin can be inhibitory or stimulatory with respect to gene expression depending on the gene. Overexpression of the hedgehog and Wnt/β-catenin pathways leads to increased proliferation and decreased differentiation associated with tumor development. The impact of hyaluronan/CD44 signaling on keratinocyte proliferation and differentiation depends both on the ligand (short HA vs long HA) and receptor (variant CD44 vs epican CD44). The short HA/CD44variant promotes proliferation and inflammation, whereas the long HA/CD44Epican promotes differentiation. VDR/$1,25(OH)_2D$ inhibits the short HA/CD44 variant pathway. The second mechanism discussed is VDR/$1,25(OH)_2D$ regulation of the expression of long noncoding RNAs. The loss of VDR

results in an increased ratio of oncogenic to tumor suppressor lncRNAs. The third mechanism involves the immune system, although the role of the immune system in epidermal carcinogenesis is not clear. However, an unbiased genomic examination of pathways associated with tumor susceptibility, inflammation, keratinocyte differentiation, and tumor formation linked these events with the VDR. $1,25(OH)_2D$/VDR promotes innate immunity but suppresses adaptive immunity. Whether this is beneficial regarding tumor development requires further study. The fourth mechanism is DNA damage response (DDR). The epidermis of VDR null mice shows impaired DDR following UVR. $1,25(OH)_2D$ accelerates DDR by what appears to be genomic and nongenomic actions. Thus the skin has developed mechanisms to protect itself from the harmful effects of UVR. Vitamin D production, metabolism, and regulation of the processes described in this chapter are likely to play key roles in this protection.

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Crosstalk Between Vitamin D and p53 Signaling in Cancer: An Update

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Abstract

It has now been convincingly shown that vitamin D and p53 signaling protect against spontaneous or carcinogen-induced malignant transformation of cells. The vitamin D receptor (VDR) and the p53/p63/p73 proteins (the p53 family hereafter) exert their effects as receptors/sensors that turn into transcriptional regulators upon stimulus. While the p53 clan, mostly in the nucleoplasm, responds to a large and still growing number of alterations in cellular homeostasis commonly referred to as stress, the nuclear VDR is transcriptionally activated after binding its naturally occurring biologically active ligand 1,25-dihydroxyvitamin D with high affinity. Interestingly, a crosstalk between vitamin D and p53 signaling has been demonstrated that occurs at different levels, has genome-wide implications, and is of high impor-
tance for many malignancies, including non-melanoma skin cancer. These interactions include the ability of p53 to upregulate skin pigmentation via POMC derivatives including alpha-MSH and ACTH. Increased pigmentation protects the skin against UV-induced DNA damage and skin photocarcinogenesis, but also inhibits cutaneous synthesis of vitamin D. A second level of interaction is characterized by binding of VDR and p53 protein, an observation that may be of relevance for the ability of 1,25-dihydroxyvitamin D to increase the survival of skin cells after UV irradiation. UV irradiation-surviving cells show significant reductions in thymine dimers in the presence of 1,25-dihydroxyvitamin D that are associated with increased nuclear p53 protein expression and significantly reduced NO products. A third level of interaction is documented by the ability of vitamin D compounds to regulate the expression of the murine double minute (MDM2) gene in dependence of the presence of wild-type p53. MDM2 has a well-established role as a key negative regulator of p53 activity. Finally, p53 and its family members have been implicated in the direct regulation of the VDR. This review gives an update on some of the implications of the crosstalk between vitamin D and p53 signaling for carcinogenesis in the skin and other tissues, focusing on a genome-wide perspective.

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Introduction

Vitamin D and p53 signaling pathways have a significant impact on spontaneous or carcinogen-induced malignant transformation of cells [1–3]. Mutations in genes of proteins of the p53 pathway represent a hallmark of many if not all types of cancer [1–3]. Low serum 25(OH)D concentrations and distinct polymorphisms (SNPs) in the vitamin D receptor (VDR) and other vitamin D-related genes are associated with an increased incidence and an unfavorable outcome of various malignancies [1]. The vitamin D receptor (VDR) and the p53 family all function typically as receptors/sensors that turn into transcriptional regulators upon stimulus, with the main difference being that VDR is transcriptionally activated after binding its naturally occurring ligand 1,25-dihydroxyvitamin D $(1,25(OH)_2D$ or calcitriol) with high affinity [1] while p53, mostly in the nucleoplasm, responds to a large and still growing number of alterations in cell homeostasis commonly referred to as stress [2–5]. In any event is the result of such activation - manifested by conformational changes and heterodimerization with retinoid X receptor (RXR) of VDR and by chemical modifications and oligomerization of the p53 family - the direct contact of these transcription factors with regulatory DNA. In both pathways the cell type and context-dependent recruitment of nuclear coregulators entails the stimulation or repression of a very large number, typically hundreds of genes [6–11]. Several of these code themselves for transcriptional regulators, adding a further level of complexity to the networks. It is obvious that transcription factor pathways may crosstalk, for instance, through the sharing of target genes or coregulators and through the engagement in interdependent regulatory loops. Indeed, all of these mechanisms, plus several others, seem to have been realized in the crosstalk of VDR with the p53 family.

Intriguingly, both pathways are critically involved in cellular processes that are important for carcinogenesis such as cell differentiation/proliferation, in the regulation of stem cell maintenance, and in cell homeostasis. While VDR controls proliferation/differentiation of many cell types, [1, 11] some members and isoforms of the p53 family, and in particular p53 itself, reduce the stem cell potential and stimulate differentiation [9]. Interestingly, on the side of the p53 family, all three members (p53/p63/p73) can be expressed as truncated isoforms capable of counteracting their siblings' transactivating effects [5]. Not too surprising, VDR and p53 have been linked to many malignancies, including non-melanoma skin cancer [1]. The present review aims at providing an overview on this interesting signaling network, with a focus on non-melanoma skin cancer and on genome-wide analyses. Before the crosstalk is discussed, the individual pathways shall be briefly outlined.

VDR and the Vitamin D Endocrine System/Regulatory Network

1,25-Dihydroxyvitamin D_3, the biologically active metabolite of vitamin D, has been shown to regulate the growth of various nonmalignant and malignant cell types, including human keratinocytes [1, 10–13]. This potent secosteroid hormone acts via binding to a corresponding intranuclear receptor (VDR), present in target tissues [1, 10–13]. VDR belongs to the superfamily of trans-acting transcriptional regulatory factors, which includes the steroid and thyroid hormone receptors as well as the retinoid X receptors (RXR) and retinoic acid receptors (RAR) [1, 10–13]. Evolutionarily, VDR is most closely related to the pregnane X receptor (PXR) that triggers xenobiotic detoxification and to the farnesoid X receptor (FXR) which governs bile acid metabolism [1, 10]. VDR binds its ligand 1,25-dihydroxyvitamin D_3 with high affinity, resulting in heterodimerization with RXR and in zinc finger-mediated binding to vitamin D-responsive elements (VDREs) in the regulatory region of target genes directly controlled by 1,25-dihydroxyvitamin D [1, 10]. As a consequence, vitamin D action in a particular cell strongly depends upon the metabolic production or delivery of sufficient concentrations of the 1,25-

dihydroxyvitamin D_3 ligand and expression of adequate VDR and RXR receptor proteins and of cell-specific programming of transcriptional responses to regulate selected genes that encode proteins that function in mediating the effects of vitamin D [1, 10]. Numerous studies, including cDNA microarray analyses of mRNAs, indicate that as many as 500–1000 genes may be regulated by VDR ligands [1, 10]. Transcriptional regulation of cell cycle regulatory proteins including p21/WAF-1 (CDKN1A) and other proteins involved in cellular growth and differentiation, including ß$_3$-integrin and fibronectin, by 1,25-dihydroxyvitamin D_3 has been shown [1, 10–12]. Keratinocytes express VDR [1, 9], whose natural ligand, 1,25-dihydroxyvitamin D_3, inhibits proliferation and induces differentiation of cultured human nonmalignant and malignant keratinocytes in vitro [1, 10–14]. Clinically, combination of 1,25-dihydroxyvitamin D_3 and isotretinoin was reported to be effective in the chemotherapy of precancerous and cancerous skin lesions, including non-melanoma skin cancer (cutaneous squamous and basal cell carcinomas) [1, 14, 15]. Additionally, it has been shown that VDR ablation sensitizes skin to chemically induced carcinogenesis [1, 14, 15].

In dependence of cell type and history, VDR signaling, like p53 signaling, can regulate cell proliferation and cell differentiation as well as apoptosis and cell survival – among many other functions that are of relevance for cancer development. The fact that VDR signaling can elicit many different responses already points to a complex regulation of this pathway and a need for integration of many additional signals that goes way beyond a simple ligand/receptor triggering of gene expression. Altogether, chemical modifications to the VDR pathway control such critical parameters as the intracellular trafficking, interaction duration of the receptor and ligand, as well as the stability and turnover of pathway-relevant proteins [1, 10]. In addition, VDR target gene regulation is controlled by microRNAs and RNA turnover [1, 10].

The p53 Family

p53, p63, and p73 are transcription factors that bind as tetramers to very closely related DNA motifs: two consecutive 10-mers (half-sites), preferentially spaced by no more than zero to two base pairs, with the consensus r,r,r,C,A/T,T,G,y,c,y in the case of p53; r,r,r,C,G,T,G,y,y,y; t/a,a/t,a,C,A/T,T,G,t,t/a,t; or r,r,r,C,A/G,T/A,G,y,y,y in the case of p63; and a/c/g,g/a,g,C,A,T,G,c/t,c,c/t in the case of p73 (r = purines; y = pyrimidines) [1, 6, 16–18]. The high degree of homology among the consensus sequences and the degeneracy of individual binding sites make it no surprise that the family members share a large number of target genes [16]. However, the actual control of a specific gene underlies the regulation by posttranslational modifications and protein/protein interactions that are specific for each transcription factor paralog. In addition, DNA binding is affected by the number of the half-sites, their orientation, their position relative to the target gene, and their possible overlap with binding sites for other transcription factors. Spacing between the 10-mers may affect protein conformation and the recruitment of coactivator or corepressor complexes [6]. Epigenetic CpG methylation does not seem to inhibit the binding to DNA strongly [1, 16, 17].

The p53 family proteins display a modular organization. Typically, a p53 relative carries an N-terminal transactivation domain (TD), a central DNA-binding domain (DBD) that with approximately 60% is the most highly conserved region among the paralogs, and C-terminal regulatory and protein/protein interaction domains [1, 5, 16]. Transcription initiation from internal promoters, alternative splicing, and alternative translation initiation sites generates a large variety of isoforms which, however, maintain the DBD in most cases. More than 10 different isoforms of p53 have so far been identified, 6 of p63 and at least 29 of p73 [5, 16, 19]. Only some of the biological functions of the minor isoforms have been identified. A whole arsenal of – partially interdependent and sequential – posttranslational modifications has evolved which, dependent

upon the presence or absence of specific protein domains, may or may not distinctly affect individual p53 family isoforms. Chemical modifications identified so far and regulating the proteins' abundance, DNA binding, level of activity as transcription factor, crosstalk with other proteins, and subcellular localization include phosphorylations, acetylations, ubiquitinations, sumoylations, neddylations, methylations, glycosylations, and oxidation/reduction [1, 5, 20, 21]. Their effects are best studied in p53, revealing an enormous degree of complexity [1, 3, 16]. For example, in the case of phosphorylations, sequentially buildup polyphosphorylation patterns at different sites (accompanied, perhaps, by other chemical changes such as acetylations), rather than single marks, establish a code that can regulate p53 function in a tissue-specific manner [1, 16, 22–24]. Active p53 regulates genes whose products serve functions in the transcription/translation of other genes; in the cell cycle, cell survival, and autophagy regulation; in the control of respiration, antioxidation, glucose metabolism, and cell adhesion/motility; in the cytoskeleton and endo-/exosome compartments; and in the control of angiogenesis [1, 6, 16].

Another example highlighting the complexity of the p53 clan regulation is the partnership between p53 and its most important negative regulators, the E3 ubiquitin ligases murine double minute 2 (MDM2) (that was originally identified as being gene-amplified on double-minute chromosomes in transformed mouse fibroblasts) and MDM4 [1, 16]. Many of the known p53-activating events act pro-p53 and anti-MDM2/4, with a large number of protein/protein interactions involved and with many modifications set on both p53 and the MDM proteins – most prominently phosphorylations and acetylations. The latter, for instance, inhibit the ubiquitination of p53 by MDM2 on at least three levels: through the inhibition of MDM2 itself, through the competitive modification of the C-terminal ubiquitin-targeted lysines on p53, and through the inhibition of p53/MDM2 binding [1, 16, 22–24]. MDM4, although not acting as a ubiquitin ligase to p53, can inhibit p53's transcriptional activity and modulate the p53/MDM2 interaction [1, 16, 21]. Since p53 can transactivate the *MDM2* gene, a negative feedback loop is formed [1, 16, 20, 21]. P63 and p73 can also stimulate *MDM2* gene transcription; however, in contrast to p53 and although both p63 and p73 are bound by MDM2, they are not ubiquitin-marked by it for degradation [1, 5, 16].

In general, cell context determines the respective function of the p53 family members. In the absence of extra stress, i.e., under physiological background stress caused, for instance, by reactive oxygen species (ROS) as a byproduct of respiration, the p53 family members are primarily involved in cell fate, differentiation, and development. These functions seem to be mainly, though not exclusively, mediated by the DNA-binding-competent yet transactivation-impaired delta-N isoforms of the proteins (ΔNp63, ΔNp73) [1]. In cells or tissues that have been additionally stressed, for example, by overt ROS production, radiation, hypoxia, hypo-/hyperthermia, metabolite shortages and imbalances, oncogene dysregulation, and virus/bacterial/parasite infections, the p53 family members, and in particular p53 itself, mainly govern repair, proliferative capacity, and survival [1]. These functions are exerted mostly by the transactivation-proficient isoforms (p53, TAp63, TAp73) [1]. Many of the damaging stresses are known to initiate and/or support cell transformation, and the earliest discovered and one of the most impressive functions of p53 is that of a tumor suppressor which can restrain damaged cells from transformation by the induction of cell cycle arrest, senescence, apoptosis, and terminal differentiation [1–3, 16, 25]. Lack of proper p53 function leads to tumors in many animals including humans; tumor-inducing viruses encode proteins that target p53; and there is quite possibly not a single tumor type existing in which the p53 pathway itself and all ascending/descending pathways are fully intact [1]. However, it has been suspected for a long time that p53 might exert functions in addition to tumor suppression, and indeed, recent discoveries have shown that it can, for instance, modulate stem cells, contribute to the general robustness and

life expectancy of humans (independently of tumor suppression), modulate mitochondrial respiration and glucose metabolism, and regulate human fertility [1, 2, 16, 26–28].

P63 and p73 are involved in tumor suppression as well, although more subtly than their cousin [1, 5, 16]. For example, while p53 as a classical tumor suppressor is frequently mutated in human cancers, p63 and p73 are not. Rather, p63 is often overproduced in tumors, which at a first glance may seem at variance with the notion of p63 being a tumor suppressor. However, in the majority of these cancers, it appears to be the p63 isoforms capable of DNA binding but incapacitated for transactivation due to lack of the transactivation domain (ΔNp63) that are predominating [1, 16, 29]. These are thought to act in a dominant-negative fashion toward transactivation-competent isoforms of the p53 family and to thereby function as oncoproteins. In contrast and like p53, transactivation-competent p63 (TAp63) can sensitize cells to apoptosis in response to DNA-damaging stress [1, 16, 30]. Moreover, in dependence of genetic background p63+/−, mice are tumor-prone, with the tumors often showing loss of heterozygosity for the remaining wild-type allele [1, 16, 31]. Loss of the transactivating isoforms of p63 or overproduction of the dominant-negative isoforms was also observed, for instance, in human carcinomas of the bladder [1, 16, 32]. Like p53 but more so than p63, p73 is a DNA damage-activated transcription factor that in response to damaging stress can stimulate many of the classical p53 target genes and also the gene for p53 itself [1, 5, 16, 33]. Mice with a specific knockout of TAp73 display genomic instability and an increased tumor incidence [1, 16, 34]. Of further note, p63 and p73 seem to have p53 independent roles in DNA repair [1, 16, 35, 36].

In contrast to p53 however, p63 and p73 are indispensable for embryonic development in all organisms studied so far [1, 16, 37]. Lack of p53 prevents mesoderm/endoderm fate determination in the frog *Xenopus* [1, 16, 38] but fails to produce obvious early phenotypes in mice or humans [1, 16, 39, 40]. At the level of tissues however,

p53 deficiency entails over-proliferation of stem cells, in accord with p53 acting as an inducer of stem cell differentiation [1, 16, 41, 42]. Another more subtle function of p53 is in mitochondrial respiration; lack of p53 here results in the reduced endurance of mice during exercise [1, 16, 43]. Contrary to p53 deficiency, overactivity of p53 does indeed have immediate and dramatic consequences in murine development – the apoptotic loss of the early embryo. One of the most remarkable functions of the p53 antagonists MDM2 and MDM4 during embryonic development lies in the prevention of this and in the keeping in check of p53-provoked apoptosis already at very early stages of development [1, 16, 44]. At later stages, for example, during neuronal development, the DNA-binding yet transactivation-defective dominant-negative isoform of p73, ΔNp73, serves as a p53 (and p63)-restraining factor by inhibiting p53 (p63)-mediated apoptosis [1, 16, 45]. Both p53 and TAp63 help shape the nervous system by inducing apoptosis; however, TAp63 is self-sufficient in this respect, while p53 depends upon TAp63 for this function [16, 46].

P63 function during development is critical not only for the efficient apoptosis of developing sympathetic neurons [1, 16, 47] but also for epithelial stem cell maintenance [1, 16, 48], squamous epithelial differentiation, and skin renewal, [1, 16, 49–51] with ΔNp63 mainly controlling the expansion of epithelial layers and TAp63 somehow pushing differentiation. Above that, TAp63 acts as the guardian of the female germ line by inducing apoptosis in damaged resting oocytes [1, 16, 52]. P73 deficiency in mice results in neuronal and olfactory dysfunctions and in chronic infection and inflammation [1, 5, 16]. Altogether, the stem cell/differentiated cell bifurcation is regulated in part by the balance between the ΔNp63 and TAp63 antagonists in the skin and – in an analogous manner – by the balance between the ΔNp73 and TAp73 antagonists in the developing nervous and immune systems.

In many respects, p73 seems to be for neuronal development and homeostasis what p63 is to the development and homeostasis of the skin, with

the transactivation defective isoform (here: ΔNp73) mostly, though not always, acting as an inhibitor of differentiation, an inhibitor of apoptosis induced by TAp63 and p53, and a promoter of proliferation [1, 16, 45, 53].

Crosstalk Between the VDR and the p53 Family in Cancer

Interestingly, an increasing body of evidence points to a crosstalk between vitamin D and p53 signaling that occurs at different levels and that might be of great importance for many malignancies, including non-melanoma skin cancer. Both p53 and VDR act as tumor suppressors in many tissues, including the skin. It turns out that much of this tumor suppressor function may be mediated through the interaction of the VDR and p53 pathways – either by activation or inhibition. This delicate interdependency shall be outlined in the follow, focusing on non-melanoma skin cancer. This form of skin cancer represents a well-characterized model. For cutaneous squamous cell carcinoma (SCC) development, UV-induced DNA damage is the most important environmental risk factor [1, 13]. Promutagenic pyrimidine dimers are the major forms of DNA damage produced directly by UV [1, 13, 54]. The predominant type of pyrimidine dimer detected after UV exposure in human skin is the thymine-thymine dimer, a cys-syn cyclobutane pyrimidine dimer (CPD), while thymine-cytosine, cytosine-cytosine bipyrimidines, and 6–4 photoproducts are less common [1, 13, 55–58]. CPDs are generated by the perturbation of the 5–6 double bonds in two adjacent pyrimidines, followed by abnormal covalent binding that connects the two pyrimidines by a stable ring configuration forming a bipyrimidine product [1, 13, 59, 60]. It is well accepted that CPD production requires the wavelengths of UVB (290–320 nm) [1, 13]. However there is some evidence for generation of thymine dimer by UVA wavelengths below 330 nm [1, 13, 58, 61–64]. UVR often causes gene mutations that may lead to cellular transformation and malignancy [1, 13, 65–

69]. DNA damage also initiates and promotes mechanisms that suppress immune surveillance responsible for detecting and eliminating transformed cells [1, 13, 70, 71]. UV exposure causes different types of DNA lesions that are produced either photochemically and directly or indirectly by UV activation of various photoreceptors that are able to alter the cellular redox equilibrium, thereby generating reactive oxygen species (ROS) [1, 13]. ROS-induced damage by UV radiation is able to cause oxidative damage to DNA and lipid peroxidation [1, 13]. Moreover, it has been shown that excess levels of nitric oxide (NO) are induced by UV-mediated upregulation of nitric oxide synthase [1, 13, 72–74] and also by UVA (320–400 nm)-mediated decomposition of NO stores in nitrosothiols and nitrite [1, 13, 75, 76]. Pathophysiologically elevated levels of NO and ROS have been demonstrated to combine to form genotoxic NO derivatives such as peroxynitrite that cause oxidative and nitrosative modifications to the sugar-phosphate scaffold and bases of DNA [1, 13].

UV irradiation causes p53 to stimulate skin pigmentation via POMC derivatives including alpha-MSH and ACTH [1, 77], thereby protecting the skin against further UV-induced DNA damage and skin carcinogenesis. On the other hand, this reduces cutaneous synthesis of vitamin D. It has also been shown that, on a second level, 1,25-dihydroxyvitamin D_3 increases the survival of skin cells after UV irradiation and that the surviving cells have no increase in DNA damage [1, 78]. In detail, the survival of keratinocytes post-UVR was significantly greater after treatment with 1,25-dihydroxyvitamin D_3 compared to vehicle ($P < 0.01$) [1, 78]. In that study, significant reductions in thymine dimers (TDs) in surviving keratinocytes after UVR were noted in the presence of 1,25-dihydroxyvitamin D_3 ($P < 0.001$) [78]. Nuclear p53 protein expression increased after UVR and was significantly higher in keratinocytes treated with 1,25-dihydroxyvitamin D_3 ($P < 0.01$), whereas NO products were significantly reduced ($P < 0.05$) [1, 78]. Both the increase in nuclear accumulation of p53 protein and reduced formation of nitric

oxide products may contribute to the reduction in TDs seen with 1,25-dihydroxyvitamin D_3 after UVR [1, 78]. Reductions in numbers of sunburn cells ($P < 0.01$) and in TDs ($P < 0.05$) were observed in that study at 24 h after UVR in skin sections from Skh-hr1 mice treated with 1,25-dihydroxyvitamin D_3 [1, 78]. The authors concluded that their results are consistent with the proposal that the vitamin D system in skin may be part of an intrinsic protective mechanism against UV damage [1, 78].

Moreover, it has been demonstrated on a third molecular level that vitamin D compounds, dependent on the presence of wild-type p53, regulate expression of the MDM2 gene [1, 79]. As outlined above, MDM2 is a p53-inducible gene and encodes a type E3 ubiquitin ligase responsible for the degradation of p53 by the 26S proteasome [1, 16]. A well-established role for MDM2 is as that of a key negative regulator of p53 activity: p53 binds to the p53-responsive elements (p53REs) in the P2 promoter of the *MDM2* gene and activates *MDM2* expression, while the subsequent increase of MDM2 protein results in its binding to p53 primarily at the N-terminal 1–52 residues and leads to p53 degradation or inhibition of its activity as a transcription factor [1, 80]. However, MDM2 also possesses numerous p53-independent activities and is also known to interact with a number of other proteins (for instance, Numb, RB, p300, insulin-like growth factor receptor, estrogen receptor, androgen receptor, etc.) involved in different cellular activities such as cell fate determination, differentiation, and signaling [1, 81–84]. Transcription of the *MDM2* gene is controlled by two distinct promoters (referred to as P1 and P2) [1, 85, 86] The P1 promoter, which is located upstream of the first exon, is responsible for the basal expression of MDM2. The P2 promoter is situated in the first intron and is responsible for inducible expression. The two transcripts initiated from the P1 and P2 promoter encode identical full-length MDM2 proteins by using the same translation start codon located in exon 2, while there are some differences in the 5′ untranslated regions (UTR) of these transcripts. A number of transcription factor binding sites have been identified in the P2 promoter region, including the two well-established p53RE sites [85, 86], AP-1/ETS [1, 87], Smad2/3 [1, 88], and an Sp1 site within a GC box cluster [1, 89]. Recently, it has been reported that RXR can bind tissue specifically to its recognition site within the P2. And of note in the context of regulation by vitamin D, there is evidence for a VDRE site in the P2 promoter region of the MDM2 gene, with experimental results indicating that MDM2 expression is regulated by 1,25-dihydroxyvitamin D3 treatment.

VDR and p53 family members act first and foremost as transcription factors, and, accordingly, much of the highly complex cross-regulation between them seems to happen at this level. For example, members of the p53 family including ΔNp63 can modulate VDR signaling through competitive binding to various VDR target genes including *p21Waf1/Cip1*. Multiple VDREs have recently been identified in the promoter region of the *p21* gene, a transcriptional target of p53, a powerful blocker of the cell cycle in G1 and G2 phase, and thereby a strong antiproliferative element of the p53 pathway [90]. Notably, the VDR binding sites are in close proximity to the p53 binding sites in the promoter of the *p21* gene [1, 90].

The skin consists of a basal layer, itself composed of self-renewing keratinocytes with limited proliferative capacity (transient amplifying cells) and stem cells with high proliferative capacity that have to be preserved, and of outwardly migrating layers of mostly resting cells at various stages of differentiation. In the skin, p53/p63 plays an important regulatory role in the maintenance of the stem cells as well as in the establishment of the differentiation gradient. The undifferentiated proliferating basal layer of the skin is ruled by dominant negatively acting ΔNp63, that binds regulatory DNA sequences, but whose transactivation is impaired. Most effects exerted by the transactivation-competent p53 family members are inhibited by it [1, 16, 91, 92]. In addition, ΔNp63 may inhibit differentiation by the blunting of VDR signaling through binding to various VDR target genes including *p21Waf1/Cip1* [1, 16, 93, 94]. TAp63 that is

minor to ΔNp63 in this proliferating compartment of the skin may become more dominant as ΔNp63 levels decrease in the course of differentiation [1, 16, 95].

Recently, a VDRE has been identified in the neighborhood of the RXR binding site and the p53 response elements within the P2 promoter of the *MDM2* gene. It is reasonable to believe that the RXR binding site has a role in the VDR response. The presence of wild-type p53 is required for the vitamin D3-mediated regulation of MDM2 expression, which may suggest an interaction between p53REs and VDRE within the P2 promoter of the *MDM2* gene. In this interaction, p53 could potentiate vitamin D action, and this could be an important feature of differentiation and the maintenance of the differentiated status. Finally, p53 family members may regulate VDR directly [1, 96, 97]. Future investigations, including whole transcriptome-including studies should provide insight into the transcripts that are initiated by VDR and p53 in concert.

In this context we have published two studies that provided further insights into the crosstalk of p53 and VDR [98, 99]. In the first study, we isolated whole-cell protein extracts of 1,25-dihydroxyvitamin D3 stimulated and UVB-irradiated vs. non-irradiated HEK 293 T cells transfected with a plasmid called pURB VDR C-Term TAP tag [98]. VDR complex was purified by tandem affinity purification (TAP). The nuclear tumor suppressor protein p53 and its negative regulator novel INHAT repressor (NIR), in addition to 43 other nuclear or cytoplasmic VDR-binding partners, were identified using nano high-performance liquid chromatography-electrospray ionization tandem mass spectrometric analysis [98]. VDR binding to p53 was confirmed by western blot analysis. It has to be noted that nuclear cofactors that contribute to vitamin D receptor (VDR)-mediated gene transcription, including retinoid X receptors, nuclear coactivators, and corepressors, have been extensively investigated, but that little is known about cytoplasmic VDR-binding partners and the physiological relevance of their interaction. Therefore, future studies are urgently needed to further elucidate the functional significance of these interactions [98].

As outlined above, the E3 ubiquitin ligase and transcriptional repressor MDM2 is a potent inhibitor of the p53 family of transcription factors and tumor suppressors. In the second investigation, we were able to show that MDM2 binds and inhibits vitamin D receptor [99]. This interaction was not affected by binding of VDR to 1,25-dihydroxyvitamin D_3, its naturally occurring ligand. VDR was ubiquitylated in the cell, and its steady-state level was controlled by the proteasome [99]. Overproduced MDM2 reduced the level of VDR, whereas knockdown of endogenous MDM2 increased the level of VDR. It is well-known that, in addition to ubiquitin-marking proteins for degradation, MDM2, once recruited to promoters by DNA-binding interaction partners, can inhibit the transactivation of genes [99]. Transient transfections with a VDR-responsive luciferase reporter revealed that low levels of MDM2 potently suppress VDR-mediated transactivation [99]. Conversely, knockdown of MDM2 resulted in a significant increase of transcript from the CYP24A1 and p21 genes, noted cellular targets of transactivation by liganded VDR [99]. Our findings suggest that MDM2 negatively regulates VDR in some analogy to p53, but future studies are needed to characterize the significance of this finding.

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Sunlight, Vitamin D, and Xeroderma Pigmentosum

16

Marie Christine Martens, Steffen Emmert, and Lars Boeckmann

Abstract

Sunlight, in particular UV-B radiation, is an important factor for endogenous vitamin D production as 80–90% of the required vitamin D needs to be photosynthesized in the skin. The active form of vitamin D, vitamin D_3 or calcitriol, binds to the ligand-activated transcription factor vitamin D receptor (VDR) for genomic and non-genomic effects. Recently, calcitriol and analogs have been shown to have antiproliferative effects in mouse and human BCC and SCC cell lines in vitro. As UV radiation plays a critical role in the photosynthesis of vitamin D, stringent sun protection, as recommended for xeroderma pigmentosum (XP) patients, may impact their vitamin D levels.

XP is a rare autosomal recessive disorder with a worldwide prevalence of 1 in 1,000,000. XP can be divided into seven different complementation groups: XP-A to XP-G. The complementation groups correspond with the underlying gene defect. Defects in these genes lead to a defective nucleotide excision repair (NER), which is necessary to remove UV-induced DNA damage such as the UV photoproducts cyclobutane pyrimidine dimers (CPD) and 6–4 pyrimidine-pyrimidone (6–4 PP) dimer. Additionally, a variant form with a mutation in the translational polymerase η gene (*PolH*), also called XP variant (XPV), exists. Patients with XPV show a defect in translesion synthesis. Due to their inability to repair UV-induced lesions, XP patients exhibit an increased risk for UV-induced nonmelanoma skin cancer (NMSC) such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) as well as melanoma. Although no curative therapy for XP exists today, numerous options for the treatment and prophylaxis of skin cancer have become available.

Keywords

Xeroderma pigmentosum (XP) · UV-induced DNA damage · Cyclobutane pyrimidine dimer (CPD) · 6–4 pyrimidine-pyrimidone (6–4 PP) dimer · Nucleotide excision repair (NER) · Polymerase η · Unscheduled DNA synthesis (UDS) · Host-cell reactivation (HCR) · Post-UV cell survival assay · Vitamin D · Sunlight

Introduction

Sunlight consists of different electromagnetic wavelengths, including light of the visible spectrum and ultraviolet (UV) radiation. UV radiation can be subdivided into three wavelength ranges: UV-C radiation (100–280 nm) is the most

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energetic and has high mutagenic potential. Due to the formation of ozone from oxygen in the stratosphere, it doesn't reach the Earth's surface. UV-B radiation (280–315 nm) and UV-A radiation (315–400 nm) can penetrate the atmosphere. UV radiation reaching the Earth's surface comprises of 90–95% UV-A radiation and only 5–10% UV-B radiation. UV-B radiation is mostly absorbed by the epidermis, while UV-A radiation penetrates into the deep dermal layers of the skin [1].

UV-Induced DNA Damage

DNA is a chromophore for UV radiation with an absorption maximum of 254 nm. Direct absorption of UV-B radiation generates DNA photoproducts by forming covalent bonds between adjacent pyrimidines on the same DNA strand. The main resulting photoproducts are cyclobutane pyrimidine dimers (CPDs) and 6–4 pyrimidine-pyrimidone (6–4 PP) dimers. While CPDs form two bonds (between carbons 4 of each pyrimidine and between carbons 5) that lead to the formation of a 4-carbon ring, 6–4 PPs form only one bond (between carbons 6 and 4 of the adjacent pyrimidines). CPDs are two times more frequent than 6–4 PPs [2–4]. CPDs are supposed to be repaired much more quickly than 6–4 PPs and therefore are said to be less mutagenic. Areas of tandem pyrimidine residues, thymine (T) or cytosine (C), are so-called hot spots for UV-induced mutations.

The mechanism behind UV signature mutation has been proposed to be explained by the "A rule." The "A rule" states that the DNA polymerase η inserts adenine (A) on the complementary DNA strand if it cannot interpret the lesion. Replication then leads to base-pair changes and therefore mutations. CPDs formed by TT usually do not result in mutations as the base on the complementary DNA strand would be A, either way. CC CPDs, on the other hand, result in a mutation to TT as two A residues are inserted opposite the dimer instead of two guanine (G) residues. Signature C to T mutations also occurs in 6–4 PPs. While the 5′ residue pairs correctly, the 3′ residue

cannot be interpreted and, thus, is replaced with an A on the opposite strand [5].

Mismatches of bases or photoproducts in the DNA lead to bulky lesions that distort the DNA backbone, thereby blocking polymerases for transcription and replication [2]. The mechanism to remove these UV-induced bulky lesions from the DNA is the nucleotide excision repair (NER). The NER identifies lesions through the distortion in the DNA structure and excises a short oligonucleotide including the lesion. The resulting gap can be repaired by replicative polymerases [4].

UV-A and UV-B radiation can also form reactive oxygen species (ROS) that alter DNA, proteins, and cell membranes through oxidation which can cause single-strand DNA breaks [6]. The best studied ROS-induced lesion is 8-oxo-deoxyguanosine (8-oxo-dG) which was shown to be premutagenic [7].

Photocarcinogenesis describes a multistep process contributing to the development of skin cancer primarily caused by UV radiation. Resulting skin cancer entities include nonmelanoma skin cancer (NMSC) such as squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), as well as melanoma [1].

Sunlight and Vitamin D

Sunlight, in particular UV-B radiation, is an important factor for endogenous vitamin D production. While 10–20% of human's requirement in vitamin D can be achieved through dietary intake, 80–90% of the required vitamin D needs to be photosynthesized in the skin [8]. During sunlight exposure previtamin D is generated from 7-dehydrocholesterol, also known as provitamin D. While all epidermal layers contain provitamin D, which can be converted to previtamin D, the highest concentration of previtamin D after sunlight exposure was observed in the *stratum spinosum* and *stratum basale* [9]. Photoisomerization of previtamin D into lumisterol and tachysterol, which are both biologically inert, occurs after prolonged sunlight exposure and prevents vitamin D toxicity. Previtamin D slowly thermally isomerizes to

vitamin D. Vitamin D binds to the transport protein vitamin D binding protein to get into the blood stream. Further metabolization of vitamin D occurs in the liver and in the kidney. The final and active form of vitamin D is 1,25-dihydroxyvitamin D, also called calcitriol or vitamin D_3. Calcitriol is transported from the kidneys to organs and tissues by the vitamin D binding protein where it binds to the vitamin D receptor (VDR) for genomic and non-genomic effects. VDR is a ligand-activated transcription factor and responsible for most of the effects of calcitriol [10].

Calcitriol influences cell proliferation, cell differentiation, and cell growth through transcriptional regulation of cell cycle regulatory proteins such as p21/WAF-1 (CDKN1A) and other proteins such as β_3-integrin and fibronectin as well as controlling the hedgehog (Hh)- and β-catenin-mediated signaling pathways [8]. Non-genomic effects of calcitriol include effects on intracellular calcium levels; regulation of many signaling pathways such as the phosphatidylinositol 3-kinase, mitogen-activated protein kinase (MAPK), and protein kinase C pathways; and the opening of calcium and chloride channels [11].

Vitamin D is involved in the calcium/phosphate homeostasis and therefore in skeletal health. Besides that well-known function, it is involved in muscle and nervous functions, cardiovascular homeostasis, and immune response [12]. But the skin is not only the site of vitamin D production but also a target tissue for calcitriol and vitamin D metabolites. Recently, the VDR was suggested to be a tumor suppressor in the skin. It was shown that these effects were partly mediated by interaction with proteins of the p53 family such as p53, p63, and p73. Non-genomic effects of vitamin D are at least partially responsible for UV-B-induced DNA damage protection. Key components of the vitamin D endocrine system, including VDR, were strongly expressed in NMSC [8]. Calcitriol and analogs have been shown to have antiproliferative effects in mouse and human BCC and SCC cell lines in vitro. Additionally, in vitro and in vivo experiments in patched (Ptch) mutant mice showed inhibited carcinogenesis and growth of BCCs [8, 13]. Further experiments revealed that calcitriol reduces Gli1 transcription and inhibits canonical Hh signaling independently of VDR signaling and downstream of Ptch. Smo seems to be the molecular target as Smo-deficient cells show no reduced Gli1 transcription in response to calcitriol [14].

The vitamin D endocrine system has been concluded to be of relevance for carcinogenesis and progression of NMSC. It has been suggested that calcitriol and analogs can be used to prevent as well as treat NMSC [8].

Xeroderma Pigmentosum: Epidemiology

Xeroderma pigmentosum (XP) is a rare autosomal recessive genetic disorder. Currently, XP can be divided into seven different complementation groups, XP-A to XP-G, as well as a variant form with a mutation in the translational polymerase η gene (*PolH*), also called XP variant (XPV). The complementation groups correspond with the underlying gene defect [15, 16].

Although the worldwide prevalence, spanning all ethnic groups, is 1 in 1,000,000, a higher prevalence can be observed in certain geographical regions. In Japan, for example, the prevalence is 1 in 22,000, and in almost 1% of the Japanese population, founder mutations in the *XPA* gene can be found. A higher prevalence of XP causing mutations can be attributed to isolation, cultural influences, or less mobility [17, 18].

The most common complementation group is XP-C with a percentage of 43% of all XP patients. Isolated regions, such as the Mayotte region in the Indian Ocean and Northern Africa, show a higher prevalence with microsatellite analysis showing a founder mutation in the Northern African population [17, 19].

The second most common complementation group is XP-D with 28% of all XP patients. A very mild form of XP is associated with a founder mutation in the *XPD* gene found among Iraqi families of Jewish decent [17, 20]. A much more common mutation hotspot in the *XPD* gene leads to an amino acid substitution at

position 683, which has substantial causal effect on disease severity [21].

The least common complementation groups are XP-E (3%), XP-G (3%), XP-B (1%), and XP-F (< 1%). Contrary to the seven complementation groups, XPV patients, representing 7% of all XP patients, do not show a defective DNA repair but a failure of error-free translesion synthesis past DNA photoproducts [17].

Xeroderma Pigmentosum: Clinical Symptoms

At least six different clinical entities are associated with mutations in XP genes: XP, XP plus neurological symptoms, trichothiodystrophy, XP plus trichothiodystrophy symptoms, XP plus Cockayne syndrome symptoms, and the cerebro-oculo-facial-skeletal syndrome [22]. Although commonly assumed, not all patients with XP show increased sun sensitivity. Only about 60% of all patients with XP develop a severe *dermatitis solaris* after low UV exposure as the first conspicuous XP symptom during the first weeks of life. The remaining patients, coming up to 40%, show no strong sun sensitivity. However, common to all patients is an early hyperpigmentation in sun-exposed areas. Additionally, signs of premature skin aging and poikilodermic skin changes can be observed as early as the age of 3–5 years. Skin cancer is common at an early age with an average of 8 years in XP patients. They develop BCCs and SCCs, as well as cutaneous melanoma [23]. The first UV-induced malignancies develop much later in the general population, at about 60 years of age. The risk for BCCs and SCCs is increased 10,000-fold in patients with XP as well as a 2000-fold increase in the risk for melanoma [24].

Ophthalmological changes have been reported to occur mostly in the UV-exposed anterior part of the eye leading to more frequently observed findings of conjunctivitis, cataract formation, and pterygium formation, as well as rare tumors [25].

Neurological symptoms with varying degrees of severity and time of first appearance develop in 25% of patients with XP. These symptoms consist of attenuated or missing tendon reflexes, progressive hearing loss, speech and gait disturbances, as well as cognitive decline. In MRI, cortical atrophy and dilated ventricles are reflective of primary neural loss. Mortality is higher in XP patients with neurological degeneration showing a median age of death at 29 years as opposed to XP patients without neurological degenerations showing a median age of death at 37 years [23, 26].

Correlations between genotype and phenotype have been reported over the last years making knowledge of the affected gene and the type and location of the mutation more important for patients and their families as well as consulting physicians [27].

Xeroderma Pigmentosum: Disease-Causing Mechanisms

To understand the molecular pathomechanism underlying XP, a closer look at the NER mechanism is necessary.

The NER is a sequential mechanism for detecting and repairing lesions in the DNA and can be subdivided into the global genome repair (GGR) and the transcription-coupled repair (TCR). During the global genome repair, the first step is the lesion sensing. The XPC complex, consisting of the three subunits, XPC, HR23B, and centrin 2, is the distortion-sensing component of the NER. The DNA-binding protein XPC binds to damaged DNA structures as well as other DNA-distorting structures preferring the stretch of non-damaged single-stranded DNA opposite a lesion. Upon DNA damage, XPC is polyubiquitinated which results in an increased DNA affinity, both damaged and undamaged. The interacting protein HR23B increases the activity of XPC in the NER and is presumably involved in the ubiquitination of XPC. The other interacting protein, centrin 2, stabilizes the XPC complex improving its activity [4].

Not all lesions distort the DNA double helix equally. While 6–4 PPs lead to a strong kink in the DNA, CPDs lead to only modest distortion. The DDB complex, a heterodimer containing

DDB1 and XPE, binds to damaged DNA. Inducing a kink in the DNA upon binding to the lesion, the XPC complex can recognize the lesion more easily. Through ubiquitination, the DDB complex increases the affinity of the XPC complex for DNA [4].

Both the GGR and the TCR need the same repair factors after lesion recognition to remove the lesion. In TCR, the RNA polymerase II (RNAPII) recognizes damage to the transcribed DNA strand and, thus, gets stalled. The transcription factor II H (TFIIH) is a ten-subunit complex consisting of the helicases XPB and XPD in a ring-shaped structure with five other subunits and a cyclin-activated kinase (CAK) complex. It is needed for both transcription initiation and DNA repair. The CAK complex, containing cyclin H, cdk7, and MAT1, constitutes a phosphorylation cascade phosphorylating RNAPII as well as several nuclear receptors. During transcription initiation, it increases the efficiency of initiation. The helicase XPB, showing a $3'$–$5'$ polarity, is important for promotor clearance by RNAPII. The helicase XPD, showing a $5'$–$3'$ polarity, is not involved in transcription initiation, but in opening a bubble of denaturation around the lesion during NER. This function also requires XPB, although more for its ATPase activity than for its helicase activity. In short, DNA unwinding around a lesion and formation of a DNA bubble is facilitated by the TFIIH complex [4, 28, 29].

The pre-incision complex is further stabilized by XPA binding to the lesion-containing DNA strand and RPA binding to the undamaged strand. These two factors also extend the bubble region while protecting the undamaged strand. XPA also inhibits the helicase activity of the seven subunit-containing TFIIH complex without the CAK complex in the presence of bulky lesions [28].

To excise the lesion from the damaged DNA strand, the endonuclease XPG and the heterodimer XPF-ERCC1 are needed with XPG cutting on the $3'$ side of the lesion and XPF-ERCC1 cutting on the $5'$ side of the lesion. Being a structure-specific endonuclease, XPG prefers the single-strand to double-strand junctions at the end of the denaturation bubble. The presence of XPG is necessary for the incision

by XPF-ERCC1. XPG also stabilizes the TFIIH complex. XPF is the endonuclease in the XPF-ERCC1 complex with ERCC1 stabilizing XPF [4]. Both subunits of the heterodimer are necessary for DNA binding with ERCC1 preferentially binding to double-stranded DNA (dsDNA) and XPF specifically recognizing single-stranded DNA (ssDNA). Thus, it was proposed that XPF binds to the non-damaged strand within the repair bubble and ERCC1 binds to the dsDNA upstream of the damage [30]. Recently, we could show that a functional XPF-ERCC1 heterodimer is necessary for ERCC1 to enter the nucleus [31].

The excised lesion containing fragment is about the size of 24–32 nucleotides. The resulting gap is then filled by replicative polymerases δ, ε, or κ, starting at the $3'$-hydroxyl generated by XPF. For recruiting polymerases δ and κ, the canonical clamp loader RFC is required, while polymerase ε requires an alternative clamp loader complex composed of the CTF18 protein and the canonical small RFC subunits. The DNA polymerase clamp protein proliferating cell nuclear antigen (PCNA) is loaded onto the primer-template junction by the clamp loaders. Ubiquitination by Rad18 and the DNA repair scaffold protein XRCC1 was needed for polymerase κ recruitment. After the gap-filling synthesis, DNA ligase I or the XRCC1-ligase III complex seals the remaining nick [32].

The XP complementation groups XP-A to XP-G, gene names corresponding with their respective complementation group, all have defects in the NER leading to the accumulation of DNA photoproducts [16]. The XPV phenotype is proficient in NER but lacks the *XPV* gene encoded polymerase η, which catalyzes accurate translesion synthesis [33]. A recent study indicates that oxidative stress takes a crucial role in UVA-induced cytotoxicity in XPV cells [34].

Xeroderma Pigmentosum: Diagnostics

The diagnosis of XP is generally a clinical one; ideally, it is assessed interdisciplinarily, combining the fields of dermatology, ophthalmology,

ENT (ear, nose, and throat), and neurology, including imaging procedures and human genetics. Due to XP being an autosomal recessive genetic disorder, it is vital to take a comprehensive family history [17].

Complementary functional tests, gene and protein expression analysis, and sequence analysis can be used to identify the affected genes. Functional DNA repair assays can help to distinguish between the eight different defective genes (*XPA–XPG* and *POLH*). Cells derived from XP patients, besides XPV, are defective in NER. To functionally measure the cellular NER capacity, a method called unscheduled DNA synthesis (UDS) is used. Using radioactively or fluorescently labeled nucleoside analogs, the amount of incorporated nucleoside analogs into the DNA after UV irradiation of patient cells can be measured. Due to a reduced NER and, hence, reduced gap-filling DNA synthesis, XP patient cells show a reduced UDS. The NER capacity of XP patient cells can, at least partially, be restored by transfection of an expression plasmid, which contains the wildtype cDNA of the respective XP gene. Increased UDS indicates successful restoration and can be used to assign XP complementation groups. Another functional assessment of the NER is host-cell reactivation (HCR). A UV-irradiated firefly luciferase coding reporter gene plasmid is transfected into XP patient cells. XP cells show decreased luciferase expression compared to wildtype cells. After wildtype cDNA transfection of the respective XP gene, the transcription-blocking UV photoproducts are repaired, and the reporter gene can be expressed. Therefore, HCR can be used to assign XP patient complementation groups as well. For the detection of the defective polymerase η in XPV, another approach is necessary as XPV is not defective in NER but in translesion synthesis. Due to translesion synthesis only occurring during S phase, caffeine needs to be added to the cell cultural medium inducing S phase. A post-UV cell survival assay should be performed. A reduced post-UV survival of XPV cells in cell cultural medium containing caffeine is proof of the defective polymerase η. A base sequence analysis should be performed to identify the exact disease-causing mutation. Cultured primary fibroblasts from skin punch biopsy or peripheral venous blood can be used to isolate patient DNA and mRNA. Expression of variant alleles can be detected through cDNA sequencing [16].

Vitamin D and Xeroderma Pigmentosum

Due to the defective DNA repair mechanisms of UV-induced DNA lesions, XP patients are advised to apply stringent sun protection. Because UV-B radiation is needed for vitamin D synthesis in the skin, the impact on vitamin D levels and vitamin D deficiency-associated disease rickets in children and osteomalacia in adults was questioned. One study found that vitamin D levels may be normal, increased, or decreased, but are not causally linked to sun-protective measures [35]. Other studies reported vitamin D deficiency and a shorter stature in XP patients [36] and vitamin D deficiency in XP-A patients [37].

Taken together, only very few studies have been conducted so far analyzing the consequences of stringent sun protection in XP patients in association with vitamin D levels. Furthermore, the few available studies revealed contradictory results. A case report about a young vitamin D-deficient XP patient also came to the conclusion that screenings of XP patients for biochemical evidence of abnormalities in vitamin D levels are needed [38]. Further investigations about vitamin D deficiency and its treatment in patients with XP should be conducted to avoid vitamin D deficiency-associated complications and allow conclusions for recommendations concerning the symptomatic treatment of XP patients.

Xeroderma Pigmentosum: Therapy

To date, there is no curative therapy for XP. Thus, an early diagnosis is essential so that systematic sun protection such as sunscreen, long-sleeved clothing, broad-brimmed hats, sunglasses, facial

protection, window foils with UV filters and UV meters, as well as quarterly dermatologic skin cancer screening can be initiated immediately [22]. Special whole-body suits are available that protect XP patients to a certain extent from UV radiation. Photo documentation of the whole skin surface is particularly important in patients with XP to detect skin cancer early on. Close-ups should be included in this documentation. Standard therapy guidelines should be applied to the treatment of premalignant lesions like actinic keratosis (AK) as well as malignant lesions in XP patients. Therapies include cryotherapy, curettage, or topical application of 5-fluorouracil [15].

Surgery is the treatment of choice for invasive skin cancer in XP patients. Because of the amount of surgeries required to be performed on the face, the excisions should be as small as possible and microangiographically controlled. Additionally, an experienced plastic surgeon should be preferred [17].

Recent case studies showed promising results for imiquimod 5% cream in the treatment of BCCs and pigmentary changes in patients with XP (Table 16.1). The most common adverse reaction was local erythema. More frequent applications led to more severe erythema and erosions in some patients [39–47]. Imiquimod is an immune response modifier that has been approved for treatment of perianal warts, AK, and superficial BCCs. While its exact mechanism of action remains largely unknown, topical application of imiquimod has been shown to induce cytokine production and to stimulate innate and adaptive immune pathways [48].

Other topical treatments, such as the application of a cream preparation of xenogenic repair enzymes (photolyase or T4 endonuclease), have been explored. Although they were shown to be effective as additional skin cancer prophylaxis in XP patients, clinical use has not been approved. However, photolyase-containing sunscreens are available from pharmacies and show beneficial effects [49–51].

BCCs have been associated with abnormal activation of the Hh signaling pathway. Hh is a ligand of the transmembrane receptor protein patched homolog 1 (PTCH1), which constitutively suppresses Hh signaling and, thus, acts as a tumor suppressor. Physiologically, PTCH inhibits migration of the transmembrane protein Smoothened (SMO) to the primary cilium of the cell. In the absence of SMO from the primary cilium, glioma-associated oncogene (GLI) transcription factors (GLI 1, GLI 2, and GLI 3) are blocked from transcription. Overexpression of GLI promotes cell division and tumorigenesis. Loss of *PTCH1* was shown in 67% of BCCs and activating mutations of *SMO* in 10% of BCCs [52, 53]. While the naturally occurring alkaloid cyclopamine acts as an Hh pathway inhibitor (HPI) by binding to SMO, its therapeutic use is limited due to suboptimal aqueous solubility and chemical stability [54]. Of the two selective small-molecule SMO inhibitors, vismodegib and sonidegib, so far only vismodegib has been used as an oral treatment for XP patients with BCCs. Adverse reactions in the two reported individuals included lack of energy, dysgeusia, muscle cramps, amenorrhea, and alopecia. Treated lesions showed partial or complete responses. Furthermore, appearance of new lesions was prevented, although for a limited time only. It was concluded that vismodegib is an acceptable treatment option; however, alternative treatment options may be more appropriate for XP patients [55, 56].

Immune checkpoint inhibitors such as programmed cell death-1 (PD-1) inhibitors have changed the clinical treatment of cancer. PD-1-targeting antibodies pembrolizumab and nivolumab were first approved for advanced melanoma in 2014. They increased overall survival as well as progression-free survival. Since then, this cancer immunotherapy has been approved for other cancer entities such as non-small cell lung cancer, head and neck squamous cell carcinoma, and bladder cancer [57]. Recent studies showed that repeated treatment with pembrolizumab reduced the size of metastases and led to regression of cutaneous carcinomas in patients with XP [58, 59].

Systemic retinoids such as isotretinoin can be used as chemopreventive compounds in the prevention of skin cancer. Retinoids, being vitamin A derivatives, facilitate and promote cellular

Table 16.1 Case studies concerning the treatment of skin cancer in XP patients with imiquimod 5% cream

Publication	Age (years), sex	Imiquimod 5% cream treatment	Treated lesions	Adverse reactions to imiquimod	Result
Weisberg and Varghese [39]	>18, M	Three times a week for a month, then twice daily	Facial BCCs	Minimal inflammatory response	Clinical resolution of two prominent nodular BCCs, fewer new lesions (less than one new clinically evident BCC per month), areas of hypopigmentation within the treated areas
	>18, M	Initially three times a week followed by a 2-week pause, afterward once monthly increased to three times a week		Severe inflammatory response (facial swelling, erosions) after 2 weeks of treatment, later on some swelling and mild erosions, then no severe inflammatory response	Large areas of hypopigmentation
Nagore et al. [40]	19, F	Once daily, five times a week for 6 weeks	Facial pigmented BCCs	Slight pruritus	Almost total clearance of tumors, improvement of pigmentary changes
Roseeuw [41]	24, F	Three times weekly in the first week, temporarily discontinued for a week, then twice weekly, again discontinued for 2 weeks, then again twice weekly for 9 weeks	Nodular BCC on the forehead	Severe erythema and erosion	Successful longtime clearance
	15, F	Three times weekly for 10 weeks, discontinued for a week after 5 weeks	Superficial multifocal lesion on the upper lip	Severe erythema and excoriation/flaking after 5 weeks	
Giannotti et al. [42]	15, M	Three times a week for 4–6 weeks, additional oral acitretin (20 mg daily)	Facial BCCs and SCCs	Mild local erythema upon application	Tumors clinically cleared, longtime clearance for at least 6 months
Nijsten et al. [43]	28, M	Three times a week	Facial BCCs and SCCs	Initially irritated skin	Softer and smoother skin, less hyperpigmented macules, suspicious lesion cleared
					Discontinuation led to recurrence of hyperkeratotic lesions
Malhotra et al. [44]	16, M	Every alternate day for 12 weeks	Facial BCCs	None	All lesions cleared completely, improved skin texture, no recurrence after 1 year

Table 16.1 (continued)

Publication	Age (years), sex	Imiquimod 5% cream treatment	Treated lesions	Adverse reactions to imiquimod	Result
Alessi et al. [45]	One patient, unknown age and sex	5–7 times per week for 8 weeks	BCC	Not specified for this patient	No recurrence after 26 months
Yang et al. [46]	30, M	Three times a week for 4 months, afterward once weekly	Facial BCCs	Mild erythema, irritation, and transient hypopigmentation	All lesions healed with minimal scarring, no recurrence after 1.5 years
Latour et al. [47]	5, M	Five consecutive days per week for 6 weeks	Facial BCC	Not specified for this patient	Completely resolved, clearing of the background pigmentation
	2, M		Facial BCC	Not specified for this patient	Improvement in background pigmentation, no new skin cancers in the following 5 years

differentiation [60]. Oral isotretinoin was shown to prevent new skin cancer formation in XP patients. After discontinuation of the treatment, tumor frequency increased again [61]. Although isotretinoin showed to be a promising chemopreventive agent, retinoid toxicity and side effects must be considered. Side effects include hyperlipidemia, hypertriglyceridemia, stiffness, retinoid dermatitis, and teratogenicity [60].

A unique prophylactic treatment with aminoglycoside antibiotics, such as geneticin and gentamicin, was proposed for patients with XP-C. Fibroblast derived from XP patients with different premature termination codons in *XPC* was treated with geneticin and gentamicin. It was shown that in some of the XP-C fibroblasts, the treatment resulted in stabilized *XPC*-mRNA, increased expression of XPC protein, and increased repair of 6–4 PPS and CPDs. Due to renal toxicity and ototoxicity of systemic treatment with aminoglycosides, it was concluded that topical treatment would be more conducive and might alleviate the cutaneous symptoms of selected XP-C patients [62, 63].

A recent study identified acetohexamide, a first-generation sulfonylurea antidiabetic drug,

as a UV sensitivity-alleviating compound in NER-deficient cells. Patient-derived XPA-deficient cells were exposed to UV irradiation, and CPD levels were measured 24 hours after UV exposure. Treatment of XPA-deficient cells with acetohexamide led to the clearance of CPDs. Further experiments led to the conclusion that acetohexamide acts via promoting the degradation of MUTYH, a DNA glycosylase known for its catalytic effects during base excision repair (BER). The authors propose that an NER-independent mechanism is responsible for removing UV-induced DNA damage in the absence of MUTYH. Inhibition of MUTYH should be further studied as it presents a potential treatment option for XP patients [64].

In the age of genome-editing tools such as TALEN (transcription activator-like effector nuclease), meganucleases, and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9, gene therapy could be a therapeutic option in the future [65]. TALEN and meganucleases were shown to efficiently correct the *XPC* founder mutation in a fibroblast cell line derived from XP-C patients having not yet reached clinical level [66]. Another study used the approach of retrovirus-based transduction of

the wild-type *XPC* gene to restore the NER in human primary XP-C keratinocytes. Normal epidermal differentiation and a functional NER were shown in organotypic skin cultures and in a preclinical murine model of human skin regeneration in vivo [67]. Our group has recently been able to use CRISPR/Cas9 for the generation of a model cell line containing a complete knockout of the *XPF* gene. This cell line will provide a great tool to further investigate the role of XP proteins and deepen the understanding of the disease and the complex genotype-phenotype correlations [31].

Neurological symptoms of XP can, unfortunately, only be observed and supported due to the lack of effective therapy [15]. Recent data suggests an involvement of the NER in the repair of endogenous DNA lesions caused by the generation of reactive species. Hydroxyl radicals react with the DNA and form 8,5-cyclopurine deoxynucleotides. These lesions are supposed to be exclusive substrates for NER and could contribute to the neurological symptoms seen in XP patients. Due to oxidative stress and cumulative oxidative DNA damage in neurons being the primary causes of neurodegeneration, antioxidant therapy with coenzyme Q_{10} in XP complementation groups prone to neurodegeneration may be beneficial, though the efficacy of coenzyme Q_{10} supplementation needs to be further evaluated. Another possible approach is the upregulation of autophagy to counteract neurodegeneration by application of rapamycin, an inhibitor of mechanistic target of rapamycin kinase (mTOR). A phase II clinical trial for rapamycin in other neurodegenerative disorders is currently realized [68]. Another research group addressed the influence of dietary restriction on aging of progeroid ERCC1-deficient mice. Dietary restriction of 30% was shown to lengthen the lifespan of ERCC1-deficient mice. Furthermore, due to the dietary restriction, 50% more neurons as well as significantly motor neurons were retained in the neocortex and in the spinal cord, respectively. A lifespan lengthening was also reported for progeroid XPG-deficient mice. Therefore, further investigation is needed to explore the effect of dietary restriction in other DNA repair-deficient disorders such as XP [69].

The National Health Service in Great Britain has implemented nationwide XP clinics and is setting an example by providing access to various specialists and nursing staff that even answer house calls from affected families (*www.guysandstthomas.nhs.uk*). Besides that, patients and their families are organized in self-help groups in many countries. The "Xeroderma Pigmentosum Society" in the USA provides education and guidance about XP and even organizes an annual "Camp Sundown" for children with XP (*www.xps.org*). Further self-help groups include groups in Great Britain (*www.xpsupportgroup.org.uk*), France (*www.enfantsdelalune.org*), and Germany (*www.xerodermapigmentosum.de*).

Conclusion

XP is a DNA repair defect syndrome caused by defective NER or defective translesion synthesis by polymerase η. UV-induced DNA damage is repaired by NER. Due to reduced DNA repair, XP patients develop basal and squamous cell carcinoma as well as melanoma demonstrating the tumor-driving effect of UV-induced DNA damage. Molecular-genetic tests can be used to reveal the underlying genetic defect and assign XP complementation groups. Although vitamin D_3 and analogs have been shown to have antiproliferative effects in mouse and human BCC and SCC cell lines in vitro, to date, no research on the effects of vitamin D in XP regarding skin cancer development or prophylaxis has been conducted. Regarding treatment of XP, no curative therapy is available, though new treatment options, topical and systemic, contribute to the treatment and prophylaxis of skin cancer and neurological degeneration.

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Part VII

Prevention and Management of Skin Cancer

Update: Solar UV Radiation, Vitamin D, and Skin Cancer Surveillance in Organ Transplant Recipients (OTRs)

17

Roman Saternus, Thomas Vogt, and Jörg Reichrath

Abstract

Although great progress has been achieved during the last decades, the clinical management of organ transplant recipients (OTRs) remains a challenge. OTRs need in general lifelong immunosuppressive therapy that is associated with an increased risk to develop skin cancer and with an unfavorable clinical outcome of these malignancies. Skin cancer prevention measures, including regular full-body examinations, are therefore necessary in OTRs to detect and treat suspicious lesions at an early stage. The frequency of aftercare depends on the individual risk factors of the patient. Patients should apply consistent sun protection with sunscreens and clothing, as well as a monthly self-examination. On the other hand, the need of UVR avoidance increases the risk of vitamin D deficiency, which itself is associated with an increased risk for many diseases, including malignancies. OTRs should therefore be monitored for 25(OH)D status and/or should take vitamin D supplements. It has to be emphasized that an interdisciplinary approach, coordinated by the transplant center, that includes regular skin examinations by a dermatologist, is needed to ensure the best care for the OTRs.

Introduction

Although great progress has been achieved during the last decades, the clinical management of organ transplant recipients (OTRs) remains a challenge [1–3]. Notably, the annual numbers of performed solid organ transplants have been continuously increasing worldwide. In the USA, while in 2003 approx. 25,000 solid organ transplantations have been performed (data from the United Network for Organ Sharing), the total number of liver, kidney, and pancreas transplantation alone was up to 27,000 in 2017 [1]. Advances in pharmacotherapy and other measures which may include regular skin examinations have led to longer graft survival and to an improvement in patient survival after organ transplantation [2, 3].

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It is well-known that solid organ transplantation is associated with an increased risk to develop cancer, compared to the general population [4–6]. London et al. estimated that the risk of developing neoplasia in the first 10 years after transplantation is 14%, rising to 40% after 20 years (compared with a 6% cumulative risk of neoplasia in an age matched control population, $p < 0.005$) [7]. While the incidence rates of many of the common tumor entities are not increased in OTRs as compared in the general population, greatly increased incidence rates for skin and lip cancers and a higher frequency of some relatively rare tumors (such as post-transplant lymphomas or Kaposi's sarcoma) have been reported after transplantation [8]. Nonmelanoma skin cancer (NMSC) is among the most common reported malignancies with a frequency from 40 to 50% of all post-transplant malignancies [5, 6, 9]. Due to their greatly increased risk of developing skin cancer, the high importance of regular dermatologic examinations in OTRs to prevent and/or to treat early manifestations of these malignancies is now generally accepted.

The development of skin cancer depends on the degree, the duration, and the intensity of immunosuppression [10]. This explains the clinical finding that the standardized incidence ratios (SRI) of different tumor entities strongly differ within the type of transplanted organ [6]. For example, heart transplant recipients, who require a relatively strong immunosuppression as compared to other OTRs, consequently develop the most cancers, followed by renal and liver transplant recipients [10]. Jensen et al. showed that heart transplant recipients have a 2.9 times higher risk (confidence interval, CI, 1.3 to 6.2) of squamous cell carcinoma (SCC) than kidney transplant recipients (cohort of 2561 kidney and heart transplant recipients) [11]. Similar results are reported by Gjersvik et al. [12]. They showed that heart transplant recipients have a significantly increased 2.8 times higher risk of developing SCC compared to kidney transplant recipients [12]. Adamson et al. reported that heart transplant recipients have a significantly higher number of skin lesions on face and scalp [13]. These patients required more cosmetic surgery and received

significantly more radiation therapy [13]. In OTRs, it has to be noted that skin cancer is not only characterized by an increased incidence, but also by an unfavorable prognosis. This clinical observation is at least in part caused by the life-long requirement of immunosuppression [9].

Increased Risk of Nonmelanoma Skin Cancer (NMSC) in Solid Organ Transplant Recipients

Nonmelanoma skin cancer (NMSC), including the most common forms basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (SCC), represents the most frequently diagnosed malignancy in the Caucasian population [14]. During the last decades, the incidence of NMSC has steadily increased [14]. In Australia, for example, the estimated incidence of NMSC increased from 555 per 100,000 person/year in 1985 to 2448 in 2011 [14]. In OTRs, the incidence of NMSC is greatly increased as compared to the general population. Data from Ireland indicate that in renal transplant patients, the standardized incidence rates for invasive NMSC and in situ carcinoma of the skin are 33-fold and 65-fold increased, respectively ($p < 0.05$) [15]. Among these patients, the risk for invasive SCC was 82-fold increased [15].

In Norway, a 65-fold increased risk for the development of SCCs among transplant recipients compared with the general population has been reported [11]. It has to be noted that, following organ transplantation, the risk to develop a cutaneous SCC is more pronouncedly increased as compared with the risk to develop a BCC. In the Netherlands, it has been reported that the overall incidence rates of SCCs and BCCs were 250 and 10 times higher after renal transplantation compared with the general Dutch population, respectively [16]. Previously published data indicate that the BCC/SCC ratio in the USA in the general population is 4:1 [17]. A recent study analyzing 8032 dermatopathology reports collected at Stanford Healthcare from 2005 to 2015 calculated an overall BCC/SCC incidence ratio of only 1.4:1 [17]. Following transplantation, the BCC/SCC ratio is reversed

[8]. In an investigation by Keller et al. analyzing skin cancers in renal transplant recipients from 2002 to 2005, the BCC/SCC ratio was 1:7 [18]. However, the authors included actinic keratosis in their calculation of SCC [18]. This may explain different ratios reported in literature. In a study from Queensland, Australia, 361 renal transplant recipients were interviewed and examined for skin tumors [19]. The authors found that the BCC/SCC ratio reversed from 3.7:1 before transplantation to 1:2 after transplantation [19]. Ferrándiz et al. analyzed 21 kidney transplant recipients [20]. They found after a median follow-up of 34 months a BCC/SCC ratio at 3.1:1 [20].

Risk Factors Associated with the Development of Nonmelanoma Skin Cancer in Transplant Recipients

In addition to the risk factors for the development of NMSC that are common in the general population, including solar or artificial ultraviolet radiation (UVR), several important risk factors, including type, dosage, and duration of immunosuppressive medication, are more specifically associated with NMSC risk in organ transplant recipients. UVR is considered to be the most important environmental risk factor for the development of skin cancer [21]. UVR may induce DNA damage, most commonly photolesions such as cyclobutane pyrimidine dimers (CPD) and pyrimidine-6,4-pyrimidone photoproducts (6-4PP) [22]. Convincing evidence now clearly indicates that exposure to solar and artificial UV both before and after transplantation increases skin cancer risk in OTRs. In a retrospective follow-up study with 36 renal transplant recipients done by Bavnick et al., the authors showed that exposure to sunlight before the age of 30 contributes more to the risk of skin cancer in renal transplant recipients than exposure after the age of 30 [23]. However, cumulative lifetime exposure to sunlight was not associated with an increased number of keratotic skin lesions in these patients [23]. Elnahas et al. analyzed 287 patients who underwent lung transplantation. They showed that independent predictors of decreased odds of NMSC were non-white race ($p = 0.002$) and body mass index <30 kg/m^2 [24].

It is well-accepted that specific p53 gene mutations are associated with human skin cancer which are induced in normal skin by solar or artificial UVR [25]. Furthermore, exposure to UVR causes immunologic changes that inhibit the host immune system from recognizing tumor cells and leads to immunologic tolerance [26]. UVR inhibits antigen-presenting cells, i.e., by reducing cutaneous Langerhans' cell density [21]. On the other hand, UVR induces suppressor T cells and soluble immunosuppressive factors (such as urocanic acid) that lead to a systemic immunodeficiency [21]. Cis-urocanic acid has immunosuppressive properties effects and may initiate intracellular reactive oxygen species (ROS) production and oxidative DNA damage, whereas trans-urocanic acid acts as a natural sunscreen [27].

Besides immunosuppressive therapy, systemic immunodeficiency may in OTRs be caused by other factors, such as pre-transplantation dialysis in renal organ transplant recipients [21]. Immunosuppressive drugs exert their effects that may lead to systemic or locally restricted/pronounced immunodeficiency, in part via inhibition of antigen-presenting cells [21]. The inhibition of antigen-presenting cells results in a local immunodeficiency [21]. Both local and systemic immunodeficiency may drive the proliferation of human papillomavirus (HPV), an important co-carcinogen for the development of various malignancies, including cutaneous SCCs [21]. OTRs with a history of HPV infection have a higher degree of susceptibility for development of skin cancer [28]. A range of investigations analyzed the role of HPV for the development of skin cancer among OTRs [29–34]. Harwood et al. analyzed the HPV status of NMSC (n = 148) in immunosuppressed and immunocompetent individuals. In the group of immunosuppressed patients, HPV DNA was detected in 84.1% of SCC, 75% of BCC, and 88.2% of premalignant skin lesions, whereas in

the group of immunocompetent, HPV DNA was detected in 27.2% of SCC, 36.7% of BCC, and 54.4% of cutaneous premalignancies [29]. Epidermodysplasia verruciformis (EV)-associated HPV types were the most frequent types in all lesions from both groups [29]. The authors concluded that the prevalence and spectrum of HPV types does not differ in SCC, BCC, or cutaneous premalignancies within the two populations [29]. Berkhout et al. demonstrated the presence of EV-associated HPV types and/or HPV types belonging to groups A2 and A4 with a higher frequency in hyperkeratotic papillomas (77.5%), SCCs (77.8%), and actinic keratoses (67.9%), as compared to BCCs (35.7%), benign skin lesions (38.5%), and clinically normal skin (32.3%) [32]. Stockfleth et al. discussed that a cutaneous infection with HPV5 or HPV8 may cause an increased risk for SCC development in transplant recipients [33]. De Villiers et al. analyzed HPV types in renal allograft patients [34]. They found that the prevailing types in malignant lesions were the EV-related HPV types 20, 23, 38, DL40, and DL267 which were present in 73% (24/33) of the malignant lesions, in 35% (6/17) of the keratoses and in 13% of the warts [34]. These HPV types may also be present in peri-lesional skin of skin tumors of immunocompetent patients [34]. The authors speculated about a possible virus/virus interaction in renal allograft patients, resulting in complementation of otherwise defective HPV types, whereas in immunocompetent individuals the immune system may inhibit the development of clinical disease associated with these HPV types [34].

It has also been shown that the incidence of NMSC increased both with the duration of immunosuppression [19] and with dose intensity of immunosuppressive drug therapy [10]. Ingvar et al. analyzed 5931 patients in a Swedish organ transplantation cohort during a follow-up period from 1970 to 1997 [35]. They found that post-transplant azathioprine treatment increases the risk of SCC. Compared with patients that never received treatment with azathioprine, those who received a high accumulated dose of azathioprine (after the entire follow-up time) had a 8.8.-fold increased risk for the development of SCC in

multivariate analysis ($p > 0.0001$). Furthermore, a high cumulative dose of corticosteroids during the follow-up period was associated with a 3.9-fold but non-significant increased risk of SCC ($p = 0.09$) compared to the lowest accumulated dose of corticosteroids [35]. Interestingly, the risk for post-transplantation development of SCC in patients who were treated with cyclosporine was not increased in that study [35]. Several mechanisms by which immunosuppressive drugs may promote or cause the development of SCCs have been described, including an impaired immune surveillance, a direct carcinogenic effect, and an increased susceptibility to other carcinogenic agents [35]. Assessing several standard immunosuppressive drug regimens in a mouse model where albino hairless mice (HRA/shk-1) were exposed to UVR (290–400 nm) for 30 weeks, Kelly et al. showed that the treatment with azathioprine or cyclophosphamide shortened the latency period for skin tumor induction and increased the rate of skin tumors [36]. In contrast, these investigators found no pronounced effect of prednisolone or cyclosporine on UVR-induced tumor development, with both immunosuppressants resulting only in a moderate reduction of the latency period for tumor induction [35, 36].

It has also been shown that the risk for development of SCC increases with the duration of immunosuppression. In a Swedish study done by Krynitz et al. (10,476 recipients transplanted from 1970 to 2008), the authors demonstrated that after 20 years the standardized incidence ratio (SIR) in all solid organ transplant recipient increased from baseline SIR 50 (95% CI, 44–56) to SIR 213 (95 CI, 194–234) [37].

Additional factors that have been reported in OTRs to be significantly associated with the development of both SCC and BCC include older age at transplantation, presence of actinic keratosis, male sex, and outdoor occupation, whereas a history of having smoked tobacco was associated with presence of SCCs but not with BCCs [38]. Molony et al. showed among renal transplant recipients a steady increase in risk of skin cancer incidence for patients older than 50 years from year 2 post-transplant, whereas the increased risk in younger renal transplant

recipients (age < 50 years) occurred later but much more significantly, reaching 200 times the risk for an age-matched non-transplanted population by year 6 post-transplant [15].

Patients with skin cancer pre-lung transplantation had higher risk of post-lung transplantation skin cancer ($p = 0.02$) [24]. Furthermore, OTRs with history of chronic sun exposure and/or sun burns and CD4 lymphocytopenia are described to have a higher degree of susceptibility for development of skin cancer [28].

In the mentoined study by Krynitz et al., it has been shown that the post-transplant risk for SCC development varied depending on the transplanted organ [37]. The post-transplant risk for SCC development is most increased among heart and/or lung recipients with a SIR of 198 (95% CI, 174–224), followed by kidney recipients with a SIR of 121 (95% CI, 116–127) and by liver recipients with a SIR of 32 (95% CI, 24–42) [37].

Also, genetic factors (such as skin type, polymorphisms in p53, arginine-arginine genotype and glutathione s-transferase) may result in the development of cutaneous carcinomas [21]. Gogia et al. demonstrated that the risk of SCC increased with each incremental decrease in Fitzpatrick skin type (FST), from FST VI to FST I (linear test for trend $p < 0.001$) [39]. Lira et al. reported that functional gene variants in the regulatory regions of COX-2 gene (PTGS2) such as COX-2 common variants (765G > C and 1195A > G) are associated with risk of NMSC [40].

The Aggressive Behavior of Nonmelanoma Skin Cancer in Transplant Recipients

It has been reported that the biologic behavior of cutaneous SCCs is more aggressive in solid OTRs as compared to the general population [41]. Lott et al. analyzed 307 patients with SCCs (OTRs: n = 153, controls: n = 154) and 246 patients with BCC (OTRs: n = 123, controls n = 123) [41]. They found that OTRs have a significant increased number of primary cutaneous SCCs

($p < 0.0001$), deep tissue involvement ($p = 0.01$), perineural ($p = 0.0001$) and lymphatic invasion ($p = 0.03$), recurrence ($p < 0.0002$), and need for radiation or chemotherapy ($p = 0.01$), whereas BCCs are in OTRs not associated with more aggressive disease when compared with the control group [41]. Comparing the clinical outcome of SCCs in OTRs with controls, neither the frequency of lymph node metastasis nor SCC-specific survival were significantly altered [41]. The authors also showed that SCCs develop in OTRs significantly at younger ages compared to the control group (mean 58.2 ± 12.4 vs. 70.4 ± 12.8; $p < 0.0001$) [41]. Carucci et al. analyzed the outcome in OTRs with in-transit metastasis from primary cutaneous SCC (15 OTRs and 6 non-transplant recipients with in-transit metastasis). The authors found that in-transit metastasis in OTRs are associated with a poor prognosis [42].

Organ Allograft Recipients Are at Increased Risk for Malignant Melanoma

OTRs are not only at an increased risk to develop NMSC. Eruptive de novo dysplastic nevi following renal transplantation have been reported [43], and a large body of evidence has now convincingly demonstrated that the risk for development of malignant melanomas is also increased in OTRs. In agreement with other investigations, Dahlke et al. reported in a systematic review, a 2.4-fold (95% confidence interval, 2.0–2.9) increased incidence of melanoma after transplantation, compared to the general population [44]. Data from Norway by Jensen et al. showed that the risk for malignant melanoma is threefold increased among OTRs compared with the general population [11]. Le Mire et al. estimated that the risk to develop a malignant melanoma was eightfold increased in the Oxford renal transplant population compared with the general population, which is the highest increase in melanoma incidence associated with OTR reported in the literature until today [45].

Data from France indicate that the prevalence for the development of melanoma in OTRs has increased in the last decades [46]. Between 1991 and 2000, the prevalence of melanomas was 0.28%, whereas between 1991 and 2015, the prevalence was 1.09%, representing a 3.9-fold increase in prevalence after 2000 [46]. The authors noted that the prevalence of SCC and BCC over the same period in the same patient group remained unchanged [46]. Risk factors for the development of melanoma in OTRs are age, a history of heavy sun exposure, fair skin pigmentation, and high number of nevi which are also known risk factors for melanoma in the general population [46]. The authors discussed that the immunosuppressive therapy may play an important role for the development of melanoma [46]. They suggested that a strong immunosuppression able to completely prevent rejection may increase the incidence of de novo cancers, including melanoma [46]. Scientific findings indicate that immunosuppressive therapy is associated with a more aggressive course of melanoma and with a higher incidence of amelanotic melanomas (frequency in immunosuppressed patients vs. controls (21% vs 5.3%; $p = 0.0175$) [47].

It has been reported that the overall survival rates are significantly lower in patients that developed a melanoma following organ transplant compared with the expected survival rate in melanoma patients that did not undergo organ transplantation, regardless of Breslow thickness or Clark level [48]. In that study, cause-specific survival due to melanoma in OTRs with melanomas of 1.51 to 3.00 mm in Breslow thickness or Clark level III or IV was significantly reduced when compared with the expected survival rate [48]. However, other investigators reported a relatively favorable clinical outcome in melanoma patients that had received an organ transplant [45]. This clinical observation may reflect the consequent implementation of secondary and/or tertiary prevention measures in the clinical management of OTRs that include close monitoring by a dermatologist and that, e.g., may result in melanoma detection at a relative early stage [45]. It has been reported that patients who developed melanoma before transplantation have neither an increased risk of recurrence nor metastasis [48]. However, the authors of this study could not exclude the bias that patients with thinner melanomas undergo organ transplantation more frequently than patients with thicker melanomas [48].

Increased Incidence and Prevalence of Other Types of Skin Cancer in Solid Organ Transplant Recipients

Less common skin cancers such as Merkel cell carcinoma (MCC) and Kaposi's sarcoma (KS) have been described to be significantly increased in OTRs [8, 11, 49–53]. For MCC, it has been reported that the overall risk is increased 23.8-fold (95% CI 19.6–28.7) [50]. The SIR of KS is 61.46 (95% CI 50.95–73.49) [51]. Jensen et al. reported an 84-fold increased risk for KS among OTRs compared with the general population [11]

Douds et al. reported a renal transplant patient taking prednisolone and cyclosporine who developed a metastatic MCC 2 years after transplantation [52]. Penn et al. analyzed the occurrence of MCC in the Cincinnati Transplant Tumor Registry [53]. Within this cohort, 41 cases of MCC were reported. Whereas in the general population, MCC typically occurred in older ages; in transplant patients, the mean age at diagnosis was 53 (range 33–78) years, and 29% of recipients were < 50 years old [53].

Only a few cases of other rare skin tumors, including angiosarcoma and other cutaneous vascular tumors, cutaneous mesenchymal tumors such as dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, adnexal gland carcinoma, and primary cutaneous lymphomas including post-transplantation lymphoma have been reported [54–57]. Hafner et al. calculated among renal transplant recipients an incidence of 156/100,000/ year (95% Cl 28–489/100,000/ year) for cutaneous malignant fibrous histiocytoma and of 78/100,000/year (95% CI 4–368/ 100,000/year) for atypical fibroxanthoma [57].

Immunosuppressive Treatment

Patients who have received a solid organ transplant usually need immunosuppressive therapy lifelong to prevent a rejection reaction. However, long-duration and high-dosed immunosuppression is associated with severe side effects, including an increased cancer risk [58, 59]. At least in part due to immunosuppressive therapy, the cancer may in OTRs be more aggressive with acceleration in growth and metastasis and subsequent lower patient survival [59]. A key target of immunosuppressive therapy is the activity of T cells. In the so-called three-signal model [60], the first step is antigen presentation by an antigen-presenting cell to the T cell via binding to the T-cell receptor (TCR/CD3) [60]. Upon activation, the transcription of genes coding for co-stimulating factors is increased via a calcineurin-mediated pathway. These co-stimulatory factors bind at signal 2 to T cells (CD28 receptor) [60]. Signals 1 and 2 are necessary for the expression of interleukin 2 (IL-2) among other mediators. Interleukin 2 binds at the IL-2 receptor on the T-cell surface, resulting in T-cell activation, mediated by mTOR among others [60].

Immunosuppressive mechanisms block the production and release of cytokines from activated T cells, downregulate and inhibit T-cell surface receptors, inhibit T-cell proliferation, or cause T-cell depletion [60]. Another important mechanism for development of skin cancer is the inhibition of DNA repair mechanism by immunosuppressive agents [22]. Several mechanisms can repair DNA photolesion and therefore prevent from development of skin cancer (including direct reversion of damage, double-strand break repair (homologous and non-homologous recombination) and excision repair (which comprises base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR)) [22]. Immunosuppressive drugs may inhibit nucleotide excision repair (NER) that results in a cellular mutator phenotype and cutaneous carcinogenesis [22]. Due to the different immunosuppressive effects of these

drugs, the carcinogenetic mechanism differs among calcineurin inhibitor, azathioprine, mycophenolate mofetil, and mTOR inhibitors [61].

Cyclosporine belongs to the group of calcineurin inhibitors (such as tacrolimus). These drugs inhibit calcineurin and therefore modulate the amplification of intracellular signals that are necessary for T-cell activation via step 2 and 3 [60]. Furthermore, data from Hojo et al. indicate that cyclosporine is able to promote cancer progression via a direct cellular effect that is independent of its effect on the host's immune cells and that cyclosporine-induced tumor growth factor beta (TGF-b) production is involved in this process [62]. Calcineurin inhibitors also block NER [22]. It has been reported that calcineurin inhibition results in a 200-fold increased skin cancer risk compared with the normal population [22].

Azathioprine is an antimetabolite that inhibits the de novo synthesis of purines and therefore blocks T-cell proliferation upon step 3 [60]. Azathioprine also inhibits NER but only the last step, i.e., gap filling [22]. This may explain the fact that skin cancer risk under azathioprine is threefold less compared with calcineurin inhibitors [22].

Mycophenolate mofetil (MMF) prevents proliferation of T and B cells via inhibition of purine synthesis [60]. Sirolimus and everolimus are mTOR inhibitors and prevent proliferation of T cells [60]. mTOR inhibitors do not reduce NER and can even inhibit the growth of already initiated tumors, resulting in a reduced skin cancer risk compared with calcineurin inhibitors, for example [22]. For example Dantal et al. analyzed kidney transplant recipients receiving calcineurin inhibitors with at least one SCC [63]. After randomization, 64 of these patients received sirolimus as a substitute for calcineurin inhibitor or maintained the immunosuppressive treatment with calcineurin inhibitors [63]. The authors showed that survival free of SCC was significantly longer in the sirolimus group than in the calcineurin inhibitor group ($p = 0.007$). Furthermore, the number of patients with new skin cancers was significantly lower in the sirolimus group compared with the calcineurin inhibitor

group (22% vs. 59% for SCC ($p < 0.001$), 34% vs. 66% for other skin cancers ($P < 0.001$) and 20% vs. 37.5% for BCC ($P < 0.05$) [63].

There is an ongoing discussion whether the broad variety of immunosuppressive drugs used in OTRs differ in their effect on cancer risk [58]. Some data indicate that tacrolimus and mycophenolate mofetil may be less carcinogenic than cyclosporine and azathioprine, respectively [10]. Prednisone is considered to have a smaller risk of promoting cancer than most other immunosuppressive drugs [10]. Data from Gallagher suggest that azathioprine- and cyclosporine-based regimens are associated with similar overall long-term cancer risks (481 patients during a median follow-up of 20.6 years were analyzed) [58]. Similar results were found by Thiel el al. from Switzerland analyzing 59 renal graft recipients receiving CyA compared with 213 patients who were initially immunosuppressed with azathioprine and prednisone (AzaP) [64]. They found no difference for the development of skin cancer among the CyA and AzaP patients [64]. Jensen et al. reported that OTRs treated with triple immunosuppression with cyclosporine, azathioprine, and prednisolone had a 2.8 times higher risk (CI, 1.4 to 5.3) of experiencing SCC relative to the historical group of recipients taking azathioprine and prednisolone [11]. Stallone et al. reported that sirolimus inhibits the progression of dermal Kaposi's sarcoma in kidney transplant recipients while providing effective immunosuppression [65].

Scott et al. analyzed the risk of NMSC with immunosuppressive drugs in patients with autoimmune disease and history of NMSC (n = 9460) [66]. They found that methotrexate use is associated with an increased risk of a second NMSC (HR, 1.24; 95%CI, 1.04–1.48 for a second NMSC occurring ≥ 1 year). Anti-TNF use may increase the risk of a second NMSC when used with methotrexate for rheumatoid arthritis [66].

Furthermore, there is an ongoing discussion if a reduction of immunosuppressive therapy will reduce morbidity and mortality from skin cancer and if a reduction of immunosuppression could play a therapeutic role in the management of patients with aggressive skin cancers [67].

However, there are no controlled trials that analyzed a reduction of immunosuppression with reduction of skin cancer incidence [67]. On the other hand, there is general consensus that in case of high-risk transplant-associated skin cancers, a reduction of immunosuppression should be considered, especially when the allograft is not lifesaving, such as renal transplantation [67]. Dantal et al. showed that halving of trough blood cyclosporine concentrations were associated with fewer malignant disorders but more frequent rejection [68].

In the last years, many other immunosuppressive drugs have been developed, such as alemtuzumab (monoclonal CD52 antibody), basiliximab (chimeric monoclonal antibody against CD25/IL-2 receptor), and belatacept (selective T-cell co-stimulation blocker that binds to CD80 and CD86 receptor on the antigen-presenting cell and prevents them from binding to CD28 on the T cell) [60, 69]. The relevance of these recently developed immunomodulatory drugs for the clinical management of OTRs, including their impact on skin cancer risk, still remains to be elucidated.

In summary, it can be emphasized that the broad variety of individual immunosuppressive drugs, which are available at present and used in OTRs, greatly differ in their impact on skin cancer risk and in the responsible mechanisms. Depending on the underlying pro-carcinogenic mechanism, potential additive or synergistic effects of UVR or other carcinogenic agents may greatly vary. It has to be noted that, while for some immunosuppressive drugs, UVB represents the most important pro-carcinogenic UV spectrum, other immunosuppressants, including azathioprine, exert additive or synergistic effects on UVA-mediated photocarcinogenesis.

Last but not least, one should keep in mind that other co-medications may also have an impact on skin cancer risk in OTRs. Recently, antihypertensive drugs, including hydrochlorothiazide, have been shown to increase skin cancer risk, presumably via increase of photosensitivity.

Vitamin D Deficiency in Organ Transplant Recipients: An Underrecognized Risk Factor for a Broad Variety of Severe Diseases

It is well-known that the vitamin D endocrine system is a major regulator of bone and calcium metabolism. Vitamin D, which is absorbed in the gut (vitamin D2 and/or vitamin D3) or synthesized in the skin (vitamin D3) by UVR, is transported in the blood to the liver where it is hydroxylated into 25-hydroxyvitamin D0 (25 (OH)D3 or 25(OH)D2, both metabolites also termed calcidiol), the main circulating metabolite of vitamin D [70]. Serum 25(OH)D is considered to be the best parameter for measuring a person's vitamin D status [71]. As a consequence, patients with liver insufficiency and/or failure are at risk for vitamin D deficiency [72].

Bound in the blood to a vitamin D binding protein (GC), 25(OH)D is then transported to the kidneys where it is hydroxylated at the C-1 position by the cytochrome P450 enzyme CYP27B1. The resulting 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$ or $1,25(OH)_2D2$, both metabolites also termed calcitriol) that is produced by renal cells is the major active form of vitamin D in the circulation [70]. During the last decades it has been shown that many other cell types also express CYP27B1 and have the capacity to produce $1,25(OH)_2D$ from 25(OH)D. However, the majority of this extrarenally produced $1,25(OH)_2D$ is not transferred to the blood but regulates locally various cellular functions independent from calcium and bone metabolism, including cellular proliferation and differentiation. Measuring of serum $1,25(OH)D$ is not recommended because it may often be normal or even increased as result of a secondary hyperparathyroidism associated with vitamin D deficiency [71]. However, because the last step of vitamin D activation takes place in renal cells, serum levels of $1,25(OH)_2D$ are reported to be positively correlated with estimated creatinine clearance and therefore for renal function [73]. Reduced 1-a-hydroxalse activity may be due to the decreased renal mass and uremic factors

[73]. Insufficient renal $1,25(OH)_2D$ production reduces intestinal calcium absorption, which consequently reduces serum ionized calcium concentrations and causes development of secondary hyperparathyroidism [74]. On the other hand, in chronic renal failure, $1,25(OH)_2D$ deficiency is reported to play a role in the stimulation of extrarenal $1,25(OH)_2D$ production by macrophages [75]. In a study done by Mehrota et al., $1,25(OH)_2D$ levels before transplantation were 102.37 ± 108.44 pmol/L, decreased to 46.20 ± 42.11 pmol/mL at 3 months and started increasing to 78.37 ± 60.12 pmol/mL at 6 months post-transplantation without vitamin D supplementation [74].

Furthermore, 25(OH)D deficiency in renal transplant recipients has been reported [76]. The well-accepted reason for 25(OH)D deficiency among OTRs is the fact that these patients are emphasized to avoid extensive solar exposure due to the increased risk for skin cancer resulting from their immunosuppression [76]. Kim et al. reported that pre-transplant 25(OH)D deficiency was significantly associated with a lower post-transplant glomerular filtration rate (GFR) [77]. They analyzed 106 patients who underwent kidney transplantation. The authors concluded that 25(OH)D may play an important role in maintaining graft function after kidney transplantation [77]. Bienaimé et al. analyzed in a prospective cohort of 634 kidney recipients between January 2005 and June 2010. They found that a low 25(OH)D concentration measured 3 months after transplantation is associated with a lower GFR 1 year after transplantation [78]. Keyzer et al. analyzed 435 stable renal transplant recipients at a median of 6 years after kidney transplantation [79]. The investigators found that low 25(OH)D is independently associated with an increased risk of all-cause mortality [79]. 25(OH) D < 12 ng/ml is associated with a rapid GFR decline [79]. The association of low $1,25(OH)_2D$ with mortality or graft failure depends on renal function [79].

Because secondary hyperparathyroidism is complicating chronic kidney disease, 1-hydroxylated vitamin D analogues, such as

paricalcitol and doxercalciferol are used to control secondary hyperparathyroidism [80].

As mentioned above, due to long-time immunosuppression of OTRs, the risk for the development of ultraviolet B radiation (UVB)-induced skin cancer is increased. To minimize the risk of potential harmful effects of UVR, it is recommended that OTRs should avoid excessive UVR exposure, cover their skin with clothes, and use sun blocker. But on the other hand, as a consequence of less UVB reaching the skin that is necessary for the cutaneous vitamin D production, the risk of vitamin D deficiency is increased [70]. For example, Stein et al. reported that among heart and liver transplant recipients at the time of transplantation, 91% of patients had vitamin D insufficiency, 55% had deficiency (25-OHD 10 to <20 ng/mL), and 16% had severe deficiency [81].

Reduced bone mineral density, osteopenia, and osteoporosis have been reported common in long-term renal transplant recipients which results in a high incidence of fractures [82, 83]. In several investigations, it has been shown that in renal transplant recipients who were treated with vitamin D, bone mineral density was significantly increased [82, 83]. Osteoporosis is considered to be a consequence of end-stage liver disease [84]. A decreased bone mass density has been observed in liver transplant patients [84].

Besides the classical role of vitamin D in the calcium homeostasis, vitamin D deficiency and inadequate doses of solar UVB radiation may be associated with various diseases and worse health outcomes [70, 85–87].

In summary, 25(OH)D is the best marker for the analysis of vitamin D status both in OTRs and in the general population. However, measurement of serum $1,25(OH)_2D$ concentration may also be of importance, e.g., in renal transplant recipients, before and after transplantation because the renal 1-a-hydroxylase activity depends on renal mass. Due to the important role of vitamin D, it is recommendable to screen for vitamin D deficiency in OTRs. Low 25(OH)D concentration (and low $1,25(OH)_2D$ in renal transplant recipients) should be supplemented with vitamin D [70, 84].

A Paradigm Shift in the Diagnosis and Management of Skin Malignancies in Solid Organ Transplant Recipients

General Principles

Robinson et al. analyzed the sun protection attitudes and behaviors in OTRs [88]. They found that 78% of OTRs and 69% of the US public believe that the appearance of a tan is attractive [88]. A greater proportion of OTRs believed that people looked "healthier" with a tan [88]. Unfortunately, 88% of OTRs were not aware of their increased risk of developing skin cancer [88].

These data highlights that an important factor in the prevention of skin cancer in OTRs is consistent sun protection and patient education. While, in the early days of transplantation, a dermatologist was only consulted when a visible skin change had formed, a multidisciplinary approach has meanwhile been established in the transplantation centers by integrating all relevant disciplines including dermatology. This includes the preventive education of the patient, regular dermatological screenings for early detection and early intervention as well as the development of strategies for the prophylaxis of malignant skin changes [89]. As mentioned above, for example, Le Mire et al. reported that in the Oxford renal transplant population, melanomas occurred at approximately eight times the rate in the general population which is the highest rate reported in the literature, but on the other hand, these patients had relatively good outcome which may be due to detection at a relatively early stage because these patients are closely examined by a dermatologist [45]. In order to enable the holistic care of patients at a transplant center to ensure this paradigm shift, dermatological facilities are being established at some centers. If a dermatology department is established at the transplant center, individual care of an OTR can be provided in terms of education on protection against UV radiation (both natural and artificial) with the right sun protection (through sun creams and textile

sunscreen) and tailored to the respective skin type, strategies for self-examination, regular screening examinations after transplantation tailored to immunosuppression and skin cancer history, and individual treatment of suspicious skin lesions [89].

Furthermore, a dermatological clinic at the transplant center can take the necessary measures in case of already locally advanced or metastasized tumors, e.g., performing a sentinel lymph node biopsy. In general, it is recommended that a dermatological examination is performed before the transplantation in order to treat possible malignant skin lesions before immunosuppression. As soon as possible after the transplantation, regular full-body examinations should be started, with a follow-up program adapted to the respective risk. At the first visit, the patient should explain the possible prevention strategies, and a regular checkup should be carried out along with a monthly self-examination.

Sun Protection

The guidelines for the management of SCC in OTRs recommend that all OTRs should receive extensive education about the risk of developing skin cancer and its associated morbidity and mortality. The patients should be educated concerning sun protection including the avoidance of sun exposure, protecting and covering the skin with cloths, and using of effective sunscreens with high sun protection factor that protect from both UVA and UVB [28]. Due to the increased risk of skin cancer under immunosuppression, all OTRs should use an appropriate sunscreen. Sunscreen creams with a sun protection factor (SPF) of at least 50 should be used. They should be applied daily on unclothed skin areas, even when no sun exposure is expected. Patients should always have access to several bottles of sunscreen and store them in different places, e.g., in the car. In addition, textile sun protection should be provided by long-sleeved trousers and tops and by wearing a sun hat.

Types of Skin Lesions

In general, skin tumors in OTRs have a similar clinical appearance as in the general population.

Actinic Keratosis and Other Intraepithelial Neoplasia

Actinic keratoses (AK) have the same clinical appearance in OTRs as in the general population. AKs usually appear on chronically light-damaged skin. It manifests itself in the form of rough, red, hyperkeratotic, scaly, mostly small herds. A large number of actinic keratoses can occur in the form of field cancerization. Actinic keratosis is usually easy to diagnose. Dermatoscopy can also be helpful. Here, the typical feature is the so-called strawberry pattern. If an invasive transformation is suspected, a punch biopsy of the lesion is recommended [90]. Boyd et al. reported that certain histopathologic features (including bacterial colonization, confluent parakeratosis, hyperkeratosis, increased mitotic activity, and verrucous changes) are more common in AKs of immunosuppressed transplant recipients and that these features may be used to distinguish AKs between those removed from otherwise healthy persons [91].

It is recommended to treat intraepithelial neoplasia including AKs, warts, and Bowen's disease (BD) to prevent the further development of invasive tumors and reduce the burden of viral infection, respectively [28]. A range of different therapy approaches is available. These include topical therapy approaches with imiquimod, diclofenac, 5-fluorouracil (5-FU), and 5-FU with salicylic acid [90]. However, these therapies require high compliance and sometimes long-term application, and not all topical therapies should be applied to OTRs. Due to the immunostimulant properties, it is recommended that imiquimod cream should be used with caution in patients receiving immunosuppressive treatment. However, an investigation by Ulrich et al. (analyzed 43 patients in 6 European transplant centers) showed that imiquimod is a safe

alternative for the treatment of multiple AKs in patients with solid organ transplants and that the efficacy was within the range previously observed in non-transplanted populations [92]. The same group analyzed in a randomized, placebo-controlled study with 32 patients the safety and efficacy of topical diclofenac gel in OTRs [93]. They concluded that diclofenac 3% gel is an efficient and well-tolerated treatment option for multiple AKs in OTRs and may prevent the development of invasive SCC [93]. For multifocal skin tumors or field cancerization, photodynamic therapy (PDT) may be another therapeutic option [94, 95]. PDT can be performed either under cooling in local anesthesia or due to possible pain under general anesthesia. In recent years, daylight PDT has been newly developed, which can also be performed at home after the patient has been informed. PDT is considered to be a safe and effective treatment for AKs in transplant recipients that may reduce the risk of transformation of AKs to invasive SCC [96]. But on the other hand, for example, de Graaf et al. reported that PDT does not appear to prevent the occurrence of new SCC in OTRs but reduces the increase of keratotic skin lesions [97]. For a good efficacy of PDT, distinct immune mechanisms are important [98]. This fact could explain the reported reduced efficacy of PDT in immunosuppressed patients [98].

However, there is evidence that PDT may be an effective therapeutic option for the treatment of AKs in OTRs, especially when compared to topical therapeutic options. For example, Perett et al. analyzed in a randomized intra-patient comparative study 5-FU with topical PDT [99]. They analyzed eight OTRs with epidermal dysplasia [99]. Compared with topical 5-FU, PDT was more effective and cosmetically acceptable [99]. In an investigation done by Dragieva et al., topical PDT of AKs and Bowen's disease in 20 OTRs has been analyzed. The authors concluded that PDT with 20% 5-ALA is an effective and safe treatment for AK and BD in OTRs [100]. The initial response rates were comparable with those in immunocompetent patients [100].

Furthermore, cryotherapy of the intraepidermal lesions is simple and efficient procedure quick to perform. Surgical procedures such as curettage are also possible. Treatment of large lesional areas basically possible, in our center however hardly carried out, is a laser therapy of actinic keratoses. A follow-up dermatologic examination of OTRs with history of AK is recommended to be done every 6 months [101]. It is recommended that warts, AKs, and porokeratosis that have an atypical clinical appearance or do not respond to appropriate therapy should be biopsied for histologic evaluation [28].

SCC

OTRs with SCC can be divided into low-risk and high-risk categories based on aggressive growth characteristics of the SCC. In general, a follow-up dermatologic examination of OTRs with history of nonmelanoma skin cancer is recommended to be done every 6 months [101].

Low-Risk SCC

For any lesion suspected for SCC, we recommend biopsy or direct total excision. In literature, it has been recommended that low-risk SCC can be managed with surgical excision or superficial ablative therapy, such as with electrodesiccation and curettage or with curettage and cryotherapy [101].

However, with ablative methods no statement can be made about histological parameters such as the resection status or the infiltration depth.

High-Risk SCC

Characteristics of high-grade SCCs are multiple, rapid recurrences, high-risk location (including forehead, temple, ear, lip), large size (>2 cm), history of aggressive growth, high grade (broders

3 or 4), and deep invasion (>4-6 mm, especially into fat, muscle, cartilage, or bone or with perineural invasion) [101]. It is recommended to perform an aggressive primary tumor resection, particularly with use of Mohs micrographic surgery [101]. Sentinel lymph node biopsy (SNLB) or staging elective lymphadenectomy in extremely high-risk cases should be considered [101]. Furthermore, an adjuvant radiation therapy should be considered, although usually reserved for metastatic disease [101]. The dose of immunosuppressive medication should be reduced, and treatment with systemic retinoid should be established [101]. Furthermore, retinoid therapy is possible as prophylaxis. For example, Bavnick et al. reported that among renal transplant recipients, acitretin 30 mg/d over 6 months had significantly more effect than placebo in the prevention of SCC and reduced the occurrence of keratotic skin lesions [102]. New therapeutic option for advanced or metastatic SCC such as immunotherapy with immune checkpoint inhibitor such as cemiplimab, a highly potent human monoclonal antibody directed against programmed death 1 (PD-1), is not possible [103]. On the one hand, immunosuppression would impair pharmacodynamic activity and efficacy of immune checkpoint therapy; on the other, immunotherapy can induce organ rejection. Immune checkpoint inhibitors increase the activation of T cells against malignant cells but also against other cells expressing foreign antigens such as allograft donor antigens [104]. Therefore, patients with immunosuppression have been excluded from studies with immune checkpoint therapy [103]. Abdel-Wahab et al. collected data from the medical records of patients with cancer and prior solid organ transplantation who received checkpoint inhibitor therapy (anti-CTLA-4 or anti-PD-1 therapy) [105]. They identified 39 patients of whom 41% allograft rejection occurred [105].

A follow-up dermatologic examination of OTRs with history of multiple nonmelanoma skin cancers or high-risk SCC is recommended to be done every 2–4 months or 3 months, respectively [101].

Metastatic SCC

The therapy of metastatic single nodal with no extra capsular spread includes the recommendation for aggressive SCC plus therapeutic lymphadenectomy and an optional radiation therapy [101]. For patients with multiple nodes or extra capsular spread, therapeutic lymphadenectomy and adjuvant radiation therapy are recommended [101]. A follow-up dermatologic examination of OTRs for these patients is recommended to be done every 2 months [101].

BCC

The clinical features of BCC may be pearly rolled borders, telangiectasia, central atrophy, or a darkly pigmented BCC [9]. The typical features in dermatoscopy are maple leaf-like structure, dirty grey pigment, telangiectasias, which often reach from the edge to the center, spoke wheels, or pigmented (blue, black, grey) ovoid bodies [106]. The surgical excision is the recommended standard care for BCC, for example, with Mohs surgery [9, 107]. Other treatment options are PDT or topical therapy such as imiquimod 5% of 5-flurouracil but usually reserved to superficial BCC [107]. Concerning topical therapy options such as imiquimod or 5-FU, there are similar concerns in OTRs as discussed in OTRs with actinic keratoses. However, it has to be noted that investigations of this therapy in OTRs with BCC do not exist so far [107].

Malignant Melanoma (MM)

Typical clinical and dermatoscopy features of melanoma are, for example, lesions that show asymmetry, curved boundaries, multiple colors, and/or multiple structures [106]. The German S3-guideline on melanoma does not provide any separate recommendations for OTRs, so the recommendations for immunocompetent should be used in general. In principle, a radical excision of the primary tumor should be carried out. The

safety distance depends on the penetration depth and ranges from 5 mm, 1 cm, or 2 cm for in situ forms, pT1/pT2 or pT3/pT4, respectively [108]. It is recommended to perform a sentinel lymph node biopsy from a penetration depth of 1 mm. In the case of additional risk factors such as ulceration, increased mitosis rate, or younger age (<40 years), the sentinel lymph node biopsy should also be performed on thinner primary tumors (0. 75–1 mm) [108]. With a maximum metastatic diameter in SNL between 0. 1 and 1 mm, a complete lymph node dissection can be offered, especially in the presence of other risk factors (maximum metastatic diameter, capsule infiltration and depth extension in the SNL, number of affected SNLs, thickness, and ulceration of the primary tumor) [108]. With a maximum metastatic diameter > 1 mm in the SNL, a complete lymph node dissection should be offered [108].

Therapeutic lymphadenectomy should be performed if there is clinical evidence of lymphogenic metastasis without indication of distant metastases [108]. After lymphadenectomy, adjuvant radiotherapy should be performed if at least one risk factor is present (three affected lymph nodes, capsule rupture, lymph node metastasis >3 cm, lymphogenic recurrence) [108]. For metastatic melanoma (stadium IV) or melanoma stadium in III, new therapeutic options with targeted therapy (BRAF inhibitors plus MEK inhibitors) and immunotherapy have been developed during the last years [109]. In the general population, these therapeutic options have markedly changed the outcomes of patients with early and metastatic melanoma [109]. Regarding immune checkpoint inhibitors, such as nivolumab or pembrolizumab (both anti-PD-1 antibodies) or ipilimumab (anti-CTLA-4 antibody), there are similar concerns in OTRs as discussed in OTRs with advanced SCC for cemiplimab [104]. Tripathi et al. reported in a small trail from OTRs with metastatic melanoma that were treated with immunotherapy or targeted therapy [104]. The authors found in two patients receiving immunotherapy a partial response, although one of these patients had a graft rejection reaction after immunotherapy [104]. It has been concluded that in OTRs, targeted therapy may be used in

BRAF V600E mutated tumors [104]. In OTRs with BRAF wild type tumors, checkpoint inhibitors may be only used with caution and frequent monitoring. The caution should be exercised with the use of checkpoint inhibitors in liver, lung, and heart transplant patients because of the potential for graft rejection [104].

A follow-up dermatologic examination of OTRs with history of melanoma or metastatic melanoma is recommended to be done every 3 months or 2 months, respectively [101].

Follow-Up

OTRs with a history of skin cancer should undergo a distinguished follow-up screening that differs from the follow-up recommendation for immunocompetent patients; see Table 17.1 [101]. The examination includes full-body inspection with dermatoscopy and lymph node palpation. In the case of palpable lymph nodes, a sonography should be connected. Locoregional lymph node sonography should be performed in the follow-up care of melanoma patients from stage IB onward and for high-risk SCC [108]. For R0 resected melanomas from stage IIC-IV onward, CT and MRT staging every 6 months is recommended [108].

Summary

The number of patients who have to take immunosuppressive drugs on a permanent basis has been growing steadily since organ transplantation began. Since then, it has become clear that malignant skin diseases, especially SCC, have high incidence rates in this population group. These lesions also behave more aggressively and metastasize earlier. This risk increases further with the duration and intensity of immunosuppression. Regular full-body examinations of OTRs are therefore necessary to detect and treat suspicious lesions at an early stage. The frequency of aftercare depends on the individual risk factors of the patient. Patients should apply consistent sun protection with sunscreens and

Table 17.1 Recommended dermatologic follow-up examination, cited from [101]

Skin cancer history	Dermatologic follow-up examination interval (in months)
No skin cancer or actinic keratosis	**12**
Actinic keratoses	**6**
One nonmelanoma skin cancer	**6**
Multiple nonmelanoma skin cancers	**2–4**
High-risk SCC or melanoma[a]	**3**
Metastatic SCC or melanoma	**2**

For R0 resected melanomas from stage IIC-IV onward, CT and MRT staging every 6 months is recommended [108]

[a]Locoregional lymph node sonography should be performed in the follow-up care of melanoma patients from stage IB onward and for high-risk SCC [108]

clothing, as well as a monthly self-examination. On the other hand, the necessary of avoidance UVR increases the risk of a 25(OH)D deficiency, which itself is associated with an increased risk of many diseases, including malignancies. OTRs should therefore be monitored for 25(OH)D status and, if necessary, be treated. Dermatologists and transplantation physicians should work closely together after the diagnosis of skin cancer to prevent progression of the tumor, especially if a reduction of immunosuppression must be considered for therapeutic or prophylactic reasons [10].

An interdisciplinary approach with a dermatology department within the transplant center ensures the best care for the OTRs.

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Sunscreens in the United States: Current Status and Future Outlook

18

Katherine S. Glaser and Kenneth J. Tomecki

Abstract

Incidence rates of nonmelanoma skin cancer and melanoma have been on the rise in the USA for the past 25 years. UV radiation (UVR) exposure remains the most preventable environmental risk factor for these cancers. Aside from sun avoidance, sunscreens continue to provide the best alternative protection. UVR directly damages DNA and causes indirect cellular damage through the creation of reactive oxygen species, the sum of which leads to cutaneous immunosuppression and a tumorigenic milieu. The current generation of sunscreens protect from UVR through two main mechanisms: absorption and deflection. In the USA, the Food and Drug Association (FDA) regulates sunscreen products which are considered over-the-counter drugs. With the release of new FDA testing and labeling requirements in 2011 and the enactment of the Sunscreen Innovation Act in 2014, sunscreen manufacturers are now required to evaluate their products not only on the sun protection factor (SPF) but also on broad-spectrum UVA protection. The *American Academy of Dermatology Association* and the *American Academy of Pediatrics* have provided specific recommendations for proper sun protection and sunscreen usage with the continual goal of increasing public awareness and compliance with appropriate sun protective measures. Antioxidants, photolyases, and plant polyphenols remain an interesting avenue of research as additives to sunscreens or stand-alone topical or oral products that appear to modulate the immunosuppressive effects of UVR on the skin. Additionally, although UVR induces endogenous cutaneous production of vitamin D, its damaging effects overshadow this positive benefit, especially in light of the ease of achieving recommended amounts of vitamin D through diet and supplementation.

Introduction

Background

Products purporting to protect from sunburns have been in existence as early as the 1940s. Sunscreens were initially developed with the sole purpose of minimizing erythema. Four people have been attributed to the invention of

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sunscreens: Milton Blake, an Australian chemist; Eugene Schueller, the founder of L'Oreal©; Franz Greiter, the founder of Piz Buin© who introduced the sun protection factor (SPF) in the 1960s; and Benjamin Green, the founder of Coppertone© [1, 2]. Sunscreens have come a long way since then, with numerous compounds that block or absorb different parts of the ultraviolet (UV) spectrum. Antioxidants, photolyases or photoreactivation enzymes, and polyphenols or plant-derived aromatic compounds have been the newest area of research due to their anti-inflammatory and anticancer properties [3, 4]. Though, information regarding new sunscreen compounds and delivery methods are closely guarded trade secrets by private companies.

Regulatory guidelines for sunscreens differ around the world. In the United States (USA), the Food and Drug Administration (FDA) began regulating over-the-counter sunscreen products in 1978. Their recommendations for safe and effective sunscreen use have transformed over the years with the most recent updates outlined in the 2011 Final Rule and the Sunscreen Innovation Act in 2014 [5]. Physicians should be aware of these up-to-date recommendations and be comfortable educating patients on currently available sun protective options.

In this chapter we will explore the effect of UV radiation (UVR) on the skin, types of sunscreens, how sunscreens are evaluated in the US, new labeling guidelines by the FDA, and proper sunscreen use as outlined by the *American Academy of Dermatology Association* and the *American Academy of Pediatrics*. We will also review the literature behind sunscreen efficacy, sunscreen and vitamin D, and polyphenols and photolyases as an ongoing area of research.

UV Radiation (UVR)

The spectrum of UVR consists of wavelengths of light from 200 to 400 nm and is separated into three bands: UVC (200–290 nm), UVB (290–320 nm), and UVA (320–400 nm). UVA makes up the vast majority of UVR that reaches the Earth's surface while UVB accounts for only about 5%. UVC is absorbed completely by the atmosphere and does not reach the Earth's surface [6]. Many factors affect the amount of UVR that reaches the Earth including the latitude, altitude, angle of the sun at its zenith, time of day, air pollution, and cloud cover among others. There is also notable individual variability of UVR penetrance into the skin which depends on the person's skin texture, color, thickness, and body site.

Solar UVR is generally strongest between 10:00 a.m. and 4:00 p.m. at equatorial latitudes and during summer months [7, 8]. UVA is not typically affected by environmental factors whereas much of the shorter wavelength UVB radiation is scattered by the atmospheric ozone layer, clouds, air pollution, and glass. Importantly, while only 5% of the UVR reaching the Earth's surface is UVB, it is considered the main cause of sunburn in humans [7, 9]. Thus, the risk of sunburn is highest midday and during summer months. Still, UVA makes up a large proportion of UVR reaching Earth and can penetrate deeper into the skin due to its longer wavelength and so has significant destructive potential [7, 9].

The UV index is a direct measurement of the level of UVR reaching the Earth's surface while accounting for cloud cover. In the USA it is provided daily by the National Weather Service and the US Environmental Protection Agency (EPA). It is measured on a continuous scale from 1 (low) to 11+ (extremely high). The higher the number, the greater the risk of sunburn [10]. Currently, the World Health Organization (WHO) recommends individuals to use sun protective measures when the UV index is 3 or above [11]. However, this index does not reliably account for the degree of UVA exposure which we now recognize as having considerable health risks. The public should be advised that the degree of UVR exposure is determined both by the UV index and the duration of time spent outdoors [12]. Ultimately, the decision to utilize sun protective measures should not be based solely on the UV index.

UVR and US Skin Cancer Incidence

Exposure to UVR is the single most important environmental risk factor for nonmelanoma skin cancer (NMSC) development. Research also suggests a direct link between UVR and malignant melanoma [13, 14]. Excessive UVR, even without an acute sunburn response, can cause significant damage to the skin including immunosuppression and carcinogenesis [13]. Several studies have shown that sun protective measures and avoidance of indoor tanning devices can prevent a substantial proportion of NMSC and melanoma [15–18]. For this reason, increasing public awareness on the risks of UVR and the importance of sun protection is crucial. The US Surgeon General released a Call to Action to Prevent Skin Cancer in 2014 which specifically outlines educational strategies [19].

Unfortunately, the incidence of all types of skin cancer continues to increase in the USA over the past 25 years. It is challenging to quantify the incidence of NMSC, namely basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), as they are not mandatory to report to the US Center for Disease Control or other cancer registries. Using US Medicare databases and national survey data, the most recent estimated NMSC incidence in 2012 was 5.4 million cases [20]. With the same databases, the incidence was previously estimated in 2006 to be 3.5 million cases [21]. In comparison, a 1994 study estimated the incidence to be 900,000 to 1.2 million cases [22]. This increasing incidence correlates to an increase in number of NMSC procedures performed and the annual cost of treating NMSCs.

Similarly, the incidence of melanoma continues to rise in the US. The incidence remains highest in non-Hispanic whites. Gender predilection varies based on age group. According to *The American Cancer Society*, excluding NMSC and in situ carcinomas, melanoma represents the fifth leading cause of cancer in men and women in 2019. The number of new cases of in 2019 is estimated at 96,480 with 7,230 melanoma deaths [14]. Comparatively, in 2012, the incidence of melanoma was 76,250 cases which is drastically different from the 28,550 cases in 2000 [23, 24]. Fortunately, the mortality rate has steadily declined on an annual basis due to earlier detection and advancements in treatment modalities.

UVR's Biochemical Effects on the Skin

Immunosuppression and Skin Cancer

The idea that immunosuppression can enhance skin carcinogenesis came from organ transplant patients who are more likely to develop skin cancer than the general population. The chronic use of immunosuppressants after transplantation leads to a decreased capacity for immune-mediated tumor surveillance as well as increased susceptibility to oncogenic viruses which increases the risk of carcinogenesis. Skin cancer is the single most common malignancy in this population with SCC and BCC comprising 90–95% [25]. The incidence of SCC is thought to be 65- to 250-fold greater in an organ transplant recipient and melanoma incidence up to eightfold greater. Cutaneous malignancy is a significant source of morbidity and mortality in these patients as they tend to act more aggressively in an immunosuppressed host [25, 26].

Several factors contribute to the likelihood of skin cancer in an organ transplant recipient including the duration, dosage, number, and choice of immunosuppressant [25, 27]. Certain immunosuppressants have been shown to have stronger links to skin cancer development and so, if possible, should be avoided. Literature suggests a higher likelihood of skin cancer if using azathioprine or cyclosporine. Voriconazole, often used as antifungal prophylaxis, is also linked with significantly higher rates of NMSC. Conversely, tacrolimus and mycophenolate mofetil seem to have lower relative risks of skin cancer development [25].

Additionally, the type of organ transplanted dictates the degree of immunosuppression required which correlates directly with the risk of skin cancer. For example, heart and lung

transplantation requires a significantly greater degree of immunosuppression compared to kidney and liver such that heart and lung transplant recipients have the highest incidences of skin cancer. Interestingly, a recent study looking at HLA antigen mismatch in solid organ transplants showed a statistically significant reduction in skin cancer risk if HLA alleles were mismatched. This reduction was only statistically significant for heart and lung transplant patients, not liver, kidney, or pancreas transplants [28]. These findings further emphasize the importance of the body's tumor surveillance mechanisms in the prevention of skin cancer.

UV Immunosuppression and Carcinogenesis

UV exposure by itself can cause local and systemic immunosuppression in healthy individuals. UVR is known to cause direct DNA damage, indirect damage through reactive oxygen species (ROS), and alterations in the body's cellular immunity. Both UVA and UVB have a role in UV-induced immunosuppression which can occur even at suberythemal doses [29]. Although the skin has some endogenous ability to repair damage, UV-induced stress responses shifts the body toward the suppression of local, memory, and systemic immunity.

Most studies evaluating UV-induced immunosuppression use contact hypersensitivity (CHS) reactions as a proxy for a robust immune response. In general, the skin is first sensitized by a potent allergen such as dinitrofluorobenzene (DNFB) and then exposed to UVR. Subsequently, DNFB is reapplied in the same area after UVR exposure and the reaction is assessed. A loss of CHS indicates immunosuppression [30].

The immunosuppressive and carcinogenic effects of UVR on the skin are complex and involve a variety of cell types. UVR can cause dysregulation of antigen-presenting cells such as Langerhans cells and dermal dendritic cells, which in turn can activate regulatory T cells to suppress the immune system [13, 31]. Activation and inhibition of these various cell populations leads to a complex cytokine milieu that inhibits the skin's immune response. Several immunosuppressive and pro-inflammatory cytokines are produced by keratinocytes including interleukin-10, TNF-a, and NF-kB which inhibit the body's "repair cytokines" that help fix UV-induced DNA damage [13, 32, 33]. To note, these cytokines function in homeostasis; both elevation and inhibition are involved in inflammation and immune suppression [32].

DNA is known to act as a chromophore in that it absorbs UV light, predominantly UVB. When UVB light is absorbed, the DNA enters an excited state resulting in the formation of cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidone photoproducts. These dimers create structural DNA damage which blocks replication and transcription of new DNA. The result is G1 phase arrest, failure to progress at S phase checkpoint, and UV-induced apoptosis [34]. Overtime, several mutations can then develop which allow the cell to undergo malignant transformation. Examples of UV-induced mutations include loss of function of the *p53* tumor suppressor gene and aberrant activation of genes in the hedgehog signaling pathway including Patched (*PTCH*), Sonic Hedgehog (*SHH*), and Smoothened (*SMO*) that have been linked to SCC and BCC development, respectively [35, 36].

Chromophores in the skin besides DNA can also absorb UV light, especially UVA, which can then transfer energy to the DNA via the formation of ROS. The ROS create indirect DNA damage via modified bases in the DNA such as the well-studied guanosine mutation, 8-hydroxy-2'deoxyguanosine (8-oxo-dG). Unrepaired 8-oxo-dG can be misread by DNA polymerase as thymine which increases the incidence of base pair mutations leading to decreased DNA integrity [37, 38]. Interestingly, CPDs can also be produced by UVA exposure through an unknown mechanism and are apparently cleared at a slower rate than those induced by UVB, suggesting that both UVA and UVB play a role in carcinogenesis [39].

Mechanisms of Sun-Induced Skin Darkening or "Tanning"

UVR on Tanning

The skin reacts phenotypically to UVR via skin darkening or "tanning," in layman's terms, as a less-than-effective protective response. Skin darkening can be immediate or delayed and is wavelength dependent. The mechanisms by which skin darkening occurs in response to UVA and UVB radiation differ.

Immediate and persistent pigment darkening results from UVA exposure. Immediate pigment darkening occurs within seconds of exposure and fades within hours, believed to be due to ROS formation and oxidation of preexisting melanin. Persistent pigment darkening results from similar mechanisms but begins 2–24 h after exposure and can last for weeks. The effects of UVA on pigment are more readily observed in darker skinned individuals, typically Fitzpatrick skin types III–VI. Importantly, this does not enhance the skin's ability to further protect against UVA as no new melanin is produced [40, 41].

Delayed pigment darkening is induced by UVB exposure and can develop with or without a preceding "sunburn" response. Delayed tanning develops approximately 72 h after exposure and is caused by increased melanocyte activity with new melanin formation. For this reason delayed tanning offers a minutely protective response, though studies show an increase in the skin's UVB resistance by an approximate SPF of 3. A "sunburn" is UVB-induced vasodilation, erythema, edema, and desquamation. It occurs more commonly in fairer skinned individuals and can be seen immediately after exposure but typically reaches its peak 24–48 h later. Different Fitzpatrick skin types have variable tendencies toward delayed tanning [7, 42].

Visible Light and Infrared Radiation on Tanning

More recent literature suggests an additional component of pigment darkening in response to visible light (400–700 nm) and infrared radiation (700–1440 nm) which make up the majority of solar energy. While UVR comprises <10% of the solar spectra reaching the Earth's surface, approximately 40% is visible light and 50% is infrared radiation. Like UVA, the visible light and infrared radiation spectrums are known to penetrate into the deep dermis and thus can cause similar damage [43, 44].

A study in 2012 demonstrated that human skin exposed to visible light has increased production of ROS, pro-inflammatory cytokines, and matrix metalloproteinase (MMP) expression. No evidence of thymine dimer formation was identified compared to the damage by UVR. The author's findings also showed that a traditional broad UVA/UVB sunscreen did not protect against the ROS from visible light exposure. The addition of an antioxidant into the sunscreen did lead to significantly reduced ROS and inflammation [43].

In 2010, Mahmoud et al. compared the pigment darkening response with UVA1 to visible light and further stratified these responses among darker skinned individuals (Fitzpatrick IV–VI) and fairer skinned individuals (Fitzpatrick II). Pigmentation responses were assessed with digital photography, diffuse reflectance spectroscopy, confocal microscopy, and skin biopsies. The results indicated a more robust and sustained pigment darkening with visible light compared to UVA1. Additionally, subtle erythema was initially seen surrounding the pigmentation following exposure to visible light which was not identified in the UVA1 group. Hyperpigmentation was only visualized in darker skinned individuals but occurred regardless of light source [44]. This is important for individuals with photodermatoses or photosensitivity.

Sunscreen

Types of Sunscreens

The term sunscreen is a misnomer as sunscreens only screen out UVR, a small part of the solar spectrum. For a compound to be considered a

sunscreen, it must block or absorb radiation in the UVR range (290–400 nm) and prevent or diminish the dose of UVR that reach the skin's surface. The regulation and availability of different sunscreens varies around the world. Though, globally, UV filters in sunscreens are classified as either physical or chemical agents. Many sunscreens on the market are blends of multiple UV filter active ingredients that work synergistically to provide protection across the UV spectrum as well as increasing the stability of individual compounds to provide longer-lasting protection.

Physical agents found in sunscreens are generally inorganic compounds that act by blocking UVR. It is accepted that inorganic compounds function by physically reflecting and scattering UVR from a film of inert metal particles, analogous to protective clothing. Recent literature also suggests some protection in the visible light range (400–700 nm) [44, 45]. There are two inorganic UV filters: titanium dioxide and zinc oxide. Titanium dioxide provides strong UVB and some UVA2 protection while zinc oxide provides good UVA1, UVA2, and UVB protection [42]. To encourage better utilization of inorganic filters and improve cosmesis, nanoparticle technology (particles <100 nm in diameter) has been widely incorporated by sunscreen manufacturers. However, the ability to protect against UVR is directly related to particle size. This is especially true for titanium dioxide which exhibits diminished UVA protection with nanosized particles [45, 46]. For this reason, zinc oxide and titanium dioxide are typically used in combination to provide broadband UV protection. Inorganic filters will likely become more commonplace in the USA with newer, though controversial, evidence that certain organic filters have been identified in water sources and aquatic animals as well as the implication of coral reef bleaching and potential endocrinology adverse effects if systemically absorbed [47].

Chemical agents found in sunscreens are typically organic compounds that actively absorb UVR, stabilizing the energy within the molecule itself which then dissipates the energy in the form of heat. There are five main categories of organic sunscreens including para-aminobenzoic acid derivatives, cinnamates, salicylates, benzophenones, and "other." Most US FDA-approved organic UV filters absorb UVB radiation, while few act in the UVA2 range. Only one FDA-approved organic UV filter, avobenzone, protects against UVA1. Another organic UV filter that works in the UVA1 spectrum, ecamsule, is only approved in one specific commercial line of products in the USA which was approved with an Individual New Drug Application process. Again, these organic filters are often combined to increase the photostability of the product and broaden the UV spectral coverage [7, 46].

Table 18.1 details the list of current FDA-approved sunscreens and their effective UV protection ranges. A total of 16 active ingredients are currently approved in the USA with an additional organic filter, ecamsule, approved only in one commercial line of products. At the time of publication, 8 UV filters are awaiting final approval by the FDA. This list is subject to change in the near future based on newly proposed FDA rules as of February 2019.

How Sunscreen Is Evaluated in the USA: Current and Upcoming Methods

Sunscreens in the USA are considered over-the-counter drugs and thus have a higher degree of regulation by the FDA compared to other countries where sunscreens may be considered cosmetic products. Due to this tight regulation, there are significantly less active sunscreen ingredients available to US citizens. In the USA, sunscreens are evaluated on their ability to protect against UVR as well as their photostability and substantivity or "water resistance." Previously, the FDA only regulated the evaluation and labeling of UVB protection through the "sun protection factor" or SPF. Since the 2011 FDA Final Rule, there are guidelines for in vitro UVA testing which allow a product to achieve a broad-spectrum designation [5, 49–51].

Table 18.1 List of current FDA-approved sunscreen active ingredients and their respective UV protection ranges. Since the absorption spectra of different sunscreens vary, each sunscreen may not block the entire range of UVR that it is listed under [5, 7, 48]

Filter category	Compound names	Absorption ranges		
		UVB (290–320 nm)	UVA2 (320–340 nm)	UVA1 (340–400 nm)
Organic	PABA[a]	X		
	Padimate O	X		
	Octinoxate	X		
	Cinoxate	X		
	Homosalate	X		
	Octisalate	X		
	Trolamine Salicylate	X		
	Octocrylene	X		
	Ensulizole	X		
	Dioxybenzone	X	X	
	Oxybenzone	X	X	
	Sulisobenzone	X	X	
	Ecamsule[b]		X	X
	Meradimate		X	
	Avobenzone		X	X
Inorganic	Titanium Dioxide	X	X	
	Zinc Oxide	X	X	X

[a]*PABA* Para-aminobenzoic acid
[b]Ecamsule is only approved in the US for one commercial line of products via an individual new drug process

UVB Protection

SPF is a laboratory measure of sunscreen efficacy against UVB. It is defined as the amount of UVR required to produce a sunburn on protected skin relative to that of unprotected skin. Figure 18.1 details the general protocol used by the FDA for evaluating the SPF of a sunscreen. The SPF testing methods in the USA were adopted from the 2010 published recommendations by the International Standards Organization (ISO) which are utilized globally and thus are quite similar worldwide [52].

Contrary to popular belief, the SPF of a product is not directly related to the duration of UV exposure before a sunburn occurs. UVR dosage depends on both the duration of exposure and the UV intensity, which is influenced by multiple factors as previously discussed. When sunscreen is applied correctly, the relationship between SPF and UVB protection is not linear. For example, a sunscreen with an SPF of 15 can filter 94% of UVB radiation, whereas an SPF of 30 provides greater than 97% protection at an equal UVB dosage [42, 50]. Some argue that increasing SPF

above 30 provides diminishing returns of protection while allowing companies to charge higher prices for their products.

In 1978, the FDA initially proposed a maximum labeled SPF value of 15 with each listed active ingredient providing at least an SPF of 2. The sunscreen monograph in 1999 increased the allowed maximum labeled SPF to 30 and provided maximum concentrations of each active ingredient that were considered safe [49]. Then in 2011, the maximum permissible SPF value was increased to 50 along with several labeling and efficacy testing guidelines [5]. Newly proposed in February 2019, among other changes, is the initiative to increase maximum allowed SPF to 60 [48]. This upward trend is in direct response to multiple published studies which show real-life sunscreen application by the consumer that differs from that recommended in SPF testing [52–54].

Testing for SPF is determined by an applied 2 mg/cm^2 density of product. In reality, consumers apply anywhere from 0.5 mg/cm^2 to 1.5 mg/cm^2 with notable variability among body

Fig. 18.1 Protocol for determination of sun protection factor (SPF). A minimum of 20 light skinned volunteers (Fitzpatrick I-III) are used and apply a sunscreen at 2 mg/cm^2 density over non-sun-exposed surfaces such as the buttocks or lower back. A xenon arc lamp delivers UVR to discrete areas either with previously applied sunscreen 15 min prior or to control areas with no applied product. The minimal erythema dose (MED) represents the minimum amount of UVR necessary to induce erythema of the skin of the control areas. SPF is determined by the UVR dose required for erythema on the sunscreen-covered skin divided by the control skin [5]
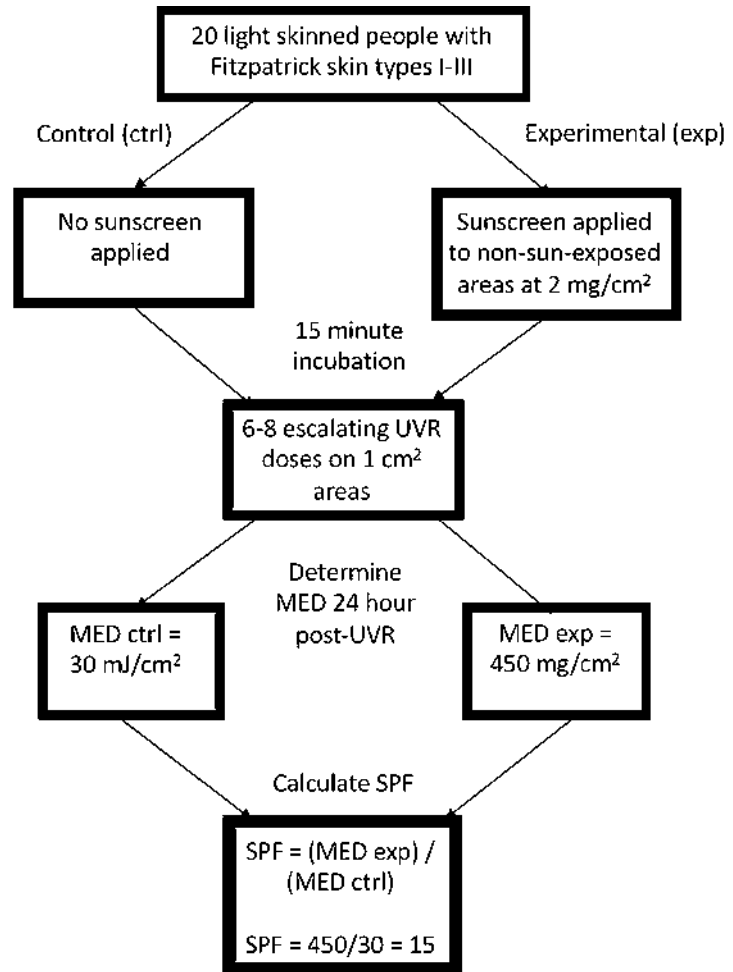
sites. This means most people achieve on average 20%–50% of the actual labeled SPF [52]. A study in 2012 supports this concept by analyzing different sunscreen products SPF 30–100 in lotion and spray formulations applied at differing densities including 0.5, 1.0, 1.5, and 2.0 mg/cm^2. The authors found a linear relationship between the application density and the labeled SPF for all tested products. At the lowest application density of 0.5 mg/cm^2, the SPF 30 product offered a mean SPF 8.8 protection, the SPF 70 product provided a mean SPF 19.3 protection, and the SPF 100 product offered a mean SPF 27.1 protection [53]. Similarly in 2018, a randomized, double-blind, split-face study measured the erythemal response after SPF 50 and SPF 100 sunscreens were applied to 200 subjects prior to snow skiing or snowboarding for 6 h. In both product groups, subjects only applied approximately half of the recommended product density and the majority reapplied 1 or fewer times. Comparatively, 55.3% of subjects were more sunburned on the SPF 50 side versus only 5% more sunburned on the SPF 100 side [54]. For this reason, there arguably is clinical utility to having higher SPF products on the market to account for the inadequate applications by the consumer.

UVA Protection

As previously discussed, UVA is the major type of UVR reaching the Earth's surface and is now known to contribute to immunosuppression,

Sunscreen UVA Absorbance Curve
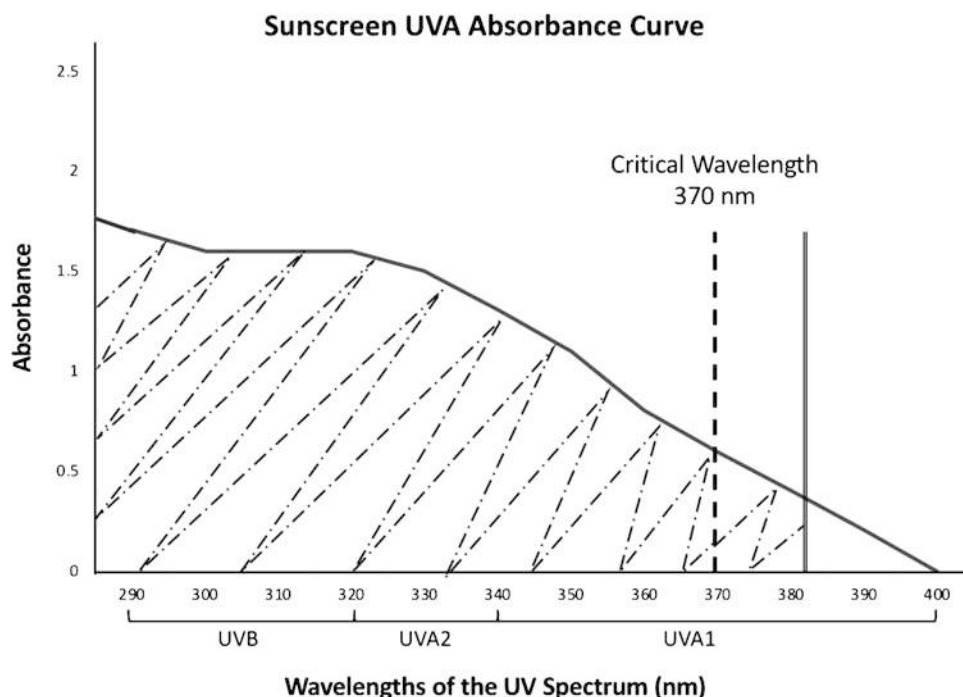
Fig. 18.2 Determination of "broad-spectrum" UV protection using in vitro critical wavelength method. Polymethylmethacrylate plates are coated with 0.75 mg/ml of sunscreen irradiated with continuous full spectrum UV. The UV transmittance is recorded by a spectrophotometer and an absorbance curve is reported. The critical wavelength is determined to be the wavelength below which 90% of the total area under the UV absorbance curve resides. A "broad-spectrum" sunscreen has CW ≥ 370 nm. Thus, the depicted sunscreen is considered "broad spectrum" [5]

carcinogenesis, and photoaging of the skin. Thus, a systematic and repeatable method of measuring a sunscreen's ability to protect against UVA is necessary. The ISO published a method for in vivo UVA testing in 2011 which was based on persistent pigment darkening assessments. However, in 2012 a more adopted in vitro method for testing UVA was published by the ISO and has since been incorporated into the FDA testing requirements [52].

For each sunscreen product, laboratory testing generates an absorbance curve within the UV spectrum from 290 to 400 nm. The area under the curve is calculated and a "critical wavelength" is established, defined as the wavelength where below which 90% of the total area under the absorbance curve resides. A sunscreen is considered "broad-spectrum" in the USA if its "critical wavelength" is greater than or equal to 370 nm.

This method of evaluation looks at the sunscreen in a pass/fail fashion and does not take into consideration the degree of protection like the SPF value [5, 42, 55]. See Fig. 18.2 for a graphical explanation of this method.

The pass/fail methods of UVA testing in the USA are thought to be more lenient than the standards utilized in Europe. The 2012 ISO guidelines, as practiced in Europe, require both critical wavelengths testing as well as a ratio of UVA protective factor to SPF of at least 1:3. In a 2017 study, 20 broad-spectrum sunscreen products from the USA (SPF 15–100) were evaluated under the European standards. Authors found that 19 of 20 products truly met the US critical wavelength guidelines and only 11 of 20 products met the European requirements. Interestingly, the higher the SPF, the less likely the product was to meet European standards. The

study suggests increasing the required critical wavelength value for higher SPF products in order to provide adequate and more reliable UVA protection in US sunscreens [56].

Substantivity

Substantivity is a sunscreen's ability to remain effective under adverse conditions such as exposure to water or sweat. Testing is in vivo and assesses the product's SPF before and after water immersion for a specific period of time. In the USA, a water-resistant product maintains the same indicated SPF value after 40 min of water immersion. Whereas a very-water-resistant product (formerly called "waterproof") maintains the same indicated SPF after 80 min of water immersion [35, 38–41].

Stability

The photostability of a sunscreen is important for long-lasting protection with continuous exposure to UV light, particularly to prevent photodegradation and ultimately decreased efficacy. The FDA has established maximum concentration levels of each UV filter allowed in sunscreen products. Some UV filters are known to be photolabile and thus are often combined with other more photostable filters to prevent photodegradation. For example, octocrylene and salicylate compounds are frequently used in combination with the photosensitive compound avobenzone to prevent its photodegradation. Another example is the FDA recommendation to avoid combinations of avobenzone with PABA or padimate O due to known photodegradation by the PABA derivatives [5, 46].

New FDA Labeling Requirements

In June 2011, the FDA released a new set of testing and labeling requirements for sunscreens and proposed further modifications to the rules for manufacturing over the counter sunscreen products. The FDA Final Rule in 2011 specified testing and labeling methods for establishing an SPF, designating broad-spectrum protection, and claiming water resistance. As of May 2012, manufacturers with more than $25,000 in sales were required to comply with these new rules by December 17, 2012 while manufacturers with less than $25,000 in sales had until December 17, 2013 [5, 57]. Figure 18.3 provides an example of a sunscreen label that complies with these new guidelines.

The FDA's SPF testing and labeling requirements are overall unchanged with the exception of a new maximum permissible labeled SPF value. Some available sunscreen products have labels with SPF values exceeding 100. Due to claims of insufficient evidence for such products, the FDA determined labels may only claim a maximum SPF value of "50+" [5, 58].

As mentioned previously, the FDA has instituted new regulations regarding UVA protection. Sunscreens that pass the new UVA laboratory evaluation with a critical wavelength above 370 nm can be labeled as "broad spectrum" although there is no indication of the degree of UVA protection [5, 55]. It is important to note that a sunscreen can be broad spectrum but still have a low or no SPF rating and vice versa.

Specific terminology is now being added in the "drug facts" portion of the sunscreen product's label to state if the product decreases the risk of skin cancer and photoaging or is only shown to prevent sunburn. This correlates to UVA/UVB coverage or only UVB coverage, respectively. All products state: "spending time in the sun increases your risk of skin cancer and early skin aging." If a sunscreen is both "broad spectrum" and has an SPF $\geq$ 15, then it can now include the following statement: "if used as directed with other sun protection measures, decreases the risk of skin cancer and early skin aging caused by the sun." For products that are not designated as broad spectrum or that have an SPF < 15, the following statement is included: "this product has been shown only to prevent sunburn, not skin cancer or early skin aging." [5, 51, 55]

Additionally, much of the previously accepted terminology has been banned. Terms like "sunblock," "waterproof," and "sweatproof" are banned as these claims are misleading. Instead, the label on the package can only state either "water resistant (40 minutes)" or "very water

Sunscreen Labeling According to 2011 Final Rule

If used as directed with other sun protection measures, this product reduces the risk of skin cancer and early skin aging, as well as helps prevent sunburn.

Only products labeled with both "Broad Spectrum" AND SPF15 or higher have been shown to provide all these benefits.

FDA

BRAND **X** Sunscreen

BROAD SPECTRUM SPF 15

WATER RESISTANT (40 MINUTES)

6.0 FL OZ (180 mL)

Drug Facts

Active Ingredients   **Purpose**
Avobenzone 3%
Homosalate 10%   Sunscreen
Octyl methoxycinnamate 7.5%

Uses
• helps prevent sunburn
• if used as directed with other sun protection measures (see **Directions**), decreases the risk of skin cancer and early skin aging caused by the sun

Warnings
For external use only
Do not use on damaged or broken skin
When using this product keep out of eyes. Rinse with water to remove.
Stop use and ask a doctor if rash occurs
Keep out of reach of children. If product is swallowed, get medical help or contact a Poison Control Center right away.

Directions
• apply liberally 15 minutes before sun exposure
• reapply:
   • after 40 minutes of swimming or sweating
   • immediately after towel drying
   • at least every 2 hours
• **Sun Protection Measures.** Spending time in the sun increases your risk of skin cancer and early skin aging. To decrease this risk, regularly use a sunscreen with a broad spectrum SPF of 15 or higher and other sun protection measures including:
   • limit time in the sun, especially from 10 a.m. – 2 p.m.
   • wear long-sleeve shirts, pants, hats, and sunglasses
• children under 6 months: Ask a doctor

Inactive ingredients
aloe extract, barium sulfate, benzyl alcohol, carbomer, dimethicone, disodium EDTA, jojoba oil, methylparaben, octadecene/MA copolymer, polyglyceryl-3 distearate, phenethyl alcohol, propylparaben, sorbitan isostearate, sorbitol, stearic acid, tocopherol (vitamin E), triethanolamine, water

Other information
• protect this product from excessive heat and direct sun

Questions or comments?
Call toll free 1-800-XXX-XXXX

Fig. 18.3 Sample sunscreen label satisfying the new FDA labeling requirements. (∗*This image has been provided by the United States FDA and is free of all copyright restrictions and available for use and redistribution without permission*)

resistant (80 minutes)." Sunscreens may no longer claim to provide "instant protection" nor can they claim to maintain efficacy for more than 2 h without reapplication. Statements like "all day protection" or wording that indicates "extended wear" that goes beyond 2 h have also been prohibited as they contradict the application instructions and cannot be substantiated currently [5, 51, 55].

Specific approved formulations for sunscreen products are listed which includes oils, lotions, creams, gels, butters, pastes, ointments, sticks, and sprays. Cosmetic products that claim to have sunscreen filters must also comply with these same rules. Wipes, towelettes, powders, body washes, and shampoos are not acceptable as sunscreen products. Combination products

with sunscreen and insect repellent are still being discussed and at the time of 2011 Final Rule still requires both FDA and EPA regulation compliance [5].

In the USA, spray formulations of sunscreens are extremely popular because of their quick and easy application. A cross-sectional study reviewed sunscreen sales in the USA from 2011 to 2016 and found a steady increase in rate of spray sunscreen purchase with up to 38% of all sunscreen sales being spray formulations [59]. For this reason, the FDA has approved spray formulations and has added specific labeling requirements to ensure adequate and safe application by the consumer. See Fig. 18.4 for an example of a spray sunscreen label with specific "warnings" and "directions."

Fig. 18.4 Sample spray sunscreen label satisfying the new FDA labeling requirements [5, 49]

Upcoming Proposed FDA Labeling Requirements

Despite the updates in labeling and testing, the FDA still has not approved a new active sunscreen ingredient since 1999. As of 2002, applications for eight new UV filters which are used around the world have been filed but are still awaiting decisions by the FDA. In an attempt to speed up this process, the Sunscreen Innovation Act (SIA) was enacted in November 2014. The SIA law amended the Federal Food, Drug, and Cosmetic Act with the purpose of establishing an expedited process for the review of safety and effectiveness data of ingredients in non-prescription sunscreens. A time frame for review was set; the deadline for a final sunscreen monograph is to be provided by the FDA by November 26, 2019 [48, 60].

As of early 2019, the FDA has begun its proposal for the final monograph in compliance with the requirements set by the SIA. The monograph establishes conditions in which sunscreens can be marketed without approved new drug applications because they are generally recognized as safe and effective (GRASE). The GRASE status of each active ingredient will be evaluated based on clinical and toxicology data including sensitization testing, phototoxicity and photoallergenicity testing, bioavailability studies, dermal and systemic carcinogenicity, reproductive toxicity, and long-term effects [48].

If sufficient data is available to support an ingredient as GRASE then it is considered Category I, currently only zinc oxide and titanium dioxide. If data exists showing the risks of an ingredient outweigh the benefits then it is considered Category II and not GRASE, currently PABA and trolamine salicylate. Data on PABA suggests a significant number of photoallergic skin reactions, cross-sensitization to similarly structured compounds, and risk of systemic absorption. Trolamine salicylate is being

considered Category II due to its known transdermal absorption which could result in systemic anticoagulation, among other salicylate acid side effects, if applied as instructed on the package. For the remaining 12 FDA-approved active ingredients where insufficient data is available, then it receives Category III designation [48]. A few of these Category III ingredients including oxybenzone, octocrylene, and octinoxate are under recent scrutiny for their potential environmental effects, i.e., their discovery in many water sources and aquatic animals globally. Oxybenzone and octinoxate have also been implicated in coral reef bleaching prompting Hawaiian legislature to pass a bill in July 2018 banning its sale in the state. Florida is considering the same action [47]. Additional data is needed before the FDA can make a final decision on the safety of these filters.

Several other updates included in the 2019 proposal are worthy of mention though are not yet finalized. Again, the maximum permissible SPF value is expected to increase to an allowable SPF 60+. Sunscreens that are shown in testing to have a range of SPF will be required to market at the lower end of the range. For example, products with SPF 50–59 during testing will be labeled as SPF 50+. Importantly, the proposal would require all sunscreens with an SPF $\geq$ 15 to also provide "broad-spectrum" protection. More stringent regulations on UVA testing are suggested so that as a product's SPF increases, the degree of UVA protection also reliably increases. To do this, the FDA will determine "broad-spectrum" protection by both achieving a critical wavelength above 370 nm and having an index of UV to UVA1 coverage $\geq$0.7 [48]. This is similar to the European requirements for UVA testing as outlined by the ISO.

Lastly, certain sunscreen formulations are being re-reviewed to determine if they are GRASE or not. Powder formulations are being considered as possibly GRASE, but additional data is required to determine a final ruling. Products with sunscreen and insect repellents will be determined not GRASE by the FDA [48].

Evidence for the Efficacy of Sunscreens

Protection from Immunosuppression

Several studies have provided evidence that indicate sunscreen use along with sun protective behaviors can prevent varying degrees of UVR's effects on the immune system [61, 62]. The level of protection is dependent on the SPF as well as UVA coverage [62]. This is not surprising since both UVA and UVB radiation have been shown to be immunosuppressive [29].

In 2003, Baron et al. published a randomized controlled trial (RCT) of 211 volunteers evaluating the protective effects of UVB sunscreens (SPF 15) compared to UVA/UVB sunscreens (SPF 15) using CHS as a model for immunosuppression. Measuring skinfold thickness versus total UV dose, the authors reported that the UVA/UVB sunscreen subjects had a greater than average skinfold thickness versus total UV dose than the UVB sunscreen subjects. The smaller the skinfold thickness, the greater the degree of immunosuppression. Though both types of sunscreen can protect against immunosuppression, the study showed that the addition of a UVA filter increased the level of protection [61].

Similar results were seen in a study published in 2007 using delayed type hypersensitivity (DTH) reactions to seven common antigens as the marker for immunosuppression. Approximately 104 healthy volunteers were divided into multiple groups; sunscreen products with the same SPF (either 9 or 25) but differing UVA protective factors were compared after exposure to indoor solar-simulated radiation with varying degrees of UVA and outdoor sunlight. All UV protocols lead to local and distant site immune suppression as marked by significantly altered DTH reactions. Sunscreens with predominately UVB coverage failed to provide adequate protection against the immunosuppressive effects of UVR at the lower and higher SPF values. Whereas, products that offered broad-spectrum coverage did yield smaller reductions in DTH

reactions after UV exposure indicating no immune system alteration [62]. The results support UVA as a major contributing factor in cutaneous immunosuppression, and the use of UVA filters can mitigate some of these effects.

Photoaging

Signs of photoaging clinically appear as wrinkles, dyspigmentation, and textural changes. Few clinical studies have examined the effects of sunscreen use on photoaging. Overall the results are promising, hence, the increasing number of cosmetic products that contain sunscreens. It is important to remember that UVA, UVB, visible light, and infrared radiation can all contribute to photoaging [43]. Wearing a broad-spectrum sunscreen daily is important to prevent photoaging as well as reverse the active signs of chronic sun damage.

In 1995, a randomized, double-blind, placebo-controlled trial involving 53 adults previously diagnosed with actinic keratosis and/or skin cancer showed that those who applied a UVA/UVB sunscreen over a 24-month period had less solar elastosis on biopsy compared with controls [63]. A similar but smaller-scale French study published in 2008 also showed a decrease in histologic findings of photoaging with use of broad-spectrum sunscreen for only 6-week duration with ongoing UV exposure. Markers of UV damage assessed on skin biopsy included epidermal thickness, decreased procollagen expression, and higher lysozyme to elastin ratios. The control group exhibited structural and molecular evidence of UV damage whereas use of a broad-spectrum sunscreen either minimized or abrogated these findings [64]. On a molecular level, broad-spectrum sunscreens have also shown promise in negating UVR's damage even at suberythemal doses. In 2007, a study showed daily suberythemal doses of UVR caused more thymine dimers, higher p53 expression, and loss of Langerhans cells in unprotected individuals [65].

Besides prevention, a broad-spectrum sunscreen is a valuable tool for those with preexisting photoaging from chronic UV exposure. A prospective, case-controlled study published in 2016 looked at 32 patients over a 52-week period while applying a broad-spectrum SPF 30 photostable product to the entire face once daily. Dermatologist's clinical evaluation and the patient's self-assessment indicated statistically significant improvements in skin texture, skin clarity, and dyspigmentation [66].

Squamous Cell Carcinomas

Several large trials offer evidence that sunscreen is effective in preventing SCC. Though, an extensive cochrane review evaluating the effects of sun protection strategies on the prevention of BCC and SCC found only one RCT study that was felt to be suitable for inclusion [67].

Published by Green et al. in 2011, the Nambour Skin Cancer Prevention Trial out of Australia was a large RCT of approximately 1600 people with a 2×2 factorial design. People were divided into four possible intervention groups: [1] regular use of broad-spectrum SPF 16 sunscreen with beta-carotene 30 mg daily supplementation, [2] regular broad-spectrum sunscreen use with placebo pill, [3] discretionary broad-spectrum sunscreen use and beta-carotene supplementation, and [4] discretionary broad-spectrum sunscreen use with placebo pill. Initial follow-up period was 4.5 years at which time 1380 people remained in the study with complete skin exams performed. A near 40% reduction in the development of SCC was identified in the regular sunscreen group compared to the discretionary group. No beneficial or harmful effects of beta-carotene were found [15]. Follow-up data after an additional 8 years of monitoring showed that regular sunscreen users continued to have a 40% lower incidence rate of SCC than controls [16]. This suggests a prolonged preventative effect of sunscreen on SCC development.

Another patient population at extremely high risk for SCC is organ transplant recipients due to their high degree of immunosuppression. In 2009, a prospective, single-center, case-controlled study looked at the preventative effects of regular sunscreen use on skin cancer development in organ transplant patients (heart, kidney, liver). Approximately 60 patients were provided a broad-spectrum SPF 50 sunscreen to apply daily to sun-exposed areas and compared to 60 patients who received only verbal and written sun protection instructions. Importantly, the groups were well-matched for degree of immunosuppression, history of skin cancer, etc. Over the course of 24 months, the sunscreen application group had significantly less new SCCs develop compared to the controls (0 versus 8, P < 0.01). Actinic keratoses were also counted at the start and completion of study. Participants initially started with similar numbers of actinic keratoses, though the group who applied sunscreen had an overall 53% reduction at study completion compared to controls who had an overall 43% increase [68].

Basal Cell Carcinomas

Although sunscreens appear to be effective in preventing actinic keratosis and SCCs, the evidence for BCCs has been inconclusive. Again, the 2016 cochrane review previously mentioned in the SCC section, found only one RCT study suitable for inclusion [67]. The Nambour Skin Cancer Prevention Trial out of Australia found no significant differences in the incidence of BCC development in the 4.5 year study period as well as the 8 year follow-up period [15, 16]. Arguably, several limitations exist in the Nambour Skin Cancer Prevention Trial [69]. Though, it remains unclear how the data would be skewed for BCCs only and not for SCCs.

The other study mentioned in the SCC section looking at organ transplant patients also found no statistically significant difference in BCC development among daily sunscreen users compared to controls [68]. Unfortunately, the authors found no other noteworthy published RCTs available at this time evaluating sunscreen use on BCCs.

Melanomas

There has long been inconsistent and contradictory evidence on melanoma prevention with sunscreens. Regardless, UVR is a known modifiable risk factor, and thus sunscreen use is strongly recommended by dermatologists.

Previously, a large meta-analysis of 18 case-controlled studies failed to show a protective association of sunscreen use with melanoma [70]. Possible confounders in some of these earlier studies include older sunscreen formulations without UVA protection, low SPF, and limited substantivity. Another consideration is "sunscreen abuse," in which the user exposes themselves to higher doses of UVR because of the perceived decreased risk of sunburn with sunscreen application. This is especially true when sun exposure was intentional to acquire a tan [71]. Individuals who burn easily or have a family history of melanoma tend to use more sunscreen which presents another confounder in case-controlled studies.

It is known that the number of nevi, atypical nevi, freckling, history of sunburns, and history of indoor tanning correlate with the development of melanoma [72]. Some studies have used the number of nevi as predictors of melanoma. A RCT performed in Canada in 2000 evaluated the number of nevi in children after using a broad-spectrum SPF 30 sunscreen regularly over a 3-year period. Of the 300 children included, only a slight decrease in the number of new nevi was noted in those who used sunscreen regularly (24 versus 28, p = 0.048) [73]. Another study looked at 630 white school-age children in Europe who wore sunscreen regularly compared to those who wore protective clothing. The authors found those in the sunscreen group had an increased number of nevi compared with the use of clothing which prevented new nevi. Likely, the regular use of sunscreen encouraged longer outdoor exposures in the population studied [74].

In 2011, Green et al. used the same cohort from the Nambour Skin Cancer Prevention Trial to instead evaluate the incidence of primary

melanoma. During the 4.5-year trial period and 10-year follow-up, fewer participants in the regular sunscreen intervention group developed primary melanoma compared with the discretionary sunscreen group (11 vs 21). The authors concluded that regular applications of a broad-spectrum SPF 16 sunscreen in white adults ages 25–75 can decrease the incidence of melanoma [17]. The study had serious limitations and critiques of the study include: [1] marginally statistically significant results (p = 0.051); [2] intervention sites were chosen for NMSC and so excluded the trunk and lower extremities where melanomas often occur; and [3] the entire body was analyzed for melanomas, not just the intervention sites [75]. Thus, the results offer some evidence to support sunscreen use in preventing melanoma but are by no means conclusive. It is possible that a similar trial, if started during childhood, would yield more statistically significant results.

More recent literature published in 2017 assessed the potential impact fraction (PIF) of sunscreen use on melanoma incidence. PIF is a tool used to determine the proportional difference between the known number of melanoma cases with current sunscreen use by the public and the expected number of melanoma cases with future higher compliance of sunscreen use. Melanoma incidences and current sunscreen use data were extracted from national surveys in two different populations, Australia and the white US population, from 2011 as a baseline. Several sunscreen implementation models were analyzed to determine the predicted number of melanomas through the year 2031. In a realistic intervention scenario where the prevalence of sunscreen use increased by 5% per year, it was estimated that 231,000 fewer melanomas would arise in the USA and 28,000 fewer in Australia. In a maximum intervention scenario where 100% of the population wore sunscreen, though unrealistic, would lead to 797,000 fewer melanomas in the USA and 96,000 fewer in Australia [76].

Current Recommendations from American Academy of Dermatology (AAD) and American Academy of Pediatrics (AAP)

The AAD and AAP are well-respected national organizations that serve as a valuable resource for dermatologists and the public. Their websites offer straightforward, easily accessible sets of recommendations on sunscreen and sun safety for adults and children [77, 78]. Table 18.2 summarizes these recommendations.

Table 18.2 Effective sun protection habits based on AAD and AAP recommendations [77, 78]

Adults and children above the age of 6 months
Seek shade or avoid sun exposure between 10AM and 4PM when the sun is the most intense
Wear sun protective clothing such as long-sleeve shirts, pants, sunglasses with UV protection, and wide-brimmed hats
Take caution around water, sand, and snow as they reflect UVR
UVR will pass through glass, fiberglass, and plexiglass unless specially treated with UV blockers
Apply a broad-spectrum water-resistant SFP 30 sunscreen daily year-round
Apply the sunscreen 15–20 min prior to outdoor exposure and reapply every 2 h while outdoors or earlier if sweating or in a wet environment
Apply enough sunscreen to cover any exposed skin, typically a shot glass full covers the full body of an adult
Do not abuse sunscreen by purposefully increasing sun-exposure due to sunscreen use.
Avoid tanning beds and recreational tanning
Children below the age of 6 months
Avoid direct sun exposure if at all possible
If sun-exposure is unavoidable, cover the child with clothing and apply broad spectrum SPF 15 sunscreen to exposed skin 15–20 min prior to sun exposure
Use a smaller amount of sunscreen than on an older child or adult with the same skin surface area
Wash or wipe off sunscreen if child will no longer be sun-exposed

Despite the many protective effects of sunscreen, the AAD strongly recommends sunscreen be used as an adjuvant to other sun protective strategies including sun avoidance and sun protective clothing. Seek shade or avoid outdoor exposure between 10:00 a.m. and 4:00 p.m., when the sun's rays are strongest, especially during the summer months. Be cautious around water, sand, and snow which reflect significant amounts of UVR. Wear protective clothing when outdoors such as long-sleeve shirts, pants, sunglasses, and wide-brimmed hats [77]. A cross-sectional study of 28,500 individuals showed that those who use only sunscreen have the highest likelihood of sunburn (62%) compared to those who practice regular sunscreen use with sun protective clothing, hats, and shade-seeking behaviors (26% in sun sensitive people, 6% in non-sun sensitive people) [79].

Sunscreen remains an important sun safety tool and should be worn year-round. Cloud cover and windows block much of the UVB radiation, but not UVA unless specially treated. Thus, sunscreen should be applied daily regardless of season or weather. Individuals above 6 months of age should look for a water-resistant, broad-spectrum sunscreen with an SPF of at least 30. Lip balm with an SPF 30 or greater is also encouraged. Avoid products sold in combination with an insect repellant [77].

The proportion of sunscreens on the market that meet the above AAD standards has increased significantly since 2011, when the new FDA labeling requirements came out. A study in 2017 assessed the number of compliant products sold at the two largest US pharmacy retailers (Walmart and Walgreens) and found that approximately 65% of the 470 sunscreen-containing products met all three standards including broad spectrum, SPF > 30, and water resistance [80].

To achieve the appropriate SPF as tested (2 mg/cm^2 application density), the AAD recommends using 1 oz. of sunscreen or enough to fill a shot glass to fully cover an adult body. Sunscreen should be applied to exposed dry skin at least 15 min before sun exposure, paying particular attention to common areas of NMSC such as the face, ears, hands, arms, and lips.

Importantly, sunscreen should be reapplied every 2 h or after swimming or heavy perspiration [77]. New FDA labeling rules indicate whether a sunscreen is effective for 40 or 80 min under wet conditions [5]. Sunscreen should not be abused for the purpose of increasing the duration of sun exposure.

The AAD, FDA, and the Surgeon General's Call to Action all explicitly recommend avoiding indoor tanning beds or recreational tanning due to the high risk of skin cancer development including melanoma and NMSC [19, 77]. In an attempt to reduce the high volume of tanning bed users, the FDA has proposed banning tanning bed access to minors under age 18 without parental consent and requiring a signed risk acknowledgment certification for adults [81]. Currently the decision is on a state-by-state basis, though many states are implementing these policies. Using the National Youth Risk Behavior Surveys, a study in 2017 found that high school students who admitted to indoor tanning also had an increased association of sunburn within the preceding year (82% versus 53%, p < 0.001) suggesting overall risky sun behaviors in those who use tanning beds. Fortunately the study also showed the prevalence of indoor tanning among high school students, a common age for tanning bed abuse, has decreased from 2011 to 2015 at a rate of 15.6% to 7.3% [82].

Within the available online AAD resources is the suggestion to use sunless self-tanning products if a tan appearance is desired but with continued use of sunscreen [83]. However, sunless tanning may inappropriately reinforce the belief system that tanned skin is more desirable. Dodds et al. performed a cross-sectional study in 2018 evaluating US adults over the age 18 who use sunless tanning products. Of those individuals, higher associations with other risky skin cancer-related behaviors were found including indoor tanning and recent sunburn but also higher rates of sunscreen use and having a full body skin exam [84]. Given this information, the solution to decrease risky tanning practices clearly requires multifactorial approaches and is a continued objective of the AAD.

Infants and toddlers are also at high risk of UV damage and skin cancer. Structurally, children's skin is thinner than that of adults and has lower melanin concentrations which allows UVR to penetrate more deeply. Animal studies suggest that the skin of children, especially infants, is immunologically immature and less able to respond to UV damage than adult skin [85]. Therefore, extra care must be taken to protect children from UV exposure.

The AAP recommends that infants under 6 months of age be kept out of direct sunlight whenever possible. If exposure cannot be avoided, a broad-spectrum, water-resistant sunscreen with an SPF > 15 should be applied to skin that is not protected by clothing or shade (e.g., hands, face) 15–30 min before sun exposure. Apply a small test spot to the infant's back to ensure no allergic reaction develops. The 2 mg/cm^2 application density does not apply to infants, rather the AAP recommends using a smaller amount than one would for an older child or adult. Be sure to wash or wipe the sunscreen off when the infant will no longer be exposed [78].

Children, adolescents, schools, and summer camps remain a target area to educate and advocate for increased compliance with sun protective behaviors as sunburns during childhood lead to significantly increased risks of skin cancer. Many schools require over-the-counter drugs to have administration regulations, meaning students must store their sunscreen in a locked cabinet typically in the nurse's station and have physician authorization to use while at school. This creates significant barriers and decreases accessibility. The AAD published a position statement in 2016 arguing that sunscreen products are considered safe and should be available to students without physician authorization. Additionally, students should be given adequate time and encouraged to apply sunscreen and protective clothing prior to outdoor exposures [86]. As of 2017, a total of 11 states have enacted legislation that allows students to possess and apply sunscreen at school without physician approval [87].

Sunscreen and Vitamin D

The skin plays an important role in the endogenous production of vitamin D. Within the skin are large quantities of 7-dehydrocholesterol, a sterol that absorbs solar radiation in the UVB range and photoconverts to previtamin D3. Thereafter, previtamin D3 is spontaneously converted to vitamin D3 through thermally induced isomerization. Vitamin D3 enters the circulation and undergoes metabolism in the liver to 25-hydroxyvitamin D3 and then in the kidney to its active form 1,25-dihydroxyvitamin D3 [88].

Deficiency of vitamin D is common and has been implicated in many processes from heart disease to bone health to cancer. There has been recent controversy regarding the use of sunscreens and vitamin D production with concern that vitamin D deficiency could be exacerbated by sunscreen use [89]. There is truth to this idea. A study in 1987 showed that in vitro application of 5% PABA to skin samples prevented photoconversion of 7-dehydrocholesterol to previtamin D3. In the same study, in vivo testing of a single application of 5% PABA sunscreen with SPF 8 on 8 healthy subjects resulted in a decreased serum vitamin D3 concentration [88]. Another in vivo study by the same authors in 1988 evaluated the vitamin D stores and circulating 25-hydroxyvitamin D3 levels in 20 long-term sunscreen users in the USA. The mean serum 25-hydroxyvitamin D3 levels were on average 51 nmol/L lower among long-term PABA users than in normal sun-exposed controls. Two of the PABA users were deficient, defined as <20 nmol/L, compared to one in the normal controls [90].

Nevertheless, the research is not completely cut and dry. A RCT conducted in Australia in 1995 observed no statistically significant difference in mean serum 25-hydroxyvitamin D3 or 1,25-dihydroxyvitamin D3 levels between placebo controls and sunscreen users with broad-spectrum SPF 17 during the summer season [91]. In 2017, a study analyzed the cutaneous vitamin D production and serum 25-hydroxyvitamin D3 levels after a single

narrowband UVB exposure with varying amounts of body surface exposed (9%, 23%, 50%, 96%) on individuals who either applied broad-spectrum SPF 50 sunscreen or no sunscreen. The results showed that sunscreen use significantly decreased the cutaneous vitamin D production regardless of the degree of body surface area exposed to UVB (by 83%, 88%, 75%, and 92%, respectively). However, the circulating levels of vitamin D were only marginally affected (by 13%, 10%, 7%, and 10%, respectively). Likely, other endogenous precursors are utilized in the absence of cutaneous previtamin D3 production [92].

Several factors influence endogenous production of Vitamin D by the skin aside from duration of UVB exposure. The amount of air pollution, distance from the sun (season and location on Earth), time of day, cloud cover, and the concentration of melanin in the skin are all believed to play a role [93]. Additionally, the amount of vitamin D3 production varies depending on the UV spectra of the sunlight. Maclaughlin et al. investigated these spectral differences in 1982. In their study, exposure of skin with specific 295 nm radiation lead to 65% conversion of 7-dehydrocholesterol to previtamin D3 compared to simulated solar UVR which lead to only 20% conversion [94].

Recent assertions have been made that claim 15 min of daily sun exposure without sunscreen is enough for physiological amounts of vitamin D production with negligible UV damage [95]. However, with so many variables involved, it is nearly impossible to determine whether this is enough sunlight or enough vitamin D to be considered appropriate [93]. Risks associated with UVR occur not only after bad sunburns but also with cumulative intermittent exposure. With the rising incidence of melanoma and NMSC, unprotected UVR exposure remains ill-advised. Fortunately, oral vitamin D is available through supplementation and many natural dietary sources such as fish, beef liver, eggs, some cheeses, fortified foods such as milk, breakfast cereal, and others. Oral intake of vitamin D serves an easy avenue for achieving adequate vitamin D levels without undue exposure to a known carcinogen [96, 97].

Polyphenols

Polyphenols have received a lot of recent press for having anti-inflammatory, anti-tumor, and antioxidant properties [3]. They are found in high concentrations in several spices, herbs, and colorful fruits and vegetables such as grapes, pomegranates, tomatoes, etc. [3]. Molecularly, polyphenols are all characterized by multiple phenol units. Their aromaticity allows them to stabilize ROS and charged metal ions. Unlike traditional sunscreens, polyphenols do not block or absorb UVR. Instead, they act to mitigate the damaging effects caused by UVR such as ROS scavenging, upregulation of DNA repair enzymes, and inhibition of stress response cell signaling pathways [3]. With overall poor compliance with routine photoprotective measures and the more recent discovery of visible light contributing to photoaging, polyphenols may serve as a useful adjunct to sunscreen. Although the research is promising for these compounds, their approval for use as a sunscreen additive is likely years in the future.

Many of the polyphenols have similar mechanisms of action and exert their effects through a number of avenues. Plant polyphenols may reduce UV-induced DNA damage by increasing the repair of CPDs or the removal of CPD-positive cells. They have also been shown to inhibit the activation of the MAPK signaling pathway, an important part of the body's UV-induced stress response [98]. A decrease in ROS production has been seen with polyphenol use via inhibition of UV-induced lipid peroxidation, pro-inflammatory factor infiltration, and nitric oxide production [99, 100]. Important to the reduction of signs of photoaging, polyphenols can decrease the expression of matrix metalloproteinases 1, 3, and 9 induced by UVR and increase the synthesis of collagen type I [100, 101].

Green tea, white tea, and black tea are commonly consumed beverages around the world and are an important, inexpensive source of polyphenols. Derived mainly from the leaves of the *Camellia sinensis* plant, green tea polyphenols (GTP) are monomeric flavonoids

called catechins [98]. Katiyar et al. showed that in vivo topical treatment of human skin with a GTP subtype before exposure to a single 4x MED dose of UVR inhibited UV-induced hydrogen peroxide and nitric oxide production, epidermal lipid peroxidation, and CD11b + inflammatory leukocyte infiltration, a source of ROS products. The study also found an increase in catalase activity and total glutathione levels and a decrease in glutathione peroxidase activity, all of which are cellular antioxidant compounds or enzymes [102]. Mantena et al. fed hairless mice water containing GTP and exposed them to a photocarcinogenesis protocol for 24 weeks resulting in lower tumor incidence (35%), multiplicity (63%), and size (55%) compared with controls [103]. Experiments done with a topical GTP subtype yielded similar results in these same three measured parameters [104].

Polypodium leucotomos is a tropical fern of the Polypodiaceae family grown in South America that is rich in phenolic compounds including caffeic, ferulic, chlorogenic, cinnamic, and vanillic acids. The antioxidant, anti-inflammatory, immunoregulatory, and photoprotective properties of *Polypodium leucotomos extract* (PLE) have led to its use as a topical and oral supplement in many parts of the world for the prevention and treatment of many dermatologic conditions. In the USA, it has been available as a dietary supplement since 2006 [100].

Several studies have demonstrated PLE's safety and effectiveness in photoprotection. Kohli et al. published a trial in 2017 evaluating the skin's response to visible light, UVA1, and UVB exposure with and without PLE taken 240 mg orally 2 h and 1 h prior to exposure. Subjects were assessed 24 h after irradiation by photography, scoring of erythema and pigmentation, MED, colorimetry, and skin biopsies. At baseline all participants developed an erythemal response to UVB but limited response to UVA1 or visible light, not uncommon in fair skinned patients. All subjects had less UV damage biomarkers on histology including CPDs, proliferating cell nuclear antigens, Ki67 positivity, cyclin D1 positivity, and inflammation measured by COX-2. The MED increased

and/or clinically detected erythemal responses decreased in 17 of 22 subjects simulating a UVB tolerance close to that of a darker skinned phenotype [99]. In 2015, Nestor et al. published a double-blind RCT out of Florida assessing 40 healthy subjects (Fitzpatrick skin types I–IV) over a 60-day period taking PLE 240 mg twice daily or placebo [105]. The PLE group overall had less sunburn episodes, a greater likelihood of an increased MED and decreased UV-induced erythemal intensity. Importantly no significant safety events were identified on physical examination or in laboratory testing with blood counts, comprehensive metabolic panels, and prothrombin time or partial thromboplastin time [104]. The efficacy of PLE against visible light and infrared irradiation has also been demonstrated in a prospective clinical trial in 2016 wherein subjects took PLE 960 mg/day for 21 days [100].

Photolyases

Photolyases are a group of natural enzymes found in most plants and animals that act by repairing UV-induced DNA damage. Mechanistically, photolyases have specific cofactors with chromophore properties which transfer energy to CPDs in the UV-damaged DNA effectively breaking them back down to monomers in a process called photoreactivation. This allows for DNA repair as well as indirect improvements in immune surveillance [4]. Humans do not have endogenous photolyases and instead rely on nucleotide excision repair mechanisms. Hence, their predisposition toward photoaging and photocarcinogenesis compared to other plant and animal species. Again, though the data is promising, there are no sunscreens in the USA that contain photolyases which are becoming available in European countries.

Few recent trials have demonstrated the utility of sunscreens with liposome encapsulated photolyases in the prevention and treatment of photodamage. A small study out of Spain evaluated 20 subjects clinically, histologically, dermatoscopically, and with confocal microscopy after 2 months of topical application of a

photolyase sunscreen twice daily. The authors found decreased clinical signs of photoaging, less atypia on confocal microscopy, and decreased histologic signs of UV damage including decreased CPDs, Ki67 expression, p21 expression, and proliferating cell nuclear antigens in all subjects [4]. Another study of 30 patients looked at the rate of actinic keratosis recurrence after one photodynamic therapy (PDT) session who were treated post-procedurally with a daily photolyase sunscreen versus a broad-spectrum sunscreen. A longer remission rate was seen in the photolyase group compared to the non-photolyase group wherein a majority required repeat PDT within the 9-month trial period.[4v]An important high-risk population with xeroderma pigmentosum was also studied. Over 12 months of treatment with photolyase sunscreen or standard sunscreen, eight subjects were monitored for the development of actinic keratoses and NMSC. In those using a photolyase sunscreen, there was a 65% reduction in new actinic keratoses (14 versus 5), 56% reduction in BCCs (6.8 versus 3), and 100% reduction of SCCs (3 versus 0) [4]. This data suggests a wide array of practical uses for photolyases in dermatology.

Conclusion

It is apparent that UVR exposure is a part of life. Knowing the many risks of UVR, it is prudent to minimize exposure whenever possible. If avoidance with shade and clothing is not feasible, sunscreens offer the best line of defense currently. In the USA, the FDA continues to update testing and labeling regulations of sunscreen with ongoing efforts and recommendations by the AAD and AAP. The current generation of sunscreens offer protection against sunburns, photoaging, NMSC (especially SCCs), and melanoma. Though, there continues to be a wider selection of sunscreen products outside the USA which in some cases have even greater efficacy. Arguments of sunscreens exacerbating the rates of vitamin D deficiency are unfounded, especially when oral supplementation is easily accessible and affordable. Trade secrets regarding new sunscreens and delivery methods in the pipelines of private companies are nearly impossible to acquire. However, future immunoprotective compounds in the form of polyphenols, antioxidants, and photolyases offer an interesting avenue of mitigating UVR-induced damage and immunosuppression.

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A Handful of Sunscreen for Whole-Body Application

19

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Abstract

Background The rule of thumb "Fill up a handful of sunscreen and spread it all over your body" has been used in several sun safety campaigns. The intention was to increase the applied sunscreen to obtain a quantity of 2 mg/cm^2 to all accessible skin. The present study is the first to investigate how this advice works in practice, evaluated by quantity of sunscreen applied and amount of covered skin.

Methods Seventeen volunteers wearing swimwear were asked to "Fill up a handful and spread it all over your body." Before and after sunscreen application, the volunteers were photographed in black light. As sunscreen absorbs black light, the darkness of the skin increases with increasing amounts of applied sunscreen, making it possible to identify skin left without coverage. The sunscreen container was weighed before and after to quantify the amount of sunscreen applied.

Results A median of 21% of the accessible skin was left completely without coverage. The 79% covered area was covered with a median of 1.12 mg/cm^2, not the expected 2 mg/cm^2.

Conclusion In practice, the advice "Fill up a handful of sunscreen and spread it all over your body" led to a better but still modest protection, compared to the intended effect.

Introduction

Adults wearing swimwear consistently use less than 1 mg sunscreen per cm^2 skin and often get sunburned in spite of sunscreen use [1–4]. Years ago, to increase the amount of applied sunscreen, the Centers for Disease Control and Prevention [5] introduced the rule of thumb: "Fill up a handful and spread it all over your body." The Agency continued: "Yes, we said `handful´. You need that much for good coverage" [5]. The goal of the recommendation is to increase the amount of applied sunscreen to 2 mg per cm^2 on sun-exposed skin, which will provide an actual sun protection factor (SPF) as labelled on the bottle [6]. This advice has subsequently been repeated in local campaigns worldwide and is believed to be easy to understand and follow [7]. To our knowledge, it has never been investigated how the recommendation works in practice.

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This study aimed to investigate the degree of coverage obtained by following the recommendation. Coverage was assessed as quantities of sunscreen applied and areas of skin left without sunscreen.

Materials and Methods

Healthy Caucasians, at least 18 years of age, were included. Exclusion criteria were skin disease, allergy to sunscreen content, pregnancy, or breastfeeding. Participants provided informed written consent and were enrolled from www.forsoegsperson.dk, a site connecting researchers and participants. During the study men wore swimming trunks, and women wore bikinis and left their ears and neck uncovered. The Committee on Health Research Ethics in the Capital Region, Denmark, concluded that no ethical approval was needed (H-1-2014-094). The study was conducted at Bispebjerg Hospital from May to July 2017. The study was registered at clinicaltrials.gov (identifier: NCT03627260).

Subjects reported their height and weight, from which we calculated the total body surface area [8]. The total area accessible for sunscreen application (i.e., not covered by clothing) was 84% for women and 81% for men [9].

The volunteers were given a sunscreen container and asked to follow the advice "Fill up a handful of sunscreen and spread it all over your body." Containers were weighed before and after the session without the subjects' knowledge. The sunscreen used was Actinica® SPF 50+ (Galderma, France).

Before and after sunscreen application, volunteers had standardized photos taken of the whole body in black light. As sunscreen absorbs black light, it was possible to investigate sunscreen distribution quantitatively, as well as missed areas, by relating photo darkness to sunscreen coverage (Fig. 19.1) [9, 10]. Missed areas were assessed in 11 skin regions: face, ears, front of neck, back of neck, arms, back of hands, front of trunk, back of trunk, thighs, lower legs, and instep. The skin areas with sunscreen applied were calculated by subtracting the missed areas from the total area accessible for sunscreen application.

The overall weighed amount of sunscreen applied was divided by (i) the total accessible skin area or (ii) the actual sunscreen-covered area, giving the quantities of sunscreen in mg/cm^2.

Quantities of sunscreen at six specific skin sites: forehead, chest, upper back, belly, back of thigh, and back of lower leg, were estimated from the skin darkness appearing in the photo [9]. Each skin site had an area of approximately 30 cm^2.

To validate the results, the total amount of sunscreen from the photos was compared to the weighed amount of sunscreen used for each body region. In regions where we did not measure the applied quantity of sunscreen, we used the measured quantity for the nearest region. The quantity of sunscreen was assumed to be even over each of the 11 regions. Adding up the amounts in the different regions gave the amount of sunscreen used by each volunteer, according to the photo analysis [9].

The needed sample size was calculated based on a study investigating sunscreen application without prior instruction, where participants applied a mean of 8.4 g (standard deviation (SD) = 4.7 g) [4]. After application of a handful of sunscreen, we expected participants to apply 15 g (SD = 6.9). 17 participants were needed to detect a difference in quantity after application without instructions and after application of a handful, with a power of 90% and a significance level of 5%. The statistical analysis was performed in IBM SPSS statistics version 22 (IBM, USA). Since data were not normally distributed, the correlation between body size and sunscreen use was tested with Spearman's rank-order correlation. Unpaired data were tested with the Mann-Whitney test. P-values <0.05 were considered significant.

Results

Seventeen volunteers, 8 men and 9 women (age range, 21–42; height range, 165–189 cm; weight range, 60–83 kg; body mass index (BMI) range,

Fig. 19.1 An example of a photo taken in black light after application of a handful of sunscreen. The photo reveals where on the body sunscreen has been applied. Skin covered with sunscreen appears dark blue or black depending on applied quantity. Missed areas appear in light blue color. The picture illustrates uneven sunscreen application

20–29), were included, and all participants completed the study. Only three volunteers could recall having heard about the rule of thumb before the study. An example of a photo taken in black light is shown in Fig. 19.1.

Participants had a median skin surface accessible for sunscreen application of 1.53 m^2 (interquartile range (IQR): 1.43–1.61). After sunscreen application participants had covered a median of 1.17 m^2 (IQR: 1.04–1.36) with sunscreen and left 21% (IQR: 14–33) of their accessible skin without coverage. The missed areas in the different body regions are presented in Table 19.1. The regions with the largest missed areas were ears, back, and instep with a median missed area of more than 38% each. Better covered were the face, back of hands, and lower legs with median missed areas of less than 6% each.

Subjects used a median of 12.4 g sunscreen (IQR: 8.35–17.8) corresponding to 0.87 mg/cm^2 (IQR: 0.59–1.20). There was no significant correlation between amount of sunscreen used and skin accessible for sunscreen application ($p = 0.1$), (Fig. 19.2). For the area actually covered by

sunscreen, it was 1.12 mg/cm^2 (IQR: 0.84–1.63). The three sites with smallest quantities of sunscreen applied were the upper back, the back of the thigh, and leg with median applied quantities below 0.45 mg/cm^2. The two sites with highest quantities of sunscreen applied were the chest and belly with median applied quantities above 1.68 mg/cm^2. See Table 19.2.

The median difference between the amount of sunscreen applied estimated from the photos compared to the weighed amount of sunscreen used was 1.7 g (IQR: −1.85 to 3.72), which was not significantly different from zero ($p = 0.1$). This confirmed that photo analysis can be used to detect quantities of sunscreen applied.

Discussion

To apply 2 mg sunscreen per cm^2, the participants in swimwear should apply a mean amount of 30.6 g. However, when asked to use a handful of sunscreen to cover their body, they used a median of 12.4 g. A median of 21% of the accessible skin was left completely without coverage. The 79% covered area was covered with a median of 1.12 mg/cm^2. In practice, the rule of thumb provided modest protection only, with many missed areas. The intention, that the entire body should be covered by 2 mg/cm^2 of sunscreen, was not reached. The present study showed no trend toward persons with larger body size applying a larger handful of sunscreen (Fig. 19.2), but all participants in the study had a normal BMI [11]. Thus, our data set is not optimal for examining such a possible correlation.

In a study from 2016 of beachgoers on Danish beaches applying sunscreen without any instructions, beachgoers applied an average of 8 g [4]. In a laboratory study from 2015, volunteers applied a median amount of 9 g when asked to apply sunscreen as they would do on the beach [9]. This is not significantly different from the actual quantity used at the beach ($p = 0.3$), indicating that sunscreen use in real-life situations

Table 19.1 Percentage of body surface left without sunscreen, missed area, after application of a handful of sunscreen

Body region	Missed area, % median (IQR)
Face	4 (3–14)
Ears	39 (6–100)
Neck, front	12 (8–22)
Neck, back	11 (4–37)
Trunk, front	6 (3–11)
Trunk, back	40 (26–47)
Arms	15 (13–31)
Hands, back	5 (1–21)
Thighs	20 (10–40)
Lower leg	5 (1–12)
Instep	42 (12–100)
Total	**21 (14–33)**

and in the laboratory is comparable. The volunteers in the present study were asked to follow the rule of thumb and used a 53% larger quantity of sunscreen than uninstructed beachgoers ($p = 0.001$) [4]. Volunteers in a laboratory have previously been asked to consecutively apply sunscreen, twice and with 20 min interval, without further instruction [9]. After two applications a median of 16 g was used corresponding to 100% more than used by the beachgoers and 33% more than by volunteers using a handful.

After a single application, as on the beach, it was found that a median of 20% of the accessible skin was left without cover [9]. After application of a handful of sunscreen, the median missed area was 21%, which means that the recommendation did not lead to a decrease in missed areas. Two consecutive applications significantly halved the median missed area to 9% ($p < 0.001$) [9].

To increase the amount of applied sunscreen and have it distributed evenly on the body, two consecutive sunscreen applications seem to be more effective than the advice "Fill up a handful of sunscreen and spread it all over your body."
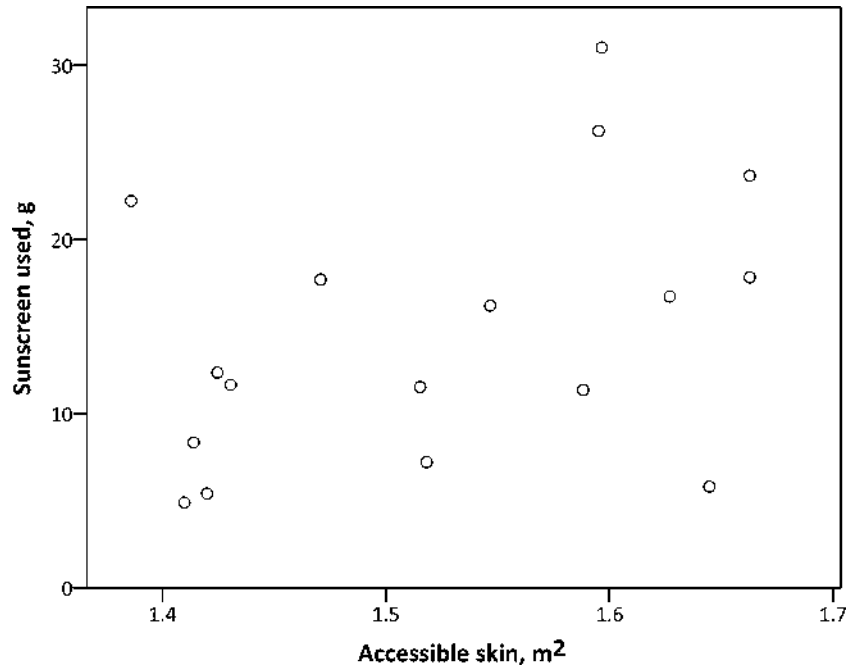
Fig. 19.2 There was no correlation between skin area accessible for sunscreen application and total amount of sunscreen used, a handful ($p = 0.1$). This means that there was no tendency toward larger people using a larger handful of sunscreen. Each volunteer is represented by a circle

Table 19.2 Quantities of sunscreen applied at specific skin sites after application of a handful of sunscreen

Skin site	Quantity of sunscreen, mg/cm^2 median (IQR)
Forehead	0.65 (0.28–1.38)
Chest	1.68 (1.34–2.46)
Belly	2.25 (0.70–2.47)
Upper back	0.21 (0.06–0.34)
Thigh, back	0.16 (0.10–0.55)
Lower leg, back	0.42 (0.23–1.81)

Conflicts of Interest None declared.

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Ultraviolet Exposure Scenarios: Balancing Risks of Erythema and Benefits of Cutaneous Vitamin D Synthesis

Ann R. Webb and Ola Engelsen

Abstract

Exposure to sunlight is a major source of vitamin D for most people. Yet public health advice has focused overwhelmingly on avoiding exposure of unprotected skin because of the risks of erythema and skin cancer. Given that there are also health risks associated with low vitamin D status, we explore the possibilities of achieving a range of targets associated with vitamin D and the accompanying erythema risk. We have calculated the exposure required to gain a number of proposed oral-equivalent doses of vitamin D, as functions of latitude, season, skin type and skin area exposed, together with the associated risk of erythema, expressed in minimum erythema doses. The model results show that a recommended daily intake of 400 IU is readily achievable through casual sun exposure in the midday lunch hour, with no risk of erythema, for all latitudes some of the year, and for all the year at some (low) latitudes. We also show that such daily, sub-erythemal doses at lunchtime during the summer months is sufficient to avoid winter-time vitamin D deficiency for the UK all-weather climate, provided that lower arms and legs are exposed in the warmer months. At the higher proposed vitamin D dose of 1000 IU, lunchtime sun exposure is still a viable route to the vitamin but requires the commitment to expose greater areas of skin and is effective for a shorter period of the year. The highest vitamin D requirement considered was 4000 IU per day. For much of the globe and much of the year, this is not achievable in a lunchtime hour and where it is possible large areas of skin must be exposed to prevent erythema. When the only variable considered was skin type, latitudinal and seasonal limits on adequate vitamin D production were more restrictive for skin type 5 than skin type 2.

Introduction

The ultraviolet (UV) region of the solar spectrum (280–400 nm) is responsible for a number of biological and chemical effects. For humans, the direct effects occur in organs that are exposed to sunlight, i.e., the skin and eyes. Here we consider only the skin. In the skin, the main competing responses to ultraviolet radiation are the synthesis

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of vitamin D (positive health benefit), damage to cells and DNA manifested as erythema and an increased probability of skin cancer (negative health risk). Previous chapters have covered the synthesis and benefits of vitamin D, and later chapters provide extensive details of current knowledge of skin cancer. Here we summarise only the points relevant to debate the relative risks and benefits of sun exposure.

Vitamin D has long been accepted as necessary for calcium metabolism and hence a healthy skeleton [1]. More recently it has also been linked with a protective effect against many life-threatening diseases, including a range of internal cancers and auto-immune diseases [2, 3]. The so-called sunshine vitamin is also available through the diet, either in a very limited set of foods (mainly fatty fish, though some foods in some countries are fortified) or as supplements. As a dietary constituent, there are recommended guidelines for ingesting vitamin D. Until the last decade, these were based only on bone health indicators and were aimed at eradicating the bone diseases of vitamin D deficiency: rickets and its adult form, osteomalacia. The guidelines ranged from zero dietary intake (assuming sunlight to provide all necessary vitamin D) to 400–600 IU (international units) per day for those with extra growth requirements (pregnant and lactating women and children) or those "at risk" through potentially reduced capacity for cutaneous synthesis (e.g. the housebound and elderly) [4, 5]. The tolerable upper intake level for oral vitamin D has been widely increased to 4000 IU per day [6–8] (IOM 2011, EFSA, 2012 COT 2014). At higher doses long-term ingestion can potentially have negative effects, although at what level is unclear. Single therapeutic doses of up to 50,000 IU are given under medical supervision to cure bone disease, but this is a very different situation to unregulated home intake of supplements. Suggestions that vitamin D benefits more than bone health has led to calls for an increase in the recommended daily intake (RDI) of the vitamin. Intakes proposed to secure all the potential benefits of vitamin D range from 1000 IU [9] to 4000 IU per day [10]. National and international bodies providing guidance on

vitamin D intake in the past decade advise, variously, 400 IU per day [11] (SACN) to 600 IU per day [6, 12] (IOM, EFSA) for the general population (excluding infants under the age of 1). It is clearly stated that these guidelines assume minimal sunlight exposure [6] and that where there is cutaneous synthesis of vitamin D, the dietary requirement will be lower, perhaps zero [12]. In addition, there are differences in the effectiveness of different forms of vitamin D. Vitamin D_3 is formed in the skin, while vitamin D_2 (frequently used in food fortification and supplements) is the plant derived form of the vitamin. There is evidence that vitamin D_3, whether made cutaneously or ingested, is the more effective for increasing vitamin D status [13–15].

Ultraviolet radiation (UV), including that in sunlight, is a recognised carcinogen. Exposure to UV increases the risk of a cancer developing, although the details of the risk mechanism differ with the type of skin cancer. For the most life-threatening form, malignant melanoma and incidents of bad sunburn, especially in childhood, seem more important than the cumulative lifetime dose of UV that is implicated for squamous cell carcinoma (SCC), while basal cell carcinoma (BCC) seems to combine elements of both sunburn and cumulative UV risks [16–18]. Current public health policy from, e.g. the UK, the USA Australia, and World Health Organisation advises against sunlight exposure of unprotected skin, especially in the middle of the day (see websites for CRUK; EPA; Sunsmart; WHO) [19–22] when the advice is to stay indoors or cover up completely.

Existing recommendations to the public are contradictory: one assumes that UV exposure will normally provide necessary vitamin D, while the other advises minimising exposure to UV. This contradiction is exacerbated if calls to raise the recommended daily intake (or equivalent cutaneous synthesis) for vitamin D are considered. Here we explore the possibilities of achieving current and suggested vitamin D status through sunlight exposure and assess the associated risks expressed in terms of erythema.

Differences Between Vitamin D Synthesis and Erythema

Vitamin D synthesis and erythema both result from exposing unprotected skin to ultraviolet radiation, but there are significant differences between the two responses.

Action Spectra

A fundamental difference is that between the two action spectra. Vitamin D synthesis is very much a response to UVB radiation (280–315 nm) [23], while erythema is elicited by both UVB and UVA (315–400 nm) [24] radiation. The action spectra for the two responses are shown in Fig. 20.1.

The biologically effective dose rate for each response is given by

$$\text{Biologically effective dose rate} = \int E_\lambda \, A_\lambda \, d\lambda$$

where E_λ is the incident radiation at a given wavelength, A_λ is the biological response at that wavelength and λ is wavelength. Since the solar spectrum is not a constant shape, especially in the UV, the ratio between erythema- and vitamin D-effective radiation is not a constant either.

Biological Endpoints

Erythema is damage to the skin, and the endpoint of the damage is visible as a reddening, and in extreme cases blistering, of the exposed skin. Vitamin D, by contrast, is synthesised in the skin but then enters the circulation and is hydroxylated in the liver to 25-hydroxyvitamin D (25OHD). It is the concentration of circulating 25OHD that is measured as an indicator of a person's vitamin D status since the hydroxylation to the active form in the kidney, 1,25 dihydroxyvitamin D (1,25), is tightly controlled by other factors. Vitamin D synthesised in all exposed skin contributes to the concentration of 25OHD in the blood, so increasing skin area exposed is one way to increase vitamin D status, rather than increasing exposure on a particular region of skin.

Other organs also have receptors for 25OHD, and the cells can make their own 1,25 for internal use; hence the argument that once bone health requirements have been met, "left over" 25OHD can be used by the body for other health benefits. To gain these benefits, the circulating 25OHD must be higher than the concentrations required simply to avoid rickets, and the associated vitamin D intake/synthesis must increase correspondingly [10].

Acute vs. Chronic Exposures

Erythema is experienced when UV exposure reaches or exceeds a personal minimum erythemal dose (MED) in a single exposure, or exposures close together. Two sub-erythemal doses gained a week apart are not additive for erythema, while the same two exposures either side of lunch on the same day could produce an erythemal response since there is inadequate time for repair processes to function [25, 26]. All exposure will contribute to cumulative lifetime dose (and hence the risk of SCC). Avoiding erythema is a main goal in skin cancer prevention and constitutes the main risk reduction for malignant melanoma.

The absolute UV dose that produces a slight reddening of the skin, i.e. an MED, is individual-dependent. It is broadly related to skin type [27] and skin colour, but neither is a very accurate predictor of MED [28]. The pigment melanin, which gives skin its brown colour, absorbs UV radiation. It therefore prevents damage to DNA, and simultaneously prevents 7-dehydrocholesterol conversion to previtamin D in the first step of vitamin D synthesis. The photochemical production of previtamin D, the first step in vitamin D synthesis, requires sufficient UVB photons incident on the skin. If the UVB requirement is met, then previtamin D accumulates by further photochemical reactions during any immediately sequential exposure(s).

Fig. 20.1 CIE erythema action spectrum [24] (bold line) and the action spectrum for the formation of previtamin D in human skin [23] (dots) with solar spectra measured at solar zenith angles of 25 and 75 degrees. The units of irradiance are $Wm^{-2}\,nm^{-1}$
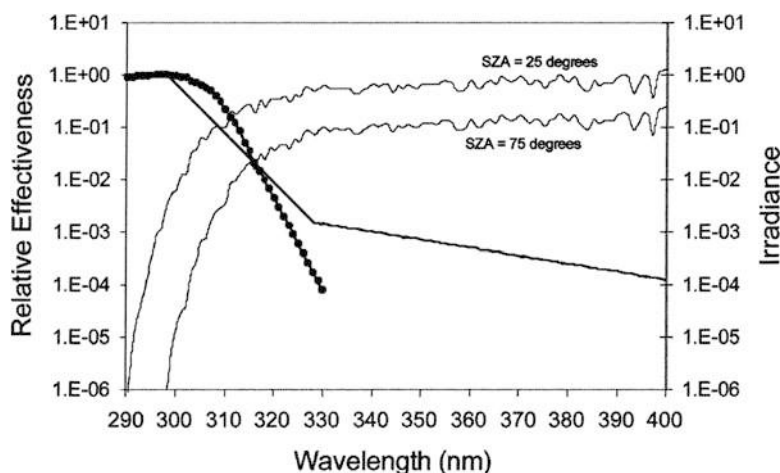
Once formed, the previtamin D can be converted into several other biologically inert isomers in reversible reactions. In sunlight the previtamin D in the isomer mixture that results from continuing exposure never exceeds about 12% [29], and the amount of previtamin D reaches a steady state. The next stage of vitamin D synthesis, thermal isomerisation of previtamin D to vitamin D, takes several hours and so does not remove previtamin D from the isomer mixture during a typical exposure period. Prolonged exposure is therefore of no benefit once there is sufficient UVB to produce the initial previtamin D because the amount of previtamin D formed is limited. Note that while avoidance of erythema is a major focus of reducing skin cancer risk, even sub-erythemal levels of UV exposure will elicit some DNA damage, although this is mostly repaired [30].

A generalised optimum exposure regime for acquiring and maintaining an adequate vitamin D status is therefore "little and often," e.g. a sub-erythemal dose of sufficiently UVB-rich radiation every day or two. The same regime is suitable for avoiding erythema, and in this respect benefit gain and risk avoidance are served by the same behaviour. Indeed, regular sun exposure has also been shown to increase survival in cases of malignant melanoma, an effect that may be mediated by vitamin D [31].

Implications for UV Exposure

Ambient Solar UV Radiation

The ambient solar UV radiation is most commonly expressed (measured or calculated) as the radiation incident on a flat, horizontal, unshaded plane. The ambient UV depends first upon latitude, day of year and time, which are factors combined to give the solar zenith angle (SZA, the angle between the local vertical and a line from the observer to the sun). The smaller the SZA (the higher the sun in the sky), the more intense is the solar radiation. This is due to two processes. When the direct solar beam strikes the surface at an oblique angle, the incident energy is spread over a larger surface area than when the radiation is normal to the surface, reducing the intensity by the cosine (SZA). In addition, as the SZA increases the pathlength of the radiation, that is, the distance it travels from the sun through the Earth's atmosphere, there is an increase in the scattering and absorption occurring along the path of the photons travelling towards the Earth. Thus the radiation reaching the surface is further reduced.

Superimposed on the very predictable cycle of SZA are the effects of components of the atmosphere, notably clouds, aerosols and ozone. These influences can change unpredictably and modify the daily and annual cycles in incident radiation.

Nonetheless, general experience and expectation is for maximum solar radiation in the summer time and in the middle of the day and minimum in winter and towards sunrise and sunset. Cloudless skies act as the default that allows maximum irradiance in the great majority of situations.

Solar Zenith Angle and the UV Spectrum

In addition to changes in incident solar energy, changes in SZA also change the shape of the solar spectrum, particularly in the UV region. This is a result of the changes in pathlength and hence the amount of atmospheric absorption and scattering. The absorption and scattering processes are wavelength-dependent, and this is particularly true of ozone absorption and Rayleigh scattering from air molecules. For clouds and aerosols, this is less so. Ozone absorbs strongly at wavelengths less than 280 nm, so much so that no radiation at these wavelengths reaches the surface. Its absorptive properties then decrease rapidly through the UVB waveband and into the UVA, until there is no appreciable absorption for wavelengths greater than about 340 nm. The ozone absorption spectrum is mirrored in the rapidly increasing spectral irradiance in the UVB (see Fig. 20.1). As the distance the radiation has to travel through the stratospheric ozone layer increases, so does the absorption, attenuating the shortest wavelengths more than the longer UV wavelengths.

The scattering of solar radiation by air molecules is accurately described by Rayleigh scattering theory. The scattering is proportional to the inverse fourth power of the wavelength (α λ^{-4}), which means that radiation at 300 nm is scattered about three times more than that at 400 nm. A major part of the ultraviolet radiation is back-scattered to space. At large SZA, most UV radiation is diffuse. Once more, increased pathlength leads to a disproportionate loss of the shorter UV wavelengths. Changes in stratospheric ozone will also alter the spectral shape, while cloud and aerosol effects are less wavelength-dependent, but the dominant influence is SZA.

This spectral dependence on SZA means that for small SZA the proportion of UVB in the total UV waveband is greater than for large SZA. Since the action spectra for vitamin D synthesis (UVB) and erythema (UVB + UVA) differ, the ratio between their two biological doses changes as the solar zenith angle changes: there is more vitamin D effective radiation per dose of erythemally effective radiation at small SZA than at large SZA. Therefore the most efficient time to gain some UV exposure (maximising vitamin D synthesis for a fixed erythemal dose) is at small SZA. For a given location, this is around noon on any day and in the summer months [32, 33].

Unprotected Skin Area

The area of skin exposed to UV radiation is extremely important in determining the resultant effect on vitamin D status since only exposed skin can synthesise vitamin D. Circulating 25OHD is the total resultant effect of vitamin D synthesis from any part of the body surface. On the other hand, skin area does not determine the severity of erythema, only the skin area that suffers from reddening, though the exposure increases cumulative lifetime dose and hence skin cancer risk. Thus the best way to increase vitamin D status while minimising the risk of erythema is to expose a large area of skin for a short period of time, rather than a small area of skin for a longer time.

Note that for either effect the skin exposed must be unprotected, i.e. free of any covering, including sunscreen and other skincare products that may contain an element of sunscreen (SPF), e.g. moisturisers and foundation creams. Face, neck and hands are the most frequently exposed skin areas (equivalent to 11.5% full body surface area). At freezing temperatures, most people only expose the face (3.5%), except in extreme cold when even that may be covered. In summer casual wear, exposed skin may increase to about 35% with lower arms and lower legs exposed, in addition to hands, face and neck, while during

workout with addition of full arms and full legs exposed, this increases to 57.5% [34] skin area.

A further consideration is the orientation of the exposed skin. The human body is made of many surfaces, with orientations that change with the motion of the person relative to the position of the sun in the sky. Unless lying flat (e.g. actively sunbathing) limited skin surfaces match the flat horizontal plane to which ambient solar radiation is referred. In our original calculations [33], the exposure of a flat horizontal plane was taken as a default exposure representing the local environment, while recognising that the exposure of any given body part will be a constantly changing fraction of this ambient UV. Later work includes transforming the horizontal ambient to a randomly oriented vertical surface (the average over vertical surfaces aligned at different angles to the direct solar beam), as more appropriate to the upright, ambulatory human body [35].

Realistic Exposure Times

A photobiological effect is the result of photons of suitable, effective wavelengths reaching target molecules in sufficient number that the resultant photochemical changes cause a noticeable biological reaction. In principle, even at very low irradiation rates, one can eventually acquire a sufficient dose of UV radiation to produce erythema or a measurable change in circulating 25OHD. In practice the time might be so long that other processes (repair or use of vitamin D) prevent a noticeable biological reaction, and there is said to be no biological effect, even though the underlying photochemical reactions have occurred to a small degree.

For long durations of sun exposure, a biological effect may become apparent but would require a devotion to sunbathing that is unrealistic or impractical. For example, at high latitudes prolonged exposure of bare skin may be uncomfortably cold. In considering the normal working adult, we have taken 1 h as the maximum period for a realistic daily exposure time, equating to a full lunch hour spent outdoors. An hour spent outdoors at times well removed from noon

(e.g. before/after the working day) will incur a much lower absolute UV dose and one that is comparatively weighted less towards vitamin D synthesis and more towards erythema than at noon. Weekends and holiday periods provide the opportunity for more extensive exposure, but it has already been established that a regime of UV exposure "little and often" is most effective and beneficial.

Assessing the Erythema Risks of Exposures for Vitamin D Synthesis

To assess the UV exposures sufficient to provide for our vitamin D requirements (and the associated erythema risks), there are several variables that have to be determined: What are the vitamin D requirements? What skin type to consider? What skin area to expose? The UV radiation at the ground can then be modelled as a function of time and place, having defined some baseline atmospheric conditions, and applied to the conditions for vitamin D synthesis and erythema. Models are used because ground-based measurements of spectral UV (required for the application of several action spectra) are not common. However, the ground-based measurements can be used to validate the models [36]. UV doses for erythema and vitamin D synthesis are illustrated for the previous day, using similar modelling and satellite data input, as a satellite product at http://www.temis.nl/uvradiation/UVdose.html

Two approaches to determining the twin erythema and vitamin D potentials of sun exposure are now discussed. The first is an idealised model that draws on experience of a small number of people exposed to artificial UV radiation and has been applied globally to illustrate the possibilities of synthesising vitamin D at a range of oral intake equivalents anywhere in the world and then the associated erythema risks assessed. The second approach is based on a series of human volunteer studies in everyday life, and all-weather climatology, and begins from the premise of limiting exposures to sub-erythemal levels and then asking if that allows sufficient vitamin D to be

synthesised in the skin. This more pragmatic assessment is only illustrated for the UK and a single target for vitamin D status.

Global Model of Vitamin D and Erythema Potential

Throughout this approach the FastRT UV simulation tool [37] was used to calculate UV irradiances on a flat, horizontal surface at sea level. The atmosphere was taken to be cloudless and the surface non-reflecting. The ozone layer thickness was fixed at 350 Dobson Units, a typical level. Aerosol was taken to be of rural type [38] with optical depth given by $\tau = \beta * \lambda^{-\alpha}$ where the Ångström coefficient α was set to 1.3 and the wavelengths (λ) are in micrometres. The Ångström coefficient β was related to 25 km visibility $R_m[km]$ using the parameterization of Iqbal (1983) [39], i.e.

$$\beta = 0.55^{1.3} \, (3.912/R_m[km] - 0.01162) \\ \times [0.02472 * (R_m[km] - 5) + 1.132].$$

In all other aspects, a US standard atmosphere [40] was assumed.

Vitamin D_3 effective doses were computed using the action spectrum for conversion of 7-DHC to previtamin D_3 in human skin [23] (CIE, 2006) (Fig. 20.1). The method used by Webb and Engelsen [33] (2006) was applied for a range of conditions. A vitamin D dose, VD (X) was defined as corresponding to the UV equivalent of an oral dose of X IU vitamin D. Since radiation is incident on the skin and the response to either irradiation or oral dosing is measured in the blood, the dose VD(X) must be qualified by the conditions of skin exposure. The relation between the UV equivalent of an oral dose and skin area exposed was based on Holick's rule [9, 41]. This equates exposure to ¼ of personal MED on ¼ skin area (hands, face and arms) to an oral dose of 1000 IU vitamin D. UV doses were calculated under reference conditions. We assumed these reference conditions to be midlatitude midday in spring (Boston, 21 March, 42.2° N, ozone = 350DU),

after Webb and Engelsen (2006) [33]. First to be calculated was the time required to acquire a ¼ MED around solar noon, using FastRT model [37] simulations. Then, using the same simulated solar spectra at the ground over the same time interval about noon but weighting with the action spectrum for previtamin D_3 synthesis [23], instead of the erythema action spectrum [24], the vitamin D_3 effective dose acquired over the same time interval was calculated. This is the VD (1000) based on exposure of ¼ body surface area for a given MED.

Holick's rule has here been generalised by linear extrapolation:

$$VD(X, C, S) = 0.25 * MED(S) * 0.25/C \\ * X/1000$$

where C is the percentage of the skin exposed to UV radiation and S is the skin type.

The calculations were performed for two MEDs equivalent to skin types 2 and 5 [27]. The calculations were also repeated for different degrees of skin exposure, as shown in Table 20.1 which lists all the variables used in the calculations.

In the example above, a person exposing hands, face and arms would now make the equivalent of 1000 IU with 1 VD(1000) and would suffer a minimal erythema after 1 MED, which by definition is four times the VD exposure under the reference conditions assumed (i.e. Boston, 21 March, 42.2° N, ozone = 350DU), but not necessarily for other conditions with a different shape to the solar spectrum at the ground.

Assumptions and Limitations

Calculations were all performed with an idealised atmosphere and receiving surface, neither of which would actually match reality in any but the rarest of cases. Nonetheless, the atmosphere represents a collection of standard conditions, and a flat surface is unambiguous even if it only represents the tops of shoulders, head and feet for the upright human body. The angle of incidence for radiation at any body site changes

Table 20.1 Variables of vitamin D dose, MED and skin area used in the model calculations

Variable	Value	Reference
X for VD(X)	400 IU	11
	1000 IU	9
	4000 IU	10
Skin type	2, MED = 250 J m^{-2}	27
	5, MED = 600 J m^{-2}	27
Skin area exposed	Face, neck and hands (11.5%)	34
	F,N,H and arms (25.5%)	34
	F,N,H, arms and legs (57.5%)	34

continuously with motion of both the body and sun: for some sites, some of the time, incident radiation will be greater than on a horizontal surface but in many cases irradiances will be lower than the case of a horizontal plane [35]. To explore different atmospheric conditions, including ozone, cloud, aerosol, surface albedo and surface elevation, the reader is directed to https://fastrt.nilu.no/VitD_quartMEDandMED_v2.html where user selected inputs can be applied to the calculations. In the majority of real-life cases, we would expect the required exposure times to exceed those produced by the model.

Three recommendations for dietary intake of vitamin D have been used, one representing the lower end of the current public health guidelines intended to prevent bone disease by dietary intake alone [11] and two suggestions for revised guidelines that, it is suggested, would confer all possible health benefits associated with vitamin D [9, 10]. No account has been taken of any sections of the population such as the elderly or pregnant who may have different vitamin D requirements [42, 43], except insofar as they are intrinsically included in current guidelines based on bone health. Nor have confounding factors such as body fat [44] been considered.

The UV equivalence to an oral dose that is at the heart of these calculations is based on the assumption that the relation between body surface area exposed and change in circulating 25OHD is linear. We have used that assumption again in assessing exposures for different skin areas in Table 20.1 but without clear proof that this linearity is anything more than sensible expectation. Additionally, the oral dose equivalence that we have used was not determined exactly, rather

the whole-body exposure to 1 MED of UV was shown to produce a rise in 25OHD that fell between that produced by oral doses of 10,000 IU and 25,000 IU [41]. From this we followed Holick [9] in approximating 1 MED, full body to 16,000 IU, and thus ¼ MED and ¼ surface area to 1000 IU, which is neat mathematically but is maybe imprecise.

Furthermore, in the original work [33, 45], the spectrum of the artificial source used in Holick's formulation was unknown and was assumed to be solar simulating and so similar to Boston sunlight. Dowdy et al. [46] have since identified the artificial source used and estimated the ratio of vitamin D effective to erythema-effective radiation for the source employed was 1.33 rather than the 1.75 in Boston spring sunlight that was used in the original calculations. This means that strictly the ¼ skin area for ¼ MED to provide 1000 IU vitamin D has to be scaled by 1.32, either for skin area or UV dose (but not both together), to provide 1000 IU in Boston spring sunlight. For example, retaining the 0.25 skin area, one would only need 3/16 rather than ¼ MED in sunlight, and therefore the times in Figs. 20.2, 20.4, 20.5, and 20.6 and Table 20.2 that follow should be divided by 1.32, although they remain illustrative and not absolute. The findings of Dowdy et al. [46] have been accounted for in the latest update to the online simulation tool to which the reader is directed throughout this chapter.

A further modification might arise from the work of Webb et al. [35] who addressed the orientation of skin with respect to the radiation incident on a horizontal surface. They assessed the time to receive the same UV dose as measured/calculated on a horizontal surface or a

Fig. 20.2 Time in hours required to synthesise the oral equivalent of 400 IU vitamin D for skin type 2 exposing hands, face, neck, arms and legs
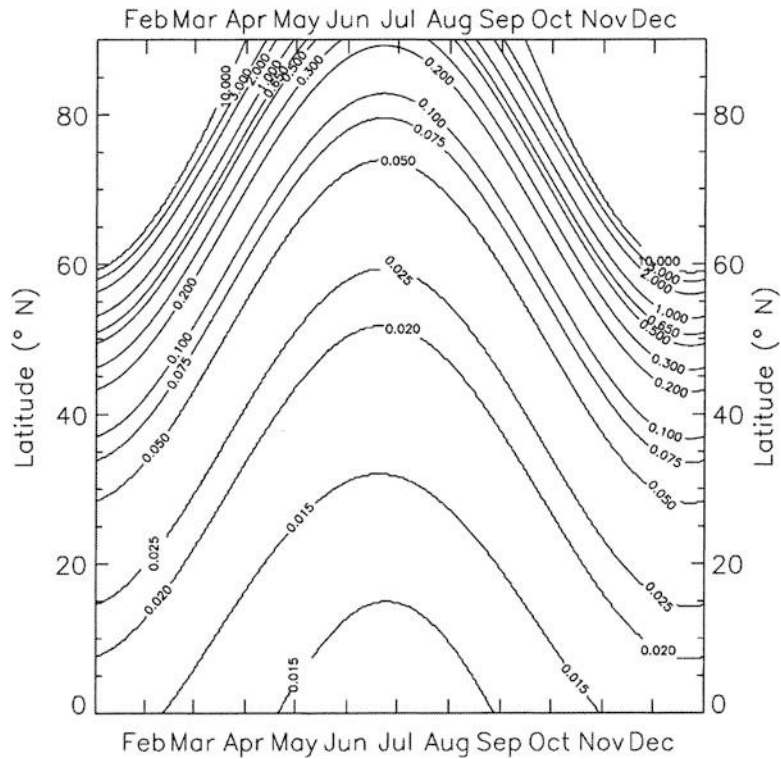
human body either prone (in which case account must be taken of turning over so all skin is exposed) or upright and moving randomly so all sides are equally exposed. This would provide a further scaling factor for the results presented here but one that is dependent on solar elevation (when the sun is high in the sky, the direct solar beam is more normal to a horizontal surface; when it is low in the sky, the direct solar beam is more normal to a vertical surface). For our reference situation (Boston at noon at the spring equinox) when the solar elevation is 48°, the conversion from horizontal surface to vertical surface exposure time is a factor of 1.15. As the solar elevation increases, this conversion factor also increases so that at noon in midsummer in Boston, it is close to 1.4 [35]. The overall result of these two adjustments (divide by 1.32 and multiply by a date dependent factor between 1.15 and 1.4) is to bring the results back close to the original calculation, at least for Boston.

Given the idealised nature of the original calculations and thus the illustrative nature of

Table 20.2 Exposure time, in minutes (to nearest half minute) and associated MED (in parentheses) for Boston at the spring equinox for all permutations of variables according to original calculation [45]. Multiply by 1.15/1.32 (0.87) to adjust to vertical surface and account for FS lamp used in underlying oral equivalency experiments

Vit. D >	400 IU		1000 IU		4000 IU	
Skin type>	2	5	2	5	2	5
Area ∨						
F,N,H (11.5%)	9 (0.21)	21 (0.21)	21.5 (0.54)	53.5 (0.54)	89.5 (2.16)	237 (2.16)
F,N,H,A (25.5%)	4 (0.09)	9.5 (0.09)	10 (**0.24**)	24 (0.24)	40 (0.97)	97 (0.97)
F,N,H,A,L (57.5%)	2 (0.04)	4 (0.04)	4 (0.10)	11 (0.10)	17 (0.43)	42 (0.43)

the results, no changes have been made to the original figures.

The action spectra used are, respectively, a mathematical fit to a collection of data, widely accepted through common use (erythema) or the only one available that is based on measurements in human skin (previtamin D synthesis). Although the latter has been challenged [47], no better candidate has currently been identified. As with the base case atmosphere, these action spectra must be understood as representative, in this case of human skin, not necessarily exact for every person.

Similarly, the MEDs used to quantify a skin type are average values from a wide range. MED and skin type are loosely related, but each skin type can encompass a wide range of MED values, and the ranges overlap.

In summary, the calculations shown here are for illustration. They should not be taken as precise recommendations for UV exposure since realistic situations will differ in many aspects from the limited range of conditions represented here. True exposure requirements depend on details of each location, the prevailing atmospheric conditions, and personal characteristics. What the calculations do allow is a comparison between the various existing or suggested recommended daily intakes of vitamin D, expressed as a UV exposure equivalent, and the associated risks of erythema in each case.

Results

A sample of the calculations for different vitamin D, skin type and skin area scenarios is used here for illustration. Note that the latitudinal and seasonal pattern seen in all plots is a function of the changing spectrum and intensity of the solar irradiance with latitude- and season-dependent solar zenith angle (represented, e.g. by noontime SZA). If the solar spectrum remained the same all day, then the permutations of dose, skin type and skin area would be simple scaling factors of a single example set, e.g. 1000 IU, skin type 1, skin area 25%. For example, a doubling of the desired dietary

equivalent intake would require twice the exposure time. Likewise, exposing twice the skin area would half the required exposure time. A skin type requiring twice as much UV radiation to get sunburnt would require twice the amount of exposure time with constant UV intensity.

However, the spectrum changes with diurnal changes in SZA, with less irradiance and disproportionately less at shorter UVB wavelengths, as SZA increases (i.e. as the sun gets closer to the horizon). The calculations start at noon and move symmetrically away, taking account of the changing irradiance in doing so. Thus an exposure time of 30 min means 15 min before noon to 15 min after noon (during which time the SZA and spectrum will not change very much). An exposure of 6 h means 3 h before noon to 3 h after noon, during which time the SZA and UV spectrum can change more significantly. Scaling for the variables will provide a reasonable approximation to the full calculation when exposure times are short and the scaling is small, so that both situations are encompassed by times close to noon when the spectrum does not change significantly. When times or scaling factors are large, the changing solar spectrum introduces increasingly large errors to any attempt to directly scale results. However, as stated previously, we take a 1 h exposure around noon to be the maximum feasible on a regular basis, and then for a given latitude and month, scaling can be applied.

Figure 20.2 shows the time required to achieve the oral equivalent of 400 IU vitamin D for skin type 2, exposing face, neck, hands and arms and legs (57.5% skin area). Even at 70° latitude, it is possible for a skin type 2 individual to synthesise the equivalent of 400 IU vitamin D in less than an hour for about 6 months of the year, by exposing all but the torso to sunlight. Whether this would be practical or desirable given the temperature is a further pragmatic consideration. Furthermore, the year-round clear skies of the model certainly never occur. The associated MEDs can be seen in Fig. 20.3. As skin area exposed decreases, exposure times increase, and the viable vitamin D season shortens. Nonetheless, 400 IU vitamin D can be achieved without erythema by less than 1 h exposure of hands, face and neck for several

Fig. 20.3 The fractional MED gained during the minimum exposure required for 400 IU vitamin D, skin type 2, skin area 57.5% (i.e. the situation in Fig. 20.2)
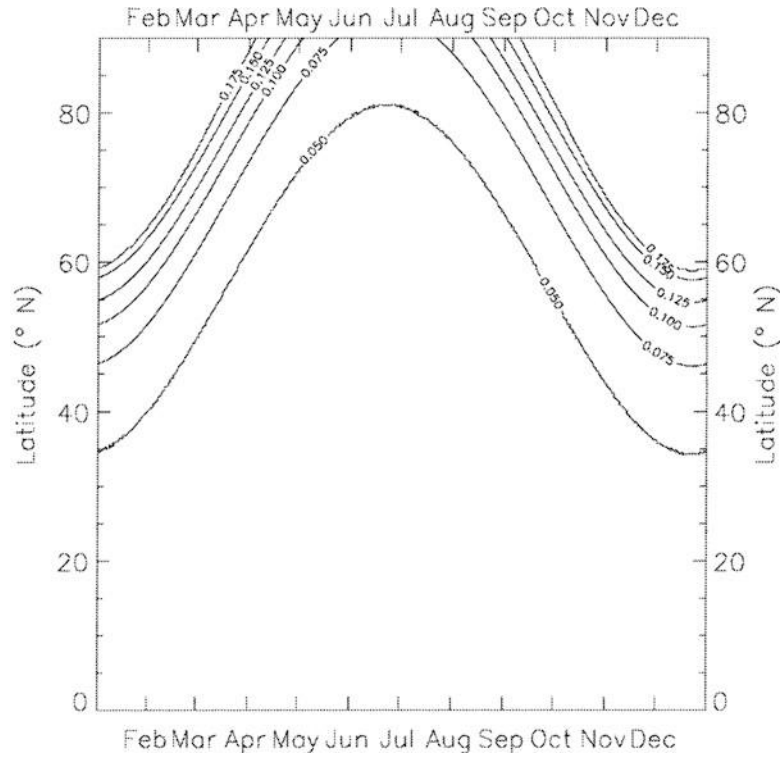
months even at 70° latitude, which we take as the limit of significant populations. 400 IU and 11.5% area exposed yields very similar results as 1000 IU and 25% area exposed [33] and is consequently not repeated here.

Figures 20.4 and 20.5 show the time required to achieve the oral equivalent of 4000 IU for skin types 2 and 5 with 11.5% skin area exposed. Figure 20.6 shows the associated MEDs acquired in the same time for skin type 2; the pattern is similar, and the smallest number of MED (1.9 in the Tropics) is the same for skin type 5. Thus, achieving 4000 IU without risk of sunburn is not possible unless large skin areas are exposed. Crude scaling of Figs. 20.2, 20.3, 20.4, 20.5 and 20.6 shows that 4000 IU can be achieved in this way in less than 1 h and without incurring erythema at low latitudes, provided that sufficient (skin type dependent) skin area is exposed.

As an example of midlatitude exposures in all situations, Table 20.2 shows exposure times and number of MEDs acquired while achieving the

stated UV dose for all permutations of variables for Boston (42°N) at the spring equinox. The MEDs for 1000 IU, skin type 2, skin area 25% is 0.25 by definition. Note that the skin area in the calculation is 25.5% so the MED is 0.24. At this time and location, all exposure times except 4000 IU for skin type 5, 11.5% skin area, are within 1 h either side of noon, so the scaling is applicable. For other permutations, or alternative values of the variables, readers may make their own calculations at https://fastrt.nilu.no/VitD_quartMEDandMED_v2.html

In summary, current conservative recommendations for daily 400 IU vitamin D supply (cutaneous or oral) are achievable through sun exposure, without risk of erythema, for all or part of the year. Supplements are readily available for the locations and periods when cutaneous synthesis is not practically possible. At the intermediate recommendation of 1000 IU vitamin D daily, sun exposure of less than 1 h can still serve as a single source at low latitudes and at middle to

Fig. 20.4 Time in hours required to synthesise the oral equivalent of 4000 IU vitamin D for skin type 2 exposing hands, face and neck
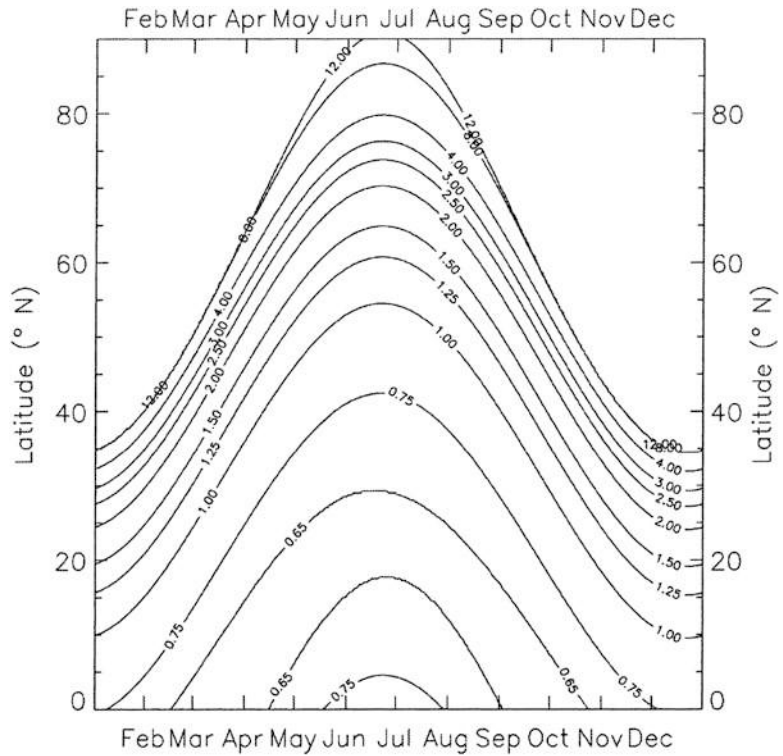
high latitudes (results not shown here) for some or all of the year for those willing to expose sufficient skin area. As latitude (or skin pigment) increases, the viable periods for sun-induced vitamin D become ever more constrained. Supplementation at these levels is now easily available commercially, and a combination of sunlight and supplementation makes this level of equivalent daily vitamin D acquisition achievable for most indigenous people. Migrant communities who have moved polewards would however be more reliant on supplements. Achieving 4000 IU equivalent of vitamin D by sun exposure becomes problematical. Where it is theoretically possible to provide this vitamin D through skin synthesis, e.g. for skin type 2 in the Tropics, the exposure time required also produces a significant erythemal dose: 1.9 MED for hand, face, neck exposure and ~ 0.4 MED when full arms and legs are also exposed. Skin type 5 can only acquire 4000 IU in less than an hour by exposing 25% or more skin area and incurring close to a full MED (at the smaller possible skin areas) even in the

Tropics (results not shown here). If a personal goal is to achieve a vitamin D supply equivalent to 4000 IU daily, then supplements of this value are now readily available, since they no longer breach the tolerable upper intake level.

UK Model for Avoidance of Vitamin D Deficiency

The second illustration inverts the approach above and takes a fixed daily exposure time (again during the noon hours or lunchtime) and then determines under what circumstances of latitude, skin type and skin area exposed that exposure time can provide for vitamin D requirements. Other differences include the use of all-weather climatology [36] (not clear skies only) and the SZA-dependent conversion of incident radiation from that on a horizontal surface to that on a randomly oriented vertical surface [35], more representative of the upright human body.

Fig. 20.5 Time in hours required to synthesise the oral equivalent of 4000 IU vitamin D for skin type 5 exposing hands, face and neck
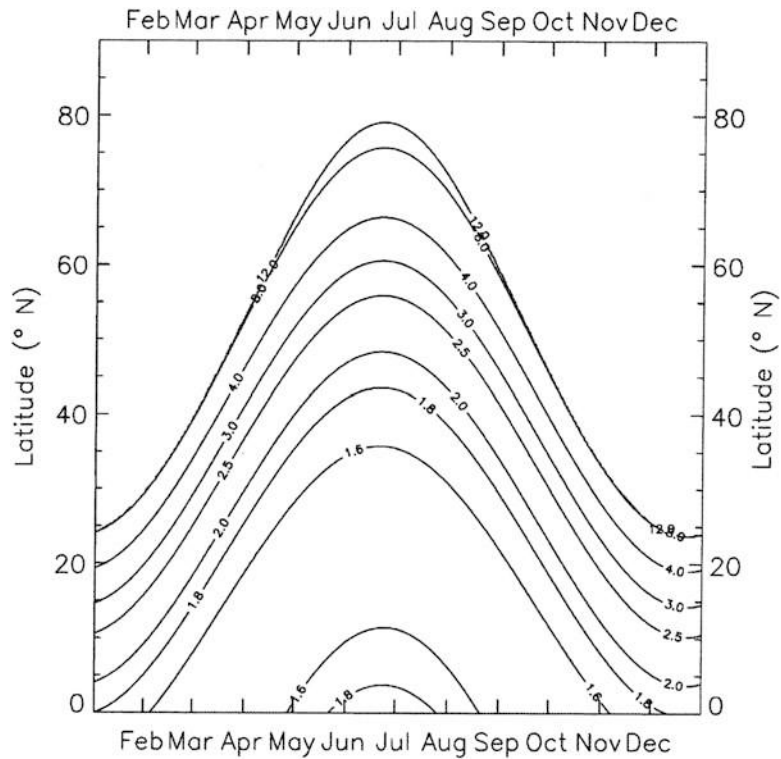
Fig. 20.6 The fractional MED gained during the minimum exposure required for 4000 IU vitamin D, skin type 2, skin area 11.5% (i.e. the situation in Fig. 20.4)
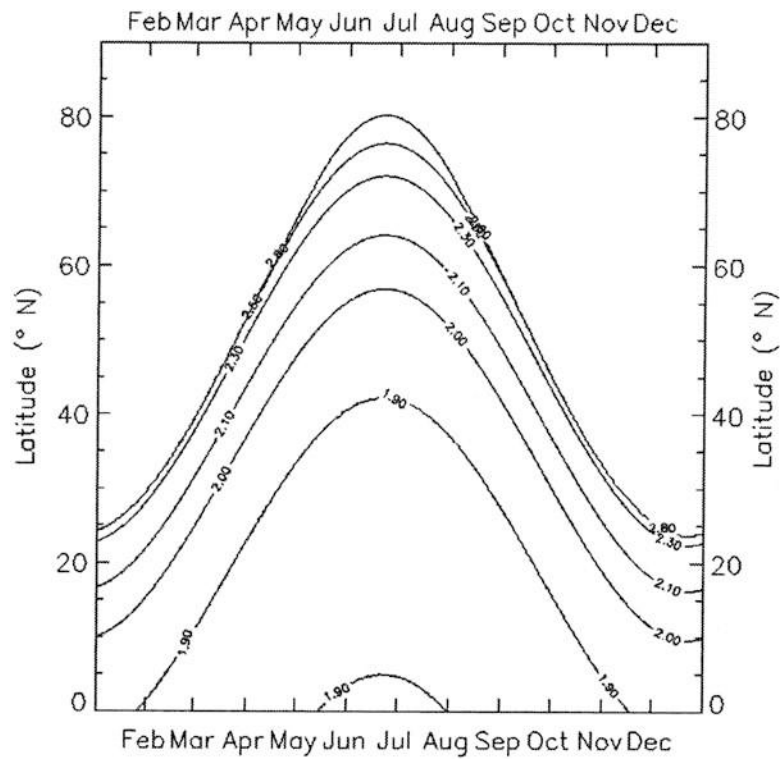
Starting from a 10-year all-weather UV climatology of the UK [36], the time required to gain a dose of one standard erythema dose (1SED = 100 Jm^{-2} erythema effective UV) on the South Coast at noon on June 21st with clear skies was calculated. This time (9 min) was taken as the constant exposure time, ensuring that a dose no greater than 1 SED would be received anywhere on any day in the UK. For the majority of the time and locations, the dose would be considerably less than this due to higher latitude, season (not midsummer) or clouds. The UV exposure on a 1° latitude by 1° longitude grid was then calculated for each day from March to September, for the constant exposure time in the noontime period, from the 10-year climatology [36], and converted to vertical incidence [35]. Finally the daily doses were summed to give an annual "little and often" cumulative UV dose. Exposure from October to February was not included as this is the period of the vitamin D winter in UK [48].

A year-round observational study of vitamin D status, diet and sun exposure in Manchester, UK [48], allowed calculation of 25OHD spend (from decrease in circulating 25OHD through the winter) and assessment of the summer 25OHD level that must be attained for 97.5% of the population to remain clear of deficiency at the end of winter [49]. End winter deficiency was defined as 25OHD < 25 nmol/L to match the Scientific Advisory Committee on Nutrition (SACN) assessment for oral intake of vitamin D [11]. The increase in circulating 25OHD that must be attained through summer sun exposure (also accounting for year-round spend) was then assessed. This was related to sun exposure through a study that gave regular doses (1.3 SED) of simulated summer sunlight to participants over a 6-week period [50] so that the long-term change in 25OHD from "little and often" exposures could be quantified. The target summer increase in 25OHD was thus converted to a target summer sun exposure (assuming this was achieved with regular, low-dose exposures). The skin area exposed during the simulated summer sunlight exposures [50] was 35%, equivalent to wearing modest shorts/skirt and T-shirt. Skin area was adjusted in the model by linear interpolation to other skin areas, as before, and the model also runs for 10% exposed skin area (hands and face only) throughout the summer and 10% in the cooler months of March–May and September plus 35% in June–August [49]. Finally, the target summer sun exposure was compared with the climatological summer sun exposure attained through 9-min exposure every day at lunchtime, with each of the three skin area scenarios. The process above covers white-skinned individuals (skin types 1–4, with human study participants predominantly skin types 2–3). The calculations were repeated for skin type 5 [51] using a simulated summer sun study of the same skin type [52].

Assumptions and Limitations

The calculations [49, 51] are based on UV climatology [36], that is, the average ambient UV that might be expected. The actual UV available from year to year will vary with the weather conditions, with "good" and "bad" summers providing some variation about this norm. Figure 20.7 shows the range of annual variation in June daily UV doses across Europe.

There are multiple and varied ways to achieve a given input to 25OHD status through dose of UV (irradiance and time variables) and skin area exposed. The strictures of constant time and lunchtime period for exposure are selected for simplicity and because noon exposure is most efficient and incurs least erythema risk per vitamin D benefit, while coinciding with lunchtime when people generally have time to step outside. Fixed skin areas are associated with acceptable seasonal dress and directly linked to the underlying studies.

While exposures for vitamin D synthesis have been translated to vertical surfaces (upright human body), the model still assumes that exposure takes place in an unshaded location with a broad horizon, e.g. in an open field or park. More limited exposures available in city streets with a small sky view factor have not been accounted for.
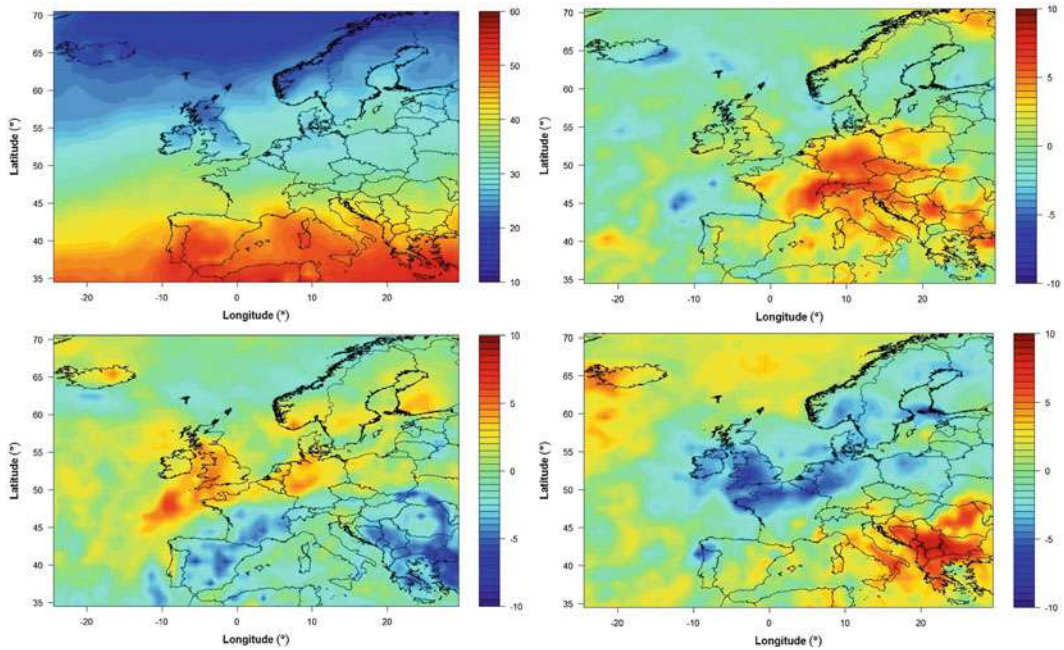
Fig. 20.7 Ten-year (2003–2012) UV climatology and annual deviations across Europe, after Kazantzidis et al., 2015. The colour scale units are SED (1 SED $= 100 \, \mathrm{Jm}^{-2}$ erythema effective UV: note different scale for top left panel) and represent the daily average UV dose for the month of June. Top left, 10-year climatology. Top right, deviation from climatology for year 2003, which brought heatwave conditions across Europe. Bottom panels show regional differences in year-to-year variations: bottom left is 2010, bottom right 2012

The human studies [48, 50, 52] on which the calculations are based did not exclude dietary vitamin D, but dietary intake was assessed, and supplement use was an exclusion criterion for the simulated sunlight studies, but not the year-round observation studies. Therefore the dietary intake from these studies (median < 130 IU per day in all studies) is inherent in the results.

The underlying skin type 5 year-round observation study [53] did not have sufficient participants with circulating 25OHD at elevated levels in summer to enable 25OHD spend and end summer 25OHD requirements to be reliably assessed. Therefore data from the white-skinned study [48] was used on the assumption that spend is independent of skin type at equivalent 25OHD status [51].

The action spectra used in this study are the same as in the global case, and the same assumption about linearity between skin area exposed and resultant change in circulating 25OHD has been made, therefore the same associated caveats apply.

Results

The constant exposure time for the white-skinned population equated to 9 min [49], while that for skin type 5 (set equivalent to 2.75 SED) was 25 min [51]. Assuming lunchtime exposure for this skin type-dependent period every day, whatever the weather, it would be possible to avoid vitamin D deficiency year-round, and across the UK (50–60° latitude) if skin area exposed was 35%. Vitamin D deficiency would also be avoided if the skin area exposed was 35% only from June–August and 10% for the remaining months. In this case the potential for vitamin D provision through sun exposure in northern Scotland becomes marginal, while in the south of England, it remains easily attainable. Exposing only hands and face (10% skin area) throughout the summer would not meet vitamin D requirements at any UK location for any skin type assessed.

While vitamin D requirements can in principle be met through sun exposure in a skin type

5 population, in practice this was not observed [53]. The need to spend 25 min per day in the sun at lunchtime and to expose 35% skin area at least in some months is both demanding and contrary to the cultural mores of this section of the Manchester population [54]. They tend to seek shade when outdoors, and women in particular tend not to expose more than hands and face in public. Dietary intake in this population group was also lower than for the white-skinned population, and 25OHD levels were consistently low throughout the year [53].

The definition of meeting vitamin D requirements is defined in this example as remaining clear of deficiency (25OHD > 25 nmol/L) throughout the year, accepting that at UK latitudes there will be a natural seasonal cycle in vitamin D status as dietary intake is low and UV-induced synthesis of vitamin D is demonstrably season-dependent. If sufficient vitamin D is accumulated during the summer, then given its half-life in the circulation and potential for storage, it can provide for needs during the winter. The nadir of the seasonal cycle is set low, recognising that to attain this wintertime target, summertime vitamin D status must be much higher. SACN [11] concluded that the dietary equivalent intake needed to remain with 25OHD > 25 nmol/L year-round, ignoring cutaneous synthesis, is 400 IU per day. Observed intake in the Manchester groups studied was less than a third of this [48, 50, 52, 53].

While the 9 min exposure at lunchtime has been calculated for the UK, it would apply to other locations at the same latitudes and with similar weather. At lower latitudes the vitamin D winter period becomes shorter, and UV irradiance is greater at noon year-round as latitude decreases. Both these effects would serve to shorten the necessary exposure time, assuming skin area exposed remains the same. Moving to higher latitudes, the opposite is true, and exposure times would need to increase as the season for viable vitamin D synthesis becomes shorter and the available UVB irradiance decreases.

These climatology and human-based studies from the UK and the idealised modelling of global UV exposures begin with different criteria but deliver consistent messages. Vitamin D requirements, stated as either remaining at 25OHD > 25 nmol/L year-round, or as equivalent to 400 IU oral intake per day, can be met by sun exposure, at clearly sub-erythemal doses and in realistic clothing and climate conditions from the equator to at least latitude 60°. In principle this also applies at higher latitudes if skin area exposed is not a restriction.

For the higher levels of vitamin D supply (equivalent to the oral intakes of 1000 or 4000 IU per day, which aim to retain a higher 25OHD status year-round), a purely sun exposure supply becomes increasingly challenging. At high latitudes these targets become impossible in any practical sense, particularly for those with pigmented skin.

Public Health and Personal Choice

One Size Does Not Fit All

It is clear from the illustrations above that there is no single, simple recommendation for sun exposure for vitamin D synthesis that will apply to all people and all locations, even should a standard target vitamin D status or daily intake be agreed. It is therefore a harder message to convey than the simple "Stay out of the sun" health policy that it contradicts. UV dose requirements, in absolute terms, will depend on skin pigment and age, as well as skin area exposed, while the time taken to achieve that dose will depend on location, season, time of day and weather conditions.

The type of recommendation made by Holick [9] of ¼ MED on ¼ surface area for a 1000 IU equivalent dose is a flexible and practical way to account, at least approximately, for both personal characteristics and time-place-weather considerations. It does require some self-knowledge and a little understanding of influences on UV or use of the UV index [55] as provided in weather forecasts. Surface areas and fractional MEDs can be changed to suit conditions or to provide the equivalent of other vitamin D requirements, within limits.

The fixed time and season-appropriate skin area exposures of Webb et al. [49, 51] are more prescriptive and therefore make for a somewhat simpler public health message by removing the need for detailed self-knowledge and daily adjustment. The guidance would need to be adjusted for different countries (or regions in large nations) to account for the local climatology.

Understanding the Options

Sunlight exposure cannot be the complete answer to vitamin D supply for the global population. For large regions of the world, it is not possible, nor practical, to achieve significant cutaneous vitamin D synthesis for several months of the year. This problem is exacerbated for highly pigmented peoples living at high latitudes. A further challenge comes from modern lifestyles, compared to the agricultural or hunter-gatherer practices of our ancestors. Sun-initiated vitamin D synthesis may be an adequate solution for a larger number of people if a seasonal cycle in Vitamin D status, as widely observed, is not detrimental to health. This would seem to be the case for bone health so long as the winter time dip in vitamin D status is not too low and does not last too long. The effect on other potential benefits of vitamin D is an unknown factor.

Where sunlight cannot provide adequate vitamin D, whether because of latitude, weather, pigment, age or culture or because the selected requirements would in many cases incur erythema, oral intake (diet and supplements) is an alternative means to uphold vitamin D status. Food fortification is one means to increase the vitamin D content of modern diets [56], but this is variable from country to country and cannot be relied upon to reach those parts of the population most in need of extra vitamin D. A vitamin supplement containing a known dose of vitamin D is the most reliable method of ensuring a steady supply of the vitamin, assuming compliance and no issues of malabsorption from the gut. Vitamin supplements must be purchased and can be costly in the long run, while sunlight is free, if not always freely available.

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Part VIII

UV-Induced Cutaneous Synthesis of Vitamin D and the Physiologic Consequences – II. Promise and Outlook

The Paleolithic Nutrition Model in Relation to Ultraviolet Light and Vitamin D

21

Reinhold Vieth

Abstract

The biology of every species has been optimized for life in the environment in which that species evolved. Humans originated in the tropics, and while some natural selection took place in response to behaviors and environments that decreased exposure to ultraviolet light, there has never been a species-wide biological accommodation. Paleolithic nutrition advocates argue that risk of disease is higher because modern diets differ from what was consumed by early humans. Early humans were the naked ape living in the tropics, exposed to high levels of ultraviolet light and vitamin D nutrition (serum 25-hydroxyvitamin D; 25(OH)D) averaging 115 nmol/L, as compared to today's population averages that are well below 70 nmol/L. Natural selection from an available gene pool cannot compensate fully to an environmental change away from the one within which the species originally evolved. Vitamin D nutrition remains a contentious area. The epidemiological evidence consistently relates lower 25(OH)D to higher disease risk. However, evidence from double-blind clinical trials looking at preventing new disease in healthy volunteers has been disappointing. But such negative trials have been the case for all nutrients except for folic acid which lowers risk of spina bifida. The Paleolithic nutrition model is based on fundamental biological concepts, but it has overlooked the environmental effects of ultraviolet light and vitamin D nutrition. This paper presents evolutionary and Paleolithic aspects of ultraviolet light and vitamin D with the aim to support pertinent research and, ultimately, public policy regarding nutrition and light exposure.

Introduction

Recent years have seen multiple debates as to what dietary policy should target in terms of circulating levels of 25-hydroxyvitamin D (25 (OH)D) [5, 46]. Dietary guidelines follow risk-benefit profiles, but they mainly focus on risk. The starting point for nutrition policy makers are intakes and levels of nutrient that are typical of people who are regarded as generally healthy. Any upward change to nutrition or sun protection

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policy requires the highest level of evidence, and that is defined as double-blind, placebo-controlled clinical trials [5, 7, 25]. However, "evidence-based medicine" does not mean that the only meaningful evidence is the pharmaceutical-licensing model of double-blind, placebo-controlled clinical trials [38]. Evidence encompasses a breadth of sources, from the bottom of the evidence pyramid upward.

The purpose of this article is to present a biologically based perspective. In the present analysis, the starting point is not what levels of vitamin D nutrition or sun exposure activity is typical among healthy members of modern societies. Instead, my contention here is that the thinking about the optimal ranges for serum 25 (OH)D and for sun exposure should start from what these were for the first humans.

The evolutionary perspective is rarely presented in medical curricula, yet it offers significant insights into understanding health and disease [19]. There have been perspectives published about the Paleolithic diet [13, 26, 50]. Recent epidemiological investigations show that Paleolithic or Mediterranean diet patterns are indeed associated beneficially with all-cause and cause-specific mortality in the United States [50]. However, I am not aware of any mention in the literature on Paleolithic nutrition, about the role of vitamin D or of ultraviolet light exposure. This would appear to be a major oversight, since biologically modern *Homo sapiens* first appeared in the horn of Africa, where exposure of skin ultraviolet light with its endocrine effects should be obvious issues that need to be addressed in the context of both understanding disease and optimizing human health.

The sun exposure experienced by the original humans should be regarded at as optimal because it was the environment for which the genome of the human species was selected, to result in what is now, its modern biological form. Therefore, it is logical that consideration should be given to reversing the traditional way that policy groups approach nutritional adequacy. Traditionally, the question is: "What is the minimal amount of nutrient needed to prevent disease?" Instead, it appropriate to ask, "At what point does human health suffer from progressively diminishing exposure to sunshine and vitamin D?". This is because the historic progression of these things has gone from abundance in the past, to the minimal levels of vitamin D and sunshine now regarded suitable for sustaining health.

Adaptation

Like all primates, humans are a species whose biology is best suited to inhabit tropical latitudes. The fitness of a species to its environment can be achieved through adaptation, or evolution. Fleagle explains that the nongenetic process of adaptation to environmental change is a characteristic that allows an organism to live and reproduce in an environment where it probably could not otherwise exist. Such adaptation can be achieved through processes other than natural selection. For example, *Homo sapiens* have adapted to environments through cultural and technological means such as clothing, shelter, heating, or air-conditioning. In contrast, natural selection is the process, whereby heritable features, be the anatomical or behavioral, that enhance the fitness of an organism relative to its peers, will increase in frequency in the population in succeeding generations [15]. Adaptation to diminishing vitamin D nutrition and sunshine happened because of human intelligence. But for present-day populations that are native to the tropics, their biology has certainly not adapted to less vitamin D and sunshine than what their ancestors experienced.

Natural Selection

Genetic variation develops in species because of the accumulation of random imperfections that occur during the replication of genes. Those imperfections are due to chemicals, radiation, or copying error. The overall assembly of genes within a species is referred to as a gene pool. Distinct differences in any specific gene from among individuals are referred to as alleles. Alleles may or may not alter the protein encoded

by a gene. But as the number of alleles proliferates, the gene pool expands so that there is potential for some alleles of certain genes to provide certain individuals in a species with specific survival advantage (fitness) over other individuals who do not possess those alleles on their genes.

Natural selection is the process by which those individuals of a species, who possess genes that confer greater fitness of those individuals to their environment. "Fitness," in the context of natural selection, pertains to the ability to produce more offspring that are viable to the extent that they will give birth to offspring of their own. Natural selection increases the proportion of a population that exhibits a genetic makeup more fit for an environment.

The means by which the skin color of human populations became lighter as humans migrated away from the tropics involved only one final aspect of evolution: natural selection. There was no evolution in the complete sense of the word. A gene pool existed among those persons migrating out of Africa tens of millennia ago, from which genes could be selected that maximized the ability to give birth and to grow healthy offspring. The vitamin D hypothesis is a widely accepted mechanism driving natural selection for lighter skin color in persons migrating away from the tropics [22, 23].

The purpose of this paper is not to debate skin color or evolution, but rather to use their principles to address the question: What level of sun exposure and/or vitamin D nutritional status (serum 25-hydroxyvitamin D; 25(OH)D) should be regarded as optimal for human life?

In this broader context of what is optimal for all aspects of health, one needs to incorporate factors that have little to do with the traditional sense of fitness in the evolutionary sense. Evolutionary fitness relates simply to maximization of the production of healthy babies [45]. What modern medicine regards as optimal extends to other aspects of life, such as prevention of osteoporosis, cancer, heart disease, and immune disorders. These latter items are not traditional aspects of biology that would confer "fitness" to a species, because they pertain largely to the phase of life

beyond the reproductive years. Cross-sectionally, there is abundant evidence that higher sunshine exposure and/or higher 25(OH)D lower mortality due to many diseases [8]. These beneficial relationships remain an undeniable fact, regardless of those who would dismiss the epidemiologically demonstrable beneficial relationships with 25(OH)D to lifestyle factors, or who would dismiss benefits for sun exposure based on perceived risks due to skin cancer. The relationships have to be due to one or the other or both sun and vitamin D. The health-policy questions remain: Exactly how much vitamin D and/or how much sun exposure should we be aiming for, to optimize health for the population? And should that advice be different for Blacks versus Whites?

It has long been known that despite technological progress, human populations have seen progressive declines in serum 25(OH)D levels over the millennia (Fig. 21.1) [43]. Those older data on serum 25(OH)D have been confirmed in recent years; however, now there is the important new observation that deeply pigmented skin does not prevent the attainment of the same high 25(OH)D levels attainable in Whites [12, 31, 32]. Our best characterization of the nature of sun exposure and, hence, vitamin D nutritional status comes from those small and hard-to-access populations in the tropics whose lives and culture approximates those of the earliest humans. Luxwolda et al. reported serum 25(OH)D in five East African traditional-living ethnic groups across the life cycle: Maasai, Hadzabe, Same Sengerema, and Ukerewe [31, 32] (Fig. 21.2). What is notable are the lower serum 25(OH)D levels of urbanized Africans living in Africa compared to the Africans living a traditional culture. Those urban African levels are a match to the 25(OH)D of Canadians who are of European ancestry. That is, the 25(OH)D levels of White and Black people are the same for urban populations if they live at the latitude of their ancestors. However, once those of African ancestry move north, to Canada, most of them have serum 25(OH)D levels that are lower than 40 nmol/L. At 40 nmol/L, half the population is deemed to possess levels that are not sufficient for sustaining the bone

Fig. 21.1 Evolutionary perspective of circulating vitamin D nutritional status boxes show quartile values for 25(OH)D of the groups represented here, whiskers show the extreme, non-outlier values. Primary references to these images have been published previously [43, 44]
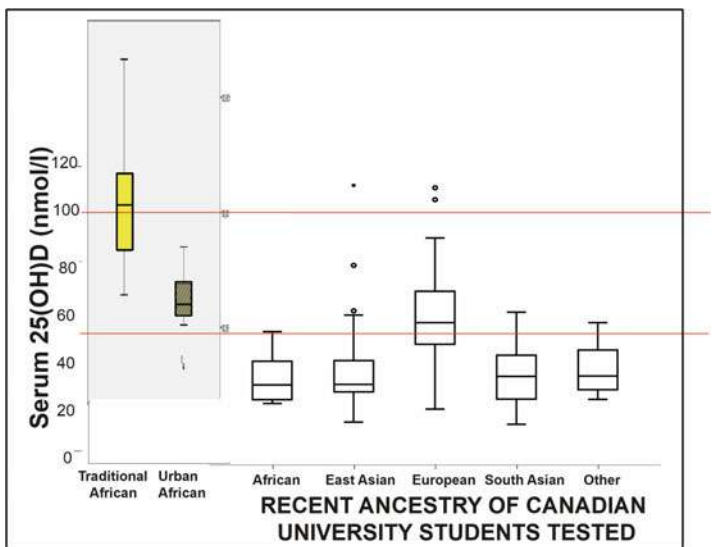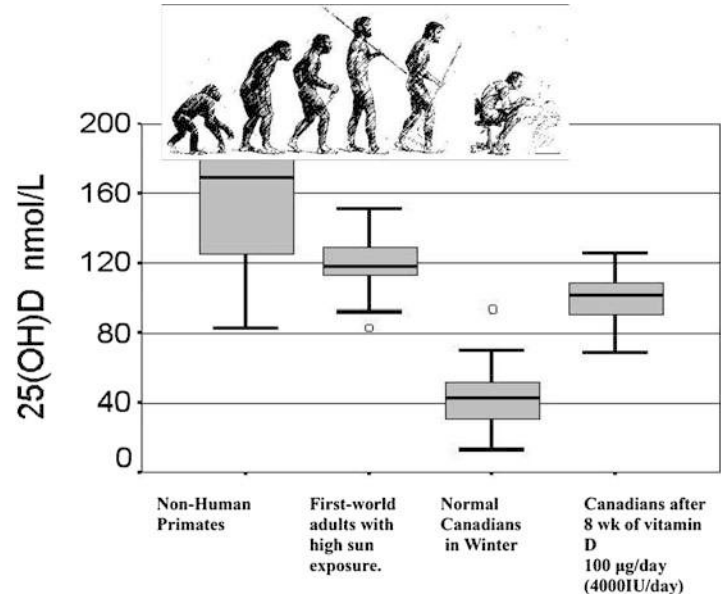
Fig. 21.2 The effect of culture, environment, and ancestry on serum 25(OH)D concentrations These results are compiled from publications that shared similar LC/MS methodology. The figure consist of boxplots which each shows the group's median boxed by the inter-quartile range, and the whiskers that show the highest and lowest values that were not outliers. To the left are data from Luxwolda et al., showing values from tropical Africa, both for Masai, who lived a traditional, pastoral lifestyle, and Bantu, who lived a modern urban lifestyle [31, 32]. To the right are data from two sample sets collected from separate cohorts of Canadian students at the University of Toronto during Winter 2007 [17] and at George Brown College during February 2012 [1]. Note the progressive decline in the African ancestry group, as they shift away from the more traditional culture and environment

health criteria advocated by the Institutes of Medicine [21].

Samples derived from pregnant and lactating women were included. The most striking observation was that despite no supplemental vitamin D, the serum 25(OH)D levels went up during the second and third trimesters of pregnancy, at which point they averaged 150 nmol/L [32]. A smaller but similar rise in serum 25(OH)D that was seen in the traditional-living African ethnic groups was also seen in urban-living African women through pregnancy in women not receiving vitamin D supplement during pregnancy [24]. This natural increase in serum 25 (OH)D during pregnancy coincides with the pregnancy-related increase in vitamin D-binding protein [6]. If total 25(OH)D levels do not increase during pregnancy, then higher concentration of serum-binding protein will result in lower concentrations of the more tissue-accessible, free 25(OH)D. In African women living in their tropical environment, without a vitamin D supplement, the sharp increase in serum 25 (OH)D far exceeds the serum levels typically seen in Western societies. In fact, in Western societies, 25(OH)D levels trend downward during pregnancy [18]. Any objective interpretation from the perspective of basic biology leads to the conclusion that declining 25(OH)D levels during pregnancy are not physiological for humans.

Optimal Paleolithic Sun Exposure and Vitamin D Status

The geographic differences in serum 25(OH)D among people of sub-Saharan African ancestry (Blacks) have also been confirmed by Durazo et al., who tested people living in urban areas [12]. They acknowledge the concept that higher 25(OH)D levels may match our Paleolithic genome as suggested by [31, 32]. Durazo et al. agree that healthy urban adult populations in equatorial Africa have mean concentrations of 25(OH)D in the range of 75–110 nmol/L (30–45 ng/mL) [12]. However, they argued that there are no vitamin D-specific adverse outcomes for northern population groups such as American

Blacks who they showed to have with significantly lower 25(OH)D values than among light-skinned persons. Those authors conclude that it is premature to assert that concentrations in the range of 30–45 ng/mL are more "genome appropriate" for humans [12]. However, they failed to account for the relationships between sun exposure, vitamin D nutritional status, and pregnancy.

It has been argued that it is normal for Blacks to have lower serum 25(OH)D levels than Whites and that the calcium biology of Black persons was an adaptation to their lower 25(OH)D [2]. This latter is an odd perspective, because its logic starts from Blacks living in the United States and works backward, with the teleological argument to explain a current situation. But the perspective ignores the fact that humans originated as Blacks in equatorial Africa and that all natural selection to adapt to temperate, northern environments has been specific to White populations, not Blacks [45].

The consequence of such low serum 25(OH)D levels during pregnancy has been elegantly shown in a post hoc analysis of a clinical trial conducted at the Medical University of South Carolina [48]. Wagner and Hollis conducted a clinical trial using 4000 IU/day vitamin D during pregnancy versus 400 IU/day. When the results were compared based on attainment of a serum 25 (OH)D threshold value of 100 nmol/L (double the 50 nmol/L recommended by the IOM), those women with serum concentrations exceeding 100 nmol/L had a 46% lower rate of preterm birth ($n = 233$, $p = 0.004$); among Hispanic women preterm birth rate was 66% lower ($n = 92$, $p = 0.01$); among African American women the preterm birth rate was 58% lower ($n = 52$, $p = 0.04$) [48]. Therefore, bringing serum 25(OH)D of pregnant women in the United States up into the range that is "normal" for traditional African women who are pregnant lowered the risk of premature birth.

In addition to the clear benefit of higher serum 25(OH)D in the context of preterm birth, [48], higher prenatal exposure to 25(OH)D levels is associated with improved cognitive development and reduced risk of ADHD and autism-related traits later in life [16]. These associations point

to a tragic and potentially high public health burden given the low vitamin D status that seems to be accepted as normal for modern societies, as compared to anthropological norms.

Simple exposure to sunshine is well recognized as being a net benefit to human health and longevity, even without implicating vitamin D nutrition. From a public health perspective, uncontested benefits of sun exposure include certain cancers, multiple sclerosis, diabetes, cardio-vascular disease, autism, Alzheimer's disease, and age-related macular degeneration [20]. One recent study estimated that approximately 12% of US deaths (340,000 persons per year) may be linked to inadequate sun exposure as reflected in serum 25(OH)D levels [8].

Furthermore, as part of the Melanoma in Southern Sweden (MISS) study Lindqvist et al. analyzed the mortality data on 29,518 women across a 20 year period [28]. Women were categorized into four groups: from sun avoiders to those seeking out high sun exposure. Compared to the high exposure group, the sun avoiders were determined to have a risk factor for death of a magnitude that is similar to that of smoking [28] More recently, the same group reported that for this Swedish cohort, living in this low-UV region, the women possessing fair phenotype exhibited 8% lower mortality than non-fair skin women, despite a moderately higher skin cancer mortality among fair skin women [29].

Our species, *Homo sapiens*, has existed in its modern form for 100,000 years. It is reasonable to assume that since that time, there has been no further species-wide evolutionary change. Among some human populations, there has been natural selection driven by geography and environment, and in particular, as driven by sun exposure. Humans first appeared in the tropics, and most of our population still resides there [27]. The large majority of medical and epidemiological research has focused on populations living in temperate regions. Consider that 57% of the world's population lives within 30° of the equator, yet with a mere 5.7% of the population lives north of 49° latitude [27], a region that has produced far more basic and epidemiological research on the topics of latitude and vitamin D. There is very little known about health or disease relationships for the biologically normal sun exposure of the tropics. In fact, most physicians and health policy makers intensely focus on giving advice that populations should minimize such exposure [39, 40]. Evidently, it is normal to assume that "normal" human populations are those that live outside the tropics.

It is not biologically natural for humans to inhabit temperate latitudes with their seasonality and to shield their skin from sunlight. Tropical sunshine, along with its higher intensities of ultraviolet light throughout the year, is the striking features of the environment in which the human species arose. With our technologies that provide shelter, heat, and clothing, we modern humans create artificial microenvironments that are only a partial substitute for life in the tropics. What is missing are substitutes for the tropical the exposure of skin to sunshine. The vast majority of modern societies that presently inhabit the tropics possess serum 25(OH)D levels that match the lower 25(OH)D levels of the inhabitants of temperate climes. Cultures of sun avoidance are now common among modern populations living in the north as well as in the tropics.

Because of migration and cultural changes, most of the human population no longer resides at the optimum environment (Fig. 21.3). For humans, biological changes due natural selection did occur among human populations that moved away from the tropical environments for which our species was originally optimized. Furthermore, as humans migrated away from the tropics, they adopted behaviors that related to sun avoidance, such as indoor living, indoor work, shade-seeking behavior, and clothing. The consequences of that environmental change are well known: lower vitamin D nutritional status and diminished exposure of skin to ultraviolet light. Recent decades have seen large changes in the ethnic demographic within first-world countries. It is well known that for non-White immigrants, sun exposure and vitamin D nutritional status are much lower than they are for Whites. Furthermore, most nutritional and epidemiological research offers minimal insight
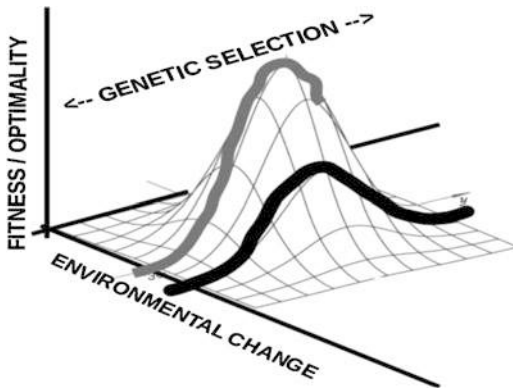
Fig. 21.3 The interactions between an environment change and natural selection as those influence the fitness of a species to its environment. The term, optimality, is used here to refer to those aspects of human health that are not likely to influence the reproductive process that relates to traditional biological sense of fitness of a species. The major premise here is that the genetic makeup of a species was achieved through the process of natural selection for those characteristics that maximize reproductive success. Hence, by definition, the biology of a species is one that is optimized for life in the environment in which the species evolved. The heavy, light-gray line represents the role of genetic variation at the optimal environment for the species. The heavy, black line represents the role of genetic variation at an environment that is substantially different from the original environment that was optimal. Natural selection cannot completely correct the deficiencies of a nonnatural environment. In other words, natural selection can achieve a local maximum, but it cannot reattain the level fitness/optimality of the original environment [33]

to the health of non-White sub-populations living in first-world countries. The classical view of evolution implies that for a given genome, natural selection within a population selects for features that produce a relative optimum for the environment (Fig. 21.3). A change in environment imposes new stresses that are accommodated through adaptation and further natural selection from within the preexisting genome, with the end result that biology largely – but not completely – adapts to the new environment. I contend that the adaptation to the new environment is an incomplete one, in that the fitness/optimality can only occur within the range of the genome available. Table 21.1 lists conditions that would support the contention illustrated by Fig. 21.3, which the biology of the human species has not fully adapted to its avoidance of sunshine, with its

environmental effects of diminished ultraviolet light and vitamin D production.

Non-vitamin D Effects of Ultraviolet Light

Absorption of ultraviolet light by the skin triggers mechanisms that defend skin integrity and that regulate homeostasis systemically. However, this is accompanied by greater risk of skin pathology (e.g., cancer, aging, autoimmune responses). These effects are consequences of transduction of UV electromagnetic energy into chemical, hormonal, and neural signals, as determined by the nature of the chromophores and tissue compartments receiving specific UV wavelengths. Ultraviolet radiation can upregulate local neuroendocrine axes, and for this, UVB is markedly more efficient than UVA. The locally induced cytokines, corticotropin-releasing hormone, urocortins, pro-opiomelanocortin peptides, enkephalins, or others can enter the circulation to produce systemic effects, including activation of the central hypothalamic-pituitary-adrenal axis, opioidogenic effects, and immunosuppression [41]. All of these effects are independent of vitamin D production. However, because the preceding effects happen in the short term, their existence begs the question, if the non-vitamin D responses to ultraviolet light are indeed biologically meaningful, then what is the consequence of prolonged deprivation of ultraviolet light exposure?

One interesting example of a likely endorphin effect is a well-being experiment conducted by Feldman et al. In that experiment, subjects were given sunlamp treatments on three occasions per week for 6 weeks: randomly and blinded, once with an ultraviolet emitting lamp, once with a lamp not emitting ultraviolet light. At the third weekly occasion, subjects could select their preference treatment for the session. At 39 of 41 cycles of this study, subjects chose to have the ultraviolet light for their session, claiming that their choice elicited a more relaxed less tense mood [14]. Light has long been known to influence brain function [11, 35, 41] and the pertinent

Table 21.1 Evidence that natural selection has failed match the levels of fitness and optimality of the tropical environment where our species originated

Latitude/environment related
Adaptation involved lighter skin pigmentation which is more susceptible to burning and skin cancer
Part of that adaptation involved a change to pelvic bone to prevent rickets and osteomalacia, but predisposing to osteoporosis – this is the vitamin D paradox that despite lower 25(OH)D of American Blacks, they possess stronger bones [45]
Prevalence of winter seasonal affective disorder increases with latitude [35]
UVB exposure to skin lowers blood pressure by releasing nitric oxide from skin [49]
Blood pressure correlates inversely with distance from the equator [36]
Risk of rheumatoid arthritis higher at higher latitudes [34, 42]
Mortality higher with sun-avoiding behavior [28]
Higher latitude relates to greater risk and prevalence of schizophrenia [11]
Vitamin D related
Mortality from multiple health conditions decreases with higher serum 24(OH)D levels [8]
Low 25(OH)D levels during pregnancy relate to poor birth outcomes [4, 9, 48]
In older adults, lower serum 25(OH)D concentrations are prospectively related to increased risk of Alzheimer's disease [30]
Higher latitude relates latitude greater risk of dementia relationship [37]
Lower serum 25(OH)D relates to greater risk of multiple sclerosis [3]
Increased prenatal exposure to 25(OH)D levels is associated with improved cognitive development and reduced risk of ADHD and autism-related traits later in life [16]
Higher prenatal serum 25(OH)D levels relate to lower risk of autism and schizophrenia [10, 11, 47]

effects of light on immediate sense of well-being may serve the functional purpose of increasing more sunshine-related behavior, including the longer-term effects of improved vitamin D nutritional status.

Conclusion

Paleolithic nutrition has focused on foods consumed, but the Paleolithic model extends beyond diet to incorporate environment, which is equally relevant to health policies in the context of sunlight exposure and vitamin D nutrition. Biologically based thinking starts from the basic premise that disease risk may have an evolutionary underpinning and that modern human cultures and environments are probably not a substitute for what is natural or optimal [19]. Natural selection is a process that optimizes the choices from the available menu of options within the genome for fitness to reproduce. Within the relatively recent evolutionary context of modern human existence, the environmental stresses due to latitude, clothing, and sun avoidance cannot have altered

biology optimally and certainly not across all population groups.

The perspective of health policy makers has been to adhere to what is prevalent among healthy populations, unless there is overwhelming evidence that more sun or more vitamin D intake produce a benefit. This approach is not well justified, because there are many adverse relationships associated with diminishing vitamin D nutrition status [8] and sun avoidance behavior [28]. Application of the Paleolithic nutrition way of thinking to vitamin D nutrition and ultraviolet light exposure is logical, both from the perspective of basic biology and from the perspective of epidemiology.

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