



Mini-review

Vitamin D and breast cancer: Emerging concepts



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ABSTRACT

The benefit of vitamin D in cancer prevention and to certain extent therapy has been well recognized. The active form of vitamin D, 1,25-dihydroxycholecalciferol (1,25(OH)₂D₃) is a natural ligand for vitamin D receptor (VDR). Since 1,25(OH)₂D₃ exerts toxic effects at a concentration that is beneficial, nearly 1500 analogs of vitamin D have been synthesized and evaluated for their efficacy in a variety of carcinogenesis and human cancer models both in vitro and in vivo. Among these only a handful of them have been approved for evaluation in clinical trials for leukemia, breast, prostate and colon cancers. The mechanism of vitamin D action is mediated by the nuclear VDR and the signaling cascade for its action is extensively reported. In this review we focus on the newer concepts for vitamin D action. These include (1) differential effects of vitamin D in maintaining cell proliferation when the cells are under stress but suppressing cell growth when the cells are transformed; (2) functional significance of VDR polymorphism in potential vitamin D responsiveness; (3) regulation of constitutive splicing of vitamin D target gene, CYP24a, by the hormone and its significance; and (4) regulation of microRNA by vitamin D in breast cancer. It is anticipated that the new work in these selective areas would expand the understanding of vitamin D in breast cancer prevention and therapy.

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1. Introduction

1.1. Vitamin D and cancer

Vitamin D is a steroid hormone from a family of 9, 10 secosteroids. It was originally discovered by Edward Mellanby in 1919 while working on rickets [1,2]. The classification of vitamin D (cholecalciferol) is derived from its differences in the structures of their side chains. Vitamin D is classified into six subclasses: vitamin D₂ is ergosterol (24 methylergosterol); D₃, cholecalciferol; D₄, 24 methylcholecalciferol; D₅ sitosterol (24-ethylcholecalciferol); D₆, Stigmasterol (24 ethylergocalciferol) and D₇, 24-cis-methylcholecalciferol [3]. Vitamin D is derived from either food (milk, fish) or by exposure to sun light. The UV rays from the sun convert 7-dehydrocholesterol to secosteroid by the cleavage of B-ring through photolysis mediated thermo-isomerization. In the past the toxicity profile of different forms has been completed. The results have shown that vitamin D₃ is the most toxic form whereas D₅ is the least toxic [4]. A general metabolism scheme has been established, which indicates that vitamin D gets metabolized to 25(OH)D₃ by CYP27A1 (25-hydroxylase) in the liver and subsequently to 1,25(OH)₂D₃ by CYP27B1 (1-hydroxylase) either in

the kidneys or in the target organs. Studies in the past several years have shown that epithelial cells where vitamin D exerts its effects express CYP27B1 and therefore metabolism by vitamin D target organs is considered feasible [5].

The primary role of vitamin D has been considered to be in calcium homeostasis in the body and is essential for bone mineralization. Over the years, it has become increasingly clear that vitamin D not only has a function in bones, but it also significantly affects cell proliferation and differentiation. In cancer cells, the active metabolite of vitamin D, 1,25-dihydroxycholecalciferol (1,25(OH)₂D₃) suppresses cell proliferation [6]. The overall role of vitamin D in cancer also is largely supported by epidemiological observations. Cancer incidence specifically for that of colon and breast cancer is lower geographically where there is increased sun light [7]. Similarly plasma levels of 25(OH)D₃ are also well correlated with the disease. Although there is no agreement on the required serum level of 25(OH)D₃ as being ideal, there are numerous reports suggesting ideal, adequate or deficient categories for vitamin D. Serum levels of 25-hydroxyD₃ of <20 ng/ml is considered as deficiency of vitamin D whereas 20–30 ng/ml as insufficiency, and 30–80 ng/ml as optimal [8]. Holick suggested the ideal concentrations to be between 75 and 150 ng/ml. The toxicity of vitamin D occurs at concentrations of >375 ng/ml [8]. These studies support case control studies where 87% of the triple negative breast cancer patients had inadequate levels of serum 25(OH)D₃. One of the recent reports indicated that serum concentration of 130 nM or approximately 50 ng/ml 25(OH)D₃ protected against breast cancer

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by nearly 50% [9]. Similarly there have been several correlative studies that have indicated positive correlation of serum vitamin D levels and protection from colon and prostate cancers [10]. These results would then provide a rationale for using vitamin D for cancer prevention or therapy.

1.2. Vitamin D analogs and breast cancer prevention and therapy

It has been well established that the mechanism of vitamin D action is mediated by the vitamin D receptor (VDR). The active hormone 1,25(OH)₂D₃ can induce a cascade of gene regulation and signaling molecules by binding to VDR [11]. This would make 1,25(OH)₂D₃ an ideal chemopreventive or therapeutic agent. However the active form of vitamin D is very toxic at concentrations that would provide a protective effect. Under these circumstances nearly 1500 analogs of vitamin D have been synthesized with expectation that altering the molecular structure of vitamin D can generate a relatively non-toxic analog retaining its cell differentiating properties that makes the vitamin D effective [12,13]. Amongst the newly synthesized analogs of vitamin D, only a handful has been followed through for their activity as low-calcemic, efficacious antiproliferating agents. These include EB1089, KH1060, 22-Oxacalcitriol, calcipotriol, 1 α (OH)D₂, hexafluoro-1,25(OH)₂D₃ (RO24-5531) and 1 α (OH)D₅ [14,15]. More recently a new class of vitamin D analogs characterized by two side-chains attached to carbon-20 (Gemini) and deuterium substituted on one side-chain have been synthesized [16]. Although these analogs do not have any adverse calcemic effects of vitamin D₃, they do induce toxicity unrelated to calcemia. These agents are in the process of development for clinical use. Finally some analogs have been synthesized that are structurally unrelated to vitamin D and yet interact with VDR [17]. They have not been evaluated in *in vivo* extensively. The basic definition and approach for chemopreventive agents include effectiveness of compounds in increasing latency period for the appearance of the first tumor, decreasing tumor incidence, and multiplicity. Over the years this approach has been modified to include molecular mechanisms, identification of surrogate endpoint markers, and enhancing or blocking certain signaling pathways. Mammary carcinogenesis studies *in vivo* has utilized two models; N-methyl-N-nitrosourea (MNU) and 7,12-dimethylbenz(a)anthracene (DMBA) induce mammary adenocarcinoma in rats [18]. The majority of the tumors induced by MNU are ER+, PR+ and VDR+ adenocarcinoma whereas DMBA induced tumors are both adenocarcinoma as well as fibroadenoma. Amongst the vitamin D analogs evaluated, RO-24-5531, 1 α (OH)D₅, MC903, EB1089, 1 α (OH)D₃, 1,25(OH)₂D₃ have shown efficacy in reducing tumor multiplicity in the MNU-induced mammary carcinogenesis model. One major problem is encountered in *in vivo* experiments is hypercalcemic activity of vitamin D analogs [19]. All the agents studied were less calcemic as compared to the 1,25(OH)₂D₃. However the studies were compromised due to increased calcium at concentrations effective for reducing tumor multiplicity for the majority of the analogs except 1 α (OH)D₅, which did not induce hypercalcemia at concentrations that reduced the tumor multiplicity and increase tumor latency. Using the DMBA-induced mammary carcinogenesis model, once again 1 α (OH)D₅ reduced tumor multiplicity in rats [20,21] with no toxic effects. In addition, two Gemini classes of vitamin D analogs have also been evaluated in the MNU-induced mammary carcinogenesis model. Gemini 0072 [1 α ,25-dihydroxy-20S-21(3-trideuteromethyl-3-hydroxy-4,4,4-trideuterobutyl)-23-yne-26,27-hexafluoro-19-nor-cholecalciferol] and Gemini 0097 [1 α ,25-dihydroxy-20R-21(3-trideuteromethyl-3-hydroxy-4,4,4-trideuterobutyl)-23-yne-26,27-hexafluoro-19-nor-cholecalciferol] administration inhibited the tumor multiplicity significantly at non-calcemic concentrations [22]. These and some other Gemini analogs are under intense evaluation for their

efficacy in suppressing cancer growth in xenograft models [23]. Since it has been extensively reported in the literature that the effects of vitamin D are mediated by vitamin D receptor (VDR), a few studies have been reported on the effects of DMBA on mammary tumor formation in intact versus VDR-KO mice. The results showed that in fact VDR was protective in the sense that VDRKO mice resulted in more aggressive tumors and multiplicity was increased [24]. This raises a question whether VDR by itself has a (function), if so then would there be any difference between VDR that is liganded with 1,25(OH)₂D₃ and the one that is unliganded. There are several reports distinguishing differences in the action of liganded and unliganded receptors including a recent report from our laboratory [25].

The premise of chemoprevention suggests that the chemopreventive agent ought to be effective in preventing transformation of normal cells or progression of transformation to a tumor. However the most crucial question is when does prevention end and therapy begin. For example the first step in identifying a chemopreventive agent in most labs is to determine its antiproliferative activity in cancer cells. This would mean that an agent that is effective at suppressing growth of cancer cells can also be used as a chemopreventive agent. Thus, most of the chemopreventive agents can also be antiproliferative for cancer cells and vitamin D and its analogs are not exceptions. A plethora of investigations have been reported in the literature investigating efficacy and mechanism of action of vitamin D and its numerous analogs in a variety of target organs and cancer types. Typically, 1,25(OH)₂D₃ a natural VDR ligand and vitamin D hormone mediates its action by binding to VDR. The vitamin D liganded VDR and unoccupied RXR associate to form a heterodimer. Once this complex is formed it recognizes VDRE in the promoter region of the vitamin D target [26]. The nongenomic rapid actions of vitamin D have been extensively reviewed in many reports and has not been considered in the main stream of vitamin D action in prevention or therapy of cancer and therefore is not reviewed here [27]. Nonetheless it is important to point out that recent development of VDR interactions revealed that VDR contains two ligand binding sites, one binding site is a bowl-like pocket or genomic pocket whereas the other one is an alternative planar pocket. The genomic pocket of VDR binds to active-vitamin D and induces gene transcription whereas the planar site is involved in rapid responses. This new concept suggests that both rapid response as well as the genomic function of vitamin D is mediated via VDR [28]. The efficacious analog of vitamin D in most cases would mediate their action in a manner similar to that of 1,25(OH)₂D₃. The effects are mediated via altering several signaling pathways, depending on the target cell, leading to either suppression of cell proliferation by inducing apoptosis, enhancing cell differentiation, blocking cell cycle, inducing expression of inhibitors of cell cycle progression, inhibiting colony formation, reducing cell inflammation, inhibiting cell invasion, or metastasis and downregulating estrogen receptor signaling pathway in breast cancer [29].

Our laboratory has mainly focused on breast and colon carcinogenesis models using 1 α (OH)D₅. This analog was synthesized, chemically characterized, and established as a non-calcemic effective vitamin D analog for mammary and colon carcinogenesis. The preclinical toxicity studies have been completed and the drug is approved for clinical trials. In this review we would like to focus on issues that are relatively new and not much work has been done. We have raised several new questions in uncharted territory of vitamin D research and will be summarized here.

1.3. Update on clinical trials with vitamin D analogs

While the serum level of 25-hydroxyvitamin D₃ is associated with cancer incidence, the clinical trials have not been very

successful due to vitamin D related toxicity at effective dose level. The clinical trial literature (www.clinicaltrials.gov) for vitamin D as single agent lists 16 studies for breast cancer clinical trials. Out of these eight studies have been completed and the others are at various stages of the trial prior to termination. Out of eight completed studies there is only one where vitamin D was used singly and the outcome is interpretable. The majority of clinical trials have utilized 1,25(OH)₂D₃ (Calcitriol) for two reasons. It is a natural hormone and a ligand for vitamin D receptors and secondly the formulation has been well worked out by the pharmaceutical companies. Both injectable (Calcijex, Abbott Pharmaceuticals) and oral (Rocaltrol, Hoffman-La Roche and DN 101 Novacea Pharmaceuticals) formulations of calcitriol are available. Many of these studies have been previously summarized [30]. The overall conclusion is that due to hypercalcemia related toxicity calcitriol could not be used at higher efficacious concentrations and it did not provide any protective efficacy at non-toxic doses. It is also concluded that calcitriol now can be safely administered if the drug is given intermittently. Although numerous analogs of vitamin D₃ have shown efficacy in experimental models, most of them have either not been evaluated for preclinical toxicity to obtain FDA approval for Phase I/II clinical trials or the studies have not been completed and/or reported. Among the most studied vitamin D analogs for breast cancer clinical trials is EB1089 (seocalcitol). One clinical trial with EB1089 was terminated due to toxicity associated with the analog while the other one showed no complete or partial response. Six patients receiving EB1089 for 90 days had exhibited stabilization of the disease [31,32]. In addition to that 1 α -hydroxyvitamin D₂, 19-nor-14-epi-23-yne-1,25-dihydroxyvitamin D₃ (inecalcitol) and 19-nor-1,25-dihydroxyvitamin D₂ (Zeplar) have been evaluated in clinical trials. Unfortunately none of them have resulted in cancer suppressing activity at relatively non-toxic concentrations. We synthesized and characterized 1 α -hydroxyvitamin D₅ and completed preclinical toxicity in rats and dogs. The analog has been approved by FDA for clinical trials. Although we have not been able to initiate a Phase I/II study in women for breast cancer, recently a Phase I clinical trial is successfully completed with 1 α -hydroxyvitamin D₅ (Card-024) for patients with cardiac conditions (R. Simpson, Cardiavent Inc. Personal communication, unpublished). This opens door for possible successful clinical trial with 1 α -hydroxyvitamin D₅ for breast cancer.

2. Emerging concepts

So far as we described above, vitamin D analogs that have been successfully used for the prevention and treatment of breast cancer in experimental models. The mechanism of action suggests clearly that vitamin D mediates its action via VDR and the VDR-1,25(OH)₂D₃-RXR complex mediates its actions by regulating cell signaling pathways. In our laboratory in addition to determining the efficacy of 1(OH)D₅, studying its mechanism of action, and ultimately taking it to a clinical trial, we also have focused on a few non-conventional issues. We posed following questions.

1. If vitamin D is supposed to maintain calcium homeostasis, maintain general tissue health and protect cells from death then why does it suppress growth of cancer cells? To attempt to address this issue, we evaluated effects of stress on normal and cancer cells and determined if vitamin D protects cells against this and if so how.
2. VDR has many polymorphic forms. The role of these polymorphic forms of VDR is controversial. Most of the studies are correlative and epidemiology-dependent. The controversial results may arise from a small sample size. Therefore we mimicked the VDR Fok1 polymorphism by transfecting selected alleles in a

cell line and asked the following question: can cells with specific Fok1 polymorphism VDRFF or VDRff be a risk factor or be predictive of better response to vitamin D?

3. It has been well established that CYP24 is a target gene for VDR. CYP24 catabolizes 1,25(OH)₂D₃ to 1,24,25(OH)₂D₃, an inactive form. There are numerous papers on the activation and inhibition of CYP24 and the mechanism of its action. Knowing this, we asked the following question, can vitamin D regulate formation of the mature form of CYP24 gene? Here we looked at the constitutive splicing of CYP24 mediated by vitamin D.
4. Finally, the literature on microRNA is building and there are a few papers now describing MiRNA regulating some vitamin D regulatory genes. We asked a question, can we identify MiRNA(s) that can regulate selective vitamin D function? While the quest on synthesizing new analogs of vitamin D is continuing and the understanding of molecular mechanisms of vitamin D action is getting intensified the questions posed above need further investigations.

2.1. Dichotomy of vitamin D action

In addition to the main function of vitamin D in promoting calcium absorption in the intestine and maintenance of serum and phosphate levels for bone mineralization, it has also been established that it has a role in cell proliferation and immune function. Therefore vitamin D has been referred to as a 'sunshine vitamin' for general health. Conversely in cancer cells, vitamin D and its analogs, as described in previous sections, suppress proliferation of transformed cells. We investigated possible mechanism for such differential role of vitamin D. Previous studies indicated that 1,25(OH)₂D₃ inhibits growth of both normal MCF12F and breast cancer cells [33]. In our laboratory we transformed MCF12F normal breast epithelial cells with MNU and DMBA and evaluated differences of vitamin D action [14]. It was observed that vitamin D analogs inhibited cell proliferation of transformed cells. Previously, it has also been reported that the active vitamin D₃ metabolite 1,25(OH)₂D₃ protects cells from cell death induced through various pathways [34]. The pretreatment of ovarian cancer cells with 1,25(OH)₂D₃ decreased apoptosis induced by TRAIL and Fas ligand. We examined the effects of vitamin D on cells that are stressed by various stress inducers (e.g., serum starvation and chemical induction) and compared the effects of 25(OH)D₃ and 1,25(OH)₂D₃ on cells that have not been stressed (control) with ones that were stressed [28]. Results showed that under stressed conditions, both 25(OH)D₃ and 1,25(OH)₂D₃ protect MCF12F breast epithelial cells from death; while they inhibited cell proliferation of control unstressed cells. This observation along with other reports in the literature indicates that vitamin D is involved in cell survival signaling. Contrary to that in cancer cells at non-toxic concentrations, vitamin D and analogs induce cell apoptosis and cell death. Therefore, it appears that vitamin D has a distinct function to maintain cellular homeostasis by protecting cells from stress and prevent uncontrolled-proliferation of normal or cancer cells (Fig. 1). The mechanism of such dual action is not currently known but can be of significant importance. Here we address an argument in support of this concept.

VDR has been identified as p53 direct target gene using *in silico* analysis [35]. At the same time, the importance of p53 induction in response to stress in determining cell fate has also been studied. The p53 protein levels are found to be elevated by stress with increased stability. Consequently, p53-responsive genes are trans-activated and cells either are arrested to allow DNA repair or to undergo apoptosis to eliminate the damaged cells. In contrast, cells disrupted for p53 are unable to repair the DNA damage, leading to uncontrolled cell proliferation and malignancy. In normal, unstressed cells, the p53 protein is short-lived (T_{1/2} ~20 min),

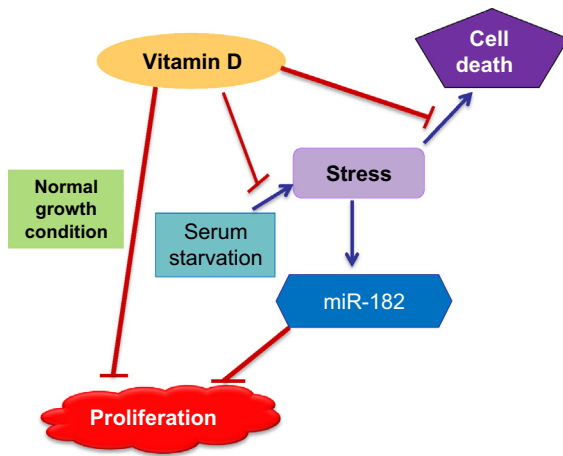


Fig. 1. Differential effects of vitamin D to suppress cell proliferation and prevent cell death. Vitamin D prevents cell death induced by serum starvation mediated stress and prevents cell proliferation of cancer cells under normal growth conditions. These effects are also correlated with stress induced miRNA182. Stress induced miRNA182 enhances cell proliferation, which is suppressed by vitamin D.

reflecting a rapid turnover through ubiquitin-mediated proteolysis [36]. Recently activating transcription factor 3 (ATF3), a stress sensor, has been found to activate p53 by blocking its ubiquitination [37]. ATF3 is rapidly induced by diverse environmental insults including genotoxic stress. Since p53 is a key molecule in stress response and carcinogenesis, we can infer that its direct target gene VDR could be significantly involved in protecting cells against transformation through its anti-stress function. To strengthen the link between p53 and VDR, VDR has further been identified as a target gene of p73, a p53 analog. In addition, VDR is also trans-activated by stress-activated protein kinases p38 and JNK, indicating that VDR is deeply involved in stress-related response [38]. Unlike p53, the VDR activity can be mediated by its ligand vitamin D, suggesting the functional role of VDR and vitamin D in stress response. Moreover some molecules involved in stress response including EGR, p70S6, VDUP1, and thioredoxin are also VDR target genes. Thus, the anti-stress action of preventing cell transformation by vitamin D can allow us to understand its chemopreventive action. This two-way approach can clarify the dual action of vitamin D in preventing cell death and inducing cell apoptosis under two separate conditions.

2.2. Differential action of VDR Fok1 polymorphism

The effects of vitamin D for the most part are correlated with nuclear VDR for the genomic actions. However several polymorphisms in VDR gene have been reported. They include Bsm1, Apa1, Taq1 restriction sites, variable PolyA length and Fok1 restriction site [39]. Of these polymorphic sites Bsm1 and Apa1 are substitutions on intron 8 whereas Taq1 brings about substitution of cytosine to thymine on exon 9. The polyA lengths have been described as 'long' or 'short' and that also occurs on 3' untranslated region of exon 9. Fok1 polymorphic VDR occurs due to a different initiation site. In VDR^{ff} the initiation occurs at the first ATG codon resulting in a 427 amino acid VDR. However due to T to C substitution the first initiation site becomes ACG and the initiation has to start at the next initiation ATG codon resulting in a 3 amino acid shorter truncated VDR. The short VDR has methionine, glutamic acid and alanine missing [40]. There have been a few epidemiological reports correlating VDR Fok1 polymorphism with breast cancer incidence and therefore risk. The results however are controversial, often due to smaller sample size. For example, VDR^{FF} allele in combination with long-Poly A was reported to be

a risk factor in the UK [41], whereas in another report Chen [42] found VDR^{ff} to be a risk factor in the Nurses' Health study in the USA. Yet another report did not find any correlation between Fok1 polymorphism and breast cancer incidence. More recently in two meta-analyses; one from 21 separate studies and large patient population and another one from 8100 control and 6300 breast cancer cases showed a positive association between VDR^{ff} and augmented risk for the disease [43,44]. In our laboratory, we generated and characterized three cell-lines from single cell clones of MCF-7 cells; MCF-7 vector control, MCF-7-VDR^{FF} and MCF-7-VDR^{ff} and determined effects of 1,25(OH)₂D₃. Results showed that cells expressing VDR^{FF} responded to vitamin D better than VDR^{ff} expressing cells [45]. Moreover ER α expression was downregulated significantly in VDR^{FF} cells as compared to VDR^{ff} cells and VDR^{ff} expressing cells exhibited increased expression of pro-inflammatory genes such as COX-2, IL-8 and CCL2. Collectively these studies indicated that the VDR^{ff} genotype plays a significant role in enhancing breast cancer risk and identifies populations that would be better suited for vitamin D treatment based on their Fok1 classification for VDR. The overall significance of all VDR polymorphisms is summarized in Fig. 2.

2.3. Vitamin D regulation of CYP24 constitutive splicing

CYP24A1 encodes for the enzyme 1,25(OH)₂D₃ 24hydroxylase, which is one of the cytochrome P450 superfamily of enzymes. Its principal function is to convert 1,25(OH)₂D₃ to 1,24,25(OH)₃D₃, an inactive form. This also is important to reduce toxicity of active vitamin D₃. CYP24A1 is a VDR target gene and has been targeted for therapy. Inhibitors of CYP24A1 are being designed and synthesized. Suppressing catabolism of 1,25(OH)₂D₃ can make more vitamin D available for its activity. Based on CYP24A1 activity to abrogate anticancer activity of 1,25(OH)₂D₃, CYP24A1 has recently been considered as a potential oncogene [46]. On the other hand, 1,25(OH)₂D₃ in the absence of CYP24A can lead to vitamin D toxicity and that can be a major concern. We have been using CYP24A1 expression as a marker for VDR activity since CYP24A1 is a target gene for VDR. In addition to evaluating CYP24A1 expression in relation to the action of vitamin D and analogs in breast cancer we also have focused on understanding about the processing of the CYP24A1 transcripts and its regulation by vitamin D.

Over the years considerable attention has been given to the significance of alternative splicing in cancer development and progression. However the splicing of a gene that converts precursor mRNA to a functional mRNA has rarely been studied. It has been assumed that the precursor immature RNA gets spliced to a mature functional RNA due to the unstable nature of the pre-mRNA. The spliceosome, a large molecular weight ribonucleoprotein then connects two exons by removing intronic segment [47]. It has also been reported that the splicing of RNA requires transcription activation through nuclear receptor coregulators. One such coregulator, NCoA62/Skip has been reported to interact with vitamin D induced transcripts obtained from GH mini-gene cassette. And therefore it could also interact with VDR and regulate vitamin D mediated splicing [48]. Using RT-PCR we observed that 1,25(OH)₂D₃ induces splicing of CYP24A1 in a time dependent fashion. In colon cancer HT29 cells the splicing began at 45 min after incubation with vitamin D and was completed by 120 min. We also investigated if the splicing of CYP24A1 was intron dependent. The results showed that the splicing at intron 1 was more efficient as compared to intron 10. Moreover the results also suggested that the vitamin D dependent splicing is not an alternative splicing but were a part of the CYP24 mRNA maturing process [49]. Interestingly, the splicing pattern correlated well with the responsiveness of cell-lines to vitamin D. CYP24A1 remained unspliced in the absence of 1,25(OH)₂D₃ and was spliced only when it was

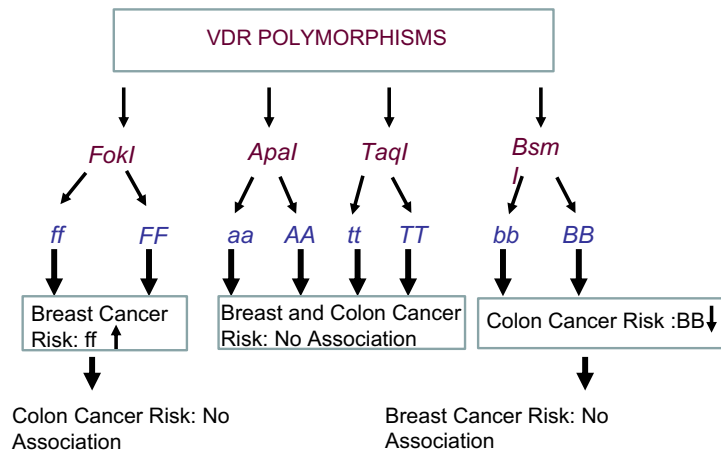


Fig. 2. VDR polymorphisms and risk association for developing colon or breast cancer.

incubated with vitamin D indicating that the splicing was dependent on vitamin D. In addition we have also observed similar regulation for splicing of CYP24A1 by 1,25(OH)2D3 in mammary epithelial cells in mammary gland organ culture (unpublished). Currently, it is premature to consider that this is a general phenomenon for all cells responsive to vitamin D or that it may be a common phenomenon for steroid receptor regulation of splicing of its most responsive gene. This would be an important observation and can provide an insight into whether splicing of a target gene is dependent upon the receptor ligand.

2.4. Vitamin D regulation of MicroRNA

Functional genomics involved transcribing genes for generating mRNA to have translational product required for signaling cascade. Only a few years back a new regulatory role for a non-coding small regulatory microRNA (miR) of about 22 nucleotides has been identified [50]. The principal function of miR is gene silencing. A single miR can regulate multiple genes. The miRs base-pair with mRNA in a perfect or near perfect fashion. This base-pairing association of miR with mRNA promotes cleavage or destruction of mRNA and induces degradation. On the other hand if the base-pairing is not perfect, it triggers translation repression and therefore mRNA is silenced. There are approximately 1500 miRs identified, however since they regulate function of several mRNAs the specific function and the regulatory role has not been fully understood. These results suggest that miR can contribute towards cancer development and may be differentially expressed in normal and cancer tissues and therefore can be exploited as key targets for cancer chemoprevention [51]. During the past few years, a role of vitamin D in targeting miRs has been reported. Earlier we reported that serum starvation altered expression of multiple miRs as determined by microarray analyses of miR including miR182 and let-7a. Incubation of cells with 25(OH)D3 decreased expression of miR182 and induced stress. Overexpression of miR182 in MCF12F cells suppressed cell proliferation [31]. Currently we do not know if vitamin D decreased miR182 is directly involved in protecting against stress induced cell death (Fig. 2). Similar results have been recently reported for colon cancer cell lines. Vitamin D upregulated expression of miR22 and thereby confirming that miR22 can be a vitamin D target [52]. This was further confirmed by reporting that silencing miR22 resulted in abolishing the effects of vitamin D mediated suppression of selective genes. As described in previous sections CYP24 is one of the major target genes regulated by vitamin D. Presence of miR125b recognition element in the 3'-untranslated region of CYP24 mRNA led to the study in MCF7 cells. It was re-

ported that miR125b regulated expression of CYP24. It was also observed that there was an inverse relationship between CYP24 protein and cancer-miR125b [53]. It is interesting to note that miR125b post-transcriptionally regulated expression of VDR in MCF-7 cells. 1,25(OH)2D3 downregulated expression of miR125b with simultaneous induction of VDR [54]. More recently it has been reported that miR498 induced by 1,25(OH)D3 decreased expression of human telomerase reverse transcriptase (hTERT). In this study also miR498 targeted 3'-untranslated region of hTERT mRNA. These results suggest that one of the mechanisms by which miRNA regulated by vitamin D may function by telomerase regulation in ovarian cancer cells [55]. Interestingly, miRNAs have also been detected in serum or plasma. In a preliminary study, the plasma profile of miRNA after supplementation with vitamin D was generated for 10 patients [56]. Results showed 136 miRNAs were detected in vitamin D treated patients after a year of supplementation with vitamin D, of which 12 miRNAs were further confirmed with qRT-PCR. The authors concluded that there was a significant correlation between serum 25(OH)D3 and miRNA 532-3p and miR 221 expression. However the authors also pointed out that this is a preliminary report on small number of samples. Nonetheless this is an emerging approach for determining vitamin D action and its altered expression of miR in plasma may provide a very useful biomarker for determining vitamin D responsiveness.

3. Conclusion

The role of Vitamin D and its non-toxic analogs has been very carefully studied for the past 20 years. It has been recognized that vitamin D has chemopreventive effects on the development and progression of cancers including breast cancer. The effects of vitamin D are mediated largely by VDR and its signaling transduction. However in this mini-review we have tried to identify new areas that may prove important for vitamin D action. These include differential role of vitamin D for normal, stressed normal and transformed breast epithelial cells, importance of VDR polymorphism in identifying patients that may better respond or not-respond to vitamin D, vitamin D regulation of mRNA may involve yet another layer of regulation through miR and finally the splicing of regulatory genes of vitamin D may require vitamin D for gene-splicing.

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