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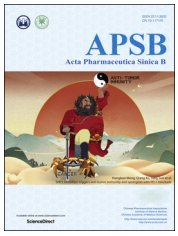

REVIEW

# Repurposing vitamin D for treatment of human malignancies *via* targeting tumor microenvironment

**Xu Wu[a,†], Wei Hu[b,†], Lan Lu[c,†], Yueshui Zhao[a], Yejiang Zhou[d], Zhangang Xiao[a], Lin Zhang[a,e], Hanyu Zhang[a], Xiaobing Li[a], Wanping Li[a], Shengpeng Wang[f], Chi Hin Cho[a], Jing Shen[a,*], Mingxing Li[a,*]**

[a]*Laboratory of Molecular Pharmacology, Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou 646000, China*
[b]*Department of Gastroenterology, Shenzhen Hospital, Southern Medical University, Shenzhen 518000, China*
[c]*Sichuan Industrial Institute of Antibiotics, Chengdu University, Chengdu 610106, China*
[d]*Department of Gastrointestinal Surgery, The Affiliated Hospital of Southwest Medical University, Luzhou 646000, China*
[e]*Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong, China*
[f]*State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China*

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**KEY WORDS**

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**Abstract** Tumor cells along with a small proportion of cancer stem cells exist in a stromal microenvironment consisting of vasculature, cancer-associated fibroblasts, immune cells and extracellular components. Recent epidemiological and clinical studies strongly support that vitamin D supplementation is associated with reduced cancer risk and favorable prognosis. Experimental results suggest that vitamin D not only suppresses cancer cells, but also regulates tumor microenvironment to facilitate tumor repression. In this review, we have outlined the current knowledge on epidemiological studies and clinical trials of vitamin D. Notably, we

*Abbreviations:* $1,25(OH)_2D_3$, 1$\alpha$,25-dihydroxyvitamin $D_3$; 25(OH)D, 25-hydroxyvitamin D; CAF, cancer-associated fibroblast; CRC, colorectal cancer; CSC, cancer stem cell; DBP/GC, vitamin D-binding protein; ESCC, esophageal squamous cell carcinoma; GI, gastrointestinal; NSCLC, non-small cell lung cancer; PC, pancreatic adenocarcinoma; PG, prostaglandin; PSC, pancreatic stellate cells; TDEC, tumor derived endothelial cell; TME, tumor microenvironment; TIC, tumor initiating cell; TIL, tumor-infiltrating lymphocyte; VDR, vitamin D receptor; VDRE, VDR element; VEGF, vascular endothelial growth factor

*Corresponding authors.
E-mail addresses: crystal_stray@126.com (Jing Shen), star.lee@hotmail.com (Mingxing Li).

†These authors made equal contributions to this work.

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Tumor-infiltrating lymphocyte;
Tumor-derived endothelial cell;
Cancer-associated fibroblast

summarized and discussed the anticancer action of vitamin D in cancer cells, cancer stem cells and stroma cells in tumor microenvironment, providing a better understanding of the role of vitamin D in cancer. We presently re-propose vitamin D to be a novel and economical anticancer agent.



## 1. Introduction

Tumor microenvironment (TME) is comprised of various stromal cells (*e.g.*, endothelia, cancer-associated fibroblasts, and immune cells) and extracellular components (*e.g.*, cytokines and extracellular matrix), which surround tumor cells and are nourished by a vascular system. The pivotal roles of TME during tumor initiation, progression, and metastasis have been recently reviewed in detail elsewhere[1,2]. Importantly, TME greatly influences therapeutic efficacy and emerges as a new target for treating tumors[3–5].

Vitamin D is a multifunctional precursor of the potent steroidal hormone calcitriol (1$\alpha$,25-dihydroxyvitamin $D_3$, $1,25(OH)_2D_3$). As most foods contain little vitamin D, there are certain diseases which need vitamin D as a dietary supplement to replenish the deficiency, especially for the elderly and children to maintain adequate vitamin D store for bone health and autoimmunity[6]. Recently, epidemiological and clinical observations strongly suggest that vitamin D deficiency or low circulating 25-hydroxyvitamin D (25(OH)D) level in serum increases the risk of developing multiple malignancies. Experimental evidence also showed that $1,25(OH)_2D_3$, the active form of vitamin D, can exhibit anticancer actions through different signaling pathways, including inhibition of proliferation, induction of cell apoptosis and differentiation, as well as suppression of metastasis and angiogenesis in various cancers. Notably, vitamin D was found to not only have profound effects on normal cancer cells, but also on cancer-associated stromal cells and cancer stem cells within the TME. Regarding the important role of TME in cancer initiation, progression, metastasis and recurrence, vitamin D might be used as a therapeutic agent targeting the TME to assist clinical treatment and prognosis for many kinds of cancer. Herein, this review summarized the anticancer action of vitamin D in the TME of cancer cells, stroma cells and cancer stem cells. We also outlined the epidemiological studies and clinical trials of vitamin D, providing a better understanding of the role of vitamin D in cancer treatment to re-propose vitamin D as a novel anticancer agent.

## 2. Potentiating vitamin D as an anticancer agent

### 2.1. Vitamin D metabolism and signaling

#### 2.1.1. Vitamin D metabolism

After absorption, vitamin D binds to vitamin D-binding protein (DBP, also named as GC) in the circulation, and is transported to the liver. There are three main enzymes, *i.e.*, CYP27A1, CYP27B1 and CYP24B1, involved in vitamin D metabolism. The metabolic process of vitamin D in human is depicted in Fig. 1. Briefly, vitamin D can be hydroxylated by CYP27A1, and converted into 25(OH)D in the liver. Further, 25(OH)D is catalyzed by CYP27B1 in the kidney, which results in the active form $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ is mainly responsible for the biological function of vitamin D in humans. However, CYP24A1 functions inversely, converting $1,25(OH)_2D_3$ into an inactive form. In fact, the expression of CYP24A1 is highly induced by $1,25(OH)_2D_3$, which is thus the limiting step of vitamin D activation. CYP24A1 is usually highly expressed in cancer tissues, and CYP24A1-induced vitamin D insufficiency might also promote cancer progression. A study of 99 CRC patients demonstrated that CYP24A1 expression was higher in cancer specimens than paired normal tissue, and high expression level of CYP24A1 strongly correlated with tumor invasion, lymph node metastasis, decreases in overall survival ($P = 0.026$) and advanced CRC recurrences ($P = 0.32$)[7]. In addition, Shiratsuchi et al.[8] has recently observed that knockdown of CYP24A1 significantly restrained lung cancer xenograft *in vivo*, suggesting that CYP24A1 could be identified as a potential oncogene in lung cancer. It was found that a protein kinase CK2 inhibitor, 4,5,6,7-tetrabromobenzimidazole, could enhance the antitumor effect of $1,25(OH)_2D_3$ on prostate cancer through directly inhibiting the CYP24A1 expression[9]. Thus, the inhibition of CYP24A1 expression or activity and exploration of CYP24A1 inhibitors might be a good way to increase the anticancer action of $1,25(OH)_2D_3$.

#### 2.1.2. Vitamin D signaling

The biological actions of $1,25(OH)_2D_3$ are mainly mediated by vitamin D receptor (VDR) *via* genomic pathway, although a $1,25(OH)_2D_3$-induced non-genomic pathway has been identified recently (Fig. 1). The binding of $1,25(OH)_2D_3$ to VDR triggers VDR translocation into the nucleus and transcriptionally induces the downstream target genes such as *CDKN1A*, *C-MYC*, *CDH1*, and *CYP24A1*. Thus, the expression level of VDR in cancers is always associated with vitamin D response. Notably, high VDR expression has been found in many cancer types, such as breast cancer and papillary thyroid carcinoma[10,11]. There was also a similar trend of CYP27B1 expression in cancers, which could provide a possible explanation for VDR overexpression in cancer cells[12,13]. High level of VDR expression has been associated with improvement in prognosis of patients with lung, prostate, pancreatic, and colorectal cancers, indicating that VDR expression might be a prognostic biomarker in these cancers[14–17]. Recently, it was demonstrated that higher expression level in stroma cells in cancer adenocarcinoma could facilitate larger actions for vitamin D, thus improving therapeutic outcome[18,19]. Moreover, we recently demonstrated that higher expression of VDR was found in gastric cancer tissue than that in normal gastric tissue, and $1,25(OH)_2D_3$ displayed inhibitory effect in gastric cancer cells without influencing normal epithelial cells[20]. Taken together with the current findings, overexpression of VDR in cancer cells and tissues or in surrounding stroma cells would pose an advantage of using vitamin D as an anticancer agent with an enhanced therapeutic window against cancer. However, although VDR expression has a predominant role for vitamin D action, VDR may potentially function as an oncogene. One study reported that knockdown of VDR evidently induced apoptosis in breast and prostate cancer cells[21], indicating a controversial role for VDR.

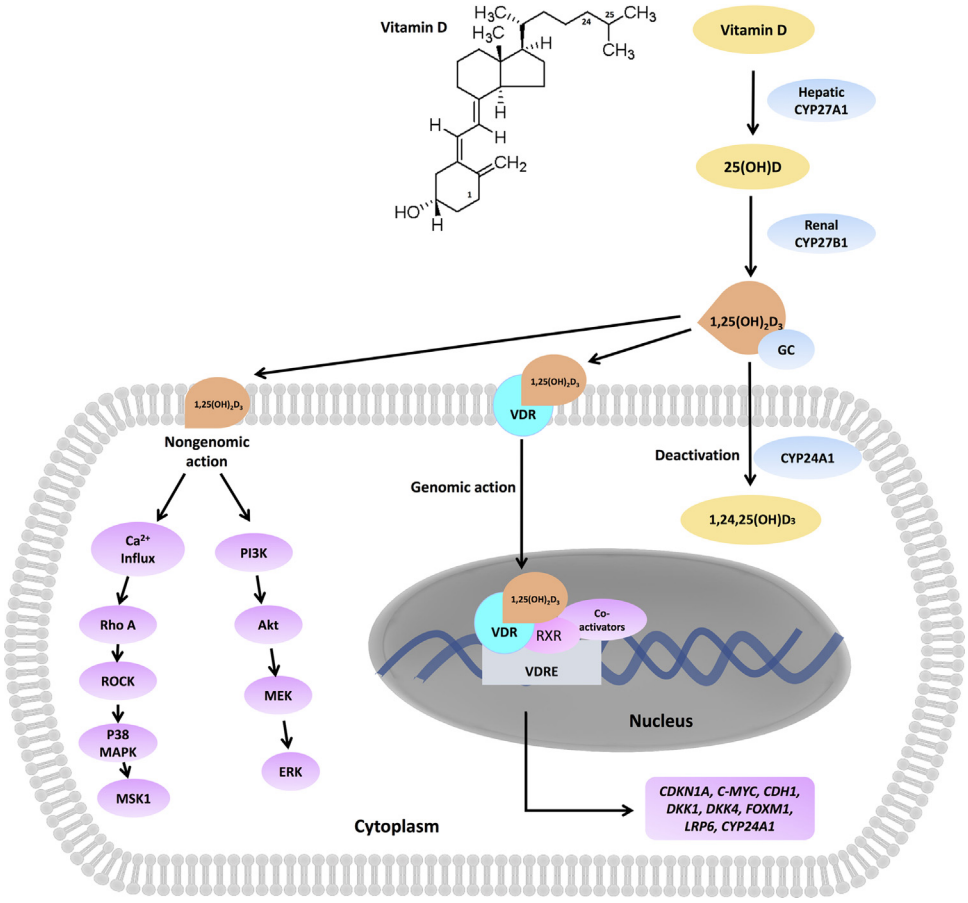


**Figure 1** Vitamin D metabolism and signaling. After absorption, vitamin D is hydroxylated by hepatic CYP27A1 into 25(OH)D, which is further catalyzed by CYP27B1 in the kidney resulting in the active form $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ triggers genomic or non-genomic actions. The binding of $1,25(OH)_2D_3$ to vitamin D receptor (VDR) triggers VDR translocation into nuclear and transcriptionally induces the downstream target genes such as *CDKN1A*, *C-MYC*, *CDH1*, *DKK4*, *FOXM1*, *LRP6* and *CYP24A1*. Alternatively, $1,25(OH)_2D_3$ induces rapid upregulation of cytosolic $Ca^{2+}$ concentration and activates Rho-ROCK-p38MAPK-MSK1 pathway. In certain cancer cells, $1,25(OH)_2D_3$ is capable of stimulating PI3K/Akt/ERK1/2/MAPK pathway.

### 2.2. *Epidemiological studies*

The serum concentration of the vitamin D metabolite 25(OH)D is commonly used as the index of vitamin D status in body. In particular, circulating 25(OH)D level in human lower than 20 ng/mL, from 20 to 30 ng/mL, and higher than 30 ng/mL indicate a deficiency, a relative insufficiency and a sufficiency of vitamin D, respectively[22,23]. Although controversial findings exist, most epidemiological studies have indicated that serum vitamin D status is correlated with multiple types of cancer risk, including colon, prostate, breast, and gastric cancers. Patients with low vitamin D levels are usually diagnosed with poorer survival and higher cancer mortality, suggesting that vitamin D might protect individuals against late stages of carcinogenesis.

#### 2.2.1. *Prostate cancer*

An early prediagnostic study demonstrated that there was a prospective decrease in prostate cancer risk in men at age above 57 years with high level of serum 25(OH)D[24]. A 13-year-follow-up study involved 129 cases and 167 controls showed that vitamin D supplement and increased plasma 25(OH)D concentration significantly correlated with prostate cancer susceptibility[25]. A recent study included 1066 men with prostate cancer within the US Physicians' Health Study between 1982 and 2000, and 1618 matched healthy men as controls[26]. Results suggested that improving vitamin D status through increased exposure to sunlight and vitamin D supplement might reduce prostate cancer risk, particularly in men with the FokI *ff* genotype[26]. However, there was a nested case-control study showing no association between serum 25(OH)D level and prostate cancer risk[27]. A meta-analysis found a significant association between higher 25(OH)D level and increased prostate cancer risk[28]. It seemed that results in prostate cancer were somewhat inconsistent, which might be due to the limitations of selected population and control. Considering that the anticancer action of vitamin D has been well elucidated in prostate cancer cells *in vitro* and *in vivo*[29], more thorough epidemiological studies on vitamin D and prostate cancer risk may be needed.

#### 2.2.2. *Breast cancer*

The relationship between vitamin D intake and breast cancer susceptibility has been well studied. Pooled analysis on breast cancer supported the inverse correlation between vitamin D and calcium intake and breast cancer risk, while some of the studies showed no association[30–33]. Although the results are complex and inclusive, it is expected that vitamin D supplement still benefits for breast cancer prevention. In a sectional study, high vitamin D levels significantly

improved clinical outcomes in Brazilian postmenopausal women with breast cancer[34]. Moreover, it seemed that the association was stronger in premenopausal *vs.* postmenopausal women[27,35,36]. Recently, a cohort study including 1666 women diagnosed with breast cancer seemed highly convincing. They found that elevations in serum 25(OH)D concentrations were associated with superior overall survival especially among premenopausal women[37], strongly suggesting the use of serum vitamin D level as a prognostic marker for breast cancer. Moreover, among African American women, data showed that vitamin D deficiency increased breast cancer susceptibility by approximately 23%[38], indicating vitamin D intake could be consider as a preventive factor for breast cancer incidence.

#### 2.2.3. *Colorectal cancer*

Epidemiological studies conducted on colorectal cancer (CRC) provided strong support for inverse association between serum 25(OH)D level and colon cancer risk in both men and women[39–42]. A cross-sectional study indicated that low concentration of 25(OH)D in serum was significantly associated with adenomas or colon polyps[43]. A meta-analysis showed that higher circulating 25(OH)D levels predicted decrease cancer death of CRC patients[44]. indicating that restoration of vitamin D level to normal levels in vitamin D deficient patients might benefit for a better prognosis of CRC. Notably, the association between vitamin D status and CRC risk sometimes varied with multiple factors. For example, a nest case-control study showed that high concentrations of plasma 25(OH)D was correlated with decreased CRC incidence in individuals with intense immune reaction but not that with lower-level immune responses (Odds ratio = 0.10; 95% CI: 0.03 to 0.35; $P < 0.001$)[45]. The association between dietary calcium and vitamin D supplementation and decreased CRC risk was also observed in a Japanese population with VDR gene polymorphism Apa I but not Bsm I and Taq I[46].

#### 2.2.4. *Upper gastrointestinal cancer*

Epidemiological studies on upper gastrointestinal (GI) cancers are limited. In the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers, a study examined the association between the level of serum 25(OH)D and upper GI cancers (esophageal and gastric cancer). In the subgroup of Asian, but no Caucasians, there was a significant decrease of GI cancer risk associated with low concentration of 25(OH)D (Odds ratio = 0.53, 95% CI: 0.31 to 0.93; $P = 0.003$)[47]. Another study only found prospective evidence in esophageal squamous cell carcinoma (ESCC) but not in gastric carcinogenesis[48]. In a study of 197 gastric cancer patients, the cancer center of Sun Yat-sen University demonstrated that vitamin D deficiency might contribute to decreases in overall survival ($P=0.019$)[49]. Until now, the relationship between vitamin D supplements and upper gastrointestinal cancer risk has not been unsubstantiated, and the protective and prognostic role of serum 25(OH)D concentrations need to be further analyzed.

#### 2.2.5. *Other types of cancer*

Some studies have reported an association of bladder cancer risk with vitamin D insufficiency, suggesting a protective role of vitamin D in bladder cancer[50–52]. In addition, the incidences of other types of cancer (*e.g.*, lung cancer, ovarian cancer, and melanoma) showed controversial outcomes or indeterminate results between 25(OH)D concentrations and cancer risk, and the association might be varied in different cancers[53–55].

Taken together, epidemiological evidence for the association between vitamin D supplements and cancer incidence are inclusive, and the beneficial role of vitamin D sufficiency is not well established in all cancer types. More well-controlled studies may be needed. Based on previous studies, additional factors such as district should be taken into consideration and further assessed. For example, there might be no statistical significance between plasma 25(OH)D levels and pancreatic cancer risk in European populations, whereas high circulating vitamin D levels showed protective effect on pancreatic cancer prognosis in USA cohorts[56,57].

### 2.3. *Clinical trials of vitamin D and its analogues for treatment of cancers*

#### 2.3.1. *Vitamin D and 1,25(OH)$_2$D$_3$*

Initial trials of vitamin D have been conducted using its original form. It has been found that 400 IU is the optimal and safe dose of vitamin D for adults to prevent cancers, while high-dose higher than 2000 IU may inversely promote cancer growth in multiple cancer types such as gastric, pancreatic, colorectal and breast cancers[58]. The selection of proper doses might be one of the reasons for the controversial role of vitamin D in cancer prevention and treatment. In one study, vitamin D intake ($\geq$ 400 IU) daily significantly decreased risks of lung cancer among non-smoking participants[59]. However, a clinical trial including 2303 subjects found that vitamin D intake (2000 IU/day) was not associated with lower cancer risk compared with the placebo control group in all cancer types[60]. Furthermore, it is seemed that high dose supplementation of calcium and vitamin D was correlated with undesirable results. For example, daily intake of calcium (1200 mg/day) and vitamin D (1000 IU/day) continuous for 3–5 years could increase the incidence of serrated adenomas in colon among the participants with one or more colon polyps[61].

Clinical trials performed in most cases were supplied with 1,25(OH)$_2$D$_3$ other than vitamin D to achieve the higher serum concentrations, expecting to produce the maximum antitumor effects of 1,25(OH)$_2$D$_3$. Clinical studies of 1,25(OH)$_2$D$_3$ plus other anticancer drugs such as docetaxel, estramustine, and carboplatin in patients with cancers indicated that the high dose (20 IU/kg) of 1,25(OH)$_2$D$_3$ was safe and well-tolerated[62–65]. Some of the trials exhibited promising antitumor activity, while some did not. A phase II trial of in androgen-independent prostate cancer with high-dose of 1,25(OH)$_2$D$_3$ (480 IU three days per week) for at least one month plus dexamethasone revealed encouraging antitumor activity[66]. It was demonstrated in a phase II study in advanced pancreatic cancer that 1,25(OH)$_2$D$_3$ might have enhanced activity in combination with docetaxel but not with gemcitabine or erlotinib[67]. However, in another phase II trial of weekly intravenous 1,25(OH)$_2$D$_3$ at dose of 2960 IU in combination with dexamethasone for castration-resistant prostate cancer, no positive outcomes were found[68,69]. DN-101, a high-dose calcitriol formulation, was developed to increase the bioavailability of 1,25(OH)$_2$D$_3$. The maximum tolerated dose (MTD) of DN-101 was 1800 IU by weekly oral administration, while hypercalcemia was observed in patients treated at 2400 IU weekly[65]. A phase II study in recurrent prostate cancer, combination therapy with weekly 1,25(OH)$_2$D$_3$ (DN-101, 1800 IU) and daily naproxen was associated with prolonged the PSA doubling time, indicating the encouraging benefit of 1,25(OH)$_2$D$_3$[70]. However, a randomized phase III trial of androgen-independent prostate cancer study of calcitriol enhancing taxotere (ASCENT, DN-101 1800 IU per week) unexpectedly revealed that shorter overall survival was found in 1,25(OH)$_2$D$_3$ treatment group compared to placebo group[71]. Consequently, even when a high dose of 1,25(OH)$_2$D$_3$ was studied, the outcomes were not

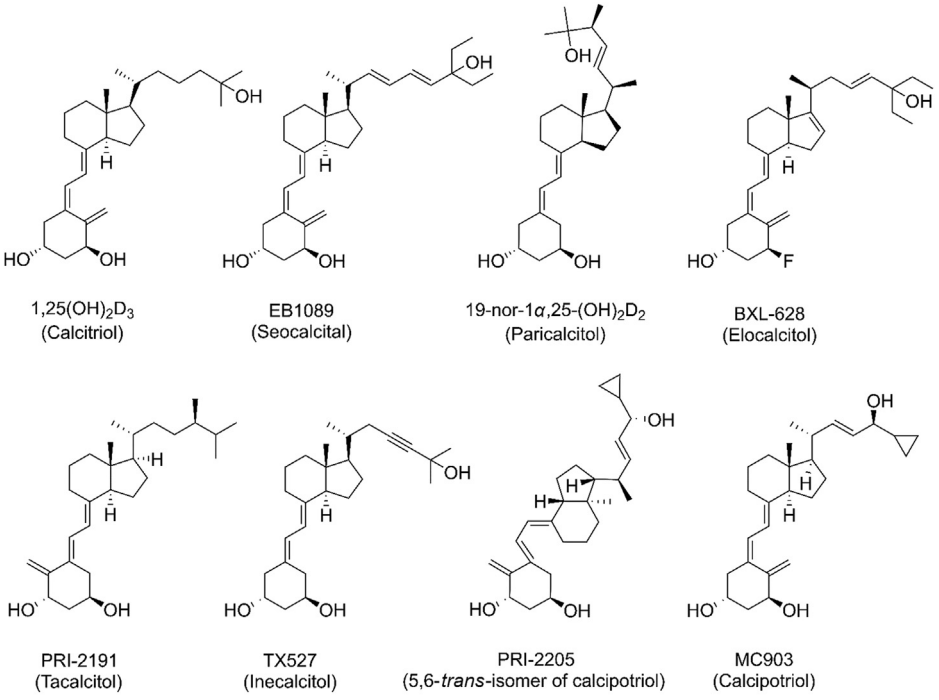


**Figure 2** Structures of vitamin D analogues.

always promising. In particular, optimization of the dosage of $1,25(OH)_2D_3$ might be critical for effective treatment of cancers.

### 2.3.2. *Vitamin D analogues*

Normally, picomolar concentrations of $1,25(OH)_2D_3$ are sufficient to maintain bone homeostasis, while vitamin D in cancer treatment sometimes necessitates relatively higher dosages, which simultaneously increases the risk of hypercalcemia and hypercalciuria. Moreover, $1,25(OH)_2D_3$ is chemically unstable, and easily converted into the inactive form by CYP24A1, limiting the efficacy of vitamin D as a therapeutic agent. To diminish the side effects and increase the therapeutic effect of $1,25(OH)_2D_3$ for cancer, vitamin D analogues (Fig. 2), including EB1089, 19-nor-1$\alpha$,25-$(OH)_2D_2$ (paricalcitol), BXL-628 (elocalcitol), calcipotriol, PRI-2191 (tacalcitol, 1,24-dihydroxyvitamin $D_3$), PRI-2205 (5,6-*trans*-isomer of calcipotriol), have been developed and investigated. The side chain attached to C17 is the main region involved in development for vitamin D analogues, because it is responsible for the binding to vitamin D receptor and is the main target for degradation by CYP24A1. Generally, vitamin D analogues showed similarly or extensively enhanced tumor effect than $1,25(OH)_2D_3$ and mostly yield less calcemic.

Among the most of these clinically studied analogues is EB1089. This drug (also named as seocalcitol) is characterized by an altered side chain structure featuring 26,27-dimethyl groups and two double bonds. It was synthesized to overcome the side effect of hypercalcemia. EB1089 was shown to inhibit prostate cancer metastasis *in vivo* with significantly weaker calcemic side effect compared with $1,25(OH)_2D_3$[72]. EB1089 has been shown to induce cell cycle arrest, cell differentiation and apoptosis in various cancer types including colon, prostate, breast and hepatocellular cancer (without hypercalcemia) both *in vitro* and *in vivo*[73–77]. Notably, EB1089 was shown to be more effective than $1,25(OH)_2D_3$ in inducing apoptosis through suppressing BCL-2 and this agent also induced autophagic cell death in breast cancer cell line MCF7[78,79]. EB1089 was also significantly less calcemic than $1,25(OH)_2D_3$ and exhibited anti-metastatic effects on prostate cancer[72]. A phase II clinical study has shown that it was well tolerated at a daily dose of 10–20 μg, which is significantly less calcemic than $1,25(OH)_2D_3$[80]. A phase II study of EB1089 conducted on patients with inoperable hepatocellular carcinoma indicated promising antitumor activity[81].

Another less calcemic vitamin D analogue, paricalcitol, was applied in a phase I/II study in advanced androgen-insensitive prostate cancer patients with *i.v.* receiving paricalcitol at escalating doses of 5 to 25 μg[82]. Serum parathyroid hormone (PTH) level, a common index of advanced prostate cancer, was significantly decreased by paricalcitol, potentiating it as a therapeutic agent for improving cancer outcome. Accordingly, oral paricalcitol was also proved to be associated with low serum PTH level in patients with metastatic breast cancer[83].

Inecalcitol, which has been identified as a VDR antagonist, more effectively activates VDR expression than $1,25(OH)_2D_3$ and exerts much stronger inhibitory effect on prostate cancer[84,85]. In a phase I study, in metastatic castration-resistant prostate cancer patients, the maximum dose of inecalcitol was 4000 μg per day combined with docetaxel, which showed antiproliferative activity and 100-fold lower hypercalcemic activity than $1,25(OH)_2D_3$[86].

These findings show that there is still limited utility of vitamin D and $1,25(OH)_2D_3$ for clinical use due to hypercalcemia induced by high-dose administration. The efficacy of low-dose vitamin D on cancer prevention or treatment requires additional future study. On the other hand, less calcemic vitamin D analogues may play an important role in cancer treatment. Moreover, new combination treatments of vitamin D analogues and chemotherapeutics for cancer therapy may be discovered. Current knowledge on clinical trials of vitamin D and its analogues has been summarized in Table 1[59,60,64,67,68,71,86–93].

**Table 1** Representative clinical trials of vitamin D intake for cancer prevention or treatment.

| Subject | Dosage regimen | Aim | Main finding | Ref. |
|---|---|---|---|---|
| 128,779 participants | Oral 400 IU vitamin D plus 1 g calcium per day | The association between vitamin D intake and lung cancer | Among non-smokers, vitamin D intake significantly benefited for decreasing risks of lung cancer when compared with control. | 59 |
| 2303 healthy women | Oral 2000 IU vitamin D plus 1500 mg calcium per day | Cancer risk | Vitamin D supplementation did not reduce cancer risk. | 60 |
| 250 patients | i.v. docetaxel 36 mg/m2 per week for a 4-week cycle in combination with 45 μg DN-101 or placebo orally per day before docetaxel | Comparison of the efficacy and safety of DN-101 and docetaxel with placebo and docetaxel on AIPC | Oral taken DN-101 prolonged survival of AIPC patients compared with placebo. | 87 |
| 19 patients | Oral DN-101 180 μg on day 1 and i.v. mitoxantrone 12 mg/m2 on day 2 every 21 days with daily prednisone 10 mg orally for a maximum of 12 cycles | To evaluate efficacy and safety of DN-101 combined with mitoxantrone and glucocorticoids in AIPC | The addition of DN-101 does not appear to increase the toxicity of mitoxantrone in AIPC. | 64 |
| 25 patients | Oral $1,25(OH)_2D_3$ 0.5 μg/kg on day 1, followed by docetaxel 36 mg/m2 i.v. on day 2 per week for three consecutive weeks, followed by 1-week non-treatment | To assess safety and efficacy of weekly high-dose oral 1,25D3 and docetaxel in patients with non-resectable, incurable pancreatic cancer | Weekly oral intake of $1,25(OH)_2D_3$ and docetaxel might prevent pancreatic cancer progression. | 67 |
| 18 patients | i.v. $1,25(OH)_2D_3$ weekly at a dose of 74 μg and dexamethasone in patients with CRPC | CRPC treatment and prevention | $1,25(OH)_2D_3$ supplement did not achieve favorable clinical outcomes. | 68 |
| 953 patients | ASCENT (45 μg DN-101, 36 mg/m2 docetaxel, and 24 mg dexamethasone weekly for 3 of every 4 weeks); control (5 mg prednisone twice daily with 75 mg/m2 docetaxel and 24 mg dexamethasone every 3 weeks) | To compare the efficacy and safety of docetaxel plus DN-101 to docetaxel plus prednisone in a phase III trial | ASCENT treatment decreased prostate cancer survival compared with control. | 71 |
| 66 patients | Daily oral supplementation of vitamin D (400, 10,000, or 40,000 IU per day) | Ki67 staining in prostate cancer tissue | Vitamin D intake decreased Ki67 level in prostate cancer tissue. | 88 |
| 23 patients | Oral administration of 0.5 μg/kg $1,25(OH)_2D_3$ in 4 divided doses over on day 1 of each treatment week, docetaxel 36 mg/m2 i.v. infusion on day 2 of each treatment week and zoledronic acid 4 mg i.v. on day 2 of the first and fifth week of each cycle | To evaluate the efficacy and safety of combination of high dose $1,25(OH)_2D_3$, docetaxel and zoledronic acid in CRPC | Prostate-specific antigen response was detected. | 89 |
| 54 patients | Inecalcitol at eight dose levels (40–8000 μg) daily combined with docetaxel | Prostate cancer treatment and prevention | Inecalcitol in combination with docetaxel encouraged PSA response of prostate cancer. | 86 |
| 1107 patients | 0.5 μg $1,25(OH)_2D_3$ plus 75 mg acetylsalicylic acid and 1250 mg calcium carbonate ($n = 209$), or placebo ($n = 218$) | Adenoma recurrence after three-year treatment | Supplement with $1,25(OH)_2D_3$ did not reduce the risk of CRC recurrence. | 90 |
| 64 cases and 64 controls | Diclofenac sodium 3% gel, $1,25(OH)_2D_3$ 3 μg/g ointment | BCC progression | Combination of diclofenac and $1,25(OH)_2D_3$ treatment inhibited BCC proliferation. | 91 |
| 104 CRC patients | Calcium (1200 mg daily) alone, vitamin D (1000 IU daily) alone and in combination or placebo | APC/$\beta$-catenin pathway in normal colorectal mucosa | Vitamin D intake significantly suppressed APC/$\beta$-catenin pathway. | 92 |
| 2259 participants with colon adenoma | Daily oral 1000 IU vitamin D or 1200 mg calcium carbonate, or both or placebo | Colon adenoma recurrence | Vitamin D prevented colon cancer recurrence among individuals with AA genotype in VDR rs7969585 polymorphism. | 93 |

AIPC, androgen-independent prostate cancer; ASCENT, AIPC Study of Calcitriol Enhancing Taxotere; BCC, basal cell carcinoma; CRPC, castration-resistant prostate cancer; DN-101, a new high-dose oral formulation of $1,25(OH)_2D_3$; i.v., intravenous administration; PSA, prostate specific antigen.

### 2.4. Vitamin D-related gene polymorphism

Polymorphisms in genes related with vitamin D metabolism, including *VDR*, *CYP27A1*, *CYP27B1*, *CYP24A1* and *GC*, have been associated with cancer risk and progression, and may function as a valuable factor in cancer prognosis. Here, we summarized the epidemiological and genetic studies performed in recent years depicting the association of gene polymorphisms with cancer risk, progression and prognosis in Table 2[57,93–119].

A meta-analysis revealed that Bsm I, Apa I, Fok I and Poly (A) polymorphisms in *VDR* gene might be related to breast cancer progression[114]. Another study which involved 3336 incident primary melanoma cases found that *VDR* single nucleotide polymorphisms (SNPs), rs1544410/Bsm I and rs731236/Taq I, significantly correlated with melanoma survival among subjects exposed to high UVB[117]. A case-control study including 528 CRC patients and 605 cancer-free controls and a follow-up study with 317 cases, which were conducted in northeast China, suggested that two polymorphisms in *CYP27B1* (rs10877012 and rs4646536) are associated with decreased CRC risk while *CYP24A1* (rs4809957) polymorphism might lead to a worse prognosis of CRC[94]. It was also reported that the *CYP24A1* variant rs964293 modulated the association between combined oestrogen-progestogen (E+P) hormone therapy and CRC risk, suggesting the important role of *CYP24A1* polymorphism in cancer progression[97]. Equal importantly, *GC* polymorphisms might be a predictive marker for outcome of chemotherapies. AA genotypes of *GC* rs4588 polymorphism was strongly associated with longer overall survival in CRC patients with treatment of irinotecan/cetuximab, whereas treatment with irinotecan/bevacizumab were associated with the converse[103]. These data support the use of specific gene polymorphisms as potential markers for cancer risk and cancer mortality.

## 3. Mechanisms of action of vitamin D within the tumor microenvironment

The antitumor activity of $1,25(OH)_2D_3$, the active form of vitamin D, has been widely studied in a number of cancer types. By binding to VDR, $1,25(OH)_2D_3$ exerted antitumor efficacy through regulating target gene expression or nongenomic actions related to different signaling pathways in both normal cancer cells and cancer stem cells. The anticancer actions of $1,25(OH)_2D_3$ include the induction of cell cycle arrest, cell differentiation, cell apoptosis, autophagic cell death, as well as inhibition of metastasis tumor angiogenesis. Importantly, vitamin D could modulate stromal cells to suppress tumor angiogenesis, progression and metastasis in TME. Recent studies also demonstrate an anti-inflammatory role of vitamin D within TME. The schematic illustration of mechanisms related to the anticancer action of vitamin D within the TME is shown in Fig. 3.

### 3.1. Tumor cells

#### 3.1.1. Regulation of proliferation

$1,25(OH)_2D_3$ has been found to inhibit cell proliferation through cell cycle arrest in most cancer cells, which plays an important role in cancer prevention[76]. The effect of $1,25(OH)_2D_3$ on cell cycle distribution was mainly dependent on induction of p21 and p27 expression. p21 and p27 were identified as the targets of $1,25(OH)_2D_3$ and were critical for G1 phase cell cycle arrest and inhibition of cancer cell proliferation[120,121]. It was reported that the activation of JNK and ERK1/2 MAPK signaling pathways by $1,25(OH)_2D_3$ were required for the induction of p21 expression[122]. Notably, p53 was involved in the regulation of p21 by $1,25(OH)_2D_3$. There were multiple binding sites for p53 located in the promoter of p21 to cooperate with VDR to regulate transcriptional activity of p21[120]. We recently found that $1,25(OH)_2D_3$ can induce p21expression and G1 phase cell cycle arrest in a mutant p53 and VDR dependent manner in gastric cancer cells[20]. At the meantime, p27 could up-regulated by $1,25(OH)_2D_3$, although no canonical VDR element (VDRE) was identified in the p27 promoter[123]. It is demonstrated that VDR could not directly bind to the p27 promoter but interacts with sp1 to regulate the promoter activity of p27[123]. Also, $1,25(OH)_2D_3$ up-regulated p27 expression through inhibition of Thr187 phosphorylation-dependent degradation in prostate cancer and increase the stability of p27 through down-regulation of ubiquitin pathway in ovarian cancer.[124,125] Furthermore, some other cyclin-dependent kinase inhibitors such as p15 and p16 may be induced by $1,25(OH)_2D_3$[121,126,127].

#### 3.1.2. Induction of apoptosis

$1,25(OH)_2D_3$ can induce cell apoptosis in several cancer cells, mainly in breast, prostate and squamous carcinoma cells, effects which often require lengthy exposures to $1,25(OH)_2D_3$. $1,25(OH)_2D_3$-mediated cell apoptosis was demonstrated to be through mitochondrial-dependent pathway, in which the release of cytochrome c and the protein of BCL-2 family were involved, resulting in the suppression of the anti-apoptotic protein (BCL-2, BCL-XL) and the induction of the apoptotic protein (such as BAX, BAK, BAD)[77,128–132]. In addition, a study demonstrated that $1,25(OH)_2D_3$ could induce apoptosis in ovarian cancer cells through down-regulation of telomerase activity and telomerase reverse transcriptase (hTERT) expression after 6 days treatment with $1,25(OH)_2D_3$, contributing to shortening the telomere length[133,134]. It was also found that suppression of hTERT expression by $1,25(OH)_2D_3$ was regulated by microR-498, showing that microRNA played an important role in vitamin D regulation in cancers[133,134]. This sheds light on a new perspective in $1,25(OH)_2D_3$-induced cancer cell apoptosis. At the meantime, $1,25(OH)_2D_3$ could accelerate chemotherapeutics-induced apoptosis, thus enhancing the antitumor activity of chemotherapeutic drugs such as paclitaxel, gemcitabine and dexamethasone[135–138]. In *in vitro* studies, it has been reported that vitamin D, in synergy with cisplatin, the histone deacetylase inhibitor trichostatin A, sodium butyrate or the methylation inhibitor 5-aza-2′-deoxycytidine, could induce apoptosis in gastric cancer cells[139,140]. This indicated that $1,25(OH)_2D_3$, the normal dietary supplement, could be considered for combination treatment for cancer.

#### 3.1.3. Induction of differentiation

Induction of differentiation has been reported to involve in $1,25(OH)_2D_3$-induced cancer suppression. $1,25(OH)_2D_3$ is considered as a differentiating agent for leukemia cells. It could promote myeloid leukemia cells differentiating into mature myeloid cells, and has been used in clinical trials for acute myeloid leukemia[141,142]. Furthermore, $1,25(OH)_2D_3$ can induce differentiation in cancer cells, mostly in colon cancer, through repression of WNT/$\beta$-catenin signaling. Activation of WNT/$\beta$-catenin signaling is commonly found in colon cancer, and is associated with undifferentiated properties and malignance of cancers[143].

**Table 2** The association of vitamin D-related gene polymorphisms with cancer risk or mortality.

| Gene | Polymorphism | Study | Main finding | Ref. |
|---|---|---|---|---|
| *CYP27B1* | rs10877012 (G > T); rs4646536 (C > T) | A follow-up study with 528 CRC patients and 605 cancer-free controls | Reduced CRC risk in GG carriers of rs10877012 and CC genotype of rs4646536; higher CRC risk in GT + TT carries of rs10877012 | 94,95 |
| *CYP27A1* | rs4674343 (G > A); rs6436086 (T > C) | 1260 men with prostate cancer and 1331 controls | Positive association of rs4674343 and rs6436086 with prostate cancer risk | 96 |
| | rs964293 | 10,835 postmenopausal women (5419 CRC cases and 5416 controls) | Modify the association between combined oestrogen-progestogen hormone treatment and CRC incidence | 97 |
| | rs6068816; rs2181874 | 426 NSCLC cases and 445 controls from China | Reduction of NSCLC susceptibility by rs6068816 in both smokers and non-smokers; reduction of NSCLC risk in smokers with mutated homozygous rs6068816; increased NSCLC risk in those with mutated homozygous rs2181874 | 98 |
| | rs4809957 (A > G) | 528 CRC cases and 605 cancer-free controls in northeast China | Worse prognosis of CRC patients with CYP24A1 A > G (rs4809957) | 94 |
| | rs2296241 | 582 ESCC patients and 569 controls in a Northern Chinese population | Positive correlation between ESCC and rs2296241 | 99 |
| *GC* | rs12512631; rs2239182 | 3566 primary melanoma cases | Longer cutaneous melanoma overall survival in those with rs12512631 and rs2239182 in GC | 100,101 |
| | rs7041 | 426 NSCLC cases and 445 controls from China; 1777 breast cancer cases and 1839 controls | Significant association of decreased NSCLC incidence with rs7041; increased risk of breast cancer in rs7041 TT genotype | 98,102 |
| | rs4588 | 522 metastatic CRC patients | Longer CRC patients overall survival in AA carriers of rs4588 | 103 |
| *VDR* | rs11568820 | 1598 patients with stage I to III CRC | Interaction of rs11568820 genotype with serum 25(OH)D concentration, modulating the risk for CRC-specific and all-cause mortality | 104 |
| | rs7299460 | 493 pancreatic cancer patients from five prospective US cohorts | Better pancreatic cancer prognosis in rs7299460, while no interaction with pancreatic cancer risk | 57 |
| | Apa I | 340 patients (201 chronic hepatitis, 47 cirrhosis and 92 HCC) and 100 healthy controls | Association of CC genotype in Apa I with HCC progression | 105 |
| | Apa I | 302 RCC patients and 302 healthy controls | Increased susceptibility of RCC in AA and AC genotypes in Apa I variants compared with CC genotypes in Chinese populations | 106 |
| | rs2228570/Fok I | 378 participants: 78 CRC cases and 230 non-cancer controls | rs2228570/Fok I associated with CRC risk in African American populations | 107 |
| | rs2228570/Fok I | Meta-analysis including 9720 prostate cancer patients and 9710 control subjects; 10,486 prostate cancer cases and 10,400 controls | rs2228570/Fok I correlated with prostate cancer risk and progression in Caucasian | 108,109 |
| | rs2228570/Fok I (C > T) | Meta-analysis with 209 tobacco-related cancers (cases) and 418 controls | Increase risk of smoking-related cancers in AA genotype of Fok I polymorphism | 110 |
| | rs2228570/Fok I (C > T) | 1820 white ovarian cancer cases and 3479 controls; 4152 cases and 6693 controls of Caucasian populations | Association of risk of ovarian cancer with rs2228570/Fok I | 111,112 |
| | rs1989969 | 330 cases and 608 controls | Association with increased gastric cancer risk and development, especially in the younger and alcohol drinking Chinese population | 113 |
| | Bsm I; Apa I; Fok I; Poly (A) | Meta-analysis with 26,372 breast cancer cases and 32,883 controls | Significant association with breast cancer susceptibility | 114 |
| | rs7975232/Apa I; rs731236/Taq I | 100 cases of Egyptian females with breast cancer; 498 patients with breast cancer with a mean age at diagnosis of 61 years from Saarland, Germany | Strong correlation of ATT genotype in ApaI and TaqI polymorphism with susceptibility of breast cancer carcinogenesis in Egyptians | 115,116 |

| | | | |
|---|---|---|---|
| rs1544410/Bsm I; rs731236/Taq I | 3336 incident primary melanoma cases | Positively association with favorable overall survival melanoma patients exposed with high UVB | 117 |
| rs1544410/Bsm I | A population-based cohort of 531 CRC patients in Newfoundland and Labrador, Canada | Significant correlation with the overall survival of CRC patients; Decreased overall survival in the G-allele in rs1544410 compared with the A-allele | 118 |
| rs7968585 | A randomized clinical trial conducted at 11 clinical centers in the United States including 2259 participants | Reduced colorectal cancer recurrence risk among patients with AA genotype of rs7968585 | 93 |
| rs1544410/BsmI; rs7975232/ ApaI; rs2228570/Fok I | Meta-analyses with total 44,165 cases from 64 studies | Associated of cancer prognosis with rs1544410/Bsm I and rs7975232/Apa I polymorphisms; Correlation of lung cancer mortality with rs2228570/Fok I polymorphism | 119 |

CRC, colorectal cancer; ESCC, esophageal squamous cell carcinoma; HCC, hepatocellular carcinoma; GC, vitamin D binding protein; NSCLC, non-small cell lung cancer; RCC, Renal Cell Carcinoma; VDR, vitamin D receptor.

$1,25(OH)_2D_3$-induced VDR translocation can bind $\beta$-catenin to the VDRE of vitamin D target genes, thereby inhibiting the transcriptional activity of WNT downstream targets[144]. It was also found that $1,25(OH)_2D_3$ inhibited WNT/$\beta$-catenin signaling through induction of E-cadherin and the WNT inhibitor DKK1 in colon cancer cells, functioning as a multilevel suppressor of WNT/$\beta$-catenin signaling pathway[145–149]. E-cadherin is associated with epithelial phenotype of cancer cells and loss of E-cadherin causes invasiveness of cancer cells. Induction of E-cadherin and other differentiated epithelial marker ZO-1 by $1,25(OH)_2D_3$ and inhibition of WNT/$\beta$-catenin downstream targets such as c-Myc and cyclin D finally restrain the cancer cell differentiation, which is also associated with tumor angiogenesis, migration and invasion.

### 3.1.4. *Inhibition of angiogenesis and metastasis*

The vitamin D signaling pathway is also associated with tumor angiogenesis, which is one of the mechanisms contributing to the anticancer action of $1,25(OH)_2D_3$. Vascular endothelial growth factor (VEGF) is a growth factor that stimulates the formation of blood vessels, and its overexpression in cancer cells promotes angiogenesis and metastasis[150]. Suppression of hypoxia-inducible factor 1 (HIF-1$\alpha$)-mediated VEGF expression and signaling by $1,25(OH)_2D_3$ was detected in many kinds of cancer cells including prostate, breast and colon cancers[151,152]. NF-$\kappa$B signaling, the nuclear protein Forkhead box M1 (FOXM1) and DKK4 have been suggested to mediate the anti-angiogenesis effects of $1,25(OH)_2D_3$. It was found that $1,25(OH)_2D_3$ inhibited angiogenesis through reduction of NF-$\kappa$B mediated interleukin-8 (IL-8) secretion in prostate cancer cells[151]. Treatments with $1,25(OH)_2D_3$ or the vitamin D analogue EB1089 or activation of VDR remarkably suppressed pancreatic ductal adenocarcinoma cells proliferation, self-renewal abilities and metastasis through down-regulation of FOXM1. The latter is an oncogene that plays a key role in cell cycle regulation and carcinogenesis[153]. In addition, DKK4, a known downstream target of vitamin D signaling and an inhibitory *Wnt* ligand, was reported to inhibit tumor angiogenesis, migration and invasion in colon cancer cells *in vitro*. These findings further support the anti-angiogenesis and anti-invasiveness roles for vitamin D in cancer prevention[147]. Finally, $1,25(OH)_2D_3$ also suppressed epithelial-mesenchymal transition of SKOV-3 ovarian cancer cells through reduction of *Slug and Snail* and upregulation of E-cadherin, thus resulting in the inhibition of migration and invasion of SKOV-3 cells[154].

### 3.1.5. *Induction of autophagy*

Induction of autophagy by vitamin D was initially studied in the immune system. Vitamin D can induce autophagy in many kinds of immune cells through the host defense peptide LL37 for bacteria eradication. Accumulating evidences (best substantiated in breast cancer) indicates that vitamin D, as well as its analogue EB1089, suppresses cancer progression through autophagic-induced cell death. Initially, it was found that EB1089 triggers massive autophagy in MCF-7 breast cancer cells by upregulating the tumor suppressor gene Beclin-1, thereby inducing breast cancer cell death[79]. Recent evidence showed that the vitamin D-induced autophagic effect was found specifically in luminal-like breast cancer cells and benefited a lot in combination with hydroxychloroquine (HCQ), an inhibitor of autolysosome acidification for suppression of breast cancer growth *in vivo*[155]. Studies also indicated that the autophagy produced by either vitamin D or its analogue EB1089 could also modulate radiation sensitivity in

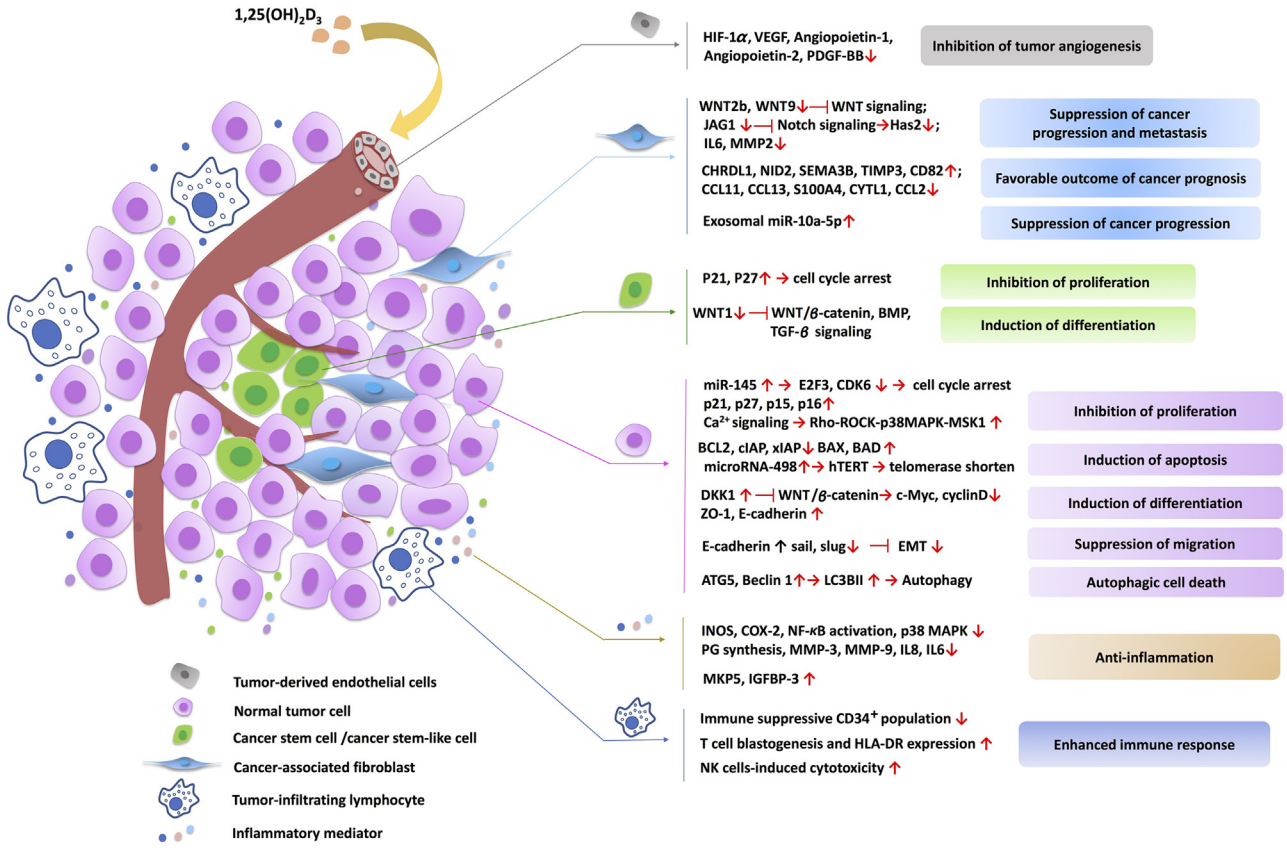


**Figure 3** Mechanisms of anticancer action of vitamin D within tumor microenvironment (TME). The active form of vitamin D, $1,25(OH)_2D_3$, not only suppresses cancer cell growth, but also regulates a range of stromal cells, including cancer-associated fibroblasts, tumor-derived endothelial cells, cancer stem cells and infiltrating immune cells, within TME to facilitate cancer suppression. $1,25(OH)_2D_3$ also exhibits anti-inflammatory effect within TME. The representative signal transduction pathways are displayed.

breast cancer and non-small cell lung cancer (NSCLC) cells. Vitamin D or EB1089 increased radiation efficiency through promotion of autophagy in p53 existing breast cancer[156] and NSCLC cells[157] but not in p53-null cells, which indicates an important role of p53 in vitamin D-induced autophagy. Above all, vitamin D functions as an autophagic switch in human health maintenance and various types of cancer resistance, providing a novel perspective by magnifying the radiation responses in human malignancies.

#### 3.1.6. *Nongenomic effect*

Nongenomic actions of $1,25(OH)_2D_3$ are rapid and not dependent on VDR-mediated transcriptional activation (Fig. 2), recently reviewed by Hii and Ferrante[158]. The most well-established nongenomic effect of $1,25(OH)_2D_3$ is the rapid intestinal absorption of $Ca^{2+}$. It has been demonstrated that alteration of intracellular $Ca^{2+}$ homeostasis is associated with tumor initiation, angiogenesis, progression and metastasis[159]. Based on this, a new mechanism of $1,25(OH)_2D_3$ in regulation of E-cadherin has been found in colon cancer cells. Rho-ROCK-p38MAPK-MSK1 activation was mediated by $1,25(OH)_2D_3$-induced upregulation of cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_{cyt}$), which was required for genes expression of CYP24A1 and E-cadherin[160]. In squamous cell carcinoma cells, $1,25(OH)_2D_3$ provoked apoptosis through the rapid nongenomic activation of PI3K/Akt/ERK1/2/MAPK signaling and inhibition of the anti-apoptotic protein cIAP and XIAP[161]. These findings dual actions of VDR and expands the understanding that the nongenomic activation of VDR might synergize with the VDR-dependent genomic pathway to produce antitumor effect of $1,25(OH)_2D_3$.

### 3.2. *Stromal cells*

The tumor stroma cell is one important part of developing tumor microenvironment, promoting expansive proliferation, metastatic capacities and chemoresistance of solid tumors. Stroma cells contain many cell types including epithelial cells, tumor-associated fibroblast, tumor-derived endothelial cells and infiltrating immune cells. More and more studies show that different stromal cells types in tumor microenvironment influence a lot of hallmarks of cancer. For example, endothelial cells trigger tumor angiogenesis, while cancer-associated fibroblasts (CAFs) are involved in activating tumor invasion and metastasis, proliferation and in resisting cell death[162,163]. Emerging evidence suggests that vitamin D participates in modulating the gene signature of tumor stroma cells to regulate tumor angiogenesis, progression, and metastasis. Such findings suggest that vitamin D is a promising therapeutic for cancer.

#### 3.2.1. *Tumor-derived endothelial cell*

Both *in vitro* and *in vivo* studies have demonstrated that $1,25(OH)_2D_3$ is able to inhibit tumor derived endothelial cells (TDECs). It is reported that $1,25(OH)_2D_3$ and its analogs inhibited TDECs proliferation without affecting normal aortic endothelial cells at concentrations

comparable to those required to suppress cancer cells[152,164]. Recently, emerging evidence showed that $1,25(OH)_2D_3$ repressed tumor angiogenesis in some *in vivo* models such as the TRAMP-2 prostate tumor models and breast cancer models. $1,25(OH)_2D_3$ inhibited TDECs in TRAMP-2 tumor with wild-type VDR but not in VDR knockout mice and enhanced tumor blood vessel density in VDR-knockout mice when compared with tumors from wild-type mice[165]. These results indicate the importance of VDR signaling on TDECs in the regulation of prostate cancer angiogenesis. It should be noted that $1,25(OH)_2D_3$ could induce apoptosis of VEGF sprouting endothelial cells, thereby decreasing the blood vessel density in breast cancer xenograft tumors[152,166]. Therefore, $1,25(OH)_2D_3$ may function as an important regulator in alteration of pathologic angiogenesis to regulate the tumor microenvironment.

#### 3.2.2. *Cancer-associated fibroblast*

The effect of $1,25(OH)_2D_3$ on CAFs has been mainly investigated in pancreatic adenocarcinoma (PC) and CRC. VDR overexpression was found in tumor stromal cells of PC including pancreatic stellate cells (PSCs) existing in the extracellular matrix and CAFs[19]. $1,25(OH)_2D_3$ suppressed pancreatic cancer metastasis through inhibiting secretion of metastasis supportive factors in PSCs including the WNT ligand (WNT2B), Notch receptor (JAG1), cytokines (IL6) and growth factors[19]. In a recent report, the activation of vitamin D signaling pathway in pancreatic CAFs suppressed the transmission of certain exosomal oncomiR such as miR-10a-5p from CAF to PC cells and thus diminished its protumoral effects on PC cells[167], which provided a new perspective on how vitamin D regulates CAFs. Furthermore, it is reported that $1,25(OH)_2D_3$ exhibited protective effect against CRC through inhibiting CAFs independently of VDR expression in CRC cells, while higher VDR expression in CAFs predicted longer overall survival in CRC patients[168]. Although promising results have been achieved on the regulation of CAFs by vitamin D, the underlying mechanisms remain to be evaluated, especially in different cancer types.

#### 3.2.3. *Tumor-infiltrating lymphocyte*

Recently, a number of clinical and preclinical studies showed that tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment play a significant role in cancer progression or tumor suppression and may be identified as a new therapeutic target for cancer treatment. Effects of vitamin D on immune cells has been well studied, especially in host defense against bacteria such as the clearance of *Mycobacterum tuberculosis*. It was reported that the antibacterial activity of vitamin D on *M. tuberculosis* was mediated by autophagy through the upregulation of LL37 and the activation of Beclin-1 and ATG5 in monocytes and macrophage[169,170]. Vitamin D supplements promoted INF-$\gamma$ related antimicrobial effects of human macrophages[171]. On the other hand, vitamin D plays a significant role in adaptive immunity, and vitamin D deficiency might disrupt CD8 effector differentiation and the response of memory CD8 T cells for viral or bacterial infection in mice[172]. It was also found that vitamin D-induced tolerogenic dendritic cells (DC) increased population of $CD4^+CD25^+Foxp3^+$ T cells, $CD4^+IL\text{-}10^+$ T cells, and $CD19^+CD5^+CD1d^+$ B cells, resulting in attenuated experimental autoimmune encephalomyelitis[173]. Moreover, vitamin D induced regulatory T cell (Treg/Th17) differentiation through TGF-$\beta$ signaling in a VDR-dependent manner, which indicated that vitamin D uptake might benefit for organ transplantation recipients[174]. Sun et al.[175] found that vitamin D could modulate IL-17 and RANKL expression through VDR-mediated NF-$\kappa$B signaling, resulting in the inhibition of Th17 cell differentiation in both *in vitro* and mice models. Vitamin D indeed plays an important role in both innate and adaptive immune responses. There is increasing evidence related to the important role of vitamin D on immune cells such as DC, NK cells, T cells, B cells as well as TILs in cancer and inflammation. For example, vitamin D can modulate tumor cell sensitivity to NK cells through suppression of miR-302c and miR-520c in breast cancer cells, resulting in increased NK cells-induced cytotoxicity and decreased breast cancer cell viability[176].

PD-1 and PD-L1 function as blockers of immune responses and anti-PD-1 immunotherapy achieves rapid and effective treatments on a variety of cancers. Although PD-L1 suppresses the anti-tumor activity of T cells through immune checkpoint blockade, the anti-immunity role of PD-L1 might show protective effect in inflammation. It was found that vitamin D directly induced PD-L1 and PD-L2 expression in human epithelial cells and myeloid cells and treatment with vitamin D significantly suppress the inflammatory cytokine expression[177], suggesting the anti-inflammatory role of vitamin D in immune responses and the protective role of vitamin D by reducing human malignancy initiation. One phase 1B study including patients with head and neck squamous cell carcinoma indicated that daily oral administration of vitamin D metabolite $25(OH)D_3$ decreased immune suppressive cells $CD34^+$ population and had favorable immune responses including promoting T-cell blastogenesis and HLA-DR expression[178]. As the functional role of vitamin D in cancer immunotherapy is still limited, more studies on vitamin D in anti-cancer immunity are needed. Since cancer immunotherapy is now becoming a hot topic and benefits increasing number of cancer patients worldwide, it is expected that vitamin D might enhance cancer immunoprevention and increase the activity of immune cells to kill cancer cells.

### 3.3. *Cancer stem cells*

Cancer stem cells (CSCs) or tumor initiating cells (TICs) are critically responsible for tumorigenesis, progression, metastasis, and tumor recurrence, but they account for only a small proportion of cancer cells. Regarding the important role of the low-proportion CSCs in cancer microenvironment, targeting CSCs might be an efficient and promising method for cancer suppression. Recent studies have demonstrated that vitamin D may suppress cancer progression through targeting CSCs compartment. The role of vitamin D in regulating CSCs in gastrointestinal cancers has been thoroughly reviewed in our previous report[179]. Here we further summarized the suppressive action of vitamin D on CSCs in other cancers such as breast and prostate cancers.

In breast cancer, CD44 was identified as a breast cancer stem cell marker and $CD44^+/CD24^{-/low}$ cells and $CD49f^+/CD24^{-/low}$ were identified as tumor-initiating cells[180]. In MCF10A ductal carcinoma *in situ* (DCIS) cells, CD44 was significantly down-regulated by a Gemini vitamin D analog, BXL1024[180]. BXL1024 reduced the breast cancer stem-like cells population including $CD44^+/CD24^{-/low}$ and $CD49f^+/CD24^{-/low}$ subpopulation of MCF10A DCIS cells, and suppressed the stem cell markers expression (CD44 and CD49f)[180]. Further study has shown that the suppressive effect of $1,25(OH)_2D_3$ or its analogue on breast CSCs in MCF10A DCIS was dependent in Hes1-mediated inhibition of Notch signaling[181]. In mammosphere, OCT4 and KLF4, which is associated with stem cell self-renewal and undifferentiated phenotype, were reduced by $1,25(OH)_2D_3$ or BXL1024[182]. It is reported that another vitamin D analogue EB1089 in cooperation with BRAC1 exhibited anti-mammosphere formating

ability in breast cancer cells[183]. Notably, Jeong et al.[184] found that $1,25(OH)_2D_3$ or oral administration with vitamin D could inhibit mouse TICs in MMTV-WNT1 mammary tumors through WNT/$\beta$-catenin signaling *in vivo*. $1,25(OH)_2D_3$ inhibited spheroids formation of mouse TICs in 3D tumor spheroid culture assay *in vitro*[184]. However, some studies showed that VDR was down-regulated in breast cancer mammosphere, potentially indicating less vitamin D action on breast CSCs[183,185]. Despite the controversy, the current findings still provided a new strategy for vitamin D targeting breast CSCs and give us a deeper understanding of vitamin D effect in cancer.

Although the anticancer effect of vitamin D on prostate cancer cells has been widely explored both *in vitro* and *in vivo*, the exact pharmacological action of vitamin D on prostate CSCs is not well understood. Existing evidence showed that the mechanism involved in the regulation of prostate CSCs by vitamin D was partly similar to that of prostate cancer cells. Maund et al.[186] demonstrated that $1,25(OH)_2D_3$ could induce p21 and p27 expression as well as cell cycle arrest in mouse prostate CSCs, and its anti-proliferative effect was mainly mediated by upregulation of IL-1$\alpha$. In addition, the micro-array data indicated that the CSCs signaling (*e.g.*, BMP and TGF-$\beta$) participated in the regulatory action of $1,25(OH)_2D_3$ on prostate CSCs[186]. Taken together, the mechanisms underlying vitamin D regulation of CSCs seem similar to that of normal cancer cells, i.e., mainly through WNT/$\beta$-catenin, BMP, Notch and TGF-$\beta$ signaling mechanisms.

### 3.4. *Inflammatory mediators*

Inflammation plays an important role in tumorigenesis[187]. Studies have indicated that vitamin D exhibits anti-inflammatory effects within TME to inhibit cancer initiation and progression[188]. Animal studies showed that dietary supplement of vitamin D significantly decreased colon cancer incidence in mice, which might be due to the decrease of MAPK and NF-$\kappa$B signaling as well as the reduction of pro-inflammatory cytokines in colonic epithelial cells[189]. It is reported that $1,25(OH)_2D_3$ may restrain inflammatory progression through downregulation of MMP-3, INOS and COX-2 levels and upregulation of inflammatory mediators including IL-1$\beta$, IL-6, IL-8, and prostaglandins (PGs) in macrophage[190,191]. COX-2 is involved in pathogenesis of many inflammatory diseases[192], and $1,25(OH)_2D_3$ is able to decrease COX-2 expression in many kinds of cancers such as breast cancer, colon cancer, ovarian cancer and prostate cancer, thereby suppressing cancer growth[193–196]. In a clinical trial, higher levels of vitamin D were correlated with lower COX-2 level in prostate cancer cells and stroma cells[197]. Moreover, inflammatory factors including TNF-$\alpha$, IL-6 and IL-8 were significantly inhibited by $1,25(OH)_2D_3$ in prostate primary epithelial cells, indicating the beneficial role of anti-inflammatory action of vitamin D in prostate cancer[197]. Above all, vitamin D exerts anti-inflammatory effects through suppression of the production and action of inflammatory mediators such as cytokines, chemokines and PGs, and inhibition of MAPK and NF-$\kappa$B signalings in cancer cells, macrophages as well as the epithelial cells, all of which might contribute to the prevention of cancer progression and inflammatory progress.

## 4. Conclusions/perspectives

Accumulating evidence strongly supports the notion that vitamin D deficiency is associated with elevated cancer risk and poor prognosis. Vitamin D supplementation can exert profound suppression of cancers, in particular *via* targeting components of the TME. Importantly, the emerging role of vitamin D in the regulation of the TME provides a mechanistic basis for its potential efficacy in treating cancer. Although the preclinical and epidemiologic data are persuasive and provide supportive evidence for continued development of vitamin D or its analogues for cancer therapy, no well-designed clinical trial of vitamin D has been carried out. In particular, the optimum dosage of vitamin D supplementation to reduce cancer risk or treat cancer is still unclear. Future clinical trials that treat cancer patients with suitable doses of vitamin D, or new analogues as well as combination therapy may finally demonstrate the promise for use of a classical vitamin with low and known toxicity in humans. Such results may lead to the development of an effective medicine for the prevention and treatment of malignancies in an economical and efficient manner.

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