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Vitamin D Pathway in Endocrine-Related Cancer: Literature Review

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Abstract

Since the seminal 1980 paper by Garland et al linking vitamin D (VitD) to cancer, abundant studies have now shown evidence that VitD can exert a wide range of oncoprotective actions. Preclinical cancer studies link VitD with modulation of inflammation, cell proliferation, cell differentiation, angiogenesis, invasive and metastatic potential, apoptosis, and epigenetic control via miRNA. New synthetic VitD analogs have also demonstrated even more potent tumor suppressive effects. However, conflicting studies show that varying serum concentrations of VitD and its analogs can greatly affect the potency of its anti-cancer role, where too little exerts no influence and too much may even induce cancer. In addition, lower serum VitD can still be compensated by stronger VDR activity and lower DBP activity. This controversy sparked more studies in how upstream effects on VitD and its activation pathway can modulate its downstream anticancer effects. The sheer vast amount of evidence linking VitD to cancer since its first discovery 30 years can be overwhelming, making it difficult to see the big picture. The objective of this literature review is to offer an up-to-date and cohesive view of both the downstream and upstream associations of VitD with cancer. The analysis will tie together all current evidence to compare the similarities and differences of how VitD affects major types of endocrine-related cancers, in the hopes of presenting a concise visual for future preclinical studies in this now vast field of research.

Keywords: Vitamin D; Endocrine-related cancer; Oncoprotective actions

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Introduction

VitD Physiology: metabolism and action (Figure 1)

VitD is derived from an inactive form, 7-dehydrocholesterol (Pro-VitD) from diet and sunlight-driven synthesis in the skin. The active molecule 1a, 25$(OH)_2D_3$, also known as calcitriol, is synthesized from a tightly regulated multistep process. Temperature dependent isomerization of ProVitD forms non-hydroxylated D3, cholecalciferol. Cholecalciferol is converted by the liver into its main circulating form, 25(OH)D, by a mitochondrial and microsomal hydroxylase encoded by the gene CYP27A1. 24(OH)D travels in circulation on a binding protein, Vitamin D binding protein (DBP) and is finally transformed into its active hormone form 1,25(OH)D in the kidney and various target cells by a second hydroxylase encoded by the gene CYP27B1. Its classic physiological function is to regulate bone metabolism and serum calcium concentrations, but current knowledge now shows abundant extra skeletal actions by VitD, including modulation of cancer onset, progression, and mortality.

After the formation of 25(OH)D3 and calcitriol, both forms travel through circulation bound to DBP, and must dissociate into its free-form to diffuse through cell membranes of its target. In the cytoplasm, 25(OH)D3 is converted to calcitriol, and calcitriol diffuses freely into the nucleus. Once inside the nucleus, calcitriol binds to a free vitamin D receptor (VDR). After binding, VDR complexes with retinoid X receptor (RXR) and other recruited co-activators molecules. This VitD VDR-RXR-Coactivator complex binds to various DNA segments of vitamin D response elements (VDRE) found in promoters of more than 750 genes, about 3% of human DNA [1] (Figure 1). In addition, the activated complex can also affect epigenetic control on additional genes via all three epigenetic control mechanisms: DNA methylation, covalent histone modifications, and miRNA

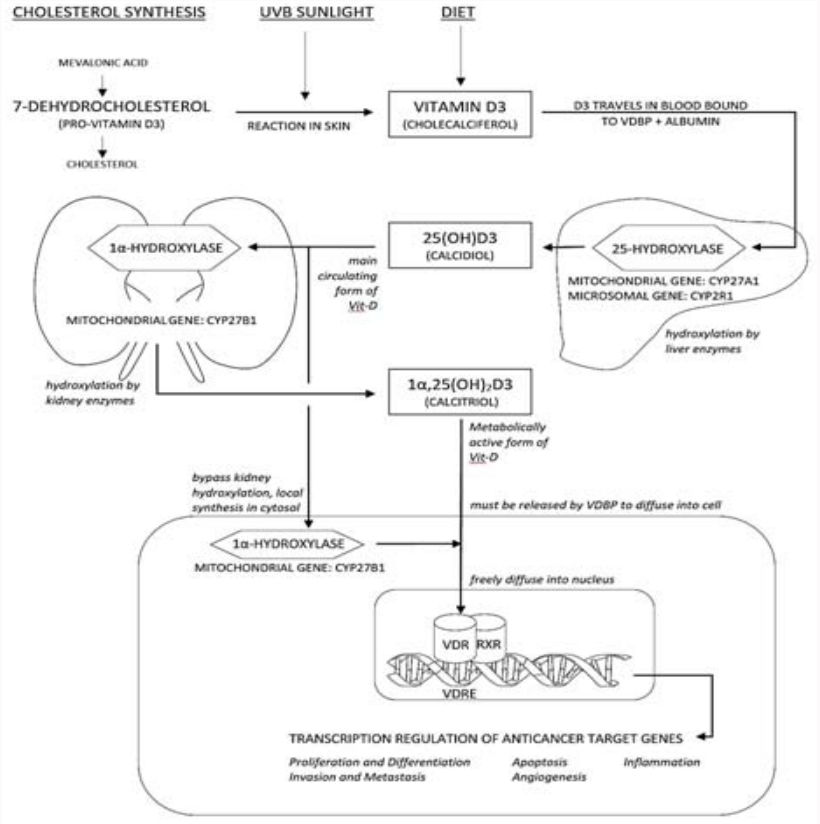

Figure 1: Vitamin D production and metabolism. VDR: Vitamin D Receptor; RXR: Retinoid X Receptor; VDRE: Vitamin D Response Element).

regulation. Each of the molecules associated with VitD, from its activating enzymes to its binding proteins all can be individually regulated. With such an involved signaling system in a wide area of effect, it is no surprise that altered VitD/low VitD can play a role in cancer cells and all the varying stages of cell transformation leading to cancer.

Vitamin D blood levels and upstream effectors

Vitamin D is acquired from dietary intake and endogenous synthesis after sun exposure, with the latter mechanism accounting for ~90% of body stores in most regions of the world. In the liver, VitD is converted to 25-hydroxyvitamin D [25(OH)D] [1], which circulates in both bound and unbound fractions and is the metabolite usually measured to determine VitD status. Serum 25(OH)D reflects the combination of exposure to sunlight and diet [2]. Part of 25(OH)D is converted by the kidneys to 1,25-hydroxyvitamin D, the biologically active form of vitamin D that acts as a hormone.

UV ray exposure and VitD: In the skin, ultraviolet rays (UV) reacts with a compound called 7 dehydrocholesterol and vitamin D is made. At higher altitudes, less UV is absorbed due to thinner atmosphere. Therefore, distance from the equator is important to maintain enough vitamin D. It also depends on the season it cold and rainy season is not suitable for enough UV rays and therefore, lacks VitD. Peak UV levels are 40% higher during summer than latitudes of the northern hemisphere. UV radiation (UVR) is insufficient to produce VitD in people living above 42 north latitude, which includes Boston, northern California, and other areas north.

Skin pigmentation: Peak UV times are 11 am and 4 pm, therefore, five minutes/day of exposure to UV rays is sufficient for white skin, whereas twenty minutes/day is sufficient for a person with darker skin.

Food intake enriched with vitamin D: Healthy diets include naturally enriched VitD foods, fortified foods, and beverages, vitamin supplements alternate to sun exposure without having a risk of extreme sun exposure to sensitive skin.

Obesity and vitamin D

Vitamin D deficiency has been implicated in a wide variety of disease states. It has been consistently shown an association of increasing BMI and lower serum 25-hydroxyvitamin D (25D) concentrations. Larger studies [3,4] showed obesity to be associated with lower 25D concentrations, high PTH, and low 1,25D concentrations. A genetic study has suggested that higher BMI leads to lower 25D, with the effects of lower 25D on BMI likely to be small [5]. The association between reduced 25D concentrations and obesity is, therefore, well-established, although the mechanisms for the lower 25D concentrations are not fully described. Possible mechanisms of low VitD are shown to be due to: 1) Lower dietary intake; 2) Reduced cutaneous synthesis: a) Altered behavior for not exposing to sunlight; b) Altered metabolism in obese women expressing the enzymes for both the formation of 25D and its active metabolite, 1,25D and for degradation of VitD may affect via adipose tissue (AT) [6]. Subcutaneous AT has also been found to have lower expression of one of the enzymes responsible for 25-hydroxylation of vitamin D (CYP2J2), as well as a tendency toward a decreased expression of the 1-α hydroxylase. These data suggest that both 25-hydroxylation and 1-α hydroxylation are impaired in obesity. Equal ultraviolet irradiation and oral doses of VitD resulted in a 57% lower increase in serum 25D concentrations in obese individuals compared to

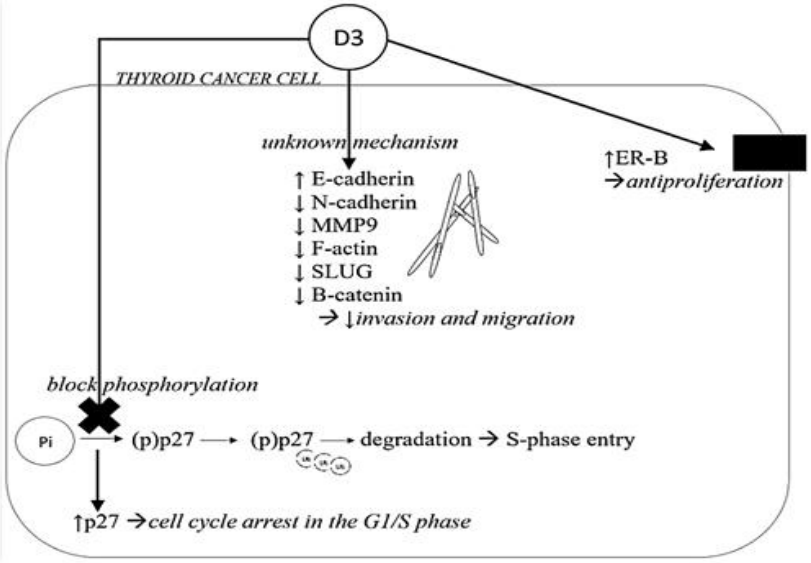

Figure 2: Vitamin D action and pathway in thyroid cancer. ER-B: Estrogen Receptor β; MMP: Matrix Metalloproteinase.

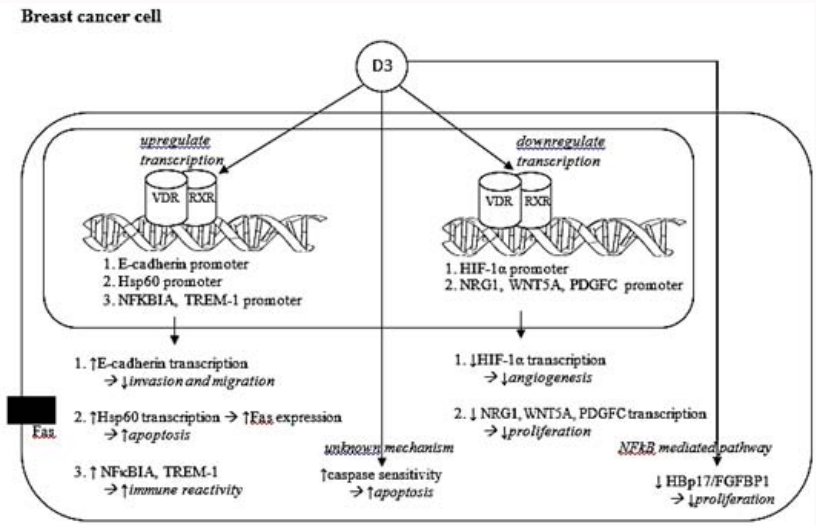

Figure 3: Vitamin D pathway in breast cancer.
VDR: Vitamin D Receptor; RXR: Retinoic Acid Receptor; NRG: Neuregulin; PGDFC: Platelet Derived Growth Factor; TREM: Triggering Receptor Expressed on Myeloid Cells.

non-obese [7]. In conclusion, 50% decreased the bioavailability of cutaneous-synthesized vitamin D_3 in the obese subjects could account for the consistent observation that obesity is associated with VitD deficiency, therefore, larger than usual oral vitamin D should be able to correct the vitamin D deficiency associated with obesity.

Ethnic differences: Darker skin pigmentation ethnic differences play a role in circulating levels of serum VitD [8]. Black Americans are more likely to have lower levels of circulating 25(OH)D than White Americans, as darker skin pigmentation is associated with a slighter increase in serum concentration after a given amount of UVB exposure [9-12]. The data showed a higher prevalence among Blacks of rs7041 polymorphism in the DBP gene associated with low levels of DBP, potentially resulting in bioavailable 25(OH)D levels similar to those in Whites, despite their lower levels of total 25(OH)D. One of the mechanisms involved is that Africans, despite VitD deficiency, can be protected from breast cancer due to a higher activity of the VitD pathway. Black Americans and Native Americans have the lower incidence of breast cancer and more negative prognostic markers. Low DBP means more release into cells, exerts more effect; therefore, DBP is an upstream regulator of VitD.

Measuring 25(OH)D has multiple factors affecting its regulation, but 1,25OH is tightly regulated and not greatly varied, therefore, 25(OH)D is the best biomarker for VitD levels clinically. 25(OH) is mostly used for epidemiology [13], while 1,25OHD is the main form of VitD used for *in vitro* experiments [14]. This presents a problem in differentiating which form of VitD carries the more potent oncoprotective actions.

Vitamin D receptor polymorphisms

- Fok1
- Cdx2
- A1012G

Table 1: Patient tumor samples.

1)	BCPAP	Female papillary thyroid cancer BRAF(V600E) mutation
2)	KTC1	Male papillary thyroid cancer BRAF(V600E) mutation
3)	TPC1	p18 papillary thyroid cancer RET/PTC1 mutation
4)	FTC133	p15 follicular thyroid cancer
5)	Hth7	p90 anaplastic thyroid cancer BRAF WT
6)	C643	p16 male anaplastic thyroid cancer HRAS (G13R) mutation

The pattern of SNP associations was distinct between AAs and EAs. In AAs, six GWAS-identified SNPs in European Americans (EA) GC, CYP2R1, and DHCR7/NADSYN1 were replicated, while nine GWAS SNPs in GC and CYP2R1 were replicated in EAs. A CYP2R1 SNP, rs12794714, exhibited the strongest signal of association in AAs. In EAs, however, a different CYP2R1 SNP, rs1993116, was the most strongly associated [15].

VitD binding protein polymorphism: Vitamin D binding protein (DBP), also known as group-specific component (Gc), has several different physiological functions, including transport of VitD and its metabolites, macrophage activation, actin scavenging, and fatty acid binding [16]. DBP is a highly polymorphic serum protein with three common alleles (Gc1F, Gc1S, and Gc2) and more than 120 rare variants. The presence of unique alleles is a useful tool for anthropological studies to discriminate and to reveal ancestral links between populations [17]. The presence of unique alleles is a useful tool for anthropological studies to discriminate and to reveal ancestral links between populations. Populations with a white skin have a relatively lower frequency of the Gc1F-allele and higher frequency (50%–60%) of the Gc1S-allele. The Gc1F-allele frequency is markedly higher among Black Americans and Black Africans. The Gc1F and Gc1S-allele frequencies display a typical geographical cline from Southeast Asia, through Europe and the Middle East, down to Africa. A common feature of all populations is the less predominance of the Gc2-allele, in comparison with the Gc1-allele. Unlike Black populations, Caucasians have a markedly higher Gc2-allele frequency. The Tuareq KelKummar population of Mali from the Southern Sahara is the only community with a complete absence of the Gc2-allele. The observed variation in the Gc-allele frequencies in different geographic areas may be correlated with skin pigmentation and intensity of sunlight exposure. Pigmented (black) and keratinized (yellowish) skin types are characterized by a lower rate of UV light penetration and a higher susceptibility to rickets. The higher frequency of Gc1F in dark skinned persons may be explained by its greater affinity for and more efficient transport of vitamin D metabolites.

Vitamin D analogs

MART-10 is a new generation analog shown to be more potent than 1α, 25(OH)2D3 in inhibiting prostate, breast, liver, head and neck and thyroid cancer growth *in vitro*. In contrast to 1α, 25(OH)2D3, this analog did not raise serum calcium *in vivo* in an animal model [18]. VitD non-calcemic analog JK 1624 F2-2 (JKF): modulates estrogen receptors in thyroid cancers [19].

Seocalcitol (EB1089) [20]: Few data exist exploring the administration of other VitD analogs as cancer therapy. Phase I and phase II studies of seocalcitol (EB1089) an analog of calcitriol have been conducted. EB-1089 was administered by mouth every day and dose limiting toxicity was determined by hypercalciuria. Using this regimen and with a phase II dose determined in this manner, no clinical activity of EB-1089 was seen in pancreatic cancer or hepatocellular carcinoma. Hypercalciuria likely is an unnecessarily conservative dose-limiting toxicity in developing an agent for the treatment of patients with advanced cancer.

Alpha vitamin D2: Wilding's group reference has carefully studied 1α hydroxyvitamin D2 (1αD2). They have completed phase I trials using a daily regimen as well as phase II trials in prostate cancer as a single agent and in combination with docetaxel. Only limited evidence of antitumor activity was seen, but, again, the daily regimen is likely to be less active than an intermittent high dose schedule.

Inecalcitol: Inecalcitol is a novel VitD analog (19 nor-, 14 epi-, 23-ync, 1,25 dihydroxy vitamin D3) which is being developed by Hybrigenics Corporation. The analog appears to express fewer propensities to induce hypercalcemia while maintaining antitumor activity. A pilot trial presented at the meeting of the American Society of Clinical Oncology in May 2009 indicates that oral doses up to 1000 mcg daily + docetaxel are safe.

Paricalcitol: Paricalcitol is neither 19 nor, 1 alpha, 25 dihydroxy vitamin D2 and has less potential for hypercalcemia than calcitriol. Paricalcitol, marketed as Zemplar® by Abbott Laboratories, appears to be superior to calcitriol in the therapy of secondary

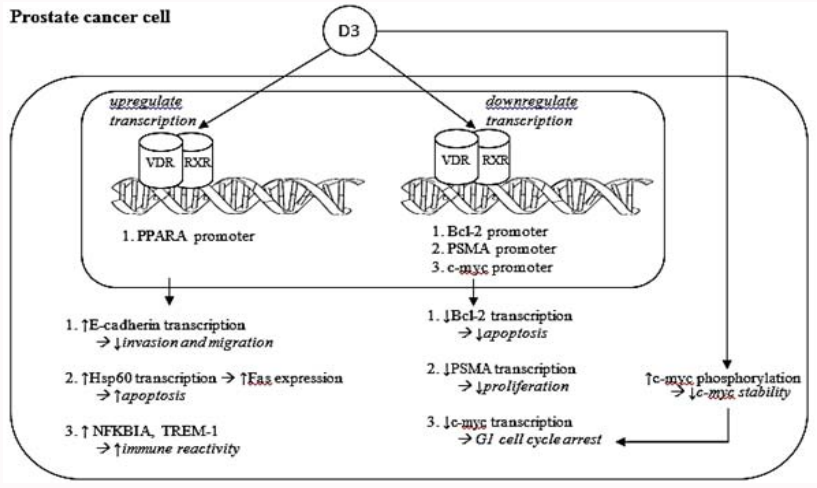

Figure 4: Vitamin D pathway in prostate cancer.
VDR: Vitamin D Receptor; RXR: Retinoic Acid Receptor; PSMA: Prostate Specific Membrane Antigen; TREM: Triggering Receptor Expressed on Myeloid Cells.

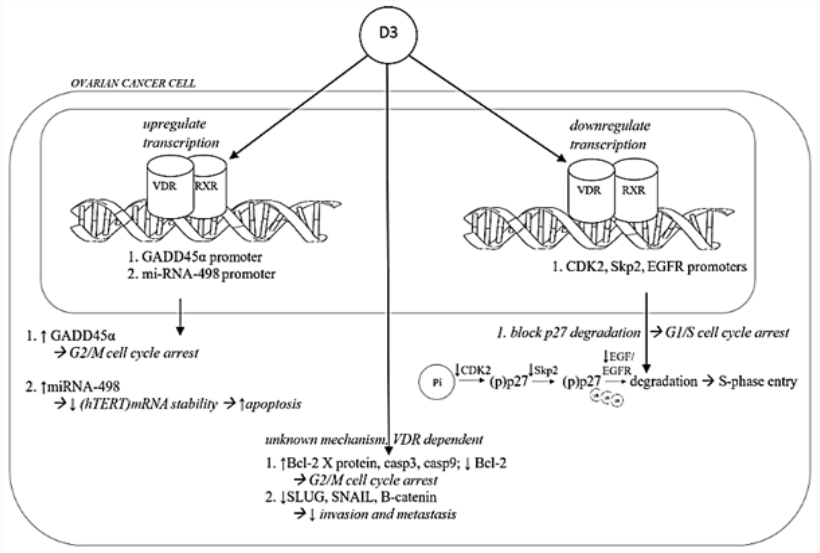

Figure 5: Vitamin D pathway in ovarian cancer.
VDR: Vitamin D Receptor; RXR: Retinoid X Receptor; EGF: Epidermal Growth Factor; CDK: Cyclin D Kinase; hTERT: Human Telomerase Reverse Transcriptase.

hyperparathyroidism and chronic renal failure. Patients receiving paricalcitol survive longer than those receiving calcitriol [86]. Schwartz and colleagues have defined a 3 × weekly intravenous regimen for men with prostate cancer. No clear responses were seen in this trial though in many men, PSA reductions were seen [87].

Vitamin D pathway in thyroid cancer (Figure 2)

The most common forms of differentiated thyroid cancer are distinguished histologically as papillary, follicular, and anaplastic. Both normal and malignant thyroid cells express VDRs and vitamin D metabolic pathway enzymes [21,22]. VDR expression varies widely in distinct thyroid cancer cell lines such as C643, BCPAP, Hth7, Hth74, K1, KAT18, SW1736 and TPC1, and cell response to calcitriol treatment *in vitro* also respond differently depending on the various degrees of differentiation [23]. Current studies have focused on VitD's effect on the proliferation, differentiation, and metastasis of thyroid cancer, as well as the upstream effects of VitD's metabolic pathway in thyroid cancer. There are yet to have studies looking at VitD's effect on angiogenesis and inflammation pathways of thyroid cancers.

Proliferation and differentiation: *In vitro* studies of cell lines NPA (a poorly differentiated papillary carcinoma with mutant p53) and WRO (a well differentiated follicular carcinoma with mutant p53), as well as xenograft tumors, treated with 1,25(OH)D3 and analog EB1089 show cell cycle arrest in the G1/S phase [24]. This effect is induced as 1,25(OH)D3 and EB1089 increase CDK inhibitor $p27^{kip1}$ (p27) in a dose-dependent manner by down regulating its degradation process through decreasing the phosphorylation of Threonine187 on p27, which in turn decreases the activity of the SCFSkp2 (Skp2), the protein complex that targets phosphorylated p27 for ubiquitin dependent proteolysis [24]. P27 is found to be significantly lower in malignant thyroid cells compared to normal, with lower levels in more poorly differentiated cells, and VitD as a direct counter to this effect may slow tumor cell proliferation [25].

1,25(OH)D3 and analog EB1089 also induce G1 cell cycle arrest through a secondary action by increasing PTEN tumor suppressor levels, which mediates cell cycle arrest via the PI3-kinase/Akt/PKB pathway [24]. However, 1,25(OH)D3 and EB1089 do not induce differentiation or apoptosis in NPA or WRO cell lines [24].

In three human thyroid cancer cell lines, ARO (anaplastic carcinoma), NPA (papillary carcinoma) and WRO (follicular carcinoma), ER-a is constitutively upregulated and aids estrogen-induced thyroid cancer cell proliferation, while ER-b is down regulated to inhibit apoptosis and cell grown inhibition [26,27]. A non-calcemic VitD analog JK1624F2-2 upregulates ER-b in ARO and WRO but down regulates it in NPA cells, promoting cancer antiproliferation through inhibition of growth stimulus.

Invasion and metastasis: In anaplastic thyroid carcinoma cell lines 8305C and 8505C, 1,25(OH)D3 and analog MART-10 can attenuate cell invasion and metastasis by several of the following methods, with all effects by MART-10 being more potent than 1,23(OH)D3 [28]. The effect includes upregulation of cell adhesion molecule E-cadherin and down regulation of invasion stimulator N-cadherin. There is also repression of secreted protease MMP-9 that degrades basement membranes' collagen type-IV and decreases amounts of movement-inducing intracellular F-actin. 1,25(OH) D3 and MART-10 also affect the EMT transdifferentiating process whereby epithelial cells transform into more invasive mesenchymal cancer cells, by repressing the associated transcription factor Slug and the signaling molecule B-catenin. With anaplastic thyroid cancer as one of the most rapidly growing tumors with the strongest metastatic potential, and MART-10 showing such potent and widespread effects, further *in vivo* studies of MART-10 application is crucial.

Vitamin D metabolic pathway: Gene expression of four main VitD metabolic enzymes, CYP27A1, CYP2R1, CYP27B1, CYP24A1, have been studied in 6 different malignant cell lines, BCPAP, KTC1, TPC1, FTC133, Hth7, and C643, as well as in patient tumor samples (Table 1).

Baseline levels of 1a-hydroxylase encoded by gene CYP27B1 are significantly higher in TPC1 papillary, BCPAP papillary, FTC133 follicular, and Hth7 anaplastic cells [29,30]. This is confirmed by a study of fresh papillary thyroid samples, with higher expression correlating to the presence of lymphocytic infiltration and no expression of CYP27B1 in normal follicular tissue [30].

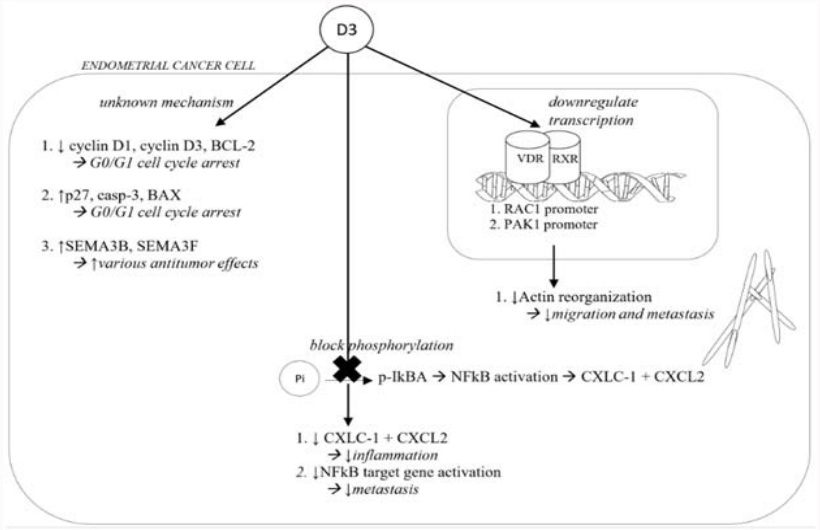

Figure 6: Vitamin D pathway in endometrial cancer.
VDR: Vitamin D Receptor; RXR: Retinoid X Receptor.

25-hydroxylases encoded by genes CYP27A1 and CYP2R1 varies with different thyroid cell lines. CYP27A1 is higher in BCPAP papillary and C643 anaplastic cells, and lower in TPC1 papillary, FTC133 follicular, and Hth7 anaplastic cells [28]. CYP27A1 is found significantly higher in patient samples of papillary carcinoma and follicular adenoma whereas CYP2R1 is only higher in Hth7 cells [31]. These baseline levels indicate the potential for malignant thyroid cells to activate VitD in many different forms, which broadens the number of therapeutic approaches. Although non-supplemented serum concentrations of 25(OH)D3 has been shown not to affect the prognosis of aggressiveness of papillary thyroid cancer, these changes of VitD's metabolic enzymes in thyroid cancers may indicate the potential to activate supplemental levels of VitD in different forms and broaden the number of therapeutic approaches [32].

On the other hand, even though VitD's main degradation enzyme encoded by CYP24A1 is consistently lower in thyroid cancer cell lines, *in vitro* treatment with 1,25(OH)D3 induces transcription of CYP24A1 in two cell lines with lowest baseline levels: TPC1 and C643 [31]. These results have also been seen in HTh7, HTh74, and SW1736 cell lines in a different study that looked at *in vitro* treatment with both 1,25OHD3 and analog DP006 [21]. This effect is consistent with VitD's negative feedback pathway that activates its own degradation, but it confers relative resistance to VitD treatment in these malignant thyroid cell lines, and it is currently unknown if this resistance can be overcome.

Vitamin D pathway in breast cancer (Figure 3)

Breast cancer is one of the most common cancers in a woman and second commonest cause of cancer deaths in woman after lung cancer. If cancer is localized in the breast only, the survival rate is 99%, however, if cancer spreads to lymph node or distant metastasis 5-year survival rates goes down to 85% and 26%, respectively. VitD deficiency is common in patients diagnosed with breast cancer, with the worst prognosis and outcome in the lowest serum concentrations [33] as well as high risk of developing breast cancer with lower serum levels. Vitamin D enters target cells and works only when it is unbound to protein carriers.

Genetics: Combined prospective studies showed no association of circulating 25(OH)D with breast cancer risk [34]. A previous study suggested that SNPs in the Retinoic acid receptor (RXRA) and PLAUR genes in the VitD pathway may contribute to breast cancer disease-free survival (DFS). In particular, SNPs in RXRA predicted for poorer or improved DFS in patients, according to the type of systemic treatment received. If it can be validated, these markers could be used for risk stratification of breast cancer patients [35].

Proliferation and differentiation: The previous study reported novel findings regarding the effects of 1α, 25-dihydroxyvitamin D3 on NFκB signaling in tamoxifen-resistant breast cancer cells and suggests that VitD might be interesting for further evaluation as a new strategy to treat antiestrogen resistant breast cancers [36]. 1,25(OH)(2)D regulates HIF-1α protein patient tumor samples regulation in MCF10A cells in contrast to through proteasomal degradation with the presence of H-ras oncogene in MCF10A cells [37]. Metaplastic carcinomas may respond to 1α, 25(OH)(2)D(3), since they express VDR and 1α, 25(OH)D(3) induces de novo E-cadherin expression in breast cancer cells by promoter demethylation [38]. The reduction of gene activation by VD(3) in breast cancer cells was caused by the interference of the transactivation potential of NF-kappaB p65 subunit [39].

1α, 25(OH)D(3) acts as an anticancer agent by increasing expression of Fas on the surface of melanoma cells through Hsp60 induction and strengthens caspase sensitivity to the Fas-mediated apoptotic pathway by NK cells [40].

Apoptosis: In adjacent normal mammary associated fibroblasts (NAF), a distinct subset of genes was induced by 1,25(OH)D, involved in anti-apoptosis, detoxification, antibacterial defense system and protection against oxidative stress, which may limit carcinogenesis. Co-expression network and interactome analysis of genes commonly regulated by 1,25D in NAFs and breast cancer associated fibroblasts (CAF) revealed differences in their co-expression values, suggesting that 1,25D effects in NAFs are distinct from those triggered in CAFs [41].

Invasion and migration, angiogenesis: Low VitD was correlated with progression and metastasis of breast cancer patients [42]. 1, α vitamin D3 was shown to inhibit invasive potentials of breast

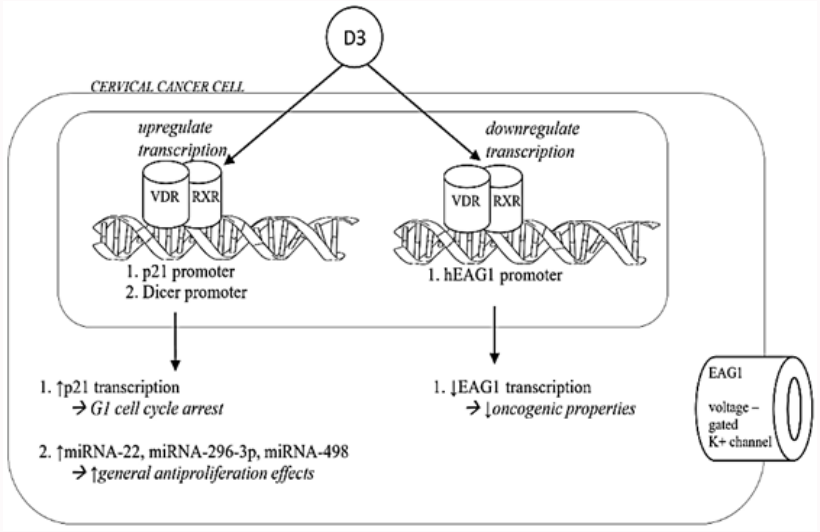

Figure 7: Vitamin D action and pathway on cervical cancer cells.
VDR: Vitamin D Receptor; RXR: Retinoid X Receptor.

cancer cell lines via inhibiting growth and migration [43]. Vitamin D [44] was shown to inhibit metastasis of MDA-MB-231 cell lines and decreased the capacity of MCF10CA1 cells survival in the bone. This action was shown via inhibiting E-Cadherin expression, which is a marker of epithelial-mesenchymal transition (EMT). MART-10 was shown to have more potent than vitamin D3 in preventing the metastatic potential of MCF-7 breast cancer cells *in vitro* [45,46].

Inflammation: Previously it was suggested that the COX-2/PGE2 pathway positively regulates the expression of CYP1B1 in breast cancer. 1,25(OH)D3 inhibits the growth of MCF-7 cells and downregulates CYP1B1 mediated by the COX-2/PGE2 pathway [47]. Based on these findings, it was concluded that 1α, 25(OH)2D3 down regulated HBp17/FGFBP-1 expression via NF-KB [48]. Classical and non-classical (novel) vitamin D analogs show pro-differentiation, antiproliferative and anticancer properties. Functional analysis revealed that in CAFs, genes associated with proliferation (NRG1, WNT5A, PDGFC) were down regulated and those involved in immune modulation (NFKBIA, TREM-1) were up regulated, consistent with anti-tumor activities of 1,25D in breast cancer.

Vitamin D metabolic pathway: The previous study showed several signaling pathways commonly regulated by vitamin D compounds and highlight their regulation on TLR4 [49]. Gemini vitamin D(3) analogue, Ro-438-3582 [Ro3582; 1 alpha,25-dihydroxy-20S,21(3-hydroxy-3methylbutyl)-23-yne-26,27-hexafluorocholecalciferol], inhibited cell proliferation and activated the BMP/Smad signaling pathway in MCF10AT1 breast epithelial cells. Inhibition of RAS activity blocked the translocation of PKC alpha to the plasma membrane and the phosphorylation of Smad1/5 induced by Ro3582, indicating that RAS is necessary for the activation of PKC alpha and Smad signaling. In conclusion, Ro3582 inhibits cell proliferation and activates BMP/Smad signaling *via* a RAS and PKC alpha pathway in breast epithelial cells [50]. siRNA and genome-wide studies both suggest that the anti-proliferative effects of 1,25D in MCF7 breast tumor cell lines do not rely on classical VitD pathway per se [51]. Antitumoral effects of calcitriol in basal cell carcinomas involve inhibition of hedgehog signaling and induction of VDR and differentiation [52]. A crosstalk between VDR and miR-214 in regulating hedgehog signaling in breast cancer cells, provides a new therapeutic target for breast cancer [53].

Vitamin D pathway in prostate cancer (Figure 4)

Prostate cancer is the second most commonly diagnosed cancer and the second leading cause of cancer deaths in males. The association of lower 1,25D with prostate cancer was found in men above the median age of 57 years at serum storage but not younger men and was similar in Black and White men [54]. It was recently shown that DBP modulates circulating VitD status in prostate cancer patients [55].

Genetics: Some evidence shows that VitD pathway SNPs, such as VDR and CYP17 were associated with prostate cancer risk and grade, but not stage. There was no evidence of an association in men with deficient VitD (measured by having low sun exposure) [56]. Recent study showed that SNP markers localized to each of four genes (GC, CYP24A1, CYP2R1, and DHCR7) previously associated with 25(OH)D were genotyped in 10,018 cases and 11,052 controls from the NCI Breast and Prostate Cancer Cohort Consortium but genetic variants related to lower 25(OH)D were associated with a decreased risk of aggressive prostate cancer [57].

Proliferation and differentiation: 20(OH)D possesses high efficacy for inhibiting cancer cell proliferation *in vitro* and is non-toxic *in vivo*, supporting its further development as a potential anticancer therapeutic agent [58]. *In vivo* tests show that while 1,25(OH)(2)D at doses as low as 0.8 μg/kg induces calcium deposits in the kidney and heart, 20(OH)D(2) is devoid of such activity even at doses as high as 4 μg/kg and 20(OH)D shows cell-type dependent antiproliferative and prodifferentiation activities through activation of VDR, while having no detectable toxic calcemic activity [59].

Apoptosis: 1,25D-mediated downregulation of the anti-apoptotic protein Bcl-2, whose expression is regulated by E2F, also is Rb-independent in prostate cancer cells [60].

Inflammation: Suppression of prostate-specific membrane antigen (PSMA) by 1,25VD occurs at the level of the PSMA enhancer and is elevated by over-expression of the VDR. This regulation involves the AR but is not dependent on the presence of androgens [61]. 1,25(OH)(2)D(3) reduces both c-Myc mRNA levels and c-Myc

protein stability to inhibit the growth of prostate cancer cells [62].

Vitamin D metabolic pathway: Utilizing 1,25(OH)D or its analogs combined with inhibition of PI3K/AKT for the treatment of prostate cancer [63]. VitD orchestrates a pattern of changes within prostate epithelial cells that limit or slow carcinogenesis [64]. 1,25(OH)D3 modulates the effects of low-dose IR (1-5 Gy) on cultured human keratinocytes [65]. 1,25-Dihydroxyvitamin D3 modulates lipid metabolism in prostate cancer cells through miRNA-mediated regulation of PPARA [66]. Dietary components, such as omega3PUFAs and VitD, have the potential to delay the progression of prostate cancer cells to an aggressive and untreatable state [67].

Vitamin D pathway in ovarian cancer (Figure 5)

Ovarian cancer is one of the most common gynecologic neoplasms among women, with an overall 5-year survival rate of 40% [68]. Endogenous VDRs have been detected in ovarian cancer cell lines, which are independently regulated by estrogen and progesterone receptors [69,70]. Research now shows the actions of 1α,25(OH)D3 in ovarian cancer cells are inhibition of proliferation, invasion and metastasis, inflammation, and induction of apoptosis [71].

Proliferation and differentiation: In OVCAR3 cells, 1,25(OH)2D3 inhibits cell growth by inducing cell cycle arrest at G1/S and G2/M checkpoints [72,73]. 1,25(OH)2D3 arrests OCa cells in G1/S phase by increasing the abundance of p27, an inhibitor of cyclin-dependent kinase (CDK) activity [72]. 1,25(OH)2D3 stabilizes p27 at the protein by decreasing mRNA transcription levels of cyclin E-associated CDK2, which phosphorylate p27 at Thr187 to mark it for ubiquitin-mediated proteolysis, and by decreasing mRNA transcription of Skp2 ligase, which ubiquitinates p27 [72]. The p27 degradation activity to commit cells to S phase entry from the G1/S checkpoint is also known to couple with mitogenic signals from EGF. New studies now show that 1α,25(OH)D3 also regulates this partnered signal by down regulating the EGFR receptor through another novel VDRE identified in intern 1 of the EGFR genome [74].

1α,25(OH)D3 -induced cell cycle arrest at the G2/M checkpoint specifically requires GADD45α which is a DNA damage-induced and p53-regulated gene that plays an essential role in cell cycle and DNA repair. GADD45α has also been implicated in the tumorigenesis of ovarian cancer [75]. 1α,25(OH)D3 upregulates GADD45α mRNA within 2 hours of treatment as an early response action, but protein accumulation requires days, suggesting other post-transcriptional regulations. Transcriptionally, 1α,25(OH)D3 complexes with functional VDR and RXR endogenous to ovarian cancer cells, and the complex then binds to a novel VDRE in the 3′ untranslated region in the fourth exon of GADD45α's promoter region [73].

The same upregulation of GADD45α to inhibit proliferation is also seen with EB1089, although unlike other types of cancer cells, while more dilute concentrations of EB1089 can elicit similar growth inhibition patterns, the final magnitude of cell growth inhibition is the same as 1,25(OH)D3, suggesting a saturation for VitD mediated antiproliferation effects in ovarian cancer cells [76]. Also unlike in other types of cancer cells, VitD-induced antiproliferation is not mediated by p21 in ovarian cancer cells [72]. These results taken together show VitD inducing antiproliferation is multiple levels, indicating very potent effects in ovarian cancer cells.

When combined with chemotherapy agents such as carboplatin, growth suppression of SKOV-3 cells is synergistic with the combination, and interestingly cell cycle arrest is proportionally more in the G2/M phase [77]. Combined treatment with Mullerian inhibiting substance on SKOV3, OVCAR3 and OVCA433 cell lines show more apoptosis with upregulation of Bcl-2 associated X protein, caspase-3, caspase-9, and down regulation of Bcl-2 [78].

Apoptosis: The quantitative reverse transcription-PCR analysis shows that 1α,25(OH)D3 induces apoptosis in OVCAR-3 cells by down regulating human telomerase reverse transcriptase (hTERT) mRNA, the catalytic subunit of telomerase [79]. The decrease is not due to transcriptional repression through the known VDRE in the 5' regulatory region of hTERT gene, because it is found not functional in ovarian cancer cells [79]. Rather, 1α,25(OH)D3 destabilizes hTERT mRNA by regulating miRNA-498 which binds to the 3′-UTR of hTERT and decreases its mRNA expression to induce overall cell death [79,80]. A novel VDRE in the 5-prime regulatory region of the miR-498 genome has been found, which is occupied by endogenous vitamin D receptor and its coactivators [80]. 1α,25(OH)2D3 can also suppress this pathway when it is induced by leptin, indicating VitD as a potential therapeutic agent for obese women with estrogen-sensitive tumors such as ovarian cancer [81].

Conflictingly in OVCAR-3 cells, microarray studies show that 1α,25(OH)D3 upregulate several apoptosis genes, especially ones involved in the extrinsic apoptotic pathway mediated through death receptors [82]. Fas, TRAIL receptor 2 (TRAIL-R2), and caspase-7 are down-regulated, whereas TRAIL and TRAIL-R4, a decoy receptor that suppresses the apoptosis response to TRAIL are upregulated. Overall, it seems 1α,25(OH)D3 suppresses apoptosis induced by TRAIL and extrinsic FAS-FasL mediated apoptosis. TRAIL is known to selectively induce apoptosis in transformed cells but not in normal cells, making it a uniquely attractive therapeutic agent for human cancers. Fortunately, the suppression of TRAIL-induced apoptosis by 1,25(OH)D3 can be overcome by siRNA for TRAIL-R4, suggesting a favorable combination for clinical therapy to include calcitriol, siRNA for TRAIL-R4, and TRAIL [82].

Invasion and metastasis: The combined potent anti- apoptotic and anti-proliferative effects correlate *in vivo* to further downstream inhibition cancer cell migration and metastatic seeding [82]. Mechanistically, 1α,25(OH)D3 suppresses the migration and invasion of SKOV-3 ovarian cancer cells by inhibiting TGF-induced EMT through down regulation of transcription factors SLUG, SNAIL, and β catenin [80]. mRNA decreases in a dose-dependent manner and directly correlates with increased expression of endogenous VDR. In addition, knockdown of VDR in OVCAR4 cells increases their ability to colonize the omentum in an *ex vivo* system in the absence of 1,25D3, showing a potential ligand-independent suppression of EOC invasion by epithelial VDR [83]. Taken together, these results reinforce the need for further preclinical studies on how to increase VDR expression in ovarian cancer cells.

Inflammation: Contrary to other VitD's anticancer effects, VitD's metabolic pathway has been shown to increase inflammation in the ovarian tumor microenvironment through increased expression of IL-37 in macrophages [84]. Ovarian tumor cells SKOV-3 secrete a tumor molecule, versican VI that induces gene expression of hCAP18/IL-37 in macrophages, which both induces and relies on VDR and CYP27B1 for downstream IL-37 production [84]. Thus, hCAP18/LL-37 generated by 1,25(OH)D3 in tumor microenvironments propagates a tumor-promoting effect that increases tumor proliferation and invasion, which will need further study to find potential points of anti-tumor intervention.

Vitamin D metabolic pathway: As the above evidence suggests, the anticancer effects of VitD are highly dependent on its metabolic pathway. Since much of VitD's response is mediated by VDR in ovarian cancer cells, it is important to know that RIPK1, enzyme acting downstream of tumor necrosis factor alpha to control cell survival and death, can decrease transcriptional activity of VDR, as well as for a complex with it to retain it in the cytoplasm [91]. As TNF-α plays a major role in tumorigenesis, this suppressive mechanism plays a major part in cancer intervention, indicating RIPK1 depletion as a potential strategy to increase the potency of VitD therapy.

In a survey of 61 ovarian tumors, 18 metastases and 10 normal ovaries poorly differentiated primary tumors and metastases show the lowest level of CYP27B1 expression, while non-metastasizing tumors show a higher CYP27B1 level than tumors that develop metastases. The expression of CYP27B1 is positively correlated with a lower proliferation rate, lower dynamism of tumor growth and tumor infiltrating lymphocyte response, suggesting that local expression of CYP27B1 in ovarian tumor cells can modify their behavior and promote a less aggressive phenotype by affecting local concentrations of active vitamin D levels within the tumor microenvironment [85]. Similarly, lower expression of 1a hydroxylase is also correlated with more malignant ovarian cancer cells [86]. In addition, as expected, 1α,25(OH)D3 and its analogs significantly increase CYP24A1 levels in OVCAR-3 cells [87,88].

Vitamin D pathway in endometrial cancer (Figure 6)

Endometrial cancer develops in about 142,000 women worldwide, with the typical age of diagnosis after menopause in the seventh decade of life [89]. The overall 5-year survival rate is 80%, but there is substantial prognostic difference between histological types of endometrial cancers. Endometrial cancers also express endogenous VDR in both human tissue samples and immortalized cell lines [90]. Because endometrial cancer development is closely related to hormonal levels, much of preclinical VitD research focuses on VitD actions in the presence of progesterone, a known highly effective endometrial cancer prevention agent.

Proliferation and differentiation: Both 1,25(OH)D3 alone and in combination with progesterone shows inhibition of cancer growth in cell lines, Ishikawa, HEC-1B, and RL-95 through induction of G0/G1 cell cycle arrest with associated down regulation of cyclins D1 and D3, as well as induction of p27 [90-92]. In addition, although no VitD-induced apoptosis has been evident, treatment both alone and in combination with progesterone show additional growth inhibition through upregulation of caspase-3 activation, upregulation of BAX, and down regulation of BCL2 [91,92]. Because apoptosis stimuli often arrest growth before inducing cell death, it is possible that longer treatment times or greater concentrations of treatment may eventually lead to apoptosis, but further research is needed for confirmation.

Class 3 semaphorins (SEMA), SEMA3B and SEMA3F, are secreted proteins that regulate angiogenesis, tumor growth, and metastasis, and have a role in the pathogenesis of multiple cancers, including breast, ovarian, and lung cancer cells [91]. SEMA3B and SEMA3F are strongly expressed in normal endometrial tissues but down regulated in malignant cells. Treatment of endometrial cell lines, Ishikawa, HEC-1B, and RL-95 with 1,25(OH)D3, shows significant upregulation of SEMA3B and SEMA3F, which is further enhanced with the combination of progesterone treatment [91]. This finding projects future therapy intervention to increase SEMA3B and SEMA3F, potentially in combination with VitD and progesterone.

Similar to other cancer cell types, treatment of HEC-1B cells shows inhibition of differentiation through down regulation of E-cadherin, although the exact mechanism of differentiation suppression is not as extensively researched in endometrial cancer. There is an associated gene Icb-1 (C1or f38) found to be necessary for VitD's induction of differentiation-associated genes, but the specific pathway is yet to be elucidated [93].

Invasion and metastasis: Invasion and metastasis not only require proteolysis of the basement membrane, but also intracellular motility mechanisms, such as actin reorganization. Actin reorganization is under control of various signaling pathways including ras-related C3 botulinum toxin substrate 1 (RAC1), p21 protein-activated kinase 1 (PAK1) and actin-related protein 2 (ARP2). A 24 h treatment of endometrial cancer cells with 1α,25(OH)2D3 can significantly decrease RAC1 and PAK1 transcript levels and activity, decreases ARP2 protein levels and depolymerize actin [94]. The same effect is even more potent with non-calcemic/VDR-independent analog PT19c, providing yet another unique anticancer effect of VitD, as well as an alternative therapeutic option for specific cells that downregulate VDR [95].

Inflammation: Treatment of Ishikawa and HEC-1B cell lines with 1,25(OH)D3 decreased expression of inflammatory cytokines CXCL1 and CXCL2 through suppression of phosphorylation on IκBα, which in turn suppresses activation of NFκB [96]. Regulation of NFκB signaling is a key component of anticancer efforts since the pathway ultimately promotes both inflammations in the tumor microenvironment and expression of NFκB-regulated metastasis genes.

Vitamin D metabolic pathway: Functional endogenous VDR and 1a-hydroxylase have been found in both normal and malignant endometrial cells, with splice variants seen in Ishikawa, RL95/2 and HEC1-A cells [96,97]. Both CYP27A1 and CYP2R1 are higher in malignant endometrial cells, and actively engage in local synthesis of 1α,25(OH)2D3 that downstream cause growth inhibition in the same cells [98]. In addition, the CYP24A1 cytosolic expression is lower in cancer cell lines Ishikawa, RL95/2, and HEC1-A, but higher in clinical samples of more aggressive grade 3 and stage III and IV tumors [98]. Fortunately, the most recent studies have found that progestins can inhibit 1α,25(OH)2D3-induced CYP24A1 expression in Ishikawa, RL95/2, and HEC1-A cells [98]. This points toward a favorable combination treatment as progestins also exert antiproliferative and anti-inflammatory effects similar to VitD, although more evidence is needed for its effects in clinical tissue samples.

Vitamin D pathway in cervical cancer (Figure 7)

Cervical cancer represents approximately 4.5% of all malignancies in women [99]. The role of VitD in cervical cancer has not been as extensively researched like in prostate or breast cancer. Much of its effects are still to be elucidated, but there are identified cell lines that contain endogenous VDR and 1α-hydroxylase [100]. SiHa and HeLa cervical cell lines are considered calcitriol-responsive because they express the VDR, whereas C33-A cervical cell line is considered calcitriol-unresponsive, because it lacks the VDR [101]. However, immunohistochemical staining in tissues of cervical cancer patients show that while expression of VDR was significantly increased in cervical cancer compared to normal cervical tissue on the protein level, it is not increased on the RNA-level, indicating that VDR

protein expression may be necessary for VitD activity but not be a prognostic factor in cervical cancer [102].

1α,25(OH)D3 treatment of HeLa cells shows VDR-dependent induction of cell cycle arrest in the G1 phase *via* down regulating transcription of HCCR-1 oncogene and upregulation of p21 transcription and promoter activity [103]. Other studies focus on VitD's effect on broadly regulated antitumor mechanisms. Human ether à go-go-1 (EAG1) is a voltage-gated potassium channel that displays oncogenic properties and is over expressed in many tumors such as cervical, breast, lung, colon and prostate cancer [104]. Inhibition of EAG1 gene expression or channel activity reduces tumor cell proliferation both *in vitro* and *in vivo* [104]. 1,25(OH) D3 treatment of SiHa and HeLa cell lines shows down regulation of EAG1 expression *via* a functional negative vitamin D response element (nVDRE) E-box type in the hEAG1 promoter [105,106]. VitD also seems to play a role in the miRNA processing machinery in cervical cancer cells. In SiHa cells, 1,25(OH)D3 increases expression of antitumor miRNAs such as miR-22, miR-296-3p, and miR-498 by upregulating the transcription of their processing enzyme Dicer and RNA helicase DDX5, through VDR-ligand complex binding on the promoter region of Dicer [101,107].

Conclusion

Vitamin D pathway is diverse in different cancer. Although many pathways have been explored to determine its action in different cancers, however, no direct relationship was detected to conclude its valid role in cancer therapy. Patients with genetic alterations may need supplemental vitamin D to get free vitamin D available to them. Many vitamin D analogs are tried in patients with cancers to combat hypercalcemia due to the high dose of vitamin D administration. It appears that vitamin D analogs can be given as a supplemental therapy for many cancers safely. Moreover, vitamin D supplements may improve chemo and radio sensitivity to cancer cells that are resistant to chemo/radiotherapy. However, more research is needed to reach this conclusion. All association studies in the past are done in random samples. However, if there is a genetic association to cancer risk, Vitamin D supplementation will be helpful to those individuals who lack free vitamin D and common VitD pathway genetic defects. Further research is needed to cluster the correlation of genetic alterations with increased cancer risk.

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