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Invited review

Vitamin D and prevention of breast cancer[1]

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Abstract

Epidemiologic data have demonstrated that breast cancer incidence is inversely correlated with indices of vitamin D status, including ultraviolet exposure, which enhances epidermal vitamin D synthesis. The vitamin D receptor (VDR) is expressed in mammary epithelial cells, suggesting that vitamin D may directly influence sensitivity of the gland to transformation. Consistent with this concept, *in vitro* studies have demonstrated that the VDR ligand, 1,25-dihydroxyvitamin D (1, 25D), exerts negative growth regulatory effects on mammary epithelial cells that contribute to maintenance of the differentiated phenotype. Furthermore, deletion of the VDR gene in mice alters the balance between proliferation and apoptosis in the mammary gland, which ultimately enhances its susceptibility to carcinogenesis. In addition, dietary supplementation with vitamin D, or chronic treatment with synthetic VDR agonists, reduces the incidence of carcinogen-induced mammary tumors in rodents. Collectively, these observations have reinforced the need to further define the human requirement for vitamin D and the molecular actions of the VDR in relation to prevention of breast cancer.

Vitamin D and cancer prevention

Although originally identified based on its ability to prevent the bone disease rickets, it is now recognized that 1a,25 dihydroxyvitamin D_3 (1,25D), the biologically active form of vitamin D_3, exerts effects in almost every tissue in the body. Recent epidemiological studies have focused attention on a possible link between vitamin D and cancer. The concept underlying this link is fairly simple: that the vitamin D receptor (VDR) and its ligand 1,25D induce a program of gene expression that contributes to maintenance of the quiescent, differentiated phenotype. This concept predicts that the vitamin D system might have relevance for both prevention and treatment of cancer. The initial identification of the VDR in breast cancer cells suggested that this receptor might represent a target for breast cancer therapy, and multiple studies have assessed the effects of vitamin D on breast cancer cells *in vitro* and tumors *in vivo*. In addition, the potential side effects of vitamin D therapy have been characterized, and a combination of trials of vitamin D compounds with standard therapies such as anti-estrogens, chemotherapeutic drugs and radiation have been reported. For the most part, these studies have been limited to animal models of breast cancer such as the NMU-induced breast cancer model in rats and human xenograft models in immunosuppressed mice.

Studies to test the role of vitamin D in breast cancer prevention have included characterization of the expression and function of the vitamin D pathway in normal mammary tissue using animal models and human cells. Studies with the VDR knockout mouse model have been highly informative in determining whether complete abrogation of vitamin D signaling alters the susceptibility of mammary tissue to cancer development. In this review, we summarize the currently available data generated from both *in vitro* and *in vivo* studies, with an emphasis on the cellular and molecular mechanisms by which vitamin D may contribute to breast cancer prevention.

Overview of vitamin D biology

The nutritional substance termed "vitamin D" comprises ergocalciferol (vitamin D_2, from plant sources) and cholecalciferol (vitamin D_3, from animal sources). Both forms can be

obtained from the diet, but very few natural foods have significant vitamin D activity[1]. In many countries, including the US, fortification of foods such as milk and orange juice with vitamin D_3 is common. Cholecalciferol can also be generated from 7-dehydrocholesterol in the epidermis upon exposure to ultraviolet (UV) radiation, thus, vitamin D is not technically a vitamin. In fact, because the concentration of vitamin D in natural foods is quite low, the majority of vitamin D in most individuals likely originates from epidermal synthesis, however there is considerable individual variation in this process[2]. In particular, epidermal synthesis is affected by skin pigmentation, sunscreen use, age, season, latitude and other lifestyle factors. Despite the fortification of vitamin D_3 in foods and endogenous synthesis, the prevalence of vitamin D insufficiency is surprisingly common, especially in populations living in northern climates and in the elderly[3-6]. Particularly relevant to the possible relationship between vitamin D and breast cancer, vitamin D deficiency has been reported in a high percentage of women, including during adolescence, pregnancy and/or lactation and after menopause, even in sunny climates[3,7-9]. These and other determinants of vitamin D status in humans are summarized in Table 1.

Table 1. Determinants of vitamin D status in humans.

DETERMINANTS OF VITAMIN D STATUS	
Dietary Sources	
-Ergocalciferol (D_2)	Natural foods
-Cholecalciferol (D_3)	Fortification
	Supplements
Factors Affecting Epidermal Synthesis	Skin pigmentation
	Latitude
	Season
	Sunscreen use
	Clothing
Endogenous Influences	Age
	Gender
	Malabsorption syndromes
	Liver or Kidney disease
	Obesity
	Rare genetic defects in CYP27B1 or VDR

The increasing number of reports of vitamin D insufficiency has prompted re-evaluation of the recommended adequate intake for vitamin D[5,10] which was directed against prevention of rickets. There is fairly compelling evidence that prolonged sub-clinical vitamin D deficiency, which may not be associated with hypocalcemia or bone disease but could limit availability of active vitamin D metabolites to tissues, contributes to chronic disease in human populations. However, relevant biomarkers of vitamin D status that reflect newly identified actions in colon, prostate and breast that are relevant for cancer prevention (as discussed below) remain to be identified.

Both naturally occurring forms of vitamin D require metabolism for biological activity, and defects in activation pathways can affect vitamin D function[11]. For simplicity, this review focuses on vitamin D_3, but the metabolism and function of vitamin D_2 is similar, although some data suggest that D_2 is less potent than D_3 in humans. Regardless of source (endogenous synthesis or diet), the initial step in metabolism of vitamin D_3 is hydroxylation at the 25 position, generating 25-hydroxyvitamin D_3 (25D). The 25D metabolite, which is mainly produced in the liver, is the major circulating form and the most accurate biomarker of overall vitamin D_3 status[6,12]. Further metabolism of 25D occurs in many tissues and leads to generation of multiple metabolites, two of these -24,25-dihydroxyvitamin D_3 (24,25D) and 1α,25-dihydroxyvitamin D_3 (1,25D), have been extensively characterized in relation to maintenance of calcium homeostasis. Generation of 24,25D is catalyzed by 24-hydroxylase (also termed CYP24A1), an enzyme present in most vitamin D target tissues. The 24,25D metabolite does not avidly bind VDR, and its production is considered the first step in degradation of 25D. Production of 1,25D, the biologically active metabolite, is mediated by 1α-hydroxylase (also termed CYP27B1), an enzyme that is highly expressed in renal proximal tubules. The importance of renal 1α-hydroxylase in maintaining systemic 1,25D has been documented in patients with end stage renal failure, who require exogenous supplementation with this metabolite to prevent renal osteodystropy. Although originally thought to be exclusively localized to the kidney, 1α-hydroxylase gene expression and activity has now been localized to multiple other tissues including mammary gland[13-16]. In extra-renal cell types, 1α-hydroxylase likely generates 1,25D, which acts locally within tissues, as circulating 1,25D is virtually undetectable in anephric individuals. The 1,25D metabolite, sometimes called *calcitriol*, is the high affinity ligand for the VDR, a nuclear transcription factor (Figure 1).

Vitamin D: cellular uptake and general mechanism of action

Vitamin D metabolites, including 25D and 1,25D, circulate as free steroids and in complex with the vitamin D

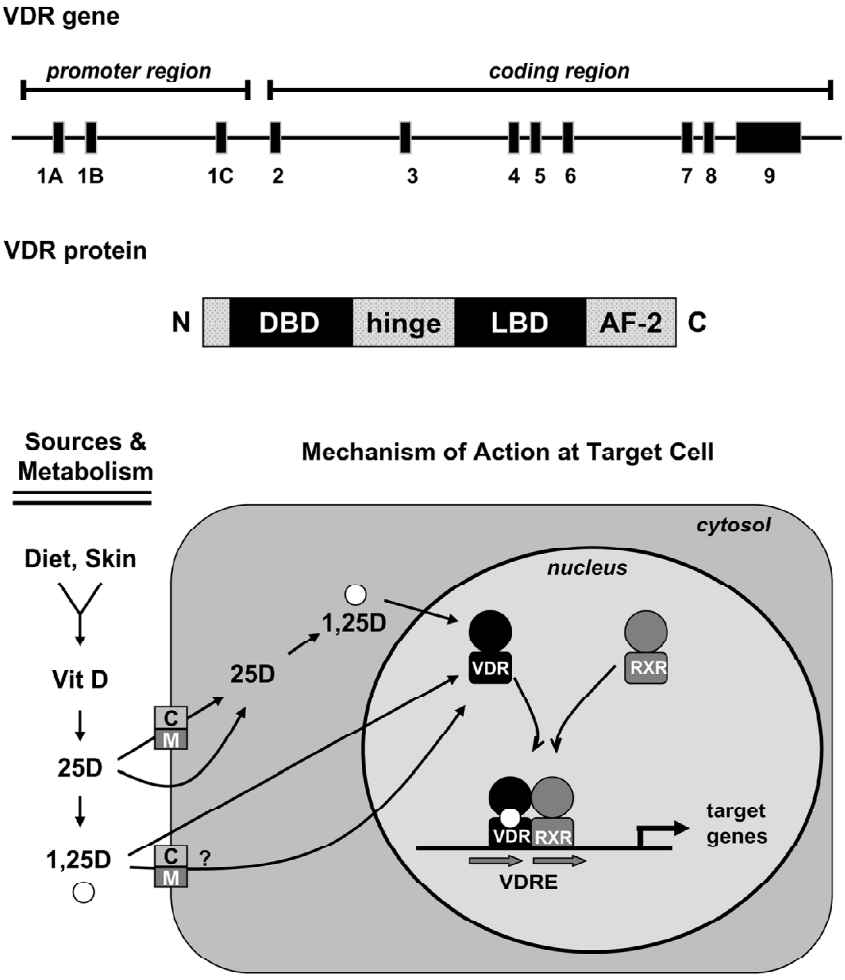

Figure 1. Overview of the vitamin D receptor (VCR) and vitamin D signaling pathway. Top, VDR gene organization. The hVDR gene is localized to chromosome 12q13.11, and consists of multiple promoter regions (A–C) followed by the coding region spanning exons 2 through 9. Middle, VDR protein domain structure. The hVDR is a 48kDa protein with a short N-terminal extension, a DNA binding domain (DBD) comprised of two zinc finger motifs, a hinge region which allows conformational flexibility, a ligand binding domain (LBD) that binds 1,25D, and an AF-2 domain where transcriptional co-factors bind. Bottom, overview of vitamin D action. Vitamin D_2 and D_3 can be obtained from dietary sources; vitamin D_3 can also be synthesized in the epidermis. Both forms are converted to 25-hydroxyvitamin D (25D) in the liver and circulate bound to the vitamin D binding protein (DBP). The relative contribution of active versus passive cellular uptake mechanisms for various vitamin D steroids (either free or bound to serum proteins) is unclear. However, it has been demonstrated that the 25D-DBP complex is internalized by target cells that express the endocytic receptors megalin (M) and cubilin (C), such as the kidney. In the renal proximal tubules, and likely also in many target cells, 25D is converted to 1,25-dihydroxyvitamin D (1,25D). 1,25D can act within the cells in which it is produced, or it can be released into the tissue microenvironment and/or the systemic circulation (in the case of the kidney). Intracellular 1,25D binds to the VDR which dimerizes with the retinoid X receptor (RXR) to bind vitamin D response elements (VDRE) in target genes. Complexity and tissue specific responses are achieved via multiple, widely spaced vitamin D responsive regions, structurally distinct VDREs, recruitment of distinct co-activator and co-repressor complexes, alternative dimerization partners, and ligand independent activities.

binding protein (DBP), a member of the albumin gene family. The 1,25D metabolite is presumed to enter cells via diffusion through the plasma membrane, however, uptake of this metabolite via active processes, either as the free steroid or in complex with DBP, has not been ruled out. The 25D metabolite binds to DBP with 20–30-fold higher affinity than does 1,25D, and the 25D-DBP complex has been shown to enter renal cells via receptor-mediated endocytosis. This process is facilitated by the megalin-cubilin endocytic receptor complex present on the renal cell plasma membrane, which is

essential for maintenance of vitamin D homeostasis *in vivo*[17]. However, the relative contribution of facilitated versus passive uptake mechanisms for physiologically significant vitamin D steroids in different cell types has yet to be thoroughly characterized, either *in vitro* or *in vivo*.

Once internalized, the metabolic fate of 25D reflects its trafficking to metabolizing enzymes and the relative expression and/or activity of the 24- and 1-hydroxylases. In cells with high levels of 24-hydroxylase, generation of 24,25D and subsequent catabolism would predominate, precluding VDR activation. However, cells with functional 1α-hydroxylase could potentially convert 25D to 1,25D, which could bind to VDR and mediate tissue-specific cell regulatory effects in an autocrine fashion. The implication of the autocrine pathway is that local cellular production of 1,25D would likely be regulated in a tissue-specific manner independently from systemic calcium homeostasis. Similarly, the actions of locally produced 1,25D would be confined to the immediate cellular environment and would not necessarily affect body calcium homeostasis. Existence of the autocrine pathway also implies that circulating 25D becomes the critical determinant of cellular vitamin D activity and necessitates re-definition of the optimal serum levels of 25D needed for the maintenance of local 1,25D generation.

Whether generated in cells from 25D or taken up from the circulation, 1,25D binds to the VDR, a member of the steroid receptor family of ligand-dependent transcription factors that modulate gene expression in a tissue-specific manner[18]. In an early study, 23 of 33 established human cancer cell lines surveyed expressed VDR[19]. Expression profiling of breast, prostate, and squamous carcinoma cells has identified 1,25D responsive gene clusters involved in the regulation of cell cycle, differentiation, cell adhesion and immune responses, indicating a diverse and broad range of VDR targets potentially involved in cell regulation[20-23]. Like other nuclear receptors, gene regulation by the liganded VDR requires dimerization, most often with the retinoid X receptor (RXR) family, and binding to specific DNA sequences in target gene promoters[24]. Although a variety of structurally distinct vitamin D responsive elements have been identified, the best characterized is a hexanucleotide direct repeat separated by three variable base pairs (DR3) to which VDR:RXR heterodimers bind[25]. Additional mechanisms of genomic VDR signaling include interacting with partners other than RXR, binding to diverse DNA sequences, and ligand independent effects[26,27]. VDR can also influence gene expression via interactions with other transcription factors such as Sp1[28]. In addition, the VDR is subject to post-translational modifications, including phosphorylation, that affect its transcriptional activity[29,30].

In addition to genomic signaling, 1,25D can exert rapid effects on signal transduction pathways, leading to biological responses at the plasma membrane or in the cytoplasm[18]. Identification of an alternative binding pocket in the VDR for ligands that mediate rapid effects suggests that the VDR mediates some of these non-genomic effects, a suggestion supported by studies with cells from VDR null mice[31,32]. Localization of the nuclear VDR protein to caveolae, specialized signaling complexes present in plasma membrane, further supports this concept[33]. Examples of non-transcriptional effects of the 1,25D – VDR complex with potential relevance to cancer cell regulation include regulation of ion channels, protein kinase C activation, interaction with β-catenin and activation of protein phosphatases PP1c and PP2Ac[34-36]. The possibility that alternative receptors for vitamin D metabolites that have been linked to rapid responses[37] may contribute to cancer cell regulation by 1,25D has yet to be thoroughly investigated. Thus, the relative contributions of genomic and non-genomic signaling in mediating the diverse biological effects of 1,25D, particularly in relation to its anti-cancer properties, remain to be fully clarified.

Effects of vitamin D on breast cancer cells and tumors

In response to the initial identification of VDR in cancer cells, numerous studies examined the effects of 1,25D on transformed cells[38]. Furthermore, a large number of structural analogs of vitamin D developed by pharmaceutical companies and academic researchers have been used to probe the mechanisms of vitamin D-mediated growth inhibition[39-41]. In the following sections, data generated with both natural vitamin D metabolites and synthetic analogs is discussed, and readers are referred to the original citations for details.

In both estrogen receptor positive and negative breast cancer cells, 1,25D induces cell cycle arrest, differentiation and apoptosis [13,42-46]. The anti-proliferative effects of 1,25D result from alterations in key cell cycle regulators thatculminate in de-phosphorylation of the retinoblastoma protein and arrest of cells in G_0/G_1[46]. The cyclin dependent kinase inhibitors p21 and/or p27 are genomic targets of the 1,25D-VDR complex in many cell types [28,47,48]. In addition to direct regulation of cell cycle modulators, 1,25D blocks mitogenic signaling, including that of estrogen, EGF, IGF-1 and KGF and upregulates negative growth factors such as TGF-β[49-52].

In some transformed cells, 1,25D induces apoptotic cell

death via generation of reactive oxygen species, dissipation of the mitochondrial membrane potential and cytochrome c release[43,53], features of the intrinsic (mitochondrial) pathway of apoptosis. Furthermore, 1,25D exerts additive or synergistic effects in combination with other triggers of apoptosis, such as radiation and chemotherapeutic agents[54-57]. In MCF-7 cells, 1,25D down regulates the anti-apoptotic protein Bcl-2 and induces the redistribution of the pro-apoptotic protein Bax from cytosol to mitochondria[43,53]. Furthermore, overexpression of Bcl-2 renders breast cancer cells resistant to 1,25D-mediated apoptosis[53]. The role of caspases and other proteases in 1,25D-mediated cell death appears to vary with cell type. Activation of caspases 3 and 9 occurs during 1,25D-induced apoptosis in some cells, and caspase inhibition can prevent some features of 1,25D-mediated apoptosis, however, caspase inhibitors do not prevent 1,25D-mediated death[43,58]. Other proteases implicated in 1,25D-mediated cell death include calpains and cathepsins[58-61]. Collectively, these studies indicate that a wide variety of different signaling pathways, apoptotic regulatory proteins and proteases may contribute to 1,25D-mediated apoptosis depending on the specific cell type and/or context.

Although cells lacking functional p53 retain sensitivity to 1,25D[53], the VDR has been identified as a transcriptional target of p53 and the related proteins p63 and p73[62,63]. These studies suggest that VDR regulated pathways may contribute to the tumor suppressive effects of the p53 family. In support of this notion, VDR and p53 mediate similar biological effects (growth arrest in G_0/G_1, apoptosis, DNA repair) via common target genes (p21, bax, GADD45). On the p21 promoter, both independent and overlapping VDR and p53 binding sites have been characterized[64].

To examine whether the anti-cancer effects of 1,25D are mediated by the nuclear VDR, we developed mammary tumor cell lines from VDRKO mice[65]. Tumors were induced in mice lacking VDR and their normal wild type (WT) littermates, and cell lines were established from these tumors. WT cell lines expressed VDR and underwent growth inhibition and apoptosis when treated with 1,25D, whereas VDRKO cell lines did not express VDR and did not exhibit growth arrest or apoptosis when treated with 1,25D or synthetic vitamin D analogs. Interestingly, cells lacking VDR retained sensitivity to retinoids, anti-estrogens and DNA damaging agents such as etoposide. These data indicate that VDR specifically mediates the growth inhibitory effects of vitamin D steroids, but is not absolutely required for the anti-cancer effects of unrelated agents. Given the known interactions between VDR and p53, however, subtle differences in the sensitivity of VDRKO cells to diverse apoptotic agents have not been ruled out. More detailed studies are clearly needed to determine whether 1,25D and the VDR exert independent effects on pathways involved in DNA damage sensing and repair, cell cycle regulation or apoptosis.

Although therapeutic use of 1,25D is precluded by dose-limiting calcemic toxicity, synthetic analogs of 1,25D that exhibit less potent calcemic effects have provided proof of the principle that VDR agonists can inhibit growth and induce regression of mammary tumors in animal models[66-68]. Furthermore, studies on xenografts derived from WT and VDRKO cells indicated that the expression of functional VDR in tumor epithelial cells (rather than in accessory cells such as fibroblasts, immune cells or endothelial cells) is necessary for the anti-tumor effects of vitamin D analogs *in vivo* (Valrance *et al*, in press). These studies definitively establish that the VDR is the mediator of the negative growth regulatory effects of vitamin D steroids *in vivo*.

Evidence for breast cancer prevention by vitamin D

In contrast to the extensive work demonstrating the effects of vitamin D on transformed mammary cells, there has been less emphasis on defining the role of vitamin D in breast cancer prevention. As noted above, the link between vitamin D and breast cancer prevention is based on the concept that 1,25D promotes or maintains the differentiated phenotype in normal mammary cells. Consistent with this concept, the VDR is expressed in normal mammary epithelial tissue *in vivo* and in non-transformed human mammary epithelial (HME) cells *in vitro*[13,69,70]. In mouse mammary gland, VDR is localized predominantly in differentiated epithelial cells, and its expression increases 100-fold during the course of pregnancy and lactation[70,71].

The function of the vitamin D pathway in HME cells has recently been evaluated. The effects of 1,25D on HME cells include growth arrest and induction of differentiation markers such as E-cadherin, but apoptosis has not been reported[13]. As in breast cancer cells, supra-physiological concentrations of 1,25D are required to elicit these effects. Notably, non-transformed mammary cells express CYP27B1, the enzyme that converts 25D to 1,25D, suggesting the possibility that 25D may be the biologically relevant metabolite in the mammary gland[13,14]. Indeed, mammary cells can bioactivate 25D to 1,25D and physiological concentrations of 25D can inhibit growth of HME cells *in vitro*[13,14]. A caveat to these studies is that very little is known about the delivery of 25D to mammary cells. As discussed earlier, 25D binds avidly to serum DBP, therefore it is likely that 25D is delivered to the

mammary gland in complex with DBP. However, whether 25D dissociates from the 25D-DBP complex or whether the 25-DBP complex is internalized intact by mammary cells is unclear. Recent studies have demonstrated that both murine and human mammary epithelial cells express megalin and cubilin, proteins required for the endocytic uptake of DBP in kidney. Furthermore, uptake of DBP occurred in mammary cells *in vitro* and was correlated with 25D-mediated transactivation of VDR[72]. However, further studies are necessary to determine whether endocytosis of the 25D-DBP complex occurs in mammary tissue *in vivo*.

Animal studies also support the concept that vitamin D signaling reduces breast cancer development. Rodents fed western style diets (low in vitamin D and calcium, high in saturated fat) developed hyperproliferation and/or enhanced rates of tumor formation in colon, prostate and mammary gland compared to rats fed adequate calcium and vitamin D[73]. In mammary gland organ culture, 1,25D inhibited hormone-driven proliferation and reduced the number of carcinogen initiated pre-neoplastic lesions during both the initiation and the promotion stages, indicating that vitamin D signaling can exert direct anti-neoplastic effects at multiple steps in the carcinogenesis process[74]. VDR agonists have also been shown to inhibit angiogenesis, invasion and metastasis indicating a potential benefit of vitamin D on later stages of cancer progression[66,75,76].

Data from VDR null mice support a role for vitamin D in cancer prevention

Mice lacking the VDR demonstrate excess proliferation and branching as well as impaired apoptosis during the reproductive cycle compared to their normal counterparts[70,71]. Comparison of gene expression in normal and VDR knockout mice has identified cyclin D1, p21, clusterin, β-catenin and TGF-β1 as potential VDR target genes in the mammary gland *in vivo* (Zinser *et al*. unpublished data). Demonstration that VDR ablation alters growth regulatory pathways in mammary gland raised the possibility that VDRKO mice might display an enhanced risk for cancer development in this tissue. Indeed, the incidence of mammary hyperplasias and the development of ER negative tumors in response to the carcinogen DMBA was higher in VDRKO mice than their WT counterparts[77]. Furthermore, on the MMTV-neu transgenic background, VDR heterozygote mice demonstrated a higher incidence of neu-driven mammary tumors than did WT mice[78]. Notably, differences in cancer susceptibility were not limited to the mammary gland, as VDRKO mice displayed increased sensitivity to tumors in the lymph nodes and skin in response to DMBA compared to WT mice[77,79]. These *in vivo* studies have provided the most direct evidence that VDR signaling can protect against cancer development. Collectively, these and other animal studies have confirmed that the effects of vitamin D signaling observed *in vitro* translate to effects on cell proliferation, differentiation and apoptosis *in vivo* that are of sufficient magnitude to affect the carcinogenic process.

Effect of transformation on the vitamin D pathway

Some transformed breast cells display limited sensitivity to 1,25D, suggesting that the vitamin D pathway may be deregulated during cancer development. Multiple mechanisms have been identified that contribute to 1,25D resistance, including loss of VDR expression, alterations in transcriptional co-regulators and overexpression of CYP24, the enzyme that catabolizes 1,25D. Overexpression of the anti-apoptotic protein bcl-2 renders cancer cells resistant to 1, 25D-mediated apoptosis and expression of certain oncogenes (including ras and SV40 large T antigen) interferes with vitamin D signaling[53,80,81]. Breast cancer cell lines have been selected for resistance to 1,25D *in vitro*[82,83]. These cell lines retain expression of VDR but exhibit changes in protein expression that favor autonomous growth signaling and downregulate the apoptotic pathway[84,85]. Amplification of the CYP24 gene was reported in human breast cancer and higher CYP24 expression was detected in tumors compared to adjacent normal tissue[14,86]. De-sensitization of breast cancer cells to growth inhibition by VDR ligands has also been associated with changes in nuclear receptor co-repressors via epigenetic mechanisms, which are potentially reversible[87,88]. These data indicate that cancer cells use multiple mechanisms to evade the negative growth regulatory effects of the vitamin D signaling pathway.

Vitamin D and breast cancer links at the population level

An evaluation of the Nurses Health Study found that intake of dairy products, dairy calcium and vitamin D were inversely associated with breast cancer risk in premenopausal, but not postmenopausal, women[89]. John *et al*[90] demonstrated that sunlight exposure and dietary vitamin D were associated with reduced risk of breast cancer, however, the association was dependent on the region of residence. A prospective analysis of breast cancer incidence in relation to vitamin D intake for over 30 000 participants in the Women's Health Study indicated that higher intake of vitamin D was moderately associated with a lower risk of pre- but

Table 2. Summary of evidence linking vitamin D signaling pathway to prevention of breast cancer.

EVIDENCE LINKING VITAMIN D SIGNALING TO BREAST CANCER PREVENTION	
Cellular Systems	• Human mammary epithelial cells express VDR, vitamin D metabolizing enzymes (CYP24A1, CYP27B1) and endocytic machinery for internalization of 25D-DBP (megalin, cubilin)
	• Both 1,25D and 25D inhibit growth of human mammary epithelial cells
Animal models	• VDR is expressed and regulated in normal mouse mammary gland
	• 1,25D inhibits hormone stimulated proliferation and branching
	• 1,25D inhibits carcinogen induced pre-neoplastic lesions in organ culture and in vivo
	• Enhanced proliferation and reduced apoptosis in mammary gland of VDRKO mice
	• Increased sensitivity to tumorigenesis in response to chemical carcinogens and oncogenes
Epidemiology	• Inverse associations between biomarkers of sunlight exposure, dairy products and/or dietary vitamin D and risk of breast cancer
	• Low serum 25D associated with enhanced breast cancer risk and/or disease activity
	• Amplification of CYP 24 in breast cancers

not post- menopausal breast cancer[91]. These data are consistent with reports of inverse association between vitamin D status and mammographic density in pre-menopausal women[92,93]. Correlation between exposure to solar radiation and breast cancer risk has also been suggested in large epidemiological studies[94,95]. A pooled analysis of studies that assessed serum 25D in relation to breast cancer demonstrated a clear dose-response relationship, with the highest quintile of serum 25D associated with a 50% reduction in breast cancer risk[96]. These data suggested that serum 25D concentrations above 50 ng/mL may be required to optimize vitamin D signaling in mammary tissue. However, studies have demonstrated that it is difficult to maintain serum 25D in this range from dietary sources, particularly when sunlight exposure is limited[97,98]. Furthermore, the amount of vitamin D present in currently available over the counter supplements (400 IU) is too low to significantly elevate serum 25D[5]. Collectively, these observations emphasize the need for re-evaluation of public health recommendations regarding sun exposure, vitamin D intake, food fortification and supplement use in relation to vitamin D status and chronic disease.

Conclusions

Table 2 summarizes the current evidence linking the vitamin D signaling pathway with breast cancer prevention. VDR is expressed in normal mammary cells, where it regulates proliferation, apoptosis and differentiation via distinct targets at different stages of development. In mice, deficiency of the VDR enhances risk for transformation in mammary gland, lymphoid tissue, skin and colon. The VDR ligand 1, 25D inhibits growth and induces apoptosis in breast cancer cells and tumors, and these effects absolutely require the VDR. VDR ligands also inhibit growth of normal human mammary epithelial cells, and evidence suggests that autocrine bio-activation of vitamin D precursors can occur within mammary cells. Thus, data from both human tissues and animal models support the concept that the VDR and its ligand induce a program of gene expression that contributes to maintenance of the differentiated phenotype in breast cells, a concept that is consistent with a role for vitamin D in both prevention and treatment of breast cancer. At the current time, however, the amount of vitamin D (either from diet or endogenous synthesis) needed to optimize growth inhibitory signaling through the VDR *in vivo* is currently undefined, and further studies are needed before guidelines or requirements for human populations can be established. Collectively, these studies emphasize that multiple components of the vitamin D signaling system are present in normal mammary cells and emphasize the need for additional research on expression and function of these proteins in intact mammary tissue *in vivo*, particularly in relation to maintenance of the differentiated phenotype.

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