Vitamin D-3 Receptor as a Target for Breast Cancer Prevention1,2
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ABSTRACT The vitamin D-3 receptor (VDR) is a nuclear receptor that modulates gene expression when complexed with its ligand 1-α,25-dihydroxycholecalciferol [1,25(OH)2-D3], which is the biologically active form of vitamin D-3. The cellular effects of VDR signaling include growth arrest, differentiation and/or induction of apoptosis, which indicate that the vitamin D pathway participates in negative-growth regulation. Although much attention has been directed in recent years toward the development of synthetic vitamin D analogs as therapeutic agents for a variety of human cancers including those derived from the mammary gland, studies on vitamin D as a chemopreventive agent for breast cancer have been quite limited. The VDR is expressed in normal mammary gland, where it functions to oppose estrogen-driven proliferation and maintain differentiation; this suggests that 1,25(OH)2-D3 participates in negative-growth regulation of mammary epithelial cells. Furthermore, preclinical studies show that vitamin D compounds can reduce breast cancer development in animals, and human data indicate that both vitamin D status and genetic variations in the VDR may affect breast cancer risk. Collectively, findings from cellular, molecular and population studies suggest that the VDR is a nutritionally modulated growth-regulatory gene that may represent a molecular target for chemoprevention of breast cancer. J. Nutr. 133: 2425S–2433S, 2003.

KEY WORDS: • vitamin D • mammary gland • breast cancer • VDR knockout mice

Biology of vitamin D and vitamin D receptors
Vitamin D: mechanism of action. Although originally identified based on their roles in calcium and bone homeostasis, the vitamin D-3 receptor (VDR)4 and its ligand 1-α,25-dihydroxycholecalciferol [1,25(OH)2-D3] are now recognized to exert effects in almost every tissue in the body (Fig. 1). Targets for vitamin D signaling include the central nervous system, skin and hair follicles, immune system and endocrine glands. At the cellular level, vitamin D signaling affects proliferation, differentiation and apoptosis of both normal and transformed cells. The VDR is a member of the nuclear receptor family of proteins that act as ligand-dependent transcription factors to modulate expression of specific genes in a tissue-specific manner (1,2). These receptors contain ligand-binding domains that selectively bind lipid-soluble ligands and DNA-binding domains that recognize and bind specific nucleotide sequences in target genes. Along with coactivators, corepressors and a variety of additional accessory nuclear proteins, the ligand-receptor complexes induce or repress target gene promoters. In the case of the VDR, gene regulation by the liganded receptor requires dimerization and most often heterodimerization with the retinoid X receptor (RXR) family. Although a variety of structurally distinct vitamin D–responsive elements are identified for vitamin D–regulated genes, the best characterized is a hexanucleotide direct repeat separated by three variable base pairs to which VDR-RXR heterodimers bind. However, the recognition that VDR also can function as a homodimer or as a heterodimer with partners other than RXR suggests enormous flexibility for the genomic pathways that are regulated by vitamin D. To add complexity to vitamin D signaling, there is increasing evidence that vitamin D can exert rapid, nongenomic effects on signal transduction pathways that induce biological responses such as calcium transport (3). Although a distinct membrane or cytosolic localized receptor that mediates the rapid effects of vitamin D was proposed, recent studies using cells from VDR-knockout (KO) mice indicate that the nongenomic effects of vitamin D in osteoblasts are abrogated in the absence of the nuclear VDR (4). These data indicate that at least in bone cells, the nuclear VDR is required for these nongenomic effects of vitamin D.

Nutritional aspects of vitamin D. The ligand for the VDR, 1,25(OH)2-D3, is derived from vitamin D (calciferol), which is...
a fat-soluble vitamin that was identified as an antirachitic factor in the early 1920s. The two naturally occurring forms of vitamin D are cholecalciferol (vitamin D-3, from animal sources) and ergocalciferol (vitamin D-2, from plant sources). For clarity, this review focuses on vitamin D-3; however, it should be noted that the potency, metabolism and function of ergocalciferol and cholecalciferol are equivalent in humans. Both forms of vitamin D can be obtained from the diet, although natural foods with the exception of fish are relatively low in calciferols. For this reason, milk is fortified with vitamin D in the U.S. and Canada. The actual vitamin D content of fortified milk is highly variable and often less than the stated 400 IU per quart (5). Vitamin D-3 also can be synthesized from the cholesterol derivative 7-dehydrocholesterol in the epidermis, but this process requires ultraviolet radiation and is dependent on sun exposure, which is highly variable (6,7).

Despite the fortification of vitamin D in milk and the ability of the body to synthesize the vitamin, vitamin D deficiency is surprisingly common, especially in populations that live in northern climates and in the elderly (7). Of patients admitted to Massachusetts General Hospital in a 1998 sample, > 50% were vitamin D deficient (8), and at the end of winter, > 40% of healthy young men in Boston had serum vitamin D levels in the insufficient range (9). Similar rates of vitamin D insufficiency are reported for middle-aged men in Finland (10). Factors associated with low vitamin D status include aging, liver or kidney disease, certain medications, poor diet and limited epidermal synthesis of cholecalciferol (reasons for which include infrequent exposure to sunlight, living in geographical areas with low solar radiation, dark pigmentation and liberal use of sunscreen).

Particularly relevant to the potential role of vitamin D in breast cancer, both aging and estrogen deficiency are associated with low vitamin D status. Aging reduces the production of cholecalciferol in the skin, and estrogen deficiency decreases both metabolic activation of vitamin D and expression of the VDR [reviewed in (7)]. Thus, postmenopausal women, the predominant target population for breast cancer, are at higher risk for vitamin D deficiency than younger women. Indeed, the recommended daily allowance for vitamin D increases with age (200 IU for those under age 50, 400 IU for ages 51–70 and 600 IU for age 71+). The chronic consequences of subclinical vitamin D deficiency in humans have yet to be thoroughly defined. However, it is important to note that in the vast majority of cases, vitamin D deficiency can easily be prevented or cured by dietary adjustment or use of a daily multivitamin supplement (9).

**Metabolism of vitamin D.** The metabolic activation of vitamin D (Fig. 2) is achieved through a series of enzymatic steps that are the same regardless of the source (dietary or endogenously produced) or chemical form (cholecalciferol or ergocalciferol) of the vitamin. The initial step is hepatic hydroxylation of vitamin D at the 25 position to generate 25-hydroxycholecalciferol [25(OH)-D3] from cholecalciferol. The major circulating form of calciferol, 25(OH)-D3 is stored in adipose tissue and is an accurate biomarker of the body’s overall vitamin D status. Further metabolism of 25(OH)-D3 generates two major metabolites: 24,25-dihydroxycholecalciferol [24,25(OH)2-D3] or 1-α,25-dihydroxycholecalciferol [1,25(OH)2-D3]. Production of 24,25(OH)2-D3 is catalyzed by the enzyme vitamin D 24-hydroxylase, which is present in the majority of vitamin D-target tissues. The 24,25(OH)2-D3 metabolite does not readily bind VDR, and its production is generally considered the first step in the pathway leading to degradation of 25(OH)-D3. Production of 1,25(OH)2-D3 (the biologically active vitamin D metabolite that binds to VDR) is mediated by the...
enzyme vitamin D 1α-hydroxylase, which is highly expressed in renal proximal tubules (11).

The vitamin D hydroxylases that are responsible for metabolism of 25(OH)-D₃ are type I (mitochondrial) cytochrome P450 oxidases that use NADPH and molecular oxygen to catalyze the hydroxylation reaction. The activities of the renal vitamin D hydroxylases are tightly regulated to coordinate activation and degradation of vitamin D within the body. Because the kidney 1α-hydroxylase produces 1,25(OH)₂-D₃ (a potent calcium-elevating hormone) for the systemic circulation, its activity is tightly regulated by the calcium status of the individual. Thus, under conditions of increased demand for calcium such as growth, pregnancy and lactation, 1α-hydroxylase expression and activity is induced and 1,25(OH)₂-D₃ is generated. The elevated levels of circulating 1,25(OH)₂-D₃ subsequently interact with VDR in target tissues such as kidney, intestine and bone to mobilize calcium. Conversely, when calcium demands are low, the activity of 1α-hydroxylase is suppressed and the activity of 24-hydroxylase is enhanced, which leads to formation of 24,25(OH)₂-D₃ and initiation of the catabolic pathway leading to excretion. Both positive (parathyroid hormone, estrogen, growth hormone) and negative [1, 25(OH)₂-D₃, calcium] regulators of the renal hydroxylases have been identified, and acute regulation of renal 1,25(OH)₂-D₃ production is mediated through the adenylate cyclase and protein kinase C pathways (12–14).

**Extrarenal activation of vitamin D.** In recent years, it has become apparent that tissues in addition to the kidney can catalyze the production of 1,25(OH)₂-D₃ from 25(OH)-D₃. Epidermal keratinocytes and activated macrophages as well as epithelial cells in prostate, breast and colon (15–17) are shown to express the vitamin D 1α-hydroxylase. Although the presence of this enzyme suggests that certain extrarenal tissues have the ability to convert 25(OH)-D₃ to 1,25(OH)₂-D₃, circulating 1,25(OH)₂-D₃ is virtually undetectable in anephric conditions. Thus, if extrarenal tissues do produce 1,25(OH)₂-D₃, it is apparently not released into the bloodstream but instead may act locally by binding to VDR that are present within the same or adjoining cells. Such local actions of 1,25(OH)₂-D₃ might include regulation of cell proliferation, differentiation and apoptosis. The implication of these autocrine or paracrine actions is that cellular production of 1,25(OH)₂-D₃ is likely regulated in a tissue-specific fashion independently from systemic calcium homeostasis. Similarly, the actions of locally produced 1,25(OH)₂-D₃ would be confined to the immediate cellular environment and would not necessarily impact body-calcium homeostasis. The emerging view of systemic versus cellular pathways of 1,25(OH)₂-D₃ production and action is presented in Figure 2. In this review, we focus on the impact of the vitamin D endocrine system at the cellular level in one particular target tissue, the mammary gland, with an emphasis on its potential role in prevention of human breast cancer. In the subsequent sections, we describe data on the functions of vitamin D in a normal mammary gland, the ability of vitamin D to suppress carcinogenesis in animal models and the influences of vitamin D status and VDR polymorphisms on breast cancer risk.

**Cellular effects of 1,25(OH)₂-D₃.** The demonstration of VDR expression in diverse tissues including the skin, pancreas, prostate and mammary gland as well as in transformed cells from a wide variety of tumor types has emphasized that the actions of 1,25(OH)₂-D₃ are not limited to regulation of calcium and bone homeostasis. At the cellular level, 1,25(OH)₂-D₃ induces cell-cycle arrest, differentiation and apoptosis in a variety of normal and transformed cell types including osteoblasts, epidermal keratinocytes and mammary epithelial cells. Extensive research is directed toward elucidation of the effects of 1,25(OH)₂-D₃ on breast cancer cells, and several excellent reviews on this topic are available (18–20). VDR expression is retained in the majority of human breast tumors, thus it represents a potential therapeutic target for established cancer. Consistently, 1,25(OH)₂-D₃ has been shown to exert negative-growth regulatory effects on breast cancer cells and tumors. The effects of 1,25(OH)₂-D₃ on breast cancer cells include modulation of cell-cycle machinery to arrest cells in G0/G1 and disruption of mitochondrial function to induce apoptotic cell death (19,21). The primary focus of the work on vitamin D and breast cancer to date was directed toward the development of synthetic vitamin D₃–based drugs for therapy of human cancers, and the progress in this area was summarized recently (22).

**Role of vitamin D in normal mammmary gland**

**Overview.** In contrast with the extensive studies on the effects of vitamin D on breast cancer cells, considerably less emphasis has been placed on the role of 1,25(OH)₂-D₃ and the VDR in the normal mammary gland and the possible role of vitamin D signaling in breast cancer prevention. We proposed (23) that the 1,25(OH)₂-D₃/VDR complex induces a program of genes that suppresses proliferation and stimulates differentiation in the normal mammary gland. This hypothesis predicts that dysregulation of VDR-mediated gene expression in the mammary gland will alter mammary gland development or function and possibly predispose cells to transformation. A corollary to this hypothesis is that optimization of VDR signaling in the mammary gland might protect against breast cancer development. In this section, we discuss the expression of VDR in the normal mammary gland and the consequences of VDR disruption on mammary gland development.

**Localization of VDR in mammary gland.** The VDR has been identified in human, rabbit and rodent mammary glands (23–26), and we recently examined the particular cell types within the mouse mammary gland that express VDR as well as its developmental regulation (27). Our data indicate that although VDR is present in all of the major cell types in the gland (basal and luminal epithelial cells, cap cells, stromal cells), its expression is not temporally or spatially uniform. During puberty, a distinct gradient of VDR expression is observed that includes weak VDR staining in proliferative populations and strong VDR staining in differentiated populations. During pregnancy and lactation, VDR expression increases in the mammary gland (26). This effect is likely mediated via lactogenic hormones (25) and suggests a role for 1,25(OH)₂-D₃ in proliferation or differentiation of the gland. This suggestion is supported by organ-culture studies that indicate the effects of 1,25(OH)₂-D₃ on calcium transport, casein expression and branching morphogenesis (27–29). Notably, involution of the gland after weaning, a process that involves extensive tissue remodeling and apoptosis, is characterized by marked upregulation of VDR in epithelial cells (Zinser and Welsh, unpublished data). The discrete compartmentalization of the VDR and its dynamic developmental regulation imply a functional role for vitamin D signaling in mammary gland in vivo.

**Effect of VDR ablation on mammary gland development.** Despite localization of the VDR in the mammary gland and in vitro data that support a role for 1,25(OH)₂-D₃ in growth regulation of mammary cells, it is difficult to demonstrate that lack of vitamin D directly affects the mammary gland in vivo. Dietary vitamin D deficiency has profound effects on calcium and bone homeostasis that confound interpretation of the role of the VDR in mammary gland differentiation. In recent
studies, we examined the role of the VDR in mammary gland development and function using the VDR-KO mouse (30). In the VDR-KO mouse model, complications such as hypocalcemia can be prevented by feeding the mice a high-calcium, high-phosphate and high-lactose diet (31). In the setting of normocalcemia, VDR-KO mice are fertile and can lactate, which indicates that mammary gland function is not grossly abnormal in the absence of vitamin D signaling (27,32).

To examine the consequences of VDR disruption on mammary gland development, we compared ductal morphogenesis in VDR-KO mice and their wild-type (WT) controls (27). Whole-mount analysis of glandular development during puberty (4–10 wk after birth) demonstrates that mammary glands from VDR-KO mice exhibit accelerated growth and branching morphogenesis as compared with glands from age- and weight-matched WT mice. In addition, glands from VDR-KO mice exhibit enhanced growth in response to exogenous estrogen and progesterone both in vivo and in organ culture as compared with glands from WT mice. In organ culture, incubation with 1,25(OH)2-D3 inhibits branching of mammary glands from WT mice but has no effect on glands from VDR-KO mice.

Because 1,25(OH)2-D3 can induce apoptosis in breast cancer cells, we also examined whether glandular involution, a process that is driven by epithelial cell apoptosis, is altered by VDR ablation. Our data indicate that glands from VDR-KO mice exhibit delayed involution as compared with WT mice, which suggests that the upregulation of the VDR detected at the peak of glandular regression (4 d after weaning) plays a functional role in physiological apoptosis (Zinser and Welsh, unpublished data). Collectively, these data provide the first in vivo evidence that 1,25(OH)2-D3 and the nuclear VDR affect ductal elongation, branching morphogenesis and glandular involution during mammary gland development, and support the concept that 1,25(OH)2-D3 and the VDR participate in negative-growth regulation of the mammary gland. Additional studies to monitor gene expression in VDR-KO and WT mice are in progress to identify transcriptional targets of the VDR that are involved in mammary gland proliferation, differentiation and apoptosis.

**Metabolism of vitamin D in mammary cells.** The data reviewed in the preceding sections highlight the anti-proliferative and prodifferentiating effects of 1,25(OH)2-D3 and the VDR in the mammary gland. As discussed earlier, emerging evidence suggests that a number of extrarenal tissues can hydroxylate 25(OH)-D3 to produce 1,25(OH)2-D3, which acts locally to induce cell-type–specific effects that are independent of calcium homeostasis. Owing to the negative-growth regulatory effects of 1,25(OH)2-D3 in the mammary gland, identification of 1α-hydroxylase and local conversion of 25(OH)-D3 to 1,25(OH)2-D3 in mammary cells has significant implications with respect to development, progression or treatment of breast cancer.

To date, the only evidence that normal mammary cells express the vitamin D 1α-hydroxylase is a preliminary report that the mRNA for the enzyme is detected in human mammary tissue (15). This report prompted us to investigate whether normal mammary cells in culture express biologically significant 1α-hydroxylase activity. We reasoned that if mammary cells are capable of converting 25(OH)-D3 to 1,25(OH)2-D3, then treatment with 25(OH)-D3 should induce similar growth-inhibitory effects as treatment with 1,25(OH)2-D3. Similar approaches were used to demonstrate the presence of active 1α-hydroxylase in normal prostate cells (33). For our experiments, we used primary human mammary epithelial (HME) cells that were immortalized by stable transfection of telomerase (TERT). Although capable of infinite population doublings in vitro, HME cells that express telomerase (HME<sup>TERT</sup>) retain the morphology and growth characteristics of normal mammary epithelial cells and are not tumorigenic in the absence of additional genetic mutations (34). Like the majority of ductal epithelial cells that are found in human mammary gland in vivo, HME<sup>TERT</sup> cells are estrogen-receptor negative.

To compare HME<sup>TERT</sup> cellular sensitivity to 25(OH)-D3 and 1,25(OH)2-D3, cultures were treated in parallel with increasing concentrations of each compound, and growth was assessed after 4 d using a crystal violet technique. For comparison, assays also were conducted with MCF-7 cells, which is a human breast cancer cell line that expresses VDR and is sensitive to 1,25(OH)2-D3–mediated growth arrest and apoptosis (21). As demonstrated in Figure 3A, HME<sup>TERT</sup> cells are sensitive to 1,25(OH)2-D3–mediated growth arrest, with ~50% growth inhibition (as compared with vehicle-treated control cells) induced by 100 nmol 1,25(OH)2-D3/L after 96 h. These data indicate that the HME<sup>TERT</sup> cells express VDR, which is confirmed by Western blotting (data not shown). Interestingly, under the same conditions, HME<sup>TERT</sup> cells are growth inhibited by the precursor metabolite 25(OH)-D3 at doses as low as 1 nmol 25(OH)-D3/L. Within the same time frame, the dose responses of HME<sup>TERT</sup> cell-growth inhibition are virtually identical for 25(OH)-D3 and 1,25(OH)2-D3, Western blotting with an antibody directed against the murine 1α-hydroxylase enzyme 3-hydroxycholecalciferol [25(OH)-D3] on growth of immortalized normal human mammary epithelial cells and MCF-7 breast cancer cells. Human mammary epithelial cells expressing telomerase (HME<sup>TERT</sup> (A) and MCF-7 cells (B) were treated with the indicated concentrations of vitamin D metabolites or vehicle control for 96 h before assessment of adherent cell numbers by crystal violet technique. Data are means ± SE of 4–6 replicates; *P < 0.05, significantly different from control
(The Binding Site, San Diego, CA) that is known to recognize the human protein identifies a protein at 56 kDa in HME\textsuperscript{TERT} cells that comigrates with 1α-hydroxylase from kidney extracts (data not shown). These studies provide provocative evidence that nontransformed human mammary epithelial cells express functional vitamin D 1α-hydroxylase, which is capable of generating 1,25(OH)\textsubscript{2}-D\textsubscript{3} from 25(OH)-D\textsubscript{3}.

In contrast with nontransformed mammary epithelial cells, MCF-7 breast cancer cells are growth inhibited by 1,25(OH)\textsubscript{2}-D\textsubscript{3} but not by 25(OH)-D\textsubscript{3} (Fig. 3B). No growth inhibition of MCF-7 cells is observed in response to 25(OH)-D\textsubscript{3} even at doses as high as 500 nmol 25(OH)-D\textsubscript{3}/L. These data are consistent with other reports (15) and imply that MCF-7 cells are not capable of generating biologically significant amounts of 1,25(OH)\textsubscript{2}-D\textsubscript{3} from 25(OH)-D\textsubscript{3}. Surprisingly, MCF-7 cells express vitamin D 1α-hydroxylase protein (data not shown) and mRNA (15), which suggests that these transformed cells express a nonfunctional 1α-hydroxylase enzyme. An alternate explanation is that MCF-7 cells express high levels of the 24-hydroxylase and convert 25(OH)-D\textsubscript{3} into the alternate metabolite 24,25(OH)\textsubscript{2}-D\textsubscript{3}, which does not have antiproliferative actions. Additional studies on function and regulation of the vitamin D hydroxylases in normal and transformed mammary cells are necessary to distinguish between these possibilities.

**Model for vitamin D metabolism and actions in normal mammary gland.** We summarized the studies discussed above that support a role for vitamin D and its receptor in normal mammary gland biology in Table 1. Despite demonstration of VDR expression and function in the mammary gland, little is known about the relative contributions of the specific cell types in the gland with respect to metabolism, activation and function of vitamin D. The mammary gland is composed of ducts arranged around a lumen into which milk is secreted during lactation (Fig. 4). The mammary ducts are composed of epithelial cells and are contained within an extensive adipose-rich stromal compartment termed the mammary fat pad that includes fibroblasts, adipocytes and extracellular matrix.

For major hormones and growth factors in the mammary gland (such as estrogen, progesterone and insulin-like growth factors), it is well known that complex interactions exist between the epithelial and stromal compartments. These epithelial-stromal interactions are crucial determinants of proliferation, differentiation and apoptosis in the mammary gland during development (35). Because vitamin D modulates proliferation, differentiation and apoptosis in the mammary gland, it is likely that epithelial-stromal interactions also are important in regulation of vitamin D function in the mammary gland.

Drawing from the information discussed above, we have compiled a working model for cell-type specific metabolism and activation of vitamin D in the mammary gland (Fig. 4). We propose that all three major cell types in the gland (epithelial cells, fibroblasts and adipocytes) express VDR and have the ability to respond to 1,25(OH)\textsubscript{2}-D\textsubscript{3}. However, we predict that relative VDR expression is dynamically regulated in a cell-type specific fashion that depends on the physiological situation. For instance, VDR is highly induced in adipocytes that are triggered to undergo differentiation (36), in fibroblasts treated with growth factors (37) and in epithelial cells exposed to lactogenic hormones (25).

Based on data presented in Figure 3A and detection of 1α-hydroxylase mRNA in normal mammary tissue (15), 1α-hydroxylase is expressed in mammary epithelial cells where it converts 25(OH)-D\textsubscript{3} to 1,25(OH)\textsubscript{2}-D\textsubscript{3}, which acts in a paracrine or autocrine manner. We also speculate that mammary fibroblasts express the vitamin D 1α-hydroxylase, but more studies are necessary to examine this possibility. Additional outstanding issues related to this model include elucidation of the molecular regulation of the mammary 1α-hydroxylase and determination of whether its regulation is distinct from that of the renal 1α-hydroxylase.

Also indicated in Figure 4, we hypothesize that like adipose tissue elsewhere in the body, the mammary fat pad has the ability to store 25(OH)-D\textsubscript{3}. This hypothesis predicts that mammary adipocytes provide a local source of 25(OH)-D\textsubscript{3} that is available to the surrounding fibroblasts and epithelial cells for conversion into 1,25(OH)\textsubscript{2}-D\textsubscript{3}. Because this concept has yet to be tested, outstanding questions include whether 25(OH)-D\textsubscript{3} can be detected in the mammary gland, and if so, what the mechanisms are by which 25(OH)-D\textsubscript{3} is transported to local intracellular sites of 1α-hydroxylation. In this respect, it is interesting to note that megalin, a protein essential for cellular uptake of 25(OH)-D\textsubscript{3} in kidney, is expressed in mammary epithelial cells (38,39).

**Role for vitamin D in breast cancer prevention**

**Overview.** Identification of 1,25(OH)\textsubscript{2}-D\textsubscript{3} and the VDR as components of a signaling network that affects proliferation, differentiation and apoptosis in the mammary gland raises the possibility that optimal vitamin D status may protect against mammary transformation. In this section, we review studies (Table 2) that used preclinical and epidemiological approaches to provide evidence that vitamin D signaling represents a target for breast cancer prevention.

**Effects of vitamin D on mammary carcinogenesis in animal models.** In contrast with the extensive number of in vivo studies that tested the effects of vitamin D and its analogs on established breast cancer, few studies assessed whether vitamin D can prevent mammary tumorigenesis. Four studies used various rodent models of chemical carcinogen-induced breast cancer to assess the role of vitamin D in prevention (40–43).

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**TABLE 1**

**Summary of studies that support a role for 1,25(OH)\textsubscript{2}-D\textsubscript{3} and VDR in mammary gland biology**

<table>
<thead>
<tr>
<th>Model</th>
<th>Observation</th>
<th>Reference citation</th>
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<tbody>
<tr>
<td>Rodent mammary gland development in vivo</td>
<td>Impaired differentiation in mammary glands from vitamin D-deficient mice</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td>VDR regulated during puberty, pregnancy and lactation</td>
<td>(26,27)</td>
</tr>
<tr>
<td></td>
<td>VDR-null mice exhibit accelerated mammary gland development</td>
<td>(27)</td>
</tr>
<tr>
<td>Mouse mammary organ culture</td>
<td>VDR regulated by lactogenic hormones</td>
<td>(25)</td>
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<tr>
<td></td>
<td>1,25(OH)\textsubscript{2}-D\textsubscript{3} induces differentiation and calcium uptake</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>VDR-dependent regulation of branching morphogenesis by 1,25(OH)\textsubscript{2}-D\textsubscript{3}</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>1,25(OH)\textsubscript{2}-D\textsubscript{3} upregulates transforming growth factor-β</td>
<td>(43)</td>
</tr>
<tr>
<td>Normal human mammary cells/tissue</td>
<td>Express vitamin D 1α-hydroxylase</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>Express VDR</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
<td>25(OH)-D\textsubscript{3} and 1,25(OH)\textsubscript{2}-D\textsubscript{3} induce growth arrest</td>
<td>Figure 3</td>
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*1,25(OH)\textsubscript{2}-D\textsubscript{3}, 1α,25-dihydroxycholecalciferol; 25(OH)-D\textsubscript{3}, 25-hydroxycholecalciferol; VDR, vitamin D receptor.*
1,25(OH)2-D3 can interact with VDR in both epithelial and stromal cells. Synthetic vitamin D analogs were shown to prevent that vitamin D inhibits the promoting effects of a high-fat diet less obvious when animals receive a low-fat diet, which suggests vitamin D. Interestingly, the effects of dietary vitamin D are (DMBA) than rats fed control diets with adequate calcium and (41,42). Vitamin D analogs were developed by the pharmaceutical industry as therapeutic alternatives, because treatment (41,42). Vitamin D analogs were developed by the pharmaceutical industry as therapeutic alternatives, because treatment analogs and antiestrogens might protect against breast cancer via independent mechanisms. Using a similar approach, Mehta et al. (42) demonstrated that rats treated with the vitamin D analog 1α-hydroxyvitamin D-5 before treatment with NMU exhibit reduced tumor incidence and multiplicity as compared with control rats. Of note, administration of the vitamin D analogs in both of these prevention studies was through dietary supplementation, thus demonstrating that oral ingestion of vitamin D–based chemopreventive agents represents a feasible approach. Also important to note is that neither vitamin D analog induced elevations in serum calcium or any other side effects when administered chronically. Both oral administration and long-term safety are clearly important issues with respect to feasibility of cancer chemopreventive agents in general.

In mouse mammary gland organ culture, pretreatment with 1,25(OH)2-D3 or the analog 1α-hydroxyvitamin D-5 reduces the incidence of DMBA-initiated preneoplastic lesions, an effect that is associated with upregulation of the negative-growth regulator transforming growth factor-β (43). The 1α-hydroxyvitamin D-5 analog is effective during both the initiation and the promotion stages of mammary lesion formation in organ culture. This is the first study to demonstrate that vitamin D compounds exert direct antineoplastic effects on the mammary gland and suggests that vitamin D compounds can inhibit both early and late events in tumorigenesis. These studies support the concept that vitamin D protects against tumor development in animal models at both the initiation and promotion stages and offer compelling evidence that the 1,25(OH)2-D3 and VDR may be important targets for breast cancer prevention.

Epidemiological studies on vitamin D status and breast cancer. The majority of women who develop breast cancer are of postmenopausal age, and estrogen deficiency and aging are often associated with vitamin D deficiency. However, few epidemiological studies have examined whether dietary intake of vitamin D per se alters breast cancer incidence in populations. A newly published evaluation of the Nurses Health Study (44) finds that intakes of dairy products, dairy calcium and total vitamin D (as measured by food-frequency questionnaires) are inversely associated with breast cancer risk in premenopausal but not postmenopausal women. These data are consistent with an earlier study that reports an inverse correlation between intake of dairy products and breast cancer risk (45). Another recent study includes evaluation of sunlight exposure in addition to vitamin D from diet and supplements in relation to breast cancer risk (46). In this study, several measures of sunlight exposure and dietary vitamin D intake are associated with a reduced risk of breast cancer; however, the associations are dependent on region of residence. Studies also report links between solar radiation (which induces epidermal synthesis of vitamin D) and breast carcinoma mortality (47,48). In two studies in which vitamin D status was measured in relation to breast cancer, low levels of 1,25(OH)2-D3 were found to be associated with increased breast cancer risk or disease progression (49,50).

VDR polymorphisms and breast cancer risk. It is increasingly apparent that genetic variability can influence individual responsiveness to dietary or pharmaceutical interventions. There is considerable interest in the genetically determined differences in the VDR signaling pathway in relation to disease susceptibility. A number of common allelic variants (or polymorphisms) in the human VDR gene were identified and these were extensively studied with respect to risk for a variety of diseases including breast cancer (51). The best-studied VDR polymorphisms include a start codon polymorphism (FokI) in exon 2, BsmI and Apal polymorphisms in an intronic region between exons VIII and IX, a TaqI variant in exon IX and a singlet (A) repeat in exon IX. Seven published reports examine the relationship between one or more VDR polymorphisms and breast cancer incidence or progression (52–58). Six of these studies identify specific alleles of the VDR that correlate with breast cancer incidence and/or metastasis (52–57), whereas one study (58) fails to detect a significant correlation.

![FIGURE 4 Model for cell-type-specific metabolism and activation of vitamin D in the mammary gland. Schematic depicts the organization of mammary ducts in vivo: epithelial cells are arranged around a central lumen and surrounded by an extracellular matrix composed of fibroblasts and adipocytes (top left). Enlarged views of individual mammary cell types depict proposed localization of vitamin D metabolites and VDR (bottom right). The model proposes that 25(OH)-D3 is stored in adipocytes of the mammary fat pad, transported into stromal and epithelial cells and converted to 1,25(OH)2-D3 by 1α-hydroxylase; 1,25(OH)2-D3 can interact with VDR in both epithelial and stromal cells to modulate proliferation, branching morphogenesis and milk secretion.](image-url)
Table 2

Summary of studies that link 1,25(OH)₂-D₃ and VDR to prevention of breast cancer

<table>
<thead>
<tr>
<th>Approach</th>
<th>Observation</th>
<th>Reference citation</th>
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<tr>
<td>Rodent chemical carcinogenesis studies</td>
<td>Dietary calcium/vitamin D modulate DMBA-induced mammary tumorigenesis</td>
<td>(40)</td>
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<td></td>
<td>Vitamin D compounds prevent DMBA-induced preneoplastic lesions in organ culture</td>
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<td>Vitamin D analogs prevent NMU-induced mammary tumors in vivo</td>
<td>(41,42)</td>
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<td>Population studies</td>
<td>Inverse associations reported between biomarkers of sunlight exposure, dairy products and/or dietary vitamin D and risk of breast cancer</td>
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<td>Low serum 1,25(OH)₂-D₃ associated with enhanced breast cancer risk and/or disease activity</td>
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<td>Genetic links</td>
<td>Amplification of vitamin D 24-hydroxylase in breast cancers</td>
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<td>VDR polymorphisms linked to breast cancer risk and/or metastatic progression</td>
<td>(52–57)</td>
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<td>FokI and singlet A repeat polymorphisms affect VDR transcriptional activity</td>
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1 DMBA, dimethylbenz-(a)-anthracence; NMU, N-methyl-N-nitrosourea.

Although these findings are certainly intriguing, the underlying basis for an association between VDR polymorphisms and breast cancer susceptibility is currently unclear. Three of the VDR polymorphisms that are linked to breast cancer susceptibility (BsmI, ApaI or TaqI variants) do not alter the amount, structure or function of the VDR protein produced (51). There is evidence, however, that two of these polymorphisms (the VDR start codon polymorphism defined by FokI and the singlet (A) repeat in exon IX) have functional significance. The FokI site dictates which of two potential translation initiation sites is used. Individuals that lack the FokI restriction site initiate translation at the first site and express the full-length VDR, which consists of 427 amino acids. In contrast, individuals with the FokI restriction site use a second ATG site and generate a VDR protein of 424 amino acids. Although no significant differences in ligand affinity, DNA binding or transactivation activity are found between these two VDR forms when studied independently (59), when the VDR start codon polymorphism is considered simultaneously with the singlet (A) repeat in exon IX, differences in VDR function are detected in vitro (60). In transient transfection assays with a VDR-D–responsive reporter gene, the shorter VDR variant is shown to interact more strongly with transcription factor IIB and display higher potency than the longer VDR variant (60). These data support the concept that functionally relevant polymorphisms in the VDR exist, and further studies are required to determine whether the VDR genotype interacts with other risk factors for breast cancer.

Outstanding research questions

In this review, we highlight epidemiological, clinical, cellular and molecular research studies that address the role of vitamin D and its receptor in the normal mammary gland and in breast cancer. Although these studies provide considerable evidence that 1,25(OH)₂-D₃ and the VDR play a role in mammary gland biology that might affect susceptibility to transformation, numerous outstanding research issues remain to be addressed. Studies to define the downstream targets of VDR in the normal mammary gland that participate in reducing susceptibility to breast cancer are essential. Of particular interest is whether critical windows of development exist during which intervention with vitamin D–based preventive strategies are most effective. Gene-profiling studies using the VDR-KO mouse model will likely prove useful in addressing this issue.

Investigations into how the transformation process affects the vitamin D–signaling pathway also are needed. Data from mammary cell lines suggest that oncogenic transformation with SV40 or ras inhibits VDR signaling and induces resistance to the growth-inhibitory effects of 1,25(OH)₂-D₃ (61,62), but additional research is needed to determine whether these interactions are relevant to human breast cancer. Data presented in Figure 3 suggest that transformation might be associated with deregulation of 25(OH)₂-D₃ metabolism in mammary cells (either loss of vitamin D 1α-hydroxylase activity and/or enhancement of 24-hydroxylase activity). In support of this concept, the vitamin D 24-hydroxylase gene recently was shown as amplified in human breast cancer (63). Additional studies are necessary to determine actual enzyme activities as a function of neoplastic progression in mammary cells and to assess whether the vitamin D hydroxylases are useful targets for breast cancer prevention or therapy.

Perhaps most important are translational studies to examine whether dietary vitamin D affects breast cancer development and how vitamin D interacts with hormonal factors such as estrogens, phytoestrogens and selective estrogen-response modifiers like tamoxifen. Large-scale intervention studies such...
as the Women's Health Initiative, which is examining the effects of calcium and vitamin D supplementation on cancer, osteoporosis and heart disease in postmenopausal women, offer the best approach toward addressing this important issue. Until more definitive answers are available, all women should be particularly attentive to their calcium and vitamin D intake to ensure that recommended daily allowances are met. This is particularly important because estrogen deficiency and aging are commonly associated with marginal vitamin D status.

Collectively, the studies described in this and other recent reviews (18–22) provide convincing evidence that vitamin D and its receptor represent targets for breast cancer prevention and therapy. Because the ligand for the VDR can be derived from dietary sources, we propose that this receptor represents a nutritionally modulated growth-regulatory gene in the mammary gland (Fig. 5). Implications of this concept are that specific dietary guidelines for breast cancer prevention might ultimately be developed for the general population, breast cancer patients or individuals with specific VDR polymorphisms. Furthermore, this concept implies that synthetic vitamin D analogs designed to trigger specific effects in the mammary gland might be effective in the prevention of human breast cancer.

LITERATURE CITED


