Vitamin D and breast cancer: insights from animal models

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ABSTRACT
1α,25-Dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$], the biologically active form of vitamin D that interacts with the vitamin D receptor (VDR), is a coordinate regulator of proliferation, differentiation, and survival of breast cancer cells. Therefore, vitamin D compounds that bind and activate VDRs offer promise as therapeutic agents for the treatment of established breast cancer. In addition, epidemiologic, clinical, and animal studies suggested that vitamin D status is important for protection against the development of breast cancer. To elucidate potential biological mechanisms through which vitamin D status might be associated with breast cancer risk, basic research studies focused on defining the molecular effects of vitamin D signaling in the normal mammary gland. Both VDR and vitamin D 1-hydroxylase, the enzyme that generates 1,25(OH)$_2$D$_3$, are expressed and dynamically regulated in the normal mammary gland. Furthermore, studies with mice lacking VDRs established that vitamin D participates in negative growth control of the normal mammary gland and that disruption of VDR signaling is associated with abnormal ductal morphologic features, increased incidence of preneoplastic lesions, and accelerated mammary tumor development. These studies support the concept that suboptimal generation of 1,25(OH)$_2$D$_3$ in the mammary gland might sufficiently deregulate VDR-mediated gene expression to sensitize mammary cells to transformation. In light of these observations, studies to define the most appropriate biomarkers of vitamin D status in relation to protection against breast cancer among human subjects are urgently needed. Am J Clin Nutr 2004;80(suppl):1721S–4S.

KEY WORDS
Vitamin D, vitamin D receptor, breast cancer, mammary gland, prevention, vitamin D hydroxylases

INTRODUCTION
Although it was originally identified on the basis of its role in calcium and bone homeostasis, it is now recognized that 1α,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$], the biologically active form of vitamin D$_3$, exerts effects in almost every tissue in the body, including the mammary gland. The effects of 1,25(OH)$_2$D$_3$ on transformed mammary cells have been well characterized and include cell cycle arrest in G$_0$/G$_1$, induction of differentiation markers, and activation of apoptosis (1–4). Clinically relevant biomarkers that are modulated by 1,25(OH)$_2$D$_3$ in human breast cancer cells include BRCA1, p21, p53, c-Myc, and cyclin D1. Of particular interest, the effects of 1,25(OH)$_2$D$_3$ are not dependent on functional p53 and are not restricted to estrogen-dependent cells. On the basis of these findings, there has been considerable interest in the therapeutic use of vitamin D receptor (VDR) agonists for treatment of breast cancer. Unfortunately, early animal studies indicated that administration of the naturally occurring 1,25(OH)$_2$D$_3$ metabolite at doses necessary to inhibit tumor growth was associated with calcemic toxicity. Those observations suggested either that circulating 1,25(OH)$_2$D$_3$ is not a physiologic regulator of mammary cell turnover or that vitamin D signaling is deregulated during cancer development. As discussed below, studies have provided support for both suggestions. Although therapeutic use of 1,25(OH)$_2$D$_3$ is precluded by dose-limiting calcemic toxicity, studies with synthetic structural analogs of 1,25(OH)$_2$D$_3$ have provided proof that vitamin D compounds can inhibit growth and induce regression of established estrogen-dependent and estrogen-independent tumors in animal models (5–7). Several excellent reviews of the efficacy of vitamin D analogs and their potential mechanisms of action are available (2–4).

VITAMIN D AND PREVENTION OF BREAST CANCER
In contrast to the extensive literature on the potential use of vitamin D-based therapeutic agents for treatment of breast cancer, there has been less research emphasis on the possibility that vitamin D signaling may protect against breast cancer. Support for this concept has been provided in both human and animal studies, however. Only a few epidemiologic studies have examined whether dietary intake of vitamin D alters breast cancer incidence in human populations. A recent evaluation of the Nurses Health Study (8) found that dairy product, dairy calcium, and total vitamin D intakes were inversely associated with breast cancer risk among premenopausal women. These data are consistent with those of an earlier study that reported an inverse correlation between intake of dairy products (which are supplemented with vitamin D) and breast cancer risk (9). Another study found that several measures of sunlight exposure and dietary vitamin D intake were associated with reduced risk of breast cancer (10). Links between solar radiation, which induces epidermal synthesis of vitamin D, and breast carcinoma mortality rates have also been described (11, 12). In 2 studies in which vitamin D status was measured in relation to breast cancer, low serum 1,25(OH)$_2$D$_3$ concentrations were associated with increased breast cancer risk or disease progression (13, 14).
Several animal studies have linked vitamin D with breast cancer prevention. Jacobson et al. (15) demonstrated that rats fed diets low in vitamin D and calcium developed significantly more mammary tumors when treated with the carcinogen 7,12-dimethylbenzanthracene (DMBA) than did rats fed adequate calcium and vitamin D. In mouse mammary gland organ culture, 1,25(OH)\(_2\)D\(_3\) reduced the incidence of DMBA-initiated preneoplastic lesions during both the initiation and promotion stages (16). That was the first study to demonstrate that vitamin D compounds exerted direct antineoplastic effects on the mammary gland, and it suggested that vitamin D signaling could inhibit both early and late events in tumorogenesis. Synthetic vitamin D analogs were also shown to extend tumor latency and reduce tumor incidence and multiplicity in chemical carcinogenesis models of breast cancer (17, 18). Furthermore, the vitamin D analog 1,25-dihydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol significantly enhanced the ability of tamoxifen to prevent mammary tumors, which suggests that vitamin D compounds and antiestrogenic compounds might protect against breast cancer through independent mechanisms (17); this concept was supported by in vitro studies with breast cancer cells (6).

Together, these studies suggest that optimal vitamin D status may protect against mammary transformation. To elucidate the potential biological mechanisms involved, we focused on defining the effects of the vitamin D signaling pathway on proliferation, differentiation, and apoptosis in the normal mammary gland.

**VITAMIN D SIGNALING IN THE NORMAL MAMMARY GLAND: STUDIES WITH VDR KNOCKOUT MICE**

The functions of 1,25(OH)\(_2\)D\(_3\) are mediated through the VDR, a ligand-dependent transcription factor (19). The VDR has been identified in human, rabbit, and rodent mammary gland (20–23), and we examined developmental regulation of the VDR in mouse mammary gland (24). The VDR is expressed in all major cell types of the gland (basal and luminal epithelial cells, cap cells, and stromal cells), and its expression is dynamically regulated during the reproductive cycle (greatest expression is found during pregnancy and lactation). The enhanced expression of VDR during pregnancy and lactation is likely mediated by lactogenic hormones (22) and suggests a role for 1,25(OH)\(_2\)D\(_3\) in differentiation of the gland. This suggestion is consistent with immunohistochemical data that showed that VDR expression was greater in differentiated cells of the gland than in proliferating cells (24). In addition, organ culture studies demonstrated effects of 1,25(OH)\(_2\)D\(_3\) on calcium transport, casein expression, and branching morphogenesis (24–26).

To examine the effects of disruption of the vitamin D signaling pathway on mammary gland development, we used VDR knockout mice (which completely lack functional VDRs) and their normal wild-type littermates (24). Analysis of glandular development during puberty (4–10 wk after birth) demonstrated that mammary glands from VDR knockout mice were heavier and exhibited accelerated growth and branching morphogenesis, compared with glands from wild-type mice. In addition, glands from VDR knockout mice exhibited enhanced growth in response to exogenous estrogen and progesterone, both in vivo and in organ culture, compared with glands from wild-type mice. In organ culture, incubation with 1,25(OH)\(_2\)D\(_3\) inhibited branching of mammary glands from wild-type mice but had no effect on glands from VDR knockout mice. In the absence of VDR, accelerated glandular development was observed during pregnancy, and postnatal involution (a process driven by epithelial cell apoptosis) was delayed (27). Together, these data provide the first in vivo evidence that 1,25(OH)\(_2\)D\(_3\) and the nuclear VDR affect ductal elongation, branching morphogenesis, and hormonal sensitivity during mammary gland development, and they support the concept that 1,25(OH)\(_2\)D\(_3\) and the VDR participate in pathways that inhibit proliferation and induce differentiation in the mammary gland.

We used two distinct mouse models of cancer to test the hypothesis that lack of VDR signaling might alter sensitivity to tumorigenesis. As discussed above, previous work showed that vitamin D\(_3\) compounds could prevent carcinogen-induced preneoplastic lesion development in organ culture and tumorogenesis in whole-animal models (15–18); therefore, our first studies involved exposure of wild-type and VDR knockout mice to the chemical carcinogen DMBA. In this model, VDR knockout mice were hypersensitive to carcinogen-induced skin proliferation and developed a variety of skin tumors, whereas wild-type mice developed minimal epidermal hyperplasia but were tumor free (28). This study provided the first in vivo data that demonstrated that VDR ablation enhanced sensitivity to tumorogenesis. We also demonstrated that the VDR was highly expressed in cells from DMBA-induced mammary tumors that developed in wild-type mice in response to DMBA (29). With this model, we observed an increased percentage of DMBA-induced preneoplastic mammary lesions in glands from VDR knockout mice, compared with wild-type mice, and the histopathologic features of mammary tumors that developed in VDR knockout mice (primarily pilar tumors) were different from those of tumors in wild-type mice (primarily myoepithelial tumors). Additional studies are in progress to determine the significance, if any, of this finding.

Studies with the mouse mammary tumor virus (MMTV)-\(\text{neu}\) transgenic mouse model of breast cancer were conducted to test whether VDR ablation would enhance sensitivity to transformation through a protooncogene that is often overexpressed in human breast cancer (30). Our studies (31) involved crossing VDR knockout mice with MMTV-\(\text{neu}\) mice and monitoring mammary gland morphologic features and tumor development, as a function of VDR gene dosage, with time. We found that VDRs were highly expressed in \(\text{neu}\)-driven mouse mammary tumors and in pulmonary metastatic foci and that loss of either one or both copies of the VDR was associated with increased incidences of preneoplastic lesions and abnormal ductal morphologic characteristics among MMTV-\(\text{neu}\) mice. Furthermore, loss of one copy of the VDR significantly enhanced \(\text{neu}\)-driven mammary tumorigenesis. These data suggest that haploinsufficiency of the VDR is associated with mammary gland pathologic lesions and sensitization of the gland to transformation in response to altered growth factor signaling. This finding is consistent with those of studies that showed that tumor suppressor genes such as \(p53\) and \(p27\) can act in a haploinsufficient manner to enhance tumorigenesis (32).

**BIOACTIVATION OF VITAMIN D IN MAMMARY CELLS**

The data discussed above confirm that 1,25(OH)\(_2\)D\(_3\) and the VDR affect mammary gland development and regulation of
breast cancer cell growth. However, little is known about delivery, uptake, or metabolism of vitamin D in mammary gland or breast cancer cells. Vitamin D itself, whether derived through gastrointestinal absorption or synthesized in the skin, is hydroxylated at the 25-position by liver vitamin D 25-hydroxylase, which generates 25-hydroxyvitamin D [25(OH)D3]. 25(OH)D3 is the major circulating form of vitamin D; it is stored in adipose tissue and is an accurate biomarker of the body’s overall vitamin D status. However, 25(OH)D3 does not bind readily to VDR and must be metabolized to 1,25(OH)2D3, the high-affinity VDR ligand. This bioactivation of 25(OH)D3 is mediated by vitamin D 1-hydroxylase, an enzyme that is highly expressed in renal proximal tubules. Several types of nonrenal cells, including epidermal keratinocytes, activated macrophages, prostatic epithelial cells, and colonocytes, are now known to express 1-hydroxylase (33, 34). Molecular characterization of the human 1-hydroxylase indicated that the enzymes expressed in the kidney and in nonrenal sites are coded by the same gene, located on chromosome 12 (35, 36). Although the presence of the 1-hydroxylase enzyme suggests that some extrarenal tissues have the ability to convert 25(OH)D3 to 1,25(OH)2D3, 1,25(OH)2D3 is virtually undetectable in serum under anephric conditions, which indicates that the kidney is the major source of circulating 1,25(OH)2D3. This observation suggests that 1,25(OH)2D3 produced in nonrenal tissues is not released into the bloodstream but instead acts locally by binding to VDRs present in the same tissues. Such local actions of 1,25(OH)2D3 likely include regulation of cell proliferation, differentiation, and apoptosis, as discussed above.

On the basis of these observations, 2 distinct pathways of vitamin D biosynthesis and action have emerged, i.e., an endocrine pathway, which generates systemic calcemic effects through circulating 1,25(OH)2D3, and an autocrine/paracrine pathway, which generates tissue-specific cell regulatory effects through local release of 1,25(OH)2D3 (37). The implication of the concept of autocrine or paracrine actions is that cellular production of 1,25(OH)2D3 would likely be regulated in a tissue-specific manner, independent of systemic calcium homeostasis. Similarly, the actions of locally produced 1,25(OH)2D3 would be confined to the immediate cellular environment and would not necessarily affect systemic calcium homeostasis.

To date, 1-hydroxylase has been identified in normal mouse mammary gland and in benign and malignant human breast tissue (27, 38). These findings support the hypothesis that normal breast tissue expresses 1-hydroxylase and generates 1,25(OH)2D3, which acts locally to inhibit growth and to maintain differentiation of mammalian epithelial cells. A major implication of this hypothesis is that circulating 25(OH)D3, rather than 1,25(OH)2D3, would be the physiologically important metabolite that maintains VDR-mediated gene expression in mammary cells. This concept is consistent with the experimental observation that the concentrations of 1,25(OH)2D3 necessary to inhibit the growth of both normal and transformed mammary cells are much higher than those found in the circulation. In contrast, data from our laboratory indicated that concentrations of 25(OH)D3 in the physiologic range (35–50 nmol/L) inhibited growth of nontransformed mammary epithelial cells (37).

Both 25(OH)D3 and 1,25(OH)2D3 can undergo additional hydroxylation reactions catalyzed by vitamin D 24-hydroxylase, with generation of 24,25-dihydroxyvitamin D3 and 1,24,25-trihydroxyvitamin D3 (metabolites that do not bind VDR and are degraded and excreted) (39). Vitamin D 24-hydroxylase is present in most vitamin D target tissues; therefore, the ultimate fate (bioactivation or degradation) of 25(OH)D3 and the half-life of 1,25(OH)2D3 depend on the relative activities of 1α-hydroxylase and 24-hydroxylase. When 1α-hydroxylase predominates, 25(OH)D3 is bioactivated to 1,25(OH)2D3, the VDR ligand that promotes growth arrest and differentiation. When 24-hydroxylase predominates, 25(OH)D3 is converted to 24,25-dihydroxyvitamin D3, an inactive metabolite that is degraded and excreted. There are no data on whether vitamin D 24-hydroxylase is expressed in the mammary gland, but it is expressed in breast cancer cells (40). Perhaps even more significantly with respect to breast cancer, the 24-hydroxylase gene was found to be amplified in human breast cancer (41). This finding is consistent with the concept that deregulation of vitamin D metabolism, which results in insufficient tissue concentrations of 1,25(OH)2D3, might be associated with the development of breast cancer. Additional studies to test this concept are underway in our laboratory, with a cell culture model of human breast cancer progression.

CONCLUSIONS

Despite vitamin D fortification of foods and the ability of the body to synthesize the vitamin, vitamin D deficiency is surprisingly common, especially among individuals living in northern climates and among elderly individuals (42). With particular relevance to the potential role of vitamin D in breast cancer, both aging and estrogen deficiency are associated with low vitamin D status. Therefore, postmenopausal women, who represent the predominant target population for breast cancer, are at higher risk for vitamin D deficiency than are younger women. The chronic consequences of subclinical vitamin D deficiency, defined on the basis of low circulating concentrations of 25(OH)D3, have not yet been thoroughly defined. It should be noted, however, that the concentrations of 25(OH)D3 that inhibit the growth of normal human mammary cells are within the range found in the human circulation (35–100 nmol/L). Because accumulation of 25(OH)D3 is highly responsive to dietary vitamin D intake and sunlight exposure, circulating 25(OH)D3 concentrations are decreased during vitamin D deficiency. Therefore, in the mammary gland, which is capable of biosynthesis of 1,25(OH)2D3 from 25(OH)D3, low concentrations of circulating 25(OH)D3 resulting from vitamin D deficiency would limit 1,25(OH)2D3 availability to the VDRs in mammary cells. In turn, suboptimal generation of 1,25(OH)2D3 in the mammary gland would result in deregulation of VDR-mediated gene expression and would possibly predispose mammary cells to transformation. In light of these observations, studies to define the most appropriate biomarkers of vitamin D status in relation to protection against breast cancer among human subjects are urgently needed.

REFERENCES


