Vitamin D and Cancer—A Review

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Abstract

Vitamin D is a steroid hormone that has traditionally been recognized for its role in calcium metabolism and skeletal homeostasis. However, there is growing recognition of its immunomodulatory and anti-tumorigenic effects both in vivo and in vitro. Vitamin D and its receptor form a nuclear receptor-ligand complex that exerts anti-proliferative effects via downstream intracellular signaling. While basic science data for the role of vitamin D in both treating and preventing malignancy have been promising, clinical and epidemiologic data in cancer patients have been mixed. In this paper, we will briefly review the basic science and clinical data for the role of vitamin D in cancer prevention and treatment for the four most common malignancies: breast, prostate, colorectal, and lung cancer.

Keywords

Vitamin D, breast cancer, colorectal cancer, lung cancer, prostate cancer, cancer prevention

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According to the American Cancer Society, in 2013 there will be an estimated 1,660,290 new cancer cases and 580,350 deaths from cancer. Lung, colorectal, breast and prostate cancer are the most common malignancies in the US, accounting for approximately 50 % of new cancer diagnoses. Given the morbidity and mortality of these four malignancies (see *Figure 1*), researchers are exploring many areas of prevention and treatment, including the role of vitamin D.

Vitamin D is a fat-soluble vitamin that regulates bone modeling and calcium metabolism. Although we absorb moderate amounts of the vitamin from foods such as milk and fatty fish, the majority of vitamin D is produced in the body when 7-dehydrocholesterol in the skin is exposed to ultraviolet (UV) B radiation to produce vitamin D3 (cholecalciferol). Vitamin D3 then undergoes two hydroxylation steps: first in the liver to form 25-dihydroxyvitamin D2, the major circulating metabolite, and then primarily in the kidney to produce 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), the biologically active form. It is estimated that 30–50 % of the adult population is vitamin D deficient.[1–3] The most commonly accepted definition of vitamin D deficiency is a serum level of 25-dihydroxyvitamin D2 less than 20 ng/mL. Vitamin D insufficiency is defined as a serum level of 25-dihydroxyvitamin D2 between 20 and 30 ng/mL and a level greater than 30 ng/mL is considered sufficient.[4]

Active vitamin D is produced in a number of tissues, including the breast, prostate, and colon. This activated vitamin D binds to the vitamin D receptor (VDR) to form a nuclear receptor-ligand complex. This forms a heterodimer with the retinoid-X-receptor (RXR) and can regulate the expression of up to 200 genes, including p21, p27, c-fos, and c-myc.[5,6] In preclinical studies, 1,25(OH)2D3 inhibited cell proliferation, induced cell differentiation, promoted apoptosis, and decreased angiogenesis (see *Figure 2*).[5,7,8,9] It is likely that the antiproliferative effects of 1,25(OH)2D3 are mediated in part by stalling the cell cycle at the G1/S checkpoint by increasing inhibitors and reducing activators of the cyclin-dependent kinase (CDK) complexes. This prevents DNA synthesis and cell growth.[10,11] In addition, the enzyme 1-α-25-dihydroxyvitamin D3 24-hydroxylase (CYP24) is the primary enzyme responsible for the catabolic inactivation of 1,25(OH)2D3 and is considered a candidate oncogene.[12,13]

In this paper, we review the recent basic science and clinical research concerning vitamin D and the four most common malignancies: lung, breast, colorectal, and prostate cancer.

Lung Cancer

Lung cancer is the leading cause of mortality from cancer worldwide with only a 10 % survival rate at 5 years.[14] There are two main pathologic types of primary lung cancer, small cell (15 % of cases) and non-small cell (NSCLC) (about 85 % of all lung cancer cases). NSCLC has three subtypes: squamous-cell carcinoma, adenocarcinoma, and large-cell lung cancer. Despite advances in early detection and standard treatment, NSCLC is often diagnosed at an advanced stage and has a poor prognosis. Small-cell lung cancer is aggressive and presents with metastatic disease in 60 % of cases. It is highly responsive to chemotherapy and radiotherapy but almost always relapses and is almost universally fatal.

There have been multiple in vitro studies that have established 1,25(OH)2D3 as an inhibitor of lung cancer growth.[15,16] Vitamin D demonstrates antiproliferative effects in several epithelial-derived stem cell lines such as the NCI-H82 and NCI-H209 small cell lung carcinoma[17] and the EBC-1 and H520 nonsmall cell carcinoma cell lines.[18] The exact mechanism of this regulation is still being elucidated. Researchers have

also demonstrated that 1,25(OH)2D3 inhibits squamous cell carcinoma (SCC) cell motility, invasion, and metastasis, partially through the promotion of E-cadherin-mediated cell–cell adhesion.[19]

1,25(OH)2D3 also has demonstrated activity in vivo. As discussed above, CYP24 is considered a candidate oncogene for its intracellular inactivation of 1,25(OH)2D3. Zhang et al. used a selective CYP24 inhibitor compound CTA091 and found that it decreased 1,25(OH)2D3 catabolism and enhanced 1,25(OH)2D3-mediated growth inhibition.[20] CYP24A1 overexpression is also associated with decreased survival in lung adenocarcinoma.[21]

Given the in vitro and in vivo data, researchers have completed several observational trials that have explored the relationship between 1,25(OH)2D3 and lung cancer survival. In a US study of 456 patients with early-stage NSCLC, patients who had 25(OH)D2 levels ≥21.6 ng/mL experienced a significant improvement in survival compared with patients with 25(OH)D2 levels ≤10.2 ng/mL.[22] Nuclear expression of the VDR is also associated with increased survival. Srinivasan et al. demonstrated that the 5-year overall survival rates were 59 % for patients with high nuclear VDR expression versus 27 % for low nuclear VDR expression.[23] This was correlated by Kim et al. by showing that patients with lung adenocarcinoma whose cells expressed higher levels of VDR had an improved survival (hazard ratio [HR] 0.80, 95 % confidence interval [CI] 0.65–0.99) with evidence of G1 cycle arrest.[24] Pilz et al. demonstrated that increased circulating 25(OH)D2 was associated with improved survival in lung cancer patients.[25] However, epidemiologic evidence for the efficacy of vitamin D is mixed. Freedman et al. found no association between 25(OH)D2 status and total or lung cancer mortality in 16,818 subjects from the third National Health and Nutrition Examination Survey.[26] On further analysis with longer follow up, they even found an increased risk for lung cancer mortality in men with higher circulating vitamin D levels.[27]

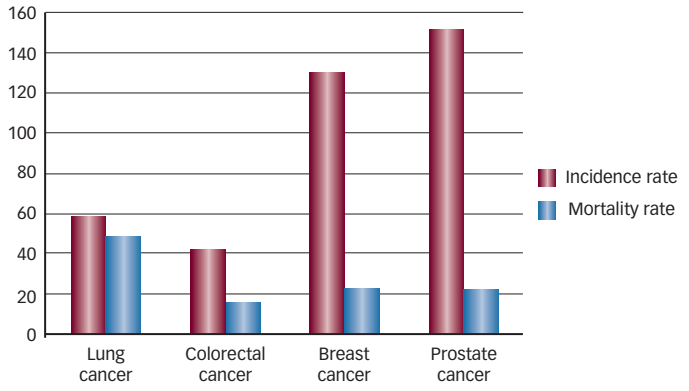
Given the intracellular effects of 1,25(OH)2D3, researchers have explored whether 1,25(OH)2D3 may have a synergistic effect with cytotoxic and targeted treatment. Several studies demonstrate that 1,25-(OH)2D potentiates the cytotoxic effect of taxane and platinum-based chemotherapy in SCC murine models.[28,29] In addition, there is evidence that the VDR directly inhibits epidermal growth factor receptor (EGFR) transcriptional activity in breast cancer cells and that is augmented after treatment with 1,25(OH)2D3.[30] This may have implications for patients with EGFR mutations that are treated with target therapies such as erlotinib.

Ultimately, while there is strong evidence that vitamin D has antineoplastic effects in vitro and in vivo, the epidemiologic evidence for improved survival is mixed. Randomized clinical trials are needed to demonstrate whether vitamin D has a clinically significant benefit for lung cancer patients both for prevention and as an adjuvant treatment.

Breast Cancer

Aside from nonmelanoma skin cancer, breast cancer is the most common cancer among women in the US. In 2009 (the most recent year for which statistics are available), 211,731 women in the US were diagnosed with breast cancer and 40,676 women died from the disease. There are several subtypes of breast cancer that affect both prognosis and treatment. In the US, 75 % of tumors are estrogen receptor positive, 20–25 % of tumors overexpress a tyrosine kinase known as Her2/neu and 10–15 % of tumors are 'triple-negative,' meaning they lack estrogen receptors and do not have an overexpression of her2/neu. These triple-negative cancers have a poorer prognosis compared with other types of breast cancer.

Figure 1: Age-adjusted US Incidence and Death Rates per 100,000 People



Incidence rates from the SEER database, death rates from the US Mortality Files.

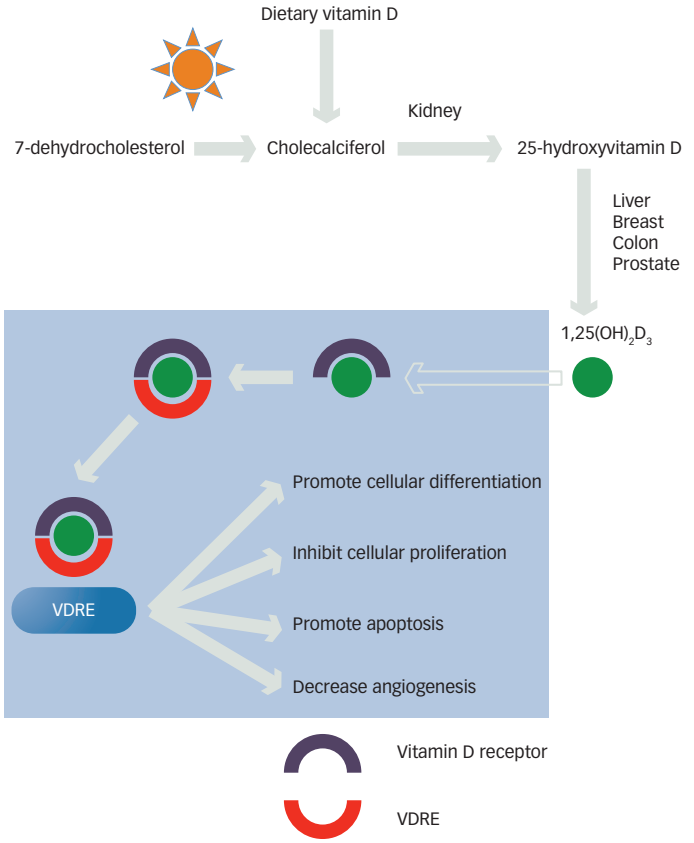
As discussed previously, multiple studies have found an in vitro effect of 1,25(OH)2D3 on breast cancer cell lines including cell-cycle arrest[31,32] and regulation of downstream signaling pathways.[6] In mice, vitamin D deficiency has also been shown to promote breast cancer metastases to bone. This suggests that 1,25(OH)2D3 may effect the bone microenvironment.[33] In addition, similar to lung cancer cell lines, 1,25(OH)2D3 increases the expression of E-cadherin which prevents both invasion and metastasis.[34]

In 1990, Garland et al. first reported an inverse association between total average annual sunlight energy that strikes the ground and age-adjusted breast cancer mortality in the US, suggesting that vitamin D may have a role in breast cancer tumorigenesis. Subsequent studies have had mixed results. Villaseñor et al. found a decreased risk for overall mortality (age-adjusted HR=0.58; 95 % CI 0.36–0.96) in women with sufficient (>30 ng/ml) levels of serum vitamin D; however, this effect was attenuated in multivariate analysis (HR=0.90; 95 % CI 0.50–1.61).[35]

This relationship has also been explored in several large cohort studies. The largest is the Nurse's Health Study, which included 88,691 women who completed surveys every 4 years including items about vitamin D intake. High vitamin D intake was associated with a statistically significant lower risk for premenopausal breast cancer (odds ratio [OR] 0.72; 95 % CI 0.55–0.94) but not for postmenopausal breast cancer.[36] A similar finding was reported in the Women's Health Initiative Study (WHI). Higher intake of vitamin D was associated with a lower risk for breast cancer in premenopausal women (OR 0.65; 95 % CI 0.42–1.00) but not in postmenopausal women (OR 1.30; 95 % CI 0.97–1.13).[37]

Researchers have also examined the relationship between serum 1,25(OH)2D3 levels and breast cancer risk, both in prospective cohort studies and case-control studies. The majority of case-control studies found a statistically significant lower risk for breast cancer among women with higher circulating vitamin D levels.[38–41] Crew et al. found

Figure 2: Vitamin D Metabolism



VDRE = vitamin D response element.

that 1,25(OH)2D3 levels above 40 ng/mL are associated with a decreased breast cancer risk (OR 0.56; 95 % CI 0.41–0.78) and this effect was greatest among postmenopausal women, in contrast to the Nurse's Health Study.[42]

At this time, there have been only two randomized clinical trials exploring vitamin D levels and the risk of breast cancer. In the WHI, there was no reduction in the risk for breast cancer in women randomly assigned to take calcium (1,000 mg) and vitamin D3 (400 IU) daily versus placebo.[43] In another trial of approximately 1,200 postmenopausal women randomized to receive 1,100 IU of vitamin D3 and calcium, calcium alone, or placebo, a 60 % lower risk for cancer of all types, including breast cancer, was observed after 4 years of supplementation with vitamin D3 and calcium (p=0.013).[44] Currently, there are several phase II studies looking at whether high-dose vitamin D supplementation (20,000 IU weekly or 30,000 IU weekly) prevents breast cancer in both pre and postmenopausal women at high risk for developing breast cancer.

Lastly, there is some data for utilizing vitamin D in breast cancer treatment. First, in vitro studies have demonstrated that 1,25(OH)2D3 suppresses the estrogen pathway by reducing the expression of the gene coding for aromatase,[45] the enzyme that converts androgens to estrogens as well as downregulating expression of estrogen receptor-α (ER-α).[46,47] In addition, 1,25(OH)2D3 potentiates the cytotoxic effects of several agents used in breast cancer treatment including adriamycin[48] and paclitaxel.[49] Several randomized controlled trials are currently enrolling women to examine the effects of high-dose 1,25(OH)2D3 in combination with taxanes.

Colorectal Cancer

Colorectal cancer is the third leading cause of cancer-related death in the US. It is the second leading cause when both sexes are combined. It is expected to cause about 50,830 deaths during 2013.[50] Globally, the age-adjusted incidence of colorectal cancer range from 30 or more cases per 100,000 people in North America, certain areas of Europe, Australia, New Zealand, and Japan to less than five cases per 100,000 people in much of Africa and parts of Asia.[51] This large disparity among the different geographic regions suggests a role for vitamin D in the development of colorectal cancer.[52]

In colorectal cancer cell lines, 1,25(OH)2D3 causes growth arrest of colorectal cancer cells. Specifically, 1,25(OH)2D3 induces the expression of CDK inhibitors p21CIP1 and p27KIP1 and represses the action of cyclins A and F.[53–56] 1,25(OH)2D3 also downregulates the c-myc oncogene via binding the vitamin D response element and by augmenting the expression and binding of intermediate proteins to regulatory regions of the gene.[57–60]

Vitamin D also modulates apoptosis in colorectal cancer cells. 1,25(OH)2D3 induces the expression of G0S2, which prevents BCL-2 from forming anti-apoptotic heterodimers with the well-known BAX protein.[56,62] Furthermore, 1,25(OH)2D3 downregulates the anti-apoptotic protein BAG1 and sensitizes colorectal cancer cells to the cytotoxic agent 5-fluorouracil by downregulating the anti-apoptotic protein survivin.[63,64] Additionally, 1,25(OH)2D3 enhances expression of several proteins linked to the formation of tight junctions, adherens junctions, and hemidesmosomes, all crucial in maintaining an epithelial phenotype.[56,65,66] This may be crucial in preventing colorectal cancer metastases.

Angiogenesis appears to be regulated by vitamin D in vitro. For example, 1,25(OH)2D3 represses the expression of hypoxia-inducible factor (HIF)-1α and regulates the expression of vascular endothelial growth factor (VEGFA).[55,67] 1,25(OH)2D3 also represses DICKKOPF-4 (DKK4), a WNT antagonist that promotes invasion and angiogenesis in colorectal carcinoma cultures.[68]

In colorectal carcinoma cultures, vitamin D has also been found to modify both phase I and II enzymes involved in reduction, oxidation, hydrolysis, and conjugation—all important for the removal of compounds that may contribute to the formation of cancer.[69] Furthermore, vitamin D has been showed to regulate the expression of members of the multidrug resistance-associated protein family that are responsible for the efflux of potentially toxic conjugates from the intracellular milieu.[70,71]

There is a substantial amount of epidemiologic literature that examines the relationship between vitamin D and the risk for colorectal cancer. Garland and Garland published a paper in 1980 that proposed an inverse relation between latitude and colorectal cancer mortality.[72] In 1989, an inverse association between vitamin D and colorectal cancer status in the US was established.[73] A recent meta-analysis of 35 independent studies confirmed a consistent inverse relationship between serum vitamin D levels and the risk for developing colorectal cancer.[74] Additionally, a systematic review of 18 prospective studies that assessed the association of vitamin D intake or serum levels of 25(OH)D2 and the risk for colorectal cancer in approximately 1,000,000 individuals was performed. They found

an inverse association between both serum 25(OH)D2 and vitamin D intake and the risk for developing colorectal cancer.[75] However, Pereira et al. notes that many epidemiologic studies do not take into account endogenous vitamin D production from UV light exposure and are limited by measurement error from the various dietary assessment methods and food composition tables used to calculate dietary intake.[62]

There is some clinical trial data on vitamin D supplementation and colorectal cancer, albeit scarce. Trivedi et al. ran the first trial with almost 3,000 older individuals receiving 100,000 IU of 1,25(OH)2D3 or placebo for four months.[76] They found no change in the incidence of colorectal cancer or mortality over 5 years. The largest clinical trial to date is the WHI. Close to 40,000 postmenopausal women were randomized to receive calcium and 400 IU/day of 1,25(OH)2D3 versus placebo. Initially, the results did not show a relationship between vitamin D supplementation and the incidence of colorectal cancer.[77] However, the dose of vitamin D was relatively low and the compliance among the women in the study was only around 80 %.[78] A re-analysis of the WHI data showed concurrent vitamin D and estrogen replacement increased the risk for developing colorectal cancer and treatment with vitamin D in the placebo group significantly decreased the risk for developing colorectal cancer.[79] Another re-analysis showed a 17 % nonsignificant reduced risk for developing colorectal cancer in the group supplemented with vitamin D.[80]

There is a lot of evidence implicating a role for vitamin D in colorectal cancer in vitro and in epidemiologic studies. While the WHI gives us some insight on vitamin D replacement in colorectal cancer, additional clinical trials are needed before vitamin D can become mainstream in colorectal cancer therapy.

Prostate Cancer

Other than skin cancer, prostate cancer is the most common cancer in US men. The American Cancer Society estimates that in 2013, about 238,590 new cases of prostate cancer will be diagnosed and about 29,720 men will die of prostate cancer.[50]

Many studies have examined the question of whether or not vitamin D plays a role in prostate cancer. This originated when the VDR was found in three cell lines of prostate cancer suggesting a role of the vitamin D pathway in prostate cancer progression.[81] Recently, high VDR expression in prostate tumors was shown to be associated with a reduced risk for lethal cancer.[82] In addition, normal prostate cells express 1-α-hydroxylase, and prostate cancer cells have been shown to lose 1-α-hydroxylase activity in vitro.[83–85] This suggests a relationship between vitamin D and progression to malignancy in prostate cancer cells.

Interestingly, in cells from the LNCaP prostate cancer cell line, which have very low levels of endogenous 1-α-hydroxylase activity, the antiproliferative action of 25(OH)D2 was much less pronounced when compared with calcitriol.[85] Barnett and Beer hypothesize in their review article that loss of local prostatic 1-α-hydroxylase may render prostate cancer cells more dependent on circulating 1,25(OH)2D3 rather than on the more abundant 25(OH)D2, which can reduce the prostate cell's ability to regulate its own growth in response to vitamin D levels.[86] This finding was demonstrated after transfection of 1-α-hydroxylase cDNA into a prostate cancer cell line increased 1-α-hydroxylase activity significantly.[87]

Given this in vitro data, other studies have been performed to examine the correlation between UV light exposure and incidence of prostate cancer or prostate cancer mortality. Of the nine studies that have been performed since 2011, seven of the studies show reduced UV light exposure increases prostate cancer risk.[88–94] Additionally, the two studies that did not show an increase in the risk for prostate cancer in the setting of reduced UV light did show a difference in mortality for the season of diagnosis.[95,96]

Given the mixed UV exposure data, we have found 15 case-cohort or case-control studies examining serum vitamin D and its relationship to prostate cancer risk or mortality. Ten of these studies did not find an association between low vitamin D levels (either 1,25(OH)2D3 or 25(OH)D2) and prostate cancer risk or mortality.[97–106] Five studies show an increase in prostate cancer risk or mortality with low vitamin D;[107–111] one of them shows a decreased risk for lethal cancer[107] and two of them demonstrate an increased risk for aggressive cancer.[110,111] Of note, two studies show an increased risk with higher levels of 25(OH)D2[108,110] and one study shows a possible increased risk for aggressive cancer with higher levels of 25(OH)D2.[103]

While the epidemiologic data is promising for vitamin D as a potential therapy in prostate cancer, the use of 1,25(OH)2D3 comes at a cost of hypercalcemia, which comes with its own set of toxicities. Unfortunately, hypercalcemia is shown in some studies to be associated with either an increase in the risk for prostate cancer/and or an increase in the risk for high-grade prostate cancer.[112–118]

In an effort to increase efficacy and decrease toxicity, the use of vitamin D and its analogues have been studied in combination with other agents. There are many phase II clinical trials that show calcitriol decreases the levels of prostate-specific antigen.[119–124] One of these studies did show an improved survival with a formulation of calcitriol in combination with cytotoxic docetaxel, although the phase II study was stopped early in the setting of excess deaths.[125]

The in vitro studies and the UV light studies show more of a link between prostate cancer and vitamin D, while the serum studies showed more mixed data. Given the high prevalence of prostate cancer in men around the world, we will likely hear more soon about vitamin D and its association with prostate cancer.

Conclusion

Lung, breast, prostate, and colorectal cancer are the four most common malignancies in the US and an estimated 279,650 people will die from these malignancies in 2013. Both basic scientists and clinical researchers are exploring whether vitamin D has a role in both cancer prevention and treatment. In vivo and in vitro studies are promising, demonstrating that the vitamin D ligand-receptor complex downregulates many intracellular pathways necessary for cellular proliferation. However, the clinical studies have been mixed. While several large studies have shown increased survival rates for breast and lung cancer patients with sufficient vitamin D levels, this has not been replicated in other studies. Vitamin D in colorectal cancer appears promising from studies in vitro, but the clinical studies are not as promising at this time. The UV light studies and serum studies that evaluate vitamin D in prostate cancer are mixed as well. Ultimately, large randomized clinical trials are necessary to demonstrate

that vitamin D decreases cancer incidence. While this study is underway for breast cancer, it may be prohibitively expensive to perform for other types of malignancies. Similarly, there are many trials currently underway to explore whether vitamin D may be used to enhance cytotoxic therapy. Ultimately, more data is needed to translate vitamin D's role in cancer from the bench to the bedside. In the meantime, achieving an adequate vitamin D status should be a goal for everyone and may prove to be beneficial in preventing malignancy. ■

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