Calcium, Vitamin D and the Vitamin D Receptor: Impact on Prostate and Breast Cancer in Preclinical Models

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Epidemiological, molecular, and cellular studies have implicated vitamin D, a fat-soluble vitamin, and the vitamin D receptor (VDR) in the development and/or progression of cancer. The activation of vitamin D in the kidney is intricately linked to dietary calcium, another nutrient that has been associated with cancer risk in epidemiological studies. While calcium and vitamin D interact in the maintenance of skeletal health, it is now recognized that these nutrients exert independent effects on cell behavior, including proliferation, differentiation, and apoptosis. In addition to the kidney, many tissues contain VDR and express vitamin D-metabolizing enzymes. The regulation of these enzymes is likely tissue specific and unrelated to calcium status. This short review will focus on data generated from animal, cell, and molecular studies that have assessed the interactions between calcium and vitamin D on breast and prostate cancer.

INFLUENCE OF DIETARY CALCIUM IN PRECLINICAL MODELS OF PROSTATE CANCER

In vitro studies have shown that 1,25(OH)\(_2\)D\(_3\) has anti-proliferative and pro-apoptotic effects in prostate cancer cell lines, including LNCaP cells. Epidemiological studies have suggested that the consumption of calcium-rich dairy products may be associated with an increased risk for prostate cancer.\(^1\)\(^-\)\(^3\) These data have led to the hypothesis that a high calcium intake may enhance the risk for prostate cancer via suppression of serum 1,25(OH)\(_2\)D\(_3\). To date, only one published study has directly tested this hypothesis in an animal model. Balaji et al.\(^4\) reported that dietary calcium did not affect the growth of subcutaneous xenografts of LNCaP cells in a nude mouse model system. Because LNCaP cells represent an established cell line originally derived from a lymph node metastasis and express a mutated androgen receptor, these xenografts may not be the ideal system to test the effects of dietary calcium. We therefore followed up the studies of Balaji et al.\(^4\) with a distinct cell line, PC346c, in both orthotopic and subcutaneous xenograft models.

PC346c cells were derived from an early stage, non-metastatic, prostate cancer and express a wild-type androgen receptor.\(^5\) In our study, nude mice were adapted to low-calcium (0.18% Ca, 0.14% P) or high-calcium (2.0% Ca, 1.5% P) diets and implanted with slow-release testosterone pellets prior to inoculation of PC346c cells in the flank (subcutaneous) or the prostate (orthotopic). Body weights and tumor volumes were monitored for 6 weeks after cell inoculation. The results indicated that dietary calcium had no effect on growth rate, final tumor size/weight, or mitotic index of either subcutaneous or orthotopic PC346c tumors. These data are consistent with those of Balaji et al.,\(^4\) and suggest that dietary calcium does not affect the growth of established prostate cancer cells in vivo.

To test the possibility that dietary calcium might have an impact on initial tumor development (rather than the growth of already transformed cells), we utilized the LPB-Tag transgenic mouse model of prostate cancer. In this model, expression of SV40 large T antigen is targeted to the dorsolateral lobes of the prostate with the large probasin promoter.\(^6\) Prostate tumors develop in these mice beginning at 5 weeks of age, and these tumors express both androgen receptor and VDR. Contrary to the hypothesis, dietary calcium had no effect on urogenital morphology, tumor weight, or histological grade in LPB-Tag transgenic mice studied at 5, 7, or 9 weeks of age.\(^7\) Collectively, these three studies have demonstrated...
that high dietary calcium does not aggravate or accelerate prostate tumor growth or progression in animal models, and therefore do not support the concept that dietary calcium per se influences prostate cancer risk.

ROLE OF VITAMIN D AND THE VITAMIN D RECEPTOR IN MODELS OF BREAST AND PROSTATE CANCER

The effects of vitamin D analogs on cancer have been examined in numerous animal systems, including xenograft, carcinogen-induced, and transgenic models of breast and prostate cancer. These trials have demonstrated that some vitamin D analogs can successfully inhibit the growth of both breast and prostate tumors with minimal calcemic toxicity. Our laboratory has taken a different approach and examined the influence of VDR ablation on tumor progression in several model systems, including the DMBA model of mammary carcinoma and the MMTV-neu and LPB-Tag transgenic models of breast and prostate cancer, respectively. These studies have demonstrated that VDR ablation enhances the incidence of mammary hyperplasia in the DMBA and MMTV-neu models and accelerates the progression of prostate cancer in the LPB-Tag model.

We also tested the effect of dietary vitamin D3 on MMTV-neu tumorogenesis using diets designed to maximize 25(OH)D3 delivery to mammary tissue without altering serum calcium. The experimental diets contained 0, 250, or 5000 IU of vitamin D3/kg diet and were fed for 6 months beginning at the age of 10 weeks (i.e., after pubertal development of the mammary gland was complete). Increasing dietary vitamin D3 did not inhibit MMTV-neu mammary tumor development despite the expected increase in serum 25(OH)D3. Measurement of the vitamin D-metabolizing enzymes cyp24 and cyp27B1 in normal mammary gland versus MMTV-neu driven tumors indicated that cyp24 was up-regulated relative to cyp27B1, which may have disrupted local metabolism of 25(OH)D3 and 1,25(OH)2D3 and precluded the tumor-suppressor effects of dietary vitamin D3. Further studies in alternative animal models of cancer are needed to determine whether vitamin D metabolism is consistently altered during tumorogenesis.

DISSOCIATION OF CALCIUM REGULATION AND ANTI-TUMOR FUNCTIONS OF THE VITAMIN D RECEPTOR

To assess the mechanisms of VDR signaling in breast cancer, we established cell lines from mammary tumors that developed in DMBA-treated wild-type (WT) and VDR knockout (VDRKO) mice. Studies in these cells have demonstrated that the anti-tumor effects of vitamin D compounds are triggered through VDR-mediated induction of growth arrest and apoptosis both in vitro and in vivo. More recently, we successfully reconstituted the growth-inhibitory VDR-signaling pathway via stable transfection of human VDR into the mouse-derived VDRKO mammary tumor cells. Growth inhibition of VDRKO cells stably expressing human VDR was detected at concentrations of 1,25(OH)2D3 as low as 10 pM. In this model system, we also stably expressed VDRs carrying point mutations that are known to cause hereditary rickets in humans, and some of these mutant VDRs retain the ability to signal growth inhibition in response to concentrations of 1,25(OH)2D3 as low as 0.1 nM. These and other studies have provided evidence for dissociation of the calcemic and growth-regulatory actions of VDR at the cellular level.

CONCLUSION

In conclusion, dietary calcium had no impact on the development or progression of prostate cancer in three distinct animal models, indicating that suppression of circulating 1,25(OH)2D3 has little influence on tumor growth. These data are consistent with epidemiologic studies reporting that serum 1,25(OH)2D3 is not predictive of prostate cancer risk, and suggest that local production of 1,25(OH)2D3, which is unlikely to be altered by dietary calcium, may be more relevant in regulation of prostate epithelial cell growth than is serum 1,25(OH)2D3. In animal models of both prostate and breast cancer, VDR agonists can suppress, while VDR ablation can accelerate, hyperplasia and tumorigenesis. Thus, VDR appears to function as a tumor-suppressor gene in epithelial tissue. However, neither supplementation nor deficiency of dietary vitamin D3 altered the course of mammary tumorigenesis in the MMTV-neu transgenic model when diets were manipulated after pubertal development of the mammary gland was complete.

These data suggest that the effects of dietary vitamin D3 may be restricted to certain developmental windows; however, additional studies are needed to test this concept. Finally, in vitro studies with cells derived from VDRKO mammary tumors have provided proof of the theory that VDR-mediated growth inhibition can be dissociated from calcemic regulation. Ongoing studies in this model should provide insight into the mechanism of action of synthetic vitamin D analogs that display enhanced anti-tumor actions yet reduced calcemic potency, as well as the molecular mechanisms by which the VDR mediates such a wide spectrum of biologic effects in vivo.
REFERENCES


