

Vitamin D and breast cancer: new approaches for hormonal therapy of breast cancer

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Epidemiologic studies have indicated that vitamin D might play a protective role against breast cancer. Incidence of breast cancer and mortality rate vary considerably worldwide and reveal a geographic pattern. The lowest rates of breast cancer generally occur in countries close to the equator. With increasing latitude reported breast cancer incidence and mortality rates also increase. A negative correlation between available sunlight and breast cancer death rates has been shown. Because sunlight exposure is a measure of vitamin D produced in the skin, it has been hypothesized that vitamin D formed in the skin may reduce the risk of breast cancer [1-3].

Breast cancer is the most frequent cause of cancer death in women in the western world. Many studies have tried to identify the causal factors responsible for the uncontrolled growth of the tumor cells. A variety of biochemical and genetic changes have been identified in breast carcinomas and have been found to be related to breast cancer growth. However, especially because of the heterogeneity of the disease on the clinical, biologic, and genetic levels, the exact mechanism of breast cancer development and progression is still unclear. During the last 15 years, it has become evident that 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the biologically most active form of vitamin D₃, exerts effects on a variety of tissues which are apparently unrelated to calcium homeostasis. 1,25(OH)₂D₃ has been shown to induce cellular differentiation and inhibit proliferation of hematopoietic cells and cancer cells. In addition, studies with animal cancer models have shown that 1,25(OH)₂D₃ application can prolong the survival of leukemic mice and suppress the growth of tumors of different origins including breast [4, 5]. These newly discovered properties suggest a possible role of the hormone in the treatment of cancer. However, a major drawback for a clinical application is that high doses are needed. These doses produce serum levels of 1,25(OH)₂D₃ for above the physiologic level, which may lead to hypercalcemia. Many investigators have tried to change the 1,25(OH)₂D₃ molecule in order to retain its antiproliferative and differentiation-inducing activity combined with a reduced effect on calcium and bone metabolism. This strategy has resulted in new synthetic vitamin D₃ analogs with clinical potential [6-8].

The expression of the vitamin D receptor (VDR) in breast cancer was first demonstrated in the human breast cancer cell line MCF-7 [9]. Further studies have extended this finding even to surgically obtained normal breast and breast tumor tissue [10]. The VDR is expressed in about 80% of human breast tumor specimens. The VDR status is not correlated to the expression of other steroid hormone receptors (estrogen receptor, progesterone receptor) [11-30] or to the clinical indices (age, menopausal status, T-stage, histology, lymph node involvement) including overall survival [12-31]. Nevertheless, two studies reported that the VDR status correlated positively with the disease-free interval [14-31]. The steroid hormone responsiveness is directly proportional to the number of corresponding receptors. Regulation of the number of VDR may affect the cellular responsiveness to 1,25(OH)₂D₃. In several different systems including MCF-7 and T47-D breast cancer cells, upregulation of the VDR by 1,25(OH)₂D₃ itself (homologous upregulation) and by hormones (estradiol) and growth factors [epidermal growth factor (EGF), insulin, insulin-like growth factor-1 (IGF-I)] has been demonstrated (heterologous upregulation) [2, 32-37]. Because VDR mediates the biological effects of calcitriol and analogs on differentiation and proliferation in target cells, VDR upregulation may indicate an increased sensitivity of breast cancer to endogenously or therapeutically applied calcitriol. Thus, a relationship between VDR level and growth inhibition has been suggested for breast cancer cells [38, 39]. Nevertheless, the presence of a functional VDR is not always combined with a growth-inhibitory response of 1,25(OH)₂D₃. A lack of growth inhibition by 1,25(OH)₂D₃ independent of the status of functional VDR has been demonstrated in breast cancer cells [40, 41]. The underlying mechanism of this VDR-independent resistance to growth inhibition is unknown.

The first studies on the effect of 1,25(OH)₂D₃ on breast cancer cells showed a biphasic growth response

of the estrogen receptor-positive T47-D human breast tumor cell line. At low concentrations (10⁻¹¹ M), a stimulation of cell growth was observed, whereas at higher concentrations (10⁻⁸ M), an inhibition was observed [10, 42, 43]. This growth-inhibitory effect of 1,25(OH)₂D₃ was confirmed in other breast tumor cell lines and shown to be independent of the estrogen receptor status [44, 45]. In the growth inhibited breast cancer cells 1,25(OH)₂D₃ and 1,25(OH)₂D₃ analogs caused an increase of the number of cells in the G₀/G₁ and occasionally in the G₂ phase together with a decrease of the number of cells in the S phase [40, 44, 46-49] indicating a cell cycle block in the G₀/G₁ phase. Apoptosis (programmed cell death), is an asynchronous cellular process with cytoplasmic and nuclear condensation, disruption of the cytoskeleton, and condensation of intermediate filaments around the nucleus and is related to the cell cycle [50]. Induction of apoptosis can be a possible way in which 1,25(OH)₂D₃ inhibits tumor cell growth. Recently it has been shown that 1,25(OH)₂D₃ induces apoptosis in various tumour cells [40, 49, 51, 52]. A central role for apoptosis in the action of 1,25(OH)₂D₃ is uncertain because growth inhibition of several breast cancer cells appeared to be independent of apoptosis [40]. Thus, the growth stimulation of MCF-7 cells, that were growth inhibited by 1,25(OH)₂D₃, after removal of 1,25(OH)₂D₃ indicates independence of growth inhibition from apoptosis [53]. Possibly in these latter cases induction of differentiation is more prominent. Treatment of breast cancer cells with 1,25(OH)₂D₃ resulted in morphologic changes, which may resemble a more differentiated status of the cells [43, 54, 55]. Induction of differentiation was recently shown in several breast cancer cells [40]. The results of these studies suggest that induction of differentiation and growth inhibition are two independent processes. The various synthetic vitamin D₃ analogs have been shown to be more potent than 1,25(OH)₂D₃ in the growth inhibition of several cancer cell types, whereas their *in vivo* calcemic activity was similar or even reduced compared with 1,25(OH)₂D₃.

Mammary tumors can be induced in rats by oral administration of the carcinogens N-nitroso-N-methylurea (NMU) or 7,12-dimethylbenz[a]anthracene (DMBA). Application of 1,25(OH)₂D₃ resulted in an inhibition of the growth of NMU- [31, 56] and DMBA-induced rat mammary tumors [57, 58], whereas Noguchi *et al.* [59] did not find an effect of 1,25(OH)₂D₃ on the incidence and growth of DMBA-induced rat mammary tumors. To achieve tumor suppression, high doses of 1,25(OH)₂D₃ (about 0.5 µg/kg BW) were needed with the subsequent development of hypercalcemia and weight loss. Thus, synthetic vitamin D₃ analogs with low *in vivo* calcemic activity have been developed. Only a few analogs have been evaluated *in vivo* for their potential use in the treatment of breast cancer. To date, two clinical studies on the effect of vitamin D₃ analogs on cancer growth in humans have been reported. Topical application of calcipotriol (MC903) in a small group of patients with locally advanced or cutaneous metastatic breast cancer showed a reduction in the size of treated lesions in 4 of 14 patients [60], whereas another study could not confirm this observation [61]. In a phase I trial the analog EB1089 is being examined in advanced breast cancer, but no detailed analyses have been published.

The antiestrogen tamoxifen is the most widely used endocrine agent in the treatment of breast cancer [62]. A major problem of tamoxifen therapy is that in case of response, the tumor almost inevitably progresses to a tamoxifen-resistant state during prolonged therapy. Furthermore, long-term tamoxifen therapy has been linked to an increased risk of endometrial cancer. Therefore, despite the efficacy of tamoxifen for breast cancer, alternative additional endocrine therapies are needed. Thus, several studies have focussed on possible future combination therapies with 1,25(OH)₂D₃ and 1,25(OH)₂D₃ analogs in estrogen receptor-positive and -negative breast cancer. A synergistic antiproliferative effect of submaximum dosages of the vitamin D analog, 22-oxacalcitriol, and tamoxifen in breast cancer cells has been described *in vitro* and *in vivo* [63]. Thus, the combined treatment with 1,25(OH)₂D₃ and tamoxifen resulted in stronger growth inhibition of MCF-7 cells than treatment with either compound alone [53]. With a number of vitamin D₃ analogs a similar effect was observed [37]. In combination with tamoxifen the cells were more sensitive to the antiproliferative action of 1,25(OH)₂D₃ and the analogs [37]. The ability of tamoxifen to reduce the total tumor burden of rats treated with the carcinogen NMU is significantly enhanced by a combination of the vitamin D₃ analog, Ro24-5531, with low doses of tamoxifen [64]. Thus, implications for the use of vitamin D analogs not only in treatment but also in the prevention of breast cancer have been indicated. Vitamin A-derivatives like fenretinide are currently being tested in clinical trials as preventive agents against recurrence of breast cancer, and animal studies point to a potential use of these compounds as therapeutic agents for breast cancer [65]. A combination therapy of retinoic acid and 1,25(OH)₂D₃ showed a synergistic growth inhibition of

breast cancer cells [66]. Furthermore, combinations with vitamin D₃ compounds and cytotoxic drugs (TNF, GM-CSF, Adriamycin, 5-FU, Carboplatin, Cisplatin) have been studied [45, 57, 67-69]. The data on combinations of 1,25(OH)₂D₃ and 1,25(OH)₂D₃ analogs with various other anticancer compounds are promising and justify further analyses. For example, the development of effective combination therapies may result in better response rates and lower dosages combined with a reduced risk of negative side-effects.

Invasion and metastasis of tumor cells are the primary causes for the fatal outcome of cancer diseases. A recent report by Mork Hansen *et al.* [70] indicated that 1,25(OH)₂D₃ may be effective in reducing the invasiveness of breast cancer cells. They have shown that 1,25(OH)₂D₃ inhibited the invasion and migration of a metastatic human breast cancer cell line (MDA-MB-231). A fact to be considered in relation to metastasis is that bone is the most frequent site of metastasis of advanced breast cancer [71]. There are some indications from clinical studies that bone metastases develop preferentially in areas with high bone turnover [72, 73]. By contrast, agents that inhibit bone resorption have been reported to reduce the incidence of skeletal metastasis [74]. As 1,25(OH)₂D₃ is an important stimulator of bone resorption and consequently of bone turnover, treatment with 1,25(OH)₂D₃ or vitamin D₃ analogs for breast cancer might increase the risk of skeletal metastases. However, Krempien [75] reported that following intraarterial injection with Walker 256 tumor cells, rats treated with 1,25(OH)₂D₃ developed significantly more bone metastases than untreated controls. In relation to this aspect, it was shown that tamoxifen, which exerts positive estrogenic effects on bone [76], considerably suppressed the bone resorption induced by 1,25(OH)₂D₃ ED1089, and KH1060 [77]. These data suggest that in vitamin D-tamoxifen combination therapy for breast cancer tamoxifen may offer protection against the bone resorption induced by vitamin D₃ compounds and thereby decreases the risk of bone metastases.

Vitamin D₃ analogs have potent antiproliferative effects on breast cancer cells *in vitro* and suppress breast cancer growth *in vivo* without marked calcemic effects. However, apart from the strong calcemic activity of 1,25(OH)₂D₃ other negative side-effects may arise, in particular immunosuppressive effects and an increased risk of bone metastases. The development of new vitamin D₃ analogs continues. In the future vitamin D₃ analogs with even stronger antiproliferative action and better selectivity may become available. A major advantage of endocrine therapy with vitamin D may be that vitamin D suppresses breast tumor growth independent of the presence of the estrogen receptor. Most established endocrine therapies are based on antiestrogenic action, and for estrogen receptor-negative tumors therapeutic choices are limited. Vitamin D treatment could theoretically be beneficial for a large group of patients, since the VDR is expressed in about 80% of human breast cancers. Another promising aspect of vitamin D treatment might be its combination with other established endocrine (tamoxifen) or cytotoxic agents. Finally, epidemiologic studies and laboratory results have suggested a role for vitamin D in the prevention of breast cancer. In the next few years, clinical studies are needed to confirm that vitamin D₃ analogs, either alone or in combination with other antitumor agents, can provide an effective treatment for breast cancer.

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