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Vitamin D and therapy of breast cancer

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Abstract: Breast cancer is one of the most widely distributed cancer diseases in women. Breast carcinomas derive from the epithelial lining of the milk ducts and the duct lobules. $1\alpha, 25$ dihydroxyvitamin D3 [$1, 25(\text{OH}) 2\text{D}_3$], the biologically active form of vitamin D that interacts with vitamin D receptor. It prevents and inhibits the growth of breast cancer cells by arresting the cancer cells replication cycles, promoting apoptosis, inhibiting invasion, metastasis and angiogenesis. The mechanism of action is not well fully identified, the vitamin D modulate the expression of myc, fos, jun genes and up regulate of p53, Rb genes. In addition, it inhibits expression of aromatase enzyme through inhibiting production of cox-2 enzyme and increase expression of 15-hydroxyprostaglandin dehydrogenase. Although much of attention has been directed in recent years to development new analogous of vitamin D3 as potential therapeutic agent in breast cancer therapy, addition of vitamin D3 to chemotherapy has been found to potentiate their anticancer effect and associated with reduction of their toxicity. In this review, I try to emphasize the role of vitamin D3 in potentiating therapy of breast cancer.

Keywords: Vitamin D, breast cancer, cancer, analogues of vitamin D, Libya

Breast cancer (BC) is widely distributed diseases among women. BC accounts for 23% of all cancers of women (1). One in every eight women develops BC. The most common type of BC is invasive ductal carcinoma which accounts for 28%. There are several risk factors linked to development of BC and some are related to the estrogens levels, others are to age, family history and genes (2). Breast carcinomas derive from the epithelial lining of the milk ducts and the duct lobules. Tumor can either be confined within the ducts or lobules, or invades the surrounding tissue and gives rise to distant metastases. Some biochemical and genetic changes were identified in breast carcinomas with several factors were shown to influence BC growth. There are several BC types differ in their capability of spreading (metasta-sizing) to other body tissues. The causes of BC are not yet fully understood

although many of risk factors were identified, however, many different types of BC exist. BC is diagnosed with physician and self-examination of breasts, ultrasound scanning, mammography and biopsy. Therapy of BC depends on cancer type and its stage (3). BC originates from breast tissue, most commonly from inner lining of milk ducts or the lobules that supply the ducts with milk. Although breast cancer predominantly occurs in female and it can affect male also (4). There are several types of BC. Some are more common than others and others are combinations of cancers. The most common BC types are ductal carcinoma in situ, invasive ductal carcinoma and invasive lobular carcinoma. Other types of BC that are less common than the above mentioned BC namely: mucinous carcinomas, mixed tumors, inflammatory BC, triple-negative breast cancers, Paget's disease of

the nipple and adenoid cystic carcinoma. However, there are other types of BC that are not commonly appeared in females as papillary carcinoma, phyllodes tumor, angiosarcoma and tubular carcinoma. Regarding risk factors, some of BC can be modified by intake of alcohol while others cannot be influenced. The chances of BC are found to be related to age, family history: the risk of BC is more correlated to the women who have relatives with such disease; individual history: having been diagnosed with BC in one breast increases the risk of cancer in the other breast or the chance of an additional cancer in the original breast; hormonal changes: women who started their menstrual cycle at younger age (< 12) or menopause (> 55) have a slightly increased risk; breast tissue: women with dense breast tissue have a higher risk of BC. Others are race, exposure to continuous previous chest radiation, use of oral contraceptives and exercise which seems to lower the risk of BC.

Biochemistry of vitamin D

Vitamin D₃ (calcitriol; vit D₃) is synthesized in skin from 7-dehydrocholesterol in reaction catalyzed by ultraviolet light or obtained from diet. Adequate amounts of vit D₃ are produced in skin with casual exposure to sunlight and, therefore, vit D₃ is not a vitamin in the sense of an essential nutritional substance. The major dietary sources of this fat-soluble vitamin are fortified dairy products, margarine, fatty fish and fish liver oils. Vit D₃ itself is biologically inert. It is metabolized in the liver to 25-hydroxyvit D₃ and then in kidney to vit D₃ which is the biologically most active form. The hydroxylation of vit D₃ in liver is catalyzed by 25-hydroxylase. Serum concentration of 25-hydroxyvit D₃ is reflection

of the vit D status of a person and summates the concentrations derived from diet and photoformation. Conversion of 25-hydroxyvit D₃ into active hormone vit D₃ in kidney by α -hydroxylase is under stringent control. Most important stimulatory factors of α -hydroxylase activity are parathyroid hormone and reduced plasma calcium and phosphate levels but α -hydroxylase activity is inhibited by 1, 25-dihydroxyvit D₃ via negative feedback loop. Other factors were implicated in regulation of renal 1, 25-dihydroxyvit D₃ synthesis are estrogen, prolactin, growth hormone and insulin. The half-life of 1, 25-dihydroxyvit D₃ is four to six hours. As a result normal circulating levels of 1, 25-dihydroxyvit D₃ is precisely controlled and fluctuates in response to the mineral needs by a person. Kidney can also convert 25-hydroxyvit D₃ to 24, 25-dihydroxyvit D₃ by enzyme 24-hydroxylase. Under circumstances of relative vit D₃ deprivation the kidney produces only 1, 25-dihydroxyvit D₃. If the supply of vit D₃ is adequate both dihydroxylated metabolites are formed.

Molecular biology of vitamin D

The biological action of 1, 25-dihydroxyvit D₃ is mediated through binding to a specific receptor (vit D receptor). This receptor is an intracellular protein with a molecular weight of 50 - 60 KD which binds to 1, 25-dihydroxyvit D₃ and DNA. Vit D receptor is member of the steroid receptor family which are nuclear transcription factors. Other members of this family are the glucocorticoid, estrogen, retinoic acid, and thyroid hormone receptor. Comparison of the amino acid sequences of vit D receptor and other members of the steroid receptor family reveals similarity in structural and functional

organization. The region that exhibits the greatest degree of amino acid homology among the members of the receptor family is the DNA binding domain (region C). This region is required for DNA sequences recognition and receptor dimerization. The

DNA binding domain consists of about 70 amino acids that fold in two so-called zinc-finger motifs. Each finger binds a zinc ion that is tetrahedrally coordinated by four cysteines.

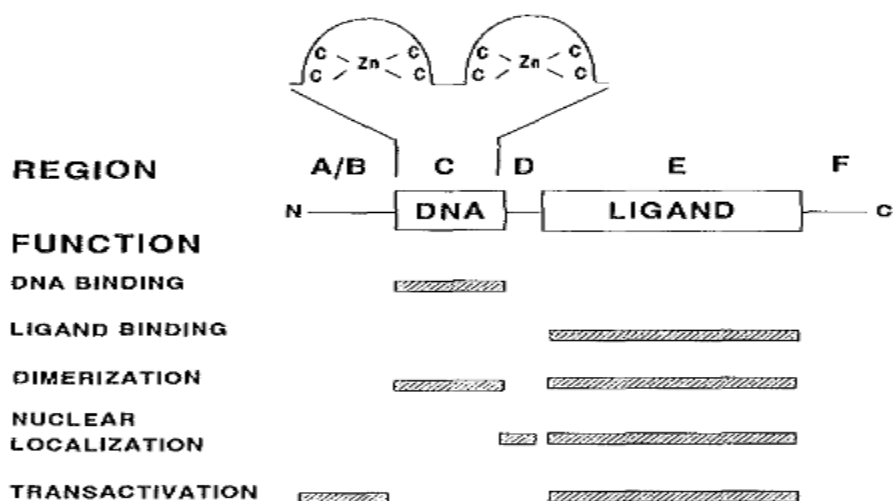


Figure 1: Structural and functional organization of steroid hormone receptors

The DNA binding domain is followed by a hinge region (region D) that links to the hormone binding domain (region E) located at the carboxy terminus of the receptor. The hormone binding domain, that encompasses about 210 amino acids, is moderately conserved and determines the steroid binding specificity of the receptor. It also participates in hormone dependent transcriptional activation and receptor dimerization.

Regional tissue distribution of the vitamin D receptors

Vit D receptor is not confined to tissues that play recognized roles in mineral metabolism, like intestine, kidney, bone and parathyroid gland. Also wide variety of tissues not

primarily related to calcium and bone metabolism were shown to express the vit D receptor. In addition to normal tissues and organs, the vit D receptor was demonstrated in a number of malignant cell types. Given the widespread tissue distribution of the vit D receptor, there are many putative target tissues for 1, 25-dihydroxy vitamin D₃ (5).

Vitamin D receptor and breast cancer

The presence of vit D receptor in BC was first demonstrated in the human BC cell line MCF-7 (6). Later studies have extended finding to other BC cell lines and to surgically obtained normal breast and breast tumors tissue (7). Vit D receptor is present in about 80% of human breast tumor

specimens. The presence of vit D receptor is not correlated to the presence of other steroid hormone receptors (estrogen receptor, progesterone receptor) (8, 9). Also, no relation was observed between vit D receptor status and clinical indices (age, menopausal status, T-stage, histology, lymph node involvement) (10, 11). However, two studies reported that the receptor status correlated positively with disease-free interval (9, 11). Regulation of vit D receptor may affect the cellular responsiveness to 1,25-dihydroxyvit D. In different systems regulation of the vit D receptor by 1, 25-dihydroxyvit D₃ itself (homologous up regulation) and by hormones and growth factors has been demonstrated (12, 13). In BC a homologous up regulation of the vit D receptor has been observed in MCF-7 cells (14) and a heterologous up regulation by serum, growth factors (EGF, insulin, IGF-I) and oestradiol was noticed in MCF-7 and T47-D cells (15, 16).

Tumor suppression by vitamin D₃

Several animal models for BC are being used. Thus, mammary tumors can be induced in rats by oral administration of the carcinogens Nitroso-Methylurea (NMU) or 7, 12-dimethylbenz[a]anthracene (DMBA). NMU- and DMBA-induced tumors form model for hormone-dependent tumors as they contain considerable amounts of estrogen receptors and regress on ovariectomy and antiestrogen treatment (17, 18). Presence of vit D receptor is demonstrated in NMU tumors. Oral or intraperitoneal intake of 1, 25-dihydroxyvit D₃ which is rapidly converted to 1, 25-dihydroxyvit D₃ in liver, resulted in an inhibition of the growth of NMU- (11, 19) and DMBA-induced rat mammary tumors (20, 21).

Vitamin D and breast cancer

Recently, researchers reported that vit D₃ prevents and inhibits the growth of many cancerous cells including BC cells by arresting the cancer cells' replication cycles, promoting apoptosis, and inhibiting invasion, metastasis and angiogenesis (22). They also reported that vit D₃ exerts several powerful anticarcinogenic effects on BC cells and helping to maintain breast epithelial cells in well differentiated state via up-regulation of the glycol-protein e-cadherin. In addition, BC cells have vit D receptors and when these receptors are activated by vit D, it triggers a series of molecular changes that can slow down cell growth, cause cells to die and make the cancer less aggressive. Furthermore, it promotes calcium absorption so it may contribute to its ability to fight cancer, since calcium has been shown to decrease proliferation.

Mechanisms for the antiproliferative action of vitamin D₃ in breast cancer cells

Vitamin D₃ regulated genes: Several vit D regulated genes have thus far been identified in different cell types and tissues from several species. These genes are associated with divergent functions, like mineral homeostasis, the metabolism of vit D, the secretion of peptide hormones, cellular proliferation and differentiation. Despite the extensive number of genes and proteins that appear to be regulated by 1, 25-dihydroxyvit D₃, evidence that the hormone directly modulates gene transcription exists only for some of these genes.

Modulation of oncogene expression:

Oncogenes affect cellular growth and differentiation through their protein products, which belong to various categories of the cell signaling machinery, such as growth factors, growth factor receptors, GTP-binding proteins, and nuclear proteins involved in transcription regulation (23, 24). Oncogene expression at inappropriate location or time period during cell cycle or maturation, over-expression of oncogenes, or mutations in oncogenes cause the transformation of cells in culture and induction of tumors in animals (23, 25). A second class of cancer-related genes, the tumor suppressor genes, normally prevents tumor growth. Mutations or deletions in these genes cause their functional inactivation, which in turn contributes to cellular transformation (26, 27).

A number of oncogenes and tumor suppressor genes were implicated in the development of BC (Table 1). A large number of reports have described amplification and/or over expression of the *erbB-2*, *int-2*, and *myc* oncogenes in a high

percentage of human BC specimens. Deletions or mutations in the DNA of human BC specimens have been associated with the tumor suppressor genes *Rb* and *p53*. Moreover, it was proposed that the activity of vit D_3 compounds play role in the tumor suppressive of onco-genes. The role of vit D_3 investigated in the leukemic cell line HL60. In these cells, 1, 25-dihydroxyvit D_3 induced a progressive down-regulation of *myc* and a stimulation of *fos* and *frns*, which was initiated after about four hours of incubation and preceded the differentiation into monocyte-like cells and loss of proliferation capacity (28, 29). In addition, 1, 25-dihydroxyvit D_3 modulated the expression of *myc*, *fos*, and *jun* in other cell types than HL60 cells (30, 31). However, so far very little data are available on the regulation of oncogenes by 1, 25-dihydroxyvit D_3 in BC cells. Thus, a study of Mathiasen et al. (32) describes decreased *myc* expression after three hours, and a transient induction of *fos* expression with a maximum after one hour, and up regulation of *p53* and *Rb* genes of 1, 25-dihydroxyvit D_3 and EB1089 treatment of MCF-7 cells.

Table1: Oncogenes and tumor suppressor genes associated with breast cancer

Oncogenes	Description of gene product
<i>erbB-2</i> or <i>HER-2</i>	related to the epidermal growth factor receptor
<i>int-2</i>	related to the fibroblast growth factor family
<i>Myc</i>	nuclear protein
<i>Ha-ras</i>	G-protein
Tumor suppressor genes	
<i>Rb</i> gene	nuclear phosphoprotein with DNA binding affinity
<i>p53</i> gene	nuclear phosphoprotein

Interaction with steroid hormones and polypeptide growth factors: BC cell growth is regulated by steroid hormones, acting via nuclear steroid hormone receptors, and polypeptide growth factors, acting via membrane receptors. Steroid hormones (estrogens, progestin) may act directly on the tumour cell to stimulate growth, or indirectly via regulation of growth factor production and growth factor receptors. BC cells secrete a number of growth factors including TGF α , TGF β , IGF-I, IGF-II, and bFGF, which may act by autocrine loops, when the cells possess the adequate receptors. Growth factors are derived from the circulation or are produced by stromal cells in the tumor and act by paracrine loops on BC cells (28, 33). 1, 25-Dihydroxyvit D₃ may affect BC cell growth by affecting these complex growth regulatory systems of steroid hormones and growth factors. 1, 25-Dihydroxyvit D₃ may inhibit secretion of stimulatory growth factors or stimulate the secretion of negative growth factors. More, 1, 25-dihydroxyvit D₃ regulate the number of growth factor or steroid receptors. Also, 1, 25-dihydroxyvit D₃ may interfere with the action of steroid hormones on nuclear level, or with the intracellular signaling pathways of membrane bound growth factors. Previously, Dernirpence et al. (34) and James and others (35) pointed to an interaction of 1,25-dihydroxyvit D₃ with oestradiol. In fact, both reports indicated that 1, 25-dihydroxyvit D₃ and, the vit D₃ analogue EB1089, suppressed the mitogenic effect of oestradiol in MCF-7 cells. It has also been shown that estrogen receptor concentration is decreased and estrogen-induced gene transcription is inhibited by the treatment with 1, 25-dihydroxyvit D₃. Vit D₃ inhibits expression of aromatase enzyme through inhibiting production of the enzyme COX-2. Expression of aromatase required for

synthesis of estrogen and therefore may play role in the prevention by vit D of estrogen receptor (ER) positive BC. Upregulation of aromatase expression in breast fibroblast increases the tissue concentration of estradiol (E₂), which then activates large number of carcinogenic genes via the estrogen receptor- α in malignant epithelial cells. Aromatase converts androgenic precursors to estrogen both in the cancerous breast epithelial cells and in (BAF) in the stroma surrounding the tumor by direct transcriptional repression of aromatase promoter II. Calcitriol lowers PG synthesis (major stimulator of aromatase transcription via promoter II) by suppressing the expression of COX-2 and increase of 15-hydroxy prostaglandin dehydrogenase that catalyze PG degradation (22, 36). There are some indications that 1, 25-dihydroxyvit D₃ can interfere with paracrine or autocrine acting growth factors in BC. A possible interaction with paracrine loop was suggested by Saez and others (21), who have observed that MCF-7 and BT-20 cells were growth stimulated by co-culture with fibroblasts, whereas this process was reversed by 1, 25-dihydroxyvit D₃ treatment. It has also been shown that the number of EGF binding sites was decreased by 1, 25-dihydroxyvit D₃ treatment in some BC cell lines (MCF-7, T47-0) but increased in other cell lines (MOA-MB-231, BT-20) (21, 37). As 1,25-dihydroxyvit D₃ reduced the growth of all these cell lines, the relation between EGF receptor regulation and growth inhibition by 1,25-dihydroxyvit D₃ is not clear yet. TGF- β is a negative growth factor that is produced by BC cell lines (38, 39). One report described stimulation of TGF- β secretion and TGF- β mRNA expression by treatment with 1, 25-dihydroxyvit D₃ and vit D₃ analogues in BT-20 BC cells (40).

Calcitriol acts through both genomic and non-genomic mechanisms: In genomic pathways, calcitriol binds to intracellular vit D receptor (VDR) which subsequently heterodimerizes with another nuclear receptor retinoid X receptor (RXR). The heterodimer binds to vit D response element in target genes and leads to gene transcription regulation. In addition, calcitriol has rapid effects that are independent of gene transcription regulation, which are defined as non-genomic effects and not mediated directly through steroid receptor-ligand-DNA interaction. However, non-genomic actions may indirectly affect gene transcription via the regulation of intracellular signaling pathways that target transcription factors. Calcitriol induces a number of non-genomic responses including rapid intestinal absorption of calcium, release of calcium from intracellular stores, opening of voltage-gated calcium and chloride channels and, the activation of protein kinase C, protein kinase A, phosphatidylinositol-3 kinase (PI3K) and phospholipase C.

Vitamin D₃ in relation to angiogenesis, invasion, and metastasis: Vit D₃ plays a role in inhibiting the processes of angiogenesis, invasion and metastasis of BC. Angiogenesis is an essential requirement for the growth of solid tumors. Inhibition of angiogenesis may contribute to tumor suppressive activity of vit D compounds. To achieve tumor suppression, high doses of 1, 25-dihydroxyvit D₃ or 1 α -hydroxyvit D₃ are needed to use. These high doses, about 0.5 gm per kg, resulted in the development of hypercalcaemia and subsequent weight loss. To overcome this problem, synthetic, vit D₃

analogues with low calcaemic activity were developed. However, only few analogues have yet been evaluated for their potential use in the treatment of BC.

Combination therapies with vitamin D₃: The present data obtained with 1, 25-dihydroxyvit D₃ and synthetic vit D₃ analogues offer promise for the use of vit D in endocrine treatment of estrogen receptor-positive and negative BC. Single agent treatment with a low calcaemic vit D₃ analogue could provide a new endocrine therapy, however, a combination therapy with established endocrine or cytotoxic agents may offer additional advantages, e.g. better response rates, lower dosages needed and thereby reducing the risk of negative side-effects. Several *in vitro* and *in vivo* studies have focused on possible future combination therapies with vit D₃ compounds. 1 α , 25-dihydroxychole-calciferol (calcitriol) has also broad spectrum anti-tumor activities as supported by numerous epidemiological and experimental studies. Calcitriol potentiates the anti-tumor activities of multiple chemotherapeutics agents including DNA-damaging agents cisplatin, carboplatin and doxorubicin; antimetabolites 5-fluorouracil, hydroxyurea, cytarabine and gemcitabine; and microtubule-disturbing agents paclitaxel and docetaxel. Calcitriol elicits anti-tumor effects mainly through induction of cancer cell apoptosis, cell cycle arrest, differentiation, angiogenesis and the inhibition of cell invasiveness by a number of mechanisms. Calcitriol enhances the cytotoxic effects of gamma irradiation and certain antioxidants and naturally derived agents (41).

Table 2: Examples of vitamin D₃ analogue

Vitamin D3 analogue	Breast cancer cell line	Estrogen receptor status	Relative potency compared to 1,25-(OH) D3	Ref.
Oct	MCF-7 ZR-7S-1 T47-0 MOA-MB-231 BT-20	ER-pos. ER-pos. ER-pos. ER-neg. ER-neg.	approx. 10	42
EBI089	MCF-7	ER-pos.	50	32
KHI060	MCF-7	ER-pos.	800	43
MC903	T47-0	ER-pos.	1	19
Ro24-S531	MCF-7 T47-0	ER-pos. ER-pos.	10-100	44
MART-10	MCF-7	ER-POS	500-1000	45

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