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. 1α, 25 dihydroxyvitamin D3 [1, 25(OH) 2D3], 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, angio-sarcoma 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 D_3 (calcitriol; vit D_3) is synthesized in skin from 7-dehydrocholest-erol in reaction catalyzed by ultraviolet light or obtained from diet. Adequate amounts of vit D_3 are produced in skin with casual exposure to sunlight and, therefore, vit D_3 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 D_3 itself is biologically inert. It is metabolized in the liver to 25-hydroxylvit D_3 and then in kidney to vit D_3 which is the biologically most active form. The hydroxylation of vit D_3 in liver is catalyzed by 25-hydroxylase. Serum concentration of 25-hydroxyvit D_3 is reflection of the vit D status of a person and summates the concentrations derived from diet and photoformation. Conversion of 25-hydroxyvit D_3 into active hormone vit D_3 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 D_3 via negative feedback loop. Other factors were implicated in regulation of renal 1, 25-dihydroxyvit D_3 synthesis are estrogen, prolactin, growth hormone and insulin. The half-life of 1, 25-dihydroxyvit D_3 is four to six hours. As a result normal circulating levels of 1, 25-dihydroxyvit D_3 is precisely controlled and fluctuates in response to the mineral needs by a person. Kidney can also convert 25-hydroxyvit D_3 to 24, 25-dihydroxyvit D_3 by enzyme 24-hydroxylase. Under circumstances of relative vit D_3 deprivation the kidney produces only 1, 25-dihydroxyvit D_3. If the supply of vit D_3 is adequate both dihydroxylated metabolites are formed.

Molecular biology of vitamin D

The biological action of 1, 25-dihydroxyvit D_3 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 D_3 and DNA. Vit D receptor is member of the steroid receptor family which are nuclear transcription factors. Other members of this family are the glucocor-ticoid, 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 cystines.

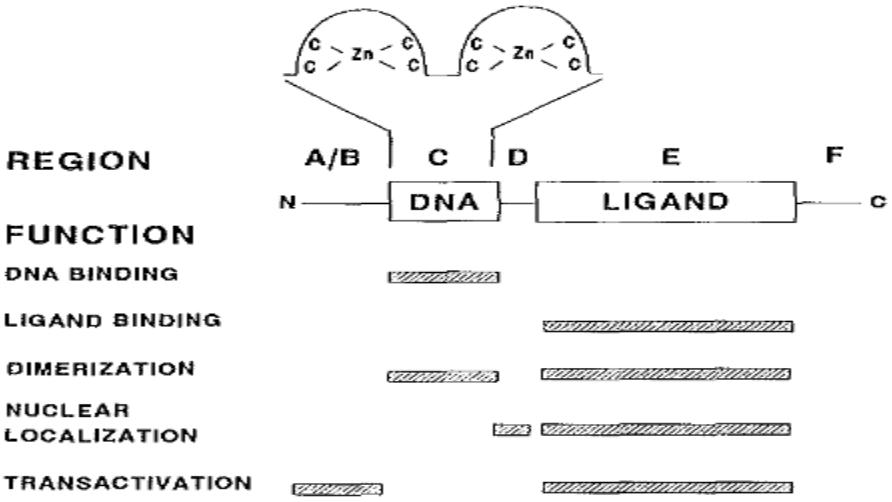

Figure 1: Structural and functional organization of steroid hormone receptors

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 specify 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 D_3 (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-dihyd-roxyvit D. In different systems regul-ation of the vit D receptor by 1, 25- dihydroxyvit D_3 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 D_3

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 ovarie-ctomy and antiestrogen treatment (17, 18). Presence of vit D receptor is demonstrated in NMU tumors. Oral or intraperitoneal intake of 1, 25-dihydroxyvit D_3 which is rapidly converted to 1, 25-dihydroxyvit D_3 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 D_3 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 D_3 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 it is ability to fight cancer, since calcium has been shown to decrease proliferation.

Mechanisms for the antiproliferative action of vitamin D_3 in breast cancer cells

Vitamin D_3 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 diver-gent functions, like mineral homeostasis, the metabolism of vit D, the secretion of peptide hormones, cell-ular proliferation and differen-tiation. Despite the extensive number of genes and proteins that appear to be regulated by 1, 25-dihydroxyvit D_3, 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 transfor-mation of cells in culture and induction of tumors in animals (23, 25). A second class of cancer-related genes, the tumor suppr-essor 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-dihyd-roxyvit D_3 induced a progressive down-regula-tion 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
erbB-2 or HER·2	related to the epidermal growth factor receptor
int-2	related to the fibroblast growth factor family
Myc	nuclear protein
Ha·ras	G-protein
Tumor suppressor genes	
Rb gene	nuclear phosphoprotein with DNA binding affinity
p53 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 TGFCI', TGF13, IGF-I, IGF-II, and PBGF, 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). l, 25-Dihydroxyvit D3 may affect BC cell growth by affecting these complex growth regulatory systems of steroid hormones and growth factors. 1, 25-Dihydroxyvit D_3 may inhibiting secretion of stimul-atory growth factors or stimulate the secretion of negative growth factors. More, 1, 25-dihydroxyvit D_3 regulate the number of growth factor or steroid receptors. Also, 1, 25-dihydroxyvit D_3 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 D_3 with oestradiol. In fact, both reports indicated that 1, 25-dihydroxyvit D_3 and, the vit D_3 analogue EB1089, suppressed the mitogenic effect of oestradiol in MCF-7 cells. It has also been shown that estrogen receptor concen-tration is decreased and estrogen-induced gene transcription is inhibited by the treatment with 1, 25-dihydroxyvit D_3. Vit D_3 inhibits expression of aroma-tase 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-alpha in malignant epithelial cells. Aromatase converts andro-genic 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. Calcitirol 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 indica-tions that 1, 25-dihydroxyvit D_3 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 D_3 treatment. It has also been shown that the number of EGF binding sites was decreased by 1, 25-dihydroxyvit D_3 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 D_3 reduced the growth of all these cell lines, the relation between EGF receptor regulation and growth inhibition by 1,25-dihydroxyvit D_3 is not clear yet. TGF-B is a negative growth factor that is prod-uced by BC cell lines (38, 39). One report described stimulation of TGF-B secretion and TGF-B mRNA expr-ession by treatment with 1, 25-dihy-droxyvit D_3 and vit D_3 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 D_3 in relation to angiogenesis, invasion, and metastasis: Vit D_3 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-dihydroxy-vit D_3 or 1α-hydroxyvit D_3 are needed to use. These high doses, about 0.5 gm per kg, resulted in the development of hyper calcaemia and subsequent weight loss. To overcome this problem, synthetic, vit D_3 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 D_3: The present data obtained with l, 25-dihydroxyvit D_3 and synthetic vit D_3 analogues offer promise for the use of vit D in endocrine treatment of estrogen receptor-positive and nega-tive BC. Single agent treatment with a low calcaemic vit D_3 analogue could provide a new endocrine therapy, however, a combination therapy with established endocrine or cytotoxic agents may offer additional advent-ages, 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 D_3 compounds. 1α, 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 chemo-therapeutics 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 D_3 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

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin. 2011, 61; 2: 69-134.
2. National Cancer Institute. Breast cancer screening. cancer.gov/cancertopics/pdq/ screening/ breast/healthprofessional (Accessed on April 16, 2012).
3. Carlson RW, Allred DC, Anderson BO, Burstein HJ, Carter WB, Edge SB, et al. Breast cancer. Clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2009, 7; 2: 122-192.
4. Sariego J. Breast cancer in the young patient. Am Surgeon. 2010, 76; 12: 1397-1401.
5. Walters NR. Newly identified actions of the vitamin D endocrine system. Endocrin Revi. 1992, 13: 719-764.
6. Eisman JA, Marlin TJ, Maclntyre I and Moseley JM. 1, 25-dihydroxyvitamin D receptor in breast cancer cells. Lancet. 1979, 2: 1335-1336.
7. Eisman JA. 1, 25-Dihydroxyvitamin D3 receptor and role of 1, 25-(OH)2 D3 in human cancer cells. In: Kumar R, ed., vitamin D metabolism: basic and clinical aspects, pp. 365-382. Martinus Nijhoff, the Hague, 1984.
8. Eisman JA, Suva LJ, Sher E, Pearce PJ, Funder JW and Martin TJ. Frequency of 1, 25-dihydroxyvitamin D3 receptor in human breast cancer. Cancer Res. 1981, 41: 5121-5124.
9. Berger U, McClelland RA, Wilson P, Greene GL, Haussler MR, Pike JW, Colston K, Easton D and Coombes RC. Immunocytochemical determination of estrogen receptor, progesterone receptor, and 1,25·dihydroxyvitamin D3 receptor in breast cancer and relationship to prognosis. Cancer Res. 1991, 51: 239-244.
10. Freake HC, Abeyasekera G, Iwasaki J, Marcoccl C, Maclntyre I, McClelland RA, Skilton, RA, Easton DF and Coombes RC. Measurement of 1, 25-dihydroxyvitamin D3 receptors in breast cancer and their relationship to biochemical and clinical indices. Cancer Res. 1984, 44: 1677-1681.

11. Colston KW, Berger U and Coombes RC. Possible role for vitamin D in controlling breast cancer cell proliferation. Lancet. 1989, 1: 188-191.
12. Pike JW. Vitamin D3 receptors: structure and function in transcription. Aunn Rev Nutr. 1991, 11: 189-216.
13. Darwish H and DeLuca HF. Vitamin D-regulated gene expression. Critical Rev. Eukaryotic Gene Expression. 1993, 3: 89-116.
14. Costa EM, Hirst MA and Feldman D. Regulation of l, 25-dihydroxyvitamine D3 receptors by vitamin D analogues in cultured mammalian cells. Endocrinol. 1985, 117: 2203-2210.
15. Krishnan AV and Feldman D. Stimulation of 1, 25-dihydroxyvitamin D3 receptor gene expression in cultured cells by serum and growth factors. J Bone Mineral Res. 1991, 6: 1099-1107.
16. Escaleira MTF, Sonohara S and Brentanl MM. Sex steroids induced up-regulation of 1, 25-(OH) 2 vitamin D3 receptors in T 47D breast cancer cells. J Steroid Biochem Molec Biol. 1993, 45: 257-263.
17. Furr BJA and Nicholson RI. Use of analogues of luteinizing-releasing hormone for treatment of cancer. J Reprod Fert. 1982, 64: 529-539.
18. Wilkinson JR, Williams JC, Singh D, Goss PE, Easton D and Coombes RC. Response of nitrosomethylurea-induced rat mammary tumors to endrocrine therapy and comparison with clinical response. Cancer Res. 1986, 46: 4862-4865.
19. Colston KW, Chander SK, Mackay AG and Coombes RC. Effects of synthetic vitamin D analogues on breast cancer cell proliferation in vivo and in vitro. Biochem Pharmacol. 1992, 44: 693-702.
20. Iino Y, Yoshida M, Sugamata N, Maemura H, Ohwada S, Yokoe T, Ishlkita T, Horiuchi R and Morishita Y. 1α-Hydroxyvitamin D3, hypercalcemia, and growth suppression of 7, 12- dimethylbenz[a] anthracene-induced rat mammary tumors. Breast Cancer Res Treat. 1992, 22: 133-140.
21. Saez S, Falette N, Gulllot C, Meggouh F, Lefebvre MF and Crepin M. 1, 25(OH) 2D3 Modulation of mammary tumor cell growth in vitro and in vivo. Breast Cancer Res Treat. 1993, 27: 69-81.
22. Steroids. 2012 Sep; 77(11):1107-12. doi: 10.1016/ j.steroids .2012.06.005. Epub 2012 Jul 16
23. Druker BJ, Mamon HJ and Roberts TM. Oncogenes, growth factors and signal transduction. N Engl J Med. 1989, 321: 1383-1391.
24. Studzinski GP. Oncogenes, growth, and the cell cycle: an overview. Cell Tissue Kin. 1989, 22: 405-424.
25. Travali S, Koniecki J, Petralia S and Baserga R. Oncogenes in growth and development. FASEB J. 1990, 4: 3209-3214.
26. Weinberg RA. Tumor suppressor genes. Science. 1991, 54: 1138-1145.
27. Hollingsworth RE and Lee WH. Tumor suppressor genes: new prospects for cancer research. J Natl Cancer Inst. 1991, 83: 91-96.
28. Reitsma PH, Rothberg PG, Astrin SM, Trial J, Bar-Shavit Z, Hall A, Teltelbaum SL and Kahn AJ. Regulation of rnyc gene expression in HL-60 leukaemia cells by a vitamin D metabolite. Nature. 1983, 306: 492-494.
29. Brelvi ZS, Christakos S and Studzinski GP. Expression of monocyte-specific oncogenes c-fos and c- fms in HL60 cells treated with vitamin D3 analogues correlates with inhibition of DNA synthesis and reduced calmodulin concentration. Lab Invest. 1986, 55: 269-275.
30. Kremer R, Bolivar I, Goltzman D and Hendy GN. Influence of calcium and 1,25-dihydroxycholecalciferol on proliferation and proto-oncogene expression in primary cultures of bovine parathyroid cells. Endocrinol. 1989, 125: 935-941.

31. Tu-Yu AH, Morris RC and Ives HE. Differential modulation of fos and jun gene expression by l, 25-dihydroxyvitamin D3. Biochem Biophys Res Commun. 1993, 193: 161-166.
32. Mathiasen IS, Colston KW and Binderup L. EBI089, a novel vitamin D analogue, has strong antiproliferative and differentiation inducing effects on cancer cells. J Steroid Biochem Molec Biol. 1993, 46: 365-371.
33. Frelss G, Prebols C and Vignon F. Control of breast cancer cell growth by steroids and growth factors: interactions and mechanisms. Breast Cancer Res Treat. 1993, 27: 57-68.
34. Demirpence E, Balaguer P, Trousse F, Nicolas JC, Pons M and Gagne D. Antiestrogenic effects of all-trans-retinoic acid and 1,25-dihydroxy vitamin D3 in breast cancer cells occur at the estrogen response element level but through different molecular mechanisms. Cancer Res. 1994, 54: 1458-1464.
35. James SY Mackay AG and Colston KW. Effects of a new synthetic vitamin D analogue, EBI089, on the oestrogen-responsive growth of human breast cancer cells. J. Endocrinol. 1994, 141: 555-556.
36. J Steroid Biochem Mol Biol. 2010, 121; 1-2: 343-8. doi: 10.1016/ j.jsbmb. 2010.02.009. Epub 2010 Feb 13.
37. Falette N, Frappart L, Lefebvre MF and Saez S. Increased epidermal growth factor receptor level in breast cancer cells treated by 1, 25-dihydroxyvitamin D3. Mol Cell Endocrinol. 1989, 63: 189-198.
38. Knabbe C, Lippman ME, Wakefield LM, Flanders KC, Kasid A, Derynck R and Dickson RB. Evidence that transforming growth factor β is a hormonally regulated negative growth factor in human breast cancer cells. Cell. 1987, 48: 417-428.
39. Arrick BA, Kore M and Derynck R. Differential regulation of expression of three transforming growth factor β species in human breast cancer cell lines by estradiol. Cancer Res. 1990, 50; 299-303.
40. Koli K and Keski-Oja J. 1,25-Dihydroxyvitamin D3 enhances the expression of transforming growth factor-β1 and its latent form binding protein in cultured breast carcinoma cells. Cancer Res. 1995, 55: 1540-1546.
41. Ma Y, Trump DL and Johnson. CSJ Cancer. 2010, 15; 1: 101-107.
42. Abe J, Nakano T, Nishii Y, Matsumoto T, Ogata E and Ikeda K. A novel vitamin D3 analog, 22-oxa-l, 25-dihydroxyvitamin D3, inhibits the growth of human breast cancer in vitro and in vivo without causing hypercalcemia. Endocrinol. 1991, 129: 832-837.
43. Binderup L, Latini S, Binderup E, Bretting C, Calverley M and Hansen K. 20-Epi-vitamin D3 analogues: a novel class of potent regulators of cell growth and immune responses. Biochem Pharmacol. 1991, 42: 1569-1575.
44. Anzano MA, Smith JM, Uskokovic MR, Peer CW, Mullen LT, Letterio JJ, Welsh MC, Shrader MW, Logsdon DL, Driver CL, Brown CC, Roberts AB and Sporn MB. 1α, 25-dihydroxy-16-ene-23-yne-26,27-hexafIuoro-cholecalciferol (R024.5531), a new deltanoid (vitamin D analogue) for prevention of breast cancer in the rat. Cancer Res. 1994, 54: 1653-1656.
45. Chiang KC, Yeh CN, Chen SC, Shen SC, Hsu JT, Yeh TS, Pang JH, Su LJ, Takano M, Kittaka A, Juang HH and Chen TC. Evid based complement Alternat. Med. 2012, 2012: 310872.