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Vitamin D and Breast Cancer: Molecular Communications

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Epidemiological studies have demonstrated that vitamin D status is inversely associated with breast cancer incidence, mortality, and recurrences, suggesting vitamin D as a potent agent to reduce the risk of breast cancer. The hormonally active metabolite of vitamin D, 1α,25-dihydroxyvitamin D (1α,25(OH)$_2$D, calcitriol), and its analogs have exerted inhibitory activity of cellular proliferation through arresting cell cycle and inducing apoptosis, and suppressive effects on the invasion, angiogenesis, and metastasis of breast cancer. In the studies of molecular basis of vitamin D activities, many upstream signaling pathways cross-talking with vitamin D signaling have been investigated. 1α,25(OH)$_2$D and its analogs regulates different signaling pathways mediated by transforming growth factor-β superfamily, epidermal growth factor receptors family, estrogen signaling-related molecules, insulin-like growth factor-binding proteins, and protein kinase C. The multipotent activities of vitamin D in signaling modulation may be efficient and effective in suppressing highly heterogeneous breast cancer.

Key words: breast cancer, epideramal growth factor receptor, estrogen, transforming growth factor-β, vitamin D

In addition to the well-known roles of vitamin D (cholecalciferol and ergocalciferol) and its metabolites in the physiological regulation of calcium/phosphate homeostasis and bone mineralization [Brown *et al.*, 1999; Dusso *et al.*, 2005; Holick, 2007], the accumulating epidemiological, preclinical, and clinical studies have demonstrated that vitamin D and/or the metabolites exert anti-tumor activity by inhibiting cell proliferation, inducing apoptosis and cell differentiation, and inhibiting cell invasion, angiogenesis, and metastasis [Garland *et al.*, 2006; Vijayakumar *et al.*, 2006; Deeb *et al.*, 2007]. These studies have suggested that vitamin D may act as a potent chemopreventive agent in several cancer types including breast cancer.

Based on the global cancer statistics (GLOBOCAN 2008), breast cancer is the most frequently occurring cancer (23% of total new cancer cases) and accounts for the highest mortality rate (14% of the total cancer death) in women worldwide [Jemal *et al.*, 2011]. Although the breast cancer incidence in Asia including Korea is relatively lower than those in Western countries, new cases of breast cancer and mortality rate in Asian countries have been gradually rising during the last decade [Jemal *et al.*, 2011]. In addition, human breast tumorigenesis has been shown to be highly heterogeneous, and it appears that there are different dominant pathways playing a critical role during tumor progression [Stingl and Caldas, 2007; Vargo-Gogola and Rosen, 2007]. Therefore, safe and effective dietary agents regulating the key signaling pathways are necessary to prevent breast carcinogenesis. This review focuses on summarizing recent studies of vitamin D on the suppression of breast cancer and molecular mechanisms of action.

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Vitamin D Sources and Metabolism

Vitamin D sources. Significant amounts of vitamin D can be obtained from dietary natural sources (salmon, cod liver oil, egg yolk etc.), fortified foods (milk, juice, cheese, etc.), and dietary supplements (Fig. 1). Vitamin Ds from dietary sources enter the blood stream through the lymphatic system after the incorporation with chylomicrons [Holick, 2007]. Vitamin D is also produced in the skin through the breakdown of 7-dehydrocholesterol into previtamin D by an ultraviolet B from the sun light, and previtamin D is then converted into vitamin D by thermal isomerization [Holick, 2007].

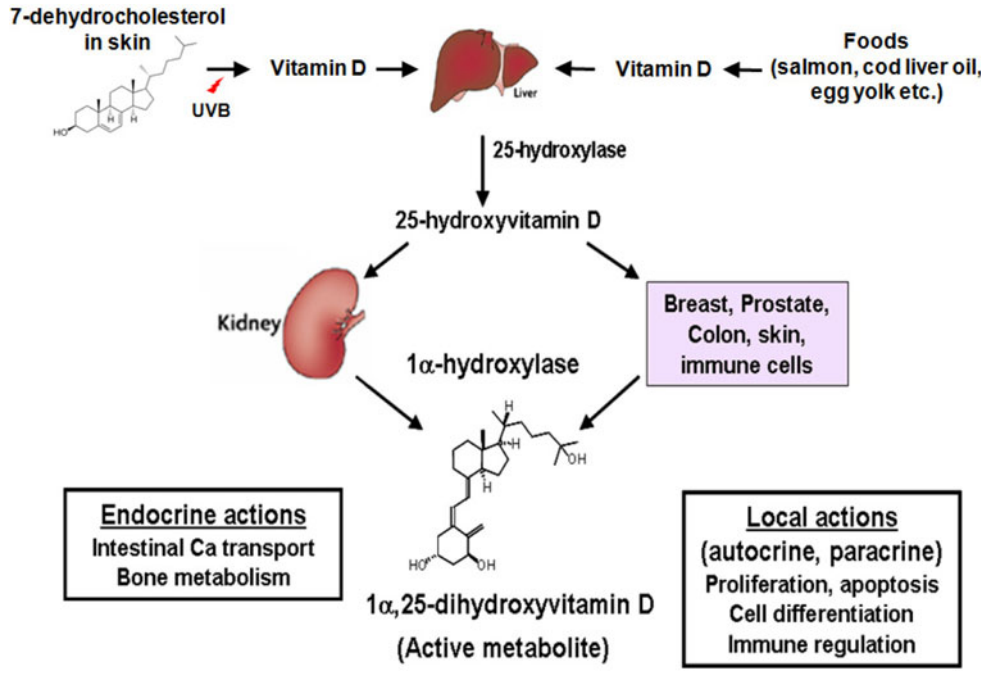

Fig. 1. Metabolism of vitamin D. Vitamin D can be synthesized through convertion of 7-dehydrocholesterol into vitamin D using the UVB energy in the skin and/or obtained from foods. The absorbed vitamin D is hydrolysed into form 25-hydroxyvitamin D by 25-hydroxylase in the liver. 1α,25-dihydroxyvitamin D (1α,25(OH)$_2$D) is produced by 1α-hydroxylase (CYP27B1) in the kidney to play a role in calcium homeostasis and bone metabolism. The local production of 1α,25(OH)$_2$D in extrarenal organs shows functions in cell proliferation, differentiation, and immune regulation.

Vitamin D metabolism. Vitamin D is metabolized into 25-hydroxyvitamin D, the major circulating metabolite, by the hepatic cytochrome P-450 monooxygenases, 25-hydroxylase (CYP27A1) in the liver (Fig. 1) [Prosser and Jones, 2004]. The second step for vitamin D activation is 1α-hydroxylation of 25-hydroxyvitamin D mainly in the kidney [Fraser and Kodicek, 1970]. The mitochondrial cytochrome P-450 enzyme, 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase) encoded by the gene CYP27B1 produces hormonally active vitamin D metabolite, 1α,25-dihydroxyvitamin D (1α,25(OH)$_2$D, calcitriol) from 25-hydroxyvitamin D [Haussler *et al.*, 1998]. Extrarenal organs including colon, breast, prostate, lung, pancreas, monocytes, and skin are also known to express 1α-hydroxylase, which converts 25-hydroxy-vitamin D into 1α,25(OH)$_2$D locally [Zehnder *et al.*, 2001; Hewison *et al.*, 2004]. However, the produced 1α,25(OH)$_2$D in those organs function as tissue-specific autocrine/paracrine factor to mediate local actions of vitamin D in cell proliferation, differentiation, and immune regulation [Dusso *et al.*, 2005; Townsend *et al.*, 2005a; 2005b], suggesting that 1α,25(OH)$_2$D does not enter the circulating track for calcium metabolism [Holick, 2007].

Vitamin D receptor (VDR). The active vitamin D metabolite, 1α,25(OH)$_2$D transduce the cellular signal by binding and activating VDR, a member of the nuclear receptor superfamily for the steroid hormones discovered in 1969 [Haussler and Norman, 1969]. VDR is known to be expressed in more than 30 tissues in human [Reichel *et al.*, 1989], and its crystal structure of the ligand binding domain (LBD) was first reported in 2000 [Rochel *et al.*, 2000]. The regulation of target genes of 1α,25(OH)$_2$D occurs by binding to VDR. The 1α,25(OH)$_2$D-bound VDR heterodimerizes with retinoid X receptor (RXR) and translocates to the nucleus, where 1α,25(OH)$_2$D-VDR-RXR complex specifically interacts with VDR element (VDRE), and modulate the transcriptional activity [Haussler *et al.*, 1998; Dusso *et al.*, 2005]. It has also been reported that non-genomic rapid response is mediated by the vitamin D receptor located in the plasma membrane in caveolae-rich environments, although the mechanism is not yet clearly understood [Norman *et al.*, 2004]. Norman's group have demonstrated that the flexibility of 1α,25(OH)$_2$D in single bonds of carbons 6 and 7 determined genomic or nongenomic rapid responses, in which 6-s-trans configuration of 1α,25(OH)$_2$D binds to the VDR for genomic regulation and 6-s-cis-conformation is used by the VDR for rapid responses [Norman *et al.*, 2004; Norman, 2006]. Although the rapid action of 1α,25(OH)$_2$D works via non-genomic regulation, it may affect the transcription indirectly by secondary messengers

inducing cross-talk with other signaling pathways such as protein kinase C (PKC) pathway [Losel and Wehling, 2003; Deeb *et al.*, 2007]

Vitamin D Status and Breast Cancer

Among vitamin D metabolites, the plasma level of 25-hydroxyvitamin D is used as a common indicator for determining vitamin D status, because 25-hydroxyvitamin D is the major circulating molecule and its serum level is known to be proportional to vitamin D intake [Holick, 1981; 2007]. The serum level of 1α,25$(OH)_2$D, however, is not appropriate for use as a vitamin D status indicator, because the renal production of 1α,25$(OH)_2$D can be elevated by parathyroid hormone (PTH) when vitamin D is deficient and calcium concentration is low [Holick, 2009]. The epidemiological studies demonstrating the association of serum 25-hydroxyvitamin D and breast cancer risk have been performed. In pooled analysis, a higher serum level of 25-hydroxyvitamin D (>52 ng/mL) showed a 50% decrease of breast cancer risk compared to the lower level (<13 ng/mL) [Garland *et al.*, 2007]. Yin *et al.* [2010] also performed a meta-analysis with four nested and five case-control studies and found significant inverse association between serum level of 25-hydroxyvitamin D and breast cancer risk in case-control studies, although this finding remained unconfirmed in nested case-control studies. In postmenopausal breast cancer patients, lower serum level of 25-hydroxyvitamin D was reported to be significantly associated with the increased risk of cancer death and distant recurrence [Vrieling *et al.*, 2011]; however, there was no significant association with breast cancer risk in the mostly premenopausal population in the Nurses' Health Study II [Eliassen *et al.*, 2011], suggesting the estrogen-related signaling could be communicating with vitamin D signaling. Interestingly, in a case-control and a case-series study, Yao *et al.* [2011] compared the serum concentration of 25-hydroxyvitamin D in different breast cancer subtypes and showed that 10 ng/mL increase of serum 25-hydroxyvitamin D correlated with a 64% lower risk of triple negative breast cancer. These accumulating study results suggest that the serum 25-hydroxyvitamin D may be deeply associated with breast carcinogenesis and 25-hydroxyvitamin D can act as a useful indicator for the breast cancer in women.

Anti-tumor Mechanisms of the Active Vitamin D Metabolite (1α,25$(OH)_2$D) on Breast Cancer

Although 1α,25$(OH)_2$D is not commonly considered as vitamin D status indicator [Holick, 2009], Caucasian women having low level of 1α,25$(OH)_2$D were reported to have five times higher risk of breast cancer than frequency-matched control in a clinic-based case-control study [Janowsky *et al.*, 1999], and serum level of 1α,25$(OH)_2$D was also negatively correlated with the progression of breast cancer to bone metastases [Mawer *et al.*, 1997]. Hormonally active vitamin D metabolite, 1α,25$(OH)_2$D binds its cellular receptor VDR, which acts as a transcription factor or cellular signaling molecule, and regulate the expression of the target genes to exert the biological activities [Dusso *et al.*, 2005]. In clinical trial, however, 20-30% of patients who were given a dose of 1.5-2.0 mg/day of 1α,25$(OH)_2$D developed hypercalcemia, a potentially life threatening situation [Osborn *et al.*, 1995]. Therefore, different vitamin D analogs have been synthesized to overcome the hypercalcemic toxicity and to enhance the anti-tumorigenic activity. 1α,25$(OH)_2$D and its analogs have been investigated for their activity in preventing and/or suppressing mammary tumorigenesis.

Inhibition of cell proliferation. 1α,25$(OH)_2$D and its analogs were reported to down-regulate cyclin D1/ cyclin-dependent kinase (CDK)s and up-regulate the CDK inhibitors p21 and p27, which leads to accumulation of the cells in the G1 phase in both estrogen receptor (ER)-positive and -negative breast cancer cells [Wu *et al.*, 1997; Verlinden *et al.*, 1998; 2000; Jensen *et al.*, 2001; Flanagan *et al.*, 2003; Hussain-Hakimjee *et al.*, 2006]. Although the gene *CDKN1A* encoding p21 is known to contain a functional VDRE in the promoter region, the cell cycle perturbation by vitamin D and its analogs can also be derived indirectly from the cross-talk with other signaling pathways including the epidermal growth factor (EGF) [Koga *et al.*, 1988; McGaffin *et al.*, 2004], the transforming growth factor-β (TGF-β) [Wu *et al.*, 1998; Yang *et al.*, 2001; Li *et al.*, 2005], and the mitogen-activated protein kinase (MAPK)-extracellular signal regulated kinase (ERK1/2) or MAPK-p38 signaling pathway [Capiati *et al.*, 2004; Rossi *et al.*, 2004; Li *et al.*, 2005]. A vitamin D analog, 1α(OH)D_5, was also reported to inhibit cell proliferation by inducing cell differentiation via VDR [Lazzaro *et al.*, 2000; Mehta *et al.*, 2000]. In combination studies, 1α,25$(OH)_2$D and its analogs enhanced the sensitivity to the treatments of adriamycin [Sundaram *et al.*, 2000], melatonin [Bizzarri *et al.*, 2003], and radiation [Chaudhry *et al.*, 2001].

Induction of apoptosis. Several studies have demonstrated that 1α,25$(OH)_2$D and its analogs induced apoptosis by regulating the mediators including anti-apoptotic BCL-2 and pro-apoptotic Bax in breast cancer cells. In MCF-7 human breast cancer cells, 1α,25$(OH)_2$D and its analogs have been reported to induce apoptosis by inhibiting BCL-2 expression, translocating Bax, and inducing cytochrome c release via the caspase-independent pathway [James *et*

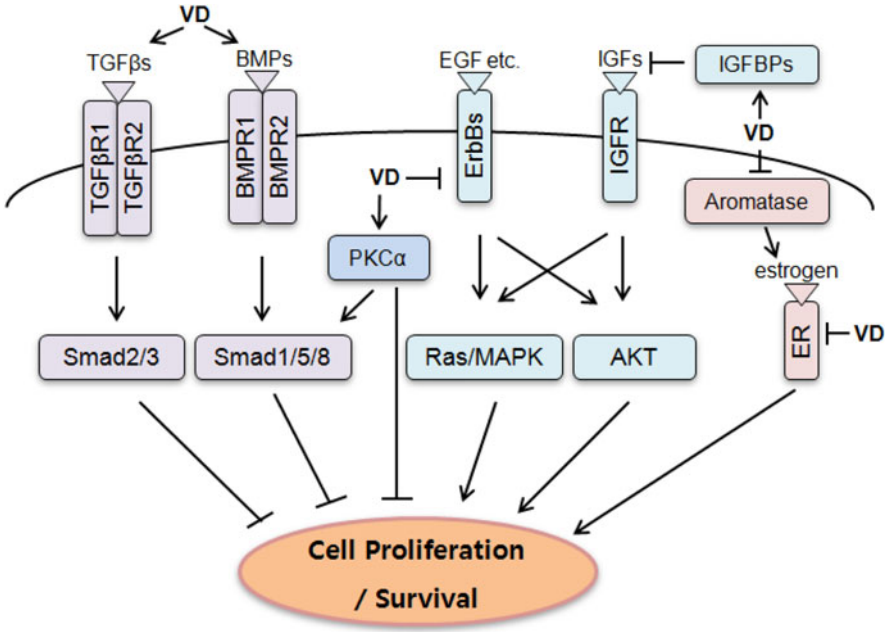

Fig. 2. Signaling pathways communicating with vitamin D signaling in breast cancer. Hormonally active metabolite, 1α,25-dihydroxyvitamin D (1α,25(OH)$_2$D) and its analogs inhibit cellular proliferation and induce apoptosis by regulating molecular targets in different signaling pathways. VD, 1α,25(OH)$_2$D and its analogs; TGFβs, transforming growth factor βs; TGFβR, TGFβ receptor; BMPs, bone morphogenetic proteins; BMPR, BMP receptor; EGF, epidermal growth factor; IGF, insulin-like growth factor; IGFR, IGF receptor; IGFBPs, IGF-binding proteins; ER, estrogen receptor; MAPK, mitogen-activated protein kinase.

al., 1996; Mathiasen *et al.*, 1999; Narvaez and Welsh, 2001]. In addition, mechanistic studies have suggested that 1α,25(OH)$_2$D and its analogs down-regulate ER [Simboli-Campbell *et al.*, 1997], activate PKC [Narvaez *et al.*, 2003], and regulate TGF-β and p38 MAPK signaling to induce apoptosis [Li *et al.*, 2005]. Furthermore, co-treatment of 1α,25(OH)$_2$D or its analogs with other agents, such as tamoxifen [Welsh, 1994], retinoids [James *et al.*, 1995], adriamycin [Sundaram *et al.*, 2000], and radiation [Sundaram and Gewirtz, 1999; Polar *et al.*, 2003] enhanced the induction of apoptosis.

Effects on cell invasion, angiogenesis and metastasis. Studies of the role of 1α,25(OH)$_2$D and its analogs on cell invasion have shown that 1α,25(OH)$_2$D and its analogs inhibit cell invasion by decreasing invasion-related serine protease and metalloproteinase, MMP-9 [Koli and Keski-Oja, 2000; Flanagan *et al.*, 2003; Sundaram *et al.*, 2006]. Moreover, Mantell *et al.* [2000] found that 1α,25(OH)$_2$D inhibited the vascular endothelial growth factor (VEGF)-induced endothelial cell sprouting and elongation *in vitro,* and also demonstrated *in vivo* suppression of vascularization in tumors. In addition, a significant decrease of tumor cell-induced angiogenesis by co-treatment of cells with 1α,25(OH)$_2$D and retinoids was reported [Majewski *et al.*, 1995]. The vitamin D analog EB1089 exerting less calcemic activity was reported to suppress bone metastases by decreasing the number of bone metastases, surface area of osteolytic lesions, and tumor burden per animal after intracardiac injection of MDA-MB-231 human breast cancer cells [El Abdaimi *et al.*, 2000].

Cross-talk of Vitamin D Signaling with Other Pathways

In addition to the gene regulation by vitamin D involved in calcium/phosphate homeostasis, 1α,25(OH)$_2$D and its analogs have been demonstrated to activate or repress the transcription of different genes in normal and tumor tissues [Katayama *et al.*, 2003; Palmer *et al.*, 2003; Guzey *et al.*, 2004; Krishnan *et al.*, 2004; Peehl *et al.*, 2004; Nagpal *et al.*, 2005; Zhang *et al.*, 2005]. However, among those genes regulated by 1α,25(OH)$_2$D and its analogs, especially the genes involved in carcinogenesis such as proto-oncogenes and tumor-suppressing genes, many do not have VDREs in their promoter regions [Deeb *et al.*, 2007]. This suggests that indirect modulation via cross-talk with other signaling pathways may play a role in deriving different cellular responses in cell proliferation, differentiation, and apoptosis (Fig. 2) [Losel and Wehling, 2003; Deeb *et al.*, 2007].

TGF-β superfamily. The TGF-β superfamily, including TGF-βs, activins, and bone morphogenetic proteins (BMPs), are multifunctional cytokines that affect inflammation, immune response, cell proliferation,

differentiation, and apoptosis [Bierie and Moses, 2006]. The nuclear receptor ligands, vitamin D analogs, have been shown to induce the synthesis of ligands and receptors for TGF-βs and BMPs in different types of cells including epithelial and leukemia cells [Hatakeyama *et al.*, 1996; Wu *et al.*, 1998; Jung *et al.*, 1999; Yang *et al.*, 2001; Bizzarri *et al.*, 2003; Li *et al.*, 2005]. In addition, Mehta *et al.* [1997] reported that the vitamin D analog, $1\alpha(OH)D_5$ significantly induced the expression of TGF-β1 and VDR in normal mouse mammary glands. Among the studies with TGF-β/BMP signaling and nuclear receptors, $1\alpha,25(OH)_2D$ has been shown to induce an interaction among Smad3, intracellular mediator transducing TGF-β signaling, and VDR in the nucleus, and potentiate VDR-dependent transcription, suggesting that Smad3 may mediate cross-talk between the vitamin D and TGF-β signaling pathways, acting as a coactivator [Yanagi *et al.*, 1999; Yanagisawa *et al.*, 1999]. $1\alpha,25(OH)_2D$ and novel Gemini vitamin D analogs are also reported to activate the BMP signaling pathway through the enhancement of the production of BMPs and suppression of the inhibitory Smad6 in human mammary epithelial cells [Lee *et al.*, 2006a; 2006b].

Epidermal growth factor receptor (EGFR) family and RAS. The EGFR family is composed of four closely related receptors, including EGFR (ErbB1), HER2/c-neu (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) [Hackel *et al.*, 1999]. The downstream signaling includes the MAPK and AKT pathways, which regulate cell growth, survival, and cell differentiation [Oda *et al.*, 2005]. Several studies have demonstrated that $1\alpha,25(OH)_2D$ and vitamin D analogs suppress cell proliferation by blocking the cell mitogenic signaling at the level of EGFR [Koga *et al.*, 1988; Tong *et al.*, 1999; McGaffin *et al.*, 2004; Deeb *et al.*, 2007]. Treatment of breast cancer cells with $1\alpha,25(OH)_2D$ and vitamin D analogs reduced the specific binding of EGF by decreasing the number of receptors [Koga *et al.*, 1988] and inhibited the mRNA synthesis of EGFR [McGaffin *et al.*, 2004]. In the mouse mammary tumor virus (MMTV)-Her2/neu transgenic mouse model, Gemini vitamin D analog inhibited mammary tumorigenesis by suppressing the phosphorylation of ErbB2 receptor, as well as that of downstream signaling molecules, AKT and ERK [Lee *et al.*, 2010]. RAS/MAPK signaling in breast cancer has drawn less attention due to low percentage (~5%) of *RAS* mutation [von Lintig *et al.*, 2000]. However, Paranjape *et al.* [2011] recently demonstrated that *KRAS* variant increased the risk of triple-negative breast cancer in premenopausal women, and Whyte *et al.* [2009] showed that abnormal activation of RAS/MAPK signaling in breast cancer suggests the involvement of RAS/MAPK signaling during mammary carcinogenesis. The modulation of MAPK including ERK, JNK, and p38 by $1\alpha,25(OH)_2D$ in breast cancer cells has also been reported [Gilad *et al.*, 2005; Cordes *et al.*, 2006; Brosseau *et al.*, 2010].

Estrogen signaling related molecules. Estrogen is the major stimulator of estrogen ER positive breast cancer, and ER antagonists, tamoxifen and raloxifene, are Food and Drug Administration (FDA)-approved agents for breast cancer preventive therapy [Cuzick *et al.*, 2011]. $1\alpha,25(OH)_2D$ has been shown to inhibit estrogen signaling by suppressing the expression of ER-α [Stoica *et al.*, 1999; Swami *et al.*, 2000]. In addition, $1\alpha,25(OH)_2D$ was involved in reducing the expression of aromatase, the enzyme that catalyzes the estrogen synthesis, which results in down-regulating the estrogen signaling [Krishnan *et al.*, 2010a; 2010b; Lundqvist *et al.*, 2011]. More importantly, $1\alpha,25(OH)_2D$ regulates aromatase activity tissue-specifically, which indicates that $1\alpha,25(OH)_2D$ inhibits the aromatase expression in breast cancer cells or the surrounding mammary adipose tissue, whereas enhances in osteoblast and fibroblasts [Enjuanes *et al.*, 2003; Yanase *et al.*, 2003; Krishnan *et al.*, 2010b]. The tissue-specific role of $1\alpha,25(OH)_2D$ in estrogen synthesis is beneficial, because estrogen is necessary for the maintenance of bone mineralization in postmenopausal women [Krishnan *et al.*, 2010b].

Insulin-like growth factor binding proteins (IGFBPs). IGFBPs, a group of six different proteins, sequester free ligands, insulin-like growth factors (IGFs), by binding with high affinity, thereby modulating the mitogenic and prosurvival IGF signaling pathway [Grimberg and Cohen, 2000]. After the studies showing plasma levels of IGF-I were significantly associated with prostate cancer risk [Chan *et al.*, 1998; Peng *et al.*, 2006; Silha *et al.*, 2006; Peng *et al.*, 2007], the roles of IGFBPs, especially IGFBP-3, in cell proliferation and tumor growth have been investigated. In breast cancer, IGFBP-3 has been suggested to acts as a tumor suppressive factor [Pazaitou-Panayiotou *et al.*, 2007; Tomii *et al.*, 2007], although the association of circulating IGFBP-3 with pre-menopausal breast cancer is still unsettled [Renehan *et al.*, 2006; Schernhammer *et al.*, 2006]. The expression of IGFBPs has been shown to be up-regulated by $1\alpha,25(OH)_2D$ and its analogs in different cancer cells including prostate [Boyle *et al.*, 2001; Stewart *et al.*, 2005], colon [Oh *et al.*, 2001; Palmer *et al.*, 2003], and breast [Swami *et al.*, 2003]. Recently, a functional VDRE has been identified in the promoter region of IGFBPs [Peng *et al.*, 2004; Matilainen *et al.*, 2005; Carlberg *et al.*, 2007], suggesting that IGFBPs may be the primary target genes of $1\alpha,25(OH)_2D$ and its analogs. Lee *et al.* [2006a; 2008] also reported that Gemini vitamin D analogs induce the

expression of IGFBP-3 in estrogen receptor (ER)-positive and -negative animal models, as well as MCF10A series of breast epithelial cells.

PKC. The PKC family of serine/threonine kinases regulates cell growth, apoptosis, differentiation, cell migration, and carcinogenesis in different types of cells [Teicher, 2006; Griner and Kazanietz, 2007]. PKCs were originally thought to be pro-mitogenic kinases, but this effect may be PKC isoform-dependent and cell-type-dependent, as many PKCs can also inhibit cell cycle progression [Griner and Kazanietz, 2007]. Among many different PKC isoforms, PKCα is known to inhibit cell proliferation via p21 induction and suppress tumor formation *in vivo* [Detjen *et al.*, 2000; Oster and Leitges, 2006]. PKC has been shown to be regulated by $1\alpha,25(OH)_2D$ and several vitamin D analogs [Buitrago *et al.*, 2003; Boyan *et al.*, 2006a; 2006b]. Boyan *et al.* [2006a; 2006b] suggested that a caveolar environment may play an important role in mediating the PKC activation by $1\alpha,25(OH)_2D$. Furthermore, Lee *et al.* [2007] reported that Gemini vitamin D analog induced VDR dependent-Smad1/5/8 phosphorylation through the activation of PKC α.

Conclusion

Many studies have been performed to demonstrate the role of vitamin D in breast cancer prevention or therapy, and it is clear that vitamin D signaling is deeply involved in human breast carcinogenesis. In the mechanistic studies, hormonally active vitamin D metabolite, $1\alpha,25(OH)_2D$ and its analogs, exerted the ability to regulate the multiple signaling pathways including TGF-β, PKC, IGFBPs, ErbBs, and estrogen signaling in a nuclear VDR-dependent and/or membrane VDR-dependent manner. Because breast cancer is a highly heterogeneous chronic disease, in which significant amounts of genetic and epigenetic changes are induced and multiple signaling pathways are also deregulated [Vargo-Gogola and Rosen, 2007], the multipotent activities of vitamin D could be adequate strategy for effective suppression of breast carcinogenesis. Recently, Perou *et al.* [2000] and Sorlie *et al.* [2001] proposed a novel classification of breast cancer based on distinct profiles of gene expression in human breast tumors, which include luminal A, luminal B, basal-like, Her-2 positive, and normal breast-like subtypes. Therefore, in future studies on the molecular targets of vitamin D *in vitro* or *in vivo* breast cancer model, it will be necessary to define the subtypes of breast cancer models and then identify the subtype-specific targets of vitamin D.

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