ACTA ABP BIOCHIMICA POLONICA

Vol. 68, No 4/2021
489–497
https://doi.org/10.18388/abp.2020_5961

Review

Vitamin D endocrine system in breast cancer*

Kinga Linowiecka[1]✉, Agnieszka Wolnicka-Głubisz[2]✉ and Anna A. Brożyna[1]✉

[1]Department of Human Biology, Institute of Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Toruń, Poland; [2]Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Vitamin D is a steroid hormone of great importance in the human body. It is produced in the skin from 7-dehydrocholesterol, upon UV radiation. In order to exert its functions, vitamin D has to be hydroxylated (*via* CYP27A1 and CYP27B1 hydroxylases), which is followed by its interaction with the vitamin D receptor (VDR) or retinoic acid-related orphan receptors α or γ (RORα and RORγ). By binding with the vitamin D response elements (VDRE) located in the promoter regions, the vitamin D ligand-receptor complex may regulate vitamin D-related genes. Recently, vitamin D has acquired a great interest for its plausible association with cancer development. This review discusses the potential role of vitamin D, its analogues, and enzymes involved in its metabolism with breast cancer incidence and outcome. According to the literature, alterations in the vitamin D endocrine system, both at the mRNA and protein level, have an impact on breast cancer incidence and prognosis. Moreover, specific enzymes participating in vitamin D metabolism may serve as therapeutic targets. Notably, treatment with vitamin D analogues also gives promising results in experimental research. However, given the fact that breast cancer is heterogenous disease, further studies are needed to thoroughly elucidate the potential of vitamin D and enzymes involved in its metabolism in breast cancer development, progression and therapy. Therefore, plausible effects of vitamin D in cancer therapy or prevention have been the principal aim of numerous studies.

Keywords: vitamin D, breast cancer, CYP27A1, CYP27B1, CYP24A1, VDR, RORα, RORγ

Received: 29 September, 2021; revised: 13 October, 2021; accepted: 14 October, 2021; available on-line: 15 November, 2021

✉e-mail: klinowiecka@umk.pl (K.L.); a.wolnicka-glubisz@uj.edu.pl (A.W.-G.); anna.brozyna@umk.pl (A.A.B).

Acknowledgements of Financial Support: This research was funded by National Science Center (grant no. 2018/29/N/NZ3/02514).
*This paper has been published on the occasion of Jubilee Conference entitled "The latest achievements in biochemistry, biophysics and biotechnology – 50 years of history of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University in Kraków" Kraków, September 23–24, 2021.

Abbreviations: 1,25(OH)2D3, cacitriol; 25(OH)D, calcidiol; CYP27A1, 25-hydroxylase; CYP11A1, cholesterol desmolase; CYP27B1, 1α-hydroxylase; CYP24A1, 1,25-dihydroxyvitamin D3 hydroxylase; VDBP, vitamin D binding protein; VDR, vitamin D receptor; VDRE, vitamin D response elements; RORα, retinoic acid-related orphan receptor α; RORγ, retinoic acid-related orphan receptor γ; RXR, retinoid X recepto.r

VITAMIN D METABOLISM

Vitamin D is a precursor of 1,25-dihydroxyvitamin D (calcitriol), a steroid hormone that plays a very important role in the body in maintaining calcium and phosphorus homeostasis. There are two major forms of vitamin D:D_2 (ergocalciferol) and D_3 (cholecalciferol). Vitamin D_2 is mainly produced by plants, and can be delivered to the body with the plant components of meals (for example mushrooms and yeast). In turn, vitamin D_3 (cholecalciferol) is of animal origin. The main source of vitamin D_3 for humans is the synthesis from 7-dehydrocholesterol that occurs in the skin exposed to the sun light, mainly to the UVB light (290–315 nm) (Tripkovic *et al*., 2012; Christakos *et al*., 2016). An additional source of this vitamin is a diet rich in fish oils, eggs or fortified foods, such as breakfast cereals and fruit juices. The two forms, D_2 and D_3, differ primarily in their side chain structure, however, they are converted in the body to the same biologically active compound – calcitriol ($1,25(OH)_2D_3$).

Vitamin D, synthesized under the influence of UVB radiation, is released from epidermal cells into the blood and lymphatic vessels located in the deeper layers of the dermis. This form of vitamin D, similarly to the one taken with food, is bound to the vitamin D binding protein (VDBP) and transported to the liver (Christakos *et al*., 2016). The liver plays a particularly important role in vitamin D first hydroxylation which is carried out by 25-hydroxylase (CYP27A1). This reaction produces calcidiol ($25(OH)D_3$) which is subsequently transported in the bloodstream as a protein-bound VDBP to the kidneys (Christakos *et al*., 2016). A transmembrane protein, megalin, present in the proximal tubules of kidneys, acts as a VDBP receptor, allowing the uptake of ($25(OH)D_3$) in tubular epithelial cells by endocytic internalization (Christakos *et al*., 2016). The second hydroxylation and formation of the active form of vitamin D_3, or calcitriol ($1,25(OH)_2D_3$), is catalyzed by 1α-hydroxylase (CYP27B1) in the kidneys (Holick, 2017).

The biological activity of calcitriol is based on its interaction with the vitamin D receptor (VDR) (Jones, 2013; Holick, 2017). After binding calcitriol, the VDR receptor heterodimerizes with the retinoid X receptor (RXR) and translocates to the nucleus. The resulting VDR-RXR heterodimer acts as a transcription factor - it can bind to a specific DNA sequence present in the promoter regions, referred to as the vitamin D response element (VDRE), which can regulate expression of the target genes (Jones, 2013; Holick, 2017).

It was indicated that only about 15% of 7-dehydrocholesterol transforms into previtamin D_3 in the UV-exposed skin. Each subsequent UV light exposure leads to an equilibrium between previtamin D_3 conversion into its further derivatives: lumisterol3 and tachysterol3, and its transformation back into 7-dehydrocholesterol. Furthermore, if vitamin D_3 produced in the skin is exposed

to UVB radiation, it can be converted into several suprasterols and 5,6-trans-vitamin D_3 as a result of absorption of this radiation. In addition, previtamin D_3 may be also transformed into several toxisterols. Therefore, regardless of individual sun exposure, there is no risk of vitamin D hypervitaminosis or toxicity due to photodegradation of excess previtamin D_3 and vitamin D_3 to products without calcemic activity (Wacker & Holick, 2013).

Concentration of the active form of vitamin D ($1,25(OH)_2D_3$) is tightly regulated by hydroxylation of carbon at position C24, carried out by CYP24A1 (1,25-dihydroxyvitamin D_3 hydroxylase) (Annalora *et al.*, 2010; Wasiewicz *et al.*, 2015). Hydroxylation of calcitriol causes a drastic decrease in its biological activity, and further oxidation by CYP24A1, resulting in urinary excretion of the newly formed metabolite – the calcitroic acid (Prosser & Jones, 2004; Wasiewicz *et al.*, 2015). Alternative pathways of vitamin D metabolism have been also identified. One of them is initiated by the CYP11A1 hydroxylase (cholesterol desmolase), where cholesterol is converted to pregnenolone to initiate steroidogenesis (Slominski *et al.*, 2012b; Slominski *et al.*, 2015). The products of this pathway are many hydroxyl derivatives, including 20-hydroxyvitamin D_3 ($20(OH)D_3$), which are biologically active and may act through VDR and alternative receptors (Slominski *et al.*, 2017a; Slominski *et al.*, 2017c). Therefore, vitamin D may undergo alternative activation pathways in the skin or other organs where CYP11A1 is expressed (Slominski *et al.*, 2017a; Slominski *et al.*, 2017c). The classical and alternative pathways of vitamin D metabolism are presented in Fig. 1.

NOVEL RESEARCH ON VITAMIN D DEFICIENCY IN BREAST CANCER

There are a lot of studies indicating that vitamin D influences inhibition of cell proliferation, invasion, metastasis and angiogenesis, as well as induction of apoptosis and tumor cell differentiation (Chakraborti, 2011). Therefore, various cancers, including breast cancer, have been studied in relation to vitamin D deficiency and cancer risk.

Breast cancer is the most common malignancy among women worldwide. Early stage disease without metastases is curable in ~70–80%, while advanced breast cancer with metastases to distant organs is considered to be terminal since currently available therapies are ineffective for those cases (Harbeck *et al.*, 2019).

Although many studies have been conducted to evaluate the relationship between vitamin D deficiency and breast cancer risk, there is still a controversy in the literature about this association. Some studies have shown that there is no association between breast cancer risk and vitamin D levels (Chlebowski *et al.*, 2008), and others show that breast cancer is associated with low vitamin D levels (Janowsky *et al.*, 1999; Abbas *et al.*, 2008; Yousef *et al.*, 2013; Alco *et al.*, 2014; Clark *et al.*, 2014; Song *et al.*, 2019). Interesting results on the association of vitamin D deficiency and breast cancer come from Pakistan, where low levels of vitamin D are detected especially among

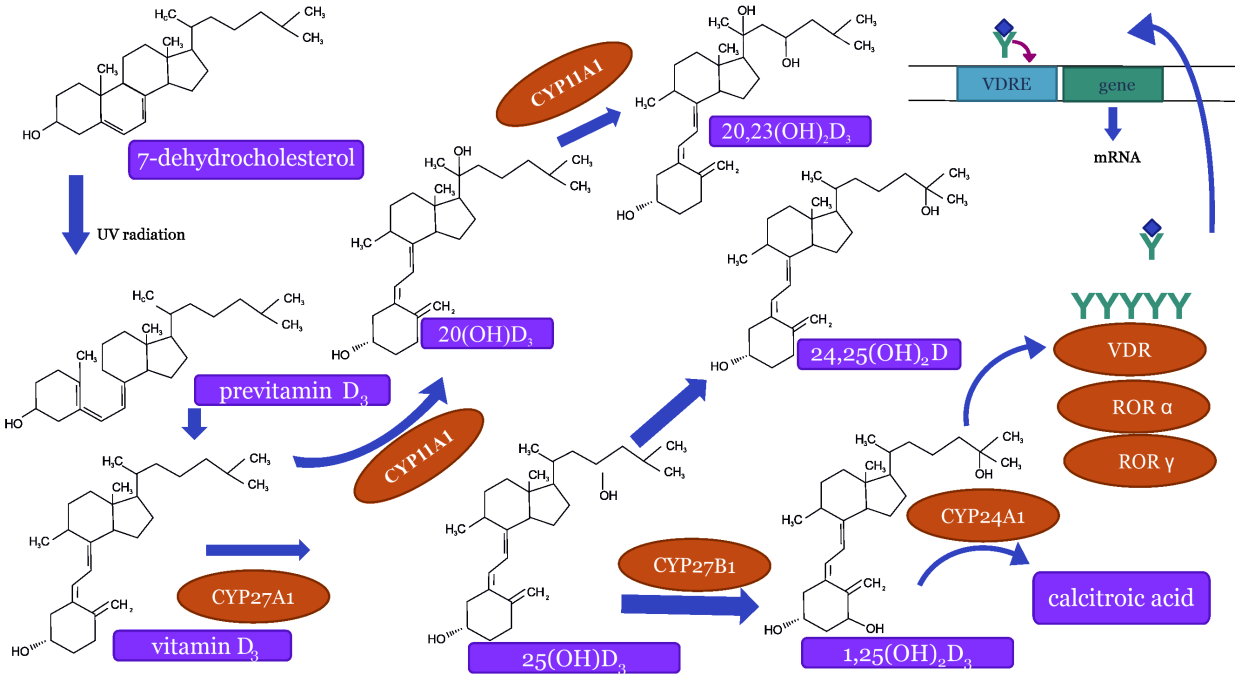

Figure 1. The classical and alternative pathways of vitamin D metabolism.
Abbreviations: CYP27A1, 25-hydroxylase; CYP11A1, cholesterol desmolase; CYP27B1, 1α-hydroxylase; CYP24A1, 1,25-dihydroxyvitamin D3 hydroxylase; VDR, vitamin D receptor; RORα, retinoic acid-related orphan receptor α; RORγ, retinoic acid-related orphan receptor γ; VDRE, vitamin D response elements; $1,25(OH)_2D_3$, cacitriol; 25(OH)D, calcidiol. Under exposure to UV radiation, 7-dehydrocholesterol transforms into previtamin D_3. After several subsequent transformations, vitamin D_3 can be converted into $25(OH)D_3$ or $20(OH)D_3$ forms, in reactions catalyzed by CYP27A1 or CYP11A1, respectively. Further hydroxylation led by CYP11A1 results in formation of the $20,23(OH)_2D_3$ derivative. $25(OH)D_3$ form can be transformed into $24,25(OH)_2D_3$ or it can be hydroxylated by CYP27B1 to active form of vitamin D, $1,25(OH)_2D_3$. It can bind to one of the receptors: VDR, RORα, or RORγ, which is followed by translocation to VDRE in the nucleus, where it can impact vitamin D-related genes. $1,25(OH)_2D_3$ can be also converted by CYP24A1 to calcitroic acid; which can be subsequently excreted.

the female population, due to body covering with clothing and non-exposure of skin to UVB. In a study conducted at Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan, vitamin D deficiency was found in 95.6% of breast cancer patients and 77% in the control group (Shaukat *et al*., 2017).

High plasma concentrations of 25(OH)D may have beneficial effects in prevention of breast cancer, especially in older women. However, the risk of developing this cancer may also be affected by the level of local conversion of 25(OH)D to $1,25(OH)_2D_3$ in the breast tissue, as well as circulating $1,25(OH)_2D_3$ in the serum (Bertone-Johnson *et al*., 2005). It is also believed that vitamin D_3 deficiency is associated with a worse prognosis in patients with breast cancer (Goodwin *et al*., 2009). The observational study evaluating the association between serum 25(OH)D levels and breast cancer risk, involving a group of 1760 individuals, found that serum 25(OH)D levels above 130 nM lead to a 50% reduction in the incidence of this cancer (Garland *et al*., 2007).

ANALYSES OF ENZYMES IMPLICATED IN VITAMIN D METABOLISM IN BREAST CANCER PATIENTS

As was mentioned above, there is an ambiguous relationship between the vitamin D level and breast cancer incidence. The enzymes which take part in vitamin D metabolism, also serve as the crucial components for maintaining vitamin D concentration in the organism. Therefore, it seems plausible that their impaired activity may be related to the breast cancer occurrence.

It has been demonstrated that in the vitamin D metabolism, enzymes belonging to the cytochrome P450 mixed-function oxidases play the major role (Sugimoto & Shiro, 2012). CYP27A1 is a mitochondrial enzyme responsible for vitamin D 25-hydroxylation. It is also involved in bile acid formation (Lorbek *et al*., 2012), since it participates in cholesterol transformation to 27-hydroxycholesterol (Kimbung *et al*., 2017). This specific metabolite serves as a selective estrogen receptor modulator (DuSell *et al*., 2008), and, therefore, all of the studies reviewed so far, did not analyze CYP27A1 in terms of its prospective implication in the vitamin D level in breast cancer patients. Nevertheless, there are several studies which investigated expression level of CYP27A1 in breast cancer patients. Kimbung and others (Kimbung *et al*., 2020) conducted a study relating to the immunohistochemical expression of CYP27A1 in breast cancer tumors. Nearly one third of breast cancer tumors expressed high CYP27A1 level. Moreover, the majority of them were high graded tumors, with larger size and without estrogen or progesterone receptors (Kimbung *et al*., 2020). According to the authors, breast cancer patients with high CYP27A1 displayed poorer overall survival and recurrence-free survival (Kimbung *et al*., 2020). Another study revealed that an increased CYP27A1 level was predominantly detected in HER2 negative (HER2(–)) breast tumors in grade II with high Ki67 and p53 (Le Cornet *et al*., 2020). This points out that high CYP27A1 expression appears to be more frequently detected in more aggressive breast cancer types. Therefore, an important question is whether the pathways underlying upregulation of CYP27A in breast tumors are related to vitamin D metabolism.

The subsequent vitamin D hydroxylation – from 25-hydroxyvitamin D ($25(OH)D_3$) to 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) – is driven by the CYP27B1 enzyme (Bikle, 2014). Since $1,25(OH)_2D_3$ is an active form of vitamin D, CYP27B1 is an evident determinant of maintaining the vitamin D level. Nevertheless, there is no general agreement about the CYP27B1 expression level in breast cancer. A breast cancer study analyzing mRNA expression of *CYP27B1* in 30 patients revealed its downregulation in comparison to normal breast tissue (Zhalehjoo *et al*., 2017). Moreover, this decrease was more profound in breast tumors in stage 2, in contrast to those in stage 1 (Zhalehjoo *et al*., 2017). Similar results were obtained in the study by Segersten and others (Segersten *et al*., 2005), where *CYP27B1* mRNA expression was significantly decreased, though the analysis was performed on only 10 breast tumors. Moreover, an in vitro study revealed that *CYP27B1* is expressed in non-transformed human mammary epithelial cells, however, after induced oncogenic transformation, its expression is significantly reduced (Kemmis & Welsh, 2008). Therefore, it is somewhat surprising that some papers indicated that expression of CYP27B1 is increased in breast tumors (Townsend *et al*., 2005; Friedrich *et al*., 2006), or not-statistically different between breast tumors and normal breast (Lopes *et al*., 2010). This demonstrated inconsistency may be linked to the molecular subtype of breast cancer and, possibly, to its own specific vitamin D metabolism. In line with this assumption, several *in vitro* studies indicated different CYP27B1 expression after exposure of vitamin D analogs in molecularly different breast cancer cell lines (Diesing *et al*., 2006; Richards *et al*., 2015). Furthermore, it cannot be excluded that changes in CYP27B1 expression may be related to CYP27B1 splice variants, since such variants were detected in the breast cancer cell lines (Cordes *et al*., 2007; Fischer *et al*., 2007).

Degradation of vitamin D (both forms, 25(OH)D and $1,25(OH)D_3$) is led by CYP24A1 (Bikle, 2014), and thus its expression is frequently analyzed along with CYP27B1 in order to obtain the complete insight into vitamin D metabolism in an organism. As it was indicated in an *in vitro* study, CYP24A1 suppression may impact growth and tumorigenic potential of breast cancer cells (Osanai & Lee, 2016). In the study by Cai and others (Cai *et al*., 2019) enrolling over 1000 patients from the TCGA-BRCA cohort, low *CYP24A1* mRNA expression was significantly correlated with poor breast cancer prognosis, overall survival and relapse-free survival. Interestingly, decreased CYP24A1 expression was also associated with the molecular subtype of breast cancer and hormonal receptors' status. Also, an in vitro study with breast cancer cells reveled that breast cultures corresponding to different molecular subtypes displayed different *CYP24A1* mRNA expression levels (Alimirah *et al*., 2010). These findings support our previous assumption that the vitamin D metabolism may differ depending on a specific subtype of breast cancer. On the other hand, another study indicated that there is a relationship between inhibition of CYP24A1 and increased anticancer influence of $1,25(OH)_2D_3$ (Sheng *et al*., 2016). This inconsistency may be potentially linked with single nucleotide polymorphisms (SNPs) of *CYP24A1*, which were reported in breast cancer patients (Cao *et al*., 2020).

There has been an increasing amount of literature on deregulation of CYP27B1 and CYP24A1 in breast cancer, suggesting that the interaction of $1,25(OH)_2D_3$ with its specific receptors may be also disturbed in the course of this malignancy. Since the main vitamin D receptor – VDR – was identified in breast epithelial cells (Zinser & Welsh, 2004), and since there are hundreds of vitamin D-related genes (Nurminen *et al*., 2019), alterations in the VDR level may be plausibly associated with

breast tumorigenesis. In fact, findings from previous papers support this hypothesis. Based on analysis of over 700 invasive breast tumors, Huss and others (Huss *et al.*, 2019) indicated that high VDR expression is strongly related to favorable prognosis: smaller size and lower grade of tumor, and a decreased mortality risk. Moreover, high VDR expression was also more frequently detected in tumors with estrogen and progesterone receptor expression (Lopes *et al.*, 2010; Huss *et al.*, 2019), which are found to have better prognosis. Comparing the VDR level among the different types of breast cancer, the highest VDR expression is observed in benign lesions, and decreases with tumor progression (Lopes *et al.*, 2010) and more aggressive phenotype (Al-Azhri *et al.*, 2017). An *in vitro* study by Kemmis and Welsh (Kemmis & Welsh, 2008) also indicated that a provoked malignant transformation of normal breast cells has significantly decreased the *VDR* expression. However, $1,25(OH)_2D_3$ supplementation to normal cells and breast cancer ones evoked *VDR* downregulation only in one healthy and in one tumorigenic cell line, with no effect in the majority of the rest of breast cultures (Beaudin *et al.*, 2015). Based on these findings, it seems that another mechanism may be implicated in regulation of the tumorigenic potential of breast cancer cells. According to Singh and Adams (Singh & Adams, 2017), several miRNAs may regulate the VDR level in breast cancer. Based on a literature review and in silico analysis, the authors proposed three mRNAs: miR-23, miR-124 and miR-125, since they play crucial roles in breast carcinogenesis. However, further work is required to establish their function in terms of the VDR level in breast cancer development.

Although vitamin D mainly interacts with VDR, there is a growing evidence of its possible transport *via* retinoic acid-related orphan receptors α and γ (RORα and RORγ). Vitamin D derivatives $20(OH)D_3$, $20(OH)D_2$ and $20,23(OH)_2D_3$ can interact with RORα and RORγ in an antagonistic or inverse agonistic manner (Slominski *et al.*, 2014c; Slominski *et al.*, 2017c). A considerable amount of literature has been published on the plausible connection between impaired expression of nuclear receptors and breast cancer development (Riggins *et al.*, 2010; Muscat *et al.*, 2013; Doan *et al.*, 2014). Although RORα was also found to be expressed in normal breast (Zhu *et al.*, 2006), both receptors are mainly investigated in breast tumors. Expression of *RORa* is reduced in breast cancer (Zhu *et al.*, 2006; Lu *et al.*, 2007), and there are several studies investigating its role in breast carcinogenesis. According to in vitro research, RORα may impact an increase in aromatase expression in breast cancer cells, thus augmenting their proliferation (Odawara *et al.*, 2009). Given that aromatase can convert androgens to estrogens, this enzyme may play central role in breast cancer development, since estrogens are involved in growth of the breast cancer cells (Saha *et al.*, 2019). The molecular mechanism underlying RORα's impact on inhibition of breast cancer cell proliferation is related to its ability to recruit transcription factors. Both, RORα and RORγ, have an ability to bind corepressors or coactivators in regulatory regions of the transcribed genes, and thus they can influence gene expression (Jetten, 2009). Another in vitro study demonstrated that RORα may bind transcription factor E2F1, which is responsible for cell cycle regulation, and hence for cell proliferation (Xiong & Xu, 2014). Moreover, RORα was also indicated as a potential breast tumor suppressor, as it can regulate the tumor suppressor microenvironmental factor: semaphorin 3F (SEMA3F) in breast cancer cells (Xiong *et al.*, 2012). Expression level of *RORγ* is also reduced in aggressive types of breast cancers, and decreases with histological grade (Muscat *et al.*, 2013; Oh *et al.*, 2016). Moreover, high RORγ is correlated with distance metastasis-free survival and better outcome of breast cancer (Oh *et al.*, 2014). The molecular mechanism associated with RORγ and breast cancer development is plausibly linked with a DNA repair pathway or TGF-β induced epithelial mesenchymal transition (EMT) pathway (Oh *et al.*, 2016), which leads to metastasis (Imamura *et al.*, 2012). The aforementioned findings suggest that RORα and RORγ may be prospective factors in breast cancer therapy.

As was mentioned above, active form of vitamin D can be hydroxylated by CYP11A1, followed by production of approximately 10 vitamin D derivatives, including $20(OH)D_3$ or $20,23(OH)_2D_3$ (Slominski *et al.*, 2014a). However, CYP11A1 is also a crucial enzyme in cholesterol metabolism, thus it can convert cholesterol to pregnenolone which is an initial step in steroid hormones' synthesis (Miller & Bose, 2011). Therefore, CYP11A1 expression in breast cancer is mainly analyzed from that point of view. Nevertheless, several studies reported that genetic polymorphisms of this gene are prospectively related to breast cancer risk (Zheng *et al.*, 2004; Setiawan *et al.*, 2006; Yaspan *et al.*, 2007; Sun *et al.*, 2012). It cannot be excluded that *CYP11A1* gene polymorphisms may be also associated with implications of vitamin D metabolism in breast cancer. However, in order to answer entirely whether CYP11A1 significantly implicates vitamin D metabolism in breast cancer, it is necessary to analyze the vitamin D_3 analogues' level.

POSSIBLE EPIGENETIC IMPACT ON CHANGES IN VITAMIN D METABOLISM OBSERVED IN BREAST CANCER PATIENTS

Epigenetic processes are proven to have an impact on transcription regulation (Weinhold, 2006). Moreover, there is a general agreement that disturbances in epigenetic mechanisms are associated with cancer initiation (Baylin & Jones, 2011). The most fundamental and widely described epigenetic processes are associated with DNA methylation and a variety of histone modifications. DNA hypomethylation occurs in many types of cancer, including breast cancer (Feinberg & Vogelstein, 1983), moreover, changes in DNA methylation are associated with molecular subtypes of breast cancer (Holm *et al.*, 2016), suggesting an important role of impaired DNA methylation in breast carcinogenesis. Additionally, it was proven that alterations in DNA methylation of *BRCA*, *p53* or *ESR1* are involved in breast cancer progression (Karsli-Ceppioglu *et al.*, 2014). Therefore, it seems plausible that genes implicated in vitamin D metabolism may be also epigenetically changed during breast cancer development. In line with this hypothesis, a comprehensive cohort study has been recently published (O'Brien *et al.*, 2018). The authors examined 198 CpG loci in or near vitamin D-related genes in women with diagnosed breast cancer or with breast cancer diagnosed in their sisters. The study indicated a significant correlation between methylation of *RORa* and 25(OH)D level with regard to breast cancer incidence. Furthermore, significant relationship was also noticed for CpG methylation of *CYP24A1*, *CYP27A1* and *VDR* (O'Brien *et al.*, 2018). Similar results were also found in a previous study, which demonstrated that VDR is significantly hypermethylated in breast tumors in comparison to normal mammary glands (Marik *et al.*, 2010). Changes in methylation of vitamin D-related genes were also detected in breast cancer cell lines.

In these studies, *CYP27B1* (Shi *et al*., 2002) and *VDR* (Marik *et al*., 2010) were found to be hypermethylated. Moreover, such changes were reversible upon treatment with 5-aza-2-deoxycytidine (5-aza-dC). Interestingly, supplementation of $1,25(OH)_2D_3$ did not impact the methylation status in breast cancer cells (Marik *et al*., 2010). However, $1,25(OH)_2D_3$ treatment in MDA-MB-231 cells was related to *Cadherin 1* demethylation, and this effect was significantly higher than after treatment with 5-aza-dC (Lopes *et al*., 2012). These findings highlight the unambiguous relationship between DNA methylation and breast cancer in terms of vitamin D metabolism.

It was conclusively demonstrated that vitamin D exerts its effect by binding in its active form to VDR. Additionally, it was indicated that VDR has an ability to form a dimer with RORα which can subsequently bind to the vitamin D response elements (VDRE) in the DNA (Cheskis & Freedman, 1994; Nishikawa *et al*., 1994). This complex impacts transcription through interactions with histone acetyltransferases (HAT), followed by chromatin changes (Campbell *et al*., 2010). An increasing body of evidence reveals that histones' modifications (including methylation and acetylation) are involved in breast cancer metastasis (extensively reviewed in Nandy *et al*., 2020; Zhuang *et al*., 2020). According to Saramäki and others (Saramäki *et al*., 2009) both the histone acetylation and methylation processes are involved in cyclic chromatin looping during regulation of *p21* expression after $1,25(OH)_2D_3$ supplementation to breast cancer cells. It should be also mentioned that the histone deacetylase inhibitors, along with $1,25(OH)_2D_3$, caused significant changes in colony formation and expression of vitamin D-related genes in breast cancer cell lines (Hossain *et al*., 2020). These data demonstrate that the active form of vitamin D may be considered as a potential epigenetic drug.

VITAMIN D AND ITS ANALOGUES AS POTENTIAL THERAPEUTIC DRUGS IN BREAST CANCER

The use of $1,25(OH)_2D_3$ at therapeutic doses is limited due to calcemic effects. Thus, the studies are focused on identification or synthesis of its derivatives showing anticancer properties and reduced calcemic effects. Already almost 30 years ago Colston and others (Colston *et al*., 1992) reported that calcipotriol, a vitamin D analogue, has significantly inhibited proliferation of breast cancer cells *in vitro*, inhibited tumor progression *in vivo* and had 100–200 folds lower hypercalcemic effects. The same group also showed that other vitamin D analogues, EB1089 and CB1093, resulted in inhibition of breast cancer growth (Colston *et al*., 1998; Xie *et al*., 1999). UVB1 and EM1, novel non-hypercalcemic vitamin D analogues, with higher binding affinity to VDR, caused a decrease in viability of cells derived from triple negative breast cancers and organoids in patient-derived xenografts (PDXs) model of breast cancer. The inhibitory effect was stronger than the one observed for calcitriol (Ferronato *et al*., 2021). BXL0124, a vitamin D analog with hypercalcemic toxicity, decreased proliferation of breast cancer cells in an *in vivo* model and inhibited the ductal carcinoma in situ progression to invasive ductal carcinoma (Wahler *et al*., 2014). Recently discovered CYP11A1-dereived hydroxyderivatives of vitamin D_3, such as mono-, dihydroxy- and trihydroxy- forms with or without the hydroxyl group at position C1α, show antiproliferative, pro-differentiation, and anti-inflammatory actions (reviewed in Slominski *et al*., 2017a; Slominski *et al*., 2017b; Slominski *et al*., 2017c; Chaiprasongsuk *et al*., 2019). The anticancer activity of these derivatives is at least as strong as that of $1,25(OH)_2D_3$ or even stronger (Zbytek *et al*., 2008; Janjetovic *et al*., 2009; Janjetovic *et al*., 2010; Li *et al*., 2010; Slominski *et al*., 2011; Slominski *et al*., 2012a; Slominski *et al*., 2013; Slominski *et al*., 2013; Slominski *et al*., 2017c; Tuckey *et al*., 2011; Lu *et al*., 2012; Lin *et al*., 2015; Lin *et al*., 2016a; Lin *et al*., 2016b; Lin *et al*., 2018; Chaiprasongsuk *et al*., 2019), while the calcemic effects are weaker or are not observed (Slominski *et al*., 2010; Slominski *et al*., 2013; Slominski *et al*., 2014a; Slominski *et al*., 2014b; Wang *et al*., 2012). The antitumor effects were observed in different cancers, including non-melanoma skin cancer (Slominski *et al*., 2020), oral squamous cell cancers (Oak *et al*., 2020), melanomas (Wasiewicz *et al*., 2015; Slominski *et al*., 2018) and others. Antiproliferative activity of a non-calcemic vitamin D derivative, $20(OH)D_3$, also displayed inhibitory effects on proliferation of breast cancer cells (Wang *et al*., 2012). In summary, these studies support the hypothesis related to the potential use of these vitamin D analogues as antitumor agents to treat breast cancers.

CLINICAL RESEARCH ON BREAST CANCER AND VITAMIN D

Since experimental studies demonstrated a very promising data, some clinical trials have been established. Currently, 84 clinical trials for breast cancers and vitamin D are registered at clinicaltrials.gov: 16 are recruiting, 5 are active but not recruiting, 8 are terminated, 48 are completed, 2 are withdrawn and for 5 the status is unknown; 13 of these trials are observational and are interventional, 18 of them have the results, but only some of them are published. Some studies showed that vitamin D supplementation did not change the mammographic density, considered as an indicator of breast cancer risk (Brisson *et al*., 2017; Alipour *et al*., 2018; Crew *et al*., 2019). These clinical trials showed that the vitamin D level was not related to the relapse-free survival, breast cancer-specific survival and overall survival (Lohmann *et al*., 2015). As Charehbili and others (Charehbili *et al*., 2016) had shown, the vitamin D serum level decreased during treatment with chemotherapy, but no effects on pathological complete response were found. On the other hand, clinical trials support the importance of vitamin D supplementation in the reduction of angiogenic growth factors, such as vascular endothelial growth factor A, angiopoietin 2 and hypoxia-inducible factor 1 in breast cancer patients (Shahvegharasl *et al*., 2020).

CONCLUSIONS

The currently available data suggest that vitamin D and its related genes may be of clinical significance in breast carcinogenesis. Deregulation of hydroxylases implicated in vitamin D metabolism may abrogate the effect of local $1,25(OH)_2D_3$ production in tumors. Moreover, enzymes involved in vitamin D metabolism in the breast tissue may be important targets for both, prevention and treatment of breast cancer, including epigenetic therapy. Therefore, the plausible effects of vitamin D in cancer therapy or prevention have been the principal aim of numerous studies. However, there is still a need for further studies in this field, especially for analysis of vitamin D-related processes in specific molecular subtypes of breast cancer, as it is possible that different bio-

logical types of breast cancer display a distinct vitamin D metabolism.

REFERENCES

Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, Chang-Claude J (2008) Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer – results of a large case-control study. *Carcinogenesis* **29**: 93–99. https://doi.org/10.1093/carcin/bgm240

Al-Azhri J, Zhang Y, Bshara W, Zirpoli G, McCann SE, Khoury T, Morrison CD, Edge SB, Ambrosone CB, Yao S (2017) Tumor expression of vitamin D receptor and breast cancer histopathological characteristics and prognosis. *Clin Cancer Res* **23**: 97–103. https://doi.org/10.1158/1078-0432.CCR-16-0075

Alco G, Igdem S, Dincer M, Ozmen V, Saglam S, Selamoglu D, Erdogan Z, Ordu C, Pilanci KN, Bozdogan A, Yenice S, Tecimer C, Demir G, Koksal G, Okkan S (2014) Vitamin D levels in patients with breast cancer: importance of dressing style. *Asian Pac J Cancer Prev* **15**: 1357–1362. https://doi.org/10.7314/apjcp.2014.15.3.1357

Alimirah F, Vaishnav A, McCormick M, Echchgadda I, Chatterjee B, Mehta RG, Peng X (2010) Functionality of unliganded VDR in breast cancer cells: repressive action on CYP24 basal transcription. *Mol Cell Biochem* **342**: 143–150. https://doi.org/10.1007/s11010-010-0478-6

Alipour S, Shirzad N, Sepidarkish M, Saberi A, Bayani L, Hosseini L (2018) The effect of vitamin D supplementation on breast density changes: a clinical trial study. *Nutr Cancer* **70**: 425–430. https://doi.org/10.1080/01635581.2018.1446088

Annalora AJ, Goodin DB, Hong W-X, Zhang Q, Johnson EF, Stout CD (2010) Crystal structure of CYP24A1, a mitochondrial cytochrome P450 involved in vitamin D metabolism. *J Mol Biol* **396**: 441–451. https://doi.org/10.1016/j.jmb.2009.11.057

Baylin SB, Jones PA (2011) A decade of exploring the cancer epigenome – biological and translational implications. *Nat Rev Cancer* **11**: 726–734. https://doi.org/10.1038/nrc3130

Beaudin SG, Robilotto S, Welsh J (2015) Comparative regulation of gene expression by 1,25-dihydroxyvitamin D_3 in cells derived from normal mammary tissue and breast cancer. *J Steroid Biochem Mol Biol* **148**: 96–102. https://doi.org/10.1016/j.jsbmb.2014.09.014

Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, Hankinson SE (2005) Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* **14**: 1991–1997. https://doi.org/10.1158/1055-9965.EPI-04-0722

Bikle DD (2014) Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol* **21**: 319–329. https://doi.org/10.1016/j.chembiol.2013.12.016

Brisson J, Bérubé S, Diorio C, Mâsse B, Lemieux J, Duchesne T, Delvin E, Vieth R, Yaffe MJ, Chiquette J (2017) A randomized double-blind placebo-controlled trial of the effect of vitamin D3 supplementation on breast density in premenopausal women. *Cancer Epidemiol Biomarkers Prev* **26**: 1233–1241. https://doi.org/10.1158/1055-9965.EPI-17-0249

Cai H, Jiao Y, Li Y, Yang Z, He M, Liu Y (2019) Low CYP24A1 mRNA expression and its role in prognosis of breast cancer. *Sci Rep* **9**: 13714. https://doi.org/10.1038/s41598-019-50214-z

Campbell FC, Xu H, El-Tanani M, Crowe P, Bingham V (2010) The Yin and Yang of vitamin D receptor (VDR) signaling in neoplastic progression: Operational networks and tissue-specific growth control. *Biochem Pharmacol* **79**: 1–9. https://doi.org/10.1016/j.bcp.2009.09.005

Cao S, Wei F, Zhou J, Zhu Z, Li W, Wu M (2020) The synergistic effect between adult weight changes and CYP24A1 polymorphisms is associated with pre- and postmenopausal breast cancer risk. *Breast Cancer Res Treat* **179**: 499–509. https://doi.org/10.1007/s10549-019-05484-6

Chaiprasongsuk A, Janjetovic Z, Kim T-K, Jarrett SG, D'Orazio JA, Holick MF, Tang EKY, Tuckey RC, Panich U, Li W, Slominski AT (2019) Protective effects of novel derivatives of vitamin D_3 and lumisterol against UVB-induced damage in human keratinocytes involve activation of Nrf2 and p53 defense mechanisms. *Redox Biol* **24**: 101206. https://doi.org/10.1016/j.redox.2019.101206

Chakraborti CK (2011) Vitamin D as a promising anticancer agent. *Indian J Pharmacol* **43**: 113–120. https://doi.org/10.4103/0253-7613.77335

Charehbili A, Hamdy N a. T, Smit VTHBM, Kessels L, van Bochove A, van Laarhoven HW, Putter H, Meershoek-Klein Kranenbarg E, van Leeuwen-Stok AE, van der Hoeven JJM, van de Velde CJH, Nortier JWR, Kroep JR, Dutch Breast Cancer Research Group (BOOG) (2016) Vitamin D (25-0H D3) status and pathological response to neoadjuvant chemotherapy in stage II/III breast cancer: Data from the NEOZOTAC trial (BOOG 10-01). *Breast* **25**: 69–74. https://doi.org/10.1016/j.breast.2015.10.005

Cheskis B, Freedman LP (1994) Ligand modulates the conversion of DNA-bound vitamin D_3 receptor (VDR) homodimers into VDR-retinoid X receptor heterodimers. *Mol Cell Biol* **14**: 3329–3338. https://doi.org/10.1128/mcb.14.5.3329-3338.1994

Chlebowski RT, Johnson KC, Kooperberg C, Pettinger M, Wactawski-Wende J, Rohan T, Rossouw J, Lane D, O'Sullivan MJ, Yasmeen S, Hiatt RA, Shikany JM, Vitolins M, Khandekar J, Hubbell FA (2008) Calcium plus vitamin D supplementation and the risk of breast cancer. *J Natl Cancer Inst* **100**: 1581–1591. https://doi.org/10.1093/jnci/djn360

Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G (2016) Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev* **96**: 365–408. https://doi.org/10.1152/physrev.00014.2015

Clark AS, Chen J, Kapoor S, Friedman C, Mies C, Esserman L, DeMichele A (2014) Pretreatment vitamin D level and response to neoadjuvant chemotherapy in women with breast cancer on the I-SPY trial (CALGB 150007/150015/ACRIN6657). *Cancer Med* **3**: 693–701. https://doi.org/10.1002/cam4.235

Colston KW, Chander SK, Mackay AG, Coombes RC (1992) Effects of synthetic vitamin D analogues on breast cancer cell proliferation *in vivo* and *in vitro*. *Biochem Pharmacol* **44**: 693–702. https://doi.org/10.1016/0006-2952(92)90405-8

Colston KW, Perks CM, Xie SP, Holly JM (1998) Growth inhibition of both MCF-7 and Hs578T human breast cancer cell lines by vitamin D analogues is associated with increased expression of insulin-like growth factor binding protein-3. *J Mol Endocrinol* **20**: 157–162. https://doi.org/10.1677/jme.0.0200157

Cordes T, Diesing D, Becker S, Fischer D, Diedrich K, Friedrich M (2007) Expression of splice variants of 1alpha-hydroxylase in mcf-7 breast cancer cells. *J Steroid Biochem Mol Biol* **103**: 326–329. https://doi.org/10.1016/j.jsbmb.2006.12.034

Crew KD, Anderson GL, Hershman DL, Terry MB, Tehranifar P, Lew DL, Yee M, Brown EA, Kairouz SS, Kuwajerwala N, Bevers T, Doster JE, Zarwan C, Kruper L, Minasian LM, Ford L, Arun B, Neuhouser M, Goodman GE, Brown PH (2019) Randomized double-blind placebo-controlled biomarker modulation study of Vitamin D supplementation in premenopausal women at high risk for breast cancer (SWOG S0812). *Cancer Prev Res* **12**: 481–490. https://doi.org/10.1158/1940-6207.CAPR-18-0444

Diesing D, Cordes T, Fischer D, Diedrich K, Friedrich M (2006) Vitamin D – metabolism in the human breast cancer cell line MCF-7. *Anticancer Res* **26**: 2755–2759

Doan TB, Eriksson NA, Graham D, Funder JW, Simpson ER, Kuczek ES, Clyne C, Leedman PJ, Tilley WD, Fuller PJ, Muscat GEO, Clarke CL (2014) Breast cancer prognosis predicted by nuclear receptor-coregulator networks. *Mol Oncol* **8**: 998–1013. https://doi.org/10.1016/j.molonc.2014.03.017

DuSell CD, Umetani M, Shaul PW, Mangelsdorf DJ, McDonnell DP (2008) 27-Hydroxycholesterol is an endogenous selective estrogen receptor modulator. *Mol Endocrinol* **22**: 65–77. https://doi.org/10.1210/me.2007-0383

Feinberg AP, Vogelstein B (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* **301**: 89–92. https://doi.org/10.1038/301089a0

Ferronato MJ, Nadal Serrano M, Arenas Lahuerta EJ, Bernadó Morales C, Paolillo G, Martinez-Sabadell Aliguer A, Santalla H, Mascaró M, Vitale C, Fall Y, Arribas J, Facchinetti MM, Curino AC (2021) Vitamin D analogues exhibit antineoplastic activity in breast cancer patient-derived xenograft cells. *J Steroid Biochem Mol Biol* **208**: 105735. https://doi.org/10.1016/j.jsbmb.2020.105735

Fischer D, Seifert M, Becker S, Ludders D, Cordes T, Reichrath J, Friedrich M (2007) 25-Hydroxyvitamin D3 1alpha-hydroxylase splice variants in breast cell lines MCF-7 and MCF-10. *Cancer Genomics Proteomics* **4**: 295–300

Friedrich M, Diesing D, Cordes T, Fischer D, Becker S, Chen TC, Flanagan JN, Tangpricha V, Gherson I, Holick MF, Reichrath J (2006) Analysis of 25-hydroxyvitamin D_3-1alpha-hydroxylase in normal and malignant breast tissue. *Anticancer Res* **26**: 2615–2620

Garland CF, Gorham ED, Mohr SB, Grant WB, Giovannucci EL, Lipkin M, Newmark H, Holick MF, Garland FC (2007) Vitamin D and prevention of breast cancer: pooled analysis. *J Steroid Biochem Mol Biol* **103**: 708–711. https://doi.org/10.1016/j.jsbmb.2006.12.007

Goodwin PJ, Ennis M, Pritchard KI, Koo J, Hood N (2009) Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. *J Clin Oncol* **27**: 3757–3763. https://doi.org/10.1200/JCO.2008.20.0725

Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, Ruddy K, Tsang J, Cardoso F (2019) Breast cancer. *Nat Rev Dis Primers* **5**: 1–31. https://doi.org/10.1038/s41572-019-0111-2

Holick MF (2017) The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord* **18**: 153–165. https://doi.org/10.1007/s11154-017-9424-1

Holm K, Staaf J, Lauss M, Aine M, Lindgren D, Bendahl P-O, Vallon-Christersson J, Barkardottir RB, Höglund M, Borg Å, Jönsson G, Ringnér M (2016) An integrated genomics analysis of epigenetic subtypes in human breast tumors links DNA methylation patterns

to chromatin states in normal mammary cells. *Breast Cancer Res* **18**: 27. https://doi.org/10.1186/s13058-016-0685-5

Hossain S, Liu Z, Wood RJ (2020) Histone deacetylase activity and vitamin D-dependent gene expressions in relation to sulforaphane in human breast cancer cells. *J Food Biochem* **44**: e13114. https://doi.org/10.1111/jfbc.13114

Huss L, Butt ST, Borgquist S, Elebro K, Sandsveden M, Rosendahl A, Manjer J (2019) Vitamin D receptor expression in invasive breast tumors and breast cancer survival. *Breast Cancer Res* **21**: 84. https://doi.org/10.1186/s13058-019-1169-1

Imamura T, Hikita A, Inoue Y (2012) The roles of TGF-β signaling in carcinogenesis and breast cancer metastasis. *Breast Cancer* **19**: 118–124. https://doi.org/10.1007/s12282-011-0321-2

Janjetovic Z, Tuckey RC, Nguyen MN, Thorpe EM, Slominski AT (2010) 20,23-dihydroxyvitamin D_3, novel P450scc product, stimulates differentiation and inhibits proliferation and NF-kappaB activity in human keratinocytes. *J Cell Physiol* **223**: 36–48. https://doi.org/10.1002/jcp.21992

Janjetovic Z, Zmijewski MA, Tuckey RC, DeLeon DA, Nguyen MN, Pfeffer LM, Slominski AT (2009) 20-Hydroxycholecalciferol, product of vitamin D_3 hydroxylation by P450scc, decreases NF-κB activity by increasing IκBα levels in human keratinocytes. *PLOS ONE* **4**: e5988. https://doi.org/10.1371/journal.pone.0005988

Janowsky EC, Lester GE, Weinberg CR, Millikan RC, Schildkraut JM, Garrett PA, Hulka BS (1999) Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. *Public Health Nutr* **2**: 283–291. https://doi.org/10.1017/s1368980099000385

Jetten AM (2009) Retinoid-Related Orphan Receptors (RORs): Critical roles in development, immunity, circadian rhythm, and cellular metabolism. *Nucl Recept Signal* **7**: nrs.07003. https://doi.org/10.1621/nrs.07003

Jones G (2013) Extrarenal vitamin D activation and interactions between vitamin D_2, vitamin D_3, and vitamin D analogs. *Annu Rev Nutr* **33**: 23–44. https://doi.org/10.1146/annurev-nutr-071812-161203

Karsli-Ceppioglu S, Dagdemir A, Judes G, Ngollo M, Penault-Llorca F, Pajon A, Bignon Y-J, Bernard-Gallon D (2014) Epigenetic mechanisms of breast cancer: an update of the current knowledge. *Epigenomics* **6**: 651–664. https://doi.org/10.2217/epi.14.59

Kemmis CM, Welsh J (2008) Mammary epithelial cell transformation is associated with deregulation of the vitamin D pathway. *J Cell Biochem* **105**: 980–988. https://doi.org/10.1002/jcb.21896

Kimbung S, Chang C-Y, Bendahl P-O, Dubois L, Thompson JW, McDonnell DP, Borgquist S (2017) Impact of 27-hydroxylase (CYP27A1) and 27-hydroxycholesterol in breast cancer. *Endocr Relat Cancer* **24**: 339–349. https://doi.org/10.1530/ERC-16-0533

Kimbung S, Inasu M, Stålhammar T, Nodin B, Elebro K, Tryggvadottir H, Ygland Rödström M, Jirström K, Isaksson K, Jernström H, Borgquist S (2020) CYP27A1 expression is associated with risk of late lethal estrogen receptor-positive breast cancer in postmenopausal patients. *Breast Cancer Res* **22**: 123. https://doi.org/10.1186/s13058-020-01347-x

Le Cornet C, Walter B, Sookthai D, Johnson TS, Kühn T, Herpel E, Kaaks R, Fortner RT (2020) Circulating 27-hydroxycholesterol and breast cancer tissue expression of CYP27A1, CYP7B1, LXR-β, and ERβ: results from the EPIC-Heidelberg cohort. *Breast Cancer Res* **22**: 23. https://doi.org/10.1186/s13058-020-1253-6

Li W, Chen J, Janjetovic Z, Kim T-K, Sweatman T, Lu Y, Zjawiony J, Tuckey RC, Miller D, Slominski A (2010) Chemical synthesis of 20S-hydroxyvitamin D_3, which shows anti-proliferative activity. *Steroids* **75**: 926–935. https://doi.org/10.1016/j.steroids.2010.05.021

Lin Z, Marepally SR, Goh ESY, Cheng CYS, Janjetovic Z, Kim T-K, Miller DD, Postlethwaite AE, Slominski AT, Tuckey RC, Peluso-Iltis C, Rochel N, Li W (2018) Investigation of 20S-hydroxyvitamin D_3 analogs and their 1α-OH derivatives as potent vitamin D receptor agonists with anti-inflammatory activities. *Sci Rep* **8**: 1478. https://doi.org/10.1038/s41598-018-19183-7

Lin Z, Marepally SR, Kim T-K, Janjetovic Z, Oak AS, Postlethwaite AE, Myers LK, Tuckey RC, Slominski AT, Miller DD, Li W (2016a) Design, synthesis and biological activities of novel gemini 20s-hydroxyvitamin D_3 analogs. *Anticancer Res* **36**: 877–886

Lin Z, Marepally SR, Ma D, Kim T-K, Oak ASW, Myers LK, Tuckey RC, Slominski AT, Miller DD, Li W (2016b) Synthesis and biological evaluation of vitamin D_3 metabolite 20S,23S-dihydroxyvitamin D_3 and its 23R epimer. *J Med Chem* **59**: 5102–5108. https://doi.org/10.1021/acs.jmedchem.6b00182

Lin Z, Marepally SR, Ma D, Myers LK, Postlethwaite AE, Tuckey RC, Cheng CYS, Kim T-K, Yue J, Slominski AT, Miller DD, Li W (2015) Chemical synthesis and biological activities of 20S,24S/R-dihydroxyvitamin D_3 epimers and their 1α-hydroxyl derivatives. *J Med Chem* **58**: 7881–7887. https://doi.org/10.1021/acs.jmedchem.5b00881

Lohmann AE, Chapman J-AW, Burnell MJ, Levine MN, Tsvetkova E, Pritchard KI, Gelmon KA, O'Brien P, Han L, Rugo HS, Albain KS, Perez EA, Vandenberg TA, Chalchal HI, Sawhney RPS, Shepherd LE, Goodwin PJ (2015) Prognostic associations of 25 hydroxy vitamin D in NCIC CTG MA.21, a phase III adjuvant randomized clinical trial of three chemotherapy regimens in high-risk breast cancer. *Breast Cancer Res Treat* **150**: 605–611. https://doi.org/10.1007/s10549-015-3355-x

Lopes N, Carvalho J, Durães C, Sousa B, Gomes M, Costa JL, Oliveira C, Paredes J, Schmitt F (2012) 1Alpha,25-dihydroxyvitamin D_3 induces *de novo* E-cadherin expression in triple-negative breast cancer cells by CDH1-promoter demethylation. *Anticancer Res* **32**: 249–257

Lopes N, Sousa B, Martins D, Gomes M, Vieira D, Veronese LA, Milanezi F, Paredes J, Costa JL, Schmitt F (2010) Alterations in Vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions. *BMC Cancer* **10**: 483. https://doi.org/10.1186/1471-2407-10-483

Lorbek G, Lewinska M, Rozman D (2012) Cytochrome P450s in the synthesis of cholesterol and bile acids – from mouse models to human diseases. *FEBS J* **279**: 1516–1533. https://doi.org/10.1111/j.1742-4658.2011.08432.x

Lu Y, Chen J, Janjetovic Z, Michaels P, Tang EKY, Wang J, Tuckey RC, Slominski AT, Li W, Miller DD (2012) Design, synthesis and biological action of 20R-hydroxyvitamin D_3. *J Med Chem* **55**: 3573–3577. https://doi.org/10.1021/jm201478e

Lu Y, Yi Y, Liu P, Wen W, James M, Wang D, You M (2007) Common human cancer genes discovered by integrated gene-expression analysis. *PLoS One* **2**: e1149. https://doi.org/10.1371/journal.pone.0001149

Marik R, Fackler M, Gabrielson E, Zeiger MA, Sukumar S, Stearns V, Umbricht CB (2010) DNA methylation-related vitamin D receptor insensitivity in breast cancer. *Cancer Biol Therapy* **10**: 44–53. https://doi.org/10.4161/cbt.10.1.11994

Miller WL, Bose HS (2011) Early steps in steroidogenesis: intracellular cholesterol trafficking: Thematic Review Series: Genetics of Human Lipid Diseases. *J Lipid Res* **52**: 2111–2135. https://doi.org/10.1194/jlr.R016675

Muscat GEO, Eriksson NA, Byth K, Loi S, Graham D, Jindal S, Davis MJ, Clyne C, Funder JW, Simpson ER, Ragan MA, Kuczek E, Fuller PJ, Tilley WD, Leedman PJ, Clarke CL (2013) Research resource: nuclear receptors as transcriptome: discriminant and prognostic value in breast cancer. *Mol Endocrinol* **27**: 350–365. https://doi.org/10.1210/me.2012-1265

Nandy D, Rajam SM, Dutta D (2020) A three layered histone epigenetics in breast cancer metastasis. *Cell Biosci* **10**: 52. https://doi.org/10.1186/s13578-020-00415-1

Nishikawa J, Kitaura M, Matsumoto M, Imagawa M, Nishihara T (1994) Difference and similarity of DNA sequence recognized by VDR homodimer and VDR/RXR heterodimer. *Nucleic Acids Res* **22**: 2902–2907

Nurminen V, Seuter S, Carlberg C (2019) Primary vitamin D target genes of human monocytes. *Frontiers Physiol* **10**: 194. https://doi.org/10.3389/fphys.2019.00194

Oak ASW, Bocheva G, Kim T-K, Brożyna AA, Janjetovic Z, Athar M, Tuckey RC, Slominski AT (2020) Noncalcemic vitamin D hydroxyderivatives inhibit human oral squamous cell carcinoma and down-regulate hedgehog and WNT/β-catenin pathways. *Anticancer Res* **40**: 2467–2474. https://doi.org/10.21873/anticanres.14216

O'Brien KM, Sandler DP, Xu Z, Kinyamu HK, Taylor JA, Weinberg CR (2018) Vitamin D, DNA methylation, and breast cancer. *Breast Cancer Res* **20**: 70. https://doi.org/10.1186/s13058-018-0994-y

Odawara H, Iwasaki T, Horiguchi J, Rokutanda N, Hirooka K, Miyazaki W, Koibuchi Y, Shimokawa N, Iino Y, Takeyoshi I, Koibuchi N (2009) Activation of aromatase expression by retinoic acid receptor-related orphan receptor (ROR) α in breast cancer cells. *J Biol Chem* **284**: 17711–17719. https://doi.org/10.1074/jbc.M109.009241

Oh TG, Bailey P, Dray E, Smith AG, Goode J, Eriksson N, Funder JW, Fuller PJ, Simpson ER, Tilley WD, Leedman PJ, Clarke CL, Grimmond S, Dowhan DH, Muscat GEO (2014) PRMT2 and RORγ expression are associated with breast cancer survival outcomes. *Mol Endocrinol* **28**: 1166–1185. https://doi.org/10.1210/me.2013-1403

Oh TG, Wang S-CM, Acharya BR, Goode JM, Graham JD, Clarke CL, Yap AS, Muscat GEO (2016) The nuclear receptor, RORγ, regulates pathways necessary for breast cancer metastasis. *EBioMed* **6**: 59–72. https://doi.org/10.1016/j.ebiom.2016.02.028

Osanai M, Lee G-H (2016) CYP24A1-induced vitamin D insufficiency promotes breast cancer growth. *Oncol Rep* **36**: 2755–2762. https://doi.org/10.3892/or.2016.5072

Prosser DE, Jones G (2004) Enzymes involved in the activation and inactivation of vitamin D. *Trends Biochem Sci* **29**: 664–673. https://doi.org/10.1016/j.tibs.2004.10.005

Richards SE, Weierstahl KA, Kelts JL (2015) Vitamin D effect on growth and vitamin D metabolizing enzymes in triple-negative breast cancer. *Anticancer Res* **35**: 805–810

Riggins RB, Mazzotta MM, Maniya OZ, Clarke R (2010) Orphan nuclear receptors in breast cancer pathogenesis and therapeutic response. *Endocr Relat Cancer* **17**: R213-231. https://doi.org/10.1677/ERC-10-0058

Saha T, Makar S, Swetha R, Gutti G, Singh SK (2019) Estrogen signaling: An emanating therapeutic target for breast cancer treatment. *Eur J Med Chem* **177**: 116–143. https://doi.org/10.1016/j.ejmech.2019.05.023

Saramäki A, Diermeier S, Kellner R, Laitinen H, Vaïsänen S, Carlberg C (2009) Cyclical chromatin looping and transcription factor association on the regulatory regions of the p21 (CDKN1A) gene in response to 1α,25-dihydroxyvitamin D3. *J Biol Chem* **284**: 8073–8082. https://doi.org/10.1074/jbc.M808090200

Segersten U, Holm PK, Björklund P, Hessman O, Nordgren H, Binderup L, Åkerström G, Hellman P, Westin G (2005) 25-Hydroxyvitamin D_3 1α-hydroxylase expression in breast cancer and use of non-1α-hydroxylated vitamin D analogue. *Breast Cancer Res* **7**: R980–R986. https://doi.org/10.1186/bcr1332

Setiawan VW, Cheng I, Stram DO, Giorgi E, Pike MC, Berg DVD, Pooler L, Burtt NP, Marchand LL, Altshuler D, Hirschhorn J, Henderson BE, Haiman CA (2006) A systematic assessment of common genetic variation in CYP11A and risk of breast cancer. *Cancer Res* **66**: 12019–12025. https://doi.org/10.1158/0008-5472.CAN-06-1101

Shahvegharasl Z, Pirouzpanah S, Mahboob SA, Montazeri V, Adili A, Asvadi I, Sanaat Z, Esfehani A, Pirouzpanah S-S, Mesgari M (2020) Effects of cholecalciferol supplementation on serum angiogenic biomarkers in breast cancer patients treated with tamoxifen: A controlled randomized clinical trial. *Nutrition* **72**: 110656. https://doi.org/10.1016/j.nut.2019.110656

Shaukat N, Jaleel F, Moosa FA, Qureshi NA (2017) Association between vitamin D deficiency and breast cancer. *Pak J Med Sci* **33**: 645–649. https://doi.org/10.12669/pjms.333.11753

Sheng L, Anderson PH, Turner AG, Pishas KI, Dhatrak DJ, Gill PG, Morris HA, Callen DF (2016) Identification of vitamin D3 target genes in human breast cancer tissue. *J Steroid Biochem Mol Biol* **164**: 90–97. https://doi.org/10.1016/j.jsbmb.2015.10.012

Shi H, Yan PS, Chen C-M, Rahmatpanah F, Lofton-Day C, Caldwell CW, Huang TH-M (2002) Expressed CpG island sequence tag microarray for dual screening of DNA hypermethylation and gene silencing in cancer cells. *Cancer Res* **62**: 3214–3220

Singh T, Adams BD (2017) The regulatory role of miRNAs on VDR in breast cancer. *Transcription* **8**: 232–241. https://doi.org/10.1080/21541264.2017.1317695

Slominski A, Janjetovic Z, Tuckey RC, Nguyen MN, Bhattacharya KG, Wang J, Li W, Jiao Y, Gu W, Brown M, Postlethwaite AE (2013) 20S-Hydroxyvitamin D_3, noncalcemic product of CYP11A1 action on vitamin D_3, exhibits potent antifibrogenic activity *in vivo*. *J Clin Endocrinol Metabol* **98**: E298–E303. https://doi.org/10.1210/jc.2012-3074

Slominski AT, Brożyna AA, Skobowiat C, Zmijewski MA, Kim T-K, Janjetovic Z, Oak AS, Jozwicki W, Jetten AM, Mason RS, Elmets C, Li W, Hoffman RM, Tuckey RC (2018) On the role of classical and novel forms of vitamin D in melanoma progression and management. *J Steroid Biochem Mol Biol* **177**: 159–170. https://doi.org/10.1016/j.jsbmb.2017.06.013

Slominski AT, Brożyna AA, Zmijewski MA, Janjetovic Z, Kim T-K, Slominski RM, Tuckey RC, Mason RS, Jetten AM, Guroji P, Reichrath J, Elmets C, Athar M (2020) The role of classical and novel forms of vitamin D in the pathogenesis and progression of nonmelanoma skin cancers. *Adv Exp Med Biol* **1268**: 257–283. https://doi.org/10.1007/978-3-030-46227-7_13

Slominski AT, Brożyna AA, Zmijewski MA, Jóźwicki W, Jetten AM, Mason RS, Tuckey RC, Elmets CA (2017a) Vitamin D signaling and melanoma: role of vitamin D and its receptors in melanoma progression and management. *Lab Invest* **97**: 706–724. https://doi.org/10.1038/labinvest.2017.3

Slominski AT, Janjetovic Z, Fuller BE, Zmijewski MA, Tuckey RC, Nguyen MN, Sweatman T, Li W, Zjawiony J, Miller D, Chen TC, Lozanski G, Holick MF (2010) Products of vitamin D_3 or 7-dehydrocholesterol metabolism by cytochrome P450scc show anti-leukemia effects, having low or absent calcemic activity. *PLoS One* **5**: e9907. https://doi.org/10.1371/journal.pone.0009907

Slominski AT, Janjetovic Z, Kim T-K, Wright AC, Grese LN, Riney SJ, Nguyen MN, Tuckey RC (2012a) Novel vitamin D hydroxyderivatives inhibit melanoma growth and show differential effects on normal melanocytes. *Anticancer Res* **32**: 3733–3742

Slominski AT, Kim T-K, Hobrath JV, Janjetovic Z, Oak ASW, Postlethwaite A, Lin Z, Li W, Takeda Y, Jetten AM, Tuckey RC (2017b) Characterization of a new pathway that activates lumisterol *in vivo* to biologically active hydroxylumisterols. *Sci Rep* **7**: 11434. https://doi.org/10.1038/s41598-017-10202-7

Slominski AT, Kim T-K, Hobrath JV, Oak ASW, Tang EKY, Tieu EW, Li W, Tuckey RC, Jetten AM (2017c) Endogenously produced nonclassical vitamin D hydroxy-metabolites act as "biased" agonists on VDR and inverse agonists on RORα and RORγ. *J Steroid Biochem Mol Biol* **173**: 42–56. https://doi.org/10.1016/j.jsbmb.2016.09.024

Slominski AT, Kim T-K, Li W, Yi A-K, Postlethwaite A, Tuckey RC (2014a) The role of CYP11A1 in the production of vitamin D metabolites and their role in the regulation of epidermal functions. *J Steroid Biochem Mol Biol* **144 Pt A**: 28–39. https://doi.org/10.1016/j.jsbmb.2013.10.012

Slominski AT, Kim T-K, Shehabi HZ, Semak I, Tang EKY, Nguyen MN, Benson HAE, Korik E, Janjetovic Z, Chen J, Yates CR, Postlethwaite A, Li W, Tuckey RC (2012b) *In vivo* evidence for a novel pathway of vitamin D_3 metabolism initiated by P450scc and modified by CYP27B1. *FASEB J* **26**: 3901–3915. https://doi.org/10.1096/fj.12-208975

Slominski AT, Kim T-K, Shehabi HZ, Tang E, Benson HAE, Semak I, Lin Z, Yates CR, Wang J, Li W, Tuckey RC (2014b) *In vivo* production of novel vitamin D_2 hydroxy-derivatives by human placentas, epidermal keratinocytes, Caco-2 colon cells and the adrenal gland. *Mol Cell Endocrinol* **383**: 181–192. https://doi.org/10.1016/j.mce.2013.12.012

Slominski AT, Kim T-K, Takeda Y, Janjetovic Z, Brożyna AA, Skobowiat C, Wang J, Postlethwaite A, Li W, Tuckey RC, Jetten AM (2014c) RORα and ROR γ are expressed in human skin and serve as receptors for endogenously produced noncalcemic 20-hydroxy- and 20,23-dihydroxyvitamin D. *FASEB J* **28**: 2775–2789. https://doi.org/https://doi.org/10.1096/fj.13-242040

Slominski AT, Li W, Bhattacharya SK, Smith RA, Johnson PL, Chen J, Nelson KE, Tuckey RC, Miller D, Jiao Y, Gu W, Postlethwaite AE (2011) Vitamin D analogs 17,20S(OH)2pD and 17,20R(OH)2pD are noncalcemic and exhibit antifibrotic activity. *J Invest Dermatol* **131**: 1167–1169. https://doi.org/10.1038/jid.2010.425

Slominski AT, Li W, Kim T-K, Semak I, Wang J, Zjawiony JK, Tuckey RC (2015) Novel activities of CYP11A1 and their potential physiological significance. *J Steroid Biochem Mol Biol* **151**: 25–37. https://doi.org/10.1016/j.jsbmb.2014.11.010

Song D, Deng Y, Liu K, Zhou L, Li N, Zheng Y, Hao Q, Yang S, Wu Y, Zhai Z, Li H, Dai Z (2019) Vitamin D intake, blood vitamin D levels, and the risk of breast cancer: a dose-response meta-analysis of observational studies. *Aging (Albany NY)* **11**: 12708–12732. https://doi.org/10.18632/aging.102597

Sugimoto H, Shiro Y (2012) Diversity and substrate specificity in the structures of steroidogenic cytochrome P450 enzymes. *Biol Pharm Bull* **35**: 818–823. https://doi.org/10.1248/bpb.35.818

Sun M, Yang X, Ye C, Xu W, Yao G, Chen J, Li M (2012) Risk-association of CYP11A1 polymorphisms and breast cancer among Han Chinese women in Southern China. *Int J Mol Sci* **13**: 4896–4905. https://doi.org/10.3390/ijms13044896

Townsend K, Banwell CM, Guy M, Colston KW, Mansi JL, Stewart PM, Campbell MJ, Hewison M (2005) Autocrine metabolism of vitamin D in normal and malignant breast tissue. *Clin Cancer Res* **11**: 3579–3586. https://doi.org/10.1158/1078-0432.CCR-04-2359

Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Hyppönen E, Berry J, Vieth R, Lanham-New S (2012) Comparison of vitamin D_2 and vitamin D_3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am J Clin Nutr* **95**: 1357–1364. https://doi.org/10.3945/ajcn.111.031070

Tuckey RC, Li W, Shehabi HZ, Janjetovic Z, Nguyen MN, Kim T-K, Chen J, Howell DE, Benson HAE, Sweatman T, Baldisseri DM, Slominski A (2011) Production of 22-hydroxy metabolites of vitamin D_3 by cytochrome P450scc (CYP11A1) and analysis of their biological activities on skin cells. *Drug Metab Dispos* **39**: 1577–1588. https://doi.org/10.1124/dmd.111.040071

Wacker M, Holick MF (2013) Sunlight and vitamin D: A global perspective for health. *Dermatoendocrinol* **5**: 51–108. https://doi.org/10.4161/derm.24494

Wahler J, So JY, Kim YC, Liu F, Maehr H, Uskokovic M, Suh N (2014) Inhibition of the transition of ductal carcinoma *in situ* to invasive ductal carcinoma by a gemini vitamin D analog. *Cancer Prev Res* **7**: 617–626. https://doi.org/10.1158/1940-6207.CAPR-13-0362

Wang J, Slominski A, Tuckey RC, Janjetovic Z, Kulkarni A, Chen J, Postlethwaite AE, Miller D, Li W (2012) 20-hydroxyvitamin D_3 inhibits proliferation of cancer cells with high efficacy while being non-toxic. *Anticancer Res* **32**: 739–746

Wasiewicz T, Szyszka P, Cichorek M, Janjetovic Z, Tuckey RC, Slominski AT, Zmijewski MA (2015) Antitumor effects of vitamin D analogs on hamster and mouse melanoma cell lines in relation to melanin pigmentation. *Int J Mol Sci* **16**: 6645–6667. https://doi.org/10.3390/ijms16046645

Weinhold B (2006) Epigenetics: The science of change. *Environ Health Perspect* **114**: A160–A167

Xie SP, Pirianov G, Colston KW (1999) Vitamin D analogues suppress IGF-I signalling and promote apoptosis in breast cancer cells. *Eur J Cancer* **35**: 1717–1723. https://doi.org/10.1016/s0959-8049(99)00200-2

Xiong G, Wang C, Evers BM, Zhou BP, Xu R (2012) RORα suppresses breast tumor invasion by inducing SEMA3F expression. *Cancer Res* **72**: 1728–1739. https://doi.org/10.1158/0008-5472.CAN-11-2762

Xiong G, Xu R (2014) RORα binds to E2F1 to inhibit cell proliferation and regulate mammary gland branching morphogenesis. *Mol Cell Biol* **34**: 3066–3075. https://doi.org/10.1128/MCB.00279-14

Yaspan BL, Breyer JP, Cai Q, Dai Q, Elmore JB, Amundson I, Bradley KM, Shu X-O, Gao Y-T, Dupont WD, Zheng W, Smith JR (2007) Haplotype analysis of CYP11A1 identifies promoter variants associated with breast cancer risk. *Cancer Res* **67**: 5673–5682. https://doi.org/10.1158/0008-5472.CAN-07-0467

Yousef FM, Jacobs ET, Kang PT, Hakim IA, Going S, Yousef JM, Al-Raddadi RM, Kumosani TA, Thomson CA (2013) Vitamin D status and breast cancer in Saudi Arabian women: case-control study. *Am J Clin Nutr* **98**: 105–110. https://doi.org/10.3945/ajcn.112.054445

Zbytek B, Janjetovic Z, Tuckey RC, Zmijewski MA, Sweatman TW, Jones E, Nguyen MN, Slominski AT (2008) 20-Hydroxyvitamin D3, a product of vitamin D_3 hydroxylation by cytochrome P450scc, stimulates keratinocyte differentiation. *J Invest Dermatol* **128**: 2271–2280. https://doi.org/10.1038/jid.2008.62

Zhalehjoo N, Shakiba Y, Panjehpour M (2017) Gene expression profiles of CYP24A1 and CYP27B1 in malignant and normal breast tissues. *Mol Med Rep* **15**: 467–473. https://doi.org/10.3892/mmr.2016.5992

Zheng W, Gao Y-T, Shu X-O, Wen W, Cai Q, Dai Q, Smith JR (2004) Population-based case-control study of CYP11A gene polymorphism and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **13**: 709–714

Zhu Y, McAvoy S, Kuhn R, Smith DI (2006) RORA, a large common fragile site gene, is involved in cellular stress response. *Oncogene* **25**: 2901–2908. https://doi.org/10.1038/sj.onc.1209314

Zhuang J, Huo Q, Yang F, Xie N (2020) Perspectives on the role of histone modification in breast cancer progression and the advanced technological tools to study epigenetic determinants of metastasis. *Frontiers Genet* **11**: 1353. https://doi.org/10.3389/fgene.2020.603552

Zinser GM, Welsh J (2004) Accelerated mammary gland development during pregnancy and delayed postlactational involution in vitamin D_3 receptor null mice. *Mol Endocrinol* **18**: 2208–2223. https://doi.org/10.1210/me.2003-0469