

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Vitamin D, Its Receptor Gene Polymorphism and Breast Cancer

Mehir un Nisa Iqbal and Taseer Ahmed Khan

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/64505>

Abstract

Vitamin D is synthesized within skin followed by the peripheral maturation in liver and kidneys. Vitamin D is most essential secosteroid produced its systemic functions via complex with steroid/thyroid nuclear receptor called vitamin D receptor (VDR). The binding of the vitamin D₃ to VDR causes conformational changes that permit VDR-RXR heterodimer formation and VDR/ SRC-1 (transcriptional co-activator proteins) interactions. Functional expression and nuclear activation of VDR is necessary to produce its effects upon binding with vitamin D response element (VDRE) on target gene where it causes transcriptional activation resulting in the prevention of breast cancer by inhibiting proliferation, impeding differentiation and stimulating pro-apoptosis. Season, latitude, pigmentation of skin, aging, sunscreen use, obesity, and smoking all affect the production of vitamin D. In case of vitamin D deficiency or VDR gene polymorphisms, vitamin D responses are altered and probably are involved in the risk of breast cancer. Since many epidemiological, observational and interventional studies have been done to illustrate the role of vitamin D and its receptor gene polymorphism in breast cancer development but controversial findings have been observed. Therefore, the role of vitamin D and its receptor gene polymorphisms in development of breast cancer are still a matter of discussion.

Keywords: breast cancer, vitamin D, VDR, vitamin D receptor gene polymorphisms, VDR gene polymorphisms

1. Introduction

1.1. Breast cancer

Breast carcinoma is one of the most frequently diagnosed cancers among women worldwide with a high frequency reported in the West [1, 2]. This highest incidence of breast cancer in American whites and in most European countries reveal the long-standing high prevalence of reproductive factors associated with increased risk of breast cancer, including early menarche, late child bearing age, few pregnancies, hormone replacement therapy and increased mammography [3, 4]. In Israel, the increased incidence of breast cancer may reflect the disproportionately high prevalence of BRCA1 and BRCA2 mutations [5, 6].

Western lifestyle is another most important factor for Britain's high number of breast cancer cases fuelled by the women overeating, too much drinking and too little exercise doing in routine life. In addition, breastfeeding is also an important factor, which reduces the chance of developing breast cancer. Eastern women do not drink alcohol than women in the United Kingdom, and obesity ratio is much lower in Asian women than in western women, whereas breastfeeding rates are much higher in Asians (<http://www.dailymail.co.uk/news/article-1301445/Western-lifestyle-blame-soaring-breast-cancer-rates.html>). Affected women with breast cancer are usually young and often present with advanced disease [7]. According to a World Health Organization (WHO) estimate, around 25.2% people are diagnosed with breast cancer annually. The exact reason why a woman develops breast cancer is still unrevealed; though certain risk factors enhance a person's probability of getting breast cancer.

The factors that play a significant role in the aetiology of breast cancer include genetic [8, 9], hormonal [10, 11], environmental [12], lifestyle [13] and reproductive factors [14]. In addition, ovarian hormones (endogenous estrogen) are the key risk factors for the development of breast cancer and their progression among post-menopausal women [15, 16]. However, it is unclear that to what degree the effects of other risk factors may be mediated by their links with circulating free estradiol. Intake of vegetables and fruits are related with a substantial decrease of breast cancer risk [17, 18]. Vegetables are rich in antioxidants and certain phytochemicals may contribute to the reduced risk of breast cancer [19–21]. Plant-based diets are also high in fibres, which can decrease serum estrogen and could, in this way, contribute to reduced risk of breast cancer [22, 23]. In addition, increased consumption of fruit and vegetables are associated with lower rates of obesity, which is a crucial risk factor for post-menopausal breast cancer [24]. High energy intake, physical sluggishness, high body mass index (BMI) and weight put on are coupled to an increased breast [25] cancer risk. Low levels of HDL-C in breast cancer patients than in control subjects have also been documented [26]. But still, data from prospective studies are very limited (Moorman, 1998). Furthermore, consanguineous marriages are common in certain racial groups, which will increase the risk of breast cancer [27].

Among these contributing factors, vitamin D and its receptor gene polymorphisms may play a pivotal role in the development of mammary gland tumorigenesis [28].

1.2. Vitamin D and vitamin D receptor (VDR)

Vitamin D and VDR are the two most important participants playing a key role in vitamin D endocrine system in the prevention of breast cancer. Vitamin D is a sunshine vitamin, which is involved in a variety of actions and also reduces the risk of many cancers [29, 30].

VDR is a member of nuclear receptor (NR) superfamily and transcription regulating factor also called NR1I1 or nuclear receptor subfamily 1, group I and member 1. VDR is a high-affinity, low-capacity receptor having a molecular weight of about 48–55 kD. VDR is expressed in majority of human tissues. But some cells have decrease or no VDR expression including RBCs, mature cardiac and skeletal muscles and cerebellar Purkinje cells [31]. Its actions are preceded by the formation of heterodimer with retinoid X receptor (RXR), which causes the conformational changes in VDR and allow the binding of vitamin D₃ at ligand binding domain (LBD). In addition, the heterodimer complex then binds with a specific sequence present in the DNA called vitamin D response element (VDRE). Genomic pathway involves the expression of genes in a tissue-specific manner [28].

1.2.1. VDR domains

VDR contain five functional domains (**Figure 1**) including:

1. A and B domains both are shortest domains contain 20 amino acids.
2. C domain (DNA binding domain or DBD) having two Zn fingers [32] motifs. Two alpha helices are found at the carboxy terminus of each Zn finger (namely helix A and B which constitutes DNA recognition and phosphate binding sites respectively).
3. Flexible hinge D domain is present in between C and E domains having the ability to change structural conformation after VDR ligand binding.
4. E domain (ligand binding domain or LBD) consists of 12 alpha helices along with 3 short beta strands, organized in a manner that it forms three dimensional hormone binding pockets of which vitamin D₃ is attached.

Both N-ter and C-ter has activation function (called AF-2) in translation [33].

1.2.2. Vitamin D/VDR actions

1. **Genomic actions:** Vitamin D₃ produces its pleiotropic effects by genomic and non-genomic actions. It mediates its genomic actions upon binding to intracellular nuclear transcription factor called VDR.
2. **Non-genomic actions:** Vitamin D also plays various non-genomic actions. Non-genomic actions are also called rapid actions, which are caused by the interaction of vitamin D with the membrane VDR to perform its biological effects through intracellular signalling pathways [35]. However, the contribution of non-genomic pathway in the development of anti-neoplastic effects on breast remains unclear.

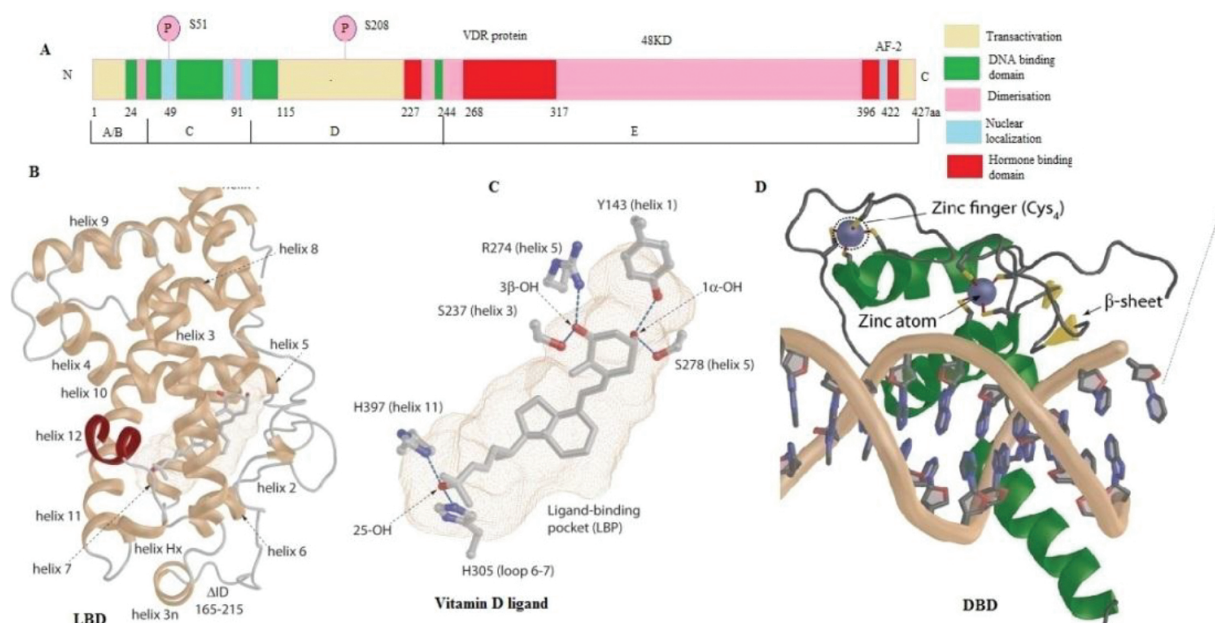


Figure 1. The crystal structure of VDR showing its functional domains [34]. (A) Schematic representation of VDR domains. (B) LBD of the VDR which contains 12 alpha helices. (C) The binding mode of Vitamin D in the hormone-binding pocket. (D) The DBD of the Vitamin D. The two zinc atoms are represented in blue in colour, whereas beta sheets are represented in yellow colour.

2. Bio-activation and metabolism of Vitamin D in normal breast

It is already known that vitamin D affects the breast cancer cell growth but limited information is available about its delivery, uptake and metabolism in mammary cells. Vitamin D is either derived from the gastrointestinal (GIT) absorption or synthesized within the skin under the exposure of UVB radiations, which then undergoes the 25-hydroxylation in liver in presence of 25-hydroxylase resulting in the production of 25(OH)D₃. 25(OH)D₃ is the precursor molecule for the synthesis of active Vitamin D₃ (1,25(OH)₂D₃). It is a major circulating form of vitamin D, which is stored in adipose tissues. It is also an accurate biomarker of vitamin D, which determines the overall status of vitamin D in the body. However, the precursor does not readily binds to the VDR and must be converted into its active form, 1,25(OH)₂D₃, which has high binding affinity to VDR. The conversion of precursor vitamin D into its active metabolite occurs in the presence of 1- α -hydroxylases. Immunohistochemistry and *in situ* hybridization studies indicated strong expression of 1- α -hydroxylase protein and mRNA in the distal convoluted tubule, the cortical and medullary part of the collecting ducts and the papillary epithelia. Lower expression was observed along the thick ascending limb of the loop of Henle and Bowman's capsule. Weaker and more variable expression of 1- α -hydroxylase protein and mRNA was seen in proximal convoluted tubules, and no expression was observed in glomeruli or vascular structures [36]. Whereas lesser expression of 1- α -hydroxylase was also observed in non-renal cells including keratinocytes, macrophages, prostatic epithelium, colonocytes [37, 38] and breast epithelium [39] to lesser extent. Kidneys and non-renal 1- α -hydroxylases are

encoded by the same gene mapped on the chromosome 12 [40]. However, the presence of this enzyme on non-renal tissues indicated that the non-renal tissues have the ability of vitamin D bio-activation, responsible to convert 25(OH)D₃ into 1,25(OH)₂D₃. 1,25(OH)₂D₃ is virtually not detected in human serum under anephric conditions, which means that kidneys are the major source of 1,25(OH)₂D₃ in circulation. These observations emphasize that 1,25(OH)₂D₃ produced by the non-renal tissues is not released in the bloodstream. However, they act locally upon binding to VDR on the same tissues from where it is synthesized. Such local actions of vitamin D are likely included in proliferation, differentiation and apoptosis, which are discussed below in later sections.

2.1. Bio-activation pathways in breast cells

The above information supports the hypothesis that two distinct pathways may be involved in the bio-synthesis and bio-activation of vitamin D in breast such as 1,25(OH)₂D₃ and 25(OH)D₃ (vitamin D precursor) pathways [41, 42].

2.1.1. Endocrine pathway

The endocrine pathway is involved with the circulation of 1,25(OH)₂D₃, which reaches the mammary tissues and produces anti-neoplastic effects through genomic pathway.

2.1.2. Autocrine/paracrine pathway

The other pathway is the autocrine/paracrine pathway involved with the 25(OH)D₃, which reaches the mammary gland and converts into 1,25(OH)₂D₃ [43] in the presence of 1- α -hydroxylase to prevent breast cancer [41]. Most of the extra-renal tissues of the body have its own 1- α -hydroxylase enzyme needed for the production of active metabolite of vitamin D [37]. The circulating level of 25(OH)D₃ seems to be the key regulator of tissue-specific synthesis of active vitamin D [37, 44]. The locally produced active vitamin D binds with VDRs of mammary epithelium in order to regulate the expression of more than 200 genes, which are involved in controlling cell proliferation, inhibit cell growth, stimulate cell differentiation, induce apoptosis and inhibit angiogenesis [45] and contribute in the prevention of breast tumorigenesis [46]. Moreover, mammary epithelial cells also contain 24-hydroxylase enzyme (CYP24), which converts active vitamin D into less active metabolites including 24,25-dihydroxyvitamin D₃ and 1,24,25-trihydroxyvitamin D₃ [43]. For this reason, we can say that breast tissues contain all the elements of vitamin D signalling axis, which involve in the local synthesis as well as metabolism of vitamin D and its signal transduction through VDRs.

3. Vitamin D signalling in the prevention of breast cancer

3.1. VDR expression in breast

Several extra-renal epithelial cells of body express VDR, for example, epithelial cells of rat, mouse and human mammary glands. VDR expression is highest in breast tissues during

puberty, pregnancy and lactation in women [47]. In mice, the expression is highest in ductal epithelium when compared to terminal end-buds epithelium of mammary gland. In human, VDR-positive cells are found in basal and luminal layer of breast epithelium [39]. Cap cells and stromal compartments of breast are also rich in VDR [48–50]. The presence of VDR in different cells of breast highlights the complexity of vitamin D signalling in breast tissues.

3.2. Mechanism of vitamin D signalling in breast cancer prevention

Despite these consistent data, the exact mechanism of breast cancer prevention by vitamin D has yet to be discerned. Both 25(OH)D₃ and 1,25(OH)₂D₃ exert its profound effects on normal VDR-positive breast epithelium such as hormone-stimulated growth inhibition, ductal elongation, ductal branching and induction of biomarkers involved in breast differentiation. The expression of VDR and 1- α -hydroxylase in mammary adipocytes also takes part in the prevention of cancer in whole tissue since adipocytes secrete diffusible signals in response to 25(OH)D₃, which constrain morphogenesis of the nearby ductal tissues [48].

Furthermore, alteration in cellular energy metabolism, immune responses and other processes of vitamin D signalling in the prevention of breast cancer on non-tumourigenic breast epithelium is described below.

3.2.1. Anti-proliferation

Vitamin D causes cell-cycle arrest by direct or indirect involvement of growth factors and does not allow the cell to enter in the S phase from G1 phase [51]. It increases the expression of cyclin-dependent kinases (CDKs) inhibitors, including p21 and p27, and reduces the expression of CDK2, CDK4, cyclin D1, cyclin A1 and cyclin E1, which results in the arrest of cell-cycle progression [52, 53]. It is also involved in the downregulation of c-myc oncoprotein and inhibits the cell proliferation [54]. However, all these consequences describe that vitamin D hampers the cell proliferation by affecting the crucial controllers of cell-cycle progression. Furthermore, vitamin D also enhances the transcription factor CCAT enhancer-binding protein alpha (C/EBP α), which mediates the anti-proliferative effects of vitamin D observed in *in vitro* study on MCF-7 cells [55]. Tumour suppressor TCF-4 also hinder cell-cycle progression [56]. Beside these, vitamin D also causes the induction of BRCA 1 (breast cancer 1) gene, which is inversely associated with the cell proliferation, promotes tumour suppression and inhibits cell-cycle progression [57].

3.2.2. Growth arrest and pro-apoptosis

Vitamin D plays an important role in the induction of apoptosis in mammary tissues, since *in vitro* conditions, such as shrinkage of cell, condensation of chromatin network and fragmentation of DNA, have been observed in MCF-7 cells upon treatment with vitamin D [58]. The mechanism by which vitamin D induced apoptosis has not been fully understood. However, the most probable mechanism is the downregulation of anti-apoptotic protein, called Bcl2 (51). Vitamin D increases the tumour necrotic factor alpha (TNF α) with or without caspase 3 activation. In the caspase 3-independent mechanism, vitamin D-mediated induction of

apoptosis in MCF-7 cells is thought to be correlated with mitochondrial disruption, which causes the release of cytochrome C and formation of reactive oxygen species (ROS) resulting in the apoptosis [59]. Other mechanism of caspase-independent apoptosis induced by vitamin D-dependent Ca^{+} absorption is most likely associated with the increased activation of lysosomal proteases [60]. Finally, vitamin D also acts a pro-oxidant for breast cancer cells, which generally increase the redox potential [61] of carcinogenic cell, may be one of the most important underlying pro-apoptotic mechanisms of vitamin D. The pro-oxidant action of vitamin D in MCF-7 cells could result from increased intra-cellular reactive oxygen species production during aerobic metabolism. Vitamin D inhibits the expression of one of the major constituents of the cellular defence system against ROS, like superoxide dismutase (SOD) [62]. This decrease could be one of the mechanisms underlying the pro-oxidant action of vitamin D. Indeed, it was previously reported that overexpression of SOD protects MCF-7 cells from being injured [63, 64]. Decrease in SOD levels would cause a shift in the balance between superoxides and hydrogen peroxide (H_2O_2). Increased levels of superoxides can, in turn, cause increased oxidative damage attributable to interaction with NO to form the highly toxic peroxynitrite [65] and to increased availability of free iron that supports hydroxyl radical formation through the Fenton reaction [66].

Changes in the redox state could translate into reversible oxidation of cysteines in major proteins that determine cell fate, such as protein kinases, protein tyrosine phosphatases and transcription factors (e.g. Sp1, activator protein-1, nuclear factor- κB and p53) [67–73]. The key components of the apoptotic process, such as mitochondrial permeability transition pores and increase caspases, are also subjected to redox regulation [74]. Oxidation of the cysteine in the active site of GAPDH may be considered a sensitive, easily accessible marker for these processes. It is noteworthy that the increase in the cellular redox potential was shown to abolish the DNA-binding ability of the transcription factors activator protein-1 and nuclear factor- κB [75] can cause apoptosis and prevent breast cancer. Notably, a recent study describes the relationship between p53 and VDR. Mutant P53 (mutp53) converts the Vitamin D pro-apoptotic activity into anti-apoptotic activity and attain oncogenic activity which demonstrate gain of function (GOF) [76].

3.2.3. *Anti-angiogenesis*

Vitamin D inhibits angiogenesis, which is another important feature for tumour growth and progression. It also has the ability to impede angiogenesis at very minute concentration [77] mediated through the downregulation of vascular endothelial growth factor (VEGF), tenascin-C, tumour growth factor α (TGF- α) and epidermal growth factor (EGF) [78, 79].

3.2.4. *Anti-invasion or anti-metastasis*

Vitamin D inhibits the invasion of tumour in nearby tissues but its deficiency promotes the growth of breast cancer cells in the bones of nude mice and alters the bone micro-environment [80]. This ability of vitamin D is supposed to be caused by the decrease expression of metalloproteinases (MMP-9) and serine proteinases (such as urokinase-type plasminogen activator and tissue-type plasminogen activator) along with the increased expression of their inhibitors

[81]. In addition, vitamin D also downregulates P-cadherin [82] and upregulates E-cadherin [83].

3.2.5. *Anti-inflammation*

Vitamin D reduces the expression of cyclooxygenase-2 (COX-2), which plays a crucial role in the synthesis of prostaglandin in many breast cancer cell lines in human. It increases the upregulation of 15-hydroxyprostaglandin dehydrogenase, an enzyme which is involved in catalysing the conversion of active prostaglandins into biologically inactive ketoderivatives [84]. Prostaglandins have been supposed to play a role in the breast cancer development and its progression [85]. Prostaglandins are secreted by the breast cancer cells or surrounding tissues promote tumour progression caused by cell proliferation, resistant to apoptosis, tumour invasion and angiogenesis [85]. An increased expression of COX-2 in breast cancer has been assumed to correlate with high-grade, large tumour size and poor prognosis [86].

3.2.6. *Anti-estrogen*

Vitamin D inhibits estrogen biosynthesis (steroidogenesis) and its biological actions [84]. Vitamin D suppresses the estrogen pathway by inhibiting the expression of gene which encodes aromatase (the enzyme which converts androgens to estrogen) [84]. Vitamin D also reduces the expression of estrogen receptor alpha (ER α -) [87]. The combined actions of vitamin D can decrease the estrogen and the receptor, which mediates their signalling in the prevention of breast cancer.

3.3. Vitamin D deficiency and breast cancer

The half-life of circulating vitamin D is approximately about 2–3 weeks which is a better indicator of blood vitamin D. Active vitamin D3 (1,25(OH)₂D₃) is locally synthesized from its precursor (25-(OH)D₃) in almost all body cells because of the universal presence of 1 α -hydroxylases in all cell type including breast [88]. So, the deficiency of 1- α hydroxylase may augment the deficiency of vitamin D and thereby associated with increased breast cancer risk and mortality [89].

Serum vitamin D concentrations and vitamin D supplementations are the independent predictors of breast cancer risk. Serum level of vitamin D of more than 50 ng/ml is associated with the 50% lower risk of breast cancer in women than serum values less than 30 ng/ml [90, 91]. In addition, breast cancer risk reduces in the pre-menopausal women who consume calcium and vitamin D orally [92]. Locally advanced breast cancer patients have more severe vitamin D deficiency than those with early-stage disease [93].

Deficiency of vitamin D is related with secondary hyperparathyroidism, which causes increased bone resorption, release of calcium from bones osteoclasts into the blood and may exacerbate osteoporosis with subsequent harsh effects on bone mineral density (BMD). In breast cancer patients, osteopenia and osteoporosis mostly occur because of early menopause and vitamin D deficiency, which is then augmented by chemotherapy and hormone replacement therapy [94]. Therefore, breast cancer patients are necessary to suffer a baseline metabolic

bone evaluation along with circulating vitamin D levels and bone mineral densitometry [94, 95].

Vitamin D deficiency is also associated with the recurrence, tumour size and death from breast cancer. It means that having enough amount of vitamin D may be able to keep a cancer from getting worse. In fact a recent meta-analysis concluded that high circulating level of vitamin D is weakly related with breast cancer incident; however, strong association was found with better breast cancer survival [89]. So, the maintenance of an optimal vitamin D status at the time of diagnosis and during 1-year follow-up period is necessary for improving survival of breast cancer patient.

There are four types of studies which illustrated whether exposure of ultraviolet B (UVB) radiations and low levels of vitamin D decrease the risk of breast cancer.

3.3.1. Geographical studies

In these studies, the geographical variation in the incidence or mortality of breast cancer is compared statistically with solar UVB radiations. The lower breast cancer incidence rate was found in the regions of high solar UVB radiations such as in Australia, China, France, Nordic countries, Spain and the United States [96].

3.3.2. Observational studies

Observational studies do comparison of vitamin D levels with the incidence of breast cancer among cases and controls. There are two categories of observational studies:

1. The studies in which vitamin D levels is measured near the time of breast cancer diagnosis are called case-control studies.
2. The studies in which vitamin D is measured at the time of women enrolment in studies prior to the breast cancer diagnosis are called nested case-control studies.

Only the case-control studies have reported that low levels of vitamin D are associated with breast cancer risk [97]. The reason why nested case-control studies have not reported the same results may be due to

1. breast cancer develops very rapidly, and
2. without supplementation, vitamin D levels tend to change little over time.

Observational studies have also documented that those females have higher vitamin D levels at the time of diagnosis live longer as compared to those with low vitamin D levels [46, 96]. In addition, the chances of mortality are higher in black women after diagnosis of breast cancer than in white women.

3.3.3. Laboratory studies

Laboratory studies have focused on the mechanisms of vitamin D in the contribution of reduced risk of breast and other cancer types. According to these studies, vitamin D allows

the cells to stay alive if they are the right type and present at the right place, or it helps the cells to commit suicide (apoptosis) if cells are not the right type or not present at the right place. Vitamin D also reduces the formation of blood vessels around tumours and decreases the ability of tumours to invade [98]. According to the randomized controlled trials, vitamin D reduced the risk of cancer, including breast cancer [99, 100].

4. Vitamin D receptor gene

The human VDR (hVDR) gene is located at long arm of chromosome 12 bands 13-14 (12q13-14) [101, 102]. The gene is 75 kb long and contains 11 exons [103]. This gene is divided into three regions: one coding region and two non-coding regions.

4.1. Non-coding regions

The 5' promoter region of VDR lacks initiator (TATA and CAAT boxes) and is rich in GC content. It provides the putative site for binding of many transcription factors [103]. The promoter region is present at exon 1(1a, 1b, 1c, 1d, 1e, 1f). The promoter region facilitates the transcription activity of VDR target gene. The 3' UTR contains poly (A) repeats, which is reported to be associated with the mRNA stability.

4.2. Coding region

Coding region comprises of exon 2–9. Exon 2, which have translation start codons, contains DNA-binding site, whereas exon 7, 8 and 9 have ligand (vitamin D) binding site [104].

5. Single nucleotide polymorphism (SNP)

Polymorphism is defined as the presence of two or more clearly different phenotypic variants of a particular DNA sequence in the same population of a species. The most common form of polymorphism is the single nucleotide polymorphism in which variation occurs at a single base pair usually present in approximately 1% of the population. These types of changes can be present in non-coding region of genes and in introns, which would not affect the translation of proteins, but these changes can affect the degree of gene expression and levels of proteins. The changes can also be present in coding regions of DNA or exons resulting in the formation of an altered protein sequence. Sometimes variation in exons do not cause the change in the structure of protein called synonymous polymorphisms.

These changes often produce or eliminate restriction sites for endonuclease to digest the DNA. As a result, fragments of DNA with a different length will be obtained which can be identified by gel electrophoresis. This process is called restriction fragment length polymorphisms (RFLPs). The produced fragments will be the undigested fragments, which is homozygous dominant, whereas the digested fragments are heterozygous and homozygous recessive.

Sometimes polymorphic alleles are linked with each other and within a population in non-random proportion is known as linkage disequilibrium (LD), [105] and the combination of alleles (blocks) or set of SNPs present on the same chromosome which tends to be inherited together is termed as haplotype. The size of these blocks is different ranging between 10 and 20 kb and could be important in determining the reason of genetic disorder.

6. SNPs in the VDR gene

The variation in the 5'-promoter region of VDR gene can change the sequence of mRNA as well as protein levels, whereas alteration in 3' UTR sequence can disturb the stability of mRNA thereby affecting the efficacy of translated protein. Some SNPs have been existed in the VDR gene, including *Cdx2* [106], *Fok1* [107], *Bsm1*, *Taq1*, *EcoRV* [108], *Apa1* [101] and *poly (A)* [109] microsatellite repeats.

6.1. *Cdx2* SNP

The VDR *Cdx2* (G-1739A) is the single nucleotide polymorphism, which was recognized by the sequence analysis of promoter region. It is an adenine to guanine (A to G) SNP situated at the promoter region of VDR gene at exon 1e. It was initially reported to be located at the 3731 bp upstream exon 1a of promoter region of VDR gene among Japanese women [106], but later identified to be located at 1739 kbp upstream of 1e exon just 2 kb away from the exon 1a among many ethnic population [110]. It is the binding region of *Cdx2* protein, a most important intestine-specific caudal-related homeodomain protein, which increases the transcription of VDR. When A allele is present in *Cdx2* promoter, the *Cdx2* protein is bound more strongly as compared to when a G allele is present. The A allele stimulates the initiation of transcription, whereas G allele inhibits [106].

6.2. GATA SNP

GATA (A-1012G) is located at exon 1a in the core sequence of DNA called AGATAT [111]. It provides the binding of GATA protein and the binding site is present in A allele and absent in G allele. The mechanism of this polymorphism is not identified yet; however, this polymorphism alters the immune responses to cancer cells. A allele is responsible to reduce cytotoxic response to cancer cells. In addition, it is also an important element that if the transcription is begun in exon 1a or 1d. In presence of G allele, exon 1d comprises an alternate start codon which results in a formation of N-ter extended protein called VDRB1. G allele is most likely associated with the VDRB1 (long) protein, whereas A allele is related with the VDRA (short) protein.

6.3. *Fok1* SNP

Fok1 polymorphism is also called start codon polymorphism (SCP). It is a thymine to cytosine (ATG to ACG) polymorphism located at the 10 bp upstream 5' end of exon 2 on the DNA-

binding domain, which results in a formation of more active transcription factor that is three amino acids shorter [103, 112]. Those individuals who have ACG sequence in the start codon, the initiation of translation occurs at the second ATG site which results in a formation of three less amino acids at NH2 terminus containing 424 amino acids. If the initiation occurs at first ATG sequence, it produces full-length VDR protein containing 427 amino acids. In the presence of restriction site, alleles are designated as 'f', whereas its absence is designated as 'F' (active form) [113]. The restriction recognition site of *Fok1* is 5'-GGATG*-3'; 3'-CCTAC*-5' and enzyme cleaves 9/13 nucleotide downstream of the recognition site.

6.4. *Bsm1-Apa1-Taq1* SNP

Most of the functional sequence variants identified near the 3' region of VDR gene were *Bsm1*, *Apa1* and *Taq1* SNP. These SNPs are in linkage disequilibrium with each other and are located in the same haplotype block. Therefore, these SNPs may have the potential to influence the mRNA stability. The *Apa1* and *Bsm1* are located at intron 8, whereas *Taq1* is located at exon 9 [114].

The presence of restriction enzyme site in these SNPs is designated as lower case letter such as b, a and t, whereas absence is designated as upper case letter including B, A, T. The restriction site for *Bsm1* is 5'-GAATGCN*-3', *Apa1* is 5'-GGGCC*C-3' and *Taq1* is 5'-T*CGA-3'.

6.5. *Poly (A)* repeats

Poly (A) tail is a variable number of tandem repeats (VNTR) or short tandem repeats (STR) containing variable numbers of adenine nucleotide present at the 3' UTR of VDR. *Poly (A)* is also linked with *Bsm1*, *Apa1* and *Taq1* polymorphisms and also involved in the mRNA stability of VDR. It varies in length and can be divided into two types:

1. The long (L) *Poly (A)* sequence in which 18–24 adenine nucleotide is present, and
2. The short (S) *Poly (A)* sequence in which 13–17 adenine nucleotide is present.

Because all four polymorphisms (*Bsm1*, *Apa1*, *Taq1* and *Poly (A)*) are present in close proximity on the VDR gene, strong linkage disequilibrium exists among them. The two most common haplotypes are:

1. baTL haplotype in which *Bsm1* and *Apa1* restriction sites are present, whereas *Taq1* site is absent along with the presence of long *Poly (A)* repeats.
2. BA tS haplotype *Bsm1* and *Apa1* restriction sites are absent, whereas *Taq1* site is absent along with the presence of short *Poly (A)* repeats [115].

The baTL haplotype is reported to be associated with the increase incidence of breast cancer [116].

7. VDR gene polymorphisms and breast cancer

VDR gene polymorphism is associated with the breast cancer risk [117–125] but insufficient data are available to find the relationship with breast cancer risk [126]. The studies have pointed out allelic variations in VDR gene, such as *Cdx2*, *Fok1*, *Bsm1*, *Taq1*, *Apa1* and *Poly A* in different ethnic groups with breast cancer incidence with contradictory results [117, 118, 121, 126].

7.1. *Cdx2* polymorphism and breast cancer

The contradictory observations were reported on the association of *Cdx2* polymorphism and breast cancer susceptibility [125]. Recently, a meta-analysis has documented that *Cdx2* polymorphism is linked with breast cancer susceptibility only in Africans [127]. However, no profound relations was observed between *Cdx2* polymorphism and breast cancer risk among Pakistani population [126].

7.2. *Fok1* polymorphism and breast cancer

Fok1 polymorphism contain large consensus sequence has no relationship with breast cancer incidence [116, 117]. But the association between *Fok1* polymorphism and breast cancer was reported in several ethnic groups [113, 120], mainly in Caucasians [128, 129]. Nemenqani et al. [121] found that *Fok1* polymorphism is associated with the ER and PR status of breast cancer and described that *Fok1* polymorphism has a significant interaction with the ER status but not with PR status of breast cancer.

7.3. *Bsm1* polymorphism and breast cancer

Bsm1 polymorphism is the most important functional VDR gene polymorphism, which is found to be associated with the risk of developing breast cancer [124]. However, it has also been documented that there is no relation between *Bsm1* and breast cancer [119]. Rollison et al. [123] describe that *Bsm1* is involved to alter the vitamin D intake and overall breast cancer risk. McCullough et al. [130] found that B allele of *Bsm1* decreases breast cancer incidence by 20%.

7.4. *Taq1* polymorphism and breast cancer

Many case-control studies suggested that *Taq1* polymorphism is not associated with breast cancer risk [119–121]. But it has been reported that *Taq1* is one of the functional polymorphisms which is linked with increased breast cancer incidence [131, 132]. A meta-analysis on large ethnic groups revealed that the *Taq1* polymorphism increases the risk of breast cancer development in Caucasians; however, no profound association was observed among Asians [133].

7.5. Other polymorphisms and breast cancer

Positive association of poly A [118] or *Apa1* [119] was found to be reported with breast cancer risk, showing a connection between polymorphism and likelihood of having a tumour.

8. Conclusion

This chapter concluded that women with breast cancer are more likely to have low vitamin D levels. Those women who do not get adequate vitamin D may be more likely to develop breast cancer later in life as compared to those who have higher vitamin D levels, who are less likely to develop breast cancer and less likely to die from breast cancer.

Because of the broad spectrum of vitamin D effects on mammary tissue, it is suggested to be a most important physiological growth regulator of mammary gland and could be a potential therapeutic agent. Additionally, due to the expression of VDR to a higher extent on breast epithelial cells, vitamin D signalling should also be monitored during breast cancer treatment. Since breast cancer is a complex disease which may or may not be associated with the decreased vitamin D levels or VDR polymorphisms. However, the functions and role of vitamin D and VDR cannot be neglected during breast cancer treatment.

Author details

Mehir un Nisa Iqbal and Taseer Ahmed Khan*

*Address all correspondence to: takhan@uok.edu.pk

Department of Physiology, University of Karachi, Karachi, Pakistan

References

- [1] Patani N, Martin LA, Dowsett M. Biomarkers for the clinical management of breast cancer: international perspective. *International Journal of Cancer*. 2013;133(1):1–13.
- [2] Stevens KN, Vachon CM, Couch FJ. Genetic susceptibility to triple-negative breast cancer. *Cancer Research*. 2013;73(7):2025–2030.
- [3] Parkin DM, Fernandez LM. Use of statistics to assess the global burden of breast cancer. *The Breast Journal*. 2006;12 Suppl 1:S70–S80.
- [4] Zahl PH, Maehlen J, Welch HG. The natural history of invasive breast cancers detected by screening mammography. *Archives of Internal Medicine*. 2008;168(21):2311–2316.
- [5] Roa BB, Boyd AA, Volcik K, Richards CS. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. *Nature Genetics*. 1996;14(2):185–187.
- [6] Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected

in case series unselected for family history: a combined analysis of 22 studies. *American Journal of Human Genetics*. 2003;72(5):1117–1130.

- [7] Ahmed R, Shaikh, H., and Hasan, S.H Is carcinoma of breast a different disease in Pakistani population? *Journal of Pakistan Medical Association*. 1997;47:114–116.
- [8] Golmard L, Delnatte C, Lauge A, Moncoutier V, Lefol C, Abidallah K, et al. Breast and ovarian cancer predisposition due to de novo BRCA1 and BRCA2 mutations. *Oncogene*. 2016;35(10):1324–7.
- [9] Peterlongo P, Chang-Claude J, Moysich KB, Rudolph A, Schmutzler RK, Simard J, et al. Candidate genetic modifiers for breast and ovarian cancer risk in BRCA1 and BRCA2 mutation carriers. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2015;24(1):308–316.
- [10] Kaaks R, Tikk K, Sookthai D, Schock H, Johnson T, Tjonneland A, et al. Premenopausal serum sex hormone levels in relation to breast cancer risk, overall and by hormone receptor status - results from the EPIC cohort. *International Journal of Cancer*. 2014;134(8):1947–1957.
- [11] Fortner RT, Eliassen AH, Spiegelman D, Willett WC, Barbieri RL, Hankinson SE. Premenopausal endogenous steroid hormones and breast cancer risk: results from the Nurses' Health Study II. *Breast Cancer Research*. 2013;15(2):R19.
- [12] Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. *American Journal of Human Genetics*. 1991;48(2):232–242.
- [13] McKenzie F, Ferrari P, Freisling H, Chajes V, Rinaldi S, de Batlle J, et al. Healthy lifestyle and risk of breast cancer among postmenopausal women in the European Prospective Investigation into Cancer and Nutrition cohort study. *International Journal of Cancer*. 2015;136(11):2640–2648.
- [14] Song N, Choi JY, Sung H, Jeon S, Chung S, Song M, et al. Tumor subtype-specific associations of hormone-related reproductive factors on breast cancer survival. *PLoS One*. 2015;10(4):e0123994.
- [15] McTiernan A, Wu L, Chen C, Chlebowski R, Mossavar-Rahmani Y, Modugno F, et al. Relation of BMI and physical activity to sex hormones in postmenopausal women. *Obesity (Silver Spring, Md)*. 2006;14(9):1662–1677.
- [16] Rinaldi S, Key TJ, Peeters PH, Lahmann PH, Lukanova A, Dossus L, et al. Anthropometric measures, endogenous sex steroids and breast cancer risk in postmenopausal women: a study within the EPIC cohort. *International Journal of Cancer*. 2006;118(11):2832–2839.
- [17] Franceschi S, La Vecchia C, Russo A, Negri E, Favero A, Decarli A. Low-risk diet for breast cancer in Italy. *Cancer Epidemiology, Biomarkers & Prevention : a publication*

of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 1997;6(11):875–879.

- [18] Longnecker MP, Newcomb PA, Mittendorf R, Greenberg ER, Willett WC. Intake of carrots, spinach, and supplements containing vitamin A in relation to risk of breast cancer. *Cancer Epidemiology, Biomarkers & Prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 1997;6(11):887–892.
- [19] Pantavos A, Ruiter R, Feskens EF, de Keyser CE, Hofman A, Stricker BH, et al. Total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk: the Rotterdam Study. *International Journal of Cancer*. 2015;136(9):2178–2186.
- [20] Miller PE, Snyder DC. Phytochemicals and cancer risk: a review of the epidemiological evidence. *Nutrition in Clinical Practice: official publication of the American Society for Parenteral and Enteral Nutrition*. 2012;27(5):599–612.
- [21] Aune D, Chan DS, Vieira AR, Navarro Rosenblatt DA, Vieira R, Greenwood DC, et al. Dietary compared with blood concentrations of carotenoids and breast cancer risk: a systematic review and meta-analysis of prospective studies. *The American Journal of Clinical Nutrition*. 2012;96(2):356–373.
- [22] Rose DP, Goldman M, Connolly JM, Strong LE. High-fiber diet reduces serum estrogen concentrations in premenopausal women. *The American Journal of Clinical Nutrition*. 1991;54(3):520–525.
- [23] Ferrari P, Rinaldi S, Jenab M, Lukanova A, Olsen A, Tjønneland A, et al. Dietary fiber intake and risk of hormonal receptor-defined breast cancer in the European Prospective Investigation into Cancer and Nutrition study. *The American Journal of Clinical Nutrition*. 2013;97(2):344–353.
- [24] Murthy NS, Mukherjee S, Ray G, Ray A. Dietary factors and cancer chemoprevention: an overview of obesity-related malignancies. *Journal of Postgraduate Medicine*. 2009;55(1):45–54.
- [25] Thune I, Brenn T, Lund E, Gaard M. Physical activity and the risk of breast cancer. *New England Journal of Medicine*. 1997;336(18):1269–1275.
- [26] Ray G, Husain SA. Role of lipids, lipoproteins and vitamins in women with breast cancer. *Clinical Biochemistry*. 2001;34(1):71–76.
- [27] Powell JE, Kelly AM, Parkes SE, Cole TR, Mann JR. Cancer and congenital abnormalities in Asian children: a population-based study from the West Midlands. *British Journal of Cancer*. 1995;72(6):1563–1569.
- [28] Jurutka PW, Whitfield GK, Hsieh J-C, Thompson PD, Haussler CA, Haussler MR. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. *Reviews in Endocrine and Metabolic Disorders*. 2001;2(2):203–216.

- [29] Bikle DD. Vitamin D and cancer: the promise not yet fulfilled. *Endocrine*. 2014;46(1): 29–38.
- [30] Shao T, Klein P, Grossbard ML. Vitamin D and Breast Cancer. *The Oncologist*. 2012;17(1):36–45.
- [31] Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *Journal of Chemical Neuroanatomy*. 2005;29(1):21–30.
- [32] Sone T, Kerner S, Pike JW. Vitamin D receptor interaction with specific DNA. Association as a 1,25-dihydroxyvitamin D₃-modulated heterodimer. *The Journal of Biological Chemistry*. 1991;266(34):23296–23305.
- [33] Orlov I, Rochel N, Moras D, Klaholz BP. Structure of the full human RXR/VDR nuclear receptor heterodimer complex with its DR3 target DNA. *The EMBO Journal*. 2012;31(2): 291–300.
- [34] Molnar F. Structural considerations of vitamin D signaling. *Frontiers in Physiology*. 2014;5:191.
- [35] Ellison TI, Dowd DR, MacDonald PN. Calmodulin-dependent kinase IV stimulates vitamin D receptor-mediated transcription. *Molecular Endocrinology*. 2005;19(9):2309–2319.
- [36] Zehnder D, Bland R, Walker EA, Bradwell AR, Howie AJ, Hewison M, et al. Expression of 25-hydroxyvitamin D₃-1alpha-hydroxylase in the human kidney. *Journal of the American Society of Nephrology*. 1999;10(12):2465–2473.
- [37] Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, et al. Extrarenal expression of 25-hydroxyvitamin D₃-1 α -hydroxylase. *The Journal of Clinical Endocrinology & Metabolism*. 2001;86(2):888–894.
- [38] Omdahl JL, Morris HA, May BK. Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. *Annual Review of Nutrition*. 2002;22:139–166.
- [39] Santagata S, Thakkar A, Ergonul A, Wang B, Woo T, Hu R, et al. Taxonomy of breast cancer based on normal cell phenotype predicts outcome. *The Journal of Clinical Investigation*. 2014;124(2):859–870.
- [40] Fu GK, Lin D, Zhang MY, Bikle DD, Shackleton CH, Miller WL, et al. Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type 1. *Molecular Endocrinology (Baltimore, Md)*. 1997;11(13):1961–1970.
- [41] Welsh J, Wietzke JA, Zinser GM, Byrne B, Smith K, Narvaez CJ. Vitamin D-3 receptor as a target for breast cancer prevention. *The Journal of Nutrition*. 2003;133(7 Suppl): 2425s–2433s.
- [42] Welsh J. Vitamin D and breast cancer: insights from animal models. *The American Journal of Clinical Nutrition*. 2004;80(6 Suppl):1721s–1724s.

- [43] Townsend K, Evans KN, Campbell MJ, Colston KW, Adams JS, Hewison M. Biological actions of extra-renal 25-hydroxyvitamin D-1 α -hydroxylase and implications for chemoprevention and treatment. *The Journal of Steroid Biochemistry and Molecular Biology*. 2005;97(1-2):103–109.
- [44] Welsh J. Targets of vitamin D receptor signaling in the mammary gland. *Journal of Bone and Mineral Research: the official journal of the American Society for Bone and Mineral Research*. 2007;22 Suppl 2:V86–V90.
- [45] Chen WY, Bertone-Johnson ER, Hunter DJ, Willett WC, Hankinson SE. Associations between polymorphisms in the vitamin D receptor and breast cancer risk. *Cancer Epidemiology, Biomarkers & Prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005;14(10):2335–2339.
- [46] Mohr SB, Gorham ED, Alcaraz JE, Kane CI, Macera CA, Parsons JK, et al. Serum 25-hydroxyvitamin D and breast cancer in the military: a case-control study utilizing pre-diagnostic serum. *Cancer Causes & Control*. 2013;24(3):495–504.
- [47] Zinser G, Packman K, Welsh J. Vitamin D(3) receptor ablation alters mammary gland morphogenesis. *Development (Cambridge, England)*. 2002;129(13):3067–3076.
- [48] Ching S, Kashinkunti S, Niehaus MD, Zinser GM. Mammary adipocytes bioactivate 25-hydroxyvitamin D(3) and signal via vitamin D(3) receptor, modulating mammary epithelial cell growth. *Journal of Cellular Biochemistry*. 2011;112(11):3393–3405.
- [49] Campos LT, Brentani H, Roela RA, Katayama ML, Lima L, Rolim CF, et al. Differences in transcriptional effects of 1 α ,25 dihydroxyvitamin D₃ on fibroblasts associated to breast carcinomas and from paired normal breast tissues. *Journal of Steroid Biochemistry and Molecular Biology*. 2013;133:12–24.
- [50] Knowler KC, Chand AL, Eriksson N, Takagi K, Miki Y, Sasano H, et al. Distinct nuclear receptor expression in stroma adjacent to breast tumors. *Breast Cancer Research and Treatment*. 2013;142(1):211–223.
- [51] Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nature Reviews Cancer*. 2007;7(9):684–700.
- [52] Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J. Inhibitory effects of 1 α ,25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. *Molecular Endocrinology*. 2001;15(8):1370–1380.
- [53] Verlinden L, Verstuyf A, Convents R, Marcelis S, Van Camp M, Bouillon R. Action of 1,25(OH)₂D₃ on the cell cycle genes, cyclin D1, p21 and p27 in MCF-7 cells. *Molecular and Cellular Endocrinology*. 1998;142(1-2):57–65.
- [54] Saunders DE, Christensen C, Wappler NL, Schultz JF, Lawrence WD, Malviya VK, et al. Inhibition of c-myc in breast and ovarian carcinoma cells by 1,25-dihydroxyvitamin D₃, retinoic acid and dexamethasone. *Anti-Cancer Drugs*. 1993;4(2):201–208.

- [55] Dhawan P, Wieder R, Christakos S. CCAAT enhancer-binding protein alpha is a molecular target of 1,25-dihydroxyvitamin D₃ in MCF-7 breast cancer cells. *The Journal of Biological Chemistry*. 2009;284(5):3086–3095.
- [56] Beildeck ME, Islam M, Shah S, Welsh J, Byers SW. Control of TCF-4 expression by VDR and vitamin D in the mouse mammary gland and colorectal cancer cell lines. *PLoS One*. 2009;4(11):e7872.
- [57] Campbell MJ, Gombart AF, Kwok SH, Park S, Koeffler HP. The anti-proliferative effects of 1 α ,25(OH)₂D₃ on breast and prostate cancer cells are associated with induction of BRCA1 gene expression. *Oncogene*. 2000;19(44):5091–5097.
- [58] Simboli-Campbell M, Narvaez CJ, Tenniswood M, Welsh J. 1,25-Dihydroxyvitamin D₃ induces morphological and biochemical markers of apoptosis in MCF-7 breast cancer cells. *The Journal of Steroid Biochemistry and Molecular Biology*. 1996;58(4):367–376.
- [59] Narvaez CJ, Welsh J. Role of mitochondria and caspases in vitamin D-mediated apoptosis of MCF-7 breast cancer cells. *The Journal of Biological Chemistry*. 2001;276(12):9101–9107.
- [60] Hoyer-Hansen M, Nordbrandt SP, Jaattela M. Autophagy as a basis for the health-promoting effects of vitamin D. *Trends in Molecular Medicine*. 2010;16(7):295–302.
- [61] Koren R, Hadari-Naor I, Zuck E, Rotem C, Liberman UA, Ravid A. Vitamin D is a prooxidant in breast cancer cells. *Cancer Research*. 2001;61(4):1439–1444.
- [62] Ravid A, Rocker D, Machlenkin A, Rotem C, Hochman A, Kessler-Icekson G, et al. 1,25-Dihydroxyvitamin D₃ enhances the susceptibility of breast cancer cells to doxorubicin-induced oxidative damage. *Cancer Research*. 1999;59(4):862–867.
- [63] Doroshov JH, Akman S, Esworthy S, Chu FF, Burke T. Doxorubicin resistance conferred by selective enhancement of intracellular glutathione peroxidase or superoxide dismutase content in human MCF-7 breast cancer cells. *Free Radical Research Communications*. 1991;12–13 Pt 2:779–781.
- [64] Manna SK, Zhang HJ, Yan T, Oberley LW, Aggarwal BB. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *The Journal of Biological Chemistry*. 1998;273(21):13245–13254.
- [65] Squadrito GL, Pryor WA. Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radical Biology & Medicine*. 1998;25(4–5):392–403.
- [66] Minotti G. Sources and role of iron in lipid peroxidation. *Chemical Research in Toxicology*. 1993;6(2):134–146.
- [67] Denu JM, Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry*. 1998;37(16):5633–5642.

- [68] Meplan C, Richard MJ, Hainaut P. Redox signalling and transition metals in the control of the p53 pathway. *Biochemical Pharmacology*. 2000;59(1):25–33.
- [69] Lander HM. An essential role for free radicals and derived species in signal transduction. *FASEB Journal: official publication of the Federation of American Societies for Experimental Biology*. 1997;11(2):118–124.
- [70] Finkel T. Oxygen radicals and signaling. *Current Opinion in Cell Biology*. 1998;10(2):248–253.
- [71] Sun Y, Oberley LW. Redox regulation of transcriptional activators. *Free Radical Biology & Medicine*. 1996;21(3):335–348.
- [72] Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. *FASEB Journal: official publication of the Federation of American Societies for Experimental Biology*. 1996;10(7):709–720.
- [73] Nakamura H, Nakamura K, Yodoi J. Redox regulation of cellular activation. *Annual Review of Immunology*. 1997;15:351–369.
- [74] Hampton MB, Fadeel B, Orrenius S. Redox regulation of the caspases during apoptosis. *Annals of New York Academy of Sciences*. 1998;854:328–335.
- [75] Clive DR, Greene JJ. Cooperation of protein disulfide isomerase and redox environment in the regulation of NF-kappaB and AP1 binding to DNA. *Cell Biochemistry and Function*. 1996;14(1):49–55.
- [76] Stambolsky P, Tabach Y, Fontemaggi G, Weisz L, Maor-Aloni R, Siegfried Z, et al. Modulation of the vitamin D3 response by cancer-associated mutant p53. *Cancer Cell*. 2010;17(3):273–285.
- [77] Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. *Circulation Research*. 2000;87(3):214–220.
- [78] Flynn G, Chung I, Yu WD, Romano M, Modzelewski RA, Johnson CS, et al. Calcitriol (1,25-dihydroxycholecalciferol) selectively inhibits proliferation of freshly isolated tumor-derived endothelial cells and induces apoptosis. *Oncology*. 2006;70(6):447–457.
- [79] Ben-Shoshan M, Amir S, Dang DT, Dang LH, Weisman Y, Mabeesh NJ. 1alpha,25-dihydroxyvitamin D3 (Calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. *Molecular Cancer Therapeutics*. 2007;6(4):1433–1439.
- [80] Ooi LL, Zhou H, Kalak R, Zheng Y, Conigrave AD, Seibel MJ, et al. Vitamin D deficiency promotes human breast cancer growth in a murine model of bone metastasis. *Cancer Research*. 2010;70(5):1835–1844.
- [81] Koli K, Keski-Oja J. 1alpha,25-dihydroxyvitamin D3 and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. *Cell Growth & Differ-*

entiation: the molecular biology journal of the American Association for Cancer Research. 2000;11(4):221–229.

- [82] Pendas-Franco N, Gonzalez-Sancho JM, Suarez Y, Aguilera O, Steinmeyer A, Gamallo C, et al. Vitamin D regulates the phenotype of human breast cancer cells. *Differentiation; research in biological diversity*. 2007;75(3):193–207.
- [83] Lopes N, Carvalho J, Duraes C, Sousa B, Gomes M, Costa JL, et al. 1Alpha,25-dihydroxyvitamin D3 induces de novo E-cadherin expression in triple-negative breast cancer cells by CDH1-promoter demethylation. *Anticancer Research*. 2012;32(1):249–257.
- [84] Krishnan AV, Swami S, Peng L, Wang J, Moreno J, Feldman D. Tissue-selective regulation of aromatase expression by calcitriol: implications for breast cancer therapy. *Endocrinology*. 2010;151(1):32–42.
- [85] Wang D, Dubois RN. Cyclooxygenase-2: a potential target in breast cancer. *Seminars in Oncology*. 2004;31(1 Suppl 3):64–73.
- [86] Ristimaki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Research*. 2002;62(3):632–635.
- [87] Stoica A, Saceda M, Fakhro A, Solomon HB, Fenster BD, Martin MB. Regulation of estrogen receptor-alpha gene expression by 1, 25-dihydroxyvitamin D in MCF-7 cells. *Journal of Cellular Biochemistry*. 1999;75(4):640–651.
- [88] Hewison M, Burke F, Evans KN, Lammas DA, Sansom DM, Liu P, et al. Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. *Journal of Steroid Biochemistry and Molecular Biology*. 2007;103(3–5):316–321.
- [89] Kim Y, Je Y. Vitamin D intake, blood 25(OH)D levels, and breast cancer risk or mortality: a meta-analysis. *British Journal of Cancer*. 2014;110(11):2772–2784.
- [90] Lowe LC, Guy M, Mansi JL, Peckitt C, Bliss J, Wilson RG, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. *European Journal of Cancer (Oxford, England: 1990)*. 2005;41(8):1164–1169.
- [91] Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiology, Biomarkers & Prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005;14(8):1991–1997.
- [92] Goodwin PJ, Ennis M, Pritchard KI, Koo J, Hood N. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. *Journal of Clinical Oncology*. 2009;27(23):3757–3763.

- [93] Palmieri C, MacGregor T, Girgis S, Vigushin D. Serum 25-hydroxyvitamin D levels in early and advanced breast cancer. *Journal of Clinical Pathology*. 2006;59(12):1334–1336.
- [94] Napoli N, Vattikuti S, Ma C, Rastelli A, Rayani A, Donepudi R, et al. High prevalence of low vitamin D and musculoskeletal complaints in women with breast cancer. *The Breast Journal*. 2010;16(6):609–616.
- [95] Camacho M, Martinez-Perez A, Buil A, Sigüero L, Alcolea S, López S, et al. Genetic determinants of 5-lipoxygenase pathway in a Spanish population and their relationship with cardiovascular risk. *Atherosclerosis*. 2012;224(1):129–135.
- [96] Moukayed M, Grant WB. Molecular link between vitamin D and cancer prevention. *Nutrients*. 2013;5(10):3993–4021.
- [97] Tretli S, Schwartz GG, Torjesen PA, Rødsahl TE. Serum levels of 25-hydroxyvitamin D and survival in Norwegian patients with cancer of breast, colon, lung, and lymphoma: a population-based study. *Cancer Causes & Control*. 2012;23(2):363–370.
- [98] Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *The American Journal of Clinical Nutrition*. 2007;85(6):1586–1591.
- [99] Bolland MJ, Grey A, Gamble GD, Reid IR. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. *The American Journal of Clinical Nutrition*. 2011;94(4):1144–1149.
- [100] Heaney RP. Guidelines for optimizing design and analysis of clinical studies of nutrient effects. *Nutrition Reviews*. 2014;72(1):48–54.
- [101] Faraco JH, Morrison NA, Baker A, Shine J, Frossard PM. ApaI dimorphism at the human vitamin D receptor gene locus. *Nucleic Acids Research*. 1989;17(5):2150.
- [102] Szpirer J, Szpirer C, Riviere M, Levan G, Marynen P, Cassiman JJ, et al. The Sp1 transcription factor gene (SP1) and the 1,25-dihydroxyvitamin D₃ receptor gene (VDR) are colocalized on human chromosome arm 12q and rat chromosome 7. *Genomics*. 1991;11(1):168–173.
- [103] Miyamoto K, Kesterson RA, Yamamoto H, Taketani Y, Nishiwaki E, Tatsumi S, et al. Structural organization of the human vitamin D receptor chromosomal gene and its promoter. *Molecular Endocrinology (Baltimore, Md)*. 1997;11(8):1165–1179.
- [104] Hughes MR, Malloy PJ, Kieback DG, Kesterson RA, Pike JW, Feldman D, et al. Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. *Science*. 1988;242(4886):1702–1705.
- [105] Wall JD, Pritchard JK. Haplotype blocks and linkage disequilibrium in the human genome. *Nature Reviews Genetics*. 2003;4(8):587–597.
- [106] Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K, et al. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in

- the human vitamin D receptor gene. *Journal of Bone and Mineral Research*. 2001;16(7):1256–1264.
- [107] Saijo T, Ito M, Takeda E, Huq AH, Naito E, Yokota I, et al. A unique mutation in the vitamin D receptor gene in three Japanese patients with vitamin D-dependent rickets type II: utility of single-strand conformation polymorphism analysis for heterozygous carrier detection. *American Journal of Human Genetics*. 1991;49(3):668–673.
 - [108] Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin. *Proceedings of the National Academy of Sciences of the United States of America*. 1992;89(15):6665–6669.
 - [109] Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. *Epidemiologic Reviews*. 2000;22(2):203–217.
 - [110] Fang Y, van Meurs JB, Bergink AP, Hofman A, van Duijn CM, van Leeuwen JP, et al. Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. *Journal of Bone and Mineral Research*. 2003;18(9):1632–1641.
 - [111] Merika M, Orkin SH. DNA-binding specificity of GATA family transcription factors. *Molecular and Cellular Biology*. 1993;13(7):3999–4010.
 - [112] Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, et al. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *Journal of Bone and Mineral Research*. 1997;12(6):915–921.
 - [113] Ingles SA, Haile RW, Henderson BE, Kolonel LN, Nakaichi G, Shi CY, et al. Strength of linkage disequilibrium between two vitamin D receptor markers in five ethnic groups: implications for association studies. *Cancer Epidemiology, Biomarkers & Prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 1997;6(2):93–98.
 - [114] Fang Y, van Meurs JB, d'Alesio A, Jhamai M, Zhao H, Rivadeneira F, et al. Promoter and 3'-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. *American Journal of Human Genetics*. 2005;77(5):807–823.
 - [115] Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL, Bell DA. Association of prostate cancer with vitamin D receptor gene polymorphism. *Cancer Research*. 1996;56(18):4108–4110.
 - [116] Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. *British Journal of Cancer*. 2001;85(2):171–175.
 - [117] Abd-Elsalam EA, Ismaeil NA, Abd-Alsalam HS. Vitamin D receptor gene polymorphisms and breast cancer risk among postmenopausal Egyptian women. *Tumour Biology*. 2015;36(8):6425–6431.

- [118] Colagar AH, Firouzjah HM, Halalkhor S. Vitamin D receptor poly(A) microsatellite polymorphism and 25-hydroxyvitamin D serum levels: association with susceptibility to breast cancer. *Journal of Breast Cancer*. 2015;18(2):119–125.
- [119] Guo B, Jiang X, Hu X, Li F, Chen X. Association between vitamin D receptor gene polymorphisms and breast cancer in a Chinese population. *International Journal of Clinical and Experimental Medicine*. 2015;8(5):8020–8024.
- [120] Mishra DK, Wu Y, Sarkissyan M, Sarkissyan S, Chen Z, Shang X, et al. Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African-American and Hispanic women. *PLoS One*. 2013;8(3):e57967.
- [121] Nemenqani DM, Karam RA, Amer MG, Abd El Rahman TM. Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. *Gene*. 2015;558(2):215–219.
- [122] Reimers LL, Crew KD, Bradshaw PT, Santella RM, Steck SE, Sirosh I, et al. Vitamin D-related gene polymorphisms, plasma 25-hydroxyvitamin D, and breast cancer risk. *Cancer Causes & Control*. 2015;26(2):187–203.
- [123] Rollison DE, Cole AL, Tung KH, Slattery ML, Baumgartner KB, Byers T, et al. Vitamin D intake, vitamin D receptor polymorphisms, and breast cancer risk among women living in the southwestern U.S. *Breast Cancer Research and Treatment*. 2012;132(2):683–691.
- [124] Shahbazi S, Alavi S, Majidzadeh AK, Ghaffarpour M, Soleimani A, Mahdian R. BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer. *Medical Oncology (Northwood, London, England)*. 2013;30(1):393.
- [125] Yao S, Zirpoli G, Bovbjerg DH, Jandorf L, Hong CC, Zhao H, et al. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. *Breast Cancer Research*. 2012;14(2):R58.
- [126] Iqbal M, Khan TA, Maqbool SA. Vitamin D receptor Cdx-2 polymorphism and premenopausal breast cancer risk in southern Pakistani patients. *PLoS One*. 2015;10(3):e0122657.
- [127] Zhou ZC, Wang J, Cai ZH, Zhang QH, Cai ZX, Wu JH. Association between vitamin D receptor gene Cdx2 polymorphism and breast cancer susceptibility. *Tumour Biology: the journal of the International Society for Oncodevelopmental Biology and Medicine*. 2013;34(6):3437–3441.
- [128] Shan JL, Dai N, Yang XQ, Qian CY, Yang ZZ, Jin F, et al. FokI polymorphism in vitamin D receptor gene and risk of breast cancer among Caucasian women. *Tumour Biology: the journal of the International Society for Oncodevelopmental Biology and Medicine*. 2014;35(4):3503–3508.

- [129] Wang J, He Q, Shao YG, Ji M, Bao W. Associations between vitamin D receptor polymorphisms and breast cancer risk. *Tumour Biology: the journal of the International Society for Oncodevelopmental Biology and Medicine*. 2013;34(6):3823–3830.
- [130] McCullough ML, Stevens VL, Diver WR, Feigelson HS, Rodriguez C, Bostick RM, et al. Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: a nested case-control study. *Breast Cancer Research*. 2007;9(1):R9.
- [131] Curran JE, Vaughan T, Lea RA, Weinstein SR, Morrison NA, Griffiths LR. Association of A vitamin D receptor polymorphism with sporadic breast cancer development. *International Journal of Cancer*. 1999;83(6):723–726.
- [132] Lundin AC, Soderkvist P, Eriksson B, Bergman-Jungestrom M, Wingren S. Association of breast cancer progression with a vitamin D receptor gene polymorphism. South-East Sweden Breast Cancer Group. *Cancer Research*. 1999;59(10):2332–2334.
- [133] Wang J, Eliassen AH, Spiegelman D, Willett WC, Hankinson SE. Plasma free 25-hydroxyvitamin D, vitamin D binding protein, and risk of breast cancer in the Nurses' Health Study II. *Cancer Causes & Control*. 2014;25(7):819–827.

