Published in final edited form as:

Mol Cell Endocrinol. 2017 September 15; 453: 88–95. doi:10.1016/j.mce.2017.04.026.

Function of the vitamin D endocrine system in mammary gland and breast cancer

JoEllen Welsh, PhD

Empire Innovations Professor, University at Albany Cancer Research Center, 1 Discovery Drive, Rensselaer, NY 12144

Abstract

The nuclear receptor for $1\alpha,25$ -dihydroxycholecalciferol (1,25D), the active form of vitamin D, has anti-tumor actions in many tissues. The vitamin D receptor (VDR) is expressed in normal mammary gland and in many human breast cancers suggesting it may represent an important tumor suppressor gene in this tissue. When activated by 1,25D, VDR modulates multiple cellular pathways including those related to energy metabolism, terminal differentiation and inflammation. There is compelling pre-clinical evidence that alterations in vitamin D status affect breast cancer development and progression, while clinical and epidemiological data are suggestive but not entirely consistent. The demonstration that breast cells express CYP27B1 (which converts the precursor vitamin D metabolite 25D to the active metabolite 1,25D) and CYP24A1 (which degrades both 25D and 1,25D) provides insight into the difficulties inherent in using dietary vitamin D, sun exposure and/or serum biomarkers of vitamin D status to predict disease outcomes. Emerging evidence suggests that the normally tight balance between CYP27B1 and CYP24A1 becomes deregulated during cancer development, leading to abrogation of the tumor suppressive effects triggered by VDR. Research aimed at understanding the mechanisms that govern uptake, storage, metabolism and actions of vitamin D steroids in normal and neoplastic breast tissue remain an urgent priority.

Introduction to Vitamin D and Breast Cancer

Breast cancer is a heterogeneous disease with multiple subtypes, each of which have distinct cells of origin and etiology (1). As such, there is no single identified cause of breast cancer, and treatment strategies are increasingly directed towards the underlying molecular defects unique to each subtype. The vast majority of breast cancers arise from epithelial cells in either the ducts or lobules that have sustained genetic and epigenetic alterations leading to aberrant growth control and disruption of intracellular signaling at the tissue level. Physiological and pathological influences such as hormonal milieu, obesity, diet, age, and inflammation contribute to disease progression through multiple mechanisms. Although survival rates are increasing for breast cancer overall, certain subtypes, such as triple negative breast cancer (TNBC) and inflammatory breast cancer (IBC), are especially

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

aggressive and associated with drug resistance leading to poor survival. Overall, breast cancer is associated with a 20% mortality rate within the first 5 years after diagnosis. The high prevalence of breast cancer (over 250,000 cases diagnosed annually in the US) warrants continued research into more effective treatment options and prevention strategies.

Nuclear receptors, such as those activated by the steroid hormones estrogen and progesterone, are critical regulators of mammary gland development and have complex roles in breast cancer etiology. As such, these receptors represent important targets for both prevention and treatment of breast cancer. The vitamin D receptor (VDR), another member of the nuclear receptor family which is highly expressed in breast tissue, is activated by its hormonal ligand 1,25-dihydroxyvitamin D (1,25D). As described in more detail below, activation of VDR by 1,25D modulates the phenotype of normal mammary cells and breast cancer cells in culture. Dietary and pharmacologic studies in animal models of breast cancer have also provided compelling evidence for tumor suppressive actions of VDR agonists (2; 3). Deletion of the VDR gene in mice enhances the development of hyperplasias and hormone independent mammary tumors in response to chemical carcinogens and sensitizes the mammary gland to tumorigenesis driven by various oncogenes. Collectively these laboratory data suggest that the VDR acts as a tumor suppressor in mammary gland. If so, then it is logical to assume that human breast cancer risk or progression would be related to vitamin D status (typically measured as serum 25-hydroxyvitamin D [25D]). Furthermore, such an association might explain, at least in part, the geography and seasonality of breast cancer, differences in disease incidence between Caucasian and African American populations and the impact of obesity, as all of these factors are known to modulate vitamin D status (4–8). More importantly, confirmation of a link between vitamin D status and breast cancer would raise the possibility that breast cancer development or survival could be modified by strategies to increase serum 25D such as food fortification, use of dietary supplements or prudent increases in sun exposure. While a substantial amount of epidemiological and clinical data support such associations, the cumulative data is inconsistent (4; 9–12). In many epidemiological studies, the impact of vitamin D status appeared to be limited to sub-groups in a cohort with specific attributes, such as pre- or post-menopausal status, presence of obesity, specific tumor subtype, race/ethnicity or genetic polymorphisms. With respect to polymorphisms, considerable effort has been directed at defining genetic determinants of serum 25D that underlie response to supplementation and how these may affect chronic disease incidence and progression. For breast cancer, extensive analyses have yielded conflicting data as recently reviewed (13).

Only a few vitamin D supplementation trials with breast cancer as a dedicated end-point have been conducted, and these have suffered from major limitations (underpowered, not specific for vitamin D, inappropriate dose, etc) as discussed by Lappe and Heaney (14). Despite these limitations, some analyses of the data from these trials support a benefit of vitamin D supplementation on breast cancer incidence or severity (15–17), although often this benefit is explained or modified by other lifestyle factors (ie, physical activity, BMI or hormone replacement therapy).

This review will focus on recent cellular and molecular studies that have demonstrated the importance of autocrine/paracrine vitamin D metabolism in breast cancer and identified

novel aspects of VDR signaling in breast cancer. Areas in which additional research efforts are needed will be highlighted.

VDR expression and function in normal and neoplastic breast tissue

Although it has long been recognized that the VDR is expressed in normal mammary tissue and in breast cancers, detailed insight into its distribution, regulation and function are still emerging. With the public availability of large genomic datasets such as The Cancer Genome Atlas (TCGA), it is now possible to evaluate the frequency of genomic *VDR* changes (mutations, amplifications, deletions and mRNA expression profiles) in large cohorts of human cancers (18). Examination of the TCGA METABRIC dataset (which contains over 2500 samples), indicates that only 4% of invasive human breast tumors exhibit alterations in *VDR* sequence or expression (Figure 1). In the few cases where VDR alterations were observed, the most frequent change was an unexpected up-regulation of VDR mRNA. While the significance of this finding is unclear, the TCGA data indicates that the majority of breast cancers do not exhibit loss of function mutations or reduced expression of the VDR gene.

Changes in VDR protein expression during development and tumorigenesis have also been extensively studied. In mouse mammary gland, VDR protein is expressed in all major cell types (basal and luminal epithelial cells, cap cells, stromal cells) but its expression is not temporally or spatially uniform (19; 20). VDR is developmentally regulated with induction during pubertal growth and peak expression during pregnancy and lactation. In the epithelial compartment of the murine gland, the strongest VDR staining is found in the differentiated luminal cells suggesting that VDR expression is inversely associated with proliferation. Similarly, high content multiplex immunofluorescent analysis of normal human breast epithelium (21) indicated that VDR positive cells are enriched in the differentiated luminal cell layer and do not co-localize with proliferating (Ki67 positive) cells. This study also examined co-localization of VDR, estrogen receptor (ER) and androgen receptor (AR) in a panel of breast cancers and correlated receptor expression with patient outcomes. VDR expression was detected in >90% of cells of ER+ and HER2+ tumors but at markedly lower frequency in TNBCs. Examination of over 3,000 human breast tumors revealed that patient outcomes were best for tumors that expressed all three hormone receptors (VDR, ER, AR) and worst for those that expressed none of the receptors. Additional experiments demonstrated that combining drugs that target several of these receptors resulted in additive responses in breast cancer cells *in vitro* indicating the translational potential of these findings.

Multiple clinical studies have examined whether breast tumor VDR expression is independently predictive of patient outcome, with conflicting results (Table 1). An early study of 136 patients with primary breast cancer reported that women with VDR negative tumors relapsed significantly earlier than women with VDR positive tumors (22). In a more recent study of >1000 human breast cancers of mixed sub-types, Al-Azhri et al (23) reported that VDR expression was inversely related to tumor size, hormone receptor negativity and TNBC subtype. This is consistent with data showing that VDR protein expression declines in highly aggressive tumors (24; 25) and is deregulated by oncogenic transformation (26–

28). However, in the Al-Azhri study, VDR expression was not correlated with overall or breast-cancer specific survival.

Mechanisms underlying the regulation of VDR expression in mammary gland and breast cancer have not been well studied. During pregnancy and lactation, VDR expression increases in mammary gland, an effect which is likely mediated via lactogenic hormones (29), although no specific mechanisms were identified. Binding of the 1,25D ligand to VDR has long been recognized to stabilize the protein against degradation (30), and 1,25D also transcriptionally up-regulates the VDR gene (31; 32). Other biological factors that have been shown to up-regulate VDR gene expression include estrogen, phytoestrogens and retinoids (33; 34). The VDR gene structure is complex, with at least four promoters and four enhancer elements described to date (31; 35). Using publicly available data from GenBank, Saccone et al (35) reported ten experimentally verified and two annotated but non-confirmed VDR transcripts, highlighting the complexity of VDR gene expression. Many of the identified transcripts are thought to be expressed in a tissue-specific fashion but the physiological or pathological significance of these variants remains to be experimentally explored. It should be noted that a caveat to the TCGA data analysis (Table 1) is that it does not necessarily distinguish these alternative VDR transcripts. To date, only one study (36) has investigated the spectrum of VDR transcript expression in breast cancer. Using primer sets specific for 5' transcript variants, this study demonstrated that the levels and splice variants of VDR are markedly different in primary breast cancer compared to normal adjacent tissue. Specifically, breast cancers expressed higher levels of shorter VDR transcripts which if translated would code for truncated (likely non-functional) VDR proteins. Interestingly, these shorter VDR transcripts were barely detectable in normal tissue suggesting their presence in breast cancer might contribute to the pathological process. Fortunately, the aberrant expression of the shorter VDR transcripts was similar in biopsy tissue and in established breast cancer cell lines, which should facilitate detailed *in vitro* analysis of their function in the context of cellular sensitivity to 1,25D.

Epigenetic regulation of the VDR by methylation, histone modification and non-coding RNAs has also been reported and a few studies have been conducted in breast cancer models. For example, Marik et al (36) used quantitative methylation-specific PCR to demonstrate hypermethylation of CpG islands of VDR promoter regions in primary breast cancers and the absence of methylation in normal breast tissue. As noted above, this group reported distinct truncated VDR transcripts in breast tumors and therefore they investigated whether promoter methylation might contribute to expression of VDR variant transcripts. Treatment of breast cancer cells with the demethylating agent 5' deoxy-azacytidine restored expression of the active longer transcripts of VDR, suggesting that reversal of VDR methylation through pharmacologic approaches may restore breast cancer cell susceptibility to 1,25D. In addition to methylation, histone modifications have been demonstrated to influence VDR expression in a tissue-specific fashion (35), but further studies to clarify the extent to which this epigenetic mechanism contributes to VDR regulation in breast tissue will be required. There is also some evidence for control of VDR expression by non-coding RNAs, one of which (miR-125b) has been experimentally confirmed to alter VDR protein levels in MCF-7 breast cancer cells (37). Collectively, these data indicate that control of VDR is complex, with environmental (ie, hormones, ligands, cytokines), genetic and

epigenetic factors interacting to potentially drive expression of distinct variants or isoforms in different tissues. Given the importance of VDR in mediating the pro-differentiating and anti-tumor effects of 1,25D, elucidation of factors that specifically regulate VDR in breast tissue should be a research priority.

Vitamin D storage and uptake in mammary gland and breast cancers

In the context of cancer, the presence of VDR in tumor cells supports the concept that vitamin D metabolites accumulate in tumors to elicit anti-cancer effects, and that reduced delivery of VDR ligands secondary to deficiency accelerates tumor growth. Indeed, dietary vitamin D deficiency is associated with increased tumor burden in animal models (38–40), but the available data in human populations is less convincing. Individual differences in handling, metabolism and action of vitamin D, all of which are highly dependent on environment and genotype, are major confounders in human studies that have been controlled in animal studies. Even in animal studies, however, the specific vitamin D metabolites that act on pre-neoplastic or transformed cells to mediate anti-tumor effects have not been precisely defined. One study indicated that chronic systemic administration of 25D via implanted mini-pumps lead to accumulation of both 25D and 1,25D in transgenic mouse mammary tumors, supporting the concept that tumors accumulate 25D and synthesize 1,25D (41). However, no studies have systematically measured accumulation of vitamin D or 25D in breast tissue or tumors as a function of dietary vitamin D or in relation to storage in normal body pools. Thus it is currently unclear whether, under states of deficiency, vitamin D steroids are preferentially trafficked to/internalized by tumor cells as opposed to being utilized to replenish physiological stores. In fact, little is known about the mechanisms by which 25D is trafficked to and/or released from stores. Circulating vitamin D metabolites are bound to the vitamin D binding protein (DBP) which is encoded by the GC gene. DBP-bound 25D has been shown to be internalized by the megalin-cubilin complex in breast cell models *in vitro* (42). If this active uptake process occurs in breast tissue *in vivo*, then genetic polymorphisms in GC (43–45), which have been shown to affect circulating 25D levels, may well affect the uptake of the 25D-DBP complex into tissues. Mechanistic studies on vitamin D disposition between tissue pools in tumor bearing animals may help to clarify some of these important issues.

Role of CYP27B1 in mammary gland and breast cancer

Normal mammary gland expresses the enzymatic machinery to both synthesize and catabolize 1,25D (46). Given that 25D is available to and likely stored in the breast, the observation that both CYP27B1 and CYP24A1 are expressed and functional in mammary epithelial cells highlights the importance of vitamin D metabolism in dictating the availability of 1,25D to the VDR. Initial evidence for mammary expression of CYP27B1 included PCR analyses, immunohistochemistry and enzyme assays to demonstrate the presence and function of CYP27B1 in mouse mammary gland, benign breast tissue, breast tumors and established human breast cancer cell lines (20; 47–49). Subsequently, Kemmis et al (50) demonstrated expression of CYP27B1 and synthesis of 1,25D from 25D in immortalized non-transformed human mammary epithelial cells (HMEC). Introduction of oncogenic mediators such as SV40 or RAS significantly reduced the expression of

CYP27B1 in mammary epithelial cells leading to abrogation of 1,25D synthesis and reduced cellular sensitivity to growth inhibition by 25D (27). These observations support the concept that 25D can mediate anti-tumor actions in breast epithelial cells *in vitro* but that significant down regulation of either VDR and CYP27B1 during transformation leads to cellular resistance to these actions. However, for the most part, the clinical data does not support the loss of CYP27B1 expression during breast oncogenesis in patients (Table 1). Typical studies have utilized biopsies to compare mRNA or protein expression in breast cancers and adjacent normal tissue or cases versus controls, with most data indicating retention of CYP27B1 in tumors, albeit often at a reduced level (24; 47; 48; 51–54). Less than 2% of breast cancers annotated in The Cancer Genome Atlas datasets exhibit genomic alterations in CYP27B1 (Table 1). However, these studies have not ruled out the possibility that altered splice variants of CYP27B1 (which have been detected in breast cancer cells) or deregulation of protein synthesis, stability or function could be associated with tumorigenesis (55; 56).

CYP27B1 expression and function (determined as conversion of 25D to 1,25D) has also been detected in adipocytes isolated from human breast and in murine mammary adipocytes grown in organoid culture (57). Co-culture experiments and characterization of adipose-specific VDR null mice have provided evidence that breast adipocytes bioactivate 25D to 1,25D, signal via VDR within the adipocytes and release soluble factors that act on adjacent breast epithelial cells (58). Given that adipose tissue is a major storage depot for vitamin D steroids, paracrine VDR signaling between breast adipocytes and adjacent epithelial cells likely plays a significant role in both normal development and carcinogenesis, as has been demonstrated for other steroid hormones active in breast.

Proof of principle that CYP27B1 in the breast confers tumor suppressive effects was recently provided in mouse models. In one study, administration of 25D to polyoma middle T antigen-mouse mammary tumor virus breast cancer model (PyMT-MMTV) elevated tumor 1,25D levels, delayed tumor development and decreased lung metastasis (41). In a second study using the same model, Li et al (59) reported that deletion of *Cyp27b1* from the mammary epithelium accelerated tumorigenesis, up-regulated cell proliferation, angiogenesis, cell cycle progression and survival markers in tumors and elevated several known oncogenic pathways (AKT, NF- κ B and STAT3).

While it is clear that CYP27B1 manipulation exerts effects on breast tumor biology, virtually nothing is known about the regulation of CYP27B1 with respect to normal physiology of the mammary gland. Like VDR, CYP27B1 expression in breast tissue is enriched during pregnancy and lactation (60), but neither the hormones nor mechanisms that trigger this up-regulation have been identified. Although the prevailing opinion is that the regulation of CYP27B1 in extra-renal tissues, including mammary gland, is distinct from that in kidney (which is primarily geared to calcium homeostasis), there is little experimental evidence to support or refute this concept.

Role of CYP24A1 in mammary gland and breast cancer

In contrast to CYP27B1, the mRNA expression of CYP24A1 is generally low in normal breast tissue. The demonstration of CYP24A1 amplification in breast cancer (61) lead to the hypothesis that CYP24A1 up-regulation would lead to excessive catabolism of 25D and 1,25D and loss of VDR tumor suppressive actions. In the large TCGA cohort (Figure 1), CYP24A1 is amplified or up-regulated in 9% of breast cancers, suggesting that enhanced vitamin D catabolism may indeed operate in a subset of tumors. Immunohistochemical analysis of biopsy samples also support this concept, with CYP24A1 protein detected in only 19% of benign breast lesions but over 50% of breast cancers (24). Using a model in which breast cancer explants are cultured *ex vivo*, Suetani et al reported that basal expression of CYP24A1 is highly variable between individuals. The heterogeneity of CYP24A1 expression in clinical samples correlates well with data from established breast cell lines (Table 1). In a comparison of six cell lines derived from normal and transformed human breast tissue, basal CYP24A1 expression was found to differ substantially (62), with high CYP24A1 expression in normal breast epithelial cell lines (hTERT-HME1 and HME) relative to the MCF10A (fibrocystic) and breast cancer cell lines such as MCF-7. In this series of cell models, higher expression of CYP24A1 did not necessarily blunt the effects of 1,25D, as cells with high CYP24A1 expression retained significant VDR activity. However, in aggressive breast cancer cells that express high levels of CYP24A1 (MB-MDA-231) Osanai and Lee demonstrated that knockdown of CYP24A1 reduced anchorage independent growth *in vitro* and abrogated tumorigenesis *in vivo* (63). Thus, more detailed analysis of CYP24A1 function in breast cancer cells (in the presence and absence of 1,25D) are warranted. The presence of CYP24A1 splice variants that may alter enzyme activity have been reported in breast cancer cell lines and tissues (64; 65), and characterization of these variants may help clarify the discrepancies noted above with respect to CYP24A1 expression in breast cancer. The regulation of CYP24A1 by factors other than 1,25D in breast cancer has not been particularly well studied, although inflammation was shown to increase, and miR125b shown to decrease, CYP24A1 *in vitro* (66; 67). Epigenetic regulation of CYP24A1 by promoter methylation has been reported in lung and prostate cancer (68; 69), but studies in breast cancer are lacking. It should also be noted that CYP24A1 was identified as a gene highly enriched in the luminal progenitor population of both human and mouse breast epithelium (70) suggesting the possibility that CYP24A1 may have an as yet unidentified role in breast biology which may or may not be VDR related.

Targets and functions of the vitamin D pathway in breast biology and carcinogenesis

Organ culture studies have demonstrated effects of 1,25D on calcium transport, casein expression and branching morphogenesis in mouse mammary gland (19; 29; 71; 72). Peng et al (49) tested the chemopreventive effects of 1,25D and 25D in mammary gland organ culture models, reporting that both vitamin D steroids suppressed the induction of precancerous lesions by the carcinogen DMBA. Demonstration of hormonal effects of 1,25D and 25D in organ culture systems indicates that vitamin D metabolites operate directly within the gland rather than secondary to systemic effects on other tissues or endocrine

pathways. More recent organ culture studies have utilized tissue from VDR null mice to demonstrate that VDR in the gland is required for the observed effects of 1,25D on branching morphogenesis (19). Collectively these data confirm that the mammary gland is a bona-fide target for canonical 1,25D-VDR signalling.

VDR ablation alters the normally tightly controlled developmental morphogenesis of the mammary gland. During the rapid pubertal growth period, elongation and branching of the mammary ducts was accelerated in VDR null mice relative to wild-type animals (19). This difference is likely attributed to increased sensitivity of VDR null tissue to hormonal stimulation, as glands from VDR null mice exhibited enhanced growth in response to exogenous estrogen and progesterone, both *in vivo* and in organ culture, compared with glands from wild-type mice. Furthermore, 1,25D inhibited growth and side branching of glands from wild-type mice but not VDR null mice. Similar differences in glandular development were observed during early pregnancy of VDR null mice (60). However, these developmental differences do not ultimately impair glandular function, as VDR null mice successfully lactate and produce milk of normal protein and calcium content. In older VDR null mice, atrophy of the mammary fat pad develops (73), leading to expansion and thickening of the ducts reminiscent of ductal ectasia in post-menopausal women. The precise mechanism for the age-related atrophy of the mammary fat pad in VDR null mice has not been established, but is not restricted to the breast, as other subcutaneous and visceral adipose depots also undergo atrophy (74).

Since the VDR is known to function as a transcription factor when bound to its ligand 1,25D, much emphasis has been placed on identification of 1,25D responsive target genes in models of breast cancer prevention or treatment. Such gene targets could potentially be useful as biomarkers of vitamin D action in the breast for epidemiological or intervention studies. The *in vitro* effects of 1,25D on gene expression have been compared in many non-transformed (ie, hTERT-HME1, HME, MCF10A) and cancer cell lines, including ER positive (MCF7 cells), ER negative (MDA-MB-231, SUM159, Hs578T) and HER2 positive (SKBR3) models. Since it is difficult to compare data generated by different labs, Beaudin et al (62) performed side-by-side comparison of the effect of 1,25D on six putative VDR target genes: CYP24A1, ITG β 3 (encodes integrin β 3), SLC1A1 (encodes a glutamate transporter), KDR (encodes a VEGF receptor), BIRC3 (encodes an apoptosis inhibitor) and GLUL (encodes glutamine synthase) in a cohort of cell lines derived from normal and pathologic human breast tissue. These six genes have diverse functions in metabolism (GLUL, SLC1A1), angiogenesis (KDR), apoptosis (BIRC3) and cell adhesion (ITG β 3) consistent with cancer preventive actions as described in more detail in Beaudin et al (62). The comparative analysis indicated that, with the exception of CYP24A1 which was induced in all cell lines, the genomic responses of immortalized cell lines derived from normal human breast were markedly distinct from that of established cell lines from diseased or cancerous breast (MCF10A, DCIS.com, SUM159PT, Hs578T, MCF-7). In general, all breast cancer cell lines were less responsive to 1,25D than the immortalized cells with respect to induction or repression of the selected genes, despite reasonably comparable VDR expression. The presence of genomic alterations such as deletions and translocations in the established cancer cell lines, which could alter the expression or function of VDR or its target genes, likely explains much of the heterogeneity in response to 1,25D. Also, since the established

breast cancer cell lines represent different molecular subtypes of the disease (75; 76), it is possible that VDR function and target gene regulation differ substantially by subtype. Not enough studies have attempted to stratify the impact of vitamin D or VDR function/ expression on tumor biology by disease subtype. In any case, these genomic profiling studies have clearly indicated that VDR target gene regulation is highly heterogeneous (2), suggesting that it may be difficult to define a vitamin D exposure “gene signature” that would be useful for breast cancer patients based solely on *in vitro* data.

These data highlight the need to assess vitamin D signaling in models that more accurately mimic the complex epithelial-stromal interactions and three dimensional morphology of breast tumors. Two groups have examined the effects of vitamin D steroids on *ex vivo* cultures of fresh biopsy tissue. Milani et al (77; 78) conducted whole genome profiling after 1,25D treatment of breast cancer slices whereas Callen’s group (79; 80) compared target gene regulation after 1,25D or 25D treatment of normal and cancerous tissue. These studies provided confirmation that breast cancers respond to 1,25D with up-regulation of CYP24A1 and reduction in cell proliferation and although CYP27B1 is expressed, cancers are less sensitive to 25D than normal tissues. Through integration of available genomic datasets from multiple breast model systems, Simmons et al (81) identified a cohort of genes regulated in explant studies that are also regulated by 1,25D in cell culture models (81). This analysis identified four genes (*CYP24A1*, *CLMN*, *EFTUD1* and *SERPINB1*) that were up-regulated by 1,25D in the tumor explants and in three breast-derived cell lines. Interestingly, there were no genes that were commonly down-regulated by 1,25D in all model systems. The four up-regulated genes, as well as *KLK6* (kallikrein-related peptidase 6) - a gene that was highly induced in both tumor explants and MCF7 cells (81) - were confirmed as 1,25D regulated in breast cancer cell lines and in a subset of human clinical samples from normal tissue and breast cancer. Observational studies have indicated that high expression of *KLK6*, *CLMN* (encodes Calmin, a membrane calponin-like protein) and *EFTUD1* (encodes elongation factor like GTPase 1, a ribosome biogenesis factor) in breast tumors promotes better survival, supporting a link between vitamin D signaling and disease outcomes (79). *SERPINB1* encodes Serpin Family B Member 1 (a proteinase which primarily functions in an anti-inflammatory capacity), but its role in breast cancer has not been well studied. As more genomic datasets become available, it should be possible to generate more extensive relevant gene signatures of vitamin D exposure that could be tested in observational and intervention studies.

Future View/Research gaps

In summary, much remains to be learned about vitamin D signaling in mammary gland and its relationship to human breast cancer. Given the heterogeneity of breast cancer and the complexity of the vitamin D endocrine system, it is not surprising that epidemiological studies have failed to find consistent correlations between vitamin D status (as measured by serum 25D) and breast cancer risk or progression. Research efforts should be directed at development of more relevant indicators of vitamin D function at the tissue level, such as specific gene signatures or local concentrations of active metabolites. Although there have been great strides in characterizing the molecular function of the VDR, the identification of novel epigenetic regulators and tissue specific alternative transcripts provides an additional

level of complexity. Detailed mapping of VDR transcripts and protein expression in the various subtypes of human breast cancer in relation to outcome may aid in identification of patient cohorts most likely to benefit from vitamin D supplementation. Other factors which have been understudied include the role of post-translational VDR modifications and the role of RXR, the heterodimer partner of VDR (in particular, the impact of retinoid ligands). Interactions between VDR and other nuclear receptors, such as ER and AR, may also be important modulators of breast cancer outcome. Combined targeting of several nuclear receptors, with or without standard therapies for each disease subtype, may prove beneficial. Better understanding of the mechanisms of vitamin D delivery, uptake, storage and metabolism in the normal mammary gland and in breast tumors is needed. If deregulation of VDR, CYP27B1 or CYP24A1 expression or function are common in human breast cancers, then approaches to prevent or reverse these disturbed pathways may be effective in some patients.

Acknowledgments

This work was funded by NIH grants CA9700, CA144963, CA166434 and CA19450 to J. Welsh.

References

1. Skibinski A, Kuperwasser C. The origin of breast tumor heterogeneity. *Oncogene*. 2015; 34:5309–16. [PubMed: 25703331]
2. Narvaez CJ, Matthews D, LaPorta E, Simmons KM, Beaudin S, Welsh J. The impact of vitamin D in breast cancer: genomics, pathways, metabolism. *Frontiers in physiology*. 2014; 5:213. [PubMed: 24982636]
3. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nature reviews Cancer*. 2014; 14:342–57. [PubMed: 24705652]
4. Yao S, Ambrosone CB. Associations between vitamin D deficiency and risk of aggressive breast cancer in African-American women. *The Journal of steroid biochemistry and molecular biology*. 2013; 136:337–41. [PubMed: 22995734]
5. Kim Y, Franke AA, Shvetsov YB, Wilkens LR, Cooney RV, et al. Plasma 25-hydroxyvitamin D3 is associated with decreased risk of postmenopausal breast cancer in whites: a nested case-control study in the multiethnic cohort study. *BMC cancer*. 2014; 14:29. [PubMed: 24438060]
6. Bilinski K, Boyages J. Association between 25-hydroxyvitamin D concentration and breast cancer risk in an Australian population: an observational case-control study. *Breast cancer research and treatment*. 2013; 137:599–607. [PubMed: 23239153]
7. Morton ML, Thompson CL. Decreasing 25-hydroxy-vitamin D levels account for portion of the effect of increasing body mass index on breast cancer mortality. *Molecular nutrition & food research*. 2013; 57:260–6. [PubMed: 23180617]
8. Shirazi L, Almquist M, Malm J, Wirfalt E, Manjer J. Determinants of serum levels of vitamin D: a study of life-style, menopausal status, dietary intake, serum calcium, and PTH. *BMC women's health*. 2013; 13:33. [PubMed: 23945218]
9. Eliassen AH, Warner ET, Rosner B, Collins LC, Beck AH, et al. Plasma 25-Hydroxyvitamin D and Risk of Breast Cancer in Women Followed over 20 Years. *Cancer research*. 2016; 76:5423–30. [PubMed: 27530324]
10. Jacobs ET, Kohler LN, Kunihiro AG, Jurutka PW. Vitamin D and Colorectal, Breast, and Prostate Cancers: A Review of the Epidemiological Evidence. *Journal of Cancer*. 2016; 7:232–40. [PubMed: 26918035]
11. Cadeau C, Fournier A, Mesrine S, Clavel-Chapelon F, Fagherazzi G, Boutron-Ruault MC. Postmenopausal breast cancer risk and interactions between body mass index, menopausal

- hormone therapy use, and vitamin D supplementation: Evidence from the E3N cohort. *International journal of cancer*. 2016; 139:2193–200. [PubMed: 27451078]
12. Shekariz-Foumani R, Khodaie F. The Correlation of Plasma 25-Hydroxyvitamin D Deficiency With Risk of Breast Neoplasms: A Systematic Review. *Iranian journal of cancer prevention*. 2016; 9:e4469. [PubMed: 27703645]
 13. Jolliffe DA, Walton RT, Griffiths CJ, Martineau AR. Single nucleotide polymorphisms in the vitamin D pathway associating with circulating concentrations of vitamin D metabolites and non-skeletal health outcomes: Review of genetic association studies. *The Journal of steroid biochemistry and molecular biology*. 2016; 164:18–29. [PubMed: 26686945]
 14. Lappe JM, Heaney RP. Why randomized controlled trials of calcium and vitamin D sometimes fail. *Dermato-endocrinology*. 2012; 4:95–100. [PubMed: 22928064]
 15. Cauley JA, Chlebowski RT, Wactawski-Wende J, Robbins JA, Rodabough RJ, et al. Calcium plus vitamin D supplementation and health outcomes five years after active intervention ended: the Women's Health Initiative. *Journal of women's health (2002)*. 2013; 22:915–29.
 16. Neuhouwer ML, Manson JE, Millen A, Pettinger M, Margolis K, et al. The influence of health and lifestyle characteristics on the relation of serum 25-hydroxyvitamin D with risk of colorectal and breast cancer in postmenopausal women. *American journal of epidemiology*. 2012; 175:673–84. [PubMed: 22362582]
 17. 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; 94:1144–9. [PubMed: 21880848]
 18. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012; 490:61–70. [PubMed: 23000897]
 19. Zinser G, Packman K, Welsh J. Vitamin D(3) receptor ablation alters mammary gland morphogenesis. *Development (Cambridge, England)*. 2002; 129:3067–76.
 20. Zinser GM, Welsh J. Accelerated mammary gland development during pregnancy and delayed postlactational involution in vitamin D3 receptor null mice. *Molecular endocrinology (Baltimore, Md)*. 2004; 18:2208–23.
 21. Santagata S, Thakkar A, Ergonul A, Wang B, Woo T, et al. Taxonomy of breast cancer based on normal cell phenotype predicts outcome. *The Journal of clinical investigation*. 2014; 124:859–70. [PubMed: 24463450]
 22. Berger U, McClelland RA, Wilson P, Greene GL, Haussler MR, et al. Immunocytochemical determination of estrogen receptor, progesterone receptor, and 1,25-dihydroxyvitamin D3 receptor in breast cancer and relationship to prognosis. *Cancer research*. 1991; 51:239–44. [PubMed: 1846309]
 23. Al-Azhri J, Zhang Y, Bshara W, Zirpoli GR, McCann SE, et al. Tumor Expression of Vitamin D Receptor and Breast Cancer Histopathological Characteristics and Prognosis. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2016
 24. Lopes N, Sousa B, Martins D, Gomes M, Vieira D, et al. 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*. 2010; 10:483. [PubMed: 20831823]
 25. Zhang Y, Guo Q, Zhang Z, Bai N, Liu Z, et al. VDR status arbitrates the prometastatic effects of tumor-associated macrophages. *Molecular cancer research: MCR*. 2014; 12:1181–91. [PubMed: 24821711]
 26. Escalera MT, Brentani MM. Vitamin D3 receptor (VDR) expression in HC-11 mammary cells: regulation by growth-modulatory agents, differentiation, and Ha-ras transformation. *Breast cancer research and treatment*. 1999; 54:123–33. [PubMed: 10424403]
 27. Kemmis CM, Welsh J. Mammary epithelial cell transformation is associated with deregulation of the vitamin D pathway. *Journal of cellular biochemistry*. 2008; 105:980–8. [PubMed: 18767073]
 28. Mittal MK, Myers JN, Misra S, Bailey CK, Chaudhuri G. In vivo binding to and functional repression of the VDR gene promoter by SLUG in human breast cells. *Biochem Biophys Res Commun*. 2008; 372:30–4. [PubMed: 18485278]

29. Mezzetti G, Barbiroli B, Oka T. 1,25-Dihydroxycholecalciferol receptor regulation in hormonally induced differentiation of mouse mammary gland in culture. *Endocrinology*. 1987; 120:2488–93. [PubMed: 3032594]
30. Santiso-Mere D, Sone T, Hilliard GMT, Pike JW, McDonnell DP. Positive regulation of the vitamin D receptor by its cognate ligand in heterologous expression systems. *Molecular endocrinology* (Baltimore, Md). 1993; 7:833–9.
31. Zella LA, Meyer MB, Nerenz RD, Lee SM, Martowicz ML, Pike JW. Multifunctional enhancers regulate mouse and human vitamin D receptor gene transcription. *Molecular endocrinology* (Baltimore, Md). 2010; 24:128–47.
32. Zella LA, Kim S, Shevde NK, Pike JW. Enhancers located within two introns of the vitamin D receptor gene mediate transcriptional autoregulation by 1,25-dihydroxyvitamin D3. *Molecular endocrinology* (Baltimore, Md). 2006; 20:1231–47.
33. Byrne IM, Flanagan L, Tenniswood MP, Welsh J. Identification of a hormone-responsive promoter immediately upstream of exon 1c in the human vitamin D receptor gene. *Endocrinology*. 2000; 141:2829–36. [PubMed: 10919269]
34. Wietzke JA, Welsh J. Phytoestrogen regulation of a Vitamin D3 receptor promoter and 1,25-dihydroxyvitamin D3 actions in human breast cancer cells. *The Journal of steroid biochemistry and molecular biology*. 2003; 84:149–57. [PubMed: 12710998]
35. Saccone D, Asani F, Bornman L. Regulation of the vitamin D receptor gene by environment, genetics and epigenetics. *Gene*. 2015; 561:171–80. [PubMed: 25682935]
36. Marik R, Fackler M, Gabrielson E, Zeiger MA, Sukumar S, et al. DNA methylation-related vitamin D receptor insensitivity in breast cancer. *Cancer biology & therapy*. 2010; 10:44–53. [PubMed: 20431345]
37. Mohri T, Nakajima M, Takagi S, Komagata S, Yokoi T. MicroRNA regulates human vitamin D receptor. *International journal of cancer*. 2009; 125:1328–33. [PubMed: 19437538]
38. Swami S, Krishnan AV, Williams J, Aggarwal A, Albertelli MA, et al. Vitamin D mitigates the adverse effects of obesity on breast cancer in mice. *Endocr Relat Cancer*. 2016; 23:251–64. [PubMed: 26817629]
39. Williams JD, Aggarwal A, Swami S, Krishnan AV, Ji L, et al. Tumor Autonomous Effects of Vitamin D Deficiency Promote Breast Cancer Metastasis. *Endocrinology*. 2016; 157:1341–7. [PubMed: 26934299]
40. Jeong Y, Swami S, Krishnan AV, Williams JD, Martin S, et al. Inhibition of Mouse Breast Tumor-Initiating Cells by Calcitriol and Dietary Vitamin D. *Mol Cancer Ther*. 2015; 14:1951–61. [PubMed: 25934710]
41. Rossdeutsch L, Li J, Luco AL, Fadhil I, Ochiatti B, et al. Chemoprevention activity of 25-hydroxyvitamin D in the MMTV-PyMT mouse model of breast cancer. *Cancer prevention research* (Philadelphia, Pa). 2015; 8:120–8.
42. Rowling MJ, Kemmis CM, Taffany DA, Welsh J. Megalin-mediated endocytosis of vitamin D binding protein correlates with 25-hydroxycholecalciferol actions in human mammary cells. *The Journal of nutrition*. 2006; 136:2754–9. [PubMed: 17056796]
43. Hansen JG, Tang W, Hootman KC, Brannon PM, Houston DK, et al. Genetic and environmental factors are associated with serum 25-hydroxyvitamin D concentrations in older African Americans. *The Journal of nutrition*. 2015; 145:799–805. [PubMed: 25716552]
44. Kuhn T, Kaaks R, Teucher B, Hirche F, Dierkes J, et al. Dietary, lifestyle, and genetic determinants of vitamin D status: a cross-sectional analysis from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany study. *European journal of nutrition*. 2014; 53:731–41. [PubMed: 24005870]
45. Brouwer-Brolsma EM, Vaes AM, van der Zwaluw NL, van Wijngaarden JP, Swart KM, et al. Relative importance of summer sun exposure, vitamin D intake, and genes to vitamin D status in Dutch older adults: The B-PROOF study. *The Journal of steroid biochemistry and molecular biology*. 2016; 164:168–76. [PubMed: 26275945]
46. 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; 97:103–9. [PubMed: 16081283]
47. Friedrich M, Diesing D, Cordes T, Fischer D, Becker S, et al. Analysis of 25-hydroxyvitamin D3-1 α -hydroxylase in normal and malignant breast tissue. *Anticancer research*. 2006; 26:2615–20. [PubMed: 16886671]
 48. Townsend K, Banwell CM, Guy M, Colston KW, Mansi JL, et al. Autocrine metabolism of vitamin D in normal and malignant breast tissue. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2005; 11:3579–86. [PubMed: 15867263]
 49. Peng X, Hawthorne M, Vaishnav A, St-Arnaud R, Mehta RG. 25-Hydroxyvitamin D3 is a natural chemopreventive agent against carcinogen induced precancerous lesions in mouse mammary gland organ culture. *Breast cancer research and treatment*. 2009; 113:31–41. [PubMed: 18205042]
 50. Kemmis CM, Salvador SM, Smith KM, Welsh J. Human mammary epithelial cells express CYP27B1 and are growth inhibited by 25-hydroxyvitamin D-3, the major circulating form of vitamin D-3. *The Journal of nutrition*. 2006; 136:887–92. [PubMed: 16549446]
 51. McCarthy K, Laban C, Bustin SA, Ogunkolade W, Khalaf S, et al. Expression of 25-hydroxyvitamin D-1 α -hydroxylase, and vitamin D receptor mRNA in normal and malignant breast tissue. *Anticancer research*. 2009; 29:155–7. [PubMed: 19331145]
 52. Segersten U, Holm PK, Bjorklund P, Hessman O, Nordgren H, et al. 25-Hydroxyvitamin D3 1 α -hydroxylase expression in breast cancer and use of non-1 α -hydroxylated vitamin D analogue. *Breast cancer research : BCR*. 2005; 7:R980–6. [PubMed: 16280049]
 53. de Lyra EC, da Silva IA, Katayama ML, Brentani MM, Nonogaki S, et al. 25(OH)D3 and 1,25(OH)2D3 serum concentration and breast tissue expression of 1 α -hydroxylase, 24-hydroxylase and Vitamin D receptor in women with and without breast cancer. *The Journal of steroid biochemistry and molecular biology*. 2006; 100:184–92. [PubMed: 16828283]
 54. Zhalehjoo N, Shakiba Y, Panjehpour M. Gene expression profiles of CYP24A1 and CYP27B1 in malignant and normal breast tissues. *Molecular medicine reports*. 2017; 15:467–73. [PubMed: 27922682]
 55. Fischer D, Seifert M, Becker S, Ludders D, Cordes T, et al. 25-Hydroxyvitamin D3 1 α -hydroxylase splice variants in breast cell lines MCF-7 and MCF-10. *Cancer genomics & proteomics*. 2007; 4:295–300. [PubMed: 17878529]
 56. Cordes T, Diesing D, Becker S, Fischer D, Diedrich K, Friedrich M. Expression of splice variants of 1 α -hydroxylase in mcf-7 breast cancer cells. *The Journal of steroid biochemistry and molecular biology*. 2007; 103:326–9. [PubMed: 17287116]
 57. 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; 112:3393–405. [PubMed: 21769914]
 58. Johnson AL, Zinser GM, Waltz SE. Loss of vitamin D receptor signaling from the mammary epithelium or adipose tissue alters pubertal glandular development. *American journal of physiology. Endocrinology and metabolism*. 2014; 307:E674–85. [PubMed: 25139050]
 59. Li J, Luco AL, Ochiatti B, Fadhil I, Camirand A, et al. Tumoral Vitamin D Synthesis by CYP27B1 1 α -Hydroxylase Delays Mammary Tumor Progression in the PyMT-MMTV Mouse Model and Its Action Involves NF-kappaB Modulation. *Endocrinology*. 2016; 157:2204–16. [PubMed: 27119753]
 60. Zinser GM, Welsh JE. Accelerated mammary gland development during pregnancy and delayed post-lactational involution in vitamin D3 receptor null mice. *Molecular Endocrinology*. 2004; 18:2208–23. [PubMed: 15178742]
 61. Albertson DG, Ylstra B, Segraves R, Collins C, Dairkee SH, et al. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. *Nature genetics*. 2000; 25:144–6. [PubMed: 10835626]
 62. Beaudin SG, Robilotto S, Welsh J. Comparative regulation of gene expression by 1,25-dihydroxyvitamin D3 in cells derived from normal mammary tissue and breast cancer. *The Journal of steroid biochemistry and molecular biology*. 2015; 148:96–102. [PubMed: 25239595]
 63. Osanai M, Lee GH. CYP24A1-induced vitamin D insufficiency promotes breast cancer growth. *Oncology reports*. 2016; 36:2755–62. [PubMed: 27600601]

64. Scheible C, Thill M, Baum S, Solomayer E, Friedrich M. Implication of CYP24A1 splicing in breast cancer. *Anti-cancer agents in medicinal chemistry*. 2014; 14:109–14. [PubMed: 23869778]
65. Fischer D, Becker S, Cordes T, Bucker B, Diedrich K, et al. Vitamin D-24-hydroxylase in benign and malignant breast tissue and cell lines. *Anticancer research*. 2009; 29:3641–5. [PubMed: 19667160]
66. Rubsamén D, Kunze MM, Buderus V, Brauss TF, Bajer MM, et al. Inflammatory conditions induce IRES-dependent translation of cyp24a1. *PloS one*. 2014; 9:e85314. [PubMed: 24416388]
67. Komagata S, Nakajima M, Takagi S, Mohri T, Taniya T, Yokoi T. Human CYP24 catalyzing the inactivation of calcitriol is post-transcriptionally regulated by miR-125b. *Molecular pharmacology*. 2009; 76:702–9. [PubMed: 19570947]
68. Deeb KK, Luo W, Karpf AR, Omilian AR, Bshara W, et al. Differential vitamin D 24-hydroxylase/CYP24A1 gene promoter methylation in endothelium from benign and malignant human prostate. *Epigenetics*. 2011; 6:994–1000. [PubMed: 21725204]
69. Ramnath N, Nadal E, Jeon CK, Sandoval J, Colacino J, et al. Epigenetic regulation of vitamin D metabolism in human lung adenocarcinoma. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*. 2014; 9:473–82.
70. Lim E, Wu D, Pal B, Bouras T, Asselin-Labat ML, et al. Transcriptome analyses of mouse and human mammary cell subpopulations reveal multiple conserved genes and pathways. *Breast cancer research : BCR*. 2010; 12:R21. [PubMed: 20346151]
71. Mezzetti G, Monti MG, Casolo LP, Piccinini G, Moruzzi MS. 1,25-Dihydroxycholecalciferol-dependent calcium uptake by mouse mammary gland in culture. *Endocrinology*. 1988; 122:389–94. [PubMed: 2827995]
72. Bhattacharjee M, Wientroub S, Vonderhaar BK. Milk protein synthesis by mammary glands of vitamin D-deficient mice. *Endocrinology*. 1987; 121:865–74. [PubMed: 3622380]
73. Welsh J, Zinser LN, Mianeki-Morton L, Martin J, Waltz SE, et al. Age-related changes in the epithelial and stromal compartments of the mammary gland in normocalcemic mice lacking the vitamin D3 receptor. *PloS one*. 2011; 6:e16479. [PubMed: 21298063]
74. Narvaez CJ, Matthews D, Broun E, Chan M, Welsh J. Lean phenotype and resistance to diet-induced obesity in vitamin D receptor knockout mice correlates with induction of uncoupling protein-1 in white adipose tissue. *Endocrinology*. 2009; 150:651–61. [PubMed: 18845643]
75. Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell*. 2006; 10:515–27. [PubMed: 17157791]
76. Kao J, Salari K, Bocanegra M, Choi YL, Girard L, et al. Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. *PloS one*. 2009; 4:e6146. [PubMed: 19582160]
77. Milani C, Katayama ML, de Lyra EC, Welsh J, Campos LT, et al. Transcriptional effects of 1,25 dihydroxyvitamin D(3) physiological and supra-physiological concentrations in breast cancer organotypic culture. *BMC cancer*. 2013; 13:119. [PubMed: 23497279]
78. Milani C, Welsh J, Katayama ML, Lyra EC, Maciel MS, et al. Human breast tumor slices: a model for identification of vitamin D regulated genes in the tumor microenvironment. *The Journal of steroid biochemistry and molecular biology*. 2010; 121:151–5. [PubMed: 20307664]
79. Sheng L, Anderson PH, Turner AG, Pishas KI, Dhatrak DJ, et al. Identification of vitamin D target genes in human breast cancer tissue. *J Steroid Biochem Mol Biol*. 2015
80. Suetani RJ, Ho K, Jindal S, Manavis J, Neilsen PM, et al. A comparison of vitamin D activity in paired non-malignant and malignant human breast tissues. *Molecular and cellular endocrinology*. 2012; 362:202–10. [PubMed: 22750718]
81. Simmons KM, Beaudin SG, Narvaez CJ, Welsh J. Gene Signatures of 1,25-Dihydroxyvitamin D3 Exposure in Normal and Transformed Mammary Cells. *Journal of cellular biochemistry*. 2015; 116:1693–711. [PubMed: 25736056]

Highlights

Observational data provide evidence that vitamin D status negatively correlates with breast cancer

Vitamin D receptor is expressed in mammary gland and breast cancer

Deregulation of vitamin D function occurs during carcinogenesis

Figure 1. Analysis of genomic alterations in VDR, CYP24A1 and CYP27B1 in human breast tumors

This oncoprint reports cases in which the indicated alterations (amplification, deep deletion, mRNA upregulation or mRNA downregulation) in VDR, CYP24A1 or CYP27B1 were detected in individual tumor samples. The TCGA dataset utilized was the Breast Cancer (METABRIC) consisting of 2509 patients. Data analysis was conducted within the cBioPortal for Cancer Genomics at <http://www.cbioportal.org/>.

Table 1

Summary of studies on vitamin D pathway expression in breast cancer.

STUDY	PATIENTS AND SAMPLES	READOUTS	RESULTS	NOTES
Zhalehjoo et al 2017 (53)	Tumor and normal adjacent tissue from 30 breast cancer patients	PCR	CYP27B1 ↓ & CYP24A1 ↑ in tumor vs normal	No correlation of CYP expression with tumor size or lymph node positivity
Lopes et al 2010 (23)	29 Normal 379 Benign lesions 189 DCIS 189 IDC	IHC PCR	VDR ↓ with tumor progression; CYP27B1 ↓ in IDC vs benign; CYP24A1 ↑ in DCIS and IDC VDR and CYP27B1 ↑ in tumors	See paper for correlation of VDR pathway to other breast cancer markers
McCarthy et al 2009 (50)	Paired tumor and normal tissue from 30 breast cancer patients 18 normal breast samples	PCR	VDR ↑ in tumor vs normal CYP27B1 not different in tumor vs normal	No correlation of VDR pathway to ER status, grade or lymph node positivity
De Lyra et al 2006 (52)	88 breast cancer 35 normal breast	PCR	VDR, CYP27B1 and CYP24A1 detected in all samples, not different in cancer vs normal	
Fischer et al 2009 (64)	4 benign tissue 4 breast cancers	PCR Western blot	CYP24A1 ↓ in tumor vs normal	Some evidence for splice variants
Friedrich et al 2006 (46)	11 benign tissue 12 breast cancer	PCR	CYP27B1 ↑ in tumor vs normal	
Townsend et al 2005 (47)	Paired tumor and normal tissue from 41 breast cancer patients	PCR IHC Enzyme assay	VDR, CYP27B1 and CYP24A1 ↑ in cancer vs normal Detection of CYP27B1 in breast tissue and tumors Higher conversion of 25D to 1,25D and 1,24,25D in cancer vs normal	
Sergersten et al 2005 (51)	19 breast cancer 10 adjacent normal breast (unpaired)	PCR IHC	CYP27B1 ↓ & CYP24A1 ↑ in tumor vs normal Detection of CYP27B1 in breast tissue and tumors	

DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma