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Comparative regulation of gene expression by 1,25-dihydroxyvitamin D₃ in cells derived from normal mammary tissue and breast cancer

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Abstract

Previous genomic profiling of immortalized, non-tumorigenic human breast epithelial cells identified a set of 1,25-dihydroxyvitamin D₃ (1,25D) regulated genes with potential relevance to breast cancer prevention. In this report, we characterized the effect of 1,25D on a subset of these genes in six cell lines derived from mammary tissue and breast cancers. Non-tumorigenic cell lines included hTERT-HME1, HME and MCF10A cells which are often used to model normal breast epithelial cells. Breast cancer cell lines included MCF7 cells (a model of early stage, estrogen-dependent disease), DCIS.com cells (a derivative of MCF10A cells that models *in situ* breast cancer) and Hs578T cells (a model of metastatic disease). All of these cell lines express the vitamin D receptor (VDR) and exhibit anti-cancer responses to 1,25D such as changes in proliferation, apoptosis, metabolism, or invasion. Our comparative data demonstrate highly variable responses to 1,25D (100nM, 24h) between the cell lines. In both hTERT-HME1 and HME cell lines, *CYP24A1*, *SLC1A1* and *ITGB3* were up-regulated whereas *KDR*, *GLUL* and *BIRC3* were down-regulated in response to 1,25D. In contrast, no changes in *SLC1A1*, *ITGB3* or *GLUL* expression were detected in 1,25D treated MCF10A cells although *KDR* and *BIRC3* were down-regulated by 1,25D. The effects of 1,25D on these genes in the breast cancer cell lines were blunted, with the DCIS.com cells exhibiting the most similar responses to the immortalized hTERT-HME1 and HME cells. The differences in cellular responses were not due to general impairment in VDR function as robust *CYP24A1* induction was observed in all cell lines. Thus, our data indicate that the genomic changes induced by 1,25D are highly cell-type specific even in model cell lines derived from the same tissue. The implication of these findings is that genomic responses to changes in vitamin D status *in vivo* are likely to be distinct from individual to individual, particularly in neoplastic tissue.

Keywords

Vitamin D; genomics; mammary gland; breast cancer

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INTRODUCTION

In most cell culture models, VDR ligands such as 1,25 dihydroxyvitamin D₃ (1,25D) induce growth arrest which may be associated with the expression of differentiation markers (1, 2) or the onset of apoptosis (3, 4). These effects of 1,25D are presumed to be mediated via VDR regulation of gene expression but the specific mechanisms remain undefined. Genomic and proteomic profiling of various cells and tissues has identified scores of VDR-regulated genes and proteins in diverse pathways, indicating a broad range of potential downstream targets (5–8). Individual reports have focused on a few specific pathways or genes linked to vitamin D effects in specific model systems but few studies have compared the effects of 1,25D on gene expression in normal and pathologic cells from the same tissue. Tissue or disease-specific VDR gene targets would represent valuable biomarkers of vitamin D action for epidemiologic and intervention studies.

Considerable attention has been directed at the effects of vitamin D on breast cancer in cell culture and animal models. 1,25D inhibits the proliferation of non-tumorigenic mammary epithelial cells and VDR ablation enhances the risk of carcinogenesis (9, 10). Many breast cancer cell lines retain expression of VDR and VDR agonists have been shown to retard tumor growth and/or induce regression in animal models (11–13). Although many vitamin D-regulated genes have been identified in model systems of breast cancer, no studies have compared the effects of 1,25D on gene expression in non-tumorigenic and tumorigenic cells. In the studies described here, we selected a panel of putative VDR target genes from our recent microarray profiling of immortalized human mammary epithelial cells and analyzed their expression in six distinct mammary cell model systems. Our results suggest that 1,25D-mediated gene expression is highly cell-type specific even in cells derived from the same tissue.

METHODS

Non-tumorigenic breast cell model systems

These studies employed three non-tumorigenic mammary epithelial cell lines (hTERT-HME1, HME and MCF10A) whose characteristics are detailed in Table 1. The hTERT-HME1 and HME cells represent similar but unrelated lines of mammary epithelial cells that were independently isolated from healthy tissue and immortalized through retroviral introduction of human telomerase reverse transcriptase (TERT). The hTERT-HME1 cell line was obtained from Clontech (originally marketed as the Infinity™ Human Mammary Epithelial Cell Line, now available from ATCC). The HME cell line was obtained from Dr. Robert Weinberg (14). The hTERT-HME1 and HME cells are diploid and non-tumorigenic. The well characterized MCF10A cell line (available from ATCC) was originally isolated from human fibrocystic mammary tissue and is often used to model “normal” mammary epithelial cells since they are non-tumorigenic (15). However, the MCF10A cell line is aneuploid (due to a gain of chromosome 8) and exhibits numerous chromosomal translocations and amplifications (Table 1). These three non-tumorigenic cell lines lack expression of the estrogen receptor but express epithelial cytokeratins and grow as cuboidal monolayers. For the studies described here, the hTERT-HME1, HME, and MCF10A cells

were maintained and treated in serum-free M171 media containing mammary epithelial growth supplements (Life Technologies, Grand Island, NY).

Breast cancer cell model systems

Three tumorigenic cell lines representative of different stages and subtypes of breast cancer were used: MCF7, DCIS.com and Hs578T cells (Table 1). MCF7 cells (obtained from ATCC) were isolated from the pleural effusion of a patient with metastatic ductal carcinoma and are often used to model early stage breast cancer since they express the estrogen receptor and retain differentiated features such as E-cadherin and epithelial cytokeratins. However, they are profoundly aneuploid with a modal chromosome number between 76–88 and have extensive chromosomal rearrangements. DCIS.com cells (obtained from Dr. Jason Herschkowitz, University at Albany Cancer Research Center, Rensselaer, NY) were derived from cell culture of a lesion formed by xenotransplantation of MCF10AT cells (a clone of MCF10A cells expressing oncogenic *Ras*). DCIS.com cells are tumorigenic when implanted into the mammary fat pad or ducts of immunocompromised mice, forming localized lesions reminiscent of human ductal carcinoma *in situ* (DCIS) that slowly progress to invasive cancer (16, 17). Hs578T cells (obtained from ATCC) were isolated from a carcinosarcoma, a subtype of triple negative breast cancer with mesenchymal features (18, 19). All three breast cancer cell lines express VDR and respond to 1,25D (see Table 1 for references).

Cell-type specific media was used to maintain the tumorigenic cell lines. MCF7 cells were cultured in α -medium essential medium (α -MEM) supplemented with 5% fetal bovine serum (FBS). DCIS.com cells were maintained in DMEM/F12 media with 5% horse serum, hydrocortisone, EGF, insulin, and antibiotics. Hs578T cells were maintained in DMEM with 10% FBS and insulin. For experiments, the breast cancer cell lines were retained in their maintenance media.

Assessment of basal and 1,25D responsive gene expression

Real-time PCR was used to evaluate a subset of putative VDR target genes previously identified by microarray profiling of hTERT-HME1 cells treated with 100nM 1,25D for 24h. These genes were chosen for follow-up based on their regulation by 1,25D and their cancer relevant functions as detailed in Table 2. For these assays, hTERT-HME1, HME, MCF10A, MCF7, DCIS.com, and Hs578T cells in 100mm dishes were treated with 100nM 1,25D or ethanol vehicle 24h after plating. RNA was isolated 24h later with the Qiagen RNeasy kit (Qiagen, Valencia, CA) and analyzed for concentration and purity on a Nanodrop 1000 Spectrophotometer. cDNA was prepared using TaqMan Reverse Transcriptase Reagents (Life Technologies, Grand Island, NY) and analyzed in duplicate using SYBR Green PCR Master Mix (ABgene - Thermo Scientific, Pittsburgh, PA) on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Primer sequences were obtained from Origene (Rockville, MD) and are listed in Supplemental Table 1. Data were calculated by the $\Delta\Delta C_t$ method and normalized against 18S. For calculation of basal and 1,25D regulated *VDR* and *CYP24A1* expression, normalized values for each cell line were expressed relative to those of the vehicle treated hTERT-HME1 cell line. For calculation of the effect of 1,25D on *ITGB3*, *SLC1A1*, *KDR*, *BIRC3* and *GLUL*, data were expressed relative to that of vehicle treatment within each cell

line which was set to 1. Basal expression of these genes was also calculated as described for *VDR* and *CYP24A1* and is available as Supplemental Figure 1. GraphPad Prism software (La Jolla, CA) was used to measure statistical significance by one-way ANOVA followed by Dunnett's multiple comparison test (p values less than 0.05 were considered significant).

RESULTS

Relative VDR expression in mammary epithelial cell lines

VDR expression, as assessed by qPCR and normalized to 18S RNA, was detected in all of the model cell lines (Figure 1A). Under basal conditions, the highest levels of *VDR* mRNA were found in hTERT-HME1 and DCIS.com cells; all other breast epithelial cell lines expressed significantly less *VDR*. In response to 24h treatment with 100nM 1,25D (Figure 1B), *VDR* expression was significantly down-regulated in hTERT-HME1 and DCIS.com cells and up-regulated in Hs578T cells, whereas no changes were observed in HME, MCF10A or MCF7 cells. These data indicate cell-type specific *VDR* expression and regulation which is unrelated to the tumorigenic potential of the cells.

Basal and 1,25D induced CYP24A1 expression

Since *VDR* expression varied in the model cell lines, we assessed expression of the *VDR* target gene *CYP24A1* under basal and 1,25D-treated conditions to better characterize relative *VDR* function in each cell line. As shown in Figure 2A, basal *CYP24A1* expression was significantly higher in the two telomerase-immortalized non-tumorigenic cell lines (hTERT-HME1 and HME) than in all other cell lines. However, in response to treatment with 1,25D (100nM, 24h), *CYP24A1* expression was robustly induced in all cell lines (Figure 1B) with the magnitude of induction ranging from ~700 fold in the HME cells to >1,200 fold in the MCF7 cells. These data indicate that despite the lower baseline expression in most cell lines, the *CYP24A1* gene remains highly sensitive to induction by 1,25D, suggesting that *VDR* transcriptional activity is fairly comparable in all six cell lines.

Comparative regulation of cancer-relevant VDR target genes in mammary epithelial cell lines

Previous microarray profiling of hTERT-HME1 cells treated with 1,25D (100nM, 24h) identified 483 entities that were significantly ($p < 0.05$) altered >1.5 fold in response to 1,25D (319 up-regulated/164 down-regulated). In this report, we have chosen a subset of these genes (Table 2) for follow-up in six breast derived cell lines with varying tumorigenic potential. Representative up-regulated (*ITGB3*, *SLC1A1*) and down-regulated (*GLUL*, *KDR*, *BIRC3*) genes that function in diverse pathways relevant to cancer biology (metabolism, angiogenesis, apoptosis and adhesion) were selected. As indicated in Table 2, we chose genes localized throughout the genome in order to minimize the possible contributions of chromosomal alterations in some of the cell lines that might alter basal or induced gene expression.

We used PCR to examine the effect of 1,25D on the expression of these five genes in the six cell lines described in Table 1. The data summarized in Table 3 indicate the relative fold change (1,25D treated vs. ethanol treated) for each gene in each cell line. The effects of

1,25D on these five genes in the two immortalized breast epithelial cells (hTERT-HME1 and HME) are highly concordant. Consistent with the microarray data, *ITGB3* and *SLC1A1* genes were up-regulated and *KDR*, *GLUL* and *BIRC3* were down-regulated in response to 1,25D treatment. Thus, despite their origin in different laboratories, both non-tumorigenic, telomerase-immortalized mammary epithelial cells demonstrated highly similar patterns of gene expression in response to 1,25D. In contrast to the comparable responses to 1,25D in the telomerase-immortalized cell lines, only two (*KDR* and *BIRC3*) of the five genes were similarly regulated by 1,25D in the MCF10A cells. Neither *ITGB3* nor *SLC1A1* were up-regulated, and *GLUL* was not down-regulated, in 1,25D treated MCF10A cells. Since *CYP24A1* was significantly induced by 1,25D in MCF10A cells, these data suggest deregulation of a subset of VDR responsive genes in this cell line rather than a general impairment in 1,25D-VDR signaling.

The effects of 1,25D on expression of these five genes in the tumorigenic breast cancer cell lines were also highly variable. Although *CYP24A1* was highly induced by 1,25D in all breast cancer cell lines (Figure 2B), the up-regulation of *ITGB3* and *SLC1A1* that was observed in hTERT-HME1 and HME cells was observed in DCIS.com cells but not in MCF7 or Hs578T cells. With respect to genes found to be down-regulated in the immortalized breast cell lines, only *KDR* in MCF7 cells and *BIRC3* in DCIS.com cells showed similar down-regulation by 1,25D. Interestingly, in contrast to the up-regulation of *ITGB3* observed in 1,25D treated hTERT-HME1, HME and DCIS.com cells, this gene was significantly down regulated by 1,25D in MCF7 and Hs578T cells. Also, it is worth noting that despite their common origin, MCF10A and DCIS.com cells exhibited highly divergent responses to 1,25D, with DCIS.com cells exhibiting gene changes more similar to hTERT-HME1 and HME cells than MCF10A cells. Only one gene, *BIRC3*, was similarly regulated by 1,25D in the MCF10A and DCIS.com cells.

In lieu of the observed differences in gene regulation by 1,25D, we analyzed the basal expression of each gene in each cell line relative to the hTERT-HME1 cells (Supplemental Table 1). Although there were some significant differences in basal gene expression, particularly for *ITGB3* and *SLC1A1*, there were no obvious correlations between basal expression and 1,25D sensitivity in this series of cells.

DISCUSSION

In this study we examined the heterogeneity of genomic responses to 1,25D in a series of cell lines derived from normal and pathologic human breast tissue. In lieu of conducting extensive whole genome profiling in multiple cell lines, we elected to study a subset of genes that were previously identified as 1,25D-regulated in an immortalized but non-transformed mammary epithelial cell line (hTERT-HME1 cells). We hypothesized that one or more of these genes, which function in various cancer-relevant pathways, were potential mediators (or markers) of the known anti-cancer effects of 1,25D in breast cancer cells. Therefore, it was of interest to assess whether one or more of these genes represent general targets of VDR signaling in breast-derived cell lines in which case they might prove useful as biomarkers of 1,25D exposure for translational studies.

In order to assess the generality of genomic responses to 1,25D, we first verified the results of our microarray profiling in two independently derived immortalized breast epithelial cell lines (hTERT-HME1 and HME cells). The responses of these cell lines to 1,25D were previously shown to be highly similar to that of primary cultures of breast epithelial cells indicating that the immortalization process per se does not affect VDR expression or function (9). Indeed the effects of 1,25D on all six genes studied (*CYP24A1*, *ITGB3*, *SLC1A1*, *KDR*, *BIRC3*, and *GLUL*) were highly comparable in these two cell lines (Table 2), and the gene alterations were consistent with the initial array results (Table 1). Thus, these genes appear to be bona-fide VDR targets in non-transformed mammary epithelial cells.

Interestingly, with the exception of *CYP24A1*, gene regulation in response to 1,25D was highly variable in the remaining four cell lines studied. This discordance was unexpected, especially in the non-tumorigenic MCF10A cells, which are commonly used as a model of “normal” mammary epithelial cells and thus would be expected to respond comparably to the telomerase-immortalized mammary epithelial cell lines. However, only two genes (*KDR* and *BIRC3*, which were both down-regulated by 1,25D) of the five genes examined were responsive to 1,25D in the MCF10A cells. Other genes that were down- (*GLUL*) or up- (*ITGB3*, *SLC1A1*) regulated by 1,25D in hTERT-HME1 and HME cells were unaffected in MCF10A despite comparable up-regulation of *CYP24A1* (700–900 fold induction) in the three cell lines. These data suggest that MCF10A cells, despite their non-tumorigenic phenotype, are not a useful model of normal breast epithelial cells with respect to VDR genomic actions.

Similar heterogeneity in 1,25D regulation of gene expression was observed in the tumorigenic breast cancer cell lines examined despite comparable *CYP24A1* induction. Of the breast cancer cell lines, the response of the DCIS.com cells was most comparable to the non-tumorigenic hTERT-HME1 and HME cells (with three of five genes displaying comparable regulation). This is surprising since the DCIS.com cells were derived from a variant of the MCF10A cells which did not respond similarly to the hTERT and HME cells. Only one gene (*BIRC3*) was similarly regulated in MCF10A and DCIS.com despite their common origin. In MCF7 cells, which have been extensively used to explore the anti-proliferative and pro-apoptotic actions of 1,25D, none of the up-regulated genes and only one of the down-regulated genes (*KDR*) was altered by 1,25D. 1,25D had no significant effects on expression of any of the genes studied in the Hs578T cells. At present, the basis for these discrepancies in gene regulation are unclear as all of the breast cancer cell lines express VDR and exhibit 700–1,200 fold induction of *CYP24A1* upon 1,25D treatment. It should be noted that all of the established cancer cell lines used in this study have substantial genomic alterations including chromosomal deletions and translocations as well as activation of oncogenes and loss of tumor suppressor proteins. These changes could conceivably de-regulate expression of the target genes in general or selectively alter VDR function (ie, via phosphorylation or other post-translational modifications). Further studies will be necessary to determine whether the altered genomes in these cell lines affect the regulatory regions of the selected genes which might alter basal expression or response to 1,25D.

It is worth noting that the differences in 1,25D responsiveness of these cell lines did not correlate with basal *VDR* mRNA expression. For example, hTERT-HME1 and HME cells displayed consistent responses to 1,25D despite significant differences in *VDR* mRNA expression. Further comparison of *VDR* mRNA expression and regulation (as some cell lines displayed up-regulation while others displayed down-regulation of *VDR* after 1,25D treatment) as well as *VDR* protein expression will be needed to determine whether subtle differences in *VDR* function could explain the differences in cellular responsiveness observed here. In addition, we have not tested whether these genes are direct 1,25D/*VDR* targets (ie, by ChIP assays), nor are there any literature reports on *VDR* binding to the genes we examined (other than *CYP24A1*). *In silico* analysis indicated that at least one of the genes tested here (*GLUL*) contains a potential vitamin D response element (20). Time course studies in hTERT-HME1 cells (unpublished) indicated that *ITGB3*, *SLC1A1*, and *KDR* showed significant changes in expression within 4 hours of 1,25D treatment, suggesting that they might represent direct *VDR* targets. One might expect direct *VDR* target genes to be similarly regulated by 1,25D in different cell lines, whereas genes that are indirectly regulated by 1,25D/*VDR* (ie, through miRNAs or signal transduction cascades) would likely be more variable in different cell lines grown under different conditions. One limitation of the current studies is that only one time point (24 hours after 1,25D treatment) has been compared. Further studies, including more extensive kinetics, are needed to determine whether the discrepancies in gene regulation after 1,25D treatment observed in these cell lines reflects the underlying mechanisms of regulation.

Consistent with the current data, previous studies of vitamin D-mediated genomic effects in breast cancer have suggested heterogeneity in *VDR* target genes. Comparison of 1,25D-mediated gene expression in MCF7 cells with that of a more aggressive breast cancer cell line (MDA-MB-231 cells) identified only seven genes (out of 2,000) that were commonly altered in both cell lines (5). Similarly, the effects of a Gemini 1,25D analog on pre-malignant (MCF10AT1) and malignant (MCF10CA1a) breast cancer cells revealed distinct gene expression profiles for each cell line (6) but considerable overlap in target genes (about 55% of the genes altered in MCF10AT1 cells were similarly altered in the MCF10CA1a cells). This degree of overlap likely reflects the fact that these cell lines share a common origin as both were derived from MCF10A cells (21). Further studies to determine whether the 1,25D target genes identified in these MCF10A derivatives are also modulated in DCIS.com cells would be of interest.

Another notable finding from this work is that basal *CYP24A1* expression differed substantially among the cell lines. The immortalized breast epithelial cell lines (hTERT-HME1 and HME) had high *CYP24A1* expression relative to the MCF10A and the breast cancer cell lines. Thus, the blunted effects of 1,25D were not secondary to enhanced catabolism. This was surprising since *CYP24A1* has been reported to be up-regulated in human breast cancer relative to benign lesions and normal tissue (22, 23). A more extensive analysis of *CYP24A1* splice variants (24) may be warranted to clarify the significance of this observation. 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 (25)

suggesting the possibility that *CYP24A1* may have an as yet unidentified role in breast biology which may or may not be VDR dependent.

Collectively, these studies indicate that even in cell culture models of breast cancer, VDR target gene regulation is highly heterogeneous. Given the complexity of human breast tumors which are composed of multiple cell types and cluster into distinct molecular subtypes (18, 26), it seems unlikely that a small set of genes will be suitable as biomarkers of vitamin D action in tumors. Thus, while identification of vitamin D regulated pathways will continue to be of interest with respect to mechanistic evaluation of growth arrest, differentiation, apoptosis and other anti-cancer actions in individual cell models, these same pathways may not be clinically relevant in the majority of tumors. Other approaches such as *ex vivo* treatment of biopsy tissue (27, 28) may offer a more relevant approach for identification of VDR target genes as markers of vitamin D exposure *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Relative VDR expression in vehicle and 1,25D treated breast cells

VDR mRNA was quantitated by qPCR in hTERT-HME1, HME, MCF10A, MCF7, DCIS.com, and Hs578T cells treated with 100nM 1,25D or ethanol vehicle for 24h. Data were calculated by the $\Delta\Delta C_t$ method, normalized against 18S and expressed relative to those of the hTERT-HME1 cell line which was set to 1. Bars represent mean $\pm$ standard deviation of triplicates. A. Basal VDR expression. * Significantly different from hTERT-HME1 value by one-way ANOVA, $p < 0.05$. B. 1,25D treatment. * Significantly different, 1,25D treated vs. control within each cell line.

Figure 2. Relative *CYP24A1* expression in vehicle and 1,25D treated breast cells

CYP24A1 mRNA was quantitated by qPCR in hTERT-HME1, HME, MCF10A, MCF7, DCIS.com, and Hs578T cells treated with 100nM 1,25D or ethanol vehicle for 24h. Data were calculated by the $\Delta\Delta C_t$ method, normalized against 18S and expressed relative to those of the hTERT-HME1 cell line which was set to 1. Bars represent mean $\pm$ standard deviation of triplicates. A. Basal *CYP24A1* expression. * Significantly different from hTERT-HME1 value by one-way ANOVA, $p < 0.05$. B. 1,25D treatment. Fold increase with 1,25D treatment

within each cell line is indicated above bars. All 1,25D treated values were significantly different from vehicle control values.

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Table 1

Characteristics of mammary epithelial cell lines used in this study

Cell Line	Origin and Characteristics	Effect of VDR Agonists
hTERT-HME1	<ul style="list-style-type: none"> Epithelial cells isolated from healthy mammary tissue Immortalized through forced expression of telomerase Karyotype: Diploid Non-tumorigenic Models normal mammary tissue 	Growth arrest, gene regulation (9)
HME	<ul style="list-style-type: none"> Epithelial cells isolated from healthy mammary tissue Immortalized through forced expression of telomerase Karyotype: Diploid Non-tumorigenic Models normal mammary tissue 	Growth arrest, gene regulation (29)
MCF10A	<ul style="list-style-type: none"> Epithelial cells isolated from fibrocystic mammary tissue Spontaneously immortalized in culture Karyotype: aneuploid (47 chromosomes) - gain of chr8, translocations t(3;9); t(5;3;9); t(6;19) and amplifications (5q23.1-qter, 8q24.21, 10q22.1) Non-tumorigenic Models pre-neoplastic lesions 	Growth arrest, regulation of gene expression, BMP signaling and glucose metabolism (30–32)
MCF7	<ul style="list-style-type: none"> Breast cancer cells isolated from pleural effusion of patient with metastases Karyotype: aneuploid (chr number ranges from 76 to 88), >50 rearrangements Tumorigenic Models early stage estrogen dependent disease 	Growth arrest and apoptosis, regulation of gene expression (4, 5, 33, 34)
DCIS.com	<ul style="list-style-type: none"> Isolated from a xenograft of ras-transformed MCF10A cells Tumorigenic, forms DCIS-like lesions that progress to carcinoma Models high-risk ductal carcinoma <i>in situ</i> 	Growth arrest, inhibition of invasion and stem cell markers (17, 35)
Hs578T	<ul style="list-style-type: none"> Epithelial cells derived from a breast carcinosarcoma; metaplastic Karyotype: aneuploid (chr number ranges from 50–77), chr17 deleted, 1 normal chr15 Tumorigenic Models metaplastic disease 	Growth arrest, apoptosis, inhibition of IGF1 and NfκB signaling (36, 37)

Table 2

List of 1,25D responsive genes identified by microarray profiling in hTERT-HME1 cells that were chosen for follow-up.

Fold Change ¹	Gene ID ²	Gene Symbol	Chromosomal Localization	Function of Gene Product
181.4	1591	<i>CYP24A1</i>	20q13	Encodes the cytochrome P450 enzyme that degrades 1,25-dihydroxyvitamin D ₃
13.9	3690	<i>ITGB3</i>	17q21	Encodes the cell surface integrin β3 that functions in cell-surface signaling and cell adhesion. Also known as <i>CD61</i> , a marker of luminal breast epithelial cells.
7.1	6505	<i>SLC1A1</i>	9p24	Encodes the plasma membrane glutamate transporter EAAT1 which mediates cellular glutamate uptake. Protects against oxidative stress.
-6.6	3791	<i>KDR</i>	4q11	Encodes the kinase insert domain receptor, a type III receptor tyrosine kinase which is one of two receptors for VEGF. Promotes angiogenesis.
-5.1	330	<i>BIRC3</i>	11q22	Encodes the baculoviral IAP repeat containing 3 protein that inhibits apoptosis. Often up-regulated in breast cancer.
-4.1	2752	<i>GLUL</i>	1q31	Encodes glutamine synthetase, a metabolic enzyme that converts glutamate to glutamine. Required for growth of basal-like breast cancer cells

¹ Data from microarray profiling of hTERT-HME1 cells treated for 24h with 100nM 1,25D. Fold change was calculated for 1,25D treated cells relative to vehicle treated control cells

² Entrez gene ID

Table 3

Comparative effects of 1,25D on selected target genes in six mammary epithelial cell lines as measured by quantitative PCR.

Gene	CELL LINES					
	hTERT-HME1	HME	MCF10A	MCF7	DCIS	Hs578T
<i>ITGB3</i>	12.41 ± 10.15	8.75 ± 0.41	1.21 ± 0.17	0.36 ± 0.04	5.60 ± 1.02	0.66 ± 0.10
<i>SLC1A1</i>	6.62 ± 4.10	13.02 ± 5.32	0.66 ± 0.06	0.79 ± 0.22	6.27 ± 0.29	1.07 ± 0.13
<i>KDR</i>	0.09 ± 0.04	0.54 ± 0.10	0.15 ± 0.01	0.53 ± 0.10	1.00 ± 0.29	0.92 ± 0.21
<i>BIRC3</i>	0.10 ± 0.03	0.45 ± 0.17	0.32 ± 0.07	0.69 ± 0.22	0.40 ± 0.10	1.00 ± 0.17
<i>GLUL</i>	0.15 ± 0.06	0.45 ± 0.10	1.06 ± 0.13	1.09 ± 0.18	0.79 ± 0.12	0.75 ± 0.14

Data represent fold change for each cell line treated with 100nM 1,25D for 24h relative to ethanol vehicle treated cells which were set to 1. Each value represents the mean ± SD of triplicates. Significance at p<0.05 is indicated by bold-face type and was assessed by one way ANOVA followed by Dunnett's multiple comparison test. hTERT-HME1, HME and MCF10A cells were grown in serum-free Media 171 with growth supplements. MCF7 cells were grown in αMEM with 5% FBS, DCIS cells in DMEM/F12 with 5% horse serum, and Hs578T cells in DMEM with 10% FBS as described in Methods.