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Review

Comparative regulation of gene expression by 1,25-dihydroxyvitamin D_3 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 D_3 (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 (100 nM, 24 h) 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.

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Contents

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1. Introduction

In most cell culture models, VDR ligands such as 1,25-dihydroxyvitamin D_3 (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.

2. Methods

2.1. 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

Table 1
Characteristics of mammary epithelial cell lines used in this study.

Cell Line	Origin and characteristics	Effect of VDR agonists
hTERT-HME1	• 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	• 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	• 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	• 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	• Isolated from a xenograft of ras-transformed MCF10A cells • Tumorigenic, forms DCIS-like lesions that progress to carcinoma • Models high-risk ductal carcinoma *in situ*	Growth arrest, inhibition of invasion and stem cell markers [17,35]
Hs578T	• 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]

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