Abstract. Background: Local synthesis of 1α,25(OH)D₃ in breast tissue may contribute to maintenance of normal cell function and could be impaired with low circulating levels of the precursor 25-hydroxyvitamin D. The aims of this study were to: i) assess the association between breast cancer risk and plasma 25OHD₃ concentration and ii) define the significance of expression of the 25OHD activating enzyme CYP27b1 in non-malignant and malignant models of breast epithelial cells. Materials and Methods: Breast cancer patients and control women were recruited and their 25OHD levels measured by enzyme-linked immunosorbent assay (ELISA). mRNA expression of CYP27b1 and the 1,25(OH)₂D₃ inactivating enzyme CYP24 were measured in breast cancer cell lines by RT-PCR and correlated with immunoblotting approaches to the translated proteins. Results: For women with 25OHD <50 nM the odds ratio for breast cancer compared with women with 25OHD >50 nM was 3.54 (CI 1.89-6.61, p<0.001). CYP27b1 and CYP24 were detected in non-malignant and malignant cell models. Protein levels of 24OHase but not 1αOHase were decreased at confluence in the cell lines. Conclusion: Impaired local generation of 1,25OHD₃ may contribute to the development of breast cancer.

There are approximately 1 million new cases of breast cancer diagnosed in the world each year. Until recently, it was the leading cause of cancer deaths in women and still comprises 18% of all cancers in women. It has been estimated that at least 1 in 10 women in the UK will develop breast cancer at some time in their lives and there are more than 14,000 breast cancer deaths in the UK annually.

The underlying causes for the development of breast cancer are still not understood, but many of the major risk factors are unavoidable e.g., being a woman, having a strong family history of breast cancer and increasing age (12). Other risk factors include aspects of reproductive history such as age at menarche, menopause and first live birth, use of the contraceptive pill and hormone replacement therapy. A strong family history of breast cancer is associated with a significant increase in a woman’s risk of developing the disease. However, familial breast cancers, linked to high penetrant genetic mutations, account for only about 5% of cases (3, 4). The vast majority of cases are sporadic and the underlying causes are poorly understood.

The relative importance of environmental, lifestyle and dietary factors to risk of breast cancer is still a matter of debate. Dietary factors associated with risk reduction for breast cancer include a high ratio of vegetable derived to total energy intake, a high fish intake (28), as well as a high dietary vitamin D intake (40). Another observed risk factor is latitude as it relates to UVB irradiation. Garland and associates (24, 25) reported that the risk of fatal breast cancer in major urban areas of the United States appears to be inversely proportional to the intensity of sunlight and that synthesis of vitamin D from sunlight exposure may be associated with low risk of fatal breast cancer.

Experimental studies have provided evidence for a link between vitamin D and protection against breast cancer. In addition to its well-established role in maintenance of calcium homeostasis, the active form of the hormone vitamin D, 1α,25 dihydroxyvitamin D (1α,25(OH)₂D₃), is known to have potent antiproliferative effects in many cancer cell types, including breast (reviewed in (13, 14)). Both normal and neoplastic breast tissues express the nuclear vitamin D receptor (VDR) through which 1α,25(OH)₂D₃ exerts both its anticancer effects and its effects on calcium homeostasis. Gene ablation studies have implicated the VDR in normal breast development (46, 53, 57). Furthermore, a number of polymorphisms have been identified in the gene encoding the VDR, some of which have been linked to risk of certain cancers including breast (8, 26, 30, 33).
Evidence suggests that any effects of dietary/environmental vitamin D restriction on risk of breast cancer are not directly related to changes in the circulating levels of 1α,25(OH)2D3. The most direct correlate of vitamin D status is the serum 25 hydroxyvitamin D3 (25OHD3) level, the major circulating metabolite of vitamin D. 1α,25(OH)2D3 is produced by hydroxylation of 25OHD3, a reaction catalyzed by the renal enzyme 25-hydroxyvitamin D-1α hydroxylase (1αOHase, encoded by the CYP27b1). The activity of the renal enzyme is regulated such that circulating concentrations of 1α,25(OH)2D3 are maintained within a relatively narrow range in the face of substantial changes in serum 25OHD3 levels (11).

Until recently mRNA expression of the 25OHD3 activating enzyme, CYP27b1, was thought to be restricted to the classical sites for endocrine synthesis of 1α,25(OH)2D3. However, the extra renal expression of CYP27b1 in non-classical target tissues has raised the possibility of an autocrine/paracrine role for the local synthesis and signalling of 1α,25(OH)2D3 (7, 15, 17, 20, 22, 23, 55). Recent studies have indicated that breast cancer cells express CYP27b1, although its role in modulating local responses to vitamin D remains to be defined (51). Thus, 25OHD3 may enter into an intracellular VDR signalling axis that co-ordinates the local synthesis, metabolism and signal transduction of 1α,25(OH)2D3. The components of the axis have been shown to be dynamically regulated as the activity of 1αOHase is repressed by 1α,25(OH)2D3 and, correspondingly, CYP24, which encodes the catabolic 25(OH)D-24-hydroxylase (24OHase) is positively regulated by 1α,25(OH)2D3. Thus, elevated levels of 1α,25(OH)2D3 appear to block its synthesis and induces its own inactivation (34, 47, 50) and, therefore, represents a classic endocrine axis.

There is a possibility that local synthesis of 1α,25(OH)2D3 in normal breast tissue may promote innate antiproliferative effects which, in turn, are repressed in cancer, possibly as a result of altered vitamin D status and specifically the circulating levels of 25OHD, the substrate for the 1αOHase enzyme. Therefore, we have hypothesised that the development of breast carcinoma is associated with both reduced local 1α,25(OH)2D3 availability, which is determined by an altered balance of CYP27b1 and CYP24 expression and activity, and VDR polymorphisms associated with disease progression.

To explore these possibilities, a series of studies were undertaken, with cell line models and primary tumour material, to assess the relationship between 25OHD3 levels and breast cancer risk in a UK population. Secondly, we aimed to further define the significance of the expression of 1αOHase in non-malignant and malignant breast epithelial cell models in order to develop a more comprehensive model with which to predict responsiveness towards 1α,25(OH)2D3.

Materials and Methods

Volunteers and measurement of circulating 25OHD. Ethical approval for this study was received from the St. George’s Hospital Ethics Committee, UK. Breast cancer patients and control volunteers were recruited as part of a study investigating the VDR genotype and risk of breast cancer. Only Caucasian women were recruited. All gave written informed consent before providing a 10-ml blood sample and answering a brief questionnaire. The questionnaire was used to determine personal information such as hormone replacement therapy (HRT) use and menopausal status. In addition to this, volunteers were asked whether they were vegetarians or vegans. No detailed dietary information was requested.

One hundred and thirty-one control volunteers were recruited from the Duchess of Kent breast-screening clinic on hospital grounds after having a negative mammogram. Forty-eight control volunteers were recruited from the same clinic where they were being investigated for benign breast disease. The breast cancer patients (n=179) were recruited from the Combined Breast Clinic in St. George’s Hospital. Breast cancer patients were matched to one control volunteer each. The main criteria for matching was the time of year the blood sample was taken, since vitamin D is derived from cutaneous synthesis in response to sunlight exposure. The year was divided into summer (April - September) and winter months (October - March). Each case was matched to a control subject where the sample was obtained in the same half of the year and also within 2 months of each other. The majority of patients were matched to a control that had the same menopausal status although 14 were not. The majority were also matched closely for age at sampling. The median difference in age at sampling was 2 years (range 0 - 12 years). None of the patients or control volunteers were vegetarians or vegans.

Aliquots of whole blood were taken for VDR genotyping as previously described (27) and the remaining blood was separated into plasma and Buffy coat by centrifugation. The plasma was then used to measure the circulating 25OHD concentration by enzyme immunoassay (ImmunoDiagnostics Services, UK). This assay detects both 25OHD3 and 25OHD2.

Analyses of VDR BsmI polymorphism. Genomic DNA was extracted from whole blood samples using a GenElute blood genomic DNA kit (Sigma). The BsmI genotype was determined by performing a PCR reaction followed by a restriction digest. PCR was carried out using a forward primer (‘5’ CAAACCAAGCTACAGTAGCGGT CAGTGA 3’) and a reverse primer (‘5’ AACAGCCGGGAAGAG GTCAAAGGG 3’). The PCR product was digested using the BsmI enzyme (BioLabs). Restriction digest products were separated on a 1.3% agarose gel and visualised using ethidium bromide.

Expression of components of the vitamin D signalling axis in breast cancer cell lines. To address the contribution of the local generation and catabolism of 1α,25(OH)2D3, the expressions of CYP27b1 and CYP24 were determined in established breast cancer cell lines. The cell lines T-47D, ZR-75-1, MCF-7, MDA-MB-231 and MCF-12A were cultured as previously described (51). Total RNA was extracted from sub-confluent cultures using the GenElute RNA extraction system (Sigma, Poole, UK) according to the manufacturer’s instructions. RNA was eluted in 30 μl of RNase-free water and stored at –70°C. Quantitative RT-PCR analysis of mRNA expression for CYP27b1, CYP24 and VDR was carried out as previously described (10).
Western immunoblot analysis. The cells were seeded at low and high density (2x10⁴/cm² and 8x10⁴/cm², respectively) and cultured until the high-density cultures reached confluency (72 h), to give sub-confluent and confluent cultures, respectively, and whole cell lysates prepared for Western immunoblot analysis. Briefly, 30 µg of total protein for each sample was subjected to SDS-PAGE and transferred onto PVDF membrane (Immobilon-P; Millipore, Bedford, MA, USA) and blocked with TBS-T containing 5% milk powder for 1 h. 25OHD3-24-hydroxylase sheep polyclonal antibody was a generous gift from Prof. Rajiv Kumar (Mayo Proteomics Research Center, Mayo Clinic and Foundation, Rochester, MN, USA). For detection of 25OHD3-1α-hydroxylase or 25OHD3-24-hydroxylase, sheep polyclonal antibodies were diluted 1:200 and 1:500, respectively, followed by secondary antibody (anti-sheep-HRP) (The Binding Site, Birmingham, UK) (55). Proteins were detected using ECL (Amersham, Amersham, Bucks, UK) and autoradiography. To ensure even loading and transfer of protein, the membranes were subsequently probed with a 1/5000 dilution of primary mouse monoclonal β-actin antibody (AC-15, Sigma), followed by anti-mouse-HRP secondary antibody (Binding Site) and the signals developed with ECL and autoradiography as above. To quantify the relative changes in protein levels, densitometry analysis was performed on triplicate sets of lysate and the values normalised to β-actin levels.

Proliferation assays in the presence of 1α,25(OH)2D3 and 1α,24(R),25(OH)2D3. 1α,25(OH)2D3 was a kind gift from Dr. Milan R. Uskokovic, (Hoffman-La Roche, Nutley, NJ, USA). 1α,24(R),25(OH)3D3 was a generous gift from Prof. Satya G. Reddy (Brown University School of Medicine, Providence, USA). All compounds were stored at 1 mM in ethanol at −20°C in the dark. The proliferation of the cell lines in the presence of vitamin D compounds was measured by colony formation in soft agar. Trypsinized and washed, single-cell suspensions were prepared from 80% confluent cultures, counted and plated onto 24-well flat-bottomed plates using a two-layer soft agar system with 1x10⁵ cells in 400 µl of media per well, as described previously (9). The support layer was prepared with agar (1%) equilibrated at 42°C. Prior to the addition of this layer to the plate, the various treatments were pipetted into the wells. After 10 days of incubation, the colonies (>50 cells) were counted using an inverted microscope. Dose response curves were constructed and the ED₅₀ values were interpolated.

Results

25-hydroxyvitamin D levels in breast cancer patients and controls.

The mean plasma 25OHD concentrations were significantly lower in breast cancer patients than matched controls (80.1 vs. 97.8 nM, p<0.001). Overall, for women with low or insufficient 25OHD levels (<50 nM), the odds ratio (OR) for breast cancer compared with those with levels >50 nM was 3.54 (95% CI 1.89-6.61, p<0.001). When the 25OHD values were separated into quartiles (quartile 1, <50 nM; quartile 2, 50-100 nM; quartile 3, 100-150 nM; quartile 4, 150-200 nM) analysis showed that, compared to women with the highest levels of 25OH-D (quartile 4), women with insufficient levels (quartile 1) had more than five times the risk of breast cancer (OR=5.83 (95% CI 2.31-14.7, p<0.001).

Table I. Odds ratios (OR) for breast cancer risk by BsmI genotype and 25OHD levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>quartile 1, all genotypes</td>
<td>3.54 (1.89 – 6.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>quartiles 2-4, all genotypes</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>quartile 1 and bb</td>
<td>6.82 (2.57 – 17.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>quartile 1 and Bb/BB</td>
<td>4.01 (1.89 – 8.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>quartile 2-4 and bb</td>
<td>2.66 (1.48 – 4.79)</td>
<td>0.001</td>
</tr>
<tr>
<td>quartile 2-4 and Bb/BB</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval.

Figure 1. Plasma 25OHD concentration (by quartile) and breast cancer risk. 25OHD values were separated into quartiles (quartile 1, <50 nM; quartile 2, 50-100 nM; quartile 3, 100-150 nM; quartile 4, 150-200 nM). The number of controls and cases in each 25OHD quartile and the odds ratio (OR) for breast cancer risk for each quartile are shown. (OR=5.8 (95% CI 2.31-14.7, p<0.001).
that for the group as a whole. However, this was of only borderline significance due to the small number of cases.

**VDR polymorphisms and breast cancer risk.** We have previously reported that the VDR gene *Bsm*I polymorphism (which in Caucasians is in linkage disequilibrium with the polyA repeat sequence) is associated with breast cancer risk in a UK population (11). Results from the present study are in agreement with our previous findings such that the odds ratio for breast cancer risk for women with the *bb* genotype was 2.02 (95% CI 1.03-3.97, \( p=0.04 \)) compared with the *BB* genotype. Furthermore, we found that women with the *bb* genotype in combination with low/insufficient 25OHD levels had an even greater risk for breast cancer than low 25OHD levels alone. While women of all genotypes with 25OHD concentrations <50 nM had an OR for breast cancer risk of 3.54, the OR for women with 25OHD levels <50 and the *bb* genotype was 6.82 (95% CI 2.57-17.1, \( p<0.001 \), (41)). These data are summarised in Table I.

**Expression of 1αOHase in cell lines.** There is evidence that breast cells contain the 1αOHase enzyme required for the autocrine production of 1α,25(OH)2D3 from circulating 25OHD3. Previously we detected CYP27b1 mRNA in breast cancer cell lines and, using immunohistochemical approaches, confirmed readily detectable staining in primary tumours and, importantly, demonstrated enzyme activity (51) (Table II). Parallel studies by Dr. Heide Cross and co-workers found that 24OHase expression was dependent upon the proliferation status. Thus, in CaCo2 colon cancer cells the levels of 24OHase were reduced upon confluence, which was associated with differentiation (6). Together, these studies support the concept that the components of the VDR signalling axis are dynamically expressed and that these processes may be corrupted in cancer.

To compliment both of these studies, we examined 1αOHase and 24OHase in a panel of cancer cell lines either during exponential proliferation or at confluence. The 1αOHase appeared relatively constant in cells which were either actively cycling or undergoing confluence-induced G1 arrest (Figure 2A). Equally, the levels appeared to be modestly reduced in the cancer cell lines, compared to T47-D cells, which reflects our previous mRNA studies (51). By contrast, in the most rapidly proliferating cells, for example in MCF-7 and MDA-MB-231 cells, there was evidence that 24OHase was indeed down-regulated upon confluence (Figure 2B). The pattern reflected the expression of Cyclin E, as a marker of S-phase cells, and the percentage of cells out of G1 of the cell cycle. For example, sub-confluent growing populations of MDA-MB-231 cells were associated with approximately 2- fold and 3-fold higher expressions of Cyclin E and 24OHase levels, respectively, and 62% of cells in S- and G2/M-phases of the cell cycle (Figure 2B). Similar levels of down-regulation were seen in MCF-7 cells. Thus, elevated 24OHase expression appeared to correlate well with cell proliferation.

1α,25(OH)2D3 and 1α,24(R),25(OH)3-D3 demonstrate a range of antiproliferative potencies against breast epithelial cells. The antiproliferative effects of 1α,25(OH)2D3 upon MCF-12A and a series of breast cancer cell lines were titrated across a broad concentration range (0.001 to 100 nM) in clonogenic assays to generate dose-response curves. An example for

| Table II. Expression and activity of components of the VDR signalling axis in breast cancer cell lines and primary material. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell type | ED50\(^1\) | ED50\(^2\) | CYP27b1\(^3\) | 1αOHase\(^4\) | Met\(^5\) | VDR\(^6\) | VDR\(^7\) | CYP24\(^9\) | 24OHase\(^10\) | Met\(^11\) |
| MCF-12a | 5 | Nd | 1 | Nd | Nd | 1 | Nd | 1 | Nd | Nd |
| T47-D | 15 | >1000 | 1.3 | ++ | ++ | Nd | 0.87 | Nd | 2.2 | ++ |
| MCF-7 | 70 | >1000 | 0.51 | ++ | ++ | + | 0.21 | Nd | 1.87 | ++ |
| ZR-75-1 | 70 | >1000 | 0.38 | ++ | ++ | Nd | 0.12 | Nd | 74 | ++ |
| MCF-7Res | >1000 | >1000 | 0.44 | Nd | Nd | 0.07 | Nd | 234.0 | Nd | Nd |
| MDAMB-231 | >1000 | >1000 | 0.36 | ++ | ++ | Nd | 0.12 | Nd | 220.0 | ++ |

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\(^1\)ED50 is the dose of 1α,25(OH)2D3 required to inhibit colony formation by 50% (1, 5, 9, 10, 14, 18, 37, 45).

\(^2\)ED50 is the dose of 1α,24(R),25(OH)3-D3 required to inhibit colony formation by 50%.

\(^3\)Measurement of relative CYP27b1 mRNA levels compared to MCF-12A cells (22, 51).

\(^4\)Detection of 1αOHase protein by immunoblotting analyses in breast cancer cell lines (22, 51).

\(^5\)Measurement of relative VDR mRNA levels compared to MCF-12A cells (32).

\(^6\)Detection of VDR protein by immunoblotting analyses in breast cancer cell lines.

\(^7\)Determination of VDR protein by immunoblotting analyses in breast cancer cell lines or by immunohistochemistry (IHC).

\(^8\)Levels of VDR protein are down-regulated in resistant variants of MCF-7 compared to parental MCF-7 cells (21).

\(^9\)Measurement of relative CYP24 mRNA levels compared to MCF-12A cells (22, 51).

\(^10\)Detection of 24OHase protein by immunoblotting analyses in breast cancer cell lines.

\(^11\)Measurement of 24OHase activity by examining conversion of radiolabelled 25(OH)D precursor (51).

Nd = Not determined, + = detected.
The responses of the cancer cell lines to 1α,25(OH)2D3 were in accordance with previous studies (9, 18, 31). In the current study, these responses were compared to those for MCF-12A [ED50 5 nM], which represents an in vitro model of non-malignant breast epithelial cells. As shown in Table II, each of the cancer cell lines had higher ED50 values than MCF-12A. However, there remained considerable variation between the sensitivity of these malignant cells with ED50 values ranging from 15 nM in the case of T-47D cells to >1000 nM in MDA-MB-231 cells (Figure 3B and Table II).

In contrast to the range of antiproliferative responses to 1α,25(OH)2D3, there was a uniform absence of antiproliferative response to 1α,24(R),25(OH)3-D3, its 24OHase-generated metabolite product (Figure 3 and data not shown). Thus, the addition of an OH group to C 24 of 1α,25(OH)2D3 appears to ablate the antiproliferative potency of the parent compound. Interestingly, in MDA-MB-231 cells, a range of proproliferative responses were observed only at low concentrations of 1α,25(OH)2D3 and 1α,24(R),25(OH)3-D3 (Figure 3B). Thus colony growth was stimulated significantly at concentrations of 0.1 nM 1α,25(OH)2D3 and 1α,24(R),25(OH)3-D3 by 152±12% (±SEM) and 146±7% (p<0.05) relative to respective control cultures.

Discussion

The central hypothesis of the current study is that the proliferation of breast epithelial cells is regulated by the local synthesis, signalling and metabolism of 1α,25(OH)2D3. The initiation and/or progression of breast cancer involves suppressed VDR signalling, which can occur by either dysregulation of the balance of synthesis and metabolism of 1α,25(OH)2D3 and/or specific polymorphisms of the VDR.

In vivo data now support a physiological role for VDR-mediated signalling to promote differentiation of normal breast epithelial cells. Proliferating hormones such as...
cortisol, prolactin and insulin up-regulate the VDR, which is initially present at low levels, in mammary gland explants. The up-regulation of the VDR is essential for gland differentiation and function as there is reduced ductal differentiation and development in the mammary gland of VDR knockout mice compared to wild-type litter-mates (46, 56, 58). Breast cancer cell lines display a spectrum of sensitivities, including complete insensitivity towards 1α,25(OH)2D3, suggesting these functions are targeted in malignancy. The mechanisms underpinning the basis of this apparent hormonal insensitivity are unclear, but it seems likely that their resolution will facilitate the clinical application of 1α,25(OH)2D3 as an anticancer agent.

Vitamin D is derived both from dietary sources and cutaneous synthesis in response to UV irradiation. Circulating concentrations of 25OHD serve as a useful index of vitamin D status and in vitamin D sufficiency serum concentrations range from 50-200 nM (14). Although several studies have documented an association between dietary/environmental intake of vitamin D and risk of breast cancer, the precise relationship between serum 25OHD levels and cancer risk has not been documented in a large cohort. Our present study has shown a significant association of low 25OHD levels with breast cancer risk such that women with the lowest levels of 25OHD (<50 nM) had over five times the risk of breast cancer compared with those with the highest levels (>150 nM). It was also found that the risk associated with low 25OHD levels was increased in combination with the ‘at risk’ VDR polymorphism such that women with the bb genotype in combination with low/insufficient 25OHD levels had an even greater risk of breast cancer than those with low 25OHD levels alone. Low vitamin D status and VDR polymorphisms have also been associated with the risk of prostate cancer (42).

The observed expression of 1αOHase in normal breast tissue suggests that paracrine production of 1α,25(OH)2D3 could be important in maintenance of normal breast cell function (51). Thus, it could be postulated that low circulating concentrations of 25OHD, arising either as a result of reduced exposure to sunlight or to dietary patterns, impair the generation of 1α,25(OH)2D3 within breast tissue, increasing the risk of tumour development. Elevated CYP24 (2, 51) may lead to overexpression of 24OHas which, in turn, attenuates the capacity for 1α,25(OH)2D3 to activate antiproliferative VDR target genes. Certainly the current data fit with a model of autocrine production of vitamin D compounds, the antiproliferative actions of which are determined in part by precursor availability and perhaps other dietary and environmental factors, as has been proposed for the colon (16).

These results of the current study, and our recent parallel ones (26, 27, 51) together indicated that 1α,25(OH)2D3 may be generated locally in breast tissue and may play a role in the maintenance of normal breast cell function. The capacity to generate 1α,25(OH)2D3 locally in the breast will be dependent on the availability of 25OHD as substrate for the 1αOHase enzyme. Little is known about the metabolism or half-life of 1α,25(OH)2D3 in normal breast or breast cancer cells. Catabolism of 1α,25(OH)2D3 is initiated via hydroxylation at the 24 position in the side-chain to produce 1α,24(R),25(OH)3-D3, a reaction catalyzed by 24OHase. The current study demonstrated that this compound has a different activity profile to the parental 1α,25(OH)2D3 and support further the significance of the concept of inappropriate inactivation of locally generated 1α,25(OH)2D3. This concept is supported further by a number of parallel studies. We targeted 24OHase via an antisense strategy in MDAMB-231 cells, which have high basal CYP24 levels (Table II) and sustained 24OHase expression in proliferating cells. These studies demonstrated a significant enhancement of the growth inhibitory effects of 1α,25(OH)2D3. Equally the MCF-7Res cells validate further the important contribution of elevated CYP24 to predict 1α,25(OH)2D3 insensitivity as these cells demonstrate significantly elevated levels of CYP24 expression relative to their parental MCF-7 cell line (132 fold, p<0.001) (Table II).

Expression and activity of 1αOHase and CYP27b1 has been detected in various extrarenal tissues and is reduced in transformed cells (19, 29, 35, 36, 38, 39, 43, 44, 48, 49, 52, 54). The current study extends these findings to breast cancer cells and supports the concept that loss of CYP27b1 expression, possibly resulting in local, tumour-specific 1α,25(OH)2D3 insensitivity, is associated with transformation. The enzymes involved in activation and catabolism of vitamin D in breast tissue may provide novel targets for prevention and treatment of breast cancer.

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