1,25-Dihydroxyvitamin D₃ Receptors in Human Epithelial Cancer Cell Lines

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ABSTRACT

Specific, high-affinity cytosolic receptors for 1,25-dihydroxyvitamin D₃ have been demonstrated in five human cancer cell lines. The cell lines were derived from tumors of breast, lung, cervix, and melanotic and amelanotic melanomas. Binding affinity (Kᵦ) of the receptors for 1,25-dihydroxyvitamin D₃ were all approximately 0.2 nM, and receptor content ranged from 21 to 174 fmol/mg cytosol protein. The receptors from all five cell lines sedimented at 3.25 on sucrose density gradients and exhibited preferential affinity for 1,25-dihydroxyvitamin D₃ compared to other vitamin D metabolites.

INTRODUCTION

The detection of steroid hormone receptors in a variety of cancers is currently being investigated as a means of ascertaining whether tumors have the potential to be hormonally dependent (20, 28). In addition, knowledge of the receptor status of tumors may provide information related to the state of tumor differentiation or the expected treatment response (21). Breast cancers have been extensively investigated for estrogen receptors (20–22, 28) and, more recently, similar studies with androgen receptors in prostate cancer (12, 15) and glucocorticoid receptors in leukemia (18) have been reported. 1,25(OH)₂D₃, the active metabolite of vitamin D₃, has a mode of action analogous to that of steroid hormones (14), and recent studies have characterized receptors in various mammalian (2, 4, 9, 16, 27) and human (8, 26) target cells. Receptors for 1,25(OH)₂D₃ have been described in osteosarcoma cells (19), breast cancer tissue (7, 10), and malignant melanoma (3). As a major regulator of calcium transport, 1,25(OH)₂D₃ action may alter multiple cell properties and may influence the behavior of a wide variety of tumors. The possible role of 1,25(OH)₂D₃ as a modulator of cancer cell growth has recently been raised (3, 10). We therefore thought that it was important to examine a number of additional malignant human cell lines for the presence of 1,25(OH)₂D₃ receptors and to compare the properties of these tumor receptors both to one another and to receptors in normal tissues. Our results indicate that such receptors are indeed present in a variety of human epithelial malignant cell lines and that the properties of these receptors are similar to those of receptors present in normal mammalian target organs.

MATERIALS AND METHODS

Sedimentation Analysis. KTEDM extracts were incubated with a saturating concentration (1.3 nM) of [³²P]-1,25(OH)₂D₃ with or without radioinert steroids for 3 hr at 0°C. Bound and free steroids were separated by dextran-coated charcoal or by hydroxyapatite (4, 9, 27). Parallel incubations contained 100-fold molar excess of radioinert [³²P]-1,25(OH)₂D₃ for determination of nonspecific binding, which was always less than 15%.

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2 To whom requests for reprints should be addressed, at Division of Endocrinology, 5-005, Stanford University School of Medicine, Stanford, Calif. 94305.
3 The abbreviations used are: 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 25-OH-D₃, 25-hydroxyvitamin D₃; 1α-OH-D₃, 1α-hydroxyvitamin D₃; 24,25(OH)₂D₃, 24,25-dihydroxyvitamin D₃; KTEDM, 300 mM KCl, 10 mM Tris-HCl, 1 mM EDTA, 5 mM dithiorthreitol, and 10 mM sodium molybdate, pH 7.4. [CANCER RESEARCH 42, 856-859, March 1982] 0006-5472/82/0042-0008$02.00

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centrifuged at 2° for 18 hr at 257,000 × g. The 14C-proteins, carbonic anhydrase (3.0S), ovalbumin (3.7S), and bovine serum albumin (4.4S) (New England Nuclear, Boston, Mass.), were used as internal markers.

**Protein Determination.** Protein concentration was assayed by the Coomassie blue dye method of Bradford (1).

**RESULTS**

**Sedimentation Analysis.** We used the technique of sedimentation analysis on sucrose density gradients to examine the 1,25(OH)₂D₃-binding components present in KTEDM extracts from 5 human malignant cell lines. The findings were qualitatively the same in each case. Extracts from breast, lung, and HeLa cells all reproducibly demonstrated a [³H]-1,25(OH)₂D₃-binding peak in the 3.2S region. The binding could be eliminated by an excess of radioinert 1,25-(OH)₂D₃ confirming the specific nature of this binding component (Chart 1). Similarly, KTEDM extracts from both the melanin-producing and amelanotic human melanoma cell lines contained a specific 3.2S binder for [³H]-1,25(OH)₂D₃. No specific binding could be demonstrated with extracts from the Cloudman mouse melanoma cell line (Chart 2).

**Saturation Analysis.** KTEDM cell extracts from various cell lines were incubated with increasing concentrations (0.065 to 1.3 nM) of [³H]-1,25(OH)₂D₃ for 3 hr at 0°. In all cell lines examined, Scatchard analysis of [³H]-1,25(OH)₂D₃ binding yielded linear plots, indicating a single class of binding sites. Two such plots, obtained with KTEDM extracts of HeLa and breast cells, are shown in Chart 3, and data from all cell lines are summarized in Table 1. The apparent dissociation constants were very similar in all the cell lines examined (~0.2 nM), and Scatchard analysis of [³H]-1,25(OH)₂D₃ binding indicated a range of receptor content from 21 to 174 fmol/mg protein. No specific binding could be detected with KTEDM extracts from WI-38 fibroblasts (lung) whereas normal human skin fibroblasts did possess high-affinity receptors for 1,25(OH)₂D₃ (Table 1).

**Specificity of 1,25(OH)₂D₃ Binding in Cancer Cells.** In order to determine the hormonal specificity of this receptor, the ability of 2 physiologically less active vitamin D₃ metabolites, 25-OH-D₃ and 24,25(OH)₂D₃, and the synthetic analog 1α-OH-D₃ to compete for [³H]-1,25(OH)₂D₃-binding sites was analyzed by sucrose density gradient techniques. KTEDM extracts of HeLa cells were incubated with 1.3 nM [³H]-1,25(OH)₂D₃ with or without a 5-fold molar excess of various vitamin D₃ metabolites. Sedimentation of the extracts bound with [³H]-1,25(OH)₂D₃ yielded a single binding peak sedimenting at 3.2S. A 5-fold molar excess of radioinert 1,25(OH)₂D₃ caused a substantial reduction of [³H]-1,25(OH)₂D₃ binding in the 3.2S peak (Chart 4). The same concentration of 25-OH-D₃, 24,25(OH)₂D₃, and 1α-OH-D₃ produced only a negligible decrease in the height of the 3.2S peak (Chart 4B). Similar results were previously found with KTEDM extracts of Hs695T melanoma cells (3). As shown

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**Table 1**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue of origin</th>
<th>Kd (nm)</th>
<th>Protein content (fmol/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hs695T</td>
<td>Melanoma (amelanotic)</td>
<td>0.18</td>
<td>174 (172–176)</td>
</tr>
<tr>
<td>G361</td>
<td>Melanoma (amelanotic)</td>
<td>0.12</td>
<td>21 (15–26)</td>
</tr>
<tr>
<td>Huk-HeLa</td>
<td>Cervical carcinoma</td>
<td>0.22</td>
<td>31 (19–43)</td>
</tr>
<tr>
<td>Hs0576T</td>
<td>Breast carcinoma</td>
<td>0.12</td>
<td>40 (35–45)</td>
</tr>
<tr>
<td>A549</td>
<td>Lung carcinoma</td>
<td></td>
<td>50 (46–54)</td>
</tr>
<tr>
<td>Normal cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human skin</td>
<td>Infant foreskin</td>
<td>0.27</td>
<td>42 (30–50)</td>
</tr>
<tr>
<td>fibroblasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WI-38</td>
<td>Normal lung fibroblast</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

*Numbers in parentheses, range.

For undetermined reasons, possibly relating to the high mucin content, we have been unable to obtain satisfactory Scatchard plots with A549 lung cells. In this case, receptor content was estimated by single-point analyses using a saturating concentration of [³H]-1,25(OH)₂D₃. No specific receptor binding could be detected in WI-38 cells.

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in Chart 5, the receptor in both the breast cancer cell line Hs0578T and the lung cancer cell line A549 also preferentially bound the dihydroxymetabolite. When KTEDM extracts of these 2 cell lines were incubated with 1.3 nM [3H]-1,25(OH)2D3, the inclusion of a 5-fold molar excess of radioinert 1,25(OH)2D3 markedly reduced the height of the 3.2S peak, whereas 25-OH-D3 was a less effective competitor at the same concentration. The profile found with the lung sample is somewhat unusual in the relatively weaker response to 5-fold 1,25(OH)2D3 and the somewhat stronger competition exhibited by 25-OH-D3.

We have attributed these findings, as well as our inability to obtain adequate Scatchard plots, to the large concentration of mucinous material synthesized by these cells (17) and present in the cytosol preparations.

**DISCUSSION**

These experiments demonstrate the presence of a specific,

![Chart 4. Sucrose gradient analysis of 1,25(OH)2D3 binding in KTEDM extracts of Huk-HeLa cells.](chart4.png)

high-affinity binder for 1,25(OH)2D3 in 5 human epithelial cancer cell lines. The 3.2S sedimentation coefficient and the preferential binding of 1,25(OH)2D3 compared to 25-OH-D3 or 24,25(OH)2D3 indicate that this binder is the 1,25(OH)2D3 receptor and not the 25-OH-D3 plasma binder found in all tissues (4). The properties, including affinity, hormonal specificity, and sedimentation coefficient, are virtually identical to those we and others have obtained for the 1,25(OH)2D3 receptor in a variety of mammalian target organs (2, 4, 16, 27), indicating a similarity between the malignant and normal cell receptors.

The concentration of binding sites in these tumors varied from 21 to 174 fmol/mg protein. However, we do not wish to emphasize the quantitative aspects of these data since recent findings in our laboratory indicate that the receptor content of cultured cells varies extensively at different points in the culture cycle (2). In that study, primary cultures of normal bone cells exhibited a 4-fold alteration in 1,25(OH)2D3 receptor content which directly correlated with the rate of cell proliferation. The cells in the present study were not examined for receptor fluctuation related to cell growth.

The demonstration of receptors in these tumor cells raises the question of whether the receptor is present in the normal counterpart or whether the findings in malignant cells represent a cancer-related alteration. We and others demonstrated 1,25(OH)2D3 receptors in normal breast tissue (4, 7). Although we have been able to show 1,25(OH)2D3 receptors in human (8) and mouse (4) skin, it is not yet clear whether normal melanocytes possess receptors. However, both cultured human skin fibroblasts and keratinocytes are receptor positive (8). Samples of total mouse lung do not contain detectable receptors (4). However, the type II alveolar cell, the origin of the tumor from which cell line A549 was derived, represents a minority fraction of the total lung cell population (24), and detection of a receptor limited to this cell would be difficult. Again, whereas total mouse uterus appears negative for receptor (4), good data are not available regarding normal cervix.

Since the origin of all 5 tumor cell lines is an epithelial layer, all may be involved in transport of calcium, which is the best understood function of 1,25(OH)2D3. It remains to be determined whether the nonmalignant counterparts of these cell types are target tissues for the hormone.

As with estrogen receptors in breast cancer, the demonstration of 1,25(OH)2D3 receptor in these tumor cells may be significant in either of 2 ways: (a) the demonstration of receptors may merely prove to be a marker of cellular differentiation providing an additional measurable protein gene product to characterize the tumor; (b) the presence of receptors indicates that the cell is potentially responsive to 1,25(OH)2D3, and thus tumor activity may be altered in the presence of the hormone. The cellular pattern of growth and metabolism may be regulated either by 1,25(OH)2D3 itself or by local shifts in calcium transport mediated by the hormone. In this regard, our studies on the effect of 1,25(OH)2D3 on the in vitro growth of malignant melanoma cell line Hs695T indicate that these melanoma cells are functionally responsive to the hormone. Inclusion of 1,25(OH)2D3 in the tissue culture medium leads to a marked inhibition of cell proliferation (3). The present findings in the Cloudman melanoma emphasize that not all melanoma cell lines are receptor positive.

These data indicate that 1,25(OH)2D3 receptors are present in several human malignant cell lines. The possible effects of...
the hormone on tumor growth and the potential value of 1,25(OH)2D3 receptor determinations in patients with these tumors warrant further study.

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REFERENCES

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