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

Kay Colston, M. Joseph Colston, A. Howard Fieldsteel, and David Feldman

Department of Medicine, Stanford University School of Medicine, Stanford, California 94305 (K. C., M. J. C., A. H. F., D. F.), and Division of Life Sciences, SRI International, Menlo Park, California 94025 (M. J. C.; A. H. F.)

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 (Kd) 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 sedimeted 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

Steroids. 1a,25-Dihydroxy[23,24-3H]cholecalciferol (82 and 120 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, Ill.). Crystalline 1,25(OH)2D3, 25-OH-D3, 1a-OH-D3, and 24,25(OH)2D3 were gifts from Dr. M. Uskokovic (Hoffmann-LaRoche Inc., Nutley, N. J.).

Cell Culture. Five human cancer cell lines were examined. Hs6855T is a breast cancer cell line of epithelial origin which produces casein and lacks estrogen receptors (13). A549 is a carcinoma of the lung which arose from type II alveolar epithelial cells and which synthesizes surfactant (17). Huk-HeLa cells were originally derived from human kidney but recently were found to be overgrown by HeLa cervical carcinoma cells as has been reported for a number of cell lines originating in many different laboratories (23). Two melanoma cell lines were also examined: G-361, a human melanoma cell line (American Tissue Culture Collection, CRL 1424) which produces melanin in vitro; and Hs695T, a metastatic amelanotic melanoma cell line, which has retained morphological properties of melanocytes (6). All the cells were grown as monolayers in glass roller bottles using Eagle's minimal essential medium or Dulbecco's modification of that medium (Grand Island Biological Co.) containing 10% fetal bovine serum. All 5 cell lines have been shown to produce tumors when injected into immunodeficient animals (5, 11) and, where such tumors have been examined histologically, their appearance was consistent with the tumor type from which the cell line was derived (5). An additional tumor cell studied was the Cloudman S91 mouse melanoma cell line (American Tissue Culture Collection; CCL 53.1, clone M-3) which was grown in Ham's medium supplemented with 20% calf serum. Several nonmalignant fibroblast cell lines were also examined. Human skin fibroblasts, derived from infant foreskin, were maintained as described previously (8). WI-38 cells, from a fibroblast cell line derived from normal human lung, were obtained from V. J. Cristofalo (Wistar Institute) and were maintained in F-17 medium (Grand Island Biological Co.) supplemented with 10% newborn calf serum.

[3H]-1,25(OH)2D3 Binding Assay. All cells were grown to confluence, and fresh medium was added 24 hr before the experiment. Cell monolayers were rinsed with phosphate-buffered saline (NaCl 8 g/liter, KCl 0.2 g/liter, Na2HPO4 0.94 g/liter, KH2PO4, 0.2 g/liter) and scraped from the glass with a rubber policeman; in some experiments, cells were detached by trypsinization. Cells were washed 3 times and then were sonicated in a hypertonic buffer (KTEDM) containing 300 mM KCl, 10 mM Tris-HCl, 1 mM EDTA, 5 mM dithiothreitol, and 10 mM sodium molybdate, pH 7.4. High-speed supernatants of the cell extracts were obtained by centrifugation at 144,000 X g for 30 min and designated KTEDM extracts. It has been shown previously that 0.3 M KCl extracts contain both cytosolic receptors and any receptor in the nuclear compartment (25). Aliquots of the KTEDM extracts (200 µl) were incubated with the appropriate 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 1000-fold molar excess of radiolabeled 1,25(OH)2D3 for determination of nonspecific binding, which was always less than 15%.

Sedimentation Analysis. KTEDM extracts were incubated with a saturating concentration (1.3 nm) of [3H]-1,25(OH)2D3 with or without radiolabeled steroids for 3 hr at 0°C. Free steroid was removed by charcoal adsorption. Aliquots (200 µl) of the KTEDM extracts were layered onto linear 5 to 20% sucrose gradients prepared in KTEDM buffer and
binding at 2°C for 18 hr at 257,000 x 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°C. 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

---

# Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue of origin</th>
<th>Kd (nM)</th>
<th>Receptor 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>AS49</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 fibroblasts</td>
<td>Infant foreskin</td>
<td>0.27</td>
<td>42 (30–50)</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 AS49 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.
in Chart 5, the receptor in both the breast cancer cell line Hs0578T and the lung cancer cell line A549 also preferentially bound the dihydroxy metabolite. When KTEDM extracts of these 2 cell lines were incubated with 1.3 nM \([^{3}H] \cdot 1,25(OH)_{2}D_{3}\), the inclusion of a 5-fold molar excess of radioinert 1,25(OH)_{2}D_{3} markedly reduced the height of the 3.2S peak, whereas 25-OH-D_{3} 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)_{2}D_{3} and the somewhat stronger competition exhibited by 25-OH-D_{3}. 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, high-affinity binder for 1,25(OH)_{2}D_{3} in 5 human epithelial cancer cell lines. The 3.2S sedimentation coefficient and the preferential binding of 1,25(OH)_{2}D_{3} compared to 25-OH-D_{3} or 24,25(OH)_{2}D_{3} indicate that this binder is the 1,25(OH)_{2}D_{3} receptor and not the 25-OH-D_{3} 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)_{2}D_{3} 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)_{2}D_{3} 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)_{2}D_{3} receptors in normal breast tissue (4, 7). Although we have been able to show 1,25(OH)_{2}D_{3} 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)_{2}D_{3}. 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)_{2}D_{3} 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)_{2}D_{3}, 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)_{2}D_{3} itself or by local shifts in calcium transport mediated by the hormone. In this regard, our studies on the effect of 1,25(OH)_{2}D_{3} 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)_{2}D_{3} 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)_{2}D_{3} 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.

ACKNOWLEDGMENTS

Cell lines Hs0578T and A549 were obtained from Dr. W.A. Nelson-Rees and were produced with support from the Division of Cancer Cause and Prevention, Biological Carcinogenesis Branch, National Cancer Institute, under the auspices of the Office of Naval Research and the Regents of the University of California.

REFERENCES

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


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/3/856

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.