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EFFECT OF 1,25-DIHYDROXYVITAMIN D_3 ON HUMAN CANCER CELLS *IN VITRO*

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Summary—1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) dependent growth and differentiation of 6 tumor cell lines has been determined by the use of the monolayer proliferation assay. Cell lines of 4 gastro-intestinal carcinomas, 1 malignant schwannoma, and 1 malignant histiocytoma have been established and characterized. Cells were incubated for 4, 7, and 11 days in the presence of 0.8 or 8 nM $1,25(OH)_2D_3$ and for control without addition of the hormone. Proliferation rates of $1,25(OH)_2D_3$ treated cells were compared with cell growth in the untreated controls. Five out of 6 cell lines showed a $1,25(OH)_2D_3$ dependent growth pattern. With 8 nM $1,25(OH)_2D_3$ they were all inhibited. With 0.8 nM, 3 of them were inhibited at any time of the test period, whereas 1 was stimulated at day 4 and inhibited at days 7 and 11. One cell line was stimulated at days 4, 7, and 11 when incubated with 0.8 nM $1,25(OH)_2D_3$. No striking morphological changes could be observed in the presence of $1,25(OH)_2D_3$. We conclude that $1,25(OH)_2D_3$ dependent cells *in vitro* are not necessarily growth-inhibited by this compound. Thus, $1,25(OH)_2D_3$ is not an exclusively proliferation inhibiting agent.

INTRODUCTION

It is widely accepted that 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) plays a key role in the regulation of calcium homeostasis [1]. The action of this steroid hormone is mediated through specific receptors, which are present in the "classical" and a number of "non-classical" target organs [2]. In 1979, Eisman *et al.*[3] reported the presence of receptors for $1,25(OH)_2D_3$ in the breast cancer cell line MCF 7. Additionally, binding or effects of $1,25(OH)_2D_3$ in cultured cells derived from an osteogenic sarcoma [4], malignant melanoma [5], mouse myeloid leukemia [6], colonic carcinoma [7], and parathyroid tumors [8] have been described. The $1,25(OH)_2D_3$ dependent modulation of growth and differentiation of cultured tumor cells is accepted to be a receptor-mediated process [5, 7] and has been reported to occur in a number of different cell lines as reviewed by Ref. [9].

In this study, the influence of $1,25(OH)_2D_3$ on growth rate and differentiation of cultured tumor cells was investigated by the use of the monolayer proliferation assay as described by Refs. [10–12]. Six cell lines, 4 of them derived from carcinomas and 2 of sarcomas, have been incubated for different times with different concentrations of $1,25(OH)_2D_3$ in order to determine hormone-induced effects. The monolayer proliferation assay was the method of choice since it uniquely allows the simultaneous investigation of the different parameters of proliferation and differentiation.

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EXPERIMENTAL

Origin of cells

Tumor material was obtained during surgery (Department of Surgery, University Hospital, Hamburg; head, Prof. Dr H. W. Schreiber) and immediately taken into culture. For characterization, a part of it was processed for paraffine histology and immunohistochemistry.

Cell culture

Tissue was minced mechanically into small pieces and digested enzymatically by a mixture of collagenase and dispase (0.1 U/0.8 U per ml) obtained from Boehringer, Mannheim. The cell suspension was centrifuged at 600 r.p.m. and the pellet and supernatant were seeded into different culture flasks. The growth medium was Leibowitz L15, supplemented with: 5% fetal bovine serum (FBS), insulin (80 IU/l), transferrin (2.5 mg/l), fetuin (6.25 mg/l), aprotinin (20,000 kU/1), glutamin (1 mM), glucose (1 g/l), $NaHCO_3$ (1.1 g/l), and gentamycin (1 mg/l). For subculture, cells were processed by differentiated trypsination with trypsin–EDTA (0.05%–0.02%, Boehringer, Mannheim). Cell cultures were obtained from 4 gastrointestinal carcinomas and 2 sarcomas. For more details see Refs. [10–12].

Immunocytochemical cell characterization

For this purpose, sections from paraffine blocks were used and the respective cells lines were grown on glass slides. Demonstration of the cytoskeletal proteins cytokeratin and vimentin was performed in all cell lines applying the ABC technique [13] or the

peroxidase–antiperoxidase method (for details see Refs. [14, 15]). The antibodies were of monoclonal origin (Boehringer; Amersham, Braunschweig). In the group of gastrointestinal tumors and cell lines derived there of, several tumor-associated antigens (CEA, CA 50, CA 19-9, and CA 125) were additionally determined. Proper controls were performed.

Monolayer proliferation assay

A defined number of cells (10^5/ml) was seeded into multiwell culture dishes (12× cluster, Costar). When cells reached the logarithmic growth phase (estimated by microscopical observation) $1,25(OH)_2D_3$ was added to the culture medium in concentrations of 0.8 and 8 nM for 4, 7 and 11 days. $1,25(OH)_2D_3$ was a generous gift of Hoffman-La Roche, Basel. Controls were grown in culture medium without addition of $1,25(OH)_2D_3$. At the beginning and the end of a test period, cells were counted in a hemocytometer. Each experiment was done in triplicate. Morphological appearance of the cells was observed on every day of the test period by phase contrast microscopy.

RESULTS

Characterization

Immunohistochemically demonstrated cytoskeleton proteins (cytokeratin, vimentin) and tumor-associated antigens (CEA, CA 50, CA 19-9, CA 12-5) were determined in both the parent tissue and the derived cultured cells. Similarity between the proportional distribution of cells positive for these substances within the tissue and the cell cultures was observed.

$1.25(OH)_2D_3$ and cell growth

Five out of 6 cell lines showed a $1,25(OH)_2D_3$ dependent growth rate when incubated at concentrations of 0.8 and 8 nM, respectively. Proliferation rate of one cell line (EPSS 86-176) was not altered by addition of $1,25(OH)_2D_3$ to the culture medium. Figure 1 summarizes the results of the changes of the proliferation rates. Except one, the "$1.25(OH)_2D_3$ dependent cell lines reacted in a dose- and time-dependent manner, such that proliferation was inhibited to different degrees. Incubation of the cell line EPG 86-26 with 0.8 nM $1,25(OH)_2D_3$ led to an increased cell growth at day 4, and an inhibition at days 7 and 11 of the test period; when incubated with 8 nM $1,25(OH)_2D_3$ however EPG 86-26 was inhibited at any time. Cell line EPG 85-257 showed a biphasic growth pattern—0.8 nM $1,25(OH)_2D_3$ stimulated proliferation up to 117% whereas 8 nM $1,25(OH)_2D_3$ inhibited cell growth down to 72% in comparison to untreated controls.

$1.25(OH)_2D_3$ and cell morphology

As determined by phase contrast microscopy, slight but not striking morphological changes of size and shape of the cells were present in the $1,25(OH)_2D_3$ cell cultures. However, no homogeneous modulation was observed.

DISCUSSION

The present study describes $1,25(OH)_2D_3$ effects on characterized cultured tumor cells *in vitro*. Congruity of the parent tissue with the respective cultured cells on the distribution pattern of a number of tumor-associated antigens and cytoskeleton proteins underlines the relevance of this *in vitro* model inasmuch as it gives evidence of a similarity between the two systems.

We clearly demonstrated a $1,25(OH)_2D_3$ dependent growth rate of 4 gastrointestinal carcinoma cell lines and of 1 malignant schwannoma cell line. This is observed at concentrations of 0.8 and 8 nM $1,25(OH)_2D_3$. It has to be emphasized that the same concentration (0.8 nM), which causes a decrease in cell growth in cell lines EPC 85-86, EPSS 86-91, and EPP 85-181, stimulates EPG 86-26 at day 4. In contrast, at this concentration (0.8 nM) EPG 86-26 was inhibited when incubated for 7 and 11 days with $1,25(OH)_2D_3$. This time-dependent biphasic growth pattern within a given concentration has not been described before and could be explained by the concept that the product of concentration and time may have differentiated effects. Growth stimulation of a certain steroid and inhibition at a higher concentration of the same compound as seen in cell line EPG 85-257 is in agreement with other reports [5, 7].

In conclusion, our results harmonize with those obtained by Colston *et al.*[5] and Frampton *et al.*[7] using malignant melanoma cells or T-47D cells, respectively, as much as concentration range and time course studies are concerned. However, we believe that results obtained with cultured tumor cells derived from different tissue origin should be compared with caution, since receptor content and affinity vary widely within different organ system [16]. Comparison of the results obtained by different authors who have been working with $1,25(OH)_2D_3$ dependent tumor cells is complicated by different experimental conditions such as the use of different concentration of charcoal-treated and non-treated serum. Frampton [7], who observed stimulation of T-47D cells in medium supplemented with 5% charcoal-extracted serum and an inhibition of cell growth at the same concentration of $1,25(OH)_2D_3$ in the presence of untreated 1% FCS, states that the residual levels of $1,25(OH)_2D_3$ in the untreated serum could be masking the effects of added hormone. We did not use charcoal-extracted serum since this leads also to an uncontrolled extraction of other compounds than $1,25(OH)_2D_3$ thus excluding the direct transmission of results obtained by the application of this method to results obtained by the use of plain serum repleted systems.

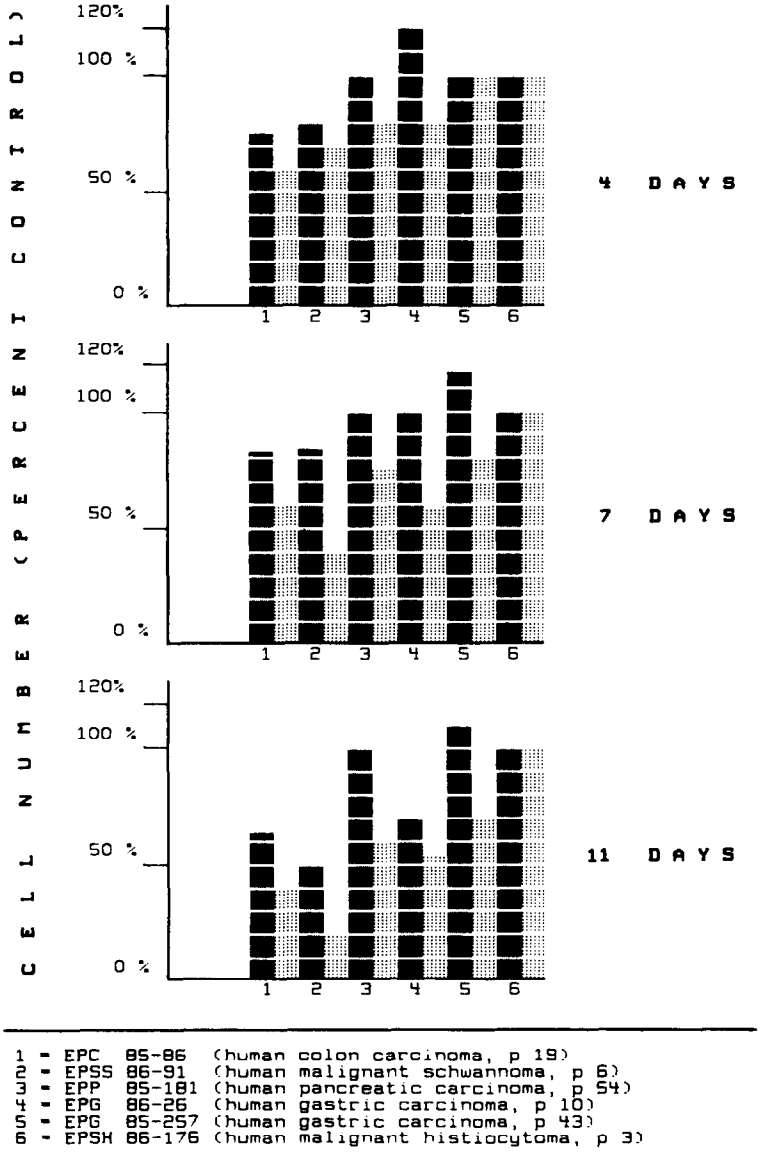

Fig. 1. Modification of cell growth in six malignant cell lines by application of 0.8 nM (■) or 8 nM (⁘) 1,25-dihydroxyvitamin D_3 during 4, 7 or 11 days.

In phase contrast microscopy the effect on differentiation of cultured HL 60 and T-47D cells as described by [6, 7] was not found in cell lines investigated here. Further studies using long-term incubations are to be undertaken to elucidate this effect of $1,25(OH)_2D_3$ on epithelial tumor cells.

In conclusion, this study shows both $1,25(OH)_2D_3$ dependent cell lines as well as one non-dependent cell line. The same concentration of the hormone can stimulate as well as inhibit cell growth. We therefore deduce that $1,25(OH)_2D_3$ has some antiproliferative potency but is not a straight growth-inhibiting agent.

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