Control of VX₂ Carcinoma Cell Growth in Culture by Calcium, Calmodulin, and Prostaglandins

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ABSTRACT

Based on our in vivo observation that growth of VX₂ carcinoma transplanted in rabbits paralleled development of hypercalcemia, we studied the regulation of VX₂ tumor growth using a clonal cell line isolated from VX₂ tumor (VX₂-L). VX₂-L cell growth was dependent on prostaglandins released by the cultured cells into the medium, since indomethacin suppressed VX₂-L growth, and prostaglandins A₂, E₁, E₂, F₁₀, and F₂₀ stimulated VX₂-L proliferation. In contrast, prostaglandins D₂ and I₂ inhibited VX₂-L proliferation. In contrast to previous reports, increases in extracellular calcium concentration promoted VX₂-L growth not only directly but indirectly through augmentation of prostaglandin E synthesis. Antagonists of the intracellular calcium binding protein calmodulin inhibited cell replication.

Increases in extracellular calcium also stimulated production of a nonprostaglandin macromolecular bone-resorbing factor. This factor may account for the hypercalcemia which we were unable to block by indomethacin.

These results suggest a close relationship between VX₂-L growth, prostaglandin production, and hypercalcemia. It is proposed that calcium blockers and anticalmodulin drugs might be powerful anticancer and/or antihypercalcemic agents for malignant cells such as VX₂-L.

INTRODUCTION

One of the animal models used to study the mechanism of hypercalcemia associated with cancer has been the VX₂ carcinoma which occurs in rabbits. Hypercalcemia seen in VX₂ tumor-bearing rabbits has several interesting characteristics. It is associated with increased osteoclastic bone resorption but occurs in the absence of metastases to and destruction of bone by tumor cells (23, 59). Excision of the tumor results in reversal of hypercalcemia (58), but hypercalcemia occurs in thyroparathyroidectomized rabbits (52). It is therefore plausible that VX₂ tumor cells release a humoral bone-resorbing factor which is unrelated to the function of the parathyroid glands. Voelkel et al. (51) demonstrated that a PG₃ synthesis inhibitor indomethacin (16) prevented the hypercalcemia in VX₂-bearing rabbits and suggested that PGE₂, a potent stimulator of osteoclastic bone resorption (28), released by the tumor cells accounts for the hypercalcemia.

We were able to reproduce similar hypercalcemia in rabbits by transplantation of clonal VX₂ cells (VX₂-L) established as described in the text and found that increases in serum calcium occurred in parallel with tumor enlargement, which led us to speculate that there may be a close correlation between serum calcium level and tumor growth. Furthermore, we confirmed that VX₂-L released significant amounts of PGE into the culture medium. There is accumulating evidence which suggests that PGs are involved in the growth of a variety of human tumors (2, 10, 24, 43) and animal tumors (17, 39, 41, 42).

In the present study, we first studied the relationship between growth of VX₂-L in culture, extracellular calcium concentration, and PGs. We found that VX₂-L growth is dependent on PGs and calcium, and PG production by the cells is influenced by calcium. We also found that the intracellular calcium receptor calmodulin is involved in calcium stimulation of growth and PG production of VX₂-L. In addition, we report that VX₂-L cells produce a macromolecular bone-resorbing factor. These results suggest that several factors, PGs, and this macromolecular factor are responsible for the hypercalcemia in VX₂-bearing rabbits.

MATERIALS AND METHODS

VX₂ Carcinoma. VX₂ tumor was kindly supplied by Dr. Yohei Ito, Department of Microbiology, Faculty of Medicine, Kyoto University. The tumor has been serially passaged in male albino rabbits weighing between 0.8 and 1.2 kg in our laboratory. Aseptically excised tumor was cut into pieces of approximately 5 mm in diameter by scalpel, and one piece for each animal was transplanted i.m. in the thigh muscles by trocar (37). The transplantation was repeated every 4 weeks.

Establishment of VX₂-L Cell Line. The tumor tissue was minced with scissors into pieces smaller than 1 mm in diameter and was placed in 35-mm plastic culture dishes (Linbro; Flow Laboratories, Inc., McLean, VA) with a drop of culture medium (see below) to initiate explant outgrowth cultures according to the method of Jensen et al. (27). After 3 weeks when the cells grew out from the tumor pieces, the primary cultures were harvested with 0.05% trypsin-0.01% EDTA solution. Five × 10⁶ cells were plated onto 35-mm plastic culture dishes in DMEM (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 5% (v/v) FBS (Grand Island Biological Co., Grand Island, NY) and 1% (v/v) penicillin-streptomycin solution (Flow Laboratories) and maintained in culture at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The culture medium was changed every 2 days. The VX₂-L clone was established by single-cell cloning in microtiter wells as described (33). The VX₂-L clone established in this manner was grown in a similar culture condition to that described above. In each experiment, FBS was dialyzed before use in order to eliminate small molecular substances including PGs and calcium.

Measurement of DNA Synthesis. VX₂-L cells were inoculated and cultured for 24 hr in DMEM supplemented with 5% FBS. The cultures were washed with ice-cold calcium- and magnesium-free PBS 3 times, and the culture medium was changed into DMEM supplemented with 0.2% dialyzed FBS and containing drugs and/or calcium as indicated. After 44 to 68 hr of culture, the culture medium was changed into fresh serum-free DMEM. The cells were then incubated with [³H]thymidine (1...
VX₂ Cell Growth and Hypercalcemia

Established VX₂ Clone. VX₂-L cells, established as described in "Materials and Methods," demonstrated an epithelial shape and proliferated rapidly (doubling time at log phase, 28 to 32 hr) with piling up to form a dense cell layer, and they have been maintained for more than 2 years in our laboratory (Fig. 1). VX₂-L cells demonstrated anchorage-independent growth by forming colonies in soft agar [number of colonies/5 × 10⁵ cells plated, 363 ± 189 (S.E.) for 6 experiments] when assayed by the method of Hamburger and Salmon (21). These results suggest that VX₂-L cells retain cancerous property even after long cultivation in vitro. Furthermore, transplantation of 1 to 3 × 10⁸ VX₂-L cells in the thigh muscles of young male rabbits resulted in formation of tumors histologically similar to VX₂ carcinoma. Although we obtained several VX₂ clones other than VX₂-L, and each clone showed epithelial shape and anchorage-independent growth in vitro and produced PGE similarly to VX₂-L, none of them, for unknown reason, formed tumors in animals. Therefore, in the following experiments, only VX₂-L cells were used. In a preliminary experiment, we examined the effect of serum concentration in the culture medium on VX₂-L growth and found that the cells were capable of growing, although the rate of proliferation was positively dependent on serum concentration added in the culture medium, under the culture conditions used in each experiment.

PGE Production and Growth of VX₂-L. VX₂-L cultured in DMEM supplemented with 0.2% dialyzed FBS for 72 hr released PGE (21 ng/10⁶ cells) into the medium. Addition of 1 μM indomethacin to the cultures resulted in a dramatic decrease in PGE production, which was accompanied by a marked reduction of DNA synthesis and cell division (Table 1). Growth of other VX₂ clones was also inhibited by 1 μM indomethacin (data not shown). Skin fibroblasts isolated from VX₂ tumor-bearing rabbits released much less PGE into the culture medium (2.1 ng/10⁶ cells) than did VX₂-L, which was inhibited by indomethacin. However, in contrast to VX₂-L, indomethacin has no effect on DNA synthesis and cell division of skin fibroblasts (Table 1). Since the number of VX₂-L cells or skin fibroblasts which had been treated with 1 μM indomethacin for 68 hr was higher than that of the cells at plating, it is unlikely that the effects of indomethacin seen in this experiment are due to cytotoxicity.

Effects of Various PGs on VX₂-L Growth. PGA₂, PGF₁α, and PGF₂α, significantly stimulated VX₂-L growth at a concentration of 10⁻⁴ M. PGE₁ and PGE₂ markedly promoted VX₂-L growth at a concentration as low as 10⁻⁶ M, and PGE₂ was more effective than PGE₁ (Chart 1). On the other hand, both PGD₂ and PGI₂ inhibited VX₂-L growth (Chart 1).

Tumor Growth and Hypercalcemia. Tumors formed by transplantation of VX₂-L were palpable after 7 to 10 days and visible after 10 to 14 days. Tumor volume calculated according to the methods described previously by Ito et al. (26) began to increase within 2 weeks after transplantation and reached approximately

Table 1

<table>
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<tr>
<th>Cells</th>
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<th>PGE production (ng/10⁶ cells)</th>
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<td>VX₂-L</td>
<td>None</td>
<td>21 ± 3¹</td>
<td>56 ± 3, 14 ± 3</td>
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<tr>
<td></td>
<td>Indomethacin (1 μM)</td>
<td>0.5 ± 0.1²</td>
<td>21 ± 2³, 6 ± 1³</td>
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</tr>
<tr>
<td>Skin fibroblast</td>
<td>None</td>
<td>21 ± 0.2</td>
<td>30 ± 3, 7 ± 1</td>
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¹ Mean ± S.E. (n = 6).
² Significantly smaller than untreated group (p < 0.01).

RESULTS

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PGE Determination. PGE content in the medium harvested from the cultures treated with drug and/or calcium as indicated in the presence of 0.2% dialyzed FBS and drugs as indicated for 44 to 68 h, they were harvested with 0.05% trypsin-0.01% EDTA solution and stained with 0.5% trypan blue. The cells which excluded trypan blue were counted on a hemocytometer.

Measurement of Cell Number. After the VX₂-L cells were cultured in the presence of 0.2% dialyzed FBS and drugs as indicated for 44 to 68 h, they were harvested with 0.05% trypsin-0.01% EDTA solution and stained with 0.5% trypan blue. The cells which excluded trypan blue were counted on a hemocytometer.

PGF₂α significantly stimulated VX₂-L growth at a concentration of 10⁻⁴ M. PGE₁ and PGE₂ markedly promoted VX₂-L growth at a concentration as low as 10⁻⁶ M, and PGE₂ was more effective than PGE₁ (Chart 1). On the other hand, both PGD₂ and PGI₂ inhibited VX₂-L growth (Chart 1).

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¹ Mean ± S.E. (n = 6).
² Significantly smaller than untreated group (p < 0.01).
VX₂ CELL GROWTH AND HYPERCALCEMIA

Chart 1. Effects of various PGs on VX₂-L growth. VX₂-L cells (1 x 10⁶/35-mm dish) were cultured in DMEM supplemented with 5% FBS for 24 hr. Then, the cells were grown in DMEM supplemented with 0.2% dialyzed FBS for 72 hr in the presence of PGA₂ (○), PGD₂ (●). PGE₁ (▲), PGE₂ (●), PGF₆ (□), PGF₆ (△), or PGI₂ (◆). The cell number of untreated VX₂-L after 72-hr culture was 3.8 ± 0.4 x 10⁵. Points, mean of 6 cultures.

Chart 2. Growth of VX₂ carcinoma transplanted in rabbits and development of hypercalcemia. The tumor was transplanted as described in the text. Tumor volume (●) was calculated by the formula ½ x length x width x thickness (25). Serum calcium level (▲) was determined using o-cresolphthalein complexone (36). Points, mean of 6 animals; bars, S.E. Inset, linear regression of the chart.

100 cu cm at 5 weeks of transplantation (Chart 2). In parallel with this, serum calcium concentrations also began to increase after 2 weeks and reached 16.8 ± 0.24 mg/dl (S.E.; n = 6) at 5 weeks (Chart 2). A highly significant correlation existed between serum calcium concentration and tumor volume (Chart 2, inset). Serum calcium concentrations in sham-operated control animals were unchanged (11.8 ± 0.3 mg/dl and 12.1 ± 0.3 mg/dl, pre- and 5 weeks of posttransplantation, respectively).

Calcium Stimulation of VX₂-L Growth. The results demonstrated in Chart 2 suggest that elevations in extracellular calcium may influence VX₂ carcinoma growth. An increase in calcium concentration in the culture medium (DMEM contains 1.8 mm calcium) from 2.5 to 5 mm resulted in a dramatic stimulation of VX₂-L DNA synthesis in a dose-dependent manner (Chart 3). However, in the presence of 1 μM indomethacin, calcium stimulation of DNA synthesis by VX₂-L was greatly impaired. Addition of 1 μM PGE₂ markedly promoted calcium stimulation of VX₂-L DNA synthesis when the calcium concentration was in the range of 1.8 to 3.5 mm. However, when the calcium concentration was above 3.5 mm, no promotion by PGE₂ of calcium stimulation of VX₂-L DNA synthesis occurred. PGE₂ completely restored indomethacin-reduced calcium stimulation of VX₂-L DNA synthesis.

Experiments in which VX₂-L cells were treated with EDTA to further confirm that calcium is necessary for VX₂-L to proliferate were unsuccessful, since the cells detached from the bottom of dishes 3 hr after the addition of EDTA.

Effects of Nifedipine and Ionophore A23187 on VX₂-L Growth. We next studied the effects of drugs known to alter the entry of extracellular calcium into cells on VX₂-L growth. The calcium blocker nifedipine (4) and divalent cation ionophore A23187 (55) were tested. As shown in Table 2, nifedipine inhibited VX₂-L growth in conjunction with inhibition of ⁴⁵Ca entry into the cells in a concentration-dependent manner. In contrast, ionophore A23187 demonstrated a stimulatory effect on VX₂-L growth as well as on ⁴⁵Ca entry into the cells.

Inhibition of VX₂-L Growth by Calmodulin Antagonists. The results shown on Table 2 suggest that changes in intracellular calcium level due to alteration of calcium entry into cells influence VX₂-L growth. It is therefore of particular interest to study the role of the intracellular calcium receptor calmodulin (8, 34) in control of VX₂-L growth. W-7, a newly synthesized anticalmodulin...
Effects of nifedipine and ionophore A23187 on VX2-L growth and {superscript}45Ca uptake

VX2-L cells were plated at a density of 5 x 10⁶ cells/17-mm dish (5.5 x 10⁶ cells/sq cm) and cultured as described in the Table 1 legend. The cells were then treated with or without the drugs for 68 hr in DMEM supplemented with 0.2% dialyzed FBS.

### Table 2

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Concentration (µM)</th>
<th>Cell no. (10⁴/17-mm dish)</th>
<th>[³H]Thymidine incorporation (dpm/mg protein x 10⁻¹⁰)</th>
<th>[³H]Ca entry (dpm/mg protein x 10⁻⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>18 ± 3</td>
<td>21 ± 3</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>0.01</td>
<td>10 ± 5</td>
<td>13 ± 3</td>
<td>12 ± 2</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>9 ± 2</td>
<td>10 ± 3</td>
<td>8 ± 3</td>
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<td>1.0</td>
<td>9 ± 1</td>
<td>10 ± 3</td>
<td>5 ± 1</td>
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<tr>
<td>Ionophore A23187</td>
<td>0.2</td>
<td>29 ± 4</td>
<td>36 ± 3</td>
<td>74 ± 4</td>
</tr>
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</table>

- °Ca entry was determined when the cells inoculated at the same time as those described in the legend above became confluent.
- ° Mean ± S.E. (n = 4).
- ° Significantly smaller than untreated group (p < 0.01).
- ° Significantly greater than untreated group (p < 0.01).

Effects of W-7 on DNA synthesis and {superscript}45Ca uptake by VX2-L

<table>
<thead>
<tr>
<th>W-7 (µM)</th>
<th>Cell no. (10⁴/17-mm dish)</th>
<th>[³H]Thymidine incorporation (dpm/mg protein x 10⁻¹⁰)</th>
<th>[³H]Ca entry (dpm/mg protein x 10⁻⁹)</th>
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<tbody>
<tr>
<td>Experiment 1</td>
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<td></td>
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<tr>
<td>0</td>
<td>15 ± 2</td>
<td>23 ± 2</td>
<td>25 ± 5</td>
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<tr>
<td>10</td>
<td>11 ± 2</td>
<td>20 ± 2</td>
<td>24 ± 3</td>
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<tr>
<td>30</td>
<td>9 ± 1º</td>
<td>12 ± 2º</td>
<td>30 ± 8</td>
</tr>
<tr>
<td>50</td>
<td>9 ± 1º</td>
<td>10 ± 1º</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>18 ± 3</td>
<td>21 ± 1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10 ± 2</td>
<td>14 ± 2º</td>
<td></td>
</tr>
<tr>
<td>30 (W-5)</td>
<td>16 ± 2</td>
<td>22 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

- ° VX2-L cells were plated, cultured, and treated as described in the legend to Table 2.
- ° Ca entry was measured as described in Table 2, Footnote a.
- ° Mean ± S.E. (n = 4).
- ° Significantly smaller than the untreated group (p < 0.01).

The anticalmodulin drug W-7 also suppressed calcium stimulation of PGE production, but to a lesser extent than did nifedipine.

**DISCUSSION**

There is accumulating evidence which indicates a significant role of calcium in the mitotic process of a variety of cells (3, 32, 57). In contrast to normal cells, it has been demonstrated that neoplastic cells require much less calcium for their growth (1, 56). Swierenga et al. (50) have shown that neoplastic cells with high tumorigenicity are able to proliferate in a low-calcium me-
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dium and suggested that the relationship between calcium concentration and growth is a simple and sensitive indicator of tumorigenicity in vivo. However, in VX₂-L cells, although the cells showed anchorage-independent growth in vitro and profound tumorigenicity in vivo, growth was stimulated by increases in extracellular calcium (Chart 3) or in the intracellular calcium level caused by stimulation of extracellular calcium influx (Table 2) and was inhibited by decreases in intracellular calcium levels caused by inhibition of extracellular calcium influx (Table 2). These results indicate that VX₂-L growth is dependent on calcium, despite its highly tumorigenic property, and thus, VX₂-L tumors are different from other neoplastic cells in this respect.

Subsequently, we have found that the intracellular calcium receptor calmodulin is involved in VX₂-L growth (Table 3; Chart 3). Involvement of calmodulin in CHO-K₁ cell proliferation has been reported (6, 22, 46). In Swiss 3T3 or normal rat kidney cells transformed by oncogenic viruses, increased intracellular calmodulin levels were found compared to their nontransformed counterparts, suggesting that calmodulin may play a role in the expression of one characteristic of transformed cells, uncontrollable growth (7). In fact, Wei and coworkers (53, 54) and Ito and Hidaka (25) have demonstrated that there is a positive correlation between the intracellular calmodulin level and hepatoma growth in vivo. Very recently, however, there are accumulating reports that indicate that calmodulin is not always implicated in the growth of neoplastic (35) and transformed (9, 13) cells. Therefore, the role of calmodulin in cell proliferation is controversial. In the present study, we demonstrated clearly that a new synthetic and specific calmodulin antagonist W-7 inhibited VX₂-L growth, suggesting a pivotal role for calmodulin in VX₂-L proliferation. It is likely that the influence of calcium and calmodulin on VX₂-L growth, at least in part, may be related to its hypercalcemia-producing activity. Thus, the hypercalcemia provides an environment favorable for VX₂-L to proliferate which, in turn, may produce further severe hypercalcemia. In addition to this, we have shown that VX₂-L released significant amounts of PGE₂, which were stimulated by the elevation of the calcium level in the culture medium and required calmodulin function (Chart 4). PGE and other families of PGs are potent bone resorbers (11, 28) and a presumable cause of hypercalcemia in VX₂-bearing rabbits. Another well-recognized action of PGE is the negative regulation of humoral and cellular immunity (20, 41). Thus, the hypercalcemia might be an essential event for the VX₂ tumor to grow and escape from the host immunosurveillance system. Finally, these results raise the possibility that calcium blockers and anticalmodulin drugs might be potent anticancer drugs in some hypercalcemia-producing tumors, such as the VX₂ tumor and many other types of malignant tumor (38), as suggested (25, 53, 54). In a preliminary experiment, we have found that nifedipine administered together with bleomycin, which has been shown recently to be a potent antihypercalcemic agent (40), prevented enlargement of the VX₂ tumor and development of the hypercalcemia, and it resulted in increased survival (data not shown).

Inhibition of VX₂-L PG production by indomethacin resulted in decreased DNA synthesis and division of VX₂-L but not of skin fibroblasts isolated from VX₂-bearing rabbits (Table 1), indicating that PG production may be specifically necessary for VX₂-L proliferation. This is supported further by the results that PGE₁, PGE₂, PGF₁α, and PGF₂α promoted VX₂-L growth (Chart 1). At present, it is not known if PG stimulation of VX₂-L growth is mediated by specific PG receptors on the plasma membrane. If so, VX₂-L cells proliferate in response to PGs secreted from the cell itself, thus providing an example of an autocrine system (48). Studies to prove this are in progress.

It is of interest that PGE₂ and PGF₂ inhibited VX₂-L growth (Table 1). Recently, antineoplastic (18, 47) and antimetastatic actions (15, 49) of PGE₂ both in vivo and in vitro have been reported. Our results support these previous reports. As far as PGF₂ is concerned, this is the first demonstration to our knowledge that PGF₂ is effective in directly inhibiting malignant cell growth. It is generally believed that the effects of PGE₂ and PGF₂ (5) as well as PGE and PGF (45) are mediated through cyclic AMP accumulation in cells. Growth of VX₂-L cells treated with the phosphodiesterase inhibitor isobutylmethylxanthine and the nonspecific adenylyl cyclase stimulator forskolin was suppressed (data not shown). It therefore seems likely that inhibition of VX₂-L growth by PGE₂ and PGF₂ might be mediated through a mechanism associated with cyclic AMP. Mechanism(s) involved in VX₂-L growth stimulation by PGE, PGE, or PGF are unknown but probably are not associated with cyclic AMP. Further studies are needed to elucidate the mechanism.

Our result that indomethacin suppressed VX₂-L growth is inconsistent with previous reports in which indomethacin had no effect on VX₂ growth (51). This discrepancy is likely to result from the difference of VX₂ strain used* and the culture condition. In the previous experiments, VX₂ cells were cultured in the presence of high concentrations of sera (15% horse and 2.5% fetal calf serum), which supply enough PGs for VX₂ cells to proliferate. In contrast, in our experiments, VX₂-L was cultured in DMEM supplemented with 0.2% dialyzed serum. In these culture conditions, endogenous PGs are the major source of PGs for VX₂-L growth.

VX₂-L released nondialyzable macromolecular factor(s) with profound bone-resorbing activity at high calcium concentration (Chart 5). Failure of indomethacin to inhibit the production of this factor suggests that a mechanism independent of PGs but directly dependent upon calcium might be involved in the production of this factor, and this factor might be responsible for the hypercalcemia which is not prevented by administration of indomethacin. In fact, the calcium blocker nifedipine suppressed the release of this factor at calcium levels below 4 mM (Chart 5). Recently, Doppelt et al. (12) demonstrated that the hypercalcemia in their variant of VX₂-bearing rabbits was not affected by treatment of indomethacin, and elevated plasma calcium levels were reduced rapidly to normal when the diet was changed from the normal to a calcium-free diet. They proposed that the hypercalcemia is dependent on dietary calcium but independent of PG. Our results fit in well with their observations, since production of the macrohormone bone-resorbing factor was dependent on extracellular calcium concentrations. Of particular interest is the relationship between PGs and this factor in producing the hypercalcemia in VX₂-bearing rabbits. At low calcium levels, no production of this factor occurred (Chart 5), although concentrations of PGE sufficient to cause bone resorption were secreted into the medium (Chart 4). We speculate that the hypercalcemia in VX₂-bearing rabbits might involve 2 steps. PGs first secreted by VX₂ tumors elevate serum calcium by causing increased bone

*J. T. Potts, personal communication.
resorption, and thus, elevated serum calcium induces PG-independent but calcium-dependent production of this factor that further elevates the serum calcium level. This idea is supported by the result of Galasko et al. (19), who found that administration of indomethacin before or within 7 days of VX2 tumor transplantation (no hypercalcemia in animals) was effective in inhibiting bone destruction, whereas indomethacin administered 14 or 21 days after transplantation (when the animals were already hypercalcemic) was unable to block osteolysis.

Calcium stimulation of VX2-L DNA synthesis was greatly impaired in the presence of indomethacin (Chart 3). This result, together with the finding that calcium stimulates VX2-L PG production (Chart 4), suggests that the stimulatory effect of calcium on VX2-L growth is mediated through PG production. Restoration of PGE2 by indomethacin-inhibited calcium stimulation of VX2-L DNA synthesis further supports this (Chart 3). However, since indomethacin failed to completely block calcium stimulation of VX2-L (Chart 3), it appears that VX2-L growth is not only dependent on PGs but directly dependent on calcium or partly on a mechanism unrelated to PGs.

The result that the anticalmodulin drug W-7 suppressed calcium stimulation of PGE production by VX2-L (Chart 4) indicates calmodulin involvement in VX2-L PGE production. It is generally accepted that the effect of calcium on many cellular functions is mediated by the ubiquitous cytosolic calcium-binding protein calmodulin (8, 34). Calmodulin is known to stimulate phospholipase A2 activity (60) that is a rate-limiting enzyme in the generation of the PG precursor arachidonic acid. Taken together, it is proposed that, in the control of VX2-L growth, calcium activates calmodulin, which stimulates phospholipase A2 activity. Elevated enzyme activity then increases production of PGE which, in turn, promotes VX2-L growth.

In conclusion, the present study demonstrates that VX2-L has a variety of capabilities including production of PGE and a macromolecular bone-resorbing factor causing the hypercalcemia and increased responsiveness to calcium and PGE. These influences may be important factors in tumor cell replication and lead to unrestrained tumor growth.

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REFERENCES


Fig. 1. Phase-contrast microscopy of VX₂-L. The cells were cultured in DMEM supplemented with 5% FBS for 10 days. Note that the cells demonstrate epithelial shape and are piling up to form a dense cell layer.
Control of VX₂ Carcinoma Cell Growth in Culture by Calcium, Calmodulin, and Prostaglandins
Toshiyuki Yoneda, Masaya Kitamura, Teruaki Ogawa, et al.


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