Induction of Alkaline Phosphatase Activity in Cultured Human Intracranial Tumor Cells

Nobuhiko Takahara, Fritz Herz, Robert M. Singer, Asao Hirano, and Leopold G. Koss

Department of Pathology and Division of Neuropathology, Montefiore Hospital and Medical Center, Albert Einstein College of Medicine, Bronx, New York 10467

In this report, we present evidence that, irrespective of their histogenetic derivation, cells of primary intracranial tumors produce a thermolabile enzyme which has the characteristics of the liver-bone-kidney form of alkaline phosphatase. In addition, we will show that some of these cells respond with increased levels of enzyme activity to inducers of term-placental alkaline phosphatase such as glucocorticoids (5, 22, 23, 35, 36).

INTRODUCTION

In humans, at least 3 different forms of alkaline phosphatase [orthophosphoric monooester phosphohydrolase (alkaline optimum) (EC 3.1.3.1)] have been recognized. They are the term-placental, intestinal, and liver-bone-kidney forms (32, 34, 38). They can be distinguished from each other by several parameters (13). It has been known for some time that the heat-stable, term-placental type is produced ectopically by a variety of human tumors and is usually referred to as "Regan" isoenzyme (11). The production of heat-labile, non-Regan isoenzymes by human tumors has been described recently (7, 18).

In cultured cancer cells, the 3 forms of alkaline phosphatase have been demonstrated (3, 24, 43). However, studies on enzyme regulation have been restricted mainly to cells producing the liver-bone-kidney enzyme form.

MATERIALS AND METHODS

As described previously (26), aliquots of surgically removed primary benign and malignant tumors of the CNS were used to initiate the cultures. The benign tumors were 7 meningiomas, 2 pituitary adenomas, and 1 hemangioblastoma. The malignant tumors were 5 glioblastomas, 3 astrocytomas of low grade, 2 astrocytomas of high grade, 1 ependymoma and one pineal region tumor. There were also 5 metastatic tumors to the brain. Of these, one was from breast, one from melanoma, one from bone, and 2 from lung. The tumor tissues were placed in Earle's balanced salt solution and immediately taken to the laboratory. Following removal of blood and the electrocoagulated portion, the tumor tissue was minced with small curved scissors and stirred at 25°C with 0.1% trypsin in a Ca²⁺- and Mg²⁺-free Earle's salt solution until most of the material was dissociated into single cells and small fragments which were decanted and washed with complete culture medium (26). Following centrifugation at 800 x g, the cells were resuspended in medium and inoculated into 25- and/or 75-sq cm plastic culture flasks. Minimum essential medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml) was used throughout. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. The medium was changed 3 times a week. None of the cultures displayed the morphological arrangements typical of normal fibroblasts. Cells were subcultured as they reached confluency (generally after 7 to 10 days) using trypsin (0.05%)-EDTA (0.02%).

Where indicated, the osmolality of the medium was increased from 284 to 384 mosmol/kg by the addition of 50 mM NaCl (from an autoclaved 3 M stock solution) 24 hr after cell transfer (22). A stock solution of 100 µg of prednisolone per ml was prepared in ethanol, and a final concentration of 0.5 µg per ml (1.4 µM) was added to the cultures 24 hr after cell transfer; ethanol (0.5%) was added to the respective controls. To parallel cultures, a final 2 mM concentration of sodium butyrate was added also 24 hr after cell transfer. In other experiments, the cultures were supplemented with various combinations of NaCl, prednisolone, and butyrate. With primary cultures, the agent(s) was added 1 day after culture initiation. Cells growing in regular medium served as controls. To study the effect of the removal of prednisolone, after 24 hr and every 24 hr thereafter, the steroid-containing medium was removed and the cultures were washed twice with and then covered with regular medium. Other cultures growing in steroid-supplemented medium were similarly treated, except that they were washed with and refed prednisolone-containing medium. Cells growing in regular medium throughout the experiment were also included. Cells from experimental and control cultures were harvested at 24-hr intervals.

Cultures for enzyme assays were washed 3 times with 15 to 20 ml of cold 0.15 M NaCl and lysed with 0.5 ml of 0.25% sodium deoxycholate (17). To optimize the recovery of lysates, the 75-sq cm flasks were centrifuged for 2 min at 50 x g in an IEC Model UV centrifuge with a...
zyme preparations were used. Extracts from cultured cancer cells of alkaline phosphatase activity (24), or frozen in liquid nitrogen. Sonicates of tumor tissues were similarly carried out at pH 7.4 using the buffer solution indicated above. The pH was computed from controls kept in the respective buffers at 4°. The variation of duplicate assays was less than 10%. Activity was expressed in units per mg of protein, the latter determined according to the method of Lowry et al. (31) using crystalline bovine serum albumin as standard.

Partial Purification of Alkaline Phosphatase. Cell lysates were diluted with 0.25 volume of NaCl (130 mM)-Tris (30 mM)-MgCl2 (1 mM) buffer solution (pH 7.4) and extracted for 15 min with 1 volume of 1-butanol (24). After centrifugation at 1500 X g for 10 min, the butanol layer was discarded and the aqueous layer was used either immediately or frozen in liquid nitrogen. Sonicates of tumor tissues were similarly extracted. Butanol extraction yielded a 10- to 15-fold purification of alkaline phosphatase activity (24).

Thermal Inactivation. Thermostability of alkaline phosphatase was investigated by incubating duplicate aliquots of 0.05 ml of cell lysates or of butanol-extracted preparations at 56° with 0.1 ml of 1 M 2-amino-2-methyl-1-propanol-HCl buffer (pH 10.6). After incubation for various lengths of time, tubes were transferred to 4° and the remaining activity was subsequently measured at 37° by the addition of 0.1 ml of 0.016 M p-nitrophenyl phosphate containing 2 mM MgCl2 (24). Enzyme reactions were stopped with 0.25 n NaOH. Thermal inactivation was also carried out at pH 7.4 using the buffer solution indicated above. The pH of the buffers was monitored during and after preincubation, and no significant changes were noted. The percentage of residual activity was computed from controls kept in the respective buffers at 4°.

Inhibition Studies. For these experiments, butanol-extracted enzyme preparations were used. Extracts from cultured cancer cells of cervical (C41) and colonic (HT-29) origin, which produce, respectively, the term-placental (23) and the intestinal (25) alkaline phosphatase forms, were included for comparison. The following specific inhibitors of migration or the diminution of activity in an isoenzyme band was used as a criterion for positive cross-reactivity (39). Following electrophoresis, enzyme activity was visualized by staining the gels according to Angelis et al. (1) with α-naphthyl phosphate as substrate and 4-amidodiphenylamine diazonium salt (Dajac Laboratories, Philadelphia, Pa.) as azo dye in 1 M propanediol buffer (pH 9.7). For inhibition studies, 20 mM L-phenylalanine was incorporated into the reaction mixture, using D-phenylalanine in the controls (39). In order to verify that the antibody was active, specimens of term-placental alkaline phosphatase of similar activity were run concurrently; retardation of migration was seen in each instance.

RESULTS

Alkaline phosphatase activity of cultured intracranial tumor cells varied over a relatively wide range, and there was no correlation with the type of tumor from which the cultures were derived. The specific activity of cultures derived from 7 different meninomas was 4.2 x 10^{-4}, 6.1 x 10^{-4}, 2.0 x 10^{-3}, 3.1 x 10^{-3}, 5.4 x 10^{-3}, 1.1 x 10^{-2}, and 7.8 x 10^{-2}, and that of cultures derived from 5 glioblastomas was 9.4 x 10^{-3}, 1.0 x 10^{-2}, 2.4 x 10^{-2}, 5.5 x 10^{-2}, and 7.8 x 10^{-2}. The specific activity of the cultures was similar to that of the original tumor specimens tested. For example, the activity of a glioblastoma was 3.1 x 10^{-2} and that of its primary culture 5.5 x 10^{-2}. After the third transfer, the activity of the cultured cells was 3.6 x 10^{-2}. Alkaline phosphatase activity in an astrocytoma was 2.0 x 10^{-3} and that of its fourth and eighth subcultures 1.0 x 10^{-3} and 1.4 x 10^{-3}, respectively. Repeatedly subcultured cells, obtained from the same patient, had essentially the same specific activity. The results with cultures initiated from the metastatic tumors were as variable as those obtained with cultures derived from primary tumors. Specific activity of the cultured cells of metastatic tumors was also similar to that of the original specimens (Table 1). The relatively low enzyme activity of some cultures cannot be ascribed to a differential effect of the harvesting procedure used, since equivalent results were seen when the cell monolayers were dispensed with a rubber policeman. The possibility of alkaline phosphatase leakage into the culture medium was considered, but upon testing used media, none was detected. Acid phosphatase measurements were included in this study because its specific activity does not vary significantly from one type of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Alkaline phosphatase activity in cultured cells derived from tumors of the brain and from tumors metastasizing to the brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity (units/mg protein)</td>
<td>78-178 (ma- lignant astro- cytoma) (x 10^{-3})</td>
</tr>
<tr>
<td>Noncultured</td>
<td>2.0</td>
</tr>
<tr>
<td>T1</td>
<td>1.5</td>
</tr>
<tr>
<td>T2</td>
<td>4.4</td>
</tr>
<tr>
<td>T3</td>
<td>3.6</td>
</tr>
<tr>
<td>T4</td>
<td>1.0</td>
</tr>
<tr>
<td>T5</td>
<td>1.2</td>
</tr>
<tr>
<td>T6</td>
<td>0.9</td>
</tr>
<tr>
<td>T7</td>
<td>1.2</td>
</tr>
<tr>
<td>T8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

_# Identification code._

_# T, transfer number._
Alkaline Phosphatase in Cultured Brain Tumor Cells

cultured cell to another (22) and it is not influenced by stimuli affecting alkaline phosphatase (36). Acid phosphatase activity of all cultures was within the same range, varying between $1.5 \times 10^{-2}$ and $3.7 \times 10^{-2}$. These values were similar to those of other cultured human tumor cells (22).

Increased levels of alkaline phosphatase activity are induced in certain human tumor cell lines by increasing the osmolality of the culture medium (22, 36) or by exposure to glucocorticoids (5, 35) or sodium butyrate (4, 17). Induction experiments were conducted on cultures derived from the primary intracranial tumors. Primary cultures and cells transferred up to 20 times were used. Each experiment with cultured cells was repeated at least twice. The experimental conditions used were those found to be optimal for enzyme induction (4, 22, 35). A response was considered positive only when the specific activity in experimental cultures was 2.5 times higher than in controls. Of the stimuli tested, hyperosmolality (384 mosmol/kg) did not increase alkaline phosphatase activity in any of the cultures examined. By contrast, prednisolone (1.4 μM) elicited induction in cultures derived from pituitary adenomas, glioblastomas, and malignant astrocytomas. Enzyme induction was also seen in primary cultures of glioblastomas (Table 2). Sodium butyrate (2 mM) induced increased activity only in cultures of pituitary adenoma and hemangioblastoma cells. Inducibility by either agent was independent of the base level activity. When prednisolone and sodium butyrate were added simultaneously, the levels of activity were higher than those obtained with each agent individually, and in some instances the effect was synergistic (Table 2). The alkaline phosphatase of cells which did not respond to each agent alone was also refractory to their combination. With respect to the cultures derived from metastases to the brain, only the enzyme of the metastatic breast and lung tumors was inducible by prednisolone; sodium butyrate had no effect on any of these cultures. The addition of the stimulus to intact cells attached to their growth surface and covered with balanced salt solution (which does not support cell growth) or to lysates did not affect enzyme activity. The possibility that prednisolone or sodium butyrate promoted the formation of enzyme-activating factors was ruled out by mixing experiments of control and experimental cell lysates. The effect of the stimuli was discernible within 48 hr and reached its maximum by 72 hr. Removal of inducer from the medium of growing cells resulted in lower specific activity (Chart 1). Hyperosmolality, prednisolone, sodium butyrate, and their combinations did not affect acid phosphatase activity (results not shown).

Thermostability is a very sensitive parameter for distinguishing isoenzymes of alkaline phosphatase (13, 21, 24). Thus, preincubation of the term-placental enzyme at 56°C in the presence of 0.67 M 2-amino-2-methyl-1-propanol-HCl buffer (pH 10.6) caused a relatively small loss of activity. By contrast, the enzyme of brain tumors and of the cultures derived therefrom was rapidly inactivated; more than 98% of the activity was lost.

Table 2
Effects of various agents on alkaline phosphatase activity of cultured cells derived from tumors of the brain and from tumors metastasizing to the brain

<table>
<thead>
<tr>
<th>Additions to medium</th>
<th>Specific activity ($\times 10^{-2}$)</th>
<th>Specific activity ($\times 10^{-2}$)</th>
<th>Specific activity ($\times 10^{-2}$)</th>
<th>Specific activity ($\times 10^{-2}$)</th>
<th>Specific activity ($\times 10^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.3</td>
<td>1.1</td>
<td>10.7</td>
<td>4.0</td>
<td>3.7</td>
</tr>
<tr>
<td>NaCl (50 mM)</td>
<td>2.8</td>
<td>0.5</td>
<td>5.4</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Prednisolone (0.5 μg/ml)</td>
<td>108.3</td>
<td>20.4</td>
<td>20.1</td>
<td>4.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Sodium butyrate (2 mM)</td>
<td>40.5</td>
<td>7.6</td>
<td>103.7</td>
<td>21.2</td>
<td>7.1</td>
</tr>
<tr>
<td>NaCl + prednisolone</td>
<td>38.9</td>
<td>7.3</td>
<td>19.6</td>
<td>4.0</td>
<td>11.6</td>
</tr>
<tr>
<td>NaCl + sodium butyrate</td>
<td>36.4</td>
<td>6.8</td>
<td>162.1</td>
<td>33.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Prednisolone + sodium butyrate</td>
<td>386.8</td>
<td>73.5</td>
<td>319.8</td>
<td>65.3</td>
<td>22.3</td>
</tr>
<tr>
<td>NaCl + prednisolone + sodium butyrate</td>
<td>197.1</td>
<td>37.2</td>
<td>330.7</td>
<td>67.5</td>
<td>82.8</td>
</tr>
</tbody>
</table>

a Identification code.

b T, transfer number; IR, activity ratio of experimental to control cultures.

c Primary culture.

---

**Chart 1.** Effect of prednisolone and its removal on alkaline phosphatase activity. Glioblastoma (79-115) cells, at the fifth passage, were dispersed as indicated in the text and inoculated into regular medium. At 24 hr (upward arrow) the cells were rinsed and covered with medium containing 0.5 μg prednisolone per ml. At 48, 72, and 96 hr, cultures in prednisolone-containing medium were either rinsed with and refed regular medium (downward arrows) or washed with and refed steroid-containing medium. Duplicate cultures of each set were harvested at 24-hr intervals with 0.25% sodium deoxycholate (17). Cell lysates were stored in liquid nitrogen until the end of the experiment. Alkaline phosphatase activity was determined as indicated. •¿, activity of cells growing in regular medium at time of harvest; O, activity of cells growing in prednisolone-containing medium at time of harvest. Specific activity is expressed in units/mg protein.

---

FEBRUARY 1982 565

Downloaded from cancersres.aacrjournals.org on April 20, 2017. © 1982 American Association for Cancer Research.
after 2 min. At pH 7.4, the inactivation was more gradual, yet after 5 min only 7% of the activity remained (Table 3). The results were the same for the enzyme of control cultures and of inducer-treated cultures with increased specific activity. The alkaline phosphatase of the cultured cells derived from metastases to the brain was equally thermolabile.

The thermolabile alkaline phosphatase was further characterized with specific inhibitors that readily distinguish the various enzyme forms (3, 13, 37). For control purpose, preparations of term-placental and intestinal enzymes were included in the tests. An example of the plots (13) used to determine the inhibitor concentration required to produce 50% inhibition is shown in Chart 2. The enzyme of the original CNS tumor specimens tested and of the cultured cells grown with or without inducer(s) was strongly inhibited by l-bromotetramisole, levamisole, and L-homoarginine. It was unaffected by L-phenylalanine, L-phenylalanylglycylglycine, and L-leucylglycylglycine (Table 4). By comparing these inhibition patterns with those of the various human alkaline phosphatase forms (3, 13, 37), it is evident that the CNS tumor cells produced the liver-bone-kidney enzyme. Na3VO4, an alkaline phosphatase inhibitor (30) not heretofore used for this purpose, also distinguished this enzyme form from the thermolabile, intestinal type.

**Table 3** Thermostability of alkaline phosphatase

Enzyme preparations from cultured human brain tumor cells grown with or without inducer were preincubated at 56°C at pH 10.6 and pH 7.4 as indicated in the text. Specific activity varied between 2.8 x 10⁻² and 8.2 x 10⁻¹. Term-placental alkaline phosphatase was included for comparison. The percentage of residual activity was computed from controls kept at 4°C. Data at pH 7.4 represent the average of 15 determinations.

<table>
<thead>
<tr>
<th>Time at 56°C (min)</th>
<th>% of residual enzyme activity</th>
<th>Term-placental</th>
<th>Brain tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 10.6</td>
<td>pH 7.4</td>
<td>pH 10.6</td>
</tr>
<tr>
<td>1</td>
<td>ND²</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>98.8</td>
<td>100</td>
<td>&lt;2</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>100</td>
<td>&lt;2</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>100</td>
<td>&lt;2</td>
</tr>
<tr>
<td>5</td>
<td>89.6</td>
<td>100</td>
<td>&lt;2</td>
</tr>
<tr>
<td>7.5</td>
<td>ND</td>
<td>100</td>
<td>&lt;2</td>
</tr>
<tr>
<td>10</td>
<td>77.5</td>
<td>100</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

* ND, not done.

**Table 4** Concentrations of inhibitors producing 50% inhibition of alkaline phosphatase activity

Assays were carried out in triplicate using p-nitrophenyl phosphate as substrate in 2-amino-2-methyl-1-propanol-HCl buffer (pH 10.6). At least 5 concentrations of each inhibitor were used. Three or more determinations were made on each specimen at each inhibitor concentration, and the average values (% of activity remaining) were used in subsequent calculations.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Cultured brain tumor cells</th>
<th>Term-placental enzyme</th>
<th>Intestinal enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Phenylalanine</td>
<td>≥50.0</td>
<td>≥5.0</td>
<td>3.0</td>
</tr>
<tr>
<td>L-Phenylalanylglycylglycine</td>
<td>≥50.0</td>
<td>0.5</td>
<td>14.0</td>
</tr>
<tr>
<td>L-Leucylglycylglycine</td>
<td>≥50.0</td>
<td>6.0</td>
<td>25.0</td>
</tr>
<tr>
<td>L-Homoarginine</td>
<td>3.7</td>
<td>40.0 (0.3</td>
<td>40.0</td>
</tr>
<tr>
<td>Levamisole</td>
<td>0.09</td>
<td>2.0</td>
<td>11.0</td>
</tr>
<tr>
<td>l-Bromotetramisole</td>
<td>0.01</td>
<td>0.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Na3VO₄</td>
<td>0.22</td>
<td>0.14</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* \( \text{IC}_{50} \) concentration of inhibitor producing 50% inhibition of alkaline phosphatase activity, obtained from graphs depicting 100/the percentage of activity remaining versus inhibitor concentrations (13).

**Fig. 1.** Isoenzyme profile of alkaline phosphatase of cultured brain tumor cells grown with and without prednisolone. Cells derived from a glioblastoma (79-115) were grown for 7 days in regular medium (A) and in prednisolone-containing medium (B). Specific activities were 3.0 x 10⁻² and 22 x 10⁻², respectively. Before electrophoresis, aliquots of each specimen were heated for 5 min at 65°C (pH 7.0) and mixed with antisem to placentral alkaline phosphatase. Nonheated aliquots were similarly mixed. Conditions of electrophoresis were as described (38), and visualization of activity was as indicated in the text. Gel 1, untreated samples; Gel 2, heated samples; Gel 3, o-phenylalanine (20 µM) in the reaction mixture; Gel 4, L-phenylalanine (20 µM) in the reaction mixture; Gel 5, unheated samples treated with antiserum to term-placental alkaline phosphatase; Gel 6, heated samples treated with antiserum.
Alkaline Phosphatase in Cultured Brain Tumor Cells

react with the antiserum to term-placental alkaline phosphatase and was not sensitive to L-phenylalanine inhibition may represent an as yet uncharacterized isoenzyme.

DISCUSSION

Alkaline phosphatase activity has heretofore not been investigated in cultured primary intracranial tumor cells. As the foregoing results indicate, there were quantitative differences among cultures initiated from aliquots of the same tumor types obtained from different individuals. These differences were observed in cultures of benign and malignant tumors, and they were in keeping with the results of earlier histochemical and biochemical investigations (8, 9, 12, 41, 42) on a variety of CNS tumors. It is of interest to note that in one histochemical study no alkaline phosphatase activity was detected in glioblastomas and astrocytomas (12). In our studies, there were no significant enzyme activity differences between the original tumor and the cultured cells derived therefrom and activity was independent of the number of transfers; cells derived from the same patient had essentially the same specific activity upon repeated subculture. These findings differ from the changes in alkaline phosphatase activity observed in other systems in which there was no correlation between the specific activity of the original tissue and of the cultured cells initiated from the same (45). It should be noted that the results obtained with cultures of tumor metastases to the brain were similar to those of cultured primary CNS tumor cells. It would be of considerable interest to ascertain whether brain metastases of primary tumors producing term-placental alkaline phosphatase and cultured cells derived therefrom would preserve the expression of this enzyme form.

The observation that the enzyme of primary intracranial tumor tissue and of tumor metastases to the brain as well as of the cultures derived therefrom was thermolabile and not inhibited by L-phenylalanine is in keeping with previous findings with meningiomas (9, 42) and cranioopharyngiomas (41). By applying a variety of specific inhibitors, we have now established that the enzyme represents the liver-bone-kidney form of alkaline phosphatase. The presence of this enzyme form in human brain has been described recently (14).

The liver-bone-kidney type of alkaline phosphatase has been found in some cultured cells of human origin, including fibroblasts (44), Chang liver cells (19), and KMK-2, a continuous line derived from a gastric carcinoma (43). However, in contrast to the enzyme of responsive CNS tumor cells, the liver-bone-kidney activity of these cells is not inducible (35, 36, 43). Of the inducers tested, prednisolone elicited increased activity levels in most cultures obtained from benign and malignant tumors of the CNS. The effect of the steroid was also observed in primary cultures, and the responsiveness of the cells persisted upon repeated subculture. This is of special interest since most studies on the control of alkaline phosphatase activity have been conducted on continuous cell lines that had been transferred many times (4, 5, 16, 22, 23, 36). Sodium butyrate, a substance known to affect a variety of other cellular processes (4, 29), was uniformly effective only with cultures from pituitary adenomas and hemangioblastomas. The reason for this selectivity is not clear. The lack of response by cultured intracranial tumor cells to hyperosmolality was in keeping with our previous findings that only term-placental (21, 22, 36) and intestinal (25) alkaline phosphatases are inducible by this factor.

The mechanisms responsible for the control of alkaline phosphatase in cultured human tumor cells have not been elucidated. Studies with HeLa cells suggest that the hormonally induced increase in activity is due to the synthesis of a modifier molecule that interacts with the enzyme to produce an alkaline phosphatase with enhanced catalytic efficiency (2). The mode of action of sodium butyrate appears to depend on the type of cells used. Thus, DNA synthesis does not seem to be necessary for enzyme induction in HeLa cells, whereas in choriocarcinoma cells it seems to require new synthesis of DNA, RNA, and protein (4). However, because alkaline phosphatase is a cell membrane-associated enzyme (28), it is possible that the increase in specific activity does not necessarily represent an effect on the enzyme and its synthesis per se but that the stimuli may elicit alterations in the enzyme microenvironment leading to increased activity. The finding that dimethyl-\(\text{L-2',3'-distearo}\)-\(\text{oxy}\)-proplyl-2'-hydroxyethylammonium acetate, a phospholipase \(A_2\) inhibitor, causes alkaline phosphatase stimulation in HeLa S3G (33) and the suggestion that 5-iodo-2'-deoxyuridine, another inducer, acts by affecting the cell membrane (16) would support such an interpretation (22).

The functional significance of alkaline phosphatase activity in brain and CNS tumors is not known. It has been suggested that in brain it may have a role in the activation and inactivation of enzymes through dephosphorylation (14). Alkaline phosphatase may be involved in the metabolism of pyridoxal phosphate, a cofactor of glutamate decarboxylase and glutamate transaminase (14). Pyridoxal phosphate is a substrate for the purified sheep brain enzyme (6). The membrane location of alkaline phosphatase could be of significance in the design of chemotherapeutic agents. For example, it has been suggested that phosphorylated derivatives of cytotoxic agents might be useful in the treatment of 6-thiopurine-resistant acute leukemia (27).

ACKNOWLEDGMENTS

We wish to thank Alexander Schermer and Elissa M. Leahy for expert technical assistance and Pearl Parsowith for her help in preparation of the manuscript.

REFERENCES


Induction of Alkaline Phosphatase Activity in Cultured Human Intracranial Tumor Cells

Nobuhiko Takahara, Fritz Herz, Robert M. Singer, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/42/2/563

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.