Progestin Regulation of Alkaline Phosphatase in the Human Breast Cancer Cell Line T47D

Diego Di Lorenzo, Alberto Albertini, and David Zava

Institute of Chemistry, School of Medicine, University of Brescia, Brescia, Italy [D. D. L., A. A.], and Peralta Cancer Research Institute and Aerol Biotechnology, San Leandro, California 94578 [D. Z.]

ABSTRACT

In T47D breast cancer cell line, progestin (R5020) induces de novo synthesis of an alkaline phosphatase enzyme. Based on inhibitor profiles and antigenic specificity, it is apparent that this enzyme belongs to the class of membrane-associated tissue-unspecific alkaline phosphatases. Enzyme induction was uniquely specific to progestins and not altered by other steroid hormones or synthetic analogues. The progestin induction of the tissue-unspecific alkaline phosphatase was time and dose dependent. The protein synthesis inhibitor cycloheximide blocks the enzyme synthesis and tunicamycin blocks the enzyme activity, showing that the induction was new synthesis of protein in its complete glycosylated form and not activation of a preexisting enzyme. To our knowledge this is the first report of progesterone-induced expression of a tissue-unspecific alkaline phosphatase gene of such magnitude (about 30- to 100-fold) in a progesterone-responsive tissue.

INTRODUCTION

Human breast cancer cell lines which grow well in monolayer culture have proved to be good model systems to investigate the receptor-mediated actions of sex steroid and peptide hormones. While the cancer cell differs from its normal counterpart in many respects it, nevertheless, often retains many of its differentiated characteristics, including sensitivity to sex steroids and peptide growth factors. T47D cells have proved a particularly ideal model to investigate the molecular actions of sex steroids (1, 2) and peptide hormones as they have been shown to contain functional receptors for and respond to estrogens, progestins, and their respective antagonists as well as to an array of peptide growth factors (e.g., epidermal growth factor, insulin, prolactin, calcitonin) all of which regulate the growth and differentiation of the normal mammary epithelium (3). Moreover, these cells contain exceptionally high titers of PR and are exquisitely sensitive to the actions of progestins in a manner quite similar to their actions in normal and neoplastic mammary epithelial cells of rodents and humans. For instance, in T47D cells, progestins (a) activate progesterone receptor to a form that is retained and processed in the nucleus, a prerequisite for gene activation; (b) down-regulate estrogen receptor; (c) induce the synthesis of milk fat (1), a specific gene product normally synthesized by fully differentiated mammary epithelium (3, 4); (d) inhibit cell proliferation at high concentrations; and (e) alter cell morphology (1, 3) that in some ways might be considered as an attempt to differentiate in vitro. We now report that progestins also induce de novo synthesis of a plasma-associated alkaline phosphatase in T47D cells. In this report we have characterized the enzyme and find that it is similar, if not identical, to the alkaline phosphatase present in the normal human breast and in human milk (4–6).

MATERIALS AND METHODS

Materials. Tissue culture reagents and supplies were purchased from GIBCO (Grand Island, NY) and Falcon (Becton Dickinson Co., Oxnard, CA). R5020 (promegestone) and [3H]leucine were provided by New England Nuclear (Boston, MA). RU486 (mifepristone) was a gift from Roussel-UCLAF (Romainville, France). 4-Hydroxymatoxifen was a gift from Stuart Pharmaceuticals-I.C.I. Division (Wilmingtom, DE). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Glass slides with 8 microwell chambers (Lab Tek 4808) were purchased from Miles Scientific (Naperville, IL). Bone alkaline phosphatase was purchased from International Enzymes, Inc. (Fallbrook, CA). Goat anti-mouse IgG was purchased from Chappel, Inc. (Cochran, IL). The ABC kit for immunostaining was purchased from Vector Laboratories, Inc. (Burlingame, CA), and mouse monoclonal antibodies (ascites preparations) against human liver alkaline phosphatase (ALP-L/SP2/64) and human term placenta (HT-7) were gifts from, respectively, Professor Harry Harris (University of Pennsylvania, Philadelphia, PA) and Dr. José Milan (La Jolla Cancer Research Foundation, La Jolla, CA).

Cell Culture Conditions. T47D cells (clone 10) were a gift from I. Keydar (Tel Aviv, Israel). They are periodically tested for Mycoplasma by the fluorescent method of Battaglia et al. (7) with the Hoechst reagent bisbenzimide 33258 and have been found to be negative. The cells are routinely cultured in RPMI medium fortified with 5% fetal calf serum, 0.2 µg/ml bovine insulin (0.2 IU/ml), penicillin (10,000 units/ml), and streptomycin (10 mg/ml). For experiments, cells were plated in 35 x 24 mm well cluster plates in the medium above and then 24 h later were switched to medium containing 1–2% charcoal-treated serum with or without hormones in a final ethanol volume of 0.1%. For routine induction of alkaline phosphatase, cells were allowed to reach near confluence and then treated for 3 days with two media changes, with 1 nM R5020 and then harvested as described above.

Preparation of Cell Extracts. Cells were either released from their growth chamber by scraping them off with a rubber policeman into phosphate-buffered saline (0.14 M NaCl, 0.01 M Na2HPO4, 0.01 M KH2PO4, and 0.003 M KCl, pH 7.4) or by releasing them from the flask by treatment with 0.5% trypsin-1 mM EDTA in Hanks' balanced salt solution for 5–10 min at room temperature. We have not observed that trypsin treatment significantly reduces the cellular content of alkaline phosphatase. With trypsin treatment the cells were resuspended in 2% charcoal-treated serum to stop the action of trypsin, and then washed twice in 10 mM sodium phosphate (pH 7.4) buffer containing 0.1 M sucrose and 10% glycerol.

Two methods were routinely used for the extraction of alkaline phosphatase from cells. In the first method the cells were lysed with TPSG at 0°C for 30 min with vigorous vortex mixing about every 5 min. Nuclei were sedimented at 2500 rpm and the supernatant was saved as cytosol. Nuclei were then extracted with 0.1 M sodium phosphate buffer, pH 7.4, containing 1 M sodium chloride (DNA assay buffer). Using this method the TPSG-soluble cytosolic fraction generally contained 20–30% of the total cellular alkaline phosphatase,
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whereas greater than 70% of the alkaline phosphatase and 95% of the cellular DNA were present in the crude nuclear pellet extracted into the DNA assay buffer. Exhaustive washing of nuclei with TPSG buffer with Triton X-100 concentrations as high as 1% did not release a significantly greater amount of protein or alkaline phosphatase from nuclei. In the second method cells were extracted at 22°C for 1 h with 20% n-butyl alcohol in 50 mM Tris-HCl, pH 7.4, 0.25 M sucrose, 2 mM MgCl₂ as described (4). The aqueous phase was separated by centrifugation for 10 min at 10,000 rpm and then dialyzed overnight at 4°C against 10 mM Tris/HCl, pH 7.4. The insoluble pellet at the interface of the butyl alcohol/aqueous phase was solubilized in DNA assay buffer and assayed for DNA as described above. The aqueous phase contained greater than 95% of the alkaline phosphatase activity and the insoluble pellet contained greater than 95% of the cellular DNA. The protein content of the cell extracts was assayed by the method of Lowry et al. (8) and the DNA solubilized in the DNA assay buffer was determined by the method of Labarca and Paigen (9) as modified in our laboratory.

Alkaline Phosphatase Assay. Alkaline phosphatase activity was determined as described by others (10, 11) and modified in our laboratory. Briefly, 1 volume of cell extract was incubated with 3 volumes of 1 mg/ml pNPP prepared in DEAM. In our experiments, 25 μl of cell extracts were incubated with 75 μl pNPP-DEAM in wells of a 96-well plate in triplicate. The enzyme reaction was allowed to proceed at room temperature until a pale yellow color appeared, at which time the reaction was stopped with 100 μl of 1 N NaOH. Ten standards of 1–10 μM p-nitrophenol were prepared in DEAM buffer and 100 μl of each were transferred to the microtiter plate. Absorbance was read in a manual microtiter spectrophotometer (Model EL-330, Bio-Tek Instruments, Inc.) at 405 nm. Alkaline phosphatase activity is expressed as pmol p-nitrophenol formed/min/μg DNA or mg protein.

EAIA. Liver (tissue unspecific) and germ cell alkaline phosphatases were distinguished in cell extracts by EAIA exactly as described in detail by Jemmerson and Fishman (12). Cluster 96-well tissue culture plates were treated with goat anti-mouse IgG followed with mouse anti-human liver or anti-placental alkaline phosphatase. Cell extracts were then added to allow binding of alkaline phosphatase to the immobilized anti-alkaline phosphatase monoclonal antibodies, and the presence of the enzyme was detected by adding the chromogenic alkaline phosphatase substrate pNPP.

Immunocytochemical Detection of Alkaline Phosphatase. T47D cells were grown on 8-well cluster glass slides (approximately 2000 cells/well), and then allowed to confluence. They were then added to allow binding of alkaline phosphatase to the immobilized anti-alkaline phosphatase monoclonal antibodies, and the presence of the enzyme was detected by adding the chromogenic alkaline phosphatase substrate pNPP.

RESULTS

Progestin Regulation of Alkaline and Acid Phosphatase Activity in T47D Cells. In normal uterine and breast epithelial cells, progestins increase the synthesis of a broad spectrum of different enzymes, including acid and alkaline phosphatase (4, 13). To determine if progestins also enhance the cellular activity of these enzymes in T47D cells, we incubated the cells with and without progestins for 3 days and then assayed cell extracts and formalin-fixed cells for these two enzymes. After removing the cells from their growth chamber with trypsin-EDTA, they were either lysed in a detergent buffer or extracted directly with a butyl alcohol buffer (4). The detergent-lysed cells were centrifuged to separate crude nuclei from cytosol. The nuclei were then either solubilized in a high salt (1 M NaCl) buffer (i.e., DNA assay buffer) or extracted with the butyl alcohol buffer.

As depicted in Table 1, we observed that progestins induced alkaline phosphatase activity greater than 30-fold. Acid phosphatase activity, although quite elevated in control cells, was not increased by progestin treatment. Alkaline and acid phosphatase activities in formalin-fixed cells were quantitatively similar to their levels in cell extracts, thus indicating that fixation for at least 15 min does not destroy the catalytic site of the enzyme. We further observed that about 70% of the alkaline phosphatase activity was resistant to extraction in the detergent buffer, but readily solubilized in the high salt DNA assay buffer or in the butyl alcohol buffer. The former solubilized both DNA and the alkaline phosphatase, whereas the latter solubilized only the phosphatase, leaving the DNA insoluble. Nearly all of the acid phosphatase activity was solubilized in the detergent buffer. Hence, we have found that by lysing cells in detergent buffer and then extracting the crude nuclei with butyl alcohol buffer a 20- to 40-fold purification of alkaline phosphatase can be achieved relative to extracting the enzyme directly into a butyl alcohol buffer as described previously for the extraction of this enzyme from milk and mammary epithelium (5, 6, 14).

Detergent-resistant and Soluble Forms of Alkaline Phosphatase. In order to determine whether the detergent-resistant form of alkaline phosphatase merely represented enzyme that was only partly soluble in the detergent buffer and slowly leached off the membrane components or if it was truly a nuclear-bound component, we examined the effect of multiple detergent extractions on the soluble (cytosolic) and insoluble (crude nuclear) bound enzyme. Progestin-treated cells were extracted sequentially 4 times with the detergent buffer and the alkaline phosphatase activity was determined in the supernatant and nuclear pellet after each wash. The results illustrated in Table 2 clearly reveal that alkaline phosphatase is avidly bound to the nuclear pellet and that alkaline phosphatase merely represented enzyme that was slowly leached off the membrane components.

Table 1 Progestin regulation of alkaline and acid phosphatase activities in different extracts and formalin-fixed T47D cells

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>Alkaline Phosphatase</th>
<th>Acid Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPSG</td>
<td>NaCl</td>
<td>NaCl</td>
</tr>
<tr>
<td>Fresh</td>
<td>6±3</td>
<td>264±18</td>
</tr>
<tr>
<td>0.1% ethanol</td>
<td>28±13</td>
<td>22±2</td>
</tr>
<tr>
<td>Control</td>
<td>12±0.4</td>
<td>7±6</td>
</tr>
<tr>
<td>R5020</td>
<td>13±5</td>
<td>42±60</td>
</tr>
<tr>
<td>Formalin</td>
<td>18±5</td>
<td>179±7</td>
</tr>
</tbody>
</table>

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Table 2 Efficiency of extraction of alkaline phosphatase from T47D cells with Triton X-100-containing buffer

T47D cells were treated with 10 nm concentrations of R5020 for 3 days and then released from their growth chamber with trypsin/EDTA as described in the text. Alkaline phosphatase activity in the cytosols and nuclear pellets were then determined as described in “Materials and Methods.” Values represent the mean of 3 determinations with less than 10% variation.

<table>
<thead>
<tr>
<th>Wash</th>
<th>TP Serg-soluble</th>
<th>TP Serg-insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.17 (0.25)</td>
<td>0.71 (28.7)</td>
</tr>
<tr>
<td>2</td>
<td>0.04 (&lt;0.05)</td>
<td>0.75 (28.8)</td>
</tr>
<tr>
<td>3</td>
<td>0.03 (&lt;0.05)</td>
<td>0.78 (28.5)</td>
</tr>
<tr>
<td>4</td>
<td>0.02 (&lt;0.05)</td>
<td>0.81 (25.2)</td>
</tr>
</tbody>
</table>

* Number of times nuclei were washed with 0.2% TP Serg.
* TP Serg soluble (i.e., nuclei solubilized in DNA assay buffer).
* TP Serg insoluble (i.e., nuclei solubilized in DNA assay buffer).
* µm pNPP formed/min/µg DNA.
* Cytosolic protein determined from TP Serg-soluble fraction.
* DNA determined from TP Serg-insoluble fraction.

Table 3 Ligand-dependent induction of alkaline phosphatase (ALP) in T47D human breast cancer cells

T47D cells were treated for 3 days with the various hormones as indicated, fixed in 10% formalin/phosphate-buffered saline, and then assayed for alkaline phosphatase activity as described in Table 1.

<table>
<thead>
<tr>
<th>Hormone treatment</th>
<th>Dose (nM)</th>
<th>ALP activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>5 ± 0.2</td>
</tr>
<tr>
<td>R5020</td>
<td>10^-6</td>
<td>220 ± 26</td>
</tr>
<tr>
<td>Progesterone</td>
<td>10^-6</td>
<td>197 ± 24</td>
</tr>
<tr>
<td>MPA</td>
<td>10^-4</td>
<td>104 ± 11</td>
</tr>
<tr>
<td>RU486</td>
<td>10^-4</td>
<td>10 ± 0.4</td>
</tr>
<tr>
<td>R5020 + RU486</td>
<td>10^-6 + 10^-4</td>
<td>10 ± 0.1</td>
</tr>
<tr>
<td>Estradiol</td>
<td>10^-6</td>
<td>5 ± 0.5</td>
</tr>
<tr>
<td>Estradiol + R5020</td>
<td>10^-9 + 10^-6</td>
<td>175 ± 19</td>
</tr>
<tr>
<td>4-Hydroxytamoxifen</td>
<td>10^-7</td>
<td>10 ± 0.3</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>10^-7</td>
<td>5 ± 0.3</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>10^-7</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>10^-7</td>
<td>4 ± 0.4</td>
</tr>
</tbody>
</table>

* µm p-nitrophenol formed/min/µg DNA.
* Mean ± SD.

Ligand Specificity of Alkaline Phosphatase Induction. Having shown that alkaline phosphatase, but not acid phosphatase, was induced by progestins in T47D cells, we then investigated if the induction was progestin specific. As presented in Table 3, all of the progestins tested (R5020, progesterone, and medroxyprogesterone acetate) induced alkaline phosphatase activity at physiological concentrations, whereas dexamethasone, dihydrotestosterone, estradiol, RU486 (a potent antiprogestin), and the antiestrogen 4-hydroxytamoxifen, all of which bind to specific receptors in T47D cells, had no effect on enzyme activity. The antiprogestin RU486, when combined with R5020 at a 10-fold molar excess, completely blocked the induction of alkaline phosphatase.

Dose- and Time-dependent Induction of Alkaline Phosphatase by Progestin. Induction of alkaline phosphatase with progesterone and R5020 was shown also to be dose dependent (Fig. 1). Maximal induction occurred at the physiological concentrations of progestins of 100 pM to 1 nM. We and others (3) have previously shown that these concentrations saturate PR sites and activate their binding to nuclei. Time course experiments revealed further that progestin induction of alkaline phosphatase activity is time dependent (Fig. 2). Trace levels of enzyme activity were evident only after about 6 h of progestin treatment; thereafter enzyme activity continued to rise until it reached maximal levels within about 2–3 days. After this time the presence of progestins in the culture medium do not increase enzyme activity any further. One possible explanation for this effect is that once the progesterone receptors present in the cell (T47D contains approximately 200,000 receptors/cell) and bound by the progestin have elicited their effect in the nucleus, their turnover, processing, and resynthesis become inadequate to support such a strong hormone action, and it decreases to a level (about 50%) that is maintained for several days.

Dependence of Progestin-induced Alkaline Phosphatase on Protein Synthesis and Glycosylation. Although the time course experiments suggested that progestins activate de novo synthesis of alkaline phosphatase, it was also possible that progestins only increased enzyme activity via some indirect mechanism by activating a preexisting enzyme rather than by increasing enzyme formation. To determine if progestin induction of alkaline phosphatase was dependent on protein synthesis, we next investigated the effect of the translation inhibitor cycloheximide. As seen in Fig. 3A, increasing doses of cycloheximide resulted in a concomitant decrease in progestin-induced alkaline phosphatase activity as well as a decrease in protein synthesis, as
phosphatase to inhibitors relative to the activities of alkaline
We compared sensitivity of the progestin-induced alkaline
investigated the sensitivity of the enzyme to specific inhibitors.
To determine to which of these categories the progestin-
tromigration, thermostability, and inhibition by specific re
their tissue of origin, gene structure, immunoreactivity, elec-
categorized as tissue-unspecific (liver, bone, kidney, breast,
milk, etc.), placental, germ cell, and intestinal types based on
induces de novo formation of the alkaline phosphatase enzyme
class of membrane-associated tissue-unspecific alkaline phos
5
B 50-
100
Cycloheximide
Tunicamycin ug/ml
Table 4 Inhibitors of alkaline phosphatases prepared from human tissues and cell
Table 4 Inhibitors of alkaline phosphatases prepared from human tissues and cell
determined by incorporation of [3H]leucine into newly synthet-
determined by incorporation of [3H]leucine into newly synthet-
Tunicamycin also blocked enzyme activity when added to
T47D cells together with R5020 over a period of 24 h (Fig.
T47D cells were incubated with R5020 and R5020 plus tunicamycin for
maximal inhibition was obtained at a tunicamycin concentration of 10 µg/
mil.

Fig. 3. A, cycloheximide inhibition of R5020-induced alkaline phosphatase and protein synthesis. T47D cells were treated with 1 nM R5020 in the absence or presence of increasing concentrations of cycloheximide as indicated. [3H]-Leucine was included at 1 µCi/ml. After 24 h alkaline phosphatase activity and trichloroacetic acid-precipitable [3H]leucine (dpm) were determined in the TPSG-soluble fraction. Enzyme activity and trichloroacetic acid-precipitable [3H]leucine are expressed relative to cells without cycloheximide. B, blockade of R5020-induced alkaline phosphatase activity by the inhibitor of glycosylation, tunicamycin. T47D cells were incubated with R5020 and R5020 plus tunicamycin for 24 h. Maximal inhibition was obtained at a tunicamycin concentration of 10 µg/ml.

Categorization of Progestin-induced Alkaline Phosphatase with Enzyme Inhibitors. Alkaline phosphatases generally are categorized as tissue-unspecific (liver, bone, kidney, breast, milk, etc.), placental, germ cell, and intestinal types based on their tissue of origin, gene structure, immunoreactivity, electromigration, thermostability, and inhibition by specific reagents. To determine to which of these categories the progesterone-induced alkaline phosphatase in T47D cells belongs, we next investigated the sensitivity of the enzyme to specific inhibitors. We compared sensitivity of the progestin-induced alkaline phosphatase to inhibitors relative to the activities of alkaline phosphatases derived from other sources of normal and malignant human tissues, including normal human breast, normal human liver, human bone (osteosarcoma), human term placenta, and HeLa cells (Table 4). Homoarginine, levamisole, and EDTA, but not phenylalanine, were potent inhibitors of progestin-induced alkaline phosphatase from T47D cells, normal breast, normal liver, and bone. In contrast, the alkaline phosphatases from human term placenta and HeLa cells were unaffected by homoarginine and EDTA, but were significantly inhibited by phenylalanine. Based on these inhibitor profiles it is apparent that the enzyme from T47D cells belongs to the class of membrane-associated tissue-unspecific alkaline phosphatase (15, 16).

Characterization of Progestin-induced Alkaline Phosphatase with Monoclonal Antibodies Directed against Human Liver and Placental Alkaline Phosphatase. Because of the obvious similarities of progestin-induced alkaline phosphatase with liver, bone, and kidney alkaline phosphatase, we next determined if monoclonal antibodies produced against human liver alkaline phosphatase (15) cross-react with the progestin-induced isoenzyme from T47D cells. Using a highly specific enzyme-antigen immunoassay (12) and monoclonal antibodies directed to liver and germ cell alkaline phosphatase, we were able to confirm that indeed the progestin-induced alkaline phosphatase from T47D cells is a tissue-unspecific alkaline phosphatase (Table 5). Conversely, the germ cell alkaline phosphatase was only recognized by its corresponding monoclonal antibody. In these experiments we also confirmed that the monoclonal antibody directed to the liver enzyme binds to alkaline phosphatase from normal human liver, bone-type enzyme from human osteosarcoma (SAOS-2), and normal breast.

Detection of Progestin-induced Alkaline Phosphatase by Immunocytochemistry. The availability of monoclonal antibodies directed against the progestin-induced alkaline phosphatase as well as the germ cell enzyme also enabled us to perform immunocytochemistry experiments. Formalin-fixed T47D cells were incubated with the anti-liver alkaline phosphatase antibody, followed by a secondary IgG goat anti-mouse peroxidase-labeled avidin-biotin method of immunocytochemistry. We observed no staining in untreated T47D cells in contrast to a heterogeneous staining pattern in progestin-treated cells (Fig. 4, A and B). Diffuse and light staining was apparent in most cells; however, in many cells, intense staining appeared to be localized at the plasma membrane and in focal regions adjacent to nuclei. Staining was not present in nuclei. As a control,
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Table 5  Enzyme-antigen immunoassay of alkaline phosphatases (ALP) from different sources by using monoclonal antibodies directed against liver- and regan-type enzymes

<table>
<thead>
<tr>
<th>Tissue-unspecific antigens</th>
<th>Anti-liver ALP</th>
<th>Anti-placenta ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bone</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Milk</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T47D cells: untreated</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T47D cells: progestin-treated</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Nor have we demonstrated that in the T47D human breast cancer cell line progestins induce the synthesis of a membrane-bound alkaline phosphatase, similar if not identical, to the enzyme present in normal breast and human milk. Enzyme induction was uniquely specific to progestins and was not altered by other steroid hormones or synthetic analogues. Moreover, progestin induction of alkaline phosphatase was dependent on time, protein synthesis, and correct glycosylation, and new synthesis of antigenic sites as determined by EAIA and immunocytochemistry. Hence, it seems reasonable to conclude that progestins induce de novo synthesis of alkaline phosphatase and are not simply activating a preexisting enzyme. Alkaline phosphatases are membrane-associated glycoproteins which, based on their tissue of origin and gene loci, are categorized in three major groups (15–19), including term placental, intestinal, and tissue-unspecific alkaline phosphatase. They may be further subgrouped according to their sensitivity to inhibitors and heat, electromigration, and antigenicity. The alkaline phosphatase purified from human milk and present in the normal human mammary gland belongs to the group of tissue-unspecific isoenzymes (17). Not surprising, the progestin-induced alkaline phosphatase we have identified in T47D cells is a member of the issue-unspecific group of isoenzymes based on its inhibitor profile and antigenicity. The term tissue-unspecific alkaline phosphatase refers to a class of isoenzyme proteins which do not have the tissue specificity exhibited by the isoenzymes of intestine, placenta, and germ cells, but share common antigenic epitopes.

We have shown that estradiol alone had no effect on alkaline phosphatase activity in T47D cells, nor did it augment the effect of progestins. This lack of response to estrogens or its augmentation of progestin action is not unexpected, since in T47D cells PR is expressed at very high levels in the absence of an apparent estrogen regulatory mechanism (3). In contrast, the expression of PR in normal mammary epithelial cells (20), as well as several breast cancer cell lines (21) in culture (e.g., MCF-7 and ZR-75-1), is dependent on estrogen priming. While alkaline phosphatase is clearly progestin/PR-regulated in T47D cells, it remains to be determined if estrogen priming is a prerequisite for progestin-regulated synthesis of alkaline phosphatase in other cancer cell lines, normal mammary epithelial cells, or hormone-dependent (i.e., ER and PR positive) breast carcinomas with an estrogen-dependent PR. Several types of hormones have been reported to enhance the cellular activity of alkaline phosphatases in other tissue types; however, not to the extent (i.e., 30- to 100-fold) we have demonstrated progestins induce this enzyme in T47D cells. Mulkins and Sussman.

DISCUSSION

We have demonstrated that in the T47D human breast cancer cell line progestins induce the synthesis of a membrane-bound alkaline phosphatase, similar if not identical, to the enzyme present in normal breast and human milk. Enzyme induction was uniquely specific to progestins and was not altered by other steroid hormones or synthetic analogues. Moreover, progestin induction of alkaline phosphatase was dependent on time, protein synthesis, and correct glycosylation, and new synthesis of antigenic sites as determined by EAIA and immunocytochemistry. Hence, it seems reasonable to conclude that progestins induce de novo synthesis of alkaline phosphatase and are not simply activating a preexisting enzyme. Alkaline phosphatases are membrane-associated glycoproteins which, based on their tissue of origin and gene loci, are categorized in three major groups (15–19), including term placental, intestinal, and tissue-unspecific alkaline phosphatase. They may be further subgrouped according to their sensitivity to inhibitors and heat, electromigration, and antigenicity. The alkaline phosphatase purified from human milk and present in the normal human mammary gland belongs to the group of tissue-unspecific isoenzymes (17). Not surprising, the progestin-induced alkaline phosphatase we have identified in T47D cells is a member of the issue-unspecific group of isoenzymes based on its inhibitor profile and antigenicity. The term tissue-unspecific alkaline phosphatase refers to a class of isoenzyme proteins which do not have the tissue specificity exhibited by the isoenzymes of intestine, placenta, and germ cells, but share common antigenic epitopes.

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