The Protein Tyrosine Phosphatase DEP-1 Is Induced during Differentiation and Inhibits Growth of Breast Cancer Cells

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ABSTRACT

Sodium butyrate-induced differentiation of breast cancer cell lines was used to identify protein tyrosine phosphatases (PTPs) involved in differentiation and growth inhibition of breast cancer cells. Of 42 PTPs analyzed, 31 were expressed in the ZR75-1 breast cancer cell line. Expression of four PTPs (DEP-1, SAP, P'TPγ, and PAC) was regulated in ZR75-1 cells undergoing differentiation. Expression of two of these PTPs (DEP-1 and SAP) was also regulated in the SKBr-3 cell line undergoing differentiation. In view of its marked induction with differentiation in an estrogen receptor (ER)-positive and an ER-negative breast cancer cell line, DEP-1 was investigated for a role in growth inhibition or induction of differentiation in breast cancer cells. A DEP-1 cDNA construct under control of a constitutively active cytomegavirus promoter was transfected into the ZR75-1, SKBR-3, and MCF-7 breast cancer cell lines, and resistant colonies were selected with G418. DEP-1 expression inhibited the development of resistant colonies by 3-5-fold in all three lines compared to transfection with vector alone. Three stable MCF-7 cell lines expressing DEP-1 under control of an inducible metallothionein promoter were then established. In these lines, induction of DEP-1 expression inhibited breast cancer cell growth by 5-10-fold. These data describe PTPs expressed and regulated in breast cancer cell lines during differentiation and identify one PTP, DEP-1, that inhibits the growth of breast cancer cells in vitro.

INTRODUCTION

Control of protein phosphorylation at tyrosine residues is a fundamental regulatory mechanism in signal transduction pathways involved in transformation and growth of breast cancer cells (1). Overexpression of type 1 receptor tyrosine kinases, such as the epidermal growth factor receptor and the c-erbB2 oncogene, has been demonstrated in breast cancers (2, 3). Furthermore, blockade and functional inhibition of c-erbB2 by monoclonal antibodies inhibits the growth of c-erbB2-induced tumors xenografted in athymic mice (4) and has induced remissions of c-erbB2-overexpressing breast cancers in humans (5). Induction of PTP activity, the natural antagonist of protein tyrosine kinase activity, has been associated with inhibition of growth in breast cancer cells. Specifically, tyrosine phosphatase activity increases when breast cancer cells in vitro are grown in the presence of the antiestrogen tamoxifen (6). This suggests that induction of PTP activity may be inhibitory to growth stimuli in breast cancer cells.

Although signaling through protein tyrosine kinases has been well studied, little is known about the role of PTPs in breast epithelial cells. In other systems, increased PTP activity has been associated with differentiation of cancer cells, and specific PTPs have been demonstrated to inhibit cellular transformation (7-9). In particular, mutant T-cell PTP inhibits focus formation by NIH 3T3 cells transformed with v-fms and v-erbB, and PTP activity is increased in murine and human leukemia cells undergoing differentiation (7-9). In light of these data, and because each PTP appears to have specificity for particular phosphotyrosine residues, we hypothesized that specific PTPs might exist, the function of which would inhibit growth or induce differentiation of breast cancer cells (10-12).

To identify specific PTPs involved in inhibition of growth and induction of differentiation in breast cancer, we analyzed the PTPs expressed and regulated during sodium butyrate-induced differentiation of breast cancer cell lines in vitro. We demonstrated expression of 31 PTPs in the ZR75-1 breast cancer cell line undergoing differentiation and found that expression of four of these PTPs (DEP-1, SAP, P'TPγ, and PAC) is regulated with differentiation (13-16). Because DEP-1 expression was markedly induced with differentiation of the SKBr-3 breast cancer cell line, we investigated its role in inhibiting growth or inducing differentiation in breast cancer cells. We demonstrated that overexpression of DEP-1 inhibits colony formation in three breast cancer cell lines and is growth inhibitory in MCF-7 breast cancer cells.

MATERIALS AND METHODS

Cells and Cell Culture. The ZR75-1 and SKBr-3 breast cancer cell lines were supplied by American Type Culture Collection. The MCF-7 cell line was a gift from Dr. E. Chu (National Cancer Institute, Bethesda, MD). ZR75-1, MCF-7, and SKBr-3 breast cancer cells were cultured at 37°C in a humidified CO2 incubator (5%) in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD), supplemented with 10% (v/v) FCS and 1% penicillin-streptomycin.

Differentiation. Cells were seeded in 100-mm tissue culture plates at 2 x 106 cells/plate. Sodium butyrate (Sigma Chemical Co., St. Louis, MO) at a final concentration 1 mm was added to cultures at 24 h with cells in exponential growth phase. At indicated times, cells were harvested with 0.05% trypsin 0.53 mm EDTA (Life Technologies, Inc., Gaithersburg, MD) and counted using a hemacytometer. Cellular lipid content was assessed by staining cytospins with Oil Red O (Poly Scientific, Bay Shore, NY) as previously described (17).

RNA Analysis. Total cellular RNA was isolated from cells using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer's recommendation. RNA (12 μg/lane) was fractionated on 1% agarose gels containing 0.675% formaldehyde, transferred overnight onto Hybond-N nylon membranes (Amersham, Cleveland, OH), and fixed to membranes by baking at 80°C for 2 h. cDNA fragments used as probes were labeled with [a-32P]dCTP using a Random Primers kit (Life Technologies, Inc., Gaithersburg, MD) to a specific activity of 500-2000 cpm/pg. Northern blots were prehybridized for 1 h at 42°C in hybridization solution (40% Formamide, 4X SSC, 7 mm Tris-HCl, 0.8X Denharts solution, 20 μg/ml sheared herring sperm DNA, 10% Dextran sulfate, 1% SDS) and then hybridized overnight at 42°C using 2 X 106 cpm of [a-32P]dCTP labeled probe per ml of hybridization solution. Filters were rinsed three times at room temperature with 2X SSC, 0.1% SDS and then washed twice at 52°C for 20 min each with 0.1X SSC, 0.1% SDS. Filters were then exposed to X-AR film (Kodak, Rochester, NY) at −70°C with intensifying screens.

RT-PCR. A series of primers that correspond to PTPs were synthesized. These primers have been used extensively in analysis of PTP expression in a number of different human, mouse, and hamster cell lines (18, 19).
sequences of these primers were based on human data when available. For those PTP thus far only cloned from nonhuman mammals, the primer sequences were chosen from the cDNA of the species where it was first cloned. Primers were designed to yield amplicons ranging in length from 162 to 494 bp with temperatures for optimal PCR between 53.3 and 60.5°C. PTP primers were chosen that amplify a specific band from cDNA but not from genomic DNA, and where the primer sequence was derived from nonhuman mammalian sequences, they were tested to ensure that they amplified a specific band from human cDNA. PTP primers used for these studies are available on request.

RT-PCR was done as described previously (20, 21). In brief, cDNAs were prepared from the ZR75-1 cell line by random hexamer-primed RT of 0.5 μg of total RNA in a 20-μl reaction volume. RT reactions were incubated at room temperature (25°C) for 20 min, at 42°C for 20 min, and at 55°C for 20 min. The RT was inactivated at 99°C for 5 min. Ten- and 100-fold dilutions of the RT mixture were made in water. Based on abundances of each amplonic in preliminary analyses, target sequences within 0.1 μl of the appropriate dilution of the cDNA reaction were amplified in 20-μl reactions containing 0.5 units of Taq DNA polymerase (Life Technologies, Inc., Gaithersburg, MD), 4 pmol each of the sense and antisense primers, 50 mM KCl, 2 mM MgCl2, 20 mM Tris, 62.5 μM each of dATP, dCTP, dGTP, and TTP, and 0.1 μl [α-32P]dCTP. All PCR reaction components except for the primers were prepared as a master mix, which was subsequently aliquoted and overlaid with mineral oil. Reactions were hot-started, and amplification of each of the primer pair/template reactions was performed in parallel, using the same master mix for each RT. The first two cycles of PCR consisted of denaturing at 97°C for 45 s, primer annealing at 54°C for 300 s, and primer extending at 72°C for 60 s. An additional 28 cycles were completed under identical conditions except that the denaturation temperature was decreased to 94°C and the annealing time was decreased to 60 s.

Following electrophoresis through 1.5% Tris-borate-EDTA agarose gels, the amplicons were detected by ethidium bromide staining and were then transferred by electroblotting to GeneScreen Plus nylon membranes (DuPont, Boston, MA) as described previously (20). DNA was fixed to the membranes by air drying and UV crosslinking. A PhosphorImager screen was briefly exposed to the membranes and the screen was then scanned on a Molecular Dynamics PhosphorImager. The images recorded by the PhosphorImager were analyzed by volume integration with the ImageQuant software. The relative level of PTP expression was assessed by comparison with the level of GAPDH in the same sample (21).

Expression Vector Constructs. A cDNA containing the entire open reading frame of DEP-1 (a gift from Dr. N. Tonks, Cold Spring Harbor Laboratory) was cloned into the CMV early promoter-based expression vector pcDNA3 (Invitrogen, Portland, OR) and into the inducible metallothionein promoter-based expression vector pMTβH-globin-neo (a gift from Dr. S. Segal, NCI Bethesda, MD; Ref. 22).

Colony-forming Assay. ZR75-1, SKBr-3, and MCF-7 breast cancer cell lines (1 x 106 per 100-mm tissue culture plate) were each transfected with 20 μg of DEP-1 pcDNA3 expression construct or 20 μg of pcDNA3 vector using the lipofectin protocol (Life Technologies, Inc., Gaithersburg, MD). Twenty-four h after transfection, cells were harvested with 0.05% trypsin 0.53 mM EDTA and split into five plates; 48 h after transfection, resistant colonies were selected with 0.418 (300-600 μg/ml). Transfection efficiency was assessed by 3-galactosidase activity at 48 h after transfection as described previously (23). All experiments were performed in triplicate and repeated at least twice.

**RESULTS**

Identification of PTPs in Differentiating Breast Cancer Cells. Sodium butyrate inhibited growth and induced a differentiated phenotype in the ZR75-1 breast cancer cell line. Differentiation was characterized by growth arrest with a block at the G1-S boundary and development of normal breast epithelial cell features, including increased cell size, increased cytoplasmic granularity, reduction in the nuclear cytoplasmic ratio, and accumulation of intracytoplasmic lipid (24). The morphological changes and increased intracytoplasmic lipid induced by sodium butyrate treatment of ZR75-1 cells are shown in Fig. 1A (top right panel) and are compared to control ZR75-1 cells (top left panel). The growth inhibition induced by sodium butyrate in ZR75-1 cells is shown in Fig. 1B (left panel). Furthermore, ZR75-1 cells grown in 1 mM sodium butyrate on an extracellular matrix developed a branching duct-like morphology reminiscent of normal mammary ducts, whereas control ZR75-1 cells grown in the absence of sodium butyrate remained in tight clumps (data not shown; Ref. 25). Sodium butyrate also induced differentiation and inhibited growth of the SKBr-3 breast cancer cell line. In contrast, an MCF-7 breast cancer cell line subclone was resistant to sodium butyrate-induced differentiation. This cell line underwent minimal differentiation changes (Fig. 1A, bottom panels) and was not significantly growth inhibited when incubated with sodium butyrate (Fig. 1B, right panel).

In the ZR75-1 and SKBr-3 cell lines, which are associated with induction of morphological differentiation by sodium butyrate, there was induction of expression of molecules associated with a differentiated breast epithelial cell phenotype (Fig. 2A). These included FAS and HMFG (26-28). FAS expression is associated with the production of mature lipids, and HMFG expression is associated with lactating breast tissue (26, 28). High levels of HMFG expression are also demonstrated in nontransformed breast epithelial cell lines (e.g., MCF10, 184B5, and primary human mammary epithelial cells) but not in breast cancer cell lines (e.g., ZR75-1, SKBr-3, and MCF-7; Fig. 2B). However, longer radiographic exposure demonstrates that there is some endogenous HMFG expression in the breast cancer cell lines. Thus, FAS and HMFG induction are indicative of progression toward a more differentiated breast epithelial cell phenotype. The two differentiating breast cancer cell lines, ZR75-1 and SKBr-3, were used to identify PTPs the expression of which was regulated with differentiation and growth inhibition of breast cancer cells. Expression of these PTPs was then analyzed in the MCF-7 breast cancer cell line that did not differentiate when treated with sodium butyrate.

RT-PCR was used to investigate expression of PTPs in ZR75-1. The repertoire of PTPs expressed by ZR75-1 (Table 1) includes 9 transmembrane PTPs, 15 intracellular PTPs, 5 dual-specificity PTPs, and 2 isoforms of a small acidic PTP. Eleven PTPs were not expressed by the ZR75-1 cell line, including 8 transmembrane PTPs and 3 intracellular PTPs.

Semi quantitative RT-PCR was used to identify PTPs the expression of which was regulated in ZR75-1 undergoing differentiation with sodium butyrate, and this regulation was confirmed by Northern analysis. With induction of differentiation, the mRNA levels of four PTPs (DEP-1, SAP, PTPμ, and PAC) changed significantly, and each changed with a different time course (Fig. 3). DEP-1 expression was

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Fig. 1. Sodium butyrate induced differentiation of breast cancer cell lines. A, ZR75-1 (top panels) and MCF-7 (bottom panels) breast cancer cells were incubated for 120 h in the presence (right panels) or absence (left panels) of 1 mM sodium butyrate. These cells were then stained with Oil Red O. In the ZR75-1 cell line, sodium butyrate-induced differentiation is demonstrated. This is characterized by increased cell size, reduction in nuclear cytoplasmic ratio, and accumulation of intracytoplasmic lipid. In contrast, in the MCF-7 cell line, minimal morphological changes and only a slight increase in intracytoplasmic lipid content are demonstrated when cells are incubated with sodium butyrate. ×400. B, growth curves for ZR75-1 (left panel) and MCF-7 (right panel) breast cancer cells grown in the presence (■) or absence (○) of 1 mM sodium butyrate. Growth of ZR75-1 cells was significantly inhibited by sodium butyrate (P < 0.01), whereas growth of MCF-7 cells was not significantly inhibited by sodium butyrate (P = 0.08).

induced at 1-2 h and increased continuously during differentiation (Fig. 3A). SAP and PTPγ expression were both absent in untreated cells. SAP mRNA expression was induced at 2 h, peaked at 8-24 h, and then declined with further differentiation, but levels always remained elevated over baseline (Fig. 3A). PTPγ expression was induced approximately 2 h into differentiation, peaked at 4 h, and was absent again by 24 h (Fig. 3A). PAC expression was constant early in differentiation and declined from 48 h on (Fig. 3B). The pattern of expression of these PTPs on Northern analysis was similar to that demonstrated by semiquantitative RT-PCR. Expression of three PTPs (PTPmeg1, hPEST, and SH-PTP2), which was not regulated in differentiating ZR75-1 by RT-PCR, was also not regulated by Northern analysis (data not shown; Refs. 29-31). In view of this result, expression of the other PTPs identified but not regulated during differentiation was not assessed by Northern analysis.

By Northern analysis, DEP-1 and SAP expression was also induced in the SKBr-3 cell line differentiated with sodium butyrate. Induction of DEP-1 expression was similar to ZR75-1 with increasing expression from the 1-h time point onward (Fig. 4). SAP was not expressed in undifferentiated SKBr-3 cells and was induced with a similar time course to ZR75-1. However, even at its peak, SAP induction was very weak (data not shown). PAC and PTPγ were not expressed in the SKBr-3 cell line. Induction of HMFG expression is shown as evidence of differentiation in SKBr-3 (Fig. 4).

In the differentiation-resistant MCF-7 breast cancer cell line, DEP-1, PTPγ and PAC mRNA were expressed but not regulated by treatment with sodium butyrate (Fig. 3, A and B). SAP mRNA was not expressed in MCF-7. Of note, the baseline level of DEP-1 expression was similar in all three breast cancer cell lines untreated with sodium butyrate.

**DEP-1 Inhibits Growth of Breast Cancer Cells.** Because DEP-1 expression was induced in both an ER-positive (ZR75-1) and an ER-negative (SKBr-3) breast cancer cell line undergoing differentiation, it was investigated for a role in growth inhibition or differentiation of breast cancer cells. A colony-forming assay was performed in which full-length DEP-1 cDNA under control of a constitutively
active CMV promoter was transfected into the ZR75-1, SKBr-3, and MCF-7 breast cancer cell lines and resistant colonies were selected with G418. At 15 days, in all three cell lines, there were 3-5-fold fewer G418-resistant colonies in DEP-1-transfected cells compared to control cells transfected with vector alone (Fig. 5). Before G418 selection, 48 h after transfection, DEP-1 mRNA was highly expressed in pooled cells; however, at 15 days of G418 selection, DEP-1 mRNA was not expressed in 20 individual colonies or in three plates of pooled colonies (data not shown). Transfection efficiency, measured by β-galactosidase assay, was similar in cell lines transfected with DEP-1 and in those transfected with vector alone. This suggested that DEP-1 expression was inhibiting the outgrowth of breast cancer cells in vitro.

To investigate whether DEP-1 expression was inhibitory to breast cancer cell growth, stable cell lines expressing DEP-1 were established. The MCF-7 breast cancer cell line was transfected with full-length DEP-1 cDNA under control of a zinc-inducible metallothionein promoter. Three cell lines were established from G418-resistant colonies (D5, D11, D23) in which DEP-1 mRNA expression was induced when the cell line was incubated with 100 μM ZnCl2 (Fig. 6A). Exogenous DEP-1 expression was induced 3-7-fold over endogenous levels by incubation with zinc in all three lines. Two control cell lines (VI1, VI2) transfected with pMTHF-globin-neo vector alone were also established. Growth curves for each cell line induced and uninduced by zinc were produced (Fig. 6B). Zinc induction of DEP-1 expression was associated with significant growth inhibition in all three cell lines compared to the same cell line without DEP-1 expression. In contrast, zinc treatment of vector-transfected control cell lines had no significant effect on their growth. Also, growth of clones D5, D11, and D23 without induction of DEP-1 expression was not significantly different from vector-transfected control cell lines (Fig. 6B).

Cell cycle analysis of zinc-induced DEP-1-expressing clones D5 and D11 at day 5 demonstrated a 58% reduction in S-phase activity in these cell lines compared to uninduced clones D5 and D11 without DEP-1 expression. Oil Red O staining of clones D5, D11, and D23, expressing DEP-1, demonstrated some increased intracytoplasmic lipid content compared to control cells. However, by Northern analysis, there was no increase in HMFG or FAS expression in DEP-1-expressing cells compared to controls (data not shown). This demonstrates that induction of DEP-1 expression causes growth inhibition of MCF-7 breast cancer cells but minimal differentiation associated changes.

**DISCUSSION**

The differentiation process induced by sodium butyrate, characterized by growth arrest and induction of a more mature breast epithelial cell phenotype, provides a useful experimental model system to identify genes and pathways capable of inhibiting growth or inducing differentiation in breast cancer cells (24, 32-34). This system was used to identify PTPs, a family of proteins that may be involved in growth arrest or differentiation of breast cancer cells (6). Of 42 PTP transcripts analyzed, 31 were expressed, and 4 of the expressed PTPs were regulated, with differentiation of the ZR75-1 breast cancer cell line. Members of all PTP families, including receptor-like transmembrane PTPs, intracellular PTPs, and dual-specificity PTPs, were expressed in ZR75-1 (Table 1; Refs. 35, 36). Because most PTPs identified have only recently been cloned and because the number of new PTPs being identified continues to increase, the expression pattern of PTPs in many tissues is unknown. In one study of murine erythroleukemia cells, 12 of 16 PTP transcripts were identified by Northern
A

Fig. 3. Northern analysis of PTPs expressed in ZR75-1 and MCF-7 treated with sodium butyrate. A, Northern blots of ZR75-1 (left panel) and MCF-7 (right panel) incubated with 1 mm sodium butyrate. RNA was harvested at the denoted time points and the blot was probed with cDNA for PTPγ, SAP, and DEP-1. GAPDH was a control for RNA loading. DEP-1 expression at baseline was similar in ZR75-1 and MCF-7. The apparent high endogenous expression level in MCF-7 is due to longer radiographic exposure. B, Northern blots of ZR75-1 (left panel) and MCF-7 (right panel) incubated with 1 mm sodium butyrate. RNA was harvested at the denoted time points, and the blot was probed with cDNA for PAC. GAPDH was a control for RNA loading.

B

Fig. 4. Northern blot demonstrating induction of DEP-1 expression in SKBr-3 undergoing differentiation. RNA was harvested from SKBr-3 incubated in 1 mm sodium butyrate at the denoted time points. Up-regulation of HMFG expression is shown as a control for differentiation in SKBr-3. GAPDH was a control for RNA loading.

Fig. 5. Colony-forming assay. There were significantly fewer G418-resistant colonies in plates transfected with DEP-1 cDNA under control of a constitutively active CMV promoter (D; ■) compared to plates transfected with pcDNA3 vector control (V; □) in three cell lines ZR75-1 (P = 0.0003), MCF-7 (P = 0.0016), and SKBr-3 (P = 0.0008).
common with the immunoglobulin superfamily of cellular adhesion molecules, including N-CAM (39, 40). It has been suggested that DEP-1 may mediate density-dependent cellular growth arrest through homophilic interaction with DEP-1 molecules on other cells (13). This function is in keeping with PTPµ, which mediates cell-cell aggregation through a homophilic binding mechanism, or with PTPβ, the expression and phosphatase activity of which increase with density-dependent growth arrest of human umbilical vein endothelial cells (41-43). Interestingly, an increase in receptor-like PTP activity is demonstrated only in contact-inhibited cells grown at high density but not in non-contact-inhibited cells grown at high density (43). We demonstrated only a slight increase in DEP-1 expression in the non-contact-inhibited ZR75-1 cell line when grown at high density4 and the increased DEP-1 expression demonstrated with differentiation of breast cancer cell lines was not associated with increased cell density. Thus, the role of DEP-1 in differentiating breast cancer cell lines is unlikely to be induction of density-dependent growth arrest.

In these experiments, transfection of the ZR75-1, SKBr-3, and MCF-7 breast cancer cell lines with DEP-1 inhibited colony formation, suggesting that DEP-1 expression was inhibitory to growth of breast cancer cells in vitro (Fig. 5). This was investigated by establishing three MCF-7 cell lines (D5, D11, and D23) with stable expression of DEP-1 under control of an inducible promoter. Induction of DEP-1 expression significantly inhibited growth of all three cell lines, confirming that DEP-1 was growth inhibitory in MCF-7 breast cancer cells (Fig. 6). However, DEP-1 expression induced only minimal features of breast epithelial cell differentiation, suggesting that it was insufficient to induce differentiation of MCF-7 breast cancer cells.

Induction of cellular PTP activity has been demonstrated in association with differentiation and growth inhibition in many systems, and specific PTPs have been identified, the expression of which is regulated during these processes (8, 9, 44-46). In particular, membrane-associated PTP activity is increased with growth inhibition of ER-positive breast cancer cells, although the specific PTPs responsible for the increased activity and the biological effect of inducing activity of a specific PTP in breast cancer cells are unknown (6). The data presented here demonstrate that induction of DEP-1 expression markedly inhibits the growth of MCF-7 breast cancer cells; however, these cells do continue to cycle at a slow rate. It is possible that higher levels of DEP-1 induction or activation of the pathways downstream of DEP-1 would completely inhibit growth of breast cancer cells. The specific phosphotyrosines dephosphorylated by DEP-1 and the pathways through which it mediates the growth inhibitory effect remain to be characterized.

The roles of the three other VFPs (SAP, PTPγ, and PAC) expressed and regulated during differentiation of breast cancer cell lines are currently being investigated. The function of SAP is unknown. However, SAP, like DEP-1, was transiently induced in both ZR75-1 and SKBr-3 during differentiation, suggesting that it also may be important in growth arrest or differentiation of breast cancer cells (Fig. 3A; Ref. 14). In ER-positive breast cancer cells, PTPγ mRNA expression is regulated through the ER, in which 17β-estradiol is found to down-regulate PTPγ expression, and tamoxifen, an antiestrogen, induces its expression (47). In our studies, PTPγ is expressed and regulated in the ER-positive ZR75-1 cell line during differentiation, but it is not expressed in the ER-negative SKBr-3 cell line during differentiation (Fig. 3A). PTPγ is also expressed but not regulated in the ER-positive MCF 7 cell line, which is resistant to differentiation (Fig. 3A). Thus in breast cancer, the induction of PTPγ expression is consistent with the function of a growth inhibitory gene regulated through the ER. PAC, in contrast with DEP-1, SAP, and PTPγ, is an intracellular PTP constitutively expressed in ZR75-1 and is down-regulated with differentiation (Fig. 3B; Ref. 15). PAC has previously been described only in cells of hematopoietic origin; however, here we demonstrate constitutive PAC expression in two breast epithelial cell lines, ZR75-1 and MCF-7 (Fig. 3B). The down-regulation of PAC expression with differentiation of breast cancer cells is consistent with T-cell findings in which PAC acts as a mitogen-activated protein kinase inhibitor and is expressed only in cycling cells (15). Future

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4 Unpublished observation.
investigation will establish the role of these PTPs in differentiating breast cancer cells. In this study, we describe the expression and regulation of PTPs in breast cancer cells undergoing differentiation, and we identify one PTP, DEP-1, that inhibits the growth of MCF-7 breast cancer cells in vitro. From structural homology and known functional studies, it is possible that the other regulated PTPs may also be involved in pathways capable of inducing growth arrest and differentiation of breast cancer cells. Investigation of DEP-1 and the other regulated PTPs may provide insight into mechanisms of control of growth and differentiation in breast epithelial cells and may identify specific molecular targets to inhibit breast cancer cell growth.

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