Glypican-1 Is Overexpressed in Human Breast Cancer and Modulates the Mitogenic Effects of Multiple Heparin-binding Growth Factors in Breast Cancer Cells

Kei Matsuda, Haruhisa Maruyama, Fang Guo, Jörg Kleeff, Jun Itakura, Yoshiro Matsumoto, Arthur D. Lander, and Murray Kore

Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Biological Chemistry, and Pharmacology [K. M., H. M., F. G., J. K., M. K.] and Department of Developmental and Cell Biology and Developmental Biology Center [A. D. L.], University of California, Irvine, California 92697, and Department of Surgery, Yamanashi Medical University, Yamanashi 409-3821, Japan [J. I., Y. M.]

ABSTRACT

Glypicans are a family of glycosylphosphatidylinositol-anchored cell surface heparan sulfate proteoglycans implicated in the control of cellular growth and differentiation. Here we show that glypican-1 is strongly expressed in human breast cancers, whereas expression of glypican-1 is low in normal breast tissues. In contrast, the expression of glypican-3 and -4 is only slightly increased in breast cancers by comparison with normal breast tissues, and glypican-2 and -5 are below the level of detection by Northern blotting in both normal and cancer samples. Treatment of MDA-MB-231 and MDA-MB-468 breast cancer cells with phosphoinositide-specific phospholipase-C abrogated the mitogenic response to two heparin-binding growth factors, heparin-binding epidermal growth factor-like growth factor and fibroblast growth factor 2. Stable transfection of these cells with a glypican-1 antisense construct markedly decreased glypican-1 protein levels and the mitogenic response to the same heparin-binding growth factors, as well as that to heregulin α, heregulin β, and hepatocyte growth factor. Syndecan-1 was also expressed at high levels in both breast cancer tissues and breast cancer cells when compared with normal breast tissues. There was a good correlation between glypican-1 and syndecan-1 expression in the tumors. However, clones expressing the glypican-1 antisense construct did not exhibit decreased syndecan-1 levels, indicating that loss of responsiveness to heparin-binding growth factors in these clones was not due to altered syndecan-1 expression. Furthermore, 8 of 10 tumors with stage 2 or 3 disease exhibited high levels of glypican-1 by Northern blot analysis. In contrast, low levels of glypican-1 mRNA were evident in 1 of 10 tumors with stage 2 or 3 disease and in 9 of 10 tumors with stage 1 disease. Taken together, these data suggest that glypican-1 may play a pivotal role in the ability of breast cancer cells to exhibit a mitogenic response to multiple heparin-binding growth factors and may contribute to disease progression in this malignancy.

INTRODUCTION

The mortality of breast cancer in the United States has recently leveled off and even decreased slightly (1, 2). However, it remains the second leading common cause of cancer death in women in the United States (1, 2). Breast cancer is expected to account for 192,200 new cancer cases in the United States in 2001, and more than 40,000 women are projected to die this year from this disease (1). A variety of molecular alterations have been reported in breast cancer. These include loss of heterozygosity (1p, 3p, 7q, 11p, 17p, 17, and 18q), mutations (BCRA1, 2, p53, and C-H-ras-1), and gene amplifications [c-myc and c-erbB-2 (3–10)]. In the case of c-erbB-2, overexpression has been correlated with aggressive disease and decreased patient survival. Furthermore, anti-erbB-2 antibodies can suppress breast cancer cell growth in vitro and decrease tumor burden in vivo (11, 12), thereby prolonging patient survival (13).

To date, a ligand that binds to c-erbB-2 has not been identified (14, 15). Instead, c-erbB-2 is capable of heterodimerizing with the other members of the EGF receptor family once these receptors bind their ligands (14–17). These ligands are either members of the EGF family that bind directly to the EGF receptor or members of the HRG family that bind erbB-3 and erbB-4 (18). In addition to EGF, the EGF family of ligands includes transforming growth factor α, HB-EGF, betacellulin, amphiregulin, and epiregulin (16). HB-EGF, amphiregulin, and HRG are heparin-binding factors. In addition, breast cancers overexpress FGF-2 and type 1–4 FGF receptors (19) as well as HGF and its receptor (c-Met; Ref. 20). Both FGF-2 and HGF are heparin-binding factors. Together, these observations suggest that multiple heparin-binding growth factors have the potential to contribute to the pathobiology of breast cancer in humans.

The binding of heparin-binding growth factors to their cell surface receptors often requires the presence of cell surface HSPGs (19, 21, 22). There are two main families of such molecules, syndecans and glypicans, which differ significantly in core protein domain structure (23, 24). Six members of the glypican family (glypican-1–6) and four members of the syndecan family (syndecan-1–4) have been reported to date (25–28). They have important functions with respect to cell behavior, including cell-cell and cell-extracellular matrix adhesion (29, 30), growth factor signaling (23, 31), and protection of growth factors such as FGF-2 from thermal denaturation and proteolytic attack (32, 33). They also regulate the interaction of several heparin-binding growth factors with their receptors and, consequently, their biological activity (34).

In view of the potential importance of heparin-binding growth factors in breast cancer and the requirement of these factors for HSPGs, in the present study we investigated the expression and action of glypicans and syndecan-1 in breast cancer. We now report that glypican-1 and syndecan-1 are overexpressed in human breast cancer and that stable expression of a glypican-1 antisense mRNA in breast cancer cells results in reduced glypican-1 protein expression, leading to an attenuated mitogenic response to FGF-2, HB-EGF, HRG-α, HRG-β, and HGF.

MATERIALS AND METHODS

Materials. The following materials were purchased from the manufacturer indicated: (a) fetal bovine serum, Leibovitz’s medium, trypsin solution, penicillin-streptomycin solution, and Geneticin (G418), Irvine Scientific (Santa Ana, CA); (b) Genescreen membranes, New England Nuclear (Boston, MA); (c) restriction enzymes, pMH vector, the random primed labeling kit, Genius 3 nanoradioactive nucleic acid detection kit, and Genius 4 RNA labeling kit; Boehringer Mannheim (Indianapolis, IN); (d) PI-PLC, Oxford Glycosciences Inc. (Bedford, MA); (e) Sequenase version 1.0 DNA Sequencing.

Received 2/25/00; accepted 5/15/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by USPHS Grants CA-40162 (to M. K.) and NS-26862 (to A. D. L.) and by an award from the Avon Products Foundation (to M. K.).
2 Recipient of a fellowship award from the University of California Research and Education grant on Gene Therapy for Cancer.
3 Present address: Department of Visceral and Transplantation Surgery, University of Bern, 3010 Bern, Switzerland.
4 To whom requests for reprints should be addressed, at Division of Endocrinology, Diabetes and Metabolism, Medical Science I, C240, University of California, Irvine, CA 92697. Phone: (949) 824-6887; Fax: (949) 824-1035.

5 The abbreviations used are: EGF, epidermal growth factor; GPI, glycosyolphosphatidylinositol; HSPG, heparan sulfate proteoglycan; PI-PLC, phosphonoinositide-specific phospholipase C; HB-EGF, heparin-binding epidermal growth factor-like growth factor; FGF, fibroblast growth factor; HRG, heregulin; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; nt, nucleotide(s); PMSF, phenylmethylsulfonyl fluoride; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

5562

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 2001 American Association for Cancer Research.
USB Specialty Biochemicals (Cleveland, OH); (f) [α-32P]-PiCTP and [α-32P]PiCTP, Amersham (Arlington Heights, IL); (g) DNA molecular weight markers and LipofectAMINE, Life Technologies, Inc. (Gaithersburg, MD); (h) nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate stock solution, Roche Molecular Biochemicals (Indianapolis, IN); (i) monoclonal mouse anti-CD138 (syndecan-1) antibody, Serotec Inc. (Raleigh, NC); (j) horseradish peroxidase-conjugated anti-rabbit antibody, Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); (k) Cy3-conjugated anti-rabbit IgG antibody, Jackson Immunoresearch (West Grove, PA); (l) pBluescript-ISK+, Stratagene (La Jolla, CA); (m) DEAE-Sepharose, Pharmacia Biotech (Piscataway, NJ); (n) enhanced chemiluminescence blotting kit, Pierce (Rockford, IL); (o) pCDNA3.1 Myc-His, Invitrogen (Carlsbad, CA); (p) Centriprep Concentrators, Amicon Inc. (Naperville, IL); (q) HRG-α, HRG-β, and HGF, R&D Systems (Minneapolis, MN); (r) [35S]SDF-1 (1000 Ci/mmol) was synthesized by incubating in a mixture at 37°C for 6 h. Incubations were terminated by the addition of 7.5 μl of 5× SDS sample buffer and heating at 95°C for 10 min. To prepare membranes, cells were homogenized in 20 mM HEPES (pH 7.4), 1.5 mM MgCl2, 1 mM EDTA, 1 mM PMSF, and 2 mM benzanilide. After centrifugation at 1,500 × g for 10 min, supernatants were collected and centrifuged at 25,000 × g for 30 min. The resulting pellets were resuspended in 20 mM HEPES (pH 7.4) containing 10 μM leupeptin and solubilized in 5× SDS sample buffer and heated at 95°C for 10 min. For reduction and alkylation with iodoacetamide, protein lysates were incubated at 95°C for 4 min in the presence of 10 mM DTT before the addition of iodoacetamide (50 mM, final concentration). Samples were then incubated at 95°C for 2 min. For syndecan-1 (40), total lysates were brought to 6 M urea and 50 mM sodium acetate (pH 4.5). They were boiled for 10 min and centrifuged to remove cell debris. DEAE-Sepharose beads were added to the supernatants, and the mixtures were rotated overnight at 4°C. The DEAE beads were washed with PBS containing 0.1% Triton X-100, and the bound proteoglycans were eluted from the beads with 1 M NaCl in PBS containing 0.1% Triton X-100 and then diluted with 20 mM Tris (pH 7.0) containing 5 mM CaCl2 to a final concentration of 0.1 M NaCl. For syndecan-1, digestion with chondroitinase Tris-hydrochloride (0.2 unit/sample) was performed at 37°C for 2 h and terminated by the addition of 5× SDS sample buffer and heating at 95°C for 10 min, subjected to SDS-PAGE and transferred to Immobilon P membranes. Membranes were incubated for 90 min with an affinity-purified rabbit antirat glypican-1 antibody or a highly specific monoclonal mouse antihuman syndecan-1 antibody, washed, and incubated with a secondary antibody against rabbit IgG or mouse IgG for 60 min. Visualization was performed by enhanced chemiluminescence.

Glypican-1 and Syndecan-1 Purification. GAG-containing forms of glypican-1 and syndecan-1 were purified by anion exchange chromatography on DEAE-Sepharose equilibrated in buffer A [50 mM Tris-HCl (pH 8.0), 0.15 M NaCl, and 0.1% Triton X-100], as reported previously (36). Cell lysates in buffer B [50 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 0.1% Triton X-100, 1 mM EDTA, 1 μg/ml peptatin A, 1 mM PMSF, and 0.1% Triton X-100] were loaded directly onto columns containing the gel using column volumes of 0.5 ml of packed gel per milligram of protein. Columns were eluted stepwise with buffer A, buffer C [50 mM Tris-HCl (pH 8.0), 0.25 M NaCl, and 0.1% Triton X-100], buffer D [50 mM Tris-HCl (pH 8.0), 6 M urea, 0.25 M NaCl, and 0.1% Triton X-100], and buffer E [50 mM sodium formate (pH 3.5), 6 M urea, 0.2 M NaCl, and 0.1% Triton X-100]. After restoring the pH with 50 mM Tris-HCl (pH 8.0) and 0.1% Triton X-100, glypican-1 or syndecan-1 was eluted from the column with buffer F [50 mM Tris-HCl (pH 8.0), 0.75 M NaCl, and 0.1% Triton X-100]. The eluted material was diluted 5-fold with 50 mM Tris (pH 8.0) and 0.1% Triton X-100 detergent, concentrated, and clarified by filtration (36). Samples were then resuspended in buffer B and analyzed by immunoblotting.

In Situ Hybridization. To carry out in situ hybridization, tissue sections (4-μm thick) were placed on 3-aminopropylmethoxysilane-coated slides, deparaffinized, and incubated at room temperature for 20 min with 0.2 N HCl and for 15 min with 50 μg/ml proteinase K at 37°C (36, 39). The sections were then postfixied for 5 min in PBS containing 4% paraformaldehyde and incubated briefly twice with PBS containing 2 mg/ml glycine and once in 50% (v/v) formamide/2× SSC for 1 h before initiation of the hybridization reaction by the addition of 100 μl of hybridization buffer. The hybridization buffer contained 0.6 M NaCl, 1 mM EDTA, 10 mM Tris-HCl (pH 7.6), 0.25% SDS, 200 μg/ml yeast RNA, 1× Denhardt’s solution, 10% dextran sulfate, 40% formamide, and 100 ng/ml of the indicated digoxigenin-labeled riboprobe (36, 39). Hybridization was performed in a moist chamber for 18 h at 42°C. The sections were then washed sequentially with 50% formamide/2× SSC for 30 min at 42°C, 2× SSC for 20 min at 42°C, and 0.2× SSC for 20 min at 42°C.
For immunological detection, the Genius 3 nonradioactive nucleic acid detection kit was used as reported previously (36) with a 1:2000 dilution of alkaline phosphatase-conjugated polyclonal sheep antidigoxigenin Fab fragment antibody. Sections were incubated with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution in a dark box for 3 h. After the reaction was stopped with 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA, the sections were mounted in aqueous mounting medium.

Cell Culture and Growth Assay. Human breast cancer cells were routinely grown in Leibovitz’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (complete medium). To perform growth assays, MDA-MB-231 and MDA-MB-468 cells were plated overnight at a density of 10,000 cells/well in 96-well plates, washed in HBSS, and subsequently incubated in serum-free medium (Leibovitz’s medium containing 0.1% BSA, 5 μg/ml transferrin, 5 mg/ml sodium selenite, and antibodies) in the absence or presence of various growth factors. For experiments with PI-PLC, cells were incubated with the indicated concentrations of PI-PLC for 1 h. Subsequently, the presence of various growth factors. For experiments with PI-PLC, cells were incubated with indicated concentrations of PI-PLC for 1 h. Subsequently, the cells were stopped with 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA, the sections were mounted in aqueous mounting medium.

Stable Transfection. Stable transfection of G1-AS-1751 into MDA-MB-231 and MDA-MB-468 cells was performed using the lipofection method as described previously (34). Briefly, after reaching confluence, cells were split 1:10 into selection medium (complete medium supplemented with 1.5 mg/ml G418 on MDA-MB-231 cells and 0.5 mg/ml G418 on MDA-MB-468 cells), and single clones were isolated after 3–4 weeks. After expansion of individual clones, cells from each individual clone were screened for expression of glypican-1 sense and antisense mRNA by Northern blot analysis and for glypican-1 protein expression by immunoblotting. Parental MDA-MB-231 cells were also transfected with an empty expression vector carrying the neomycin resistance gene as a control. Positive clones were routinely grown in selection medium.

Statistics. Student’s t test was used for statistical analysis of the experiments. P < 0.05 was taken as the level of significance. Results of MTT cell growth assays are expressed as the SE of at least three separate experiments.

RESULTS

Expression of Members of the Glypican Family and Syndecan-1 in Human Breast Tissue. Northern blot analysis was performed on total RNA isolated from 20 normal breast tissues and 20 breast cancer samples. The 3.7-kb glypican-1 mRNA transcript was of relatively low abundance in 6 of 20 normal breast tissue samples and below the level of detection in the other 14 samples. In contrast, 10 of 20 breast cancer samples exhibited moderate to high levels of glypican-1 mRNA. The glypican-3 mRNA transcript was expressed at moderate to high levels in 5 of 20 normal breast tissue samples. In the breast cancers, it was expressed at moderate to high levels in 6 of 20 samples. The glypican-4 mRNA transcripts were below the level of detection in the normal samples and present at low levels in 7 of 20 breast cancer samples. The 3.4- and 2.6-kb syndecan-1 mRNA transcripts were present at low levels in all 20 normal breast tissue samples. In contrast, in the breast cancers, both syndecan-1 transcripts were expressed at moderate to high levels in 9 of 20 samples. A representative Northern blot is shown in Fig. 1. Glypican-2 and -5 mRNA transcripts were below the level of detection in both normal and cancer samples. The same cDNAs used in present study are able to detect the presence of the corresponding glypican-2 and -5 mRNA transcripts in human brain RNA (36), indicating that the failure to detect these transcripts in breast tissues was not due to technical difficulties with these cDNAs.

Densitometric analysis of all of the autoradiographs indicated that in comparison with normal breast tissues, there was a 6.4-fold increase (P < 0.005) in glypican-1 mRNA levels in the breast cancer tissues (Fig. 2). There was also a 4.0-fold increase in glypican-3 mRNA levels, but this difference failed to achieve statistical signifi-
cance (P = 0.0583). However, a subgroup of patients had relatively high levels of glypican-1 (10 cases; P < 0.001) or glypican-3 (6 cases; P < 0.01). There was also a 3.5-fold increase (P < 0.005) in syndecan-1 mRNA levels in the breast cancer samples (Fig. 2). Furthermore, a subgroup of patients had relatively high levels of syndecan-1 (nine cases; P < 0.001). Interestingly, 8 of 10 tumor samples that exhibited high glypican-1 mRNA levels (Fig. 2, a) also exhibited high syndecan-1 mRNA levels (Fig. 2, b). Furthermore, 8 of 10 tumors with stage 2 or 3 disease exhibited high levels of glypican-1 and syndecan-1 by Northern blot analysis. In contrast, low levels of glypican-1 mRNA were evident in only 1 of 10 tumors with stage 2 or 3 disease, but in 9 of 10 tumors with stage 1 disease.

Immunohistochemistry and in Situ Hybridization. Immunohistochemical analysis of five normal tissues using the same highly specific anti-glypican-1 antibody did not reveal any glypican-1 immunoreactivity (Fig. 3A). In contrast, five of five cancer tissues exhibited moderate to strong glypican-1 immunoreactivity in the intraductal carcinoma cells. This immunoreactivity was most marked in cancer cells that had a distorted morphology with prominent nuclei and abundant cytoplasm (Fig. 4A, outlined by arrowheads). In contrast, the small cancer cells that had a more differentiated lobular architecture exhibited faint glypican-1 immunoreactivity (Fig. 4A). Moderate to strong glypican-1 immunoreactivity was also present in the fibroblasts surrounding the cancer cells.

In situ hybridization analysis using a highly specific riboprobe was next carried out on serial sections in the same tissue samples to delineate the sites of expression of glypican-1. Ductal cells in the normal breast tissue did not exhibit an in situ hybridization signal (Fig. 3B). In contrast, a weak to moderate glypican-1 mRNA in situ hybridization signal was evident in the intraductal carcinoma cells (Fig. 4B). The surrounding fibroblasts also exhibited a faint glypican-1 mRNA signal, whereas the cancer cells that exhibited the more differentiated lobular architecture exhibited a more intense glypican-1 mRNA in situ hybridization signal (Fig. 4B). A second example of a well-differentiated cancer sample (H&E staining) is shown in Fig. 4D. In situ hybridization of serial sections revealed moderate glypican-1 mRNA signal in the cancer cells, whereas the surrounding fibroblasts exhibited a weak glypican-1 mRNA signal (Fig. 4E). A glypican-1 sense probe did not reveal any specific signal (Fig. 4, C and F).

Glypican Expression in Human Breast Cancer Cell Lines. To determine whether cultured breast cancer cells express any glypicans,
GLYPICAN-1 IS OVEREXPRESSED IN HUMAN BREAST CANCER

Effects of PI-PLC on Growth Factor Action in Breast Cancer Cells. To determine whether glypicans regulate growth factor action in breast cancer cells, MDA-MB-231 and MDA-MB-468 cells were incubated in the absence or presence of PI-PLC. This enzyme cleaves glypicans and other proteins that associate with membranes via a covalent GPI lipid linkage. In both cells (Fig. 8), IGF-I, HB-EGF, and FGF-2 induced cell proliferation. Preincubation of either cell line with PI-PLC (0.5 unit/ml) and subsequent incubation with the same concentrations of each growth factor in the presence of PI-PLC (0.1 unit/ml) completely blocked the stimulatory effect of HB-EGF and FGF-2. In contrast, in both cell lines, PI-PLC had no significant effect on the growth-stimulatory actions of IGF-I, which is not a heparin-binding growth factor (Fig. 8).

Effects of Glypican-1 Antisense Levels on Growth Factor Responsiveness. Because PI-PLC can remove many GPI-anchored proteins from the cell surface, we next sought to determine whether it is possible to modulate responsiveness to heparin-binding growth factors by altering endogenous glypican-1 protein levels. Accordingly, we transfected MDA-MB-231 and MDA-MB-468 cells with a glypican-1 antisense construct (G1-AS-1751). Northern blot analysis of total RNA using a glypican-1 sense riboprobe revealed high levels of glypican-1 antisense mRNA in MDA-MB-231 and MDA-MB-468 clones, whereas the parental cells and sham-transfected MDA-MB-231 cells did not exhibit a glypican-1 antisense mRNA transcript (Fig. 9A). Analysis with the antisense probe revealed that glypican-1 mRNA was expressed in parental and sham-transfected cells but was present at very low levels in the antisense-transfected clones (Fig. 9B). There was also a marked decrease in the 55-kDa glypican-1 protein in these clones, as determined by immunoblotting (Fig. 9C). On the other hand, the transfection of glypi-
can-1 antisense did not change the level of syndecan-1 on Northern blot analysis (Fig. 9D). Other glypican family members were below the level of detection by Northern blot analysis in the parental cells, sham-transfected cells, and all clones. The decreased expression of glypican-1 was associated with a marked attenuation of the growth-stimulatory effects of HB-EGF, HRG-α, HRG-β, FGF-2, and HGF (Fig. 10). In contrast, the growth-stimulatory action of IGF-I was similar in parental, sham-transfected, and glypican-1 antisense-transfected MDA-MB-231 and MDA-MB-468 cells (Fig. 10).

DISCUSSION

HSPGs are present on the surfaces of nearly all adherent cells, where they bind a number of heparin-binding proteins, including growth factors, extracellular matrix molecules, cell-cell adhesion molecules, and molecules involved in several degradative pathways (42–44). HSPGs belong primarily to two families of molecules, syndecans, which are transmembrane proteins, and glypicans, which are attached to the plasma membrane via GPI anchors (25). In breast cancer, there are several reports that HSPGs could be responsible for differences in their proliferative and invasive properties (45, 46). HSPGs are known to interact with FGF-2, keratinocyte growth factor, vascular endothelial growth factor, HB-EGF, and HGF (47–53). HGF and FGF are known to regulate the morphogenesis and differentiation of mammary epithelial cells, and HSPGs are likely to be important regulators of the development of the gland (54, 55). Moreover, HSPGs exhibit a differential pattern of distribution in normal and malignant breast epithelial cells, and this difference in HSPG distribution correlates with differences in sensitivity to FGF-2 (45, 56).

Together, these observations suggest that altered expression and function of HSPGs may contribute to the aberrant growth of breast cancer cells. In this study, we determined that a significant percentage of human breast cancers express relatively high levels of several glypicans and syndecan-1. By Northern blot analysis, there was a significant 6.4-fold increase in glypican-1 mRNA levels in the cancer tissues by comparison with the normal controls. There was also a slight increase in glypican-3 and -4 mRNA levels in the cancer samples. The overall increase in glypican-3 mRNA levels in the cancer samples almost achieved statistical significance. Furthermore, a subgroup of the breast cancer samples exhibited a significant increase in glypican-3 mRNA levels by comparison with the mean level in the normal samples. There was also a slight increase in glypican-3 and -4 mRNA levels in the cancer samples. The overall increase in glypican-3 mRNA levels in the cancer samples almost achieved statistical significance. Furthermore, a subgroup of the breast cancer samples exhibited a significant increase in glypican-3 mRNA levels by comparison with the mean level in the normal samples. Glypican-2 and -5 mRNA transcripts were below the level of detection in both the normal and cancer samples. Syndecan-1 mRNA levels were also significantly increased (3.5-fold) in the cancer tissues by comparison with the normal controls. There was a remarkably good correlation between glypican-1 and syndecan-1 expression in the tumors. Thus, 8 of 10 tumors with high glypican-1 mRNA levels also exhibited high syndecan-1 mRNA levels. Furthermore, 8 of 10 tumors with stage 2 or 3 disease exhibited high levels of glypican-1 and syndecan-1 by Northern blot analysis. In contrast, low levels of glypican-1 and syndecan-1 mRNA were evident in only 1 tumor with stage 2 disease, but in 9 of 10 tumors with stage 1 disease. Taken together, these observations suggest that glypican-1 and...
syndecan-1 may have the potential to contribute to the growth advantage of breast cancer cells in patients with a more advanced stage of this disease.

By immunohistochemistry, moderate to strong glypican-1 immunoreactivity was present in the poorly differentiated intraductal cancer cells and the adjacent fibroblasts, whereas faint glypican-1 immunoreactivity was present in the more well-differentiated lobular carcinoma cells. In sharp contrast with the immunostaining results, a moderate to strong glypican-1 mRNA in situ hybridization signal was present in the well-differentiated lobular cancer cells, whereas a weak in situ hybridization signal was present in the more poorly differentiated cancer cells and the adjacent fibroblasts. These observations suggest that glypican-1 synthesis is decreased in the more poorly differentiated breast cancer cells by comparison with the more well-differentiated cells and that breast cancer cells are able to secrete glypican-1 in vivo. However, the released glypican-1 may preferentially associate with the poorly differentiated cancer cells and the neighboring fibroblasts. In support of this hypothesis, both breast cancer cell lines examined in the present study were found to express and secrete glypican-1. Alternatively, glypican-1 degradation by these cell types may be markedly attenuated. Syndecan-1 may also be secreted by the breast cancer cells in vivo, because both cell lines also secreted syndecan-1 and because syndecan-1 immunoreactivity is abundant in the stroma surrounding infiltrating ductal breast carcinomas (46). Thus, the present findings are somewhat different from our previous results with pancreatic ductal adenocarcinomas, in which the cancer cells often exhibit strong glypican-1 immunoreactivity as well as a strong in situ hybridization signal (36).
MDA-MB-231 and MDA-MB-468 breast cancer cell lines expressed glypican-1 on the cell surface, as determined by immunoblotting of solubilized membrane preparations. The presence of glypican-1 on the cell surface suggested that it may enhance the interaction of heparin-binding growth factors with their high-affinity receptors. Indeed, treatment of cells with PI-PLC, an enzyme that removes surface-bound glypicans by cleaving GPI-anchored proteins, abrogated the mitogenic effects of HB-EGF and FGF-2 in both cell lines. These observations suggest that the mitogenic effects of these growth factors are dependent on the presence of surface-bound glypican-1. However, PI-PLC might also remove other GPI-anchored proteins from the cell surface. Therefore, we also generated clones of stably transfected MDA-MB-231 and MDA-MB-468 cells expressing a glypican-1 antisense construct. These clones exhibited a marked decrease in endogenous glypican-1 mRNA and protein level and a marked attenuation of the mitogenic response to several heparin-binding growth factors (HB-EGF, HRG-α, HRG-β, FGF-2, and HGF) that act through four distinct tyrosine kinase receptors (57). In contrast, IGF-I, which is not a heparin-binding growth factor, exerted similar mitogenic effects in parental, sham-transfected, and glypican-1 antisense-transfected cells.

Two lines of evidence suggest that syndecan-1 is not as crucial as glypican-1 for the activation of mitogenic signaling by heparin-binding growth factors in breast cancer cells. First, syndecan-1 is not a GPI-anchored molecule and is therefore not removed from the cell surface by PI-PLC treatment. However, the presence of syndecan-1 after treatment with PI-PLC was not capable of conferring responsiveness to HB-EGF and FGF-2 in either MDA-MB-231 or MDA-MB-468 cells. Second, MDA-MB-231 and MDA-MB-468 clones expressing the glypican-1 antisense construct did not exhibit a decrease in syndecan-1, yet they were no longer responsive to multiple heparin-binding growth factors. The extracellular domain of syndecan-1 directly regulates cell motility and invasiveness (40), and induction of stromal syndecan-1, coupled with decreased syndecan-1 expression in malignant cells, may promote the metastatic phenotype of infiltrating ductal breast carcinoma (46). Together, these observations suggest that syndecan-1 may play a critical role in motility and invasiveness in breast cancer, whereas glypican-1 may act to enhance the growth-promoting effects of heparin-binding growth factors in breast cancer cells.

Our findings raise the possibility that pharmacological or molecular interventions that interfere with glypican-1 and syndecan-1 function or expression may have a therapeutic role in breast cancer. In addition, the ability of breast cancer cells to synthesize and secrete glypican-1 and syndecan-1 at high levels raises the possibility that glypican-1 and
syndecan-1 may be present in the serum of breast cancer patients and may serve as a tumor marker for this malignancy.

REFERENCES

Glypican-1 Is Overexpressed in Human Breast Cancer and Modulates the Mitogenic Effects of Multiple Heparin-binding Growth Factors in Breast Cancer Cells


*Cancer Res* 2001;61:5562-5569.

Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/14/5562

Cited articles

This article cites 54 articles, 25 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/14/5562.full.html#ref-list-1

Citing articles

This article has been cited by 24 HighWire-hosted articles. Access the articles at:
/content/61/14/5562.full.html#related-urls

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.