Overexpression of the c-erbB-2 Gene Enhanced Intrinsic Metastasis Potential in Human Breast Cancer Cells without Increasing Their Transformation Abilities

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ABSTRACT

Overexpression of the c-erbB-2 gene-encoded p185 has been reported in approximately 30% of human breast cancers and has been correlated with lymph node metastasis and poor prognosis in breast cancer patients. To investigate whether overexpression of p185 can enhance the metastatic potential of human breast cancer cells, we have introduced the human c-erbB-2 gene into the very low p185-expressing MDA-MB-435 human breast cancer cells and established 435.eB transfectants that express higher levels of p185. In this study, we compared the metastatic phenotypes of the parental MDA-MB-435 cells and the 435.eB transfectants. In vivo experimental metastasis assays in which we injected MDA-MB-435 parental cells or 435.eB transfectants into the tail veins of ICR-SCID mice demonstrated that mice injected with p185-overexpressing 435.eB transfectants formed significantly more metastatic tumors than the mice injected with parental and control cells. The changes in experimental metastatic potential in vivo were accompanied by increased invasiveness in vitro. In addition, the secretion of basement membrane-degradative enzymes, which is an important step in the invasion and metastasis process, was also increased in the p185-overexpressing 435.eB transfectants. These results indicated that p185 overexpression can enhance the metastatic potential of MDA-MB-435 human breast cancer cells. To investigate whether enhanced metastatic potential in the p185-overexpressing 435.eB transfectants was the result of increased cancer cell growth and transformation potential, we compared the growth rate, anchorage-independent growth ability, and tumorigenicity of the 435.eB transfectants with that of the parental cells. The transfectants and the parental cells all had similar growth rates and anchorage-independent growth abilities and demonstrated similar tumorigenic potential. These findings suggest that c-erbB-2 is a metastasis-promoting gene for breast cancers that is distinct from other tumor-promoting genes in that the c-erbB-2 gene can enhance the intrinsic metastatic potentials of MDA-MB-435 cells without increasing their transformation abilities.

INTRODUCTION

Breast cancer metastasis is the major cause of death for breast cancer patients and represents the most serious challenge to therapeutic intervention. Metastasis is a complex pathophysiological process involving numerous interactions between tumor cells and host characteristics (1). The metastatic phenotypes of a tumor cell may involve enhanced cell motility, the ability to degrade basement membrane components and to invade tissues, and autonomous growth at a second site (1). Little is known about the molecular mechanisms of breast cancer metastasis, particularly how genetic alterations (e.g., specific oncogenes) can contribute to the progression and metastasis of breast cancer. Therefore, studies on the molecular mechanisms involved in breast cancer invasion and metastasis are vitally important in that they may lead to the identification of specific factors or genes that augment or suppress the metastatic or invasive potential of breast cancer cells.

The c-erbB-2 gene (also known as HER-2, neu, or NGL) encodes a transmembrane glycoprotein of Mr 185,000 (p185), which has extensive sequence homology to epidermal growth factor receptor (2, 3). Similar to epidermal growth factor receptor, p185 is a growth factor receptor with intrinsic tyrosine kinase activity (4–6). Amplification or overexpression of the c-erbB-2 gene has been found in approximately 20–30% of human breast cancers (7). Patients whose breast tumors overexpress the c-erbB-2 gene have a significantly lower survival rate and a shorter time to relapse than patients without c-erbB-2 gene overexpression. Also, high levels of expression of p185 have been positively correlated with lymph node metastasis in breast cancers (8–10). In addition to the reported clinical correlation, the mutation-activated (Val to Glu at position 659) c-erbB-2 gene was shown to increase metastatic potential in murine colon adenocarcinoma cells in experimental metastasis assays (11). We and other investigators have demonstrated that the mutation-activated rat neu is a potent oncogene (12) and is sufficient to induce higher metastatic potential in NIH3T3 cells (13). Furthermore, experiments using transgenic mice indicate that introduction of the mouse c-erbB-2/neu gene into mice can induce mammary tumors and metastases (14–17). It appears likely, therefore, that c-erbB-2 overexpression plays an important role in human breast cancer metastasis.

However, the role c-erbB-2 overexpression plays in human breast cancer metastasis has not been settled because: (a) the mouse 3T3 cells and murine colon adenocarcinoma cells used in the abovementioned study are different from human breast cancer cells, and what is found in the rodent fibroblast cell line cannot be extrapolated to the human breast cancer cell line; (b) the mutation-activated c-erbB-2 gene was used in these studies, but amplification and overexpression of the normal c-erbB-2 gene is a frequent event in human breast cancers, and mutation activation has not been found in the c-erbB-2 gene in primary human breast cancer or cell lines (18, 19); (c) there is still dispute among clinical studies on whether c-erbB-2 overexpression correlates with poor prognosis and higher metastatic potential (20). Therefore, systematic studies measuring the metastatic potential of the human breast cancer cell lines that overexpress normal c-erbB-2 gene are critical to determining whether the c-erbB-2 gene can induce higher metastatic potential in human breast cancers.

In this study, we investigated the role of the c-erbB-2 gene in human breast cancer metastasis by transfecting the normal c-erbB-2 gene under a cytomegalovirus strong promoter to overexpress the normal p185 in MDA-MB-435 human breast cancer cells, which are poorly p185 expressing. We then studied whether these p185-overexpressing transfectants exhibited increased metastatic potential compared with the low p185-expressing parental MDA-MB-435 cells. We also compared the growth rate and transformation properties of these transfectants with that of the parental cells. Our results demonstrated that overexpression of the normal c-erbB-2 gene can promote the intrinsic metastatic potentials of MDA-MB-435 human breast cancer cells without increasing their transformation abilities.
MATERIALS AND METHODS

Cell Lines and Culture. The MDA-MB-435 cell line, obtained from Dr. Janet Price at The University of Texas M. D. Anderson Cancer Center, is an estrogen receptor-negative cell line isolated from the pleural effusion of a patient with breast carcinoma (21). The cells were grown in DMEM/F12 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FBS. The 435.eB transfectants were generated by transfection of the pCMVerbB-2 plasmid containing the 4.4-kb full-length human normal c-erbB-2 cDNA (3) and the pSV2-neo plasmid carrying the neomycin-resistance selection marker gene into MDA-MB-435 cells (22). The control 435.neo and 435.neo.pool cell lines were established by transfecting the pSV2-neo plasmid alone into MDA-MB-435 cells (22). All of the transfectants were grown under the same conditions as the MDA-MB-435 cells, except that G418 (500 \mu g/ml) was added to the culture medium. The relative levels of p185 expression in these transfectants were determined by quantitation of the p185 protein bands on Western blot with the Personal Densitometer 50371 (Molecular Dynamics, Sunnyvale, CA), as described previously (22).

Experimental Metastasis Assay. This assay was performed according to our procedures published previously (13, 23). Eight-week-old pathogen-free female inbred nude mice (Taconic, Germantown, NY) were quarantined for 1 week and then used for the assays. The injections of 1 \times 10^6 cells in 0.2-ml PBS were given to 9 or 10 mice per experimental group via the lateral tail vein at day 0. Mice were euthanized using CO2 90 days after injection, and the numbers of lung metastatic nodules were determined by instillation of India ink according to the method of Wexler (24). Only those lung nodules >0.5 mm in diameter were counted. The differences in the numbers of lung nodules in mice injected with control cell lines or 435.eB transfectants were analyzed using one-tailed Student’s t test analysis. Animals were also examined for extrapulmonary metastases, and tumor nodules >0.5 mm in diameter were counted.

In Vitro Chemoinvasion Assay. To assay in vitro invasiveness, we used the procedure of Albini et al. (25) with minor modifications (26). Chemoinvasion was measured by using 24-well BioCoat Matrigel invasion chambers (Becton Dickinson Labware, Bedford, MA) with an 8-\mu m pore size polycarbonate filter coated with Matrigel. The lower compartment contained 0.6 ml of laminin (Becton Dickinson Labware) at different concentrations in DMEM/F12 as chemoattractants or DMEM/F12 as a negative control. The cells (1 \times 10^6 cells/0.1 ml DMEM/F12 containing 0.1% BSA) were placed in the upper compartment and incubated for 72 h at 37°C in a humidified 95% air, 5% CO2 atmosphere. After incubation, the filters were fixed with 3% glutaraldehyde in PBS and stained with Giemsa (Fisher Scientific, Orangeburg, NY). The cells on the upper surface of the filter were removed by wiping with a cotton swab, and chemoinvasive activity was determined by counting the number of cells per HPF\(^\text{2}\) (×200) that had migrated to the lower side of the filter. The Matrigel-invaded cells were counted by at least three HPFs per filter. Each sample was assayed in triplicate, and assays were repeated at least twice. The differences in the invasion rates between control cell lines and 435.eB transfectants were analyzed using two-tailed Student’s t test analysis.

Chemotaxis Assay. The chemotaxis assay was performed essentially as described previously (23, 27) using BioCoat cell culture inserts (Becton Dickinson Labware) with an 8-\mu m pore size polycarbonate filter. Each lower compartment of the insert contained 0.6 ml laminin at different concentrations in DMEM/F12 as chemoattractants or DMEM/F12 as a negative control. The cells (3 \times 10^6 cells/0.1 ml DMEM/F12 containing 0.1% BSA) were placed in the upper compartment and incubated for 6 h at 37°C in a humidified 95% air, 5% CO2 atmosphere. The cells were then fixed, stained, and counted as described above for the chemoinvasion assay. All of the assays were repeated at least twice.

Zymography of Type IV Collagenase Activity. This assay was performed as described previously with minor modifications (23). Equal numbers of cells (2 \times 10^6) were seeded onto six-well tissue culture plates in DMEM/F12 containing 1% FBS and cultured overnight. The cells were washed three times with DMEM/F12 and incubated in DMEM/F12 without serum for 30 h. The culture supernatants were collected, and cell debris was spun off and concentrated using Amicon spin columns (Amicon, Beverly, MA). Concentrated samples were then subjected to 10% SDS-PAGE (gel containing 1.5% gelatin) without reducing agent. After electrophoresis, the gel was washed with 2.5% Triton X-100 in 50 mM Tris-HCl buffer (pH 7.5) containing 0.05% sodium azide; incubated at 37°C for 16 h in 0.15 M sodium chloride, 10 mM calcium chloride, 50 mM Tris-HCl buffer (pH 7.5) containing 0.05% sodium azide; and then stained by Coomassie Brilliant Blue and destained.

In Vitro Growth Rate and Colony Formation Assay. Cells (4 \times 10^4 cells/well) were plated into 24-well plates. The cells were harvested and counted on different days for 11 days to generate the in vitro growth curves. Each sample was assayed in triplicate. The cell numbers are presented as mean value. For the colony formation assay, the cells (1 \times 10^3 cells/well) were plated into a 24-well plate in culture medium containing 0.35% agarose (Life Technologies, Inc., Gaithersburg, MD) overlaying a 0.7% agarose bottom layer. The cells were then incubated at 37°C for 5 weeks, after which the plates were stained with p-iodonitrotetrazolium violet (1 mg/ml) for 48 h at 37°C. Colonies greater than 1 mm were counted. Experiments were repeated three times for each cell line.

Tumorogenicity Assay. The cells in log-phase growth were trypsinized, washed twice with PBS, and centrifuged at 250 × g. The viable cells were then counted, of those, 1 × 10^3 cells in 0.2 ml of Matrigel were injected into the m.f.p. of each 8-week-old female ICR-SCID mouse under aseptic conditions. Tumor volumes were measured by calipers in two orthogonal diameters, and tumor volumes were calculated using the formula for a prolate ellipsoid: Volume (mm\(^3\)) = L × W\(^2\)/2, where L and W are the major and minor diameters (in millimeters), respectively. Ten mice were injected for each cell line. The tumor volumes are presented as mean values.

RESULTS

Enhanced Metastatic Potential of 435.eB Transfectants in Vivo. To examine whether c-erbB-2-encoded p185 plays an important role in determining the metastatic potential of the human breast cancer cells, we needed to have a panel of cell lines with the same genetic background but expressing different levels of p185. Differences in metastatic potential among these cells should be due to the differences in p185 expression level. To achieve this, we chose the human breast cancer cell line MDA-MB-435 as recipient for transfection of the c-erbB-2 gene because it contains only one copy of the c-erbB-2 gene per haploid, expresses low levels of p185, forms tumors in the m.f.p. in nude mice after m.f.p. injection, and develops metastatic tumors in these mice (28). We transfected into MDA-MB-435 cells the pCMVerbB-2 plasmid containing the 4.4-kb full-length normal human c-erbB-2 cDNA (3) and the pSV2-neo plasmid carrying the neomycin-resistance selection marker gene and thereby generated p185-overexpressing 435.eB transfectants (22). The 435.eB1, 435.eB2, and 435.eB4 transfectants express p185 at 258-, 149-, and 165-fold, respectively, that of the parental MDA-MB-435 cells (22). It is notable that the p185 level in the 435.eB transfectant is comparable to that in the SKBR-3 breast cancer cells established from a different patient, which express c-erbB-2 mRNA at 128-fold (29) and p185 at 262-fold of that of the MDA-MB-435 cells (22). Thus, the 435.eB stable transfectants express differently increased amounts of p185 that are parallel to the c-erbB-2 gene expression levels in cell lines established from primary human breast cancers. The control 435.neo and 435.neo.pool cell lines were established by transfecting the pSV2-neo plasmid alone into MDA-MB-435 cells, and they expressed p185 at levels similar to that of the MDA-MB-435 cells (22).

Our first effort to determine the metastatic potential of these cells expressing different levels of p185 was to test the ability of these cells to extravasate the circulatory system, to invade normal tissue, and to colonize at different organ sites in vivo by experimental metastasis assays. Single-cell suspensions of each cell line were injected into the lateral tail veins of 8-week-old female ICR-SCID mice. The mice were killed 90 days after injection, and the number of pulmonary and extrapulmonary metastases were examined. The sizes of the meta-

\(^2\) The abbreviations used are: HPF, high power field; FBS, fetal bovine serum; m.f.p., mammary fat pad; MMP, matrix metalloprotease.
static lung nodules were found to be similar in all of the mice injected with the 435.eB transfectants, the parental MDA-MB-435 cells, and 435.neo control cells (Table 1). However, the numbers of lung nodules were significantly increased in those mice injected with 435.eBl parental MDA-MB-435 cells (Table 1). Moreover, the frequencies of effectants that expressed higher levels of p185 compared with the 435.eB transfectants, the parental MDA-MB-435 cells, and the control 435.neo cells, and the parental MDA-MB-435 cells, were found to be similar in all of the mice injected with 435.eBl, 435.eB2, and 435.eB4 cells also increased compared with the low p185-expressing MDA-MB-435 cells and 435.neo control cells (Table 1). This result clearly demonstrated that increased p185 expression in the MDA-MB-435 human breast cancer cells can enhance metastatic potential in vivo.

**Increased Chemoinvasion of 435.eB Transfectants in Vitro.** To investigate the mechanisms that lead to the increased metastatic potential in p185-overexpressing 435.eB transfectants, we examined these tumor cells for their basement membrane invasion ability, which is an important step in malignant cell extravasation from the circulation. We compared the in vitro invasiveness of the 435.eB transfectants, the control 435.neo cells, and the parental MDA-MB-435 cells to determine whether increased p185 expression can facilitate the MDA-MB-435 cells to penetrate the layer of reconstituted basement membrane (Matrigel; Refs. 25, 27, and 30). Because laminin is one of the major basement membrane structural proteins, it was used as a chemoattractant to stimulate cells to penetrate through the Matrigel and to migrate through the filters. The invasion assay revealed prominent differences between 435.eB transfectants and the parental MDA-MB-435 cells or 435.neo cells (Fig. 1). Significantly higher rates of invasion were observed with 435.eB transfectants that expressed higher levels of p185 than the invasion rates of the 435.neo-pool control cells (P < 0.01) when the laminin concentration reached 30 μg/ml (Fig. 1). These results demonstrated that increased p185 expression in the human breast cancer MDA-MB-435 cells can facilitate tumor cell invasion in vitro, which is an important property necessary for the formation of metastasis.

**Similar Cell Motility and Adhesion Ability in 435.eB Transfectants, Parental, and Control Cells.** Tumor cell invasion is a complicated process involving a sequential series of critical steps, including tumor cell adhesion to microvessel endothelial cells and the subendothelial basement membrane mediated by extracellular matrix molecules (31) and tumor cell migration toward basement membrane facilitated by chemotactic factors (31, 32). To further examine which of these invasion steps is affected by increased expression of p185, we compared the 435.eB transfectants, parental MDA-MB-435 cells, and the control 435.neo cells for their ability to migrate toward chemoattractants (chemotaxis) and to adhere to extracellular matrix molecules.

We compared the migration rates of 435.eB transfectants, parental MDA-MB-435 cells, and the control 435.neo cells using laminin as the chemoattractant, which is one of the major extracellular matrix proteins in the basement membrane. We did not observe significant differences in migration rates among these cell lines expressing different cell lines expressing different cell lines expressing different concentrations as chemoacttractants, and the difference in migration rate remained insignificant (data not shown). We also used other major extracellular matrix proteins, such as collagen IV and fibronectin, at different concentrations as chemoattractants, and the difference in migration rate remained insignificant (data not shown).

To compare the ability of 435.eB transfectants, parental MDA-MB-435 cells, and the control 435.neo cells to attach to the basement membrane, we performed adhesion assays to measure the ability of these cells to adhere to the extracellular matrix molecules laminin, fibronectin, collagen IV, vitronectin, and Matrigel. The 435.eB transfectants, the parental MDA-MB-435 cells, and the control 435.neo cells demonstrated similar adhesion ability (data not shown).
results indicated that under our assay conditions, there is no significant increase of chemotaxis and adhesion to extracellular matrix molecules in p185-overexpressing 435.eB transfectants.

**Higher Enzyme Activities of Type IV Collagenases in 435.eB Transfectants.** Highly metastatic cancer cells synthesize various classes of membrane-degradative enzymes at higher levels than their counterparts (33). To examine whether the 435.eB transfectants secreted higher amounts of protease than did parental MDA-MB-435 cells and 435.neo control cells, we measured the activities of the basement membrane-degrading type IV collagenases in these cells. In our zymographic analysis of the conditioned medium from these cells, we detected significantly higher levels of secreted Mr 92,000 type IV collagenase (MMP-9 or gelatinase B) and Mr 72,000 type IV collagenase (MMP-2 or gelatinase A) in the conditioned medium of the 435.eB transfectants than that of the parental MDA-MB-435 cells and 435.neo control cells (Fig. 3). These data suggested that increased p185 expression in 435.eB cells can lead to increased secretion of type IV collagenases, which may contribute to the enhanced invasion and metastatic potential of 435.eB cells.

**Transformation Potential of MDA-MB-435 Cells and 435.eB Transfectants.** Our results demonstrated that overexpression of the c-erbB-2 gene indeed can enhance metastatic potential of human breast cancer MDA-MB-435 cells by enhancing metastasis-associated properties (Table 1; Figs. 1 and 3). Our next question was whether c-erbB-2 enhanced metastatic potential of MDA-MB-435 cells by promoting tumor cell growth and increasing the transformation ability that may facilitate autonomous growth of these cells at the metastatic organ sites. We, therefore, examined the in vitro growth rate of the 435.eB transfectants, the control 435.neo cells, and the parental MDA-MB-435 cells. As shown in Fig. 4A, there were remarkable overlaps among the growth curves of the p185-overexpressing 435.eB transfectants, the low p185-expressing 435.neo control cells, and the parental MDA-MB-435 cells. These results indicated that increased p185 expression in MDA-MB-435 cells did not significantly affect the growth rate of these cells. We then compared among these cells their ability for anchorage-independent growth in soft agar, which is an in vitro indicator of the transforming potential. Again, there was no significant difference in the numbers of soft agar colonies between high p185-expressing cells and low p185-expressing cells (Fig. 4B). We further compared the in vivo tumorigenicity, a more stringent parameter of transformation potential, of these cell lines. The tumor cells were premixed with an equal volume of liquid Matrigel and then inoculated into the m.f.p. of the ICR-SCID mice (34). Tumor formation was measured for mice injected with the high p185-expressing 435.eB transfectants, the low p185-expressing 435.neo control cells, and the parental MDA-MB-435 cells. The results showed that the tumor growth curves overlapped significantly (Fig. 5), which indicated that overexpression of the c-erbB-2 gene in human breast cancer
Fig. 4. A, similar cell growth rate among the 435.eB transfectants, the MDA-MB-435 parental cells, and the 435.neo control cells. Cells (4 × 10^4 cells/well) were plated in 24-well plates. The cells were harvested and counted on different days for 11 days to generate the in vitro growth curves. Each sample was assayed in triplicate; the cell numbers are presented as mean values; bars, SD. B, similar colony formation ability among the 435.eB transfectants, the MDA-MB-435 parental cells, and the 435.neo control cells in soft agarose. Cells (1 × 10^3 cells/well) were plated in a 24-well plate in culture medium containing 0.35% agarose (Life Technologies, Inc., Gaithersburg, MD) overlying a 0.7% agarose bottom layer. The cells were then incubated at 37°C for 5 weeks, after which the plates were stained with p-iodonitrotetrazolium violet (1 mg/ml) for 48 h at 37°C. Colonies greater than 1 mm were counted. For each cell line, experiments were repeated three times; bars, SD.

MDA-MB-435 cells did not increase the growth rate and transformation potential of these tumor cells.

DISCUSSION

Our study has demonstrated that overexpression of the normal c-erbB-2 gene can enhance the metastatic potential of MDA-MB-435 human breast cancer cells by increasing invasion ability, which may be caused by higher activities of MMP. On the other hand, c-erbB-2 overexpression in MDA-MB-435 cells did not increase the growth rate, transformation potential, or tumorigenicity of these cells. These findings indicated that c-erbB-2 can enhance intrinsic metastatic potentials of MDA-MB-435 human breast cancer cells without increasing their cell growth rate and transformation abilities. The results suggest that metastasis and transformation are related but separable properties that may be regulated by different molecular mechanisms (35, 36). Furthermore, these results imply that the signal transduction pathways of cell growth, cell transformations, and tumor cell metastasis may not completely overlap.

The changes in metastasis and transformation phenotype induced by overexpressing the normal human c-erbB-2 gene in human breast cancer cells are quite different from those induced by introduction of the mutation-activated rat neu gene into mouse NIH3T3 cells (13). The latter has led to increased transformation ability, including higher cell growth and DNA synthesis rate, increased colony formation in soft agar, and tumorigenicity. In addition, the mutation-activated rat neu gene has enhanced all of the metastasis/invasion-associated properties in NIH3T3 cells, including increased cell adhesion, cell motility, and secretion of membrane-degrading enzymes. However, overexpression of the normal human c-erbB-2 gene in human breast cancer cells did not lead to further enhancement of transformation potential and did not increase cell adhesion and cell motility under similar assay conditions. These differences may first be due to the difference in cell types, i.e., MDA-MB-435 human breast cancer cells versus NIH3T3 mouse fibroblasts. MDA-MB-435 are highly metastatic tumor cells that already possess all of the genetic changes needed or cellular properties required for metastasis, but NIH3T3 are nontumorigenic and nonmetastatic cells. Therefore, even without increasing the cell adhesion and cell motility beyond that of the parental cells, overexpression of the normal human c-erbB-2 gene in MDA-MB-435 cells was able to further enhance their metastatic potential by...
promoting cell invasion via activation of the type IV collagenases. Secondly, the differences may be due to the transfected genes, i.e., normal human c-erbB-2 gene versus the mutated rat neu gene; the latter is a more potent transforming gene and has higher tyrosine kinase activity than the former. Hence, the mutated rat neu gene may super-activate the transformation signals in addition to enhancing signals leading to metastasis, whereas overexpression of the wild-type p185 might not produce a signal as strong as that of the oncogenic neu to activate more on the transformation of the already highly tumorigenic MDA-MB-435 cells.

Despite the differences, we have clearly demonstrated that c-erbB-2 overexpression in human breast cancer cells enhanced the in vivo metastatic potential and in vitro invasion ability that is accompanied by increased secretion of basement membrane-degrading MMP. Tumor cells create localized defects in the extracellular matrix using a variety of proteases to traverse connective tissue barriers. MMPs are zinc-containing, metallo-enzymes with a high degree of specificity for degradation of extracellular matrix components at neutral pH (37). A direct correlation has been observed between the production of MMPs by tumor cells and the invasive and metastatic behavior of those cells (38–41). Similarly, we found previously that secretion of collagenase IV is increased in neu oncogene-transformed NIH3T3 cells and c-erbB-2 gene transfected human non-small cell lung carcinoma cells (26). In this study, we observed the same phenomenon in the p185-overexpressing 435.eB transfectants. The results indicate that one of the molecular mechanisms by which p185 overexpression-enhanced metastatic potential of the MDA-MB-435 human breast cancer cells was by promoting this important step of the invasion process.

Up-regulation of collagenase IV activity was suggested to be mainly due to transcriptional up-regulation (42–45). Transfection of an activated Hsras in the OVCAR-3 human ovarian cancer cells was shown to increase the Mr 92,000 collagenase IV activity, and this requires multiple transcription factor binding sites in the Mr 92,000 collagenase IV promoter, including a PEA3/ets motif, AP-1 sites, a NF-κB consensus sequence, and a GGT box (46). Interestingly, overexpression of the c-erbB-2 receptor may lead to increased tyrosine kinase activity, which is required for association with and tyrosine phosphorylation of Shc (47, 48). Phosphorylation of Shc in turn leads to binding to Grb2/Sos and triggers signal transduction through the ras-raf-MAP kinase pathway. We hypothesize, therefore, that ras activation may also be involved in the increased collagenase IV activity and enhanced metastatic potential induced by c-erbB-2. Currently, we are investigating the possible links between c-erbB-2 signal transduction, ras activation, and known cis-elements within Mr 92,000 collagenase IV promoter to further understand the detailed molecular mechanisms involved in up-regulation of collagenase IV by c-erbB-2 overexpression.

Overexpression of the c-erbB-2 gene has been correlated with poor clinical outcome in breast cancers (7, 49). Our recent studies on c-erbB-2 gene-transfected MDA-MB-435 breast cancer cells have provided reasonable explanations for the reported clinical correlation. We demonstrated in this study that overexpression of the c-erbB-2 gene in breast cancer cells confers increased resistance to the chemotherapeutic drug Taxol via mdr-1-independent mechanisms (22). Based on these findings, we propose that overexpression of p185 may: (a) lead to increased metastatic potential in human breast cancers, which may result in more metastatic lesions; and (b) lead to increased resistance to certain chemotherapeutic agents in human breast cancers, which may cause p185-overexpressing breast tumors to have a poor response to therapy. The combined consequence is that patients with breast tumors that overexpress p185 will have a poor clinical outcome. Therefore, more aggressive therapy will be beneficial to those patients whose tumors express high levels of c-erbB-2-encoded p185.

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