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ABSTRACT

Previously, we and others have reported high levels of expression of the c-erbB-2/neu gene in non-small cell lung cancer cell lines and primary tumors. We have also found that expression of c-erbB-2/neu-encoded p185neu was correlated with lymph node metastasis in lung squamous cell carcinomas. To investigate the potential role of the c-erbB-2/neu gene in lung cancer metastasis systematically, we introduced the human c-erbB-2/neu gene into very low p185neu-expressing NCI-H460 human non-small cell lung cancer cells and then examined the experimental metastatic potentials among the parental NCI-H460 cells and stable transfectants with increased expression of p185neu. Compared with the parental NCI-H460 cells, the NCI-H460 transfectants overexpressing p185neu produced significantly more pulmonary and extrapulmonary metastatic tumors in nude mice. The changes in experimental metastatic potential in vivo were accompanied by increased invasiveness in vitro. In addition, important steps in the invasion and metastasis process, such as secretion of basement membrane-degradative enzymes and migration through reconstituted basement membrane (Matrigel), were also increased in the NCI-H460 transfectants overexpressing p185neu. Moreover, scanning electron microscopy revealed that the p185neu-overexpressing NCI-H460 transfectants had significantly more microvilli and membrane protrusions than the parental cells, correlating with the increased invasive properties of these cells. The results demonstrate that overexpression of p185neu can enhance the experimental metastatic potential of NCI-H460 human lung cancer cells by promoting invasion and the other steps in the metastatic cascade.

INTRODUCTION

Lung cancer is the leading cause of death from all malignancies in the United States (1). NSCLC is among the most threatening of these types of malignancy, because of its high incidence and because it is usually resistant to chemotherapy and is frequently disseminated at the time of diagnosis. To provide potential diagnostic, prognostic, and therapeutic reagents for lung cancers, intensive efforts have been made to identify the relationship between specific gene alterations and the clinical behavior of lung cancers. We and others have reported the abnormal expression of c-erbB-2/neu-encoded p185neu in cell lines and primary tumors from human NSCLC (2—7). In addition, the extent of p185neu expression in primary NSCLC is correlated with poor clinical prognostic indicators including lymph node metastasis (3, 7). These clinical correlations suggest that p185neu may play an important role in malignancy of the human NSCLC.

The c-erbB-2/neu gene (also known as HER-2 or NGL) encodes a transmembrane protein of Mr 185,000, p185neu, with extensive sequence homology to epidermal growth factor receptor (8—10). Similar to epidermal growth factor receptor, p185neu is a transmembrane glycoprotein with intrinsic tyrosine kinase activity (11—13), and it is a member of the growth factor receptor tyrosine kinase gene family (14). Amplification and overexpression of the c-erbB-2/neu gene has been found in many different human primary tumors including NSCLC, and it is postulated to be important in human carcinogenesis (2—5, 15—19). In addition, expression levels of p185neu have been positively correlated with lymph node metastasis in breast cancers (20—22). Recently, we provided direct experimental evidence for the important role of the neu (denotes the rat version of c-erbB-2/neu gene) oncogene in metastasis by demonstrating that the mutation-activated rat neu oncogene is sufficient to induce higher metastatic potential in 3T3 cells (23). The mechanism by which the neu gene induced higher metastatic potential was by promoting the multiple adhesion and invasion steps of the metastatic cascade (24). These results from primary human cancer to the mouse 3T3 cell model system suggested that the c-erbB-2/neu gene may play an important role in human cancer tumorigenicity and malignancy by modifying properties important in metastasis. Here we examined the role of p185neu in human NSCLC metastasis by comparing metastasis-associated properties between the low p185neu-expressing NCI-H460 human NSCLC cell line (2, 25) and its c-erbB-2/neu transfectants expressing high levels of p185neu. We found that p185neu indeed enhanced metastatic potential in human lung cancer cells by induction of metastasis-associated properties.

MATERIALS AND METHODS

Cell Lines and Culture. NCI-H460 cells, obtained from American Type Culture Collection, were originally derived from the pleural fluid of a male with large cell lung carcinoma (25) and were grown in DMEM/F12 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum. The H460 transfectants were grown under the same conditions, except that G418 (500 µg/ml) was added to the culture medium.

DNA Transfection. All DNA transfections were carried out using the modified calcium phosphate precipitation procedure (26) with minor modifications as previously described (27). H460 cells (2 x 10⁶ cells in 10-cm dishes) were transfected with 10 µg of the pSV2erbB-2 plasmids that contain full-length (4.4 kilobases) human c-erbB-2/neu complementary DNA [a generous gift from Dr. Tadashi Yamamoto (8)] along with 1 µg of pSV2-neo plasmid DNA carrying the neomycin resistance marker gene (28). The transfectants were selected in selection medium containing 500 µg/ml of G418.

Immunoblotting. Immunoblot analyses were performed as previously described (27, 29). The primary monoclonal antibodies used were c-neu-Ab-3 against the c-erbB-2/neu-encoded p185neu protein (OncoGene Science, Inc., Manhasset, NY).

In Vitro Growth Rate Analysis. The in vitro growth rates of the cell lines were assessed by measuring increases in cell number with the 2,3-bis(2-...
methoxy-4-nitro-5-sulfophenyl-2H-tetrazolium-5-carboxanilide inner salt (XTT) assay (30) with minor modifications as described previously (27).

[^3H]Thymidine Incorporation Assay. This assay was performed as previously described (27).

**Experimental Metastasis Assay.** This assay was performed according to our previous procedures (23, 24). Seven-week-old pathogen-free female nude mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were quarantined for 1 week and then used for the assays. Injections of 4 × 10^6 cells in 0.1 ml phosphate-buffered saline were given to 5 mice per experimental group via the lateral tail vein at day 0. Mice were euthanized using CO2 49 days after injection, and the numbers of experimental lung metastases were determined by instillation with India ink according to the method of Wexler (31). Only those lung nodules >0.5 mm in diameter were counted. Animals were also examined for extrapulmonary metastases.

**In Vitro Chemoinvasion Assay.** To assay in vitro invasiveness we used the procedure of Albini et al. (32) and Repesh (33) with minor modifications (23, 34). Chemoinvasion was measured by using 24-well Transwell units with an 8-μm pore size polycarbonate filter (Costar Corp., Cambridge, MA). Each filter in the Transwell unit was coated with 0.1 ml of a 1:30 dilution (20 μg/filter) of Matrigel in cold DMEM/F12 medium to form a thin, continuous layer on top of the filter. The lower compartment contained 0.6 ml of LECM as chemoattractants or DMEM/F12 medium as a negative control. The cells (1 × 10^5/0.1 ml of DMEM/F12 containing 0.1% bovine serum albumin) were placed in the upper compartment and incubated for 72 h at 37°C in a humidified 95% air, 5% CO2 atmosphere. After the incubation, the filters were fixed with 3% glutaraldehyde in phosphate-buffered saline and stained with Giemsa. Using a Nikon microscope, we determined chemoinvasion activity by counting the number of cells per HPF (×200) that had migrated to the lower side of the filter. At least four HPFs were counted per filter. Each sample was assayed in triplicate, and assays were repeated at least twice.

**Zymography of Gelatinolytic Activity.** Cells (2 × 10^6) were seeded onto 6-well tissue culture plates in DMEM/F12 medium supplemented with 1% fetal bovine serum and cultured for 24 h. The cultures were washed three times with DMEM/F12 and incubated for 24 h in serum-free DMEM/F12. The culture supernatants were collected and sequentially centrifuged at 800 × g for 10 min and then at 18,000 × g for 10 min. Aliquots (150 μl) of the supernatants were analyzed by zymography using SDS-PAGE containing 1.5% gelatin prepared according to previously published procedures (24, 35).

**Chemotaxis Assay.** The chemotaxis assays were performed essentially as described previously using Transwell units (36) with minor modifications (24). Each lower compartment of the Transwell contained 600 μl of LECM as chemoattractants or DMEM/F12 as a negative control. Cells (4 × 10^5/0.1 ml DMEM/F12/0.1% bovine serum albumin) were placed in the upper compartment of the Transwell unit 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 the assays were done in triplicate, and the assays were repeated at least twice.

**Scanning Electron Microscopy.** Scanning electron microscopic examination of the morphological differences between the NC-I-H460 parental cell lines and H460 transfectants was performed as previously described, with minor modifications (37). Briefly, the parental and transfected cells were washed with 0.125 M sodium cacodylate buffer, pH 7.3, 310 mosM at 37°C, and fixed at this temperature with modified Karnovsky's fixative, pH 7.5, for at least 30 min. The cells were then rinsed three times in 0.125 M sodium cacodylate buffer for 5 min and postfixed in 3% glutaraldehyde in a mixture of 80:20% in a Hitachi model S520 scanning electron microscope.

**RESULTS**

**Increased Expression of p185**<sub>neo</sub>**in NCI-H460 c-erbB-2 Transfectants.** To examine whether the different increased amounts of p185<sub>neo</sub>e in H460 cell lines with the same genetic background but expressing different levels of p185<sub>neo</sub>e. Differences in metastatic potential among these cells should be due to the differences in p185<sub>neo</sub>e expression. To achieve this, we used the NCI-H460 human NSCLC cell line that expressed very low levels of p185<sub>neo</sub>e and expressed metastatic phenotype as the recipient for transfection studies (2, 25). We cotransfected the pSV2erbB-2 plasmid containing full-length (4.4 kilobases) human c-erbB-2/neu complementary DNA (8) and the pSV2-neo plasmid carrying the neomycin resistance selection marker gene into NCI-H460 cells. G418-resistant clones were selected and expanded into cell lines, which were designated H460.eB cell lines. The same approach was used to select control H460.neo cell lines, in which the pSV2-neo plasmid alone was transfected into H460 cells. It is possible that some of the stable transfectants selected by such a cotransfection strategy may only harbor the neomycin resistance gene but not the c-erbB-2 gene. Therefore, to identify those H460.eB transfectants that integrated the transfected c-erbB-2 gene and actually produced p185<sub>neo</sub>e protein, immunoblot analysis with c-neu Ab-3 antibodies were performed (Fig. 1). Three of the H460.eB transfectants (H460.eB1, H460.eB2, and H460.eB3) expressed different increased amounts of p185<sub>neo</sub>e protein compared with the parental H460 cell line; the control H460.neo cell line, as expected, did not express increased amounts of p185<sub>neo</sub>e protein. The amounts of p185<sub>neo</sub>e in the H460.neo cells and the H460.eB1, H460.eB2, and H460.eB3 transfectants are 1.2-, 30-, 41-, and 43-fold, respectively, that of the parental H460 cells, as determined by quantitation with the personal densitometer (Molecular Dynamics, Sunnyvale, CA). Thus, we established a panel of stable transfectants that express different increased amounts of p185<sub>neo</sub>e protein. The H460.neo cells were used as a control cell line to make sure that the changes in biological properties, if any, in the H460.eB transfectants were not due to the selection process or to transfection of the plasmids and the pSV2-neo gene.

**Growth Characteristics of the H460 c-erbB-2 Transfectants.** To examine what increased expression of p185<sub>neo</sub>e in H460 human lung cancer cells can lead to changes in growth characteristics of the cells, we examined their in vitro growth properties and rate of DNA synthesis. As shown in Fig. 2A, there was significant overlap among the growth curves of the p185<sub>neo</sub>e-overexpressing H460.eB transfectants, the control H460.neo cell line, and the parental H460 cell line. The results indicated that increased p185<sub>neo</sub>e expression in H460 cells did not significantly affect the growth rate of these lung cancer cells versus the control and the parental cells (Fig. 2A).

[^3H]Thymidine incorporation into DNA generally correlates well with overall DNA synthesis rate and has been used as another measure of cell growth. Measurement of the DNA synthesis rate by[^3H]thymidine incorporation assays revealed that the p185<sub>neo</sub>e-overexpressing
H460.eB transfectants had similar levels of \(^{3}H\)thymidine incorporation as that of the control H460.neo cell line and the parental H460 cell line (Fig. 2B). These results indicate that overexpression of p185\(^{neu}\) in H460 NSCLC cells did not induce significant changes in DNA synthesis rate.

Enhanced Metastatic Potential of H460.eB Transfectants in Vivo. After we established and characterized the H460.eB stable transfectants that express increased levels of p185\(^{neu}\), our first effort in determining the metastatic potential of these cells was to test the ability of these transfectants and the parental NCI-H460 cells to extravasate the circulatory system, to invade normal tissue, and to colonize organ sites in vivo. Thus, we performed experimental metastasis assays with the H460.eB and H460.neo transfectants, as well as NCI-H460 parental cells (31). Single-cell suspensions of each cell line were injected into the lateral tail veins of 8-week-old nude mice. The mice were killed 7 weeks after injection, and the number of pulmonary and extrapulmonary metastasis was examined. Although the frequencies of lung metastases and sizes of the metastatic lung nodules were found to be similar in all of the mice injected with either the H460.eB transfectants or the parental NCI-H460 cells or H460.neo cells, the median numbers of lung nodules was significantly increased in those mice given the H460.eB2 and H460.eB3 cells that expressed higher levels of p185\(^{neu}\) (Table 1). Statistical analysis demonstrated that the numbers of lung nodules in the H460.neo cells compared with the H460 parental cells were not significantly different (\(P > 0.1\)); whereas the numbers of lung nodules in the H460.eB transfectants were higher than that of the H460 parental cells (marginally significant for H460.eB1, \(P < 0.1\); significant for H460.eB2, \(P < 0.02\); very significant for H460.eB3, \(P < 0.01\)). Moreover, all three of the H460.eB transfectants yielded extrapulmonary metastases (in the ribs, mesentery, ovary, and stomach) in some mice, whereas neither the parental NCI-H460 cells nor the control H460.neo cells gave rise to any extrapulmonary metastases. The results clearly demonstrated that increased p185\(^{neu}\) expression in the human NCI-H460 NSCLC cells can enhance metastatic potential in vivo.

**Increased Chemoinvasion of H460.eB Transfectants in Vitro.** To investigate the mechanisms that lead to the increased metastatic potential in p185\(^{neu}\)-overexpressing H460.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 H460.eB transfectants, the parental NCI-H460 cells, and the control H460.neo cells to determine whether increased p185\(^{neu}\) expression can increase the invasive properties of the NCI-H460 NSCLC cells through a layer of reconstituted basement membrane (Matrigel) (32, 33, 36). Because all mice injected with either H460.eB transfectants or their parental NCI-H460 cells or H460.neo cells developed experimental lung metastases, conditioned medium from lung microvesSEL endothelial cells (LECM) (34) was used as a chemoattractant to stimulate penetration of the cells. The assay revealed prominent differences in invasiveness between the H460.eB transfectants and the parental NCI-H460 cells or H460.neo cells (Fig. 3). The H460.eB2 and H460.eB3 transfectants showed very high rates of invasion approximately 20- to 30-fold the invasion rate of the parental NCI-H460 cells or H460.neo cells (Fig. 3). The H460.eB1 line that displayed moderate increase in p185\(^{neu}\) expression showed only a slightly increased rate of invasion (2- to 4-fold the parental cells). These results demonstrate that increased p185\(^{neu}\) expression in the human NCI-H460 NSCLC cells can facilitate invasion through reconstituted basement membrane.

**Table 1 c-erbB-2 gene enhanced in vivo experimental metastatic potential of H460 human NSCLC cells**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Pulmonary metastasis</th>
<th>Extrapulmonary metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (mice with metastases/ mouse injected)</td>
<td>No. of nodules* [range (median)]</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>H460</td>
<td>4/5</td>
<td>0-2 (1)</td>
</tr>
<tr>
<td>H460.neo</td>
<td>3/5</td>
<td>0-11 (3)</td>
</tr>
<tr>
<td>H460.eB1</td>
<td>5/5</td>
<td>1-3 (3)</td>
</tr>
<tr>
<td>H460.eB2</td>
<td>5/5</td>
<td>2-84 (14)</td>
</tr>
<tr>
<td>H460.eB3</td>
<td>5/5</td>
<td>6-108 (15)</td>
</tr>
</tbody>
</table>

* Lung nodules >0.5 mm in diameter were counted in the assay.

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\(\text{Absorbance (490 nm)}\)

**Fig. 2.** A, growth rate of the H460.eB transfectants, the NCI-H460 parental cells, and the control H460.neo cells. 2, 3-bis(2-Methoxy-4-nitro-5-sulfophenyl-2H-tetrazolium-5-carboxanilide inner salt assays were performed at the indicated days after plating. Results were analyzed by regression analysis. Experiments were repeated three times. B, \([3H] \text{Thymidine incorporation of the H460.eB transfectants, the NCI-H460 parental cells, and the control H460.neo cells.} \ ([3H] \text{Thymidine (1 µCi/well) was added to cells at the indicated times to label those cells that were synthesizing DNA prior to harvest. Radioactivities of individual samples were counted by scintillation counter. Column (bars), average cpm (±SD) calculated from 10 replicate samples. Experiments were repeated three times.}**

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\(\text{R}_{\text{ex}}\)
onstrate that increased p185<sup>neu</sup> expression in NCI-H460 NSCLC cells can enhance chemotaxis of these cells.

**Structural Features of H460.eB Transfectants.** Since p185<sup>neu</sup> expression in NCI-H460 NSCLC cells enhanced cell motility and p185<sup>neu</sup> has been shown to associate with microfilaments in mammary carcinoma microvilli, structures important for cell motility (42), we assessed whether increased p185<sup>neu</sup> expression in the H460.eB transfectants could induce morphological changes in the NCI-H460 cells. We examined the morphologies of the H460.eB transfectants, the parental NCI-H460 cells, and the control H460.neo cells by scanning electron microscopy. As shown in Fig. 6, the NCI-H460 cells and the...
Fig. 6. Morphology of the NCI-H460 parental cells (A), the control H460.neo cells (B), the H460.eB3 transfectants (C), and the H460.eB2 transfectants (D) under scanning electron microscopy. Original magnifications, ×1500.

control H460.neo cells were flat, had close cell contacts, and had relatively smooth upper cell surfaces (Fig. 6., A and B). The morphologies of the H460.eB transfectants were dramatically different from that of the NCI-H460 cells and the control H460.neo cells. The most obvious changes were observed in the H460.eB2 and H460.eB3 cell lines that expressed high levels of p185 Neu (Fig. 6, C and D). The H460.eB2 cells had a more rounded shape and had numerous membrane protrusions, membrane ruffling, and microvilli; the H460.eB3 cells had significantly more and enlarged microvilli. Membrane protrusions, membrane ruffling, and microvilli are cell surface structures involved in cell motility; therefore, our results that increased p185 Neu expression in NCI-H460 NSCLC cells can lead to morphological changes are consistent with the increased cell motility seen in these cells.

DISCUSSION

To study the relationship between p185 Neu expression and human NSCLC metastasis, we established a panel of stable transfectants from the NCI-H460 NSCLC cell line that expressed different increased levels of p185 Neu. Although increased expression of p185 Neu in NCI-H460 NSCLC cells did not significantly change cell growth characteristics, increased expression of p185 Neu enhanced the experimental metastatic potential in NCI-H460 NSCLC cells. It should be recognized that the parental NCI-H460 NSCLC cells are metastatic tumor cells that possess all of the gene activation needed or cellular properties required for metastasis, and increased expression of p185 Neu augmented the metastatic signal or accelerated the transduction of metastatic signal. Noticeably, the increase in metastatic potential correlated with the level of p185 Neu expression, i.e., the H460.eB2 and H460.eB3 cell lines that expressed the highest levels of p185 Neu also showed the most significant increase in metastatic potential. Although the level of p185 Neu expression in H460.eB1 cells was significantly higher than the control H460.neo and the parental NCI-H460 cells, the increase in metastatic potential of H460.eB1 cells was not as significant, suggesting that a threshold level of p185 Neu may be required for enhancing metastatic potential in the NCI-H460 cells. Previous studies support this notion because they have demonstrated that enhanced p185 Neu expression to the level of $3-4 \times 10^5$ molecules/cell did not transform NIH3T3 cells, but NIH3T3 cells became highly transformed when p185 Neu expression increased to the level of $1-2 \times 10^6$ molecules/cell, indicating that a threshold level of p185 Neu may be required for transformation (43-46). Additionally, a striking feature of the pattern of metastasis in the H460.eB transfectants was that they all developed extrapulmonary metastasis, whereas neither the NCI-H460 parental cells nor the control H460.neo cells did so. Formation of extrapulmonary metastasis requires tumor cells to grow in a less favorable nonorthotopic microenvironment; therefore, the H460.eB transfectants may have gained ability to overcome organ selectivity of
growth (47). Taken together, the results from our studies have provided convincing evidence that increased p185(neu) expression can enhance the metastatic potential of human NSCLC cells.

Tumor cell invasion of basement membranes is a crucial event in the complex multistep process that leads to metastasis. It has been hypothesized that the invasion process consists of three steps: (a) tumor cell adhesion to endothelial cells and attachment to the matrix; (b) tumor cell secretion of hydrolytic enzymes, which can locally degrade the matrix; and (c) tumor cell locomotion into the region of the matrix modified by proteolysis (38, 48, 49). In this study, we have shown that the enhanced metastatic potential in vivo by p185(neu)-overexpressing H460.eB transfectants is accompanied by increased invasion ability in vitro. In addition, secretion of basement membrane-degrading enzymes (gelatinase IV) and cell migration were increased in the p185(neu)-overexpressing H460.eB transfectants. These observations indicate that the molecular mechanism by which the increase of p185(neu) expression enhanced metastatic potential of NSCLC cells was by promoting important steps of the invasion process. The results also provided further support of our previous work with the NIH3T3 cells, in which we demonstrated that transfection of the activated rat neu oncogene can induce the metastatic phenotype and promote invasion of extracellular matrix (23, 24). Interestingly, we have previously found that secretion of gelatinase IV is increased in neu oncogene-transformed NIH3T3 cells, and here in the p185(neu)-overexpressing H460.eB transfectants, we observed a similar phenomenon. These results indicate that p185(neu) may activate gelatinase IV activity or increase gelatinase expression via certain molecular pathways. It will be important to further examine how p185(neu) enhanced gelatinase IV activity.

p185(neu) has been reported to localize at the brush border of kidney cells and at intercellular plasma membrane protrusions of carcinoma cells (50). More recently, there was an interesting report that p185(neu) was associated with microfilaments via a large transmembrane glycoprotein complex in mammary carcinoma microvilli and that this p185(neu)-associated complex was involved in the organization of microfilaments and cell surface proteins in tumor cell microvilli (42). We observed remarkable morphological changes in H460.eB transfectants that expressed high levels of p185(neu). The most striking feature of H460.eB transfectants was increased and enlarged microvilli, increased membrane protrusions, and membrane ruffling, which are structural features of motile cells. Because we also detected increased cell motility with these H460.eB transfectants, we speculate that the increased p185(neu) expression in these cells may result in the formation of transmembrane complexes in microvilli and, thus, promote cell movement. Therefore, it will be interesting to examine whether the increase in expression of p185(neu) proteins results in increased localization of p185(neu) proteins in the microvilli of H460.eB transfectants. Our results suggest a tight link between (a) increased expression of the c-erbB-2-encoded p185(neu), (b) subcellular structural changes involved in cell motility, and (c) increased cell motility, an important property for cancer cell invasion and metastasis.

Overexpression of c-erbB-2/neu-encoded p185(neu) protein has been found in many types of human cancers and has been correlated with poor clinical outcome in breast cancers, ovarian cancers, and lung cancers (2–5, 15–17). However, there has been a long-running argument whether p185(neu) overexpression is the result of a more malignant tumor or is a causative factor in more aggressive tumors. Our recent work with human ovarian carcinoma cells indicates that p185(neu) overexpression can lead to more malignant phenotypes in human ovarian carcinoma cells (27). Here we demonstrated that p185(neu) overexpression can enhance metastatic potential by promoting multiple steps in the invasion and metastasis process. These findings support the notion that the c-erbB-2/neu oncogene plays a critical role in certain human malignancies and that overexpression of c-erbB-2/neu-encoded p185(neu) may lead to a greater degree of malignancy in these human cancers. Therefore, in the case of NSCLC, more aggressive therapy might be beneficial to those patients whose tumors express high levels of p185(neu) and may be at higher risk for metastasis.

REFERENCES


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