Interrelationships between Cellular Nucleotide Excision Repair, Cisplatin Cytotoxicity, HER-2/neu Gene Expression, and Epidermal Growth Factor Receptor Level in Non-small Cell Lung Cancer Cells

Chun-Ming Tsai,1, 2, 4 Kuo-Ting Chang,1 Lan Li,3 Reury-Perng Perng2 and Li-Ying Yang3

1Section of Thoracic Oncology, 2Chest Department, Veterans General Hospital-Taipei, Shih-pai, Taipei, 11217 Taiwan, 3Department of Medicine, School of Medicine, National Yang-Ming University, Shih-pai, Taipei, 11217 Taiwan and 4Division of Laboratory Medicine, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA

Nucleotide excision repair (NER) is a major repair mechanism for DNA lesions induced by cisplatin. Overexpressions of epidermal growth factor receptor (EGFR) and HER-2/neu have been reported to affect the sensitivity of certain human cancer cells to cisplatin, presumably by modification of DNA repair activity through interference with NER. Using an in vitro repair assay, we investigated NER activity of cisplatin-induced DNA lesions in a panel of 16 non-small cell lung cancer (NSCLC) cell lines. The interrelationships between NER activity, cisplatin sensitivity, HER-2/neu expression and EGFR level, were also analyzed. The results showed that high NER activity was closely correlated with cisplatin resistance and high levels of HER-2/neu expression (P ≤ 0.05).

Analysis of the relationships between EGFR level and each of the other three parameters revealed no statistically significant correlations (all P values were > 0.05 by Spearman rank correlation), but a trend of association (all the values of proportion of accordance were ≥ 62.5% by using a 2×2 contingency table). These results suggest that NER activity may play an important role in the cisplatin resistance of NSCLC cells and there may be an association between enhanced NER activity and high levels of p185neu and probably EGFR in NSCLC cells. The finding that high levels of EGFR showed very little influence on the relationship between p185neu and cisplatin resistance suggests that EGFR may be a less crucial factor in modulating the chemoresistance of NSCLC cells when compared with HER-2/neu.

Key words: Nucleotide excision repair — Cisplatin resistance — Epidermal growth factor receptor — HER-2/neu — Non-small cell lung cancer

The erbB gene family includes erbB-1 (the gene encodes epidermal growth factor receptor, EGFR), erbB-2 (also known as HER-2/neu), erbB-3 and erbB-4 genes. These four genes encode for receptor-type tyrosine-protein kinases and are usually co-expressed in various combinations in a variety of human tissues.1, 2) Constitutive overexpression of EGFR gene and/or HER-2/neu gene is a frequent event in a variety of human tumors, including non-small cell lung cancer (NSCLC), but not small cell lung cancer.3–15) In some types of tumors, aberrations of the genes may predict a poor prognosis and shorter survival.7–15) In recent years, many studies have explored the effects on chemosensitivity resulting from altered expression and activation of the HER-2/neu receptor and the EGFR, indicating that alteration of the activation status of growth factor receptors not only can lead directly to the perturbation of growth regulation, but also may affect the sensitivity of cancer cells to chemotherapeutic agents.

We and others have linked a high level of HER-2/neu protein product, p185neu, to chemoresistant phenotype in human NSCLC and breast cancer cell lines.16–22) Tumor cell lines overexpressing p185neu have been shown to be more resistant to cisplatin, taxol, etoposide, doxorubicin, melphalan, mitomycin-C, and carmustine.16–20) A direct correlation between p185neu and chemoresistance has been demonstrated by desensitizing HER-2/neu-transfected cancer cells to anticancer drugs through elevation of the levels of p185neu.18–21) and sensitizing p185neu-overexpressing cancer cells to anticancer drugs by inhibiting p185neu tyrosine kinase activity.22, 23) or with p185neu-specific antibodies.24–27) While the mechanism by which p185neu confers intrinsic chemoresistance remains unclear, several lines of evidence suggest that high-p185neu-expressing cancer cells may possess an enhanced DNA repair capacity.25–29) However, the connection between HER-2/neu gene overexpression and intrinsic chemoresistance profiles in human cancers is controversial. Pegram et al. demonstrated that HER-2/neu overexpression was not sufficient to induce intrinsic, pleomorphic drug resistance in vitro and in vivo,
and changes in chemosensitivity profiles resulting from HER-2/neu transfection observed in vitro were cell line-specific.30)

The association between over-expression of EGFR and chemosensitivity, however, is less consistent. Activation and elevation of EGFR have been shown to sensitize human cancer cells of breast, ovary, head and neck, cervix, colon, pancreas and prostate, as well as NSCLC to several classes of anticancer agents, including cisplatin and carboplatin, 5-fluorouracil, melphalan, and taxol.31–33) The modulating effect of epidermal growth factor (EGF) on anticancer agents has been demonstrated to be dependent on the number of EGFR and associated with downregulation of a form of DNA repair or inhibition of this activity.32, 33) In contrast, several reports have demonstrated that elevation of EGFR levels in human breast cancer cells might lead to increased chemoresistance to doxorubicin, vinblastine, and cisplatin, as well as 5-fluorouracil.34) Reduction of receptor tyrosine kinase by EGFR receptor-blocking antibodies resulted in enhanced antitumor activities of cisplatin and doxorubicin in human breast and squamous cancer cells in vitro, and in some cases in vivo, by a mechanism involving downregulation of DNA repair.35–37)

The approximate 50% sequence homology between genes of p185neo and EGFR39) suggests that the biological functions of these two genes may be related. After binding to the receptors erbB-3 and erbB-4, heregulin or neu differentiation factor can transactivate p185neo by inducing heterodimeric associations and cross-phosphorylation.39, 40) Similarly, EGFR ligands (EGF, transforming growth factor-α, amphiregulin) can also transactivate p185neo.41, 42) The transactivation of p185neo by the EGFR is biologically relevant since it stoichiometrically alters p185neo levels in human breast cancer cells and probably EGFR level and HER-2/neu expression. Our results in the present study demonstrate an association between cisplatin resistance and enhanced NER activity, overexpression of HER-2/neu gene, and probably EGFR level in the tested NSCLC cells. The results of the statistical analysis suggested that the correlation between the chemoresistance and constitutive expression of EGFR was less significant than the correlation between the chemoresistance and the expression of HER-2/neu in NSCLC.

MATERIALS AND METHODS

Cell lines and cell culture We studied the following 16 NSCLC cell lines, which were established and characterized in the NCI-Navy Medical Oncology Branch (Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) from tumor specimens obtained from previously untreated patients: ten adenocarcinomas (NCI-H23, H322, H358, H441, H522, H820, H388, H1355, H1435 and H1437); two adenosquamous carcinomas (H125 and H647); three large cell carcinomas (H460, H1155 and H1299), and one squamous cell carcinoma (H226).16, 17, 29 All of the cell lines had been maintained in RPMI-1640 medium supplemented with 5% heat-inactivated fetal bovine serum for >6 months before being tested.

In vitro chemosensitivity testing for cisplatin The tetrazolium dye colorimetric assay was used to evaluate the chemosensitivity for cisplatin (Farmitalia Carlo Erba, Milano, Italy) of the cell lines. The optimal seeding density of individual cell lines was predetermined, as in our previous studies.16, 17) Cisplatin was first dissolved in phosphate-buffered saline to 1 mM, and then diluted in culture medium to the desired concentrations. The duration of drug exposure was 96 h. The percentage of control absorbance was considered to represent the surviving fraction of cells and the IC50 values were defined as the concentrations of drug which produced 50% reduction in control absorbance. The experiments were performed in triplicate.

Nucleotide excision repair assay The in vitro nucleotide excision repair assay of Hansson and Wood48) was adopted and has been detailed previously.49) A cisplatin-damaged plasmid was used as the substrate for the repair proteins in
whole-cell extracts to quantify the DNA resynthesis activity. The essentials of this assay are summarized below.

**Plasmids** The plasmid substrate for the repair assay was a 2959-bp pBS (pBluescript KS⁺; Stratagene, La Jolla, CA) pretreated with cisplatin to induce DNA cross-links. Plateination of pBS was performed according to the method of Hansson and Wood, using a drug-to-nucleotide molar ratio of 0.005. A 3738-bp undamaged plasmid, pHM14, was used as a negative control to determine the background noise due to nonspecific incorporation of radioactive [α-32P]dCTP.

**Cell extracts** Whole cell extracts from each cell line were prepared according to the method of Manley with minor modifications as described by Shivi et al.50

**Repair reaction** Cell extracts (100 mg) were incubated in a standard 50 µl reaction mixture containing 300 ng each of cisplatin-damaged pBS and nondamaged pHM in 45 mM Hepes-KOH (pH 7.8), 70 mM KCl, 7.4 mM MgCl₂, 0.9 mM dithiothreitol, 0.4 mM EDTA, 2 mM ATP, 40 mM phosphocreatine, 2.5 mg of creatine phosphokinase, 3.4% glycerol, 18 mg of bovine serum albumin, 20 µM each of dGTP and dTTP, and 2 µCi of [α-32P]dCTP. The reaction was carried out at 30°C for 5 h before being terminated by the addition of 20 mM EDTA. Plasmid DNAs were purified, linearized by HindIII, and subjected to electrophoresis. The DNA content in ethidium bromide-stained bands was quantified by densitometry using photographic negatives and compared to the standard. The incorporation of radioactive nucleotides was quantified with a Betascope (Betagen, Waltham, MA). DNA repair synthesis, expressed as specific incorporation of [α-32P]dCTP, was calculated by subtraction of nonspecific incorporation by the nondamaged pHM control from the total incorporation by the cisplatin-damaged pBS substrate. The experiments were performed in triplicate.

**Quantitative measurement of the gene expression** Nearly confluent cells in the logarithmic growth phase were harvested, and 100 µg of protein from each sample was electrophoresed on 7.5% sodium dodecyl sulfate (SDS) polyacrylamide gels. Detection of EGFR and HER-2/neu gene expression was carried out by standard western blotting analysis using specific antibodies. The primary monoclonal antibodies used for immunoblot analyses were b-3 (1:250) against p185neu protein (Oncogene Science, Inc., Uniondale, NY) and anti-EGFR (1:250) recognizing EGFR (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Immunoblot analysis of EGFR was performed first. After stripping of the EGFR signals, the same blot was immunoblotted against anti-p85β protein. Immunoblotting for β-actin was performed last after stripping of the p185neu signals. The intensity of signals was quantitated by soft laser densitometry (Model SLR-2D/1D; Biomed, Fullerton, CA). The signal intensity of the cell line H23 was arbitrarily assigned a value of 1 unit, and the signals of the other cell lines were quantitated relatively to the signal.

### Table 1. Summary of the IC₅₀ Values of Cisplatin, NER Activity, and Levels of p185neu and EGFR in the Tested NSCLC Cell Lines

| Cell line | Cell type | Cisplatin IC₅₀ (µM) | NER activity (dCMP, fmol) | p185neu (relative units) | EGFR (relative units) |
|-----------|-----------|---------------------|--------------------------|--------------------------|------------------------|
| H1155     | LC        | 1.75±0.06           | 54.7±9.3                 | 0.47±0.25                | 0.75±0.30              |
| H23       | A         | 0.51±0.01           | 57.3±8.3                 | 1.00                     | 1.00                   |
| H226      | S         | 5.00±0.17           | 62.0±13.5                | 1.87±0.46                | 6.28±0.65              |
| H1299     | LC        | 2.27±0.38           | 44.0±19.3                | 1.93±1.36                | 1.93±0.34              |
| H460      | LC        | 0.45±0.02           | 69.7±16.3                | 2.60±0.81                | 2.49±0.19              |
| H125      | AS        | 1.83±0.17           | 17.0±3.0                 | 3.15±0.55                | 3.14±0.04              |
| H358      | AS        | 1.10±0.34           | 84.3±14.5                | 4.35±1.71                | 1.46±0.19              |
| H838      | AS        | 2.18±0.23           | 37.1±19.2                | 6.82±2.31                | 1.95±0.08              |
| H322      | A         | 1.92±0.18           | 79.2±9.5                 | 6.89±1.76                | 4.95±0.19              |
| H1437     | A         | 2.93±0.08           | 88.0±18.4                | 7.62±2.71                | 2.53±0.64              |
| H1355     | A         | 7.66±2.18           | 222.2±19.8               | 8.02±1.67                | 1.14±0.07              |
| H441      | A         | 3.74±1.11           | 40.0±10.8                | 9.66±1.23                | 2.47±0.42              |
| H647      | AS        | 5.63±0.52           | 129.0±15.7               | 9.86±2.49                | 4.61±0.55              |
| H820      | A         | 5.53±0.46           | 64.3±5.2                 | 11.95±1.95               | 7.17±1.40              |
| H1435     | A         | 20.45±0.80          | 120.5±29.0               | 13.47±3.21               | 2.69±0.89              |
| H522      | A         | 5.27±0.64           | 93.3±10.2                | 14.97±2.47               | 1.30±0.35              |

*α) The results are the means (± SE) of three independently performed assays.

b) A, adenocarcinoma; AS, adenosquamous cell carcinoma; LC, large cell carcinoma; S, squamous cell carcinoma.
intensity of H23. The protein levels were also normalized by densitometry of the β-actin signal. The experiments were performed in triplicate.

Data analysis The Spearman rank correlation was used for correlation analyses. The relationship of each pair of observed parameters was also examined by using a 2×2 contingency table, from which the value of proportion of accordance (POA) was calculated. According to the value (level) of each parameter, the 16 cell lines tested were divided into two equal groups, i.e., high- and low-level groups. POA=[(number of the cell lines which had high levels of both parameters A and B + number of the cell lines which had low levels of both parameters A and B)/16]×100%. For example, the POA value of NER activity vs. cisplatin chemosensitivity=(5+5)/16=62.5% (Fig. 3A). POA≥62.5% was arbitrarily chosen as a cut-off point for identification of an association between the two observed parameters.

RESULTS

Cisplatin sensitivity The chemosensitivity to cisplatin (expressed as IC_{50} value) was determined in 16 NSCLC cell lines and the results are shown in Table I. The IC_{50} value of the most resistant cell line H1435 (20.45 µM) was 45-fold higher than that of the most sensitive cell line H460 (0.45 µM).

NER activity NER activity was determined by means of the in vitro NER assay. Fig. 1 demonstrates the NER activity of cisplatin-modified DNA with cell-free extracts from 8 of the 16 cell lines tested. Table I shows the mean value

![NER activity](image)
of three experiments; the range of NER activity in this panel of 16 cell lines was from 17 to 222 fmol dCMP incorporation (H125 and H1355, respectively), with a 13-fold difference.

Constitutive levels of p185<sup>neu</sup> and EGFR The mean values of three experiments in which either p185<sup>neu</sup> or EGFR was quantitated by western blotting and densitometry were expressed as relative units (Table I). One example of three immunoblot analysis experiments is shown in Fig. 2 to illustrate the levels of EGFR and HER-2/neu expression of the entire panel of cell lines. Table I shows that the highest level of p185<sup>neu</sup> in H522 (14.97) was 32-fold higher than the lowest level in H1155 (0.47) (Table I). In this panel of 16 cell lines, the level of HER-2/neu gene expression has been determined by northern blotting,<sup>10</sup> antibody-sandwich enzyme-linked immunosorbent assay (ELISA),<sup>17, 29</sup> and western blotting (the present study). The results obtained from the three different methods are closely correlated with each other (r=0.85–0.91 and P≤0.001 by the Spearman rank correlation test). There was a larger gap in the levels of EGFR expression (between 2.53 and 4.61). The highest level of EGFR in H820 (7.17) was 9.6-fold higher than the lowest level in H1155 (0.75) (Table I). Four cell lines, H647, H322, H226 and H820, expressed a level of EGFR>4.5 relative units (Table I).

Statistically significant correlation between each pair of cisplatin chemoresistance, NER activity, and p185<sup>neu</sup> The Spearman rank correlation analysis showed that the correlations between the level of NER activity and the IC<sub>50</sub> value of cisplatin (r=0.524, P=0.043), and the level of

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Fig. 3. Interrelationships between cisplatin sensitivity, NER activity, HER-2/neu gene expression and EGFR gene expression. In vitro cisplatin sensitivity assay was performed using the tetrazolium colorimetric assay. NER activity was determined by using an in vitro NER assay (see “Materials and Methods” and Fig. 1). Immunoblot analyses were performed to detect the expression of HER-2/neu gene and the EGFR gene. The intensity of the signals was quantitated with a soft laser densitometer (Model SLR-2D/1D; Biomed) (Fig. 2). The results were the means of three independently performed experiments. The R and P values calculated by using the Spearman rank correlation test and the values of POA (see “Data analysis”) are labeled. The dashed line (horizontal or vertical) is applied to separate the cell lines in two equal groups (high- and low-level groups) according to the corresponding parameter (see “Data analysis”).
p185
\textsuperscript{neu} \ (r=0.556, \ P=0.031) \ were \ statistically \ significant: \ the \ greater \ the \ repair \ activity \ the \ higher \ the \ IC_{50} \ values \ and \ the \ higher \ the \ values \ of \ p185\textsuperscript{neu} \ (Fig. \ 3, \ A \ and \ B, \ respectively). \ The \ present \ finding \ that \ there \ was \ a \ close \ correlation \ between \ the \ IC_{50} \ value \ of \ p185\textsuperscript{neu} \ and \ the \ values \ of \ IC_{50} \ (r=0.747, \ P=0.004; \ Fig. \ 3C) \ confirms \ our \ previous \ reports\textsuperscript{16, 17}) \ that \ the \ higher \ p185\textsuperscript{neu} \ expressing \ NSCLC \ cells \ are \ more \ cisplatin-resistant. \ These \ correlations \ indicate \ that \ cisplatin-resistant \ NSCLC \ cell \ lines \ express \ a \ high \ level \ of \ p185\textsuperscript{neu} \ and \ have \ a \ greater \ NER \ activity. \ The \ data \ were \ also \ analyzed \ by \ the \ use \ of \ a \ 2\times2 \ contingency \ table. \ The \ relationships \ between \ NER \ activity \ and \ the \ IC_{50} \ value, \ between \ NER \ activity \ and \ the \ level \ of \ p185\textsuperscript{neu}, \ and \ between \ p185\textsuperscript{neu} \ and \ the \ IC_{50} \ value \ showed \ POA \ values \ of \ 62.5\%, \ 75.0\%, \ and \ 87.5\%, \ respectively \ (Fig. \ 3, \ A–C).

**Relationships between EGFR expression and cisplatin chemoresistance, NER activity, and p185\textsuperscript{neu}** Although Spearman rank correlation analyses did not show a significant correlation between the levels of EGFR expression and NER activity (r=0.18, P=0.49), cisplatin chemoresistance (r=0.38, P=0.14), and the HER-2/neu gene expression (r=0.36, P=0.16), the results of the 2\times2 contingency table analysis showed that all the values of POA were \(\geq 62.5\%\) \ (62.5\%, 75\% and 75\%, respectively) \ (Fig. \ 3, \ D–F). \ Therefore, it seemed that there was an association between the level of EGFR and each of the other three parameters (i.e., NER activity, cisplatin chemoresistance, and p185\textsuperscript{neu}). \ These \ results \ suggested \ that \ high \ EGFR-expressing \ NSCLC \ cells \ may \ be \ associated \ with \ high \ levels \ of \ NER \ activity \ and \ p185\textsuperscript{neu} \ expression \ and \ greater \ resistance \ to \ cisplatin.

**Very little influence on the relationship between p185\textsuperscript{neu} and cisplatin chemoresistance by high levels of EGFR**

To assess whether co-expression status of EGFR and p185\textsuperscript{neu} \ would \ modulate \ the \ chemosensitivity \ constitutively, \ the \ 4 \ cell \ lines \ with \ EGFR \ levels>4.6 \ units \ were \ excluded \ from \ the \ 16 \ cell \ lines \ (there \ was \ a \ larger \ gap \ between \ 2.53 \ and \ 4.61 \ units \ in \ the \ levels \ of \ EGFR \ expression \ and \ the \ group \ mean \ value \ was \ 2.75). \ Table \ I) \ to \ see \ whether \ there \ was \ any \ change \ in \ the \ relationship \ between \ p185\textsuperscript{neu} \ and \ the \ IC_{50} \ values \ of \ cisplatin. \ As \ shown \ in \ Fig. \ 4, \ the \ regression \ line \ of \ the \ IC_{50} \ value \ of \ cisplatin \ vs. \ the \ level \ of \ p185\textsuperscript{neu} \ derived \ from \ the \ remaining \ 12 \ cell \ lines \ (dashed \ line; \ r=0.81, \ P=0.007) \ is \ very \ similar \ to \ and \ almost \ superimposable \ on \ that \ derived \ from \ the \ entire \ panel \ of \ 16 \ cell \ lines \ (solid \ line; \ r=0.75, \ P=0.004). \ These \ findings \ indicate \ that \ the \ relationship \ between \ the \ level \ of \ p185\textsuperscript{neu} \ and \ the \ chemosensitivity \ is \ very \ little \ influenced \ by \ even \ high \ levels \ of \ EGFR.

**DISCUSSION**

Several \ reports \ have \ presented \ experimental \ evidence \ for \ direct \ involvement \ of \ DNA \ repair \ in \ HER-2/neu \ induced \ drug \ resistance.\textsuperscript{25–27} \ Cancer \ cells \ that \ constitutively \ overexpressed \ HER-2/neu \ or \ had \ been \ engineered \ to \ overexpress \ human \ HER-2/neu \ were \ found \ to \ be \ resistant \ to \ anticancer \ drugs \ such \ as \ cisplatin, \ doxorubicin, \ etopo- \ side, \ and \ taxol. \ They \ also \ showed \ an \ enhanced \ overall \ DNA \ repair \ capacity.\textsuperscript{27, 52} \ In \ NSCLC, \ elevated \ HER-2/neu \ gene \ expression\textsuperscript{16–18} \ and \ DNA \ repair\textsuperscript{52} \ have \ been \ linked \ to \ intrinsic \ chemoresistance. \ Using \ a \ panel \ of \ cell \ lines, \ in \ the \ present \ study, \ we \ demonstrated \ a \ tight \ association \ between \ each \ pair \ of \ cisplatin \ resistance, \ NER \ activity, \ and \ HER-2/neu \ expression, \ suggesting \ that \ HER-2/neu-overexpressing \ NSCLC \ cells \ may \ have \ greater \ NER \ activity \ that \ leads \ to \ attenuation \ of \ the \ cytotoxicity \ of \ cisplatin. \ A \ constitutive \ level \ of \ p185\textsuperscript{neu} \ is \ a \ predictor \ for \ intrinsic \ chemoresistance \ to \ DNA-damaging \ agents \ including \ cisplatin, \ doxorubicin, \ etoposide, \ Carmustine, \ melphalan \ and \ mitomycin-C. \ The \ cytotoxic \ effects \ of \ these \ agents \ are \ closely \ correlated \ in \ NSCLC \ cells.\textsuperscript{36} \ In \ addition \ to \ cisplatin, \ the \ sensitivity \ of \ the \ panel \ of \ cell \ lines \ to \ etoposide \ has \ been \ tested \ and \ showed \ a \ borderline \ association \ between \ the \ IC_{50} \ value \ of \ etoposide \ and \ NER \ activity \ (r=0.474, \ P=0.14, \ data \ not \ shown; \ and \ IC_{50} \ eto- \ poside \ vs. \ p185\textsuperscript{neu}, \ r=0.832, \ P=0.0013).\textsuperscript{53} \ These \ findings \ suggest \ that \ elevated \ NER \ activity \ may \ be \ associated \ with \ intrinsic \ chemoresistance \ in \ NSCLC \ cells.
Although the effects of EGFR on chemosensitivity are less consistent and may be specific to particular tumor cell lines or cell types or to particular drugs, in the present study, we demonstrated a trend that a high constitutive level of EGFR may be related to cisplatin resistance and NER activity, indicating that activating the EGFR signal transduction pathway may induce chemoresistance rather than chemosensitivity in NSCLC cells. This result is in accordance with our previous finding that EGFR can induce resistance to cisplatin and etoposide in most of the NSCLC cell lines tested (10/12) and this effect was accompanied and correlated with the inhibitory effect of EGFR on cell proliferation.\(^5\) While an inverse correlation between EGFR and HER-2/neu expression has been reported in bladder cancer tissues,\(^4\) we found a positive but moderate association between the levels of these two \textit{erbB} gene products in this study (POA value = 75\%). The finding that high levels of EGFR showed very little influence on the relationship between p185\(^{\text{neu}}\) and cisplatin resistance suggested that in the whole panel of tested cell lines the constitutive level of EGFR is a relatively less crucial biomarker for intrinsic chemoresistance when compared to HER-2/neu. Whether alteration of the activity of p185\(^{\text{neu}}\) or EGFR (elevated by gene transfection or blocked by specific antibodies) affects NER activity requires further investigation.

Evidence is mounting that the molecular basis for the increased susceptibility of cancer cells to anticancer drugs, and the development of treatment resistance, may originate from genetic lesions which alter apoptotic pathways. A pathway of apoptosis initiated by DNA damage is wild type p53-dependent and inactivation of p53 can lead to resistance to DNA-damaging agents (refs. 55–57 and others cited therein). Since our study demonstrated a close correlation of intrinsic multiple drug resistance to the expression of HER-2/neu gene in these 16 NSCLC cell lines in which mutation of p53 gene was a common (87\%) event,\(^6\) it may be conjectured that the \textit{erbB} genes function through a p53-independent pathway to modulate the apoptotic pathway, thus determining the fate of injured NSCLC cells. In breast cancer cells, Yu \textit{et al.} recently reported that overexpression of p185\(^{\text{neu}}\) by transfection transcriptionally upregulates p21\(^{\text{Cip1}}\), which associates with p34\(^{\text{Cdc2}}\), inhibits taxol-mediated p34\(^{\text{Cdc2}}\) activation, delays cell entrance to the G2/M phase, and thereby inhibits taxol-induced apoptosis.\(^5\) Moreover, \textit{erbB} genes may associate with,\(^5\) interact with or trigger some other factors to affect the status of chemoresistance.\(^2,5,6\)

The effects of overexpressing EGFR and HER-2/neu on sensitivity of tumor cells to drugs seem to be intricate, involving some step(s) of an interconnected network of cell signaling pathways that determine malignant phenotypic characteristics of tumor cells, including metastatic potential as well as intrinsic chemoresistance. Current efforts to characterize the role of \textit{erbB} gene products and their regulatory mechanisms will open opportunities to develop and to design specific inhibitors and strategies to overcome tumor metastasis and intrinsic chemoresistance. A clinical trial using Herceptin (a humanized monoclonal antibody directed against p185\(^{\text{neu}}\)) plus chemotherapeutic agents for treatment of metastatic breast cancer patients whose tumors overexpress p185\(^{\text{neu}}\) has demonstrated marked improvements in tumor response rate and remission time.\(^6\) The success of Herceptin is a critical point in translational research, proving the paradigm that if we understand what are the genetic aberrations in human cancer, we can target them.

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