Enhancement of Chemosensitivity by Tyrphostin AG825 in High-p185<sup>neu</sup> Expressing Non-Small Cell Lung Cancer Cells<sup>1</sup>

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ABSTRACT

The HER-2/neu gene product, p185<sup>neu</sup>, is a membrane-bound receptor with tyrosine kinase activity. High levels of p185<sup>neu</sup> is correlated with intrinsic chemoresistance of non-small cell lung cancer (NSCLC) cell lines. We investigated the effects of tyrphostin AG825, a selective tyrosine kinase inhibitor preferentially inhibiting HER-2/neu kinase, on the chemosensitivities and on the drug-induced cell cycle changes of NSCLC cell lines that expressed different levels of p185<sup>neu</sup>. Compared to the low-p185<sup>neu</sup> expressing cell lines, we found that the high-p185<sup>neu</sup> expressing cell lines were more resistant to doxorubicin, etoposide, and cis-diamminedichloroplatinum(II) but more sensitive to AG825. AG825 was able to significantly enhance the chemosensitivities of the high-p185<sup>neu</sup> expressing cell lines, whereas it had little effect on the chemosensitivities of the low-p185<sup>neu</sup> expressing cells, with a few exceptions in which minor antagonistic effects were observed. Although high concentrations of AG825 could reduce the drug-induced G<sub>2</sub> arrest that was accompanied by the activation of phosphorylated p34<sup>cdc2</sup>, we failed to find any remarkably differential effects of AG825 on drug-induced G<sub>2</sub> arrest and the accompanying phosphorylation status of p34<sup>cdc2</sup> of the high- and the low-p185<sup>neu</sup> expressing cell lines. In summary, tyrphostin AG825 can enhance chemosensitivity in high- but not in low-p185<sup>neu</sup> expressing NSCLC cell lines. This differential effect cannot be explained by the alterations of drug-induced cell cycle changes by AG825. Our results provide a rationale to develop p185<sup>neu</sup>-specific tyrphostin and to test them in combination with anticancer agents in vivo and in clinical trials.

INTRODUCTION

The HER-2/neu gene encodes a transmembrane glycoprotein (p185<sup>neu</sup>), which is a member of the EGF<sup>2</sup> receptor subgroup of the protein tyrosine kinase superfamily (1, 2). Amplification and/or expression of the HER-2/neu gene have been detected in subpopulations of many types of human cancers and have been linked to the presence of extensive disease and poor patient survival (3–7). In lung cancer, overexpression of the HER-2/neu gene is encountered in a subgroup of all types of NSCLC but not in small cell lung cancer (5–7). A high level of p185<sup>neu</sup> has been linked to shortened survival in adenocarcinomas (6, 7). Using a panel of NSCLC cell lines as an experimental model, we have demonstrated that the intrinsic chemoresistance of NSCLC cells correlates well with the expression of p185<sup>neu</sup> (8, 9). By transfection of HER-2/neu CDNA into a human NSCLC cell line expressing a very low level of p185<sup>neu</sup>, we (10) have demonstrated that increasing the expression of p185<sup>neu</sup> significantly enhances the chemoresistance to doxorubicin, cisplatin, mitomycin C, and etoposide in the transfected clones. The levels of the increased chemoresistance in the HER-2/neu transfecants correlates well with the level of p185<sup>neu</sup> as well as the level of p185 chemosensitivity. Since increased tyrosine kinase activity of p185<sup>neu</sup> has been implicated in the oncogenic signal transduction (11, 12), it is possible that multiple drug resistance of NSCLC cells is also conferred by the increased tyrosine phosphorylation activity of p185<sup>neu</sup>. In addition, we (13) demonstrated previously that caffeine enhances the chemosensitivities of high-p185<sup>neu</sup> expressing NSCLC cell lines to anticancer agents and that the magnitude of enhancement correlates with the level of p185<sup>neu</sup>. Caffeine has been shown to override the drug-induced cell cycle arrest at checkpoints, in particular G<sub>2</sub>-M (14, 15). It is generally accepted that G<sub>2</sub> arrest results from the inactivation of tyrosine kinase that phosphorylates p34<sup>cdc2</sup>, thus inducing premature mitosis, leading to enhanced cell killing (14, 18). Our findings (13), therefore, raise the possibility that high levels of p185<sup>neu</sup> may enhance chemoresistance by potentiating DNA repair either by enhanced phosphorylation of p34<sup>cdc2</sup> and/or by enhanced enzyme activity (or activities) for DNA repair.

To further investigate whether the HER-2/neu gene expression is correlated to the chemoresistance of NSCLC cells and examine whether p185<sup>neu</sup> is involved in the regulation of p34<sup>cdc2</sup> phosphorylation during drug exposure, we have applied a novel approach to inhibit specifically the tyrosine kinase activity of p185<sup>neu</sup>. We intended to monitor the effects of p185<sup>neu</sup> blockade on the chemosensitivity, drug-induced cell cycle changes, and alterations of p34<sup>cdc2</sup> phosphorylation in the high- and the low-p185<sup>neu</sup> expressing NSCLC cell lines. To achieve that goal, we have used a protein tyrosine kinase inhibitor, tyrphostin AG825, which has been shown to selectively block p185<sup>neu</sup> kinase in cell-free systems (19, 20). In this study, we investigate the differential effects of tyrphostin AG825 on the chemosensitivities for three commonly used anticancer drugs, doxorubicin, cis-diaminedichloroplatinum(II) (cisplatin), and etoposide, of the high- and one low-p185<sup>neu</sup> expressing cell lines. To further monitor the effect of AG825 on the etoposide-induced cell cycle changes and the accompanying alterations of the phosphorylation status of p34<sup>cdc2</sup>.

MATERIALS AND METHODS

Cell Lines. Six NSCLC cell lines, five adenocarcinomas NCI-H23, NCI-H1155, NCI-H1355, NCI-H1435, and NCI-H1437, and one large cell carcinoma, NCI-H1299, were studied. All lines were established and characterized from previously untreated patients (9, 21) and expressed a minimal level of EGF receptor (data not shown). All these cell lines contain point mutations in the p53 gene except the NCI-H1299 cell line, which contains an intragenic deletion in the gene (22). All the lines had been maintained in RPMI 1640

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<sup>3</sup>The abbreviations used are: EGF, epidermal growth factor; NSCLC, non-small cell lung cancer; cisplatin, cis-diaminedichloroplatinum(II); CI, combination index.
supplemented with 5% heat-inactivated fetal bovine serum for at least 6 months before being tested.

**Quantitative Measurement of the p185e Protein.** The nearly confluent cells were harvested by scraping, followed by centrifugation to form a cell pellet. Cells were incubated in lysis buffer [10 mM Tris-HCl (pH 7.6), 1.5 mM EDTA, 10% glycerol, and 0.1% sodium azide] and Dounce homogenized. After homogenization, the proteins were extracted in detergent. After centrifugation, supernatants were collected, and protein concentrations were determined. The immunoblot for detection and quantitation of p185e was a sandwich assay utilizing monoclonal antibodies NB-3 (coated onto microtiter wells) and TA-1 (biotin labeled) (human neu assay kits; Oncogene Science, Inc., Uniondale, NY; Ref. 23). The biotin-labeled TA-1 was detected using a streptavidin-horseradish peroxidase conjugate in PBS (pH 7.4), 1% BSA, and 0.1% chloroacetamide. After the addition of O-phenylenediamine substrate, dissolved in DMSO to 40 mM. All the agents then were subsequently diluted in DMSO (0.025—0.20%), serving as vehicle controls in the absence or concurrent presence of etoposide at the optimal concentration, were tested. After 24-h incubation, cells were collected for analyses of the cell cycle phase fraction, p185e, and the phosphorylation status of p34cdc2 kinase. For experiments on synchronized cells, cells were synchronized and treated with etoposide at the optimal concentration (3 μM) and DMSO (0.025—0.20%), serving as vehicle controls in the absence or concurrent presence of etoposide at the optimal concentration (3 μM; see “Results”) for 1/18 doubling time (i.e., H1299 for 50 min and H1355 for 2 h). After removal of etoposide by washing twice with PBS, AG825 (40 μM) was added in the tested dishes for continuous exposure. Samples of etoposide-un-treated and -treated cells without and with AG825 exposure were collected before treatment and for various time periods, starting from the beginning of etoposide exposure. Asynchronous and synchronized cells were stained with propidium iodide (50 μg/ml) plus 0.1% Triton X-100 and RNase (180 units/ml), and DNA fluorescence distributions and the proportion of cells in G1, S, and G2-M of the cell cycle were analyzed by flow cytometry as described previously by Chiu et al. (27).

**Immunoblotting.** Immunoblot analyses were performed to detect the expression of p185e and the effects of AG825 on the expression of p185e and p34cdc2 phosphorylation in the absence and concurrent presence of etoposide. Cell extracts were prepared, and 100 μg of protein from each sample were electrophoresed and Western blotted against the appropriate antibody. The primary monoclonal antibodies used for immunoblot analyses were Ab-3 (1:250) against p185e protein (Oncogene Science, Inc.; Ref. 28) and anti-cdc2 H5 (1:200) recognizing human cdc2 homologue (Upstate Biotechnology, Inc.; Lake Placid, NY; Ref. 29).

**RESULTS**

**Effects of AG825 on the Expression of p185e.** The levels of p185e of the six cell lines were determined by immunoblotting as well as antibody-sandwich ELISAs as shown in Fig. 1. The cell lines H1155, H23, and H1299 were classified as the low-p185eexpressors, and the cell lines H1437, H1435, and H1355 were classified as the high-p185eexpressors. The levels of p185e of the high-p185eexpressing H1355 were reduced by AG825 exposure at the concentrations ≥60 μM for 24 h. (Fig. 2A). The addition of the optimal etoposide concentration at 3 μM (see below) itself had little

**Fig. 1. Immunoblot of p185e of the six cell lines tested. The nearly confluent cells in logarithmic growth phase were harvested. Cell lysates (100 μg/lane) were immunoblotted with anti-p185e (Ab-3) antibody. The different mobilities of p185e in the different cell lines is likely due to protein phosphorylation, which has been shown to produce heterogeneous protein bands in similar immunoblots (41). Numbers in parentheses are the levels of p185e determined by ELISAs using NB-3 (coated onto microtiter wells) and TA-1 (biotin-labeled) monoclonal antibodies. The results are the mean ± (SE) values of three independent experiments (each experiment was performed in duplicate wells), which are expressed as a human neu unit/μg protein.
Fig. 2. A, effects of AG825 on the expression of p185\textsuperscript{ner} of the high-p185\textsuperscript{ner} expressing H1355 cell line. After being plated at the appropriate seeding density for 24 h, cells were incubated without (Lane 1) or with AG825 (10–100 \textmu M; Lanes 2–7) for 24 h; then cell lysates (100 \mu g/lane) were immunoblotted with anti-p185\textsuperscript{ner} antibody (Ab-3). AG825 at the concentration \geq 60 \textmu M reduced the levels of p185\textsuperscript{ner}. B, effects of AG825 treatment on the expression of p185\textsuperscript{ner} (Lane 2) but appear to facilitate the inhibitory effect of AG825 on the expression of p185\textsuperscript{ner}. In the presence of etoposide, AG825 reduced the expression of p185\textsuperscript{ner} in a dose-dependent manner in the range of 10 to 60 \textmu M (Fig. 2B).

**Cytotoxic Effects of Doxorubicin, Cisplatin, Etoposide, and AG825.** Anticancer agents doxorubicin, etoposide, and cisplatin were tested for their cytotoxic effects on the designated NSCLC cell lines. Their dose-effect curves are shown in Fig. 3. The three high-p185\textsuperscript{ner} expressing cell lines (Fig. 3, A-C, \textit{solid lines}) were apparently more resistant to the chemotherapeutic agents than the three low-p185\textsuperscript{ner} expressing cell lines (Fig. 3, A-C, \textit{dotted lines}). When tyrphostin AG825 was tested for its cytotoxic effect, the IC\textsubscript{50}s were very similar (34.1 to 44.3 \textmu M) for the cell lines tested. However, AG825 alone at low concentrations (2.15–21.5 \textmu M) had a greater inhibitory effect on the high- than on the low-p185\textsuperscript{ner} expressing cell lines (Fig. 3D).

**Effects of Tyrphostin AG825 on the Chemosensitivities of High- and Low-p185\textsuperscript{ner} Expressing Cell Lines.** Mean CIs was signified to represent the interaction between AG825 and the anticancer agents. The mean CI at the 50% effect level of the three AG825-containing regimens against the six cell lines are depicted in Fig. 4. AG825 significantly enhanced the chemosensitivities for doxorubicin and etoposide of the three high-p185\textsuperscript{ner} expressing cell lines (all \textit{P} \leq 0.05) and significantly enhanced the chemosensitivity for cisplatin (\textit{P} = 0.012) of the high-p185\textsuperscript{ner} expressing H1355 cell line. But AG825 had little effect on the cytotoxicity of cisplatin against the other two high-p185\textsuperscript{ner} expressing cell lines. In contrast, AG825 had no statistically significant effects on the chemosensitivities of the three low-p185\textsuperscript{ner} expressing cell lines, with the exceptions that AG825 significantly attenuated the cytotoxicities of doxorubicin and cisplatin against H1299 (CI\textsubscript{D}, 1.103 ± 0.036; CI\textsubscript{TC}, 1.231 ± 0.086; \textit{P} < 0.005) and the cytotoxicity of etoposide against H23 (CI\textsubscript{EC}, 1.093 ± 0.023; \textit{P} < 0.05). Our results clearly demonstrate that AG825 enhances the chemosensitivities of the high-p185\textsuperscript{ner} expressing cell lines, whereas AG825 had only a small effect on the chemosensitivities of the low-p185\textsuperscript{ner} expressing cell lines, with a few exceptions that minor antagonistic effects were observed (Fig. 4).

There was a statistically significant correlation between the level of p185\textsuperscript{ner} and CITD (\textit{r} = −0.943, \textit{P} = 0.035). The correlations between the level of p185\textsuperscript{ner} and CI\textsubscript{TC} and between the level of p185\textsuperscript{ner} and CI\textsubscript{TC} were marginally significant (\textit{r} = −0.771, \textit{P} = 0.085 and \textit{r} = −0.829, \textit{P} = 0.064, respectively). These findings suggest that the...
more the cells express p185<sup>neu</sup>, the greater the chemosensitivities might be enhanced by AG825 (Figs. 1 and 4).

**Effects of AG825 on the Cell Cycle Progression of Asynchronous Cells in the Absence or Presence of Etoposide.** Since the cytotoxic effect of etoposide was enhanced by AG825 to the greatest degree in our panel of high-p185<sup>neu</sup> expressing cell lines as compared to the other two anticancer agents (Fig. 4), we selected etoposide for further cell cycle analyses and biochemical studies of the effects of AG825 on the H1299 and H1355 cell lines. H1299 possesses a homozygous deletion of the p53 gene (27) and expresses low levels of p185<sup>neu</sup> (Fig. 1). H1355, which contains an endogenous mutant p53 and expresses p185<sup>neu</sup> at a steady high level (Fig. 1), yielded the most significant responses to the AG825-containing regimens (Fig. 4). Continuous exposure to 3 μM etoposide for 24 h resulted in a maximal G<sub>2</sub> arrest to these two cell lines (Fig. 5, A and B). Etoposide at the concentration of 3 μM was, therefore, selected as the optimal concentration for the succeeding cell cycle and biochemical experiments. Although AG825 itself had little effect on cell cycle progression of the H1299 and the H1355 cell lines (Fig. 5, C and D), AG825 reduced the etoposide-induced G<sub>2</sub> arrest of both H1299 and H1355 in a dose-dependent fashion. Accompanying the reduction of G<sub>2</sub> fraction, both cell lines demonstrated a greater increase of the G<sub>1</sub> fraction than the S fraction at the high concentrations (≥60 μM) of AG825 (Fig. 5, E and F). However, at the low concentrations (≤40 μM) of AG825, the reduction of G<sub>2</sub> fraction was accompanied by a predominant increase of the S fraction in H1299 (Fig. 5E) but a predominant increase of the G<sub>1</sub> fraction in H1355 (Fig. 5F). These results suggested the possibility that AG825 at low concentrations delayed S-phase progression of the etoposide-treated H1299 cells and prevented them from reaching G<sub>2</sub>. In contrast, AG825 throughout the tested concentrations might circumvent etoposide-induced G<sub>2</sub> arrest in a dose-dependent fashion in H1355. The differential effects of low concentrations of AG825 on the etoposide-induced cell cycle changes of H1355 and H1299 cells in a 24-h time period might, however, simply result from the inherently different proliferating activities (doubling times) of these two cell lines. Experiments, therefore, were carried out with synchronized cells to examine this possibility (see below).

**Effects of AG825 on Phosphorylation of p34<sup>cdc2</sup> in the Presence of Etoposide.** As noted, etoposide-induced G<sub>2</sub> arrest was associated with the accumulation of phosphorylated p34<sup>cdc2</sup> kinase that was independent of the level of p185<sup>neu</sup> expression of the cell lines (Fig. 6, A and B, Lane 2). With the addition of AG825, a marked change in the etoposide-treated H1355 and H1299 cells was a shift in p34<sup>cdc2</sup> from the slower-migrating (phosphorylated) isoform to the faster-migrating (dephosphorylated) isoform of p34<sup>cdc2</sup> at the high concentrations of AG825 (Fig. 6, A and B, Lanes 6–8). In contrast, the etoposide-induced G<sub>2</sub> arrest and hyperphosphorylation of p34<sup>cdc2</sup> of both cell lines remained little affected at the low doses of AG825 (Fig. 6, A and B, Lanes 3–5). These findings were compatible with the findings of the cell cycle study (Fig. 5, E and F), indicating that AG825 at higher concentrations could reduce etoposide-induced G<sub>2</sub> arrest through dephosphorylation (activation) of phosphorylated p34<sup>cdc2</sup>. However, AG825 throughout the tested concentrations may not have remarkably differential effects on the phosphorylation regulation of the etoposide-induced G<sub>2</sub> arrest of the high- and the low-p185<sup>neu</sup> expressing cell lines.

**Effects of AG825 on the cell cycle progression of synchronized cells with or without etoposide treatment.** Cells were synchronized as described in "Materials and Methods." In both H1355 and H1299, AG825 (40 μM) alone delayed S-phase progression to a minimal degree. In the etoposide-treated cells, however, the addition of AG825 further enhanced the etoposide-induced S-phase delay (Fig. 7, A and B). Following the slow progression of S phase, the etoposide-treated cells progressed into and arrested at G<sub>2</sub>-M in the absence or presence of AG825 (Fig. 7, C and D). In H1299 and H1355, the time-S-phase fraction curve as well as the time-G<sub>2</sub>-M phase fraction curve of the etoposide+AG825-treated cells paralleled those of the etoposide-treated cells, with a marked progress delay of the etoposide+AG825-treated cells. In the presence of AG825, no reduction of the etoposide-induced G<sub>2</sub> arrest could be detected in these two cell lines. Our findings indicated that AG825 (40 μM) had no effect on the etoposide-induced G<sub>2</sub> arrest, irrespective of the level of p185<sup>neu</sup> expressed by the cells.

**DISCUSSION**

The chemosensitivity of neoplastic cells to DNA-damaging agents may depend on the fidelity of cell cycle checkpoints (30). DNA damaged cells delay cell cycle progress at checkpoints to allow DNA repair (31). Evidence revealed that p53 is involved in the control of the G<sub>1</sub>-S checkpoint. Following DNA damage, p53 acts to suppress growth by inducing cell cycle arrest at G<sub>1</sub> while the cell attempts...
DNA repair or to promote apoptosis if optimal repair is impossible (32, 33). In lung cancer, introduction of the wild-type p53 gene into a human p53-null NSCLC cell line was able to enhance chemosensitivity (34). Cell cycle arrest in G2 for DNA repair is also a universal response to DNA damage (16, 17). Nevertheless, a much longer G2 phase in the presence of severe DNA damage may lead to cell death. Cells with normal p53 genes usually respond to DNA damage by a delay in both G1 and G2 phases, whereas tumor cells with deficient p53 arrest only at G2 (32, 35, 36). Caffeine has been shown to override cell cycle checkpoints, particularly the G2-M checkpoint (14, 15). We have reported previously that caffeine enhances the chemosensitivities of NSCLC cells that overexpress p185<sup>nu</sup> to a greater degree as compared to low-p185<sup>nu</sup> expressing cells (13). Mutation of the p53 gene was a common event (14 of 16) in the cell lines selected for the study. This finding led us to propose that high-p185<sup>nu</sup> expressing, chemoresistant NSCLC cells might utilize the repair machinery at the G2-M checkpoint more effectively.

In the present study, we have selected the cell lines with defective p53 and examined the modulation of the action of DNA-damaging agents by the selective p185<sup>nu</sup> inhibitor, tyrphostin AG825 (19, 20). Our results show that treatment with AG825 reduces the levels of p185<sup>nu</sup>. In the presence of etoposide, the negative regulation of AG825 was augmented and behaved in a dose-dependent fashion in the high-p185<sup>nu</sup> expressing H1355 cell line. By testing the effects of AG825 against six NSCLC cell lines that expressed minimal EGF receptor protein (data not shown), we show that AG825 alone or in combination with doxorubicin, etoposide, or cisplatin has enhanced cytotoxic effects against high-p185<sup>nu</sup> expressing cell lines as compared to low-expressing lines (Fig. 3). The magnitude of AG825-induced enhancement parallels the level of p185<sup>nu</sup> expression. AG825 had little effect on the cytotoxicities of DNA-damaging agents on the low-p185<sup>nu</sup> expressors, with a few exceptions in which minor antagonistic effects were observed (Fig. 4). We also found that high concentrations of AG825 were able to reduce the etoposide-induced G2 arrest, which was accompanied by the activation of p34<sup>cdc2</sup>-<sup>2</sup>. However, this effect was not p185<sup>nu</sup> specific. On the other hand, AG825 at low concentrations markedly retarded the etoposide-induced S-phase delay, whereas it had no definite effect on the etoposide-induced G2 arrest in both high- and low-p185<sup>nu</sup> expressing cell lines. It seems, therefore, that p185<sup>nu</sup> is not involved in the phosphorylation regulation of p34<sup>cdc2</sup> kinase during drug exposure.

The finding that AG825 enhances chemosensitivity of high-p185<sup>nu</sup> expressing cells further supports the view that p185<sup>nu</sup> plays a role in determining the chemoresistance of NSCLC cells. Although the mechanism by which p185<sup>nu</sup> confers multiple chemoresistance remains unclear at this time, several lines of evidence have emerged to...

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**Figure 5.** Effects of 24 h treatment of etoposide alone (0.1—10 µM; A and B) and AG825 (10—80 µM) in the absence (C and D) or concurrent presence (E and F) of etoposide (3 µM) on the cell cycle fractions of the asynchronous H1299 (upper panels) and H1355 (lower panels) cell lines (see "Materials and Methods"). Etoposide were calculated by using the etoposide combination effects of AG825 plus etoposide versus cycle fractions were calculated by using the cells with no tested agent exposure as control (C and D). The etoposide-induced G1 arrest could be detected in these two lines (A and B). The effects of AG825 on the cell cycle fractions were calculated by using the cells with no tested agent exposure as control (E and F). Results were the mean values of three separate experiments; bars, SE.

**Figure 6.** Effects of 24-h treatment of AG825 on cell cycle-regulated protein p34<sup>cdc2</sup> in etoposide-induced G2 arrest cells. After being plated for 24 h, asynchronous H1299 (A) and H1355 (B) cells were treated without (−) or with etoposide 3 µM (+). AG825 at a concentration of 0, 10, 20, 40, 60, 80, or 100 µM was added immediately before the addition of etoposide, and the incubation concurrently continued for 24 h. Then cell lysates (100 µg/lane) were immunoblotted with anti-p34<sup>cdc2</sup> monoclonal antibody.

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**Table 1.** Cell cycle fractions of asynchronous H1299 (A) and H1355 (B) cells treated without (−) or with etoposide 3 µM (+) addition of etoposide, and the incubation concurrently continued for 24 h. Then cell lysates (100 µg/lane) were immunoblotted with anti-p34<sup>cdc2</sup> monoclonal antibody.
suggest that high levels of p185_{neu} promote DNA repair (13, 37–39). Two recent reports (38, 39) have shown that p185_{neu}-specific antibodies may enhance the cytotoxicity of cisplatin against the high-p185_{neu} expressing human breast and ovarian cancer cells by interfering with DNA repair. We have demonstrated that caffeine (13) and gemcitabine (37) enhance the chemosensitivities to a greater degree in high- than in low-p185_{neu}-expressing NSCLC cells. Caffeine is known to enhance chemosensitivity of cancer cells by shortening the time for DNA repair (14, 15, 18). Gemcitabine is a novel pyrimidine analogue that has been shown to be able to inhibit DNA synthesis and repair (40). It seems that high-p185_{neu} expressing cancer cells possess a more effective DNA repair system and that this survival advantage can be overcome by caffeine, gemcitabine, and p185_{neu}-specific antibodies, as well as p185_{neu}-specific tyrophostins. The possible role of p185_{neu} in modulating DNA repair is in accordance with the finding of its localization in the nucleus (41).

Drug resistance is one of the major obstacles to successful chemotherapy and is a major challenge for cancer researchers and therapists. At the time of diagnosis, NSCLC tumors usually are more refractory to chemotherapy than small cell lung cancer tumors. Aberration of the HER-2/neu gene has been detected in 30% or more of NSCLC (5–7). Because activation of the HER-2/neu gene may contribute to metastatic (42) and chemoresistant (8–10) malignant phenotypes and normal critical tissues show little or undetectable expression of the gene (43), the HER-2/neu gene and its protein product are attractive targets for therapeutic approach. Inhibition of the activity of p185_{neu} by p185_{neu}-specific tyrophostins, in combination with DNA-damaging agents, could be of potential clinical utility for the treatment of tumors that express high levels of p185_{neu}. Our findings provide a rationale to test p185_{neu}-specific, tyrophostin-containing regimens in vivo and in clinical trials. The p185_{neu}-specific tyrophostins can also be utilized to explore the mechanism that is responsible for the enhancement of chemoresistance in high-p185_{neu} expressing tumor cells.

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