Expression of Oncogenic Epidermal Growth Factor Receptor Family Kinases Induces Paclitaxel Resistance and Alters β-Tubulin Isotype Expression*

Received for publication, February 1, 2000, and in revised form, March 30, 2000
Published, JBC Papers in Press, April 3, 2000, DOI 10.1074/jbc.M000968200

R. Bruce Montgomery‡§, Junitta Guzman‡, Donald M. O'Rourke¶, and William L. Stahl

From the Departments of Pharmacy, University of Washington, Seattle, Washington 98108 and the Department of Neurosurgery, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Oncogenic transformation confers resistance to chemotherapy through a variety of mechanisms, including suppression of apoptosis, increased drug metabolism, and modification of target proteins. Oncogenic epidermal growth factor receptor family members, including EGFRvIII and HER2, are expressed in a broad spectrum of human malignancies. Cell lines transfected with EGFRvIII and HER2 are more resistant to paclitaxel-mediated cytotoxicity, and tubulin polymerization induced by paclitaxel is suppressed compared with cells expressing wild type epidermal growth factor receptor. Because differential expression of β-tubulin isotypes has been proposed to modulate paclitaxel resistance, we analyzed β-tubulin isotypes expressed in cell lines transfected with different oncogenes. EGFRvIII- and HER2-expressing cells demonstrated equivalent total β-tubulin protein compared with cells transfected with wild type receptor or untransfected controls. EGFRvIII-expressing cells demonstrated increases in class IVa (2.5-fold) and IVb (3.1-fold) mRNA, and HER2-expressing cells showed increases in class IVa (2.95-fold) mRNA. Expression of oncogenic Ha-Ras did not change class IV RNA levels significantly. Inhibition of EGFRvIII kinase activity using a mutant allele with an inactivating mutation in the kinase domain decreased expression of class IVa by 50% and partially reversed resistance to paclitaxel. Expression of oncogenic epidermal growth factor receptor family members is associated with modulation of both β-tubulin isotype expression and paclitaxel resistance in cells transformed by expression of the receptor. This effect on tubulin expression may modulate drug resistance in human malignancies that express these oncogenes.

De novo or acquired resistance to chemotherapeutic agents can be induced by increased clearance mediated by membrane transporter proteins or by modification of molecular targets. In human tumors, a combination of these mechanisms may be responsible for clinical tumor progression. The taxanes paclitaxel and docetaxel, which are used extensively in clinical oncology, stabilize microtubules by binding to the β-tubulin component of α/β-tubulin heterodimers, blocking cells in G2/M leading to cell death (1, 2). Modification of β-tubulin by mutation or differential expression of isotypes have been proposed as mechanisms for either de novo or acquired taxane resistance (3, 4). At least six β-tubulin isotypes have been identified, constituting five evolutionarily conserved isotype classes. These differ primarily in the carboxyl-terminal 15 amino acids (5). The conservation of distinct isotype classes has suggested that the different β-tubulins contribute unique functional properties to cell structure and function. Recent evidence suggests that these isotype classes vary in their paclitaxel binding affinities. Microtubules selectively enriched for isotype class III and IV β-tubulin are significantly more resistant to paclitaxel suppression of microtubule dynamics than are microtubules composed of unfractionated tubulin (6). Tumor cell lines rendered paclitaxel-resistant by exposure to high concentrations of drug also demonstrate substantial increases in class III and IV β-tubulin (7–9). Prostate carcinoma lines resistant to the tubulin-active agent estramustine demonstrate selective increases in class III and IV tubulin isotypes (10). Clearly, one response of tumor cells to in vitro selection with microtubule-stabilizing agents is to increase the proportion of taxane-resistant tubulin. Evidence for modulation of isotype expression by paclitaxel exposure is also found in patients treated with taxanes. Paired samples from patients with advanced ovarian cancer that developed clinical paclitaxel resistance showed increases in β-tubulin isotype classes I (3.6-fold), III (4.4-fold), and IVa (7.6-fold) (8).

The epidermal growth factor receptor (EGFR2 or erbB) family of receptor tyrosine kinases plays a role in oncogenic transformation in tissues where it is overexpressed or coexpressed with one of its ligands (11, 12). Transformation occurs by mutation, overexpression, and synergistic activation of different family members. Gene amplification and overexpression of intact or wild type (wt)EGFR have been found in a variety of epithelial and neuronal neoplasms including squamous cell carcinomas, melanoma, and glioblastoma multiforme and portend a poor prognosis for patients with these tumors (13–16). Overexpression of EGFR has been associated with a relative resistance to chemotherapy in cell lines and in patients with breast, ovarian, and esophageal cancer (17–21). HER2 is a closely related member of the EGFR family which is oncogenic when overexpressed in several cell contexts as a result of transactivation of HER2 complexes (22). HER2 plays a role in oncogenesis in breast, ovarian, gastric, and lung carcinoma (23). A

* This work was supported by Veterans Affairs Merit Review and National Institutes of Health Grant CA60782 (to R. B. M. and W. L. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom reprint requests should be addressed: Oncology Division, Veteran Affairs Puget Sound HCS (111ONC), 1660 S. Columbian Way, Seattle, WA 98108. Tel.: 206-764-2709; Fax: 206-764-2851; E-mail: rbmontgo@u.washington.edu.

¶ The abbreviations used are: EGFR, epidermal growth factor receptor, wtEGFR, wild type EGFR, PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR.

1 The abbreviations used are: EGFR, epidermal growth factor receptor, wtEGFR, wild type EGFR, PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR.
EGFR Modulation of Tubulin Isotypes

mutant EGFR with constitutive kinase activity has been identified in a variety of human tumors (24). This mutant, referred to as EGFRVIII (also EGFRM, Δ EGFR, de-2-7 EGFR), activates signaling through multiple pathways and acts as a dominant oncogene when overexpressed (25–29). EGFRVIII also reduces spontaneous apoptosis when transfected into glioblastoma cell lines, suggesting that it may play a significant role in protecting tumor cells from drug-induced cytotoxicity (30). Activation of mutant EGFRVIII is ligand-independent and recapitulates the chronic autocrine or paracrine stimulation of EGFR in tissues that overexpress EGFR. Because of its broad prevalence and likely clinical significance in human tumors, we wished to determine the effects of EGFRVIII and the related oncoprotein HER2 on chemotherapy-induced cytotoxicity and potential mechanisms of resistance to these agents. This report demonstrates that expression of constitutively active, oncogenic EGFR and HER2 induce paclitaxel resistance and increase class IVa and IVb β-tubulin RNA. This modulation of β-tubulin isotype expression may demonstrate one mechanism by which oncogenic transformation mediates intrinsic resistance to paclitaxel in tumors that overexpress these oncogenes.

EXPERIMENTAL PROCEDURES

Materials—Antibodies to EGFRVIII were kindly provided by Albert Wong, Kimmel Cancer Center, and were prepared as described (31). Enhanced chemiluminescence (ECL) reagents and pan-β-tubulin antibody (N357) were from Amersham Pharmacia Biotech. Antibodies to β2 were from Santa Cruz Biotechnology. Antibody to HER2 was from Transduction Laboratories (E19420). Precast SDS-PAGE gels were from NOVEX. GeneAmp PCR amplification kits were from PerkinElmer, and Taq polymerase was from Promega. Paclitaxel and α-tubulin antibody were from Sigma.

Cell Culture—HC2 20d2c (HC2) and CO12 20d2b (CO12) were generated from transfected NIH3T3 and maintained as described (27). NIH3T3, HER2-expressing, and Ras (Val-12)-transfected NIH3T3 were provided by Dr. Michael Lilly, University of Washington. H(T96) were derived by transfection of HC2 cells with the HER2/new T691stop mutant as described previously (32, 33). All cell lines were maintained in Dulbecco’s modified Eagle’s medium, 10% fetal calf serum, 200 units/ml penicillin, 200 μg/ml streptomycin, HC2 and CO12 medium also contained 250 μg/ml G418, and HC2 and T96 (HC2) cells were selected and maintained in 13 μM aminopterin.

Paclitaxel Cytotoxicity Assay—2 × 106 cells were plated on day 0, grown for 1 day, and exposed to 0–100 nM paclitaxel for 4 days. Cells were trypsinized and assayed for viability by trypan blue staining. Triplicate flasks were counted for each concentration.

Tubulin Polymerization Assay—Quantitation of tubulin polymerization was carried out essentially as described previously with minor modifications (4). Cells were grown to confluence in 25-cm2 flasks, and monolayers were washed twice with phosphate-buffered saline and lysed at 37 °C in the dark with 300 μl of 20 mM Tris-HCl (pH 6.8), 1 mM MgCl2, 2 mM EGTA, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, leupeptin, and soybean trypsin inhibitor, normalized for protein concentrations, and resolved by SDS-PAGE on 8–16% gels. Proteins were transferred to nitrocellulose and incubated with 5% nonfat dry milk for 1 h followed by immunoblotting antibody in the same solution for 4 h. Filters were extensively washed in TBS, 0.05% Tween 20, and bound antibodies were detected by addition of conjugated protein A-horseradish peroxidase at 1:5000 for 1 h followed by ECL following the manufacturer’s directions. Experiments were performed at least 3 times and representative data are shown.

RESULTS

Oncogenic EGFR Expression Induces Resistance to Paclitaxel Cytotoxicity—HER2 and EGFRVIII undergo ligand-independent autophosphorylation, mediating oncogenic signaling and transformation. Growth factor receptor stimulation may circumvent drug-induced apoptosis and cytotoxicity. As a model system that mimics these effects, we determined the effect of EGFR expression on sensitivity to paclitaxel in transfected fibroblast cell lines. Cell lines were exposed to 0–100 nM paclitaxel, and viability was determined after 4 days of exposure. Table I demonstrates the effect of the different EGFR constructs on sensitivity to paclitaxel. Cells expressing the EGFR mutant (HC2) and HER2 were 2–3-fold more resistant to paclitaxel compared with the parental cell line. This level of resistance is modest compared with that induced by selection in tubulin-active agents (8, 10, 34), but it correlates well with other parameters of oncogenicity associated with expression of EGFRVIII, such growth in serum-free medium (27, 36).

Paclitaxel-induced Tubulin Polymerization Is Reduced in Cells Expressing EGFRVIII and HER2—The major mechanism of paclitaxel cytotoxicity is stabilizing microtubule arrays that blocks effective cell division and growth. Because both EGFRVIII and HER2 induce tubulin polymerization, we measured paclitaxel-induced polymerization activity using a cell-free assay system. This assay separates measurement of polymerization from cell-mediated functions such as membrane transport (4). In the absence of paclitaxel, tubulin is relatively evenly distributed between soluble and insoluble forms in extracts derived from all cell lines (Fig. 1). With the addition of

| Transfectant | Paclitaxel ED50 | Vincrewine ED50 |
|--------------|----------------|-----------------|
| NIH3T3       | 12             | 11              |
| wtEGFR (CO12)| 12.5           | 10              |
| EGFRVIII (HC2)| 40            | 14              |
| HER2         | 23             | 9               |

TABLE I

Paclitaxel cytotoxicity for transfected NIH3T3 cells

NIH3T3, CO12 (wt EGFR-expressing 3T3), HER2, and HC2 (EGFRRVIII-expressing 3T3) cells were grown for 1 day and exposed to paclitaxel (0–100 nM) for 4 days. Cell viability was quantitated by trypan blue staining of cells. Data were expressed as IC50, the drug concentration that inhibits cell number by 50% after 4 days. Average cell counts at 4 days for controls were NIH3T3, 2.3 × 106; CO12–2.51 × 106; HER2, 1.73 × 106; HC2, 2.94 × 106.
wtEGFR-expressing cells showed similar amounts of HER2, and HC2 were grown to confluence and lysed in 400 μl of lysis buffer. The supernatants (SN) and pellets were mixed with equal amounts of sample buffer and boiled, and 30 μl of lysate was separated on 7.5% SDS-PAGE, transferred to nitrocellulose, and probed with anti-α-tubulin antibodies.

\begin{table}[h]
\centering
\caption{EGFR Modulation of Tubulin Isotypes}
\begin{tabular}{|c|c|c|c|}
\hline
Tubulin isotype class & Gene & Forward primer & Reverse primer & Product size (bp) \\
\hline
β\textsubscript{b} & Mβ\textsubscript{b} & 5’-CGGTACCTACATGGAACC-3’ & 5’-AGAGATTCGTCGTCGTC-3’ & 194 \\
β\textsubscript{b} & Mβ\textsubscript{b} & 5’-CAAGACCCACCCGCTCTC-3’ & 5’-TCCTCTCCGCGTCGTC-3’ & 171 \\
β\textsubscript{b} & Mβ\textsubscript{b} & 5’-AGAGACGCGCAATCGTGTC-3’ & 5’-GGCTAAATGGGGAGGACACG-3’ & 164 \\
β\textsubscript{b} & Mβ\textsubscript{b} & 5’-GCTGTCCGCGAGTGGCC-3’ & 5’-CGCTATCTGAGTGTTAGTG-3’ & 157 \\
β\textsubscript{b} & Mβ\textsubscript{b} & 5’-TTCACTACAGGCCACACGG-3’ & 5’-GAGAGTCTACTGATCCCATGA-3’ & 145 \\
β\textsubscript{g} & Mβ\textsubscript{g} & 5’-ATTCCCCCAGACTGAGACTG-3’ & 5’-CTCGATCCGAGAGCAGTGTC-3’ & 86 \\
\hline
\end{tabular}
\end{table}

paclitaxel at 0.2–0.4 μg/ml, the amount of soluble tubulin decreases substantially with a proportionate increase in insoluble tubulin in extracts from cells expressing wtEGFR. In contrast, there was no apparent increase in tubulin polymerization in cells expressing EGFRvIII or HER2, demonstrating diminished sensitivity to paclitaxel microtubule stabilization.

\textit{β-Tubulin Isotype Expression Is Modulated by Expression of Oncogenes}—The observed resistance to paclitaxel-induced polymerization in cells expressing EGFRvIII could be due to changes in total tubulin levels, to mutation in the tubulin molecule itself, or to changes in the relative proportions of paclitaxel-sensitive β-tubulin isotypes (3, 7, 37). To distinguish among these possibilities, total β-tubulin protein was analyzed from NIH3T3 cell lines expressing wtEGFR, EGFRvIII, Ras, and HER2. Total β-tubulin levels differed no more than 15% among the different cell lines (Fig. 2). In order to estimate the quantitative contribution of distinct β-tubulin isotypes to the total protein levels, we performed quantitative PCR assays. Relative RNA levels for class I-IVb were determined using RT-PCR with primers previously described by Haber et al. (34).

Tubulin isotype RNA levels were normalized to PCR products for β\textsubscript{2}-microglobulin and 18 S RNA in each gel. Relative ratios of amplified products to each of these standards were similar for β-tubulin isotypes among the different cell lines. The parental NIH3T3- and wtEGFR-expressing cells showed similar amounts of β-tubulin I-IVb with very low levels of class III (Fig. 3, data for type III not shown). EGFRvIII-expressing cells showed a 2.5-fold increase in class IVa and a 3.1-fold increase in class IVb compared with cells expressing wtEGFR. HER2 expression resulted in a 2.95-fold increase in IVa and 2.1-fold increase in IVb. Ras expression induced insignificant changes compared with the parental line.

\textit{Expression of EGFRvIII and HER2 Does Not Affect p21\textsuperscript{Cip1} Expression}—Yu et al. (38) have recently shown that taxane resistance of HER2-expressing cells correlates with increased expression of p21\textsuperscript{Cip1}. Suppression of p21 production blocked resistance to taxane, suggesting that this may be a general mechanism of taxane resistance in HER2-expressing cells. To test this hypothesis in our experimental system, we determined levels of p21 in immunostaining of NIH3T3 cells expressing EGFR family members and Ras proteins. As shown in Fig. 4 and in contrast to the results of Yu et al. (38), expression of oncogenic EGFR family members did not alter p21\textsuperscript{Cip1} expression in this specific cell type.

\textit{Inhibition of EGFRvIII Kinase Activity Suppresses Class IV Tubulin Production}—EGFR signaling is not mediated exclusively by tyrosine kinase activity since various downstream kinases may be activated despite functional inactivation of the EGFR kinase domain (39). To determine whether kinase activity is important to induction of taxane resistance, HC2 cells, expressing EGFRvIII, were transfected with a HER2/neu construct, T691stop, which contains an inactivating mutation in the kinase domain (32, 33). This mutant HER2 construct inhibits EGFRvIII signaling and kinase activity as a result of dimerization of T691stop with EGFRvIII (33). The resultant doubly transfected cells, designated H(T691), were analyzed for expression of EGFRvIII, the 115-kDa T691 protein construct, and for effects on EGFRvIII autophosphorylation (Fig. 5A).

H(T691) cells maintained their expression of EGFRvIII and demonstrated significant expression of the T691 protein; however, autophosphorylation of EGFRvIII was suppressed, reflecting decreased kinase activity. H(T691) cells were then tested for paclitaxel sensitivity in the same fashion as was used to generate results shown in Table I. In these assays the ED\textsubscript{50} for HC2 was 40 nm, whereas the ED\textsubscript{50} for H(T691) was 20 nm. H(T691) cells demonstrate decreased resistance to paclitaxel and decreased expression of the paclitaxel-resistant β-tubulin class IVa (Fig. 5B), although levels of IVb were not affected (data not shown). This suggests that induction of tubulin isotype IVa is a direct result of kinase activity, and induction of isotype IVb is not kinase-dependent. The constitutive kinase activity of EGFRvIII is responsible for induction of paclitaxel resistance and increase in class IV tubulin expression.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig1.png}
\caption{\textbf{Fig. 1.} Paclitaxel effect on tubulin polymerization. CO12, HER2, and HC2 were grown to confluence and lysed in 400 μl of lysis buffer. The supernatants (SN) and pellets were resuspended in 300 μl of lysis buffer. The amount of soluble tubulin was separated on 7.5% SDS-PAGE, transferred to nitrocellulose, and probed with anti-α-tubulin antibodies.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig2.png}
\caption{\textbf{Fig. 2.} β-Tubulin expression in transfected cell lines. 40 μg of cell lysates from NIH3T3, CO12, HC2, Ras, and HER2 expressing 3T3 cells were separated on 10% SDS-PAGE, transferred to nitrocellulose, and probed with pan-β-tubulin antibody.}
\end{figure}
DISCUSSION

Resistance to antineoplastic agents can be mediated by many different mechanisms, including differential uptake and retention of drug controlled by membrane transporter proteins (1). The expanding use of taxanes in clinical practice has made the ability to predict primary or secondary resistance to these agents a potentially important aspect to treatment planning. Although de novo resistance is relatively rare, some malignancies will exhibit intrinsic resistance to taxanes, and the majority of patients with advanced disease will ultimately develop progressive disease despite continued therapy. Overexpression of EGFR, EGFRvIII, and HER2 is found in ovarian and breast carcinomas, tumors that are perhaps the best defined indications for taxane therapy (1, 24). EGFR and HER2 overexpression is inversely correlated with response to chemotherapy and prognosis, and resistance is often accompanied by an increase in receptor expression (24, 40–42). Poor response rates could result from intrinsic drug resistance mediated by these oncogenes.

We analyzed the effect of EGFRvIII and HER2 expression on cell sensitivity to paclitaxel, since these oncogenes induce constitutive signals for proliferation and transformation. Both receptors confer relative resistance to paclitaxel and confer resistance to tubulin polymerization. RT-PCR assays of RNA levels suggest that expression of EGFRvIII increases levels of class IVa and IVb 3-fold. The changes in isotype class IV are consistent with studies previously demonstrating an increase in type III or IV tubulin expression associated with paclitaxel resistance (7–9). Since class III and IV tubulins are relatively resistant to paclitaxel-induced stabilization of microtubules,
cells expressing these isotypes should have a selective growth advantage. The majority of published studies have demonstrated increases in IVa class rather than IVb, although there is no evidence that these isotypes differ in their sensitivity to paclitaxel (8–10). Conditional overexpression of IVb in Chinese hamster ovary cells by Blaude et al. (43) did not induce paclitaxel resistance, suggesting that IVb levels do not mediate taxane resistance, at least in the cell system used in their study. In our studies, the failure to suppress isotype IVb following transfection with the T691 mutant also suggests that expression of different EGFR family members combined with assay of isotype expression on paclitaxel resistance comes from the use of antisense oligonucleotides to inhibit production of specific antisense oligonucleotides specific to isotype class III β-tubulin are capable of blocking class III expression while reversing taxane resistance in drug-resistant lung carcinoma cells (53). Extending these studies through transient expression of EGFR family members combined with assay of isotype expression and taxane resistance will be required to confirm the general relevance of these findings. More detailed characterization of tumors from patients that overexpress oncogenic EGFR and correlation of isotype expression with clinical drug resistance will be important correlates to this work.

Acknowledgment—We thank Dr. William Schubach for careful reading and commentary on the manuscript.

REFERENCES

1. Goldspiegl, B. R. (1997) Pharmacotherapy 17, 110–125
2. Horvitz, S. B. (1994) Ann. Oncol. 5, 3–6
3. Giannakakou, P., Sackett, D. L., Kang, Y. K., Zhan, Z., Buters, J. T., Pojis, J., and Forouheshy, M. S. (1997) J. Biol. Chem. 272, 17118–17125
4. Minatti, A. M., Barlow, S. B., and Cabrini, F. (1991) J. Biol. Chem. 266, 3937–3994
5. Joshi, H. C., and Cleveland, D. W. (1990) Cell Motil. Cytoskeleton 16, 159–163
6. Derry, W. B., Wilson, L., Khan, I. A., Luduena, R. P., and Jordan, M. A. (1997) Biochemistry 36, 3554–3562
7. Jaffezo, J., Dumontet, C., Derry, W. B., Duran, G., Chen, G., Tsuchiya, E., Wilson, L., Jordan, M. A., and Sikic, B. I. (1995) Oncol. Res. 7, 517–527
8. Kavallaris, M., Kuo, D. Y. S., Burkhardt, C. A., Reit, D. L., Norris, M. D., Haber, M., and Horwitz, S. B. (1997) J. Clin. Invest. 100, 1282–1293
9. Ranganathan, S., Benetatos, C. A., Colarussso, P. J., Dester, D. W., and Hudes, G. R. (1998) Br. J. Cancer 77, 562–566
10. Ranganathan, S., Dester, D. W., Benetatos, C. A., Chapman, A. E., Tew, K. D., and Hudes, G. R. (1996) Cancer Res. 56, 2584–2589
11. Knaus, B., Schirmacher, V., and Lichtner, R. B. (1993) Cancer Metastasis Rev. 12, 255–274
12. Fiore, P. P., Derry, W. B., Reid, E., Flemmig, T. P., Hazan, R., Ullrich, A., King, C. R., Schlessinger, J., and Aarson, S. A. (1987) Cell 51, 1063–1070
13. Hirono, Y., Togawa, K., Fushida, S., Ninomiya, I., Yoneuma, Y., Miyazaki, I., Endou, Y., Tanaka, M., and Sasaki, T. (1995) Oncology 52, 182–188
14. Yes, M. R., Moosy, J., Donovan-Peluco, M., and Locker, J. (1992) J. Neuropathol. Exp. Neurol. 51, 44–40
15. Schlegel, J., Merdes, A., Stum, G., Albert, P. K., Forsting, M., Hynes, N., and Kiiellin, M. (1994) Int. J. Cancer 56, 72–77
16. Veale, L., Kerr, N., Gibson, G. J., Kelly, P. J., and Harris, A. L. (1993) Br. J. Cancer 68, 162–165
17. van Dam, P. A., Vergote, I. B., Lowe, D. G., Watson, J. V., van Damme, P., van der Auwera, J. C., and Shepherd, J. H. (1994) J. Clin. Pathol. 47, 914–919
18. Scambia, G., Benedetti-Pichler, M., Ferradina, D., Distefano, M., Salerno, G., Romanini, M. E., Fogotti, A., and Mancuso, S. (1995) Br. J. Cancer 72, 361–366
19. Porter-Jordan, K., and Lippman, M. E. (1994) Hematol. Oncol. Clin. N. Am. 8, 73–100
20. Long, B., McKibbon, B. M., Lynch, M., and van den Berg, H. W. (1992) Br. J. Cancer 65, 865–869
21. Dassonville, O., Formento, J. L., Francoual, M., Ramaiali, A., Santini, J., Schneider, M., Demard, F., and Milan, G. (1995) J. Clin. Oncol. 11, 1873–1878
22. Fiore, P. P., Derry, W. B., Kraus, M. H., Segatto, O., King, C. R., Schlessinger, J., and Aarson, S. A. (1987) Science 237, 178–182
23. Hung, M. C., and Lai, Y. L. (1999) Semin. Oncol. 26, 51–59
24. Moscatello, D. K., Montgomery, R. B., Sundaresan, P., McDaniel, H., Wong, M. Y., and Wong, A. J. (1999) Oncogene 14, 85–96
25. Moscatello, D. K., Montgomery, R. B., Sundaresan, P., McDaniel, H., Wong, M. Y., and Wong, A. J. (1996) Oncogene 13, 85–96
26. Moscatello, D. K., Montgomery, R. B., Duffield, D. B., and Shalit, W. L. (1997) Biochem. Biophys. Res. Commun. 232, 111–116
27. Moscatello, D. K., Montgomery, R. B., Sundaresan, P., McDaniel, H., Wong, M. Y., and Wong, A. J. (1996) Oncogene 13, 85–96
28. Romanini, M. E., Fagotti, A., and Mancuso, S. (1995) J. Biol. Chem. 270, 2927–2935
29. Chao, X., O’Rourke, D. M., Zhao, H., and Greene, M. I. (1996) Oncogene 13, 2149–2157
30. O’Rourke, D. M., Ade, E. J., Davis, J. G., Wu, C., Lee, A., Murali, R., Zhang, H. T., Xian, Q., Xian, C. O., and Greene, M. I. (1996) Oncogene 16, 1187–1207
31. Haber, M., Burkhardt, C. A., Reit, D. L., Madafiglio, J., Norris, M. D., and Horwitz, S. B. (1995) J. Biol. Chem. 270, 32169–32175
32. Ceresney, N. (1987) Anal. Biochem. 162, 156–159
33. Kavanagh, K., Long, B., O’Rourke, D. M., and Sikic, I. (1996) Cell Motil. Cytoskeleton 35, 49–58
34. Yu, D., Jing, T., Liu, B., Yao, J., Tan, M., McDonnell, T. J., and Hung, M. C. (1998) Mol. Cell. 2, 581–591
35. Wright, J. D., Reuter, C. W., and Weber, M. J. (1995) J. Biol. Chem. 270, 12085–12093
36. Fischer-Colbrie, J., Witt, A., Heinzl, H., Speiser, P., Czerwenka, K., Seveda, P., and Zeilinger, R. (1997) Anticancer Res. 17, 613–619
EGFR Modulation of Tubulin Isotypes

41. Klijn, J. G., Berns, E. M., and Foekens, J. A. (1993) Cancer Surv. 18, 165–198
42. Nicholson, S., Sainsbury, J. R., Halcrow, P., Chambers, P., Farndon, J. R., and Harris, A. L. (1989) Lancet 1, 182–185
43. Blade, K., Menick, D. R., and Cahal, F. (1999) J. Cell Sci. 112, 2213–2221
44. Sisodia, S. S., Gay, D. A., and Cleveland, D. W. (1990) New Biol. 2, 66–76
45. Kremser, T., Guzewski, K., Schulz, R. A., and Renkawitz-Pohl, R. (1999) Dev. Biol. 216, 327–339
46. Jinno, Y., Merlino, G. T., and Pastan, I. (1988) Nucleic Acids Res. 16, 4957–4966
47. Yen, T. J., Gay, D. A., Pachter, J. S., and Cleveland, D. W. (1988) Mol. Cell. Biol. 8, 1224–1235
48. Bachurski, C. J., Theodorakis, N. G., Coulson, R. M., and Cleveland, D. W. (1994) Mol. Cell. Biol. 14, 4076–4086
49. Theodorakis, N. G., and Cleveland, D. W. (1992) Mol. Cell. Biol. 12, 791–799
50. Serrano, N., Brock, H. W., and Maschat, F. (1997) Development 124, 2527–2536
51. Narisige, T., Blade, K. L., Ishibashi, Y., Nagai, T., Humawaki, M., Menick, D. R., Kuppuswamy, D., and Cooper, G., IV (1999) J. Biol. Chem. 274, 9692–9697
52. Oblinger, M. M., and Kast, S. A. (1994) Brain Res. Dev. Brain Res. 77, 45–54
53. Kavallaris, M., Burkhart, C. A., and Horwitz, S. B. (1999) Br. J. Cancer 80, 1020–1025