Abstract

The prognostic significance of epidermal growth factor receptor (EGFR) expression in lung cancer and, more importantly, its ability to predict response to anti-EGFR therapies, are currently subjects of active research. In a meta-analysis, EGFR overexpression confirmed a worse prognosis (HR 1.13) in eight studies using immunohistochemistry, although cutoff values were generally selected arbitrarily by investigators. Most applied clinical research on the EGFR has been focused on the overexpression of the receptor, whereas less research has addressed the potential role of other mechanisms of increased signaling or of nonmembrane-bound events. The emerging concept of EGFR signaling reveals a multilayered network that allows for horizontal interactions and permits multiple combinatorial responses that may explain the specificity of cellular outcomes to receptor activation. New technologies such as nucleotide arrays and proteomics will help to elucidate the issue by providing information on how EGFR signaling may affect the expression of genes and proteins in cancer cells.

INTRODUCTION

The family of epidermal growth factor receptors (EGFR; HER1, HER2/neu, HER3, and HER4) includes cell membrane receptors with intrinsic tyrosine kinase activity that trigger a cascade of biophysiological signaling reactions in response to the binding of different ligands. These receptors play a key role in the behavior of malignant cells in a variety of human tumors, inducing increased proliferation, decreasing apoptosis, and enhancing tumor cell motility and angiogenesis.

EGFR in Bronchial Preneoplastic Tissues.
Some studies have investigated EGFR expression in bronchial preneoplastic lesions (1, 2, 3, 4). In normal bronchial mucosa, as well as in hyperplastic and metaplastic areas, EGFR is significantly expressed in the basal cell layer, whereas there is no EGFR expression in the luminal cells. Increased EGFR expression in metaplastic tissue compared with normal mucosa has been reported (2), and Piythilake et al. (4) demonstrated a statistically significant stepwise increase in EGFR expression from normal bronchial mucosa to epithelial hyperplasia to cancer, suggesting a progressive EGFR involvement in lung carcinogenesis (Fig. 1). In contrast, no significant increase was observed for HER2, indicating a less significant involvement in lung carcinogenesis.

![Histology vs. EGFR](http://clincancerres.aacrjournals.org/content/10/12/4227s#page2)

**Fig. 1.**

Epidermal growth factor receptor (EGFR) expression in bronchial epithelium. EGFR score: 1 = normal EGFR expression, 2 = significant overexpression (detailed in Ref. 5). Histology scores 1 and 2 = normal and basal cell hyperplasia; 3 and 4: metaplasia and mild dysplasia; 5 and 6: moderate-and severe dysplasia; 7 and 8: carcinoma in situ and invasive cancer.

**EGFR Expression in Lung Tumors.**

The epidermal growth factor receptor is commonly overexpressed in non-small cell lung cancer (NSCLC; reviewed in Ref. 5) and head and neck cancers (6). In NSCLC, overexpression ranges from 43% to 89%. The differences in reported EGFR expressions from one study to another most likely reflect differences in the assessment techniques, definition of the level of overexpression, and differences in the study populations. It is well known that overexpression is more likely seen in squamous cell carcinoma (70%) followed by adenocarcinoma (50%) and, to a lesser extent, large cell carcinoma. EGFR expression is rare in small cell lung cancer. There is increasing evidence for an especially high EGFR expression in bronchoalveolar carcinoma.
one preliminary study has demonstrated a significant correlation between nonmucinous BAC histology and EGFR expression, whereas mucinous BAC histology was more frequently related to HER2 overexpression (8).

The mechanism responsible for EGFR overexpression is largely unknown, and gene amplification is only rarely observed in NSCLC (9). Gene copy numbers and protein status of EGFR were investigated recently in microarrayed tumor tissue from 183 NSCLC patients. Protein expression was assessed by immunohistochemistry (IHC) on a scale from 0 to 400 (percentage of positive cells × staining intensity), and EGFR gene and chromosome 7 copy numbers were identified by fluorescent in situ hybridization. The prevalent fluorescent in situ hybridization patterns were balanced disomy (40%) and trisomy (38%) for EGFR gene and chromosome 7 (40%), whereas balanced polysomy was seen in 13% and gene amplification was seen in 9% of the patients. Gene copy number correlated with protein expression ($r = 0.4; P < 0.001$; Ref. 7).

**EGFR as a Prognostic Factor in NSCLC.**

The lack of a consistent method of evaluating levels of EGFR has caused a disparity in reports of the EGFR as a prognostic factor; Table 1<ref> summarizes the most important studies in which EGFR and other members of epidermal growth factor family of receptors or ligands were investigated by IHC (10, 11, 12, 13, 14). For some tumors, EGFR is a strong prognostic indicator associated with a more aggressive disease and reduced survival (6).

A meta-analysis published recently (15) compared 11 studies involving >2000 patients. IHC was the most frequently used method: a wide range of monoclonal antibodies was used with different dilutions as well. In the eight studies using IHC, EGFR overexpression confirmed a worse prognostic significance (HR 1.13), although cutoff values usually result from arbitrary choices by investigators. In the review by Nicholson et al. (16), EGFR overexpression confirmed its prognostic value in multiple tumor types, but evidence was weaker in NSCLC. However, the true prognostic significance of EGFR might be underestimated by the fact that in the published studies EGFR is assessed as total cellular level rather than its activated form, which is probably the only one affecting prognosis (16, 17). An additional bias is the lack of standardized cutoff points of normal receptor levels and the inclusion of both early and late disease stages in the patient population.

The heterogeneity of available reports could also be explained by differences in interpreting the intensity of expression and the localization of receptors and by the wide range of methods in use for EGFR detection. IHC relies on subjective judgment, which represents an intrinsic limitation of the technique: with IHC some authors have reported only cell membrane staining (18) as opposed to cytoplasmic staining (11), whereas others did not report any preferential localization of the receptor (19). As already seen from HER2/neu studies (20, 21), differences in results may arise by using IHC, fluorescent in situ hybridization, or mRNA expression. Even a quantitative detection cannot fully define the impact of EGFR in driving tumor proliferation in vivo. However, by using real-time PCR measuring mRNA, a prognostic implication of EGFR and HER2/neu expression in NSCLC.
has been reported (22) . Standardization of techniques to determine EGFR overexpression must, therefore, become a priority in the near future; IHC remains in our opinion currently the best choice for routine clinical use, even if a universal scoring system is still needed to better compare research results. The role of automated computerized analysis of the IHC results needs to be validated in the future.

**Biology of EGFR at the Input Layer.**

Under physiological conditions, a variety of EGFR family ligands drive the formation of homo- or heterodimeric complexes among the four ErbB receptors, which provide signal amplification and diversification. NSCLC tumors have also been demonstrated to synthesize transforming growth factor α and amphiregulin, which are ligands for EGFR. These growth factors seem to form an autocrine feedback loop with EGFR and, as a result, play an important role in tumorigenesis (12, 23) . Among EGFR-positive primary lung adenocarcinomas, overall survival was significantly worse for patients with high levels of transforming growth factor α compared with EGFR-positive cancers that were negative for transforming growth factor α (24).

Whereas the prognostic role of EGFR expression in lung tumors remains a matter of debate, and no correlation has yet been found between EGFR overexpression and sensitivity to anti-EGFR therapies, as evaluated by changes in intracellular downstream signaling pathways, the relationship in the tumors among coexpression of EGFR, its physiological ligands, and the identification of a subset of patients that potentially may benefit more from anti-EGFR therapies has not been yet substantially considered.

The ability of ErbB ligands to induce not only receptor homodimers but also receptor heterodimers expands the ErbB signaling potential. The most common partner of EGFR for heterodimerization is HER2: the available evidence strongly supports EGFR-HER2 cross-talk in vivo and high expression of HER2 as mechanisms that can potentiate EGFR signals and EGFR-mediated tumor progression by increasing epidermal growth factor binding affinity, stabilizing and recycling EGFR/HER2 heterodimers, and expanding the repertoire of receptor-associated substrates and signaling responses (25, 26). In the laboratory, EGFR/HER2 heterodimerization has been shown to induce a stronger and more sustained proliferative signal than EGFR homodimers (27). Interestingly, lung cancers that coexpress EGFR and HER2 appear to possess more virulent clinical behavior (22). On the basis of recent clinical trial results, the significance of these biological findings has been reviewed recently (5, 28).

The best-described and most common ErbB1 mutation, EGFRvIII, yields a constitutively active receptor that is not down-regulated by endocytosis and is potently transforming (29). This mutant receptor is detected in 40% of high-grade gliomas, where it frequently exhibits gene amplification and does so less frequently in NSCLC and other solid tumors (30, 31, 32). In a study at the University of Colorado, lung cancer tumors, NSCLC cell lines, and normal bronchial epithelial cell cultures have all been analyzed, and no EGFRvIII could be demonstrated; 10 truncated variants were demonstrated around the EGFRvIII region, but they did not correspond by sequence analysis to EGFRvIII (33). These new variants may not be specific to tumor, as some of them were also found in normal bronchial epithelial cell lines.

**Biology of EGFR at the Signal Processing Layer.**
Ligand binding causes autophosphorylation of the adjacent intracytoplasmic domains (trans-autophosphorylation) and activation of the intracellular tyrosine kinase activity. Phosphorylation is accompanied by recruitment of downstream signal transduction molecules, leading to activation of different pathways. Activation of the EGFR pathway is not limited to members of the EGFR family but can occur via cross-talk with other signaling pathways, such as mitogenic G protein-coupled receptors (34) or platelet-derived growth factor receptor (35), and cooperates in a synergistic manner with pp60c-src, Jak-2, and the deletion of PTEN (36). The relevance of this phenomenon, known as EGFR transactivation, in lung cancer has yet to be assessed.

One of the main pathways is the ras pathway (Fig. 2). After EGFR activation, the autophosphorylated receptor binds to the SH2 domain of the adaptor protein growth factor receptor-bound protein 2. Through its SH3 domains, growth factor receptor-bound protein 2 is bound to SOS, and this molecule serves as a docking site for activated ras. The downstream signaling effects of ras activation include the activation of the effector protein serine/threonine kinase raf, the mitogen-activated protein kinase kinases 1 and 2, and the mitogen-activated protein kinases ERK1 and ERK2. Activation of this pathway leads to activation of several nuclear proteins, including cyclin D1, a protein required for cell cycle progression from G1 to S phase (37). Cyclin D1 might play a connecting role between the EGFR and the retinoids (38), based on the suggestion that retinoids produce a transcriptional down-regulation of EGFR (1). Other studies have similarly described the effect of retinoids on the transcriptional regulation of EGFR (39, 40, 41, 42).
Another important target in EGFR signaling is phosphatidylinositol 3'-kinase and the downstream protein serine/threonine kinase Akt (Fig. 2). Akt has a strong antiapoptotic effect by phosphorylating various targets. The most significant direct activation of the phosphatidylinositol 3'-kinase pathway in tumors comes from deletions of the tumor suppressor gene PTEN (phosphatase and tensin homologue). PTEN expression was evaluated by IHC in tissue samples from 125 patients with early stage NSCLC, and PTEN promoter methylation status was assessed by methylation-specific PCR in 20 microdissected PTEN-negative primary tumors, as well as in a panel of 16 NSCLC cell lines. Thirty of the 125 (24%) specimens showed a lack of staining for PTEN. PTEN methylation was detected in 7 of the 20 (35%) PTEN-negative NSCLC samples and none of the 10 PTEN-positive NSCLC samples that were microdissected.

Using the EGFR-overexpressing breast cancer cell line MDA-468, it has been shown that the resistance to gefitinib was caused by PTEN loss with consequent hyperactivation of Akt and uncoupling of the Akt pathway from EGFR. Reconstitution of PTEN in these cells reestablishes EGFR-driven Akt signaling and restores gefitinib sensitivity, confirming the essential role of Akt signaling in mediating response and resistance to gefitinib. Constitutive activity of Akt/PKB has been demonstrated in the vast majority of a panel of 17 NSCLC cell lines; in cells with high levels of Akt activity, the addition of a phosphatidylinositol 3'-kinase inhibitor or the transfection of kinase-dead Akt resulted in a dramatic increase of sensitivity to chemo- and radiotherapy. Moreover, in NSCLC cell lines, limited antiproliferative activity and absence of apoptosis after gefitinib treatment was associated with persistent ERK and Akt activity. Very exciting in vitro results have been published recently, demonstrating constitutive Akt activity in premalignant and malignant human bronchial epithelial cells, but not in normal human bronchial epithelial cells; furthermore, the phosphatidylinositol 3'-kinase/Akt pathway can be modulated for lung cancer prevention and treatment.

The signal transducers and activators of transcription (STAT) factors seem to be closely linked to the EGFR signaling pathway. The STATs are composed of a family of cytoplasmic transcription factors that transmit signals usually generated at cell surface receptors that, when activated, dimerize, translocate to the nucleus, and bind to members of the γ-activated site family of enhancers and thereby regulate gene expression. Whereas the STATs are not known to contribute directly to cell cycle checkpoint regulation or DNA repair, they contribute to tumorigenesis through their intimate connection to growth factor signaling, apoptosis, and angiogenesis. Studies using human tumor cell lines have demonstrated constitutive activation of STATs, especially STAT3, in lung cancer. After the dimeric STAT has entered the nucleus and has bound its target DNA, it is eventually dephosphorylated by a still-unidentified tyrosine phosphotase and shuttled back to cytoplasm. These interactions suggest still additional mechanisms by which the normal regulation of STAT signaling might be lost in tumorigenesis. Antisense reagents and dominant-negative constructs directed at STAT3 hold the promise of...
tumor-specific growth inhibition and appear to be useful against a range of cancer cells (50). A novel approach to blocking STAT3 function has been developed recently, using a phosphopeptide tethered to a protein transduction domain (51).

CONCLUSION

Several mechanisms to explain the increased receptor signaling in cancer have been described, including overexpression of the receptor, activation of EGFR mutations, increased coexpression of receptor ligands, and heterodimerization with other members of the EGFR family as well as with heterologous receptors systems.

Most of the applied clinical research on the EGFR has been focused on the overexpression of the receptor, whereas less research has addressed the potential role of other mechanisms of increased signaling, as well as nonmembrane bound events in the modulation of specific biological behaviors. The prognostic significance of EGFR expression in lung cancer and, more importantly, its ability to predict response to anti-EGFR therapies, are currently subjects of active research. New technologies such as nucleotide arrays and proteomics will help to elucidate the issue by providing information on how EGFR signaling may affect the expression of genes and proteins in cancer cells.

OPEN DISCUSSION

Dr. Thomas Lynch: What is your take on the MAP kinase observations that Dr. Gandara made this morning in terms of the relative potential of MAP kinase as a marker of erlotinib or gefitinib activity?

Dr. Scagliotti: I am now a little disoriented, because I was previously excited when I saw the data published by Dr. Josè Albanell and coworkers on the skin biopsy, showing decreased expression of MAP kinase after the administration of gefitinib. The model was extremely elegant. Then when we shifted to the clinical setting, we were completely unable to show any meaningful correlation. It seems to me that MAP kinase is a part of a multilayered system, and so looking just at one or two markers is not enough. We need to spend some days going around the table to reach a consensus to define in the prospective study how to look if this kind of information will be prognostic.

Dr. Alan Sandler: Dr. Rowinsky mentioned the concept of doing multiple biopsies in patients. Although it is a lofty goal, it is very difficult to get done, and until we are able to do these assays successfully and reproducibly in paraffin, it is going to be very difficult to prove or disprove.

Dr. Lynch: Do people agree with Dr. Rowinsky’s comment? At meetings, particularly when we are asked by various pharmaceutical companies to give advice, we tell them how important it is to design trials that collect tissue before and after treatment. We give it a lot of lip service, but do we actually do it?

Dr. Bruce Johnson: I don’t think it is practical in a big trial to get tissue on all the patients, even archival tissue. This has been looked at time and time again for decades. Unless you believe that there is going to be a change
in human behavior, it isn’t going to happen. I do think that it is very worthwhile to do so in a subset at centers where it is feasible and safe to do the sampling. Pretreatment is not as much of a problem as post-treatment. For post-treatment, you probably only need a few biopsies to validate that you are actually hitting the target.

**Dr. Paul Bunn:** It is obvious that the level of phospho-MAP kinase before treatment is not going to predict sensitivity to these drugs. It is highly likely that a decrease in baseline level would be much more likely to occur in responders, but of what value is that? Are there any data doing either an FDG or an FLT PET scan, say, 3 days after you give gefitinib? If you are going to try to predict response and then select those patients to continue, it might be actually more realistic to do an imaging study than sequential biopsies. Every paper in the literature suggests that a decline in FDG PET predicts survival.

**Dr. Ramaswamy Govindan:** There is an ongoing study looking at gefitinib with a ZD6474. We are doing PET scan before and afterward at five different time points. Unfortunately, because of it is so complicated, it is elective, not mandatory. So, when the study is done, we have to see how many actually got all those PET scans.

**Dr. Johnson:** But, you make the assumption that having that information is going to be useful to the patient. In terms of clinical utility, are you hurting the patient by giving him 6 weeks of gefitinib versus 3 days; are you assuming you have something better to give him if it doesn’t work?

**Dr. Lynch:** Dr. Bunn made the statement that there is no reason to think that levels of phospho-MAP kinase taken beforehand will predict for survival. I thought Dr. Gandara told us that it did.

**Dr. Roy Herbst:** I think it depends on the situation. Dr. Gandara did show the predictive levels of the positive biopsy before treatment. Dr. Khuri might want to speak about his model, which was very good in the preoperative setting in answering the question “Are we hitting the target?” But these are two different questions: Is it predictive versus are you hitting the target.

**Dr. Fadlo Khuri:** We did it with the FTI in head and neck cancer, and in fact Dr. Herbst did it with endostatin, which is much harder, in patients with metastatic disease. The easiest model is the induction therapy model if you have a surgical sample—although nothing is easy in terms of getting serial biopsies—but you can get a baseline and follow-up. Again, it is going to be hard to do in larger randomized trials. None of us ever said you cannot do it in small selected trials and targeted populations.

**Dr. David Gandara:** One comment on the serial biopsies: in the data that have been presented so far on serial biopsies with EGFR inhibitors, the responses compared with the post-therapy changes were absolutely inconsistent.

**Dr. Eric Rowinsky:** We can pat ourselves on the back for all the nice small serial biopsies studies we’ve done, but that is not going to get us anywhere in large populations, unless it is with an agent like imatinib where you have a target, a high response rate, and robust assays. If we are looking at unenriched patient populations with response rates less than 10% and nonvalidated assays, it becomes a numbers game and we’re not going to have those numbers. I think those numbers can be achieved by getting pretreatment assays. You can ask why we don’t have a centralized system where every patient with a tumor has blood and tumor biopsy sent in to
some central depository so the genomic and proteomic studies can be done. That's the only way it is going to work.

**Dr. Khuri:** Putting data in a consortium is problematic for junior faculty, since academia is structured to reward people who publish; frankly, the only people who could do it are senior people and people in industry.

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**Footnotes**


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