Predictive factors for epidermal growth factor receptor inhibitors—The bull's-eye hits the arrow

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Studies have shown that epidermal growth factor receptor (EGFR) signaling is important to normal development and neoplastic transformation, and that EGFR inhibition reduces cancer cell proliferation. The promising response rates of the EGFR inhibitor gefitinib in patients with chemotherapy-refractory non-small cell lung cancer (NSCLC) led to its approval for clinical use. However, there was little understanding of why gefitinib was effective in only some NSCLC patients. Two recent studies have identified somatic mutations in EGFR that confer its sensitivity to gefitinib in vitro and correlate strongly with patients' clinical response to the inhibitor.

Since the pioneering studies of Cohen on growth factors (Cohen, 1986) and the identification of its homology to the transforming protein encoded by the avian oncogene v-erbB, the epidermal growth factor receptor (EGFR) has been the subject of intense investigation. Numerous studies have documented the apparent importance of EGFR signaling on normal development and neoplastic transformation and progression. Most importantly for cancer therapeutics development, blocking receptor signaling by antibodies to the extracellular ligand binding domain and small molecules to the tyrosine kinase (TK) leads to the inhibition of cancer cell proliferation. Unfortunately, clinical trials of EGFR targeting agents have yielded mixed results to date. Objective tumor responses rates of 10%–18% have consistently been reported from phase 2 studies of TK inhibitors gefitinib and erlotinib in patients with non-small cell lung cancer (NSCLC); however, the mechanisms leading to tumor regression in this subset of patients and the means to identify these patients prior to initiating therapy have been elusive. Recently, two papers have described the identification of genetic abnormalities in the EGFR kinase domain that predict tumor response to gefitinib (Lynch et al., 2004; Paez et al., 2004). That both groups identified mutations in the same region of the receptor only heightens the excitement that the elusive goal of targeting gefitinib, and possibly other EGFR inhibitors, to patients most likely to benefit may be achieved.

EGFR signaling in cancer

EGFR, a 170 kDa membrane-spanning glycoprotein, is one of four members of the human EGFR (HER) family. EGFR plays an important role in organogenesis and in neoplastic processes of cell proliferation, inhibition of apoptosis, angiogenesis, and metastatic spread. Stimulation of the receptor through ligand binding leads to receptor oligomerization at the plasma membrane, activating the receptor TK and thereby causing transphosphorylation of tyrosine residues in the intracellular part of the receptor. These residues are docking sites for proteins containing Src homology domain 2 and phosphotyrosine binding domains that, in turn, trigger a chain of biochemical reactions through several signaling cascades that include the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) Akt pathways, which transmit the growth stimuli to the cell nucleus (Yarden and Sliwkowski, 2001).

The specificity and potency of the signaling output from activated EGFR are highly dependent on the activating ligand as well as the cellular levels of the coreceptors HER2/*neu* (*erb*B2), HER3 (*erb*B3), and HER4 (*erb*B4), all of which can oligomerize with the EGFR. EGFR, as well as other family members and ligands, is commonly expressed in epithelial carcinomas and gliomas (Yarden and Sliwkowski, 2001). Receptor expression by tumor cells has been linked with disease progression, poor survival, resistance to chemotherapy, and poor response to therapy. Because of these characteristics, and because agents that specifically inhibit receptor signaling also inhibit tumor cell proliferation, EGFR has been viewed as a rational target for antitumor therapy.

EGFR is the protein product of gene containing 28 exons spanning approximately 200 kb found on chromosome 7p11.2 (Reiter et al., 2001). EGFR is the cellular protooncogene homolog of the avian erythroblastosis virus v-*erb*B oncogene (Downward et al., 1984). Aberrant signaling through EGFR can lead to neoplastic transformation of human cells. For example, expression of high levels of EGFR and its ligand, transforming growth factor-α (TGFα), has been shown to induce transformation in vitro (Yarden and Sliwkowski, 2001). These studies have suggested that overexpression of the wild-type receptor leads to transformation only with ligand stimulation. Although EGFR is widely expressed in human cancers, mutations in the receptor are not common except in brain tumors. EGFR gene amplification is detected in 40% of human glioblastomas, where a significant proportion also exhibits EGFR gene rearrangements (Ekstrand et al., 1991). Other activating mutations have been created in laboratory models (Sorokin, 1995), but the identification of similar mutations in clinical cancers has been lacking. Thus, the most frequent abnormality of EGFR reported in human cancers to date is receptor overexpression.

EGFR in lung cancer

EGFR overexpression is frequently seen in non-small cell lung cancer (NSCLC), the most common cause of cancer-related death in the Western world. NSCLC collectively refers to the subhistologies of squamous cell carcinoma (SCC), adenocarcinoma (ADC), and large cell carcinoma, as well as the more indolent bronchoalveolar carcinoma (BAC). After decades of research, treatments for patients with metastatic NSCLC remain unsatisfactory. Although combination chemotherapy regimens improve survival, the effects are measured in weeks

rather than many months or years. Only a handful of drugs induce responses in more than 10% of patients, and responses are rarely durable.

Given the poor outcomes with standard systemic therapies, interest in identifying targets for therapeutics development in lung cancer is intense, and EGFR appeared to be an appropriate target due to the frequency of expression of the receptor and ligand. EGFR protein overexpression was observed in 32% to 79% of NSCLC (Hirsch et al., 2003; Mukohara et al., 2004; Rusch et al., 1997) and occurred more frequently in SCC and BAC than ADC or large cell carcinomas (Hirsch et al., 2003; Mukohara et al., 2004; Rusch et al., 1997). Gene amplification was seen in 9% of the patients by fluorescence in situ hybridization. Coexpression of TGFα and EGFR was reported in 29% and 38% of specimens, respectively. Interestingly, EGFR and TGFα overexpression was observed in all tumor stages and histological types, but was most frequently seen in SCC. The prognostic value of EGFR for survival of patients with lung cancer remains controversial; however, the results of a meta-analysis of 14 studies suggest that it does not correlate with this outcome (Meert et al., 2002).

Gefitinib in lung cancer

Given the lack of therapeutic options and the frequency of overexpression of EGFR and its ligands in NSCLC, it is not surprising that evaluating gefitinib (Iressa, AstraZeneca) in this common and highly lethal disease was a priority. Initial phase 1 dose-find and toxicity trials documented an objective response rate of 10% among NSCLC patients (Baselga et al., 2002; Herbst et al., 2002). This result was considered promising, because the agent was thought to most likely inhibit proliferation, thus delaying tumor progression rather than inducing tumor shrinkage, and because the agent had induced objective responses in a group of patients refractory to standard therapies.

As a result of these encouraging results, clinical development progressed quickly. Two large phase 2 studies comparing two doses of gefitinib were initiated; one accruing NSCLC patients with one prior treatment, conducted predominately in Japan and Europe, and the other trial conducted mainly in the United States (US). In parallel, phase 1 trials confirmed the safety of combining gefitinib with standard chemotherapy regimens in newly diagnosed patients with advanced and metastatic disease. These preliminary trials led to phase 3 trials comparing the addition of gefitinib to chemotherapy. The trials completed accrual within months, evidence of the perception that this agent would likely be a therapeutic breakthrough.

Unfortunately, results of these studies were mixed. In randomized phase 2 trials, response rates of 9.6% and 19% were reported in the US- and Japanese-led trials, respectively (Fukuoka et al., 2003; Kris et al., 2003). Although response rates were low, they were often rapid in onset and durable. Interestingly, differences in response rates for the 250 and 500 mg doses were not apparent. Despite evidence of modest single agent activity, phase 3 trials evaluating the addition of gefitinib to chemotherapy as first-line therapy in patients with metastatic NSCLC failed to show an advantage in response rate, progression-free survival, or overall survival compared to standard treatment (Giaccone et al., 2004; Herbst et al., 2004). The consistent response rates in patients with chemotherapy-refractory disease and favorable safety profile reported from the single agent studies led to the approval of the gefitinib in Japan in 2002 and the United States in 2003 (Cohen et al., 2004).

At the time of approval of gefitinib, there was little understanding of the predictive markers of drug activity. Retrospective analyses of patients receiving single agent gefitinib showed that responses were more frequent among patients who had never smoked, women, and patients with BAC or ADC with bronchoalveolar features (Fukuoka et al., 2003; Janmaat et al., 2003; Kris et al., 2003; Miller et al., 2004; Nishiwaki et al., 2004). However, there was no correlation between the intensity of immunohistochemical staining of the tumor for EGFR and the presence or absence of a response, and no obvious candidate biomarker to select patients for treatment with gefitinib.

The discovery of markers of gefitinib sensitivity

Research into the identification of biomarkers that correlate to clinical benefit or resistance to gefitinib has been intense. The first reports of a predictive marker for drug sensitivity have recently been published (Lynch et al., 2004; Paez et al., 2004). Both groups started with the simple hypothesis that patients with dramatic responses to gefitinib were likely to have tumors harboring genetic alterations in specific kinases, as is the case for imatinib mesylate treatment of c-kit mutant gastrointestinal stromal tumors and trastuzumab treatment of HER2 amplified breast carcinoma. Both groups sequenced the EGFR gene from lung cancer specimens from patients treated and not treated with gefitnib as well as normal lung tissue to identify mutations, and subsequently evaluated the biochemical effects of the mutations in vitro. Results from these groups are consistent and complementary.

Paez and colleagues (Paez et al., 2004) started their search by sequencing the exons encoding the activation loops of 47 of 58 human receptor TK genes from a subset of 58 NSCLCs and subsequently narrowed their evaluation to the EGFR TK domain. The identified mutations were found in 15 of 58 unselected tumors from Japan and one of 61 from the United States (Paez et al., 2004). These mutations were heterozygous, and more common in women, ADC, and Japanese patients. Mutations were identified in 5/5 patients who responded and 0/4 patients who did not respond to gefitinib.

Lynch and colleagues sequenced the entire coding region of the EGFR gene from tumor specimens from nine patients treated with gefitinib with clinical responses, then screened additional tumor specimens and cell lines for mutations (Lynch et al., 2004). Somatic mutations were identified in the TK domain of the EGFR gene in eight of nine patients with gefitinib-responsive lung cancer, as compared with none of the seven patients without a response (Lynch et al., 2004). Similar mutations were detected in tumors from two of 25 patients with primary NSCLC not exposed to gefitinib. However, mutations were not identified in primary breast, colon, kidney, pancreas, and brain tumor specimens or in 108 cancer cell lines.

In both studies, mutations were identified in the EGFR TK domain, with the majority of abnormalities clustering within exons 19 and 21. Mutations were either small, in-frame deletions or amino acid substitutions clustered around the adenosine triphosphate (ATP) binding pocket. Substitution mutations changing leucine 858 to arginine (L858R), guanine 719 to serine (G719S), and leucine 861 to glutamine (L861Q), as well as multiple deletion mutations clustered in the region spanning codons 746 to 759 within the kinase domain, were reported. These alterations cluster around the active site of the kinase, with the substitution mutations laying in the activation loop and glycine-rich P loop, which are important for autoregulation (Lynch et al., 2004; Paez et al., 2004).

The biological consequences of some of these mutations

were investigated in vitro. Paez and colleagues found that the H3255 cell line, which harbors the L858R mutations, was 50-fold more sensitive to gefitinib than other adenocarcinoma cell lines, with an IC_{50} of 40 nM for cell survival. Treatment with 100 nM gefitinib completely inhibited EGFR autophosphorylation, as well as phosphorylation of downstream targets extracellular signal-regulated kinase 1/2 (ERK1/2) and AKT kinase. Lynch and colleagues reported similar results from studying Cos-7 cells transiently expressing wild-type and mutant EGFR. In the absence of serum, neither wild-type nor mutant EGFR demonstrated autophosphorylation. However, activation of mutant EGFRs was greater in the presence of EGF stimulation, and mutant EGFR downregulation was significantly slower compared to wild-type EGFR. Compared to wild-type receptors, mutant receptors were more sensitive to inhibition by gefitinib with IC_{50} and IC_{100} of 0.1 μM and 2.0 μM versus 0.015 μM and 0.2 μM, respectively.

What conclusions may be drawn from these two papers? First, mutations clustering around the EGFR TK domain appear to augment ligand-induced EGFR autophosphorylation and confer increased sensitivity to gefitinib. These results suggest that agents such as antibodies, which inhibit ligand binding as well as small molecule ATP-mimetics that, like gefitinib, bind to the ATP pocket, may benefit patients with tumors that harbor the EGFR mutations. Second, within the limits of the number of specimens analyzed, it appears that most responses (totaling 13/14 between the two papers) are associated with these mutations. Third, EGFR mutations are more common in ADC and BAC histologies, nonsmokers, and Japanese patients, suggesting a much different etiology for lung cancers developing in these groups compared to squamous cell carcinomas, smokers, and non-Japanese patients.

Future directions

Many questions remain to be answered regarding the biochemical and cellular effects of the various mutations, the prevalence and natural history of EGFR mutated lung cancers in ethnically diverse populations, and the responsiveness of EGFR-mutated and -nonmutated lung cancers to gefitinib and other EGFR-targeting small molecules and antibodies.

There are two main modes of signal attenuation defined for EGFR (Carpenter, 2000; Schlessinger, 2003). The first is ligand-induced internalization and subsequent degradation, a process that occurs over the 15–30 min after EGFR ligand binding and persists for hours. The second involves rapid dephosphorylation of activated EGFR within seconds to minutes, which reverts the receptor to an inactive state in the absence of persistent ligand (Carpenter, 2000; Schlessinger, 2003). Both phenomena attenuate EGFR signaling and may be influenced by not only the presence of mutations around the EGFR TK domain, but also the types of mutations in these areas. Thus, specific mutations may result in different strengths of EGFR signaling, as well as different responses to gefitinib and other EGFR inhibitors. Further structure-activity studies will likely identify the important features of the interactions between mutated EGFR and EGFR inhibitors and may lead to the design of more potent inhibitors.

The higher frequency of mutations in lung cancer patients from Japan versus the United States is interesting. Lung cancer is the leading cause of death among cancer patients in Japan as well as the United States. However, its age-adjusted mortality and incidence rates are still less than two-thirds of those in the US (Stellman et al., 2001; Yoshimi et al., 2003). Studies from Japan and Western countries suggest that while smoking shows a dose-response relationship with all lung cancer cell types, its association is less predominant for ADC than for SCC (Barbone et al., 1997; Kabat, 1996), and particularly for ADC in women (Marugame et al., 2004). It has been postulated that specific Japanese dietary factors may protect against cancer development in subsets of patients (Takezaki et al., 2001). However, these factors may not protect against the development of lung cancers with EGFR mutations. Clearly, the etiologic factors predisposing to EGFR-mutated lung cancer specifically and lung cancers in nonsmokers as a group require further elucidation.

The correlation between sensitivity to EGFR inhibitors and nonsmoking status also suggest that chemoprevention strategies based on EGFR inhibition in high risk smokers or former smokers may not be as effective as previously hoped. Nonetheless, EGFR overexpression occurs early in the development of smoking-related premalignant lesions, and thus, evaluating EGFR inhibitors in precancerous disease, where inhibition may have a greater impact on progression to cancer, should not be abandoned prematurely.

The frequencies of these EGFR mutations, as well as their biological and clinical consequences, will also need clarification. Results from the studies to date suggest that EGFR mutations and objective tumor responses are less common in non-Japanese patients, smokers, and non-ADC histologies, but more accurate estimates of the frequencies of mutations among these diverse populations, and frequencies among patients with different stages of disease are required. In addition, the natural history of EGFR-mutated lung cancer and its response to standard therapies, gefitinib, and other EGFR inhibitors need to be determined to optimize treatment strategies.

In addition to more accurately determining the frequency and types of mutations in patients with lung cancer, it will be important to determine the robustness of the correlation between the mutations and patient benefit. The reported frequencies of the EGFR mutations in random tumor specimens assessed to date are low relative to the response rates from trials of gefitinib in metastatic disease. This apparent discrepancy may, in part, be due to the mutations appearing more frequently among patients with recurrent and/or metastatic disease compared to newly diagnosed, resectable patients. For example, if the tumor specimens analyzed in the studies by the groups were predominately from resected patients with early-stage disease, and the mutations increase the risk of relapse, then mutations may be more common among patients with advanced metastatic disease. Alternatively, these mutations may not identify all patients who may benefit from gefitinib, which seems likely, given that at least one of the nine responding patients assessed by Lynch and colleagues did not have an identifiable mutation. Conversely, the presence of these mutations may not correlate as strongly with objective tumor responses as appears from these initial studies if additional abnormalities modify the enhanced EGFR signaling conferred by mutant EGFR. Thus, the true predictive value of the presence/absence of the different mutations and clinical benefit of treatment with gefitinib and other EGFR inhibitors will require additional evaluation. If there is a strong correlation between the presence of mutated EGFR and clinical benefit, and the magnitude of benefit is comparable or superior to standard chemotherapy, then gefitinib would be a reasonable alternative therapy for these patients.

At the moment, it appears that the presence of the described EGFR mutations will identify NSCLC patients with

the highest likelihood of responding to gefitinib. However, other as yet unidentified factors may confer sensitivity to gefitinib resulting in either responses or delayed progression. Erlotinib, another small molecule EGFR TK inhibitor, was recently found to extend survival compared to placebo among metastatic NSCLC patients (Genentech Press Release, Tarceva Extends Survival of Patients with Relapsed Non-Small Cell Lung Cancer, April 25, 2004, http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=7387). This result suggests that EGFR inhibition may confer a survival advantage in a patient population unselected for the presence of EGFR mutations. If benefit from treatment were limited only to patients with the reported EGFR mutations, currently estimated to occur in 8% or less of NSCLC patients, either the magnitude of the effect in this subset of patients would have to be very large to lead to a positive result in an unselected patient population, or there are other factors determining benefit to additional patients. If tumor specimens from patients enrolled in this trial can be retrieved and analyzed, the frequency as well as prognostic and predictive importance of the EGFR mutations, and possibly other biomarkers, can be identified.

The investigators identifying the first biomarkers predictive of sensitivity to gefitinib are to be congratulated. By detailed investigation of the target, they have identified a subset of lung cancer patients that may substantially benefit from treatment with the EGFR inhibitor. Identification of other markers of sensitivity and resistance to EGFR inhibitors relevant to lung cancer as well as other epithelial cancers and brain tumors will undoubtedly identify treatments and treatment strategies to maximize the benefit to patients.

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