Epidermal Growth Factor Receptor Kinase Domain Mutations in Esophageal and Pancreatic Adenocarcinomas

Eunice L. Kwak, Janusz Jankowski, Sarah P. Thayer, Gregory Y. Lauwers, Brian W. Brannigan, Patricia L. Harris, Ross A. Okimoto, Sara M. Haserlat, David R. Driscoll, David Ferry, Beth Muir, Jeff Settleman, Charles S. Fuchs, Matthew H. Kulke, David P. Ryan, Jeff W. Clark, Dennis C. Sgroi, Daniel A. Haber, and Daphne W. Bell

Abstract

Purpose: Specific activating mutations within the epidermal growth factor receptor (EGFR) identify a subset of non–small cell lung cancers with dramatic sensitivity to the specific tyrosine kinase inhibitors (TKI), gefitinib and erlotinib. Despite the abundant expression of EGFR protein in a broad range of epithelial cancers, EGFR mutations have not been reported in a substantial fraction of other cancers. Given recent reports of TKI-responsive cases of esophageal and pancreatic cancer, this study was designed to determine the prevalence of EGFR mutations in these gastrointestinal cancers.

Experimental Design: We sequenced exons 18 to 21 of EGFR from 21 cases of Barrett’s esophagus, 5 cases of high-grade esophageal dysplasia, 17 cases of esophageal adenocarcinoma, and 55 cases of pancreatic adenocarcinoma. Subsets of esophageal (n = 7) and pancreatic cancer cases (n = 5) were obtained from patients who were subsequently treated with gefitinib or erlotinib-capecitabine, respectively.

Results: Mutations of EGFR were identified in two esophageal cancers (11.7%), three cases of Barrett’s esophagus (14.2%), and two pancreatic cancers (3.6%). The mutations consisted of the recurrent missense L858R and in-frame deletion delE746-A750, previously characterized as activating EGFR mutations in non–small cell lung cancer. We also identified the TKI drug resistance–associated EGFR T790M mutation in an untreated case of Barrett’s esophagus and the corresponding adenocarcinoma.

Conclusion: The presence of activating mutations within EGFR in both esophageal and pancreatic adenocarcinomas defines a previously unrecognized subset of gastrointestinal tumors in which EGFR signaling may play an important biological role. EGFR mutations in premalignant lesions of Barrett’s esophagus also point to these as an early event in transformation of the esophageal epithelium. The role of genotype-directed TKI therapy should be tested in prospective clinical trials.

Somatic mutations in epidermal growth factor receptor (EGFR) seem to define a specific subset of non–small cell lung cancers (NSCLC), ~10% of cases, which are most commonly adenocarcinomas and bronchoalveolar carcinomas arising in nonsmokers, with an increased prevalence in women and individuals of Asian ethnicity (1–4). The mutations are clustered around the ATP-binding pocket of the receptor, and ~80% consist of either a single missense mutation (L858R) or nested in-frame deletions (delE746-A750 and variants thereof). Experimental studies have shown both qualitative and quantitative alterations in downstream signaling by mutant EGFR, and suggested that NSCLC cells with these mutations may be dependent on the altered signals for survival, a phenomenon described as “oncogene addiction” (5–9). Indeed, these cells seem to be exquisitely sensitive to the reversible inhibitors of EGFR, gefitinib and erlotinib. Most clinical studies have shown that NSCLC with EGFR mutations, responses are often limited by the relatively rapid acquisition of drug resistance (25–27). Acquired resistance to these TKIs in previously responsive cases has been
linked to a secondary somatic mutation in EGFR, T790M, which reduces binding by reversible inhibitors, gefitinib and erlotinib, but may be overcome by a new class of irreversible EGFR inhibitors (28–31).

Although the rapid and dramatic responses to gefitinib and erlotinib seen in ~10% of NSCLC have focused attention on the subset of lung cancers with EGFR mutations, some responsive tumors have also been noted in other tumor types (32–39). With the exception of rare case reports, most responses to these TKIs in other tumor types have been modest, and correlative studies with EGFR mutation analysis are incomplete. In ovarian cancer, two EGFR mutations have been reported (3.5% of cases analyzed) and these were observed in a gefitinib-responsive case, as well as an untreated case (32). In head and neck cancers, in which 10% of cases show responses to gefitinib, no mutations were noted in a U.S. study, but 7% of Asian tumors were found to harbor such mutations (40, 41).

Among gastrointestinal cancers, six EGFR mutations (<1%) were reported in colorectal cancer, three mutations in cholangiocarcinoma (13%), and a rare mutation (S768I) in an esophageal cancer cell line (42–46). Data on TKI responsiveness was only available for a single case of colorectal cancer with a rare G857R mutation that progressed during treatment (44). In esophageal and pancreatic cancers, combination treatments including TKI with chemotherapy have led to some partial responses, but EGFR mutational analyses have not been undertaken (35–39). Given the postulated role of EGFR signaling in gastrointestinal cancers, the limited therapeutic options available in these cancers, and the potential for genotype-directed trials of more potent irreversible EGFR inhibitors, we undertook a mutational analysis of EGFR in esophageal and pancreatic cancers.

Materials and Methods

Clinical specimens

Esophageal cancer. Archival specimens of esophageal adenocarcinoma (n = 17) and tissue biopsies of Barrett’s esophagus (n = 21) or high-grade dysplasia (n = 5) were obtained from patients diagnosed at Massachusetts General Hospital or the University of Leicester, United Kingdom. Seven esophageal adenocarcinomas were resected from patients prior to gefitinib treatment (500 mg/d) at the University of Leicester. Response was assessed by computed tomography scan, as well as endoscopic visualization of the gastroesophageal junction.

Pancreatic cancer. Formalin-fixed, paraffin-embedded tumor specimens were obtained from 55 patients treated for pancreatic cancer at Massachusetts General Hospital. This series included tissue from 50 unselected surgical cases, as well as an additional 5 cases that were treated with erlotinib (150 mg orally on days 1-21) in combination with capcitabine (1,000 mg/m² orally twice a day on days 1-14) as second line therapy within a phase II clinical trial for metastatic disease (36). Treated patients were selected because they remained on the study >100 days. Response to erlotinib-capcitabine was assessed by physical exam, laboratory measures (including CA19-9 tumor marker), and imaging (usually computed tomography scan), using Response Evaluation Criteria in Solid Tumors guidelines. All clinical specimens were procured in accordance with institutional guidelines.

Mutational analysis of EGFR

Automated nucleotide sequencing was used to determine the genotype of EGFR. Unselected pancreatic adenocarcinomas were not microdissected prior to DNA isolation. In all other cases, tissue sections were cut from formalin-fixed paraffin-embedded tumor blocks, and an H&E stained section was reviewed by a pathologist. Regions of tissue with at least 50% tumor cell content were microdissected prior to DNA isolation. DNA was extracted using the PureGene kit according to the manufacturer’s instructions (Gentra Systems, Inc., Minneapolis MN). Exons 18 to 21 of EGFR were amplified by nested PCR using primers and conditions described previously (23). Amplified products were purified with exonuclease I (United States Biochemical, Cleveland OH) and shrimp alkaline phosphatase (United States Biochemical), diluted in water, and subjected to bidirectional sequencing using BigDye Terminator v 1.1 mix (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Capillary sequencing was done on an ABI 3100 genetic analyzer (Applied Biosystems). Sequences were evaluated for the presence of mutations using Sequence Navigator and Factura software (Applied Biosystems). All reported mutations were scored as reproducible, having been identified in at least two independent PCR amplifications.

Determination of EGFR copy number

TaqMan quantitative real-time PCR using genomic DNA was used to determine the copy number of EGFR relative to PCDH7, an unrelated gene on another chromosome, as previously described (23). Only cases treated with an EGFR-TKI were evaluated for gene copy number.

Results and Discussion

Esophageal cancer. Analysis of 17 esophageal adenocarcinomas revealed two cases (11.7%) with a somatic heterozygous EGFR mutation (Table 1). Both mutations were previously reported “recurrent” mutations, the in-frame deletion delE746-A750 and the missense L858R, that are predictive of EGFR-TKI responsiveness in NSCLC. Neither case had amplification of the mutant EGFR as measured by quantitative real-time PCR analysis. However, one additional esophageal cancer had 16-fold amplification of wild-type EGFR. The two esophageal cancers with an EGFR mutation had not responded to gefitinib treatment, but the case with amplification had a partial response.

Esophageal adenocarcinoma typically arises in Barrett’s esophagus, following replacement of the normal stratified epithelium by specialized intestinal epithelium, and progressive transformation from metaplasia to low-grade dysplasia, high-grade dysplasia, and finally adenocarcinoma (reviewed in ref. 47). To determine the timing of EGFR mutations within this malignant transformation, we analyzed 5 cases of high-grade dysplasia as well as 21 cases of Barrett’s esophagus. In 8 of 21 cases, paired specimens of Barrett’s esophagus and adenocarcinoma were available for analysis. Three of 21 cases (14%) of Barrett’s esophagus had an EGFR mutation (Table 1): two had the delE746-A750 sensitizing EGFR mutation, whereas the third had the T790M drug-resistance mutation. This mutation is of particular interest because it was first identified as a secondary EGFR mutation associated with acquired resistance to gefitinib by reducing drug binding within the ATP pocket (28–31). However, we have recently reported the T790M mutation in the germ line of a family with inherited susceptibility to bronchoalveolar lung cancer, and the mutation has also been found in previously untreated cases of NSCLC (48). Thus, the T790M mutation may mediate altered functional properties of the receptor in addition to conferring drug resistance.

Among specimens of Barrett’s esophagus with EGFR mutations, a matched specimen of esophageal carcinoma was only available for the case with the T790M mutation. The mutation
was detected in the corresponding adenocarcinoma, contained within a 3.0 × 2.5 × 0.7 cm lesion classified as T2-N1-Mx with lymphatic invasion, suggesting that the tumor is likely to have arisen from this premalignant clone in the Barrett’s esophagus. The presence of EGFR mutations in premalignant esophageal lesions is consistent with findings in NSCLC, where such mutations have been reported in histologically normal bronchial and bronchiolar epithelium adjacent to the adenocarcinoma (49). Taken together, these observations indicate that EGFR mutations may arise as an early genetic event in esophageal transformation, and are present in a subset of adenocarcinomas of the esophagus.

Pancreatic cancer. Somatic heterozygous EGFR mutations were detected in 2 of 55 (3.6%) pancreatic cancers. Both cases had the recurrent delE746-A750 mutation. We note that many pancreatic cancer specimens had a predominance of reactive stromal components, and microdissection of tumor cells was required to ensure analysis of the tumor cell population. The number of treated cases analyzed was too small to permit any meaningful correlation with clinicopathologic features.

Both pancreatic cancers with EGFR mutations were identified from five cases that exhibited disease stabilization in response to therapy with erlotinib and capecitabine. It is noteworthy that TKIs, the presence of EGFR mutations in Barrett’s esophagus Deletion of nucleotides 2235-2249 19 DelE746-A750
3 Barrett’s esophagus Deletion of nucleotides 2235-2249 19 DelE746-A750
4 Barrett’s esophagus Deletion of nucleotides 2235-2249 19 DelE746-A750
5 Barrett’s esophagus Substitution of T for C at nucleotide 2369 20 T790M

EGFR Mutations in Gastrointestinal Tumors

Table 1. EGFR mutations in esophageal adenocarcinoma (n = 17), dysplasia (n = 5), and Barrett’s esophagus (n = 21)

<table>
<thead>
<tr>
<th>Case</th>
<th>Histology</th>
<th>EGFR mutation</th>
<th>Exon</th>
<th>Effect on protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adenocarcinoma</td>
<td>Deletion of nucleotides 2235-2249</td>
<td>19</td>
<td>DelE746-A750</td>
</tr>
<tr>
<td>2</td>
<td>Adenocarcinoma</td>
<td>Substitution of G for T at nucleotide 2573</td>
<td>21</td>
<td>L858R</td>
</tr>
<tr>
<td>3</td>
<td>Barrett’s esophagus</td>
<td>Deletion of nucleotides 2235-2249</td>
<td>19</td>
<td>DelE746-A750</td>
</tr>
<tr>
<td>4</td>
<td>Barrett’s esophagus</td>
<td>Deletion of nucleotides 2235-2249</td>
<td>19</td>
<td>DelE746-A750</td>
</tr>
<tr>
<td>5</td>
<td>Barrett’s esophagus</td>
<td>Substitution of T for C at nucleotide 2369</td>
<td>20</td>
<td>T790M</td>
</tr>
</tbody>
</table>

Table 2. EGFR mutations in pancreatic cancer (N = 55)

<table>
<thead>
<tr>
<th>Case</th>
<th>Histology</th>
<th>EGFR mutation</th>
<th>Exon</th>
<th>Effect on protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adenocarcinoma</td>
<td>Deletion of nucleotides 2235-2249</td>
<td>19</td>
<td>DelE746-A750</td>
</tr>
<tr>
<td>2</td>
<td>Adenocarcinoma</td>
<td>Deletion of nucleotides 2235-2249</td>
<td>19</td>
<td>DelE746-A750</td>
</tr>
</tbody>
</table>

was detected in the corresponding adenocarcinoma, contained within a 3.0 × 2.5 × 0.7 cm lesion classified as T2-N1-Mx with lymphatic invasion, suggesting that the tumor is likely to have arisen from this premalignant clone in the Barrett’s esophagus. The presence of EGFR mutations in premalignant esophageal lesions is consistent with findings in NSCLC, where such mutations have been reported in histologically normal bronchial and bronchiolar epithelium adjacent to the adenocarcinoma (49). Taken together, these observations indicate that EGFR mutations may arise as an early genetic event in esophageal transformation, and are present in a subset of adenocarcinomas of the esophagus.

Pancreatic cancer. Somatic heterozygous EGFR mutations were detected in 2 of 55 (3.6%) pancreatic cancers. Both cases had the recurrent delE746-A750 mutation. We note that many pancreatic cancer specimens had a predominance of reactive stromal components, and microdissection of tumor cells was required to ensure analysis of the tumor cell population. The number of treated cases analyzed was too small to permit any meaningful correlation with clinicopathologic features.

Both pancreatic cancers with EGFR mutations were identified from five cases that exhibited disease stabilization in response to therapy with erlotinib and capecitabine. It is noteworthy that DNA analyzed from these five cases were isolated from specimens that could not be subjected to microdissection (for technical reasons). This was due to the fact that DNA analyzed from these five cases were isolated from specimens that could not be subjected to microdissection (for technical reasons). This may have limited our ability to evaluate EGFR mutation status in unselected cases, as these specimens had a predominance of reactive stromal components. In support of this idea, we were unable to detect characteristic KRAS mutations in a subset (n = 10) of nonmicrodissected cases. Given the heterogeneity of tumor specimens, the actual frequency of EGFR mutations in pancreatic cancer may be appreciably greater than the 3.6% observed in this study (Table 2). KRAS mutations were detectable in three of five microdissected tumors, suggesting that unlike NSCLC, these mutations might coexist with EGFR mutations in pancreatic cancer. None of the five responsive cases had amplification of EGFR as measured by quantitative real-time PCR. The response in pancreatic cancer was defined by stable disease that allowed continued therapy for 100 days, and hence, does not fall within the same category of dramatic responses seen in NSCLC. Nonetheless, these observations raise the possibility that EGFR mutations may identify a more tractable subset of pancreatic cancer.

Conclusion

In the U.S., esophageal and pancreatic cancers have 5-year survival rates of only 12% and 4%, respectively, according to the American Cancer Society statistics for 2004. Pancreatic cancer is the fourth leading cause of cancer death among men, and the fifth among women, whereas esophageal cancer is the sixth leading cause of cancer deaths in men. Hence, there is an urgent need for improved forms of treatment for these two malignancies. Our observation that EGFR mutations are present in a subset of esophageal and pancreatic cancers offers an initial insight into genetic pathways that may subclassify these gastrointestinal cancers, and suggests that the EGFR pathway may be implicated in tumorigenesis. Of note, previous studies have shown that 8% to 30% of esophageal cancers have an amplification of EGFR (50, 51). Among other gastrointestinal cancers, we did not find EGFR mutations in 18 gastric cancer cell lines (data not shown), and mutations in colon cancer seem to occur in <1% of cases (42–44). Thus, among gastrointestinal cancers, all of which express EGFR, esophageal and pancreatic cancers are unique in having a significant fraction with genetic markers of EGFR deregulation. In addition to highlighting possible therapeutic options in these established cancers using TKIs, the presence of EGFR mutations in Barrett’s lesions raises the interesting possibility of chemoprevention in patients with severe EGFR mutant preneoplastic lesions.

Although our data suggests that EGFR mutations may serve as a molecular classifier in esophageal and pancreatic cancers, we cannot draw firm conclusions about their association with TKI responsiveness in these diseases. Unlike NSCLC, in which a subset of cases with immediate and dramatic TKI response was clinically evident, no such “hyperresponsive” fraction has been reported in erlotinib or gefitinib trials of esophageal and pancreatic cancer. Nonetheless, ~10% of cases with advanced
esophageal and pancreatic cancers do show some degree of responsive or stable disease following treatment with these TKIs, either alone or in combination with chemotherapy (35–39). Molecular characterization of these clinical studies has not been reported, although an EGFR mutant TKI–hypersensitive esophageal cell line has recently been described (46). A number of molecular considerations may in fact distinguish EGFR mutant NSCLC from similar mutations in gastrointestinal cancers. It is possible that in these gastrointestinal, EGFR mutations are of secondary importance and are not hallmarks of “oncogene-addicted” cancers, as they seem to be in NSCLC. Additional genetic lesions, such as KRAS mutations that are present in 90% of pancreatic adenocarcinomas, could blunt their dependence on mutant EGFR-derived survival signals. It is also possible that TKI resistance mechanisms, including altered receptor trafficking and the T790M secondary EGFR mutation, may limit the effect of gefitinib and erlotinib in these cancers. In this context, irreversible EGFR inhibitors, such as HKI-272, which seems to circumvent mechanisms of resistance to the reversible TKIs, gefitinib and erlotinib (30), may warrant investigation in genotyped esophageal and pancreatic tumors.

In conclusion, whereas only modest responses have been observed following EGFR inhibition in nongenotyped cases of pancreatic and esophageal cancer, prospective studies, including carefully collected and analyzed tumor specimens, are required to determine the value of targeting the EGFR pathway in these gastrointestinal cancers.

References


22. Adelstein DJ, Rybicki LA, Carroll MA, Rice TW, Mekhail T. Phase II trial of gefitinib for recurrent or metastatic esophageal or gastroesophageal junction (GEJ) cancer. ASCO 2005.


Cancer Therapy: Clinical


www.aacrjournals.org

Downloaded from clinicanoncology.aacrjournals.org on July 14, 2017. © 2006 American Association for Cancer Research.
Epidermal Growth Factor Receptor Kinase Domain Mutations in Esophageal and Pancreatic Adenocarcinomas


Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/14/4283

This article cites 46 articles, 32 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/14/4283.full#ref-list-1

This article has been cited by 18 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/12/14/4283.full#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.