Role of epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of esophageal carcinoma and the suggested mechanisms of action (Review)

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Abstract. Cumulative evidence indicates that epidermal growth factor receptor (EGFR) is one of the most commonly altered genes in human cancer, via overexpression, amplification and mutation. Targeted inhibition of EGFR activity suppresses signal transduction pathways which control tumor cell growth, proliferation and resistance to apoptosis. Small molecule tyrosine kinase inhibitors (TKIs) are among the most common EGFR-targeting agents and have been used clinically to treat various malignancies. This review discusses the mechanism of action and clinical data that are relevant to the use of EGFR-TKIs in the treatment of esophageal carcinoma. The clinical and basic scientific experience of these agents thus far have implications for the future of therapeutic targeting of EGFR.

Contents

1. Introduction

Esophageal carcinoma is one of the most significant causes of cancer-related mortality among malignancies worldwide. The incidence of esophageal carcinoma has quadrupled in the last three decades, with the 5-year survival rate less than 15%. In the United States, the estimated incidence was 16,640 new cases and 14,500 mortalities in 2010 (1). Surgery is the main treatment option in localized, non-metastatic esophageal carcinoma (2,3), while the benefits of chemotherapy or radiotherapy as adjuvant treatment are controversial (4,5). The main site of treatment failure is locoregional recurrence. A minority of patients with locoregional failure may be salvaged with surgery or irradiation. With the improvements in locoregional control, distant metastasis is becoming more prevalent. The prognosis for patients with advanced esophageal carcinoma or recurrence is extremely poor, with a 5-6-month median overall survival (OS) time (6,7). Although a number of regimens have been tested in randomized studies, there is uncertainty with regard to the choice of chemotherapy regimens. These observations have emphasized the need to develop new effective therapeutic approaches.

There is substantial evidence suggesting that epidermal growth factor receptor (EGFR) plays a key role in esophageal carcinoma. Increased EGFR expression may influence multiple aspects of tumor biology, including survival, proliferation of cells, motility, invasiveness and resistance to treatment (8-10). The success of active small molecule tyrosine kinase inhibitors (TKIs) targeted against EGFR in treating non-small cell lung cancer (NSCLC) has prompted research into their clinical benefits in numerous other solid tumors. This review discusses the role of EGFR-TKI in esophageal carcinoma, describes the characteristics of EGFR and discusses the treatments that target it and are currently available for esophageal carcinoma patients.

2. EGFR and Barrett's esophagus

Barrett's esophagus (BE) is the only known pre-malignant condition of esophageal adenocarcinoma and is associated with a >40-fold increased risk of developing cancer (11). However, the origin of BE cells and the mechanism by which the condition develops is poorly understood. EGFR plays a key role in the process of normal esophageal epithelial cell carcinogenesis. BE tissue may originate from esophageal squamous cells (12). Long-term exposure of squamous cells to bile and gastric

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acid causes the cells to differentiate to columnar cells. EGFR expression in esophageal preneoplastic tissues was found to be higher compared with that in normal epithelium (13) and caused uncontrolled proliferation by activating the autocrine growth pathway. Autonomous activation of EGFR amplifies tumorigenic processes, from metaplasia, low-grade dysplasia, high-grade dysplasia to adenocarcinoma (14). NF-κB-CDX2 axis activation (15) and COX-2 upregulation (8) have been observed in this process. EGFR-positive expression was observed in 60% of Barrett's-associated adenocarcinoma (16). EGFR overexpression enhanced tumor cell migration, mediated by the relocalization of p120 from the cytoplasm to the membrane, upregulation of MMP1 and increased interaction with E-cadherin (17). The rate of EGFR amplification in high-grade dysplasia/esophageal adenocarcinoma patients was 8-11% (18,19) and may serve as diagnostic marker.

Although EGFR expression and amplification have been found to be relatively common in Barrett's adenocarcinoma, the possibility of genetic heterogeneity should be considered. According to the study by Owonikoko *et al* (20), more EGFR gene amplification was detected in the tumor tissue center than in the periphery. In another study (15), identical EGFR amplification status was found in Barrett's adenocarcinoma primary tumors and corresponding lymph node metastases.

3. EGFR polymorphisms in esophageal carcinoma

Polymorphisms in EGFR have been identified to be functional and influence gene expression and promote EGFR activity and protein production. EGFR intron 1 has a highly polymorphic CA single sequence dinucleotide repeat region (CA-SSR1, typically consisting of 14-22 repeats) whose length has been found to inversely correlate with transcriptional efficiency (21). Short CA repeats were associated with increased EGFR transcript level and elevated tumor response to anti-EGFR therapy (22). *In vitro*, longer CA repeats were correlated with better clinical prognosis in lung cancer (23), pancreatic cancer (24) and head and neck cancer (25). The length of CA repeats also influence DNA bendability and the binding of repressor proteins (26,27). Another notable finding was the consistently identical length of CA repeats analyzed in matched tumor and normal tissues (28), indicating that this was a stable polymorphism that does not change over time and may be easily measured in normal and cancer tissues.

Lee *et al* performed a retrospective analysis of 148 Chinese patients who received cisplatin-based concurrent chemoradiotherapy (29). A long CA allele was defined as length of the CA repeats ≥20, whereas alleles <20 CA repeats in length were classified as short alleles. The authors found that patients with the homozygous short allele of EGFR intron 1 had worse response and decreased survival time.

Another polymorphism EGFR G497A in EGFR, which attenuates the binding of EGF, has also been reported. The association between EGFR G497A and the risk of esophageal carcinoma was reported by Upadhyay *et al* in a study of an Asian population (30). No association of EGFR G497A genotype was observed (OR, 1.48; P=0.067), but the EGFR +61A/A genotype was significantly associated with risk of esophageal carcinoma (OR, 1.65; P=0.025), particularly in males (OR, 1.76; P=0.031). Similarly, another study has revealed a negative correlation of EGFR G497A with clinical outcome in esophageal carcinoma patients treated with radiotherapy with or without chemotherapy (31).

4. EGFR mutation in esophageal carcinoma

In NSCLC, retrospective studies have consistently demonstrated clinical predictors of response to the EGFR-TKIs, including Asian ethnicity, female gender, adenocarcinoma histology and non-smoking history (32). Furthermore, patients who exhibit significant success with EGFR-TKI treatment have been reported to have genetic mutations in the EGFR gene. EGFR-TKI treatment sensitivity is highly dependent on EGFR mutations, especially in exons 18, 19, 20 and 21 (33,34). Several studies have investigated the status of EGFR mutations in esophageal carcinoma.

Kwak *et al* sequenced exons 18 to 21 of EGFR from 21 cases of Barrett's esophagus, 5 cases of high-grade esophageal dysplasia and 17 cases of esophageal adenocarcinoma (35). This screening for mutations in exons 18 and 21 was performed as nearly all the mutations have been found in these regions. Somatic heterozygous EGFR mutations were identified in 2 of 21 esophageal cancers. One was the recurrent missense L858R and the other was an in-frame deletion, delE746-A750. Both were characterized as sensitizing EGFR mutations in NSCLC. However, the two patients with the EGFR mutation had not responded to EGFR-TKI (gefitinib) treatment. The study also reported EGFR mutations within this precancerous lesion. Three of 21 cases (14%) of BE had an EGFR mutation. Two had the delE746-A750 sensitizing EGFR mutation, whereas the third had the T790M drug-resistance mutation.

Pühringer-Oppermann *et al* analyzed the sequences of exons 19 and 21 in 105 patients with esophageal adenocarcinoma (36). The majority of the samples were of the wild-type genotype and only one silent mutation in exon 19 was identified. Similarly, Sudo *et al* investigated the existence of EGFR mutations in esophageal cancer (37). They found that one of the 50 patients had an EGFR mutation in codon 719, resulting in an amino acid substitution from glycine to aspartic acid. In addition, the study analyzed EGFR mutation coding in 17 esophageal cancer cell lines. Three of the 17 cell lines had the same silent mutation at nucleotide 2607, a G-to-A substitution in exon 20. However, not all studies have confirmed this result. Janmaat *et al* investigated 36 patients with advanced esophageal cancer treated with gefitinib (38) and no EGFR mutation was observed.

Although there are limitations to the studies presented, including relatively small numbers of patients and a retrospective nature, the studies appear to suggest that EGFR mutations in esophageal carcinoma are rare but do exist. The association between EGFR mutation and esophageal carcinoma was reported by Kaneko *et al*, who analyzed the overexpression of EGFR protein and mutations in EGFR in esophageal squamous cell carcinoma patients who received chemoradiotherapy (39). The authors identified a silent mutation at codon 787 of exon 20 of the EGFR gene in 19 patients (33%). The OS rate of patients with the EGFR mutation in exon 20 was lower than that of the patients without the mutation (OR, 2.347; 95% CI, 1.183-4.656; P=0.015). The EGFR mutation in exon 20 was associated with decreased OS.

Table I. Gefitinib in clinical trials for patients with esophageal carcinoma.

First author (ref.)	No. of patients	Line of therapy	Phase/treatment model	Grade 3/4 toxicities (%)	Results response (%)	Survival
Janmaat (38)	36	Second	Phase II gefitinib	Diarrhea (8.3) Rash (2.8) Increased AST (2.8) Increased ALT (2.8) Vomiting (2.8)	PR (2.8) SD (27.8)	MPFS, 59 days MOS, 164 days
Ferry (45)	27	Second	Phase II gefitinib	Diarrhea (11.1) Rash (18.5)	PR+SD (37)	MPFS, 1.9 months
Javle (46)	6	First	Phase I/II gefitinib + oxaliplatin + RT	Diarrhea 1/6 Vomiting 1/6 Fatigue 1/6 Constipation 2/6	CR 1/6 PR 1/6 SD 1/6	MOS, 10.8 months MPFS, 8.4 months
Rodriguez (47)	80	First	Phase II gefitinib + 5-FU/Cis + RT + Surgery	Vomiting (1.3) Mucositis (2.6) Hematological neutropenic fever (5) ANC <1000/μl (3.8)	pCR (7.5) pPR (33.8)	3-year OS, 42% 3-year LC, 76%
Sunpaweravong (48)	37		Phase II gefitinib + 5-FU/Cis ± RT	Hematological neutropenia (19) Leucopenia (19) Anemia (16)	Stage II-IVa: CR (15) PR (63) SD (11) Stage IVb: PR (50) SD (30)	NS

RT, radiotherapy; PR, partial response; SD, stable disease; CR, complete response; pCR, pathological complete response; pPR, pathological partial response; MPFS, median progression-free survival; MOS, median overall survival; OS, overall survival; LC, locoregional control; NS, not stated. AST, aspartate transanimase; ALT, alanine transanimase; ANC, absolute neutrophil count.

5. Clinical studies

Gefitinib and erlotinib are oral TKIs against EGFR. In 2005, a phase III clinical trial comparing gefitinib and placebo in advanced recurrent NSCLC failed to demonstrate increased survival (40), prompting the US FDA to restrict the use of gefinitib to patients who had previously benefited or continue to benefit from the treatment. Nevertheless, gefitinib is approved for use in a number of other countries, in certain cases as a first-line therapy. Erlotinib is FDA-approved for the treatment of patients with locally advanced or metastatic NSCLC, either following failure of at least one prior chemotherapy or, more recently, as maintenance therapy for patients whose disease has not progressed after four cycles of platinum-based chemotherapy. Erlotinib has also been approved for use with gemcitabine in the first-line treatment of pancreatic cancer, based on a phase III trial that demonstrated a small improvement in OS for the combination over gemcitabine alone, without significant improvement in the objective response rate (ORR) (41). EGFR-TKIs used as single-agent or combined with other treatments are being studied in esophageal carcinoma.

6. Gefitinib

In vitro, gefitinib exhibited a dose-dependant inhibition of cellular proliferation in esophageal carcinoma cells. Gefitinib inhibited EGF-induced autophosphorylation of EGFR and the downstream signaling pathways, Ras/Raf/MAPK and PI3K/Akt, and caused G1 arrest of cell cycle (42). Inhibition was also correlated with TRAIL-induced apoptosis enhancement via activation of caspase 3 and caspase 9 and inactivation of Bcl-xL (43). Taira *et al* reported that the combination of gefitinib and radiotherapy showed a synergistic effect and an additive effect in human esophageal carcinoma cell lines (44). Several studies have attempted to define the response rate and clinical outcomes of a treatment of using gefitinib in esophageal carcinoma patients. In 2006, Janmaat *et al* performed a phase II of 36 patients who had failed one line of prior chemotherapy and were treated with gefitinib (500 mg/day) (38). In this study, gefitinib showed a modest activity in second-line treatment of advanced esophageal carcinoma. Of the 36 enrolled patients, only one (2.8%) achieved a partial response. The disease control rate was 30.6%, which was less than previous results. The study also attempted to identify the

Table II. Erlotinib in clinical trials for patients with esophageal carcinoma.

First author (ref.)	No. of patients	Line of therapy	Phase/treatment model	Grade 3/4 toxicities (%)	Results response (%)	Survival
Dobelbower (49)	11	First	Phase I erlotinib + 5-FU/Cis + RT	Rash 6/11 Vomiting 1/11 Dehydration 3/11 Esophagitis 2/11 Hematological: WBC 4/11 Hemoglobin 1/11 Platelet 1/11	NS	NS
Ilson (50)	30	Second	Phase II erlotinib	Rash 10%	PR (6.7)	MOS, 10.3 months
Li (51)	24	First	Phase II erlotinib + Pac/Cis + RT	Rash 4.2% Esophagitis 20.8% Hematological: WBC 16.7% Platelet 8.3%	CR (45.8) PR (45.8)	2-year OS, 70.1% 2-year LC, 87.5%

RT, radiotherapy; PR, partial response; CR, complete response; MPFS, median progression-free survival; NS, not stated; MOS, median overall survival; OS, overall survival; LC, locoregional control; WBC, white blood cell count.

patients who were likely to benefit from gefitinib treatment. EGFR, PI3K and K-ras gene mutations were investigated. K-ras mutation was identified in two patients with progressive disease. Squamous cell carcinoma histology, female gender and EGFR protein overexpression may be predictors of response to gefitinib. Furthermore, diarrhea was a common adverse effect (58.3%). During the treatment, four patients had a dose reduction for severe diarrhea and rash.

Similarly, in a phase II study, 27 patients with advanced inoperable adenocarcinoma of the esophagus underwent first-line target therapy with gefitinib (500 mg/day) until progression or unacceptable toxicity (45). Preliminary results revealed that three patients had a partial response and a 37% disease control rate with an acceptable toxicity profile. The study analyzed the change of gene expression of tumor tissue biopsy before and after the target treatment. The change in p-EGFR levels, although not statistically different, indicated that in a few patients there was suppression of p-EGFR expression following gefitinib therapy.

To date, one phase I/II study has assessed the safety and efficacy of gefitinib combined with chemoradiotherapy. In a previous trial (46), six patients with immunohistochemistry EGFR scores of 0 or 1+ were enrolled in the study. Preoperative radiotherapy was delivered in 1.8 Gy per fraction once daily over 28 days to a dose of 50.4 Gy. Intravenous oxaliplatin (85 or 100 mg/m^2) was begun on the first day of radiation. Gefitinib (250 mg/day) was administered for 1 year. This combination treatment was well tolerated but showed limited efficacy. Three patients (50%) experienced progressive disease during treatment. Median overall and disease-free survival times were 10.8 and 8.4 months, respectively. The lower dose of gefitinib (250 mg/day) and the lower EGFR expression (5 patients with EGFR 1+ and 1 patient 0+) may have led to this lower clinical activity.

Although gefitinib had a modest response rate in the above studies, an OS benefit was exhibited in a phase II clinical trial (47). This was an open label phase II study of patients with locoregionally advanced esophageal (34 patients) or gastroesophageal junction (GEJ) carcinoma (46 patients). Gefitinib (250 mg/day) was administered with preoperative concurrent chemoradiotherapy (CCRT; RT, 30 Gy, 1.5 Gy, BID; CT, cisplatin, 20 mg/m^2/day and fluorouracil, 1,000 mg/m^2/day) for 4 weeks and restarted with postoperative therapy for 2 years. The estimated 3-year OS rate for patients receiving and not receiving gefitinib was 42 and 28%, respectively (P=0.06). The estimated 3-year OS rate for patients with and without diarrhea was 52 and 30%, respectively (P=0.006).

Gefitinib has also been combined with 5-FU/cisplatin in a phase II evaluation of patients with stage II-IVb esophageal cancer, with preliminary data presented. Patients with stage IVb disease received gefitinib/5-FU/cisplatin while those with stage II-IVa disease also received concurrent radiation. Of the 25 evaluable patients with stage II-IVa disease, 4 underwent esophagectomy and 1 (4% of patients enrolled or 25% of those who underwent surgery) was found to have a pathological complete response. Of 5 evaluable patients with stage IVb disease, the ORR was 50% (48).

7. Erlotinib

Only three studies have evaluated the safety of erlotinib with or without chemoradiotherapy. Dobelbower *et al* (49) reported safety and tolerability of erlotinib delivered at 50, 100 or

150 mg/day with concurrent 5-FU, cisplatin and thoracic radiation in a phase I study of esophageal carcinoma. A total of 11 patients with squamous cell carcinoma or adenocarcinoma of the esophagus were enrolled in this study. Two patients were discontinued from the study due to toxicities unrelated to erlotinib. There were no erlotinib dose reductions and no treatment delays. All patients experienced esophagitis during treatment (grade 1, 55%; grade 2, 32%; grade 3, 9%; grade 4, 9%). Radiation treatment was interrupted in two patients and resumed after 3 days when esophagitis improved. The other major toxicities were grade 1 or 2 diarrhea, skin rash, nausea and dehydration. The study by Ilson *et al* (50) evaluated the feasibility and efficacy of erlotinib in patients with previously treated esophageal cancer. A total of 30 patients with esophageal and GEJ carcinoma received 150 mg erlotinib daily. Patients continued therapy until disease progression or unacceptable toxicity. EGFR overexpression was observed in 24 patients (80%). The response rate was 6.7%, with response duration 5.5-7.0 months. Of the 30 patients, 13% had grade 2 diarrhea. Grade 3 hematological toxicity was observed in 10% of patients and 67% of patients experienced grade 3 skin rash. The authors concluded that erlotinib had limited activity in patients with esophageal cancer, requiring further evaluation in squamous cell carcinoma. More recently, another phase II study (51) investigated the feasibility and efficacy of CCRT in combination with erlotinib for locally advanced esophageal carcinoma. A total of 24 patients with locally advanced esophageal carcinoma were treated with concurrent chemotherapy (paclitaxel 135 mg/m^2 days 1 and 29; cisplatin 20 mg/m^2 days 1-3 and 29-31) and chest radiation (60 Gy, 2.0 Gy/day). All patients were treated with 150 mg erlotinib daily. Partial response was observed in 11 patients (45.8%) and complete response in 11 patients (45.8%), as confirmed by chest CT scan and barium swallow. The 2-year OS, local-regional control and relapse-free survival rates were 70.1% (95% CI, 50.4-90), 87.5% (95% CI, 73.5-100) and 57.4% (95% CI, 36.3-78.7), respectively. The incidence of acute grade esophagitis toxicities was 20.8%. Hematological toxicity and skin rash were also significant but manageable.

Overall, gefitinib and erlotinib have little single-agent activity in the first- or second-line setting in esophageal carcinoma. Although no specific conclusions may be drawn, it may be that patients who are more likely to respond have tumors that have squamous cell carcinoma histology and EGFR overexpression. The toxicities in all these trials were similar. In general, therapy was well tolerated, with diarrhea and skin rash being the major toxicities. These occurred in 30-70% and 47-86% of patients, respectively, but were mostly grade 1/2 (52).

8. Conclusion

Medical development in cancer therapeutics has been transformed by our increasing understanding of the cellular mechanisms of carcinogenesis. Multidisciplinary studies, including use of the novel targeted therapies described in this review as single agents or in combination with chemotherapy or chemoradiotherapy, to identify patient- and tumor-specific markers predictive for response and/or toxicity remain urgently needed for esophageal carcinoma.

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