Epidermal Growth Factor Receptor Is a Prognosis Predictor in Patients With Esophageal Squamous Cell Carcinoma

Wencheng Zhang, MD,* Hongxia Zhu, MD,* Xiao Liu, MD, Qifeng Wang, MD, Xun Zhang, MD, Jie He, MD, Kelin Sun, MD, Xiangyang Liu, MD, Zongmei Zhou, MD, Ningzhi Xu, MD, and Zefen Xiao, MD

Department of Radiation Oncology, Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center of Cancer, Tianjin; and Laboratory of Cellular and Molecular Biology, State Key Laboratory of Molecular Oncology, and Departments of Radiation Oncology, Pathology, and Thoracic Surgery, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Background. Our previous study indicated the survival rate for esophageal squamous cell cancer (ESCC) patients in stage III and positive lymph node groups with postoperative radiation therapy was significantly increased compared with surgery alone. But a predictive biomarker was needed to identify the patients who would benefit from postoperative radiotherapy. This study aims to evaluate epidermal growth factor receptor (EGFR) as an indicator to predict the prognosis of ESCC and to identify the patients who would benefit from postoperative radiotherapy.

Methods. Tissue samples were collected from our previous randomized study: 243 in the surgery alone group and 198 in the surgery plus radiotherapy group. Expression of EGFR was analyzed by immunohistochemical staining.

Results. The expression of EGFR is correlated with depth of tumor invasion ($p = 0.005$), lymph node metastasis ($p < 0.001$), and pathologic stage ($p < 0.001$). The survival rate of patients with high EGFR expression is significantly lower than that of patients with low EGFR expression ($p = 0.000$). Notably, in stage IIA cases, the 5-year survival rate is 57.6% in the low EGFR expression group and 36.6% in the high expression group ($p = 0.020$). EGFR is one of the independent variants that influence the prognosis. Moreover, for high EGFR expression patients the survival rate of the surgery plus radiotherapy group is higher than that of the surgery alone group ($p = 0.034$).

Conclusions. Expression of EGFR can be a prognostic predictor for ESCC. Patients with high expression of EGFR may benefit from postoperative radiation therapy.


Esophageal cancer is a highly aggressive disease. Worldwide, it is the sixth most common cause of cancer death [1]. Surgery remains the first choice for medically fit patients with resectable disease. Despite advances in surgical techniques and treatments, the prognosis of esophageal cancer remains poor: the 5-year survival rate after surgery alone is approximately 40%, and only 10% for patients with locally advanced disease [2, 3]. To improve those rates, preoperative therapy has been proposed. However, several randomized trials of preoperative chemotherapy/chemoradiotherapy found that patients had disparate pathologic responses (complete response or no complete response) and survival rates for the same treatment [4–8].

The Union for International Cancer Control–American Joint Committee on Cancer (UICC-AJCC) clinical staging system [9] is the most frequently used criterion for predicting treatment outcome, although the survival rates are usually different even among patients with the same disease stage undergoing the same treatment. For stage IIA patients, 5-year survival after curative resection including different operative methods such as transhiatal or transthoracic esophagectomy and an extended trans-thoracic approach ranges from 22% to 84% [10–13]. Such a great difference in prognostic outcome for the same stage patients indicates that other prognostic indicators are urgently needed. In our prospective study of prophylactic radiotherapy after curative resection of esophageal squamous cell carcinoma (ESCC), approximately 35% of stage III esophageal cancer patients benefited from postoperative radiotherapy. The survival rate was significantly increased compared with that for surgery alone [11, 14]. However, a predictive biomarker is needed to identify the patients who would benefit from postoperative radiotherapy.

Recently, much progress has been made in identifying molecular makers and developing targeted therapy. Aberrant expression of EGFR has been found in many

Accepted for publication March 13, 2014.

*Wencheng Zhang and Hongxia Zhu contributed equally to this paper.

Address correspondence to Dr Xiao, Department of Radiation Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing 100021, China; e-mail: xiaozefen2013@163.com.
human cancers, including brain, head and neck [15, 16], lung [17, 18], breast [19], and colorectal [20], as well as esophageal cancer [21]. In esophageal cancer, overexpression of EGFR detected by immunohistochemistry analysis is very common, occurring in approximately 80% of patients with adenocarcinoma and squamous cell carcinoma [22]. A number of studies have shown that increased EGFR expression was associated with poor survival among patients with esophageal cancer [23–25]. An EGFR mutation was rarely detected in esophageal carcinoma [26–29].

In the present study, we examined EGFR expression retrospectively for 549 patients in our previous study who had received randomized radical resection alone or surgery plus postoperative radiation therapy. The purpose of this study was to identify patients who could benefit from postoperative radiation therapy and to explore whether EGFR expression could be used as a prognostic indicator for ESCC.

**Material and Methods**

**Samples**

Samples were collected from our previous study executed between September 1986 and December 1997. In that study, patients with esophageal carcinoma who had undergone curative resection were randomly allocated, using the envelope method, to two groups: those who underwent radical esophagectomy only (surgery [S]) and those who underwent esophagectomy plus postoperative radiation therapy (S+RT) [11, 14]. We retrieved 441 formalin-fixed and paraffin-embedded archival tissue samples, with 243 in the S group and 198 in the S+RT group (including 17 patients who received ≤40 Gy RT). The Institutional Review Board of Cancer Hospital, Chinese Academy of Medical Science, approved the study protocol.

**Patient Follow-Up**

Patients were instructed to return for follow-up, included clinical examination, barium swallow, chest radiography, abdominal ultrasonography, and thoracic computed tomography (CT), at 3-month to 6-month intervals for 1 year, and then 6 to 12 months thereafter. If ultrasonography results of the abdomen were suspicious, abdominal CT was performed. Local failure was determined by positive pathologic diagnosis or radiographic evidence of mediastinal lesions revealed by CT scan. Signs or symptoms of vocal cord paralysis or tracheal compression combined with mediastinal lesions shown on CT were also considered local failures. Patients unable to return for follow-up were instructed to report to their local hospitals or clinics for examination and completion and return of study data forms. Although some of these reports were unclear, especially when the patients died, all patients were followed through the end of December 2010.

**Immunohistochemistry**

Samples were deparaffinized and rehydrated in water. Antigen retrieval was achieved in citrate buffer (0.01 M, pH 6.0) using a microwave-based method. The tissue section slides were incubated with peroxidase blocking reagent (ready-to-use 3% H2O2 solution) for 10 minutes...
followed by washing in phosphate-buffered saline (PBS) solution twice to block the endogenous peroxidase. The PV-9000 Polymer Detection System (GBI Labs, Mukilteo, WA) was used for immunohistostaining. Briefly, the primary anti-EGFR antibody (NCL-EGFR-384; Novocastra, Newcastle Upon Tyne, UK) was incubated with slides in a humidified chamber overnight at 4°C. After being washed with PBS three times, the slides were incubated with a polymer helper in a moist chamber at room temperature for 20 minutes. The slides were washed again in PBS buffer, and then incubated with polymer of horse–radish peroxidase anti-mouse/rabbit secondary antibody in a moist chamber for 30 minutes. The slides were then stained using 3', 3'-diaminobenzidine (DAB [ZLI-9018; Zhongshan, Beijing, China]).

The scoring was performed by two experienced pathologists who were blinded to the clinical outcome data. The slides were evaluated, and EGFR expression was scored in at least five microscopic fields, and the mean values of the five fields were obtained. The immunostaining of EGFR was semiquantitatively evaluated based on the color and the percentage of cells showing membrane staining. The intensity was classified as follows: 0, negative staining; 1, weak staining; 2, moderate staining; 3, strong staining. Then the rate of positive cells (1, 2, 3, and 4). For data analysis, scores of less than 8 were defined as low expression and scores of 8 or more, as high expression (Fig 1).

Statistical Methodology

Statistical analysis was performed using SPSS 13.0 software (SPSS, Chicago, IL). The survival rate was examined using the Kaplan-Meier method, and any differences were assessed using the log rank test. Correlations were analyzed by Pearson’s χ² test using SPSS 13.0 software. The survival curve was plotted using GraphPad Prism (GraphPad Software, San Diego, CA). Survival time is from time of surgery to time of death or time of last follow-up. Disease-free survival rate was calculated from the date of surgery to the first date of local or distant recurrence, or last date of follow-up.

Results

Patient Characteristics

Table 1 shows the clinical characteristics of 441 patients. The population was predominantly male (78.2%), with a median age of 56 years (range, 28 to 68). Three hundred thirty-five patients (76%) had high expression of EGFR and 106 (24%) had low expression. The expression of EGFR is correlated with depth of tumor invasion (p = 0.005), lymph nodes metastasis (p < 0.001), and pathologic stage (p < 0.001). However, the expression of EGFR is not correlated with the tumor location, tumor length, histologic grade, or patients’ age and sex.

Relationship Between EGFR Expression and Survival

After analyzing EGFR expression level with overall survival time of ESCC patients, we demonstrated that EGFR expression of tumor tissues can indicate their outcomes. For the low EGFR expression group, the overall 5-year survival rate was 41.5% with a median survival time of...
and for the high EGFR expression group, the overall 5-year survival rate was 18.2% with a median survival of 18 months. The difference in overall survival rate between the two groups was highly statistically significant ($\chi^2 = 18.127, p = 0.000$; Fig 2A1). The disease-free 5-year survival rates were 38.7% and 17.6% in low and high EGFR expression groups, respectively. The difference between the two groups was also significant ($\chi^2 = 15.171, p = 0.000$; Fig 2A2).

To minimize the interference of other factors on the interpretation of results, we examined the EGFR expression only from patients who had no lymph nodes metastasis, including T1 to T4 stage patients. Of 213 cases, 72 (33.8%) displayed low EGFR expression, and 141 (66.2%) displayed high EGFR expression. The 5-year survival rate was 54.2% with a median of 68.4 months in the low EGFR expression group, and 28.4% with a median of 26.4 months in the high EGFR expression group. The difference in survival rate between the two groups is significant ($\chi^2 = 10.299, p = 0.001$; Fig 2B1).

Whereas the overall survival rate is correlated with expression of EGFR, it is not correlated with T stage in this study (T1 to T4N0M0) because we found that the 5-year overall survival rates for T1$+$T2, T3, and T4 were 41.4%, 37.2%, and 32.3%, respectively, and there was no significant difference between them ($\chi^2 = 2.351, p = 0.309$; Fig 2B2).

Furthermore, to exclude the interference of staging, we examined EGFR expression only in stage IIA (according to the AJCC classification, seventh edition, 2009) cases. Among 74 cases, 33 (44.6%) had low EGFR expression,
and 41 (55.4%) had high EGFR expression. The 5-year survival rate is 55.4% with a median of 81 months in the low EGFR expression group, and 33.6% with a median of 36 months in the high EGFR expression group. The difference between the two groups was statistically significant ($\chi^2 = 5.437, p = 0.020$; Fig 2C).

**EGFR High Expression Is Potential Indicator of Positive Outcome of Postoperative Radiation Therapy**

Based on our findings, we wanted to determine whether EGFR could be a potential indicator for postoperative radiotherapy for ESCC. To do so, we examined EGFR expression and survival rate between the S group and the S+R group. For high EGFR expression patients, the 5-year overall survival rate was 15.0% for the S group and 22.3% for the S+R group; and the 5-year disease-free survival rate was 14.4% for the S group and 21.6% for the S+R group. There were significant differences between the S group and the S+R group in overall survival rate as well as in disease-free survival rate ($p = 0.034$ and $p = 0.01$, respectively; Fig 3A). For low EGFR expression patients, the 5-year overall survival rate and disease-free survival were 39.5% and 37.5% in the S group and 44% and 40% in the S+R group, respectively. The differences between the two groups in overall survival rate and disease-free survival rate were not statistically significant ($p = 0.165$ and $p = 0.304$, respectively; Fig 3B). The results strongly suggest that ESCC patients who have high expression of EGFR are more likely to benefit from postoperative radiation therapy.

**Multivariate Analysis**

We performed multivariate analysis of overall survival and disease-free survival. As shown in Table 2, age, sex, pathologic T and N stage, EGFR expression, histologic grade, and treatment modality (S versus S+RT) are all independent factors that affect prognosis.

**Comment**

Based on the present study, we confirm that overexpression of EGFR is common in ESCC patients, and EGFR expression is correlated with tumor T staging, lymph node metastatic status, and TNM staging (AJCC 2009). We also show that EGFR in ESCC may become a favorable marker to indicate the subtype of patients who are more likely to benefit from postoperative radiation therapy.

Expression of EGFR as a prognostic indicator in ESCC has been reported previously [21–25]. In our current study, the results show that the survival rate for ESCC patients with high EGFR expression is significantly lower than that for patients with low EGFR expression. Furthermore, after multivariate analysis, for the first time...
we have shown that EGFR, like TNM stage and histologic grade, is also an independent factor that influences the prognosis of ESCC. Additionally, EGFR is also a prognostic indicator for head and neck, lung, and breast cancers [21–25]. More notably, consistent with what we find here is the study reported by Maurizi and colleagues [24]. They showed that EGFR level determined by a radioligand receptor assay correlated very well with the 5-year survival rate of 140 primary laryngeal squamous cell carcinoma patients. The 5-year survival rate for patients with EGFR-negative tumors was 81%, whereas for patients with EGFR-positive tumors it was 25%. Also, the 5-year relapse-free rate was 77% for patients with EGFR-positive tumors compared with 24% for patients with EGFR-negative tumors. Therefore, our data, with others, strongly support that EGFR is an effective biomarker for predicting prognosis of ESCC. The potential mechanism for the association of EGFR positivity with benefit from adjuvant radiotherapy is unclear. We are trying to get clues from preclinical research. The UICC-AJCC classification [30] is the best system for predicting treatment outcome and indicating clinical prognosis of cancer patients. However, because of the complexities and heterogeneities of human cancers, most clinicians, if not all, realize and agree that it is not satisfactory for them to predict the prognosis of their patients, one by one, by relying solely on the UICC-AJCC classification.

We [11, 14] reported previously that the 5-year survival rate for ESCC patients with stages I and IIa who underwent surgery plus postoperative radiotherapy is approximately 50%, whereas the other patients die of recurrence and metastasis. However, it is difficult to predict clinically who will have a good prognosis and who will not. Furthermore, the difficulties in predicting the prognosis for the same stage, such as stage IIa patients or lymph node negative patients, hinder the decision when choosing the best treatment modality. Moreover, Reeh and associates [31] reported that there is poor discrimination between different stages, such as stages Ib and IIa, stages IIIa and IIIb, and stages IIIC and IV, judging by the overall survival of 605 esophageal carcinoma patients from 1992 to 2009, even based on the seventh edition of the UICC classification. Therefore, the investigators “strongly propose that the next revision of the UICC classification should reduce the stages to groups with similar survival, without defining complex subgroups” [31].

Our present results not only support this notion, but also provide one more indicator, EGFR, of prognosis to ensure better prediction than solely depending on TNM staging. Based on detection of EGFR expression and by means of hierarchical analysis, we can further divide a single stage, such as IIa, into two subgroups: overall survival better, or overall survival worse. For example, among patients who had no lymph node metastasis, the survival rates for those with low or high EGFR expression were significantly different ($p = 0.001$). Furthermore, for stage IIa only, survival rates for patients with low or high EGFR expression were also statistically significant ($p = 0.020$). Therefore, our results demonstrate that there are indeed survival differences between EGFR high and low expression even within the same IIa staging group (AJCC 2009). Therefore, we suggest that EGFR expression should be determined in all ESCC patients, especially those in stage IIa and those with no lymph node metastasis, to predict the prognosis more accurately.

The role of neoadjuvant chemoradiotherapy for esophageal cancer has been debated for several decades. Recently, treating patients with preoperative chemotherapy or chemoradiotherapy has been favored, especially patients with potentially curable esophageal cancer, as it can improve their overall survival [32]. Nevertheless, in China, there are so many patients who have undergone surgery first, and then postoperative radiation therapy may still be needed afterward. Moreover, we previously showed that approximately 35% of stage III esophageal cancer patients could benefit from postoperative radiation therapy and that the survival rate with postoperative radiation therapy was significantly increased compared with surgery alone [11, 14]. In our present study, we confirm further that in ESCC patients with high EGFR expression, the overall survival of the surgery plus radiation therapy group is significantly higher than that of the surgery alone group ($p = 0.034$). Therefore, our results demonstrate that EGFR is not only a prognostic predictor for ESCCC, but also a beneficial indicator for postoperative radiotherapy. We also noted the results, similar to ours, reported by Gotoh and colleagues [33]. In their study of 62 ESCC patients who underwent radical chemoradiotherapy with subsequent

### Table 2. Multivarient Analysis of Overall Survival Rate and Progression-Free Survival Rate

<table>
<thead>
<tr>
<th>Variables</th>
<th>Overall Survival</th>
<th>p Value</th>
<th>Disease-Free Survival</th>
<th>HR (95% CI)</th>
<th>p Value</th>
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<tbody>
<tr>
<td>HR (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td>HR (95% CI)</td>
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<tr>
<td>Age 1.246 (1.105–1.406)</td>
<td>0.000</td>
<td></td>
<td>1.221 (1.083–1.376)</td>
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<tr>
<td>Sex 0.753 (0.588–0.965)</td>
<td>0.025</td>
<td></td>
<td>0.777 (0.607–0.993)</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>T stage 1.253 (1.049–1.497)</td>
<td>0.013</td>
<td></td>
<td>1.199 (1.005–1.429)</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>N stage 1.246 (1.105–1.406)</td>
<td>0.000</td>
<td></td>
<td>1.475 (1.126–2.470)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Histologic grade 1.192 (1.013–1.402)</td>
<td>0.034</td>
<td></td>
<td>1.246 (1.062–1.462)</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>EGFR expression 1.452 (1.137–1.855)</td>
<td>0.003</td>
<td></td>
<td>1.351 (1.057–1.728)</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>Treatment, S or S+RT 0.735 (0.600–0.901)</td>
<td>0.003</td>
<td></td>
<td>0.689 (0.563–0.844)</td>
<td>0.000</td>
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CI = confidence interval; EGFR = epidermal growth factor receptor; HR = hazard ratio; RT = radiotherapy; S = surgery.
evaluations of primary site endoscopic response and survival, they showed that among 21 EGFR-positive cases, 13 of 21 (62%) had a complete response at the primary site; however, of 41 EGFR-negative cases, only 14 of 41 (34%) had a complete response. The difference is significant ($p = 0.037$). Taken together with our findings, we propose that a prospective and randomized trial of large size should be undertaken to further validate the role of EGFR as an indicator for determining the treatment modality for ESCC.

In conclusion, EGFR expression could be a prognostic predictor for ESCC, especially for patients without lymph node metastasis and in stage IIa. Moreover, patients with high EGFR expression could benefit from postoperative radiation therapy.

We thank Professor Xiaohui Lin for editing the manuscript.

This work was supported by the Capital Foundation for Medical Research and Development (2007–2012); Science and Technology Project of Beijing (Z121107001012004); National Natural Science Foundation of China (81272512, 81021061, 39925020, and 39925020); Foundation of CMAS (LC2010A02); and Chinese Hi-Tech R&D Program (2006AA02A403; 2012AA02A503).

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