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Prognostic value of EGFR family expression in lymph node-negative esophageal squamous cell carcinoma patients

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ABSTRACT

The human epidermal growth factor receptor (EGFR) family has been widely studied in cancer, however, the prognostic role of EGFR family expression in lymph node-negative esophageal squamous cell carcinoma (ESCC) patients have not been invalidated. This study was designed to determine the prognostic value of EGFR family expression in a population of lymph node-negative ESCC patients treated with curative resection. EGFR family protein expression was examined by immunohistochemical analysis of tissue microarrays of 94 patients with lymph node-negative ESCC after radical esophagectomy with three-field lymphadenectomy. Survival differences were compared using Kaplan–Meier analysis. Cox regression analyses were performed to determine the prognostic factors for overall survival and disease-free survival (DFS). ErbB4 expression was found to be an independent prognostic factor for DFS in patients without lymph node metastasis; increased ErbB4 expression was associated with decreased DFS. Additionally, patients with high ErbB4 expression tended to have worse overall survival. EGFR, ErbB2 and ErbB3 expression were not significantly associated with survival in lymph node-negative ESCC patients. Increased ErbB4 immunohistochemical expression was associated with poor prognosis in lymph node-negative ESCC patients.

1. Introduction

Worldwide, esophageal cancer (EC) is one of the most common malignant tumors, and esophageal squamous cell carcinoma (ESCC) is the main histological type in eastern countries, such as China, where it accounts for more than 90% of EC cases [1]. Lymph node metastases in ESCC represent an important prognostic indicator after surgical resection [2]. Although patients with regional lymph node-negative ESCC have better overall survival (OS) than those with nodal metastases, many lymph node-negative patients suffer tumor relapses despite curative resection [3,4]. Thus, it is necessary to investigate the prognostic value of molecular factors associated with disease progression in staged lymph node-negative ESCC patients, as these data may help individually tailor adjuvant therapies.

The human epidermal growth factor receptor (EGFR) family, which comprises four members: EGFR (ErbB1), ErbB2, ErbB3 and ErbB4, has been widely studied in cancer [5]. All EGFR members have an extracellular ligand-binding region, a hydrophobic transmembrane domain and an intracellular domain with intrinsic protein-tyrosine kinase activity. Ligand binding induces the formation of receptor homo- and heterodimers and activates intrinsic kinase activity, resulting in phosphorylation of specific tyrosine residues within the cytoplasmic tail. These phosphorylated residues serve as docking sites for a range of proteins, the recruitment of which activates intracellular signaling pathways [6].

The EGFR pathway plays pivotal roles in the growth and maintenance of numerous tissues by regulating cell division, apoptosis, differentiation and migration [5,6]. Studies have shown that dysregulated EGFR signaling is connected to cancer tumorigenesis and progression [7]. Furthermore, a significant association between EGFR

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overexpression and lymph node involvement, metastasis and poor OS has been found in ESCC [8,9]. ErbB2 gene amplifications and increased protein expression are also associated with clinical characteristics and overall survival in ESCC [10,11]. Compared with EGFR and ErbB2, investigations into the role of ErbB3 and ErbB4 in ESCC are rare. In this study, we investigated levels of EGFR, ErbB2, ErbB3 and ErbB4 protein expression in primary ESCC tumors using immunohistochemistry (IHC) to determine the prognostic value of EGFR family proteins expression in patients with lymph node-negative ESCC who had undergone curative resection.

2. Materials and methods

2.1. Study population

Patients diagnosed with thoracic ESCC who underwent radical esophagectomy with three-field lymphadenectomy at Fudan University Shanghai Cancer Center between 2001 and 2009 were enrolled in this study, which was approved by the institutional review board. The inclusion criteria were as follows: (1) ESCC was confirmed by histopathology and classified by the 7th edition of the TNM-UICC/AJCC recommendation; (2) N0 confirmed by pathological examination; (3) only one primary tumor; (4) not receiving preoperative chemotherapy and/or radiotherapy; (5) having undergone radical esophagectomy with three-field lymphadenectomy with ≥15 total lymph nodes retrieved; (6) R0 resection. No patients died from postoperative complications. Patients whose paraffin specimens were unavailable were excluded. As a result, 107 patients were selected. Among these patients, 13 were excluded from analysis because they were lost to follow-up. Thus to a total of 94 patients were included in the final analyses; the clinicopathological characteristics of the final study population are summarized in Table 1.

Regular follow-up was performed in all patients at three-month intervals after the operation in the first two years, six-month intervals in the third year and at yearly intervals thereafter, as described previously [12]. Follow-up evaluation included physical examination, barium esophagram, enhanced computational tomography (CT) of the chest and neck, and ultrasonographic examination of the upper abdomen. Esophageal recurrence was confirmed by endoscopy and biopsy. The median follow-up for all patients was 63.7 months, with a range of 3.1–127.2 months.

Table 1
Clinicopathological characteristics of study population (n = 94).

Characteristics	No. of patients (%)
Gender	
Male	69 (73.4)
Female	25 (26.6)
Age (years)	
Median	58 (range 37–74)
< 60	54 (57.4)
≥60	40 (42.6)
Tumor location	
Upper	7 (7.4)
Middle	64 (68.1)
Lower	23 (24.5)
Tumor length (cm)	
< 4	50 (53.2)
≥4	44 (46.8)
Tumor differentiation	
Well	14 (14.9)
Moderate	57 (60.6)
Poor	23 (24.5)
Pathologic T stage	
T1	5 (5.3)
T2	36 (38.3)
T3	48 (51.1)
T4	5 (5.3)
Vascular invasion	
Negative	86 (91.5)
Positive	8 (8.5)
Perineural invasion	
No	84 (89.4)
Yes	10 (10.6)
No. of lymph nodes retrieved	
Median	26 (range 16–74)
≤26	47 (50)
> 26	47 (50)
Adjuvant therapy	
No	50 (53.2)
Yes	44 (46.8)

2.2. IHC to evaluate expression

Tissue microarrays (TMAs) were constructed as previously described [13]. Briefly, standard hematoxylin and eosin-stained slides were reviewed from each section of ESCC tissues. Then, three representative tumor tissue cores (1 mm in diameter) and one adjacent normal esophageal tissue core (1 mm in diameter) from the same patient were removed from the original formalin-fixed paraffin-embedded tissue blocks and rearranged on a recipient paraffin block. IHC staining was performed on serial 4-μm thick sections from TMA blocks using the standard Envision method with a panel of antibodies: EGFR (rabbit mAb clone EP38Y; 1:100; Abcam, Cambridge, UK); ErbB2 (rabbit mAb EP1045Y; 1:50; Abcam); ErbB3 (nouse mAb clone RTJ2; 1:200; Abcam); ErbB4 (rabbit mAb 83B10; 1:150; Cell Signaling Technology, Danvers, MA, USA).

Tissue sections were independently assessed by three blinded histopathologists, and discrepancies were resolved by consensus. A modified semi-quantitative H-score was used to evaluate IHC staining [14]. The score was generated by multiplying the percentage of positive-stained cells (0%–100%) and the intensity of staining for each tissue core. For EGFR, the staining intensity was classified as follows: 0, no staining; 1+, partial membrane staining; 2+, weak, complete membrane staining; 3+, moderate, complete membrane staining; and 4+, strong, complete membrane staining. For ErbB2, ErbB3 and ErbB4, IHC staining intensity was classified as follows: 0, negative; 1+, weak; 2+, moderate; and 3+, strong. Thus, overall H-scores ranged from 0 to 400 (EGFR) or 0–300 (ErbB2, ErbB3 and ErbB4).

2.3. Statistical analysis

Statistical analyses were conducted with SPSS v17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were summarized by descriptive statistics, such as medians and ranges. Categorical variables were tabulated by frequency and percentage. Spearman rank correlation tests were used to evaluate the relationships among protein expression. OS was defined as the time from the date of surgery to the date of death from any cause. Patients who were still alive at the date of the last follow-up were censored. Disease-free survival (DFS) was defined as the time from the date of surgery to disease relapse (any site), or death from any cause, whichever comes first. Patients who were alive without tumor recurrence were censored at the date of last follow-up. Evaluated patterns of failure were first failure (local, regional, or distant). If recurrences occurred within 60 days of each other, they were counted as simultaneous. OS and DFS rates were estimated using the Kaplan–Meier method, and compared using the log-rank test. Optimal cut-off points for H-scores of biomarkers were determined as the value of the maximum Yuden index by receiver operating characteristic (ROC) analysis. High expression was defined as an H-score ≥ the cut-off point, and low expression as an H-score < the cut-off point. Cox regression models were performed to estimate the hazard ratio (HR)