RESEARCH ARTICLE

Relationship between EGFR Over-expression and Clinicopathologic Characteristics in Squamous Cell Carcinoma of the Esophagus: A Meta-analysis

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Abstract

Over-expression of epidermal growth factor receptor (EGFR) has been identified as a common feature associated with clinical outcome in many types of cancer, including squamous cell carcinoma of the oesophagus (SCCO). However, the clinical importance of EGFR over-expression in SCCO remains unsettled as conflicting results exist. Therefore we carried out the present meta-analysis of published studies for clarification. A total of 13 studies including 1, 150 patients were enrolled. EGFR over-expression was positive in 722 of these cases. With EGFR over-expression, patients had higher depth of invasion, vascular invasion, and poor prognosis. However, expression had no relation with degree of differentiation, histological grade, lymph node metastasis, clinical stage or lymphatic invasion. EGFR over-expression is probably a valuable predictor for the T stage, vascular invasion and OS, and it could be used as a poor prognosis indicator for the esophageal SCC patients. Targeting therapy to EFGR should be considered to the combined treatment in SCCO.

Keywords: EGF receptor - SCC of the oesophagus - survival - metastasis - meta analysis

Introduction

Squamous cell carcinoma of the oesophagus (SCCO) is one of the most aggressive malignant tumors in humans. Despite improvements in surgical techniques, radiotherapy and chemotherapy, the prognosis remains poor (Ando et al., 2000). Its unfavorable prognosis is largely explained by biological characteristics of the tumor. Improving the survival of SCCO patients is nowadays a challenge for practitioners confronted with this pathology. Epidermal growth factor receptor (EGFR), its over-expression has been identified as a common feature of many types of cancer including SCCO, and its association with clinical outcome in patients with malignant diseases has been studied because of the roles of EGFR signaling not only in cell proliferation but also anti-apoptosis, metastasis and angiogenesis (Woodburn., 1999). It seems to be noticeably interesting target for therapy, and this led us to wonder about its implication in SCCO patients and potential use as a therapeutic target in this pathology. However, the clinical importance of EGFR over-expression remains unsettled as the conflicting results still exist, so we carried out this meta-analysis of published studies to clarify it.

Materials and Methods

Search strategy and selection criteria

Systematic computerized searched of the PubMed and Meadline databases (up to December 20, 2013) were performed. The following MESH headings, keywords and text words were used: (1) esophagus or esophageal squamous cell carcinoma or neoplasm or carcinoma, and (2) epidermal growth factor receptor or EGFR or EebB1. The reference lists of retrieved articles and previous narrative reviews were scanned for other potentially relevant articles; investigators of eligible studies were contacted for supplement of additional data relevant to meta-analysis. When multiple articles pertained to overlapping populations of patients, only the newest, largest or most informative single article was selected.

Studies were included in the meta-analysis if they met the following criteria: (1) expression of EGFR was evaluated in the primary SCCO tissue as opposed to sera or metastatic tissue or in tissue adjacent to the tumor; (2) the expression of EGFR was measured by immunohistochemistry (IHC) of protein only; (3) analysis of the association between EGFR expression and

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clinicopathological parameters or overall survival (OS); (4) studies were published as a full paper in English; (5) Median follow-up time exceeded 2 years in studies for survival analysis. Reviews and conference abstracts were excluded because of limited data for evaluation.

Quality assessment

The quality of each study was assessed from the following aspects: (1) Whether the inclusion criteria of cases and their basic characteristics were clear; (2) Whether the experimental design was scientific; (3) Whether the processing factors and methods were accurate; (4) Whether statistical methods were appropriate; (5) Whether the bias were discussed in the study. The above five items, meet one was 1 points. Score≥3 was regarded as reliability.

Data extraction

Data was carefully extracted independently by two investigators and resolved controversies by discussion according to the criteria listed above. If they could not reach an agreement, an expert was invited to the discussion. The following information was extracted from each study: first author, year of publication, number of eligible patients, and clinicopathologic features.

Statistical analysis

The impact of EGFR over-expression on clinicopathological parameters was estimated for each study by odds ratio (OR) with its 95% confidence interval (CI), respectively. According to clinical characteristics, moderate and poor differentiation were combined; T1 and T2 were combined; T3 and T4 were combined; stage I and stage II were combined; stage III and stage IV were combined.

For the quantitative aggregation of OS results, the impact of EGFR over-expression on OS was estimated for each study by the hazard ratio (HR), with its 95% CI, respectively (Parmar et al., 1998). In summary, when the estimated HR and its CI were described in the publications, we obtained these values directly; when these statistical variables were not given explicitly in an article, they were calculated directly using two of the following parameters: the log-rank statistic, its *p*-value or the O-E statistic (difference between numbers of observed and expected events); when those data were not available, the following were studied: the total number of events, the number of patients at risk in each group and the log-rank statistic or its *p*-value, allowing calculation of an approximation of the HR estimate; when the only available data were in the form of graphical representations, they were calculated from Kaplan-Meier survival curves; the Kaplan-Meier curves were read by two persons using Engauge Digitizer 2.11 version (Mark Mitchell, Boston, USA) independently to reduce inaccuracy in extracted survival rates. By convention, an observed HR of >1 implied a worse OS for the group with EGFR over-expression.

A χ^2-test-based Q statistic test for the between study heterogeneity was used with *p*-value of 0.05 to determine statistical significance. A *p*-value greater than 0.1 indicates a lack of heterogeneity among studies, so the fixed-effects model was used to combine OR or HR.
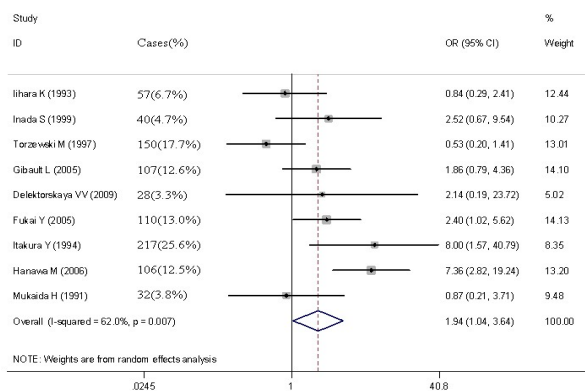

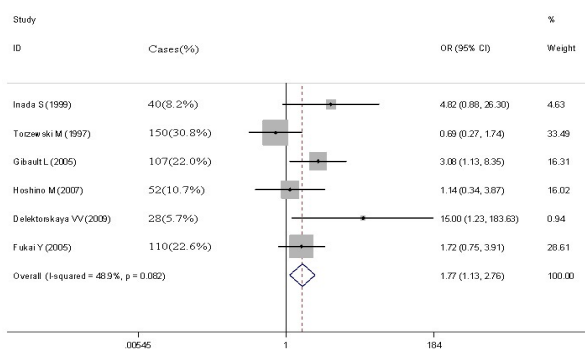**Figure 1. Forest Graph about the Relationship between Positive Expression of EGFR and T Stage**

Figure 2. Forest Graph about the Relationship between EGFR Expression and Vascular Invasion

Otherwise, the random-effects model was used. Evidence of publication bias was evaluated by the funnel plot with Begg's test and Egger's linear regression asymmetry test. For these analyses, a *p* value below 0.05 was considered representative of statistically significant publication bias.

All statistical analyses were performed by STATA 11.0 software (Stata Corporation, College Station, TX, USA) and P values for all comparisons were two-tail and statistical significance was defined as *p*<0.05.

Results

The retrieval results and quality evaluation

Initially, a total of 56 papers were detected. After reading their abstracts, thirty-eight literatures with repetitive, animal experiment or review were deleted. To further read the whole passage, eventually 13 literatures were involved in our study. A total of 1150 cases of patients were recruited, and the positive expression rate of EGFR was 62.8%. Basic characteristics of the literatures were demonstrated in table 1.

Analyzed results
The relationship between positive expression of EGFR and T stage

A total of 9 literatures were selected, including 267 cases of T1+T2 patients, and 580 cases of T3+T4 patients. Because of statistical heterogeneity between the two groups (*p*=0.007<0.05), random effect model was used. There was significant difference between T1+T2 group and T3+T4 group (OR=1.941, 95% CI: 1.037-3.636, *p*=0.038). It showed that with enhanced expression of EGFR, invasion depth increased in SCCO (Figure 1).
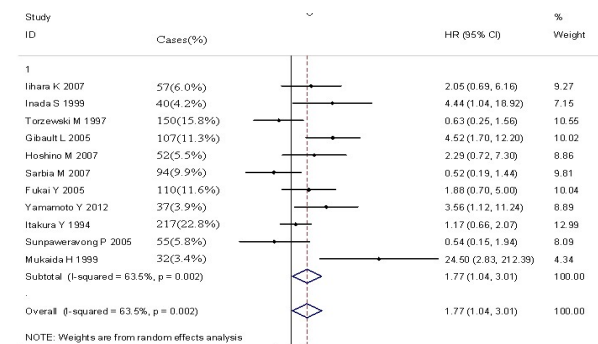
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Table 1. Basic Characteristic of the Literatures

Author	Year	Positive expression case (%)	Clinicopathologic parameters	Quality assessment				
				1	2	3	4	5
Mukaida et al	1991	18 (47.4%)	differentiation, T, LM, HG, OS	1	1	1	1	0
Iihara et al	1993	22 (46.8%)	LM, HG, DM, T, stage, OS	1	1	1	1	0
Itakura et al	1994	154 (71.0%)	differentiation, T, LM, stage, OS	1	1	1	1	0
Torzewski et al	1997	124 (82.7%)	HG, T, LM, LI, VI, OS	1	1	1	1	0
Inada et al	1999	17 (42.5%)	differentiation, LI, VI, LM, T, stage, OS	1	1	1	1	0
Gibault et al	2005	73 (68.2%)	differentiation, LM, T, VI, OS	1	1	1	1	0
Fukai et al	2005	77 (70.0%)	HG, T, LM, DM, stage, VI, LI, OS	1	1	1	1	0
Sunpaweravong et al	2005	26 (48.1%)	differentiation, stage, OS	1	1	1	1	0
Hanawa et al	2006	53 (50%)	T	1	1	1	1	0
Hoshino et al	2007	37 (71.2%)	differentiation, stage, LM, LI, VI, OS	1	1	1	1	0
Sarbia et al	2007	57 (61.1%)	OS	1	1	1	1	0
Delektorskaya et al	2009	24 (85.7%)	differentiation, T, LM, DM, stage, VI	1	1	1	1	0
Yamamoto et al	2012	40 (75.5%)	HG, OS	1	1	1	1	0

*LM, lymphatic metastasis; HG, histological grading; DM, distant metastasis; LI, lymphatic-vessel invasion; VI, vascular invasion

Table 2. Correlation between EGFR Expression and Degree of Differentiation, Histological Grading, Lymphatic Metastasis, Clinical Stages, Lymphatic-vessel Invasion

Clinicopathology	Literature number	Cases number		Heterogeneity	Effect model	OR	95%CI		P
degree of differentiation	7	poor	287	No (p=0.656)	fixed	1.429	0.955	2.139	0.083
		moderate + well	235						
histological grading	5	G1+G2	231	Yes (p=0.022)	random	0.955	0.393	2.319	0.919
		G3+G4	155						
lymphatic metastasis	9	(-)	353	No (p=0.148)	fixed	1.322	0.959	1.823	0.089
		(+)	480						
clinical stages	7	I+II	216	No (p=0.130)	fixed	0.995	0.576	1.719	0.986
		III+IV	334						
lymphatic-vessel invasion	4	(-)	157	No (p=0.225)	fixed	1.028	0.603	1.753	0.919
		(+)	192						

Figure 3. Forest Graph about the Relationship between EGFR Expression and OS

Table 3. Publication Bias between EGFR Expression and the Clinicopathologic Parameters

Clinicopathologic parameters	t	95% CI		P
degree of differentiation	0.34	-1.783	2.321	0.75
Histological grading	2.49	-0.447	8.758	0.064
T stage	0.19	-4.553	5.352	0.854
lymphatic metastasis	0.99	-1.653	4.032	0.355
clinical stages	-0.4	-6.068	4.443	0.707
lymphatic-vessel invasion	2.2	-2.758	8.707	0.155
vascular invasion	1.65	-1.747	6.907	0.173
OS	1.6	-1.162	5.843	0.129

The relationship between EGFR expression and vascular invasion

A total of 5 literatures were included, including 286 positive cases, 201 negative case, and no significant heterogeneity was found between the two groups (*p*=0.082>0.05), so we used the fixed effect model of Meta analysis. The results showed that there was statistical significance between the two groups (OR=1.771, 95% CI: 1.134-2.764, *p*=0.012), indicating that EGFR may promote vascular invasion in SCCO (Figure 2).

The relationship between EGFR expression and OS

A total of 11 literatures were included, and the groups had statistically significant heterogeneity (*p*=0.002<0.05), so the random effect model was used. Results showed that HR value was 1.768 (95% CI: 1.039-3.007), and through

Z test, it had statistically significance (*p*=0.036<0.05), indicating that with higher expression of EGFR, OS reduced, and the prognosis was more poor (Figure 3).

The relationship between EGFR expression and degree of differentiation, histological grading, lymphatic metastasis, clinical stages, lymphatic-vessel invasion

According to the heterogeneity, corresponding effect model was selected. It showed that there was no correlation between EGFR and degree of differentiation, histological grading, lymphatic metastasis, clinical stages, lymphatic-vessel invasion (Table 2).

Publication bias analysis

Funnel plot analysis was used and its symmetry was analyzed by Egger's z test. It showed that there were no publication bias existed between EGFR expression and the clinicopathologic parameters such as histological grading, degree of differentiation (Table 3).

Discussion

China is a high-incidence area of SCCO, accounting for 53.9% of morbidity and 49.3% of deaths in the world, and in China the incidence and mortality have been the sixth and fourth of all malignant tumors, respectively (Chen et al., 2011). At present, surgical operation, radiotherapy, and chemotherapy are the main treatments for SCCO. Although the diagnosis and treatment have made great progress, its incidence and mortality are still high, so it is extremely urgent to explore new therapy.

With the rapid development of drugs targeting research into clinic, targeted therapy plays an important role in advanced solid tumors. EGFR is found the most important target molecules to date, and a number of studies have confirmed that EGFR is over-expressed in many carcinomas such as breast cancer, non-small cell lung cancer, colorectal cancer and head and neck neoplasm, closely related to proliferation, invasion, metastasis, angiogenesis and apoptosis (O-charoenrat et al., 2002; Matkovic et al., 2008; Molaei et al., 2009; Abusail et al., 2013). But as for SCCO, the studies with respect to EGFR expression, clinicopathological characteristics and prognosis are lack of large sample clinical and pathological data to discuss, and the conclusions are not consistent. According to the inclusion and exclusion criteria, only 13 high quality English literatures can be analyzed. From table 1, it is seen that 13 studies score were 4 points which showing high quality. The positive rate of EGFR in SCCO was between 42.5% and 85.7%, and the studies covered many aspects, such as histological grade, degree of differentiation, T stage, lymph node metastasis, OS, which could provide comprehensive data to explore the relationship between EGFR over-expression and the clinicopathological characteristics of SCCO.

A former Meta-analysis consisted of 9 articles, including 802 cases of SCCO, and EGFR positive expression rate was 61.8% (Yu et al., 2011). Its results showed that EGFR was closely related to not only lymph node metastasis, prognosis, but also the differentiation and T staging. Our Meta-analysis included 13 papers including 1150 cases, and the positive rate of EGFR was 62.8%. Our results showed that over-expression of EGFR in SCCO had correlation with the degree of T stage, vascular invasion and OS, so there was predictive value for the above indexes, but no relation to histological grading, lymphatic metastasis, clinical stages and lymphatic-vessel invasion was found. EGFR over-expression was inclined to reduce prognosis through increasing the depth of invasion, vascular invasion. Although compared with that of the former study, the conclusion of this Meta-analysis was not consistent, the number of included studies and cases in our study were increased, especially in the overall survival, literature involved in this study from 5 to 11, so the conclusion should be more convincing. The consistent results of the two Meta-analysis is that EGFR expression is one of the factors of poor prognosis in patients with SCCO, and have relation to the depth of tumor invasion, therefore theoretically anti-EGFR targeted therapy has potential application value.

Considering the original publication bias exists in a certain extent, and it may affect the reliability of meta-analysis results, the 13 articles involved in this Meta-analysis were also investigated by statistical analysis. Egger analysis showed that the funnel plot was symmetric ($p>0.05$), which indicated the bias was small, at least, the potential bias had no substantial influence on the final conclusion, which further increased the reliable conclusion that EGFR over-expression had effect on the prognosis of the patients with SCCO. However, the Meta-analysis has limitations from the published literature, because the adopted literatures were all openly published, and language only in English, so the unpublished literatures and existing language bias may also affect the results of this Meta-analysis. Additionally, despite the expression of EGFR were all detected by immunohistochemical method, but the antibody manufacturer, dilution, judgment standard are not identical, which may also influence the results.

In conclusion, this study showed high expression of EGFR in SCCO which increased T stage, vascular invasion risk and a poor prognosis, so anti-EGFR targeted therapy may have certain curative effect or generate breakthrough for treatment in SCCO. It has been reported that compared with chemotherapy alone, cetuximab plus chemotherapy could more obviously improve the curative effect for metastatic SCCO (Lorenzen et al., 2009). Because the number of cases in this Meta analysis is still insufficient, conclusions were inconsistent with the previously one, it still need to continue to accumulate clinical pathological data, observe prognosis, and do a more comprehensive and further study on EGFR over-expression correlates with SCCO, so as to provide a more sufficient theoretical basis for anti-EGFR target therapy in SCCO.

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