ORIGINAL ARTICLE

High expression of cyclooxygenase 2 is an indicator of prognosis for patients with esophageal squamous cell carcinoma after Ivor Lewis esophagectomy

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Abstract

Background: The poor prognosis of esophageal squamous cell carcinoma (ESCC) is attributed to a high recurrence rate after surgery. Cyclooxygenase 2 (COX2) is an important regulator of cell growth, differentiation, apoptosis, and transformation. COX2 overexpression is significantly associated with the tumorigenesis and progression of diverse cancers; however, its expression and significance in ESCC remains unclear.

Methods: We enrolled 118 patients with ESCC who had undergone Ivor-Lewis esophagectomy. The expression profile of COX2 was examined by immunohistochemistry.

Results: A high expression of COX2 correlated with a higher T staging ($P = 0.014$), lower differentiation degree ($P = 0.002$), lymph node metastasis ($P = 0.009$), recurrence status ($P = 0.004$), and tumor node metastasis (TNM) stage ($P = 0.001$). Cox regression analysis showed that TNM stage ($P = 0.001$), differentiation degree ($P = 0.001$), and high COX2 expression ($P = 0.004$) were independent risk factors of prognosis.

Conclusion: Our data indicated that COX2 expression level is associated with key clinicopathological features and could be an effective biomarker to predict ESCC prognosis.

Introduction

Esophageal squamous cell carcinoma (ESCC) is a common digestive malignancy worldwide.[1] The the first treatment choice for resectable ESCC is surgery. Although great advances have been made in combined therapies for ESCC, the prognosis of patients remains poor, with a five-year survival rate of merely 20–30%.[2,3] More than half of the patients develop recurrence within two to three years after surgery.[4] As far as we know, the prognosis of ESCC patients is tumor node metastasis (TNM) stage specific. However, we cannot only estimate prognosis using TNM stage, because TNM stage lacks sensitivity and accuracy. Therefore, finding a biomarker may assist in identifying high-risk patients, which may significantly guide further treatment. Much literature exists on the biomarkers of prognosis of ESCC patients; however, no specific biomarker has been adopted and routinely applied.[5] Therefore, more accurate biomarkers to predict prognosis for ESCC patients are imminently required.

The cyclooxygenase (COX) system plays a crucial role in the carcinogenesis of esophogeal carcinoma by promoting the process from esophagitis to dysplasia and carcinoma.[6] COX2, one of the two iso-enzymes of COX, is absent or low in normal adult tissues and has been involved in increased proliferation. As an important regulator of cell growth, differentiation, apoptosis, and transformation, COX2 overexpression is significantly associated with the tumorigenesis and progression of diverse cancers, including squamous cell carcinomas (SCCs) of the head and neck, lung, vagina, and uterine cervix.[7–9] The overexpression of COX2 has been proven to correlate with a dismal prognosis in gastrointestinal tumors as it decreases apoptosis and increases invasiveness.[10] However, few reports have investigated COX2 expression in ESCC; thus, the clinicopathological significance and prognostic value of COX2 in ESCC is still unclear. In this study, we aimed to investigate the expression of COX2 in 118 ESCC patients and explore whether COX2 expression can predict ESCC prognosis after surgery.

Materials and methods

Ethics statement

Approval for this study was obtained from the Research Ethics Committee of the Provincial Hospital affiliated to Shandong University. Signed informed consent was provided by all participants.

Patients and tissues

The most common tumor location was the midthoracic esophagus.[2] In order to eliminate tumor location bias, we assessed 91 male and 27 female patients with midthoracic ESCC, ranging in age from 38–74 years (mean 60 years). All patients who received Ivor Lewis esophagectomy at the Department of Thoracic Surgery at the Provincial Hospital affiliated to Shandong University from October 2006 to September 2009 were enrolled in this study. Inclusion criteria were: (i) patients received Ivor-Lewis esophagogastrectomy with thoracic and abdominal two-field lymph node dissection, performed to achieve complete resection, according to 2009 Union for International Cancer Control standards for midthoracic ESCC; (ii) no preoperative chemotherapy or radiotherapy; (iii) no residual cancer cells under the upper and lower cutting edge and lateral margin without residual focus (R0), proven by pathological examination; (iv) no severe preoperative complications existed; (v) minimum complete three-year follow-up review and record of first recurrence location; and (vi) more than 12 lymph node dissections.

We obtained a pair of samples from surgical specimens of each selected patient. Each pair consisted of ESCC tissue and corresponding healthy esophageal mucosa (CHEM). CHEM was obtained at a distance of more than 5 cm away from the ESCC margin, which was proven free of tumor, deterioration, and necrosis by light microscope examination.

Immunohistochemistry

Formalin-fixed and paraffin-embedded sections were dewaxed in xylene. Sections were then rehydrated using graded alcohol and placed in an endogenous peroxide block for 10 minutes. The sections were then incubated with ultravision protein block for five minutes to lower nonspecific background staining. After the specimens were incubated with rabbit monoclonal antihuman COX2 (1:500) and Ku80 (1:500; Abcam Ltd., Cambridge, UK) primary antibody overnight at 4°C, they were washed with phosphate-buffered saline (PBS), followed by incubation with primary antibody amplifier quanto for 10 minutes and horseradish peroxidase polymer quanto for 10 minutes. After the sections were washed with PBS and distilled water, they were incubated with 3,3-diaminobenzidin and counterstained with hematoxylin. In the negative control, the primary antibody was substituted with PBS. The immunohistochemical score (IHS) was calculated by combining the proportion score (percentage of positive stained cells) with the staining intensity score. The proportion score ranged from 0–4, as follows: 0 (<5%), 1 (5–24%), 2 (25–49%), 3 (50–74%), and 4 (≥75%). Staining intensity was scored as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The proportion and staining intensity scores were then multiplied to generate the IHS for each case. A case with IHS ≥4 was considered high expression. Two experienced experts who were blinded to patient data independently scored the samples and reached agreement by re-analysis and discussion.

Follow-up

Patients were examined regularly every three to six months after surgery, including thorough physical examination, chest and upper abdomen contrast-enhanced computed tomography (CT) scan, abdomen ultrasonography, positron emission tomography (PET), bone scintigraphy, and cerebral CT. The biopsy was performed based on specific imageological and clinical examination. Lymph node enlargement after surgery was diagnosed as lymph node recurrence and if new lesions appeared in other organs after exclusion of the primary tumor, metastatic ESCC was clinically diagnosed. Follow-up concluded in December 2013; the longest follow-up period was 60 months, with a median of 40 months.

Statistical analysis

All statistical analyses were performed with SPSS version 19.0 (IBM Corp., Armonk, NY, USA). Kaplan–Meier method was used to calculate the survival rate and the log-rank test was performed to compare survival differences. Independent prognostic factors were judged using Cox regression multivariate analysis. When the *P* value reached <0.05, the difference was regarded as significant.

Results

Patients' characteristics

According to inclusion criteria, 118 patients were enrolled in this study. None of the patients received chemotherapy or radiotherapy before surgery. The median age was 60 years (38–74 years), and 91 (77.1%) of the patients were men. Of the 118 patients, 32 (27.1%) had a tumor greater than 50 mm, while the remainder had tumors sized 50 mm or less. The clinical data of all patients is shown in Table 1.

Table 1 Correlation of COX2 expression with clinicopathologic features of ESCC patients

Characteristics	Cases (118)	COX2 expression level		P
		Low (30)	High (88)	
Age (years)				0.309
<50	31	10	21	
≥50	87	20	67	
Gender				0.348
Male	91	25	66	
Female	27	5	22	
Tumor size				0.124
<30 mm	20	7	13	
30–50 mm	66	19	47	
≥50 mm	32	4	28	
Differentiation degree				0.002
High	18	9	9	
Moderate	52	16	36	
Low	48	5	43	
pT				0.014
T1	8	4	4	
T2	30	12	18	
T3+T4	80	14	66	
pN				0.009
N0	30	13	17	
N1+N2	88	17	71	
Recurrence				0.004
Yes	80	14	66	
No	38	16	22	
pTNM				0.001
I+II	35	16	19	
III+IV	83	14	69	

P values <0.05 were considered significant. Statistical analysis was performed using the chi-square test. COX, cyclooxygenase; ESCC, esophageal squamous cell carcinoma; TNM, tumor-node-metastasis.

Table 2 Relationship between COX2 and Ku80 expression

Cases (118)		COX2 expression level		P
		Low	High	
Ku80 expression level		30	88	0.002
Low	43	18	25	
High	75	12	63	

COX, cyclooxygenase.

Correlations between COX2 overexpression and clinicopathological characters

Cyclooxygenase 2 overexpression was significantly associated with histological differentiation ($\chi2 = 12.219$, $P = 0.002$), lymph node metastasis status ($\chi2 = 6.805$, $P = 0.009$), tumor status ($\chi2 = 8.559$, $P = 0.014$), and TNM stage ($\chi2 = 10.805$, $P = 0.001$). However, there was no statistically significant correlation with gender, age, or tumor size (Table 1).

Relationship between COX2 and Ku80 expression

Our previous studies demonstrated that Ku80 was overexpressed in ESCC.[11,12] In this study, we found the expression of COX2 was correlated to the expression level of Ku80 in ESCC ($\chi2 = 9.640$, $P = 0.002$) (Table 2).

Correlation between COX2 protein overexpression and prognosis of esophageal squamous cell carcinoma patients

Follow-up data were available for all patients. Kaplan–Meier analysis revealed an overall five-year survival rate of 20.34%; patients with COX2 protein overexpression had a five-year survival rate of 10.22% compared to 50% of those without COX2 protein overexpression (Figs 1, 2). Multivariate analysis indicated that TNM stage, histological differentiation, and COX2 protein overexpression were independent prognostic factors (Table 3).

Discussion

Esophageal squamous cell carcinoma is a malignant tumor with strong invasion, a high probability of lymph node metastasis, frequently located in the middle thoracic esophagus, and has a high incidence rate in China. The five-year survival rate of ESCC patients after surgery is merely 30–50%.[2] It has been demonstrated that nearly half of ESCC patients develop recurrence within two to three years after surgery, with two dominating recurrence patterns: locoregional lymphatic metastatic and hematogenous metastatic recurrence.[13] However, the mechanism of metastasis is still indistinct.

Cyclooxygenase 2 can be used as a molecular marker for diagnosis or an independent prognostic factor for many malignant tumors. Chang *et al.* demonstrated that COX2 plays a significant role in the regulation of angiogenesis in normal mammary tissue, which is an important biomarker of breast cancer.[14] Hahm *et al.* reported that overexpression of COX2 can attenuate the degree of atrophic gastritis and promote gastric carcinogenesis, concluding that COX2 is a potential tumor marker for gastric cancer.[15] However, the expression of COX2 has not been explored in ESCC. Furthermore, the relationship between COX2 and clinicopathological characteristics and survival of ESCC patients is still unclear.

In this study, we investigated whether COX2 expression could reveal clinicopathological significance in ESCC. We found the expression of COX2 was upregulated in ESCC, compared with normal esophageal members. Furthermore, we determined that COX2 overexpression was correlated

Table 3 Comparison of Kaplan–Meier survival curves

|	B	SE	Wald	P	HR	95% CI
Differentiation	0.661	0.200	10.901	0.001	1.936	1.308~2.865
pT	0.060	0.251	0.057	0.811	1.062	0.649~1.736
pN	0.190	0.464	0.169	0.681	1.210	0.488~3.002
pTNM	1.432	0.430	11.099	0.001	4.187	1.803~9.723
COX2	0.841	0.293	8.216	0.004	2.319	1.305~4.122

CI, confidence interval; COX, cyclooxygenase; HR, hazard ratio; SE, standard error; TNM, tumor node metastasis.

with ESCC differentiation degree ($\chi 2 = 12.219$, $P = 0.002$). The expression level of COX2 was higher in poorly differentiated tumors than in well differentiated tumors. There were also significant correlations between COX2 expression and lymph node metastasis ($\chi 2 = 6.805$, $P = 0.009$) and TNM stage ($\chi 2 = 10.805$, $P = 0.001$). COX2 overexpression was discovered in 71 (80.68%) patients with lymph node metastasis compared to 17 (56.67%) patients without lymph node metastasis ($\chi 2 = 6.805$, $P = 0.009$). In addition, the expression level of COX2 was higher in patients with late ESCC TNM stage than those with early TNM stage ($\chi 2 = 10.805$, $P = 0.001$).

Our previous studies determined that Ku80 is an important prognostic factor in ESCC.[11,12] Recent reports suggest that Ku80 is implicated in the development and progression

of ESCC, making Ku80 a significant candidate target for ESCC.[16,17] In this study, we found a positive relationship between the expression of COX2 and Ku80, revealing the diversity and complexity of COX2 in ESCC; thus, our current study added a new mechanism to the function of COX2. All of our evidence demonstrates the potential for developing Ku80 as a useful therapeutic target in some key stages of ESCC progression.

The impact of COX2 on the prognosis of patients has been reported in many studies. In our study, the statistical results of survival analysis indicated that the five-year survival rate of patients with COX2 overexpression was lower than those without COX2 expression. The overall five-year survival rate of the 118 ESCC patients was 20.34%. Patients with COX2 overexpression had a five-year survival rate of 10.24% com-
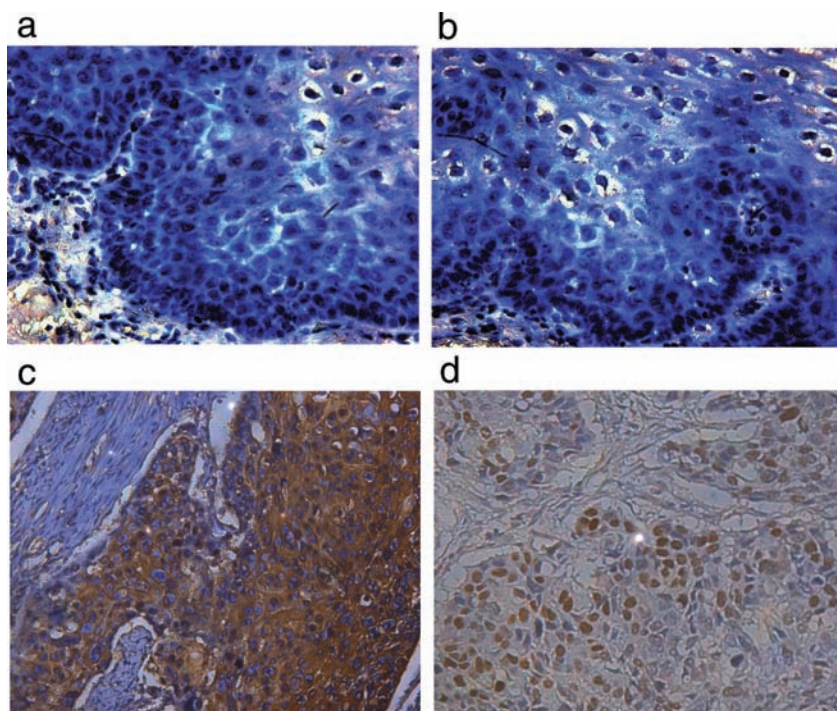
Figure 1 Immunohistochemistry assay of cyclooxygenase (COX)2 and Ku80 in esophageal squamous cell carcinoma (ESCC) and corresponding healthy esophageal mucosa (CHEM). (**a**) Representative negative expression of COX2 in CHEM (×400). (**b**) Representative negative expression of Ku80 in CHEM (×400). (**c**) Representative high expression of COX2 in ESCC tissues (×400). (**d**) Representative high expression of Ku80 in ESCC tissues (×400).
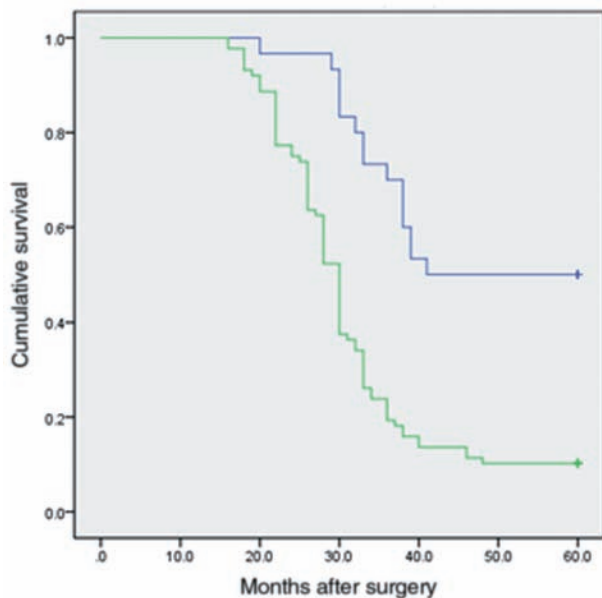
Figure 2 Overall survival of esophageal squamous cell carcinoma patients according to cyclooxygenase (COX)2 expression level.

pared to 50% for those without COX2 expression ($P < 0.05$). The results of Cox regression multivariate analysis illustrated that COX2 overexpression was an independent prognostic factor ($P = 0.004$).

The specific mechanism of COX2 overexpression in ESCC is unclear. It may be involved in the activation of oncogenes, cell apoptosis, or the regulation of tumorigenesis and metastasis-associated genes. The exact mechanism, especially with respect to signaling pathways and molecular mechanisms, is undetermined.

In summary, we demonstrated that COX2 overexpression was significantly associated with key adverse clinicopathological features. Cox regression analysis indicated that the high expression of COX2 protein is an independent risk factor for prognosis. These results suggest that COX2 must be a prognostic factor and might be a new potential therapeutic target for ESCC. As the exact mechanism of COX2 overexpression in ESCC remains unclear, further research exploring COX2 inhibitors and whether these inhibitors could work to ameliorate prognosis is required. We urgently need more translational and clinical trials to illustrate the potential roles of COX2 inhibitors in the treatment of ESCC.

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Disclosure

No authors report any conflict of interest.

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