• ESOPHAGEAL CANCER •

Expression of MUC1 in esophageal squamous-cell carcinoma and its relationship with prognosis of patients from Linzhou city, a high incidence area of northern China

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Abstract

AIM: To further characterize the possible relationship between the molecular changes and prognosis of ESC and to elucidate the possible mechanisms involved.

METHODS: 114 specimens of ESC were collected from Linzhou city, and all patients were followed up for more than 5 years after resection. Histopathological analysis and immunohistochemical staining (ABC) were employed to detect the alteration of MUC1.

RESULTS: The positive immunostaining rate for MUC1 was 79 % (90/114), and the high-expression rate was 63 % (72/114). The mean survival periods (months) of those with high- and low-expression rates of MUC1 were 41 (95 % CI: 35, 47) and 52 (95 % CI: 45, 59), respectively. Patients in the low-expression group obviously survived longer than those in high-expression group, and the difference was significant (*P*<0.05). The expression of MUC1 protein in the esophageal carcinoma specimens with metastasis was stronger than those without metastasis, the difference was also significant (*P*<0.05). The stepwise multivariate analysis showed that "differentiation", "expression of MUC1" and "TNM staging" were the most important factors affecting the prognosis of esophageal carcinoma patients (*P*<0.05).

CONCLUSION: A good correlation between the alteration of MUC1 and the regional lymph node metastasis was observed. Furthermore, high-expression of MUC1 was associated with poor prognosis for esophageal cancer patients. These results indicated that MUC1 is a promising biomarker for predicting lymph node metastasis and prognosis in esophageal cancer.

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INTRODUCTION

Esophageal squamous-cell carcinoma (ESC) is one of the most common malignant diseases in northern China, and Linzhou city (formerly Linxian) had being the highest incidence area[1, 2]. The five-year survival rate for early esophageal cancer patients is more than 90 %. However, for the patients at late or advanced stage, the five year survival rate is only 10-15 %[1,2]. So far, the conventional traditional prognostic markers, such as cancer stage based on metastasis and pathological grade are still used to evaluate the prognosis of esophageal cancer patients. But, it has been well recognized that there is discordance between the conventional prognosis biomarkers and the actual prognosis. For example the patients with well differentiated cancer may have a worse prognosis than those with poorly differentiated ones, indicating the limitation of those markers for predicating.

With the development of molecular biotechnology, many new measurements have been applied in cancer prognosis research. Studies on ESC prognosis have been expanded in recent years; however, the molecular mechanisms involved in prognosis of esophageal cancer, especially the survival analysis on whom from high-incidence area of esophageal carcinoma was very limited. We followed up the ESC patients from Linzhou city and determined the alteration of MUC1 expression and its relationship to the prognosis, to further characterize the possible relationship between them so as to elucidate the possible mechanisms of ESC carcinogenesis, and to determine the alteration of MUC1 and prognosis with histopathological and immunohistochemical methods.

MATERIALS AND METHODS

Patients

One hundred and fourteen patients with ESC, who had undergone esophagectomy at the Esophageal Carcinoma Hospital of Linzhou City between 1993 and 1996 were enrolled in this study. All the patients were local residents of Linzhou city and had not received radiation therapy or chemotherapy prior to the surgery. There were 67 men and 49 women. The mean age was 53.5±8.1 (range 37-72) years for males and 53.6±7.8 (range 40-69) years for females, respectively. All specimens were confirmed by pathology as ESC.

Follow-up

All patients were followed up until March 2001, at which the patients had survived for more than 5 years or died within that period after surgical treatment. 57 patients survived less than 5 years died of recurrence or metastasis.

Tissues processing

All tumor specimens were fixed with formalin and embedded with paraffin. Each block was sectioned serially at 5 um, one of which was stained with hematoxylin and eosin for histopathological analysis by two pathologists and the others were used for immunostaining.

Histopathological analysis
Histopathological diagnoses were made according to the previously established criteria[3].

Immunohistochemical staining
Anti-MUC1 antibody was a mouse monoclonal anti-serum directed at a hexapeptide in the tandem repeat region of the protein core of MUC1 (clone Ma552; Novocastra, Burlingame, CA), which was kindly provided by Dr. Yongqin Li (College of Medicine, Harvard University). The avidin-biotin-peroxidase complex (ABC) method was used for MUC1 immunostaining. In brief, after dewaxing, quenching endogenous peroxidase activity with 3 % H_2O_2, and blocking cross-reactivity with normal serum (Vectastain Elite Kit; Vector, Burlingame, CA), the tissues were incubated overnight at 4 ℃ with primary antibodies (1:400 for MUC1). Location of the primary antibodies was achieved by subsequent use of a biotinylated anti-primary antibody, an avidin-biotin complex conjugated to horseradish peroxidase, and 3',5'-diaminobenzidine (Vectasitain Elite Kit). Normal serum blocking and omission of the primary antibody were used as negative controls.

Evaluation of immunostaining
Clear cytoplasm and cell membrane staining was the criterion for a positive reaction. The staining was graded by the percentage of positively stained neoplastic cells as follows: -, <5 %; +, 5-50 %; ++, >50 % of the neoplastic cells stained. For statistical analysis, the examined cases were divided into 2 groups: the low-expression group, composed of the "-" and "+" groups (less than 50 % of neoplastic cells stained) and the high-expression group, the "++" group (over 50 % of the neoplastic cells stained)[4].

Statistical analysis
Chi-squared test was performed to evaluate the relevance of regional lymph node metastasis and expression of MUC1 protein, Kaplan-Meier was used for survival analysis, and multivariate analysis for screening prognostic factors. The significant difference was considered when the *P* value was less than 0.05.

RESULTS
Results of follow up
Among the follow up of 114 ESC patients followed up, 33 % (33/114) survived 5 years, 50 % (57/114) died within 5 years after surgical treatment and 17 % (20/114) cases were censored during the follow-up.

Expression of MUC1 and its relationship with survival of ESC
Among the 114 surgically resected ESC specimens examined, the positive immunostaining for MUC1 was observed in 90 cases (78.9 %), and high-expression was seen in 72 cases (63.2 %) and low-expression was in 42 cases (36.8 %). The mean survival period (months) and 95 % confidence interval of esophageal carcinoma patients with high- and low-expression of MUC1 were 41(35, 47) and 52(45, 59), respectively. Patients in the low-expression group obviously survived longer than those in high-expression group, and the difference was significant (*P*<0.05, Table 1 and Figure 1).

Relationship between the expression of MUC1 and regional lymph node metastasis
According to the status of the regional lymph nodes with or without metastasis, all specimens were divided into two groups, with metastasis and without metastasis. The expression of

MUC1 protein in the ESC specimens with metastasis was obviously stronger than those without metastasis, and the difference was significant (*P*<0.05, Table 2).

Table 1 Survival analysis of high-expression and low-expression of MUC1 in ESC

Expression of MUC1	No. of specimens examined	No. of death	Mean survival period (month) x (95% CI*)
Low-expression	42	17	52(45, 59)
High-expression	72	40	41(35, 47)
Total	114	57	

*: Confidence Interval; Log-rank: $\chi^2=5.11$, *P*=0.0238.

Table 2 Relationship between the expression of MUC1 and regional lymph node metastasis

Group	No. of specimens examined	Expression of MUC1 protein			
		- n(%)	+ n(%)	++ n(%)	+++ n(%)
Without metastasis	77	19(24.7)	29(37.7)	7(9.1)	22(28.5)
With Metastasis	37	0(0)	4(10.8)	5(13.5)	28(75.7)
Total	114	19	33	12	50

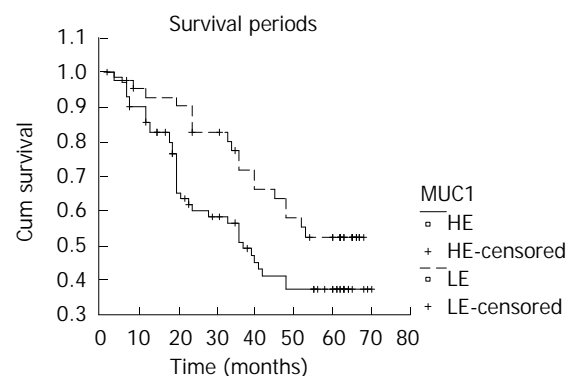Chi-squared test: $\chi^2=27.4693$, *P*<0.05.

Figure 1 Comparison of survival period between high-expression and low-expression of MUC1 in ESC. HE: High-expression; LE: Low-expression.

Cox model analysis
Ten parameterss, including sex, age, invading depth, regional lymph node metastasis, metastasis of other organs, TNM stage, differentiation and MUC1, were used as independent variables, survival periods of ESC patients were used as dependent variables, and all variables were ranked into Cox model analysis (Table 3). The stepwise multivariate analysis showed that "differentiation", "expression of MUC1" and "TNM staging", were the most important factors affecting the prognosis of ESC patients (*P*<0.05), RR values for each parameter were 2.2382, 1.9409 and 1.8621, respectively (Table 4).

Table 3 All factors employed by Cox regression model

Factors	Variable	
Sex	X1	0=male, 1=female
Age	X2	0=<45 years, 1=45~55 years, 2=>55 years
Invading depth	X3	0=lamina propria or submucosa, 1=muscularis propria, 2=adventitia, 3=adjacent structures
Regional lymph node metastasis	X4	0=no, 1=yes
Distant metastasis	X5	0=no, 1=yes
TNM stage	X6	0=0, 1=I, 2=II, 3=III, 4=IV
Differentiation	X7	0=high, 2=moderate, 3=low
Expression of MUC1	X8	0=low, 1=high

Table 4 Results of Cox model stepwise regression analysis

Variable	Parameter Estimated	Standard error	R	RR
X6	0.6217	0.1549	0.1717	1.8621
X7	0.8057	0.1961	0.1763	2.2382
X8	0.6632	0.3075	0.0311	1.9409

DISCUSSION

Both patients and doctors monitored the patients' survival period after surgery. So far, it is still controversial about evaluating. Out study on the expression of MUC1 in ESC tissues showed that the patients in the low-expression group obviously survived longer than those in high-expression group, and the difference was significant ($P<0.05$). It is therefore indicated that detective of expression of MUC1 may be of value in assessing the prognosis of ESC patients.

The epithelial mucin coded by the MUC1 gene is a transmembrane molecule, which is expressed in most glandular epithelial cells. The molecule was first identified in human milk, as a large molecular weight glycoprotein rich in serine, threonine and proline carrying a high percentage of *O*-linked carbohydrate[5]. MUC1 is widely expressed by normal glandular epithelial cells, and the expression is dramatically increased when the cells become malignant[6,7], and its relationship with the prognosis of several carcinomas have been already reported[4, 8-11]. Changes in the expression levels of MUC1 have also been described in esophageal lesions[12]. Our finding showed that MUC1 was expressed in all surgical specimens with lymph node metastasis. Its high-expression rate reached 89 %, and was significantly different from the specimens without lymph node metastasis ($P<0.05$). The lymph node and lymphatic vessel invasion has been reported as poor prognosis factor[13, 14].

Mucins are heavily glycosylated glycoproteins that have protective and lubricating functions[15, 16]. MUC1 expressed in tumors may function as an anti-adhesion molecule, which inhibits cell-cell adhesion, inducing a release of cells from tumor nests. Thus, MUC1 expression may be related to invasion or metastasis of carcinoma cells[17-19]. MUC1 can down-regulate the expression of E-cadherin, which is a calcium-dependent adhesion molecule, functioning in the cell-cell adhesion, while the low-expression of E-cadherin increased the invading ability of tumor cells[20-22]. Our previous studies found that MUC1 was expressed in 50 primary ESC cells and the metastasized cancer cells of the matched lymph nodes. In addition, it was found that the coincidence of positive immunostaining between primary tumor and its matching lymph node was observed in 28 cases (56.0 %), while the coincidence of negative immunostaining was observed only in 2 cases (1.0 %)[23]. It therefore indicated that the expression of MUC1 might play an important role in the invasion or metastasis of ESC cells, which might be one of the mechanisms involving in poor prognosis. But a discordant view was held by Japanese authors who argued that the expression of MUC1 was not significantly associated with metastasis of human esophageal carcinomas[24]. Further study is still necessary.

Considering the complexity in cancer, it is difficult to define it useful prognostic and predictive factors[25-28]. In the present study, we use multivariate analytic method for screening the prognostic factors in combination with clinical data. The stepwise multivariate analysis shows that "differentiation", "expression of MUC1" and "TNM staging" are the most important factors affecting the prognosis of ESC patients ($P<0.05$), our findings also indicate that high expression of MUC1 is related to poor prognosis of ESC.

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