Original paper

Mucin 1 expression correlates with metastatic recurrence in postoperative patients with esophageal squamous cell cancer

Zhi-Gang Sun[1], Li Yu[2], Fei Yang[3], Wei Gao[3], Zhou Wang[4], Liang-Ming Zhu[4]

[1]Department of Thoracic Surgery, Jinan Central Hospital Affiliated to Shandong University, Shandong University, Jinan, Shandong Province, China
[2]Department of Otorhinolaryngology, Jinan Central Hospital Affiliated to Shandong University, Shandong University, Jinan, Shandong Province, China
[3]Department of Pathology, Jinan Central Hospital Affiliated to Shandong University, Shandong University, Jinan, Shandong Province, China
[4]Department of Thoracic Surgery, Provincial Hospital Affiliated to Shandong University, Jinan, Shandong Province, China

Mucin1 (MUC1) expression correlates with invasion and metastasis and poor survival in some cancers. The purpose of the study was to investigate the clinical significance of MUC1 expression and the risk of tumor metastatic recurrence in patients with esophageal squamous cell cancer (ESCC) after curative resection. A total of 108 ESCC patients were enrolled in this study. MUC1 expression was detected in ESCC tissues from 70 patients by immunohistochemistry (IHC). The expression of MUC1 in the cancerous tissue group was significantly higher than that in the paracancerous normal tissue group (65.4%:10.0%, $p < 0.01$). MUC1 expression correlated with pT (< 0.05), pN ($p < 0.01$) and pTNM stage (< 0.01). The 5-year survival rate of the patients was 39.8%. The 5-year tumor metastatic recurrence rate of the patients was 74.1%, and it was associated with pT ($p < 0.01$), pN ($p < 0.01$), pTNM stage ($p < 0.01$) and MUC1 expression ($p < 0.01$). Multivariate analysis confirmed that pN and MUC1 expression were independent predictive factors. In conclusion, MUC1 expression correlates with tumor metastatic recurrence in postoperative ESCC patients.

Key words: Mucin1, esophageal squamous cell cancer, metastatic recurrence, immunohistochemistry.

Introduction

Esophageal cancer is one of the most common malignancies in China. The major histological type is esophageal squamous cell carcinoma (ESCC). Esophagectomy has remained the most effective modality for ESCC patients. However, the long-term outcome is unsatisfactory, and the 5-year survival rate is only 20-30% [1]. More than half of the postoperative patients developed recurrence within 2-3 years, and 80% would eventually die from tumor recurrence [2, 3]. To date, TNM staging is the main parameter for predicting recurrence and prognosis, even though it lacks sensitivity and accuracy. Therefore, the discovery of a sensitive reliable biomarker identifying the high-risk patient with recurrence is crucial for improvement of the survival rate [4].

Recent studies have revealed that several genes are involved in the origin and progression of ESCC. It has been reported that Mucin1 (MUC1) plays important

roles in development and progression of some cancers [5, 6]. MUC1 is a structural membranous bound mucin, expressed on the apical surface of normal glandular epithelial cells in normal tissues [7]. In neoplastic tissues, MUC1 expression could be up-regulated and could be expressed on the entire cell surface. MUC1 expression correlates with invasion, metastasis and poor prognosis in some cancers.

However, few reports have confirmed the correlation between MUC1 expression and clinicopathological characteristics in patients with ESCC. Thus, the present study was designed to investigate the clinical significance of MUC1 expression and its correlation with the risk of tumor metastatic recurrence in patients with ESCC after curative resection by both univariate and multivariate analysis. In the study, MUC1 expression was detected by immunohistochemistry (IHC).

Material and methods

Patients

There were 162 consecutive ESCC patients who underwent esophagectomy at the Department of Thoracic Surgery, Jinan Central Hospital Affiliated to Shandong University; and the Department of Thoracic Surgery East Ward, Provincial Hospital Affiliated to Shandong University from August 2008 to July 2009. A total of 108 patients were enrolled in the study. The inclusion criteria were as follows: (1) patients accepted no preoperative radiotherapy and chemotherapy; (2) middle and lower thoracic esophageal cancer patients accepted curative resection via left posterolateral thoracotomy incision; (3) postsurgical pathology confirmed ESCC with stage I-III and no residual malignant cells on the upper and lower incisal edges; (4) no seriously surgical contraindication; (5) cases kept well preserved. There were 89 men and 19 women in the study and the patients' age ranged from 49 to 76 years (Table I). The TNM staging was according to the International Union Against Cancer (UICC) in 2009. The study was approved by the Ethics Committee of Shandong University.

Immunohistochemistry

All the ESCC specimens were obtained from the 108 patients. Twenty corresponding normal tissue samples, which came from the 108 patients' normal esophagus (6 cm away from ESCC) randomly, were used as controls. The tissue specimens were fixed in 10% neutral buffered formalin and processed routinely. Hematoxylin and eosin (HE)-stained slides as well as immunohistochemical reactions were performed on paraffin-embedded and formalin-fixed tissue using primary antibodies against MUC1 (1 : 100 dilution; Fuzhou Maxim Inc., Fuzhou, Fujian, China) and visualized by the Envision System (Dako). MUC1 was located in the tumor cell cytoplasm. MUC1 protein expression is indicated by bright yellow, brown yellow or brown granules focally or diffusively distributed. The expression of MUC1 was scored as follows: 0, 1 = < 5% of cells; 2 = 5-29% of cells;

Table I. Correlation between MUC1 expression and clinical features of the 108 ESCC patients

Clinical characteristics	Patients (n = 108)	MUC1 expression		P value[A]
		(–)	(+)	
		38	70	
Gender				0.290*
Male	89	29	60	
Female	19	9	10	
Age, years				0.548*
< 60	52	20	32	
≥ 60	56	18	38	
Smoking				1.000*
Yes	49	17	32	
No	59	21	38	
Tumor length, cm				> 0.05
< 3	12	6	6	
3-5	52	21	31	
> 5	44	11	33	
Tumor location				0.100*
Middle	66	19	47	
Lower	42	19	23	
Differentiation				> 0.05
Well	15	8	7	
Moderately	70	25	45	
Poorly	23	5	18	
pT				< 0.05
pT1	10	7	3	
pT2	59	20	39	
pT3	39	11	28	
pN				0.004*
–	65	30	35	
+	43	8	35	
pTNM				< 0.01
pI	37	21	16	
pII	48	13	35	
pIII	22	4	19	

*P value[a]: χ^2 test, *Fisher's exact probability test.*

3 = 30-60% of cells; 4 = > 60% of cells. Cancers were regarded as positive when the score was ≥ 3, according to previous reports [8, 9].

Follow-up

In our cases, 12 patients received postoperative radiotherapy alone. Postoperative chemotherapy was given to 44 patients in more than three cycles (mainly 5-fluorouracil and cisplatin/carboplatin), and 26 patients received combined chemoradiotherapy. Patients were routinely examined every 3 months during the first 3 years and every 6 months thereafter. During each follow-up visit, the patient underwent a thorough physical examination, chest roentgenography, ultrasonography of the neck and abdomen, chest CT, and endoscopic examination. Some patients even underwent positron emission tomography combined with computed tomography (PET/CT) examination. The location and time of tumor relapse were recorded. Patients who died of tumor were enrolled in the prognostic analysis.

Statistical analysis

The frequency data were analyzed using Fisher's exact probability test or the χ^2 test. Univariate analysis was performed by Kaplan-Meier survival curves. The log-rank test was performed to compare the recurrence difference. Multivariate analysis was carried out using the Cox proportional hazard model. Differences were considered significant when the P value was less than 0.05. The statistical data were obtained using the SPSS software package (SPSS 13.0 Inc., Chicago, IL, USA).

Results

Correlation between MUC1 expression and clinical characteristics

MUC1 protein was detected by IHC in ESCC tissues from 70 patients. The diagnostic sensitivity was 65.4% (70/108). The expression of MUC1 in the cancerous tissue group was significantly higher than that in the paracancerous normal tissue group (65.4%:10.0%, p < 0.01). (Fig. 1, Table II). As

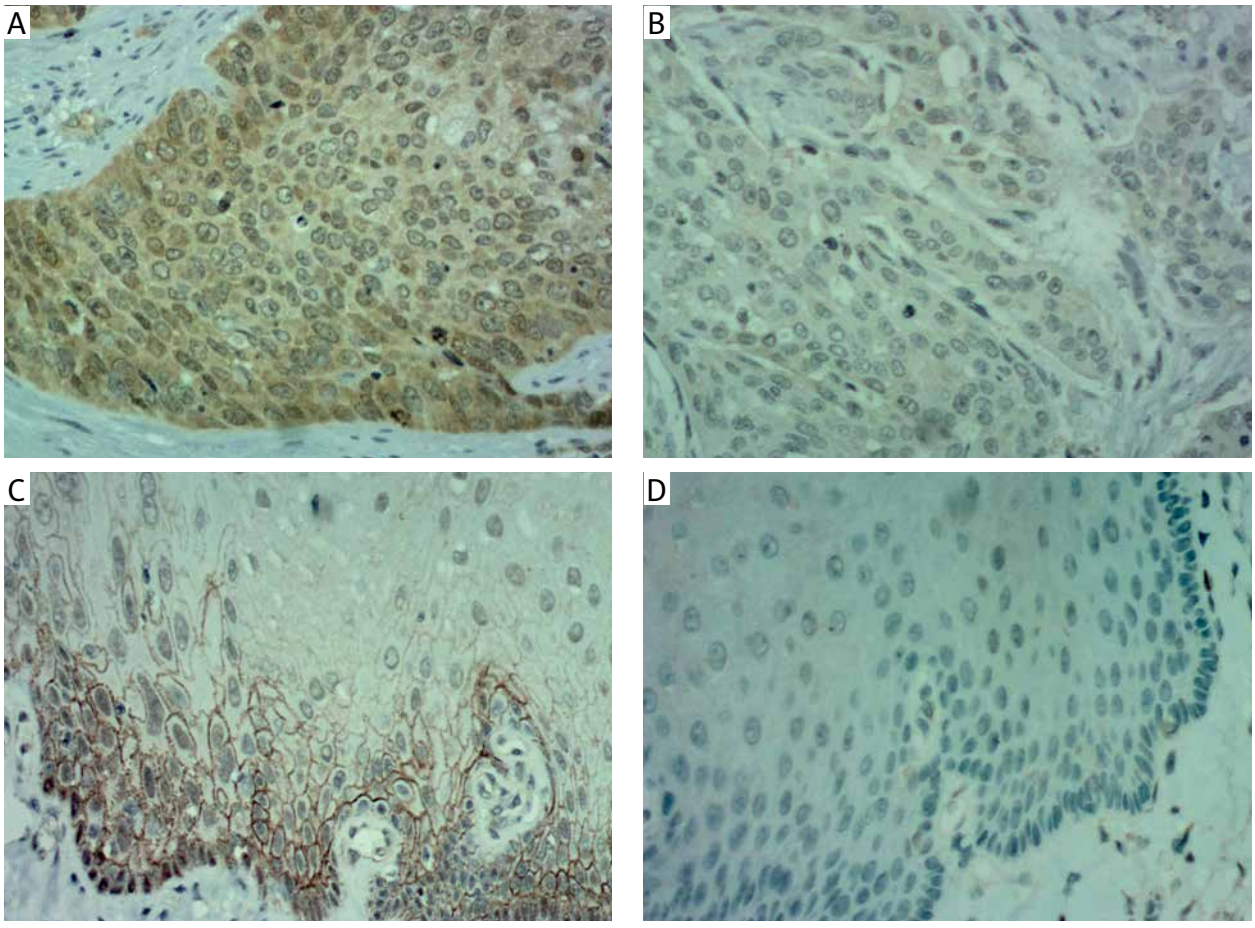

Fig. 1. A) Immunohistochemical staining of human ESCC tissue sections demonstrating MUC1 protein. The MUC1 staining was confined to the cytoplasm, and photomicrographs showed human ESCC specimen with high MUC1-positive tumor cells (> 3). Original magnification 200×. B) Photomicrographs showing ESCC specimen with low MUC1-positive tumor cells (< 3). Original magnification 200×. C) Photomicrographs showing the corresponding normal tissue specimen with MUC1-positive tumor (> 3). Original magnification 200×. D) Photomicrographs showing the corresponding normal tissue specimen with no MUC1-positive tumor (< 3). Original magnification 200×

shown in Table I, MUC1 expression correlated with pT (< 0.05), pN ($p < 0.01$) and pTNM (< 0.01). No other clinicopathological parameter was related to MUC1 expression.

Correlation between MUC1 expression and tumor metastatic recurrence

The Kaplan–Meier method indicated that the 5-year survival rate of the 108 patients was 39.8% (Fig. 2). The first recurrence exhibiting lymph nodal metastasis was recognized in 80 patients (74.1%) in the first 5 years after the operation (Fig. 3). The patients had the first recurrence in cervical, supraclavicular, mediastinal, and celiac lymph nodes (Table III). Forty-one patients (51.25%) developed lymph node recurrence; 24 patients (30.00%) developed a hematogenous recurrence and 15 patients (18.75%) had lymph node and hematogenous recurrence. In univariate analysis by the log-rank test (Table IV), the 5-year tumor metastatic recurrence rate in ESCC patients after the operation was significantly associated with pT ($p < 0.01$), pN ($p < 0.01$), pTNM stage ($p < 0.01$) and MUC1 expression ($p < 0.01$) (Figs. 4-7). No other clinicopathological parameter was re-

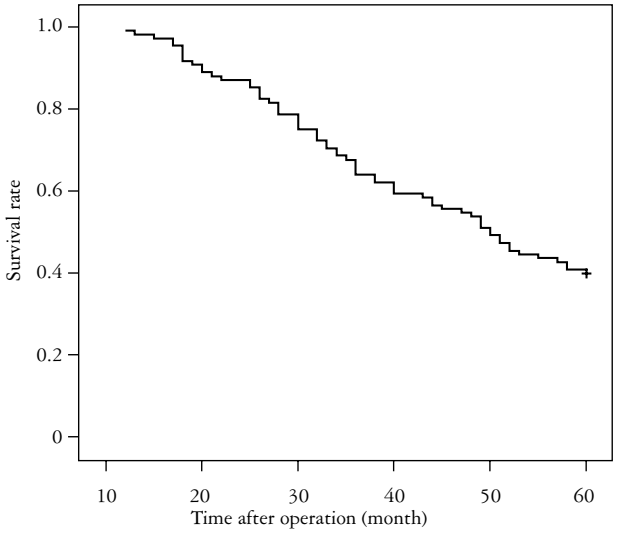

Fig. 2. Kaplan-Meier analysis of overall survival after the operation

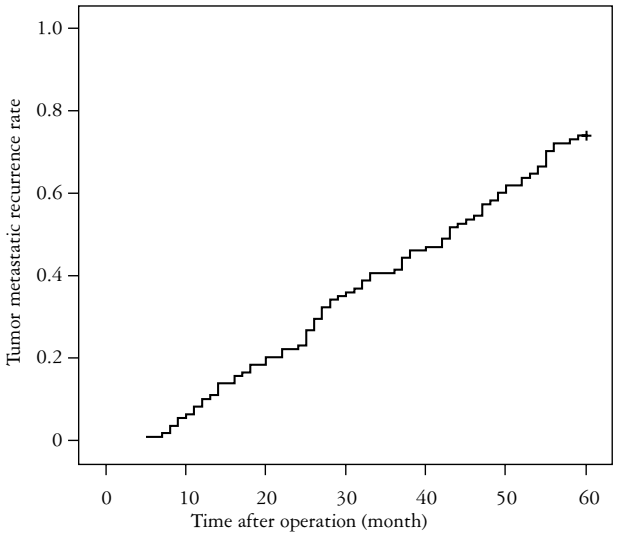

Fig. 3. Kaplan-Meier analysis of tumor metastatic recurrence rate after the operation

Table II. MUC1 expression in cancerous tissue group and paracancerous normal tissue group

CASE	NUMBER	MUC1 EXPRESSION		
	128	– (N = 56)	+ (N = 72)	P VALUE[B]
Cancerous tissue group	108	38	70	0.001
Normal tissue group	20	18	2	

P value[b]: Fisher's exact probability test.

Table III. Sites of tumor metastatic recurrence in 80 ESCC patients

SITES OF TUMOR METASTATIC RECURRENCE	NO. OF PATIENTS (%)
Lymph node recurrence	41 (41/80) (51.25)
Cervical/supraclavicular lymph node	3
Mediastinal lymph node	21
Abdominal lymph node	6
Mediastinal and cervical lymph node	5
Mediastinal and abdominal lymph node	6
Hematogenous recurrence	24 (24/80) (30.00)
Brain	2
Lung	6
Esophagus	4
Stomach	4
Liver	5
Bone	3
Hematogenous and lymph node recurrence	15 (15/80) (18.75)
Lung and mediastinal lymph node	4
Lung, pleura and mediastinal lymph node	1
Stomach and mediastinal lymph node	3
Liver and abdominal lymph node	4
Brain, liver and abdominal lymph node	1
Liver, lung and mediastinal lymph node	1
Bone, liver and abdominal lymph node	1

Table IV. Univariate analysis with respect to 5-year metastatic recurrence rate

Clinical characteristics	Patients	5-year metastatic recurrence rate (%), 80 (74.1)		P value[c]
	108	n	(%)	
Gender				0.249
Male	89	67	75.3	
Female	19	13	68.4	
Age, years				0.974
< 60	52	38	73.1	
≥ 60	56	42	75.0	
Smoking				0.507
–	59	43	72.9	
+	49	37	75.5	
Tumor length, cm				0.063
< 3	12	7	58.3	
3-5	52	36	69.2	
> 5	44	37	84.1	
Tumor location				0.865
Middle	66	48	72.7	
Lower	42	32	76.2	
Differentiation				0.395
Well	15	10	66.7	
Moderately	70	52	74.3	
Poorly	23	18	78.3	
pT				0.002
pT1	10	2	20.0	
pT2	59	45	76.3	
pT3	39	33	84.6	
pN				<0.01
–	65	38	58.5	
+	43	42	97.7	
pTNM				<0.01
pI	37	16	43.2	
pII	48	41	85.4	
pIII	23	23	100	
Chemotherapy				0.301
–	38	25	65.8	
+	70	55	78.6	
Radiotherapy				0.085
–	70	44	62.9	
+	38	36	94.7	
MUC1 expression (IHC)				0.001
–	38	23	60.5	
+	70	57	81.4	

P value[c]: log-rank test.

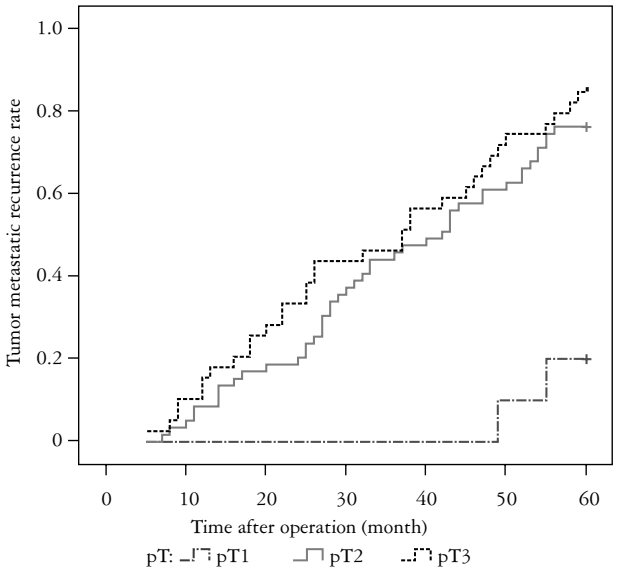

Fig. 4. Kaplan-Meier analysis of tumor metastatic recurrence rate after the operation in patients with pT stage

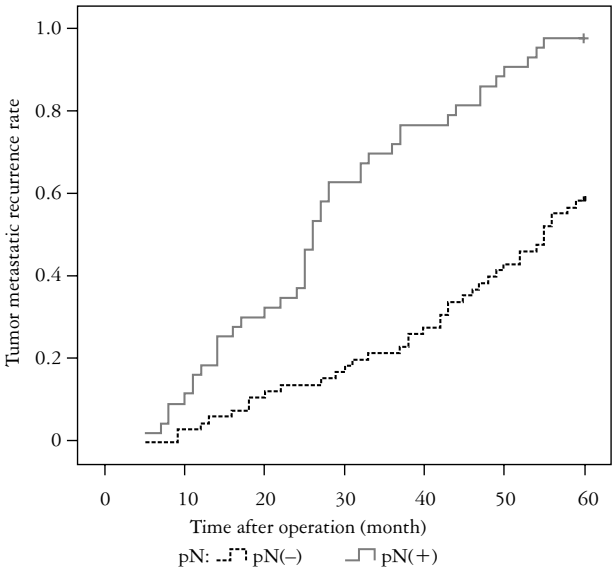

Fig. 5. Kaplan-Meier analysis of tumor metastatic recurrence rate after the operation in patients with pN(–) and pN(+)

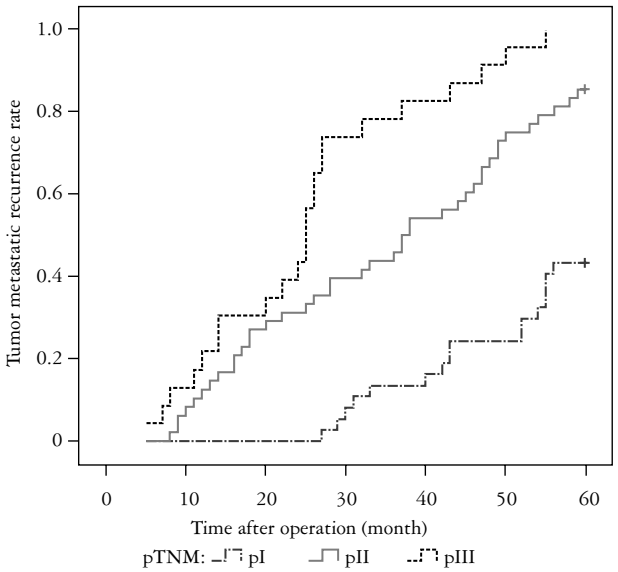

Fig. 6. Kaplan-Meier analysis of tumor metastatic recurrence rate after the operation in patients with pTNM stage

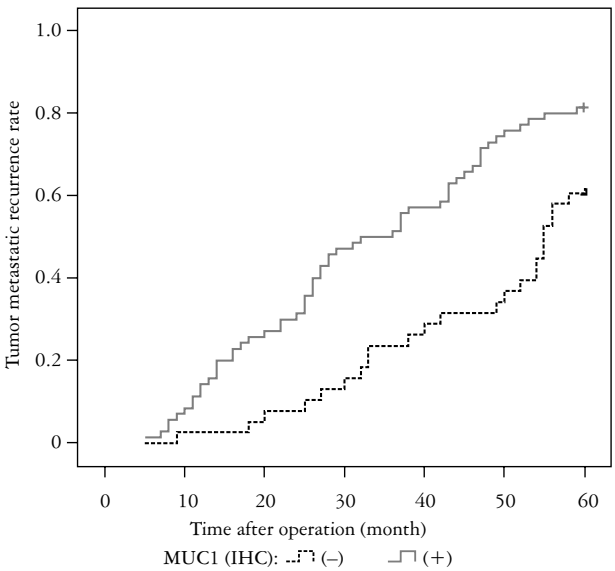

Fig. 7. Kaplan-Meier analysis of tumor metastatic recurrence rate after the operation in patients with positive and negative MUC1 expression, respectively

lated to 5-year tumor metastatic recurrence rate. The results of Cox regression multivariate analysis confirmed that pN and MUC1 expression were independent predictive factors (Table V).

Discussion

MUC1 expression correlated with invasion and metastasis and poor survival in some cancers. It was reported that MUC1 was overexpressed in breast cancer, and was absent or expressed at a low level in normal mammary gland. MUC1 might be a potential target in breast cancer immunotherapy [10, 11]. In gastric cancer, MUC1 was not only expressed in metastatic disease, but also found to be highly expressed in primary tumor, indicating that it might be promoting initial spread [12, 13]. High MUC1 expression was also associated with lymph node metastasis and vascular invasion in liver [14] and pancreas [15] cancer and oral squamous cell carcinoma [16]. MUC1 was also associated with higher grade tumors and shorter metastasis-free survival in renal cell carcinoma [17], thyroid cancer, and lymphomas [18]. These studies revealed a strong link between MUC1 expression and metastatic progression.

Only a few studies have reported the clinicopathological characteristics of MUC1 in ESCC patients, and their correlation remains controversial. Guillem *et al.*

Table V. Results of Cox regression multivariate 5-year tumor metastatic recurrence rate analysis

	B	SE	Wald	P	HR	95.0% CI for HR
Gender	–0.040	0.335	0.015	0.904	0.960	0.498-1.852
Age	–0.277	0.235	1.394	0.238	0.758	0.478-1.201
Smoking	0.214	0.245	0.757	0.384	0.1.238	0.765-2.003
Tumor length, cm	0.212	0.190	1.254	0.263	1.237	0.853-1.793
Tumor location	–0.351	0.274	1.641	0.200	0.704	0.411-1.204
Differentiation	0.051	0.233	0.049	0.825	1.053	0.667-1.660
pT	0.600	0.329	3.322	0.068	1.822	0.956-3.472
pN	1.318	0.487	7.322	0.007	3.735	1.438-9.699
pTNM	0.217	0.375	0.336	0.562	1.243	0.596-2.591
Chemotherapy	–0.140	0.266	0.278	0.598	0.869	0.517-1.463
Radiotherapy	0.126	0.254	0.248	0.618	1.135	0.690-1.866
MUC1 expression	0.569	0.264	4.632	0.031	1.766	1.052-2.966

B – regression coefficient; SE – standard error; Wald – Wald value; HR – hazard ratio; CI – confidence interval

[19] and Kahkhaie *et al.* [20] evaluated MUC1 expression in ESCC at the mRNA level. They both found that MUC1 expression correlated with tumor progression in ESCC. Song [21] reported that 78.9% of ESCC patients expressed MUC1 at the protein level. Also, MUC1 expression was correlated with lymph node metastasis, and high expression of MUC1 correlates with poor survival in ESCC patients. Kijima *et al.* [22] used immunohistochemistry to detect MUC1 protein in ESCC patients and found that MUC1 expression was an early event in cancer progression; however, it was not significantly associated with metastasis of human esophageal carcinomas. Sagara *et al.* [23] used different anti-MUC1 monoclonal antibodies examining MUC1 expression in 192 ESCC and obtained a different result on prognosis. These findings could be explained by the use of different analytic methods, different inclusion criteria, and variable treatments and follow-up protocols. Up to now, no reports have been found in PubMed on the correlation between MUC1 expression and tumor metastatic recurrence in patients with ESCC. In our study, the expression of MUC1 was detected by IHC in 65.42% of ESCC patients. MUC1 expression correlated with tumor invasion (pT), lymph node metastasis (pN) and pTNM. The 5-year tumor metastatic recurrence rate of the patients with MUC1 expression in tumor issue was significantly higher than that of the patients without expression in a univariate analysis. To eliminate the impact of mixed factors correlated with the result on statistical analysis, Cox regression multivariate analysis was performed to determine the independent predictive factors. pN and MUC1 expression were the independent predictive factors for tumor metastatic recurrence. The examination of MUC1 expression in ESCC would become a useful marker to predict tumor metastatic recurrence.

In conclusion, the expression of MUC1 is related to tumor invasion, lymph node metastasis and pTNM in ESCC. MUC1 expression correlates with tumor metastatic recurrence in ESCC patients.

The authors declare no conflict of interest.

References

1. Sun ZG, Wang Z, Zhang M. Correlation between vascular endothelial growth factor C expression and prognosis in patients with esophageal squamous cell carcinomas after Ivor-Lewis esophagectomy. Asia Pac J Clin Oncol 2012; 8: e68-76.
2. Kitadai Y, Amioka T, Haruma K, et al. Clinicopathological significance of vascular endothelial growth factor (VEGF)-C in human esophageal squamous cell carcinomas. Int J Cancer 2001; 93: 6662-6671.
3. Hsu PK, Wang BY, Huang CS, et al. Prognostic factors for post-recurrence survival in esophageal squamous cell carcinoma patients with recurrence after resection. J Gastrointest Surg 2011; 15: 558-565.
4. Kleespies A, Bruns CJ, Jauch KW. Clinical significance of VEGF-A, -C and -D expression in esophageal malignancies. Onkologie 2005; 28: 281-288.
5. Pillai K, Pourgholami MH, Chua TC, et al. MUC1 has prognostic significance in malignant peritoneal mesothelioma. Int J Biol Markers 2013; 28: 303-312.
6. Situ D, Wang J, Ma Y, et al. Expression and prognostic relevance of MUC1 in stage IB non-small cell lung cancer. Med Oncol 2011; 28 Suppl 1: S596-604.
7. Zeng W, Gouw AS, van den Heuvel MC, et al. Hepatocellular carcinomas in cirrhotic and noncirrhotic human livers share angiogenic characteristics. Ann Surg Oncol 2010; 17: 1564-1571.
8. Matsuda K, Masaki T, Watanabe T, et al. Clinical significance of MUC1 and MUC2 mucin and p53 protein expression in colorectal carcinoma. Jpn J Clin Oncol 2000; 30: 89-94.

9. Ajioka Y, Allison LJ, Jass JR. Significance of MUC1 and MUC2 mucin expression in colorectal cancer. J Clin Pathol 1999; 49: 560-564.
10. Lavrsen K, Madsen CB, Rasch MG, et al. Aberrantly glycosylated MUC1 is expressed on the surface of breast cancer cells and a target for antibody-dependent cell-mediated cytotoxicity. Glycoconj J 2013; 30: 227-236.
11. Yang E, Hu XF, Xing PX. Advances of MUC1 as a target for breast cancer immunotherapy. Histol Histopathol 2007; 22: 905-922.
12. Yonezawa S, Kitajima S, Higashi M, et al. A novel anti-MUC1 antibody against the MUC1 cytoplasmic tail domain: use in sensitive identification of poorly differentiated cells in adenocarcinoma of the stomach. Gastric Cancer 2012; 15: 370-381.
13. Wang XT, Kong FB, Mai W, Li L, Pang LM. MUC1 Immunohistochemical Expression as a Prognostic Factor in Gastric Cancer: Meta-Analysis. Dis Markers 2016; 2016: 9421571.
14. Guo Q, Tang W, Inagaki Y, et al. KL-6 mucin in metastatic liver cancer tissues from primary colorectal carcinoma. Hepatogastroenterology 2009; 56: 960-969.
15. Mizumoto M, Honjo G, Kobashi Y, et al. Molecular profile of apomucin and p53 protein as predictors of malignancy in intraductal papillary mucinous neoplasms of the pancreas. Hepatogastroenterology 2011; 58: 1791-1795.
16. Hamada T, Nomura M, Kamikawa Y, et al. DF3 epitope expression on MUC1 mucin is associated with tumor aggressiveness, subsequent lymph node metastasis, and poor prognosis in patients with oral squamous cell carcinoma. Cancer 2012; 118: 5251-5264.
17. Leroy X, Zerimech F, Zini L, et al. MUC1 expression is correlated with nuclear grade and tumor progression in pT1 renal clear cell carcinoma. Am J Clin Pathol 2002; 118: 47-51.
18. Kaira K, Murakami H, Serizawa M, et al. MUC1 expression in thymic epithelial tumors: MUC1 may be useful marker as differential diagnosis between type B3 thymoma and thymic carcinoma. Virchows Arch 2011; 458: 615-620.
19. Guillem P, Billeret V, Buisine MP, et al. Mucin gene expression and cell differentiation in human normal, premalignant and malignant esophagus. Int J Cancer 2000; 88: 856-861.
20. Kahkhaie KR, Moaven O, Abbaszadegan MR, et al. Specific MUC1 splice variants are correlated with tumor progression in esophageal cancer. World J Surg 2014; 38: 2052-2057.
21. Song ZB, Gao SS, Yi XN, et al. 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. World J Gastroenterol 2003; 9: 404-407.
22. Kijima H, Chino O, Oshiba G, et al. Immunohistochemical MUC1 (DF3 antigen) expression of human esophageal squamous cell carcinoma. Anticancer Res 2001; 21(2B): 1285-1289.
23. Sagara M, Yonezawa S, Nagata K, et al. Expression of mucin 1 (MUC1) in esophageal squamous-cell carcinoma: its relationship with prognosis. Int J Cancer 1999; 84: 251-257.

Address for correspondence

Liang-Ming Zhu
Department of Thoracic Surgery
Jinan Central Hospital Affiliated to Shandong University
Shandong University, Jinan 250013
Shandong Province, China
tel. (0086)15318816207
e-mail: zlm1655@126.com