Expression of E-cadherin and MMP-9 protein in esophageal squamous cell carcinoma and their clinical pathological significances

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Abstract: To detect the expression of E-cadherin and MMP-9 protein in esophageal squamous cell carcinoma (SCC) and investigate their correlations with the occurrence and development of esophageal SCC, SP immunohistochemical method was used to detect the expression of E-cadherin and MMP-9 protein in 124 cases of esophageal SCC, 62 cases of normal esophageal epithelium. The expression of E-cadherin and MMP-9 protein were closely correlated with the infiltration and lymph node metastasis of esophageal SCC ($P < 0.05$), but were not correlated with the tumor grade, age or gender of the patients ($P > 0.05$). The expression rate of E-cadherin protein was much higher in normal esophageal epithelium (90.3%) than in esophageal SCC (43.5%). There was significant difference between them ($P < 0.01$). However, the expression rate of MMP-9 protein was 72.6% in esophageal SCC, 33.9% in normal esophageal epithelium. And there was significant difference between them，too($P < 0.01$). The expression of E-cadherin protein was negatively correlated with MMP-9 expression ($P<0.01$). Conclusively, E-cadherin and MMP-9 may play important roles in the infiltration, metastasis and carcinomatous changes of mucosal epithelium in esophageal carcinoma. United detection of them may be used in the early diagnosis and prognosis judgment of esophageal SCC.

[Yongxia Wang, Zheng Tang, Yu Wang, Xinlai Qian. **Expression of E-cadherin and MMP-9 protein in esophageal squamous cell carcinoma and their clinical pathological significances.** *Life Sci J* 2013;10(2):3006-3009] (ISSN:1097-8135). http://www.lifesciencesite.com. 415

Key Words: E-cadherin; Matrix metalloproteinase-9; esophageal squamous cell carcinoma; Immunohistochemistry; Invasion; Metastasis

Introduction

Esophageal cancer (EC) is one of the most frequent malignant tumors in digestive system, and accounts for approximately 500,000 new cases and 300,000 deaths per year worldwide, half of which occur in China, especially in West and North of China. And 90% of EC in China is squamous cell carcinoma (SCC) [1, 2]. Invasion and metastasis are the important biological hallmarks of esophageal carcinoma and also the main cause for the death of patients with the carcinoma [3, 4]. The mechanisms of tumor metastasis represent a great challenge for both researchers and clinicians. Therefore, it is of great interest to also better understand the molecular mechanisms underlying the development and progression of esophageal SCC. E-cadherin is one of the significant members of the cadherin family, which plays an important role in the invasion and metastasis of tumors by mediating the cell adhesion of the same kind [5]. Extra cellular matrix (ECM) also plays a significant role in the tumor's invasion and metastasis [6], during the process of the invasion and metastasis of the tumor cells need to demolish the ECM. Matrix metalloproteinases (MMPs) are important in destroying the enzymes in ECM, among which MMP-9 keeps the closest link with the tumors' invasion and metastasis because it can resolve the normal tissue matrix and lead to hyperplasia and metastasis of carcinoma cells [7]. This paper tends to explore the expression of E-cadherin and MMP-9 in esophageal carcinoma (124 cases) and normal mucous of esophagus (62 cases) by Immunohistochemistry and investigate their correlations with the development and progression of esophageal squamous cell carcinoma.

Material and Methods

general material

All the samples were taken from the stored paraffin blocks of esophageal specimens by surgical resection in the Third Teaching Hospital of Xinxiang Medical University. Preoperatively, all the cases had no chemotherapy, radiotherapy and immunotherapy history. The age of 62 patients with esophageal carcinoma, 33 males and 29 females, varied from 38 to 75 (average age 60.6±9.5). The HE staining had confirmed that all the cases belongs to squamous cell carcinoma, with 30 cases in level Ⅰ, 50 cases in Level Ⅱ and 44 cases in level Ⅲ.Lymphatic metastasis was found in 84 cases, but was not found in other 40 cases. Low infiltration is found in 18 cases, which lied in or under mucous layer. Deep infiltration was found in 106 cases, which was in muscular layer or theca externa. Besides, 62 cases of normal mucosa of oesophagus were taken in this study for the control group.

Main Agent and Method

SP immunohistochemistry was performed in this study. Rabbit anti-human E-cadherin polyclonal antibody (ready for use) fluid was the product of American Santa Cruz, purchased from Beijing Zhong Shan Jin Qiao Biological Company. Monocolonal mouse anti-human MMP-9 protein was purchased from Beijing Zhong Shan Jin Qiao Biological Company, at a 1:100 dilution. The experiment was conducted according to the kit introduction. Diaminlbenzidin (DAB) was used for colour development, and PBS was used to replace monoclonal antibody as negative contrast.

Result Judgment Standard

The positive staining is brown or brown yellow particles. E-cadherin positive expression shows strong colouring in normal cell membrane, and shows colouring of cell membrane and endochylema in carcinoma. MMP-9 positive expression mainly shows in endochylema. Each slide was randomly observed in 10 visual fields and counted the number of positive cells. It was grouped as positive cases when the number of positive cells ≥10% and negative ones when the number of positive cells ＜ 10%.

Statistical Analysis

Statistical analysis was performed with SPSS software (version 10.0).The comparation of rate used $\chi 2$ test. Associations for E-cadherin and MMP-9 protein expression were assessed using Spearman's rank correlation test. Differences were considered significant at $P < 0.05$.

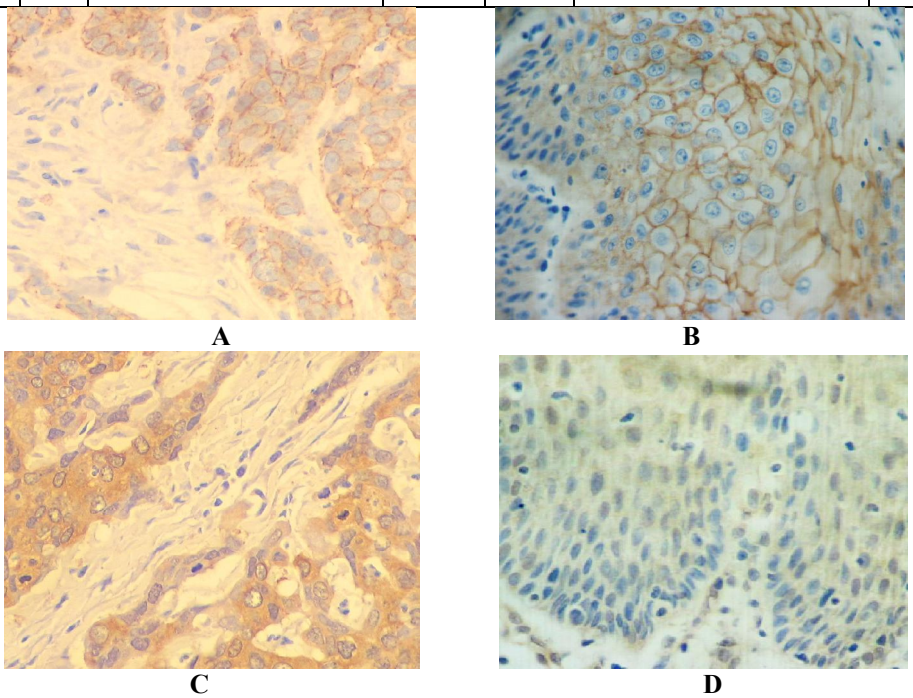
Results

Expression of E-cadherin and MMP-9 in esophageal ECC and normal mucosa

The positive expression of E-cadherin in normal tissues showed strong colouring in the cell membrane, and colouring endochylema and cell membrane in carcinoma, which is much lighter than in normal tissues（Fig. 1A, Fig. 1B）.The expression rate increased in normal esophageal epithelium than in esophageal ECC (Table 1).The positive expression of MMP-9 protein mainly demonstrates strong colouring of endochylema (Fig. 1C, Fig. 1D), the expression rate in carcinoma than in normal membrane decreased (table 1).

Table 1. Expression of E-cadherin and MMP-9 in esophageal ECC and normal mucosa

Tissue types		E-cadherin			MMP-9		
		Positive number（%）	$\chi 2$	*P*	Positive number（%）	$\chi 2$	*P*
ECC	124	54（43.5）	37.422	0.000	90（72.6）	25.738	0.000
Normal	62	56（90.3）			21（33.9）		



A **B** **C** **D**

Figure 1. Immunohistochemical staining of E-cadherin and MMP-9. Expression of E-cadherin in **A** esophageal squamous cell carcinoma (ESCC)and **B** normal mucoma; Expression of MMP-9 in **C** ESCCand **D** normal mucoma;

Expression of E-cadherin and MMP-9 in esophageal squamous cell carcinoma and its relationship with clinicopathologic features

The positive expression of E-cadherin and MMP-9 protein in ESCC was closely correlated with the infiltration and lymph metastasis (P<0.05), but had no correlation with the differentiation degree and patients' age or sex (p>0.05) (Table 2).

Table 2. Relationship between the expression of E-cadherin、MMP-9 and the clinicopathologic features of esophageal SCC

Clinicopathologi-cal features		Expression of E-cadherin			Expression of MMP-9		
		Positive number	χ2	P	Positive number	χ2	P
Gender							
Male	66	30	0.209	0.648	52	2.732	0.098
Female	58	24			38		
Age							
≥60	72	34	0.943	0.332	56	2.330	0.127
＜60	52	20			34		
Differentiation degree							
Ⅰ	30	10	3.694	0.158	26	4.841	0.089
Ⅱ	50	20			32		
Ⅲ	44	24			32		
Infiltration depth							
Superficial layer	18	16	17.608	0.000	6	16.298	0.000
Deep layer	106	38			84		
Lymph node metastasis							
Positive	84	24	23.760	0.000	76	38.108	0.000
Negative	40	30			14		

Correlation between E-cadherin and MMP-9 in esophageal squamous cell carcinoma

According to the spearman analysis, The expression of E-cadherin and MMP-9 shows negative correlation in esophageal SCC (p<0.01) (Table 3).

Table 3. Relationship between between E-cadherin and MMP-9 in tumor tissues

Immunohistochemical index		MMP-9		R	P
		+	—		
E-cadherin	+	24	30	-0.554	0.000
	—	66	4		

Discussion

Studies have shown that the infiltration and metastasis are main causes of the death of patients with esopheageal carcinoma, which is one of the most common malignant tumors. The infiltration and metastasis of the tumor is a complex pathological process, including tumor cells dropping from primary lesion, adhesion with extra cellular matrix, depredating local matrix by oncocyte secreting protease, moving to the dissolved region by protease by pseudopodium [8].

E-cadherin is an important adhesion molecule to mediate the gathering of cells because it has the adhesion function of calcium-dependent cells of the same kind, which makes the adjorning epithelial cells adhere together closely so as to prevent the exfoliation of cells. When carcinoma occurs, the E-cadherin expression will lower and the adhesion between cancered cells will be weakened, so it is easy for cells to exfoliate from the original site and form the tumour's local infiltration and remote metastasis. Therefore, E-cadherin gene is an important anti-metastasis gene, and the mutation is the critical event in tumour's infiltration and metastasis [9.10]. The results of the research showed E-cadherin gene had expression in normal esophageal tissues; and the expression in carcinoma was significantly lowered and the differences between them had statistical significance. Meanwhile, the study showed that the positive expression of E-cadherin in deep-infiltration group and lymph metastasis group was obviously lower than that in low-infiltration and non-lymph metastasis group. These results imply that the esophageal squamous cell carcinoma with low E-cadherin expression has much stronger capability of infiltration and metastasis.

MMP family is the main enzyme to dissolve the

extracellular matrix, which plays a significant role in tumor's infiltration and metastasis. Gelatinase is the only enzyme which can dissolve the three-screw structures in ECM and IV collagen. And it can be divided into many subtypes. MMP-9 is one of them. It can not only dissolve and destroy the extracellular matrix and basement membrane to make the cancer infiltrate towards surrounding regions by defected basilar membrane but also push the growth and invasion of tumors by affecting new vessels [11]. The study showed the expression of MMP-9 was extremely low in normal esophageal membrane but increased in carcinoma, which gave the hint that the high expression of MMP-9 was closely connected with the occurrence of esophageal squamous cell carcinoma. However, the expression of MMP-9 had no correlation with the sex, age of the patients and tumor's histological grading but had obvious correlation with infiltration degree and non-lymph metastasis; this showed the high expression of MMP-9 enhanced the infiltration and metastasis of esophageal squamous cell carcinoma.

This study also showed that the expression of E-cadherin and MMP-9 had negative correlation. This coincide with the previous finding that MMP can significantly prohibit the expression of E-cadherin[12], which demonstrates that E-cadherin is one of the inhibition targets for MMP-9。

In general, E-cadherin and MMP-9 play important roles in the occurrence, infiltration and metastasis of esophageal squamous cell carcinoma. Meanwhile, E-cadherin as a major inhibition target for MMPs, is closely connected with MMP-9. Therefore, the combination detection of E-cadherin and MMP-9 is expected to be one of the molecule indexes to the diagnosis of early esophageal squamous cell carcinoma and judgment of prognosis.

References

1. Mariette C, Balon JM, Piessen G, et a1.Pattern of recurrence following complete resection of esophageal carcinoma and factors predictive of recurrent disease[J]. Cancer, 2003, 97(7):1616-1623.
2. Enzinger PC, Mayer RJ. Esophageal cancer [J]. N Engl J Med, 2003, 349: 2241-2252.
3. Douglas Hanahan, Robert A. Hallmarks of Cancer: The Next Generation [J].Cell, 2011, 144(5):646-674.
4. Morales CP, Souza RF, Spechler SJ. Hallmarks of cancer progression in Barrett's esophagus[J]. Lancet, 2002, 360(8):1587-1589.
5. Meyer T, Hart IR. Mechanisms of tumor metastasis [J]. Eur J Cancer, 1998, 34(4): 214-221.
6. Davies B, Waxman J, Wasan H, et a1.Levels of matrix metalloproteinase in bladder cancer correlate with tumor grade and invasion[J]. Cancer Res, 1993, 53(22): 5365-5369.
7. Yao J, Xiong S, Klos K ,et al. Multiple signaling pathways involved in activation of matrix metalloproteinase-9 by heregulin-betal in human breast cancer cells[J]. Oncogene, 2001, 20(12):8066-8074.
8. Kohn EC, Liotta LA. Molecular insights into cancer invasion: strategies for prevention and intervention [J]. Cancer Res, 1995, 55(9): 1856-1862.
9. Kim JM, Noh EM, Kwon KB,et al. Suppression of TPA-induced tumor cell invasion by sulfuretin via inhibition of NF-κB-dependent MMP-9expression[J]. Oncol Rep, 2012, 38 (10):2012-2218.
10. Parfiniewicz B, Pendzich J, Gruchlik A,et al. Impact of celecoxib on soluble intercellular adhesion molecule-1 and soluble e-cadherin concentrations in human colon cancer cell line cultures exposed to phytic acid and TNF-alpha[J]. Acta Pol Pharm, 2012, 69(6):1283-1290.
11. Gong J, Zhu SG, Wu CY, et al. Expression and significance of MMP-9, PR, Ki-67 and Survivin in multiple meningiomas [J]. Zhonghua Yi Xue Za Zhi, 2012, 92(45):3218-3220.
12. Laco J, Ryska A.The use of immunohistochemistry in the differential diagnosis of thyroid gland tumors with follocular growth pattern[J].Gesk Patol, 2006, 42(3):120-124.

6/22/2013