

The expression and clinical significance of metastasis suppressor gene and matrix metalloproteinase-2 in esophageal squamous cell carcinoma

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Abstract: To investigate the expression and clinical significance of metastasis suppressor gene and matrix metalloproteinase-2 in esophageal squamous cell carcinoma. choose 30 cases of specimens of esophageal squamous cell carcinoma which are removed in surgery and confirmed by pathology and 30 cases of specimens of normal esophageal mucosa. Use immunohistochemistry SP method to detect the expression of nm23-H1, MMP-2 protein in esophageal squamous cell carcinoma and normal esophageal mucosal. The positive rate of nm23-H1 protein in esophageal squamous cell carcinoma was 43.3% (13/30), while that in normal esophageal mucosa was 100% (30/30), which has a significant difference between them ($\chi^2=22.083$, $P<0.05$). The positive rate of MMP-2 protein in esophageal squamous cell carcinoma was 90.0% (27/30), while that in normal esophageal mucosa was 33.3% (10/30), and there is a significant difference between them ($\chi^2=28.370$, $P<0.05$); For the expression of nm23-H1 and MMP-2 in esophageal squamous cell carcinoma, there was nothing to do with sex, age and tumor size ($P>0.05$), but it was related to the degree of tumor differentiation, depth of invasion and lymph node metastasis ($P<0.05$); The expression of nm23-H1 is related to the cut end of residual cancer ($P<0.05$), while the expression of MMP-2 has nothing to do with the cut end of residual cancer ($P>0.05$); The expression of nm23-H1 and MMP-2 in esophageal squamous cell carcinoma was negatively correlated. nm23-H1 and MMP-2 have played a role in the development of esophageal cancer, which can promote the occurrence of distant metastasis; The loss of expression of nm23-H1 may be related to cut end residual cancer; nm23-H1 and MMP-2 may be as an indicator for esophageal cancer metastasis and prognosis.

Keywords: Esophageal squamous cell carcinoma; metastasis suppressor gene; matrix metalloproteinase-2; immunohistochemistry

INTRODUCTION

Esophageal cancer is a common malignancy, with high invasiveness, which was found when it was in the middle and late time in the most time with the rapid development and low survival rate. So it has attracted the attention of most people. nm23 gene is a transfer and suppressor gene discovered recently. There are nm23-H1 and nm23-H2 in Human gene group, and nm23-H1 has a more closely relationship with the metastasis of cancer cells. Matrix metalloproteinase -2 (MMP-2) is one of the important members of the MMP family, which can degrade basement membrane collagen and intercellular matrix, promoting the spread of cancer metastasis (Sawa *et al* and Fung *et al*, 1994). The study examined the expression of nm23-H1 and MMP-2 in esophageal squamous cell carcinoma and the normal esophageal mucosa by immunohistochemistry method and explored its clinical significance.

MATERIALS AND METHODS

General information

Collected 30 cases of specimens of esophageal squamous

cell carcinoma, which are removed in surgery and confirmed by pathology from February 2013 to February 2015 in our hospital. All patients with complete data, were not chemotherapy or radiotherapy before drawn. All specimens were identified and diagnosed by pathology expert. Among them, there are 21 males and 9 females; who were aged from 50 to 66 years and mean years were (86±6) years old. Resected tissue, were fixed by 10% formalin, with paraffin-embedded sections. There are 13 cases with regional lymph node metastasis, 17 cases without lymph node metastasis. All the patients were divided into ~ χ period according to the standard of TNM classification. In this study, there were 6 cases of + α , 24 cases of $\beta+\chi$. Select normal esophageal mucosa specimens of 30 patients: 18 males and 12 females; Aged from 50 to 70 years old and the mean age was (55±5) years old. Remove to serum specimens. The difference of age and gender of patients was not statistically significant ($P>0.05$).

The main instruments and reagents

MMP-2 ELISA kit (United States R & D Company); nm23-H, a monoclonal antibody (mouse anti-human, Cruz company). American Bio-rad 505 micro plate reader; micropipette (Eppendorf US company); small high-speed low-temperature centrifuge (Sigma,

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Germany); micro-oscillator (Jintan City, Jiangsu Province Universal Scientific Instrument Factory).

MMP-2 detected

(1) Remove frozen specimens, thawed at room temperature, and provided the calibration curve of the antibody standard concentrations. (2) join standards solution of antibody with a required dilution and samples to the appropriate wells, used plate sealing tape to seal the wells, and incubated at room temperature for 120 min. (3) Press washer according to washing method. In addition to the blank hole, every hole was added to 100 μ L secondary antibodies with require dilution of biotin-labeled according to the instructions, sealed wells, and incubated at room temperature for 90 min. (4) Press washer according to washing method. (5) In addition to the blank hole, every hole was added to 100 μ L Enzyme Conjugate has diluted as required, and sealed the wells at room temperature for 30 min. (6) Press washer according to washing method. (7) Added substrate A and B into each per well 50 μ L, and put in dark for 20 min. (8) Added end solution into each per 50 μ L, immediately measured absorbance of sample by micro plate reader at 450 nm, draw the standard curve, and calculated the level of MMP-2 samples. Detected were done by the same examiner operations, and steps were done in strict accordance with the instructions, with strict quality control. Put all reagents at room temperature before use them. All of the tested samples, standards and controls required to control parts of the double to determine.

nm23-H1 detection

slides were coated with rubber APES (50mL of acetone was added APES 1mL) for retaining part after conventional treatment, then they were drying through oven. Specimens were embedded in paraffin, and then sliced spare. *Immunohistochemistry*: Sections were dewaxed in xylene. After graded alcohol to water, the experimental group plus nm23-H1. I antibody (dilution is 1:50) placed in a wet box overnight at 4 $^{\circ}$ C. Then added α antibody (goat anti-mouse LgG) reaction at room temperature for 40min. Next plus III antibody and reacted at room temperature for 40min. Finally, DAB colored for 2~8 min and color development was observed under a microscope. *Experimental control*: omitting the first antibody as a negative control in the reaction. Immunohistochemical staining strictly in accordance with SP kit instructions. According to the method described by Shiozaki *et al* (1991) to do qualitative and semi-quantitative classification for protein expression.

Results criteria

The expression of nm23-H1 and MMP-2 are located in the cytoplasmic, showing yellow-brown particles or clumps. Select a representative microscopic field of view of 5 (400 times) and the total count is more than 1000 cells. The percentage of positive cells were divided by percentage: positive cells <25% named negative (-) and positive cells >125% named positive (+).

STATISTICAL ANALYSIS

Use SPSS 13.0 packages for χ^2 test, Fisher exact test to analyze, and the correlation were analyzed by χ^2 test analysis between columns linking data.

RESULTS

The expression of nm23-H1 and MMP-2

The positive rate of nm23-H1 protein in esophageal squamous cell carcinoma was 43.3% (13/30), and that in normal esophageal mucosa was 100% (30/30), with a significant difference between them ($\chi^2=22.083$, $P<0.05$). nm23-H1 The positive rate of MMP-2 protein in esophageal squamous cell carcinoma was 90.0% (27/30), and that in normal esophageal mucosa positive rate was 33.3% (10/30), with a significant difference between them ($\chi^2=28.370$, $P<0.05$).

The relationship between the expression of nm23-H1, MMP-2 and the clinical pathological data of esophageal squamous cell carcinoma

As shown in table 1. The expression of nm23-H1 and MMP-2 in esophageal squamous cell carcinoma has nothing to do with sex, age and tumor size ($P>0.05$), while it has something to do with the degree of tumor differentiation, depth of invasion and lymph node metastasis ($P<0.05$); The expression of nm23-H1 was related with the cut end of residual cancer ($P<0.05$), while the expression of MMP-2 was unrelated to the residual of cut end of cancer ($P>0.05$).

Correlation of expression between nm23-H1 and MMP-2 in esophageal squamous cell carcinoma

The expression of nm23-H1 and MMP-2 in esophageal squamous cell carcinoma was negatively correlated (contingency coefficient = -0.353, $P=0.007$).

DISCUSSION

nm23 is separated by the US National Cancer Institute in 1988 and confirmed that it is the inhibition of tumor metastasis-related genes. nm23-H1 gene is one of the gene family to six members (Janica, 1994; Vallbohmer and Lenz, 2006; Lee *et al.*, 2014), which has the most closely relationship between tumor metastasis and attracted more and more attention. The mechanism of action in different tumors are different and more complicated. Now the nm23-H1 gene is considered as the encoded product of nucleoside diphosphate kinase (NDPK), involved in the collection and decomposition of microtubules, changed cytoskeleton morphology, affected activity and adhesion of cells, and restrained tumor metastasis (Xuan *et al.*, 2015; Yu *et al.*, 2015; Szumilo *et al.*, 2002; Wang *et al.*, 1998). nm23-H1 gene mutation or deletion, resulting in inhibition of cell movement disorders and loss of meiosis, cell differentiation and

Table 1: The relationship between the expression of nm23-H1, MMP-2 and the clinical pathological data of esophageal squamous cell carcinoma (n)

	Pathological data	N	The expression of nm23-H1		The expression of MMP-2	
			Positive	Negative	Positive	Negative
Sex	Male	21	9	12	19	2
	Female	9	4	5	8	1
	χ^2 /P Value		0.036/0.849		0.302/0.583	
Age	< 45 years old	18	8	10	15	3
	\geq 45 years old	12	5	7	8	4
	χ^2 /P Value		0.115/0.735		0.802/0.370	
The degree of Differentiation	Low	14	4	10	14	0
	Medium and high	16	9	7	13	3
	χ^2 /P Value		5.031/0.025		4.446/0.035	
Tumor size	< 5 cm	20	9	11	18	2
	\geq 5 cm	10	4	6	9	1
	χ^2 /P Value		0.071/0.790		0.229/0.632	
Depth of invasion	Mucosa-superficial muscle 12		7	5	9	3
	Deep muscle-the whole layer 18		6	12	18	0
	χ^2 /P Value		6.281/0.012		7.752/0.005	
Cut off residual cancer	Yes	5	1	4	5	0
	No	25	12	13	22	3
	χ^2 /P Value		4.446/0.035		1.005/0.316	
Lymph node	Yes	13	3	10	13	0

development promote the development of tumor (You and Gao, 2007; Liu *et al.*, 2010; Janica, 1994). The relationship between abnormal expression of nm23-H1 protein in esophageal squamous cell carcinoma and occurrence, development and the transfer of tumors are slightly different in various reports. Studies suggest that expression of nm23 in well-differentiated carcinoma was significantly lower than that in low esophageal cancer, and the expression in the lymph node metastasis was significantly lower than those without lymph node transfer (He *et al.*, 2003; Wan, 2010; Zhang *et al.*, 2012). Related studies have found that the expression of nm23-H1 protein in well-differentiated esophageal cancer is higher than that in poorly differentiated carcinoma, but the difference was not statistically significant, which considered that nm23-H1 is unrelated to the degree of differentiation in esophageal cancer (Qi *et al.*, 2005). Recent studies have found that the expression of nm23-H1 protein is related to depth of invasion esophageal cancer, lymph node metastasis and so on. The experiments showed that the deletion expression of nm23-H1 gene is closely associated with the occurrence of esophageal squamous cell carcinoma, which may play an important role in the incidence and development of esophageal squamous cell carcinoma. The reducing or no expression of nm23-H1 gene can promote tumor genesis, invasion and metastasis, but also may lead to the residual of surgical end cancer. It could be used as one of indicators to diagnose esophageal squamous cell carcinoma

metastasis and prognosis in clinical (Sun and Zhao, 2009; Luo *et al.*, 2008; Lee and Kim, 2009; Wang *et al.*, 2013; Li *et al.*, 2015). MMP-2 is an important member of the family of MMPs, with degrade extra cellular matrix and basement membrane function, and high expression play an important role in dip run and metastasis in tumor cells (Du *et al.*, 2011). The experimental results showed that the expression of MMP-2 is unrelated to gender, age, residual cut-side cancer and tumor size, while it is related to the degree of tumor differentiation, depth of invasion and lymph node metastasis, and it is basically related to the literature (Liu *et al.*, 2011), showing MMP-2 is related to the occurrence and the development of esophageal cancer. High expression not only can promote local invasion and distant metastasis of tumor, but also could be used as molecular markers of esophageal squamous cell carcinoma prognosis. Loss expression of nm23-H1 and high expression of MMP-2 can cause the occurrence and metastasis of esophageal cancer, abnormal expression of both may have a synergistic effect (Wang *et al.*, 2008).

CONCLUSION

nm23-H1 and MMP-2 expression in esophageal carcinoma was negatively correlated, respectively. nm23-H1 and MMP-2 as an inhibitory factor and contributing factor to promote the spread of cancer, respectively. They play a role in the occurrence and development process in the esophagus cancer. The simultaneous detection of the

expression of two markers in esophageal squamous cell carcinoma, which is a more accurate predictor of tumor metastasis and prognosis and the interaction mechanism between the two remains need to be further study.

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