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Studies on the expression and relationship between MMP-9 and EGFR in laryngeal squamous cell carcinoma.

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Abstract

Objective: To discuss the expression and relationship between matrix metalloproteinase-9 and epidermal growth factor receptor in laryngeal squamous cell carcinoma.
Methods: Using the method of immunohistochemical to detect the matrix metalloproteinase-9 (MMP-9) and epithelial growth factor receptor (EGFR) in 58 cases of laryngeal squamous carcinoma tissues and 16 cases of normal laryngeal tissues of expression.
Results: MMP-9 in 58 cases of laryngeal squamous carcinoma tissue and EGFR positive expression rate is 68.9% and 77.6%, respectively, significantly higher than 16 cases of normal laryngeal tissue (P<0.05). MMP-9 and the expression of EGFR and tumour TNM stages, differentiate degree and lymph node metastasis (P<0.05). MMP-9, and the expression of EGFR was positively related to (chi-square=6.68, r=6.68, P<0.05). MMP-9 and EGFR is closely related to the prognosis of patients (P<0.05).
Conclusion: The expression of EGFR and the level of the expression of MMP-9 can be used as judgment index of laryngeal squamous carcinoma, malignant degree and prognosis.

Keywords: Epidermal growth factor receptor, S-P method, Matrix metalloproteinase-9, Laryngeal squamous cell carcinoma.

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Introduction

Laryngeal squamous cell carcinoma is a malignant tumour, which derives from the epithelium tissue of laryngeal mucosa. The most essential feature of laryngeal squamous cell carcinoma is invasion and metastasis of cancer cells, which is also the leading cause of death in cancer patients. Studies have shown that laryngocarcinoma accounts for 7.9~35% of head and neck malignant tumours and accounts for 1.2~1.6% of whole body malignant tumours. Among them, laryngeal squamous cell carcinoma is 96~98% [1,2]. In recent years, with the continuous progress of science and technology, our research on the mechanism of tumour invasion and metastasis is also developing steadily. Matrix metalloproteinase-9 (MMP-9) as a proteolytic enzyme plays a major role in the degradation of basement membrane and extracellular matrix components, which is the main route of invasion and metastasis of malignant tumour. Epidermal growth factor receptor (EGFR) closely related to the growth, differentiation, and metastasis of cancer cells. EGFR and MMP-9 play an important role in tumorigenesis and progression [3-6]. In this study, the expression of matrix metalloproteinase-9 and epidermal growth factor receptor (EGFR) in 58 cases of laryngeal squamous cell carcinoma (LSCC) were summarized and the relationship between MMP-9, EGFR and invasion and metastasis of laryngeal squamous cell carcinoma was analysed, which lay a foundation for the diagnosis, treatment and prognosis of laryngeal squamous cell carcinoma.

Materials and Methods

Materials

Specimen: The specimens were selected from the affiliated large hospital of Hubei Institute for Nationalities, 2010~2016, and 58 pieces of paraffin specimens proved pathologically to be squamous cell carcinoma of larynx were removed surgically. There were 57 males and 1 female respectively, age 31~69 years old, the median age was 49 years. All patients had complete follow-up data. No radiotherapy and chemotherapy were performed before operation. The follow-up period was as follows May 2014. The computational method of the survival of the patient: from the date of operation to the end of the patient's follow-up period, there is no possibility of recurrence, metastasis or death. Histological classification of laryngeal squamous cell carcinoma was according to WHO 1997 classification criteria for laryngeal cancer. According to pathological classification criteria: 11 patients with G1 grade, 21 patients with grade G2, 26 patients with grade G3; according to the TNM staging criteria, there were 36 cases of N0 and 22 cases of N1~2; there were 25 cases with stage I, 14 cases with stage III, 14 cases with stage II, 5 cases with stage IV. The normal laryngeal tissues or vocal cord polyps were chosen in the control group.

Reagents and instruments: Rat anti human MMP-9 monoclonal antibody was purchased from Shanghai

biochemical reagent Co., Ltd. Murine anti human EGFR monoclonal antibody was purchased from Novus. The S-P immunohistochemical staining kit was purchased from Shanghai Tian Cheng medical Polytron Technologies Inc. Poly lysine and DAB chromogenic agents were purchased from Shanghai Biological Technology Co., Ltd.

Methods

S-P immunohistochemical method: Slice the specimen (4~5 μm), normal dewaxing. 10 min was incubated with 3% hydrogen peroxide, and antigen repair was performed. One antibody, two resistant, was incubated with 12 h and 20 min at 4 and 25 degrees centigrade respectively. HRP labelled with streptavidin was incubated with 20 min. The phosphate acid buffer solution for cleaning, DAB staining, Hematoxylin staining, sealing are used for each step. The negative control was replaced by phosphate buffer, and the positive control group was provided by the company.

Conclusions: The standard of the result is determined by the observation of the degree of cell colouring under the high power microscope. The positive cell of MMP-9 protein in laryngeal squamous cell carcinoma is brownish yellow, showing moderate and strong positive expression. A total of 200 cancer cells were selected from all the 4 different field of view (high power microscope). The percentage of positive cells was the criterion for the expression of MMP. EGFR staining positive standard: EGFR positive products are brownish yellow particles of uniform thickness, which distributed on the cell membrane and cytoplasm. Semiquantitative analysis was based on colour intensity and percentage of positive cells. The criteria are shown in Table 1.

Statistical treatment: SPSS17.0 statistical analysis software was used for statistical analysis, Multivariate analysis was performed by radit analysis, Immunohistochemical data were taken by chi-square criterion. P<0.05 was statistically significant.

Table 1. *Expression of MMP-9 and EGFR judgement standard.*

	Negative (-)	Weakly positive (+)	Moderately positive (++)	Strong positive (+++)
Expression of MMP-9	No positive cell	Light yellow, and Positive cell<30%	Brownish yellow, and positive cells were 30%~50%	yellowish-brown, and Positive cell>50%
EGFR staining positive standard	achromatic colour	Positive cell ≤ 25%, straw yellow	The positive cells were 30%~50%, Brownish yellow	Positive cell>75%, brown

Results

Expression of MMP-9 protein in laryngeal squamous cell carcinoma

At high magnification, the positive expression of MMP-9 protein is brown granules. There are more tumours in cytoplasm. karyon is non-staining. There were more positive tumour cells in the frontal edge of the invasion. Compared with the positive proteins in laryngeal squamous cell carcinoma tissues, the expression of MMP-9 positive proteins in normal laryngeal tissues were significantly lower. The lymph node metastasis group was significantly higher than that without lymph node metastasis. Moreover, with the increase of invasion depth of cancer tissue, the expression of MMP-9 in cancer cells increased gradually (P<0.05) (Tables 2-4).

Expression of EGFR in laryngeal squamous cell carcinoma

At high magnification, EGFR protein is brownish yellow in laryngeal squamous cell carcinoma tissues, and is positive expression; and mostly located in the cell membrane (chromatin) of tumour cells. The expression of EGFR was significantly correlated with the degree of differentiation (P<0.05).But its expression was not related to histopathological type. The expression of EGFR in lymph node metastasis is higher than that in patients without lymph node metastasis. The difference was significant (P<0.05) (Tables 3 and 4).

Table 2. *Judgement standard of MMP-9 in normal laryngeal tissue and laryngeal squamous carcinoma tissues.*

	n	Expression of MMP-9				
		-	+	++	+++	Positive rate (%)
Normal laryngeal tissue	16	10	6	0	0	37.5
Laryngeal squamous cell carcinoma	58	13	8	12	25	77.6
Lymphatic metastasis group	42	4	8	8	22	90.5
No lymphatic metastasis group	16	7	3	6	0	56.2

Table 3. *Judgement standard of EGFR in normal laryngeal tissue and laryngeal squamous carcinoma tissues.*

	n	Expression of MMP-9				
		-	+	++	+++	Positive rate (%)
Normal laryngeal tissue	16	16	0	0	0	0
Laryngeal squamous cell carcinoma	58	27	7	9	15	53.4
Lymphatic metastasis group	42	23	6	7	6	45.2
No lymphatic metastasis group	16	11	2	1	2	31.2

Correlation between expression of MMP-9 and EGFR

The patients of Laryngeal squamous cell carcinoma whose expression of MMP-9 and EGFR is positive or negative at the

same time accounted for 77.59%, which indicated a positive correlation ($\chi2$=6.68, r=0.524, P<0.05), the results are shown in Table 5.

Table 4. *Positive expression and the clinical pathological features of MMP-9 and EGFR in laryngeal squamous carcinoma tissues.*

	n	MMP-9 positive expression (%)	χ2	P	Positive expression of EGFR (%)	χ2	P
TNM staging							
Phase I-II	20	8 (40.0)	8.9	#	10 (50.0)	5.82	#
III	38	32 (84.2)			23 (60.5)		
Differentiation							
Well-differentiated	25	15 (60.0)	8.6	#	9 (36.0)	5.91	#
Middle and low differentiation	32	25 (78.1)			24 (75.0)		

Note: # said compared two groups of data, the difference was statistically significant, P<0.05.

Table 5. *The relationship of MMP-9 and EGFR in laryngeal squamous carcinoma tissues.*

MMP-9	n	EGFR	
		Positive expression number (%)	Negative representation (%)
Positive expression number	40	33 (56.90)	7 (12.07)
Negative representation	18	12 (20.69)	6 (10.34)

Note: The expression of MMP-9 and EGFR is closely related, $\chi2$=6.68, P<0.05

Discussion

The occurrence, development and metastasis of tumour is a complex process. It is involved in multiple genes and multiple proteins. Changes in regional carcinogenesis, proteins and molecules in tumours of the head and neck not only limited the cancerous tissue to the area, but also caused the cancerization of the tissue outside the area. Identification of the changes in protein expression as a major marker of regional carcinogenesis is important for early detection of tumour, prediction of tumour recurrence and metastasis of tumour [7]. The invasion and metastasis of tumour is a complicated pathological process with many factors coexisting. Numerous studies show that MMP-9 and EGFR have different roles in the metastasis of laryngeal squamous cell carcinoma. In the process of infiltrating and distant metastasis, the cancer cells need to pass through the natural barrier formed by ECM and basement membrane and produce proteases to degrade ECM. Research shows that MMP can degrade all the ingredients in ECM. MMP-9 is the main enzyme that degrades ECM and type IV collagen in the human body [8]. This study shows that, the up regulation of MMP-9 was positively correlated with invasion and metastasis of laryngeal squamous cell carcinoma. Therefore, MMP-9 is a sign of malignancy of lung cancer. Reports show that the results of *in vitro* experiments showed that only EGFR positive cancer cell lines were invasive [9,10]. MMP-9 is the largest molecular weight enzyme in MMPs [11]. The results of this study show that, with the decrease of the differentiation degree of cancer cells and the depth of tumour invasion, the expression intensity of MMP-9 in tumour cells gradually increased (P<0.05). And the results show that the expression rate of MMP-9 in lymph node metastasis group was significantly higher than that in negative group. Thus, MMP-9 is a proteolytic enzyme that accelerates tumour invasion and metastasis [12]. Canning and other [13] research reports show that there were more positive MMP-9 in the cytoplasm of macrophages and tumour cells infiltrated by fibroblasts. Other scholars' research reports [14] find that MMP-9 has something to do with patient survival and the level of MMP-9 assessed by immunohistochemistry can be used as a screening for specific enzyme inhibitors.

Research reports on the mechanism of MMP production have shown that EGF has a positive regulatory effect on a variety of MMP [15]. And research has confirmed that EGF is one of the most important regulatory factors in the synthesis stage of MMP zymogen. They can inducible a high level of MMP mRNA and affect its half-life [16]. This study shows that, GFR and MMP-9 protein were highly expressed in laryngeal squamous cell carcinoma tissues, which is closely related to laryngeal squamous cell carcinoma. The combined detection of EGFR and MMP-9 expression can more accurately reflect the biological characteristics of laryngeal squamous cell carcinoma. EGFR and MMP-9 proteins can be used as molecular markers for the diagnosis and prognosis of squamous cell carcinoma of larynx. Therefore, it may have a good prospect in the prevention and treatment of laryngeal squamous cell carcinoma.

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