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Original Article

High expression of glioma-associated oncogene homoglog-1 and matrix metalloproteinase-11 in esophageal squamous cell carcinoma correlates with invasion and metastasis

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Abstract: Aims: The present study is to investigate the expression of glioma-associated oncogene homoglog-1 and matrix metalloproteinase (MMP)-11 in esophageal squamous cell carcinoma (ESCC) tissues, tumor-adjacent tissues and normal tissues. In addition, the expression and clinical significance of Gli-1 and MMP-11 proteins in ESCC were studied. Methods: A total of 36 patients who underwent surgery resection from November 2012 to November 2013 at the Affiliated Hospital of Taishan Medical College were included in the present study. The clinical and pathological data included age, gender, tumor diameter, site of lesion, tumor stage, nodal status and histopathological differentiation. The expression of MMP-11 and Gli-1 proteins in ESCC tissues, tumor-adjacent tissues and normal esophageal tissues was tested using immunohistochemical staining. The associations of Gli-1 and MMP-11 expression with clinicopathological characteristics were analyzed by Chi-square test. Spearman rank correlation analysis was used to evaluate the correlation between the expressions of Gli-1 and MMP-11. Results: Expression of Gli-1 and MMP-11 in ESCC tissues was significantly higher than that in adjacent tissues and normal tissues. The expression of Gli-1 and MMP-11 in T1/T2 stages was lower than that in T3/T4 stages. Similarly, the expression of Gli-1 and MMP-11 in clinical stages I/II was lower than that in III/IV stages. The expression of Gli-1 and MMP-11 proteins in nodal metastasis group was higher than that in patients without nodal metastasis. The expression of Gli-1 was positively correlated with MMP-11 expression in ESCC. Conclusions: The present study demonstrates that Gli-1 and MMP-11 are associated with invasion and metastasis of ESCC. Up-regulation of these proteins may be involved in the development of ESCC, and they may play a role in promoting invasion and metastasis of ESCC. The expression of Gli-1 and MMP-11 may help determine the malignant degree of ESCC.

Keywords: Esophageal squamous cell carcinoma, glioma-associated oncogene homoglog-1, matrix metalloproteinase-11, invasion, metastasis

Introduction

Esophageal squamous cell carcinoma (ESCC) is a sub-type of esophageal cancer that is common in developing countries according to the 2014 World Cancer Report by World Health Organization. ESSC has low early diagnosis rate and rapidly increasing incidence and hence, becoming a common cause of death [1]. At present, it has been confirmed that Hedgehog signaling pathway plays a key role in the process of embryo development, as well as the growth, survival and differentiation of cells [2]. In addition, Hedgehog signaling pathway is also correlated with invasion and metastasis of many tumors [3]. Cancer invasion and metastasis is one of the major causes of death and poor prognosis of a variety of human carcinomas.

Gli-1 is a transcription factor downstream of Hedgehog signaling pathway. Interference of Gli-1 expression by RNAi inhibits the develop-

ment of liver cancer, pancreatic cancer, prostate cancer, and small-cell lung cancer [4]. After the integration of Hedgehog signaling with RTK or Wnt/β-catenin signaling, the expression of Gli-1 is overexpressed, facilitating the transformation of epithelial tissues into interstitial tissues, and enhancing the invasiveness and metastatic ability of tumor cells. In addition, Gli-1 induces autocrine growth factors by up-regulating RTK, and effectively inhibits apoptosis. A study shows that Gli-1 participates in the growth, invasion, and metastasis of breast cancer cells by up-regulating matrix metalloproteinase (MMP)-11 [5]. MMPs are zinc-dependent endopeptidases involved in extracellular matrix degradation and tissue remodeling [6]. On one hand, MMPs inhibit apoptosis through the decomposition and release of biologically active molecules; on the other hand, MMPs stimulate the infiltration of cancer cells via the degradation of extracellular matrix [7]. MMP-11 is a particular member of MMPs. Recent efforts show that MMP-11 plays a very important role in matrix degradation, tissue remodeling and tumor invasion [5]. At present, the combined detection of MMP-11 and Gli-1 expression in ESCC is rarely reported. The present study aims to investigate the expression of MMP-11 and Gli-1 proteins in ESCC, tumor adjacent tissues and normal esophageal tissues, as well as its correlation with clinical and pathological characteristics of ESCC.

Materials and methods

Patients

A total of 36 patients who underwent surgery resection from November 2012 to November 2013 at the Affiliated Hospital of Taishan Medical University were included in the present study. The clinical and pathological data included age, gender, tumor diameter, site of lesion, tumor stage, nodal status and histopathological differentiation. There were 7 cases with poorly differentiated cancer, and 29 cases with moderately and highly differentiated cancer. The tumors were located in the upper segment of esophagus (6 cases), and middle and lower segment of esophagus (30 cases). There were 15 cases of tumors with a diameter more than 3 cm. According to the Sixth Edition of TNM staging standard established in 2002 [8], the patients were classified into tumor stage [I/II (13 cases), III/IV (23 cases), T1/T2 (16 cases), T3/T4 (20 cases)] and nodal metastasis [N0 (21 cases), and N1 (15 cases)]. All procedures were approved by the Ethics Committee of Taishan Medical College. Written informed consents were obtained from all patients or their families.

Immunohistochemical staining

All specimens were fixed in 10% formaldehyde. After paraffin embedding, specimens were cut into serial 5-μm-thick sections. Immunohistochemical experiments were performed using EliVision™ super Kit (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China) according to the manufactures' protocol. Paraffin-embedded tissue sections were dewaxed and hydrated before high-pressure antigen retrieval, and then endogenous peroxidase activity was quenched. Sections were incubated with goat anti-rabbit Gli-1 (1:100; Santa Cruz Biotechnology, Dallas, TX, USA) and goat anti-rabbit MMP-11 (1:50; Santa Cruz Biotechnology, Dallas, TX, USA) at 4°C overnight. Then, the sections were incubated in horseradish peroxidase-conjugated secondary antibodies for 2 h at 37°C. Subsequently, the sections were incubated with 0.05% (v/v) 3',3'-diaminobenzidine tetrahydrochloride (DAB) in H_2O_2 for 5 min to generate brown reaction product. Afterwards, the sections were counterstained by hematoxylin (Shanghai Huyu Biotechnology Co., Ltd., Shanghai, China), and dehydrated in graded alcohol before being mounted. The sections were stained back to blue by lithium carbonate, followed by washing with gradient alcohol, dehydration and drying. After being treated with xylene, the sections were sealed with neutral gum before observation under the microscope (BX-53, Olympus China Co., Ltd., Shanghai, China).

According to the method reported by Yoshikawa et al. [9], two pathologists independently counted cell number under the microscope, without knowing the clinical pathological features. Positive staining was indicated by yellow or brown yellow granules in the nucleus and (or) cytoplasm of the cells. The results of expression were graded by the number of stained tumor cells as follows: (-), no obvious staining or 5% of the tumor cells had staining; (+), ≥ 5% of the tumor cells had staining. The optical densities of Gli-1 and MMP-11 were counted in 5 randomly selected high-power fields under the microscope (40×) for each section.

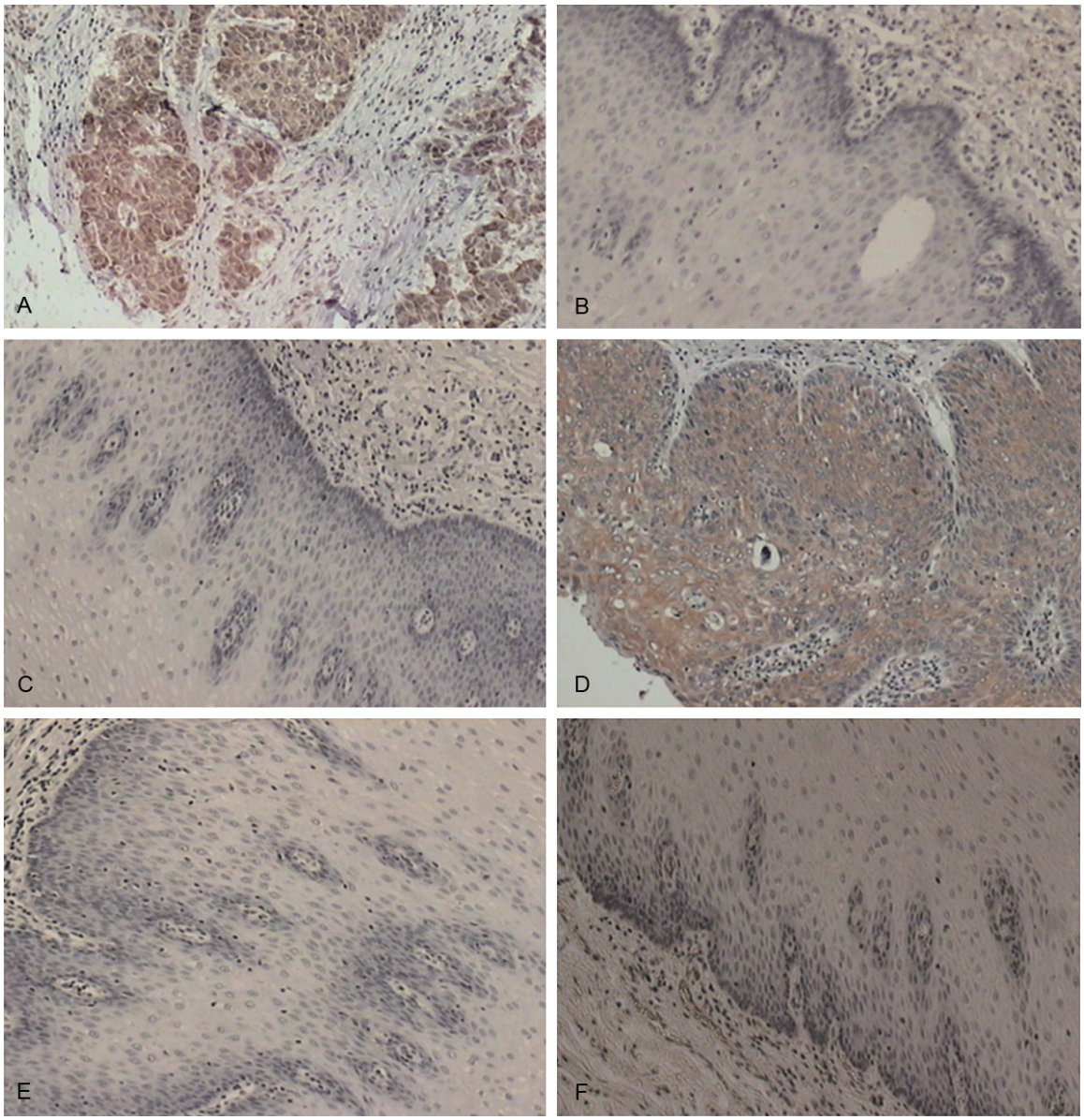

Figure 1. Expression of Gli-1 and MMP-11 proteins detected by immunohistochemistry. A. Positive expression of Gli-1 protein in ESCC tissues; B. Negative expression of Gli-1 in tumor-adjacent normal tissues; C. Negative expression of Gli-1 in normal esophageal tissues; D. Positive expression of MMP-11 in ESCC tissues; E. Negative expression of MMP-11 in tumor-adjacent normal tissues; F. Negative expression of MMP-11 in normal esophageal tissues.

Statistical analysis

Data were analyzed using SPSS 18 statistical software (IBM, Armonk, NY, USA). Values were presented as means ± SEM. The associations of Gli-1 and MMP-11 expression with clinicopathological characteristics were analyzed by Chi-square test. Spearman rank correlation analysis was used to evaluate the correlation between the expressions of Gli-1 and MMP-11. $P < 0.05$ was considered statistically significant.

Results

Expression levels of Gli-1 and MMP-11 in esophageal cancer tissues are higher than that in tumor-adjacent tissues or normal esophageal tissues

To detect the expression of Gli-1 and MMP-11, immunohistochemistry was employed. Gli-1 protein was expressed mainly in the nucleus/cytoplasm (**Figure 1A**). The positive expression rates of Gli-1 in ESCC tissues, tumor-adjacent

Table 1. Gli-1 and MMP-11 expression in esophageal cancer tissues, tumor-adjacent tissues and normal esophageal tissues

	n	Expression of Gli-1			Expression of MMP-11			χ^2	P
		-	+	Positive expression rate (%)	-	+	Positive expression rate (%)		
Normal esophagus	9	8	1	11.1	7	2	22.2	16.384 (Gli-1)	0.000
Tumor-adjacent	36	31	5	13.9	27	9	25.0	16.425 (MMP-11)	0.000
Squamous cell carcinoma	36	16	20	55.6	11	25	69.4		

Table 2. The relationship between the expression of Gli-1 and MMP-11 in ESCC and clinicopathological characteristics

Clinicopathological characteristics	n	No. of patients with positive expression of Gli-1	Positive expression rate of Gli-1 (%)	P	No. of patients with positive Expression of MMP-11	Positive expression rate of MMP-11 (%)	P
Age (year)							
≥ 55	26	14	53.8		19	73.1	
< 55	10	6	60.0	1.000	6	60.0	0.454
Gender							
Male	20	12	60.0		15	75.0	
Female	16	8	50.0	0.737	10	62.5	0.483
Diameter (cm)							
> 3	15	9	60.0		11	73.3	
≤ 3	21	11	52.4	0.741	14	66.7	0.729
Degree of differentiation							
Moderate/high	29	18	62.1		21	72.4	
Low	7	2	28.6	0.204	4	57.1	0.650
Site of lesion							
Middle/lower	30	18	60.0		22	73.3	
Upper	6	2	33.3	0.374	3	50	0.621
Clinical staging							
I/II	13	4	30.8		5	38.5	
III/IV	23	16	69.6	0.038	20	87.0	0.006
T-staging							
T1/T2	16	5	31.3		8	50.0	
T3/T4	20	15	75.0	0.017	17	85.0	0.034
Lymph-node metastasis							
Positive	21	16	76.2		18	85.7	
Negative	15	4	26.7	0.006	7	46.7	0.025

tissues, and normal esophageal tissues were 55.6% (20/36) (**Table 1** and **Figure 1A**), 13.9% (5/36) (**Table 1** and **Figure 1B**), and 11.1% (1/9) (**Table 1** and **Figure 1C**), respectively. There was significant difference among groups (χ^2 = 16.384, P < 0.05; **Table 1**). MMP-11 protein was expressed mainly in the cytoplasm (**Figure 1D**). The positive expression rates of MMP-11 in ESCC tissues, tumor-adjacent tissues, and normal esophageal tissues were 69.4% (25/36) (**Table 1** and **Figure 1D**), 25% (9/36) (**Table 1** and **Figure 1E**), and 22.2% (2/9) (**Table 1** and **Figure 1F**). There was significant difference among groups (χ^2 = 16.425, P < 0.05; **Table 1**). The positive expression rates of MMP-11 tumor-adjacent tissues and normal tissues were significantly lower than that of ESCC tissues (P < 0.05, **Table 1**). No significant

Table 3. Relationship between Gli-1 and MMP-11

Expression of Gli-1	n	Expression of MMP-11		γ	P
		+	-		
+	20	16	4	0.375	0.024
-	16	7	9		

Note: Spearman rank correlation analysis was used to analyze the correlation between the expression of Gli-1 and the expression of MMP-11 in esophageal squamous cell carcinoma.

differences in the expression of Gli-1 and MMP-11 were detected between tumor-adjacent tissues and normal esophageal tissues ($P > 0.05$; **Table 1**). These results suggest that expression levels of Gli-1 and MMP-11 in esophageal cancer tissues are higher than that in tumor-adjacent tissues or normal esophageal tissues.

Expression of Gli-1 and MMP-11 is correlated with clinical staging, T-staging and lymphatic metastasis status

To investigate how the expression of Gli-1 and MMP-11 is correlated with clinicopathological characteristics, Chi-square test was performed. The data showed that the expression of Gli-1 and MMP-11 was not dependent on age, gender, tumor diameter, degree of differentiation or site of lesion ($P > 0.05$). The positive expression rates of Gli-1 and MMP-11 in stages I and II were significantly lower than those in stages III and IV stage ($P < 0.05$). In addition, the positive expression rates of Gli-1 and MMP-11 in stages T1 and T2 were significantly lower than those in stages T3 and T4 ($P < 0.05$). Moreover, the positive expression rates of Gli-1 and MMP-11 in patients with lymphatic metastasis were significantly higher than those in patients without metastasis ($P < 0.05$) (**Table 2**). These results indicate that expression of Gli-1 and MMP-11 is correlated with clinical staging, T-staging and lymphatic metastasis status.

Expression of Gli-1 is positively correlated with the expression of MMP-11 in ESCC

To study the correlation between the expressions of Gli-1 and MMP-11, Spearman rank correlation analysis was used. The data showed that the correlation coefficient for the expression of Gli-1 and the expression of MMP-11 in ESCC was 0.375 ($P = 0.024$) (**Table 3**). The result suggests that the expression of Gli-1 is positively correlated with the expression of MMP-11 in ESCC.

Discussion

The occurrence of esophageal carcinoma is a process involving many factors. Its invasion and metastasis are multi-link processes that relate to signaling pathways. Emerging evidence indicates that Hedgehog signaling is activated in many human tumors. Gli-1 is a very important protein in Hedgehog pathway. It not only reflects the activation of Hedgehog pathway, but also closely relates to gene transcription. In addition, basement membrane and extracellular matrix degradation is one of the key factors in cancer metastasis. Of note, MMPs are one of the main substances caused by basement membrane and extracellular matrix degradation.

Accumulating evidence suggests that Gli-1 expression is up-regulated at various degrees in esophageal cancer [10], liver cancer [11], gastric cancer [12], colon cancer [13], and pancreatic cancer [14]. This suggests that the Hedgehog signaling pathway is common in the occurrence and development of tumors, and is related to the abnormal expression of Gli-1. Previous reports also show that expression of MMP-11 is up-regulated in esophageal cancer [15], gastric cancer [16], colon cancer [17], pancreatic cancer [18] and other malignant tumors of the digestive system. All these indicate that Gli-1 and MMP-11 play important roles in the occurrence and development of tumor.

In the present study, we find that the expression of Gli-1 and MMP-11 in tumor-adjacent tissues and normal esophageal tissues is lower than that in ESCC tissues. In addition, the expressions of Gli-1 and MMP-11 show positive correlation. The expression of Gli-1 and MMp-11 in ESCC is closely related to clinical staging and T staging, with higher stages having higher expression of Gli-1 and MMp-11. Our results also reveal that the expression of Gli-1 and MMp-11 in patients with lymph node metastasis is higher than those without metastasis. At the same time, our results suggest that tumors with higher expression of Gli-1 and MMP-11 have stronger invasion ability and can lead to poorer prognosis. Therefore, the combined detection of the expression of Gli-1 and MMP-11 in ESCC provides a theoretical basis to predict the prognosis of patients. In summary, Gli-1 and MMP-11 participate in the occurrence

and development of ESCC, and are related to tumor invasion and metastasis. Detection of the expression of Gli-1 and MMP-11 in ESCC not only helps determine the malignant degree, but also provides theoretical basis for the diagnosis and clinical therapy of ESCC.

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Disclosure of conflict of interest

None.

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