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Correlation between periostin and SNCG and esophageal cancer invasion, infiltration and apoptosis

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ABSTRACT

Objective: To investigate the correlation between periostin and SNCG and esophageal cancer invasion, infiltration and apoptosis. **Methods:** A total of 78 cases esophageal surgical resection specimens were collected, expression of periostin and SNCG in esophageal cancer were detected. Effect of periostin and SNCG in esophageal carcinoma invasion and infiltration was analyzed. **Results:** The upregulated rate of periostin had significant difference in esophageal cancer tissues (39.74%), adjacent tissues (17.86%) and normal tissues (0.00%); The positive expression rates of SNCG had significant difference in esophageal cancer tissues (61.54%), adjacent tissues (32.14%) and normal tissues (1.96%); The upregulated rate of periostin had a significant correlation with lymph node metastasis, adventitia invasion, TNM stage; The positive expression rates of SNCG had a significant correlation with differentiation degree, lymph node metastasis, adventitia invasion, TNM stage; Apoptosis index of the positive of expression of SNCG of esophageal cancer tissue (4.541±2.267) was significantly lower than that of the negative expression (7.316±2.582) (P<0.05). **Conclusions:** SNCG may play an important role in invasion, infiltration and apoptosis of esophageal cancer and serve as target spots in the targeted therapy of esophageal cancer.

1. Introduction

Esophageal cancer is one of the common malignant tumors in the digestive system, there is high mortality of esophageal cancer in China, invasion and metastasis are the main causes of death in patients[1]. Esophageal invasion, infiltration and metastasis are the result of a complex process with multi-stage, multi-step and multiple genes. The study found the mRNA and protein expression of periostin in tumor tissue and normal tissue are significantly different, which has a close correlation with tumor occurrence, development and prognosis[2]. An abnormal increase of SNCG expression has been found in digestive system neoplasms such as the gastric cancer, liver cancer and pancreatic cancer[3]. In this study, we ollected specimens of 78 cases of esophageal surgical resection in our hospital from January 2010 to June 2012, and investigate the correlation between periostin and SNCG and esophageal cancer invasion, infiltration and apoptosis.

2. Materials and methods

2.1. General information

Specimens of 78 cases of esophageal cancer who underwent surgical resection were collected, including 45 males and 33 females, aged 42-81 years old, and the average age was 63.5±2.1; Among them there were 10 cases with upper, 51 middle and 17 lower esophageal carcinoma; 21 cases with high differentiation cancers,

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40 cases with moderate differentiation cancers, 17 cases with low differentiation cancers. There were 37 cases in medullary type, 33 ulcer type and 8 sclerotic type; 41 cases without lymph node metastasis, 37 cases with lymph node metastasis; 21 cases without adventitia infiltration, 57 cases with adventitia infiltration. According to the TNM standards for esophageal cancer, 4 cases were in stage Ⅰ, 35 cases in stage ⅡA, 10 cases in stage IIB, 28 cases in stage Ⅲ,1 case in stage Ⅳ.

A total of 51 cases with normal tissue were obtained with the distance beyond 5 cm from the cancer tissue, 28 cases of adjacent tissues were obtained within the distance of 1.0-1.5 cm from the cancer tissue as control group. All patients had no treatment for the tumor before tissue specimens were obtained.

2.2. Methods

2.2.1. Periostin expression detection

All specimens were fixed in 10% formalin, embedded in paraffin, cut into sections with 4 μm thickness. Full RNA was extracted from the esophageal cancer tissue and normal esophageal tissues, and the adjacent tissues and esophageal non-cancerous tissue, reverse transcribed into cDNA. Periostin were detected by RT-PCR, and the quantitative expression of periostin was detected by real-timePCR. C_t is the reaction cycles, corresponding to the exponential amplification of the middle portion of the PCR reaction, the average value were obtained of three reactions. $\triangle\triangle C_t$=2exp (C_t tumor-C_t normal), T/N is $\triangle\triangle C_t$, which showed differences of periostin between cancer tissues and non-cancer tissues. T/N=4 is the critical value.

2.2.2. Immunohistochemical assay

SNCG expression of esophageal cancer tissues and normal esophageal tissues and cancer adjacent tissues were detected by immunohistochemical assay. SNCG expression was detected by concentrated SNCG monoclonal mouse anti-human antibody. The results were analyzed by double blind method. Five different visual fields were randomly selected for each slice under the optical microscope (× 400), the percentage of positive cells in 100 tumor cells were calculated. The percentage of the staining intensity and positive cell can be used to evaluate the result of SNCG positive expression, that is, light yellow 1 point, brown 2 point, dark brown 3 points; Positive cells <5% 0 point, 5% to 25% 1 point, 26% to 50% 2 points, >50% 3 points. If the total score of staining intensity and positive cell <3, it was negative, ⩾3 positive.

2.2.3. Tumor cell apoptosis by TUNEL

Apoptosis was detected by cell apoptosis detection kit, all measure operation steps were carried out according to the manual of the kit. Sections were observed under the microscope of low magnification (×100), areas with good mark effect and evenly distributed positive cells were selected. Five different horizons were selected under high power field (×400), the percentage of positive cells in 100 tumor cells was calculated as an expression of apoptosis index (AI) of the esophageal cancer cell.

2.3. Statistical analysis

Data was analyzed with SPSS software, the result was expressed by mean±SD. Date were analyzed by t test, the enumeration data were compared with the χ^2 test. P<0.05 was considered as statistical significance.

3. Results

Periostin T/N> 4 was up-regulated in tissues, 31 cases in esophageal carcinoma tissue (39.74%), 5 cases in adjacent tissues (17.86%), and no case in normal tissue (0.00%). The difference of the upregulated rate of periostin in various tissues was significant (P <0.05).

There were 48 cases with positive SNCG expression in esophageal carcinoma tissue (61.54%), 9 cases in adjacent tissues (32.14%), and 1 case in normal tissue (1.96%). The difference was significant (P <0.05).

The up-regulated expression of periostin had a significant correlation with lymph node metastasis, adventitia invasion and TNM stage (P<0.05), but no significantly correlation with age, sex, tumor location and the degree of differentiation (P>0.05) (Table 1).

SNCG positive expression had a significantly correlation with the degree of differentiation, lymph node metastasis, adventitia invasion and TNM stage (P<0.05), but no significant correlation with age, sex and tumor location (P>0.05) (Table 2).

4. Discussion

Malignant tumor does great harm to people's health. Invasion and infiltration are important markers of the tumor. It is a multi-step, multi-factor dynamic process with the complex interactions between extracellular matrix and host cell. The same as other malignancies, invasion and infiltration are the biological characteristics of esophageal cancer[4,5]. Interstitial specific genes play an important role in the regulation of tumor invasion and infiltration, the genes are expressed by interstitial cells. Molecules with different

Table 1

Relationship between periostin expression and clinical pathological features.

Groups		*n*	T/N>4	T/N≤4	χ^2	*P*
Age	≥60	40	19	21	2.063	>0.05
	<60	38	12	26		
Sex	Male	45	19	26	0.273	>0.05
	Female	33	12	21		
Tumor location	Upper and middle thoracic segment	61	24	37	0.019	>0.05
	Lower thoracic segment	17	7	10		
Differentiation	Well-differentiated	21	11	10	1.916	>0.05
	moderate or poor differentiation	57	20	37		
Lymph node metastasis	Without lymph node metastasis	41	6	35	22.755	<0.05
	With lymph node metastasis	37	25	12		
Adventitia infiltration	Without adventitia infiltration	21	3	18	7.777	<0.05
	With adventitia infiltration	57	28	29		
TNM stage	Ⅰ-Ⅱ	49	4	45	54.885	<0.05
	Ⅲ-Ⅳ	29	27	2		

Table 2

Relationship between SNCG positive expression and clinical pathological features.

Groups		*n*	Positive	Negative	χ^2	*P*
Age	≥60	40	26	14	0.416	>0.05
	<60	38	22	16		
Sex	Male	45	27	18	0.106	>0.05
	Female	33	21	12		
Tumor location	Upper and middle thoracic segment	61	37	24	0.092	>0.05
	Lower thoracic segment	17	11	6		
Differentiation	Well-differentiated	21	9	12	4.237	<0.05
	Moderate or poor differentiation	57	39	18		
Lymph node metastasis	Without lymph node metastasis	41	19	22	8.434	<0.05
	With lymph node metastasis	37	29	8		
Adventitia infiltration	Without adventitia infiltration	21	6	15	13.195	<0.05
	With adventitia infiltration	57	42	15		
TNM stage	Ⅰ-Ⅱ	49	23	26	11.869	<0.05
	Ⅲ-Ⅳ	29	25	4		

structures and functions are the expression product of such genes, including extracellular matrix proteins and secreted proteins. Periostin is an interstitial-specific gene. Hydrolysis of the extracellular matrix is closely related to tumor invasion and infiltration. The biological characteristics of the tumor are directly affected by the protease inhibitors in matrix and the balance state of proteases. SNCG belongs to the family of neural synuclein, it showed significant tissue specificity in the normal status, and might have a certain impact to the balance state.

Periostin plays an important role in the carcinoma growth, angiogenesis, invasion and infiltration. When Michaylira[6] cultured the three-dimensional tissue of esophageal squamous cell carcinoma he found that, because periosti is the cell adhesion molecules which could highly express cancer cell, it can be used as molecular markers for tumor invasion. By semi-quantitative RT-PCR and gene expression analysis, Kwon and others[7-10] proved that periostin showed high expression in esophageal squamous cell carcinoma at the mRNA level. By the stimulation of cancer cells, the interstitial cells around the cancer cells can express and secrete periostin. The periostin can trigger related signal transduction pathway network, create a living micro-environment which is beneficial to the growth

and development of the cancer cell, resulting in invasion and infiltration. This study compared the up-regulated expression rate of periostin in esophageal carcinoma tissues, adjacent tissues and normal tissues; it is found the difference in various tissues was significant. The up-regulated expression rate of periostin has a significant correlation with lymph node metastasis, adventitious invasion and TNM stage. The result showed periostin overexpression in esophageal carcinoma, and it can promote cancer cells survival, angiogenesis, invasion and infiltration. SNCG is a family member of synuclein. Its structure, may be the same as apolipoprotein, has a function of exchangeable lipid binding. SNCG expression is affected by AP-1 and Oncostatin.AP-1 SNCG can regulate during the transcription process, in order to increase or inhibit the expression of SNCG. And oncostatin can affect the transcriptional level of SNCG,thereby inhibited the protein expression of SNCG. By immunohistochemistry, Singh[11] detected the SNCG protein expression of 438 cases with breast cancer. He found that SNCG protein expression has correlation with pathological factors such as lymph node metastasis, tumor size and tumor stage, and follow-up found the survival rate of patient with SNCG positive protein expression were significantly lower than the patients with negative expression. In this study, the positive expression of SNCG was significantly different in the esophageal cancer tissues, adjacent tissues and normal tissues. The positive rate is significantly higher in esophageal carcinoma tissues. The SNCG positive protein expression has a significant correlation with differentiation degree, lymph node metastasis, adventitious invasion and TNM stage, which is consistent with the reported results. Shi[12] found that the SNCG can block signaling pathway of the mitotic control point, the over-expression will cause BubR1 protein losing its function, inhibit enzyme activity of caspase 3 and caspase 9, thereby inhibiting the apoptosis of tumor cells. By analyzing the relationship between SNCG and apoptosis, this study found that the positive expression SNCG AI values in esophageal cancer tissues was significantly lower than the negative expression, showed SNCG over-expression may cause tumor cell apoptosis inhibition, which was beneficial to cell proliferation and can lead to esophageal cancer invasion and infiltration.

In summary, the high expression of periostin and SNCG in esophageal cancer tissues was closely related to the invasion, infiltration and apoptosis of esophageal cancer, can be used as an important indicator for the judgments of esophageal cancer metastasis and prognosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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