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The expression of OCT4 and its clinical significance in laryngeal squamous carcinoma tissues

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Abstract. – OBJECTIVE: To investigate the expression of OCT4 and its clinical significance in laryngeal squamous carcinoma tissues.

PATIENTS AND METHODS: Immunohistochemical staining was used to analyze the expression of OCT4 in 61 cases of laryngeal squamous carcinoma and 10 cases of the adjacent normal laryngeal tissues.

RESULTS: The expression of OCT4 was not detected in normal laryngeal tissues, but could be detected in the nucleus of laryngeal carcinoma. The positive expression rates of OCT4 in well-moderately differentiated and poorly differentiated laryngeal squamous carcinoma tissues were 25.6% (11/43) and 66.7% (12/18) respectively, and there were significant differences ($p < 0.01$). The expression of the OCT4 protein was related to lymph node metastasis and TNM stage ($p < 0.05$), but not to gender, age and position of the tumor ($p > 0.05$).

CONCLUSIONS: OCT4 is expressed in laryngeal squamous carcinoma tissues and is closely related to the cell differentiation of laryngeal carcinoma, lymph node metastasis and clinical stage.

Key Words:
OCT4, Laryngeal squamous carcinoma, Cancer stem cells.

Introduction

Malignant tumor has become the leading cause of death in China. Laryngeal carcinoma is one of the most common malignant tumors in the head and neck. In recent years, the incidence rate has increased significantly. With the development of molecular biology and immunology, the researchers found that the tumor cells with abnormal excessive active proliferation characteristics were highly similar to the nature of stem cells, so "Tumor stem cell theory" was proposed[1]. It is believed that cancer stem cells are the key to the occurrence and development of malignant tumors. OCT4 gene is one of the molecular markers of stem cells. It is more and more studied. However, the expression of OCT4 in laryngeal carcinoma tissue is less studied. The expression of OCT4 in laryngeal carcinoma and its clinical significance was studied in this paper.

Patients and Methods

Clinical Data

The surgical blocks were selected for patients with complete medical records from April 2012 to November 2015 in Hebei General Hospital. Cases of laryngeal squamous cell carcinoma (n = 61) and cases of adjacent normal laryngeal tissues (n = 10) were confirmed by pathological examination (The cancerous tissue and at least 0.5 cm normal tissue surrounding carcinoma were cut off during surgery, and there was no cancer cell in above 2 cm laryngeal mucosa cut of the relatively large residual risk by pathological examination). The selected specimens from male (n = 51) and female (n = 10) cases, with age ranging from 45-68 years old and median age of 57 years old; supraglottic cases (n = 22), glottic cases (n = 34), and subglottic cases (n = 5); middle and high differentiation (n = 43), and low differentiation (n = 18); lymph node metastasis (n = 19), without lymph node metastasis (n = 42); the staging criteria proposed by UICC (Union for International Cancer Control) in 2002 was used in TNM (Tumor, Node, Metastasis) staging, with cases of period I, II (n = 35), and cases of period III, IV (n = 26). All patients did not receive any anti-tumor treatment before surgery.

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This study was approved by the Ethics Committee of Hebei General Hospital. Signed written informed consents were obtained from all participants before the study.

Detection of OCT4 Protein Expression

Streptavidin peroxidase method (SP method) was used in immunohistochemical staining. Light microscopy was used to analyze positive sections as a positive control, and phosphate buffered saline (PBS) instead of first antibody anti-OCT4 as a negative control and positive staining cells were counted in the field of view of 400 times, and the average of five fields was taken. Rabbit anti-human OCT4 monoclonal antibody was purchased from Epitomics, and SP kit and DAB color reagent were purchased from Zhongshan Gold Bridge Biological Technology Co., Ltd. (Beijing, China).

Immunohistochemical Staining of OCT4

According to Zhou et al's reported method[2], OCT4 positive cells appeared in the nucleus of brown yellow granules, and the final assessment was the sum of a percentage score accounted for the total number of the same kind of cells and the score of color intensity of positive cells. According to the proportion of positive cells score: No positive cell count as 0 points, positive cells 1-25% as 1 point, 26-50% as 2 points, 51-75% as 3 points, > 75% as 4 points. According to the color intensity of cells: no color as 0 points, weak staining as 1 point, medium staining as 2 points, strong staining as 3 points. According to the product of the two indexes, the results were divided into 4 grades: 0 points as (-), 1-4 points as weakly positive (+), 5-8 points as moderately positive (+ +), 9-12 points as strongly positive (+ + +). +-+++ was counted as a positive expression.

Statistical Analysis

SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for χ^2-test. $p < 0.05$ indicated that the difference was statistically significant.

Results

Expression of OCT4 Protein

As shown in Figure 1, OCT4 was not expressed in the normal laryngeal mucosa (0/10). The expression of laryngeal carcinoma was mainly expressed in the nucleus, and the positive expression rate was 25.6% in the middle and high differentiation (11/43). Poorly differentiated laryngeal cancer tissue was 66.7% (12/18). There was a significant difference in the different differentiation of laryngeal carcinoma ($X^2 = 9.119$, $p = 0.003$).

Relationship Between the Expression of OCT4 Protein and Clinical Pathological Characteristics of Laryngeal Carcinoma

As shown in Table I, OCT4 expression was not related to gender, age, and tumor location ($p > 0.05$), but related to lymph node metastasis and TNM stage. The higher lymph node metastasis

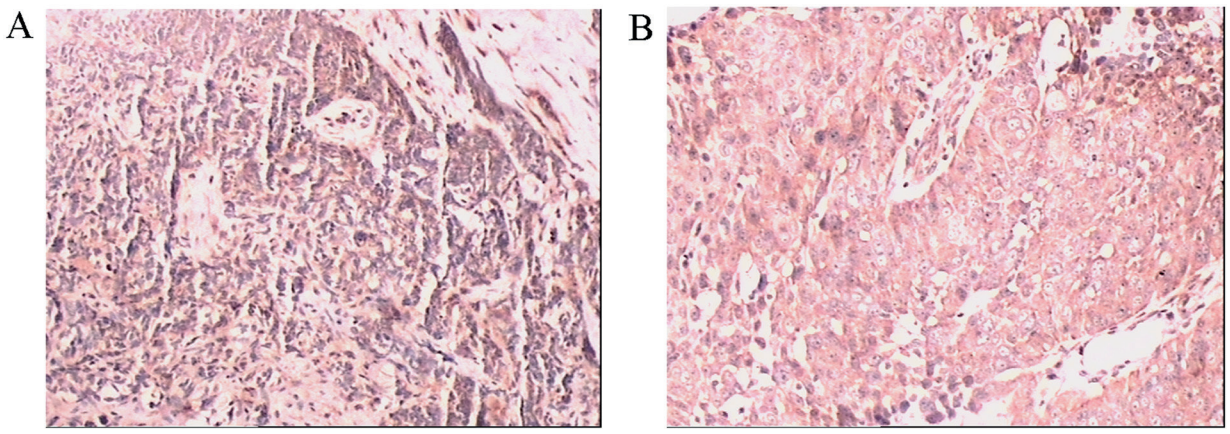

Figure 1. Immunohistochemical staining of OCT4. ***A,*** The strong positive expression of cell nuclei in poorly differentiated squamous cell carcinoma. ***B,*** Positive expression of cell nuclei in middle and high differentiated squamous cell carcinoma (SP×100).

Table I. Relationship between the expression of OCT4 protein and clinical pathological characteristics of laryngeal carcinoma.

Clinical pathological characteristics	Cases (n)	OCT4		χ^2	*p*
		+-+++	-		
Gender					
Male	51	21	30	0.822	0.365
Female	10	2	8		
Age (years old)					
< 60	34	16	18	2.861	0.091
≥ 60	27	7	20		
Anatomic location					
Supraglottic	22	6	16	4.817	0.090
Glottic	34	13	21		
Subglottic	5	4	1		
Degree of differentiation					
Middle and high differentiation	43	11	32	9.119	0.003
Poorly differentiated	18	12	6		
Lymph node metastasis					
No	42	11	31	7.611	0.006
Yes	19	12	7		
TNM staging					
I, II	35	9	26	5.026	0.025
III, IV	26	14	12		

positive and TNM stage, the higher the OCT4 protein positive expression rate, and the differences were statistically significant (p = 0.006, 0.025).

Discussion

More than 90% of the pathological type of laryngeal carcinoma is squamous cell carcinoma, and the spread is mainly the local spread and invasion, as well as regional lymph node metastasis. Although the treatment of head and neck cancer is getting better and, at present, there is progress in surgery which is the main treatment of laryngeal cancer, the 5-year survival rate of patients with laryngeal cancer has not significantly improved. In recent years, with the development of "cancer stem cell theory", there is a gradual increase in research on OCT4 protein which is the marker of embryonic stem cell.

According to the theory of cancer stem cells, there is a small group of cell colony that are cancer stem cells (CSC), which are similar to stem cells in tumor tissue, and they are the origins of tumor recurrence and metastasis[3,4]. CSC-like normal stem cells have both self-renewal and multilineage differentiation potential, with relatively unlimited proliferation and migration ability, but there is no corresponding regulation mechanism of CSC's self-renewal and differentiation, which makes the proliferation get out of control and does not have the ability to differentiate into mature cells. There is no self-stable characteristic of stem cell, which leads to abnormal cell proliferation, tumor occurrence, and development. In the course of treatment, CSC is often in a relatively static state and has a strong ability to repair DNA damage, which can escape the killing effect of chemotherapy[5], and become the basis of tumor recurrence and metastasis.

OCT4 gene, also known as OCT3, POU5F1, OTF3, or OTF4, is a member of the POU transcription factor family, and found so far the earliest and most important key gene in embryonic stem cell self-renewal and differentiation potential, with SOX2 and Nanog, as markers of stem cells. In previous studies, the OCT4 gene was expressed in embryonic and germ cell tumors, and the higher expression of OCT4 was also found in tumor tissues and cells of the reproductive system, such as skin squamous cell carcinoma[6], hepatocellular carcinoma[7], gastric cancer[8], esophageal cancer and breast cancer. It suggests that abnormal expression of OCT4 gene may be the molecular mechanism of tumor cell formation, and it is also one of the causes of stem cell carcinogenesis. Many works have found that OCT4 can maintain embryonic

stem cells and adult stem cells pluripotency and self-renewal, OCT4 has reencoding activation function in embryonic stem cell nucleus, and this function depends on DNA replication and cell division[9]. The high expression of OCT4 is related to the maintenance of the cell, and its down-regulated expression is related to the differentiation[10]. OCT4 gene silencing can make murine and human embryonic stem cells differentiated to trophoblast cells[11]. But there are few papers that have proposed the opposite conclusion. Mouse adult stem cells which lack the OCT4 gene can maintain self-renewal[12,13]. Therefore, the OCT4 gene remains to be further studied in the maintenance of adult stem cell multi potential and self-renewal. This work showed that OCT4 was not expressed in normal tissues, but expressed in laryngeal carcinoma, and with a decrease in differentiation, lymph node metastasis and increased TNM stage, the positive rate increased, observations similar to results of most types of cancer research. The results suggested that there were OCT4 positive cells in the presence of stem cell markers in laryngeal carcinoma, and they were closely related to the degree of differentiation, stage of laryngeal carcinoma and lymph node metastasis.

Conclusions

The sample size of this study is small, but the results suggest that OCT4 has high expression and is a specific gene of embryonic stem cells in laryngeal carcinoma of low differentiation, lymph node metastasis, and higher clinical stage, and confirm the theory of cancer stem cells that tumor stem cell may become a new target for future therapy of laryngeal carcinoma.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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