Expression and Meaning of VEGF-C in Oral Squamous Cell Carcinoma

WANG Yao-zhong[1], YUE Jin[2], JIA Mu-yun[2△], YUAN Rong-tao[2], DENG Yue[1△]

(1 Department of Oral Maxillofacial Surgery, Qingdao Stomatological Hospital, Qingdao 266003, China;

2 Department of Oral Maxillofacial Surgery, the Affiliated Hospital of Qingdao University Medical College, Qingdao 266003, China)

ABSTRACT Objective: To investigate the expression of vascular endothelial growth factor C (VEGF-C) in oral squamous cell carcinoma (OSCC) and adjacent tissues, and to investigate the effect of the VEGF-C in the proliferation, invasion and metastasis of OSCC. Methods: Image analysis system was used to detect the expression of VEGF-C in OSCC. Adjacent normal tissues in 60 patients were determined by immunohistochemical staining, then the relationship with lesion, size, pathologic grade, clinical stage and neck lymph node metastasis was analyzed. Results: There was significant difference between the OSCC and the adjacent tissues in the expression of VEGF-C($u=7.747$, $P < 0.01$), which indicated there was close relation between the VEGF-C expression intensity and the proliferation, invasion and metastasis of OSCC. Conclusion: The VEGF-C expressed by OSCC plays an important role in regional lymph node metastasis by inducing Cancer-week lymphatic hyperplasia expansion.

Key words: Vascular endothelial growth factor C; Oral; Neoplasms; Squamous Cell; Immunohistochemistry

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Introduction

The VEGF-C, which is also called lymphatic vessel growth factor is a member of VEGF family, the main functions of which are to enhance the chemo taxis of the lymphatic endothelial cells, to proliferate cells and to increase the lymphatic vessels' diameter and number. Studies shows that the VEGF-C whose gene is differentially expressed gene of OSCC is higher expressed in OSCC[1,2], which needs further studies for clinical significance. Then, in this paper, we detect the expression of VEGF-C in OSCC by immunohistochemistry to probe its clinical significance.

1 Materials and methods

1.1 Case Selection

Sixty patients were selected, who had OSCC and received surgical treatment in the department of oral and maxillofacial surgery, the Affiliated Hospital of Qingdao University Medical College from January 2005 to May 2008. Their OSCC corresponding normal tissues and OSCC tissues were sampled. All subjects with OSCC included 34 men and26 women between 29 and 85 years old with the median age of 62 years old. 24 patients were with tongue carcinoma, and the other 36 were with carcinoma in different sites including 7 in check, 13 in gingival, 6 in mouth, 3 in palate and 7 in lip. The pathologic grading was as follows: 37 cases in grade I, 16 cases in grade II and 7 cases in grade III. The clinical stages were: 30 cases in stage I-II and 30 cases in stage III-IV. There were 20 cases with lymph node metastasis and 40 without the metastasis. All primary tumor and cervical lymph node metastases were confirmed by pathology. The criteria of bringing in the cases: ①No reception of preoperative radiotherapy, chemotherapy or any biological treatment; ②The carcinoma corresponding tissues are located outside the edge of the tumor for 0.2cm with no canceration confirmed by pathology. Exclusion criteria: non-primary oral cavity metastatic carcinoma (excluding multiple primary carcinoma).

1.2 Main Reagents and Apparatus

Rabbit anti-human polyclonal antibody (Beijing Zhongshan Goldenbridge Biotechnology Co., Ltd.); PV6000 two-step immunohistochemistry detection kit (Zymed Company, U.S.A.); Sample PCI Image Analysis System (Compix Company, U.S.A.)

1.3 Immunohistochemical Staining

All pathology specimens are conventionally fixed by neutral formaldehyde solution with 40g/L, dehydrated by ethanol grade by grade, paraffin-embedded, thick sliced in 3um and stained by immunohistochemical universal two-step method. The time and temperature are strictly controlled among these steps. In each staining, positive and negative control are set, DAB coloring is conducted and the coloring time is controlled under the microscope. After dehydration, transparency, seal and observation with the microscope, the positive expression is shown as the brown-yellow granules.

1.4 Quantitative Analysis of Images

The results of immunohistochemical staining were measured by Sample PCI Image Analysis System for the absorbance, and were transferred to the average absorbance value (value A) for the quantitative analysis. For each slice, five views were taken for counting the average value, which was taken for the determined value. The strength of the slice's positive expression has positive correlation with value A.

1.5 Statistical Analysis

SPSS10.0 statistical software package was applied. Wilcoxon

Auther introduction: WANG Yao-zhong(1983-), male, master, research field: tumor of oral maxillofacial region, 13792835745

△Corresponding auther: JIA Mu-yun, professer, tutor for graduate, E-mail: jiamuyun@gmail.com

Corresponding auther: DENG Yue, associate professer, tutor for graduate, E-mail: dy71alveolar@yahoo.com.cn

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method were carried out to investigate OSCC corresponding tissues and OSCC for comparison (variance arrhythmia). OSCC corresponding normal mucosa, well-differentiated OSCC and poorly differentiated OSCC (variance arrhythmia) were investigated by Mann-Whitney U method. The relationship between VEGF-C and clinicopathological factors was analyzed by spearman correlation analysis.

2 Results

2.1 VEGF-C Expression in OSCC and Its Corresponding Tissues

The positive expression of VEGF-C was shown as the brown-yellow granules, which are contained in the cytoplasm. In OSCC corresponding tissues, the positive reaction was weak, which lied in the epithelial stratum basale. In OSCC tissues, the reaction extent of the positive expression of tumor cells increases, with the diffuse, focal and sporadic distribution. The value A of VEGF-C corresponding tissues was 0.0842±0.0018, while in OSCC tissues the value was 0.1315±0.0052. Among OSCC tissues, the value of well-differentiated OSCC was 0.1267±0.0069 and poorly differentiated OSCC was 0.1389± 0.0078. VEGF-C expression in OSCC tissues was higher than the OSCC corresponding tissues (u=7.747, P<0.01). The VEGF-C expressions in well-differentiated OSCC and poorly differentiated OSCC were all higher than the OSCC corresponding tissues (u=6.335, 6.339 P<0.01).

2.2 Relationship between VEGF-C Expression in OSCC

Tissues and Clinicopathological Parameters VEGF-C was positively correlated with the clinical stage and lymph node metastasis(r=0.564, 0.706, P<0.01), but has no obvious correlation with tumor size, lesion site and pathological grade. See Table I.

Table 1 Clinicopathological parameters analysis

Clinicopathological parameters n	n	VEGF-C	r
Tumor size			
T1-T2	39	0.1318± 0.0356	0.152
T3-T4	21	0.1253± 0.0343	
Growth site			
Tongue	24	0.1416± 0.0475	0.143
Other sites	36	0.1247± 0.0337	
Pathological grade			
I class	37	0.1267± 0.0348	0.229
II、III class	23	0.1389± 0.0363	
Clinical stage			
I-II phase	30	0.1090± 0.0237	0.564*
III-IV phase	30	0.1540± 0.0411	
Lymph node metastasis			
Negative	40	0.1112± 0.0231	0.706*
Positive	20	0.1720± 0.0366	

Note: * P<0.01

3 Discussion

With the only lymphatic endothelial cell-stimulating factor have been found[3], VEGF-C can combine with the flt-4 (VEGFR-3) receptors which are specifically distributed on lymphatic endothelial cells to function, and it can also promote the generation of lymphatic vessels both in vivo and in vitro. Recent researches confirmed that VEGF-C had high expression in carcinoma tissues of carcinoma of colon, breast, prostate, lung, etc., and the high expression was positively correlated with the increasing of lymphatic vessels and lymph node metastasis of carcinoma corresponding tissues[4-7]. The study of Chen WT indicated that VEGF-C was the differentially expressed gene of OSCC [8]. Duan LQ found that VEGF-C was closely related to OSCC by applying real-time quantitative PCR to verify the increase of its expression in OSCC[9]. The study showed that VEGF-C expression in OSCC tissues was obviously higher than the corresponding tissues, and increased with the extent of malignant tumor strengthens, but it has no relationship with the tumor size.

As to the method that tumer cells enter the lymphatic vessels, there are different points of view. One view is that tumor cells invade into the lymphatic vessels that have existed in the edge of the tumor and the metastasis occurs. While another view holds that the tumor can promote the generation of new lymphatic vessels,

and the metastasis occurs by invading these new lymphatic vessels[10-12]. The results showed that VEGF-C high expression was closely related to cervical lymph node metastasis of OSCC, and its possible mechanisms are as follows: ① The tumor cells secrete VEGF-C through paracrine and some autocrine to promote the significant increase of the density of lymphatic vessels in carcinoma cells and especially those in corresponding tissues, which caused the increase of the cervical lymph node metastasis rate of OSCC. ②VEGF-C chemokine changes the expression of adhesion molecules in lymphatic endothelial cells and the original functions of the lymphatic vessels, and makes the chemotaxis of tumor cells, the invasion and spread of the lymphatic vessels become active. The chemical vitality-increasing function with good chemotaxis may mediate the migration of tumor cells[13].

At present, the significances of the VEGF-C in the occult lymph node metastasis of the early OSCC patients (T1/T2N0) was still controversial [14-15]. 87 cases of clinical T1-T2N0M0 and cases of carcinoma of tongue or mouth floor with pN+ or pN0 which were chosen by Faustino and other experts were analyzed by immunohistochemistry[15]. Among 64 patients received the neck dissection surgery, 24 cases had lymph node metastasis. There was no statistical significance of VEGF-C between the metastasis and non-metastasis groups, which was different from the conclusion of the previous studies. Therefore, further studies are needed[14].

In conclusion, the over-expression of VEGF-C in OSCC indicates that it is one of important factors for the regional lymph node metastasis by inducing the proliferation and expansion of carcinoma corresponding lymphatic vessels. The VEGF-C is expected to be the indicator for the early judgment and prediction of cervical ly mph node metastas is. It is possible to reduce the tumor metastasis and to improve the prognosis by early antagonizing the expression or the receptor blockade of the VEGF-C.

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VEGF-C 在口腔鳞状细胞癌中的表达及其意义

王耀钟[1] 岳 金[2] 贾暮云[2△] 袁荣涛[2] 邓 悦[1△]

(1 青岛市口腔医院口腔颌面外科 山东 青岛 266003, 2 青岛大学医学院附属医院口腔颌面外科 山东 青岛 266003)

摘要 目的:检测血管内皮生长因子-C(VEGF-C)在口腔鳞状细胞癌(鳞癌)和癌旁组织中的表达情况,探讨 VEGF-C 在口腔鳞癌的增殖、浸润和淋巴转移中的作用。方法:采用免疫组化方法检测 60 例口腔鳞癌病人癌组织和癌旁组织中 VEGF-C 的表达,应用图像分析系统进行分析,用 Spearman 相关分析研究其与病变部位、肿瘤大小、病理分级、临床分期及颈淋巴结转移之间的关系。结果:口腔鳞癌组织 VEGF-C 的表达明显高于癌旁组织(u=7.747,P<0.01),其表达强度与临床分期及淋巴结转移密切相关(r=0.564、0.706,P<0.05),与病变部位、大小、病理分级无关。结论:口腔鳞癌细胞分泌 VEGF-C 诱导癌周淋巴管增生扩张是发生区域淋巴结转移的重要因素之一,VEGF-C 有望作为早期临床判断和预测颈淋巴结转移的指标之一。

关键词:血管内皮生长因子-C;口腔;肿瘤;鳞状细胞;免疫组织化学

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作者简介:王耀钟(1983),男,硕士,主要从事口腔颌面外科的临床工作,13792835745

△通讯作者:贾暮云,教授,硕士研究生导师,E-mail:jiamuyun@gmail.com

邓悦,副教授,硕士研究生导师,E-mail:dy71alveolar@yahoo.com.cn

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