Journal of Hainan Medical University

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Correlation of Slug gene expression with lymph node metastasis and invasion molecule expression in oral squamous cell carcinoma tissue

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ARTICLE INFO

Article history:
Received 11 Oct 2017
Received in revised form 15 Oct 2017
Accepted 18 Oct 2017
Available online 28 Oct 2017

Keywords:
Oral squamous cell carcinoma
Lymph node metastasis
Epithelial-mesenchymal transition
Slug
Invasion

ABSTRACT

Objective: To study the correlation of Slug gene expression with lymph node metastasis and invasion molecule expression in oral squamous cell carcinoma tissue. **Methods:** Oral squamous cell carcinoma tissue surgical removed in Affiliated Stomatological Hospital of Nanjing Medical University between March 2015 and April 2017 was selected and divided into the oral squamous cell carcinoma tissue with neck lymph node metastasis and the oral squamous cell carcinoma tissues without lymph node metastasis according to the condition of lymph node metastasis. The expression of Slug, epithelial-mesenchymal transition molecules and invasion molecules in the oral squamous cell carcinoma tissue were detected. **Results:** Slug, N-cadherin, Vimentin, CD147, OPN, GRP78, SDF-1 and CXCR4 protein expression in oral squamous cell carcinoma tissue with neck lymph node metastasis were significantly higher than those in oral squamous cell carcinoma tissue without lymph node metastasis while E-cadherin, P120ctn and ZO-1 protein expression were significantly lower than those in oral squamous cell carcinoma tissue without lymph node metastasis; N-cadherin, Vimentin, CD147, OPN, GRP78, SDF-1 and CXCR4 protein expression in oral squamous cell carcinoma tissue with high Slug expression were significantly higher than those in oral squamous cell carcinoma tissue with low Slug expression while E-cadherin, P120ctn and ZO-1 protein expression were significantly lower than those in oral squamous cell carcinoma tissue with low Slug expression. **Conclusion:** The highly expressed Slug in oral squamous cell carcinoma tissue can promote the epithelial-mesenchymal transition and invasion of the cells to participate in the lymph node metastasis of tumor cells.

1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the common malignant tumors of head and neck, the blood supply of oral and maxillofacial region is very rich, so the growth and metastasis of oral squamous cell carcinoma are very quick, and it is prone to lymph node metastasis and distant metastasis, and with poor prognosis and low survival rate[1−3]. At present, the regulation mechanism of lymph node metastasis and distant metastasis of oral squamous cell carcinoma has not been fully elucidated. Epithelial mesenchymal transition (EMT) is an important biological process for the cancer cells to obtain invasion and migration ability. Epithelial phenotype transition to mesenchymal phenotype can significantly promote cell invasion and migration[4,5]. Slug is the key transcription factor regulating the process of EMT, it has been confirmed to be highly expressed in oral squamous cell carcinoma[6,7], but it has not been reported whether the Slug participates in the lymph node metastasis of oral squamous cell carcinoma. In the following studies, we specifically analyzed the correlation of Slug gene expression with lymph node metastasis and invasion molecule expression in oral squamous cell carcinoma tissue.

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Fund Project: Funding Project of Jiangsu University Preponderant Discipline Construction Project No: 2014-37.

2. Research subjects and methods

2.1 General information of research subjects

Patients with oral squamous cell carcinoma who received surgical resection in Affiliated Stomatological Hospital of Nanjing Medical University between March 2015 and April 2017 were selected as the research subjects, and all patients were with squamous cell carcinoma confirmed by postoperative pathology examination, and received preoperative chemoradiotherapy and immunotherapy. According to the condition of lymph node metastasis, the surgically removed oral squamous cell carcinoma tissue was divided into the oral squamous cell carcinoma tissue with cervical lymph node metastasis and the oral squamous cell carcinoma tissue without lymph node metastasis. There were 38 patients with oral squamous cell carcinoma with cervical lymph node metastasis, including 23 male cases and 15 female cases that were 38-61 years old; there were 52 patients with oral squamous cell carcinoma without lymph node metastasis, including 31 male cases and 21 female cases that were 41-60 years old. There was no significant difference in the general data between patients with different lymph node metastasis (P>0.05).

2.2 Research methods

2.2.1 Tissue collecting and storing

After surgical resection, the oral squamous cell carcinoma tissue was collected, cleaned with saline water for 3-5 times to ensure that all residual blood was removed, then briefly frozen in liquid nitrogen for 10-15 min, taken out and placed in -80 ℃ refrigerator.

2.2.2 Gene and molecule expression testing

Oral squamous cell carcinoma tissue was taken and added in protein lysate RIPA to get tissue protein homogenate, the homogenate was placed in 4 ℃ centrifuge and centrifuged for 15 min at 12 000 r/min to separate supernatant, and enzyme-linked immunosorbent assay kit was used to detect Slug, N-cadherin, Vimentin, E-cadherin, P120ctn, ZO-1, CD147, OPN, GRP78, SDF-1 and CXCR4 protein expression.

2.3 Statistical methods

SPSS 16.0 software was used for statistical analysis, and the median of Slug protein expression was calculated and used to divide the oral squamous cell carcinoma tissue into the tissue with low Slug expression and the tissue with high Slug expression. Measurement data analysis between two groups were by t test and P<0.05 indicated statistical significance in differences in test results (P<0.05).

3. Results

3.1 Slug expression in oral squamous cell carcinoma tissue

Slug protein expression in oral squamous cell carcinoma tissue with neck lymph node metastasis was (6.58±0.93) ng/mL and Slug protein expression in oral squamous cell carcinoma tissue without lymph node metastasis was (2.36±0.36) ng/mL. After t test, Slug protein expression in oral squamous cell carcinoma tissue with neck lymph node metastasis was significantly higher than that in oral squamous cell carcinoma tissue without lymph node metastasis.

3.2 Epithelial-mesenchymal transition molecule expression in oral squamous cell carcinoma tissue

Analysis of epithelial-mesenchymal transition molecules N-cadherin (ng/mL), Vimentin (ng/mL), E-cadherin (ng/mL), P120ctn (pg/mL) and ZO-1 (pg/mL) expression in oral squamous cell carcinoma tissue was as follows: N-cadherin and Vimentin protein expression in oral squamous cell carcinoma tissue with neck lymph node metastasis were significantly higher than those in oral squamous cell carcinoma tissue without lymph node metastasis while E-cadherin, P120ctn and ZO-1 protein expression were significantly lower than those in oral squamous cell carcinoma tissue without lymph node metastasis.

Analysis of epithelial-mesenchymal transition molecules N-cadherin (ng/mL), Vimentin (ng/mL), E-cadherin (ng/mL), P120ctn (pg/mL) and ZO-1 (pg/mL) expression in oral squamous cell carcinoma tissue with different Slug expression was as follows: N-cadherin and Vimentin protein expression in oral squamous cell carcinoma tissue with high Slug expression were significantly higher than those in oral squamous cell carcinoma tissue with low Slug expression while E-cadherin, P120ctn and ZO-1 protein expression were significantly lower than those in oral squamous cell carcinoma tissue with low Slug expression.

Table 1.

Comparison of epithelial-mesenchymal transition molecules in oral squamous cell carcinoma tissue with different lymph node metastasis.

Lymph node metastasis	n	N-cadherin	Vimentin	E-cadherin	P120ctn	ZO-1
With metastasis	38	1.85±0.22	1.37±0.17	1.13±0.14	178.65±20.35	127.54±15.86
Without metastasis	52	1.02±0.15	0.62±0.08	2.04±0.29	394.51±52.39	305.96±42.83
t		8.498	11.274	9.273	13.049	15.685
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

Comparison of epithelial-mesenchymal transition molecules in oral squamous cell carcinoma tissue with different Slug expression.

Slug expression	n	N-cadherin	Vimentin	E-cadherin	P120ctn	ZO-1
High Slug expression	19	1.97±0.24	1.41±0.19	1.02±0.13	185.61±21.38	130.12±17.12
Low Slug expression	19	0.98±0.11	0.57±0.07	2.27±0.35	387.76±46.78	289.76±39.58
t		10.039	16.585	12.475	11.038	13.486
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Comparison of invasion molecules in oral squamous cell carcinoma tissue with different lymph node metastasis.

Lymph node metastasis	*n*	CD147	OPN	GRP78	SDF-1	CXCR4
With metastasis	38	2.39±0.32	6.84±0.83	1.37±0.18	332.82±46.58	274.84±32.59
Without metastasis	52	1.16±0.18	3.52±0.58	0.69±0.09	158.62±17.48	132.67±16.58
t		10.984	9.273	9.885	11.348	12.408
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 4.

Comparison of invasion molecules in oral squamous cell carcinoma tissue with different Slug expression.

Slug expression	*n*	CD147	OPN	GRP78	SDF-1	CXCR4
High Slug expression	19	2.51±0.37	6.94±0.89	1.32±0.17	352.31±49.62	279.21±33.59
Low Slug expression	19	1.02±0.15	3.31±0.51	0.73±0.10	147.64±15.96	126.58±14.85
t		15.498	11.329	8.589	14.428	13.328
P		<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Invasion molecule expression in oral squamous cell carcinoma tissue

Analysis of invasion molecules CD147 (ng/mL), OPN (ng/mL), GRP78 (ng/mL), SDF-1 (pg/mL) and CXCR4 (pg/mL) expression in oral squamous cell carcinoma tissue was as follows: CD147, OPN, GRP78, SDF-1 and CXCR4 protein expression in oral squamous cell carcinoma tissue with neck lymph node metastasis were significantly higher than those in oral squamous cell carcinoma tissue without lymph node metastasis.

Analysis of invasion molecules CD147 (ng/mL), OPN (ng/mL), GRP78 (ng/mL), SDF-1 (pg/mL) and CXCR4 (pg/mL) expression in oral squamous cell carcinoma tissue with different Slug expression was as follows: CD147, OPN, GRP78, SDF-1 and CXCR4 protein expression in oral squamous cell carcinoma tissue with high Slug expression were significantly higher than those in oral squamous cell carcinoma tissue with low Slug expression.

4. Discussion

The oral squamous cell carcinoma is with high incidence of lymph node metastasis and distant metastasis, the prognosis of patients with metastatic oral squamous cell carcinoma is poor and the 5-year survival rate is low[8,9]. The distant metastasis of oral squamous cell carcinoma is closely related to cancer cell migration, invasion, epithelial-mesenchymal transition and other biological links, the epithelial-mesenchymal transition is the first step for cancer cells to obtain the movement and migration ability, and it is valuable to decide the distant metastasis of cancer cells[10]. Slug is a key transcription factor that controls the cellular epithelial-mesenchymal transition, which identifies the epithelial marker gene E-box and prevents gene expression to make the cellular epithelial phenotype weaken and transit to mesenchymal phenotype[11]. It has been reported that the expression of transcription factor Slug significantly increases in oral squamous cell carcinoma tissue and Slug is involved in regulating the epithelial-mesenchymal transition of oral squamous cell carcinoma cell lines[12,13]. The epithelial-mesenchymal transition mediated by Slug can enhance the cancer cell migration and invasion within the oral squamous cell carcinoma lesion, but there is no report whether the Slug is related to the lymph node metastasis and distant metastasis of oral squamous cell carcinoma. In this study, the analysis of the relationship between lymph node metastasis and Slug expression in oral squamous cell carcinoma tissue showed that Slug protein expression in oral squamous cell carcinoma tissue with neck lymph node metastasis was significantly higher than that in oral squamous cell carcinoma tissue without lymph node metastasis. This indicates that the high expression of Slug in oral squamous cell carcinoma is closely related to the lymph node metastasis of tumor cells.

Slug is a transcription factor that regulates the cellular epithelial-mesenchymal transition, which can promote the epithelial-mesenchymal transition and enhance cell migration and invasion. N-cadherin and Vimentin are the markers of the mesenchymal phenotype, and their functions are to reduce the intercellular polarity and improve the movement and migration of cells, which facilitate the invasion and metastasis of cancer cells[14,15]. E-cadherin is a marker of epithelial phenotype, which forms catenin-cadherin complex with catenin P120ctn to maintain intercellular polarity and form tight junction between cells as well as between cells and extracellular matrix; ZO-1 is an important tight junction protein that participates in the formation of intercellular tight junction structures, increases the intercellular adhesion and inhibits the invasion and migration of cells[16,17]. In the study, analysis of the correlation between above epithelial-mesenchymal transition molecules in oral squamous cell carcinoma and lymph node metastasis showed that N-cadherin and Vimentin protein expression increased significantly while E-cadherin, P120ctn and ZO-1 protein expression decreased significantly in oral squamous cell carcinoma tissue with neck lymph node metastasis. It indicates that excessive epithelial mesenchymal transition in oral squamous cell carcinoma is closely related to lymph node metastasis of cancer cells. Further analysis of the effect of Slug in oral squamous cell carcinoma tissue on epithelial mesenchymal transition showed that N-cadherin and Vimentin protein expression were higher while E-cadherin, P120ctn and ZO-1 protein expression were lower in oral squamous cell carcinoma tissue with high Slug expression. This indicates that the high expression of Slug in the oral squamous cell carcinoma tissue can promote the epithelial mesenchymal transiton of cancer cells and thus participate in the lymph node metastasis of cancer cells.

On the basis of obtaining movement and migration ability, the cancer cells in the oral squamous cell carcinoma lesions need to degrade the extracellular matrix and basement membrane to invade the adjacent tissues. CD147, OPN, GRP78 and SDF-1/CXCR4 are important molecules that regulate the invasion of cells in the

oral squamous cell carcinomas. CD147 is the transmembrane glycoprotein in the immunoglobulin superfamily that can stimulate the expression of a variety of MMPs[18], OPN is a type of secreted glycosylated non-collagen protein that can make a variety of MMPs activate[19,20], and the CD147 and OPN can promote extracellular matrix degradation and cell invasion via downstream MMPs molecules; GRP78 is a class of membrane protein that can promote cell invasion through downstream Smad2/3 and MAPK pathways[21]; SDF-1/CXCR4 axis has regulatory effect on the invasion of cells, and SDF-1 can recognize the CXCR4 on the cell membrane to promote cell invasion[22]. In the study, analysis of the correlation between above invasion molecules in oral squamous cell carcinoma and lymph node metastasis showed that CD147, OPN, GRP78, SDF-1 and CXCR4 protein expression in oral squamous cell carcinoma tissue with neck lymph node metastasis were significantly higher than those in oral squamous cell carcinoma tissue without lymph node metastasis. This indicates that the high expression of multiple invasion molecules in oral squamous cell carcinoma is closely related to lymph node metastasis of cancer cells. Further analysis of the effect of Slug in oral squamous cell carcinoma tissue on invasion molecule expression showed that CD147, OPN, GRP78, SDF-1 and CXCR4 protein expression in oral squamous cell carcinoma tissue with high Slug expression were significantly higher than those in oral squamous cell carcinoma tissue with low Slug expression. This indicates that the high expression of Slug in oral squamous cell carcinoma tissues can promote the expression of multiple invasion molecules, and then participate in the lymph node metastasis of cancer cells.

The high expression of Slug in oral squamous cell carcinoma is associated with lymph node metastasis of tumor cells. Slug has promoting effect on the epithelial mesenchymal transiton and invasion of cells in oral squamous cell carcinoma tissue, and then can promote the lymph node metastasis of tumor cells through the changes in epithelial mesenchymal transiton and invasion.

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