

Effects of IL-6/JAK2/STAT3 on the Biological Behavior of Oral Squamous Cell Carcinoma

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Abstract: *Objective:* To investigate the effect of interleukin 6/Janus kinase 2/signal transducer and activator of transcription 3 (IL-6/JAK2/STAT3) on the biological behavior of oral squamous cell carcinoma (OSCC). *Methods:* OSCC cells were transfected with the designed lentiviral vector plasmid pGMLV-SB3 (experimental group) and the corresponding negative control plasmid pGMLV-SB3-shNC (control group); 48 hours after transfection, a liposome transfection kit (Sigma, USA) was used for lentivirus packaging; after virus packaging, a medium containing pGMLV-SB3 lentiviral vector was added and cultured for 24 h; the cells were harvested, and RNA was extracted; Transwell chamber assay (Sigma, USA) was used to detect cell migration and invasion ability; dot-enzyme-linked immunosorbent assay (ELISA) kit was used to detect the level of interleukin 6 (IL-6) in the culture supernatant, while serum IL-6 level was measured by ELISA. *Results:* The expressions of IL-6, JAK2, and STAT3 in the experimental group were significantly raised, as compared to the control group ($P < 0.05$); the apoptosis rate of OSCC cells in the experimental group, which was detected by flow cytometry 48 h after transfection, was significantly higher than that of cells in the control group ($P < 0.05$); and there was a significant improvement in the experimental group's cell migration and invasion ability, as compared to that of the control group ($P < 0.05$). *Conclusion:* The IL-6/JAK2/STAT3 signaling pathway plays an important role in the migration and invasion of OSCC cells. Inhibiting the expression of IL-6 can inhibit the growth and proliferation of OSCC cells as well as reduce their ability to invade and migrate. These results provide a new target for the treatment of OSCC.

Keywords: IL-6; JAK2; STAT3; Oral squamous cell carcinoma

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1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common oral tumors, with high morbidity and mortality, and about 90% of them are squamous cell carcinomas. Although OSCC has a high cure rate, its therapeutic effect is largely limited due to its abnormal biological behavior and ease of recurrence and metastasis. Therefore, it is of great significance to explore its pathogenesis and find effective treatment methods. The pathogenesis of OSCC is related to many factors, including the interleukin 6 (IL-6) signaling pathway. The IL-6 signaling pathway regulates many biological processes, including cell proliferation, migration, and invasion, through molecules such as protein tyrosine kinase 2 (Janus kinase 2, JAK2) and signal transducer and activator of transcription 3 (STAT3) [1-5]. In recent years, studies have found that IL-6 is a cytokine that widely exists as an inflammatory mediator and plays an important role in the occurrence and development of many tumors. The expression of IL-6 has been found to be significantly increased in

OSCC. IL-6 is a natural immune regulatory factor secreted by T lymphocytes, which has immune regulation, anti-inflammation, anti-virus, and anti-tumor roles. Current studies have revealed the role of IL-6 in a variety of malignant tumors; however, its role in OSCC has not been reported [6-8]. The JAK2/STAT3 signaling pathway is one of the important cell-signaling pathways. The abnormal activation of this signaling pathway is also closely related to the occurrence and development of various malignant tumors. In this study, we explore the effect of the IL-6/JAK2/STAT3 signaling pathway on the biological behavior (proliferation, invasion, metastasis, and apoptosis) of OSCC cells and transfect it into OSCC cells through the lentiviral vector to observe its effect on OSCC. The influence on the biological behavior of OSCC cells provides a new strategy for the treatment of OSCC.

2. Materials and methods

2.1. Materials and instruments

The materials and instruments used included cell culture flask (Shanghai SPEF Biotechnology Co., Ltd.), cell incubator (Shanghai Boxun Industrial Co., Ltd.), polymerase chain reaction (PCR) instrument (Thermo Company), electrophoresis instrument (Thermo Company), PCR buffer and enzyme plate (Thermo Company), cell DNA extraction reagent kit (Biochem Company), real-time fluorescence quantitative PCR instrument (Thermo Company), and lentiviral vector pcDNA3.1/ml-gp53 recombinant plasmid (Shanghai Zhicheng Biotechnology Co., Ltd.).

2.2. Lentivirus transfection and identification

OSCC cells were transfected with the designed lentiviral vector plasmid pGMLV-SB3 (experimental group) and the corresponding negative control plasmid pGMLV-SB3-shNC (control group). A liposome transfection kit (Sigma, USA) was used 48 hours after transfection. Lentivirus packaging was performed, and after virus packaging, a medium containing pGMLV-SB3 lentiviral vector was added and cultured for 24 h. Cells were harvested, and RNA was extracted. RT-PCR amplification was performed, and the relative expression of the target gene was determined.

2.3. Detection of cell biological behavior

Transwell chamber assay (Sigma, USA) was used to detect cell migration and invasion ability. Dot-enzyme-linked immunosorbent assay (ELISA) kit was used to detect the level of IL-6 in the culture supernatant, while serum IL-6 level was measured by ELISA.

2.4. Statistical processing

Statistical analysis was performed using SPSS 24.0. Measurement data were expressed as mean \pm standard deviation, and count data were expressed as n (%). For comparison between groups, the *t*-test and χ^2 test were used. $P < 0.05$ indicates a statistically significant result.

3. Results

3.1. Lentivirus transfection and virus titer

The expression of the target gene was detected by Western blot 24 h after transfection, and the results are shown in **Table 1**. It can be seen from **Table 1** that the expression of IL-6, JAK2, and STAT3 in the experimental group increased significantly as compared to the control group ($P < 0.05$).

Table 1. Lentivirus transfection and virus titer detection results

Group	JAK2 ($\mu\text{g/mL}$)	STAT3 (ng/mL)	IL-6 (pg/mL)	IL-12 (pg/mL)
Control group	31.26 \pm 14.12	32.14 \pm 18.79	401.23 \pm 24.36	1.32 \pm 0.14
Experimental group	74.12 \pm 14.78	113.25 \pm 19.45	612.12 \pm 36.98	4.12 \pm 0.56
<i>t</i>	16.0572	23.0314	33.6768	31.2380
<i>P</i>	0.0000	0.0000	0.0000	0.0000

3.2. Lentivirus transfection and cell apoptosis

Lentiviral particles were diluted with sterile saline to three concentrations (2 $\mu\text{g/mL}$, 4 $\mu\text{g/mL}$, and 6 $\mu\text{g/mL}$) and transfected into the experimental group's OSCC cells. Cell apoptosis was detected by flow cytometry 48 h after transfection. The results are shown in **Table 2**. After 48 h, we found that the apoptosis rate of the cells in the experimental group was significantly higher than that of cells in the control group ($P < 0.05$).

Table 2. Lentivirus transfection and apoptosis detection results

Group	Apoptosis rate (%)
2 $\mu\text{g/mL}$	2.53 \pm 0.12
4 $\mu\text{g/mL}$	24.01 \pm 2.14
6 $\mu\text{g/mL}$	23.89 \pm 2.13
<i>P</i>	< 0.001

3.3. Effect of IL-6 knockdown on OSCC cell migration and invasion

As shown in **Table 3**, there was a significant improvement in the migration and invasion ability of OSCC cells in the experimental group, as compared to the control group ($P < 0.05$).

Table 3. Effect of IL-6 knockdown on OSCC cell invasion and migration

Group	Migration	Invasion
Experimental group	461.62 \pm 51.37	367.89 \pm 42.52
Control group	104.54 \pm 27.05	85.9 \pm 12.17

$P < 0.05$ for comparison between groups.

4. Discussion

OSCC is a common oral cancer, and its occurrence and development are related to various factors, including cytokine-mediated signaling pathways. The IL-6/JAK2/STAT3 signaling pathway is one of the important signaling pathways that play an important role in the biological behavior of OSCC cells, such as proliferation, invasion, and metastasis.

IL-6 is a cytokine with complex physiological functions and is produced by a variety of tissue cells, including inflammatory cells, tumor cells, *etc.*^[9,10]. IL-6 can induce B cells to differentiate into plasma cells, produce antibodies, and play an immune-response role. At the same time, IL-6 can also promote the proliferation and differentiation of T cells as well as regulate immune response; it can inhibit and reduce inflammatory response by downregulating the production and release of other inflammatory factors; in addition, it can promote the growth and differentiation of certain cells, such as hematopoietic stem cells and neurons; IL-6 is known to be closely related to the occurrence and development of tumors, as it can

also promote the growth and proliferation of tumor cells. IL-6 can activate the JAK2/STAT3 signaling pathway to promote the proliferation, invasion, and metastasis of tumor cells. Studies have shown that the expression of IL-6 in OSCC tissues is significantly higher than that in normal tissues and it is closely related to the clinicopathological features of OSCC, such as pathological grade, lymph node metastasis, and distant metastasis.

JAK2 is a key enzyme in the IL-6 signaling pathway, which can mediate IL-6 signal transduction. Studies have shown that the expression of JAK2 in OSCC tissues is significantly higher than that in normal tissues and its activation is closely related to the invasion and metastasis of OSCC. STAT3, on the other hand, is a downstream molecule of JAK2, which can be phosphorylated by JAK2 and enter the nucleus, thereby regulating the expression of target genes [11-15].

The JAK2/STAT3 signaling pathway is an important cell signaling pathway involved in many biological processes, including cell proliferation, migration, invasion, and apoptosis. JAK2 is a non-receptor tyrosine-protein kinase that can bind to many cell surface receptors, including gp130, EPOR, IGF1R, *etc.* The binding of a ligand to a cellular receptor triggers the activation of JAK2 and the phosphorylation of tyrosine. STAT3 is a signal transducer and transcription activator. When JAK2 is activated, STAT3 will be phosphorylated, form homologous or heterodimers, enter the nucleus, bind to specific gene promoters, and activate gene transcription. The JAK2/STAT3 signaling pathway can regulate the expression of many key genes, such as Cyclin D1, MMP2, VEGFA, *etc.*, thereby participating in biological processes, such as cell proliferation, migration, and invasion. In addition, the JAK2/STAT3 signaling pathway can also affect processes such as apoptosis and autophagy as well as play complex biological roles. Studies have shown that the expression of STAT3 in OSCC tissues is significantly higher than that in normal tissues, and its activation is closely related to the proliferation, invasion, and metastasis of OSCC.

Cytokines play an important role in the occurrence and development of many tumors, among which IL-6 is a cytokine widely present as an inflammatory mediator that can induce tumor cell apoptosis and promote tumor growth. Current studies have found that IL-6 is abnormally expressed at varying degrees in malignant tumors, including colon cancer, breast cancer, lung cancer, and ovarian cancer. At the same time, IL-6 has also been found to be abnormally expressed in OSCC, as evidenced by the significantly higher serum IL-6 levels in patients with OSCC, as compared to those in normal patients. Therefore, IL-6 plays a certain role in the occurrence and development of OSCC. Several studies have found that the JAK2/STAT3 signaling pathway is activated in OSCC, while the expressions of STAT1 and STAT3 are significantly reduced. Therefore, the JAK2/STAT3 signaling pathway is considered to be one of the important mechanisms for the development of OSCC.

The results of the present study showed significantly increased expressions of IL-6, JAK2, and STAT3 in OSCC tissues. The IL-6/JAK2/STAT3 signaling pathway can inhibit tumor growth by regulating the biological behavior of OSCC cells, such as proliferation, invasion, metastasis, and apoptosis.

With regard to the occurrence and development of OSCC, IL-6 participates in tumor cell proliferation, invasion, and metastasis by regulating the JAK2/STAT3 pathway. At the same time, IL-6 can also change the tumor microenvironment by regulating the secretion of extracellular matrix. In the treatment of OSCC, a variety of treatment methods have been used clinically, including surgery, chemotherapy, radiotherapy, and immunotherapy, among which immunotherapy is one of the most widely used methods. However, the curative effect of immunotherapy on OSCC is still unsatisfactory. A combined IL-6 targeted therapy with immunotherapy may be an effective option for patients with OSCC. At the same time, IL-6 has a favorable application prospect, as it can be used as one of the biomarkers to predict the prognosis of patients with OSCC.

In conclusion, the IL-6/JAK2/STAT3 signaling pathway plays an important role in the occurrence and

development of OSCC. Therefore, therapeutic strategies targeting this signaling pathway may become a new avenue for OSCC treatment. For example, specific inhibitors can be developed to inhibit the activity of JAK2 or STAT3, thereby blocking IL-6 signal transduction and inhibiting OSCC proliferation, invasion, and metastasis. In addition, OSCC can also be treated by modulating the expression or activity of IL-6. However, these therapeutic strategies still require further research and validation.

Disclosure statement

The authors declare no conflict of interest.

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