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The Effect of MMP-9 Inhibitors on the Biological Behavior of Human Oral Squamous Cell Carcinoma SCC15 Cell Line Through PI3K/Akt Signaling Pathway

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Abstract: *Objective:* To investigate the effect of MMP-9 inhibitor (Mki67) on the biology of human oral squamous cell carcinoma SCC15 cell line and to explore its mechanism of action through PI3K/Akt signaling pathway. *Methods:* SCC15 cells were extracted, and the supernatant was discarded. The cells were then rinsed twice with PBS, and 0, 2.5, 5, and 10 μL of Mki67 (50 mg/mL) were added to the culture respectively. The inhibition rate of cell proliferation was detected by 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) method, and the cell migration was measured by Transwell chamber test. The cell apoptosis rate was detected by cytometry, and the p-Akt protein content in the cells of each group was determined by a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit. *Results:* The cell proliferation rates of the 2.5 μL, 5 μL, and 10 μL dose groups were all lower than the 0 μL group ($P < 0.05$) before treatment, and the cell proliferation rates in the 2.5 μL, 5μL, and 10μL dose groups decreased overtime ($P < 0.05$). After 24 h, with the increase of Mki67 concentration, the number of migration and invasion gradually decreased ($P < 0.05$), and the number of apoptosis gradually increased ($P < 0.05$); besides, the relative expression of MMP-9, PI3K, and Akt mRNA decreased gradually ($P < 0.05$), and the expression level of Akt mRNA was not statistically significant ($P > 0.05$). *Conclusion:* MMP-9 inhibitor (Mki67) can inhibit the proliferation and migration of SCC15 cell line and induce apoptosis, and its mechanism of action may be related to the inhibition of PI3K/Akt signaling pathway.

Keywords: MMP-9; PI3K/Akt; Human oral squamous cell carcinoma

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1. Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer, and its incidence rate is increasing year by year. In fact, its incidence rate is second only to lung cancer and gastric cancer, accounting for 10% to 15% of all malignant tumors. Matrix metalloproteinases (MMPs) are a class of enzymes related to the degradation of extracellular matrix and play an important role in the process of tumor invasion and metastasis. Among them, MMP-9 is a member of the MMPs family, which is closely related to the progression and prognosis of OSCC. The most common way of squamous cell carcinoma metastasis is lymph node and distant metastasis, accounting for about 80% to 90% of all metastatic cases [1]. At present, the treatment of human OSCC in our country is mainly surgery, along with radiotherapy, chemotherapy,

and other adjuvant treatments. Although surgical resection is the gold standard for the radical cure of OSCC, in recent years, studies have found that surgical resection alone cannot reduce the risk of recurrence and metastasis in patients [2-5]. Therefore, it is particularly important to carry out comprehensive treatment for OSCC patients to improve the prognosis. In our previous research, we found that the MMP-9 inhibitor (Mki67) could significantly inhibit the proliferation of SCC15 cells, induce cell apoptosis, and down-regulate the expression levels of proteins related to the PI3K/Akt signaling pathway, thus influencing the biological behavior of human oral squamous cell carcinoma SCC15 cell line. Therefore, exploring the effects of MMP-9 inhibitors on OSCC cell lines is of great significance for the development of treatment methods of OSCC.

2. Materials and methods

2.1. Experimental materials

The human SCC15 cell line was provided by Shanghai Institute of Cell Biology, Chinese Academy of Sciences. The cell culture was inoculated in 6-well culture plate at 2×10^6/well, 200 μL per well. After culturing in an incubator at 37 °C for 24 h, the cells were collected, the supernatant was discarded, washed twice with PBS, and then cultured with 0, 2.5, 5, and 10 μL of Mki67 (50 mg/mL), respectively. Then add 100 μL DMEM to each well and incubate overnight at 37 °C.

2.2. Detection methods

2.2.1. MTT assay

To measure the cell viability by colorimetry, the proliferation of cells in each group was detected after Mki67 was treated for 24, 48, and 72 hours, respectively. The inhibition rate of cell proliferation was detected by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) method, the cell migration was detected by Transwell chamber test, and the apoptosis rate was detected by flow cytometry.

2.2.2. Western blot method

The double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit was used to measure the p-Akt protein content in the cells of each group (the target protein was mixed with the corresponding negative control protein, and the corresponding concentration was 1/10 dilution).

2.3. Statistical methods

The measurement data was represented by mean ± SD, and the count data was represented by *n* (%). The data of the two groups were compared using *t* and χ^2 tests, $P < 0.05$, and the standard of statistical significance was analyzed by using SPSS 24.0 software.

3. Results

3.1. Comparison of cell proliferation rate among the four groups

The cell proliferation rates of the 2.5 μL, 5 μL, and 10 μL dose groups were all lower than the 0 μL group ($P < 0.05$) before treatment, and the cell proliferation rates in the 2.5 μL, 5μL and 10μL dose groups decreased overtime ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of cell proliferation rates in the four groups (mean ± SD, $n = 3$)

Group	24 h	48 h	72 h
0 μL	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
2.5 μL	87.25 ± 9.23	79.48 ± 9.11	72.11 ± 8.03

5 μL	75.12 ± 8.25	53.11 ± 6.32	25.35 ± 3.25
10 μL	47.15 ± 5.21	32.36 ± 4.11	18.17 ± 2.11

Note: $P < 0.05$ for pairwise comparison between the 0 μL and the other three groups.

3.2. Comparison of cell migration, invasion and apoptosis among the four groups

After 24 hours, as the concentration of Mki67 increased, the number of migration and invasion gradually decreased ($P < 0.05$), and the number of apoptosis gradually increased ($P < 0.05$), as shown in **Table 2**.

Table 2. Comparison of cell migration, invasion, and apoptosis between the four groups (mean ± SD, $n = 3$)

Group	Migration	Invasion	Apoptosis
0 μL	121.33 ± 33.21	124.12 ± 26.35	24.36 ± 23.41
2.5 μL	97.25 ± 9.23	9 9.48 ± 9.11	5 2.11 ± 8.03
5 μL	55.12 ± 8.25	53.11 ± 6.32	7 5.35 ± 3.25
10 μL	27.15 ± 5.21	22.36 ± 4.11	9 8.17 ± 2.11

Note: $P < 0.05$ for pairwise comparison between the 0 μL and the other three groups.

3.3. Comparison of the relative expression levels of MMP-9, PI3K and Akt mRNA between the four groups of cells

After 24 hours, the relative expression of MMP-9 and PI3K gradually decreased ($P < 0.05$), and the expression of Akt mRNA was not statistically significant ($P > 0.05$), as shown in **Table 3**.

Table 3. Comparison of the relative expression levels of MMP-9, PI3K and Akt mRNA in the four groups of cells (mean ± SD, $n = 3$)

Group	MMP-9	PI3K	Akt mRNA
0 μL	0.98 ± 0. 23	1.12 ± 0.12	1.06 ± 0. 11
2.5 μL	0.89 ± 0.07	0.97 ± 0 11	0.89 ± 0.03
5 μL	0.47 ± 0.04	0.71 ± 0.04	0.81 ± 0.0 5
10 μL	0.24 ± 0.04	0.34 ± 0.06	0.88 ± 0.11

Note: $P < 0.05$ for pairwise comparisons of MMP-9 and PI3K between the 0 μL and the other three groups.

4. Discussion

Tumor invasion and metastasis are the main causes of death in patients with malignant tumors, and they are also one of the difficulties in clinical treatment. Therefore, finding methods that can effectively inhibit tumor growth and metastasis and improve the survival rate of patients has become a popular research topic. Among many anticancer drugs, MMP-9 is an extracellular matrix degrading enzyme highly expressed in various tumor tissues. MMP-9 exerts its biological function by degrading various extracellular matrix components and adhesion molecules [6-7]. Studies have shown that Mki67 inhibitor inhibits cell proliferation, migration, and invasion by inhibiting MMP-9 activity [8-10]. The PI3K/Akt signaling pathway is an important signaling pathway discovered in recent years that regulates biological behaviors such as tumor proliferation, apoptosis, and metastasis [11-13]. Studies have found that abnormal activation of the PI3K/Akt pathway can cause malignant transformation of tumor cells and tumor metastasis [14]. In addition, the PI3K/Akt signaling pathway plays an important role in the regulation of apoptosis, the expression of apoptosis inhibitory factor (BAFF), and the regulation of cell cycle progression [15]. Studies have confirmed that

abnormal activation of the PI3K/Akt pathway is an important reason for the poor prognosis of patients with non-small cell lung cancer [16]. Abnormal activation of PI3K/Akt pathway has been associated with tumor metastasis and treatment resistance.

MMP-9 is involved in the degradation and reorganization of extracellular matrix and plays an important role in cell signal transduction and tumor metastasis. MMP-9 also has an important function in OSCC. Studies have shown that the expression level of MMP-9 is usually higher in OSCC, which may be related to the progression of the disease and the prognosis of patients. The expression level of MMP-9 can affect the degradation of extracellular matrix (ECM) in OSCC, thereby promoting tumor invasion and metastasis. Therefore, MMP-9 can also be used as one of the indicators for the diagnosis and treatment of OSCC. The treatment methods for MMP-9 include drug therapy for inhibiting MMP-9 and antibody therapy targeting MMP-9. Several MMP-9 inhibitors have been evaluated in clinical trials to explore their therapeutic effect on OSCC. In addition, some antibodies against MMP-9 are also in experimental research. These antibodies can target specific regions of MMP-9, thereby inhibiting its function.

PI3K is an intracellular phosphatidylinositol kinase involved in biological processes such as cell proliferation, differentiation, migration, and survival. PI3K can convert phosphatidylinositol into phosphatidylinositol triphosphate (PIP3), and PIP3 can act as an intracellular signaling molecule to activate downstream signaling pathways, including Akt and mitogen-activated protein kinases (MAPKs). PI3K is often abnormally activated in cancer cells, which may be related to the mutation or overexpression of oncogenes. Abnormal activation of PI3K can lead to abnormal cell proliferation and survival, thereby promoting the occurrence and development of cancer. Therefore, PI3K has become one of the important targets in cancer therapy. Therapeutic methods targeting PI3K include drug therapy for inhibiting PI3K and antibody therapy targeting PI3K. Some PI3K inhibitors have been approved to treat certain types of cancer, such as breast cancer and renal cell carcinoma. In addition, some antibodies against PI3K are also in experimental research. These antibodies can target specific regions of PI3K, thereby inhibiting its function.

Akt is a serine/threonine protein kinase (also known as protein kinase B [PKB]) that plays an important role in cell proliferation, differentiation, survival and migration. Akt can be activated by various signaling pathways, including PI3K pathway, mammalian target of rapamycin (mTOR) pathway, etc. Akt is often abnormally activated in cancer cells, which may be related to the mutation or overexpression of oncogenes. Abnormal activation of Akt can promote the occurrence and development of cancer, so Akt has become one of the important targets in cancer therapy. The treatment methods for Akt include Akt -inhibiting drug therapy and Akt-targeting antibody therapy. Some Akt inhibitors have been approved for the treatment of certain types of cancer, such as breast cancer, renal cell carcinoma, etc. In addition, some antibodies against Akt are also under research. These antibodies can target specific regions of Akt, thereby inhibiting its function. In addition, Akt can also affect the occurrence and development of cancer by regulating cell metabolism. The activation of Akt can promote the absorption and utilization of nutrients by cells, thereby promoting the growth and survival of cancer cells. Therefore, modulating the effect of Akt on cellular metabolism could also be a strategy in cancer therapy.

Mki67 also has an important role in human SCC. The expression level of Mki67 is usually higher in SCC, which is related to the progression of the disease and the survival rate of patients. Therefore, Mki67 can also be used as one of the indicators for the diagnosis and treatment of OSCC. In OSCC, the expression level of Mki67 is related to cell proliferation and differentiation. OSCC cells with high expression of Mki67 usually proliferate faster and are more prone to metastasis. Therefore, the expression level of Mki67 can be used to predict the degree of malignancy of OSCC and the prognosis of patients. In addition, some researches have also been done on therapeutic approaches targeting Mki67 in OSCC treatment. For example, some drugs can inhibit the function of Mki67, thereby inhibiting the proliferation and survival of OSCC cells. In addition, some antibody therapies against Mki67 are also still under research. These treatments are

designed to target OSCC cells with high expression of Mki67, causing them to die or lose their ability to proliferate.

In this study, Western blot was used to detect the downregulation of the expression levels of PI3K/Akt signaling pathway-related proteins in SCC15 cell line by Mki67. This suggests that MMP-9 inhibitors may inhibit the proliferation, migration and invasion of human oral squamous cell carcinoma SCC15 cell lines through the PI3K/Akt signaling pathway, rather than down-regulating the proliferation, migration and invasion of human oral squamous cell carcinoma SCC15 cell lines.

Based on the previous research work, this study further clarified the molecular mechanism of MMP-9 inhibitor Mki67 inhibiting the proliferation, migration and invasion of human oral squamous cell carcinoma SCC15 cell line, and provided an experimental basis for the further development of targeted therapy drugs for human oral squamous cell carcinoma.

Shortcomings of this study: this study is a single-centered experiment, and only the effects of MMP-9 inhibitors on the proliferation, migration, invasion, and apoptosis of human oral squamous cell carcinoma SCC15 cell line were detected. Therefore, further research multiple-center research with large samples is still needed and to clarify the mechanism of MMP-9 inhibitor, Mki67, in inhibiting human oral squamous cell carcinoma SCC15 cell line.

5. Conclusion

In summary, the MMP-9 inhibitor (Mki67) can reduce the expression level of MMP-9 by inhibiting the PI3K/Akt signaling pathway, thereby inhibiting the migration and invasion of human oral squamous cell carcinoma SCC15 cell line. At the same time, MMP-9 inhibitors can also induce cell apoptosis, thereby inhibiting tumor growth. Therefore, MMP-9 inhibitors may become effective drugs for the treatment of human oral squamous cell carcinoma.

Disclosure statement

The authors declare no conflict of interest.

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