IL-6 Enhances the Viability and Invasion Ability of Prostate Cancer Cells

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Abstract. Prostate cancer is a slow-growing cancer whose incidence increases with age. IL-6 expression is significantly increased in a variety of malignant tumor stroma, which is a key factor involved in the association between inflammation and tumor. So as to investigate how prostate cancer works, recombinant IL-6 was used to stimulate human prostate cancer cells (PC-3). After treatment for 24, 48, 72h, the viability of cancer cells was detected by CCK-8 experiment. The effect of recombinant IL-6 on the invasion of prostate cancer cells was analyzed by Transwell experiment. The results showed that IL-6 can increase the viability of prostate cancer cells and facilitate the invasion of prostate cancer cells.

Keywords. Prostate cancer, IL-6, cell invasion

1. Introduction

Prostate cancer is a common urinary system disease in elderly men. At present, there are more than 1 million new cases of prostate cancer in the world every year, with the incidence ranking first in male malignant tumors and the mortality ranking second, ranking second in the world's most commonly known cancers\cite{1}. The clinical symptoms of prostate cancer are similar to those of prostate hyperplasia. The main treatment methods for prostate cancer are radical resection, which can reduce the mortality of prostate cancer patients by removing the prostate gland and surrounding seminal vesicles, ejaculatory ducts and other surrounding tissues\cite{2}. Some patients with prostate cancer have metastases when they are discovered and diagnosed, and the prognosis is not good. The prognosis of prostate cancer is determined by many factors, and even the pathological diagnosis and Gleason score cannot accurately judge the clinical prognosis\cite{3}. Therefore, we need to further explore the mechanism of prostate cancer and have a more comprehensive understanding of prostate cancer. Serum Interleukin-6 (IL-6), a cytokine in the chemokine family, is significantly increased in a variety of malignant tumor stroma, and the proliferation and differentiation of tumor cells can be stimulated by affecting cell adhesion and tumor-specific antigen expression\cite{4-5}. In addition, cancer cells are protected from treatment-induced DNA damage, apoptosis, and oxidative stress by IL-6\cite{6}.

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In this study, we selected human prostate cancer cells: PC-3 for the experiment; Recombinant IL-6 was used to stimulate prostate cancer cells for 24, 48, 72 h and CCK-8 experiment was used to detect the viability of cancer cells. The effect of recombinant IL-6 on the invasion of prostate cancer cells was analyzed by Transwell experiment.

2. Methods

2.1. Cell culture

Using MEM complete culture medium containing 10% fetal bovine serum and 1% penicillin streptomycin, prostate cancer cells (PC-3) were cultured, the condition of cell incubator is 37°C and 5% CO₂.

2.2. The CCK-8 experiment detected cell proliferation

Cells from the logarithmic growth stage of PC-3 were digested and centrifuged, and the cell density was adjusted to 5×10⁴ cells /ml. The PC-3 cell suspension of logarithmic growth stage was added to the 96-well plate, and then cultured for 24 hours. The control group and the experimental group were subjected to CCK-8 experiment after 24, 48 and 72 hours of intervention. Add 10μl CCK8 solution to each well, and there should be no bubbles when adding the sample. The 96-well plates were placed in the incubator and continued incubation for 1 hour. The absorbance (A) of each well sample at 450nm was measured by enzyme-labeler, and cell viability was calculated.

2.3. Transwell experiment was used to detect cell invasion ability

At the same time, the sterile 24-well plate and Transwell chamber were pre-cooled in the refrigerator at 4°C and operated on the ice. 100μl Matrigel was laid flat at the bottom of the Transwell chamber and kept overnight at 4°C, so that Matrigel could be levelled in the chamber. The 24-well plates were taken out and a 1h incubation period is required to coagulate Matrigel. After the cells of logarithmic growth stage were digested by pancreatic enzymes, the cell density was counted and centrifuged at 1800rpm for 5min. The supernatant was discarded and a certain volume of medium without serum was added, adjust the cell density to 5×10⁵ cells/ml. In a 24-well plate, Transwell chambers were placed, Medium containing 100 μl cell suspension and 600μl 10% fetal bovine serum were added to the upper chamber and lower chamber respectively. Upper chamber cells were incubated for 24h. After the chamber was removed, Upper chamber cells were gently wiped off with cotton swabs and cleaned with ultra-pure water three times. Add 0.5ml methanol to each hole of the 24-well plate, fix the Transwell chamber in the 24-well plate chamber containing methanol for 30min, clean with ultra-pure water for 3 times, each time for 3min. To each hole, add 0.5ml of crystal violet solution and place the Transwell chamber in the hole containing crystal violet dyeing solution. After 30min, the ultrapure water was cleaned for 3 times, 5min each time, the chamber was inverted on the
filter paper, dried naturally, and photographed under the optical microscope with a magnification of 20×.

2.4. Statistics

Used IBM SPSS 17.0 to perform the statistical analysis. One-Way ANOVA analysis module was used to conduct variance analysis on the original data obtained from the experiment, in terms of mean ± standard deviation (X±SD) and the experimental group had a significant difference from the control group based on p <0.05.

3. Results

3.1. Recombinant IL-6 can improve the viability of prostate cancer cells

In order to observe the effect of recombinant IL-6 on the viability of PC-3 cells in prostate cancer cells, we verified it by CCK-8 experiment. A distinct difference in cell viability was observed between groups treated with IL-6 and those that were not, it has been suggested that exogenous recombinant IL-6 could enhance PC-3 cell viability in prostate cancer, as shown in Figure 1.

![Fig. 1 Effect of IL-6 on the viability of prostate cancer cells (*p<0.05, **p<0.01)](image)

3.2. Recombinant IL-6 promotes invasion of prostate cancer cells

So as to verify the recombinant IL-6 effect on the invasion ability of PC-3 cells in prostate cancer cells, Transwell assay was performed. As can be seen from Fig2, the PC-3 cell number in the microscope field (20×) increased significantly after the addition of exogenous recombinant IL-6 compared with the control group. As can be seen from Fig3, the number of PC-3 cells was approximately doubled then the control group. It has been suggested that exogenous recombinant IL-6 can improve the invasion ability of PC-3 in prostate cancer cells.
Prostate cancer is more common in the elderly. With the aging of the population, the incidence of prostate cancer shows an increasing trend year by year. Clinical etiology of prostate cancer is not clear, the related reasons are genetic, age, sex hormones and so on. Surgical resection is an effective means to treat prostate cancer. Prostate cancer survivors and their quality of life can be improved through resection of diseased tissue. Some patients with prostate cancer have poor prognosis after operation, such as metastasis and recurrence. IL-6 is a proinflammatory cytokine with a relative molecular weight of 26000 and composed of 185 amino acids produced by immune cells[7-8], tumor-related cells and other cells, it can exert various biological activities through receptor pathway. IL-6 can promote the expression of Interleukin-2r (IL-2r) on T cell surface by stimulating T cells and activating B cell proliferation. Interleukin-1 (IL-1) and Tumor necrosis factor (TNF) enhance the mitotic function of T helper cells (TH) and promote cell proliferation and growth in the body.
Studies have shown that there is a tendency of overexpression of IL-6 in the serum of prostate cancer patients. IL-6 can regulate the process of cell growth and proliferation through the phosphatidylinositol kinase-protein kinase B signaling pathway, regulate the proliferation and differentiation of tumor cells, improve the infiltration and invasion ability of tumor cells, and aggravate the occurrence and development of prostate cancer. IL-6 is an important participant in the chronic prostatitis response and the progression of prostate cancer, and is a key mediator for the occurrence of prostate tumors, growth stimulation, Prostate cancer metastasis and phenotypic induction, progression to castration-resistant state, and chemotherapy resistance. IL-6 expression not only activates the IL-6 autocrine ring and paracrine IGF signaling axis, but also activates the downstream JAK-STAT3 signaling pathway, reprogramming the expression of prostate cancer genes and enhancing the inflammatory response in local tissues and periprostatic adipose tissue, suggesting that IL-6 is an autonomous prostate cancer gene. Induces prostate cancer by amplifying the local inflammatory response[9].

The results of this study show that IL-6 intervention in PC-3 prostate cancer cells can improve the activity of prostate cancer cells and promote the invasion of prostate cancer cells. Analysis of the reasons: IL-6 can regulate the process of cell growth and proliferation through the phosphatidylinositol kinase-protein kinase B signaling pathway, regulate the proliferation and differentiation of tumor cells, and improve the infiltration and invasion ability of tumor cells. IL-6 expression also activates the downstream JAK-STAT3 signaling pathway, reprogramming the expression of prostate cancer genes, enhancing the inflammatory response in local tissues and periprostatic adipose tissue, and exacerbating the occurrence and development of prostate cancer.

In summary, we used 20ng/ml recombinant IL-6 to intervene in PC-3 prostate cancer cells, and verified the activity and invasion ability of IL-6 on prostate cancer cells through CCK8 and Transwell experiments. The experimental results showed that IL-6 could improve the activity in prostate cancer cells and promote the invasion of prostate cancer cells.

5.Conclusions

The results of this study showed that recombinant IL-6 could improve the activity of and the invasion ability of PC-3 prostate cancer cells. It can provide some reference for the prevention and drug development of prostate cancer.

Reference


