IL-9 PROMOTES THE GROWTH OF PANCREATIC CANCER CELLS BY ACTIVATING THE JAK/STAT3 PATHWAY

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ABSTRACT

Objective: *To explore the effect and mechanism of interleukin 9 (IL-9) on the growth of pancreatic cancer cells by activating Janus kinase (JAK)/signal transduction and transcription activator 3 (STAT3) pathway.*

Methods: *Human pancreatic cancer cell line PANC-1 was collected for routine passage, freezing, and resuscitation. PANC-1 cells were then randomly divided into an NC group, a 5 ng/mL IL-9 group, a 10 ng/mL IL-9 group, and a 20 ng/mL IL-9 group. The NC cell group was a blank control without any treatment, while the 5 ng/mL IL-9 group, 10 ng/mL IL-9 group, and 20 ng/mL IL-9 group cells were respectively treated with 5, 10, and 20 ng/mL concentration IL-9 for 24 hours. The cell proliferation rate, apoptosis rate, IL-9R mRNA expression level, and expression levels of STAT3 signaling pathway-related proteins such as p-STAT3 and STAT3 were compared across the groups. The remaining PANC-1 cells were randomly divided into a 0 μmol/L AG490 (a JAK/STAT3 signaling pathway blocker) group, a 20 μmol/L AG490 group, a 40 μmol/L AG490 group, and an 80 μmol/L AG490 group, which respectively received 0, 20, 40, and 80 μmol/L AG490 treatment for 24 hours. Differences in cell proliferation rate and expression levels of STAT3 signaling pathway-related proteins such as p-STAT3 and STAT3 were then compared.*

Results: *The proliferation rate of PANC-1 cells in the 10 ng/mL IL-9 group and 20 ng/mL IL-9 group was significantly higher than that in the NC group ($P<0.05$). The proliferation rate of PANC-1 cells in the 5 ng/mL IL-9 group was not statistically different from that of the NC group ($P>0.05$). The apoptosis rate of PANC-1 cells in the IL-9 concentration group was not statistically different from cells of the NC group ($P>0.05$). The expression levels of IL-9R mRNA in PANC-1 cells of the 10 ng/mL IL-9 group and 20 ng/mL IL-9 group were significantly higher than in cells of the NC group ($P<0.05$). The IL-9R mRNA expression level of PANC-1 cells in the 5 ng/mL IL-9 group was not significantly different from that of cells in the NC group ($P>0.05$). The expression levels of p-STAT3 protein in PANC-1 cells of 10 ng/mL IL-9 group and 20 ng/mL IL-9 group were significantly higher than in the NC group ($P<0.05$). The expression level of STAT3 protein in PANC-1 cells in IL-9 concentration group was not significantly different from that of cells in the NC group ($P>0.05$). The PANC-1 cell proliferation rate and p-STAT3 protein expression level in the 80 μmol/L AG490 group were significantly lower than those in the 0 μmol/L AG490 group ($P<0.05$). There was no significant difference in the expression level of STAT3 protein in PANC-1 cells of the AG490 concentration group ($P>0.05$).*

Conclusion: *IL-9 can promote the growth of pancreatic cancer cells, which may be achieved by activating the JAK/STAT3 signaling pathway.*

Keywords: *IL-9, STAT3, pancreatic cancer, IL-9R.*

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Introduction

Pancreatic cancer is an illness of the digestive system with a very high degree of malignancy, and it is the main cause of tumor-related deaths in developed countries[(1)]. Epidemiological surveys show that in 2012, there were 338,000 new cases of pancreatic cancer and more than 330,000 deaths worldwide. It is expected that the disease will surpass colorectal cancer and breast cancer in 2030 and become the second most common cause of tumor-related deaths, and the 5-year survival rate is only 6%.

Although the incidence of pancreatic cancer in my country is lower than in developed countries, it is still increasing year by year[(2)]. Pancreatic cancer is mainly divided into pancreatic ductal adenocarcinoma and pancreatic endocrine tumors; the former accounts for 85% of the total incidence of

pancreatic cancer, with the latter making up less than 5%[3]. Clinical reports show that 18.4% of pancreatic cancer patients are in stage I or II at the time of diagnosis, while 81.6% of patients are in stage III or IV and have local invasion and metastasis, resulting in unsatisfactory clinical effects and poor prognosis[4].

About 20% of pancreatic cancer patients can undergo surgical treatment. However, the risk of recurrence of this disease after surgery is extremely high, and radiochemotherapy and biological targeted therapy can only be used as auxiliary means to improve the quality of life of patients[5]. It is currently known that genetics, diabetes, dietary structure, and other factors can all cause pancreatic cancer, but its specific pathogenesis is not yet clear, especially its high invasiveness. Therefore, uncovering the molecular mechanisms and regulatory pathways involved in the onset and progression of pancreatic cancer is of great significance for the prevention and treatment of the disease. Phosphorylated Janus kinase (JAK)/signal transducers and activators of transcription 3 (STAT3) have important roles in transmitting many growth factors and cytokine signaling pathways. It can mediate many processes such as cell proliferation, migration, and invasion, as well as regulate the development of many diseases[6].

Interleukin 9 (IL-9) is one of the cytokines that clinicians have paid close attention to in recent years. Previous studies have shown that IL-9 plays a key role in the pathogenesis of many chronic inflammatory diseases and autoimmune diseases[7].

However, there are few reports on IL-9 and pancreatic cancer, so this study will analyze the biological effects of IL-9 on pancreatic cancer cells and the changes in the JAK/STAT3 pathway during this process. The reports are as follows.

Materials and methods

Subject

Human pancreatic cancer cell line PANC-1 was purchased from Shenzhen Haodi Huatuo Biotechnology Co., Ltd.

Main reagents and instruments

Reagents

Recombinant human interleukin 9 was purchased from Nanjing Saihongrui Biotechnology Co., Ltd.; 0.25% trypsin digestion was purchased from Wuhan Hualianke Biotechnology Co., Ltd.; RNA reverse transcription kit was purchased from Beijing Huada Protein R&D Center Co., Ltd.; a real-time PCR kit was purchased from Wuxi Xinrun Biotechnology Co., Ltd.; PCR primers were purchased from Shanghai Kanglang Biotechnology Co., Ltd.; a CCK-8 kit was purchased from Shanghai Yanxi Biotechnology Co., Ltd.; the primary antibody dilution was purchased from Beijing Kairuiji Biotechnology Co., Ltd.; RIPA protease lysate was purchased from Anhui Jingke Biotechnology Co., Ltd.; a BCA protein quantification kit was purchased from Wuhan Yunclo Diagnostic Diagnostics Research Institute Co., Ltd.; AG490 rabbit anti-human was purchased from Shanghai Qiaoyu Biotechnology Co., Ltd.; GAPDH internal reference antibody was purchased from Xiamen Huijia Biotechnology Co., Ltd.; rabbit anti-human STAT3 was purchased from Shanghai Jianglai Biotechnology Co., Ltd.; rabbit anti-human phosphorylated p-STAT3 (Y-705) antibody was purchased from Shanghai Yanmu Industrial Co., Ltd.; goat anti-rabbit secondary antibody was purchased from Beijing Taize Jiaye Technology Development Co., Ltd.

Equipment

A sterile ultra-clean workbench was purchased from Jinan Bohang Scientific Instrument Co., Ltd.; a flow cytometer was purchased from Changzhou Bida Biotechnology Co., Ltd.; a fluorescent quantitative PCR amplification instrument was purchased from Bunsen (Tianjin) Health Technology Co., Ltd. ; a -80°C ultra-low temperature refrigerator was purchased from Shanghai Shiwei Experimental Instrument Technology Co., Ltd.; an aseptic ultra-clean workbench was purchased from Shanghai Shiwei Experimental Instrument Technology Co., Ltd.; an inverted microscope was purchased from Dongguan Spectrum Standard Experimental Equipment Technology Co., Ltd.

Grouping and methods

• The human pancreatic cancer cell line PANC-1 with good growth status was taken for routine digestion, followed by cell passage, once every two days, and whether to change the medium was determined according to the color of the medium. Cells in the logarithmic growth phase were collected, frozen and placed in liquid nitrogen for long-term storage. The PANC-1 cells were recovered in a constant-temperature water bath at 37°C, and the cells were passaged again when they were covered with wallpaper to about 80%.

• Digest PANC-1 cells with 0.25% trypsin, select the PANC-1 cells in logarithmic growth phase after centrifugation, add a certain amount of medium to resuspend them, adjust the cell density to 1×10(5) cells/mL, and collect 100 μL of the single cell suspension planting in a 96-well plate, 1×10(5) per well. Some PANC-1 cells were randomly divided into an NC group, a 5 ng/mL IL-9 group, a 10 ng/mL IL-9 group, and a 20 ng/mL IL-9 group. The NC group cells were blank controls without any treatment. Cells in the 5ng/mL IL-9 group, 10 ng/mL IL-9 group, and 20 ng/mL IL-9 group were respectively treated with 5, 10, and 20 ng/mL IL-9 for 24 hours. The CCK-8 method, flow cytometry, the real-time fluorescence quantitative PCR method, and western blotting were used to detect the cell proliferation rate and apoptosis rate, as well as the IL-9R mRNA and STAT3 signaling pathway-related protein (p-STAT3, STAT3) expression levels of each group.

• The remaining PANC-1 cells were randomly divided into a 0 μmol/L AG490 (a JAK/STAT3 signaling pathway blocker) group, a 20 μmol/L AG490 group, a 40 μmol/L AG490 group, and an 80 μmol/L AG490 group, which received treatment with AG490 at their respective concentrations for 24 hours. The cell proliferation rate and the expression levels of STAT3 signaling pathway related proteins (p-STAT3, STAT3) were detected by the CCK-8 method and western blotting, respectively.

Statistical methods

The measurement data of PANC-1 cell proliferation rate and apoptosis rate of each group are expressed by ($\bar{x}$±s), the comparison between the two groups is tested by the LSD method, and the comparison between multiple groups is made with single-factor analysis of variance. All the data in this study are used SPSS 18.0 processing. P<0.05 is considered statistically significant.

Results

Comparison of PANC-1 cell proliferation rate and apoptosis rate in each group

The proliferation rate of PANC-1 cells in the 10ng/mL IL-9 group and the 20ng/mL IL-9 group was significantly higher than that in the NC group (P<0.05). The proliferation rate of PANC-1 cells in the 5ng/mL IL-9 group was not statistically different from that in NC group (P>0.05). The apoptosis rate of PANC-1 cells in the IL-9 concentration group was not statistically different from that in the NC group (P>0.05). See Table 1.

Group	Proliferation Rate (%)	Apoptosis Rate (%)
NC Group	1.00±0.01	4.80±1.01
5 ng/mL IL-9 Group	1.13±0.15	4.79±1.03
10 ng/mL IL-9 Group	1.28±0.11*	4.11±1.11
20 ng/mL IL-9 Group	1.45±0.12*	4.03±1.00

Table 1: Comparison of PANC-1 cell proliferation rate and apoptosis rate in each group ($\bar{x}$±s).
*Note: compared with the NC group, *P>0.05.*

Comparison of IL-9R mRNA expression levels in PANC-1 cells of each group

The expression level of IL-9R mRNA in PANC-1 cells of the 10 ng/mL IL-9 group and 20 ng/mL IL-9 group was significantly higher than that of NC group (P<0.05). The IL-9R mRNA expression level of PANC-1 cells in the 5 ng/mL IL-9 group was not significantly different from that of cells in the NC group (P>0.05). See Table 2.

Group	IL-9R
NC Group	1.00±0.01
5 ng/mL IL-9 Group	1.77±0.05
10 ng/mL IL-9 Group	1.99±0.11*
20 ng/mL IL-9 Group	2.88±0.19*

Table 2: Comparison of IL-9R mRNA expression levels in PANC-1 cells of each group ($\bar{x}$±s).
*Note: compared with NC group, *P>0.05.*

Comparison of p-STAT3 and STAT3 protein expression levels of PANC-1 cells in each group

The expression levels of p-STAT3 protein in PANC-1 cells of the 10 ng/mL IL-9 group and 20 ng/mL IL-9 group were significantly higher than in cells of the NC group (P<0.05). The expression level of STAT3 protein in PANC-1 cells in the IL-9 concentration group was not significantly different from that of cells in the NC group (P>0.05). See Table 3 and Figure 1.

Group	p-STAT3	STAT3
NC Group	1.11±0.04	1.09±0.02
5 ng/mL IL-9 Group	1.25±0.01	1.02±0.04
10 ng/mL IL-9 Group	1.78±0.06*	1.15±0.06
20 ng/mL IL-9 Group	1.96±0.05*	1.21±0.05

Table 3: Comparison of p-STAT3 and STAT3 protein expression levels of PANC-1 cells in each group ($\bar{x}$±s).
*Note: compared with the NC group, *P>0.05.*

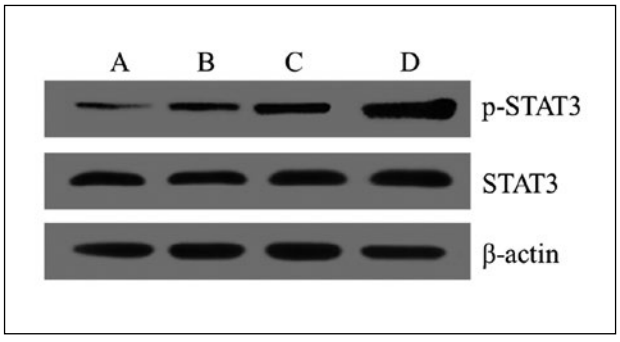

Figure 1: Comparison of p-STAT3 and STAT3 protein expression levels of PANC-1 cells in each group.
Note: A: NC group; B: 5 ng/mL IL-9 group; C: 10 ng/mL IL-9 group; D: 20 ng/mL IL-9 group.

Comparison of PANC-1 cell proliferation rate, p-STAT3 and STAT3 protein expression levels in each group

The PANC-1 cell proliferation rate and p-STAT3 protein expression level in the 80 μmol/L AG490 group were significantly lower than those in the 0 μmol/L AG490 group ($P<0.05$). There was no significant difference in the expression level of STAT3 protein in PANC-1 cells in the AG490 concentration group ($P>0.05$). See Table 4 and Figure 2.

Group	Proliferation Rate (%)	p-STAT3	STAT3
0 μmol/L AG490 Group	1.35±0.15	1.75±0.26	1.00±0.11
20 μmol/L AG490 Group	1.31±0.14	1.68±0.20	1.15±0.15
40 μmol/L AG490 Group	1.28±0.15	1.50±0.19	1.01±0.10
80 μmol/L AG490 Group	0.99±0.11*	1.15±0.05*	0.98±0.09

Table 4: Comparison of p-STAT3 and STAT3 protein expression levels of PANC-1 cell proliferation rate in each group ($\bar{x}\pm s$).
*Note: compared with the NC group, *P>0.05.*

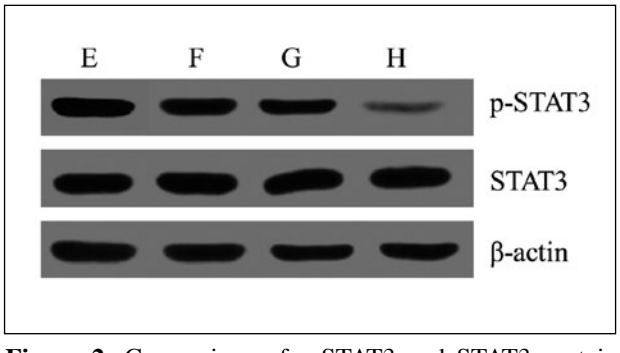

Figure 2: Comparison of p-STAT3 and STAT3 protein expression levels of PANC-1 cell proliferation rate in each group.
Note: E: 0 μmol/L AG490 group; F: 20 μmol/L AG490 group; G: 40 μmol/L AG490 group; H: 80 μmol/L AG490 group.

Discussion

Pancreatic cancer has a unique microenvironment, which is mainly composed of extracellular matrix and non-tumor cells. It has the characteristics of immunosuppression and immune escape and can promote tumor growth and metastasis. This process mainly includes the release of immunosuppressive factors and local obstacles to tumor-infiltrating lymphocytes[8]. Clinical reports have confirmed that pancreatic cancer cells can release relevant immunosuppressive cytokines, which in turn play a role in immune surveillance, such as IL-10. Another report shows that IL-22RA1 is upregulated in pancreatic cancer tissues, and can exert self-renewal, tumorigenicity, and metastasis, which are similar to stem cell functions, and IL-22RA1 expression level is negatively correlated with the prognoses of pancreatic cancer patients. In addition, IL-22RA1 has the function of promoting the growth of pancreatic stem cells, and its mechanism of action may be achieved through the IL-22RA1/STAT3 signaling pathway. Therefore, finding a molecular mechanism that can reveal the onset of pancreatic cancer is of great significance to prolonging patients' survival time and improving their quality of life.

IL-9 was first discovered in 1988 and is considered to be a T cell growth factor. With deeper research, it was found that the IL-9 amino acid clone sequence is not the same as other T cell growth factors, and it can also act on lymphocytes and bone marrow cells[9]. Relevant research on malignant pleural effusions of lung cancer shows that the number of Th9 cells in patients with malignant pleural effusions is significantly higher than that of peripheral blood, and IL-9 and IL-17 can significantly promote the proliferation and migration of lung cancer cells in the state of STAT3 signal activation. Another report shows that IL-9 plays an important role in the progression of chronic gastritis to gastric cancer, and studies have found that its expression level in gastric precancerous lesions is closely related to mast cells, but its mechanism of action has not been fully elucidated. In addition, in gastric precancerous lesions with Helicobacter pylori infection, the positive rate of IL-9 expression was positively correlated with the degree of tissue inflammation, atrophy, and intestinal metaplasia[10]. Studies related to diffuse large B lymphoma show that the expression level of IL-9 in tumor tissues increases, and each concentration of IL-9 can promote the

proliferation of tumor cells and hinder apoptosis[11]. In this study, we intervened with PANC-1 pancreatic cancer cells using different concentrations of IL-9 and found that IL-9 can only promote tumor cell proliferation, while the effect on apoptosis is not obvious. This may be due to different mechanisms of IL-9 in the microenvironment. In addition, the expression level of IL-9R mRNA in PANC-1 cells rose with the increase of IL-9 concentration[12]. This suggests that the expression of IL-9 is increased in pancreatic cancer cells. STAT3 is currently known to be a carcinogen for many epithelial malignancies, and clinical studies have shown that STAT3 expression levels are abnormally increased[13]. Some scholars found that IL-6 has the function of promoting pancreatic intraepithelial neoplasia to pancreatic cancer, which may be achieved through the STAT3/SOCS3 signaling pathway. Later, the scholar found that in an animal model of pancreatic cancer, inhibition of IL-6R expression can increase the clinical treatment effect of pancreatic ductal adenocarcinoma[14-15].

In this study, we found that the expression level of p-STAT3 protein increased in each group of PANC-1 cells after IL-9 intervention, but the total STAT3 protein expression level did not change significantly. In order to reveal the relationship between IL-9 and the JAK/STAT3 signaling pathway, this study selected AG490 to treat PANC-1 cells. The results showed that the proliferation rate and expression level of p-STAT3 protein of PANC-1 cells in the 80μmol/L AG490 group were significantly lower than in the 0μmol/L AG490 group.

In summary, IL-9 can promote the growth of pancreatic cancer cells, which may be achieved by activating the JAK/STAT3 signaling pathway.

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