Insulin Promotes Proliferative Vitality and Invasive Capability of Pancreatic Cancer Cells via Hypoxia-inducible Factor 1α Pathway*

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Summary: This study examined whether insulin-stimulated hypoxia-inducible factor 1α (HIF-1α) expression plays a crucial role in promoting the proliferative vitality and invasive capability in human pancreatic cancer cells. PANC-1 cells were divided into three groups: Control group, insulin group and insulin+YC-1 (a pharmacological inhibitor of HIF-1α) group in terms of different treatments.

Cells in the insulin group or insulin+YC-1 group were treated with insulin (0.1, 1, 10 and 100 nmol/L) alone or combined with 3-(5’-hydroxymethyl-2’-furyl)-1-benzyl indazole (YC-1, 0.1, 1, 10 and 100 μmol/L). HIF-1α mRNA and protein expression in PANC-1 cells was determined by real-time RT-PCR and Western blotting respectively. Cell proliferation and invasion were measured by using growth curve and invasion assay, respectively. Western blot analysis demonstrated that insulin dose-dependently increased the HIF-1α protein expression, and YC-1 could dose-dependently block this effect. However, neither insulin nor YC-1 altered HIF-1α mRNA levels in PANC-1 cells. Moreover, insulin could enhance the proliferation and invasion of PANC-1 cells, while YC-1 could weaken this effect. It was concluded that the malignant proliferation and local invasion of pancreatic cancer cells may be related to high-insulin microenvironment. The tumor biological behavior change resulting from high-insulin microenvironment may be associated with the increased expression of HIF-1α protein.

Key words: pancreatic cancer cell; HIF-1α; YC-1; tumor microenvironment; proliferation; invasion

Pancreatic cancer, 90% of which is ductal adenocarcinoma (PDA), is considered to be one of the most aggressive human cancers[1]. At early stage, PDA has a predisposition to aggressively invade peripheral tissues. When the diagnosis of PDA is clarified, local invasion has taken place in more than 40% of patients who tend to lose the opportunity of radical surgical resection.

In the pancreas, the blood leaving endocrine islets streams through permeable vessels and delivers insulin into the interstitial fluid[2]. When PDA occurs, pancreatic cancer cells are exposed to high-insulin microenvironment, which is remarkably different from the other malignant carcinomas. For insulin can stimulate the proliferation and substantially enhance the invasive activities of pancreatic cancer cells in vitro[3], high-insulin microenvironment was proposed to play an important role in promoting growth and invasion of PDA. However, the regulation involved in this process remains unknown.

Previous studies have shown that the intensive expression of HIF-1α protein in PDA, which has been shown to be regulated by oxygen level, various growth factors and hormones[4, 5], was associated with tumor angiogenesis, invasion and metastasis[6–9]. According to some previous study, the expression level of HIF-1α in anoxic tumor cells in the center of pancreatic cancer was not higher than that in relatively well-oxygenated tumor cells at invasive margins, which indicated an oxygen-independent regulation of HIF-1α[10]. As insulin could enhance the expression of HIF-1α protein[11], we deduce that insulin up-regulates the HIF-1α protein expression in the tumor cells at invading margins and then substantially enhances the invasiveness of pancreatic cancer.

In this study, the effects of insulin on the HIF-1α mRNA and protein expression and biological behaviors of human pancreatic cancer cell line PANC-1 were examined. YC-1, which is a pharmacological inhibitor of HIF-1α, was used to inhibit HIF-1α protein expression. Our findings showed that up-regulation of HIF-1α is the key molecular mechanism in insulin-induced proliferation and invasion of pancreatic cancer cells.
1 MATERIALS AND METHODS

1.1 Cell line and Reagents
The human pancreatic cancer cell line PANC-1 was obtained from ATCC, USA. RPMI1640 was purchased from Gibco, USA. Fetal bovine serum was provided by Sijiqing Inc, China. Insulin and YC-1 were bought from Sigma, USA. The antibody to HIF-1α, GAPDH and Matrigel were procured from BD Bioscience, USA. The HRP-conjugated secondary antibodies were products from Santa Cruz, USA.

1.2 Cell Culture
The PANC-1 cells were cultured in RPMI1640 supplemented with 10% (v/v) heat inactivated fetal bovine serum, 100 μg/mL penicillin and 100 μg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO2.

1.3 Western Blotting
Cells were chilled to 4°C, washed with ice-cold phosphate-buffered saline and solubilized with lysis buffer for 20 min at 4°C. The lysate was centrifuged for 5 min at 12 000 r/min at 4°C. Extracts were separated on a 10% SDS-PAGE gel and then transferred to a polyvinylidene difluoride membrane (Amersham Biosciences, USA). After blocked in 5% defat milk, the membrane was incubated with primary antibodies (mouse anti-HIF-1α, 1:2000; goat anti-GAPDH, 1:10000) at 4°C overnight. After washing in TBST, the membrane was incubated with appropriate HRP-conjugated secondary antibodies (rabbit anti-mouse-HRP, 1:5000; rabbit anti-goat-HRP, 1:5000) at room temperature for 1 h. The ECL system (Amersham Biosciences, USA) was used for detecting bands. Quantification of band’s density was performed by using BioRad Software Quantity One. We used the expression ratio (HIF-1α/GAPDH) to evaluate HIF-1α protein expression. Each experiment was carried out in triplicate.

1.4 Quantitative Real-time RT-PCR
Total cellular RNA was prepared from 5×10^6 cells by Trizol (Invitrogen, USA) according to the manufacturer’s instructions. One μg of RNA was reverse-transcribed by using a Roche First-Strand cDNA synthesis kit. Real-time PCRs were performed with an Applied Biosystems 7000 SDS, by using SYBR green for detecting bands. Quantification of band’s density was performed by using BioRad Software Quantity One. We used the expression ratio (HIF-1α/GAPDH) to evaluate HIF-1α protein expression. Each experiment was carried out in triplicate.

2 RESULTS

2.1 Effects of Insulin on the HIF-1α Expression in PANC-1 Cells
PANC-1 cells were incubated with different concentrations of insulin (0, 0.1, 1, 10 and 100 nmol/L) for 4 h, and then they were harvested. Western blotting and real-time RT-PCR was used to analyze the level of HIF-1α protein and mRNA expression respectively. The results showed that HIF-1α protein was weakly expressed in PANC-1 cells which were cultured in the absence of insulin. The HIF-1α protein expression was dose-dependently increased in insulin-treated PANC-1 cells (P<0.05) (fig. 1A). However, no difference was found in HIF-1α mRNA expression between PANC-1 cells cultured in control media and in insulin-contained media (P>0.05) (fig. 1B).

2.2 Effects of YC-1 on the HIF-1α Expression in PANC-1 Cells
PANC-1 cells were incubated with 100 nmol/L insulin and different concentrations of YC-1 (0, 0.1, 1, 10 and 100 μmol/L) for 4 h, and then cells were harvested. PANC-1 cells cultured in normal condition acted as control. Western blotting and real-time RT-PCR were used to analyze the level of HIF-1α protein and mRNA expression respectively. The results showed that YC-1 could reduce the expression of HIF-1α protein induced by the insulin in a dose-dependent manner. The decreased HIF-1α protein expression in PANC-1 cells could be detected in the presence of 0.1-μmol/L YC-1 (P<0.05). When PANC-1 cells were treated with 100-nmol/L insulin and 10-μmol/L YC-1, the expression of HIF-1α protein of the cells was almost the same as control cells.