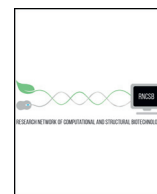




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Hyperglycemia aggravates microenvironment hypoxia and promotes the metastatic ability of pancreatic cancer

Wei Li^{a,#}, Han Liu^{b,#}, Weikun Qian^a, Liang Cheng^a, Bin Yan^a, Liang Han^a, Qinhong Xu^a, Qingyong Ma^{a,*}, Jiguang Ma^{c,*}

^a Department of Hepatobiliary Surgery, First Affiliated Hospital of Xi'an Jiaotong University, 277 West Yanta Road, Xi'an 710061, China

^b Department of Hepatobiliary Surgery, Qilu Hospital of Shandong University, Shandong 250012, China

^c Department of Anesthesiology, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China

ARTICLE INFO

Article history:

Received 22 August 2018

Received in revised form 7 October 2018

Accepted 12 October 2018

Available online 29 October 2018

Keywords:

Hyperglycemia

Hypoxia

HIF-1 α

Metastasis

Pancreatic cancer

ABSTRACT

Background: Diabetes mellitus and pancreatic cancer are intimately related. Our previous studies showed that high levels of blood glucose promote epithelial-mesenchymal transition of pancreatic cancer. In this study, we evaluated the relationship between hyperglycemia and hypoxic tumor microenvironments.

Methods: HIF-1 α expression was evaluated by immunohistochemistry in clinical pancreatic cancer tissues with or without diabetes mellitus. Statistical analysis was performed to explore the relationship between HIF-1 α expression and pathological features of patients with pancreatic cancer. In vivo and in vitro models was established to detect whether a hyperglycemia environment could cause hypoxia in the pancreatic parenchyma and promote pancreatic cancer. In addition, we also tested the effect of HIF-1 α siRNA on the high glucose-induced invasive and migratory abilities of BxPC-3 cells in culture.

Result: Our data showed that pancreatic cancer patients with diabetes had a higher level of HIF-1 α expression as well as biliary duct invasion and larger tumor volumes than individuals in the euglycemic group. Diabetic nude mice treated with streptozotocin (STZ) exhibited larger tumors and were more likely to develop liver metastasis than control mice. Acinar cells of the pancreas in diabetic mice showed an obvious expansion of the endoplasmic reticulum and increased nuclear gaps as well as chromatin close to the cellular membrane in some acinar cells. The expression area for Hypoxyprobe-1 and HIF-1 α in the diabetic orthotopic xenograft group was larger than that in the control group. The expression level of HIF-1 α in the BxPC-3 cancer cell line increased in response to high glucose and CoCl₂ concentrations. The high glucose-induced invasive ability, migratory capacity and MMP-9 expression were counter-balanced by siRNA specific to HIF-1 α .

Conclusion: Our results demonstrate that the association between hyperglycemia and poor prognosis can be attributed to microenvironment hypoxia in pancreatic cancer.

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1. Background

Pancreatic cancer is one of the deadliest types of malignant carcinoma and is typically diagnosed at a late stage, with a 3% 5-year survival rate in the US [1]. In China, pancreatic cancer is the sixth leading cause of cancer death, and the estimated numbers of newly diagnosed cases and deaths were 80,344 and 72,723, respectively, in 2011 [2]. Surgical

resection remains the best chance at long-term survival for pancreatic cancer. However, most patients are diagnosed with the unresectable form because of metastasis [3]. The causes of pancreatic cancer are still not known, although certain risk factors have been identified, including smoking, obesity, and diabetes mellitus [4].

Evidence is accumulating that diabetes is associated with pancreatic cancer development and progression as well as death in pancreatic cancer patients [5,6]. However, the mechanism of this phenomenon remains unknown. Our previous studies found that high glucose levels could promote pancreatic cancer proliferation and invasion as well as epithelial-mesenchymal transition (EMT) and metastasis [7,8]. Recent studies have proven that hyperglycemia can induce cellular hypoxia and generate mitochondrial reactive oxygen species [9].

Pancreatic tumors are generally hypoxic due to their avascular morphology [10]. In a hypoxic environment, pancreatic cancer cells express high levels of the hypoxia-inducible factor 1 α (HIF-1 α). The target

Abbreviations: EMT, epithelial-mesenchymal transition; HIF-1 α , hypoxia-inducible factor 1 α ; STZ, streptozotocin; TEM, transmission electron microscopy; CoCl₂, cobalt chloride; GDNF, glial cell line-derived neurotrophic factor; EGF, epidermal growth factor; SOD, superoxide dismutase; H₂O₂, hydrogen peroxide; PNI, perineural invasion; PSC, pancreatic stellate cells; ECM, endothelial cells, extracellular matrix; CCL2, chemical chemokine 2; VEGF, vascular endothelial growth factor.

* Corresponding authors.

E-mail addresses: qyma56@mail.xjtu.edu.cn (Q. Ma), jgma86@mail.xjtu.edu.cn (J. Ma).

Contributed equally.

<https://doi.org/10.1016/j.csbj.2018.10.006>

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genes of HIF-1 α encourage an aggressive phenotype, promoting tumor growth, invasion and metastasis [11]. Hyperglycemia and cellular hypoxia are intimately related. In tumor cells, high glucose levels are known to promote HIF-1 α expression under both normoxic and hypoxic conditions [12]. In this study, we assumed that hyperglycemia promotes the progress of pancreatic cancer by inducing a hypoxic microenvironment by regulating HIF-1 α .

To test this hypothesis, the expression of HIF-1 α and the clinical and pathological features of patients with pancreatic cancer in the First Affiliated Hospital of Xi'an Jiaotong University were analyzed. The diabetes orthotopic xenograft model was also established to detect whether a hyperglycemia environment could cause hypoxia in the pancreatic parenchyma and lead to pancreatic cancer. In addition, we also tested the effect of HIF-1 α siRNA on the high glucose-induced invasive and migratory abilities of BxPC-3 cells in culture. By investigating the underlying mechanism, we hope to find evidence to explain how diabetes facilitates pancreatic cancer progression.

2. Methods

2.1. Collection of tissues

From January 2012 to December 2014, 105 pancreatic ductal carcinomas were subjected to clinical examination at the First Affiliated Hospital of Xi'an Jiaotong University, China. Most of the patients were diagnosed at an advanced stage and were not suitable for surgical resection. Only 46 patients, who received a radical curative pancreatic operation with a pathologic diagnosis, and 8 normal pancreases were included in this study. The pancreatic specimens were divided into two groups according to the fasting blood glucose levels of the patients (an average long-term glucose level for patients with a history of diabetes mellitus and an average three-day glucose level after hospitalization): (1) euglycemia and (2) hyperglycemia. The study protocol and consent forms conform to the Declaration of Helsinki and were approved by the Ethical Review Board (ERB) Committee of The First Affiliated Hospital of Xi'an Jiaotong University, China. Written informed consent was obtained from all the participants.

2.2. Cell culture and reagents

The BxPC-3 cell line, obtained from the American Type Culture Collection (Manassas, VA, USA), was cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% dialyzed, heat inactivated, fetal bovine serum (FBS) and 1% penicillin-streptomycin (Sigma Aldrich, St Louis, MO, USA) in a 95% air/5% CO₂ humidified atmosphere at 37 °C. Both DMEM and FBS were purchased from HyClone (Logan, UT, USA). Streptozotocin (STZ) was acquired from Sigma Aldrich (St. Louis, MO, USA). The primary antibodies against HIF-1 α and MMP-9 were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Millicell Transwells for the invasion assays were obtained from Millipore (Billerica, MA, USA). Matrigel was from BD Biosciences (Bedford, MA, USA). Other reagents were purchased from common commercial sources. All drug solutions were freshly prepared on the day of testing.

2.3. Diabetes mouse model and orthotopic tumor model

The 5-week-old BALB/c athymic nude mice (male) were purchased from the Shanghai Experimental Animal Center. Animal care and experiments were carried out in accordance with the guidelines of the Xi'an Jiaotong University. The nude mice were grouped into euglycemic and hyperglycemic groups ($n = 10$). The hyperglycemic mice each received an intraperitoneal injection of STZ (dissolved in sodium citrate buffer) at a dose of 175 mg/kg body weight. Blood glucose levels were determined with an ACCU-CHEK Active meter. BxPC-3 cells (1×10^8) were injected in a total volume of 50 μ l PBS into the body of the pancreas. The mice were sacrificed after 8 weeks, and the tumors, livers and pancreases

were collected and analyzed. All experimental protocols were approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China.

2.4. Immunohistochemistry

Formalin fixed and paraffin embedded pancreas and pancreatic cancer tissue samples were used for the immunohistochemistry tests. In brief, the tissue sections were incubated with primary antibodies (HIF-1 α , 1:50; pimonidazole, hypoxyprobe-1 Mab, 1:100) overnight at 4 °C and then incubated with the appropriate biotinylated secondary antibody. The results were visualized using DAB, and the slides were counterstained with hematoxylin. The densitometry analysis of the immunohistochemical staining was performed using the Image-Pro Plus 6.0 software.

2.5. Transmission electron microscopy (TEM)

For transmission electron microscopy analyses, pancreas samples were initially fixed in 2.5% glutaraldehyde in PBS at 4 °C overnight, post-fixed in 1.0% osmium tetroxide for 2 h, dehydrated and embedded in Epon 812 resin, and then cut into semithin sections (1–2 μ m). Following methylene blue staining, the semithin sections were cut into thin sections (70 nm) using an ultrathin microtome. Finally, thin sections were lightly counterstained with uranyl acetate and lead citrate and examined with TEM (H-7650, Hitachi, Tokyo, Japan).

2.6. Transwell matrigel invasion assay

The 8.0 μ m pore inserts were coated with 30 μ l of Matrigel. BxPC-3 cell suspensions (5×10^4) were added to the upper chambers in DMEM containing 1% FBS. DMEM containing 20% FBS was placed in the lower chambers. After being incubated for 48 h, the non-invading cells were removed by scraping with a wet cotton swab, and the invading cells were stained with crystal violet. The invasion ability was determined by counting the stained cells on the bottom surface.

2.7. Wound healing assay

BxPC-3 cells were seeded in 24-well plates (1.0×10^5 cells/500 μ l). A sterile pipette tip was used to produce a wound line between cells after the cells grew to 90–100% confluence. Then, the cells were allowed to migrate for 24 h. Images were taken at time 0 and 24 h post-wounding using a Nikon Diaphot TMD inverted microscope ($\times 10$).

2.8. RNAi transfections

siRNA against HIF-1 α (5'- CCA CCA CUG AUG AAU UAA ATT -3', 5'- UUU AAU UCA UCA GUG GUG GTT') and a negative control siRNA (NC: 5'- UUC UCC GAA CGU GUC ACG UTT -3', 5'- ACG UGA CAG GUU CGG AGA ATT -3') were purchased from GenePharm (Shanghai, China).

2.9. Real-time quantitative PCR (qRT-PCR)

Total RNA extracted from BxPC-3 cells (Fastgen200 RNA isolation system, Fastgen, Shanghai, China) was reverse-transcribed into cDNA using the PrimeScript RT reagent Kits (TaKaRa, Dalian, China). The primer sequences were as follows:

MMP-9-F: 5'- GCA ATG CTG ATG GGA AAC CC -3'.

MMP-9-R: 5'- AGA AGC CGA AGA GCT TGT CC -3'.

β -actin-F: 5'-GAC TTA GTT GCG TTA CAC CCT TTC T -3'.

β -actin-R: 5'- GAA CGG TGA AGG TGA CAG CAG T -3'.

Table 1
Tumor size and invasion of pancreatic cancer patients with or without diabetes.

	Hyperglycemia(18)	Euglycemia(28)	P
Peripancreatic fat tissue invasion	12 (66.7%)	16 (57.1%)	0.518
Lymph node metastasis	5(27.8%)	4 (14.3%)	0.284
Vascular invasion	9 (50%)	12 (42.9%)	0.635
Biliary ducts invasion	4 (22.2%)	0 (0%)	0.019
Tumor size(cm)	2.95 ± 0.73	2.09 ± 0.70	< 0.001

The PCR reactions consisted of 30 s at 95 °C, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 30 s. The relative gene expression was calculated using the previously described $2^{-\Delta\Delta Ct}$ method [13].

2.10. Western blot analysis

Proteins were electrophoretically resolved on a denaturing SDS-polyacrylamide gel and were electrotransferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) for 2 h and then probed with HIF-1 α and MMP-9 antibodies at 4 °C overnight. After being blotted with the secondary antibody for 2 h at 37 °C, the complexes were visualized using the ECL Western blotting substrate and photographed by ChemiDoc XRS imaging system (Bio-Rad, USA).

2.11. Statistical analysis

Statistical analysis was performed using SPSS software (version 17.0, SPSS Inc., Chicago, USA). Data are presented as the means \pm SD of three replicate assays. Differences between the groups were analyzed by the Chi-square test, Student's *t*-test or analysis of variance (ANOVA). Statistical significance was set at $P < .05$. All experiments were repeated independently at least three times.

Table 2
The expression level of HIF-1 α in pancreatic cancer specimens.

HIF-1 α expression	Hyperglycemia(18)	Euglycemia(28)	P
-/+	5	13	
++	5	12	
+++	8	3	< 0.05

3. Results

3.1. Diabetes promotes the progression of pancreatic cancer in patients and HIF-1 α protein synthesis

A total of 105 pancreatic cancer patients were collected in the First Hospital of Xi'an Jiaotong University. Among these, 46 patients (43.8%) were treated with surgical resection and had complete pathological information. Patients were divided into hyperglycemic and euglycemic groups. The tumor size, invasion and lymph node metastasis of these patients are listed in Table 1. Of these 46 patients, 18 (39.1%) were diagnosed with diabetes, and 28 (60.9%) had normal glucose levels. We observed that patients in the hyperglycemic group had slightly higher levels of peripancreatic fat tissue invasion (66.7% vs 57.1%), vascular invasion (50% vs 42.9%) and lymph node metastasis (27.8% vs 14.3%) than those in the euglycemic group. We also found that pancreatic cancer patients with diabetes had significantly more biliary duct invasion and greater tumor volumes than patients in the normal blood glucose group. Our clinical data indicated that pancreatic cancer was more invasive in hyperglycemic patients than in euglycemic patients.

Immunohistochemical staining was used to detect the expression of HIF-1 α in normal pancreas specimens and in pancreatic cancer specimens from patients with or without diabetes (Fig. 1). A higher HIF-1 α expression was detected in the hyperglycemic group than in the normal pancreas specimens (Fig. 1A, B). Representative immunohistochemical staining images of the expression of HIF-1 α in specimens from

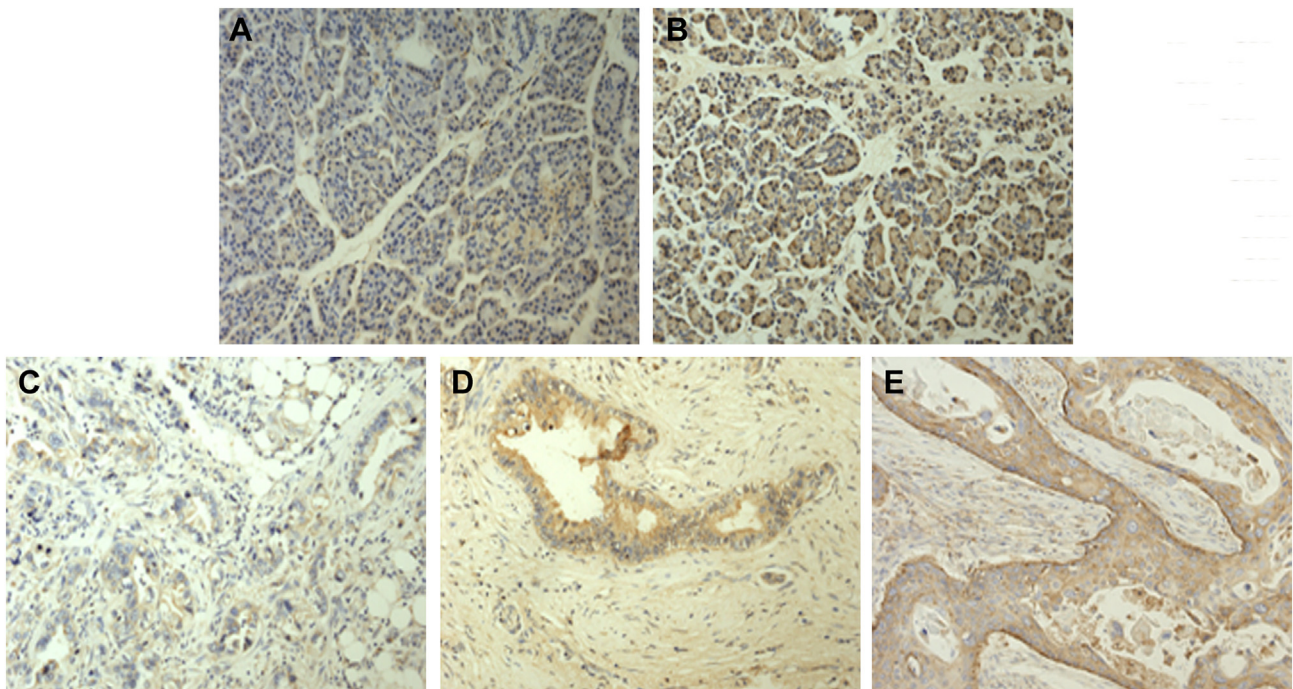


Fig. 1. Representative images showing HIF-1 α expression in normal pancreas specimens and in pancreatic cancer specimens. Immunohistochemical staining of normal pancreas specimens for HIF-1 α . A, the euglycemic group and B, the hyperglycemic group; C, D, and E show different expression levels of HIF-1 α in pancreatic cancer specimens. C, a low expression level of HIF-1 α (+), D, an intermediate expression level of HIF-1 α (++) and E, a high expression level of HIF-1 α (+++). (HIF-1 α antibody concentration 1:200; 200 \times magnification).

Table 3
Effect of hyperglycemia on nude mice weight and tumor growth, ascites and liver metastasis.

Mice	Hyperglycemia(10)	Euglycemia(10)	P
Blood Glucose (2w) *	24.59 ± 2.30	5.52 ± 1.33	< 0.001
Blood Glucose (8w) *	22.58 ± 2.24	5.48 ± 1.47	< 0.001
Weight (8w) *	20.24 ± 1.85	25.72 ± 1.38	< 0.001
Tumor volume (mm ³) *	746.50 ± 210.76	1824.70 ± 878.36	0.001
Ascites	6	1	0.057
Liver metastasis *	5	0	0.033

pancreatic cancer patients are shown in Fig. 1C-E. The HIF-1 α expression levels in pancreatic cancer specimens from the hyperglycemic group and the euglycemic group are summarized in Table 2. There are 3 patients with negative HIF-1 α in euglycemia group and 1 in hyperglycemia group.

These results indicated that pancreatic cancer patients with diabetes were more likely to experience tumor progression. Hyperglycemia might promote HIF-1 α protein synthesis and a hypoxic microenvironment.

3.2. The effect of hyperglycemia on microenvironment hypoxia

Based on the clinical findings, we next conducted experiments to ascertain whether hyperglycemia promotes microenvironment hypoxia

that then aggravates pancreatic cancer invasion in vivo. To induce diabetes in mice, 175 mg/kg STZ was injected into the peritoneal cavity of nude mice. The weight and blood glucose of the mice were monitored. As shown in Table 3, the STZ injection group showed higher levels of blood glucose from 2 to 8 weeks. After 8 weeks, the mice were sacrificed, and the pancreatic tissues were collected and embedded. HE staining showed morphology changes in diabetic mice, including swelling in pancreatic acinar cells and broadening in the lobule intervals, compared with those in the control group (Fig. 2A, B). Transmission electron microscopy was used to identify the morphology of cell subsets (Fig. 2C, D). Pancreatic acinar cells in diabetic mice showed an obvious expansion of the endoplasmic reticulum, increased nuclear gaps and chromatin close to the cellular membrane in some acinar cells. The immunohistochemistry staining showed that the HIF-1 α expression level was higher in the hyperglycemic group than in the euglycemic group (Fig. 2E, F). Our results indicated that a hyperglycemic environment might induce microenvironmental hypoxia.

3.3. Hyperglycemia promotes pancreatic cancer progression and tumor microenvironment hypoxia in nude mice

Our clinical data indicate that pancreatic cancers are more aggressive in patients who have hyperglycemia. To verify our clinical findings, an orthotopic xenograft model was used with or without

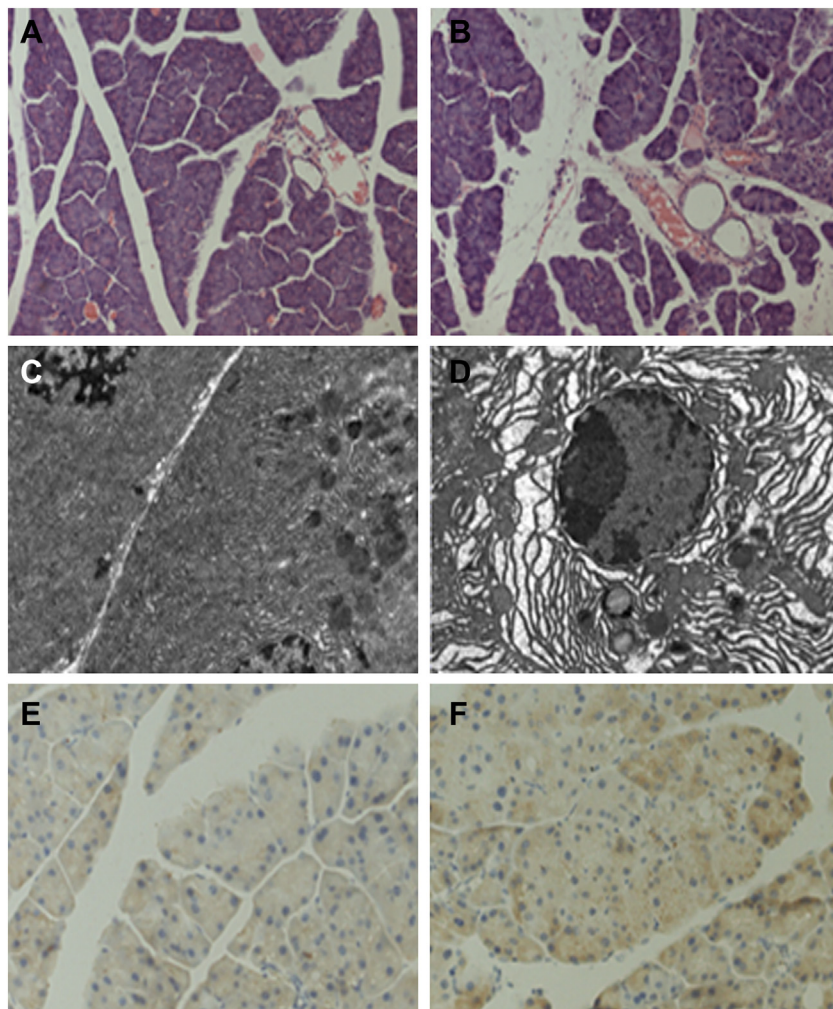


Fig. 2. The effect of hyperglycemia on the normal pancreas. To induce hyperglycemia, 175 mg/kg STZ was injected into the peritoneal cavity of each mouse. Mice were sacrificed after 8 weeks. The H&E staining and transmission electron microscopy showed the morphology changes in the pancreas (A, C, the euglycemic group and B, D, the hyperglycemic group). Immunohistochemical staining of normal pancreas specimens for HIF-1 α : E, the euglycemic group and F, the hyperglycemic group.

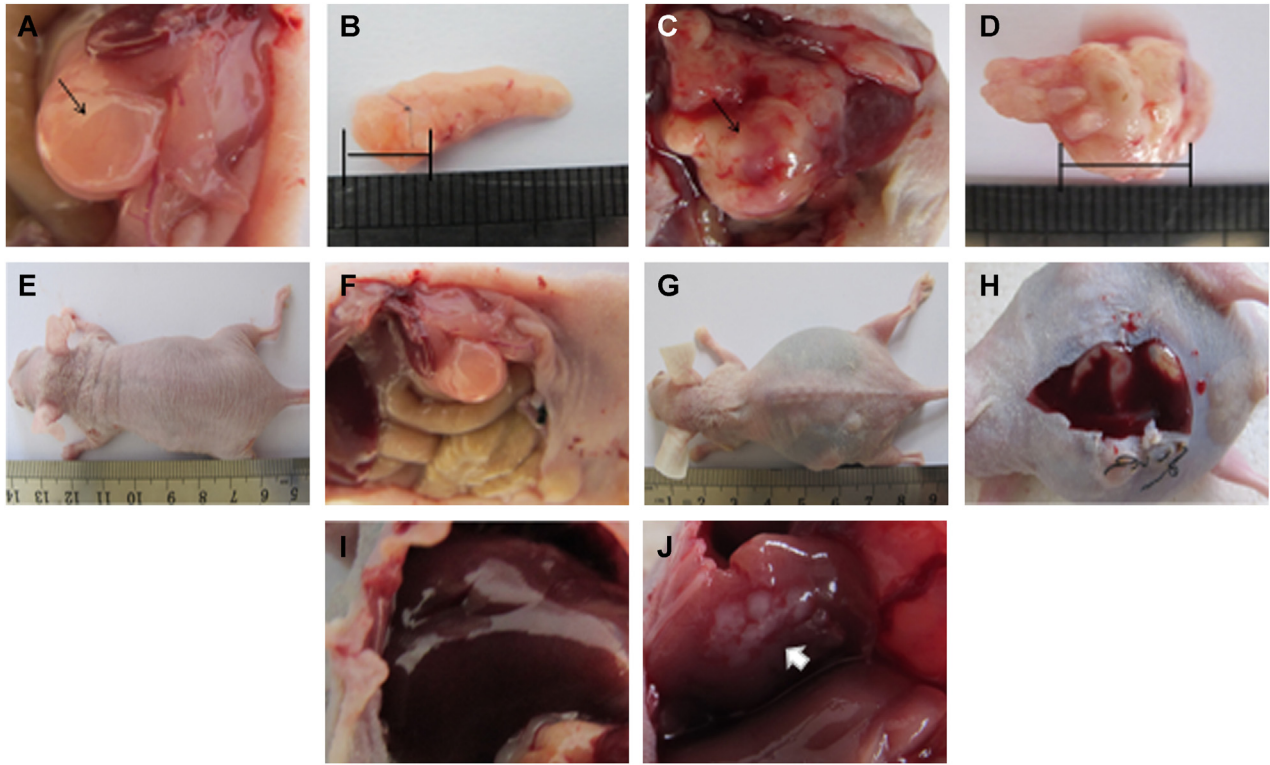


Fig. 3. The effects of hyperglycemia on tumor growth, ascites generation and liver metastasis in nude mice. (Macroscopic appearance of solid tumors after the mice were sacrificed: A, B, euglycemic group and C, D, the hyperglycemic group. Ascites generation and liver metastasis in nude mice: E, F, I, the euglycemic group and G, H, J, the hyperglycemic group.)

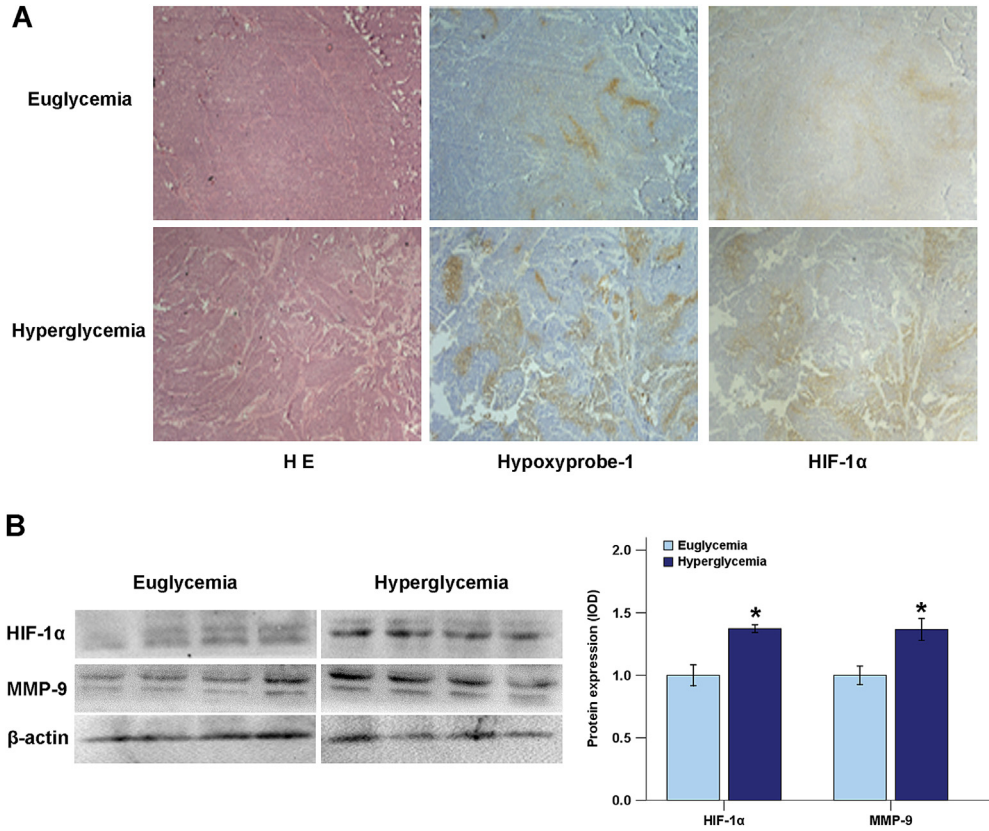


Fig. 4. Effects of hyperglycemia on microenvironment hypoxia in pancreatic cancer. A, Immunohistochemistry was performed to compare the expression of Hypoxyprobe-1 and HIF-1α in the euglycemic group and hyperglycemic group of orthotopic nude mice. B, The protein levels of HIF-1α and MMP-9 in pancreatic tumor tissues with different serum glucose levels were analyzed using Western blotting. * $P < .05$ compared to the euglycemic group.

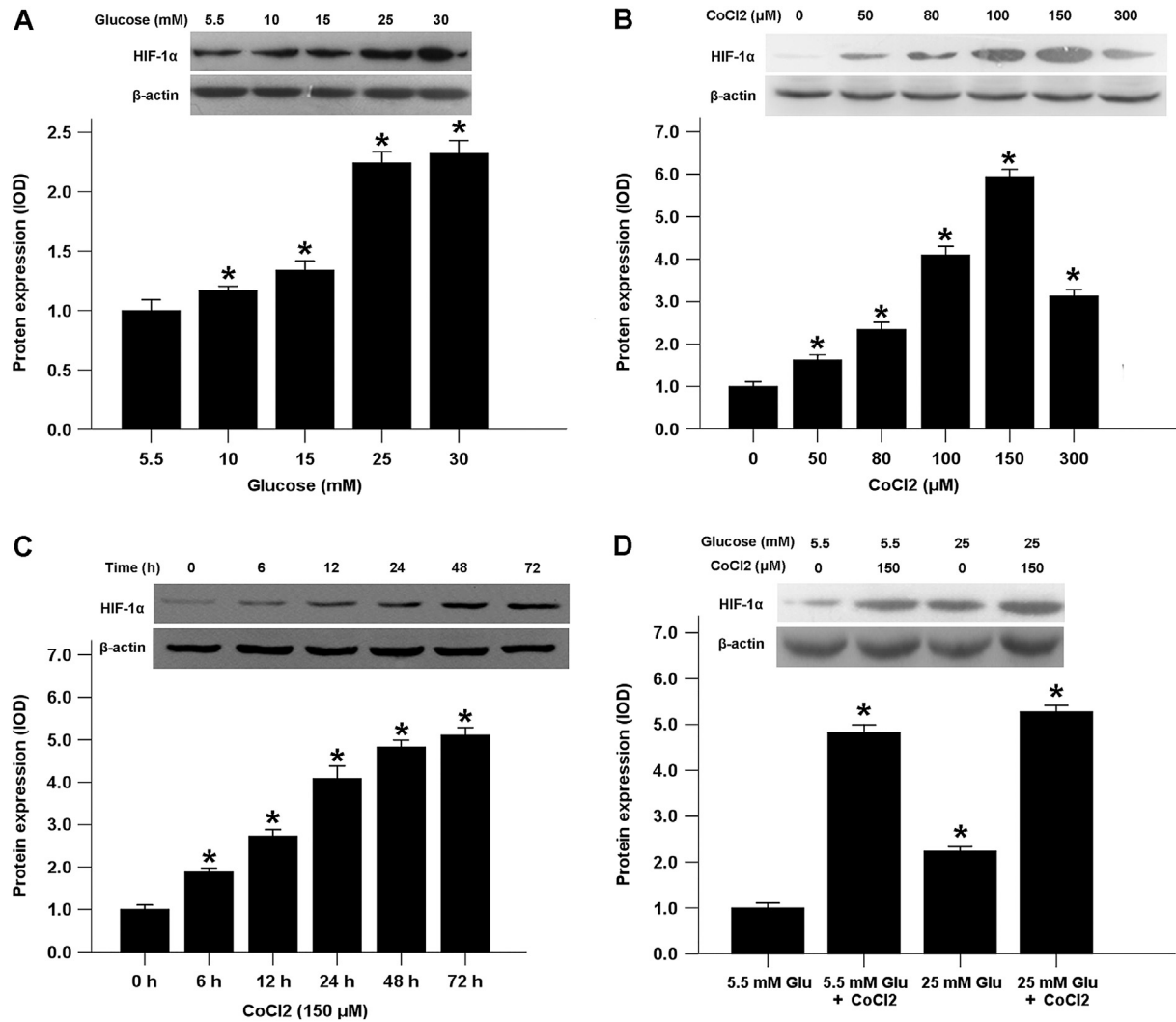


Fig. 5. Effects of high glucose and CoCl₂ on HIF-1α expression in BxPC-3 cells. **A**, The effect of glucose on the protein level of HIF-1α. BxPC-3 cells were treated with different concentrations of glucose for 72 h, and the protein level of HIF-1α was evaluated. **B**, The effect of CoCl₂ on the protein levels of HIF-1α. **C**, The effect of 150 μM CoCl₂ on the protein level of HIF-1α at the indicated times. **D**, The effect of CoCl₂ on HIF-1α expression in both normal glucose and high glucose conditions. **P* < .05 compared to the control group.

intraperitoneal injection of STZ. The average tumor volume was greater in diabetic mice than in euglycemic mice (Table 3, Fig. 3A–D). Diabetic mice were more likely to combine ascites (Fig. 3E–H) with liver metastasis (Fig. 3I, J) than mice in the control group.

Hypoxyprom-1, a type of pimonidazole hydrochloride, was used as a specific marker for hypoxia [14]. The immunohistochemistry results showed that the expression areas for Hypoxyprom-1 and HIF-1α (Fig. 4A) in the diabetic, orthotopic xenograft group were larger than in the control group. Immunoblotting results revealed that the expression of HIF-1α and MMP-9 in the diabetic group was higher than in the control group (Fig. 4B). These data suggested that a hyperglycemic environment could exacerbate hypoxia and promote tumor progression in the pancreatic orthotopic xenograft model.

3.4. The effect of high glucose and CoCl₂ on the expression of HIF-1α in pancreatic cancer cells

To explore the possible relationship between hyperglycemia and hypoxia, we examined the effects of high glucose concentrations and the expression of HIF-1α in pancreatic cancer cells. Our results showed that the expression levels of HIF-1α in BxPC-3 cancer cells increased in a dose-dependent manner

in response to high glucose concentrations compared with normal physiological glucose levels (5.5 mM glucose) (Fig. 5A). Cobalt chloride (CoCl₂), a mimetic agent used to induce cellular responses mediated by hypoxia in vitro, stabilizes HIF-1α by inhibiting prolyl hydroxylase enzymes [15]. Both hypoxia and CoCl₂ induce the transcriptional responses of HIF-1α to reduced oxygen tension [16]. Incubation with 0 to 150 μM CoCl₂ induced the expression of HIF-1α in BxPC-3 cells in a dose-dependent manner. Although 200 μM CoCl₂ also increased HIF-1α expression, the efficiency had declined (Fig. 5B). Therefore, a treatment concentration of 150 μM CoCl₂ was used in the subsequent experiments. As shown in Fig. 5C, incubation for 6 to 72 h with 150 μM CoCl₂ promoted the time-dependent expression of HIF-1α. Interestingly, our results indicated that a low glucose concentration augmented HIF-1α production in CoCl₂-treated BxPC-3 cells compared with a high glucose concentration (Fig. 5D).

3.5. HIF-1α is involved in high glucose-induced pancreatic cancer invasion and migration

To confirm that high glucose-induced HIF-1α influences the invasive and migratory abilities of pancreatic cancer cells, we used

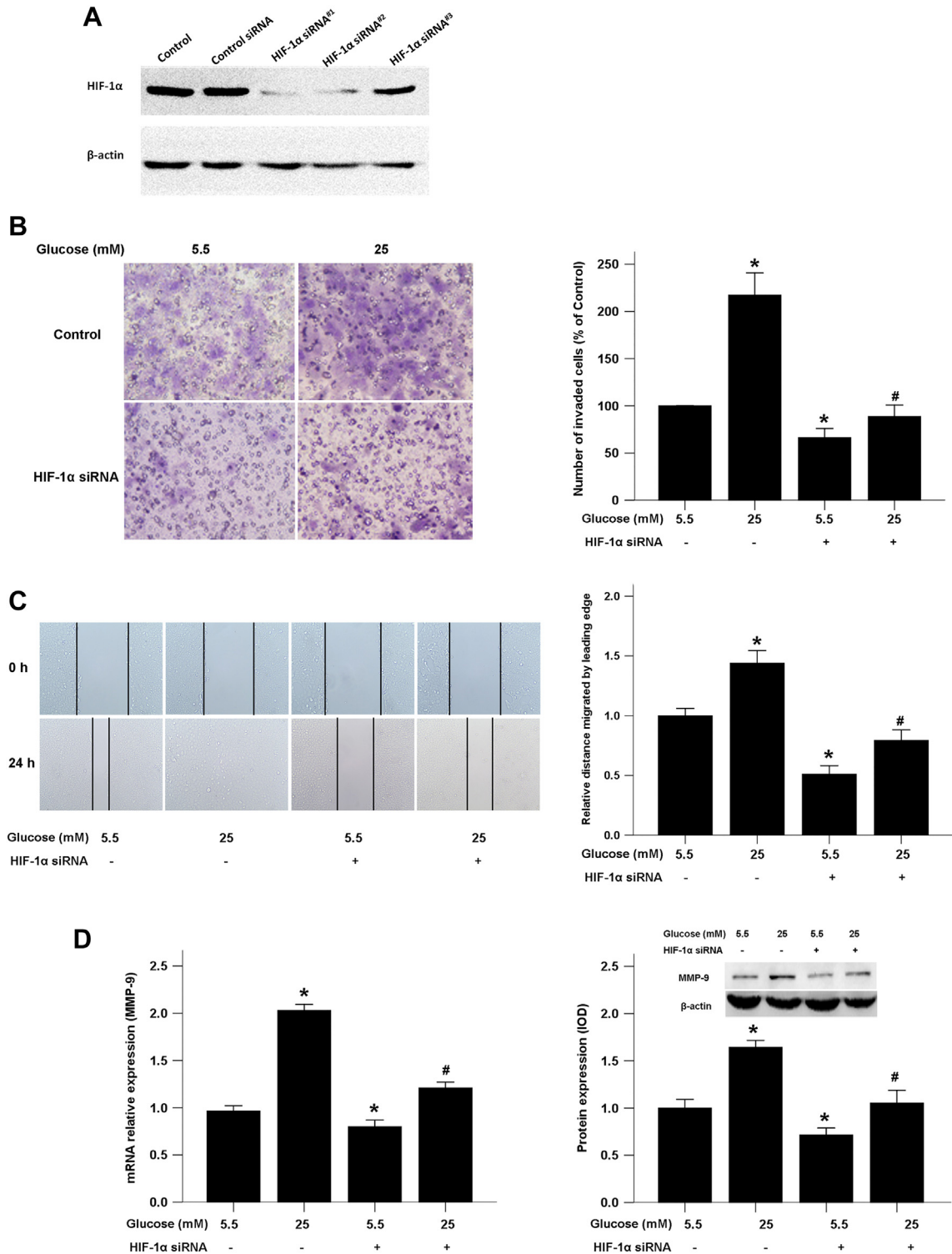


Fig. 6. HIF-1α siRNA abolished the effects of high glucose-mediated invasion and migration of pancreatic cancer cells. A, Western blotting were used to evaluated the efficiency of siRNAs targeting HIF-1α in BxPC-3cells. B, The effect of HIF-1α knockdown on BxPC-3 cell invasion. The images show the bottom side of the filter inserts with stained cells that migrated through the filter pores at 48 h. The number of migrated cells was quantified by counting the cells from 10 random fields at ×200 magnification. C, The effect on cancer cell migration in response to HIF-1α knockdown. The confluent monolayer was wounded with a sterile pipette tip, and the cells were allowed to migrate for 24 h. D, qRT-PCR and Western blotting were used to test the effect of the HIF-1α knockdown on MMP-9 expression at both mRNA and protein levels.

HIF-1α siRNA to knock down HIF-1α. Three siRNA sequences were designed and efficiency of these siRNAs was evaluated by Western blotting (Fig. 6A). And HIF-1α siRNA^{#1} was chosen for further experiments for its high quality effect on knockdown HIF-1α expression. We found that the increased BxPC-3 cell invasion and migration in the presence of high glucose was significantly

inhibited by the HIF-1α knockdown (Fig. 6B, C). Additionally, high glucose induced-MMP9 production was down-regulated in the HIF-1α siRNA group compared with the control siRNA group (Fig. 6D). These data suggest that a high glucose level contributes to increased pancreatic cancer invasion and migration in a HIF-1α-dependent manner.

4. Discussion

Pancreatic cancer is among the deadliest types of malignant digestive carcinoma. The 5-year relative survival rate is currently 8% because pancreatic cancer is commonly diagnosed in an advanced stage [17]. The total deaths due to pancreatic cancer are projected to surpass breast, prostate and colorectal cancers to become the second leading cause of cancer-related deaths by 2030 [18]. Identification of etiological factors could enable the early detection of this fatal disease so that it would be more amenable to treatment. Diabetes mellitus is among the small number of known potential risk factors for pancreatic cancer. A recent prospective study of 0.5 million Chinese adults showed that diabetes was associated with an almost two-fold increased risk of pancreatic cancer and that every 1 mmol/l increase in blood glucose level led to an increase of 15% in the incidence of pancreatic cancer [19].

Hyperglycemia and hyperinsulinemia are related factors that promote dysplasia and neoplasia in the pancreas [5]. Diabetes and the accompanying hyperglycemia lead to chronic inflammation and increased cancer risk [20]. Our previous studies showed that hyperglycemia could promote the progression of pancreatic cancer. Our studies demonstrated that high glucose *in vitro* might be regarded as an accelerator to increase cancer cell proliferation by enhancing the glial cell line-derived neurotrophic factor (GDNF)/RET or epidermal growth factor (EGF)/EGFR signaling pathways [21,22]. We also showed that high glucose could enhance the migratory and invasive abilities of pancreatic cancer cells through superoxide dismutase (SOD)-induced production of hydrogen peroxide (H_2O_2) via the activation of the ERK and p38 MAPK signaling pathways [23]. Our studies proved that hyperglycemic mice contained a higher plasma H_2O_2 level, which induced epithelial-mesenchymal transition (EMT) and promoted the metastatic activity of pancreatic orthotopic transplantation tumors, than euglycemic mice [7]. In addition, our results showed that a hyperglycemic tumor microenvironment induced perineural invasion (PNI) in pancreatic cancer through the impairment of nerve construction, formation of a low-resistance pathway of PNI by cancer cells and enhancement of the interaction between tumor cells and nerves [8].

A recent study has shown that hyperglycemia can induce cellular hypoxia and the production of mitochondrial reactive oxygen species, which in turn promote hyperglycemic damage in a coordinated manner [9]. Hypoxia exists in the microenvironment of solid tumors, including pancreatic cancer, and it plays an important role in tumor progression and metastasis [11]. In the current study, we observed that hyperglycemia could induce microenvironment hypoxia in pancreatic normal parenchyma and in tumor tissue. Hyperglycemia promotes the growth, invasion and metastasis of pancreatic cancer. The expression of HIF-1 α in pancreatic tumors was higher in hyperglycemic conditions than in euglycemic conditions. The role of high glucose concentrations in promoting pancreatic cancer invasion involves the up-regulation of HIF-1 α . Inhibition of HIF-1 α by siRNA reversed the effect of high glucose on pancreatic cancer cells.

Hypoxia is not only a consequence of unrestrained fast tumor growth; it also plays a vital role in promoting tumor proliferation, invasion, EMT, distant metastasis and resistance to therapy [11–24]. The tumor stroma is a dynamic environment, which includes inflammatory cells, pancreatic stellate cells (PSC), endothelial cells, extracellular matrix (ECM), growth factors and cytokines. Hypoxia could affect stromal cells by promoting the activation of PSCs and the secretion of specific ECM components that induce the generation of the extensive desmoplastic stroma characteristic of pancreatic cancer [25]. HIF-1 α is the most important transcription factor induced by intratumoral hypoxia, and it predicts poor outcomes for pancreatic cancer patients [26]. The expression of HIF-1 α is also linked with pancreatic cancer progression, angiogenesis, EMT and metastasis [24]. HIF-1 α is able to recruit monocytes and macrophages by promoting chemical chemokine 2 (CCL2) secretion, which accelerates the activation of PSCs and enhances tumor progression [27].

Hyperglycemic conditions and cellular hypoxia are intimately related. In tumor cells, high glucose levels promote HIF-1 α expression under both normoxic and hypoxic conditions [12]. In the STZ-induced diabetic mouse model, glomerular mesangial cells showed a significant increase in HIF-1 α expression in the nucleus. In cultured mesangial cells, high glucose levels could also enhance the expression of HIF-1 α [28]. Yan et al. showed that high glucose (30 mM) up-regulated the protein level of HIF-1 α , increased the transcriptional activity of HIF-1 α and promoted the expression of vascular endothelial growth factor (VEGF), a downstream gene of HIF-1 in the endothelial cells [29]. Our results showed that hyperglycemia could induce hypoxia and increase the expression of HIF-1 α in the normal pancreas and in pancreatic cancer.

5. Conclusion

In summary, our results indicate that pancreatic cancer, specifically the tumor within the pancreatic parenchyma, is more aggressive in a microenvironment combining hypoxia with hyperglycemia. High glucose levels can up-regulate HIF-1 α expression, which promotes metastasis of pancreatic cancer. Our findings may provide new insight into the relationship between diabetes mellitus and pancreatic cancer. Managing hyperglycemia-induced HIF-1 α might be a novel strategy for the treatment of pancreatic cancer. Our findings warrant further investigation of this possibility.

Ethics approval and consent to participate

The study protocol and consent forms conform to the Declaration of Helsinki and were approved by the Ethical Review Board (ERB) Committee of The First Affiliated Hospital of Xi'an Jiaotong University, China. Written informed consent was obtained from all the participants. Animal experimental protocols were approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China.

Conflict of interest

All authors have declared no conflicts of interest.

Funding

This work was supported by grants from the National Natural Science Foundation of China (81472248, 81672434 and 81702916) and Scientific Fund of Shandong Province, China (ZR2017BH059).

Authors' contributions

WL, HL, QM, JM designed the study; WL, HL, WQ, LC, BY, LH, QX, QM, JM carried out experiments. WL, HL, JM analyzed the data. WL, JM wrote the manuscript. WL, QM, JM supervised and revised the manuscript. All authors had final approval of the submitted and published versions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.csbj.2018.10.006>.

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