High-glucose microenvironment promotes perineural invasion of pancreatic cancer via activation of hypoxia inducible factor 1α

LUN ZHANG¹, WUNAI ZHANG¹, XIN ZHANG², YIHE MIN³, YANG ZHAO², BAOFENG WANG², WEI LI¹, SHUAI MAO⁴ and WEILI MIN²

¹Department of Hepatobiliary Surgery, First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shaanxi 710061; ²Department of Oncology, Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shaanxi 710004; ³Chongqing Three Gorges Medical College, Chongqing 404100; ⁴Department of Medicinal Chemistry, School of Pharmacy, Xi’an Jiaotong University, Xi’an, Shaanxi 710061, P.R. China

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Abstract. Pancreatic cancer (PC) is one of the most lethal diseases, with a 5-year survival rate of <9%. Perineural invasion (PNI) is a common pathological hallmark of PC and is correlated with a poor prognosis in this disease. Hyperglycemia has been shown to promote the invasion and migration of PC cells; however, the effect of hyperglycemia on the PNI of PC and its underlying mechanism remains unclear. In the present study, Western blotting was utilized to detect the expression of hypoxia inducible factor 1α (HIF1α) and nerve growth factor (NGF). Transwell and wound-healing assays were performed to detect the influence of hyperglycemia on the invasion and migration ability of PC cells. An in vitro PC-dorsal root ganglion (DRG) co-culture system and an in vivo PC sciatic nerve-infiltrating tumor model were used to evaluate the severity of PNI in hyperglycemic conditions. In the results, hyperglycemia promoted the invasion/migration ability and elevated the expression of NGF in PC by upregulating HIF1α. Moreover, in vitro short-term hyperglycemia caused little damage on the DRG axons and accelerated both the PNI of the PC and the outgrowth of the DRGs by increasing the expression of NGF via activation of HIF1α. Indeed, in vivo long-term hyperglycemia promoted the infiltration and growth of PC, and then disrupted the function of the sciatic nerve in a HIF1α-dependent manner. In conclusion, a high-glucose microenvironment promotes PNI of PC via activation of HIF1α.

Introduction

Pancreatic cancer (PC), with a 5-year survival rate of <9%, is one of the most lethal diseases, and it is the fourth leading cause of cancer-related death in the United States (1). Most patients with PC are diagnosed in an advanced stage and miss their best opportunity for surgery (2). The lack of any effective adjuvant chemotherapies also hinders their survival. Our current limited understanding of the risk factors and the tumorigenesis mechanism is an important reason for the poor prognosis of PC (3). Hence, additional studies, including research exploring the etiology of PC and searching for possible pro-tumor factors, should be performed to improve the prognosis of PC.

Pancreatic ductal adenocarcinoma (PDAC) is the most common pathological type of PC, and its high proliferation rate, abundant desmoplastic reactions, and propensity for perineural invasion (PNI) are its main pathological features (4). Previous studies have shown that the incidence of PNI in PC is nearly 100% and that it is closely related to the poor prognosis of PC (4,5). PNI is defined as the cancer cells surrounding the nerves and/or invading into the spaces of the epineurium, perineurium or endoneurium (6-8). Both in vitro and in vivo studies found that PC cells, and even precancerous pancreatic cells, can approach and invade the nerves, and then migrate/disseminate along the nerves to distant organs or tissues; in addition, PC cells also hide in nerves, which can cause in situ recurrences of PC (9,10). The interaction of the tumor cells and nerves promotes the formation of PNI. However, the underlying mechanism of PNI and its role in PC development remain unclear.

Increasing amounts of evidence have indicated that hyperglycemia is a risk factor for PC, and patients with long-standing type 2 diabetes mellitus have a two-fold increased risk of PC (11-13). In addition, apart from its pro-initiation effect, hyperglycemia also facilitates the progression of PC via modulation of its proliferation and invasion/migration, and by inducing chemoresistance among PC (14). Notably, studies found that increased PNI of PC was correlated with hyperglycemia both in animals and in patients (15,16). The possible reason for this phenomenon may be that hyperglycemia...
supports the malignant behavior of PC and modulates the expression of nerve growth factor (NGF), a prominent neurotrophic factor involved in PNI, and then promotes the outgrowth of nerve axons (17,18).

The present study evaluates the effect of hyperglycemia on the migration ability and PNI of PC, trying to explain the underlying mechanism linking hyperglycemia and PNI in PC.

Materials and methods

Cell lines and cultures. The PC BxPc-3 and Panc-1 cell lines were obtained from the Type Culture Collection of the Chinese Academy of Sciences and cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (FBS; HyClone; Cytiva) and 1% penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in a standard incubator with a 5% CO₂ atmosphere at 37°C. To induce normal or high-glucose environments, 0, 5.5 or 25 mM glucose was added to DMEM (mannitol was used to balance the osmotic pressure). After grouping and intervention, relevant assays were performed on the 0-mM glucose group, the euglycemia group, the hyperglycemia group (5.5 mM) and the hyperglycemia group (25 mM), and then the total protein was extracted by RIPA buffer (Beyotime Institute of Biotechnology) from the cells as required.

Animal experiments. All nude mice and newborn rats were reared in a specific pathogen-free chamber, with the temperature controlled at 24°C and a 12/12 h light/dark cycle, at the Animal Center of Xi'an Jiaotong University (Xi'an, China). The relative humidity in the chamber was 50-80%. An ultraviolet lamp installed in the chamber was used to keep sterile conditions. Food, water and bedding used by the nude mice was provided ad libitum and newborn rats were all sterilized. A total of 18 male nude mice (6 mice per group) and 6 male newborn rats (1 day old, weighing ~1.5 g) were used to perform this study. The average weight of the nude mice at the start of the experiment was ~20 g (age, 6 weeks). The experimental protocols were approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiaotong University. This study was conducted in accordance with the Declaration of Helsinki, and all protocols were approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiaotong University.

In vitro PNI co-culture model. The PNI co-culture model can mimic the peripheral nerve microenvironment of PC tumor tissues (7). Briefly, the construction of this model is separated into three parts, the induction of hyperglycemia in the PC cells toward the DRG was defined as parameter α and the relative outgrowth of the DRGs axons toward the PC cells was defined as parameter β. The PC cell invasion index=α/γ, and the DRG outgrowth index=β/γ (Fig. 2A). These parameters were measured by the image processing/analysis software ImageJ (version 1.52a; National Institutes of Health).

In vivo PNI sciatic nerve infiltrating tumor model. As described previously (19,20), the sciatic nerve infiltrating tumor model was adopted to research the influence of hyperglycemia on PNI of PC in vivo. Briefly, the construction of this model is separated into three parts, the induction of hyperglycemia in mice, the transplanting of PC cells into the mouse sciatic nerve and the relative intervention strategy.

Induction of hyperglycemia in mice. Female 4-week-old BALB/c nude mice were purchased from and reared in the Experimental Animal Center of Xi'an Jiaotong University. The mice were separated into three groups (6 mice/group); two groups (control group and 2-ME group) received an intraperitoneal injection of streptozotocin (STZ; MedChem Express; dissolved in sodium citrate buffer, 150 mg/kg) to induce hyperglycemia, while the third group received an intraperitoneal injection of sodium citrate buffer alone as the euglycemic control. After 7 days, the blood glucose levels of each mouse were measured to confirm establishment of the model.

Transplantation of PC cells into the mouse sciatic nerve. First, the mice were anesthetized with 1% pentobarbital sodium (50 mg/kg, intraperitoneal injection) and the sciatic nerve of the left lower extremity was exposed. Next, 5 µl Panc-1 cell suspension (1x10⁵ cells/well) was slowly (over ~2 min) injected into the perineurium of the sciatic nerve using a 10-µl
with 100 nM siRNA (50 pmol/well) using the Lipofectamine® GenePharma Co., Ltd. Glucose pretreated cells or the CAGGUUCGGAAATT-3' were purchased from Shanghai National Institutes of Health).

Considering that the sciatic nerve innervates the hind limb paw muscles, a sciatic nerve score [the nerve function was graded from 4 (normal paw function) to 1 (total paw paralysis)] based on the hind limb paw response to manual extension of the body and the distance of the paw span (the length between the first and fifth toes of the hind limbs) were used as the evaluation indices of sciatic nerve function. Finally, the nude mice were euthanized with CO2, with a chamber volume displacement rate of 10-30% per minute. Consequently, the generated tumors were extracted to calculate their volume.

Small interfering (si)RNA transfections. HIF1α-specific siRNA (si-HIF1α; sense, 5'-CCACCAGUAGAUUAATT-3' and anti-sense, 5'-UUUAAUUCACAGUGGTTT-3') and a negative control scrambled siRNA (si-NC; sense, 5'-UUCUCCGAACGUGACACGUUTT-3'; anti-sense, 5'-ACGUACAGUAGCAATT-3') were purchased from Shanghai GenePharma Co., Ltd. Glucose pretreated cells or the non-treated cells seeded in 6-well plates were transfected with 100 nM siRNA (50 pmol/well) using the Lipofectamine® RNAi MAX Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 25°C according to the manufacturer's instructions. The cells were used in subsequent experiments for 24 h after the transfection.

Cell invasion assay. Transwell Matrigel invasion assays were adopted to measure the invasive ability of the PC cells. First, 8-mm pore Millicell inserts were coated with 25 μl Matrigel at 37°C for 8 h. PC cells (BxPc-3 and Panc-1) were pretreated appropriately according to the design of the study, and cell suspensions (5x10^4/well) were added to the upper chambers in DMEM containing 1% FBS. Next, 500 μl DMEM containing 20% FBS was placed in the lower chambers. After being incubated for 48 h, the non-invading cells were removed by cotton swab and the invading cells were stained with crystal violet at room temperature for 15 min. The invasion ability was determined by counting the stained cells on the bottom surface of the insert under a light microscope.

Cell migration assay. Wound-healing assays were adopted to measure the migration ability of the PC cells. PC cells (BxPc-3 and Panc-1) were pretreated appropriately according to the design of the study. The PC cells were serum-starved during the assay. A sterile pipette tip was used to produce a wound line across the plate after the cells had been grown to 90-100% confluence. The cells were allowed to migrate for 48 h. Images were captured at 0 and 48 h post-wounding using a light microscope and the relative migration area was measured by image processing/analysis software ImageJ (version 1.52a; National Institutes of Health).

Western blot assays. PC cells (BxPc-3 and Panc-1) were pretreated appropriately according to the design of the experiment and total protein was extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology). Next, western blotting assays were performed as previously described (21). Primary antibodies against HIF1α (1:1,000; catalog no. ab51608; Abcam), NGF (1:1,000; catalog no. ab52918; Abcam) and β-actin (1:5,000; catalog no. 20536-1-AP; ProteinTech Group, Inc.) were incubated at 4°C for 12 h. Next, the goat anti-rabbit HRP-conjugated secondary antibody (catalog no. A0208; Beyotime Institute of Biotechnology) was diluted at 1:10,000 and incubated for 1 h at room temperature. Subsequently, ECL luminescence reagent (Beyotime Institute of Biotechnology) was applied for ~5 min. Images of the immunopositive bands were captured with a ChemiDoc XRS imaging system (Bio-Rad Laboratories, Inc.) and Image Lab (version 3.0 Beta 3; Bio-Rad Laboratories, Inc.) was used for densitometry analysis. Grayscale analysis was performed using ImageJ (version 1.52a; National Institutes of Health).

Bioinformatics analysis. Two online bioinformatics analysis websites, GEPIA (http://gepia.cancer-pku.cn/) and Kaplan-Meier Plotter (http://www.kmplot.com) were used to perform the bioinformatics analysis in this passage. Firstly, the Kaplan-Meier Plotter website was adopted to analysis the relapse-free survival and overall survival rates with regard to HIF1α and pancreatic cancer. A PDAC dataset (n=177) in the pan-cancer database (mRNA RNA-Seq sub-database) was divided based on the median value. Next, on the GEPIA website, the PAAD dataset was adopted to analyze the expression level of HIF1α in pancreatic cancer (179 cases) and normal pancreas (171 cases). |Log2FC| and P<0.01 were used as the cut-offs. The correlation between HIF1α and NGF/BDNF in pancreatic cancer was performed via automatic analysis on the GEPIA website.

Statistical analysis. The data are presented as the mean ± SD. Using the GraphPad Prism software package (version 6.0; Graphpad Software, Inc.), the differences in the in vivo data were assessed with Student's t-test (unpaired) when there were 2 groups. Dunnett's post hoc test in conjunction with one-way ANOVA was performed to compare the differences between the control group and other groups when there were 3 groups. Pearson's correlation test was applied for bioinformatics analysis. All tests were two-sided, and a P<0.05 was considered to indicate a statistically significant difference.

Results

Hyperglycemia promotes the invasion and migration of PC cells and has little effect on the outgrowth of DRGs. The necessary condition for PNI is a high invasion/migration ability of PC cells. Hence, before the research on the PNI of PC, the influence of hyperglycemia on the invasion/migration ability of the PC cells was first evaluated. Transwell assays showed that 5.5 mM glucose (euglycemia) had little effect on the invasion ability of PC cells compared to the normal media group; however, 25 mM glucose (hyperglycemia) promoted PC cell invasion (Fig. 1A and B). Wound-healing assays also revealed that both euglycemia (5.5 mM glucose) and hyperglycemia (25 mM glucose) accelerated the migration ability of the PC
cells compared with the normal media group (Fig. 1C and D). These phenomena revealed that hyperglycemia promoted the invasion/migration of PC cells, which indicated that hyperglycemia may participate in the PNI of PC.

Next, a PC cell-DRG direct co-culture system was used to explore the association between hyperglycemia and PNI. The main focus was placed on the influence of hyperglycemia on PC, so different concentrations of glucose (0, 5.5 and 25 mM) were first used to culture the DRGs alone and the outgrowth of the DRG axons was observed. The results showed that short-term intervention (<3 days) exposure to glucose had no marked influence on the outgrowth of the DRGs (Fig. 1E); however, as the time was extended (>3 days; data not shown), hyperglycemia began to suppress the growth of the axons, which may be due to its destruction of the Schwann cells (16).

All of these findings suggested that hyperglycemia promoted the invasion and migration of PC cells and had little effect on the outgrowth of DRGs; thus, the PC cell-DRG direct co-culture system can be adopted for in vitro PNI research.

Hyperglycemia increases NGF expression and accelerates the PNI of PC. As shown in Fig. 2A, to evaluate the severity of PNI in the PC cell-DRG co-culture model, the initial distance (~2 mm) between the edge of the cell island and the DRG was defined as parameter γ; the relative migration distance of the PC cells toward the DRG as parameter α (PC invasion index=α/γ) and the relative outgrowth of the DRG axons toward the PC cells as parameter β (DRG outgrowth index=β/γ). Considering that NGF is an essential member of the neurotrophic family that sustains the PNI of PC, the influence of hyperglycemia on the
expression of NGF in PC was measured and hyperglycemia was found to result in increased NGF expression in both BxPc-3 and Panc-1 cells (Figs. 2B and S1A). By applying the PC cell-DRG co-culture model, it was also found that hyperglycemia significantly accelerated the invasion of the PC cells toward the DRG axons (Fig. 2C and E); in addition, hyperglycemia also promoted the outgrowth of the DRG axons (Fig. 2D and E). The promotion effect of hyperglycemia on the DRGs was much weaker than that on the PC cells, and this discrepancy may be due to the fact that hyperglycemia not only promotes the secretion of NGF by PC to stimulate the growth of axons, but it also has a certain toxic effect on nerve axons.

Hyperglycemia upregulates HIF1α, which promotes the invasion and migration of PC cells. A previous study demonstrated that HIF1α mediates PC cell metastasis in hyperglycemia-induced hypoxic conditions (22). Hence, the present study aimed to explore whether HIF1α was participating in PC cell invasion and migration. First, PC cells were cultured with different concentrations of glucose (0, 5.5 and 25 mM) and it was found that hyperglycemia upregulated HIF1α (Figs. 3A and S1B), which is consistent with the previous findings shown in Fig. 2B. Considering that HIF1α is unstable under normal conditions and is stabilized under hypoxia (22), we hypothesize that hyperglycemia aggravated the hypoxic microenvironment of PC.

Next, using two online bioinformatics analysis websites, GEPIA (http://gepia.cancer-pku.cn/) and Kaplan-Meier Plotter (http://www.kmplot.com), HIF1α expression was found to be high in PC and associated with a poor prognosis (Fig. 3B-D). In addition, HIF1α was positively correlated with the expression of neurotrophic factor NGF (Fig. 3E). To measure the
influence of HIF1α on PC cell invasion and migration, 2-ME (10 µM) was adopted as an inhibitor of HIF1α (23). The data revealed that hyperglycemia had a similar function with regard to hypoxia as to the expression of NGF and HIF1α, and HIF1α inhibition suppressed the invasion and migration of the PC cells (Figs. 3F-K and S1C). In conclusion, it was demonstrated that HIF1α is essential for the invasion and migration of PC cells.

Hyperglycemia facilitates the invasion and migration of PC cells via HIF1α. As aforementioned, HIF1α was demonstrated to promote the invasion and migration of the PC cells. Next, the PC cell-DRG co-culture model was adopted to explore whether HIF1α was essential for the PNI of PC. First, si-HIF1α was used to inhibit the expression of HIF1α, and consistent with previous findings, it was found that the expression of NGF was also decreased in both hypoxic and normoxic conditions (Figs. 4A and S1D). Next, si-HIF1α- or si-NC-treated PC cells were cultured with DRGs, and after 48 h of co-culture, both PC cell invasion and DRG outgrowth were suppressed after HIF1α downregulation (Fig. 4B-D). Combined with the aforementioned results, it can be concluded that HIF1α is
essential for PC cell invasion/migration and PNI. However, the present study also found that hyperglycemia promoted the invasion/migration/PNI of PC cells and that hyperglycemia can upregulate HIF1α. Hence, it was next demonstrated that the promoting effect of hyperglycemia on invasion/migration was HIF1α-dependent. Specifically, si-HIF1α or si-NC was used to influence the expression of HIF1α in the PC cells, which were then treated with 25 mM glucose. As shown in Fig. 4E-G, hyperglycemia promoted the invasion/migration of the PC cells, but inhibiting HIF1α abrogated this effect. In conclusion, hyperglycemia facilitated the invasion and migration of PC cells via HIF1α.

Hyperglycemia facilitates the PNI of PC by activating HIF1α in vitro. The aforementioned results revealed that HIF1α plays a vital role in the hyperglycemia-induced invasion and migration of PC cells. Therefore, the present study next aimed to analyze the role of HIF1α in the PNI of PC in a background of hyperglycemia. si-HIF1α or si-NC were used to influence the expression of HIF1α in the PC cells, which were then cultured with DRGs...
in normal or high-glucose media. After co-culture for different time points, the PC cell invasion and DRG outgrowth of these groups was measured, and hyperglycemia was found to promote the PNI of PC; however, this effect was abrogated by the inhibition of HIF1α (Fig. 5A-C). In western blot assays, it was found that hyperglycemia upregulated the levels of both HIF1α and NGF; however, consistent with the above findings, after inhibition of HIF1α, the expression of NGF was downregulated in both hypoxic and normoxic conditions (Figs. 5D and S1E). Hence, taken together, all of these findings indicated that hyperglycemia promoted the PNI of PC in a HIF1α-dependent manner (Fig. 5E).

**Hyperglycemia promotes the PNI of PC in a HIF1α-dependent manner in vivo.** The present study demonstrated that hyperglycemia promoted the PNI of PC in a HIF1α-dependent manner in vitro; however, whether this phenomenon occurred in actual organisms was still unclear. Hence, by using STZ injections and sciatic nerve grafts, an in vivo PNI sciatic nerve infiltrating
tumor model (Fig. 6A) was established to explore the influence of hyperglycemia on the PNI of PC.

First, the blood glucose levels of the mice were detected on the 7th day after injecting 150 mg/kg STZ or sodium citrate buffer, and STZ treatment was found to effectively induce hyperglycemia in the mice (Fig. 6B). Next, the sciatic nerve function in the three groups of mice was monitored and evaluated using a sciatic nerve score and the distance of the paw span, and it was found that hyperglycemia significantly affected the function of the sciatic nerve; however, HIF1α inhibition improved this phenomenon (Fig. 6C and D).

After the sciatic nerve and the surrounding tumor tissue were dissected out, hyperglycemia was found to promote the infiltration of the PC cells and tumor growth, while HIF1α inhibition partially abrogated this effect (Fig. 6E and F). In conclusion, the present study demonstrated that hyperglycemia promoted the PNI of pancreatic cancer cells in a HIF1α-dependent manner in vivo.

Discussion
PC is one of the most highly malignant cancers, with a 5-year survival rate of <9% (1). Difficulties in its early diagnosis and rapid progression, and a lack of therapeutic strategies are the main clinical features of PDAC, which together lead to its poor prognosis (24). In total, <20% of patients with PC have the opportunity to undergo radical excision, leaving only certain adjuvant chemotherapies, such as gemcitabine, S-1 and FOLFIRINOX, for treatment; however, even among patients who undergo surgery, 80% still rapidly progress to the late-stage of disease (25). Hence, early prevention and
diagnosis, rather than finding ways to treat PC, is a crucial strategy to improve its poor prognosis.

As the main pathological feature of PDAC (8), PNI is closely associated with its progression and poor prognosis (26-28). The cross-talk between tumor cells and nerves promotes PNI. Specifically, on the one hand, PC cells secrete neurotrophic factors, such as NGF, that promote the growth of nerves toward them, while on the other hand, the activation of the migration and invasion-related pathways of the PC cells themselves triggers them to migrate toward and invade nerves (19). Once PC cells invade into the nerves, they trigger an inflammatory response that further destroys the structure of the nerves, and neurotrophic factors (such as NGF-activating transient receptor potential cation channel subfamily V member 1) secreted by PC increase the sensitivity of the nerves to stimulation, causing cancer-related pain; this entire process induces high rates of cancer metastasis and cancer recurrence (28,29).

It is important to keep in mind that cancer cells and nerves do not have an isolated existence in tumor tissues; they are surrounded by pancreatic stellate cells (PSCs), immune cells, endothelial cells and other elements, such as the extracellular matrix. These elements form a complex tumor microenvironment (TME) that promotes the PC to invade the nerves (4,30-32). Indeed, as aforementioned, after they invade the nerves, the inflammation caused by the PC cells induces immune cell infiltration, PSC activation and a series of mesenchymal remodeling reactions to build an even more tumor-promoting TME (7,33,34). Such a cascade-amplifying loop exacerbates the progression of PC and PNI. Notably, in transgenic mouse models of PC, researchers have demonstrated that PNI is an early precancerous lesion of PC and that anti-PNI treatment is beneficial for the prognosis (5,26,35,36).

As a clear risk factor of PC, hyperglycemia promotes the progression of PC by modulating proliferation/invasion/migration, inducing apoptotic/autophagy and increasing the chemoresistance of PC (14,20,22). However, although the PNI-promoting effect of hyperglycemia has been previously reported (16,37), the underlying mechanism remains unclear. The present study demonstrated that hyperglycemia promoted the invasion/migration of PC cells, and the expression of NGF was elevated. All of these phenomena were HIF1α-dependent. HIF1-α can be activated by a tolerable level of reactive oxygen species (ROS) (38), and ROS can develop into oxidative stress, which is beneficial for the maintenance of a tumorigenic condition (39). Therefore, more research about HIF1-α, ROS and oxidative stress is necessary. Indeed, in the present study, short-term hyperglycemia caused little damage to the DRGs axons and promoted both PC cell PNI and DRG outgrowth in a HIF1α-dependent manner. However, with prolongation of the exposure to high glucose, the outgrowth of the DRGs was suppressed, and this may be caused by the hyperglycemia damaging the structure of the axons, such as the Schwann cells, a necessary cellular element for the growth of axons (16). Long-term hyperglycemia increases the secretion of pro-inflammatory factors, such as interleukin-6, tumor necrosis factor-α and cyclooxygenase-2, and these factors may be closely related to the development of tumors; however, they will destroy the nerve structure and inhibit the growth of axons (14). Nevertheless, we consider that the nerve damage caused by hyperglycemia is not inconsistent with the occurrence of PNI. Instead, PC cells are more likely to penetrate damaged nerves in a localized inflammatory environment. Consistent with this, the present study also found that long-term hyperglycemia does promote the infiltration and growth of PC in vivo. This may be related to its activating effect on HIF1α. In the study by Li and Ma (39), it was suggested that the hyperglycemic conditions increased the expression of cytokines by PC, and caused demyelination and axonal degeneration of nerves. This is consistent with the present results showing that NGF generated by PC can promote the PNI of PC. More detailed research needs to be conducted to clarify the specific mechanisms involved in this phenomenon.

In conclusion, all of the findings of the present study revealed that a high-glucose microenvironment enhances the invasion/migration ability of PC. In addition, hyperglycemia upregulated the expression of NGF and promoted the PNI of PC by activating HIF1α. However, the underlying mechanism requires additional investigation.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable request.

Authors’ contributions

WM and WZ performed the experiments, and XZ and YM helped analyze the data. YZ and BW helped to design the project. WL, SM, WM and LZ analyzed the data and wrote the manuscript. All authors have read and approved the manuscript. WM, WZ, XZ, YM, YZ, BW, WL, SM and LZ have seen and confirmed the authenticity of the raw data generated during the study.

Ethics approval and consent to participate

All animal experiments were conducted in strict accordance with the Declaration of Helsinki, and all protocols were approved by the Ethical Committee of the First Affiliated Hospital of Xi’an Jiaotong University (Xi’an, China).

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

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