Resveratrol inhibits hypoxia-driven ROS-induced invasive and migratory ability of pancreatic cancer cells via suppression of the Hedgehog signaling pathway

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Abstract. A hypoxic microenvironment is commonly found in the central region of solid tumors, including pancreatic cancer. Our previous study revealed that resveratrol plays an important role in suppressing the proliferation and EMT of pancreatic cancer cells. However, whether resveratrol could suppress hypoxia-induced cancer progression and the underlying mechanisms have not been fully elucidated. The aim of the present study was to evaluate whether resveratrol affects hypoxia-induced reactive oxygen species (ROS) production and the activation of the Hedgehog (Hh) signaling pathway as well as the invasion of pancreatic cancer. The human pancreatic cancer cell lines, BxPC-3 and Panc-1, were subjected to a hypoxic condition and three different concentrations of resveratrol. The intracellular ROS were determined using 2,7-dichlorodihydrofluorecein diacetate. Wound healing and Transwell invasion assays were used to detect the migratory and invasive potential of the cancer cells. Metastatic-related and Hh signaling-related factors were detected by qRT-PCR and western blot analysis. Immunofluorescence staining was used to test the nuclear translocation of GLI1. The results showed that the hypoxia-induced production of ROS was decreased by resveratrol in a concentration-dependent manner. Resveratrol significantly inhibited the hypoxia-stimulated invasion and migration of pancreatic cancer cells. Resveratrol inhibited hypoxia-induced HIF-1α protein expression. Resveratrol also suppressed hypoxia-induced expression of metastatic-related factors, uPA and MMP2. In addition, resveratrol markedly inhibited hypoxia-mediated activation of the Hh signaling pathway. Furthermore, the antioxidant N-acetylcysteine (NAC) significantly suppressed the invasive and migratory ability of pancreatic cancer cells during hypoxia. Taken together, these data indicate that resveratrol plays an important role in suppressing hypoxia-driven ROS-induced pancreatic cancer progression by inhibiting the Hh signaling pathway, providing evidence that resveratrol may be a potential candidate for the chemoprevention of cancer.

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Introduction

Pancreatic cancer is a highly malignant tumor with an extremely poor prognosis, partially due to the lack of early diagnosis and treatment options (1). Although surgery remains the only way to cure this severe disease, the majority of patients present at an advanced inoperable stage and only 20% of patients are with localized disease amenable for surgery (2). Even those seemingly resectable pancreatic tumors often fail to be cured due to the microscopic systemic spread of the cancer that occurs before the surgical intervention (3). Understanding the molecular basis of the disease is highly desirable for developing new strategies to prevent and treat pancreatic cancer.

Low oxygen tension is most commonly presented in the microenvironment of solid tumors (4). Tumor hypoxia is associated with enhanced tumor invasiveness, angiogenesis and distant metastasis (5,6). Hypoxia-inducible factor-1 (HIF-1), which belongs to the basic helix-loop-helix-periodic acid-Schiff domain transcription factor family, is the most important transcription factor as a result of intratumoral hypoxia (7). HIF-1 consists of two subunits, HIF-1α and HIF-1β. Only the expression and activation of HIF-1α is tightly regulated by the cellular oxygen concentration (8). In pancreatic cancer, the level of HIF-1α expression is overexpressed and is associated with tumor progression, angiogenesis, cell migration and hepatic metastasis (9,10). Our previous study identified that Hedgehog (Hh) signaling modulated hypoxia-induced pancreatic cancer epithelial to mesenchymal transition (EMT) and invasion (11).

The Hh signaling pathway, which is considered to play an important role in vertebrate development, the homeostatic

process and tumorigenesis (12), is normally quiescent in the adult pancreas and has been shown to be very active in pancreatic cancer (11). The Hh signaling pathway, initiated through the binding of secreted Hh ligands to the membrane receptor patched 1 (PTCH1), results in smoothened (SMO) dissociation, nuclear translocation and activation of the transcription factors of the GLI family (11,13). The expression of SMO and GLI1 is presumed to be markers of Hh pathway activation (11). Our previous study confirmed that hypoxia-induced invasion and the EMT process is intimately related with the Hh signaling pathway (11). In addition, inhibition of Hh signaling also enhanced vascular density and delivery of gemcitabine in a mouse model of pancreatic cancer (14).

Reactive oxygen species (ROS) generated by the mitochondrial respiratory chain, consist of a number of chemically reactive molecules derived from oxygen, including hydrogen peroxide (H_2O_2). Malignant tumor cells commonly have increased levels of ROS, which plays a significant role in cancer progression (15,16). Our recent study showed that hypoxia-induced ROS production is intimately related with pancreatic stellate cell (PSC) activation and pancreatic cancer cell invasion (17).

Resveratrol (*trans*-3,4',5-trihydroxystilbene), a natural polyphenolic phytoalexin, is widely found in plants (such as grape skin, red wine, berries and peanuts) and in traditional Chinese medicines (such as *Rheum officinale* Baill. and *Polygonum cuspidatum*) (18). Recent studies have shown that resveratrol has many biological and pharmaceutical properties, including anti-inflammatory, antioxidant, anti-aging, neuroprotective and antitumorigenic capabilities (19-21). Our previous study demonstrated that resveratrol plays an important role in suppressing the proliferation and EMT of pancreatic cancer cells via the PI-3K/Akt/NF-κB signaling pathway (22). In addition, we also confirmed that resveratrol inhibited the growth of human pancreatic cancer cells *in vitro* by inhibiting cell proliferation and promoting cell apoptosis via inhibition of the Hh signaling pathway (23).

In the present study, we tested the hypothesis that resveratrol is able to inhibit hypoxia-induced ROS production and the invasive and migratory ability of pancreatic cancer cells. We also investigated the effect of resveratrol on hypoxia-induced activation of the Hh pathway. Results from the present study suggest that resveratrol treatment may be a novel option for the therapy of pancreatic cancer via inhibition of the Hh signaling pathway.

Materials and methods

Preparation of chemicals. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). Resveratrol (>99% pure) was acquired from Xi'an Chongxin Natural Additive Company (Xi'an, China). N-acetylcysteine (NAC) was purchased from Sigma. Millicell Transwells for the invasion assays were obtained from Millipore (Billerica, MA, USA). Matrigel was from BD (Biosciences, Bedford, MA, USA). Primary antibodies against HIF-1α, MMP-2, uPA, SHH, SMO as well as GLI1 were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Nitrocellulose membranes were from Millipore. The BCA assay kit and the chemiluminescence kit were from Pierce (Rockford, IL, USA). Other reagents were purchased from common commercial sources. All drug solutions were freshly prepared on the day of testing.

Cell cultures and treatments. The human pancreatic cancer cell lines, BxPC-3 and Panc-1, were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in DMEM containing 10% dialyzed heat-inactivated FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37˚C. In experiments designed to assess the role of hypoxia, cells were first cultured in normoxic conditions to obtain the desired subconfluence level (65-70%), and were then incubated in strictly controlled hypoxic conditions (1% O_2). Exponentially growing cells in complete medium were pretreated for 1 h with different concentrations of resveratrol, followed by continual incubation in normal culturing conditions or hypoxic conditions for the indicated time intervals according to the aim of the experiment.

Measurement of intracellular ROS. The level of intracellular ROS was measured using the ROS assay kit. In brief, cells were incubated with 2,7-dichlorodihydrofluorecein diacetate (DCFDA) for 30 min, washed in phosphate-buffered saline (PBS) 3 times, and fluorescence intensity was measured using a fluorometer (Becton-Dickinson, USA) with excitation at 488 nm and emission at 525 nm.

Wound healing assay. Cell migratory ability was detected by a wound-healing assay. Pancreatic cancer cells were seeded into 24-well plates ($1.0x10^5$ cells/500 μl). After the cells grew to 90-100% confluency, a sterile pipette tip was used to produce a wound line between the cells. Cellular debris was removed by washing with PBS and then allowed to migrate for 24 h. Images were captured at time 0 and 24 h post-wounding under a Nikon Diaphot TMD inverted microscope (magnification, x10). The relative distance traveled by the leading edge from 0 to 24 h was assessed using Photoshop software (n=5).

Transwell Matrigel invasion assays. The invasion of pancreatic cancer cells was performed in Transwell chambers. The 8.0-μm pore inserts were coated with 25 μl Matrigel. The cell suspensions ($5x10^4$) were added to the upper chambers in DMEM containing 1% FBS. Simultaneously, 500 ml of DMEM containing 20% FBS was placed in the lower chambers. The cells were allowed to migrate for 48 h at 37˚C. The non-invading cells were removed from the upper surface by scraping with a wet cotton swab. After rinsing with PBS, the filter was fixed and stained with crystal violet. Invasion ability was determined by counting the stained cells. The invasion ability was determined by counting the stained cells on the bottom surface. Three random fields were captured at a magnification of x20 (n=3).

Real-time quantitative PCR (qRT-PCR). Total RNA was extracted from the pancreatic cancer cells using the Fastgen200 RNA isolation system (Fastgen, Shanghai, China) according to the manufacturer's protocol. Total RNA was reverse-transcribed into cDNA using a PrimeScript RT reagent kit (Takara, Dalian, China). The primer sequences

were as follows: HIF-1α-F, 5'-AAG TCT AGG GAT GCA GCA-3' and HIF-1α-R, 5'-CAA GAT CAC CAG CAT CAT G-3'; MMP-2-F, 5'-GAT GAT GCC TTT GCT CGT GC-3' and MMP-2-R, 5'-CAA AGG GGT ATC CAT CGC CA-3'; uPA-F, 5'-TAA GAG CTG GTG TCT GAT TG-3' and uPA-R, 5'-TTG GAT GAA CTA GGC TAA AA-3'; SHH-F, 5'-TCC AGA AAC TCC GAG CGA TTT AAG-3' and SHH-R, 5'-CAC TTC CTG GCC ACT GGT TCA-3'; SMO-F, 5'-ACG AGG ACG TGG AGG GCT G-3' and SMO-R, 5'-CGC ACG GTA TCG GTA GTT CT-3'; GLI1-F, 5'-GGG ATG ATC CCA CAT CCT CAG TC-3' and GLI1-R, 5'-CTG GAG CAG CCC CCC CAG T-3'; β-actin-F, 5'-GAC TTA GTT GCG TTA CAC CCT TTC T-3' and β-actin-R, 5'-GAA CGG TGA AGG TGA CAG CAG T-3'. The PCR reactions consisted of 30 sec at 95˚C followed by 40 cycles at 95˚C for 5 sec, at 60˚C for 30 sec and at 72˚C for 30 sec. After each qRT-PCR, a dissociation curve analysis was conducted. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method as previously reported (24).

Western blotting. Proteins were electrophoretically resolved on a denaturing SDS-polyacrylamide gel and electrotransferred onto nitrocellulose membranes. The membranes were initially blocked with 5% non-fat dry milk in Tris-buffered saline (TBS) for 2 h and then probed with antibodies against HIF-1α, MMP-2, uPA, SHH, SMO, GLI1 or β-actin (loading control). After co-incubation with the primary antibodies at 4˚C overnight, the membranes were blotted with the secondary antibody for 2 h at 37˚C. The results were visualized using the ECL western blotting substrate and photographed by GeneBox (SynGene).

Immunofluorescence microscopy. After the designated treatment, pancreatic cancer cells were fixed with 4% paraformaldehyde for 10 min at room temperature, permeabilized in 0.5% Triton X-100 for 10 min and blocked in 1% BSA for 1 h. Fixed cells were then incubated with primary antibody against GLI1 (1:100) at 4˚C overnight. Cells were washed and incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG for 1 h in a darkroom. Nuclei were stained with DAPI for 5 min. The cells were visualized by a fluorescence microscope (Nikon, Japan) using appropriate excitation and emission spectra at a magnification of x400.

Statistical analysis. Statistical analysis was performed using SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA). Data are presented as the means ± SEM of three replicate assays. Differences between groups were analyzed by analysis of variance (ANOVA). Statistical significance was set at $P<0.05$. All experiments were independently repeated at least three times.

Results

Resveratrol decreases hypoxia-induced production of ROS in pancreatic cancer cells. The intracellular ROS levels in BxPC-3 and Panc-1 cells treated with different concentrations of resveratrol under hypoxia conditions were determined using cell-permeable and redox-sensitive compound DCFDA by flow cytometry. Our previous study confirmed that the 50% inhibitory concentration (IC_{50}) for both BxPC-3 and Panc-1 cells is

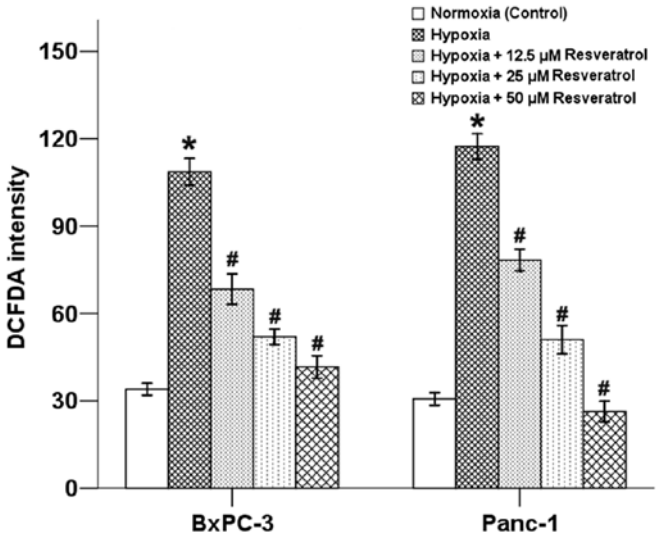

Figure 1. Effects of resveratrol on hypoxia-induced ROS production. BxPC-3 and Panc-1 cells were treated with the indicated concentrations of resveratrol (12.5, 25 or 50 μM), respectively, for ROS detection under a hypoxic condition. *P<0.05 as compared with the control group (normoxia); #P<0.05 as compared with the hypoxia group.

~50 μM of resveratrol, which exhibits no cytotoxic effects on BxPC-3 and Panc-1 cells (22). Therefore, treatment concentrations of 12.5, 25 and 50 μM of resveratrol on the cells were used for the present experiments. As shown in Fig. 1, a hypoxia condition significantly increased intracellular levels of ROS, and resveratrol suppressed these effects in a concentration-dependent manner after incubation for 24 h.

Resveratrol suppresses hypoxia-induced invasive ability of pancreatic cancer cells. A vital step of cancer metastasis is invasion of cancer cells through the basement membrane. In order to confirm whether resveratrol influences hypoxia-induced cancer cell invasive ability, we used a Transwell invasion assay. As shown in Fig. 2, hypoxia exposure significantly increased pancreatic cancer invasive ability, while the average cell number that invaded into the lower chamber decreased as the resveratrol concentration increased from 12.5 to 50 μM.

Resveratrol inhibits hypoxia-induced wound closure of pancreatic cancer cells. Migration and invasion are two important aspects that lead to the ability of cancer cells to form metastases. The effect of resveratrol on hypoxia-induced pancreatic cancer cell motility was determined using a wound-healing assay. Results showed that a hypoxic condition caused a significant increase in the migration of both BxPC-3 and Panc-1 cells after incubation for 24 h. Resveratrol suppressed these effects in a dose-dependent manner (Fig. 3). This finding revealed that resveratrol may be an effective inhibitor of hypoxia-induced migration and invasion of pancreatic cancer cells.

Resveratrol inhibits the expression of hypoxia-induced HIF-1α and metastatic-related factors. Previous studies have demonstrated that the effect induced by hypoxia is mainly mediated by HIF-1α (25). In order to investigate the effect of

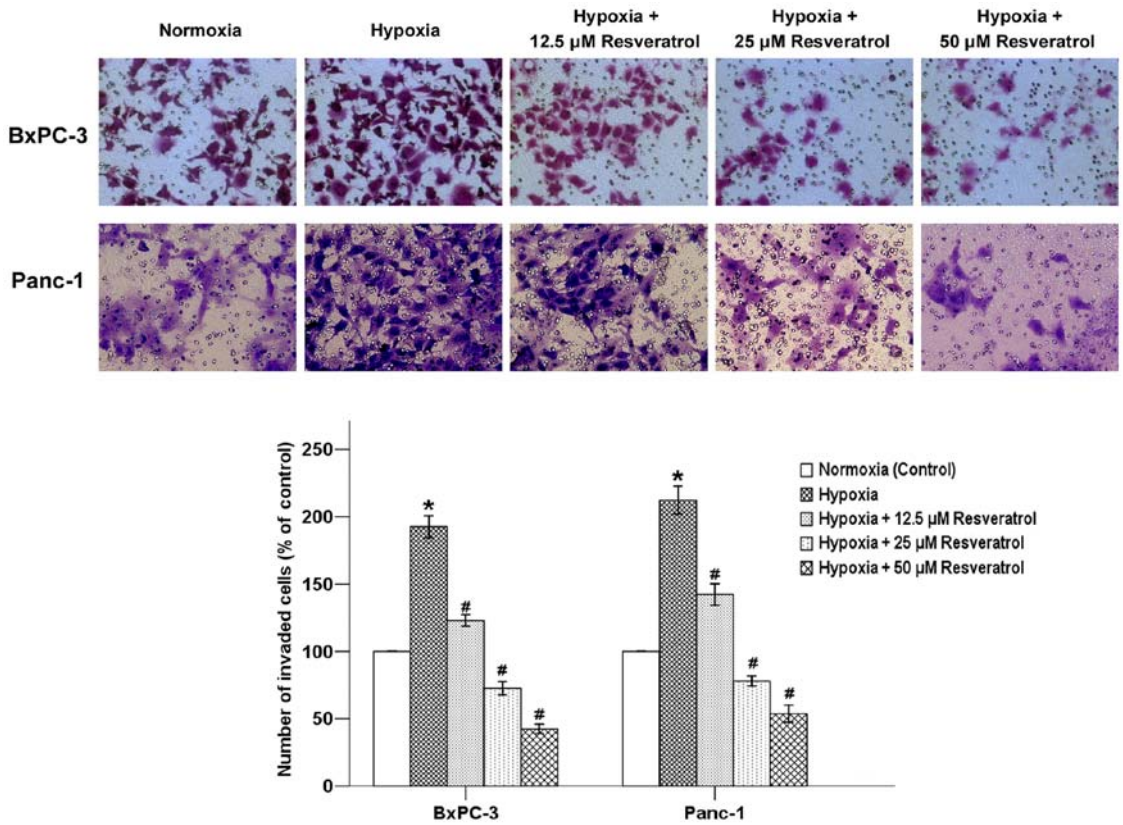

Figure 2. Effects of resveratrol on hypoxia-induced invasive ability of pancreatic cancer cells. The images show the bottom side of the filter inserts with the stained cells that had migrated through the filter pores after 48 h. The invasive ability of both BxPC-3 and Panc-1 cells was promoted under a hypoxic condition, whereas treatment with resveratrol reduced the invasion of the pancreatic cancer cells. *P<0.05 as compared with the control group (normoxia); #P<0.05 as compared with the hypoxia group.

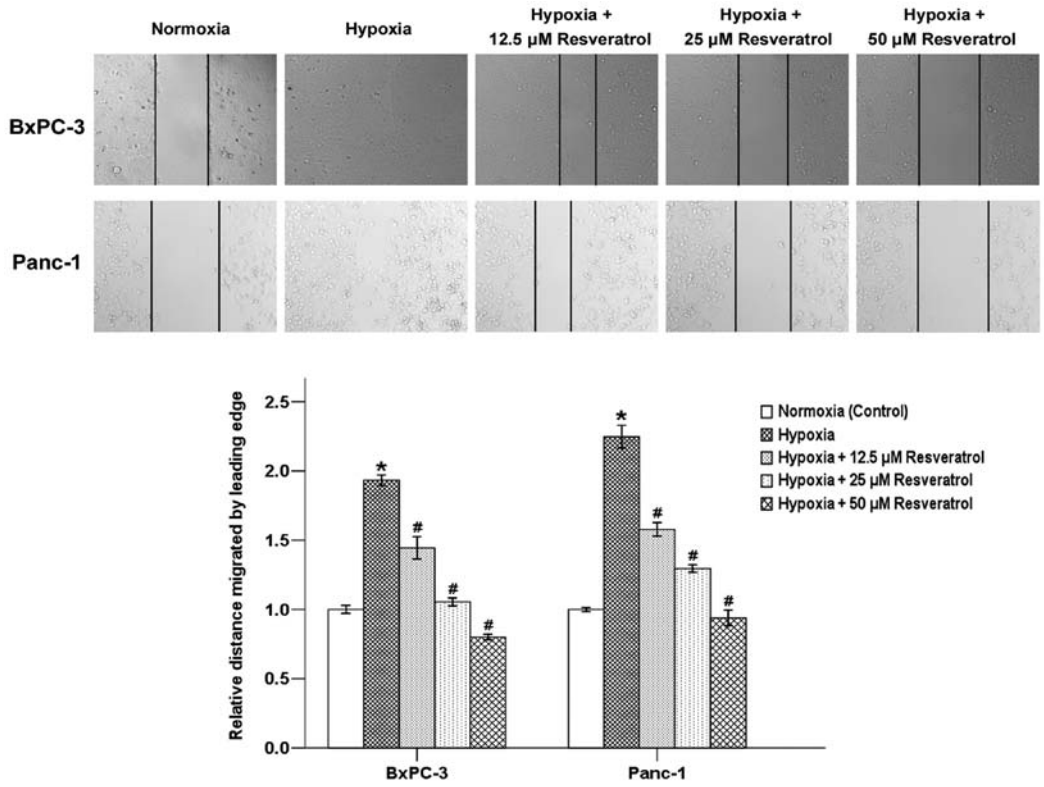

Figure 3. Effects of resveratrol on hypoxia-induced migratory ability of pancreatic cancer cells. The confluent monolayer was wounded with a sterile pipette tip, and the cells were allowed to migrate for 24 h. The migratory ability of BxPC-3 and Panc-1 cells was promoted under a hypoxic condition, whereas treatment with resveratrol reduced the migration of the pancreatic cancer cells. *P<0.05 as compared with the control group (normoxia); #P<0.05 as compared with the hypoxia group.

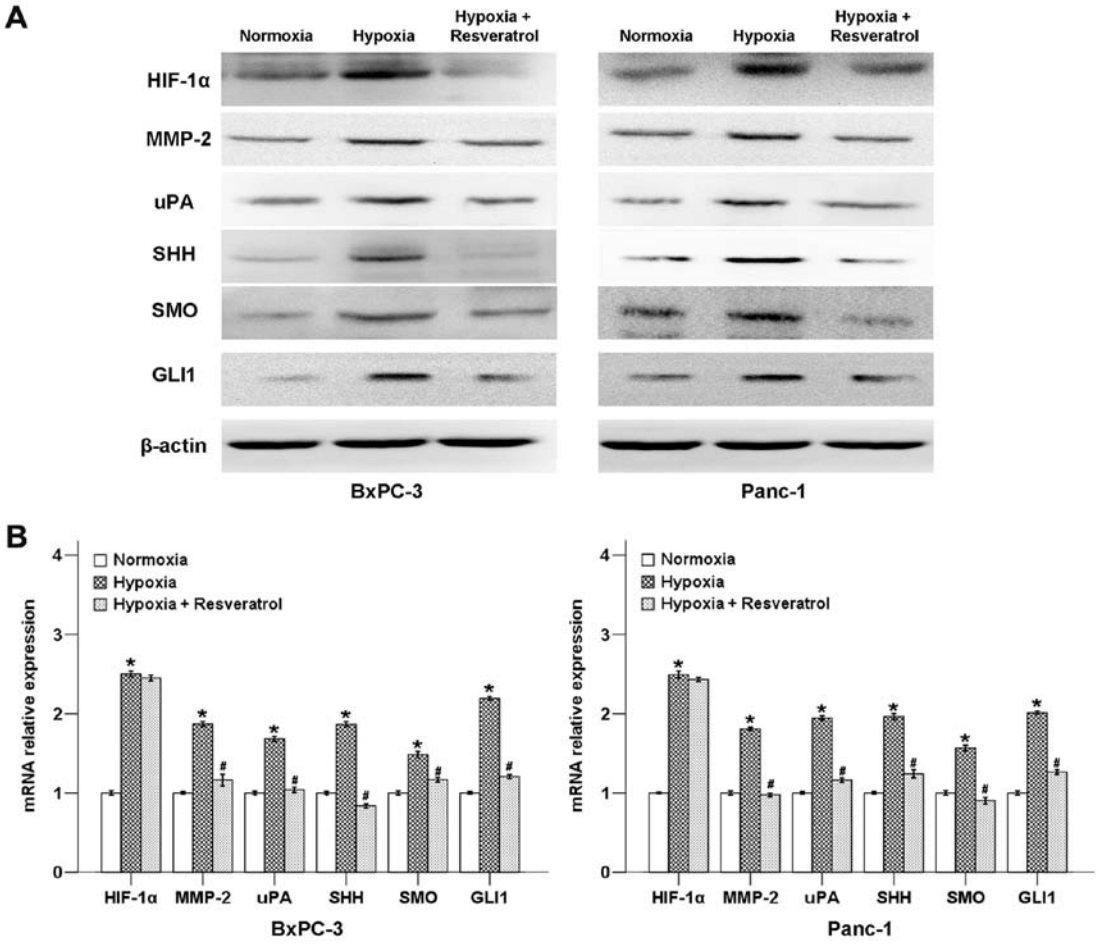

Figure 4. Effects of resveratrol on expression levels of hypoxia-induced metastatic-related factors and Hh signaling activation in pancreatic cancer cells. (A) Treatment with resveratrol diminished the effects of hypoxia-induced expression of HIF-1α, MMP-2, uPA, SHH, SMO and GLI1 at the protein level in the BxPC-3 and Panc-1 cells as determined by western blotting. (B) Treatment with resveratrol also diminished the effects of the expression of hypoxia-induced metastatic-related factors and Hh signaling activation at the mRNA level in both cancer cell lines, as determined by qRT-PCR. *P<0.05 as compared with the normoxia group; #P<0.05 as compared with the hypoxia group.

hypoxia on pancreatic cancer cells, both BxPC-3 and Panc-1 cells were exposed to hypoxic conditions (1% O_2) for up to 48 h. As shown in Fig. 4, the expression levels of HIF-1α, uPA and MMP-2 were markedly increased in both cell lines, compared with normoxic conditions. Resveratrol decreased hypoxia-induced HIF-1α protein expression (Fig. 4A), while no apparent changes in HIF-1α mRNA were observed in both the BxPC-3 and Panc-1 cells (Fig. 4B), which indicated that resveratrol inhibits hypoxia-induced HIF-1α protein expression through a post-transcriptional mechanism as metastatic-related factors, uPA and MMP-2, have been implicated in cancer invasion and metastasis. Our results showed that resveratrol suppressed hypoxia-induced uPA and MMP-2 expression at both the mRNA and protein levels (Fig. 4).

Resveratrol downregulates the hypoxia-activated Hh signaling pathway. Hh signaling plays an important role in the initiation and progression of pancreatic cancer (26). As shown in Fig. 4, the mRNA and protein expression levels of SHH, SMO and GLI1 were significantly increased in both the BxPC-3 and Panc-1 cancer cells, compared with the normal controls, which indicated that Hh signaling was activated in both cell lines under hypoxic condition. Resveratrol markedly decreased hypoxia-induced expression levels of SHH, SMO and GLI1. In addition, immunofluorescence staining of these treated cells also confirmed that a hypoxic condition could induce GLI1 expression in the nucleus of BxPC-3 and Panc-1 cells, while resveratrol obviously decreased the nuclear translocation of GLI1 (Fig. 5).

NAC suppresses hypoxia-induced ROS generation as well as the invasive and migratory ability of pancreatic cancer cells. NAC, a precursor of L-cysteine, is thought to be a scavenger of free radicals such as hydroxyl radical, H_2O_2 and superoxide. To explore whether hypoxia-induced invasive and migratory ability of pancreatic cancer cells is related with ROS production, we cultured the cells under a hypoxic condition in the presence or absence of 20 mM NAC. The results showed that NAC efficiently reduced ROS levels under a hypoxic condition in both the BxPC-3 and Panc-1 cells (Fig. 6A). The average cell number that invaded into the lower chamber decreased with NAC treatment (Fig. 6B). The cell migration ability (Fig. 6C) was also significantly inhibited 24 h after the addition of NAC. Additionally, the mRNA expression of MMP-2, uPA and GLI1 was downregulated by NAC under a hypoxic condition (Fig. 6D). Taken together, our

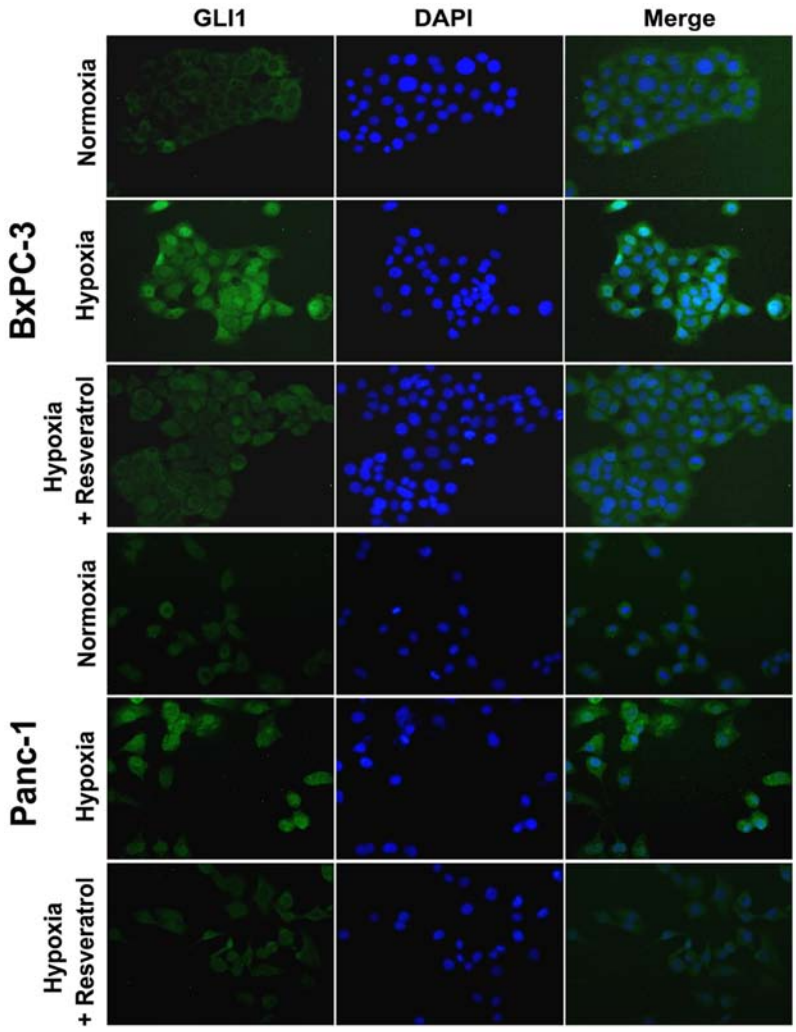

Figure 5. Immuofluorescence staining of GLI1 in pancreatic cancer cells. A hypoxic condition induced GLI1 expression in the nucleus of BxPC-3 and Panc-1 cells, while resveratrol obviously decreased the nuclear translocation of GLI1. Green represents GLI1 staining. Blue signal represents nuclear DNA staining by DAPI.

results demonstrated that resveratrol inhibits hypoxia-driven ROS-induced cancer progression via suppression of the Hh signaling pathway in both the BxPC-3 and Panc-1 cells.

Discussion

Pancreatic cancer is a malignant carcinoma of the digestive system with an extremely high mortality rate, due to both the inherently aggressive biology of the disease and its late diagnosis in most cases (2). A hypoxic microenvironment is commonly found in the central region of solid tumors, including pancreatic cancer (11). Tumor hypoxia not only increases the metastatic capacity of cancer cells, but also leads to resistance to chemotherapy and radiotherapy. Hypoxia can also induce altered transcription and translation of a number of DNA damage response and repair genes, which further leads to inhibition of recombination-mediated repair of DNA double-strand breaks. In addition, hypoxia can increase the rate of mutation (27). Overexpression of HIF-1α has been shown in many human cancers and their metastases and is closely associated with a more aggressive tumor progression (28). Our previous study demonstrated that resveratrol inhibits the growth of human pancreatic cancer cells *in vitro* by inhibiting cell proliferation and promoting cell apoptosis via inhibition of the Hh signaling pathway (23). In the present study, we focused on whether resveratrol is able to suppress hypoxia-induced cancer invasive and migratory ability and its underlying mechanism.

Our data showed that a hypoxic condition could significantly increase the production of ROS and the expression of HIF-1α as well as cancer metastatic-related factors, uPA and MMP-2, in BxPC-3 and Panc-1 cells, which further enhanced the capacity of the pancreatic cancer cells to migrate and invade the extracellular matrix. Resveratrol was able to abrogate these effects of a hypoxic condition. A previous study confirmed that hypoxia activates canonical Hh signaling through accumulation of HIF-1α (29). In the present study, we tested the effects of a hypoxic condition and resveratrol on the activation of SHH, SMO and GLI1. The data showed that a hypoxic condition significantly increased the expression levels of SHH, SMO and GLI1 in both the BxPC-3 and Panc-1 cancer cells, whereas the addition of resveratrol to the cell culture

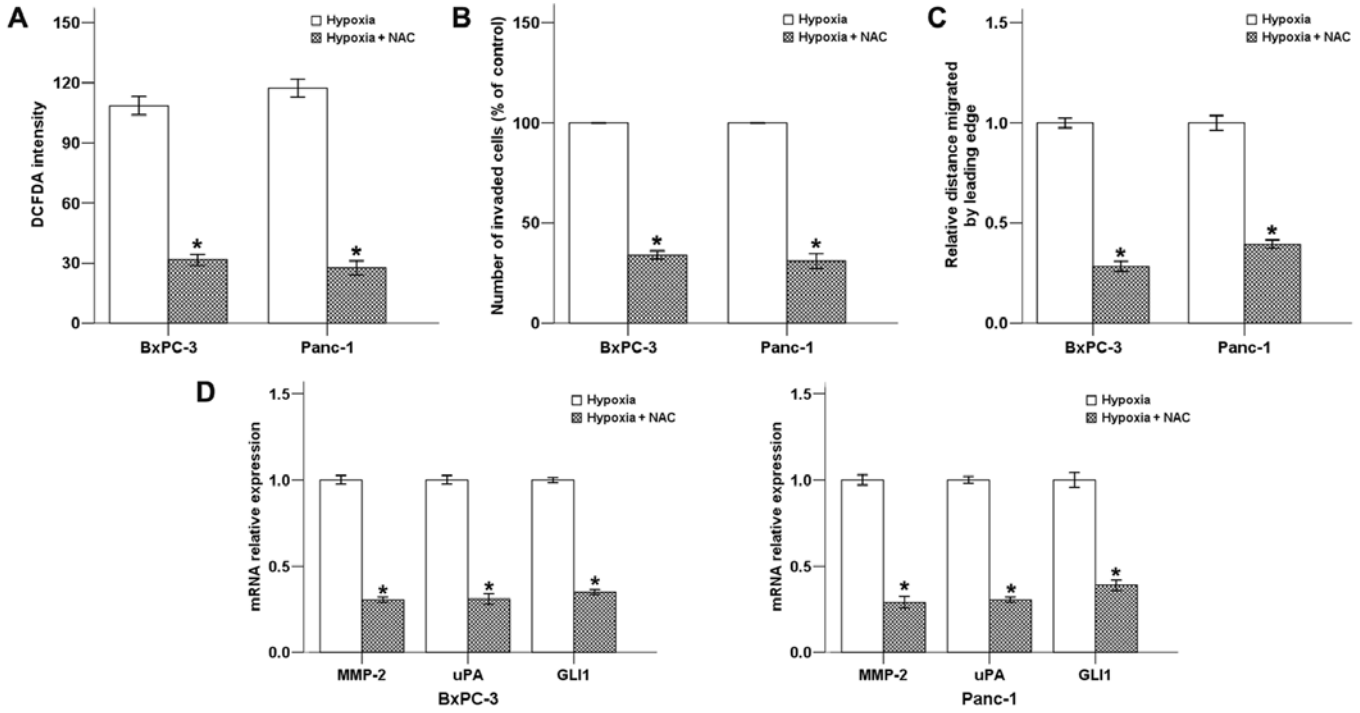

Figure 6. Effects of NAC on ROS generation and the invasive and migratory ability of pancreatic cancer cells under a hypoxic condition. (A) The cells were treated with hypoxia for 24 h with or without 20 mM NAC, and ROS production was evaluated using the cell-permeable and redox-sensitive compound DCFDA by flow cytometry. (B) NAC (20 mM) exposure for 48 h inhibited the invasion of the BxPC-3 and Panc-1 cancer cells under a hypoxic condition. (C) NAC (20 mM) reduced the migration of pancreatic cancer cells, as determined by a wound-healing assay. (D) Treatment with NAC diminished the expression of MMP-2, uPA and GLI1 at the mRNA level under a hypoxic condition in both cancer cell lines as determined by qRT-PCR. *P<0.05 compared with the control group.

resulted in a decrease in these Hh pathway-related factors. In addition, the hypoxia-enhanced nuclear translocation of GLI1 was decreased by resveratrol.

ROS generated by the mitochondrial respiratory chain, consist of a number of chemically reactive molecules derived from oxygen. As a double-edged sword, excess ROS production can kill cancer cells, whereas sublethal concentrations of ROS can stimulate tumor progression by promoting cell proliferation, survival, invasion and metastasis (30). Our previous studies confirmed that both a hyperglycemic condition and SOD-induced ROS production were able to promote the invasive and migratory activity of pancreatic cancer (15,16). Our recent study also showed that hypoxia-induced ROS production is intimately related with pancreatic stellate cell (PSC) activation and pancreatic cancer cell invasion (17). In the present study, we found that hypoxia-induced ROS production was suppressed by resveratrol in a concentration-dependent manner.

Resveratrol and its analogues have been proven to inhibit the invasion of many tumor types, including pancreatic cancer (31). Recent studies have focused on the relationship between resveratrol and hypoxia-induced cancer progression. Wu *et al* (8) demonstrated that the anti-metastatic effect of resveratrol was associated with the restriction of invasion, mobility, adhesion and MMP expression under both normoxic and hypoxic conditions in colon carcinoma. Mitani *et al* (32) showed that dietary resveratrol inhibited β-catenin-mediated androgen receptor function by decreasing the expression of HIF-1α protein in hypoxic LNCaP cells and consequently suppressed prostate cancer cell growth *in vivo*. They also confirmed that resveratrol suppressed hypoxia-induced resistance to cytotoxicity of doxorubicin and repressed the expression of CBR1 in breast cancer cells (33). In the present study, we also found that resveratrol was able to decrease hypoxia-induced pancreatic cancer invasion and migration, which may be attributed to the reduction of ROS.

Resveratrol can inhibit tumor biological behavior through multiple signaling pathways. Our previous study indicated that resveratrol plays an important role in suppressing the proliferation, migration and invasion of pancreatic cancer cells *in vitro* by modulating EMT-related factors via the PI-3K/Akt/NF-κB signaling pathway. We also showed that resveratrol was able to suppress the migration and invasion of pancreatic cancer cells by inhibiting TGF-β-mediated EMT (22). Ji *et al* (34) confirmed that resveratrol downregulated MALAT1 and decreased nuclear localization of β-catenin, which in turn attenuated the Wnt/β-catenin signaling pathway leading to the inhibition of invasion and metastasis of colorectal cancer cells. Sun *et al* (35) showed that resveratrol activated SIRT1, which further hampered lung cancer cell metastasis *in vivo*. In addition, resveratrol also inhibited hypoxia-induced HIF-1α accumulation and vascular endothelial growth factor (VEGF) expression in both human tongue squamous cell carcinomas and hepatoma cells via the suppression of ERK1/2 and Akt signaling pathway (36).

Hh signaling activation is a common event in pancreatic cancer. The Hh signaling pathway is composed of patched (PTCH), a 12-transmembrane receptor, smoothened (SMO),

a 7-transmembrane receptor and the GLI transcription factor family. A previous study demonstrated that hypoxia could activate canonical Hh signaling through accumulation of HIF-1α *in vitro* and *in vivo* (26). Our recent study also confirmed that accumulated HIF-1α could also trigger non-canonical Hh signaling to facilitate hypoxia-induced EMT and invasion processes in pancreatic cancer (11). Qin *et al* (23) recently indicated that resveratrol inhibited pancreatic cancer cell proliferation and promoted cell apoptosis via inhibition of the Hh signaling pathway. Gao *et al* (37) also demonstrated that resveratrol was able to inhibit gastric cancer cell invasion and metastasis *in vitro* by inhibiting the Hh signaling pathway and EMT. In addition, resveratrol could not only effectively downregulate interleukin-6-stimulated SHH signaling in human acute myeloid leukemia (38), but also inhibited both SHH signaling and Bcr-Abl expression in human chronic myeloid leukemia cells (39), indicating that resveratrol may have potential as a treatment for myeloid leukemia. In the present study, we showed that resveratrol was able to suppress hypoxia-induced activation of the Hh pathway and thus inhibited pancreatic cancer cell invasive and migratory ability.

In conclusion, the present study demonstrated that resveratrol plays an important role in suppressing hypoxia-induced migration and invasion of pancreatic cancer cells *in vitro* by inhibiting the Hh signaling pathway. These results suggest that resveratrol may be a potential anticancer agent for the treatment of pancreatic cancer.

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