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Original Article

Angiopoietin-1 alleviates LPS-induced inflammatory injury by up-regulation of miR-126 in pancreas cell line HPDE6-C7

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Abstract: Background: Acute pancreatitis is an inflammatory disorder of the pancreas, leading to multiple organ dysfunction syndrome in severe cases. Angiopoietin-1 (Ang-1), a Tie-2 receptor agonist, and microRNA-126 (miR-126) have been reported to be involved in angiogenesis and anti-inflammatory functions. In the present study, we explored the effects of Ang-1 and miR-126 on lipopolysaccharide (LPS)-induced inflammatory injury in pancreatic cells, HPDE6-C7. Methods: The immortalized human pancreatic duct epithelial cell line, HPDE6-C7, was treated with LPS (10 μg/mL) to induce cell injury. Ang-1 was used at 300 ng/mL concentration. Cell viability was measured using CCK-8 assay and apoptosis was assessed using flow cytometry. Quantitative real time polymerase chain reaction (RT-PCR) was used to measure the mRNA expressions of different proteins. Enzyme linked immunosorbent assay (ELISA) was used to measure the concentrations of the pro-inflammatory cytokines. Luciferase activity assay was done to identify the target of miR-126. Western blot was used to measure the expressions of different proteins. Results: Ang-1 promoted LPS-suppressed cell viability and inhibited LPS-induced cell apoptosis, pro-inflammatory cytokine (TNF-α, IL-1β, IL-6, and IL-8) production, and activation of NF-κB and JNK pathways in HPDE6-C7 cells. Furthermore, Ang-1 promoted the expression of miR-126, which in turn protected PDE6-C7 cells against LPS-induced injury. PDCD4 was identified as a direct target of miR-126 and was negatively regulated by miR-126. Mechanistic study revealed that overexpression of PDCD4 reversed miR-126-mediated inhibition of LPS-induced activation of NF-κB and JNK pathways. Conclusion: Ang-1 alleviates LPS-induced inflammatory injury by up-regulation of miR-126 and down-regulation of PDCD4 in pancreatic cell line HPDE6-C7.

Keywords: Pancreatitis, Angiopoietin-1, miR-126, PDCD4, inflammation, LPS

Introduction

Acute pancreatitis is an acute inflammatory disorder of the pancreas, often with involvement of other organs. Acute pancreatitis usually presents with upper abdominal pain with vomiting and abdominal tenderness [1]. Activation of pancreatic enzymes in the pancreas leads to autodigestion, edema, hemorrhage, and necrosis of pancreas [2]. In the high-income countries, the annual incidence of acute pancreatitis ranges from 13 to 45 per 100,000 populations [3]. Acute pancreatitis is divided into mild acute pancreatitis and severe acute pancreatitis. About 80% of acute pancreatitis cases are mild; such patients recover without complications, with a very low mortality rate (1%). However, 20% patients may experience the severe form of the disease leading to multiple organ dysfunction syndrome and even death [4-7]. Treatment strategies for acute pancreatitis include fluid therapy, short-term intravenous feeding, and pain management with narcotics for severe pain or non-steroidal anti-inflammatory drugs for mild pain [5]. Despite improvements in treatment strategies in the last few decades, the mortality rate for acute pancreatitis has not declined much [7, 8].

Several pro-inflammatory factors are reported to contribute to multi-organ failure in severe acute pancreatitis. Malmstrom et al showed

that interleukin 6 (IL-6) and IL-8 levels were significantly higher in patients with renal, respiratory, and circulatory failure, and in patients with multi-organ failure; and tumor necrosis factor α (TNF-α) was significantly higher in all types of organ failures, except for intestinal failure [9]. In a prospective clinical study, soluble IL-2 receptor (sIL-2R), IL-10, and IL-6 were significantly higher in patients with multi-organ failure [10]. Therefore, it is very important to explore the mechanism of inflammatory injury in pancreatic cells.

Angiopoietin-1 (Ang-1), an agonist of TEK tyrosine kinase (Tie-2) receptor, promotes vascular development in embryos and increases endothelial survival, migration, and differentiation. Ang-1 has potent vascular protective effects mediated via suppression of plasma leakage, inhibition of vascular inflammation, and prevention of endothelial death [11]. In an *in vivo* study in rats, administration of Ang-1-transfected mesenchymal stem cells significantly decreased pancreatic injury and inflammation, as reflected by decrease in pancreatitis severity scores and serum levels of amylase, lipase, and pro-inflammatory cytokines (TNF-α, interferon-γ, IL-1β, and IL-6), and also promoted pancreatic angiogenesis. These findings suggest that Ang-1 plays an important role in severe acute pancreatitis [12].

MicroRNAs (miRNAs) constitute a class of endogenous, non-coding, small (about 20-25 nt) RNAs that regulate the post-translational expression of human protein encoding genes. MiRNAs are involved in several physiological and pathological processes, including cell differentiation and development, metabolism, inflammation, and immunity [13-15]. Studies have identified several miRNAs as diagnostic and prognostic biomarkers for acute pancreatitis. Such miRNAs include miR-216, miR-126, miR-217, miR-375, etc [16-19]. MiR-126 is highly expressed in endothelial cells, and it promotes angiogenesis and inhibits vascular inflammation in endothelial cells [20]. In an observational study, a significant increase in miR-126-5p was observed in patients with severe acute pancreatitis [18]. However, the specific role of miR-126 in acute pancreatitis has not yet been investigated. In the present study, we evaluated the effects of Ang-1 and miR-126 on lipopolysaccharide (LPS)-induced inflammatory injury in pancreatic cells, HPDE6-C7, and also studied underlying mechanisms.

Materials and methods

Cell culture and treatment

The immortalized human pancreatic duct epithelial cell line, HPDE6-C7, was maintained in keratinocyte serum free medium, supplemented with 25 mg/500 mL bovine pituitary extract and 2.5 μg/500 mL epidermal growth factor (GIBCO Invitrogen Corporation, Grand Island, NY, USA). Cells were grown without antibiotics in an atmosphere of 5% CO_2, 99% relative humidity, and 37°C temperature. LPS was used at 10 μg/mL concentration to induce cell injury. Ang-1 was used at the concentration of 300 ng/mL.

CCK-8 assay

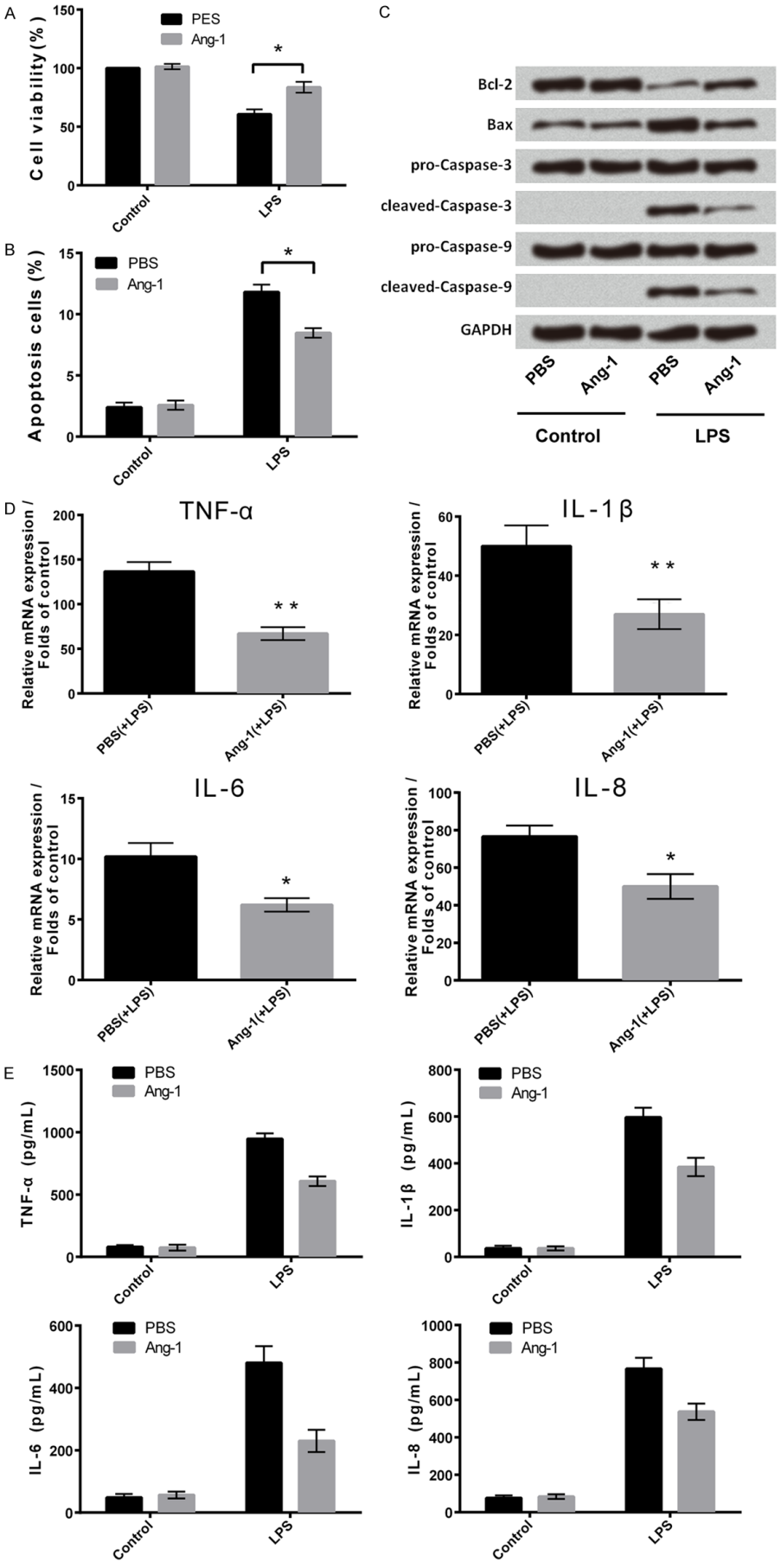
Cell proliferation was assessed by a cell counting kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD). Cells were seeded in a 96-well plate with 5000 cells/well. After stimulation, the CCK-8 solution was added to the culture medium, and the cultures were incubated for 1 hour at 37°C in humidified 95% air and 5% CO_2. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA).

Apoptosis assay

Flow cytometry analysis was performed to identify and quantify apoptotic cells using Annexin V-FITC/PI apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China). Cells (1,00,000 cells/well) were seeded in a 6 well-plate. Treated cells were washed twice with cold phosphate buffered saline (PBS) and re-suspended in buffer. The adherent and floating cells were combined and treated according to the manufacturer's instruction and measured with flow cytometer (Beckman Coulter, USA) to differentiate apoptotic cells (Annexin-V positive and PI-negative) from necrotic cells (Annexin-V and PI-positive).

Enzyme-linked immunosorbent assay (ELISA)

Culture supernatant was collected from 24-well plates and concentrations of inflammatory


A
Cell viability(%)
PES
Ang-1
Control
LPS
B
Apoptosis cells (%)
PBS
Ang-1
Control
LPS
C
Bcl-2
Bax
pro-Caspase-3
cleaved-Caspase-3
pro-Caspase-9
cleaved-Caspase-9
GAPDH
PBS
Ang-1
PBS
Ang-1
Control
LPS
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TNF-α
IL-1β
IL-6
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Relative mRNA expression / Folds of control
PBS(+LPS)
Ang-1(+LPS)
E
TNF-α (pg/mL)
IL-1β (pg/mL)
IL-6 (pg/mL)
IL-8 (pg/mL)
PBS
Ang-1
Control
LPS

Figure 1. Ang-1 alleviated LPS-induced inflammatory injury in pancreatic cells. Pancreatic duct cells, HPDE6-C7, were treated with LPS (10 μg/mL) + PBS or LPS + Ang-1 (300 ng/mL), and then (A) Cell viability was measured using CCK-8 assay; (B and C) Cell apoptosis was measured using flow cytometry and western blot; and (D and E) mRNA expressions and concentration levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) were measured using qRT-PCR and ELISA, respectively. *P<0.05, **P<0.01. Ang-1: Angiopoietin-1; CCK-8: cell counting kit-8; ELISA: enzyme-linked immunosorbent assay; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; IL: interleukin; LPS: lipopolysaccharide; PBS: phosphate buffered saline; qRT-PCR: quantitative reverse transcription polymerase chain reaction; TNF-α: tumor necrosis factor alpha.

cytokines were measured by ELISA using protocols supplied by the manufacturer (R&D Systems, Abingdon, UK) and normalized to cell protein concentrations.

MiRNAs transfection

MiR-126 mimic, miR-126 inhibitor and negative control (NC) were synthesized by GenePharma Co. (Shanghai, China). Cell transfections were done using Lipofectamine 3000 reagent (Invitrogen) as per the manufacturer's protocol.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated from transfected cells using Trizol reagent (Invitrogen) and treated with DNaseI (Promega). Reverse transcription was performed using MultiScribe® Reverse Transcriptase Kit (Applied Biosystems) and random hexamers or oligo (dT). The following reverse transcription conditions were used: 10 min at 25°C, 30 min at 48°C, and a final step of 5 min at 95°C.

Dual luciferase activity assay

The 3'UTR target site was generated by PCR. The luciferase reporter constructs with the PDCD4 (programmed cell death 4) 3'UTR carrying a putative miR-126-binding site into pMiR-report vector were amplified by PCR. Cells were co-transfected with the reporter construct, control vector and miR-126 or scramble using Lipofectamine 3000 (Life Technologies, USA). Reporter assays were done using dual-luciferase assay system (Promega) as per the manufacturer's instructions.

Western blot

The proteins used for western blotting were extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified using BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. Primary antibodies were prepared in 5% blocking buffer at a dilution of 1:1000. Primary antibody was incubated with the membrane at 4°C overnight, followed by wash and incubation with secondary antibody marked by horseradish peroxidase for 1 hr at room temperature. After rinsing, the polyvinylidene difluoride membrane-carried blots and antibodies were transferred into Bio-Rad ChemiDoc™ XRS system, and then 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

Statistical analysis

All experiments were repeated three times. The results of multiple experiments are presented as mean ± standard deviation. Statistical analyses were performed using SPSS 19.0 statistical software. *P* values were calculated using a one-way analysis of variance. *P* value of <0.05 was considered to indicate a statistically significant result.

Results

Ang-1 alleviated LPS-induced inflammatory injury in pancreatic cells

We treated HPDE6-C7 cells with LPS (10 μg/mL) + PBS or LPS + Ang-1 (300 ng/mL), and measured cell viability using CCK-8 assay, apoptosis using flow cytometry and western blot, and mRNA expressions and concentration levels of pro-inflammatory cytokines using qRT-PCR and ELISA, respectively. Results showed that LPS decreased cell viability and increased apoptosis, but Ang-1 reversed these effects by

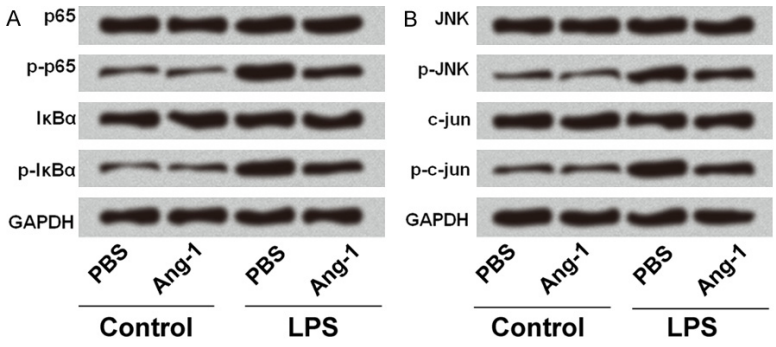

Figure 2. Ang-1 inhibited LPS-induced activation of NF-κB and JNK pathways. A and B. HPDE6-C7 cells were treated with LPS + PBS or LPS + Ang-1, and then the expression of NF-κB and JNK pathways proteins (IκBα, P65, JNK, c-jun) was measured using Western blot. Ang-1: Angiopoietin-1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; JNK: janus kinase; LPS: lipopolysaccharide; NF-κB: nuclear factor-kappa B; PBS: phosphate buffered saline.

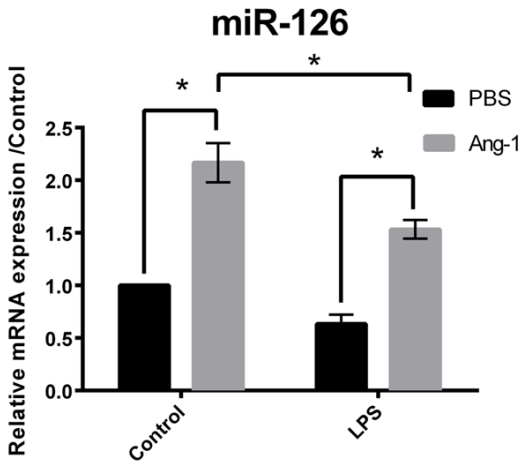

Figure 3. Ang-1 promoted the expression of miR-126. qRT-PCR was used to measure the relative mRNA expression of miR-126 in HPDE6-C7 cells. Cells in the control group were treated with PBS or Ang-1; cells in the LPS group were treated with LPS + PBS or LPS + Ang-1. *P<0.05. Ang-1: Angiopoietin-1; LPS: lipopolysaccharide; PBS: phosphate buffered saline; qRT-PCR: quantitative reverse transcription polymerase chain reaction.

significantly increasing cell viability (P<0.05; **Figure 1A**) and decreasing apoptosis (P<0.05; **Figure 1B**) compared to only LPS-treated cells. Western blot analysis of apoptosis-related proteins (**Figure 1C**) revealed that Ang-1 increased the expression of anti-apoptotic protein (Bcl-2) and decreased the expression of pro-apoptotic proteins (Bax, cleaved caspase-3 and cleaved caspase-9). Furthermore, LPS increased the mRNA expressions and concentration levels of TNF-α, IL-1β, IL-6, and IL-8 in the cells, but Ang-1 inhibited these effects by significantly decreasing the mRNA expressions and concentrations of TNF-α (P<0.01), IL-1β (P<0.01), IL-6 (P<0.05), and IL-8 (P<0.05) compared to only LPS-treated cells (**Figure 1D** and **1E**). These findings indicate that Ang-1 protected HPDE6-C7 cells from LPS-induced inflammatory injury by increasing cell viability, decreasing apoptosis, and decreasing the production of pro-inflammatory cytokines.

Ang-1 inhibited LPS-induced activation of nuclear factor-kappa B (NF-κB) and Janus kinase (JNK) pathways

Next, HPDE6-C7 cells were treated with LPS + PBS or LPS + Ang-1, and then the expressions of NF-κB and JNK pathways proteins (IκBα, P65, JNK, c-jun) were measured using western blot. As shown in **Figure 2A** and **2B**, LPS increased the expression of these proteins, but Ang-1 inhibited the increased expressions. These findings indicate that Ang-1 inhibits the LPS-induced activation of NF-κB and JNK signaling pathways.

Ang-1 promoted the expression of miR-126

qRT-PCR was used to measure the relative mRNA expression of miR-126 in HPDE6-C7 cells. For this test, the cells were divided into two groups: control group and LPS-treated group. Cells in the control group were treated with PBS or Ang-1; cells in the LPS group were treated with LPS + PBS or LPS + Ang-1. In both control and LPS-treated groups, Ang-1 significantly increased the mRNA expression of miR-126 compared to PBS treated control or LPS group (P<0.05; **Figure 3**). However, miR-126 expression was significantly lower in the Ang-1 and LPS-treated group compared to the control group treated only with Ang-1 (P<0.05; **Figure 3**). These findings indicate that Ang-1 increases the expression of miR-126.

Overexpression of miR-126 alleviated LPS-induced inflammatory injury

We then determined the effects of miR-126 on LPS-induced inflammatory injury in HPDE6-C7

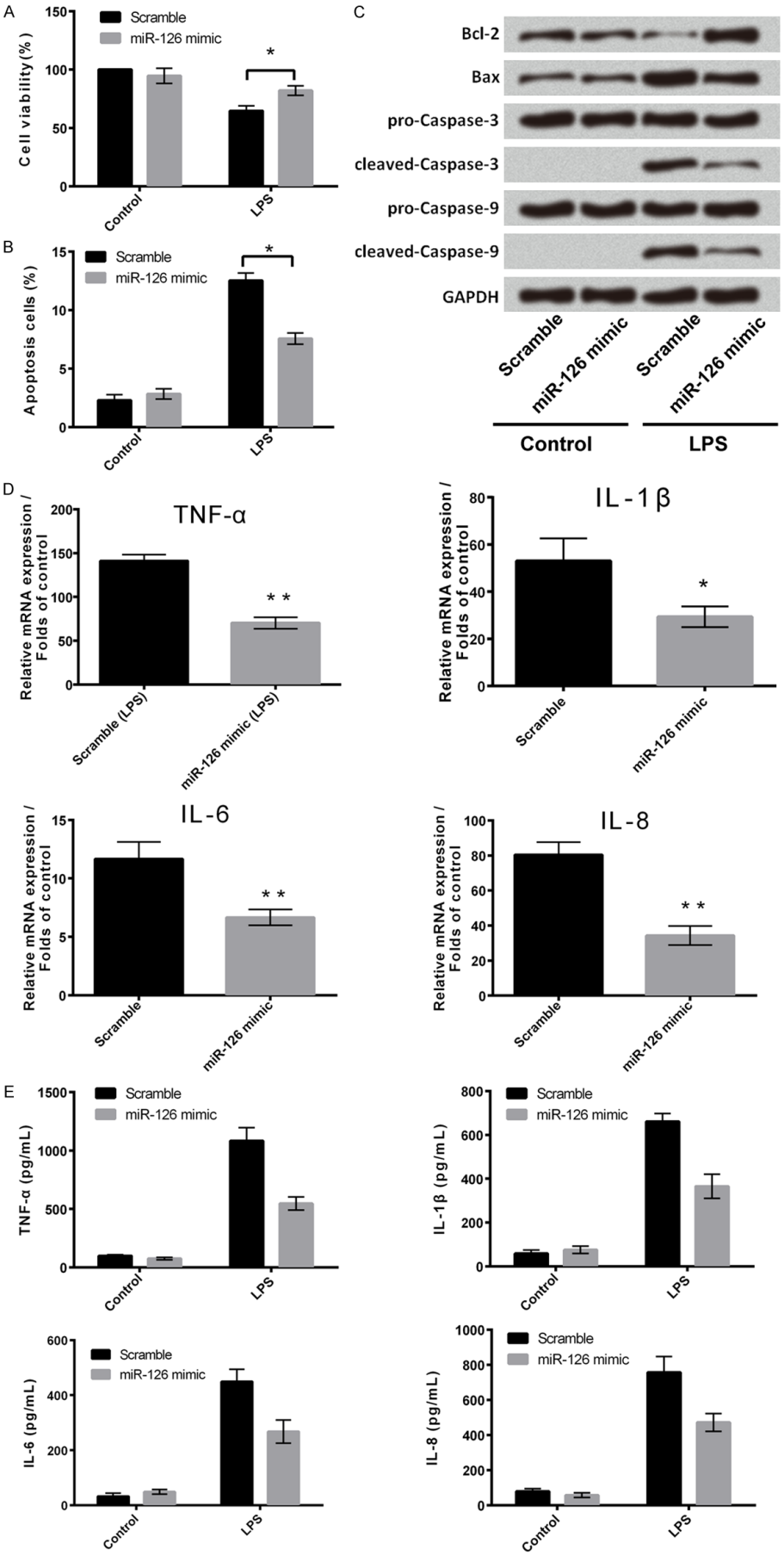
A
Cell viability (%)
Scramble
miR-126 mimic
Control
LPS
B
Apoptosis cells (%)
C
Bcl-2
Bax
pro-Caspase-3
cleaved-Caspase-3
pro-Caspase-9
cleaved-Caspase-9
GAPDH
Scramble
miR-126 mimic
Control
LPS
D
TNF-α
IL-1β
IL-6
IL-8
Relative mRNA expression / Folds of control
Scramble (LPS)
miR-126 mimic (LPS)
E
TNF-α (pg/mL)
IL-1β (pg/mL)
IL-6 (pg/mL)
IL-8 (pg/mL)

Figure 4. Overexpression of miR-126 alleviated LPS-induced inflammatory injury. HPDE6-C7 cells were treated with LPS + scramble or LPS + miR-126 mimic, and then (A) Cell viability was measured using CCK-8 assay; (B and C) Cell apoptosis was measured using flow cytometry and western blot; and (D and E) mRNA expressions and concentration levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) were measured using qRT-PCR and ELISA, respectively. *P<0.05, **P<0.01. CCK-8: cell counting kit-8; ELISA: enzyme-linked immunosorbent assay; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; IL: interleukin; LPS: lipopolysaccharide; PBS: phosphate buffered saline; qRT-PCR: quantitative reverse transcription polymerase chain reaction; TNF-α: tumor necrosis factor alpha.

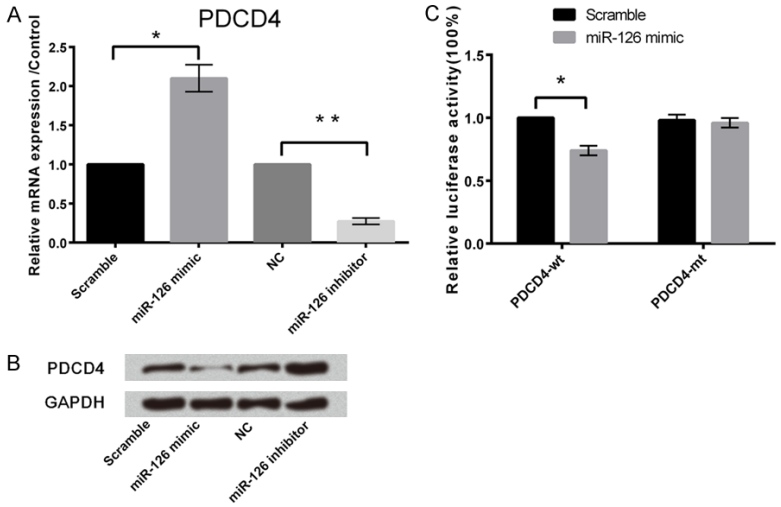

Figure 5. miR-126 negatively regulates PDCD4. A and B. The relative mRNA expression and protein expression of PDCD4 was measured using qRT-PCR and western blot, respectively. HPDE6-C7 cells in the miR-126 mimic group were transfected with scramble or miR-126 mimic, and the cells in the miR-126 inhibitor group were transfected with NC or miR-126 inhibitor. C. Dual luciferase reporter assay was performed to confirm PDCD4 as a target of miR-126. Two types of vector were constructed: PDCD4-wild-type (PDCD4-Wt) and PDCD4-mutated-type (PDCD4-Mt). Then scramble and miR-126 mimic cells were transfected with both the vectors separately, and luciferase activity was measured. *P<0.05, **P<0.01. NC: negative control; PDCD4: programmed cell death 4; qRT-PCR: quantitative reverse transcription polymerase chain reaction.

cells. We transfected HPDE6-C7 cells with either scramble or miR-126 mimic and treated those cells with both PBS and LPS separately. Cell viability was measured using CCK-8 assay, apoptosis was assessed using flow cytometry and western blot, and mRNA expressions and concentration levels of pro-inflammatory cytokines were estimated using qRT-PCR and ELISA, respectively. Despite treatment with LPS, miR-126 significantly increased cell viability (P<0.05; **Figure 4A**); decreased apoptosis (P<0.05; **Figure 4B**); increased the expression of Bcl-2 and decreased the expressions of Bax, cleaved caspase-3 and cleaved caspase-9 (**Figure 4C**); it also significantly decreased the mRNA expressions (P<0.01; **Figure 4D**) and concentrations (**Figure 4E**) of TNF-α (P<0.01), IL-1β (P<0.05), IL-6 (P<0.01), and IL-8 compared to the LPS-treated scramble cells. These findings indicate that miR-126 protected HPDE6-C7 cells from LPS-induced inflammatory injury by increasing cell viability, decreasing apoptosis, and decreasing the production of pro-inflammatory cytokines.

miR-126 negatively regulated PDCD4

Next, we measured the effects of altered expression of miR-126 on the expression of PDCD4. There were four groups of cells namely, scramble (positive control), NC (negative control), miR-126 mimic (cells transfected with miR-126 mimic), and miR-126 inhibitor (cells transfected with miR-126). qRT-PCR results (**Figure 5A**) showed that miR-126 mimic significantly decreased the mRNA expression of PDCD4 compared with scramble (P<0.05), whereas miR-126 inhibitor significantly increased the expression of PDCD4 compared with the NC (P<0.01). Similar results were obtained in the western blot analysis (**Figure 5B**). These findings suggest that miR-126 negatively regulates the expression of PDCD4.

To further confirm that PDCD4 is a target of miR-126, dual luciferase reporter assay was performed. For this assay, two types of vector were constructed: PDCD4-wild-type (PDCD4-Wt) and PDCD4-mutated-type (PDCD4-Mt). Then scramble and miR-126 mimic groups of cells were transfected with both the vectors separately. As shown in **Figure 5C**, luciferase activity was significantly decreased in the miR-126 mimic cells transfected with PDCD4-Wt

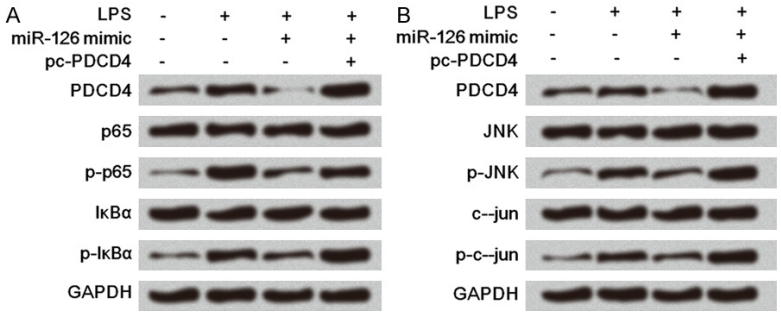

Figure 6. Overexpression of PDCD4 suppressed effects of miR-126 by activation of NF-κB and JNK pathways. A and B. HPDE6-C7 cells were transfected with LPS, LPS + miR-126 mimic, or LPS + miR-126 mimic + pc-PDCD4, and then the expression of NF-κB and JNK pathways proteins (IκBα, P65, JNK, c-jun) was measured using western blot. GAPDH: glyceraldehyde 3-phosphate dehydrogenase; JNK: janus kinase; LPS: lipopolysaccharide; NF-κB: nuclear factor-kappa B; PDCD4: programmed cell death 4.

compared to the scramble group of cells transfected with PDCD4-Wt ($P<0.05$), but no significant changes were observed following transfection of scramble and miR-126 mimic groups of cells with PDCD4-Mt vector. These findings indicated that PDCD4 is a target of miR-126.

Overexpression of PDCD4 suppressed the effects of miR-126 by activation of NF-κB and JNK pathways

Lastly, we evaluated the effects of LPS, miR-126, and PDCD4 on the NF-κB and JNK pathways using western blot. For this, we transfected HPDE6-C7 cells with LPS, LPS + miR-126 mimic, or LPS + miR-126 mimic + pc-PDCD4; un-transfected cells served as control. As shown in **Figure 6A** and **6B**, LPS and PDCD4 increased the expression of IκBα, P65, JNK, and c-jun, but miR-126 suppressed expressions of those proteins. These findings indicate that overexpression of PDCD4 can suppress the effects of miR-126 by activation of the NF-κB and JNK pathways.

Discussion

In the present study, we investigated the effects of Ang-1 and miR-126 on LPS-induced inflammatory injury in pancreatic cells (HPDE6-C7). We found that Ang-1 promoted LPS-suppressed cell viability and inhibited LPS-induced cell apoptosis, pro-inflammatory cytokine (TNF-α, IL-1β, IL-6, and IL-8) production, and activation of NF-κB and JNK pathways in HPDE6-C7 cells. Furthermore, Ang-1 promoted the expression of miR-126, which in turn protected the HPDE6-C7 cells against LPS-induced injury and down-regulated the expression of PDCD4. Lastly, the mechanistic study revealed that overexpression of PDCD4 reversed miR-126 mediated inhibition of LPS-induced activation of NF-κB and JNK pathways.

Ang-1 and Tie-2 receptor are crucial modulators of normal and pathological angiogenesis. Ang-1 inhibits apoptosis and inflammatory responses and promotes differentiation, sprouting, and migration in cultured endothelial cells [11]. Abdel-Malak et al reported that Ang-1 promotes endothelial cell proliferation and migration via AP-1-dependent autocrine production of IL-8 [21]. In an *in vitro* study, Ang-1 at 250 ng/mL concentration increased Tie-2 activation and inhibited cell apoptosis and promoted angiogenesis in myocardial cells [22]. Daly et al reported that Ang-1 inhibits forkhead transcription factor-induced apoptosis in endothelial cells [23]. In severe acute pancreatitis, the production of inflammatory mediators and chemokines majorly contributes to the inflammatory injury [24]. It has been reported that Ang-1 inhibits the production of pro-inflammatory cytokines. Hua et al showed that Ang-1 decreases the serum levels of TNF-α, interferon-γ, IL-1β, and IL-6 [12]. Likewise, in our study, Ang-1 at 300 ng/mL concentration increased cell viability and inhibited LPS-induced apoptosis and production of TNF-α, IL-1β, IL-6, and IL-8 in pancreatic cells. These findings suggest that Ang-1 promotes cell survival and protects pancreatic cells from LPS-induced inflammatory injury.

Many intracellular signal transduction pathways have been reported to be involved in inflammatory response in pancreatitis, including NF-κB. It has been shown that inhibition of these signals reduces inflammation and improves the prognosis of pancreatitis [25, 26]. He et al reported that Ang-1 inhibits TNF-α, IL-6 and IL-8 production in inflammatory lung injury via suppression of the NF-κB and p38 MAPK pathways [27]. Similarly, Jeon et al suggested

that Ang-1 exerts anti-inflammatory activity via suppression of NF-κB [28]. Oxidative stress activates many signal transduction pathways, including JNK that induce apoptosis. Murakami et al showed that Ang-1 inhibits H_2O_2-induced JNK activation in vascular endothelial cells [29]. Likewise, in our study, Ang-1 inhibited the LPS-induced activation of the NF-κB and JNK pathway proteins (IκBα, P65, JNK, c-jun). These findings suggest that Ang-1 inhibits apoptosis and alleviates inflammatory injury via inactivation of the NF-κB and JNK pathways.

The microRNA miR-126 is highly expressed in the endothelial cells and has the potential to promote endothelial cells proliferation [30, 31]. In addition, miR-126 also has anti-inflammatory effects on endothelial cells [20]. Sessa et al showed that down-regulation of miR-126 and up-regulation of p85β inhibited the biological functions of Ang-1 in endothelial cells [32]. Likewise, in our study, Ang-1 promoted the expression of miR-126 in pancreatic cells. Additionally, we showed that overexpression of miR-126 in pancreatic cells increased cell viability and inhibited LPS-induced apoptosis and production of TNF-α, IL-1β, IL-6, and IL-8. Similar findings have been reported in previous studies. Li et al showed that miR-126 increased viability and inhibited apoptosis of acute myeloid leukemia cells [33]. Zhu et al showed that levels of miR-126 were negatively correlated with levels of TNF-α, IL-1β and IL-6 in patients with inflammatory acute cerebral infarction [34].

We identified PDCD4 as a direct target of miR-126 using Luciferase activity assay. PDCD4 gene is known as a tumor suppressor gene, which inhibits cell proliferation, migration and invasion and promotes apoptosis in tumors [35]. Many studies have evaluated the interaction between PDCD4 and several miRNAs, such as miR-21 and miR-184 [35, 36]. To the best of our knowledge, we are the first to report the interaction between PDCD4 and miR-126. We showed that PDCD4 is negatively regulated by miR-126. In the mechanistic study, we found that overexpression of PDCD4 reversed miR-126-mediated inhibition of LPS-induced NF-κB and JNK activation.

In conclusion, Ang-1 and miR-126 are positively correlated, and both promote LPS-suppressed pancreatic cell viability and inhibit LPS-induced apoptosis and increased production of pro-inflammatory cytokines via inhibition of LPS-induced activation of NF-κB and JNK pathways. The effects of miR-126 were mediated via negative regulation of PDCD4. Our novel findings indicate the potential protective roles of Ang-1 and miR-126 in acute pancreatitis.

Disclosure of conflict of interest

None.

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