Inhibition of pancreatic stellate cell activity by adipose-derived stem cells

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BACKGROUND: Pancreatic stellate cells (PSCs) play a critical role in the development of pancreatic fibrosis. In this study we used a novel method to isolate and culture rat PSCs and then investigated the inhibitory effects of adipose-derived stem cells (ADSCs) on activation and proliferation of PSCs.

METHODS: Pancreatic tissue was obtained from Sprague-Dawley rats for PSCs isolation. Transwell cell cultures were adopted for co-culture of ADSCs and PSCs. PSCs proliferation and apoptosis were determined using CCK-8 and flow cytometry, respectively. α-SMA expressions were analyzed using Western blotting. The levels of cytokines [nerve growth factor (NGF), interleukin-10 (IL-10) and transforming growth factor-β1 (TGF-β1)] in conditioned medium were detected by ELISA. Gene expression (MMP-2, MMP-9 and TIMP-1) was analyzed using qRT-PCR.

RESULTS: This method produced $17.6\pm6.5\times10^3$ cells per gram of the body weight with a purity of 90%-95% and a viability of 92%-97%. Co-culture of PSCs with ADSCs significantly inhibited PSCs proliferation and induced PSCs apoptosis. Moreover, α-SMA expression was significantly reduced in PSCs+ADSCs compared with that in PSC-only cultures, while expression of fibrinolytic proteins (e.g., MMP-2 and MMP-9) was up-regulated and anti-fibrinolytic protein (TIMP-1) was down-regulated. In addition, NGF expression was up-regulated, but IL-10 and TGF-β1 expressions were down-regulated in the co-culture conditioned medium compared with those in the PSC-only culture medium.

CONCLUSIONS: This study provided an easy and reliable technique to isolate PSCs. The data demonstrated the inhibitory effects of ADSCs on the activation and proliferation of PSCs *in vitro*.

(***Hepatobiliary Pancreat Dis Int 2015;14:215-221***)

KEY WORDS: pancreatic stellate cells; isolation; mesenchymal stem cells; pancreatic fibrosis; fibrinolytic protein

Introduction

Chronic pancreatitis is characterized by chronic inflammation and altered normal structure and functions of the pancreas. The long-standing inflammation of the pancreas leads to pancreatic fibrosis as the end-stage of the disease although it may take years to develop.[1] A great number of clinical and experimental studies have confirmed that activated pancreatic stellate cells (PSCs) are the main effector cells in the process of pancreatic fibrosis[1] and therefore, PSCs may play a dominant role in the pathogenesis of pancreatic fibrosis.[2, 3] PSCs, also known as myofibroblast-like cells, are able to switch between the quiescent and activated phenotypes, like hepatic stellate cells (HSCs), to participate in tissue repair, and to secrete extracellular matrix. As a result, PSCs have caught attention from researchers, and targeting of these cells could provide novel strategies for anti-pancreatic fibrosis therapy.[2-7] With regards to this, it is an essential need to isolate and obtain purified and dynamic PSCs with high quality as a key and fundamental basis for the study of pancreatic fibrosis. Indeed, since 1998, there have been continuous studies in the development of improved methods to isolate PSCs from animal and human pancreas.[4, 5] However, to date, the isolation of PSCs is still obviously hysteretic compared with the isolation of HSCs, although PSCs are nearly identical to HSCs, with presumably the same origin.[8] Therefore, in

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doi: 10.1016/S1499-3872(14)60283-6
Published online July 17, 2014.

this study, we first provided an easy and reliable method to isolate and culture rat PSCs. This method combined and improved previous techniques from PSCs separation and HSCs isolation. After that, we explored a potential cell-therapy strategy of anti-pancreatic fibrosis by targeting PSCs *in vitro*. A previous study[9] demonstrated the immunomodulatory effect of bone marrow-derived mesenchymal stem cells on activated HSCs, since the bone marrow-derived mesenchymal stem cells have been shown to prevent the development of liver fibrosis in a number of pre-clinical studies. We, therefore, determined the inhibitory effects of adipose-derived stem cells (ADSCs) on the regulation of these PSCs activity and proliferation through co-culture with ADSCs *in vitro*, and our data could provide a hopeful possibility for stem cell transplantation for future treatment of pancreatic fibrosis.

Methods

Animals, materials and reagents

Sprague-Dawley rats weighing 300-400 g were obtained from Wenzhou Medical College Animal Center (Wenzhou, China). Transwell inserts containing semi-permeable membranes with a size of 0.4 μm and plastic six-well culture plates were purchased from Millipore Corporation (Billerica, MA, USA); type IV collagenase and DNase I from Sigma (St. Louis, MO, USA); Optiprep® gradient centrifugation medium from Axis-Shield Company (Oslo, Norway). Trypsin, Hank's balanced salt solution (HBSS), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Gibco-Invitrogen (Carlsbad, CA, USA). A rat anti-desmin monoclonal antibody and a mouse anti-α-smooth muscle actin (α-SMA) monoclonal antibody were from Boster Bio-Engineering Co., Ltd. (Beijing, China).

Isolation and cultivation of PSCs and ADSCs

The rats were anesthetized with ether. An abdominal midline incision was made to expose the abdominal aorta. Next, the hilar hepatic artery, splenic artery, left gastric artery, and right gastroepiploic blood vessels were isolated and ligated. After ligation of the abdominal aorta of the upper truncus coeliacus behind the esophagus, the abdominal aorta of the lower truncus coeliacus was intubated. After the blood collection, the portal vein was cut. The pancreas was perfused with 37 ℃ preheated HBSS containing 0.3 mg/mL type IV collagenase in 5-7 mL/min. The pancreas became swollen and shiny and easy to be located. After removing all adipose tissues, the pancreas was quickly collected and cut into pieces with a scissor. The fresh pancreatic tissues were then added to 15 mL HBSS containing 1 mg/mL type IV collagenase and 20 μg/mL DNase I and incubated at 37 ℃ for 45 minutes. After that, cell suspension was filtered through 200 mesh filtration followed by 30×g centrifugation for 5 minutes at room temperature. After discarding the sediment, the supernatant was centrifuged at 450×g for 7 minutes, and the sediment was then thoroughly mixed with 6 mL of 15% Optiprep®. Next, 6 mL of 11.5% Optiprep® and 6 mL of HBSS were carefully and sequentially added into the cell solution followed by 1400×g centrifugation for 18 minutes at 4 ℃ and the interface layer of the cells was carefully extracted and resuspended in 5 mL HBSS, followed by 450×g centrifugation for 8 minutes. After discarding the supernatant, the sediment was resuspended in DMEM supplemented with 20% FBS and antibiotics and then cultivated in flasks. Cultured for 3-5 passages, PSCs were prepared for the study of their activation and proliferation. The purity of PSCs was identified by its morphology. The viability of PSCs was tested by trypan blue staining. Similarly, ADSCs were isolated and purified from rat celiac fat tissues as described previously.[10] After culturing for 3-5 passages, cell surface markers, such as CD90, CD29 and CD45, were analyzed for characterization of the ADSCs.

Immunocytochemistry

Primary cultured PSCs were immunostained for desmin (Boster, Beijing, China) and α-SMA (Boster, Beijing, China). Primary PSCs were fixed using 2% paraformaldehyde for 10 minutes at 48 ℃. The cell membranes were disrupted with 0.5% Triton X-100 (Sangon Biotech, Shanghai, China) for 10 minutes. The PSCs were incubated with rat monoclonal anti-α-SMA (1:500) and rabbit anti-desmin (1:500) antibodies. Labeled cells were incubated with secondary antibody, an antibody binding analysis was performed using a DAB kit. Finally, the cells were observed and quantified under a light microscope.

Suppression of PSCs activity and proliferation through co-culture with ADSCs

To suppress PSCs activity and proliferation, we co-cultured PSCs with ADSCs in a 6-well plastic culture plate with Transwell inserts. This culture system separated PSCs from ADSCs, but ADSC-conditioned medium could affect PSCs as described previously.[11] In addition, this Transwell co-culture permitted these two cell populations to grow together, but prevented direct cell-to-cell contact. In each co-culture well, 3×10^4 cells of both PSCs and ADSCs were seeded in the bottom and top well, respectively. In parallel wells, PSCs replace ADSCs as a negative control (PSCs+PSCs group) and PSCs were cultured without Transwell as a blank control (PSCs group).

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