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Research Article

Isolation and characterization of human islet stellate cells

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ABSTRACT

Background and aims: We have previously demonstrated that islet stellate cells (ISCs) exhibiting a similar phenotype to classical pancreatic stellate cells (PSCs) could be isolated from rat islets, where they may contribute to islet fibrosis in type 2 diabetes mellitus (T2DM). This study was designed to determine whether human islets also contain ISC.

Materials and methods: Using standard explants techniques, human ISCs were enriched from freshly isolated human islets. Immunofluorescence visualization of markers for PSCs(α-smooth muscle actin;α-SMA), desmin, vimentin, glial fibrillary acidic protein (GFAP) was used to characterize the human ISC. Cell counting kit-8 (CCK-8) was used to assess the proliferation of ISC. The wound-healing assay and the transwell migration were used to assess the migration capacity of ISC. Immunofluorescence against collagen typesI (col-I), collagen typesIII (col-III) and fibronectin (FN) was performed to identify extracellular matrix (ECM) component synthesized by ISC. Adipogenic and osteogenic differentiation were tried to detected stem cell potential.

Results: In culture, ISC with triangular shape grow out from human islets. The passaged ISC expressed α-SMA, desmin, vimentin, GFAP and was positive for col-I, col-III and FN. The proliferation and migration ability of ISC was significantly slower than those of PSC. And both the human PSC and ISC were able to differentiate in vitro into adipocyte- and osteoblast-like cells.

Conclusion: Similar to our previous rat experiment, the current study shows that human islets also contain ISC which is phenotypically similar but not identical to human PSC.

1. Introduction

Type 2 diabetes mellitus (T2DM) is caused by a combination of genetic and environmental factors that result in decreased insulin sensitivity of its target tissues, and a reduced ability of pancreatic beta cells to elevate insulin secretion in response to increased blood glucose levels, which together lead to the progressive development of glucose intolerance and, eventually, overt diabetes [1]. Although major progress has been achieved in our understanding of the pathogenesis of T2DM, the mechanisms underlying β-cell failure in T2DM are not yet fully understood.

Islet fibrosis has been observed in the late stage of T2DM in both humans and in animal models [2–8], which could further aggravate β-cell failure. Several recent studies identified activated pancreatic stellate cells (PSC) in the fibrotic islets, suggesting that PSCs might play an important role in this process [5,9–12]. In health, PSCs are quiescent and characterized by cytoplasmic lipid droplets rich in vitamin A [13]. However PSCs can be activated in response to pancreatic injury and inflammation, or by exposure to inflammatory cytokines or oxidant stress [14–17], which induces increased proliferation; morphological changes into myofibroblast-like cells; up-regulation of the expression of α-smooth muscle actin (α-SMA); and increased synthesis and secretion of extracellular matrix (ECM) components [18].

Numerous in vivo and in vitro studies have demonstrated a central role for PSCs in the pancreatic fibrogenesis associated with chronic pancreatitis and pancreatic cancer [17–24], but much less is known about the role(s) in the endocrine pancreas of islet-specific stellate cells. We have previously reported the use of standard explant techniques to isolate and characterize a population of islet stellate cells (ISC) from rat pancreatic islets [25]. The rat ISCs were morphologically similar to rat PSCs, with both populations expressing α-SMA, vimentin, glial fibrillary acidic protein (GFAP), and the ECM components col-I,col-III and FN. However ISCs differed from PSCs by being more rapidly activated, and

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having reduced rates of proliferation and migration in vitro, demonstrating that they are phenotypically similar to, but not identical with PSCs. In view of the established role of PSC in pancreatic fibrosis, we suggested that ISCs may contribute to islet fibrosis in T2DM.

In the current study we have extended our observations to clinically relevant human islets to determine whether they contain an endogenous population of ISCs (hISCs) and, if so, to characterize the similarities and differences between hISCs and classical human PSCs.

2. Materials and methods

2.1. Human pancreatic tissue

Human pancreatic tissue was isolated from surgical samples (patients without T2DM), which was approved by the Zhongda Hospital Ethics Committee (Zhongda Hospital, Southeast University, Jiangsu, China). Each of the human samples was cut into 2 pieces, the one was used to isolate PSCs, the other one was used to isolate ISCs.

2.2. Isolation and culture of human islets

Human islets were isolated from pancreatic tissue by digestion using collagenase P. Briefly, pancreatic tissue was cut into small tissue blocks and digested with 1.5 mg/ml collagenase P (Roche) at 37 °C. Individual human islets were be characterized by dithizone (DTZ) staining (0.1 g/l, Sigma) [26] and handpicked under a stereomicroscope, as described previously [27]. Freshly isolated islets were subsequently cultured in cell culture dishes with 10 ml Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12, 1:1 v/v) with 10% fetal bovine serum (FBS, Gibco) at 37 °C in a 5% CO_2-air humidified atmosphere.

2.3. Isolation and culture of human PSC

Human PSCs were isolated from pancreatic tissue using a previously described explant technique [28]. Briefly, human pancreatic tissue was cut into small tissue blocks (0.5–1 mm^3), which was seeded into cell culture dishes in the presence of 10% FBS in DMEM/F12. Tissue blocks were cultured at 37 °C in a 5% CO_2-air humidified atmosphere.

2.4. Immunostaining

Human ISC and PSC were fixed in 4% PFA in PBS for 20 min at room temperature, followed by immunofluorescent staining for α-SMA, desmin, vimentin, GFAP, Col-I, Col-III and FN. Cells were incubated overnight at 4 °C with primary antibody (Abcam, dilution rate: α-SMA desmin, vimentin and GFAP, 1:100; Col-I, Col-III and FN, 1:200), followed by a 1 h treatment with secondary antibody (Jackson ImmunoResearch Laboratories, 1:100). The sections for negative control group were incubated with PBS instead of primary antibody. Morphometric analyses were performed using Image J software.

2.5. CCK-8 assay

Cell proliferation was determined using the CCK-8 kit (Keygen Biotech). Cells were suspended at a final concentration of 5×10^3 cells/well and cultured in 96-well microplates for 24, 48 and 72 h, after which CCK-8 reagent (10 μl) was added to each well containing 100 μl of culture medium and the plate was incubated for 1 h at 37 °C. Viable cells were evaluated by A ($A_{450\ nm}$) using auto microplate reader (Wellscan), such that $A_{450\ nm}$ was proportional to the rate of cell proliferation. All experiments were performed in quintuplicate on three separate occasions ($n=15$).

2.6. Cell migration assays

The transwell migration assay was performed in 24-transwell plates using inserts with a pore size of 8 μm (Corning). 5×10^3 stellate cells were serum-starved for 24 h, after which cells were cultured in serum-free medium in the inner compartment of the transwell insert, while the plate wells which received the inserts contained DMEM/F12 supplemented with 10% FBS. The cells were incubated for 24 h at 37 °C, then fixed with 2% glutaraldehyde and stained with 1% crystal violet for 10 min. The cells that had not migrated through the membrane were removed. Cells adhering to the lower side of the insert membrane were visualized by light microscopy and counted manually ($n=5$).

For the wound-healing assay, 5×10^5 cells were seeded in 6-well culture plates and grown to reach confluence. After serum starvation for 24 h, the monolayers were wounded by scraping off a strip of cells with a 10 μL pipette tip. After 24 h cells migrating into the wound boundaries were counted manually under a phase contrast microscope ($n=5$).

2.7. Adipogenic and osteogenic differentiation

Adipogenic differentiation was induced by cultivation of confluent cultures in DMEM/F12 containing 20% (vol./vol.) FBS, 2.5 μg/ml insulin, 100 μmol/l indomethacin, 5 μmol/l rosiglitazone and 10 nmol/l dexamethasone. For osteogenic differentiation, confluent cultures were cultivated in DMEM/F12 containing 10% (vol./vol.) FBS, 10 mmol/l β-glycerophosphate, 5 μg/ml ascorbic acid and 10 nmol/l dexamethasone (Sigma). Cultures were maintained in differentiation media for 1 month with medium changes twice a week. Cell differentiation was analyzed by staining with Oil Red O or Alizarin Red S (Sigma) for adipogenic and osteogenic differentiation, respectively [29].

2.8. Statistical analysis

Data were presented as the means ± SE. Statistical significance was determined by ANOVA or unpaired student's *t*-test, as appropriate, and differences between group were considered to be statistically significant when $P < 0.05$. All of the statistical analyses were performed using the Statistical Product and Services Solutions (SPSS) package (Version 11.5, SPSS Science).

3. Results

3.1. Isolation and culture of human ISC from isolated human islets

After about 24 h in culture, human islets adhered to the bottom of culture dishes, after which an increasing number of cells with an approximately triangular morphology and large nuclei began to grow out as adherent monolayer from the edge of human islets. As they migrated from the islet, these cells assumed a stellate appearance without any detectable lipid droplets in the cytoplasm, as shown in Fig. 1. The migration of these hISCs from human islets was similar to our previous reports of stellate cells migrating out of rat isolated islets in vitro [25].

3.2. Characterization of human ISC

Immunofluorescence visualization of markers for PSC(α-SMA, desmin, vimentin, GFAP) was used to characterize the human ISC.