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Differentiation of Pancreatic Duct-Derived Stem Cells of Rats into Insulin-Secreting Cells with Function of Down-Regulating Blood Sugar Level after Transplantation

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Abstract: To induce Pancreatic Duct-derived Stem Cells (PDSCs) of rats into insulin-secreting cells and to determine the *in vitro* and *in vivo* function of the insulin-secreting cells. PDSCs were obtained through *in situ* collagenase digestion of rat panrease and *in vitro* adherent cultivation, with the phenotype identified and then PDSCs were induced into Islet-like Cell Clusters (ICCs) through serum-free culture with their response to glucose challenge determined. Furthermore, ICCs were transplanted under the kidney capsule of diabetic nudes, with their blood sugar level monitored to determine the *in vivo* function of ICCs on regulating blood sugar. PDSCs of rats resembled Mesenchymal Stem Cells (MSCs) with CD34 and CD45 positive and CD90 and CD105 negative on phenotype, simultaneously with the expression of PDX-1 and nestin. PDSCs could differentiated into insulin-secreting ICCs through serum-free cultivation. After transplantation under the kidney capsule, ICCs could just softly down-regulated the blood sugar level of diabetic recepient nudes, showing the immaturity of ICCs. PDSCs of rats resembled MSCs, with the expression of PDX-1 and nestin which could differentiated into insulin-secreting ICCs *in vitro* and down-regulated the blood sugar level of diabetic recepient nudes after transplantation.

Key words: Stem cell, pancreatic stem cell, pancreatic duct-derived stem cell, mesenchymal stem cell, islet transplantation, diabetes

INTRODUCTION

Diabetes mellitus, caused by either an absolute insulin deficiency due to the inability of insulin-secreting β-cells (type 1) or a relative insulin deficiency due to insulin resistance (type 2) is a devastating disease prevalent throughout the world afflicting over 200 million people. In despite of insuffiency of donors, recent success in pancreatic islet transplantation and stem cell research has energized the field to discover an alternative source of stem cells with differentiation potential to β-cells. Excitingly, the generation of insulin-secreting cells from stem cells isolated from a wide variety of tissues such as embryo (Jiang *et al*., 2007), bone marrow (Marappagounder *et al*., 2013), umbilical cord blood (Kadam *et al*., 2012), liver (Li *et al*., 2012) and pancreas (Eberhardt *et al*., 2006; Lin *et al*., 2006; Merani and Shapiro, 2006; Ouziel-Yahalom *et al*., 2006; Seeberger *et al*., 2006; Zhou *et al*., 2008; Dominguez-Bendala, 2009; Lee *et al*., 2009; Bar-Nur *et al*., 2011; Smukler *et al*., 2011) has been reported. The stem/precursor cells within adult pancreas, putatively termed Pancreatic Stem Cells (PSCs) having been revealed to reside within exocrine ducts (Bonner-Weir *et al*., 2000; Lin *et al*., 2006; Seeberger *et al*., 2006) and endocrine islets of Langerhans (Zulewski *et al*., 2001; Davani *et al*., 2007) are considered to own promising prospects. Among PSCs of different sources, Pancreatic Duct-derived Stem Cells (PDSCs) might become particularly useful for therapies that target cell replacement in diabetic patients, in that duct cell types are abundantly available in pancreas and clinically, they are routinely discarded after islet isolation.

To date, however, researchers are still ignorant of the exact nature of PDSCs. For one thing, the methodological characterization of PDSCs is still ambiguous, with no gernerally accepted markers for PDSCs confirmed; for another, the origin and role of PDSCs in β-cell neo/regeneration are remained controversial. Here, researchers present a study investigating PDSCs of adult

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rats including isolation and identification of PDSCs, differentiation of insulin-secreting Islet-like Cell Clusters (ICCs) from PDSCs *in vitro* and transplantation of ICCs into diabetic nudes under the capsule of kidney to determine the *in vivo* effect on regulating blood sugar level of ICCs.

MATERIALS AND METHODS

Cell isolation and expansion cultivation: Ductal cells were isolated from pancreas of adult male Wistar rats weighing 200~300 g perfused through the common bile duct with collagenase V (Sigma, USA). The pancreatic tissue was surgically procured and digested *ex vivo* and exocrine tissue was purified on a Ficoll 400 (Sigma, USA) discontinued gradient. The pellet of islet-depleted tissue fraction (with purity of islets <2% determined by Dithizone (DTZ) staining) underwent filtration through a 600 μm grit and subsequently was transferred to 125 mm^2 untreated plastic tissue culture flasks (Jet Biofil, Canada) at an average cell density of 20 cellular aggregates/cm^2, in expansion medium RPMI 1640 (HyClone, USA) supplemented with 10% heat-inactivated Fetal Calf Serum (FCS) (HyClone, USA), 2 mmol L^{-1} L-glutamine, 10 mmol L^{-1} HEPES, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (all Solarbio, China) and cultured at 37°C in a 5% CO_2-humidified atmosphere. Omission of FCS was chosen as control. Medium was changed twice a week, with suspended cells removed during each medium change. After reaching confluence greater than 80%, adherent cells were subcultured by trypsinization and reseeded with a density of 2×10^5 cells/cm^2. Consecutive passages were performed so that sufficient cells could be harvested for characterization and differentiation culture.

Differentiation cultivation: Differentiation cultivation was initiated when attached PDSCs reached confluence >90%. PDSCs were overlaid with Matrigel (BD Bioscience, USA), a commercial preparation of murine basement membrane, as per instructions of supplier for Thin Coating Method with the exception of an increased gelling temperature. Briefly, PDSCs were cultured on Matrigel (Invitrogen, CA)-coated plates and incubated at 37°C for 1 h. Then, the differentiation medium containing DMEM/F12 (1:1) (HyClone, USA), 10 mmol L^{-1} nicotinamide (Sigma, USA), 2 g L^{-1} BSA (Solarbio, China), 10 ng mL^{-1} Keratinocyte Growth Factor (KGF) (Roche, USA), 20 ng mL^{-1} of basic Fibroblast Growth Factor (bFGF) (Peprotech, USA) and 10 nmol L^{-1} exendin-4 (Sigma, USA) was added. For some samples, 10% FCS was added to examine the effect of serum on differentiation.

Flow cytometry: To determine the cell surface antigen expression of PDSCs, single cells obtained from passage 5 was analyzed by Fluorescence Activated Cell Sorting (FACS). Briefly, 1×10^6 mL^{-1} cells in Phosphate-Buffered Saline (PBS) (Hyclone, USA) were transferred into polystyrene tubes and incubated with immunofluorescence antibodies at 4°C for 1 h.

After washing twice with PBS, labeled cells were resuspended in 100 mL PBS then analyzed for fluorescence using a FACSCalibur flow cytometry (BD Biosciences, USA) with results calculated by CellQuest Software. The antibodies used in investigation were as follows: Fluorescein Isothiocyanate (FITC) anti-rat CD34, CD105 (Sigma, USA), FITC anti-rat CD45, CD90 (eBioscience, USA).

Electron microscopy: PDSCs and ICCs were examined by transmission electron microscopy to identify secretory vesicles within cells as previously described (Korbutt *et al*., 1996).

Immunocytochemistry: PDSCs were seeded into 6 well plates with a density of 2×10^5 cells/cm^2, in which 20 mm coverslips were preset and cells were further cultured for 4-5 days until a great number of cells attached to the surface of coverslips while pre-cultured cell aggregates and ICCs were transferred on Poly-L-Lysine-coated coverslips in 6 well plates. Primary antibodies used were as follows: cytokeratin-19 (CK-19) (rabbit polyclonal, 1:100, eBioscience, USA), pancreatic and duodenal homeobox gene-1 (PDX-1) (Santa, USA). The negative controls were carried out with the secondary antibody in the absence of primary antibodies (PBS as the substitute).

RT-PCR: Cells were dissolved in 1 mL of Trizol reagent (Bio Basic, Canada) and total RNA was extracted according to the manufacturer's protocol. The 1 μg of total RNA was reverse transcribed into cDNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, USA) as recommended in the manufacturer's instructions. The PCRs were performed in 50 μL reaction volume using Taq DNA Polymerase (Invitrogen, Canada). Amplification was carried out with the program of 94°C for 5 min to denature, 43, 45 or 57°C for 30 sec for primer annealing, 72°C for 30 sec to elongate the PCR product for 30 cycles and a final polymerization at 72°C for 7 min followed by a 4°C hold. β-actin was used as control. Primers were as follows: CK-19, (f) 5'-CTG GGT GGC AAT GAG AAG AT-3', (r) 5'-TCA AAC TTG GTC CGG AAG TC-3'; PDX-1, (f) 5'-GGT GCC AGA GTT CAG TGC TAA-3', (r) 5'-CCA GTC TCG GTT CCA TTC G-3'; nestin, (f) 5'-GAGTGGGGTAGATGGGGATT-3', (r) 5'-CAGGGAGG

AA GAGAGGAACA-3'; Insulin, (f) 5'-CCG TCG TGA AGT GGA G-3', (r) 5'-CAG TTG GTA GAG GGA GCA G-3'; β-actin, (f) 5'-AGA ACA TCA TCC CTG CAT CC-3', (r) 5'-ACC CTG TTG CTG TAG CCA TA-3'.

Detection and quantitation of insulin: Dithizone (DTZ), which stains insulin-containing cells bright red, was used to quickly assess the presence of insulin within cells. Researchers performed insulin content analysis of the ICCs by Radioimmunoassay (RIA) and the values were normalized to the DNA content. PDSCs or differentiated ICCs were plated onto a Matrigel-coated 48 well plate in triplicate with low-glucose (2.8 mmol L^{-1}) DMEM medium (Hyclone, USA). After 4 h at 37°C, supernatant were collected and kept at -20°C until assayed (The supernatants were collected again and both low and high-glucose-treated ICC supernatants were stored at -20°C until they were analyzed). Then, medium was changed for high-glucose (24.8 mmol L^{-1}) DMEM medium (Hyclone, USA) with cells incubated for 4 h at 37°C, supernatant collected and kept as described earlier. Insulin level was measured by using a RIA kit for insulin (AXSYM, USA). Additionally, ICCs were treated with cold acid-ethanol (0.1 M hydrochloric acid in absolute ethanol) and kept at 4°C overnight to examine the clear supernatants for the intracellular insulin content. The values obtained were normalized relative to the total DNA content. For DNA quantitation, the ICCs were lysed in 0.5% Triton X-100 TE buffer and sonicated. A Quant-iT PicoGreen dsDNA Reagent kit (Invitrogen) was used to determine DNA concentration according to the manufacturer's recommendation. The Secreting Index (SI) of insulin was calculated to further show the function and responsiveness to glucose of ICCs, according to the formula: SI = Insulin level at high-Glucose (24.8 mmol L^{-1}) condition/Insulin level at low-glucose (5.6 mmol L^{-1}) condition.

Preparation of diabetic nudes model: The female BALB/C nudes aged 2-3 weeks (n = 30) obtained from Animal Center of China Medical University were induced by Streptozocin (STZ, i.p., 60 mg kg^{-1}) and diabetes was defined as the non-fasting blood glucose level was >16.8 mmol L^{-1} on two consecutive measurement.

Transplantation of ICCs into diabetic nudes: The 300~400 ICCs after 2 weeks differentiation cultivation were transplanted into diabetic nudes (n = 10) under the capsule of the kidney, 5~10×10^6 PDSCs of passage 5 and 300~400 IEQ naive islets were transplanted as the control (n = 10, respectively). Blood glucose level were monitored daily after transplantation.

Statistical analysis: All values were shown as mean±SE. For comparison, t-test and ANOVA analysis was used. A $p<0.05$ was considered a significant difference.

RESULTS

Morphology of PDSCs and ICCs: After primary culture for 2-3 days, attached cells as PDSCs could be clearly observed. Morphologically, they had remarkable size, most with one nucleus and examination by light microscopy analysis revealed a fusiform or spindle-shape similar to typical fibroblast-like cells whereas no if any adhered cells were present in serum-free group.

The number of fibroblast-like cells increased over time into monolayer. About 10 days later, confluence could nearly be achieved (Fig. 1A). Subcultivation led to higher degree of homogeneity in morphology of attached PDSCs and no significant morphological difference was distinguished among cells of different passages. Under differentiation culture condition, the aggregation ability of PDSCs in Matrigel gel was shown at day 3 after differentiation cultivation and culture of 12-14 days led to the formation of 3D sphere-like ICCs (Fig. 1B). Various sizes of cellular aggregates were produced in these cultures, among which a small number of ICCs reached up to 250-350 μm in diameter and failed to attach to the culture dish surface and grew in suspension. A close

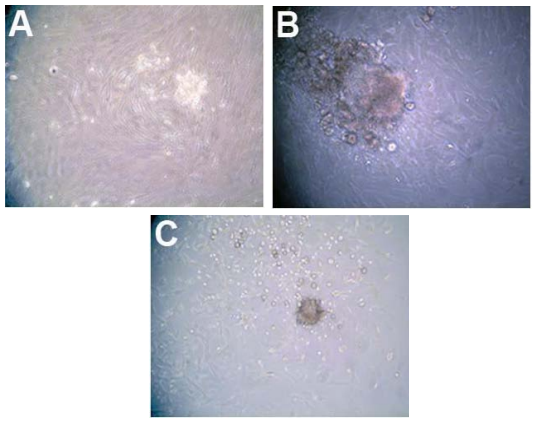

Fig. 1: Morphology of PDSCs and ICCs. A) At day 10 of primary culture, attached PDSCs appeared a fusiform-shape similar to typical fibroblast-like cells and grew into monolayer; B) Culture of 12-14 days led to the formation of 3D sphere-like ICCs; C) Many single round-shaped cells were observed at the outside margin which proliferated in suspension and had completely different morphologic features from the original adherent cells

morphological observation of ICCs through light microscopy revealed striking similarities to native islets. Furthermore, many single round-shaped cells were observed at the outside margin which proliferated in suspension and had completely different morphologic features from the original adherent cells (Fig. 1C). The number of outgrowing ICCs was augmented over time. On the contrary, the serum-containing group gave rise to no ICCs.

Transmission electron micrographs: When further characterized by transmission electron microscopy, no secretory vesicles were seen within the PDSCs of passage 3 (Fig. 2A) while the PDSC-derived ICCs after 2 weeks differentiation culture were shown to contain some cells with numerous secretory vesicles (Fig. 2B) and many of these cells contained granules with a morphological appearance conforming to adult islet β-cells.

FACS: Analysis by flow cytometry demonstrated that PDSCs of passage 5 express cell surface antigens CD90 (90.43%) and CD105 (92.72%) but not CD34 (7.26%) or CD45 (9.44%) (Fig. 3) which were generally used to define MSCs.

RT-PCR: What had been indicated by immunocytochemistry was further supported by RT-PCR at mRNA level. The expression of PDX-1 and nestin was sustained throughout the whole course of *in vitro* cultivation including in pre-cultured cells, PDSCs and ICCs whereas the expression of CK-19 and insulin was restricted within pre-cultured cell aggregates and ICCs after 2 weeks differentiation cultivation, respectively (Fig. 4). The sequence of gene expression changes observed in these experiments suggests the emergence of definitive endoderm followed by pancreatic endoderm formation with further differentiation to endocrine pancreatic cells.

Immunocytochemistry: PDSCs of passage 3 appeared insulin negative (Fig. 5a) and PDX-1 positive, with PDX-1 expression within mainly nucleus, although some cytoplasmic PDX-1 staining was also observe (Fig. 5b). After differentiation culture of 2 weeks, the insulin positive cells were present within ICCs (Fig. 5c).

Detection of insulin by DTZ staining: ICCs were stained bright red by DTZ after 2 weeks of differentiation culture (Fig. 6), confirming the presence of insulin within cells thus the presence of β-cells within ICCs. The majority of the stained cells appeared in the smaller cell clusters.

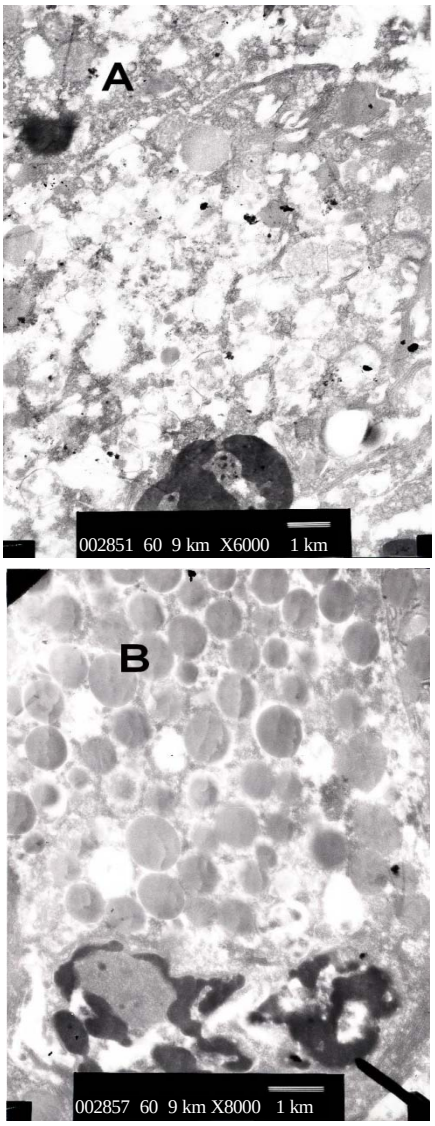

Fig. 2: Transmission electron micrographs of PDSCs and ICCs. A) PDSCs of passage 3, no secretory vesicles were seen within the cells, X6000; B) ICCs after 2 weeks differentiation culture, numerous secretory vesicles could be seen within the cells, X8000

Measurement of insulin level by RIA: Accordingly, insulin level was measured by RIA in the supernatant of both low-glucose and high-glucose medium of ICCs at day 14 of differentiation culture, with a significant difference from that of undifferentiated PDSCs of passage 5 (Fig. 7a). The insulin level of ICCs, however, was significantly lower than that of naive islets, no matter in low-glucose or high-glucose condition (Fig. 7b). In addition, the Secreting Index (SI) of insulin of ICCs was

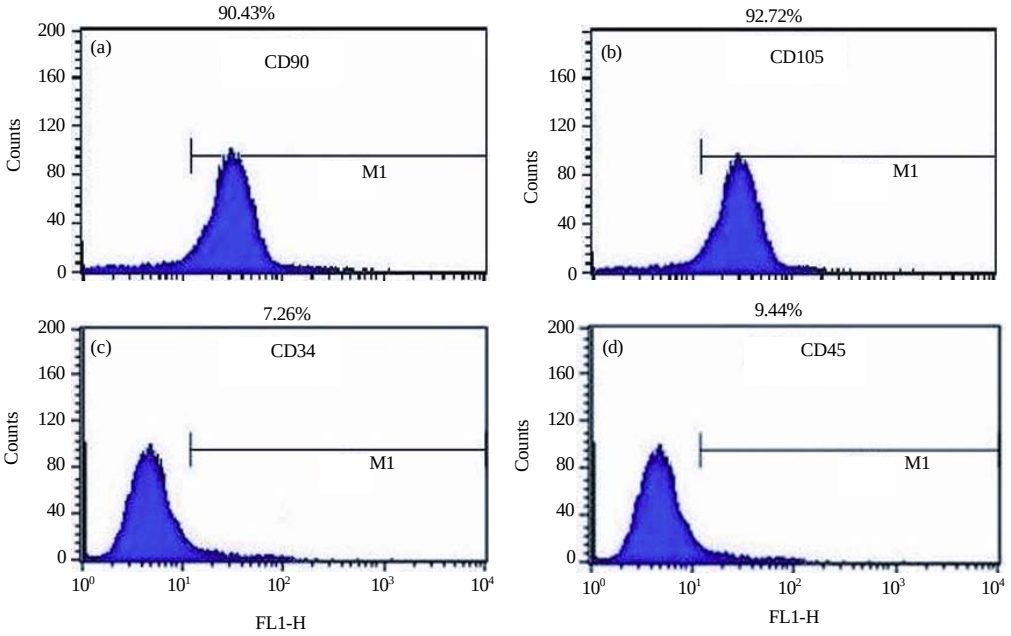

Fig. 3: Identification on cell surface antigen of PDSCs by FACS. PDSCs of passage 5: the cells expressed CD90 (90.43%) and CD105 (92.72%) but not CD34 (7.26%) or CD45 (9.44%)

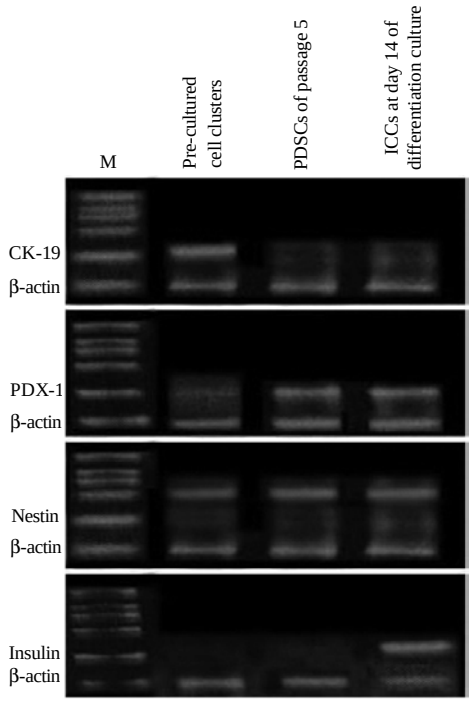

Fig. 4: Expression analysis by RT-PCR. M: Marker; 1: pre-cultured cell clusters; 2: PDSCs of passage 5; 3: ICCs at day 14 of differentiation culture

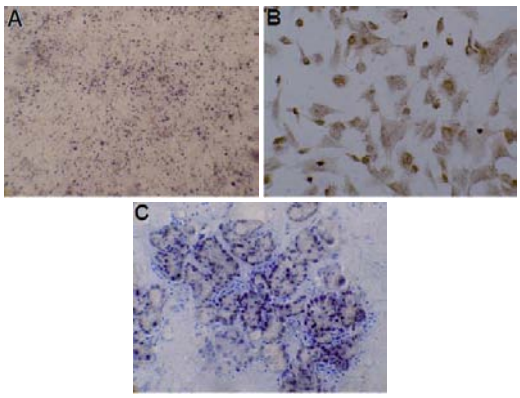

Fig. 5: Immunocytochemistry; A) PDSCs of passage 3 appeared insulin negative; B) PDX-1 positive, with PDX-1 expression within both cytoplasm and nucleus and C) After differentiation culture of 2 weeks, the insulin positive cells were present within ICCs

significantly lower than that of naive islets (2.40±0.59 vs. 3.41±0.69, $p<0.01$) but it was suggested that the ICCs generated by the protocol were able to secrete insulin in response to high concentration of glucose. Subsequently, the intracellular insulin content was quantitated and normalized, resulting in the values to be 0.13±0.02 ng mg^{-1} protein and 11.12±2.01 ng mg^{-1} protein, for PDSCs and ICCs, respectively with a significant difference ($p<0.01$).

Blood glucose level monitoring after transplantation: Normoglycemia (4.84±0.41 mM) was attained 3 days after

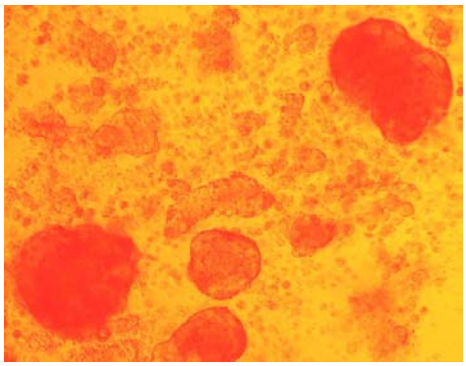

Fig. 6: Detection of insulin by DTZ staining. At day 14 of differentiation culture, ICCs were stained bright red by DTZ

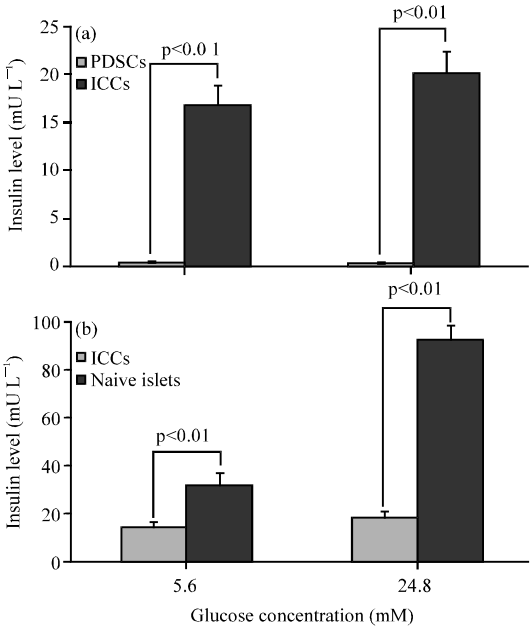

Fig. 7: Measurement of insulin level by RIA. A) There is a significant difference (p<0.01) between PDSCs of passage 5 and ICCs at day 14 of differentiation culture at insulin level, in the supernatant of either low-glucose or high-glucose medium; B) There is a significant difference (p<0.01) between ICCs and naive islets at insulin level, in the supernatant of either low-glucose or high-glucose medium

transplantation of ICCs into the diabetic recipients while the recipients that received PDSCs played still hyperglycemia (24.65±1.35 mM) with a significant difference between the two groups (p<0.01) (Fig. 8).

Type 1 and 2 diabetes are characterized by a 0-98 and 0-65% defect in β-cells, respectively (Butler *et al*., 2007)

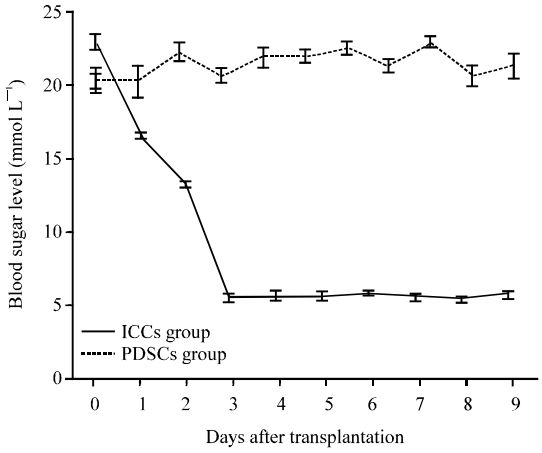

Fig. 8: Blood sugar level monitering of diavetic recipient nudes after transplantation. The blood glucose level of the diabetic recipient restored to normal 3 days after ICCs transplantation while the recipient that received PDSCs played still hyperglycemia with a significant difference between the two groups (p<0.01)

which acts as the pathophysiological basis for cell-replacement therapy, namely pancreatic islet transplantation that was even regarded as a cure in some way. It is necessary to emphasize that islet transplantation could also be potentially applied not only to type 1 diabetes but also to all types of insulin-dependent conditions resulting in glycemic dysregulation including type 2 diabetes. Answers to the frustrating efforts to develop sources of islet tissue other than limited human cadavaric islets may be the elusive stem cells, among which putative PSCs, especially PDSCs, are now gaining credibility.

MSCs have been reported to be ubiquitously found throughout the entire body such as Bone Marrow (BM), adipose, dermis, muscle, peripheral blood and compact bone, etc. (Bonner-Weir, 2000; Ferber *et al*., 2000; Hui *et al*., 2001; Abraham *et al*., 2002; Dominguez-Bendala and Ricordi, 2012). Whether or not MSCs from disparate sources are one and the same is still subject to debate. Proliferation rates vary greatly between clones even if obtained from the same tissue (Prabakar *et al*., 2012). To complicate things even more, clones not only from the same tissue but also from the same donor, also exhibit substantial variability (Paredes *et al*., 2011). The criteria for identification of MSCs are (Zuk *et al*., 2002; Danner *et al*., 2007). They adhere to surface of plastic culture dishes when maintained in serum-containing culture conditions;

express CD105, CD73 and CD90 but lack expression of CD45, CD34, CD14 or CD11β, CD79α or CD19 and differentiate *in vitro* into osteocytes, adipocytes and chondrocytes.

In this present study, therefore, based on their adherence, rapid expansion in serum-containing medium, expression of CD90 and CD105 but not CD34 or CD45, the PDSCs researchers obtained resembled MSCs, in agreement with results of others (Lin *et al*., 2006; Seeberger *et al*., 2006). Alternatively, pancreatic islet-derived stem cells obtained through *in vitro* culture have also been reported to resemble MSCs (Davani *et al*., 2007). Taken together, PSCs resemble MSCs.

Today, the origin of MSCs derived from pancreas has been unclear. Epithelial to Mesenchymal Transition (EMT) was considered to take place in the pancreas. It thus proposed the dedifferentiation of fully differentiated epithelial cells into pluripotent stem cells with a mesenchymal phenotype which, in turn, redifferentiate into endocrine cells by reprogramming in a new location such as within islets. In this study, the expression of CK-19 was dominant within pre-cultured cell aggregates but disappeared within passaged PDSCs, suggesting that PDSCs were possibly derived from pancreatic ductal epithelium, undergoing dedifferentiation from CK-19 positive to negative for which EMT might act as a plausible explanation. Of note, until now there is no evidence for EMT of β-cells *in vitro* (Gershengorn *et al*., 2004; Ouziel-Yahalom *et al*., 2006; Seeberger *et al*., 2006), implying that MSCs should be derived from non-β-cells. On the other hand, for pancreatic islet-derived stem cells, given that pure islets could not be obtained through isolation with purity of approximately 90% at best, the possibility that miscellaneous cells around islets especially pancreatic duct epithelial cells or PDSCs, took the responsibility to give rise to MSCs should not be excluded.

An open question concerns specific markers for PSCs. Unfortunately, there have been no reliable markers ascertained; indeed, their very identity has been obscure. PDX-1 (pancreatic and duodenal homeobox gene-1), a homeodomain protein, is expressed by various pancreatic precursor cells and mature islet cells. It is a critical transcription factor involved in the early pancreatic endocrine development. The presence of PDX-1 may have been instrumental for the subsequent evolutionary accumulation of β-cells in pancreas and it's elaborately involved in development of islets of Langerhans as a β-cell specific transcriptional regulator of glucose responsive genes (Stoffers *et al*., 1997; Madsen, 2007). Lack of PDX-1 in vertebrates cause pancreas agenesis (Jonsson *et al*., 1994; Offield *et al*., 1996; Stoffers *et al*., 1997). Most if not all cells in the common pancreatic duct rapidly respond to a partial pancreatectomy by replication that is followed by a transient increase in the expression of PDX-1 (Sharma *et al*., 1999). And it has been shown that the expression of PDX-1 is sufficient by itself to induce the expression of insulin in liver cells (Ferber *et al*., 2000) and in pancreatic ductal cells (Hui *et al*., 2001). These results indicate that the PDX-1 expressing pancreatic ductal cells might be facultative stem cells that are capable of redifferentiating into cells of all pancreatic lineage, if necessary.

In this study, PDX-1 was expressed at mRNA level throughout the whole course of *in vitro* cultivation indicating the presence of PDX-1 positive cells within pre-cultured cell clusters. The reason may be that the damage during isolation induced the expression of PDX-1 by mature cells after dedifferentiation. The high-expression of PDX-1 by PDSCs showed the property of stem cell while the expression of PDX-1 within the ICC suggesting its immaturity. So, researchers respeculated that PDSCs express PDX-1 but PDX-1 was not special enough to identify PDSCs.

Nestin, an intermediate filament protein expressed by neuronal stem cells, was reported as a marker for endocrine progenitor cells in the early 2000s (Zulewski *et al*., 2001). Nestin-expressing cells in the islets and ducts of the pancreas, termed Nestin-positive Islet-derived Progenitor cells (NIPs) were found to differentiate into insulin-secreting cells *in vitro* (Bonner-Weir *et al*., 2000; Abraham *et al*., 2002). The study demonstrated the sustained expression of nestin in PDSCs throughout the whole course of *in vitro* cultivation. But within pancreas, whether nestin is specially expressed in PSCs remains controversial.

It was shown in the study that ICCs expressed insulin at both mRNA and protein level after 2 weeks cultivation indicating that ICCs owned the capability of synthesizing insulin. Furthermore, ICCs possessed the ability of releasing insulin into extracellular fluid which was confirmed by that there was insulin detected within the culture medium and that the blood sugar level of recipient nude was down-regulated to normal after ICCs transplanted. So, ICCs could synthesize and release insulin, supporting that they were a kind of insulin-secreting cells.

Compared with naive islets, however, the ability of ICCs either to synthesize or to release insulin was weak which was shown by the results of insulin releasing test by gulcose challenge, with the Secreting Index (SI) of insulin to be 2.40±0.59 for ICCs and 3.41±0.69 for naive islets ($p<0.01$) suggesting the insensitive response of ICCs to glucose challenge. Thus, it has been shown that

in addition to an immature β-cell phenotype acquired by such ICCs, the fuction of these cells such as synthesis and release of insulin and reaction to glucose challenge was weak.

When exposed to different biological factors or microenvironments, PDSCs can give rise to ICCs which resemble native islets morphologically and express multiple endocrine hormones. It was reported that islet architecture is islet-size dependent (Kilimnik *et al*., 2012) and it was consumed that insulin-producing cells are predominantly localized within the small ICCs (Jiang *et al*., 2007). Here, researchers show that PDSCs had capability of highly viable proliferation in long-term culture during which serum seemed to be essential for the adherence and expansion of PDSCs. It's known that the serum contains: nutritive substances such as amino acids, lipids, nucleic acid derivatives, vitamins, minerals, etc., hormones and growth factors such as insulin, Adrenal Cortical Hormones (ACH), steroid hormones and basic Fibroblast Growth Factor (bFGF), Epidermal Growth Factor (EGF), Platelet Growth Factor (PGF), etc. binding proteins such as albumin, transferrin, etc. factors promoting contact and extension which play key roles in the course of adherence and colony formation. Here in, serum was inevitably used in expansion culture of nearly all kinds of stem/precursor cells. Given that no ICCs formed in the presence of serum, it appeared that something within serum inhibited the differentiation of PDSCs. Now it's accepted that nutritive substances, hormones, growth factors and binding proteins such as transferrin could promote differentiation of stem cells, so factors promoting contact and extension in serum might be the things that inhibit differentiation. With migration of stem/precursor cells *in vivo* presumed, it could be speculated that PSCs might proliferate and differentiate in different microenvironment, namely serum present and absent or some biological factors within serum activated and inactivated, respectively. Certainly, further investigations need to be carried out to solve the problems.

CONCLUSION

Researchers obtained stem cells from rat pancreatic ducts, as Pancreatic Duct-derived Stem Cells (PDSCs) which resembled MSCs simultaneously with the expression of PDX-1 and nestin and possessed the capability of differentiating into Insulin-secreting islet Cell Clusters (ICCs) that could down-regulate blood sugar level of diabetic recipient nude after transplantation, in spite of the immaturity of ICCs. Thus, results presented in this study provide credence that the differentiation of PDSCs to pancreatic β-cells is indeed possible and can be used as a potential source for transplantation into diabetic patients to regulate glucose homeostasis. PDSCs were worth further exploiting as a novel potential source of islet tissue.

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