

Rat Pancreatic Stromal Cells (PSC) affect Differentiation of Human Mesenchymal Stem Cells (hMSC) into Insulin-Producing Cells (IPCs) *In vitro*

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Abstract

The use of different sources of stem cells including embryonic and mesenchymal stem cells is a novel therapy for diabetic patients. However the efficiency of differentiation is not enough to complete treatment. An important point in the induction of stem cells into IPCs *in vitro* is the role of the pancreatic niche (which includes the stromal and epithelial niche). It can physically contact to adjacent cells and influence stem cell behavior via close range signaling. In this respect, we hypothesized that Pancreatic Stromal Cell (PSC) as a fundamental factor of the stromal niche may have an effective role in the generation IPCs (i.e, the efficiency of differentiation and function of newly-formed β -cells) *in vitro*. Therefore in this study, MSCs derived from umbilical cord (UC-MSCs) vein and bone marrow (BM-MSCs) was selected to differentiate into IPCs in co-culture with rat PSCs.

Our results have demonstrated that only BM-MSCs were able to differentiate into IPCs. Cells in Islet-like clusters with (out) co-cultured with rat pancreatic stromal cells, produced insulin and C-peptide and released them to culture medium at the end of the induction protocol; however they did not respond to glucose challenges very well. The presence of rat pancreatic stromal cells, up-regulated the expressions of insulin, Glut2, and Nkx2.2 in IPCs. These results suggested that rat PSCs possibly affect MSCs differentiation into IPCs by increasing the number of immature β -cells.

Keywords: Bone marrow; Mesenchymal stem cells; Insulin producing cells; Trans-differentiation; Umbilical cord vein

Introduction

Type I diabetes mellitus is a chronic disease resulting from the selective autoimmune destruction of pancreatic insulin-producing β -cells [1]. It is also an important factor to accelerate the hardening and narrowing of arteries; leading to strokes, coronary heart disease, and other blood vessel diseases [2]. Over the past two decades, transplantation of pancreatic islet cells as a potential cure for type I diabetes mellitus has become the subject of intense interest [3,4], however the problem of the worldwide shortage of transplant-ready islets has yet to be resolved. Moreover, islet transplantation has been hampered by immune rejection and recurrent attacks against islets by underlying autoimmunity [4]. Many studies have tried to find an alternative therapy by inducing insulin-secreting cells from stem cells through either gene modifications or different induction conditions or both [5-7], but their application has some limitations because of ethical considerations. Among adult stem cells, Mesenchymal Stem Cells (MSCs) are particularly attractive for clinical therapy because of their high self-renewal capacity and differentiation potential [8]. It has recently been reported that bone marrow derived MSCs (BM-MSCs) can be induced into Insulin-Producing Cells (IPCs) [9]. However, the source of bone marrow (BM) is limited and the amount of BM-MSCs and their differentiation capacity decrease with age [10]. Alternative sources for MSCs are the subject of intensive studies. The umbilical cord (UC) represents a promising alternative source easily obtained without causing pain to donors, and the procedure avoids ethical and technical issues. Recently it has been shown that UC-derived MSCs, including Wharton's Jelly-MSCs [11,12] and UC blood-MSCs [13] were able to differentiate into IPCs *in vitro*. Yet the potential of UC vein MSCs (UC-MSCs) have not been investigated.

The pancreatic niche (which includes the stromal and epithelial niches) has important role in the generation of IPCs, *in vivo* [14]. An important point to consider in the induction of stem cells into IPCs *in vitro* is the role of the pancreatic niche in the generation of IPCs. The stromal niches can be thought of as discrete anatomical sites containing niche support cells that physically contact adjacent cells and influence stem cell behavior via close range signaling [14]. In this respect, Duvillie et al. [15] reported that the mesenchyme of the pancreas controls the timing of β -cell proliferation and differentiation. Moreover, Choi et al. [16] and Lee et al. [17] have shown that Rat Pancreatic Extracts (RPE) could stimulate MSC differentiation into IPCs and increase insulin secretion, however, it may not be sufficient to induce functionally mature pancreatic cells.

According to the role of PSCs in pancreas development, and attempts to overcome the remaining challenges in generating IPCs (i.e., the efficiency of differentiation and function of newly-formed β -cells); in this study, UC-MSCs have been induced to differentiate into IPCs and then compared with BM-MSCs for their pancreatic differentiation

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potential. Moreover, we have differentiated MSCs into IPCs in co-culture with rat PSCs to investigate the effect of the pancreatic stromal niche on generating IPCs from MSCs.

Materials and Methods

Isolation and culture of human BM-MSCs and UC-MSCs

Human BM-MSCs were obtained from the Royan Cell Bank and passages 3-7 were used for all *in vitro* experiments. Cells were cultured in α -MEM (Gibco, Germany), supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin, and 15% FBS. Cells were re-plated for weekly passaging.

Human UCs were collected after normal deliveries. Each mother signed a consent form according to a protocol approved by the Royan Cord Blood Bank (RCBB). The UC-MSCs isolated and cultured as previously described [18]. After 3 weeks, the culture medium was replaced with α -MEM (Gibco) and supplemented with 20% fetal calf serum (HyClone), 100 IU/ml penicillin, 100 mg/ml streptomycin (Invitrogen; USA) and 2 mM L-glutamine (Invitrogen; USA).

Isolation and culture of rat PSCs

To isolate rat PSCs, the pancreases of 7-day postnatal Wistar rats (n=5) were removed under a protocol approved by the Institutional Review Board and Institutional Ethical Committee at Royan Institute. In the first step, pancreas tissue was diced using sterile blades into 1 mm³ pieces in RPMI 1640 that contained 1 mg/ml collagenase (type 1a; Sigma, Germany) and incubated at 37°C for 90 min. After enzymatic digestion, the collagenase solution was inactivated with RPMI 1640 supplemented with 15% FBS, and the tissue was passed through a nylon mesh filter (100 mm) to remove cell clumps and undissociated tissue. Cell suspension was centrifuged for 5 min at 800 g and the cell pellet was resuspended in RPMI 1640 (Sigma, Germany), supplemented with 10% FBS (Gibco; USA), 100 IU/ml penicillin, 100 mg/ml streptomycin (Invitrogen; USA) and 2 mM L-glutamine (Invitrogen; USA). Resuspended cells were plated onto 25 cm² culture bottles (Cellstar, Greiner, Germany). After 2 days of culture, the medium was changed and non-adherent cells were removed. When the cells reached confluence they were trypsinized (5 mg trypsin/ml PBS), washed in PBS, resuspended in 20 ml medium, replated onto 75 cm² bottles (Cellstar, Greiner, Germany) and cultured.

Flow cytometry analysis

Cells were harvested by treatment with 0.25% trypsin (Gibco, Germany), washed with PBS (pH=7.4) and labeled directly with anti-human CD90-FITC (fluorescein isothiocyanate), CD105-PE (phycoerythrin), CD73-PE, CD44-PE, CD34-PE, CD45-FITC and anti-rat CD90-FITC, CD44-FITC, CD45-PE, and CD11b-PE. Next, cells were washed in PBS (pH=7.4) and fixed with 4% paraformaldehyde for 20 min and washed twice in PBS. The specific fluorescence of 20000 cells was analyzed by FACSCalibur (Becton Dickinson, Temse, Belgium) using WinMDI 2.9 software.

Osteogenic and adipogenic differentiation assays

Osteogenic differentiation of UC-MSCs, BM-MSCs and rat PSCs were induced by 3 weeks of culturing in DMEM that contained 10% FBS, 50 mg/ml ascorbic acid 2-phosphate, 10 nM dexamethasone and 10 mM β -glycerol phosphate (all purchased from Sigma; Germany). Differentiation was confirmed by observation of extracellular matrix calcification using Alizarin red staining.

For adipogenic differentiation of UC-MSCs, BM-MSCs, and rat PSCs, DMEM-high glucose supplemented with 10% FBS, 60 mM indometacin, 10 nM dexamethasone and 10 mg/ml acid ascorbic (all from Sigma; Germany) were used. Media were changed every 3 days. After 3 weeks, cells were fixed with cold 10% formalin for 1 h, then washed twice with water and stained with oil-red solution (Sigma, Germany) for 2 h at room temperature, to reveal intra-cellular lipid droplets in the cytoplasm. Cells were washed twice and observed under an optical microscope.

In vitro pancreatic differentiation

Three previously published methods were utilized in the attempt to differentiate UC-MSCs and BM-MSCs into IPCs. These methods shall be referred to as the Shi [7], Gao [11], and Chao [13] methods. The culture medium in all protocols was high glucose DMEM. It has been revealed that glucose has a critical role for adult stem cell trans-differentiation into insulin-producing cells [19,20]. The schematic of protocols illustrated in Figure 1.

Direct co-culture of MSCs with rat PSCs

To investigate the effect of rat PSCs on the differentiation of BM-MSCs into IPCs, expanded rat PSCs from passage 3 were plated in 6-well plates and allowed to reach 80%-90% confluency. Mitomycin C (Sigma-Aldrich), at a concentration 25 μ g/ml, was then added to the culture medium to prevent stromal cell proliferation. The next day, BM-MSCs from passage 3 were seeded on the stromal cell layer at a ratio of (1:1). After an hour, pancreatic differentiation based on the Shi protocol was performed for the co-culture group.

Immunofluorescence analysis

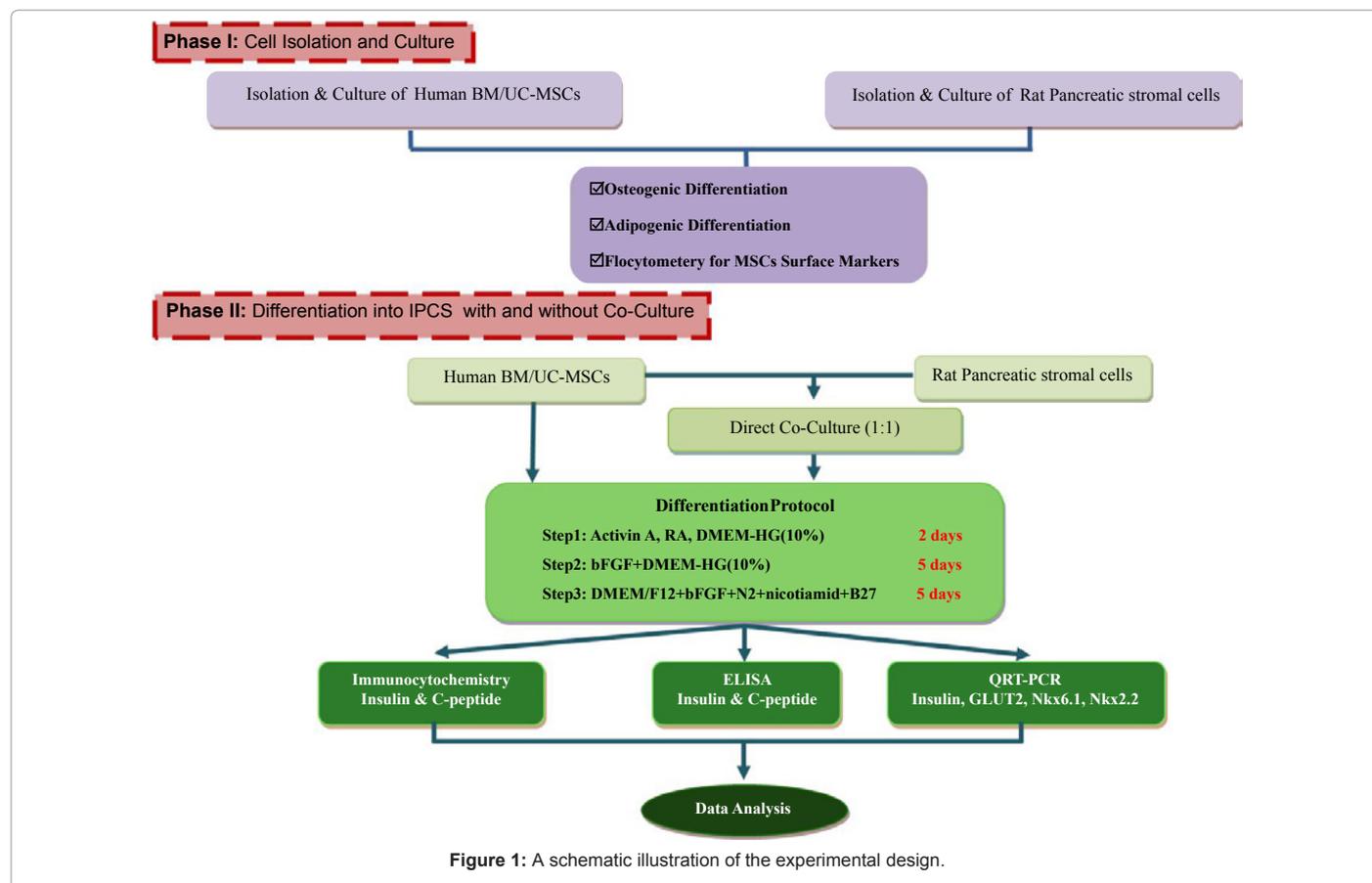
The cells were fixed with 4% paraformaldehyde in PBS for 20 min at 4°C, washed several times in PBS, and blocked for 30 min in normal goat serum 10% in PBS. Cells were then incubated overnight at 4°C with primary antibodies, that included mouse anti-human insulin 1:500 (Sigma-Aldrich) and mouse anti-human C-peptide 1:250 (Abcam, Cambridge, UK). Cells were then washed 3 times with PBS. A fluorescence-labeled second antibody (1:100, FITC-conjugated goat anti-mouse; Sigma, Germany) was applied for 1 h at 37°C and followed by PBS washes. Nuclei were counterstained with DAPI and cells were visualized using a fluorescence microscope (Olympus BX51, Japan).

Real-time quantitative PCR

Cells were harvested from differentiating plates, and total RNA was isolated from triplicate samples with an RNA extraction kit (TaKaRa, Japan) and 100-500 ng was used for reverse transcription with a Prime Script II strand cDNA synthesis kit (TaKaRa, Japan). PCR reactions were run in duplicate using 1/40th of the cDNA per reaction and 400 nM forward and reverse primers with SYBR Green master mix (TaKaRa, Japan). Real-time PCR was performed using the Rotor Gene 3000 (Corbett Research). Quantitative RT-PCRs were performed in duplicate for each sample primer set, and the mean of the three experiments was used as the relative quantification value. Relative gene expression was analyzed using the comparative Ct method, $2^{-\Delta\Delta Ct}$. All samples were normalized to the levels of GAPDH, which was used as the loading control. Primer sequences are listed in Table 1.

ELISA

The human insulin and C-peptide levels in culture supernatants were measured by Human insulin ELISA Kit and Human C-peptide



ELISA Kit (Merckodia, Switzerland). After pre-incubation with Krebs-Ringer buffer at 37°C for 60 min, the differentiated cells were first incubated with Krebs-Ringer buffer containing 5 mM glucose for 1 h to determine the basal level of insulin and C-peptide. The same IPCs were subsequently incubated with 27.5 mM glucose for an additional 1 h to determine glucose-stimulated insulin and C-peptide release. Then the respective conditioned supernatant was collected and analyzed.

Statistical analysis

Numerical values are presented as the mean ± SE. Each experiment was repeated three times. Statistical significance was determined using the two way analysis of variance (ANOVA) and repeated measure test.

Annealing temperature (°C)	Sequence (5'-3')	Strand	Gene
60	CTCATTTCCTGGTATGACAACGA	F	GAPDH
	CTTCCTCTTGTTGCT	R	
60	AAGAGGCCATCAAGCAGATCA	F	Insulin
	CAGGAGGCGCATCCACA	R	
60	GTTTGAGGATTCCTGAGTGTG	F	Glut2
	GGAAATGTCAAAGGAGACGAT	R	
60	CGATATTGTCAGCCGCTTCTAA	F	Nkx2.2
	TGCCACCAGTTGTCAGAA	R	
60	GACAAAGACGGGAAGAGAA	F	Nkx6.1
	GGAACACAGACCTTGACCT	R	

Table 1: Primers used for real-time quantitative PCR.

To compare two differentiation groups (BM-MSCs with and without co-culture) nonparametric Mann-Whitney test were used. A p value less than 0.05 was considered to be statistically significant.

Results

Isolation and characterization of human UC-MSCs, BM-MSCs and rat PSCs

As shown in Figure 1, the first step of our protocol was the isolation and characterization of MSCs from different sources (mainly, BM-MSCs and CU-MSCs). Human BM-MSCs were obtained from the Royan Cell Bank and were cultured over three passages. Under an inverted microscope, undifferentiated MSCs were typically adherent spindle and fibroblast-like cells (Figure 2A). Flow cytometric analysis of the BM-MSCs at the 3rd passage showed that these cells expressed high levels of CD44, CD105, CD90, and CD73 and low levels of CD34 and CD45 (Figure 2B). Moreover the most of the cells were positive for alizarin red and oil red after differentiation into osteocytes and adipocyte at 3rd passage, respectively (Figure 2A).

In another attempt, the mesenchymal stem cells were successfully isolated from human umbilical cord vein which named UC-MSCs. In the initial passage of culture, the cells proliferated slowly and gave rise to confluence in 21-30 days. After sub-culturing, the heterogeneous cell populations changed into a homogeneous one with flat and fibroblast-like shape (Figure 2A). They expressed MSC specific markers CD73, CD44, CD90 and CD105 at 3rd passage and were negative for the hematopoietic cell marker CD11b (Figure 2B). To investigate the

osteogenic and adipogenic differentiation potential of UC-MSCs, the 3rd passage of cells was directed towards osteocytes and adipocytes. The data revealed that unlike BM-MSCs, UC-MSCs could not differentiate into adipocytes (Figure 2B) when stained by oil red dye. However the most of the cells were positively stained by alizarin red after osteogenic maturation (Figure 2B).

To obtain PSCs, pancreases of 7-day postnatal Wistar rats were used. Adherent cells showed typical fibroblast-like structure that rapidly reached confluence (7-10 days). The cells were trypsinized and seeded at a density of 1000 cells/cm² into adherent plastic flasks for expansion. Fibroblast-like cells isolated from rat pancreas show a similar phenotype to MSC isolated from the human BM (Figure 2A). The expression pattern of PSCs was similar to BM-MSCs. Actually expressed low levels of CD45 and CD11b and high levels of CD44 and CD90 (Figure 2B). They simply differentiated into osteocyte, but not adipocyte when tested for differentiation (Figure 2A).

In vitro differentiation of UC-MSCs and BM-MSCs into insulin-producing cells

To find the most efficient protocol for the differentiation of MSCs into Insulin Producing Cells (IPCs), BM-MSCs were first induced into IPCs using the three previously published protocols, refereed here as Shi [7], Gao [11], and Chao [13] method. The methods are described in materials and methods and the results are summarized in Table 2. As shown in Table 2 just Shi protocol successfully differentiated BM-MSCs into IPCs at the last stage of differentiation.

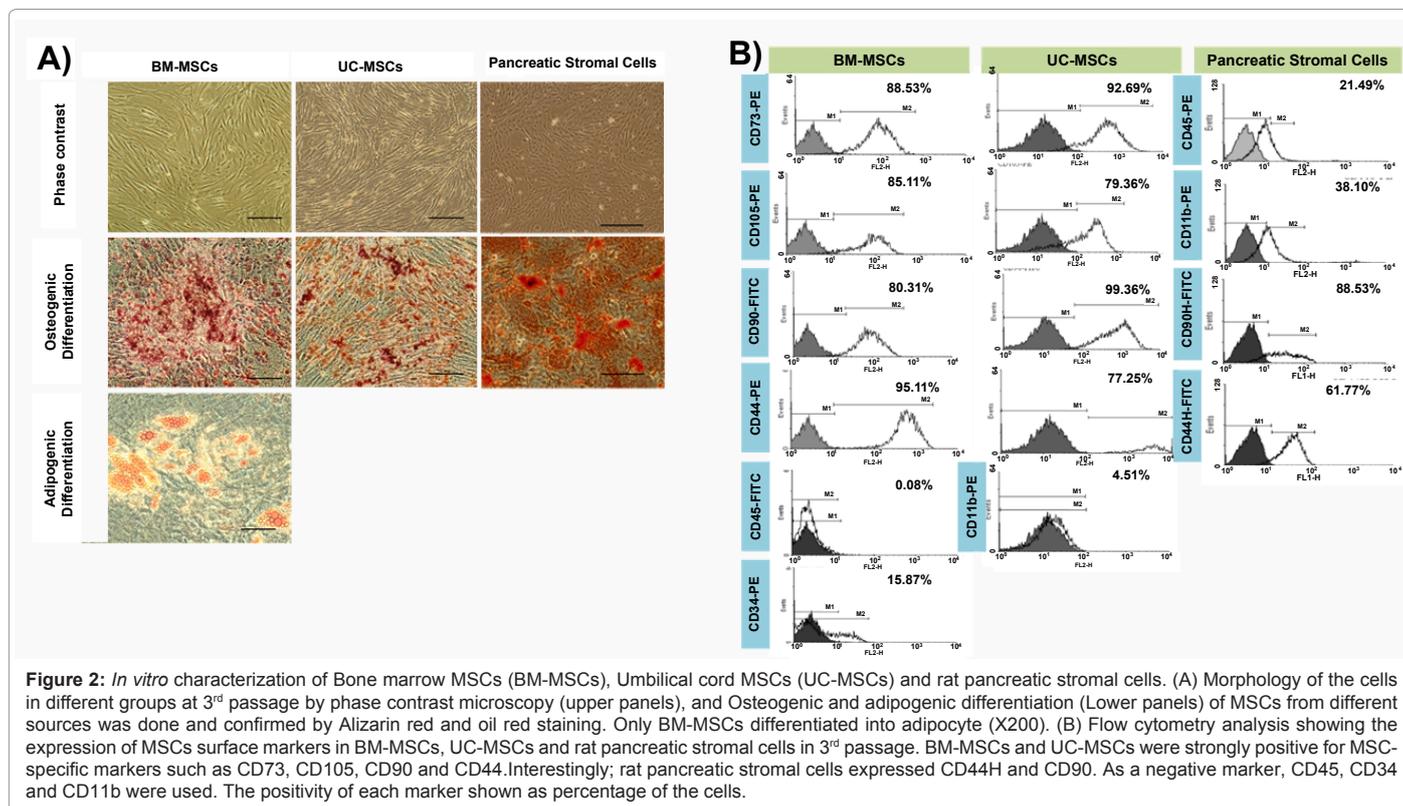
During induction of BM-MSCs with Shi method, changes in BM-MSCs morphology at step 1 could not be observed. However with prolongation of differentiation time, spindle-like cells became short and changed into round and epithelial-like cells. In other hand, the rate

of cell proliferation became slower. At the end of step 3, some cells gathered together and formed the pancreatic islet-like structure (Figure 3B). To determine whether UC-MSCs were able to differentiate into IPCs, these cells were also induced by using Shi protocol. However, they were negative for insulin and C-peptide at protein level (Figure 3D) as well as mRNA level and insulin secretion (data not shown). Hence, our results revealed that UC-MSCs were not potent to differentiate into IPCs *in vitro*. The pancreatic biopsies from cadaver and MSCs without any treatment were used as positive and negative control, respectively (Figure 3A,E).

Investigation of the effect of the co-culture of rat PSCs on differentiation of BM-MSCs into IPCs

To investigate the effect of rat PSCs as an effective factor in the pancreas niche on the differentiation of BM-MSCs into IPCs, MSCs were induced into IPCs in direct co-culture with rat PSCs by using the Shi protocol. There was no significant difference in morphology, insulin and C-peptide expression at protein level between BM-MSCs in co-culture with rat PSCs and without rat PSCs (Figure 3C).

The results of mRNA level at different stage of differentiation illustrated in Figure 4. Actually, undifferentiated MSCs (step 0) and cells at step 2 of differentiation did not expressed *insulin*, *Nkx2.2*, *Nkx6.1*, and *Glut2*; as pancreatic β cell-related genes. Interestingly, cells at step three up regulated the mRNA level of *insulin*, *Glut2*, and *Nkx2.2* in both differentiation groups (with and without co-culture). However the expression of the *insulin*, *Glut2*, and *Nkx2.2* was significantly superior in co-culture group ($p < 0.05$) (Figures 4A, 4B, and 4D) and the mRNA level of *Nkx6.1* was not significantly up-regulated in step 3 of either differentiation group (Figure 4C). The mRNA level of GAPDH was used as positive control.



Differentiation protocol	Cell Type	Duration of Differentiation	Result		
			ICC*	ELISA	RT-PCR
Shi et al.	BM-MSCs	12 days	+	+	+
	UC-MSCs		-	-	-
Chao et al.	BM-MSCs	28 days	-	-	-
Gao et al.	BM-MSCs	15 days	-	-	-

*Immunocytochemistry

Table 2: Result of differentiation of MSCs into IPCs with 3 different protocols.

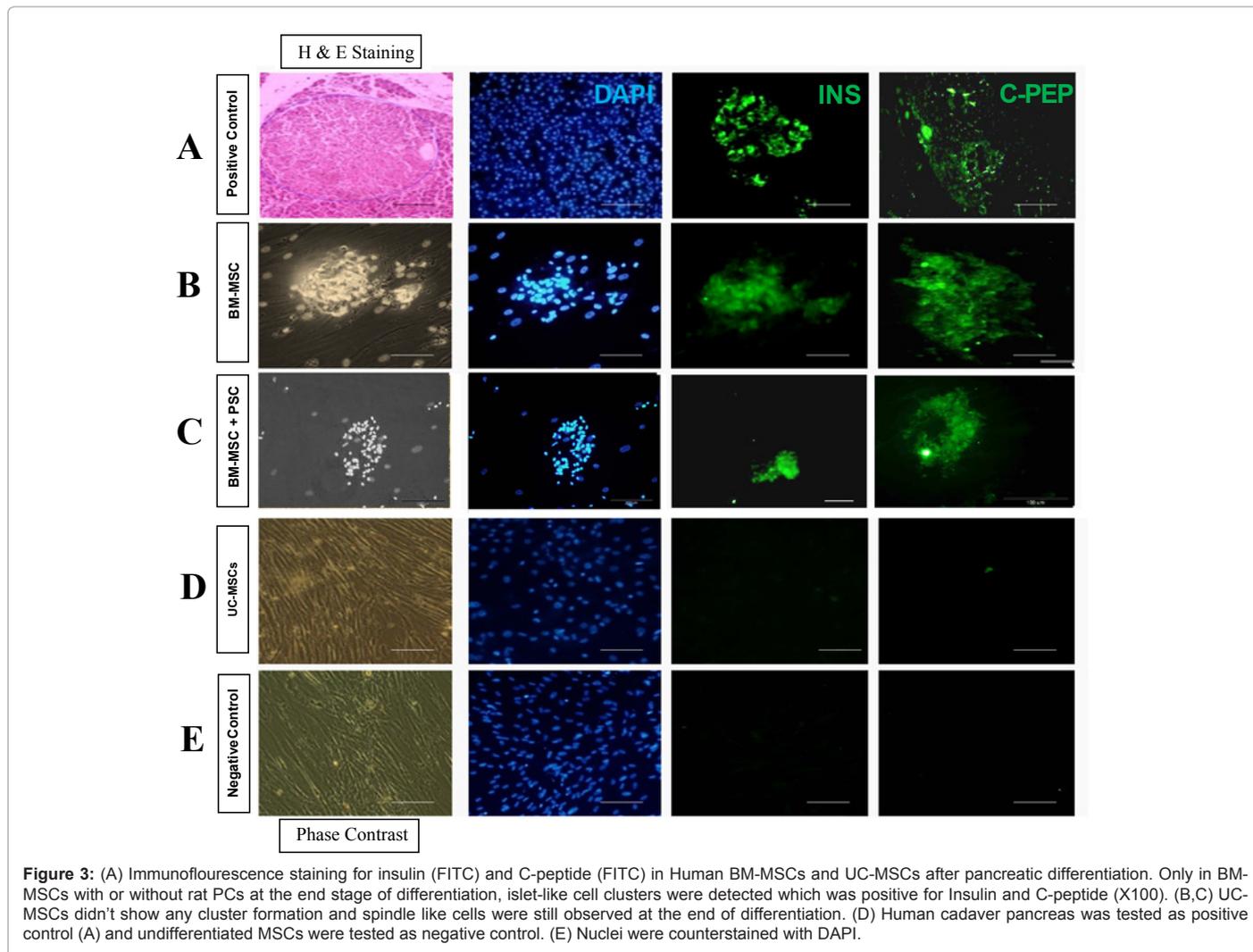


Figure 3: (A) Immunofluorescence staining for insulin (FITC) and C-peptide (FITC) in Human BM-MSCs and UC-MSCs after pancreatic differentiation. Only in BM-MSCs with or without rat PCs at the end stage of differentiation, islet-like cell clusters were detected which was positive for Insulin and C-peptide (X100). (B,C) UC-MSCs didn't show any cluster formation and spindle like cells were still observed at the end of differentiation. (D) Human cadaver pancreas was tested as positive control (A) and undifferentiated MSCs were tested as negative control. (E) Nuclei were counterstained with DAPI.

In next step we determined whether IPCs could synthesize and release insulin in a glucose-dependent manner, therefore, the ability of MSC-derived IPCs in both differentiation groups (with and without co-culture) was tested for human insulin and C-peptide production. The supernatants of differentiated cells at step 3 were analyzed by ELISA after sequential treatment with low (5 mM) and high (27.5 mM) concentrations of glucose (Figures 5A and 5B). The supernatant of differentiation without any glucose stimulation was measured for C-peptide release.

Although, cells in both groups (with and without PCS) released high level of insulin and C-peptide compare undifferentiated cells (data not shown), but, unexpectedly, the highest levels of insulin and

C-peptide secretion were observed in response to the 5 mM glucose concentration supernatants and did not increase with higher glucose concentrations in both differentiated groups.

Discussion

Recent studies have demonstrated the possibility of generating IPCs from various cellular sources including embryonic stem cells [7], Induced Pluripotent Stem Cells (IPS cells) and MSCs [11-13]. In spite of their promising potential, there are still some significant challenges, such as the efficiency of differentiation and the normal function of the newly formed β -cells, especially in synthesis and secretion of insulin in response to glucose stimulations. Stromal niches can influence stem cell behavior via close range signaling [14]. To overcome challenges

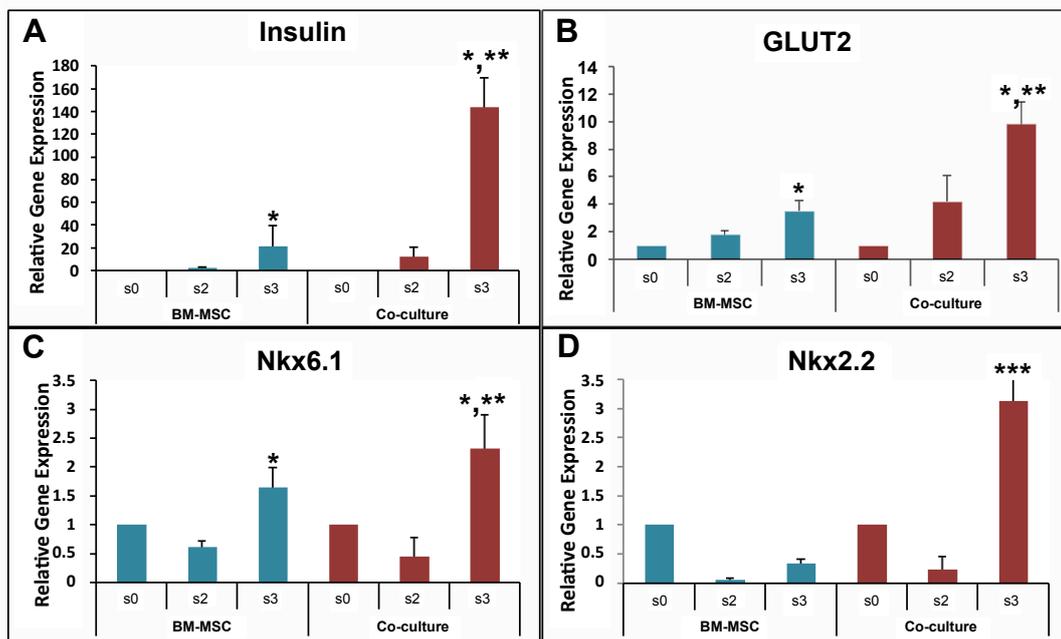


Figure 4: Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of expression of Insulin, Glut2, Nkx6.1 and Nkx2.2. Total RNA was extracted from BM-MSCs at the end of 3 steps of differentiation (S0, S2 and S3) in two different groups; BM-MSCs with and without co-culture. qRT-PCR detected the up-regulation of mRNA expression for insulin, Glut-2 and Nkx2.2 in last step differentiation comparing to step 0 and step 2. But the up-regulation of mRNA expression of Nkx6.1 did not detect in step 3 of any groups. For each sample, relative expression levels were normalized to undifferentiated GAPDH and undifferentiated BM-MSCs served as a negative control.

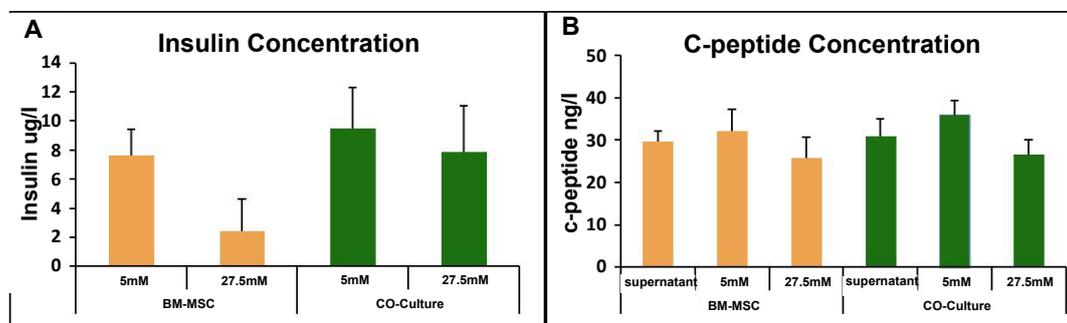


Figure 5: Insulin(A) and C-peptide(B) release in response to low (5 mM) and high (27.5 mM) glucose concentrations of clusters in both with and without co-culture groups was measured after 1 h incubation. C-peptide secretion in supernatant was also detected. The difference between insulin and C-peptide secretion in response to low and high glucose concentrations in both groups was not statistically significant. All data are presented as means \pm SE from three independent experiments.

regarding the role of the pancreatic niche, especially the stromal cells in pancreas development, in this study we differentiated MSCs into IPCs alone or in presence of rat PSCs to investigate the effect of the pancreatic stromal cells on generating IPCs. The presence of insulin and other pancreatic β cell-related genes, such as *Nkx2.2* and *Glut2* were confirmed by qRT-PCR. The functionality of the *in vitro* generated IPCs was tested by measuring insulin and C-peptide release in response to glucose challenges. On the other hand; we compared IPCs differentiation potential of human UC-MSCs, which are easily obtained from cord, manipulated and free of ethical problems, with BM-MSCs using the same protocol.

Our results demonstrated that all cells isolated from these two sources exhibited typical MSC characteristics phenotypically: a fibroblast-like morphology, plastic-adherent capability, and the expression of a typical set of surface proteins which also previously

reported [8]. In addition, bi-potential differentiation capacity (osteogenic and adipogenic) was observed for BM-MSCs; however UC-MSCs easily differentiated to osteocytes and was limited to produce adipocytes. Therefore we assume that MSCs isolated from different tissues might exhibit diverse differentiation capacities according to tissue sources [8]. Also ontogenetic age of MSCs might affect their potential [21].

In order to find the most efficient method for the differentiation of BM and UC-MSCs into IPCs, we examined three different induction methods (Gao, Chao, and Shi) on BM-MSCs, and then tried the best one on the UC-MSCs. In contrast to the Gao and Chao methods, only by use of Shi method, BM-MSCs could differentiate into IPCs which were positive for C-peptide and insulin and released them in response to physiological level of glucose. *Insulin* and other pancreatic β cell-related genes, such as *Nkx2.2* and *Glut2*, were expressed in the islet-

like cell clusters. By comparing the three differentiation methods, we suggested that Activin A and all-trans RA are two main key factors in the Shi induction method which are not used in the two other protocols. Activin A belongs to the TGF- β super-family and is important for early endoderm development *in vivo* [22] as well as definitive endoderm cells form embryonic stem cells *in vitro* [23]. It can also improve insulin secretion in cultured human pancreatic islets [24].

Meanwhile, *retinoic acid* is an important signaling molecule in the development of the early embryonic pancreas in addition to its functions on induction of ectoderm and mesoderm development [25]. It can induce *pdx1+* endoderm formation when added on the fourth day of mouse ESC differentiation [26].

After selecting the most efficient method, human UC-MSCs were induced to IPCs with the Shi protocol, which were not successful in IPCs differentiation. Although several studies demonstrated that MSCs derived from Wharton's Jelly [11,27] and umbilical cord blood [13,28] can differentiate into IPCs, our findings indicated that MSCs derived from the UC vein were not capable of differentiating into adipocytes as well as IPCs which may be related to the site of derivation of cells.

Finally, we investigated the effect of the pancreatic stromal niche on generating IPCs from MSCs, using directly co-cultured system. In compare BM-MSCs alone, cells in co culture group expressed *insulin*, *Nkx2.2*, and *Glut2* genes more than MSCs alone. Previous studies have also demonstrated that rat pancreas extract could induce phenotypic pancreatic differentiation [16,17].

Because of apoptotic cells can take up exogenous insulin from the culture medium, we measured insulin as well as C-peptide protein to demonstrate *de novo* synthesis of insulin. C-peptide was not detected in the pancreatic differentiation medium before differentiation (day 0, data not shown); and was positive and secreted just after differentiation. Both differentiation groups (BM-MSCs with and without co-culture) secrete insulin and C-peptide in response to glucose stimulations. However, there was no significant difference between two groups. Moreover, IPCs in both groups released insulin and C-peptide in response to minimal glucose stimulation (5 mM glucose) and not in presence of high glucose stimulation (27.5 mM glucose). Therefore we suggested that differentiated IPCs release all the insulin and C-peptide after stimulation with a low dose of glucose, and have no insulin reserve to release upon re-stimulation with a higher dose of glucose. The time to synthesize pro-insulin and to process insulin may exceed over 1-hour by IPCs. Thus, differentiated IPCs may have released all of intracellular insulin without resynthesize it again when exposure to the high glucose concentration. Moreover, it seems that differentiated IPCs were not matured enough; therefore, the number of glucose receptors was not sufficient, causing to have abnormal function in response to glucose stimulations. More extensive studies are needed to test the secretion of other pancreatic hormones specially glucagon in differentiated cells. Finally, we found that, the pancreatic stromal cells were not sufficient to induce functionally mature pancreatic cells as previously reported by Lumelsky et al. [29] because of the short term culture of the cells. The prolongation of culture time let to translate mRNA to protein and activate signaling pathways for proper glucose responses.

Taken together, the data presented in this study indicated that human UC-MSCs, despite their fascinating advantages such as a large potential donor pool, rapid availability, absence of discomfort to the donor, and low risk of rejection, were not able to differentiate into IPCs *in vitro*, and had lower differentiation potential than BM-MSCs. Our

results suggested that rat PSCs up-regulated the expression of β cell specific markers such as *insulin*, *Glut2*, and *Nkx2.2* at the mRNA level, however, did not affect protein level increment. We suggest that PSCs cause an enhancement in the number of immature pancreatic β -cells, but not in the number of mature cells. It is possible that the pancreatic stromal niche may control the timing of β -cell differentiation by increasing the number of progenitor cells. However more research is needed to determine the role of other factors of the pancreatic niche on the differentiation of β -cells in generating IPCs *in vitro*.

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R.KH researched data, contributed to discussion, wrote and edited the manuscript. M.E contributed to discussion, wrote and reviewed the manuscript. E.MB contributed to discussion. N.A contributed to discussion. F.S researched data. H.B contributed to discussion.

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