Liver, Pancreas and Biliary Tract

In vitro differentiation into insulin-producing β-cells of stem cells isolated from human amniotic fluid and dental pulp

Gianluca Carnevale, Massimo Riccio, Alessandra Pisciotto, Francesca Beretti, Tullia Maraldi, Manuela Zavatti, Gian Maria Cavallini, Giovanni Battista La Sala, Adriano Ferrari, Anto De Pol

A Department of Surgery, Medicine, Dentistry and Morphological Sciences with Interest in Transplant, Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy

b Department of Medical and Surgical Sciences, IRCCS Arcispedale Santa Maria Nuova, University of Modena and Reggio Emilia, Reggio Emilia, Italy

c Department of Biomedical, Metabolic and Neuroscience, Children Rehabilitation Special Unit, IRCCS Arcispedale Santa Maria Nuova, University of Modena and Reggio Emilia, Reggio Emilia, Italy

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Aim: To investigate the ability of human amniotic fluid stem cells and human dental pulp stem cells to differentiate into insulin-producing cells.

Methods: Human amniotic fluid stem cells and human dental pulp stem cells were induced to differentiate into pancreatic β-cells by a multistep protocol. Islet-like structures were assessed in differentiated human amniotic fluid stem cells and human dental pulp stem cells after 21 days of culture by dithizone staining. Pancreatic and duodenal homebox-1, insulin and Glut-2 expression were detected by immunofluorescence and confocal microscopy. Insulin secreting from differentiated cells was tested with SELDI-TOF MS and by enzyme-linked immunosorbent assay.

Results: Human amniotic fluid stem cells and human dental pulp stem cells, after 7 days of differentiation started to form islet-like structures that became evident after 14 days of induction. SELDI-TOF MS analysis revealed the presence of insulin in the media of differentiated cells at day 14, further confirmed by enzyme-linked immunosorbent assay after 7, 14 and 21 days. Both stem cell types expressed, after differentiation, pancreatic and duodenal homebox-1, insulin and Glut-2 and were positively stained with dithizone. Either the cytosol to nucleus translocation of pancreatic and duodenal homebox-1, either the expression of insulin, are regulated by glucose concentration changes. Day 21 islet-like structures derived from both human amniotic fluid stem cells and human dental pulp stem cell release insulin in a glucose-dependent manner.

Conclusion: The present study demonstrates the ability of human amniotic fluid stem cells and human dental pulp stem cell to differentiate into insulin-producing cells, offering a non-pancreatic, low-invasive source of cells for islet regeneration.

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1. Introduction

Diabetes is a chronic disease characterized by hyperglycemia resulting from defects in insulin secretion or insulin action on cells [1]. Although exogenous use of insulin remains the most successful therapy, it fails to prevent hyperglycemia, resulting in acute episodes of hypoglycemic and/or chronic complications like nephropathy, retinopathy, neuropathy, impotence and cardiovascular diseases [2,3]. For these reasons, over the past two decades the allogeneic transplantation of pancreatic islet cells has become a subject of intense interest and activity as a potential cure for diabetes [4]. Unfortunately, islet transplantation faces a great number of side effects. Particularly, the difficulty in obtaining sufficiently large numbers of purified islets due to shortage donor organs and to the loss during the purification process [5], limits their therapeutic usage thus prompting a search for alternative sources of islet cells. The use of stem cells, a potential renewable source of pancreatic β-cells, is being currently investigated as an alternative therapeutic option for the treatment of diabetes mellitus. Recently, attention has been aroused on embryonic [6], hematopoietic [7], pancreatic islet [8], human biliary tree [9] and mesenchymal stem cells [10] isolated from foetal and adult tissues, cultured and differentiated in vitro. Concerning mesenchymal stem cells, several reports have focused great interest on human bone marrow-derived stem cells (hBMSCs), dental pulp stem cells (hDPSCs) and amniotic fluid...
stem cells (hAFSCs). Under appropriate experimental conditions and chemical stimuli, hBMSCs [11] and hDPSCs [12] can differentiate into insulin-producing cells. On the contrary, Trovato et al. [13] demonstrated that under experimental conditions, used in their study, cultured hAFSCs failed to differentiate into β-cells. Only Li et al. [14] demonstrated that hAFSCs can be differentiated into functional insulin-producing cells by knocking down neural restrictive silencing factor. Despite their differentiation potential, the invasive procedures required to isolate hBMSCs as well as the scarce percentage of these cells in bone marrow can limit their use. On the contrary, hDPSCs show a large pool of donors and are easily accessible. Regardless of hAFSCs, they can be obtained following human amniocentesis backup and, as described by De Coppi et al. [15], are pluripotent stem cells capable of giving rise to multiple lineages including representatives of all three embryonic germ layers.

For these reasons the aim of the study is to develop a simple method as well as to improve the differentiation of hDPSCs and hAFSCs in pancreatic islet cells producing insulin and to compare their ability along the differentiation pathways. Particularly, we demonstrated that hDPSCs and hAFSCs, under appropriate stimuli, express genes related to pancreatic β-cell development and function, such as pancreatic and duodenal homebox-1 (PDX-1) and insulin. Therefore, hDPSCs and hAFSCs are able to differentiate in insulin-producing cells. The two different stem cell lines could represent a promising tool for the treatment of diabetes mellitus.

2. Materials and methods

2.1. Cell culture isolation and seeding

Human AFSCs were isolated and cultured as previously described [15,16]. Supernumerary amniocentesis samples were provided by the Laboratorio di Genetica, Ospedale Santa Maria Nuova (Reggio Emilia, Italy). Back-up human amniocentesis cultures were harvested by trypsinization and subjected to c-Kit immunoselection by MACS® technology (Miltenyi Biotec).

Human DPSCs were isolated from third molar of adult subjects (18 and 35 years of age) and were subjected to immunoselection by magnetic cell sorting to obtain the CD34+/c-Kit+/STRO-1+ population as previously described by Riccio et al. [17].

All samples were collected with informed consent of the patients according to Italian law and guideline of ethical committee of Modena and Reggio Emilia University.

2.2. In vitro multilineage differentiation

To induce osteogenic, adipogenic, myogenic and neurogenic differentiation, hAFSCs and hDPSCs were cultured in the appropriate induction media as previously described [18].

In order to verify adipogenic differentiation cells were stained with oil red O solution and counterstained with Harris haematoxylin for 1 min.

Myogenic differentiation was verified by double immunofluorescence staining, using an anti-human nuclei antibody (Ab) (anti-hNu; Millipore, Billerica, MA, USA) and an anti-Myosin Ab (Sigma–Aldrich), in order to verify the formation of hybrid myotubes between hDPSCs or hAFSCs and murine C2C12 cells. The expression of β3-Tubulin was analyzed by immunofluorescence staining as marker of neurogenic differentiation.

2.3. In vitro β-cells differentiation of hAFSCs and hDPSCs

Differentiation of hAFSCs and hDPSCs was carried out in three stages. Briefly, cells were seeded at the density of 10,000 cell/cm² and cultured until reaching 80% of confluence. On day 1 culture medium was replaced with Pre-induction medium [L-DMEM (Euroclone), supplemented with 5% FBS, 5 μM trans-retinoic acid (RA) and 0.5 mM β-mercaptoethanol (BME)] for two days. On the third day, the pre-induction medium was replaced with the induction medium [H-DMEM (Euroclone), serum-free, plus 10 mM nicotinamide (NA), 5 μM RA, 0.5 mM BME]. On the fifth day the medium was replaced with maintaining-1 medium (H-DMEM supplemented with 5% FBS, 10 mM NA, 10 μM zinc sulphate and 10 μM selenium). The maintaining-1 medium was replaced at day 7 with maintaining-2 medium (L-DMEM supplemented with 5% FBS, 10 mM NA, 10 μM zinc sulphate and 10 μM selenium). The maintaining-1 and the maintaining-2 media were alternated every 2 days until day 21. A time table of treatments is shown in Fig. 1. Cells cultured in medium without inducers were used as controls. All chemicals were purchased from Sigma–Aldrich unless otherwise indicated.

2.4. Medium protein profile by SELDI-TOF MS

Proteomic analyses to evaluate the presence of insulin in differentiating and control medium were performed using SELDI-TOF MS (Bio–Rad). Particularly after 14 days of differentiation, the proteomic profiles, between maintaining-1 medium of hAFSCs, hDPSCs and the medium from control cells were compared to the proteomic profiles obtained from maintaining-1 medium supplemented with 21 pg of recombinant human insulin.

Chip preparation and sample application were performed according to the manufacturer’s instructions using the Biomek 3000 Laboratory Automation Workstation (Beckman Coulter, Fullerton, CA, USA). Briefly, 3 μl of each medium was loaded on the spot of gold ProteinChip arrays and incubated for 15 min at room temperature.

After air drying, 1 μl of matrix (saturated sinapinic acid solution in 50% acetonitrile in water containing 1% trifluoroacetic acid) was applied to each spot twice. Spot adsorbed protein patterns were analyzed on a ProteinChip Reader (Series 4000, Bio–Rad Laboratories, Hercules, CA, USA) and spectra detection was performed with ProteinChip Data Manager software (Bio–Rad Laboratories Inc., Hercules, CA, USA).

The obtained spectra were baseline subtracted, normalized for total ion current (TIC) in the range of mass-to-charge ratios (m/z) between 4000 and 10,000 Da and finally mass aligned.

2.5. Immunofluorescence and confocal microscopy

Undifferentiated or differentiated hAFSCs and hDPSCs were fixed for 20 min in 4% ice-cold paraformaldehyde in phosphate-buffered saline (PBS), permeabilized with 0.1% Triton X-100 in PBS for 5 min and then processed as previously described [19]. The following primary Abs were used: rabbit anti-c-Kit, mouse anti-CD34, mouse IgM anti-STRO-1, rabbit anti-β3-Tubulin, rabbit anti-β3-Tubulin, rabbit anti-myosin, Sigma–Aldrich; mouse anti-human nuclei, Millipore; diluted 1:100. Secondary Abs (goat anti-mouse Alexa405, goat anti-rabbit Alexa488, goat anti-rabbit Alexa546, Molecular Probe; goat anti-mouse IgM Cy3, Jackson) were diluted 1:200. Nuclei were stained by 1 μg/ml 4',6-diamidino-2-phenylindole (DAPI) in PBS. Negative controls consisted of samples not incubated with the primary Ab. The multi-labelling immunofluorescence experiments were carried out avoiding cross-reactions between primary and secondary Abs.

Confocal imaging was performed by a Nikon A1 confocal laser scanning microscope as previously described [20]. The confocal serial sections were processed with ImageJ software to obtain
three-dimensional projections and image rendering was performed by Adobe Photoshop Software.

2.6. Determination of insulin release and dithizone staining

Insulin concentrations were measured in the culture media of hAFSCs and hDPSCs at 0, 7, 14 and 21 days of differentiation. Moreover, after 21 days of differentiation the total release of insulin was detected following the stimulation with 25.0 mM glucose.

Briefly, cells were gently washed with PBS once and then were incubated with L-DMEM and H-DMEM containing 5.5 or 25.0 mM glucose respectively, at 37 °C for 3 h. After 3 h of incubation, supernatants from wells were harvested and the levels of insulin from cells exposed to low or high glucose were measured. Media from undifferentiated cells were used as control.

The quantitative measurement of human insulin concentration was determined using the Insulin Human enzyme-linked immunosorbent assay (ELISA) kit (AbCam Cambridge, UK) according to the manufacturer’s instructions. Islet-like structures were assessed in hAFSCs and hDPSCs after 21 days of culture following Dithizone staining [21].

2.7. Western blotting

Whole cell lysates were obtained from undifferentiated and differentiated hAFSCs and hDPSCs at 7, 14 and 21 days of differentiation, and were processed as described by Pisciotta et al. [18]. 40 μg of total proteins from each sample were separated by 12% SDS-PAGE and then transferred to PVDF membranes.

Blots were incubated with primary Abs (goat anti-PDX-1, AbCam; rabbit anti-Glut-2, rabbit anti-PARP, Santa Cruz) diluted 1:1000, revealed by HRP-conjugated secondary Abs (anti-goat; anti-rabbit; Pierce Antibodies, Thermo Scientific) diluted 1:300. All membranes were revealed by using Enhanced Chemio Luminescence (Amersham). Anti-actin Ab was used as control of protein loading in timing experiments. Densitometry was performed on three independent experiments by NIS software (Nikon). An equal area was selected inside each band and the mean of grey level (in a 0–256 scale) was calculated. Data were then normalized to values of background and of control actin band.

2.8. Data analysis

Values are reported as the mean ± SD obtained by groups of 4–5 samples each. Differences between two experimental samples were analyzed by paired, Student’s t test. Three or more experimental samples were analyzed by ANOVA followed by Dunnett’s test (GraphPad Prism Software version 5 Inc., San Diego, CA, USA). In any case significance was set at p < 0.05.

3. Results

3.1. In vitro multilineage differentiation

In order to verify the phenotype of isolated hAFSCs and hDPSCs surface immune-labelling was performed by using c-Kit, CD34, and STRO-1 specific Abs. Isolated hAFSCs showed a clear expression of c-Kit confirming that the correct population was obtained. In the same manner hDPSCs showed positivity to c-Kit, CD34, and STRO-1 Abs, by co-expressing these antigens on their surface (Fig. 2A).

Multi-differentiation experiments were also performed for both cell types to confirm multipotent properties of isolated stem cells.

After 3 weeks of culture with osteogenic medium, both hAFSCs and hDPSCs showed nodular structures and the presence of a dense extracellular matrix. Particularly, extracellular matrix showed a positivity to the Alizarin Red staining (Fig. 2B).

The adipogenic potential of hAFSCs and hDPSCs was assessed by treating cells with adipogenic medium for 21 days. Particularly, lipid vacules were noticeable under light microscopy as early as 10 days after adipogenic induction and visualized by staining with Oil Red O after 21 days (Fig. 2B).

The ability of hAFSCs and hDPSCs to differentiate towards myogenic lineage was verified by co-culturing the two human stem cell types with C2C12 mouse myoblasts. After 14 days of co-culture myotubes formation was observed in both co-cultures. Particularly, myotubes appear multi-nucleated indicating that cell fusion occurs. Double staining with anti-hNu and anti-myosin Abs indicates that mature hybrid myotubes were formed between mouse C2C12 and hAFSCs or hDPSCs. Myotubes not labelled by anti-hNu Ab and therefore formed only by C2C12 cells were also present (Fig. 2B).

After 10 days of addition of neurogenic medium, most of hAFSCs and hDPSCs showed a typical neuronal appearance. Furthermore, cells with neuron-like morphology showed a positive staining against the β3-Tubulin neuronal marker (Fig. 2B).

3.2. β-Cell differentiation

hAFSCs and hDPSCs were cultured in differentiation medium for 21 days according to the multistep protocol described in Fig. 1. The induced cells were examined daily for morphological changes under light microscope. Particularly, undifferentiated hAFSCs and hDPSCs showed a typical fibroblast-like morphology. Conversely,
the peaks with mass-to-charge ratio (m/z) 5800 Da that matched with the detection peak obtained in the medium supplemented with insulin (Fig. 3B). Moreover, as shown in Fig. 3B, the spectra analysis of undifferentiated hAFSCs and hDPSCs media did not show any peaks with m/z 5800 Da. This result suggests that after 14 days of culture in differentiation media the two stem cell types are already committed towards the pancreatic β-cells and are able to produce and release insulin.

3.3. Insulin release assay and immunocytochemistry assay

To further clarify the function of differentiated hAFSCs and hDPSCs, insulin concentrations in the culture media were quantitatively measured by using the Human Insulin ELISA kit after 0, 7, 14 and 21 days of induction.

As shown in Fig. 4A, 7 days after differentiation the insulin secretion from hAFSCs was significantly higher (p < 0.05) when compared to the respective control (time 0). Furthermore, the insulin release statistically increased after 14 and 21 days of differentiation (30.7 ± 5.5 and 42.0 ± 4.1 μU/ml) p < 0.01 and p < 0.001 respectively (Fig. 4A).

Likewise, after 7 days of induction the insulin amount in hDPSCs medium was significantly higher in comparison to the undifferentiated hDPSCs (time 0; p < 0.05; Fig. 4A). Moreover, after 14 and 21 days of differentiation the insulin concentration in the medium increased in significant manner (27.6 ± 4.7 and 42.8 ± 0.9 μU/ml) p < 0.01 and p < 0.001 respectively (Fig. 4A).

Furthermore, at day 14 the expression of PDX-1, insulin and Glut-2 in undifferentiated and differentiated hAFSCs and hDPSCs was evaluated by immunofluorescence assay. The results showed that differentiated hAFSCs and hDPSCs after 14 days of culture were intensely labelled by anti-PDX-1, anti-insulin and anti-Glut-2 Abs (Fig. 4B). Furthermore, undifferentiated hDPSCs express PDX-1 showing a weak staining for anti-PDX-1 and anti-Glut-2 Abs, and are not labelled by anti-insulin Ab. The same results were found in undifferentiated hAFSCs (data not shown).

To confirm immunofluorescence data, the presence of PDX-1 and Glut-2 was evaluated by Western blotting (WB) in hAFSCs and hDPSCs at three different times of differentiation (7, 14 and 21 days). Densitometric analysis was carried out on the WB bands to obtain a semi-quantitative analysis. In differentiating culture, both cell types expressed PDX-1 and Glut-2 with the immunoreactive bands corresponding to 31 kDa and 62 kDa respectively (Fig. 4C). Particularly, densitometric analysis revealed that in undifferentiated cells, the protein bands corresponding to PDX-1 and Glut-2 were weakly expressed, while in differentiating hAFSCs and hDPSCs the expression of PDX-1 and Glut-2 was significantly increased (p < 0.001).

3.4. Dithizone staining

After 21 days of induction islet-like structures were stained by DTZ. Particularly, differentiated hAFSCs and hDPSCs cultures showed the typical “crimson red” staining. Undifferentiated cells were not stained by DTZ (Fig. 4D).

3.5. Effect of glucose on PDX-1 translocation and insulin secretion

In order to investigate the intracellular localization of PDX-1 and the expression of insulin in response to the changes of glucose concentration, islet-like structures formed by hAFSCs and hDPSCs, at day 21, were incubated in media at two different glucose concentrations: 5.5 mM and 25.0 mM. Immunofluorescence assay revealed that in hAFSCs and hDPSCs maintained in 5.5 mM glucose PDX-1 was mainly localized in the cytoplasm of the cells forming the islets.
(Fig. 5A,C; G,I). Under the same condition cells were very weakly stained by anti-insulin Ab (Fig. 5B,C; H,I).

After 3 h of incubation in high glucose concentration (25.0 mM), PDX-1 translocated from cytosol to nucleus. As a matter of fact, as showed in Fig. 5D and J the nuclei of both hAFSCs and hDPSCs were strongly stained by anti-PDX-1 Ab. Under these conditions the intracellular expression of insulin increased (Fig. 5E, F; K,I).

Moreover, in order to confirm whether the amount of insulin secreted in the medium increased in response to glucose stimulation, ELISA test was performed on media from hAFSCs and hDPSCs maintained in the two glucose concentrations (5.5 mM or 25.0 mM). Particularly, as shown in Fig. 5M and N, the insulin release values in both hAFSCs and hDPSCs were low at 5.5 mM glucose. Insulin values increased in a significant manner (46.2 ± 4.1 μU/ml in hAFSCs and 47.6 ± 11.3 μU/ml in hDPSCs) when cells were maintained in 25.0 mM glucose for 3 h. On the other hand, insulin concentration in media from undifferentiated hAFSCs and hDPSCs was very low under both glucose concentrations.

In order to verify whether the differentiation protocol used in this study influenced cell viability, Poly(ADP-ribose) polymerses (PARP) expression was evaluated in islet-like structures formed by both cell types. Fig. 5O shows WB analysis of PARP in hAFSCs and hDPSCs maintained in 5.5 mM or 25.0 mM glucose. Densitometric analysis revealed that the native form of PARP was clearly expressed while the cleaved form (cPARP), involved in apoptotic process, was undetectable. Conversely, positive control (etoposide treated HL60) clearly showed the band corresponding to cPARP, therefore demonstrating that cell death did not occur in hAFSCs and hDPSCs samples.

4. Discussion

The aim of this study is to explore the potential of hAFSCs and hDPSCs as a source of insulin-producing cells. Particularly we developed a simple multistep method, through a non-viral
Fig. 4. Characterization of hAFSCs and hDPSCs β-cell differentiation. A: Insulin release in the medium from 0 to 21 days differentiating cultures, detected by a specific ELISA assay (ANOVA followed by Dunnett’s test: *p < 0.05, **p < 0.01, ***p < 0.001, vs 0 days). B: Immunofluorescence staining of 14 days differentiating hAFSCs and hDPSCs by DAPI (blue), anti-PDX-1 (green), anti-Insulin (red) and anti-Glut-2 (green) Abs. Undifferentiated hDPSCs used as control show a scarcely detectable signal from anti-Insulin Ab. The same result was obtained for hAFSCs (not shown). Bar: 10 μm. C: Western blot analysis of PDX-1 and Glut-2 expression in β-cell differentiating hAFSCs and hDPSCs. Actin bands were presented as control of the protein loading. Densitometric analysis is reported in the bottom (ANOVA followed by Dunnett’s test: ***p < 0.001 diff hAFSCs vs undiff hAFSCs; **p < 0.001 diff hDPSCs vs undiff hDPSCs). D: Phase contrast images of thioflavine staining (crimson red) of islet-like structures formed by hAFSCs and hDPSCs as indicated.

degomeric reprogramming approach, to direct the differentiation of these two stem cell types as a source of glucose-regulated insulin-producing cells. In the first step we used a medium that included trans RA and BME. As demonstrated by Micallef et al., trans-RA promotes expression of the endogenous PDX-1 gene and therefore provides a reliable marker of cells within the pancreatic endoderm differentiation pathway [22]. In the second step the low glucose medium was replaced with the medium at high glucose concentration and supplemented with NA, zinc and selenium. It is well known that high-glucose condition is a crucial factor for stem cells differentiation into insulin-producing cells [23]. Zinc is an essential micronutrient required for insulin synthesis, secretion, signalling and packaging [24]. An integral part of insulin forms crystals for 2-Zn-insulin hexamer, as well as free ionized zinc in the extragranular space that acts as a reservoir for granular zinc [24–26]. Moreover, selenium stimulates pancreatic β-cell gene expression and enhances islet function [27]. In the second step NA was also used. NA is a PARP inhibitor that can induce islet formation from pancreatic progenitor cells, trans-differentiation and maturation of liver stem cells into insulin-producing cells [28,29]. NA is able to induce the differentiation and increase β-cell mass in cultured human foetal pancreatic cells [28], as well as to protect cells from prolonged exposure to large amounts of glucose [29].

Our results are in accordance to these findings. Particularly, after 7 days of differentiation hAFSCs and hDPSCs morphology changed rapidly by forming islet-like structures after 14 and 21 days of induction. Moreover, after just one week of differentiation the expression of PDX-1 and Glut-2 in hAFSCs and hDPSCs as well as the secretion of insulin in the media was observed. As a matter of fact, the transcription factor PDX-1 is the first molecular marker that temporally correlates with the pancreatic commitment and plays a critical role in pancreas development, β-cells differentiation and maintenance of β-cells function [5,30]. The expression of PDX-1 as well the expression of Glut-2 was maintained and temporally increased indicating that both cell types were committed to β-cells differentiation and improved glucose receptor expression. Besides, also insulin concentrations increased throughout the time of differentiation, although both cell types were alternatively cultured at high glucose and low glucose concentrations. No cellular death was observed according to the possible mechanisms that explain the protective effects of NA. Therefore, PARP inhibitors prevent β-cell death, induce and maintain insulin secretion [31]. The main role of pancreatic β-cells is the secretion of insulin in response to glucose and although we did not transplant the insulin-producing cells into experimental diabetic animals, we then studied whether or not insulin-producing hAFSCs and hDPSCs were able to respond to the change of glucose concentration in vitro, by simulating the physiological stimulus. Particularly, our results suggest that both differentiated hAFSCs and hDPSCs are able to secrete insulin in response to a glucose change thus maintaining their function. These data are supported by the nuclear translocation of PDX-1 after high glucose stimulation. Particularly, under low glucose culture, PDX-1
Fig. 5. Effect of glucose on PDX-1 translocation and insulin secretion. A–L: Immuno-staining by DAPI (blue), anti-PDX-1 (green) and anti-Insulin (red) Abs of islet-like structures formed by hAFSCs (A–F) and hDPSCs (G–L), exposed to 5.5 mM (A–C; G–I) and 25.0 mM (D–F; J–L) glucose. M–N: Insulin release in the medium from glucose exposed islet-like structures formed by hAFSCs (M) and hDPSCs (N), detected by a specific ELISA assay (paired t-test: *p < 0.05, 25.0 mM glucose vs 5.5 mM glucose). O: Western blot analysis of PARP in hAFSCs and hDPSCs cultured in 5.5 mM and 25.0 mM glucose. HL60, treated with etoposide, were loaded as positive control of the presence of cleaved PARP (cPARP). Actin bands were presented as control of the protein loading. Densitometric analysis is reported in the right side: no significant differences were observed in c-PARP levels comparing glucose treated cells vs controls (ANOVA followed by Dunnett’s test).
was mainly localized into the cytoplasm in both hAFSCs and hDPSCs, while in response to high glucose stimulation translocated to the nucleus. Previous studies indicated that nuclear translocation of PDX-1 occurs as a consequence of glucose concentration changes [32,33]. In the nucleus PDX-1 binds to the insulin gene [34,35] thus leading to insulin gene transcription [36]. Moreover, nuclear PDX-1 expression prevents deleterious effect of glucose and consequently preserves survival and function of β-cells [33]. These observations represent a very important consideration for the future islets transplantation programme by using hAFSCs and hDPSCs. Even though other stem cell types, such as adipose derived stem cells and biliary tree stem cells [9,37], appear appropriate for this purpose, hAFSCs and hDPSCs present the advantage to be easily collected from routine medical practice and to be rapidly expanded in culture. Moreover the possibility to culture hDPSCs in human serum containing media, maintaining an higher proliferative potential, represents a further advantage for the application to human cell therapy [18].

In conclusion, the present study confirms the potential and the ability of the two stem cell types, isolated from human amniotic fluid and dental pulp, to differentiate into insulin-producing cells. Furthermore, no substantial differences have been observed between the two stem cell types along the differentiation pathways, thus offering another non-pancreatic, low-invasive source of cells for islet regeneration and a possible new therapeutic target for the treatment of diabetes mellitus.

Conflict of interest statement
No conflicts of interest exist.

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