Roles in the propagation of pancreatic β cells. Earlier studies have shown the researcher to produce β cells from other cell types. Almost all β cells are destroyed in patients with established T1DM, stimulating tissue has been proposed as the best strategy to treat diabetes. Almost the availability of pancreas donors. Thus, β cell regeneration from auto be disregarded. Moreover, transplantation on a large scale is limited by T1DM [4,5]. However, the side-effects of immunosuppressants cannot limitation of insulin therapy has been well documented [2]. Therefore, immunological destruction of insulin-secreting pancreatic β cells [1]. The diabetes. T1DM is characterized by absolute insulin deficiency due to a path to β cell manipulation would benefit the efficient differentiation of ADSCs into pancreatic β cells. Our technology could provide a path to β cell differentiation and novel cell replacement-based therapies for T1DM.

Abstract

Background: Mesenchymal stem cells (MSCs), including adipose tissue-derived mesenchymal stem cells (ADSCs), are multipotent and can differentiate into various cell types, including pancreatic β cells. Therefore, ADSCs present a potential cell source for the treatment of type 1 diabetes mellitus (T1DM). However, current in vitro protocols are insufficient to induce fully matured insulin-producing β cells. In this study, we assessed the effectiveness of overexpression of BETα2 (NeuroD1), a member of the basic helix-loop-helix transcription factor family, with murine insulinoma cell line-derived conditioned medium (MIN6-CM) to improve the differentiation capacity of ADSCs into insulin-producing cells.

Method: Murine ADSCs were isolated from C57BL/6 mice, transduced with several transcriptional factors (TFs), and stable transfectants were established. MIN6-CM was prepared. Syngeneic recipient mice were rendered diabetic by a single injection of streptozotocin, and differentiated cells were transplanted under the kidney capsule of recipient mice. Next, blood glucose levels were monitored.

Results: CM alone was sufficient to induce insulin mRNA expression in vitro. However, other TFs were not detected. ADSCs cultured with MIN6-CM induced insulin expressions in vitro, but other β cell-related TFs were been detected. However, BETα2 transduction in MIN6-CM resulted in robust expression of multiple β cell phenotypic markers. Moreover, insulin content analysis revealed insulin protein expression in vitro. Furthermore, in vivo transplant studies revealed the effectiveness of the simultaneous use of BETα2 transduction with the CM.

Conclusion: These results suggest that the balance of cytokines and growth factors in addition to gene manipulation would benefit the efficient differentiation of ADSCs into pancreatic β cells. Our technology could provide a path to β cell manipulation and novel cell replacement-based therapies for T1DM.

Keywords: Adipose tissue-derived stem cell (ADSC); BETα2 (NeuroD1); Conditioned medium; Transcription factors; β cells
Abbreviations: ADSC: Adipose Tissue-derived Mesenchymal Stem Cell; CM: Conditioned Medium; iPS: Induced Pluripotent Stem

Introduction

Type 1 diabetes mellitus (T1DM) typically manifests in childhood and has been estimated to account for 5–10% of all diagnosed cases of diabetes. T1DM is characterized by absolute insulin deficiency due to immunological destruction of insulin-secreting pancreatic β cells [1]. The limitation of insulin therapy has been well documented [2]. Therefore, β cell replacement therapy is required for treatment of such patients [3]. Pancreas or islet transplantations are viable treatment options for T1DM [4,5]. However, the side-effects of immunosuppressants cannot be disregarded. Moreover, transplantation on a large scale is limited by the availability of pancreas donors. Thus, β cell regeneration from auto tissue has been proposed as the best strategy to treat diabetes. Almost all β cells are destroyed in patients with established T1DM, stimulating the researcher to produce β cells from other cell types.

Cellular plasticity has been extensively investigated for possible roles in the propagation of pancreatic β cells. Earlier studies have shown that ectopic expression of pancreatic and duodenal homeobox-1 (pdx1) is sufficient to induce expression of insulin in murine liver cells [6]. More recently, since the introduction of induced pluripotent stem cells (iPS), researchers are making a concerted effort again to demonstrate

*Corresponding authors: Hiroaki Nagano, MD, PhD, Associate Professor, Department of Surgery, Osaka University, Graduate School of Medicine, Suita, Yamadaoka 2-2, Osaka 565-0871, Japan, Tel: +81-(0)-6-6879-3251; Fax: +81-(0)-6-6879-3259; E-mail: h-nagano@gensu.med.osaka-u.ac.jp

Received July 10, 2014; Accepted July 26, 2014; Published July 28, 2014

Citation: Kawamoto K, Yabe S, Konno M, Ishii H, Nishida N, et al. (2014) Murine Insulinoma Cell-Conditioned Medium with BETα2/NeuroD1 Transduction Efficiently Induces the Differentiation of Adipose-Derived Mesenchymal Stem Cells into β-Like Cells both In Vitro and In Vivo. J Stem Cell Res Ther 4: 221. doi:10.4172/2157-7633.1000221

Copyright: © 2014 Kawamoto K, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
the plasticity of terminally differentiated cells [7]. In the case of pancreatic cell reprogramming, Melton et al demonstrated that acinar cells could be reprogrammed into β cells using a specific combination of three transcription factors (TFs), namely Pax1, neurogenin 3 (Ngn3), and v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) [8]. Furthermore, glucagon-producing pancreatic α cells have also been shown to be reprogrammable into β cells [9,10]. These results demonstrated that various cell types present potential cell sources for the treatment of T1DM. However, in vivo reprogramming strategies have several limitations, such as the risk of viral transmission and low reprogramming efficiency for clinical use.

Stem cells, such as embryonic stem cells, as iPSCs, present another attractive source for tissue engineering because they have the capability of self-renewal. Originally, embryonic stem cells held promise as a source of renewable β cells, but their potential has proven more difficult than expected. Alternatively, pancreatic progenitor cells present as possible candidates [11]. Multipotent mesenchymal stem cells (MSCs), which represent a nonhematopoietic cell population, can also differentiate into mesenchymal tissues (i.e., bone, cartilage, or fat) [12]. MSCs were first isolated from bone marrow and later from non-marrow tissues, including umbilical cord blood and adipose tissue, and are clinically applicable because of easy accessibility and large scale preparation. Thus, adipose tissue-derived mesenchymal stem cells (ADSCs) have been also explored as a possible source of pancreatic β cells.

During vertebrate embryogenesis, the development of pancreas is regulated by the sequential expression of a molecular network of TFs [13]. It is generally accepted that several defined factors are needed in the differentiation of insulin-producing cells [13]. Among them, Pax1 is thought to be a key transcriptional regulator of endocrine, acinar, and ductal cell development. PDX-1-deficient mice die rapidly after birth due to pancreatic insufficiency [14]. Previously, our research group demonstrated that Pax1-expressed ADSCs can mature in vivo [15]. The combined expression of Pdx1 and MafA with either Ngn3 or NeuroD are required for both exocrine cell reprogramming and staging differentiation [16]. However, simple transduction of several TFs could not induce β cell maturation. NeuroD1 is a member of the NeuroD family and binds to the E element of the insulin gene [17] to modulate the expression of genes, such as SUR1, which forms K+ channels with Kir6.2 to regulate insulin secretion [18]. The BETA2/NeuroD protein, a class B bHLH TF, has been cloned as a transcriptional activator of the insulin gene [4] and neurogenic factor in Xenopus embryos [2].

Several studies have already found that MSCs have an immunomodulatory effect by both contact-dependent and -independent mechanisms [19-24], and they secrete or release immunomodulatory effect by both contact-dependent and -independent mechanisms [25]. We hypothesized that the cocktail of cytokine and growth factors produced to reduce the toxic effects of ischemia reperfusion injury [25]. We hypothesized that the cocktail of cytokine and growth factors produced to reduce the toxic effects of ischemia reperfusion injury [25]. We hypothesized that the cocktail of cytokine and growth factors produced to reduce the toxic effects of ischemia reperfusion injury [25].

For mRNA detection, total RNA from both pre- and post-treated cells were extracted in TRIzol Reagent (Invitrogen), and cDNA was amplified by qRT-PCR. β-actin was used as an internal control for normalization. All reactions were performed along with negative controls. For immunocytochemical analysis, cells were plated in a 60-mm dish, washed twice with D-PBS, and fixed with 4% paraformaldehyde for 20 min at room temperature. Then, the dishes were washed three times with D-PBS and treated with 0.5% Triton X-100 in PBS for 15 min. The cells were treated with 3% BSA–PBS for 60 min and stained with C-peptide (1:400 dilution; Cell Signaling Technology #4593, Danvers, MA, USA) for 16 h at 4°C, and then treated with a secondary antibody (AlexaFluor 647) for 60 min at room temperature. Nuclei were counter-stained with 4′,6-diamidino-2-phenylindole (DAPI).
Insulin content measurements and glucose-stimulated insulin release (GSIS)

For insulin content and GSIS analysis, cells were seeded in a 6-well plate, cultured for 16 h, and then placed for 30 min in medium containing 3 mM glucose (Lo glucose), which was then replaced with a media containing the various constituents indicated and incubated again for an additional 60 min. The supernatant was frozen for later use. The cells were then lysed with acid ethanol at 4°C for 16 h. Cell lysates were collected and stored at -80°C. Insulin levels were then determined in both GSIS and intracellular contents using the ultra-sensitive "PLUS" mouse insulin ELISA kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan). TMB (3,3',5,5'-tetramethylbenzidine) solution was added to each well, and the plates were then incubated for
40 min, until the reaction was terminated by the addition of an equal volume of stopping solution; optical density was determined at 450/630 nm (PerkinElmer, Waltham, MA, USA).

FACS analysis

Cells were treated with Accutase reagent (EMD Millipore, Billerica, MA, USA) to dissociate the cells, which were then resuspended in staining media [DPBS supplemented with FBS (1%) and EDTA (2 mM)]. Next, the cells were stained with fluorescein isothiocyanate-conjugated anti-mouse CD71 (Clone C2; BD Pharmingen, Franklin Lakes, NJ, USA), phycoerythrin-conjugated anti-mouse CD31 (eBioscience, San Diego, CA, USA), APC-anti mouse CD140a (Clone APA5; BioLegend, San Diego, CA, USA), eFluor450 anti-mouse CD45, and allophycocyanin-conjugated anti-mouse CD90.2 for 30 min on ice.

Retroviral preparation

Pdx1 cDNA was obtained as previously described [15]. The open reading frame of the human Pdx1 gene was integrated into the retrovirus vector pMSCV-puro. To prepare the retroviruses, PT67 cells were cultured in DMEM supplemented with 10% FBS and PSM with puromycin. Eugene 6 transfection reagent (Promega, Madison, WI, USA) was diluted with 500 μL of DMEM and incubated for 5 min at room temperature. Plasmid DNA (2.5 μg) was added to the mixture, which was incubated for an additional 15 min at room temperature. Then, the culture media was replaced with fresh DMEM supplemented 10% FBS and the DNA/Eugene 6 mixture was added dropwise onto the HEK-293Ta cells. The medium was replaced after 24 h. After an additional 48 h, virus-containing supernatants, derived from the HEK-293Ta cultures, were filtered through a 0.22-μL cellulose–acetate filter additional 48 h, virus-containing supernatants, derived from the HEK-293Ta cells. The medium was replaced after 24 h. After an additional 48 h, virus-containing supernatants, derived from the HEK-293Ta cultures, were filtered through a 0.22-μL cellulose–acetate filter and used.

Sphere formation

To assess the impact of TFs and CMs for the induction or maintenance of β-like cells, the sphere formation protocol was employed. In brief, control or TF-transduced ADSCs were placed in an ultra-low attachment 96-well round bottom plate with hydrogel (Costar 7007) at a density of 1000 cells/well. Colonies were picked up on day 2 and plated on either gelatin-coated or uncoated Petri dishes (Costar 7007) at a density of 1000 cells/well. Colonies were picked up on day 2 and plated on either gelatin-coated or uncoated Petri dishes (Sansyo Co., Ltd., Tokyo, Japan).

Transplantation to diabetic mice

Eight–12-week-old female B6 mice were housed in an air-conditioned environment under a 12-h light–dark cycle. Freshly dissolved STZ (Nacalai Tesque) was used to make a concentration of 10 mg/mL stock solution. Then, the recipient mice were rendered diabetic by a single intraperitoneal injection of 200 mg/kg STZ prior to transplantation. Next, blood glucose levels were monitored every other day and confirmed for hyperglycemia (400 mg/dL). The cells were dissociated with Accutase reagent (EMD Millipore, Billerica, MA, USA) to dissociate the cells, which were then resuspended in staining media [DPBS supplemented with FBS (1%) and EDTA (2 mM)]. Next, the cells were stained with fluorescein isothiocyanate-conjugated anti-mouse CD71 (Clone C2; BD Pharmingen, Franklin Lakes, NJ, USA), phycoerythrin-conjugated anti-mouse CD31 (eBioscience, San Diego, CA, USA), APC-anti mouse CD140a (Clone APA5; BioLegend, San Diego, CA, USA), eFluor450 anti-mouse CD45, and allophycocyanin-conjugated anti-mouse CD90.2 for 30 min on ice.

Statistical analyses

Data are presented as means ± standard errors of the mean of independent experiments. Statistical testing was performed using the Student’s t-test to detect differences between groups. In cases of multiple groups testing, analysis of variance was conducted followed by a posteriori t-test. Differences were considered statistically significant at p<0.05 (*) and <0.001 (**).

Results

CM with transcription factor BETA2-induced efficient differentiation of ADSCs into multiple TF-positive β-like cells

Figures 1A and 1B describe the experimental protocol used in this study. MIN6-CM was prepared as described in the Materials and Methods section. First, we evaluated the morphological changes of ADSCs and their subclones cultured with MIN6-CM. As shown in Figure 1C, the morphological differences were minimal. However, there was a trend of round morphology among the cultured BETA2-ADSCs. When ADSCs were cultured with CM from the human pancreatic carcinoma cell line MIAPaCa-CM, ADSCs displayed massive apoptosis within 9 days (Data not shown). Next, we assessed the impact of PANC-CM. In contrast to MIAPaCa-CM, ADSCs can grow with PANC-CM, with both epithelial-like and neuron-like colonies (Figure 1D). However, ins2 message was not detected in PANC-CM-ADSC compared to MIN6 (Figure 1E). Next, we assessed the effect of MIN6-CM on ADSC. Surprisingly, CM culture for 1 week was sufficient for insulin detection by qRT-PCR (Figure 2A). These results suggest that MIN6-CM would be useful for induction of β cell differentiation. However, other TFs, such as pax6 and kir6.2, were not detected in this group during the overall 5-week culture period. Next, we assessed the differentiation efficiencies of MIN6-CM in combination with several TFs. As presented in Figure 2A, Pdx1-ADSC with CM was not effective to induce multiple TFs. However, BETA2-ADSCs with MIN6-CM resulted in efficient differentiation into insulin-producing cells. As early as culture day 3, both pax6 and kir6.2 were detected, and their expressions were retained. These results clearly demonstrate that BETA2 would be the best partner for CM-mediated differentiation. We further confirmed the effectiveness of this combination by qRT-PCR. Pdx1 overexpression with CM resulted in a synergistic upregulation of insulin messaging (Figure 2B). Furthermore, BETA2-ADSC with CM resulted in more effective induced insulin expression.

We next used nicotinamide for further maturation of β-like cells. As shown in Figure 2A, nicotinamide did not further enhance mRNA levels of Pax6, Kir6.2, or Ins2. Thus, nicotinamide addition was not very effective for further maturation of β-like cells. Then, we selected nicotinamide-free culture media for further experiments. For Pdx1-ADSC without MIN6-CM, insulin expression was not detected (control group), which was in accordance with the results of our previous report [12]. Next, we evaluated the MEF-feeder effect. As shown in Figure 2C, the MEF feeder impaired differentiation of β-like cells. These results suggest that MEF seemed to not partner well with MIN6-CM. An interesting observation was that no passaged group produced functional insulin [27]. Immunocytochemical analysis showed that C-peptide was undetectable (Figure 3A). Neither Pdx1-ADSC nor PN-ADSC with MIN6-CM expressed detectable insulin levels by immunocytochemical analysis (Data not shown). However, there were clearly significant differences in insulin content between the controls parental ADSCs and MIN6-CM-cultured BETA2-ADSCs (Figure 3B) under both low and high glucose conditions (p<0.05).
Next, we measured glucose-dependent insulin release by ADSCs in vitro. As shown in Figure 3C, MIN6-CM-cultured BETA2-ADSCs did not release insulin in response to a step-wise increase of glucose to 25 mM (high glucose). These results showed that insulin was detectable by insulin content, but these cells did not release insulin in response to physiologically relevant glucose concentrations in vitro.

Next, we analyzed the expression of MSC markers, including CD71, CD90.2, CD140a, and Sca-1. As shown in Figure 3D, the expression...
level of CD71, CD90.2, and CD140a were downregulated, whereas that of Sca-1 was upregulated. These results suggest that the MIN6-CM culture induced downregulation of mesenchymal markers. In contrast, Sca-1 seemed to be a maturation marker in our protocol.

**Transplantation to in vivo STZ-diabetic mice**

We investigated whether the BETA2-ADSCs with CM exhibited relevant *in vivo* function for the treatment of diabetes. Thus, we employed...
a STZ-induced diabetic mouse model through the administration of 200 mg/kg STZ into the peritoneum of 8–12-week-old female B6 (Figure 4A). We attempted to determine whether BETA2-ADSCs with CM were capable of controlling blood glucose levels in diabetic mice. First, we assessed the diabetogenic effect of STZ. As shown in Figure 4C, STZ-treated mice showed elevated blood glucose levels. Next, we evaluated whether the transplanted cells could control STZ-induced diabetes of recipient mice. As shown in Figure 4C, the recipient mice showed better blood glucose maintenance. Moreover, the beneficial effect of CM-cultured BETA2-ADSC transplantation also demonstrated a trend of better survival compared with the control group. There was no overt tumor in sacrifice mice. Thus, these transplanted ADSCs apparently did not participate in tumor formation.

**Discussion**

Here we report a novel combination of two techniques (BETA2 transduction and MIN6-CM culture) with a great potential to refine the β-like cell differentiation protocol. The balance of cytokines and growth factors in CM plays important roles in both efficient differentiation and functional maintenance of certain cell types. In this study, we found that ADSCs could express detectable insulin levels by qRT-PCR using parental MIN6-CM culture in vitro. Compared to our previous experience with Pdx1-ADSCs without CM, CM treatment only was enough to facilitate insulin expression in vitro. These results suggest that CM would be a useful induction agent for β cell differentiation. Furthermore, the forced expression of the BETA2 gene in murine ADSCs in the presence of MIN6-CM resulted in robust differentiation of these cells into insulin-producing cells. Using this novel technology, multiple TFs, including pax6 and kir6.2, were detected as early as culture day 3. Moreover, insulin expression of BETA2-ADSC with MIN6-CM was confirmed by insulin content analysis. Furthermore, these cells could control hyperglycemia of STZ-induced diabetic mice. These results clearly demonstrated that this combination is a promising candidate for refinement of current β cell differentiation protocols. Of note, these effects of CM were observed only in the parenteral MIN6 cell line. When the subclones MIN6-m9 and MIN6-m14 were used, this phenomenon was not observed (data not shown). These results suggest that the selection of effective CM-producing cell offers potentially important advancements in β cell replacement therapy.

In previous studies, we reported that Pdx1-transfected ADSCs with standard bovine serum-based medium lacked expression of pancreatic marker genes, including the two non-allelic insulin genes insulin 1 and insulin 2 [15]. These results suggested that the transduction of a single TF would be insufficient for in vitro maturation of the insulin gene. However, Pdx1-ADSCs can produce insulin after in vivo maturation for 30 days when administered systemically [15]. These results suggest that in vivo maturation may contribute for further β cell maturation, at least, in part, in our previous model. In the present study, we confirmed that Pdx1-ADSCs and BETA2-ADSCs lack ins2 expression (Figure 2). Also, the same can be said about the results of the present study from the point of the importance of in vivo maturation because BETA2-ADSC with MIN6-CM resulting in insulin protein was detected only by the insulin content assay. It is noteworthy that there was a trend in the BETA2-ADSCs with CM in lower glucose levels compared with Pdx1-ADSCs (historical control). In our previous study, we used intravenous tail vein cell transfer. In the present study, we employed subrenal capsular transplant, which is a more efficient site for transplantation of β-like cells. Although a proper mechanism for the effectiveness of MIN6-CM has not been clarified at present, the balance of cytokine and growth factor content may be critical for efficient β cell differentiation. As expected, BETA2-ADSCs with MIN6-CM lost expression of the mesenchymal markers CD71, CD90, and CD140a. Recently, we
reported that CD90-Hi ADSCs were reprogrammed more efficiently compared with CD90-Lo ADSCs [28]. The importance of Sca-1 has already been suggested in ductal progenitor theory [29]. In this study, Sca-1 expression was upregulated, perhaps because Sca-1 is a marker of stem and pancreatic progenitor cells. Therefore, the upregulation of Sca-1 may reflect the upregulation of progenitor properties.

A possible limitation of this study is that normoglycemia was
Atkinson MA, Maclaren NK (1994) The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 331: 1428-1436. [PubMed]
Bluestone JA, Herold K, Eisenbarth G (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 464: 1293-1300. [PubMed]
Limbert C, Fiorina P, Shapiro AM, Ricordi C, White SA, Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from adult pancreatic cells using a non-viral method would present a powerful option for future clinical use. Moreover, chemicals that induce late-stage β-cell differentiation have been reported [34]. Therefore, these technologies, along with our method, will further advance functional β-cell induction technology.

In summary, we successfully treated STZ-induced diabetic mice by ADSC transplantation, suggesting that this technology may open new avenues toward clinical applications of ADSCs for T1DM treatment.

References

1. Akkinson MA, Maclaren NK (1994) The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 331: 1428-1436. [PubMed]
2. Bluestone JA, Herold K, Eisenbarth G (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 464: 1293-1300. [PubMed]
3. Limbert C, Päht G, Jakob F, Seuffert J (2008) Beta-cell replacement and regeneration: Strategies of cell-based therapy for type 1 diabetes mellitus. Diabetes Res Clin Pract 79: 389-399. [PubMed]
4. White SA, Shaw JA, Sutherland DE (2009) Pancreas transplantation. Lancet 373: 1808-1817. [PubMed]
5. Fiorina P, Shapiro AM, Ricordi C, Secchi A (2008) The clinical impact of islet transplantation. Am J Transplant 8: 1990-1997. [PubMed]
6. Ferber S, Halkin A, Cohen H, Ber I, Einav Y, et al. (2000) Pancreatic and duodenal homeobox 1 gene induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 6: 568-572. [PubMed]
7. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 683-696. [PubMed]
8. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to b-cells. Nature 455: 627-632. [PubMed]
9. Colombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, et al. (2009) The ectopic expression of Pdx4 in the mouse pancreas converts progenitor cells into alpha and subsequently β cells. Cell 138: 449-462. [PubMed]
10. Thornell N, Nöpfe V, Avril I, Kohno K, Desgraz R, et al. (2010) Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 464: 1149-1154. [PubMed]
11. Xu X, D’Hoker J, Stange G, Bonné S, De Leu N, et al. (2008) B cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132: 197-207. [PubMed]
12. Konno M, Hamabe A, Hasegawa S, Ogawa H, Fukusumi T, et al. (2013) Adipose-derived mesenchymal stem cells and regenerative medicine. Dev Growth Differ 55: 309-318. [PubMed]
13. Arda HE, Benitez CM, Kim SK (2013) Gene regulatory networks governing pancreatic development. Dev Cell 25: 5-13. [PubMed]
14. Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371: 606-609. [PubMed]
15. Kajiyama H, Hamazaki TS, Tokuhara M, Masui S, Okabayashi K, et al. (2010) Pdx1-transfected adipose tissue-derived stem cells differentiate into insulin-producing cells in vivo and reduce hyperglycemia in diabetic mice. Int J Dev Biol 54: 699-705. [PubMed]
16. Xu H, Tsang KS, Chan JC, Yuan P, Fan R, et al. (2013) The combined expression of Pdx1 and MafA with either Ngn3 or NeuroD improves the differentiation efficiency of mouse embryonic stem cells into insulin-producing cells. Cell Transplant 22: 147-158. [PubMed]
17. Naya FJ, Stellrecht CMM, Tsai MJ (1995) Tissue-specific regulation of the insulin promoter by a classic helix-loop-helix transcription factor. Genes Dev 9: 1009-1019. [PubMed]
18. Kim JW, Seghers V, Cho JH, Kang Y, Kim S, et al. (2002) Transactivation of the mouse sufonylurea receptor 1 gene by B2/NeuroD. Mol Endocrinol 16: 1097-1107. [PubMed]
19. Yeung TY, Seeberger KL, Kin T, Adesida A, Jomha N, et al. (2012) Human mesenchymal stem cells protect human islets from pro-inflammatory cytokines. PLoS One 7: e38189. [PubMed]
20. Tan J, Wu W, Xu X, Liao L, Zheng F, et al. (2012) Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. JAMA 307: 1169-1177. [PubMed]
21. Blanc KL, Mougiakakos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 12: 383-396. [PubMed]
22. Peng Y, Ke M, Xu L, Liu L, Chen X, et al. (2013) Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. Transplantation 95: 161-168. [PubMed]
23. Reinders ME, de Fijter JW, Roelofs H, Bajema IM, de Vries DK, et al. (2013) Autologous bone marrow-derived mesenchymal stromal cells for the treatment of alloraft rejection after renal transplantation: results of a phase I study. Stem Cells Transl Med 2: 107-111. [PubMed]
24. González MA, Gonzalez-Rey E, Rico L, Büsscher D, Delgado M (2009) Adipose-derived mesenchymal stem cells overexpress CD105, CD73 and PECAM1 compared to bone marrow-derived mesenchymal stromal cells. PLoS One 4: e5093. [PubMed]
25. Du Z, Wei C, Cheng K, Han B, Yan J, et al. (2013) Mesenchymal stem cell-conditioned medium reduces liver injury and enhances regeneration in reduced-size rat liver transplantation. J Surg Res 183: 907-915. [PubMed]
26. Ohmura Y, Tanemura M, Kawaguchi N, Machida T, Tanida T, et al. (2010) Combined transplantation of pancreatic islets and adipose tissue-derived stem cells enhances the survival and insulin function of islet grafts in diabetic mice. Transplantation 90: 1366-1373. [PubMed]
27. Hansson M, Tonnion A, Frandsen U (2004) Artifactual insulin release from differentiated embryonic stem cells. Diabetes 53: 2603-2609. [PubMed]
28. Kawamoto K, Konno M, Nagano H, Nishikawa S, Tomimaru Y, et al. (2013) CD90- (Thy-1-) high selection enhances reprogramming capacity of murine adipose-derived mesenchymal stem cells. Dis Markers 35: 573-579. [PubMed]
29. Rovira M, Scott SG, Liss AS, Jensen J, Thayer SP, et al. (2010) Isolation and characterization of centroacinar/terminal ductal progenitor cells in adult mouse pancreas. PNAS 107: 75-80. [PubMed]
30. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S (2008) Generation of mouse induced pluripotent stem cells without viral vectors. Science 322: 949-953. [PubMed]
31. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, et al. (2011) Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell 8: 633-638. [PubMed]
32. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, et al. (2011) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8: 376-388. [PubMed]
33. Noguchi H, Bonner-Weir S, Wei FY, Matsushita M, Matsumoto S (2005) BET2/NeuroD protein can be transduced into cells due to an arginine- and lysine-rich sequence. Diabetes 54: 2859-2866. [PubMed]
34. Sakano D, Shiraki N, Ikawa K, Yamaozoe T, Kataoka M, et al. (2014) VMAT2 identified as a regulator of late-stage β-cell differentiation. Nat Chem Biol 10: 141-148. [PubMed]