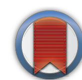
Sci. Bull. (2015) 60(20):1744–1751
DOI 10.1007/s11434-015-0844-6



www.scibull.com
www.springer.com/scp

Review

Life & Medical Sciences

Cell therapy in diabetes: current progress and future prospects

Ying Wang · Tang Hai · Lei Liu · Zhonghua Liu · Qi Zhou

Received: 1 March 2015 / Accepted: 29 May 2015 / Published online: 19 July 2015
© Science China Press and Springer-Verlag Berlin Heidelberg 2015

Abstract Diabetes mellitus, characterized by the impaired metabolism of insulin secretion in β cells, is becoming one of the most prevalent diseases around the world. Recently, cell replacement based on differentiation of various pluripotent stem cells, including embryonic stem cells, induced pluripotent stem cells and multipotent stem cells, such as bone marrow mesenchymal stem cells, adipose-derived stem cells and gnotobiotic porcine skin-derived stem cells, is becoming a promising therapeutic strategy. Cells derived from pancreatic tissues or other tissues that are relevant to β cell differentiation have also been used as cell source. However, in spite of hopeful experimental results, cell therapy in diabetes still confronts certain obstacles, such as purity of cells, functional differentiation of stem cells and possible tumorigenesis, which, in turn, lead to the seeking of new-generation tools, such as xenogenetic materials. In this review, we will summarize the current knowledge and future prospects of cell therapy in diabetes mellitus.

SPECIAL TOPIC: Stem cell, Basis and Application

Electronic supplementary material The online version of this article (doi:10.1007/s11434-015-0844-6) contains supplementary material, which is available to authorized users.

Y. Wang · Z. Liu (✉)
College of Life Sciences, Northeast Agricultural University of China, Harbin 150030, China
e-mail: liu086@126.com

Y. Wang · T. Hai · L. Liu · Q. Zhou (✉)
State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
e-mail: zhouqi@ioz.ac.cn

Keywords Diabetes · Cell therapy · Signaling pathway · Xenotransplantation

1 Introduction

According to the data of International Diabetes Federation (IDF), in 2011 there were 366 million people with diabetes, and the number is expected to rise to 552 million by 2030 [1]. The report emphasized that diabetes will become a global epidemic disease as influenza. Diabetes is a blood sugar metabolic disorder owning to lack of insulin secretion in β cells and increases risk of several long-term chronic complications, including cardiovascular disease, stroke, and micro-vascular damage to retina, kidney and nerves. Two major types of diabetes were defined based on their pathogenic mechanisms. Type I diabetes is caused by an autoimmune system dysfunction, which results in destruction of pancreatic β cells and then insulin deficiency. Unlike type I, type II diabetes is more complicated by relating to genetic and environmental factors, which results in loss of glucose homeostasis due to impaired insulin secretion of β cells in response to elevated blood sugar. As both types are closely related to β cells, cell or tissue replacement therapy appears to be the most effective way of curing diabetes.

During the past 20 years, islet transplantation has been proved to be an efficient strategy to treat diabetes and relieve the risk of complications. However,insufficient supply of immune-compatible donors becomes the major obstacle suppressing its widespread usage. Thus, exploration of new therapeutic strategy, such as cell therapy was needed. Cell derivations used in the replacement study must possess the character of β cells, i.e., producing insulin. Pluripotent stem cells (PSCs), such as embryonic

stem (ES) cells and induced pluripotent stem (iPS) cells, have the potential to differentiate into all kinds of cells in the body, including β-like cells and their progenitors. Multipotent stem cells, such as bone marrow mesenchymal stem cells (BMSCs) [2, 3], adipose-derived stem cells (ADSCs) [4] and gnotobiotic skin-derived stem cells (gSDSCs) [5], can also be reassembled into insulin-producing cells. Moreover, several other pancreatic endocrine cells and non-pancreatic tissue were enhanced to differentiate into β cells by using genetic modification and small chemical compounds. However, certain obstacles lie in the way of bench-to-bedside translation in diabetes cell replacement. Lack of knowledge about β cell differentiation and unsuccessful differentiation of β cells was considered the most important obstacle to break through. As a new strategy, xenogenetic cell therapy is becoming a hot topic in clinical application also. Here, we review the current knowledge about differentiation of β cells from various types of cells, the signaling pathways explored in induction of cell differentiation and the obstacles lies ahead of cell therapy and xenotransplantation studies in diabetes.

2 Derivation of β-like cells from various origins

In the process of embryonic pancreas formation, ventral bud is fused with dorsal bud to form the early pancreas structure. Insulin-producing β cells, derived from progenitor cells arising along the exocrine and ductal tissues [6], form the scattered islets of Langerhans along with other endocrine cells, including glucagon-producing α cells, somatostatin-producing δ cells and pancreatic polypeptide-producing PP cells [7] (Fig. 1).

ES cells, which are derived from inner cell mass (ICM) of blastocyst stage embryos, possess the abilities of self-renewal and differentiating into all types of cells. iPS cells, which are similar to ES cells in terms of developmental pluripotency, can be acquired by ectopic expression of transcription factors. Both ES and iPS cells are powerful research tools that could provide significant evidence in preclinical research [8, 9]. Several studies have been reported to differentiate these pluripotent stem cells into β-like cells in mouse and human (Table S1). According to the studies, strategies were divided into two types. One was to generate pancreatic lineage cells by activating or inhibiting pathways in embryonic bodies (EBs) using a few compounds, and then, following the development of pancreas, the cells were sequentially differentiated into posterior foregut, pancreatic endoderm, pancreatic endocrine progenitors, and insulin-expressed cells [10–12]. Another was to overexpress markers of definitive endoderm (DE) [13–16], the origin of pancreatic, by transgenetic technology, such as Pdx1 [17, 18], Ngn3 [19], Sox17 [20], Pdx1 and Foxa2 [21], Pdx1 and Ngn3 [22], Pdx1 and MafA either with Ngn3 or NeuroD [23, 24], to enhance the efficiency of pancreatic differentiation. To mimic the in vivo structure of pancreatic islet, in recent years, β-like cells with normal function in modulating blood glucose have been produced from human pluripotent stem cells by inducing signaling pathways in 3D biomaterial environment system [25].

Parthenogenetic stem cells (pSCs) can be derived from parthenogenetically activated oocytes and share similar functional and differentiation abilities with ES cells and iPS cells. The HLA-homozygous human pSCs are histocompatible with significant portion of human population and may reduce the risk of immune rejection after transplantation. Trichostatin A (TSA) pretreatment in differentiation process of human pSCs was reported to increase the proportion of definitive endoderm cells [26].

Bone marrow-derived stem cells (BMSCs) [2, 3, 27, 28] and adipose-derived stem cells (ADSCs) [4] are multipotent stem cells that can be the alternative sources for obtaining pancreatic hormone-producing cells through in vitro or in vivo induction. Insulin-secreting ability of differentiated cells could be evaluated through genetic modifications, such as transfecting or infecting segments of Pdx1 [29], Pdx1 and β cellulin [30], IPF1, HLXB9 or FOXA2 [31]. However, some modifications got insulin (INS) gene silenced [32]. In addition, gnotobiotic porcine skin-derived stem cells (gSDSCs) treated with bone morphogenetic protein 4 (BMP-4) were reported to be reprogrammed and subsequently differentiated into insulin-producing cells [5]. It was proved that some cells derived from cord blood (CB) [33], hepatic oval [34, 35], splenocyte [36], labia minora dermis-derived fibroblasts [37] and skin fibroblasts [38] could also contribute to further therapies in diabetes.

Although β cells were generated from preexisting β cells rather than from pluripotent stem cells during postnatal and adult life in vivo [39], lots of evidence has shown that cells derived from pancreatic tissue can also be directly differentiated into β-like cells in vitro, such as pancreas-derived multipotent precursor (PMP) cells [40], conophylline in pancreatic endocrine cells [41], pancreatic-derived pathfinder (PDP) cells [42], islet-enriched fractions (IEFs) [43, 44], pancreatic duct cells [45, 46] and exocrine cells [47]. In addition, during the development of pancreas, Pax4, a common transcription factor between α and β cells, could convert α cells into β cells [48–51].

3 Mimic signaling pathways of pancreas development by soluble factors

Pancreas is induced along the anterior–posterior axis (A–P axis) of DE-derived primitive gut. Epithelial–mesenchymal interaction plays a key role in early pancreatic development.

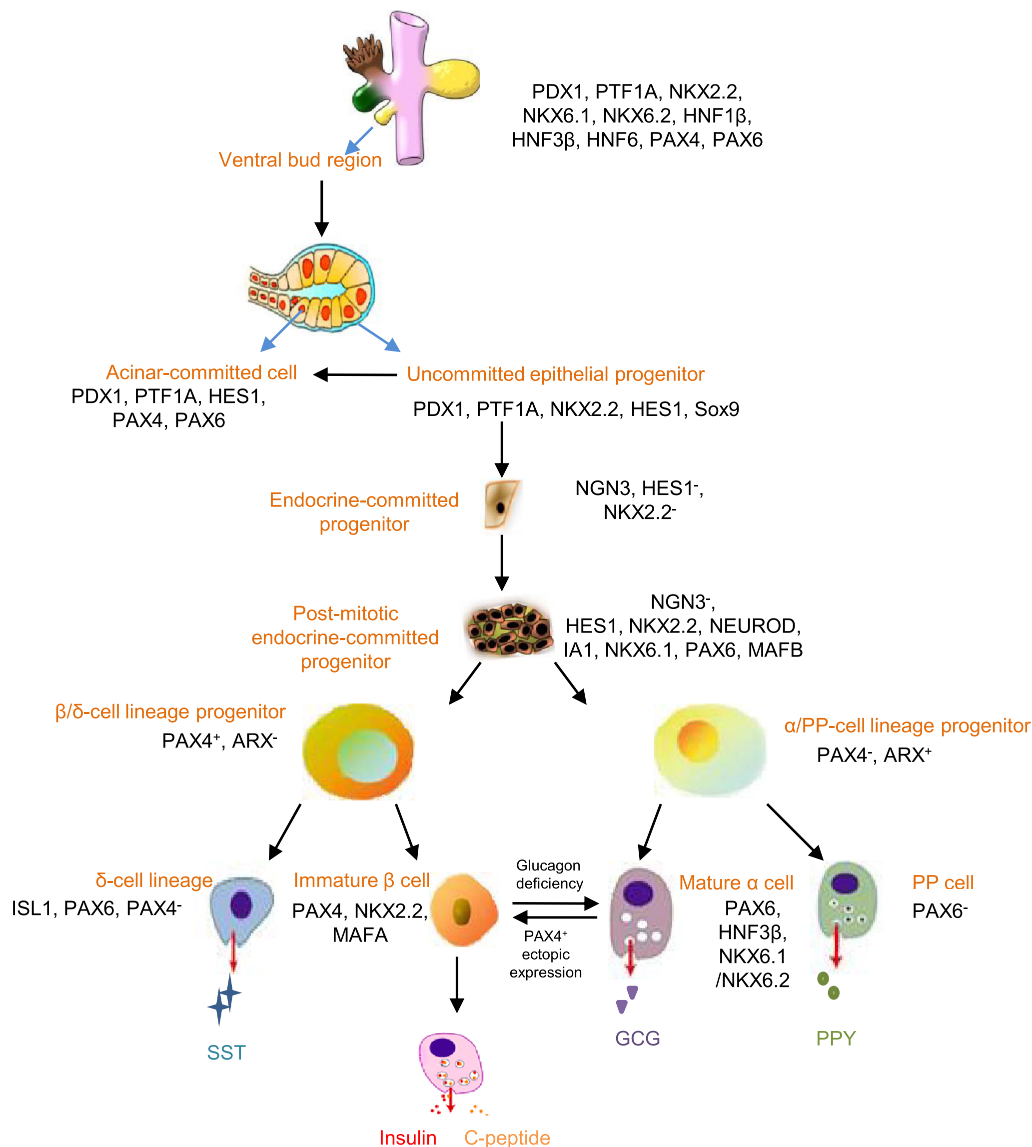


Fig. 1 Schematic representation of pancreatic lineages and their transcription factor

Soluble factors were added in these processes to explore the mechanisms of β cells production and secretion of insulin.

Fibroblast growth factor (FGF) signaling is associated with several signal transduction pathways in early development of pancreas, such as phosphatidylinositol-3 kinase (PI3K) and mitogen-activated protein kinases (MAPKs), ERK1/2. Thus, FGF receptor tyrosine kinase inhibitor SU5402, ERK inhibitor U1026 and PI3K inhibitor LY294002 served as tools to investigate the process of pancreatic transduction from hESC-derived cells. Compared with the PI3K pathway, the MAPK pathway was more necessary for FGF2-mediated induction of PDX1 expression [52].

Nodal signals are activated in the epiblast epithelium, along with other growth factors such as FGF, bone morphogenetic protein (BMP) families and WNT. However, activin A, as a replacement for recombinant Nodal in vitro [53], could induce ESCs to differentiate into definitive endoderm cells, increase insulin secretion in cultured human islets [37] and stimulate differentiation of pancreatic endoderm cells and increase insulin [54]. BMP4 can upregulate genes expressed during the early development of the mouse PSCs as well as DE-specific genes. WNT signaling has been validated in pancreatic specification in the foregut of *Xenopus*. In parallel, the defects of Pdx1-Wnt1 and Pdx1-Wnt5a [55] or Wnt7b [56] transgenic mice have confirmed the suppressing role of WNTs during pancreatic development.

Retinoic acid (RA) is a representative signaling molecule which plays important role in neuroectoderm and mesoderm in vertebrates. It was also reported to participate in regulation of embryonic endoderm differentiation to

enhance insulin expression in β cells. In addition, evidence indicated that signaling of activin A and RA might be specific in pancreatic β cell development and maturation by detecting some specific pancreatic markers [57].

Notch signaling was reported to have an ability of controlling and maintaining progenitors during pancreas development. Notch activation induces expression of Hes1, a basic helix-loop-helix (bHLH) transcription repressor which inhibits Neurogenin3 (Ngn3) and Neuro D, thus leading to activation of insulin and other islet-specific genes [42]. Sim1 temporally responds to Ngn3 in developing pancreas. Thus, Notch signaling inhibition and Ngn3 activation, to some extent, improve the expression of pancreatic hormone, including insulin and somatostatin [19].

EGF, as a super family of growth factors, was also found to be related to expanding efficacy of pancreatic progenitors with PDX1-positive cells [58]. Furthermore, members of the family, especially EGFL7, respond to the signaling pathway of pancreatic development by cross talking with factors secreted by endothelial cells. Overexpression of EGFL7 in vivo could increase the proliferation of pancreatic progenitor and differentiation of endocrine cells in vivo [59].

Constructing blood vessel is essential for all embryonic organogenesis in mammals. Blood vascular endothelium can provide growth factors that induce differentiation of pancreatic endocrine cells. VEGF, a secretion factor from vascular endothelium, was found to anchor the region of growing pancreas [60].

4 Preclinical study: obstacles lie ahead and strategies taken

Diabetes is a disease of metabolic disorder, which forms hyperglycemia caused by insulin resistance and insulin insufficiency. Owing to the enigmatic mechanisms in diabetes, treatment options are complex, with patients starting with oral anti-diabetic agents, mostly soon proceeding to insulin infections. Many of them also take drugs, such as angiotensin-(1–7) [61], that reduce insulin resistance, and some even take insular allograft. Concerning the risks of insular allograft, cell therapy will be safer, which in turn presses the research and development of cell therapy.

Considering the safety of therapies, it is necessary to do some trials in animal for preclinical applications. As mentioned above in the cell derivation, most trials depend on mouse and rat, even human for cell donors (Table 1). For recipients, immune-deficient NOD/SCID mice [62], NOD/SCID/β2M^{null} (8–10 weeks old), adult mice (6–8 weeks old) treated with streptozotocin (STZ) [63] or RIP-CreER; Dicer1$^{LoxP/LoxP}$ mice [66] are made as non-obese diabetic models. Most transplanted sites are renal capsule (direct injection), and some are epididymal fat pad (co-encapsulated in silk with MSCs) [67] or dorsal subcutaneous space (engraftment treated with liraglutide) [68] (implanted with matrigel) [64] or peritoneal cavity [4] (encapsulated on PU-PVP-IPN/Gelfoam disks [65]) or superficial temporal vein. During recent years, biomaterial 3D niches have been used to support the growth of islet and facilitate vascularization [69]. Depending on the different transplanted sites or means, the density of β-like cells for transplantation varied from 5×10^4 to 1×10^7/mice. After transplantation, blood glucose monitoring and immunohistochemistry analysis were taken for evaluating the efficiency. Furthermore, some novel protocols are proved to be able to promote the functional maturation of transplantation; for example, angiotensin II type 2 receptor is critical for the development of pancreatic endocrine lineage [70]. bFGF-treated rat proliferating islet progenitor cells (PIC) in vitro were ameliorated post-transplantation [71].

Considering optimal niche for supporting cell proliferation, xenogeneic transplantation carves another way for cell therapy. Porcine, as an ideal animal tissue donor, has several advantages in physiology, including similar organic structural properties to humans, only one amino acid difference with human insulin and sufficient availability [72]. Most importantly, it has been confirmed that porcine islet could meet the metabolic need of monkey after xenotransplantation [73]. However, porcine islet was prone to be damaged after exposure to human blood, indicating the important role of humoral immunity [74], which makes progress in xenogeneic transplantation slow. To overcome this limitation, human islet-like cell clusters are encapsulated in polytetrafluorethylene devices (TheraCyte) and transplanted into diabetic mice, resulting in robust long-term compatibility and allograft survival [75, 76]. Magnetic resonance technology was used to track and guide intraportal delivery of xenogeneic transplantation, making it possible to inspect the efficiency of islet transplantation [77]. Inhibiting the pathways of immunorejection, particularly the CD154-specific antibody-induced thromboembolic events, could achieve more than 100 days of diabetic reversal in cynomolgus macaques after transplantation with porcine islet without Gal-specific antibody [78]. Controversially, rhesus recipients treated with a CD28–CD154 costimulation blockade regimen achieved sustained insulin independence >140 days after porcine islets transplantation [79]. To reduce the immunorejection, immune-privileged transplanted sites are a better choice. Anterior chamber of eye can support autonomic nerves and blood vessels for a comfort microenvironment for sustaining islets [80]. Currently, immunomodulation with FasL chimeric with streptavidin (SA-FasL) can effectively prevent rejection from autoimmune attack [81]. These treatments will further highlight the allograft therapeutic method.

Table 1 Cell therapy in diabetes

Cell type	Density/animal	Transplantation site	Recipients	Normal/survival time	References
ICAs	1–1.2 × 10^3	Peritoneal cavity	STZ-induced male Swiss albino mice (8–10 weeks old)	Within 2 weeks	[4]
IPCCs	300 islets	Renal capsule of left kidney	STZ-induced CBA mice	>20 days	[11]
ES-PPPs	1–2.5 × 10^6	Epididymal fat pad/dorsal subcutaneous	NOD/SCID mice (8–10 weeks old)	12 weeks	[13]
ESCs-ADE	5–10 × 10^4	Kidney capsule	Adult 129 mice	At least 4 weeks	[15]
Pdx1-ES	2 × 10^6	Left subcapsular renal space	C.B-17/Icr-scid/scid Jcl male mice (10 week old)	2 weeks	[17]
SOX17-hES-derived endoderm	5 × 10^5	Left kidney capsule	SCID mice	Nearly 3 weeks	[20]
Human CB MNCs	10^7	Vein	Newborn NOD/SCID/β_2m^{null} (100 cGy) mice	2 months	[33]
hLMDFs	2 × 10^6	Right renal capsule	STZ-induced Balb/nu female mice (6 weeks old)	Nearly 33 days	[37]
PMPs spheres	~400–500 (mouse) or ~350–400 (human)	Kidney capsule	STZ-induced BalbC mice or NOD/SCID mice	10 weeks	[40]
PDP cells	1.5 × 10^6 male rat or female human	Tail vein	STZ-induced female C57Bl/6 mice	89 days	[42]
IPLCs	1–2 × 10^6	Renal capsule	NOD/SCID mice	6 weeks	[43]
Insulin-producing cells	1 × 10^6	Left renal capsule	STZ-induced 129 male mice (6–8 weeks old)	Nearly 1 week	[55]
BM-MSCs by infection	3 × 10^6	Left kidney capsule	STZ-induced SCID female mice (6–10 week old)	5 weeks	[62]
pMSCs	2.5 × 10^5 pMSCs and islets	Kidney capsule	STZ-induced C57Bl/6 mice	3 weeks	[63]
hES-PE	0.5–1 × 10^7 or 3–7 × 10^6	EFP abdominal cavity or kidney capsule	STZ-induced mice	>100 days	[64]
Islets	1800 islets	Peritoneal cavity	STZ-induced male Balb/c mice (6–8 weeks)	>7 months	[65]

5 Prospects

Major achievements in the isolation, culture and differentiated into β cells prompt hopes that it will one day be possible to replenish β cell mass in patients with diabetes using insulin-producing cells. Despite the progress, many questions remain on cell therapy. First, although there are various ways to derive β cells, the efficiency of targeted differentiation/transdifferentiation is still at a low level. Optimizing the conditions of differentiation/transdifferentiation in vitro to increase the number of insulin-secreting β cells should continue to be explored. Second, the developmental mechanisms of pancreas and pathogenic mechanisms of diabetes are still not clear. Transplanted cells still cannot integrate into in situ organ and reconstruct the new communication as normal. For example, neurons and blood vasculars are necessary for organism integrity and blood glucose adjustment. Furthermore, limited knowledge is known about the different mechanisms of diabetes and whether cells with insulin-secreting ability are attacked or imposed by host environment. Third, teratogenic potential of stem cells and other original materials has not been fully evaluated in cell replacement therapy for diabetes. How to avoid or relieve the risk of tumorigenesis is a very important safety issue in regenerative medicine. Finally, targeted intervention to reverse diabetes is required not only for ameliorating communications between cell and solid organ, but also for eliminating many immune responses for prolonged regimens. Therefore, based on technical optimization and overcoming immune rejection, xenogeneic cell therapy will play a major role in treating diabetes.

Acknowledgments This work was supported by the National Basic Research Program of China (2013CB967102) and the National Natural Science Foundation of China (31201112). We thank Yangjie Lu

from Institute of Zoology, Chinese Academy of Sciences, for her valuable comments on our manuscript writing.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Whiting DR, Guariguata L, Weil C et al (2011) IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 94:311–321
2. Wang X, Ge S, Gonzalez I et al (2006) Formation of pancreatic duct epithelium from bone marrow during neonatal development. Stem Cells 24:307–314
3. Thatava T, Ma B, Rohde M et al (2006) Chromatin-remodeling factors allow differentiation of bone marrow cells into insulin-producing cells. Stem Cells 24:2858–2867
4. Chandra V, Swetha G, Phadnis S et al (2009) Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. Stem Cells 27:1941–1953
5. Yang JH, Lee SH, Heo YT et al (2010) Generation of insulin-producing cells from gnotobiotic porcine skin-derived stem cells. Biochem Biophys Res Commun 397:679–684
6. Meier JJ, Bhushan A, Butler PC (2006) The potential for stem cell therapy in diabetes. Pediatr Res 59:65r–73r
7. Van Hoof D, D'Amour KA, German MS (2009) Derivation of insulin-producing cells from human embryonic stem cells. Stem Cell Res 3:73–87
8. Bock C, Kiskinis E, Verstappen G et al (2011) Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell 144:439–452
9. Liu X, Wang Y, Li Y et al (2013) Research status and prospect of stem cells in the treatment of diabetes mellitus. Sci China Life Sci 56:306–312
10. Lumelsky N, Blondel O, Laeng P et al (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292:1389–1394
11. Boyd AS, Wu DC, Higashi Y et al (2008) A comparison of protocols used to generate insulin-producing cell clusters from mouse embryonic stem cells. Stem Cells 26:1128–1137
12. Segev H, Fishman B, Ziskind A et al (2004) Differentiation of human embryonic stem cells into insulin-producing clusters. Stem Cells 22:265–274
13. Sui L, Mfopou JK, Chen B et al (2013) Transplantation of human embryonic stem cell-derived pancreatic endoderm reveals a site-specific survival, growth, and differentiation. Cell Transpl 22:821–830
14. Jiang J, Au M, Lu K et al (2007) Generation of insulin-producing islet-like clusters from human embryonic stem cells. Stem Cells 25:1940–1953
15. Morrison GM, Oikonomopoulou I, Migueles RP et al (2008) Anterior definitive endoderm from ESCs reveals a role for FGF signaling. Cell Stem Cell 3:402–415
16. Tateishi K, He J, Taranova O et al (2008) Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J Biol Chem 283:31601–31607
17. Shiraki N, Yoshida T, Araki K et al (2008) Guided differentiation of embryonic stem cells into Pdx1-expressing regional-specific definitive endoderm. Stem Cells 26:874–885
18. Bernardo AS, Cho CH, Mason S et al (2009) Biphasic induction of Pdx1 in mouse and human embryonic stem cells can mimic development of pancreatic beta-cells. Stem Cells 27:341–351
19. Treff NR, Vincent RK, Budde ML et al (2006) Differentiation of embryonic stem cells conditionally expressing neurogenin 3. Stem Cells 24:2529–2537
20. Wang P, Rodriguez RT, Wang J et al (2011) Targeting SOX17 in human embryonic stem cells creates unique strategies for isolating and analyzing developing endoderm. Cell Stem Cell 8:335–346
21. Lavon N, Yanuka O, Benvenisty N (2006) The effect of overexpression of Pdx1 and Foxa2 on the differentiation of human embryonic stem cells into pancreatic cells. Stem Cells 24:1923–1930
22. Kubo A, Stull R, Takeuchi M et al (2011) Pdx1 and Ngn3 overexpression enhances pancreatic differentiation of mouse ES cell-derived endoderm population. PLoS One 6:e24058
23. Xu H, Tsang KS, Chan JC 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 Transpl 22:147–158
24. Kaitsuka T, Noguchi H, Shiraki N et al (2014) Generation of functional insulin-producing cells from mouse embryonic stem cells through 804G cell-derived extracellular matrix and protein transduction of transcription factors. Stem Cells Transl Med 3:114–127
25. Pagliuca FW, Millman JR, Gurtler M et al (2014) Generation of functional human pancreatic beta cells in vitro. Cell 159:428–439
26. Turovets N, D'Amour KA, Agapov V et al (2011) Human parthenogenetic stem cells produce enriched populations of definitive endoderm cells after trichostatin A pretreatment. Differentiation 81:292–298
27. Urban VS, Kiss J, Kovacs J et al (2008) Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. Stem Cells 26:244–253
28. Khorsandi L, Nejad-Dehbashi F, Ahangarpour A et al (2015) Three-dimensional differentiation of bone marrow-derived mesenchymal stem cells into insulin-producing cells. Tissue Cell 47:66–72
29. Yuan H, Liu H, Tian R et al (2012) Regulation of mesenchymal stem cell differentiation and insulin secretion by differential expression of Pdx-1. Mol Biol Rep 39:7777–7783
30. Li L, Li F, Qi H et al (2008) Coexpression of Pdx1 and betacellulin in mesenchymal stem cells could promote the differentiation of nestin-positive epithelium-like progenitors and pancreatic islet-like spheroids. Stem Cells Dev 17:815–823
31. Moriscot C, de Fraipont F, Richard MJ et al (2005) Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation in vitro. Stem Cells 23:594–603
32. Wilson LM, Wong SH, Yu N et al (2009) Insulin but not glucagon gene is silenced in human pancreas-derived mesenchymal stem cells. Stem Cells 27:2703–2711
33. Yoshida S, Ishikawa F, Kawano N et al (2005) Human cord blood–derived cells generate insulin-producing cells in vivo. Stem Cells 23:1409–1416
34. Li Y, Zhao LJ, Xia FZ et al (2012) Transdifferentiation of hepatic oval cells into pancreatic islet beta-cells. Front Biosci (Landmark Ed) 17:2391–2395
35. Li H, Li X, Lam KS et al (2008) Adeno-associated virus-mediated pancreatic and duodenal homeobox gene-1 expression enhanced differentiation of hepatic oval stem cells to insulin-producing cells in diabetic rats. J Biomed Sci 15:487–497
36. Kodama S, Kuhtreiber W, Fujimura S et al (2003) Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science 302:1223–1227
37. Kim B, Yoon BS, Moon JH et al (2012) Differentiation of human labia minora dermis-derived fibroblasts into insulin-producing cells. Exp Mol Med 44:26–35

38. Pennarossa G, Maffei S, Campagnol M et al (2013) Brief demethylation step allows the conversion of adult human skin fibroblasts into insulin-secreting cells. Proc Natl Acad Sci USA 110:8948–8953
39. Dor Y, Brown J, Martinez OI et al (2004) Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429:41–46
40. Smukler SR, Arntfield ME, Razavi R et al (2011) The adult mouse and human pancreas contain rare multipotent stem cells that express insulin. Cell Stem Cell 8:281–293
41. Kawakami M, Hirayama A, Tsuchiya K et al (2010) Promotion of beta-cell differentiation by the alkaloid conophylline in porcine pancreatic endocrine cells. Biomed Pharmacother 64:226–231
42. Stevenson K, Chen D, MacIntyre A et al (2011) Pancreatic-derived pathfinder cells enable regeneration of critically damaged adult pancreatic tissue and completely reverse streptozotocin-induced diabetes. Rejuvenation Res 14:163–171
43. Ta M, Choi Y, Atouf F et al (2006) The defined combination of growth factors controls generation of long-term-replicating islet progenitor-like cells from cultures of adult mouse pancreas. Stem Cells 24:1738–1749
44. Choi Y, Ta M, Atouf F et al (2004) Adult pancreas generates multipotent stem cells and pancreatic and nonpancreatic progeny. Stem Cells 22:1070–1084
45. Xu X, D'Hoker J, Stange G et al (2008) Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132:197–207
46. Sancho R, Gruber R, Gu G et al (2014) Loss of Fbw7 reprograms adult pancreatic ductal cells into alpha, delta, and beta cells. Cell Stem Cell 15:139–153
47. Lemper M, Leuckx G, Heremans Y et al (2014) Reprogramming of human pancreatic exocrine cells to beta-like cells. Cell Death Differ. doi:10.1038/cdd.2014.193
48. Collombat P, Xu X, Ravassard P et al (2009) The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 138:449–462
49. Liu Z, Habener JF (2009) Alpha cells beget beta cells. Cell 138:424–426
50. Chung CH, Hao E, Piran R et al (2010) Pancreatic beta-cell neogenesis by direct conversion from mature alpha-cells. Stem Cells 28:1630–1638
51. Habener JF, Stanojevic V (2012) Alpha-cell role in beta-cell generation and regeneration. Islets 4:188–198
52. Ameri J, Stahlberg A, Pedersen J et al (2010) FGF2 specifies hESC-derived definitive endoderm into foregut/midgut cell lineages in a concentration-dependent manner. Stem Cells 28:45–56
53. Teo AK, Ali Y, Wong KY et al (2012) Activin and BMP4 synergistically promote formation of definitive endoderm in human embryonic stem cells. Stem Cells 30:631–642
54. Li L, Yi Z, Seno M et al (2004) Activin A and betacellulin: effect on regeneration of pancreatic beta-cells in neonatal streptozotocin-treated rats. Diabetes 53:608–615
55. Mfopou JK, De Groote V, Xu X et al (2007) Sonic hedgehog and other soluble factors from differentiating embryoid bodies inhibit pancreas development. Stem Cells 25:1156–1165
56. Afelik S, Pool B, Schmerr M et al (2015) Wnt7b is required for epithelial progenitor growth and operates during epithelial-to-mesenchymal signaling in pancreatic development. Dev Biol 399:204–217
57. Shi Y, Hou L, Tang F et al (2005) Inducing embryonic stem cells to differentiate into pancreatic beta cells by a novel three-step approach with activin A and all-trans retinoic acid. Stem Cells 23:656–662
58. Zhang D, Jiang W, Liu M et al (2009) Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. Cell Res 19:429–438
59. Kao DI, Lacko LA, Ding BS et al (2015) Endothelial cells control pancreatic cell fate at defined stages through EGFL7 signaling. Stem Cell Rep 4:181–189
60. Lammert E, Cleaver O, Melton D (2001) Induction of pancreatic differentiation by signals from blood vessels. Science 294:564–567
61. He J, Yang Z, Yang H et al (2015) Regulation of insulin sensitivity, insulin production, and pancreatic beta cell survival by angiotensin-(1-7) in a rat model of streptozotocin-induced diabetes mellitus. Peptides 64:49–54
62. Karnieli O, Izhar-Prato Y, Bulvik S et al (2007) Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. Stem Cells 25:2837–2844
63. Sordi V, Melzi R, Mercalli A et al (2010) Mesenchymal cells appearing in pancreatic tissue culture are bone marrow-derived stem cells with the capacity to improve transplanted islet function. Stem Cells 28:140–151
64. Kroon E, Martinson LA, Kadoya K et al (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26:443–452
65. Schneider S, Feilen PJ, Brunnenmeier F et al (2005) Long-term graft function of adult rat and human islets encapsulated in novel alginate-based microcapsules after transplantation in immunocompetent diabetic mice. Diabetes 54:687–693
66. Matveyenko AV, Singh I, Shin BC et al (2010) Differential effects of prenatal and postnatal nutritional environment on ss-cell mass development and turnover in male and female rats. Endocrinology 151:5647–5656
67. Hamilton DC, Shih HH, Schubert RA et al (2015) A silk-based encapsulation platform for pancreatic islet transplantation improves islet function in vivo. J Tissue Eng Regen Med. doi:10.1002/term.1990
68. Nishimura R, Ushiyama A, Sekiguchi S et al (2013) Effects of glucagon-like peptide 1 analogue on the early phase of revascularization of transplanted pancreatic islets in a subcutaneous site. Transpl Proc 45:1892–1894
69. Coronel MM, Stabler CL (2013) Engineering a local microenvironment for pancreatic islet replacement. Curr Opin Biotechnol 24:900–908
70. Leung KK, Liang J, Ma MT et al (2012) Angiotensin II type 2 receptor is critical for the development of human fetal pancreatic progenitor cells into islet-like cell clusters and their potential for transplantation. Stem Cells 30:525–536
71. Li G, Huang LS, Jiang MH et al (2010) Implantation of bFGF-treated islet progenitor cells ameliorates streptozotocin-induced diabetes in rats. Acta Pharmacol Sin 31:1454–1463
72. Dufrane D, Gianello P (2012) Pig islet for xenotransplantation in human: structural and physiological compatibility for human clinical application. Transpl Rev (Orlando) 26:183–188
73. Lee JI, Shin JS, Jung WY et al (2013) Porcine islet adaptation to metabolic need of monkeys in pig-to-monkey intraportal islet xenotransplantation. Transpl Proc 45:1866–1868
74. van der Windt DJ, Marigliano M, He J et al (2012) Early islet damage after direct exposure of pig islets to blood: has humoral immunity been underestimated? Cell Transpl 21:1791–1802
75. Lee SH, Hao E, Savinov AY et al (2009) Human beta-cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. Transplantation 87:983–991
76. Bruin JE, Rezania A, Xu J et al (2013) Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice. Diabetologia 56:1987–1998
77. Barnett BP, Arepally A, Karmarkar PV et al (2007) Magnetic resonance-guided, real-time targeted delivery and imaging of

magnetocapsules immunoprotecting pancreatic islet cells. Nat Med 13:986–991
78. Hering BJ, Wijkstrom M, Graham ML et al (2006) Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. Nat Med 12:301–303
79. Cardona K, Korbutt GS, Milas Z et al (2006) Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. Nat Med 12:304–306
80. Speier S, Nyqvist D, Cabrera O et al (2008) Noninvasive in vivo imaging of pancreatic islet cell biology. Nat Med 14:574–578
81. Yolcu ES, Zhao H, Shirwan H (2013) Immunomodulation with SA-FasL protein as an effective means of preventing islet allograft rejection in chemically diabetic NOD mice. Transpl Proc 45:1889–1891