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TOPIC HIGHLIGHT

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Present and future cell therapies for pancreatic beta cell replenishment

Juan Domínguez-Bendala, Camillo Ricordi

Juan Domínguez-Bendala, Camillo Ricordi, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, Miami, FL 33136, United States
Juan Domínguez-Bendala, Camillo Ricordi, Department of Surgery, University of Miami Miller School of Medicine, Miami, FL 33136, United States
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Correspondence to: Juan Domínguez-Bendala, PhD, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, 1450 NW 10th Ave, Miami, FL 33136, United States. jdominguez2@med.miami.edu
Telephone: +1-305-2434092 Fax: +1-305-2434404
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Abstract

If only at a small scale, islet transplantation has successfully addressed what ought to be the primary endpoint of any cell therapy: the functional replenishment of damaged tissue in patients. After years of less-than-optimal approaches to immunosuppression, recent advances consistently yield long-term graft survival rates comparable to those of whole pancreas transplantation. Limited organ availability is the main hurdle that stands in the way of the widespread clinical utilization of this pioneering intervention. Progress in stem cell research over the past decade, coupled with our decades-long experience with islet transplantation, is shaping the future of cell therapies for the treatment of diabetes. Here we review the most promising avenues of research aimed at generating an inexhaustible supply of insulin-producing cells for islet regeneration, including the differentiation of pluripotent and multipotent stem cells of embryonic and adult origin along the beta cell lineage and the direct reprogramming of non-endocrine tissues into insulin-producing cells.

Key words: Human embryonic stem cells; Induced pluripotent stem cells; Mesenchymal stem cells; Beta cell differentiation; Reprogramming; Islet transplantation

Peer reviewers: Dr. Tamara Vorobjova, Department of Immunology, University of Tartu, Ravila, 19, Tartu 50411, Estonia; Dr. Hui-Kang Liu, National Research Institute of Chinese Medicine, Li-Nung street section 2, Taipei 112, Taiwan, China; Ilker Tasci, Professor, Department of Internal Medicine, GATA Ic Hastaliklari B.D., Ankara 06018, Turkey; Ezio Laconi, Professor, University of Cagliari, Via Porcell, 4, 09125 Cagliari, Italy

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INTRODUCTION

Type 1 diabetes is an autoimmune disease characterized by the targeted destruction of the insulin-producing beta cells within the pancreatic islets. This is a chronic condition that requires daily insulin administration to maintain blood glucose levels within acceptable limits. However, because of the impossibility of exogenous insulin to accurately mimic islet function in the long run, diabetes often progresses with the development of debilitating complications, including kidney failure, blindness and vascular degeneration. Whole pancreas transplantation is an effective means to permanently correct hyperglycemia, but because of the risks inherent to any major surgery is rarely indicated as a treatment for diabetes. Islet transplantation is a less invasive procedure based on

the isolation of islets from their surrounding tissue and subsequent implantation in the recipient's liver[1-8]. The method entails the enzymatic and mechanical separation of the islets from the rest of the organ. Since islets have a different density than acinar tissue, centrifugation can be used to enrich for layers of high purity that are infused intraportally into the liver of the patient, where they lodge and revascularize in a matter of weeks[9-13]. The evolution of this technology has followed a typical pattern of innovation[14] in which the hype elicited by an early milestone (the invention of the isolation method[9]) ballooned with a technical achievement (the development of an steroid-free immunosuppression protocol that allowed for long-term graft survival[15]), only to burst with the realization that the long-term outcome was not nearly as good as expected (only 20% of the patients remained insulin-independent five years after the procedure[16]). As it is also commonly seen in most innovations, a dry "trough of disillusionment" ensued, during which researchers had to struggle with a hostile scientific and financial environment fed by the perception that islet transplantation was a therapeutic *cul-de-sac*. This has been so until very recently, when new developments (such as reports of novel T-cell depleting strategies that prolong graft survival and support function at rates that stand comparison to those seen when transplanting the entire organ[17]) have shifted the perception again. Of course, the expectations have now been adjusted to the reality, and few would contend now that this therapy represents the future treatment of choice for diabetes. However, the advent of stem cells in the clinical arena has helped refocus the goals of islet transplantation, which is now seen as an invaluable testing ground for the next generation of cell therapeutics rather than as the next breakthrough in the fight against diabetes. Indeed, it is expected that stem cell-derived insulin-producing cells will take over islets in the near future, making this therapy available to millions (as opposed to hundreds) of patients. It is reasonable to expect that the transition to stem cells, if not seamless, will be easier and faster than for other conditions for which there is no cell therapy today. From adult and embryonic stem cells to somatic tissue engineering and islet regeneration, this review will describe the best positioned candidates to lead this transition in the next decade.

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) are ubiquitously found throughout the entire body. Tissues from which they can be commonly isolated and expanded include the stroma of the bone marrow, the adipose tissue and the umbilical cord[18]. Whether or not MSCs from disparate sources are one and the same is still subject to debate. Indeed, to be considered MSCs they must share a number of characteristics such as adherence to plastic, capacity to differentiate along the bone, cartilage and fat lineages, presence of some surface markers (CD73, CD90 and CD105) and absence of others (chiefly those of the hematopoietic lineage). However, some MSCs express markers of pluripotency (Oct4, Nanog) whereas others do not. Proliferation rates vary greatly between clones even if obtained from the same tissue, as evidenced by a recent example in which division rates of umbilical cord blood-derived MSCs ranged anywhere from 36 h to nearly 9 d[19]. To complicate things even more, clones not only from the same tissue, but also from the same donor, also exhibit substantial variability[20]. While these lines of evidence would suggest that MSCs comprehend a far too diverse collection of cell types to make common assumptions about their true identity and therapeutic potential, these differences ultimately reside in epigenetic signatures that -as shown time and again after the advent of reprogramming techniques- are anything but irreversible. From this perspective, even if some subsets of MSCs (such as those residing in the umbilical cord blood) would appear to be more adept than others at acquiring beta cell characteristics, considerations such as the ease of procurement and expansion or the ability to derive them from the prospective recipient (as would likely be the case when considering fat-derived MSCs) may ultimately weigh more than their apparent developmental potential at the time of isolation. This same consideration applies to what many perceive as an ontological limitation of MSCs to become pancreatic islets, since beta cells and MSCs arise from different embryonal germ layers. While this is true, the epigenetic landscape has proven in recent years to be much more pliable than previously thought. Be it by direct reprogramming into pancreatic endocrine tissues or by de-differentiation followed by re-education into insulin-producing cells, there is a growing consensus in the field of diabetes that MSCs may after all be of more use than that of providing a beneficial immunomodulatory[21] and pro-angiogenic[22] microenvironment for other cells.

Most approaches described until now to coax MSCs into beta-like cells are based on the sequential addition to their culture medium of soluble factors known to have an effect on the progression of pancreatic development. Partial success has been reported in recent years with MSCs derived from a wealth of different sources, including pancreatic ductal and acinar tissues[23-25], fat[26,27], amniotic fluid[28], umbilical cord blood and placenta[19,29-33], bone marrow[34-37], endometrium[38,39], and even from the very islets of Langerhans[40-44]. However, unlike for human embryonic stem (hES) cells[45-47], researchers in the field of MSCs still have failed to define a "gold standard" method of differentiation that results in bona fide, mature or otherwise, beta cells. Efforts may have been dispersed by the parallel pursuit of many different MSC sources, each one purportedly superior to the others, with an overall lack of focus on the description of robust protocols that may result in successful beta cell differentiation from all of them. Only recently have steps been taken to adopt the strategies developed for hES cells to the differentiation of primitive populations of MSCs[19]. These caveats aside, the use of MSCs seems to be solidly ingrained in the pipeline of cell therapies diabetes. As

mentioned earlier, even in the unlikely case that all our efforts at converting them into cells with the ability to regulate glucose *in vivo* were to fail, MSCs are known for their ability to foster a favorable microenvironment for other cells to engraft. They do this by secreting a plethora of trophic agents such as nerve growth factor, basic fibroblast growth factor, vascular endothelial growth factor, brain-derived neurotrophic factor, insulin-like growth factor-1, and hepatocyte growth factor[48]. There is no reason why the favorable results observed when co-transplanting islets with MSCs[49-53] could not be extended to stem cell-derived beta-like cells.

NON-MESENCHYMAL ADULT STEM CELLS

Because of their shared provenance, hematopoietic cells from the bone marrow and umbilical cord blood cell are often mistaken with MSCs. Although some studies have shown that there are multipotent stem cells in the hematopoietic compartment of these tissues, in most cases the use of stromal (or mesenchymal) cells would be for non immune-related regeneration (see above), whereas hematopoietic cells would be primarily used to treat immune-related disorders. Examples of the latter are the decades-old practice of bone marrow transplantation or the recent attempts to reset the immunological clock of diabetes by autologous transplantation of bone marrow-derived stem cells[54,55]. More recently, some groups have tested the administration of bone marrow-derived hematopoietic cells directly into the pancreas of the subject, an approach that has yielded promising results in type 2 diabetic patients when combined with hyperbaric oxygen therapy[56]. Local injection of hematopoietic stem cells has also been tested clinically for the treatment of limb ischemia and diabetic neuropathy[57]. In general, the mechanism by which these cells might exert their action is likely related to their ability to stimulate vascular regeneration (which may in turn result in enhanced islet function when injected in the pancreas) rather than to their direct differentiation into beta cells.

As for potential stem/progenitor cells residing in the pancreas, the ongoing debate about their existence is beyond the scope of this manuscript and has been already reviewed in[58]. Whether or not they exist and have an active role on the physiological regeneration of the organ, to this date the only evidence that true pancreatic progenitors can be isolated and expanded *in vitro*[59,60] is very preliminary and needs independent confirmation. The findings by Cardinale *et al*[61] on stem cell populations in the adult extrahepatic biliary tree can be propagated *ex vivo* and give rise to hepatocytes, cholangiocytes or pancreatic islets are also very promising but warrant similar caution.

EMBRYONIC STEM AND INDUCED PLURIPOTENT STEM CELLS

Mouse embryonic stem (ES) cells have been a staple of developmental biology laboratories for the most part of the thirty years since they were first isolated[62,63]. Their human counterparts, however, are a much more recent addition[64]. When cultured according to very precise specifications, these unique cells proliferate at high speeds (typical population doubling times are in the range of 24-48 h) and in an indefinite manner, while retaining the potential to differentiate into derivatives of all three embryonic layers (endoderm, ectoderm and mesoderm). It is not difficult to appreciate in the evolution of this technology, as applied to human therapy, the same pattern previously described for islet transplantation: (1) a technology trigger, namely the initial characterization of ES cells by the team of James Thomson in 1998: A field whose main pursuit for more than two decades had been to perfect ES cell isolation techniques from non-murine species with the goal of generating higher animal models of human disease, suddenly became the new, potentially most powerful weapon to combat it; (2) The peak of inflated expectations, coincident with the description of what appeared to be a most simple method, easy to translate from mouse to human cells, to obtain insulin-producing beta cells[65]; (3) The trough of disillusionment, which came about with the realization that such method was in reality generating not beta but neuroectodermal cells and that their purported insulin staining obeyed to an artifactual uptake of insulin from the culture medium rather than to its synthesis and secretion[66]; (4) The slope of enlightenment, a methodical path of research that ended up with the unraveling of the perfect combination of factors leading to the specification of definitive endoderm, the first and critical step along the beta cell lineage[67,68]; and (5) The current plateau of productivity, in which protocols that elaborate upon that initial milestone[45,46,69,70] have reached the pre-clinical level[47]. Normoglycemia is now routinely attained when transplanting hES cell-derived beta cell progenitors into immunodeficient diabetic mice. The approach of transplanting cells halfway their differentiation course is based on the observation that beta cells differentiate better and acquire full functionality when allowed to mature *in vivo*, due to factors that remain largely unknown. The well documented risk of tumorigenesis posed by hES cells (heightened when transplanting non-terminally mature derivatives) is currently being addressed as part of the strict controls required by regulatory agencies prior to the approval of their use in clinical trials. These include the separation of pancreatic progenitors from the rest of the population[47] and the also antibody-based selective ablation of tumorigenic cells[71]. The leading company pushing for hES cell-based clinical trials for diabetes (Viacyte, Inc.) complements the above strategies with that of encapsulating the cells inside an immunoisolation device that would double as a physical barrier to contain potential tumor-forming escapees. In fact, if this barrier allowed for the total elimination of immunosupresion in the recipient, in theory such escapees would be recognized as allografts by the host and thus promptly rejected.

Induced pluripotent stem (iPS) cells are a much newer

addition to the regenerative medicine armamentarium - one that has been saluted by many as an ethically sounder alternative to hES cells[72-79]. The incredible pace at which this field of research progresses is evidenced by the fact that, merely four years after the first report on the simple reprogramming technology that allowed us to make cells "go back in time" to pluripotency without the need of destroying human embryos[72-77,79-82], iPS cells have had time not only to rise to the heights of the Pantheon of therapeutic candidates, but also to start falling[80,83]. Seen at first as a combination of the best features of adult and embryonic stem cells (pluripotency coupled to the potential for autologous isolation), it is now that we are starting to see some worrisome signs that even the most drastic reprogramming may leave traces of the original epigenetic memory of the cell[84] or cause the iPS derivates to age faster[85]. Much worse than that, reprogramming can also induce mutations or activate oncogenes[86-88] regardless of the method used, i.e., the effect is not necessarily associated to viral insertional mutagenesis. In this context, the successful adaptation to iPS cells of a beta cell differentiation protocol originally designed for hES cells[89] represents only a preliminary proof of concept that it does not in any way get us closer than we are with hES cells to clinical therapies. Moreover, the potential advantages of using patient-derived iPS cells for type 1 diabetes is dubious inasmuch as, this being an autoimmune disorder, the recipient's immune system would be expected to swiftly reject any neogenic beta cells derived through this means.

LATERAL REPROGRAMMING

While "vertical" reprogramming would cause a terminally differentiated cell to go back in time and re-acquire full pluripotency, the notion of lateral reprogramming is based on the observation that the epigenetic signature of any differentiated cell can also be overridden in a manner that ultimately leads to the adoption of another terminally differentiated phenotype. In this sense, the older term "transdifferentiation" might be more appropriate to describe this type of horizontal reprogramming. As with iPS cell reprogramming, this young field started using DNA-integrating approaches (chiefly by means of viral vehicles), but is expected to follow the steps of the former in the use of non-integrative methods such as protein transduction[90,91], episomal constructs[78], DNA minicircles[92], synthetic mRNAs[93] and even small molecules identified through high-throughput screening[94].

Earlier attempts at using reprogramming to obtain beta cells used the liver as a substrate. The choice of this organ is hardly surprising, since numerous studies confirm that liver and pancreas are susceptible of interconversion under multiple experimental, physiological or pathophysiological circumstances, including dietary depletion of copper[95-97], spontaneous tumoral processes or administration of chemicals such as diethylnitrosamine[98] or dexamethasone[99]. In fact, fully differentiated hepatocytes and pancreatic beta cells share common molecular mechanisms for glucose sensing[100,101], with glucokinase being a prominent marker or both. From an evolutionary perspective, the existence of a single hepatopancreas in many invertebrates indicates that the two organs started as one. In vertebrates, liver and pancreas arise from the same progenitor cell pool[102-110], and their separation is dictated by signals secreted by the developing heart[103,104,111,112]. More to the point, in adult mice and humans alike, the extrahepatic biliary tree harbors progenitor cells with the ability to differentiate into hepatic and pancreatic tissues[61,113].

The systemic delivery of the master pancreatic regulator Pdx1[114,115] to mice was arguably the experiment that inaugurated the field of lateral reprogramming at the beginning of the century[116,117]. The authors of those pioneering studies reported the appearance of insulin-positive cells in the liver of the animals, in sufficient amounts as to restore normoglycemia when the recipients were diabetic. Of even higher significance was the ulterior finding that reprogramming was not contingent to the ectopic expression of the gene, but persistent in time long after the expected clearance of the adenovirus used to deliver it[118]. Similar results were independently confirmed by other groups, using either Pdx1 alone or in combination with other genes, such as NeuroD or MafA, known to act synergistically with the former[119-126]. These reports were received with a mixture of expectation (the total liver-to-pancreas transdifferentiation described in transgenic frogs that expressed Pdx1 under the control of an early liver promoter[127] was nothing short of extraordinary) and vague disappointment. This is because with the exception of the above transgenic setting (which obviously did not have any clinical applicability), none of the other strategies had yielded cells that could unequivocally be defined as true beta cells. When conducted *in vitro*, transdifferentiation resulted in cells that, at best, had a hybrid hepatocyte-beta cell phenotype and were incapable of regulating insulin secretion according to changes in glucose concentration. And when done *in vivo*, the observation that the inflammation elicited by the use of adenoviruses could be as important to induce reprogramming as the actual genes -if not more-, was a troubling discovery that caused legitimate concerns about how "clean" the strategy actually was[120].

After an impasse of a few years, during which the excitement that first welcomed the advent of this new technology had started to wane, the impact of a recent report[128] renewed the enthusiasm of the scientific community. The choice of genes (Pdx1, Ngn3 and MafA) was not particularly novel, as comparable combinations of factors had already been assayed on liver[101,103,121,129-131] with all the aforementioned limitations. What was novel was the use of pancreatic acinar tissue as the substrate for reprogramming. Perhaps because of the even closer developmental ties between the beta cells and the surrounding acinar parenchyma that engulfs the islets in the native organ, the observed transdifferentiation to beta cells upon direct injection of these genes in the pancreas

of recipient mice was convincing enough to reawaken excitement about the clinical prospects of reprogramming.

CONCLUSION

Upon reviewing the diversity of options potentially available to us in the near future, one may get the impression that current efforts are somewhat dispersed. Clinicians and patient advocates alike often voice their concern that such dispersion may stand in the way of an effective allocation of manpower and resources to the strategy that has the highest translational potential. But who is to say which strategy this is at this early stage of the game? Indeed, based on the trends observed in the field, it is plausible that the approach with the highest chance of success will be a multi-pronged one, possibly involving more than one cell type and addressing both replacement and immunomodulation.

hES cells are well positioned from the standpoint of the robustness of the described differentiation methods and the heavy private and public investment bestowed upon the team that leads these efforts. ViaCyte received last year more than $20 million from the California Institute for Regenerative Medicine to speed up pre-clinical studies that could lead in the very near future to an Investigational New Drug application to the FDA. However, unlike other prospective therapies based on the use of adult stem cells (heavily represented in clinical trials), hES cell-based ones still have to break ground from a regulatory perspective. The recent withdrawal of Geron's phase I clinical trials for spinal cord injury (which pioneered the use of hES cells in the clinical arena) represents an unfortunate turn of events that is not likely to help foster a more favorable climate for new trials. Fortunately, recent advances at immunoisolation[132,133], coupled with refinements at enriching for the fraction of cells with therapeutic potential[47,71] are likely to surmount the main concerns posed by regulatory agencies. Despite the initial impression that iPS cell-based protocols could be largely swapped with those designed for hES cells, developments already discussed in the relevant section urge caution about their clinical use, which, if ever realized, will undoubtedly lag behind that of hES cells. The potential advantage of using autologous cells for type 1 diabetes is dubious at best and logistically impractical. In fact, even if autoimmunity was not a consideration and we still wanted to obtain pluripotent cells as closely matched to the recipient as possible, an alternative is human leukocyte antigen (HLA)-homozygous hES cells, which have been derived by parthenogenesis. One of these cell lines is reported to carry the most widespread HLA haplotype in North America[134,135].

Adult stem cells, especially those with known ontogenic potential to become definitive endoderm, have been remarkably difficult to identify - let alone isolate and expand. The recent discovery of such potential populations in the adult extrahepatic biliary tree[61,113] or the islets themselves[60] may open promising avenues of research, but until these early results are validated, MSCs are likely to keep the lion's share of attention and funding. Easy to derive, characterize and expand, their apparent genealogic disconnect with the endoderm from which organs such as the liver or the pancreas arise has not been an obstacle for those who champion them in the context of diabetes. Progress at developing methods to coax these cells into insulin-producing cells, albeit slow, is starting to pick up speed with the description of more primitive MSC populations and better differentiation methods. The burgeoning field of reprogramming is also likely to have a say on whether the developmental potential of MSCs is intrinsically limited to mesodermal lineages. Although none of the 11 currently listed MSC-based clinical trials for the treatment of either type 1 or type 2 diabetes makes use of MSCs previously differentiated into beta-like cells *in vitro* (www.clinicaltrials.gov), their immunomodulatory and niche-forming properties are now widely acknowledged and could be the basis of both combination (such as islets and MSCs[49]) and stand-alone therapies in the short term.

Lateral transdifferentiation, finally, may not necessarily take a backseat to the above approaches, provided that non DNA-based reprogramming techniques are refined to the extent required for the process to be efficient. Indeed, progress in this direction may effectively leapfrog years of research on stem cell differentiation, and end up in contention to become the therapy of choice for type 1 diabetes. While *ex vivo* reprogramming of acinar tissue may not represent a viable strategy in the long run (pancreatic biopsies from the prospective patients would be risky, and the use of acinar tissue from deceased donors would present the same scarcity problems that afflict organ donation in general), recent advances in "DNA-free gene therapy"[136,137] may soon change the perception that *in vivo* reprogramming of cell fate is a clinical implausibility. More so, because one commonly cited shortcoming of these approaches -i.e., the fact that reprogramming is seldom complete, invariably leaving traces of the original fate- might in fact work in our benefit if the autoimmune response seen in type 1 diabetes is elicited only by true beta cells, but not the reprogrammed ones. While this is only a speculation at this point, the potential therapeutic implications of such finding would be incommensurable. Finally, it is necessary to emphasize that most of the approaches herein listed could also be potentially applied not only to type 1 diabetes, but also to all types of insulin-dependent conditions resulting in glycemic dysregulation, including type 2 diabetes.

In the next few years, we expect to see a progressive shift from islet transplantation to the transplantation of hES cells in an immunoisolation setting. Parallel efforts will be made at using MSCs and hematopoietic stem cells for immunomodulation, which will be accompanied by an emphasis on the exploration of endogenous regeneration pathways. A cure is likely to ultimately arise from a combination of many, if not all, of the above approaches.

REFERENCES

1 **White SA**, Manas DW. Pancreas transplantation. *Ann R Coll Surg Engl* 2008; **90**: 368-370
2 **Burke GW**, Ciancio G, Sollinger HW. Advances in pancreas transplantation. *Transplantation* 2004; **77**: S62-S67
3 **Ricordi C**, Strom TB. Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol* 2004; **4**: 259-268
4 **Inverardi L**, Kenyon NS, Ricordi C. Islet transplantation: immunological perspectives. *Curr Opin Immunol* 2003; **15**: 507-511
5 **Merani S**, Shapiro AM. Current status of pancreatic islet transplantation. *Clin Sci (Lond)* 2006; **110**: 611-625
6 **Murdoch TB**, McGhee-Wilson D, Shapiro AM, Lakey JR. Methods of human islet culture for transplantation. *Cell Transplant* 2004; **13**: 605-617
7 **Pileggi A**, Alejandro R, Ricordi C. Clinical islet transplantation. *Minerva Endocrinol* 2006; **31**: 219-232
8 **Seung E**, Mordes JP, Greiner DL, Rossini AA. Induction of tolerance for islet transplantation for type 1 diabetes. *Curr Diab Rep* 2003; **3**: 329-335
9 **Ricordi C**, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. *Diabetes* 1988; **37**: 413-420
10 **Korsgren O**, Lundgren T, Felldin M, Foss A, Isaksson B, Permert J, Persson NH, Rafael E, Rydén M, Salmela K, Tibell A, Tufveson G, Nilsson B. Optimising islet engraftment is critical for successful clinical islet transplantation. *Diabetologia* 2008; **51**: 227-232
11 **Carlsson PO**, Mattsson G. Oxygen tension and blood flow in relation to revascularization in transplanted adult and fetal rat pancreatic islets. *Cell Transplant* 2002; **11**: 813-820
12 **Carlsson PO**, Palm F, Mattsson G. Low revascularization of experimentally transplanted human pancreatic islets. *J Clin Endocrinol Metab* 2002; **87**: 5418-5423
13 **Lammert E**, Gu G, McLaughlin M, Brown D, Brekken R, Murtaugh LC, Gerber HP, Ferrara N, Melton DA. Role of VEGF-A in vascularization of pancreatic islets. *Curr Biol* 2003; **13**: 1070-1074
14 **Fenn J**, Raskino M. Mastering the hype cycle: how to choose the right innovation at the right time. Boston, Mass.: Harvard Business Press, 2008
15 **Shapiro AM**, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230-238
16 **Ryan EA**, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; **54**: 2060-2069
17 **Bellin MD**, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm SH, Ansite JD, Witson J, Bansal-Pakala P, Balamurugan AN, Papas KK, Sutherland DE, Moran A, Hering BJ. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant* 2008; **8**: 2463-2470
18 **Mosna F**, Sensebé L, Krampera M. Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. *Stem Cells Dev* 2010; **19**: 1449-1470
19 **Prabakar KR**, Domínguez-Bendala J, Molano RD, Pileggi A, Villate S, Ricordi C, Inverardi L. Generation of glucose-responsive, insulin-producing cells from human umbilical cord blood-derived mesenchymal stem cells. *Cell Transplant* 2012; **21**: 1321-1339
20 **Paredes B**, Santana A, Arribas MI, Vicente-Salar N, de Aza PN, Roche E, Such J, Reig JA. Phenotypic differences during the osteogenic differentiation of single cell-derived clones isolated from human lipoaspirates. *J Tissue Eng Regen Med* 2011; **5**: 589-599
21 **Abdi R**, Fiorina P, Adra CN, Atkinson M, Sayegh MH. Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes* 2008; **57**: 1759-1767
22 **Wu Y**, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 2007; **25**: 2648-2659
23 **Lin HT**, Chiou SH, Kao CL, Shyr YM, Hsu CJ, Tarng YW, Ho LL, Kwok CF, Ku HH. Characterization of pancreatic stem cells derived from adult human pancreas ducts by fluorescence activated cell sorting. *World J Gastroenterol* 2006; **12**: 4529-4535
24 **Seeberger KL**, Dufour JM, Shapiro AM, Lakey JR, Rajotte RV, Korbutt GS. Expansion of mesenchymal stem cells from human pancreatic ductal epithelium. *Lab Invest* 2006; **86**: 141-153
25 **Baertschiger RM**, Bosco D, Morel P, Serre-Beinier V, Berney T, Buhler LH, Gonelle-Gispert C. Mesenchymal stem cells derived from human exocrine pancreas express transcription factors implicated in beta-cell development. *Pancreas* 2008; **37**: 75-84
26 **Timper K**, Seboek D, Eberhardt M, Linscheid P, Christ-Crain M, Keller U, Müller B, Zulewski H. Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun* 2006; **341**: 1135-1140
27 **Okura H**, Komoda H, Fumimoto Y, Lee CM, Nishida T, Sawa Y, Matsuyama A. Transdifferentiation of human adipose tissue-derived stromal cells into insulin-producing clusters. *J Artif Organs* 2009; **12**: 123-130
28 **Trovato L**, De Fazio R, Annunziata M, Sdei S, Favaro E, Ponti R, Marozio L, Ghigo E, Benedetto C, Granata R. Pluripotent stem cells isolated from human amniotic fluid and differentiation into pancreatic beta-cells. *J Endocrinol Invest* 2009; **32**: 873-876
29 **Pessina A**, Eletti B, Croera C, Savalli N, Diodovich C, Gribaldo L. Pancreas developing markers expressed on human mononucleated umbilical cord blood cells. *Biochem Biophys Res Commun* 2004; **323**: 315-322
30 **Sun B**, Roh KH, Lee SR, Lee YS, Kang KS. Induction of human umbilical cord blood-derived stem cells with embryonic stem cell phenotypes into insulin producing islet-like structure. *Biochem Biophys Res Commun* 2007; **354**: 919-923
31 **Gao F**, Wu DQ, Hu YH, Jin GX, Li GD, Sun TW, Li FJ. In vitro cultivation of islet-like cell clusters from human umbilical cord blood-derived mesenchymal stem cells. *Transl Res* 2008; **151**: 293-302
32 **Gao F**, Wu DQ, Hu YH, Jin GX. Extracellular matrix gel is necessary for in vitro cultivation of insulin producing cells from human umbilical cord blood derived mesenchymal stem cells. *Chin Med J (Engl)* 2008; **121**: 811-818
33 **Hu YH**, Wu DQ, Gao F, Li GD, Yao L, Zhang XC. A secretory function of human insulin-producing cells in vivo. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 255-260
34 **Wu XH**, Liu CP, Xu KF, Mao XD, Zhu J, Jiang JJ, Cui D, Zhang M, Xu Y, Liu C. Reversal of hyperglycemia in diabetic rats by portal vein transplantation of islet-like cells generated from bone marrow mesenchymal stem cells. *World J Gastroenterol* 2007; **13**: 3342-3349
35 **Paz AH**, Salton GD, Ayala-Lugo A, Gomes C, Terraciano P, Scalco R, Laurino CC, Passos EP, Schneider MR, Meurer L, Cirne-Lima E. Betacellulin overexpression in mesenchymal stem cells induces insulin secretion in vitro and ameliorates streptozotocin-induced hyperglycemia in rats. *Stem Cells Dev* 2011; **20**: 223-232
36 **Moriscot C**, de Fraipont F, Richard MJ, Marchand M, Savatier P, Bosco D, Favrot M, Benhamou PY. 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* 2005; **23**: 594-603

37 **Phadnis SM**, Joglekar MV, Dalvi MP, Muthyala S, Nair PD, Ghaskadbi SM, Bhonde RR, Hardikar AA. Human bone marrow-derived mesenchymal cells differentiate and mature into endocrine pancreatic lineage in vivo. *Cytotherapy* 2011; **13**: 279-293

38 **Harris DT**, Badowski M, Ahmad N, Gaballa MA. The potential of cord blood stem cells for use in regenerative medicine. *Expert Opin Biol Ther* 2007; **7**: 1311-1322

39 **Harris DT**, Rogers I. Umbilical cord blood: a unique source of pluripotent stem cells for regenerative medicine. *Curr Stem Cell Res Ther* 2007; **2**: 301-309

40 **Zulewski H**, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Müller B, Vallejo M, Thomas MK, Habener JF. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 2001; **50**: 521-533

41 **Huang H**, Tang X. Phenotypic determination and characterization of nestin-positive precursors derived from human fetal pancreas. *Lab Invest* 2003; **83**: 539-547

42 **Zhang L**, Hong TP, Hu J, Liu YN, Wu YH, Li LS. Nestin-positive progenitor cells isolated from human fetal pancreas have phenotypic markers identical to mesenchymal stem cells. *World J Gastroenterol* 2005; **11**: 2906-2911

43 **Ouziel-Yahalom L**, Zalzman M, Anker-Kitai L, Knoller S, Bar Y, Glandt M, Herold K, Efrat S. Expansion and redifferentiation of adult human pancreatic islet cells. *Biochem Biophys Res Commun* 2006; **341**: 291-298

44 **Eberhardt M**, Salmon P, von Mach MA, Hengstler JG, Brulport M, Linscheid P, Seboek D, Oberholzer J, Barbero A, Martin I, Müller B, Trono D, Zulewski H. Multipotential nestin and Isl-1 positive mesenchymal stem cells isolated from human pancreatic islets. *Biochem Biophys Res Commun* 2006; **345**: 1167-1176

45 **D'Amour KA**, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 2006; **24**: 1392-1401

46 **Kroon E**, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol* 2008; **26**: 443-452

47 **Kelly OG**, Chan MY, Martinson LA, Kadoya K, Ostertag TM, Ross KG, Richardson M, Carpenter MK, D'Amour KA, Kroon E, Moorman M, Baetge EE, Bang AG. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. *Nat Biotechnol* 2011; **29**: 750-756

48 **Xu YX**, Chen L, Wang R, Hou WK, Lin P, Sun L, Sun Y, Dong QY. Mesenchymal stem cell therapy for diabetes through paracrine mechanisms. *Med Hypotheses* 2008; **71**: 390-393

49 **Johansson U**, Rasmusson I, Niclou SP, Forslund N, Gustavsson L, Nilsson B, Korsgren O, Magnusson PU. Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization. *Diabetes* 2008; **57**: 2393-2401

50 **Fang B**, Li N, Song Y, Li J, Zhao RC, Ma Y. Cotransplantation of haploidentical mesenchymal stem cells to enhance engraftment of hematopoietic stem cells and to reduce the risk of graft failure in two children with severe aplastic anemia. *Pediatr Transplant* 2009; **13**: 499-502

51 **Lee ST**, Maeng H, Chwae YJ, Oh DJ, Kim YM, Yang WI. Effect of mesenchymal stem cell transplantation on the engraftment of human hematopoietic stem cells and leukemic cells in mice model. *Int J Hematol* 2008; **87**: 327-337

52 **Ball LM**, Bernardo ME, Roelofs H, Lankester A, Cometa A, Egeler RM, Locatelli F, Fibbe WE. Cotransplantation of ex vivo expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. *Blood* 2007; **110**: 2764-2767

53 **Noort WA**, Kruisselbrink AB, in't Anker PS, Kruger M, van Bezooijen RL, de Paus RA, Heemskerk MH, Löwik CW, Falkenburg JH, Willemze R, Fibbe WE. Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. *Exp Hematol* 2002; **30**: 870-878

54 **Voltarelli JC**, Couri CE, Stracieri AB, Oliveira MC, Moraes DA, Pieroni F, Coutinho M, Malmegrim KC, Foss-Freitas MC, Simões BP, Foss MC, Squiers E, Burt RK. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA* 2007; **297**: 1568-1576

55 **Couri CE**, Voltarelli JC. Autologous stem cell transplantation for early type 1 diabetes mellitus. *Autoimmunity* 2008; **41**: 666-672

56 **Estrada EJ**, Valacchi F, Nicora E, Brieva S, Esteve C, Echevarria L, Froud T, Bernetti K, Cayetano SM, Velazquez O, Alejandro R, Ricordi C. Combined treatment of intrapancreatic autologous bone marrow stem cells and hyperbaric oxygen in type 2 diabetes mellitus. *Cell Transplant* 2008; **17**: 1295-1304

57 **Huang P**, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. *Diabetes Care* 2005; **28**: 2155-2160

58 **Domínguez-Bendala J**. Pancreatic stem cells. 1st ed. New York: Humana Press, 2009: 1-254

59 **Seaberg RM**, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, Wheeler MB, Korbutt G, van der Kooy D. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol* 2004; **22**: 1115-1124

60 **Smukler SR**, Arntfield ME, Razavi R, Bikopoulos G, Karpowicz P, Seaberg R, Dai F, Lee S, Ahrens R, Fraser PE, Wheeler MB, van der Kooy D. The adult mouse and human pancreas contain rare multipotent stem cells that express insulin. *Cell Stem Cell* 2011; **8**: 281-293

61 **Cardinale V**, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, Berloco PB, Cantafora A, Wauthier E, Furth ME, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid L. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* 2011; **54**: 2159-2172

62 **Evans MJ**, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981; **292**: 154-156

63 **Martin GR**. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* 1981; **78**: 7634-7638

64 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147

65 **Lumelsky N**, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001; **292**: 1389-1394

66 **Hansson M**, Tonning A, Frandsen U, Petri A, Rajagopal J, Englund MC, Heller RS, Håkansson J, Fleckner J, Sköld HN, Melton D, Semb H, Serup P. Artifactual insulin release from differentiated embryonic stem cells. *Diabetes* 2004; **53**: 2603-2609

67 **D'Amour KA**, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol* 2005; **23**: 1534-1541

68 **Kubo A**, Shinozaki K, Shannon JM, Kouskoff V, Kennedy

M, Woo S, Fehling HJ, Keller G. Development of definitive endoderm from embryonic stem cells in culture. *Development* 2004; **131**: 1651-1662
69 **McLean AB**, D'Amour KA, Jones KL, Krishnamoorthy M, Kulik MJ, Reynolds DM, Sheppard AM, Liu H, Xu Y, Baetge EE, Dalton S. Activin a efficiently specifies definitive endoderm from human embryonic stem cells only when phosphatidylinositol 3-kinase signaling is suppressed. *Stem Cells* 2007; **25**: 29-38
70 **Basford CL**, Prentice KJ, Hardy AB, Sarangi F, Micallef SJ, Li X, Guo Q, Elefanty AG, Stanley EG, Keller G, Allister EM, Nostro MC, Wheeler MB. The functional and molecular characterisation of human embryonic stem cell-derived insulin-positive cells compared with adult pancreatic beta cells. *Diabetologia* 2012; **55**: 358-371
71 **Tang C**, Lee AS, Volkmer JP, Sahoo D, Nag D, Mosley AR, Inlay MA, Ardehali R, Chavez SL, Pera RR, Behr B, Wu JC, Weissman IL, Drukker M. An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. *Nat Biotechnol* 2011; **29**: 829-834
72 **Lewitzky M**, Yamanaka S. Reprogramming somatic cells towards pluripotency by defined factors. *Curr Opin Biotechnol* 2007; **18**: 467-473
73 **Nakagawa M**, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 2008; **26**: 101-106
74 **Okita K**, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 2008; **322**: 949-953
75 **Takahashi K**, Okita K, Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc* 2007; **2**: 3081-3089
76 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872
77 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676
78 **Yu J**, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 2009; **324**: 797-801
79 **Yu J**, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; **318**: 1917-1920
80 **Izpisúa Belmonte JC**, Ellis J, Hochedlinger K, Yamanaka S. Induced pluripotent stem cells and reprogramming: seeing the science through the hype. *Nat Rev Genet* 2009; **10**: 878-883
81 **Narazaki G**, Uosaki H, Teranishi M, Okita K, Kim B, Matsuoka S, Yamanaka S, Yamashita JK. Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. *Circulation* 2008; **118**: 498-506
82 **Okita K**, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007; **448**: 313-317
83 **Mason C**, Manzotti E. Induced pluripotent stem cells: an emerging technology platform and the Gartner hype cycle. *Regen Med* 2009; **4**: 329-331
84 **Bar-Nur O**, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. *Cell Stem Cell* 2011; **9**: 17-23
85 **Feng Q**, Lu SJ, Klimanskaya I, Gomes I, Kim D, Chung Y, Honig GR, Kim KS, Lanza R. Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. *Stem Cells* 2010; **28**: 704-712
86 **Gore A**, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, Canto I, Giorgetti A, Israel MA, Kiskinis E, Lee JH, Loh YH, Manos PD, Montserrat N, Panopoulos AD, Ruiz S, Wilbert ML, Yu J, Kirkness EF, Izpisua Belmonte JC, Rossi DJ, Thomson JA, Eggan K, Daley GQ, Goldstein LS, Zhang K. Somatic coding mutations in human induced pluripotent stem cells. *Nature* 2011; **471**: 63-67
87 **Pera MF**. Stem cells: The dark side of induced pluripotency. *Nature* 2011; **471**: 46-47
88 **Hussein SM**, Batada NN, Vuoristo S, Ching RW, Autio R, Närvä E, Ng S, Sourour M, Hämäläinen R, Olsson C, Lundin K, Mikkola M, Trokovic R, Peitz M, Brüstle O, Bazett-Jones DP, Alitalo K, Lahesmaa R, Nagy A, Otonkoski T. Copy number variation and selection during reprogramming to pluripotency. *Nature* 2011; **471**: 58-62
89 **Tateishi K**, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* 2008; **283**: 31601-31607
90 **Kim D**, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* 2009; **4**: 472-476
91 **Zhou H**, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Schöler HR, Duan L, Ding S. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 2009; **4**: 381-384
92 **Jia F**, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, Panetta NJ, Chen ZY, Robbins RC, Kay MA, Longaker MT, Wu JC. A nonviral minicircle vector for deriving human iPS cells. *Nat Methods* 2010; **7**: 197-199
93 **Warren L**, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 2010; **7**: 618-630
94 **Shi Y**, Desponts C, Do JT, Hahm HS, Schöler HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell* 2008; **3**: 568-574
95 **Rao MS**, Dwivedi RS, Subbarao V, Usman MI, Scarpelli DG, Nemali MR, Yeldandi A, Thangada S, Kumar S, Reddy JK. Almost total conversion of pancreas to liver in the adult rat: a reliable model to study transdifferentiation. *Biochem Biophys Res Commun* 1988; **156**: 131-136
96 **Rao MS**, Reddy JK. Hepatic transdifferentiation in the pancreas. *Semin Cell Biol* 1995; **6**: 151-156
97 **Rao MS**, Subbarao V, Reddy JK. Induction of hepatocytes in the pancreas of copper-depleted rats following copper repletion. *Cell Differ* 1986; **18**: 109-117
98 **Lee BC**, Hendricks JD, Bailey GS. Metaplastic pancreatic cells in liver tumors induced by diethylnitrosamine. *Exp Mol Pathol* 1989; **50**: 104-113
99 **Shen CN**, Slack JM, Tosh D. Molecular basis of transdifferentiation of pancreas to liver. *Nat Cell Biol* 2000; **2**: 879-887
100 **Nordlie RC**, Foster JD, Lange AJ. Regulation of glucose production by the liver. *Annu Rev Nutr* 1999; **19**: 379-406
101 **Kim HI**, Ahn YH. Role of peroxisome proliferator-activated receptor-gamma in the glucose-sensing apparatus of liver and beta-cells. *Diabetes* 2004; **53** Suppl 1: S60-S65
102 **Melton D**. Signals for tissue induction and organ formation in vertebrate embryos. *Harvey Lect* 1997-1998; **93**: 49-64
103 **Deutsch G**, Jung J, Zheng M, Lóra J, Zaret KS. A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development* 2001; **128**: 871-881
104 **Jung J**, Zheng M, Goldfarb M, Zaret KS. Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* 1999; **284**: 1998-2003
105 **Lemaigre F**, Zaret KS. Liver development update: new embryo models, cell lineage control, and morphogenesis. *Curr*

Opin Genet Dev 2004; **14**: 582-590
106 **Tremblay KD**, Zaret KS. Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues. *Dev Biol* 2005; **280**: 87-99
107 **Yoshitomi H**, Zaret KS. Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. *Development* 2004; **131**: 807-817
108 **Zaret KS**. Hepatocyte differentiation: from the endoderm and beyond. *Curr Opin Genet Dev* 2001; **11**: 568-574
109 **Zaret KS**. Liver specification and early morphogenesis. *Mech Dev* 2000; **92**: 83-88
110 **Wells JM**, Melton DA. Vertebrate endoderm development. *Annu Rev Cell Dev Biol* 1999; **15**: 393-410
111 **Gualdi R**, Bossard P, Zheng M, Hamada Y, Coleman JR, Zaret KS. Hepatic specification of the gut endoderm in vitro: cell signaling and transcriptional control. *Genes Dev* 1996; **10**: 1670-1682
112 **Douarin NM**. An experimental analysis of liver development. *Med Biol* 1975; **53**: 427-455
113 **Cardinale V**, Wang Y, Carpino G, Alvaro D, Reid L, Gaudio E. Multipotent stem cells in the biliary tree. *Ital J Anat Embryol* 2010; **115**: 85-90
114 **Jonsson J**, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 1994; **371**: 606-609
115 **Ahlgren U**, Jonsson J, Jonsson L, Simu K, Edlund H. beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev* 1998; **12**: 1763-1768
116 **Ferber S**, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I, Barshack I, Seijffers R, Kopolovic J, Kaiser N, Karasik A. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. *Nat Med* 2000; **6**: 568-572
117 **Ber I**, Shternhall K, Perl S, Ohanuna Z, Goldberg I, Barshack I, Benvenisti-Zarum L, Meivar-Levy I, Ferber S. Functional, persistent, and extended liver to pancreas transdifferentiation. *J Biol Chem* 2003; **278**: 31950-31957
118 **Meivar-Levy I**, Sapir T, Gefen-Halevi S, Aviv V, Barshack I, Onaca N, Mor E, Ferber S. Pancreatic and duodenal homeobox gene 1 induces hepatic dedifferentiation by suppressing the expression of CCAAT/enhancer-binding protein beta. *Hepatology* 2007; **46**: 898-905
119 **Tang DQ**, Lu S, Sun YP, Rodrigues E, Chou W, Yang C, Cao LZ, Chang LJ, Yang LJ. Reprogramming liver-stem WB cells into functional insulin-producing cells by persistent expression of Pdx1- and Pdx1-VP16 mediated by lentiviral vectors. *Lab Invest* 2006; **86**: 83-93
120 **Wang AY**, Ehrhardt A, Xu H, Kay MA. Adenovirus transduction is required for the correction of diabetes using Pdx-1 or Neurogenin-3 in the liver. *Mol Ther* 2007; **15**: 255-263
121 **Kaneto H**, Matsuoka TA, Nakatani Y, Miyatsuka T, Matsuhisa M, Hori M, Yamasaki Y. A crucial role of MafA as a novel therapeutic target for diabetes. *J Biol Chem* 2005; **280**: 15047-15052
122 **Kaneto H**, Miyatsuka T, Fujitani Y, Noguchi H, Song KH, Yoon KH, Matsuoka TA. Role of PDX-1 and MafA as a potential therapeutic target for diabetes. *Diabetes Res Clin Pract* 2007; **77** Suppl 1: S127-S137
123 **Kaneto H**, Miyatsuka T, Shiraiwa T, Yamamoto K, Kato K, Fujitani Y, Matsuoka TA. Crucial role of PDX-1 in pancreas development, beta-cell differentiation, and induction of surrogate beta-cells. *Curr Med Chem* 2007; **14**: 1745-1752
124 **Matsuoka TA**, Kaneto H, Stein R, Miyatsuka T, Kawamori D, Henderson E, Kojima I, Matsuhisa M, Hori M, Yamasaki Y. MafA regulates expression of genes important to islet beta-cell function. *Mol Endocrinol* 2007; **21**: 2764-2774
125 **Miyatsuka T**, Kaneto H, Kajimoto Y, Hirota S, Arakawa Y, Fujitani Y, Umayahara Y, Watada H, Yamasaki Y, Magnuson MA, Miyazaki J, Hori M. Ectopically expressed PDX-1 in liver initiates endocrine and exocrine pancreas differentiation but causes dysmorphogenesis. *Biochem Biophys Res Commun* 2003; **310**: 1017-1025
126 **Kojima H**, Fujimiya M, Matsumura K, Younan P, Imaeda H, Maeda M, Chan L. NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat Med* 2003; **9**: 596-603
127 **Horb ME**, Shen CN, Tosh D, Slack JM. Experimental conversion of liver to pancreas. *Curr Biol* 2003; **13**: 105-115
128 **Zhou Q**, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* 2008; **455**: 627-632
129 **Aramata S**, Han SI, Kataoka K. Roles and regulation of transcription factor MafA in islet beta-cells. *Endocr J* 2007; **54**: 659-666
130 **Aramata S**, Han SI, Yasuda K, Kataoka K. Synergistic activation of the insulin gene promoter by the beta-cell enriched transcription factors MafA, Beta2, and Pdx1. *Biochim Biophys Acta* 2005; **1730**: 41-46
131 **Zhao L**, Guo M, Matsuoka TA, Hagman DK, Parazzoli SD, Poitout V, Stein R. The islet beta cell-enriched MafA activator is a key regulator of insulin gene transcription. *J Biol Chem* 2005; **280**: 11887-11894
132 **Lee SH**, Hao E, Savinov AY, Geron I, Strongin AY, Itkin-Ansari P. Human beta-cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. *Transplantation* 2009; **87**: 983-991
133 **Fort A**, Fort N, Ricordi C, Stabler CL. Biohybrid devices and encapsulation technologies for engineering a bioartificial pancreas. *Cell Transplant* 2008; **17**: 997-1003
134 **Lin G**, OuYang Q, Zhou X, Gu Y, Yuan D, Li W, Liu G, Liu T, Lu G. A highly homozygous and parthenogenetic human embryonic stem cell line derived from a one-pronuclear oocyte following in vitro fertilization procedure. *Cell Res* 2007; **17**: 999-1007
135 **Revazova ES**, Turovets NA, Kochetkova OD, Agapova LS, Sebastian JL, Pryzhkova MV, Smolnikova VI, Kuzmichev LN, Janus JD. HLA homozygous stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells* 2008; **10**: 11-24
136 **Melnikova I**. RNA-based therapies. *Nat Rev Drug Discov* 2007; **6**: 863
137 **Osman EY**, Yen PF, Lorson CL. Bifunctional RNAs targeting the intronic splicing silencer N1 increase SMN levels and reduce disease severity in an animal model of spinal muscular atrophy. *Mol Ther* 2012; **20**: 119-126

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