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CONVERTING HUMAN PLURIPOTENT STEM CELLS INTO BETA CELLS: RECENT ADVANCES AND FUTURE CHALLENGES

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Abstract

Purpose of review—The transplantation of insulin producing β -cells derived from human embryonic stem cells and induced pluripotent stem cells (collectively termed pluripotent stem cells; PSCs) holds great promise for therapy of diabetes mellitus. The purpose of this review is to summarize recent advances in this area, emphasizing the importance of studies of endocrine pancreas development in efforts to direct PSC differentiation into endocrine cells, as well as to outline the major challenges remaining before transplantation of PSC-derived β -cells can become a reality.

Recent findings—Although several protocols to generate glucose-responsive pancreatic β -cells *in vitro* have been described, the most successful approaches are those that most closely mimic embryonic development of the endocrine pancreas. Until recently, cells generated by these methods have exhibited immature pancreatic endocrine phenotypes. However, protocols that generate more functional β -like cells have now been described. In addition, small molecules are being used to improve protocols to direct differentiation of PSCs into endoderm and pancreatic lineages.

Summary—Advances over the past decade suggest that generating function β -cells from human PSCs is achievable. However there are aspects of β -cell development that are not well understood that are hampering generation of PSC-derived β -cells. In particular the signaling pathways that instruct endocrine progenitor cells to differentiate into mature and functional β -cells are poorly understood. Other significant obstacles remain including the need for safe and cost-effective differentiation methods for large-scale generation of transplantation quality β -cells, methods to prevent immune rejection of grafted tissues, and amelioration of the risks of tumorigenesis.

Keywords

Human embryonic stem cells; induced pluripotent stem cells; diabetes; cell replacement therapy; β -cells

Introduction

Diabetes mellitus is a major worldwide health crisis, affecting more than 200 million people. In the US, nearly 25 million people have been diagnosed with diabetes, and it is predicted that this figure will increase to nearly 50 million by the year 2050 [1]. Diabetes is characterized by

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aberrant blood glucose regulation resulting in prolonged hyperglycemia associated with significant long-term health complications. Type 1 diabetes results from autoimmune destruction of the insulin-producing β -cells of the islets of Langerhans; whereas type 2 diabetes is characterized by peripheral insulin resistance and the inability to produce enough insulin to overcome this resistance. Less common forms of diabetes such as gestational diabetes, maturity onset diabetes of the young, neonatal diabetes mellitus and loss of islets in pancreatitis are also associated with impaired insulin production.

At the core of current type 1 diabetes treatment is injection of exogenous insulin, which provides some level of control over blood glucose levels and has significantly reduced diabetes morbidity [2]. Even in the best cases, glucose monitoring and insulin injections cannot fully compensate for loss of β -cells. While technological advances in glucose monitors and insulin pumps will likely improve efforts to deliver exogenous insulin [3], a more physiological solution is the replacement of β -cells by whole pancreas or islet transplantation [4]. Transplantation-based approaches such as the Edmonton protocol have proven effective [5], however the widespread use of pancreas or islet transplantation is impossible due to a chronic shortage of donors. Consequently, human pluripotent stem cells [6] are a promising, renewable source of cells from which β -cells can be derived for replacement therapy (FIGURE 1) [7,8].

In this review we will summarize the recent progress in generation of β -cells from human PSCs and outline some of the major practical challenges remaining before the widespread clinical use of PSC-derived β -cells can become a reality.

Using Developmental Principles to Guide Differentiation of PSCs to Functional β -Cells

Pluripotent stem cells exhibit stable self-renewal in culture and have the potential to differentiate into all somatic cell types, including pancreatic β -cells. The challenge in directed differentiation of PSCs into β -cells is to specifically generate only one of the ~210 somatic cell types. In the past decade, numerous approaches to generate insulin-producing β -cells from embryonic stem cells have been reported. Initial attempts using mouse [9] and human embryonic stem cells (hESCs) [10,11] included spontaneous *in vitro* differentiation following selection of cells positive for nestin [12] or coupled with ectopic expression of transcription factors known to be important for β -cell development *in vivo* [13-15]. These early studies often resulted in some insulin positive cells, but there was little evidence that these were *bona-fide*, functional endoderm-derived pancreatic β -cells. The limited success using these protocols prompted a more physiologically-based approach that utilized signaling pathways that are required during embryonic β -cell development *in vivo* [16-19]. The more successful of these efforts have directed hESC differentiation in a stepwise fashion that recapitulates all the major stages of β -cell development [20-23], and have resulted in the production of definitive endoderm-derived, mature, glucose responsive β -cells (FIGURE 1). The developmental basis of this approach is described below and reviewed in [24].

β -cell development can grossly be broken down into four steps: endoderm formation, pancreas specification, endocrine specification and beta-cell maturation [24-26] (FIGURE 1). Differentiation into definitive endoderm (DE) is the first obligatory step in generating pancreatic endocrine cells from PSCs. DE is generated *in vivo* by the process of gastrulation, where naïve cells are instructed to form the three primary germ layers: the ectoderm, mesoderm and endoderm. The molecular control of endoderm formation is highly conserved across vertebrate species and involves the Nodal signaling pathway. Nodal is a TGF β ligand whose activity initiates a series of downstream signaling events that culminates in the activation of an evolutionarily conserved transcriptional network that regulates DE development (reviewed

in [27]). Protocols to differentiate hESCs into DE utilize the nodal related protein activin, which is commercially available, is highly bioactive, and mimics nodal activity [28-31].

Definitive endoderm gives rise to a diverse array of cells and tissues that contribute to vital organs including the pancreas, liver, lungs, stomach and the epithelial lining of the alimentary tract. Remarkable progress has been made in defining the embryonic processes that direct a subset of endodermal cells into the pancreatic progenitors that arise from the posterior embryonic foregut. Signaling pathways that direct regionalization of the foregut in mouse involves FGF, BMP and retinoic acid (RA) signaling [32-35]. FGF2 and activin mediate suppression of sonic hedgehog signaling in the posterior foregut, which is required for initiation of pancreas gene expression [36]. Efficient differentiation of hESC-derived DE into the pancreatic lineage has been accomplished by temporally manipulating the FGF, BMP, RA and hedgehog-signaling pathways, thereby directing DE cells first into a foregut (HNF1 β , HNF4) and then into a pancreatic fate (Pdx1, Nkx6.1, Hnf6, Sox9)(FIGURE 1) [21,22].

The next stages of pancreas development involve the proliferation of pancreatic progenitors and their segregation into either exocrine or endocrine cell types, required for digestion and glucose homeostasis, respectively. Expansion of pancreatic progenitor cells involves FGF10 signaling [37] and the decision to become an endocrine or exocrine cell requires the Notch signaling pathway, and endocrine progenitor specification is marked by the expression of the transcription factor NGN3 [38,39]. The expansion and organization of endocrine cells into islets *in vivo* involves EGF signaling [40]. In successfully generating and expanding pancreatic endocrine cells from hESCs, FGF ligands (FGF7 and 10) [21,22] were used to expand pancreatic progenitors, inhibition of notch signaling was used to generate pancreatic endocrine progenitor cells [21], and EGF was used to expand endocrine progenitors [23].

One of the final stages of pancreas development involves maturation of endocrine progenitors to mature hormone-producing cells. Engrafting hESC-derived pancreatic endocrine cells into mice promotes their maturation into β -like cells [22], demonstrating that these cells have the intrinsic ability to form insulin-expressing cells with some functionality. However, *in vitro* attempts to differentiate pancreatic endocrine cells into glucose-responsive, insulin-secreting cells have had mixed success, in part due to our lack of understanding the signaling pathways that direct β -cell maturation *in vivo*. Most of the focus has been placed on signals that are known to affect postnatal β -cell function such as the incretin signaling pathway mediated by GLP1. Activation of this pathway with Extendin 4, which mimics the activity of GLP1, results in an increase in the formation of insulin-positive cells *in vitro* [21]. However these cells are largely fetal/neonatal in character and do not appear to be *bona fide* β -cells. It has recently been shown that islet innervation [41] correlates with the maturation of fetal β -cells. Moreover, β -cells express the receptor for nerve growth factor (NGF) [42] and NGF promotes the maturation of fetal β -cells [43]. This serves to highlight why a better understanding of fetal β -cell maturation *in vivo* will continue to guide efforts to direct maturation of PSCs into β -cells *in vitro*.

Perspective and Future Prospects

Although the concepts and framework for the directed differentiation of β -cells from pluripotent human stem cells are well developed, significant hurdles remain before this approach can be widely used for diabetes therapy. In the following section we will discuss some of these remaining challenges and outline potential solutions to some of these concerns.

Using Small Molecules to Direct PSC Differentiation

Current protocols rely on the use of up to 10 recombinant proteins (cytokines and inhibitors) for directed differentiation of PSCs [21,23]. Given the manufacturing cost of generating high

quality recombinant proteins with consistent biological activity, this approach is prohibitive to generate sufficient numbers of cells for human transplantation [44]. As such, a focus has been shifted to the possible use of small molecules for large-scale, reproducible, directed PSC differentiation under good manufacturing practice (GMP) conditions.

Recent progress has been made with the discovery of small molecules that can modulate various stages of β -cell differentiation from PSCs. Borowiak *et al.* (2009) screened a chemical library and identified compounds that would mimic activin A and induce an endoderm-specific Sox17 promoter in mouse ESCs [45]. The two compounds (IDE1 and IDE2) induced mouse and human ESC differentiation into definitive endoderm cells. IDE1 and IDE2 were shown to induce SMAD2 phosphorylation, indicating that these compounds promote DE formation via the nodal/activin pathway. Furthermore, DE generated using IDE1 and IDE2 initiated pancreatic differentiation in response to RA, FGF10 and cyclopamine (an inhibitor of hedgehog signaling). Another screen of chemical libraries identified Indolactam V, which promoted pancreatic progenitor formation from DE [46].

These findings demonstrate that the availability of chemically diverse small molecule libraries and robust biological assays can lead to the discovery of compounds that can either replace recombinant proteins or enhance their activity [47] in the directed differentiation of PSCs to β -cells. The use of chemical compounds instead of recombinant proteins will facilitate development of GMP protocols for generating therapeutic quality β -cells.

The risk of tumor formation in PSC-derived β -cells

Of the safety concerns associated with transplantation of pluripotent stem cell-derived tissues (reviewed by [48]), one main issue is tumorigenicity. Teratomas, which are benign growths containing tissue types from all three germ layers, have been reported in numerous PSC-derived cell transplantation studies. Studies using hESC-derived insulin-producing cells in some cases reported teratomas [22] where as others observed no tumors [17]. These teratomas are largely thought to derive from undifferentiated hESCs that persist in the differentiated cultures, and cell-sorting based approaches to remove hESCs have helped to reduce the incidence of teratoma formation [49]. Additional approaches to eliminate undifferentiated cells include the use of molecules or antibody-toxin conjugates that selectively kill undifferentiated PSCs. In all cases, PSC-derived and sorted cells will need to be tested for residual tumor generating cells. The current approach, teratoma formation assay in immunocompromised mice, may not be sensitive enough to detect low level PSC contamination and may not recapitulate all the unique biological contexts of different transplantation sites (reviewed in [50]). Given these limitations, the development of additional *in vitro* and *in vivo* techniques that detect residual undifferentiated cells will be critical. While there is little evidence for malignant cell types arising from PSCs, this is something that needs to be fully investigated using animal models.

Where is the ideal anatomical site for PSC-derived β -cell transplant?

The ideal β -cell transplantation site would be one that supports the long-term function and survival of grafted cells and is easily accessible for maximal patient safety (for review see [51]). Current clinical practice is to transplant islets into the liver *via* the portal vein, with the rationale that the majority of insulin released from the pancreas is utilized in the liver and the easy accessibility of this site by a minimally invasive procedure. However, half of the beta-cells die shortly after transplantation [52], and this is thought to be due to low oxygen tension, an active immune response, and high levels of toxins and drugs in the liver. In addition, the instant blood mediated inflammatory reaction (IBMIR) encapsulates transplanted islets in a fibrin clot and enhances the immune reaction against the graft [53]. Therefore, several alternative sites for islet transplantation have been tested including the kidney capsule, omentum, and subcutaneous, which may be best for patient safety but not ideal from a

functional perspective due to systemic release of insulin [53]. An improved understanding of the optimal anatomical sites for islet transplant is important for enhancing the survival and function of islet or PSC-derived β -cells following transplantation.

Requirements for transplant-related immune suppression

Despite reports that hESCs and their differentiated progeny may be non-immunogenic [54, 55], recent studies have documented the development of immune responses against transplanted hESC-derived tissues in immune competent mice [56,57]. Therefore, patients receiving allogeneic hESC-derived β -cells will require life-long immunosuppressive therapy to prevent graft rejection. Additionally, almost all commonly used immunosuppressive drugs are deleterious to β -cell function, replication and survival [58]. Furthermore, although the evolution of induced pluripotent stem cell (iPSC) technology is a promising strategy for generating autologous, patient-specific β -cells (as discussed below), even autologous transplantation for type I diabetes will require the suppression of the preexisting autoimmunity. One promising treatment approach for combating autoimmunity is the use of targeted immunotherapies such as CD3-specific antibodies for induction of long-term tolerance to auto-antigens [59].

In the case of transplant of allogeneic PSC-derived β -cells, several strategies to minimize or eliminate the requirement for immunosuppression are under investigation, including microencapsulation techniques to protect grafted tissues from the immune system (reviewed by [60]), and transplantation of cells into immune privileged sites such as the anterior chamber of the eye or the testis. However, recent studies have demonstrated rejection of islets transplanted in the testis of immunocompetent mice [61,62], indicating that this approach may still require immunosuppression. Furthermore, it is unclear whether these locations will meet the requirements for β -cell transplantation sites discussed above.

Another strategy would be to generate large banks of hESCs for the purpose of matching their HLA-phenotype to recipient transplant patients. This would require generating hundreds to potentially thousands of discrete HLA-typed hESC lines to ensure adequate matching for most individuals [63,64]. This approach would require enormous regulatory oversight and stringent quality control analyses to test for pluripotency and that each line is competent to generate functional β -cells before it could be widely used for cell replacement therapies [65].

Generation of β -cells from induced pluripotent stem cells (iPSCs)

The discovery that human somatic cells can be reprogrammed into an embryonic stem cell-like state that appears to be phenotypically and functionally equivalent to hESCs represents a seminal moment for regenerative medicine [66,67]. Induced pluripotent stem cells (iPSCs) may be an ideal source for cell replacement therapies because they can be derived from patients, including those with diabetes [68,69], and eventually used to generate autologous therapeutic β -cells for transplantation (FIGURE 1). In fact two recent studies describe the generation of insulin producing cells from human iPSCs, supporting the feasibility of this approach to generate patient-specific β -cells for autologous transplantation [23,69].

Original protocols for human iPSC generation employed the retroviral or lentiviral-mediated expression of four transcription factors involved in stem cell pluripotency [66,67]. This approach is not suitable for generation of therapeutic quality cells because of risks from insertional mutagenesis and the use of the oncogene *cMyc*, which caused tumorigenesis in chimeric mice derived from these cells [66,67]. Subsequent studies have reported iPSC generation using non-integrating methods of gene delivery such as plasmid transfection [70], episomal plasmid transfection [71], the *piggyBac* transposon system [72], adenoviral transduction [73,74], Cre-excisable viral vectors [75,76], and most recently by direct

introduction of membrane soluble reprogramming factor proteins [77,78]. With proper, rigorous quality controls [48] and the rapid advances in iPSC technology it is likely that iPSCs will factor heavily into future efforts for cell replacement therapy for diabetes.

Conclusion

There has been significant progress in our understanding of normal pancreas development and this information has been central to recent successes at directing the differentiation of PSCs to β -cells *in vitro* [79]. Further research, particularly into mechanisms of β -cell maturation *in vivo*, will likely prove essential for generation of glucose-responsive, mature β -cells suitable for cell replacement therapy for diabetes. Continued advances in establishing GMP-compliant protocols, enhanced transplantation techniques, and methods for regulation of the immune response will also factor into the success of these endeavors. Moreover, rapid progress in induced pluripotent stem cell technology is likely to prove transformational in future efforts in generating patient-specific β -cells.

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References

- [1]. Narayan KM, Boyle JP, Geiss LS, Saaddine JB, Thompson TJ. Impact of recent increase in incidence on future diabetes burden: U.S., 2005-2050. *Diabetes care* Sep;2006 29(9):2114–6. [PubMed: 16936162]
- [2]. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England journal of medicine* Sep 30;1993 329(14):977–86. [PubMed: 8366922]
- [3]. Sherr, J.; Tamborlane, WV. *The Mount Sinai journal of medicine, New York*. Vol. 75. Aug. 2008 Past, present, and future of insulin pump therapy: better shot at diabetes control; p. 352-61.
- [4]. Nanji SA, Shapiro AM. Advances in pancreatic islet transplantation in humans. *Diabetes, obesity & metabolism* Jan;2006 8(1):15–25.
- [5]. Shapiro AM, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *The New England journal of medicine* Jul 27;2000 343(4):230–8. [PubMed: 10911004]
- [6]. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* Nov 6;1998 282(5391):1145–7. [PubMed: 9804556]
- [7]. Wells JM. Genes expressed in the developing endocrine pancreas and their importance for stem cell and diabetes research. *Diabetes Metab Res Rev* 2003;19:191–201. [PubMed: 12789652]
- [8]. Wells JM, Melton DA. Vertebrate endoderm development. *Annu Rev Cell Dev Biol* 1999;15:393–410. [PubMed: 10611967]
- [9]. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 2000;49(2):157–62. [PubMed: 10868930]
- [10]. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. *Diabetes* Aug;2001 50(8):1691–7. [PubMed: 11473026]
- [11]. Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of human embryonic stem cells into insulin-producing clusters. *Stem Cells* 2004;22(3):265–74. [PubMed: 15153604]

- [12]. 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(5520):1389–94. [PubMed: 11326082]
- [13]. Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, et al. Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100(3):998–1003. [PubMed: 12525695]
- [14]. Levinson-Dushnik M, Benvenisty N. Involvement of hepatocyte nuclear factor 3 in endoderm differentiation of embryonic stem cells. *Mol Cell Biol* 1997;17(7):3817–22. [PubMed: 9199315]
- [15]. Vincent R, Treff N, Budde M, Kastenbergs Z, Odorico J. Generation and characterization of novel tetracycline-inducible pancreatic transcription factor-expressing murine embryonic stem cell lines. *Stem Cells & Development* 2006;15(6):953–62. [PubMed: 17253956]
- [16]. Jiang J, Au M, Lu K, Eshpeter A, Korbitt G, Fisk G, et al. Generation of insulin-producing islet-like clusters from human embryonic stem cells. *Stem Cells* Aug;2007 25(8):1940–53. [PubMed: 17510217]
- [17]. Jiang W, Shi Y, Zhao D, Chen S, Yong J, Zhang J, et al. In vitro derivation of functional insulin-producing cells from human embryonic stem cells. *Cell Res* April;2007 17(4):333–44. [PubMed: 17426693]
- [18]. Phillips BW, Hentze H, Rust WL, Chen QP, Chipperfield H, Tan EK, et al. Directed differentiation of human embryonic stem cells into the pancreatic endocrine lineage. *Stem cells and development* Aug;2007 16(4):561–78. [PubMed: 17784830]
- [19]. Shim JH, Kim SE, Woo DH, Kim SK, Oh CH, McKay R, et al. Directed differentiation of human embryonic stem cells towards a pancreatic cell fate. *Diabetologia* Jun;2007 50(6):1228–38. [PubMed: 17457565]
- [20]. D'Amour KA, Agulnick AD, Eliazar S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol* Oct 28;2005 23:1534–41. [PubMed: 16258519]
- [21]. D'Amour KA, Bang AG, Eliazar S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* Nov;2006 24(11):1392–401. [PubMed: 17053790]
- *[22]. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazar S, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol*. Feb 20;2008 This work demonstrated that developmental principles can be used to direct formation of pancreatic endocrine cells from human ES cells that could restore euglycemia in diabetic mice
- *[23]. Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, et al. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell research* Apr;2009 19(4):429–38. [PubMed: 19255591] In this paper, human iPSCs were differentiated into hormone expressing cells with several functional properties of β -cells.
- [24]. Spence JR, Wells JM. Translational embryology: Using embryonic principles to generate pancreatic endocrine cells from embryonic stem cells. *Developmental Dynamics* 2007;236(5):3218–27. [PubMed: 17973329]
- [25]. Gittes GK. Developmental biology of the pancreas: a comprehensive review. *Dev Biol* Feb 1;2009 326(1):4–35. [PubMed: 19013144]
- [26]. Murtaugh LC. Pancreas and beta-cell development: from the actual to the possible. *Development* Feb;2007 134(3):427–38. [PubMed: 17185316]
- [27]. Zorn AM, Wells JM. Molecular basis of vertebrate endoderm development. *Int Rev Cytol* 2007;259:49–111. [PubMed: 17425939]
- [28]. Gamer LW, Wright CV. Autonomous endodermal determination in *Xenopus*: regulation of expression of the pancreatic gene *XIHbox 8*. *Dev Biol* Sep;1995 171(1):240–51. [PubMed: 7556900]
- [29]. Henry GL, Brivanlou IH, Kessler DS, Hemmati-Brivanlou A, Melton DA. TGF-beta signals and a pre-pattern in *Xenopus laevis* endoderm development. *Development* 1996;122:1007–15. [PubMed: 8631246]

- [30]. Green JB, Smith JC. Graded changes in dose of a *Xenopus* activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature* Sep 27;1990 347(6291):391–4. [PubMed: 1699129]
- [31]. Thomsen G, Woolf T, Whitman M, Sokol S, Vaughan J, Vale W, et al. Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. *Cell* 1990;63:485–93. [PubMed: 2225062]
- [32]. Jung J, Zheng M, Goldfarb M, Zaret KS. Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* Jun 18;1999 284(5422):1998–2003. [PubMed: 10373120]
- [33]. Molotkov A, Molotkova N, Duester G. Retinoic acid generated by Raldh2 in mesoderm is required for mouse dorsal endodermal pancreas development. *Dev Dyn* Apr;2005 232(4):950–7. [PubMed: 15739227]
- [34]. Rossi JM, Dunn NR, Hogan BL, Zaret KS. Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. *Genes Dev* Aug 1;2001 15(15):1998–2009. [PubMed: 11485993]
- [35]. Wang Z, Dolle P, Cardoso W, Niederreither K. Retinoic acid regulates morphogenesis and patterning of posterior foregut derivatives. *Dev Biol.* 2006
- [36]. Hebrok M, Kim SK, Melton DA. Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes Dev* Jun 1;1998 12(11):1705–13. [PubMed: 9620856]
- [37]. Bhushan A, Itoh N, Kato S, Thiery JP, Czernichow P, Bellusci S, et al. Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development* Dec;2001 128(24):5109–17. [PubMed: 11748146]
- [38]. Apelqvist A, Li H, Sommer L, Beatus P, Anderson DJ, Honjo T, et al. Notch signalling controls pancreatic cell differentiation. *Nature* 1999;400(6747):877–81. [PubMed: 10476967]
- [39]. Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* 2002;129(10):2447–57. [PubMed: 11973276]
- [40]. Miettinen PJ, Huotari M, Koivisto T, Ustinov J, Palgi J, Rasilainen S, et al. Impaired migration and delayed differentiation of pancreatic islet cells in mice lacking EGF-receptors. *Development* Jun; 2000 127(12):2617–27. [PubMed: 10821760]
- [41]. Burris RE, Hebrok M. Pancreatic innervation in mouse development and beta-cell regeneration. *Neuroscience* Dec 12;2007 150(3):592–602. [PubMed: 18006238]
- [42]. Kanaka-Gantenbein C, Dicou E, Czernichow P, Scharfmann R. Presence of nerve growth factor and its receptors in an in vitro model of islet cell development: implication in normal islet morphogenesis. *Endocrinology* Jul;1995 136(7):3154–62. [PubMed: 7789343]
- [43]. Navarro-Tableros V, Fiordelisio T, Hernandez-Cruz A, Hiriart M. Nerve growth factor promotes development of glucose-induced insulin secretion in rat neonate pancreatic beta cells by modulating calcium channels. *Channels (Austin, Tex Nov-Dec;2007* 1(6):408–16.
- [44]. Zaret KS. Using small molecules to great effect in stem cell differentiation. *Cell Stem Cell* May 8;2009 4(5):373–4. [PubMed: 19427285]
- *[45]. Borowiak M, Maehr R, Chen S, Chen AE, Tang W, Fox JL, et al. Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. *Cell Stem Cell* Apr 3;2009 4(4):348–58. [PubMed: 19341624] This work describes the discovery of small molecules that can direct PSC differentiation into definitive endoderm.
- *[46]. Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nature chemical biology* Apr;2009 5(4): 258–65. This work describes the discovery of small molecules that can direct PSC-derived definitive endoderm differentiation into pancreatic endoderm.
- [47]. Zhu S, Wurdak H, Wang J, Lyssiotis CA, Peters EC, Cho CY, et al. A small molecule primes embryonic stem cells for differentiation. *Cell Stem Cell* May 8;2009 4(5):416–26. [PubMed: 19427291]
- [48]. Carpenter MK, Frey-Vasconcells J, Rao MS. Developing safe therapies from human pluripotent stem cells. *Nat Biotechnol* Jul;2009 27(7):606–13. [PubMed: 19587662]
- [49]. Chung S, Shin BS, Hedlund E, Pruszk J, Ferree A, Kang UJ, et al. Genetic selection of sox1GFP-expressing neural precursors removes residual tumorigenic pluripotent stem cells and attenuates tumor formation after transplantation. *J Neurochem* Jun;2006 97(5):1467–80. [PubMed: 16696855]

- [50]. Knoepfler PS. Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. *Stem Cells* May;2009 27(5):1050–6. [PubMed: 19415771]
- [51]. Merani S, Toso C, Emamaullee J, Shapiro AM. Optimal implantation site for pancreatic islet transplantation. *The British journal of surgery* Dec;2008 95(12):1449–61. [PubMed: 18991254]
- [52]. Fiorina P, Shapiro AM, Ricordi C, Secchi A. The clinical impact of islet transplantation. *Am J Transplant* Oct;2008 8(10):1990–7. [PubMed: 18828765]
- [53]. van der Windt DJ, Echeverri GJ, Ijzermans JN, Cooper DK. The choice of anatomical site for islet transplantation. *Cell Transplant* 2008;17(9):1005–14. [PubMed: 19177837]
- [54]. Drukker M, Katchman H, Katz G, Even-Tov Friedman S, Shezen E, Hornstein E, et al. Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells* Feb;2006 24(2):221–9. [PubMed: 16109762]
- [55]. Li L, Baroja ML, Majumdar A, Chadwick K, Rouleau A, Gallacher L, et al. Human embryonic stem cells possess immune-privileged properties. *Stem Cells* 2004;22(4):448–56. [PubMed: 15277692]
- [56]. Grinnemo KH, Kumagai-Braesch M, Mansson-Broberg A, Skottman H, Hao X, Siddiqui A, et al. Human embryonic stem cells are immunogenic in allogeneic and xenogeneic settings. *Reproductive biomedicine online* Nov;2006 13(5):712–24. [PubMed: 17169186]
- [57]. Swijnenburg RJ, Schrepfer S, Govaert JA, Cao F, Ransohoff K, Sheikh AY, et al. Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proc Natl Acad Sci U S A* Sep 2;2008 105(35):12991–6. [PubMed: 18728188]
- [58]. Robertson RP. Islet transplantation as a treatment for diabetes - a work in progress. *The New England journal of medicine* Feb 12;2004 350(7):694–705. [PubMed: 14960745]
- [59]. Chatenoud L, Bluestone JA. CD3-specific antibodies: a portal to the treatment of autoimmunity. *Nature reviews* Aug;2007 7(8):622–32.
- [60]. Fort A, Fort N, Ricordi C, Stabler CL. Biohybrid devices and encapsulation technologies for engineering a bioartificial pancreas. *Cell Transplant* 2008;17(9):997–1003. [PubMed: 19177836]
- [61]. Nasr IW, Wang Y, Gao G, Deng S, Diggs L, Rothstein DM, et al. Testicular immune privilege promotes transplantation tolerance by altering the balance between memory and regulatory T cells. *J Immunol* May 15;2005 174(10):6161–8. [PubMed: 15879112]
- [62]. Schneider M, Barrou B, Morin S, Bitker MO, Debre P, Richard F. Pancreatic islet transplantation: failure to prolong intratesticular islet allograft survival. *Transplantation proceedings* Dec;2000 32(8):2739. [PubMed: 11134779]
- [63]. Rao MS, Auerbach JM. Estimating human embryonic stem-cell numbers. *Lancet* Feb 25;2006 367(9511):650. [PubMed: 16503462]
- [64]. Taylor CJ, Bolton EM, Pocock S, Sharples LD, Pedersen RA, Bradley JA. Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. *Lancet* Dec 10;2005 366(9502):2019–25. [PubMed: 16338451]
- [65]. Osafune K, Caron L, Borowiak M, Martinez RJ, Fitz-Gerald CS, Sato Y, et al. Marked differences in differentiation propensity among human embryonic stem cell lines. *Nat Biotechnol* Mar;2008 26(3):313–5. [PubMed: 18278034]
- [66]. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* Nov 30;2007 131(5):861–72. [PubMed: 18035408]
- [67]. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* Dec 21;2007 318(5858):1917–20. [PubMed: 18029452]
- *[68]. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. *Cell* Sep 5;2008 134(5):877–86. [PubMed: 18691744] This was the first reported evidence that human somatic cells isolated from patients with common diseases could be reprogrammed into pluripotent stem cells.
- [69]. Maehr R, Chen S, Snitow M, Ludwig T, Yagasaki L, Goland R, et al. Generation of pluripotent stem cells from patients with type 1 diabetes. *Proc Natl Acad Sci U S A*. Aug 31;2009
- *[70]. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 2008;322(5903):949–53. [PubMed: 18845712]

In this work, plasmid transfection was used to generate iPSCs, demonstrating that reprogramming could be done safely without the use of DNA-integration.

- [71]. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* May 8;2009 324(5928):797–801. [PubMed: 19325077]
- [72]. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hamalainen R, et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* Apr 9;2009 458(7239):766–70. [PubMed: 19252478]
- *[73]. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* Nov 7;2008 322(5903):945–9. [PubMed: 18818365]
In this work, adenoviral vectors were used to generate iPSCs, demonstrating that reprogramming could be done safely without the use of DNA-integrating viruses.
- [74]. Zhou W, Freed CR. Adenoviral Gene Delivery Can Reprogram Human Fibroblasts to Induced Pluripotent Stem Cells. *Stem Cells*. Aug 20;2009
- [75]. Chang CW, Lai YS, Pawlik KM, Liu K, Sun CW, Li C, et al. Polycistronic lentiviral vector for “hit and run” reprogramming of adult skin fibroblasts to induced pluripotent stem cells. *Stem Cells* May; 2009 27(5):1042–9. [PubMed: 19415770]
- *[76]. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, et al. Parkinson’s disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* Mar 6;2009 136(5):964–77. [PubMed: 19269371] These findings represent an important translational advance because they demonstrate the feasibility of deriving patient-specific iPSCs that are free of integrated reprogramming factors yet maintain the ability to differentiate into neurons.
- *[77]. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* Jun 5;2009 4(6):472–6. [PubMed: 19481515] This report describes a DNA-free approach to reprogram human somatic cells using a protein transduction-based system.
- [78]. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* May 8;2009 4(5):381–4. [PubMed: 19398399]
- [79]. Baetge EE. Production of beta-cells from human embryonic stem cells. *Diabetes, obesity & metabolism* Nov;2008 10(Suppl 4):186–94.

to the right of the center column. Efficient differentiation is indicated by co-expression of these markers at the protein level in a high proportion of cells. Evolving iPSC technologies are currently being used for reprogramming patient-derived cells into autologous PSCs. When efficient, therapeutic-quality differentiation protocols are established, iPSCs converted to mature beta cells could be used for cell replacement therapy for people with diabetes (right column). PI3K, Phosphoinositide 3-kinase; RA, retinoic acid; HH, hedgehog; GLP1, glucagon-like peptide 1; d panc, dorsal pancreas; v panc, ventral pancreas. Schematics in the left panel have been adapted from previous reviews [7,8] with permission.