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Stem cell approaches for the treatment of type 1 diabetes mellitus

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About 23.6 million people or 7.8% of the population in the USA are afflicted with diabetes. Another 57 million have prediabetes, people who are prone to develop frank diabetes in the coming years. The total costs of diagnosed diabetes were estimated to be $174 billion in 2007, which included $116 billion for direct medical costs and $58 billion for indirect costs (resulting from disability, work loss, and premature mortality).[1] Type 2 diabetes accounts for some 90% of patients whereas type 1a (autoimmune) diabetes accounts for most of the other diabetic patients. Individuals with type 1 diabetes have absolute insulin deficiency, whereas those with type 2 disease, relative insulin deficiency usually in the presence of insulin resistance. Insulin injections are the standard therapy for type 1 diabetes. Unfortunately, insulin secretion is exquisitely sensitive to the minute to minute changes in blood glucose, and glycemia stimulated insulin secretion (GSIS) cannot be mimicked by exogenous insulin injections. Pancreas transplantation is an effective mode of therapy, but is associated with considerable operative morbidity and mortality. Whilst the initial success with the Edmonton protocol2 popularized pancreatic islet transplantation as a form of treatment, this option has been found to have some severe limitations3. Donor availability limits the number of transplants that can be done; the need for lifetime immunosuppression is another problem. Moreover, though short-term experiments indicate that in good centers, up to ~80% of transplant recipients can become free of insulin injections shortly after the procedure, long-term follow up indicates that the vast majority of them are back on insulin again within five years.4 These difficulties have prompted research into the development of innovative methods to generate new β cells in the body.

There are two experimental approaches to generate new β cells: [1] reprogramming of non-β cells to β cells using gene therapy in vivo, or [2] reprogramming of isolated non-β cells to β cells in vitro. To determine if true reprogramming has occurred, stringent criteria must be fulfilled to exclude hybdrid cells or cell mimicry.[5] Whilst insulin production is a necessary criterion, it is not sufficient. The newly reprogrammed cells should be fully characterized to determine how closely they resemble functional β cells by their morphology, expression profile, and whether they exhibit GSIS. Not all non-β cells are equally susceptible to reprogramming. Below we review the different strategies and types of cells that have been studied with respect to the generation of new β cell by reprogramming.

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Directed Differentiation/Transdifferentiation of Pancreatic Cells into β Cells

One theme that has been extensively explored by researchers is to create new β cells from existing pancreatic cells. The rationale behind this approach is that since these cells are either β cell precursors or developmentally related to β cells, the barrier to reprogramming them into functional β cells may be lower than from cells that are not as closely related developmentally.

Pancreatic Islet Progenitor Cells

Under normal conditions, β cells beget β cells, and β cell proliferation contributes to most, if not all, normal growth and expansion of β cells during life and after partial pancreatectomy.[6,7] During fetal development, pancreatic islet progenitor cells are the source of all cells of the endocrine pancreas. It is unclear whether pancreatic islet progenitor cells are also a source of cells that repopulates the pancreas after injury. Whilst the transcription factor, Pdx1 marks early pancreatic endoderm cells, another transcription factor, Ngn3, is the lineage-defining transcription factor that has been used as an endocrine precursor cell marker.8 Ngn3 is necessary for the development of all endocrine islet cell types from the Pdx1-positive progenitor cells; inactivation of Ngn3 in mice leads to complete absence of these hormone-producing cells in the pancreas of these animals.9 Ngn3 is expressed in these early fetal cells and then almost completely disappears as the endocrine cells mature.10 There is recent evidence, however, that Ngn3 mRNA and protein may be expressed at a low level in adult pancreatic endocrine cells, and play a role in β cell maintenance in mice during adulthood.[11] Ngn3-positive cells are readily found in the embryonic pancreas, during regeneration and after pancreatic ductal ligation. The capacilty of Ngn3-positive ductal cells to differentiate into b-like cells was demonstrated in a recent study in which microinjection of Ngn3-positive ductal cells isolated from the injured pancreas into early embryonic pancreas explants (to provide the fetal pancreas microenvironment) led to the development of insulin-producing cells.[12] Although this study indicates that ductal cells are a potential source of β cells, at the time of this review article, whether ductal cells contribute in any substantive way to new β cell formation following injury-induced loss of β cell mass remains controversial13 (see section on *Pancreatic Ductal Cells* below).

Beta Cells

B cells have been shown to regenerate during normal growth, after partial pancreatectomy, and during pregnancy[14]. Lineage tracing has demonstrated that newly generated β cells in the adult pancreas come directly from mature β cells.[6,7] Natural β cell regeneration has been explored as an option to obtain more β cells to rescue diabetes. Most studies in this area have only focused on determining whether or not β cells can regenerate themselves, and at what rate regeneration occurs. Unfortunately, natural regeneration has been found to occur at an extremely slow rate15 and seems to be a relatively rare event.[6,7] Understanding the regulatory mechanism controlling this event holds the key to create more β cells from β cells. Different molecules have been found to stimulate β cell proliferation via vastly different mechanisms. For example, treatment with glucagon-like peptide 1 (GLP-1) stimulates β cell proliferation[16], which has become a drug target for type 2 diabetes.17 However, the regulation process of β cell regeneration and proliferation still remains relatively unknown. Lack of information on how this process is regulated makes it difficult to use this method to generate a sufficient amount of new β cells to restore β cell function.

Alpha Cells

It was demonstrated recently that under conditions of extreme β cell loss, pancreatic α cells, which normally produce glucagon, can give rise to β cells in vivo.18 Although the mechanism was not explored, the observation suggests that appropriate manipulation of alpha cells can generate β cells. That it is possible to direct the differentiation of α cells to β cells was

demonstrated by Collombat P et al[19] who expressed the transcription factor Pax4 in α cells in mice, and managed to reprogram them to become β cells. That led to an increase in the formation of new α cells, requiring the re-expression of Ngn3 apparently in response to the glucagon deficiency, which new α cells were again reprogrammed into additional β cells. The animals developed greatly expanded β cell mass, but for unknown reasons, eventually developed diabetes. The reprogramming of α cells to β cells using a transgenic animal approach is evidently not a therapeutic option, however, the report indicates that under the right conditions, exploitation of this α-to-β reprogramming may constitute one way of generating new β cells. Perhaps such an approach can be used to advantage in type 2 diabetes, in which reduction of relative β cell mass (in the context of an increased demand for β cells) is accompanied by an increased complement of α cells.

Pancreatic Ductal Cells

Recently Inada A et al.[20] used an inducible carbonic anhydrase II-Cre marker, specific for pancreatic duct cells, to track the duct cells that appeared after duct ligation and showed that several weeks later, the marked cells seemed to have become β cells.[21] On the other hand, Solar M et al.[22], using a different lineage tracing method, concluded that, whereas pancreatic duct cells contribute to β cells during embryogenesis, they fail to do so after birth. Another recent study by Kopinke and Murtaugh[23] came to the same conclusion. It is noteworthy that the lineage tracing techniques used in all these studies have limitations[13] and the issue whether ductal cells are a natural source of β cells post-natally remains an open question. It is clear, however, that under conditions of pancreatic ductal ligation, Ngn3-positive duct cells appear, and under the right in vitro conditions, can become insulin-producing cells.[12]

Pancreatic Exocrine Cells

The pancreatic exocrine cells constitute the major cell type in the pancreas, and are also derived from Pdx1-positive progenitor cells. Since they are abundant and share a similar lineage history with endocrine cells during embryogensis, it is reasonable to speculate that these cells could be reprogrammed to pancreatic β cells. Under appropriate conditions, exocrine cells can be induced to assume phenotypic characteristics of β cells.[24,25] Zhou et al. recently showed that reprgogramming into β cells can be effected in vivo by transferring the genes for three transcription factors, Pdx1, Ngn3 and MafA, into exocrine cells.[26] Lineage tracing confirmed that the insulin-positive cells were derived from cells that once expressed carboxypeptidase A1, supporting transdifferentiation from a cell of pancreatic exocrine origin. The treatment improved streptozotocin-induced diabetes in the treated mice. A concern in translating this strategy to humans using this approach is the risk of pancreatitis caused by direct injections of adenovirus particles into pancreatic tissues.

Transdetermination of Liver Progenitor Cells into β Cells

The liver and pancreas both originate from the gut endoderm and share common progenitors. 27 The liver has natural regeneration capabilities and is also an accessible target organ for gene therapy and thus a potential source of cells for induced islet neogenesis, if cells in the liver can be reprogrammed to become β cells. Normally, hepatocytes regenerate themselves after partial hepatectomy. However, when hepatocytes are treated with certain chemicals that prevent them from proliferating, a liver progenitor cell population known as oval cells repopulates the liver by differentiating into both hepatocytes and biliary duct cells. These oval cells originate in the periportal region of the liver at the canals of Hering.28 Hepatic oval cells may be related to pancreatic progenitor cells and have been shown to become insulin-producing cells under appropriate circumstances.29 When cultured in vitro in a high glucose environment for prolonged periods, oval cells were found to acquire the capable to produce insulin.[30] Oval cell induction in rodents using special diets (e.g., 3,5-diethoxycarbonyl-1,4-dihydrocollidine or

DDC) followed by STZ treatment was found to lead to low level insulin production by the newly formed oval cells.[29]

Ferber et al.31 first targeted the liver using first generation adenovirus (FGAd) to deliver the Pdx1 gene to the liver of STZ-diabetic mice and saw the appearance of rare insulin-positive cells in the liver of treated mice. The dose of Pdx1 delivered was limited by the toxicity of FGAd and only a miniscule amount of insulin was produced.32 The origin of these insulin-positive cells was unclear. Kojima et al.33 also delivered Pdx1 to the liver of STZ-diabetic mice and induced insulin production in the liver of treated mice. However, instead of FGAd they used helper-dependent adenovirus (HDAd) to deliver the transgene, because of the much higher efficiency and markedly reduced toxicity of HDAd as compared to FGAd. They observed that, with high level Pdx1 expression, the animals treated with HDAd-Pdx1 died of fulminant hepatitis because of the simultaneous expression of pancreatic exocrine enzymes in the same cells that produced insulin. In the absence of organized pancreatic ducts draining the enzymes into the gut, the exocrine enzymes caused autodigestion of the liver cells killing the treated animals. In retrospect, this finding was not unexpected, because Pdx1 is expressed by pancreatic progenitor cells during early fetal development, which give rise to both exocrine and endocrine cells. Hepatic expression of Pdx1 in transgenic mice was also found to cause severe hepatic dysmorphogenesis and premature death.34 To circumvent this undesirable effect of Pdx1, Kojima et al. elected to use B2/NeuroD, a transcription factor downstream of Pdx1, to induce in the liver robust islet neogenesis that encompassed cells that produced all four major islet hormones (insulin, glucagon, somatostatin and pancreatic polypeptide).33 The insulin secretion from the liver exhibited GSIS and stably reversed the diabetes in the STZ-treated mice. However, the origin of the newly formed insulin-producing cells following HDAd-B2/NeuroD treatment was not addressed in this study.

Recently, Yechoor et al. used HDAd to deliver the genes for Ngn3 and βcellulin (a β cell growth factor) to the liver of STZ-diabetic mice and induced neo-islet formation in the periportal region of the treated animals. The islets contained cells that produced the four major islet hormones, and the robust insulin production exhibited GSIS and stably reversed diabetes. Lineage tracing in combination with immunocytochemical and histological analyses indicate that the insulin-producing cells came from hepatic oval cells[35]. These newly formed β-like cells exhibited an ultrastructure as well as a transcription profile very similar to those of authentic β cells.36 The process of differentiation of an adult stem cell population into terminally differentiated cells from adult stem cells of a developmentally related organ is known as transdetermination.37, 38 This treatment is effective in reversing diabetes in essentially 100% of STZ-mice. The proof-of-concept experiment in mice is valuable, and may be a starting point for further research exploiting the process of transdetermination to treat diabetes.

Generation of β Cells from Embryonic and Induced Pluripotent Stem Cells

In parallel with the directed differentiation of the different types of cells discussed above, there are alternative sources of progenitor cells that are susceptible to induced differentiation into β-like cells in vitro. One potential cell population that has been used for this purpose is the mesenchymal stem cell (MSC). Friedenstein first described the isolation of multipotential stromal cells from the bone marrow as colony-forming unit fibroblasts (CFU-Fs),39 and showed that they can differentiate into adipocytes, chondrocytes and osteocytes, both in vitro and in vivo.40 These cells have been called MSCs, and others have reported the induced "transdifferentiation" of these cells into cells from unrelated germline lineages.41 Although the induced differentiation of MSCs into insulin-producing cells in vitro has been reported by different groups (reviewed by Vija et al.,42; also see references therein), most studies used insulin production as the only criterion and did not perform the analyses needed to document that these are b-like cells in terms of their gene expression profile, ultrastructure and capacity

for GSIS. Furthermore, there is growing concern that the name MSC has been used loosely, i.e., MSCs represent different cells to different people, and much uncertainty remains with respect to the identity and characterization of MSCs,[43,44] e.g., the ability of these cells to generate multiple lineages by a single MSC has not been demonstrated. Recent information indicates that a perivascular population of MSCs has a capacity to regenerate skeletal muscle and has potent trophic and immunomodulatory properties when injected in vivo.[45-48] It is unclear if these better defined perivascular MSCs are capable of undergoing induced differentiation into β-like cells.

In contrast to MSCs, there are two well studied sources of progenitor cells that can be expanded in large amounts in vitro: embryonic stem cells (ESCs) and their functional equivalent: induced-pluripotent stem cells (iPSCs). The major benefit of using ESCs or iPSCs to generate functional islet cells is their unique ability to divide indefinitely in culture while maintaining an undifferentiated fate in a process known as self-renewal. In theory this property endows ESCs with the potential of being an unlimited source for any cell type in the human body, a rather profound prospect that drives the enthusiasm behind developing therapeutic applications for ESCs. For this prospect to be realized, one must first undertake the arduous task of establishing differentiation conditions necessary for deriving specific cell types.

Embryonic Stem Cells (ESCs)

After the isolation and propagation of the first mouse ESC lines in 1981 by Martin Evans and Matthew Kaufmann, developmental biologists immediately recognized ESCs potential as a tool for gene targeting in the mouse.[49] This breakthrough led to Martin Evans being named a co-recipient of the Nobel Prize in Physiology or Medicine in 2007. As researchers witnessed first-hand the ability of ESCs to give rise to tissues of each germ layer (ectoderm, mesoderm, and endoderm) as well as the germ cell lineage, a feature referred to as “pluripotency”, questions regarding their therapeutic potential were raised. It was to this end that James Thomson became the first to isolate and propagate a human ESC line in culture in 1998.[50] Widely regarded as a benchmark for the field, the generation of the first human ESC line also raised public awareness due in part to ethical issues stemming from the research. Since then, advances in the field of ESC research have led to a better understanding of self-renewal and differentiation, allowing for the nascent field of induced pluripotency/somatic cell reprogramming to emerge.

At the molecular level, ESCs maintain self-renewal primarily through the regulation of transcriptional networks that either promote or repress pluripotency. Many different pathways are known to influence ESC pluripotency, however typically they all do so by acting on the transcription factors: Oct4, Nanog, and Sox2; for a detailed review see (Boiani *et al.* 2005).[51] In addition to exhibiting embryonic lethal phenotypes, genetic studies of these factors found that each supports self-renewal in culture, and upon genetic ablation pluripotency is either impaired or lost altogether.[52-55] Later studies found that these factors function *in vivo* as heterodimeric/trimeric complexes to directly promote expression of factors that permit self-renewal, and repress expression of factors that promote differentiation.[56-58] Importantly, the Oct4/Nanog/Sox2 complex mediates their own expression as well, thereby forming a robust autoregulatory feed-forward loop to ensure the integrity of the primary pluripotency axis.[56]

Proper differentiation of ESCs requires repression and silencing of the primary pluripotency axis occurring coordinately with induction of a specific differentiation program. The orphan nuclear receptor *Germ Cell Nuclear Factor* (GCNF) plays an important role in suppressing pluripotency by initiating the silencing of the Oct4 gene.[59,60] During differentiation GCNF is recruited to the Oct4 proximal promoter where it oversees the repression of Oct4 gene expression, as well as Oct4 silencing by promoting DNA methylation. Aberrant re-expression of Oct4 is not a trivial concern as Oct4 is known to be a potent oncogene.[61]

Despite the regenerative potential of ESCs, several caveats exist which may make the cells less suitable as a source for tissue transplantation. The tumorigenic potential of ESCs is one noteworthy issue.[62] A standard assay in ESC research is teratoma formation in which undifferentiated ESCs are injected into SCID-nude mice to investigate their ability to differentiate into tissues of the three germ layers. Over the course of only weeks, the small population of ESCs gives rise to large tumors, which when analyzed may contain organ-like structures, teeth, and hair. Any future protocol for differentiating ESCs for transplantation must take measures to ensure that only a pure population of differentiated cells is being implanted so as to prevent teratoma formation in the recipient. Techniques such as cell sorting may be helpful in addressing this, but often the cell quantity or quality may be affected by such techniques. Employing "suicide genes" to kill off undifferentiated cells has been presented as another possible measure to address this issue, however this method requires genomic integration which itself is undesirable.[63] Interestingly, it was observed in human ESC differentiation studies that differentiation occurs symmetrically to give rise to two differentiated daughter cells rather than asymmetrically which a is characteristic of many adult stem cells.[64] This feature of ESCs ought to enhance the differentiation signal in culture and may help ameliorate their teratogenic potential.

Another major caveat to using ESCs for therapeutic applications is the issue of immune rejection of host cells in the recipient. Using any existing ESC line as a source for tissue transplantation invariably means having to cope with decreased transplant efficiency, and likely a requirement for immunosuppressive drugs. While increasing the genetic diversity of hESC lines may make it possible to tailor future treatments for some diseases, ideally any cell line that serves as a source for tissue transplantation ought to be the exact genetic complement of the recipient. This prospect can only be realized by therapeutic cloning, use of multipotent adult stem cells, or iPSC generation.

Induced Pluripotent Stem Cells (iPSCs)

The term "induced pluripotency" refers to the reprogramming of a somatic cell into a cell with a pluripotent phenotype. Although the field of iPSC is still in its infancy, the concept of somatic cell reprogramming is not. The idea was demonstrated half a century ago in Xenopus studies in which clonal embryos were derived after somatic cell nuclei were injected into an enucleated oocyte.[65] This procedure is referred to as somatic cell nuclear transfer (SCNT) and was later performed to generate "Dolly" the sheep, the first cloned mammal in 1996.[66] However, SCNT is not likely to be utilized as a therapeutic source, as the derivation of human ES cell lines has not yet been demonstrated, and the procedure requires human oocytes; the derivation of which is both invasive and brings with it additional ethical considerations and clinical risks.

A promising method of *in vitro* iPSC generation has been established by Shinya Yamanaka in which somatic cells can be directly reprogrammed in culture through ectopic expression of defined factors,[67,68] for a detailed review see (Jaenisch 2008)[69]. While iPSCs generated by this method are not identical to ESCs,[70,71] they do exhibit many ESC characteristics such as high telomerase activity, hypomethylation of endogenous pluripotency gene promoters, as well as reactivation of the X-chromosome in XX cell lines.[68,72] Most importantly, they share the same developmental potential as ESCs to give rise to each somatic cell type of the organism. This has been illustrated in the mouse by demonstrating germ-line transmission of iPSCs[73], as well as the creation of viable mice from iPS cells via tetraploid embryo complementation, [74-76] providing the most compelling evidence for pluripotency.

The generation of iPSCs *in vitro* will likely be favored over using ESCs for tissue transplantation in the future due to three major benefits. Firstly, the iPSCs are patient specific thereby minimizing the likelihood of immune rejection after transplantation. Secondly, the procedure is both relatively quick and becoming increasingly efficient. Additionally it does

not present the same technical limitations that SCNT or ESC fusion does. And finally, the procedure does not require oocytes or the destruction of an embryo therefore two major ethical concerns are circumvented.

The direct reprogramming of somatic cells *in vitro* was first established in the mouse using embryonic and adult fibroblasts.[67] The study involved screening the function of 24 candidate factors expressed in ESCs, for their ability to facilitate reprogramming. Among those candidates, the pool was narrowed to four transcription factors found to be sufficient for iPSC formation: *Oct4, Sox2, Klf4,* and *c-Myc*. Although iPSCs from the initial study were not germ-line competent, they were able to differentiate into tissues of each germ layer in teratoma formation assays. Only a year later did the same group demonstrate reprogramming in human dermal fibroblasts using a similar protocol.[68] After retroviral transduction of *Oct4, Sox2, Klf4,* and *c-Myc*, targeted fibroblasts were plated on mouse embryonic fibroblasts (MEFs) and cultured under standard ESC conditions. After 3-4 weeks, hESC-like colonies emerge that can be picked and expanded *in vitro*. The derived human iPSC lines expressed many hESC markers, exhibited high telomerase activity, differentiated into tissues of the three germ layers upon teratoma formation, and could be directly differentiated into neural and cardiac cells.[68] These two studies published simultaneously invigorated the field as they confirmed the generation of human iPSCs by transduction with the same four transcription factors as in mice, or by substituting *Nanog* and *Lin28* for *Klf4* and *c-Myc*.[77,78] In the years since, hundreds of studies have come out describing various methods and combinations of factors sufficient for reprogramming, as well as characterizations of different somatic cells that are more efficiently reprogrammed; reviewed in (Kiskinis 2010).[79] Importantly, iPSC lines have also been generated from patients suffering from a variety of neuro-degenerative disorders as well as type-1 diabetes lending a proof-of-principle towards future iPSC treatments.[80-82]

The potential use of iPSCs in the clinic also raises unique hurdles, which must be overcome before they can be successfully incorporated into any therapeutic regimen. As described with ESCs, iPSCs also exhibit enormous tumorigenic potential.[83] This will require that stringent quality control measures be implemented on any tissue derived from iPSC prior to transplantation. In addition to employing cell sorting techniques and suicide genes to enrich the target population, a broadening of our understanding of differentiation through basic research will garner improved differentiation protocols and will likely prove most effective in overcoming this issue.

Another drawback to using iPSCs in the clinic is that reprogramming typically requires genomic integration to drive exogenous gene expression. The fact that three out of the four original reprogramming factors: c-Myc, Oct4, and Klf4, are known oncogenes or have oncogenic potential makes it all the more risky to apply the unmodified approach to generate iPSCs.[62,84,85] Although it is reported that the transgenes undergo silencing after reprogramming has occurred,[67] the risk of reactivation and subsequent transformation of transplanted tissue is just too great. Viral mediated genomic integration itself is not desirable either as there is potential for integrating at genomic loci that may disrupt essential signaling axes that may not be apparent until well after reprogramming. Fortunately, recent reports have addressed this issue by using episomal vectors to reprogram, which do not require genomic integration,[86] through *piggyBac* transposition to remove transgenes post-reprogramming,[87] or by using recombinant proteins to direct reprogramming[88]. Establishing a highly efficient protocol for reprogramming that preserves the genomic landscape, perhaps by incorporating small molecules and inhibitors, is currently a major objective for the field.[89,90]

Differentiation of ESCs into functional pancreatic endocrine cells

Establishing viable differentiation protocols to progress from a pluripotent ES cell to a functional somatic cell is a major focus of research in the ESC field. Though much work remains, considerable progress has been made in defining conditions necessary for cardiomyocyte differentiation,[91] neuronal derivation,[92] and pancreatic endocrine differentiation in both the ESC and the iPSC.[93-95] Of particular therapeutic interest is the development of a protocol for the differentiation of pluripotent cells to β-cells of the pancreas. Because insulin can be expressed in cell lineages besides β-cells, insulin production alone is insufficient as a marker for β-cell differentiation. The successful differentiation of ESCs to β-cells requires that differentiated cells synthesize and secrete physiologically appropriate amounts of insulin that is subject to GSIS. To date this benchmark is yet to be fulfilled in a stringent manner either in culture or in vivo despite promising developments.

The progress made thus far in elucidating a differentiation scheme to generate cells with a β-cell phenotype are as much a triumph of developmental biology as of stem cell biology. This is evident in the fact that the most successful protocol for β-cell differentiation entails a stepwise differentiation process that recapitulates pancreatic development; for a detailed review see (Spence *et al.* 2007).[96] Early attempts at differentiating ESCs to β-cells focused on selecting for nestin-positive population of cells after embryoid body (EB) differentiation.[97] While this method was successful at generating a subpopulation of cells that express the pancreatic hormones insulin and glucagon, overall the cells were very heterogeneous as nestin is also a marker for neuronal progenitors.98

Two pivotal studies that describe the differentiation of ESCs to pancreatic endoderm reported in (D'Amour *et al.* 2006;[93] and Kroon *et al.* 2008;[99] and highlighted below) sought to recapitulate four stages of embryonic development in order to obtain glucose responsive insulin secreting cells *in vitro.* The four developmental stages simulated are: 1) differentiation to definitive endoderm, 2) establishment of the primitive gut endoderm, 3) patterning of the posterior foregut, and 4) specification and maturation of pancreatic endoderm and endocrine precursors.[93,99]

1) Differentiation to definitive endoderm

During gastrulation, the definitive endoderm is specified from mesendoderm at the node, which directs high levels of expression of *Nodal*, a transforming growth factor-β (TGFP) ligand. 100 Nodal is believed to act as a morphogen within the embryo to direct cells to the endoderm fate at high doses, and mesoderm fate at lower doses.101 The differentiation of ESCs to definitive endoderm is achieved by culturing pluripotent cells in monolayer at a low concentration of fetal bovine serum (FBS) for 3 days in the presence of a high concentration of activin A, a TGFp ligand that mimics the action of nodal. Wnt3a is additionally added for the first day of differentiation to promote mesendoderm specification prior to definitive endoderm formation, which can be characterized by expression of *Sox17*, *Foxa2*, *Cerberus (Cer)*, and *CXCR4*.[93] The differentiation of pluripotent cells to definitive endoderm is a critical step for efficient differentiation of pancreatic endoderm, therefore future protocols may benefit from incorporating the small molecules IDE1 and IDE2, which are known inducers of definitive endoderm in ESCs.[102]

2) Establishment of the primitive gut endoderm

In vivo the formation of primitive gut endoderm from definitive endoderm stems largely from morphogenetic restructuring of the embryo. *Sonic hedgehog (Shh)* plays a role in primitive gut endoderm development as well, as it exhibits broad expression throughout the gut tube, however Shh must later be repressed in the posterior foregut in order for the pancreatic buds

to be specified.[103,104] *In vitro* this process was recreated by adding back FBS and removing activin A from the culture for 2-4 days. The removal of activin A is reported to be essential for the transition from definitive endoderm to gut endoderm to occur.[93] While activin-A removal alone is sufficient to induce the gut tube markers *HNF1B* and *HNF4A*, it was observed that addition of the growth factor FGF10 and hedgehog signaling inhibitor KAAD-cyclopamine at this stage significantly promotes β-cell differentiation manifested in end-stage differentiated cells by a massive increase in insulin production.[93] However, later reports observe that supplementing media with kertinocyte growth factor (KGF) at this stage can replace both FGF10 and cyclopamine without decreasing the differentiation efficiency.[99]

3) Patterning of the posterior foregut

The foregut endoderm will give rise to the pancreas, stomach, lungs, liver, and thyroid, therefore proper patterning of the foregut is essential for directing organogenesis.[105] Two signaling pathways are known to play an important role in patterning the foregut prior to pancreatic development. The first identified was the repression of Shh in the nascent pancreatic endoderm by the adjacent notochord.[103] Later genetic studies of the *retinaldehyde dehydrogenase-2 (Raldh2)* knockout mouse identified a role for retinoic acid (RA) signaling in specifying the posterior foregut in the mouse.[106,107] They report that loss of RA signaling *in vivo* causes aberrant development of the pancreas and stomach, both of which are derived from the posterior foregut. This is consistent with the previous finding that RA signaling from the lateral plate mesoderm is sufficient to induce expression of the pancreatic marker gene *Pdx1* in pancreatic endoderm.[108] To recreate this signaling environment *in vitro*, the previously differentiated cells were cultured for 2-4 days in serum-free conditions with supplement. In addition to FGF10 and cyclopamine, the cells were treated with a high concentration of RA.[93] Later reports indicate that Noggin, a TGFp inhibitor, is an effective substitute for FGF10.[99] Properly differentiated cells at this stage express significant levels of *Pdx1, HNF6* and *Sox9*. Importantly, it is noted that RA treatment at this stage is required for later expression of *insulin*, and *Ngn3*.[93]

4) Specification and maturation of pancreatic endoderm and endocrine precursors

The specification and maturation of both acinar and endocrine cells of the pancreas *in vivo* is less clear. Studies suggest that the *Notch* signaling pathway may play a role suppressing the specification of these cell types in order to temporally regulate their differentiation, perhaps until other mechanisms promoting specification are functional.[109,110] One such mechanism may be TGFp signaling. A study in pancreatic-bud explants found that treatment with activin-like TGFp ligands stimulates SMAD2/3 and results in enhanced endocrine specification with fewer acini.[111] Alternatively, treatment with follistatin, an activin antagonist, causes enhanced acinar development.[112] The specification and maturation of endocrine precursors *in vitro* is also somewhat lacking. The initial protocol for pancreatic endocrine cell differentiation calls for posterior foregut-like cells to be cultured in serum-free conditions with supplement, and the inclusion of y-secretase inhibitors, growth factors IGF1 and HGF, as well as exendin-4 to potentiate insulin secretion in response to glucose;[93] however a more recent report refined this protocol and found that the inclusion of these factors is not necessary for efficient differentiation, and that continued serum free culture is sufficient.[99] This suggests that an innate program for β-cell differentiation is functional in these differentiating cells, however the program is stochastic and results in a heterogeneous population of pancreatic endoderm cell types.[99] Others report that EGF treatment facilitates the expansion of pancreatic progenitors,[94] however, this does little to address our impaired ability to efficiently differentiate cells to a single fate. Broader study of the mechanisms of endocrine specification will eventually yield the necessary tools to allow for direct differentiation towards specific endocrine cell types.

The four-stage differentiation process has been reported to give rise to cells with an endocrine phenotype. Many of the differentiated cells are characterized by expression of the β-cell transcription factors *Pdx1*, *Nkx6-1*, and *MafA*, a marker for glucose-responsive mature β-cells.[99] Morphologically, these cells resemble mature islets in their ability to form secretory granules as well. Interestingly, the *in vitro* differentiated cells have an impaired ability to respond to glucose stimulation in culture, however 1-3 weeks after engraftment into a mouse the cells begin to secrete human insulin and C-peptide in response to high glucose. Furthermore, the vast majority of mice that underwent engraftment of human cells exhibited protection from streptozotocin (STZ) induced hyperglycemia.[99] The discrepancy in glucose response between engrafted cells, and cells in culture, is perhaps due to the failure to differentiate mature β-cells *in vitro*. The described differentiation protocol may be sufficient for establishing a sub-population of naïve β-cells that more closely reflects fetal β-cells rather than mature β-cells. This is supported by the similar phenotype between the *in vitro* differentiated β-cells and fetal β-cells. Both cell types, though initially are unresponsive to glucose, gradually acquire the ability to respond to glucose after transplantation suggesting that further differentiation occurs *in vivo*.[113]

Summary

Much progress has been made in the programming of non-β cells to β cells. However, as they currently exist, the protocols for deriving pancreatic endocrine cells reviewed above are unlikely to be immediately applicable in the clinic. On the other hand, the field is rapidly advancing and new discoveries have been appearing in rapid succession. We are optimistic that directed differentiation of non-β cells to β cells will in time become a practical strategy for treating type 1, and possibly type 2, diabetes.

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References

1. Economic costs of diabetes in the U.S. In 2007. Diabetes Care 2008;31:596–615. [PubMed: 18308683]
2. Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230–8. [PubMed: 10911004]
3. Harlan DM, Kenyon NS, Korsgren O, et al. Current advances and travails in islet transplantation. Diabetes 2009;58:2175–84. [PubMed: 19794074]
4. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318–30. [PubMed: 17005949]
5. Rizzino A. A challenge for regenerative medicine: proper genetic programming, not cellular mimicry. Dev Dyn 2007;236:3199–207. [PubMed: 17685475]
6. Dor Y, Brown J, Martinez OI, et al. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004;429:41–6. [PubMed: 15129273]
7. Teta M, Rankin MM, Long SY, et al. Growth and regeneration of adult beta cells does not involve specialized progenitors. Dev Cell 2007;12:817–26. [PubMed: 17488631]
8. Murtaugh LC. Pancreas and beta-cell development: from the actual to the possible. Development 2007;134:427–38. [PubMed: 17185316]
9. Gradwohl G, Dierich A, Lemeur M, et al. Neruogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci USA 2000;97:1607–11. [PubMed: 10677506]

10. 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;129:2447–57. [PubMed: 11973276]
11. Wang S, Jensen JN, Seymour PA, et al. Sustained Neurog3 expression in hormone-expressing islet cells is required for endocrine maturation and function. Proc Natl Acad Sci U S A 2009;106:9715–20. [PubMed: 19487660]
12. Xu X, D'Hoker J, Stange G, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008;132:197–207. [PubMed: 18243096]
13. Kushner JA, Weir GC, Bonner-Weir S. Ductal origin hypothesis of pancreatic regeneration under attack. Cell Metab 2010;11:2–3. [PubMed: 20085728]
14. Bonner-Weir S, Sharma A. Are there pancreatic progenitor cells from which new islets form after birth? Nature Clinical Practice Endocrinology & Metabolism 2006;2:240–1.
15. Teta M, Long SY, Wartschow LM, et al. Very slow turnover of beta-cells in aged adult mice. Diabetes 2005;54:2557–67. [PubMed: 16123343]
16. Perfetti R, Zhou J, Doyle ME, et al. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology 2000;141:4600–5. [PubMed: 11108273]
17. Brubaker PL. Minireview: Update on Incretin Biology: Focus on Glucagon-Like Peptide-1. Endocrinology. 2010 en.
18. Thorel F, Nepote V, Avril I, et al. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature. 2010
19. Collombat P, Xu X, Ravassard P, et al. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 2009;138:449–62. [PubMed: 19665969]
20. Inada A, Nienaber C, Katsuta H, et al. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proc Natl Acad Sci U S A 2008;105:19915–9. [PubMed: 19052237]
21. Bonner-Weir S, Inada A, Yatoh S, et al. Transdifferentiation of pancreatic ductal cells to endocrine beta-cells. Biochem Soc Trans 2008;36:353–6. [PubMed: 18481956]
22. Solar M, Cardalda C, Houbracken I, et al. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. Dev Cell 2009;17:849–60. [PubMed: 20059954]
23. Kopinke D, Murtaugh LC. Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. BMC Dev Biol 2010;10:38. [PubMed: 20377894]
24. Baeyens L, De BS, Lardon J, et al. In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia 2005;48:49–57. [PubMed: 15616797]
25. Zhao M, Amiel SA, Christie MR, et al. Insulin-producing cells derived from human pancreatic non-endocrine cell cultures reverse streptozotocin-induced hyperglycaemia in mice. Diabetologia 2005;48:2051–61. [PubMed: 16132961]
26. Zhou Q, Brown J, Kanarek A, et al. In vivio reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008;455:627–32. [PubMed: 18754011]
27. Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. Science 2008;322:1490–4. [PubMed: 19056973]
28. Oertel M, Shafritz DA. Stem cells, cell transplantation and liver repopulation. Biochim Biophys Acta 2008;1782:61–74. [PubMed: 18187050]
29. Kim S, Shin JS, Kim HJ, et al. Streptozotocin-induced diabetes can be reversed by hepatic oval cell activation through hepatic transdifferentiation and pancreatic islet regeneration. Lab Invest 2007;87:702–12. [PubMed: 17483848]
30. Yang L, Li S, Hatch H, et al. In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc Natl Acad Sci USA 2002;99:8078–83. [PubMed: 12048252]
31. Ferber S, Halkin A, Cohan H, et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 2000;6:568–72. [PubMed: 10802714]

32. Perl S, Hirshberg B, Harlan DM, et al. How much insulin is enough? A quantitative assessment of the transdifferentiaton potential of liver. Diabetologia 2007;50:690–2. [PubMed: 17221213]
33. Kojima H, Fujimiya M, Matsumura K, et al. NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. Nat Med 2003;9:596–603. [PubMed: 12704384]
34. Miyatsuka T, Kaneto H, Kajimoto Y, et al. Ectopically expressed PDX-1 in liver initiates endocrine and exocrine pancreas differentiation but causes dysmorphogenesis. Biochem Biophys Res Commun 2003;310:1017–25. [PubMed: 14550306]
35. Yechoor V, Liu V, Espiritu C, et al. Neurogenin3 is sufficient for in vivo transdetermination of hepatic progenitors cells into islet-like structures but not transdifferentiation of hepatocytes. Dev Cell 2009;16:358–73. [PubMed: 19289082]
36. Yechoor V, Liu V, Paul A, et al. Gene therapy with neurogenin 3 and betacellulin reverses major metabolic problems in insulin-deficient diabetic mice. Endocrinology 2009;150:4863–73. [PubMed: 19819964]
37. Hadorn E. Problems of determination and transdetermination. Brookhaven Symp Biol 1965;18:148–61.
38. Hadorn E. Transdetermination in cells. Sci Am 1968;219:110–4. [PubMed: 5684924]
39. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 1970;3:393–403. [PubMed: 5523063]
40. Friedenstein AJ, Chailakhyan RK, Latsinik NV, et al. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 1974;17:331–40. [PubMed: 4150881]
41. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008;8:726–36. [PubMed: 19172693]
42. Vija L, Farge D, Gautier JF, et al. Mesenchymal stem cells: Stem cell therapy perspectives for type 1 diabetes. Diabetes Metab 2009;35:85–93. [PubMed: 19230736]
43. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. Cell Stem Cell 2008;2:313–9. [PubMed: 18397751]
44. Prockop DJ. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther 2009;17:939–46. [PubMed: 19337235]
45. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3:301–13. [PubMed: 18786417]
46. Caplan AI. Why are MSCs therapeutic? New data: new insight. J Pathol 2009;217:318–24. [PubMed: 19023885]
47. Corselli M, Chen CW, Crisan M, et al. Perivascular ancestors of adult multipotent stem cells. Arterioscler Thromb Vasc Biol 2010;30:1104–9. [PubMed: 20453168]
48. Ankrum J, Karp JM. Mesenchymal stem cell therapy: Two steps forward, one step back. Trends Mol Med 2010;16:203–9. [PubMed: 20335067]
49. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981;292:154–6. [PubMed: 7242681]
50. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–7. [PubMed: 9804556]
51. Boiani M, Scholer HR. Regulatory networks in embryo-derived pluripotent stem cells. Nat Rev Mol Cell Biol 2005;6:872–84. [PubMed: 16227977]
52. Nichols J, Zevnik B, Anastassiadis K, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 1998;95:379–91. [PubMed: 9814708]
53. Avilion AA, Nicolis SK, Pevny LH, et al. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev 2003;17:126–40. [PubMed: 12514105]
54. Mitsui K, Tokuzawa Y, Itoh H, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell 2003;113:631–42. [PubMed: 12787504]
55. Chambers I, Colby D, Robertson M, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell 2003;113:643–55. [PubMed: 12787505]

56. Boyer LA, Lee TI, Cole MF, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 2005;122:947–56. [PubMed: 16153702]
57. Loh YH, Wu Q, Chew JL, et al. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat Genet 2006;38:431–40. [PubMed: 16518401]
58. Masui S, Nakatake Y, Toyooka Y, et al. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat Cell Biol 2007;9:625–35. [PubMed: 17515932]
59. Fuhrmann G, Chung AC, Jackson KJ, et al. Mouse germline restriction of Oct4 expression by germ cell nuclear factor. Dev Cell 2001;1:377–87. [PubMed: 11702949]
60. Gu P, LeMenuet D, Chung AC, et al. Orphan nuclear receptor GCNF is required for the repression of pluripotency genes during retinoic acid-induced embryonic stem cell differentiation. Mol Cell Biol 2005;25:8507–19. [PubMed: 16166633]
61. Gidekel S, Pizov G, Bergman Y, et al. Oct-3/4 is a dose-dependent oncogenic fate determinant. Cancer Cell 2003;4:361–70. [PubMed: 14667503]
62. Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Adv Cancer Res 2008;100:133–58. [PubMed: 18620095]
63. Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective ablation of human embryonic stem cells expressing a “suicide” gene. Stem Cells 2003;21:257–65. [PubMed: 12743320]
64. Zwaka TP, Thomson JA. Differentiation of human embryonic stem cells occurs through symmetric cell division. Stem Cells 2005;23:146–9. [PubMed: 15671139]
65. Gurdon JB, Uehlinger V. “Fertile” intestine nuclei. Nature 1966;210:1240–1. [PubMed: 5967799]
66. Campbell KH, McWhir J, Ritchie WA, et al. Sheep cloned by nuclear transfer from a cultured cell line. Nature 1996;380:64–6. [PubMed: 8598906]
67. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76. [PubMed: 16904174]
68. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72. [PubMed: 18035408]
69. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell 2008;132:567–82. [PubMed: 18295576]
70. Chin MH, Mason MJ, Xie W, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 2009;5:111–23. [PubMed: 19570518]
71. Stadtfeld M, Apostolou E, Akutsu H, et al. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. Nature 2010;465:175–81. [PubMed: 20418860]
72. Maherali N, Sridharan R, Xie W, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 2007;1:55–70. [PubMed: 18371336]
73. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448:313–7. [PubMed: 17554338]
74. Zhao XY, Li W, Lv Z, et al. iPS cells produce viable mice through tetraploid complementation. Nature 2009;461:86–90. [PubMed: 19672241]
75. Boland MJ, Hazen JL, Nazor KL, et al. Adult mice generated from induced pluripotent stem cells. Nature 2009;461:91–4. [PubMed: 19672243]
76. Kang L, Wang J, Zhang Y, et al. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. Cell Stem Cell 2009;5:135–8. [PubMed: 19631602]
77. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008;451:141–6. [PubMed: 18157115]
78. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917–20. [PubMed: 18029452]
79. Kiskinis E, Eggan K. Progress toward the clinical application of patient-specific pluripotent stem cells. J Clin Invest 2010;120:51–9. [PubMed: 20051636]
80. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 2008;321:1218–21. [PubMed: 18669821]
81. Park IH, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. Cell 2008;134:877–86. [PubMed: 18691744]

82. Maehr R, Chen S, Snitow M, et al. Generation of pluripotent stem cells from patients with type 1 diabetes. Proc Natl Acad Sci U S A 2009;106:15768–73. [PubMed: 19720998]
83. Miura K, Okada Y, Aoi T, et al. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol 2009;27:743–5. [PubMed: 19590502]
84. Knoepfler PS. Myc goes global: new tricks for an old oncogene. Cancer Res 2007;67:5061–3. [PubMed: 17545579]
85. Rowland BD, Bernards R, Peeper DS. The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. Nat Cell Biol 2005;7:1074–82. [PubMed: 16244670]
86. Yu J, Hu K, Smuga-Otto K, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324:797–801. [PubMed: 19325077]
87. Woltjen K, Michael IP, Mohseni P, et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009;458:766–70. [PubMed: 19252478]
88. Kim D, Kim CH, Moon JI, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 2009;4:472–6. [PubMed: 19481515]
89. Silva J, Nichols J, Theunissen TW, et al. Nanog is the gateway to the pluripotent ground state. Cell 2009;138:722–37. [PubMed: 19703398]
90. Huangfu D, Maehr R, Guo W, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 2008;26:795–7. [PubMed: 18568017]
91. Mauritz C, Schwanke K, Reppel M, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. Circulation 2008;118:507–17. [PubMed: 18625890]
92. Zhang SC, Wernig M, Duncan ID, et al. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol 2001;19:1129–33. [PubMed: 11731781]
93. D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 2006;24:1392–401. [PubMed: 17053790]
94. Jiang J, Au M, Lu K, et al. Generation of insulin-producing islet-like clusters from human embryonic stem cells. Stem Cells 2007;25:1940–53. [PubMed: 17510217]
95. Zhang D, Jiang W, Liu M, et al. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. Cell Res 2009;19:429–38. [PubMed: 19255591]
96. Spence JR, Wells JM. Translational embryology: using embryonic principles to generate pancreatic endocrine cells from embryonic stem cells. Dev Dyn 2007;236:3218–27. [PubMed: 17973329]
97. Lumelsky N, Blondel O, Laeng P, et al. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 2001;292:1389–94. [PubMed: 11326082]
98. Lendahl U, Zimmerman LB, McKay RD. CNS stem cells express a new class of intermediate filament protein. Cell 1990;60:585–95. [PubMed: 1689217]
99. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443–52. [PubMed: 18288110]
100. Tremblay KD, Hoodless PA, Bikoff EK, et al. Formation of the definitive endoderm in mouse is a Smad2-dependent process. Development 2000;127:3079–90. [PubMed: 10862745]
101. Vincent SD, Dunn NR, Hayashi S, et al. Cell fate decisions within the mouse organizer are governed by graded Nodal signals. Genes Dev 2003;17:1646–62. [PubMed: 12842913]
102. Borowiak M, Maehr R, Chen S, et al. Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell Stem Cell 2009;4:348–58. [PubMed: 19341624]
103. Hebrok M, Kim SK, Melton DA. Notochord repression of endodermal Sonic hedgehog permits pancreas development. Genes Dev 1998;12:1705–13. [PubMed: 9620856]
104. Hebrok M, Kim SK, St JB, et al. Regulation of pancreas development by hedgehog signaling. Development 2000;127:4905–13. [PubMed: 11044404]
105. Wells JM, Melton DA. Vertebrate endoderm development. Annu Rev Cell Dev Biol 1999;15:393–410. [PubMed: 10611967]
106. Martin M, Gallego-Llamas J, Ribes V, et al. Dorsal pancreas agenesis in retinoic acid-deficient Raldh2 mutant mice. Dev Biol 2005;284:399–411. [PubMed: 16026781]
107. Wang Z, Dolle P, Cardoso WV, et al. Retinoic acid regulates morphogenesis and patterning of posterior foregut derivatives. Dev Biol 2006;297:433–45. [PubMed: 16806149]

108. Kumar M, Jordan N, Melton D, et al. Signals from lateral plate mesoderm instruct endoderm toward a pancreatic fate. Dev Biol 2003;259:109–22. [PubMed: 12812792]

109. Apelqvist A, Li H, Sommer L, et al. Notch signalling controls pancreatic cell differentiation. Nature 1999;400:877–81. [PubMed: 10476967]

110. Hald J, Hjorth JP, German MS, et al. Activated Notch1 prevents differentiation of pancreatic acinar cells and attenuate endocrine development. Dev Biol 2003;260:426–37. [PubMed: 12921743]

111. Sanvito F, Herrera PL, Huarte J, et al. TGF-beta 1 influences the relative development of the exocrine and endocrine pancreas in vitro. Development 1994;120:3451–62. [PubMed: 7821214]

112. Miralles F, Czernichow P, Scharfmann R. Follistatin regulates the relative proportions of endocrine versus exocrine tissue during pancreatic development. Development 1998;125:1017–24. [PubMed: 9463348]

113. Castaing M, Peault B, Basmaciogullari A, et al. Blood glucose normalization upon transplantation of human embryonic pancreas into beta-cell-deficient SCID mice. Diabetologia 2001;44:2066–76. [PubMed: 11719839]