Cell transplantation therapy for diabetes mellitus: endocrine pancreas and adipocyte

Junji Fujikura1), Kiminori Hosoda2) and Kazuwa Nakao1)

1)Division of Endocrinology and Metabolism, Kyoto University Hospital, Kyoto 606-8507, Japan
2)Department of Human Health Science, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan

Abstract. Experimental transplantation of endocrine tissues has led to significant advances in our understanding of endocrinology and metabolism. Endocrine cell transplantation therapy is expected to be applied to the treatment of metabolic endocrinopathies. Restoration of functional pancreatic beta-cell mass or of functional adipose mass are reasonable treatment approaches for patients with diabetes or lipodystrophy, respectively. Human induced pluripotent stem (iPS) cell research is having a great impact on life sciences. Doctors Takahashi and Yamanaka discovered that the forced expression of a set of genes can convert mouse and human somatic cells into a pluripotent state [1, 2]. These iPS cells can differentiate into a variety of cell types. Therefore, iPS cells from patients may be a potential cell source for autologous cell replacement therapy. This review briefly summarizes the current knowledge about transplantation therapy for diabetes mellitus, the development of the endocrine pancreas and adipocytes, and endocrine-metabolic disease-specific iPS cells.

Key words: Transplantation, Diabetes mellitus, Pancreas, Adipocyte, Development

Transplantation in endocrinology

In 1683, Conrad Brunner (1653-1727) removed the pancreas from a dog and noted the resulting polydipsia and polyuria, two hallmark symptoms of diabetes [3]. In 1889, Oscar Minkowski (1858-1931) transplanted canine pancreases into pancreatectomized dogs and noticed a decrease in the severity in the symptoms. This experiment demonstrated that the pancreas is responsible for the regulation of blood glucose [4]. The first recorded human pancreatic xenotransplantation was performed in 1893. Minkowski transplanted pieces of freshly slaughtered sheep’s pancreas into a diabetic 15-year-old boy; however, the boy died three days after the operation. Concern about the detrimental effects of exocrine acinar cells on graft function and viability has stimulated the development of islet cell isolation and transplantation procedures.

In addition to research on diabetes mellitus, transplantation experiments involving other tissues and organs have increased our understanding of endocrinology. In 1849, Adolph Berthold (1803-1861) reported on the transplantation of testes in cockerels [3]. He transplanted testes from young cockerels into the abdominal cavity of the castrated cockerels and found that the recipients continued to retain the normal secondary sexual characteristics. Moritz Schiff (1823-1896) showed that intra-abdominal transplantation of the thyroid gland could prevent the fatal results of total thyroidectomy [5]. George Murray (1865-1939) provided the first account of a human patient with myxedema given substitution with subcutaneous thyroid tissues, which had a beneficial effect [6]. Early in the 20th century, several investigators showed that transplantation of the parathyroid gland prevented the development of neuromuscular symptoms in parathyroidectomized animals [7].

These studies suggested that endocrine cells may be suitable for transplantation, because they are individual functional units that sense extracellular stimuli and secrete hormones.

Pancreas and islet transplantation in diabetes mellitus

The pancreas plays a key role in the maintenance of nutritional homeostasis through its exocrine and endo-
Pancreas transplantation is currently the only known therapy for T1DM that reliably establishes a long-term euglycemic state [20]. It is effective in that patients remain insulin independent for more than 10 years [21]. After transplantation, the normal glucagon response to hypoglycemia is restored, and hypoglycemic episodes are uncommon [22]. However, transplantation requires major surgery and has a surgical complication (repeat laparotomy) rate between 10% and 20% [23].

Pancreatic islet transplantation is safe and reproducible. In this procedure, isolated islets are embolized into the liver through a catheter placed into the main portal vein of the recipient. However, insulin independence rarely extends beyond two years [24-27]. The necessary immunosuppressive regimen, including a calcineurin inhibitor or mTOR inhibitor, and the process of islet isolation itself may contribute to the low islet viability [28-30]. Both pancreas and islet transplantation require immunosuppressive therapy, carry the threat of recurrence of autoimmunity, and are limited by donor shortages [31-35].

Patient-derived induced pluripotent stem (iPS) cells may provide an unlimited supply of transplantable cells for beta-cell replacement therapy in diabetic patients. Autotransplantation avoids the risks associated with allograft rejection and the need for immunosuppressants. Previous studies have demonstrated clearly that the most efficient and reproducible method to generate a given cell type from stem cells is to recapitulate embryonic development \textit{in vitro} [36, 37]. This would be the case for both the endocrine pancreas (Fig. 1) and adipocytes [38, 39]. Further understanding of the developmental processes enables us to design more robust and reliable differentiation protocols for the treatment of diabetes mellitus.

![Fig. 1 Simplified representation of the signaling pathways regulating pancreatic development](image-url)
**Endocrine pancreatic development**

*Endodermal origin of the pancreas*

The origin of pancreatic endocrine cells from the neuroectoderm (neural crest) was proposed around 1970 [40]. Pancreatic endocrine cells and neurons share common biochemical properties, morphological features, and molecules; for example, neuron-specific enolase, synaptophysin, chromogranin A, Pax4, Pax6, NeuroD, Nkx2.2, Nkx6.1,Isl1, MafB, and MafA [41, 42]. However, fate-mapping studies using quail-chick chimeras provided evidence against a neuroectoderm origin for pancreatic endocrine cells [43-45]. Furthermore, *ex vivo* organ culture experiments demonstrated that a rat embryo without the neuroectoderm can form a normal pancreas, indicating that all types of pancreatic cells are derived from endoderm progenitors [46].

*Definitive endoderm (DE) formation*

The endoderm is divided into two types: the visceral endoderm (VE), which derives directly from the inner cell mass; and the other is the DE [47]. The VE forms the yolk sac but rarely contributes to the embryo proper [48-51]. The DE forms during gastrulation, when DE progenitors ingress into the anterior primitive streak and migrate into and replace the VE layer. The DE and VE share common transcription factors such as Foxa2 and Sox17, but only the DE expresses chemokine receptor (Cxcr)-4 [52]. Wnt/beta-catenin signaling is detected in the primitive streak [53]. Wnt3- or beta-catenin-knockout mice lack a primitive streak [54, 55]. Nodal, a TGF-beta family member, is expressed in the anterior primitive streak and is required for the specification of the anterior DE [56, 57]. Induction of DE differentiation from embryonic stem (ES)/iPS cells is accomplished by adding TGF-beta ligands (Nodal, Activin A) and either Wnt molecules (Wnt3a) or a GSK3-beta inhibitor [52, 58-63].

*Pancreatic specification*

During organogenesis, the DE forms the epithelial lining of the primitive gut tube from which the digestive tract, thyroid, liver, and pancreas develop [64]. The pancreas develops from the ventral and dorsal buds of the endoderm expressing the pancreatic-duodenal homeobox gene (Pdx1) [65]. In the Pdx1-knockout mouse, the pancreas fails to develop beyond the bud stage [66, 67].

The retinoic acid (RA)-synthesizing enzyme, retinaldehyde dehydrogenase 2 (Raldh2), is expressed in the somitic mesoderm dorsal to the primitive gut tube. Raldh2-knockout mice lack expression of Pdx1 in the dorsal endoderm, but administration of RA rescues the loss of Pdx1 expression [68, 69]. Treatment of ES cell-derived endoderm cells with RA induces the expression of Pdx1 as well as other important transcription factors, such as pancreatic transcription factor-1a and neurogenin 3 (Ngn3) [70, 71].

The Hedgehog (Hh) family of proteins controls cell growth, survival, and fate, and patterns almost every aspect of the vertebrate body plan [72]. The Hh family of proteins also plays a role in the maintenance of many adult structures that include proliferating cell populations [73]. Sonic hedgehog (Shh) is expressed throughout the embryonic gut tube, except for the pancreatic bud endoderm [74, 75]. Forced expression of Shh from the Pdx1 promoter inhibits pancreatic development [74]. The Shh inhibitor cyclopamine expands the endodermal region where pdx1 expression starts [76]. Thus, the absence of Shh provides a permissive condition leading to pancreatic specification. Reciprocal antagonism between Hh and RA signaling has been suggested [77].

In addition to RA, several other mesodermal signals are critical for patterning the embryonic endoderm into the pancreas. The notochord, the future backbone, is located proximal to the dorsal prepancreatic endoderm. Notochord-derived signals (fibroblast growth factor 2 (Fgf2) and Activin) can suppress endodermal Shh expression and initiate pancreatic differentiation [75, 78, 79]. After notochordal contact with the prepancreatic endoderm, endothelial signals from the nearby aorta promote further dorsal pancreatic specification [80, 81]. The lateral plate mesoderm that lies beneath the ventral prepancreatic endoderm also sends instructive signals that establish the ventral pancreatic domain [82].

There is a bipotential precursor population for the pancreas and liver within the embryonic endoderm [83]. Bone morphogenetic protein (BMP) from cardiogenic mesoderm adjacent to the prehepatic endoderm induces hepatic genes and excludes the pancreatic fate [84, 85]. BMP inhibition by Noggin or other inhibitors at this step is the common basis for the *in vitro* pancreatic differentiation protocols from ES/iPS cells [71, 86-91].
**Pancreatic epithelial cell growth and endocrine commitment**

The Fgf7 subfamily is unique among FGFs because its members (Fgf7, Fgf10, and Fgf22) are expressed exclusively in the mesenchyme and interact specifically with the Fgf receptor 2b [92]. Fgf7 and 10 are expressed in the mesenchyme adjacent to the prepancreatic buds, and Fgf receptor 2b is expressed in the pancreatic epithelium; addition of each fgf to organ cultures promotes the proliferation of pancreatic epithelial cells [93-95]. In the Fgf10-knockout mouse, pancreatic hypoplasia and the absence of islet cells are evident [93]. Conversely, transgenic overexpression of Fgf10 in Pdx1-expressing progenitor cells increases the proliferation of epithelial cells and blocks differentiation by activating Notch signaling [95, 96].

Notch signaling is known to be responsible for the maintenance of neuronal stem cell populations by inhibiting their differentiation [97]. Targeting of Notch pathway genes in mice results in the upregulation of Ngn3 and premature endocrine differentiation at the expense of progenitor cell proliferation, suggesting that activation of the pathway plays a role in maintaining the progenitor cell state in the early pancreatic epithelium [98-100]. Ngn3 is a basic helix-loop-helix transcription factor that regulates the development of hypothalamic neurons [101]. In the Ngn3-knockout mouse, all pancreatic endocrine cell lineages and endocrine cell-related transcription factors (such as Isl1, Pax4, Pax6, and NeuroD) are lost [102].

Conditional knockout mice lacking Smad4 (a common transcriptional coactivator in the pathway) in Pdx1-expressing pancreatic progenitors can generate a normal pancreas, suggesting that TGF-beta/BMP signaling is dispensable after pancreatic commitment [103]. Inhibition of TGF-beta and BMP signaling has been reported to increase commitment to endocrine lineage [89].

**Pancreatic beta-cell differentiation and maturation**

A complex cascade of many transcription factors, such as Isl1, Pax4, Pax6, NeuroD, Nkx2.2, Nkx6.1, Isl1, MafB, and MafA is involved in the differentiation and maturation of pancreatic beta-cells, but little is known about the extrinsic signals regulating this process [104]. Many differentiation protocols for inducing insulin-producing cells from human ES/iPS cells have been reported [71, 87-90, 105-110]. However, most insulin-producing cells generated are immature, produce multiple hormones and low levels of insulin, and have poor responses to secretory stimuli. In addition, all types of pancreatic cells, not just beta-cells, are differentiated at the same time in most protocols. Further studies are now trying to obtain more fully differentiated beta-cells in the presence of specific cues.

**Adipocyte transplantation**

In mammals, adipose tissue comprises white adipose tissue (WAT), primary site of energy storage and mobilization in the form of triglyceride and brown adipose tissue (BAT), which specializes in energy dissipation as thermogenesis.

Obesity is an excess adiposity and is linked to T2DM, cardiovascular, pulmonary, liver, and kidney diseases and certain types of cancer [111, 112]. The anatomical distribution of WAT influences the risks associated with obesity. Obese individuals with a high waist-to-hip ratio, indicating increased visceral fat, have a higher risk for metabolic abnormalities than do individuals with a low waist-to-hip ratio [113]. Subcutaneous fat differs intrinsically from visceral fat and is thought to protect from metabolic disorders [114].

Lipodystrophy is an abnormal loss of adiposity and is characterized by loss of body fat and insulin resistance [115]. Lipodystrophy is accompanied by diabetes mellitus, hypertriglyceridemia, and hepatic steatosis. Leptin treatment or transplantation of wild-type but not leptin-deficient BAT rescued the phenotype of A-ZIP/F-1 lipodystrophic mice, suggesting that leptin deficiency is the major contributor to the metabolic complications of lipodystrophy [116-119]. Several studies have shown that intraperitoneal transplantation of BAT improves glucose tolerance and insulin sensitivity in mice with other conditions besides lipodystrophy [114, 120].

BAT is a major site of energy dissipation because of its high mitochondrial content; in BAT mitochondria, oxidative phosphorylation is uncoupled from adenosine triphosphate (ATP) production as a result of proton leak catalyzed by uncoupling protein 1 (UCP1) [121]. Studies of mice lacking BAT or UCP1 have demonstrated the ability of BAT thermogenesis to protect against diet induced obesity [122, 123]. Transplantation of BAT into the visceral cavity in mice improved glucose tolerance, increased insulin sensitivity, reduced body weight and fat mass, and reversed high-fat diet-induced insulin resistance [124]. Recent studies have detected metabolically active BAT in the neck and upper thorax of normal humans [125].

Therapeutic adipogenesis is an exciting frontier of
metabolic medicine, but our understanding of adipocyte development is still poor compared with that of pancreatic development [38, 39].

**Adipocyte development**

**White adipocytes**

White adipocytes can be generated from both mesoderm and neuroectoderm through mesenchymal stem cells (MSCs) [126]. MSCs are defined by plastic-adherent growth and the potential to give rise to multiple mesenchymal cell lineages including osteocytes, chondrocytes, and adipocytes [127, 128]. MSCs reside in the vascular stroma of adipose tissue, the bone marrow and many other tissues [129, 130]. Several factors that commit or inhibit the conversion of MSCs to the adipocyte lineage have been identified. BMP-2 and -4 signaling supports white adipocyte differentiation [131-134]. Wnt signaling acts as an activator of lineage commitment from MSCs to white adipocytes and later as an inhibitor of the differentiation program [135-138]. Shh signaling has an inhibitory effect on adipocyte differentiation [139]. About 10% of adipocytes are renewed annually at all adult ages in humans [140].

**Brown adipocytes**

BAT originates from the paraxial mesoderm [141]. Brown adipocytes and skeletal muscle develop from a common progenitor, which expresses Myf5 [142]. BMP-7 triggers commitment of progenitor cells to a brown adipocyte lineage by inducing the regulator PRD1-BF1-RIZ1 homologous domain containing 16 (PRDM16) and peroxisome proliferator-activated receptor (PPAR) -gamma-coactivator-1 alpha [143]. PRDM16 specifies the brown adipocyte lineage from the Myf5-expressing progenitors by activating the transcriptional function of PPAR-gamma and by suppressing myogenic factors [144, 145].

**Patient-derived iPS cells for mitochondrial disease modeling**

The ability to generate iPS cells from patients allows one to obtain genetically identical cells of clinical interest for pathogenesis modeling. Patient-specific iPS cell lines have been derived from individuals with several endocrine and metabolic diseases (Table 1). In some cases, *in vitro* differentiation of iPS cells to the affected cell types has been reported, and some of which successfully recapitulate disease-associated abnormalities. A deeper understanding in cell differentiation and function will be needed for iPS cell-based disease modeling.

We recently generated iPS cells from patients with the A3243G mitochondrial DNA (mtDNA) mutation (Mt-iPS cells) [146]. mtDNA is present inside mitochondria and codes for enzymes for ATP production [147]. In mtDNA disease, wild-type and mutant mtDNA coexist in the same cell in a state called heteroplasmy. The tRNA (Leu) A3243G mutation is observed frequently in mtDNA diseases and is associated with diabetes mellitus, hearing loss, and cardiomyopathy [148]. The mode of inheritance of mtDNA diseases is maternal because sperm-derived paternal mtDNA disappears during early embryogenesis, [149]. However, the penetrance of mtDNA disease is variable, and it is not possible to predict the phenotypes of a child from the mother’s heteroplasmy level [150]. This is also the case for somatic cells: it is not possible to predict to which cell types the mutant mtDNA will dominantly migrate during development.

A striking feature of these Mt-iPS cells was their bimodal levels of heteroplasmy (Fig. 2). The mtDNA mutation frequencies decreased to undetectable levels in about half of the clones, whereas the levels of mutation heteroplasmy were higher in the other half of the clones compared with those in the patients’ original fibroblasts. The mtDNA content did not differ significantly between mutation-free and mutation-rich Mt-iPS cells.

To date, there is no specific therapy or cure for mitochondrial diseases. Efforts to understand the mtDNA diseases have been hampered by the lack of a disease model. Mutation-rich Mt-iPS cells may provide a suitable source of cells for human mitochondrial disease modeling *in vitro*. In addition, mutation-free iPS cells could provide an unlimited supply of disease-free cells for autologous transplantation therapy.

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<th>Disease</th>
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<th>Age/Gender</th>
<th>Differentiation into affected cell types: Functional Analysis</th>
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Fig. 2  mtDNA mutation frequencies in Mt-iPS cells

A3243G mtDNA mutation frequencies in the blood cells, original fibroblasts, and Mt-iPS clones from two diabetic patients with the A3243G mtDNA mutation.

References


Hebrok M, Kim SK, St Jacques B, McMahon AP,


85. Mfopou JK, Chen B, Mateizel I, Sermon K, Bouwens L, et al. (2006) Receptor specificity of the fibroblast growth factor family. The complete mamma-


