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Transdifferentiation of hepatic oval cells into pancreatic islet beta-cells

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1. ABSTRACT

Insulin production by beta-cells derived from hepatic oval cells is a promising new approach for the treatment of diabetes. Hepatic oval cells can be redirected to the beta-cell linage by an appropriate combination of high extracellular glucose, specific extracellular matrix proteins (laminin and fibronectin), cytokines (activin A), and the expression of several differentiation-related transcription factors (Pdx-1, Ngn-3, MafA). We review the process of hepatic oval cell transdifferentiation into pancreatic islet beta-cells and the cellular signaling pathways involved.

2. INTRODUCTION TO PROSPECTS OF HEPATIC OVAL CELLS IN THE TREATMENT OF DIABETES

The pancreatic endocrine compartment is composed of the islets of Langerhans, which are clusters of four cell types that synthesize insulin (beta-cells), glucagon (alpha-cells), somatostatin (delta-cells), or pancreatic polypeptide (pp-cells). In mature rodent pancreatic islets, beta-cells are located in the core, surrounded by alpha-, delta-, and pp-cells. The pancreatic beta-cell responds to minor increases in plasma glucose concentration by releasing insulin, which stimulates glucose uptake by liver, muscle, and adipose tissue, and thereby maintains blood

glucose levels within a very narrow range (1). Progressive destruction of pancreatic beta-cells leading to decreased insulin production and hyperglycemia is observed in all forms of diabetes mellitus (DM). Therefore, a reduction in the number of functional beta-cells is the central pathological event in the development and progression of DM. Currently, the most effective long-term treatment for diabetes is islet transplantation through a procedure known as the Edmonton protocol (2). There is currently a severe shortage of pancreas donors, however, creating a need for new strategies to generate pancreatic beta-cells either *in vitro* or *in vivo*.

Hepatic oval cells are thought to have the potential to transdifferentiate into pancreatic endocrine cells, as both the liver and the pancreas originate from appendages of the upper primitive foregut endoderm (3). It has been suggested that the late separation of the liver and the pancreas during organogenesis in primitive ventral endoderm might have left both tissues with pluripotent cells that are capable of giving rise to both hepatic and pancreatic lineages (4,5). Indeed, as early as in 2002, Yang *et al.* (6) demonstrated that hepatic oval cells cultured in high-glucose media could differentiate into pancreatic endocrine cells *in vitro*. Subsequent studies determined that high glucose, the protein composition of the extracellular matrix, specific cytokines, and the expression of differentiation-related transcription factors influenced transdifferentiation.

3. HEPATIC OVAL CELLS IN BETA-CELL NEOGENESIS

In adult humans, liver progenitor cells are not well defined, but hepatic oval cells are thought to retain pluripotency. Normally, these hepatic oval cells are destined to become hepatocytes and cholangiocytes, but can be redirected to the related pancreatic islet lineage, including beta-cell neogenesis, under appropriate conditions.

Hepatic oval cells are closely related to the pancreatic progenitors that differentiate into islet beta-cells. During fetal development, cells of the distal foregut endoderm that gives rise to pancreatic endocrine islets can differentiate into liver progenitors under the influence of fibroblast growth factor and bone morphogenetic protein from the cardiac mesoderm and the septum transversum, tissues that normally give rise to liver progenitors (7-10). In theory, it may be possible to redirect hepatic oval cells toward the pancreatic islet lineage if the salient developmental signals and microenvironment are mimicked *in vitro* (Figure 1).

4. FACTORS INFLUENCING THE DIFFERENTIATION OF HEPATIC OVAL CELLS INTO ISLET BETA-CELLS

4.1. High glucose promotes hepatic oval cell differentiation into islet beta-cells

Glucose is both an ubiquitous cell nutrient and a growth factor that can stimulate stem cells to differentiate into mature beta-cells. Rat hepatic oval cells can be induced to regenerate by 2-acetoaminofluorene (2-AAF) and can subsequently differentiate into islet cells in the presence of high glucose *in vitro*. These cells express insulin, glucagon, and transcription factors, including pancreatic duodenal homeobox 1 (Pdx-1, also known as insulin promoter factor-1), paired box gene (Pax) 4, and Pax6 (6). Moreover, these cells decreased blood glucose levels when implanted into streptozocin (STZ)-induced diabetic mice (6). Tang *et al* (11) and Kojima *et al* (12) induced hepatic oval cells to differentiate into functional beta-cells in the presence of high glucose *in vitro* and *in vivo*. Studies aimed at uncovering the mechanisms of high glucose-stimulated transdifferentiation of hepatic oval cells into beta-cells indicated that glucose can regulate Pdx-1 protein phosphorylation through the protein kinase B (PKB)/glycogen synthase kinase 3 (GSK3) pathway. Glycogen synthase kinase 3 can phosphorylate Pdx-1, targeting it for proteolysis, but PKB serves to inhibit GSK3 activity. Under high glucose, PKB activity is upregulated and GSK3-mediated Pdx-1 phosphorylation is inhibited, enhancing Pdx-1 stability and leading to insulin synthesis and release (13). Long periods of high glucose, however, lead to reduce glucose sensitivity and beta-cell apoptosis (14). Therefore, the concentration of glucose is usually controlled between 20–30 mmol/L (15).

4.2. Extracellular matrix matrigel induces hepatic oval cells to differentiate into islet beta-cells

Matrigel is a soluble basement membrane matrix secreted by EHS mouse sarcoma cells that includes a variety of extracellular matrix (ECM) proteins, including laminin (LM), collagen IV, fibroblast growth factor (FGF), and tissue plasminogen activator. Leite *et al.* found that LM combined with fibronectin (FN) induced hepatic oval cells to differentiate into islet beta-cells. In addition, LM plus FN induced hepatic oval cells to express Pdx-1, Pax6, insulin, and glucagon, while LM alone induced the expression of somatostatin and glucose transporter 2 (Glut-2).

4.3. The cytokine activin A is involved in the differentiation of hepatic oval cell into islet b cells

Activin A is a member of transforming growth factor-b (TGF-b) family. When bound to its receptor on alpha- and delta-cells of embryonic and adult islets, activin A can control the proliferation, differentiation, and apoptosis of these cells. In addition, activin A can cause endodermal progenitor cells to differentiate into pancreatic cells (16). When hepatic oval cells were cultured in serum-free medium with activin A, expression of glucokinase, NeuroD, and Nkx2.2 were increased, while Pax2.6 expression was decreased, an expression pattern shared by beta-cells (17). In addition, insulin secretion of these hepatic oval cells was increased by 33-fold and the secreted insulin could regulate glucose levels *in vivo* (17).

4.4. Differentiation-related transcription factors

4.4.1. Pdx-1

Pancreatic duodenal homeobox 1 (Pdx-1) is also known as insulin promoter factor-1 (IPF) and somatostatin transcription factor (STF-1) (18). The Pdx-1 protein is

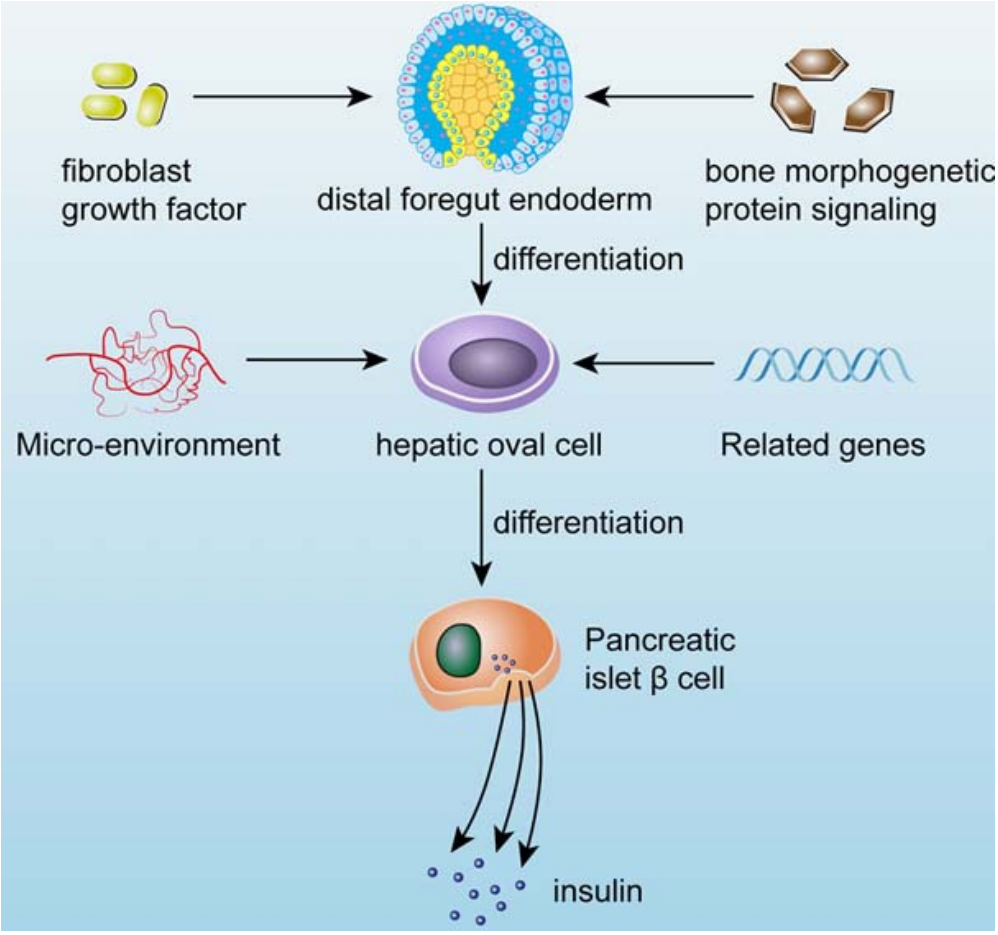

Figure 1. Hepatic oval cells in beta-cell neogenesis. During fetal development, cells of the distal foregut endoderm that gives rise to pancreatic endocrine islets can differentiate into liver progenitors under the influence of fibroblast growth factor and bone morphogenetic protein from the cardiac mesoderm and the septum transversum, tissues that normally give rise to liver progenitors. It may be possible to redirect hepatic oval cells toward the pancreatic islet lineage if the salient developmental signals and microenvironment are mimicked *in vitro*.

encoded by a 2 exon gene located on human chromosome 13, mouse chromosome 5, and rat chromosome 12 (19). A 6.5 kb 5-untranslated region constitutes a regulatory region. In the region from -2.5 kb to -3.5 kb, hepatocyte nuclear factor (HNF)1a/HNF3b/Pdx-1 trimers, or HNF1a/HNF3b/SP (1/3) trimers bind, while the region from -2.8 kb to -1.7 kb, called the PH region, has a highly homologous conserved sequence in both human and mice endowing beta-cell-specific transcriptional activity. The Pdx-1 protein can bind to PH1 region elements and co-activate transcription with HNF3b (20). Furthermore, Pdx-1 protein can infiltrate into other cells through an antennae-like protein domain. The function of exogenous Pdx-1 is similar to endogenous Pdx-1; it is a complex transcriptional and co-activating factor that binds to the proximal promoter of the insulin gene and directly regulates insulin transcription (21). In addition, Pdx-1 can non-selectively activate the expression of several differentiation-related genes in pancreatic exocrine cells. When transfected into hepatic oval cells, Pdx-1 can activate the expression of Ngn3, neurogenic differentiation factor, and Nkx6.1, transcription factors which control the early development of islet beta-cells (22,23). Zalzman *et al.* found that human fetal liver progenitor cells transfected *in vitro* with rat Pdx-1 differentiated into functional islet beta-cells that secreted insulin in response to glucose (24). When Pdx-1 was transfected into diabetic rat liver, blood glucose levels decreased and insulin-positive cells were found in liver tissue that originated from hepatic oval cells (25).

4.4.2. Ngn3

The Ngn3 protein (also known as neurogenin 3) is an member of the basic-helix-loop-helix (bHLH) superfamily of transcription factors that both regulate and suppress the expression of target genes. The expression of bHLH-target genes is negatively regulated by hairy/enhancer of split (HES) proteins that bind to E-box motifs in target gene promoters (26). There is a CANNTC E-box in the Pdx-1 gene promoter than binds Ngn3, resulting in enhanced insulin promoter activity and insulin expression (27). The promoter of Ngn3 contains three HES-1 binding sites near the TATA box, and HES-1 can

inhibit Ngn3 expression through the D111/Notch1/HES-1 signal transduction pathway. This reduced Ngn3 expression maintains the pancreas in an undifferentiated state (28). In addition, Ngn3 promotes the differentiation of pancreatic progenitor cells into islet cells. Damage to the Ngn3 gene in mice inhibited the maturation of endocrine cells and the mice died from diabetes in 1 to 3 days. In contrast, overexpression of Ngn3 in transgenic mice led to increased numbers of endocrine cells. Furthermore, when transfected into hepatic oval cells in vitro, Ngn3 induced transdifferentiation into pancreatic beta-cells and promoted the expression of beta-cell genes, including NeuroD, Pax4, Pax6, Nkx2.2, and Nkx6.1 (29,30). Under electron microscopy, the neo-islet cell clusters displayed the typical ultrastructure of pancreatic beta-cells (30). They secreted insulin in response to glucose elevation in a manner indistinguishable from the pattern seen in nondiabetic mice, a response that could have occurred only through intact stimulus-secretion coupling (29).

4.4.3. MafA

Macrophage activating factor A (MafA) contains a acidic transcription activating domain (TAD) in the N-terminal and a basic 1eucine zipper (bLZ) structure. MafA is an effective activator of insulin gene transcription that is expressed only in mature pancreatic beta-cells (31). The insulin gene contains three conserved cis-regulatory elements, E1 (-100 bp to -9l bp), A3 (-201 bp to -196 bp), and RIPE3b/CI (-126 bp to -101 bp). Binding of MafA to the RIPE3b/CI site can activate insulin gene expression (32). MafA is an important transcription factor that can participate in glucose-stimulated insulin secretion (GSIS) through both an ionic and a non-ionic signaling pathway. MafA-/- mice exhibited impaired insulin secretion as well as decreased expression of Pdx-1, Bete2, and Glut-2. Ectopic expression of MafA in chicken embryos induced insulin secretion and the formation of islet-like structures (22). Moreover, insulin expression was increased synergistically by Ngn3 and MafA, and insulin expression was dependent on the C-terminal DNA binding domain of MafA (22). In STZ-induced diabetic mice, adenovirus-mediated MafA transfection activated the insulin promoter and induced the synthesis of endogenous insulin; furthermore, this response was enhanced by Pdx-1 (27). In addition, MafA had a synergistic effect on the expression of the insulin gene in liver by inducing the expression Glut-2 and prohormone-converting enzyme 2 (PC2).

5. CONCLUSIONS

Insulin production by beta-cells derived from hepatic oval cells holds great potential for the treatment of diabetic patients. An appropriate microenvironment induces transcription factors that promote the differentiation of hepatic oval cells into pancreatic islet beta-cells. Further studies are required to establish the most efficient methods for the generation of beta-cells from hepatic oval cells in vitro and in vivo and to further examine the functional similarities between endogenous beta-cells and those derived by transdifferentiation of hepatic oval cells.

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Abbreviations: ECM: extracellular matrix; 2-AAF: 2-acetoaminofluorene; Pdx: pan-creatic duodenal homeobox; ST: streptozocin; PKB: protein kinase B; GSK3: glycogen synthase kinase 3; LM: laminin; FN: fibronectin; GluT-2: glucose transporter 2; IPF: insulin promoter factor-1; STF-1: somatostatin transcription factor; HNF: hepatocyte nuclear factor; bHLH: basic-helix-loop-helix; HES: hairy/enhancer of split; Maf: Macrophage activating factor; TAD: transcription activating domain;bLZ: basic 1eucine zipper; GSIS: glucose-stimulated insulin secretion

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