COMMENTARY

New sources of β -cells for treating diabetes

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Abstract

The treatment of diabetes by islet transplantation is presently hampered by the shortage of organ donors. The generation of insulin-producing cells is therefore a major objective in the long-term goal of curing diabetes. Alternative sources of pancreatic β -cells include existing pancreatic cells, embryonic stem cells, and cells from other tissues such as liver. This

commentary considers evidence for two new sources of β -cells: intrahepatic biliary epithelial cells and gall bladder epithelium. These observations raise the possibility that a patient's own cells may be used as a source of insulin-producing cells for cell replacement in diabetes.

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Introduction

Cell-replacement therapy involves treating diseases with functional cells derived from sources including the body's own cells. Successful cell replacement therapy in diabetes requires sufficient numbers of insulin-producing cells to be transplanted into diabetic individuals so as to achieve normal circulating glucose concentrations. However, the success of islet cell grafts is limited by two major factors. First, the immunosuppressive regimen required may have adverse effects on graft viability. Second, the number of cells as well as the number of transplants that each patient has to undergo for successful reversal of diabetes far outweighs the number of donors available. Although allogeneic transplantation of cadaveric human pancreatic islets (Shapiro *et al.* 2000) has been till now the only successfully used cell-replacement therapy for diabetes, limited availability of cadaveric human pancreas restricts transplantation procedures to only a few diabetic individuals. The generation of insulin-producing cells has therefore become an important objective in the development of a cure for diabetes (Bonner-Weir & Weir 2005, Miszta-Lane *et al.* 2006, Gangaram-Panday *et al.* 2007). Four possible cell sources have been considered for obtaining insulin-producing cells: existing β -cells from the other pancreatic cell types (endocrine, acinar or ductal), provoking regeneration from human embryonic stem cells, recapitulating the normal sequence of developmental events that leads to β -cell differentiation (D'Amour *et al.* 2006); and finally from other tissue sources, particularly those of endodermal origin (e.g. the liver), by transdifferentiation (Bonner-Weir & Weir 2005).

Pancreas, liver, and biliary system share a common developmental origin

During development, many organs such as the liver, lung and pancreas, arise from the foregut endoderm in response to signals from adjacent germ layers (Wells & Melton 2000). The liver (including extrahepatic biliary system) and ventral pancreas arise from the same region of the ventral foregut endoderm (Zaret & Grompe 2008). The common ancestry of these tissues raises the intriguing possibility that it may be possible to convert one cell type to another through the process of transdifferentiation. Transdifferentiation is based on the theory that tissues derived from the same region of the developing embryo share many transcription factors but differ in the expression of only a single or a few key transcription factors known as master switch genes. Understanding the differences in the expression of these master switch genes may allow reprogramming of stem cells towards differentiated cell types or alternatively permit directed differentiation of adult cells. Based on this idea, the liver, pancreas, and gall bladder share many common features (Fig. 1) in terms of the transcription factors expressed during embryonic development and it may therefore be possible to transdifferentiate one cell type into another.

Conversion of liver cells to pancreatic β -cells

There is now a growing body of evidence to suggest that it may be possible to induce the conversion of liver cells (hepatocytes, intra-/extrahepatic biliary epithelial cells, and gall-bladder epithelium) to pancreatic lineages. Transdifferentiation has

Figure 1 A schematic demonstrating the overlap of transcription factors expressed in liver, gall bladder, and pancreas during mouse embryonic development. During embryonic development, the ventral bud stems from the common biliary duct that then extends from the duodenal ampulla to the liver and gall bladder. Interestingly, one of the major developmental regulators of pancreatic development; Pdx1, is observed in gall bladder and common biliary duct (but not liver) during embryonic development. These overlapping profiles of transcription factors in liver (Costa *et al.* 2003, Duncan 2003, McLin & Zorn 2006), gall bladder (Clotman *et al.* 2002, Coffinier *et al.* 2002, Kalinichenko *et al.* 2002, 2003, Krupczak-Hollis *et al.* 2004, Hunter *et al.* 2007, Burlison *et al.* 2008), and pancreas (Madsen *et al.* 1996, Wells & Melton 1999, Edlund 2001*a,b*, Wilson *et al.* 2003, Habener *et al.* 2005) suggest the potential of resident progenitors to interconvert into specialized cell types that characterize the adult organs.

been demonstrated in a number of models including: overexpression of pancreas and duodenal homeobox gene 1 (pdx1) in liver cells (Ferber *et al.* 2000, Horb *et al.* 2003, Zalzman *et al.* 2005). Sumazaki *et al.* (2004) also demonstrated the conversion of the developing biliary system to pancreatic tissue in Hes1 null mice. These mice display gallbladder and cystic duct agenesis and conversion of the common bile duct to pancreatic tissue. The ectopic tissue possesses the full complement of pancreatic cell types: endocrine cells expressing glucagon (α -cells), insulin (β -cells), somatostatin (δ -cells), and pancreatic polypeptide (PP cells) in addition to exocrine cells expressing amylase and carboxypeptidase A. These results indicate a previously unknown role for Hes1 in the maintenance of the biliary phenotype and the inhibition of the pancreatic differentiation pathway. Unfortunately, use of the common bile duct as a source of tissue for the generation of β -cells is precluded, as it is essential for normal liver function. While the common bile duct may not be useful as a source of pancreatic β -cells, the gall bladder may offer an alternative starting material. Recent studies have demonstrated that gall bladder (Sahu *et al.* 2008) as well as human biliary duct epithelial cells contain endocrine pancreatic hormone-producing cells. Insulin, glucagon, PP, and somatostatin

proteins were observed by immunocytochemistry (Sahu *et al.* 2008). However, the level of insulin gene transcripts by quantitative TaqMan-based real-time PCR was at least 3000-fold lower than that observed in adult human islets. These observations support the idea that population of human gall bladder or biliary epithelial cells have the potential to transcribe and produce insulin. Altogether, these findings suggest that other endoderm-derived tissues may express or retain the potential to produce endocrine pancreatic hormones, thereby providing newer sources of pancreatic progenitor cells. There is now a new addition to the list of cell types for use in β -cell therapy. Recent data suggest that the intrahepatic biliary cells may be used as a source of β -cells.

Intrahepatic biliary epithelial cells can be expanded and converted to β -like cells

In a recent issue of the *Journal of Endocrinology*, Gordon Weir *et al.* demonstrate for the first time that a sub-population of intrahepatic biliary epithelial cells (IHBECs) can be induced to a β -like phenotype (Nagaya *et al.* 2009). The IHBECs were initially expanded using a novel collagen matrix protocol.

The cells maintained their biliary phenotype in culture. Following ectopic expression of Pdx1, NeuroD or Pdx1-VP16 (a modified form of Pdx1 containing the VP16 transactivation domain from Herpes simplex), β -cell genes (ins 1 and 2, PC1 and 2) were expressed as well as other islet hormones including glucagon and somatostatin (albeit at lower levels than in the control islets and MIN6 cells). The presence of β -like cells in these cultures was also confirmed by the expression of C-peptide, demonstrating the correct processing of the insulin protein. The β -cells also appeared to be functional: as they secreted insulin in response to glucose. This study raises some interesting questions regarding the use of liver-derived cells for diabetes therapy:

1. The β -cell phenotype was only present in a sub-population of IHBECs. This observation suggests that not all IHBECs are able to transdifferentiate towards a β -cell phenotype. It is important to determine the cellular origin of the 'transdifferentiated' cells and whether the cells with the highest transdifferentiation potential could be separated from the other biliary cells as endocrine progenitors.
2. The levels of insulin gene transcripts appear to be similar to islets, especially considering a subpopulation that express the relevant proteins. This may mean that either all cells express low levels of transcripts or that the subpopulation expresses a very high level of insulin gene transcripts. To distinguish these possibilities, single cell PCR could be performed on control and transdifferentiated cells.
3. While the data from animal experiments are encouraging, the question arises as to whether the same protocol can also be applied to human liver material.

Summary

Several studies discussed herein illustrate the potential of biliary duct and gall bladder epithelial cells to differentiate into islet hormone-producing cells. The possibility of obtaining biliary epithelial cells via endoscopy or gall bladder epithelia after cholecystectomy may indeed provide a source of pancreatic progenitor cells for treating diabetes. However, all these studies would be more meaningful, if protocols to expand and differentiate these cells into therapeutically significant numbers is achieved. Further studies need to be carried out to understand the potential of such progenitors for cell replacement therapy in diabetes.

Declaration of interest

None to declare.

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