New organs from our own tissues: liver-to-pancreas transdifferentiation

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Recent advances in pancreatic islet transplantation emphasize the potential of this approach for the long-term control of blood glucose levels in diabetic patients. However, tissue-replacement therapy will become widely available as a treatment for diabetes only when new sources of islets and insulin-producing cells are found. Here, we review recent evidence that documents the potential of mature liver as a source of tissue for generating a functional endocrine pancreas, by ectopic expression of pancreatic transcription and differentiation factors. When key events in the transconversion process have been identified, using the liver as a source of pancreatic tissue might provide a valuable approach for replacing impaired β cell function in diabetics.

Following the discovery of insulin in 1921 by Banting and Best, insulin therapy has saved the lives of T1DM (see glossary) and many T2DM patients. However, 50% of diabetics develop chronic diabetes-related complications that appear years after the onset of diabetes (including blindness, renal failure, myocardial infarction and vascular problems that lead to limb amputation) [1,2]. These complications damage the quality of life of patients and represent an annual multibillion-dollar burden on health care systems in industrialized countries [3,4].

Several clinical trials have demonstrated that strict glycemic control can slow and even prevent the progression of diabetic complications [5]. However, intensive insulin therapy increases the incidence of hypoglycemic episodes by a factor of three, and is suitable only for selected patients [6].

Pancreas transplantation and islet-cell implantation are efficient in replacing the function of the impaired tissue and, thus, inducing continuous normoglycemia [7]. These grafts bring freedom from the burden of injections, glucose testing, dietary restrictions and, importantly, continuous normoglycemia protects against the complications of diabetes [8]. However, these treatments require extensive, life-long suppression of the immune system and are restricted by the limited tissue supply from cadaveric donors. Generally, it is accepted that islet-cell implantation will become widely available only when new sources of islets or pancreatic β cells are found.

Differentiated β cells cannot be expanded *in vitro* [9] and, therefore, alternative approaches are being explored to generate insulin-producing cells. These include either genetically engineering β-cells [10,11] or utilizing potential β-cell-precursor cells. Results with embryonic stem cells [12–16], adult pluripotent cells from the pancreas [17–19] and bone-marrow derived-cells [20] have been interesting. Most of these approaches have been reviewed recently [21–23], and, in some cases, questions have been raised about the monitoring of their capacity to produce and secrete insulin [24].

In this article we review the potential of liver to provide a source of tissue to generate a functional endocrine pancreas, and the capacity of pancreatic transcription and differentiation factors to control the developmental switch between the two tissues [25–30]. This approach might generate custom-made 'self surrogate β cells for the treatment of diabetes, and bypass both the shortage of tissue from cadaveric donors and the need for life-long immunosuppression.

Why use liver to generate endocrine pancreas?

Unlike pancreatic β cells, liver regenerates efficiently, mainly by proliferation of mature hepatocytes [31]. It is

Glossary

Developmental redirection: a process in which partially committed, pluripotent cells differentiate into cells that are not usually associated with the organ they populate. This allows a mature tissue to gain extra functions in a distinct subpopulation of cells that are derived from its committed stem cells, without losing the original function of the mature cells in the organ.
Euglycemia: normoglycemia, normal range of fasting blood glucose. In humans this is 70–120 mg dl^{-1}.
FGAD: first generation E1-deleted recombinant adenoviruses.
GLUT-2: glucose transporter type 2.
HDAD: 'gutless', helper-dependent recombinant adenovirus.
Hyperglycemia: high blood-glucose levels. An abnormally high concentration of fasting glucose in the blood (>140 mg dl^{-1} in humans).
NeuroD1: beta 2, neurogenic differentiation 1.
Oval cells: small cells with ovoid nucleus, that reside in the terminal bile ductules of the adult rodent liver. These cells are believed to be bipotential (at least) and to give rise to hepatocytes and bile-duct cells.
***Pdx1*:** pancreatic and duodenal homeobox gene 1, also known as *IUF1 STF1, IDX1* and *IPF1* (*IPF1* is the official nomenclature according to HUGO Gene Nomenclature Committee (www.gene.ucl.ac.uk/nomenclature).
STZ: streptozotocin, a β-cell-specific toxin that destroys insulin-producing cells in the pancreas and is used to induce experimental diabetes.
T1DM: type 1 diabetes mellitus, also known as insulin-dependent diabetes mellitus, is caused by specific autoimmune destruction of insulin-producing cells.
T2DM: type 2 diabetes mellitus, also known as noninsulin-dependent diabetes mellitus, is caused, in part, by ablated insulin action.
Transdifferentiation: an irreversible switch of one type of differentiated cell into a different differentiated cell by changing the repertoire of gene expression. Intra-organ separation between the gained and host function occurs [59].

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the largest organ in our body with a large amount of functional redundancy [32].

Transconversion between liver and pancreas is conceivable, and has both a developmental and a phylogenic rationale. Liver and pancreas are related developmentally because both are derived from appendages of the upper primitive foregut endoderm. It has been suggested that the late separation of liver and 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 [33]. Both tissues have many characteristics in common, including responsiveness to glucose, and both express a large group of specific transcription factors [34–36]. Transconversion between pancreatic acinar cells and hepatocytes in both rodents and humans has been reported under experimental, pathological and malignant conditions [37–39].

Comparing the development of liver and pancreas in other species further emphasizes the close relationship between these two organs. In lower organisms, such as worms and fish, the two organs do not separate [40,41] and the 'hepato–pancreas' functions as both liver and pancreas [33,40].

Expression of the gene that encodes insulin is leaky in rodent liver. Surprisingly, although insulin-gene expression is generally restricted to pancreatic β cells in mammals, it is also expressed at low levels in 20% of normal mouse liver [26] and in STZ hyperglycemic mice [28]. This leakage of insulin expression might indicate that there is a more 'permissive' compaction of chromatin in the region of insulin-encoding genes in liver [42], or the presence of specific signaling pathways that support insulin-gene expression in some liver cells [43].

Developmental redirection of hepatic pluripotent and embryonic cells toward pancreas

The close developmental relationship between liver and pancreas is also demonstrated by the capacity to redirect the developmental fate of hepatic progenitors toward β-cell characteristics and function. When hepatocyte-damaging agents impair the proliferative ability of surviving hepatocytes, a biliary-stem-cell system generates new liver cells [44]. The bile-duct-derived progenitors, called OVAL CELLS, are characterized in rodents, but their human equivalents have not been found [44]. These cells were documented previously to possess several developmental options, including hepatocytes, biliary epithelium, intestinal epithelium and pancreatic acinar epithelium [31]. Yang *et al.* have demonstrated that exposure to high levels of glucose and nicotinamide *in vitro* causes oval cells to acquire an additional developmental option: that of insulin-producing cells [29]. These cells express several genes that encode pancreatic transcription factors, including *Pdx1* [29]. This study provides an example of the DEVELOPMENTAL REDIRECTION of hepatic pluripotent cells toward pancreatic insulin-producing cells that is induced by environmental factors.

The capacity to redirect the developmental fate of immortalized, human fetal liver cells by ectopic expression of *Pdx1* has been demonstrated recently [30]. Cells that express *Pdx1* ectopically produce, store and secrete processed insulin in a glucose-regulated manner, even though GLUT-2 and glucokinase activities are not induced. The modulated cells are functional, and restore and maintain EUGLYCEMIA in diabetic, immunodeficient mice [30]. This study demonstrates the capacity of *Pdx1* to shift the developmental route of committed cells in human fetal liver and endow them with many characteristics and functions of pancreatic β cells [30].

Reprogramming of cells in mature liver *in vivo*: the role of 'master regulators'

Surprisingly, master regulators that dictate the developmental fate of pluripotent cells during embryogenesis have also been shown to alter the differentiation fate of mature tissues. This phenomenon could represent a significant step forward for regenerative medicine, allowing the development of ectopic organs using the patient's own tissues. The best-known example is the induction of myogenesis by the myogenic master regulator, MyoD. Ectopic expression of *MyoD* leads to the expression of muscle-specific proteins in primary fibroblasts, differentiated melanoma, neuroblastoma, liver and several cell lines *in vitro* [45]. The few additional examples of developmental fate redirection that have been reported more recently indicates that this process is either rare or an unexplored phenomenon [46]. Some mature tissues might undergo the process of developmental shift more readily than others. For example, whereas ectopic expression of *Pdx1* alters the developmental state of mature liver cells [25], earlier studies show that *Pdx*1 does not induce the expression of pancreatic-specific genes in the digestive tract, which is also developmentally related to the pancreas [47].

The action of many transcription factors guides differentiation of the pancreas during embryogenesis [48]. These factors act at specific spatial and temporal points, and their position in the cascade of events that leads to pancreas organogenesis has been established mainly by loss-of-function studies and tracing analyses [49]. Although we anticipate that the relative position of each factor will reflect its role in ectopic pancreas formation, gain-of-function analyses might reveal additional information, as demonstrated for ectopic expression of *NeuroD1* in liver [28]. Ectopic expression of pancreatic transcription factors in permissive extrapancreatic tissues, such as liver, might provide valuable information about the role of these factors in organogenesis of the pancreas, and their potential use in engineering an 'ectopic surrogate pancreas'. The first pancreatic transcription factor to be analyzed by such an approach in mature liver *in vivo* is PDX1 [25–28].

PDX1

PDX1 has central roles in regulating organogenesis of the pancreas in embryos and β-cell function in adults [50–54]. PDX1 is involved in regulating the expression of multiple genes in β cells, including those that encode insulin, glucokinase, islet amyloid polypeptide, *Pdx1* itself (in an autoregulatory loop) and, possibly, *Glut-2* [55]. It plays a key role in pancreatic morphogenesis, as demonstrated by impaired pancreatic development in mice and humans

with homozygous mutations in *Pdx1* [50–52,56]. In humans, heterozygous mutation of *Pdx1* causes maturity-onset diabetes of the young and, probably, T2DM [57,58]. Heterozygous *Pdx1*-knockout mice have impaired glucose tolerance and an accelerated rate of β-cell apoptosis [54].

The role of PDX1 in inducing liver-to-pancreas transdifferentiation

The capacity of PDX1 to direct pancreas development has been demonstrated in mature, fully differentiated liver *in vivo*, and was initially attributed to the presence of pluripotent cells in the mature organ [25]. Recent studies indicate that this also occurs in mice and *Xenopus*, possibly via a process of TRANSDIFFERENTIATION [26–28]. Transdifferentiation describes the irreversible conversion of one type of differentiated cell to another differentiated cell [59].

Transient, ectopic expression of *Pdx1* in liver following systemic administration of FGAD (Box 1) induces expression of a wide repertoire of pancreatic genes. Surprisingly, despite the short-term expression of the *Pdx1* transgene in liver, it causes long-lasting production and secretion of processed, biologically active insulin [25,26]. PDX1 also induced its own expression in liver (auto-induction), which explains the irreversible nature of the 'liver-to-pancreas' transdifferentiation process (Figure 1).

The insulin produced by the liver following *Pdx1* administration using FGAD is functional; not only does it restore euglycemia in STZ-induced diabetic mice [25], it also prevents STZ-induced HYPERGLYCEMIA, even eight months after the initial treatment [26] (Box 1, Figure 1).

These data indicate the irreversibility of the process. They also show that the cells that produce insulin in the liver resist β-cell-specific toxins and, therefore, might not acquire all the characteristics of β cells [26].

When expressed ectopically in mature, fully differentiated liver, PDX1 functions as a pancreatic differentiation factor that has the capacity to induce the expression of many pancreas-specific genes. These genes include several that are not considered immediate targets, such as those that encode glucagon, P48 and elastase [26].

Irreversible *Pdx1*-induced liver-to-pancreas transdifferentiation by the FGAD system occurs in only $<1\%$ of liver cells, despite the initial random expression of the transgene in 30–40% of cells in liver. Cells that transdifferentiate are located close to central veins, and may be predisposed to the process ([26] Figure 1). The heterogeneity of cells in liver [60] could cause particular cells to respond differently to the pancreatic transgene. Liver cells might differ from each other in terms of ultrastructural modifications in chromatin compaction, the presence of silencing effects and different complements of transcription factors that work in concert with the ectopic gene [42,43,49,61].

Partially overcoming the hepatic predisposition

To overcome some of the barriers to *Pdx1*-induced liver-to-pancreas transdifferentiation and, thus, increase the number of liver cells that transdifferentiate, Horb *et al.* [27] created transgenic tadpoles that expressed the frog homolog of *Pdx1* fused to the transcriptional activation domain of VP-16 (*Xlhbox8–VP16*), a regulatory protein from the Herpes simplex virus. Activation of PDX1 by VP-16 in transgenic tadpoles converts most of the liver into exocrine and endocrine pancreas. This massive transformation of tadpole liver into pancreas indicates that activation of PDX1 might eliminate the requirement for cofactors that are thought to work in concert with PDX1 in activating pancreatic gene expression [62]. Alternately, VP-16 might alter chromatin condensation and remodeling of PDX1-target genes in the host genome [63,64]. That activation of PDX1 overcomes some of the barriers to transdifferentiation in hepatic cells strengthens the notion that the predisposition of hepatic cells is caused by complementing, pre-existing transcription factors and, possibly, by distinct chromatin structure.

The transgenic tadpole model suggests that *Pdx1* induces a transdifferentiation process when ectopically expressed in liver: (i) it induced a massive liver transformation; (ii) it converted mature liver cells into mature insulin-producing cells – the *Xlhbox8-VP16* transgene driven by the *Transthyretin* promoter (gene usually expressed in mature hepatocytes) was not expressed until a day after the liver bud had begun expressing hepatic markers [27]; and (iii) the *Pdx1*-mediated process involved the down-regulation of hepatic-marker expression, which usually occurs in a transdifferentiation process [27].

Therefore, this model provides an important experimental proof of principle of the potential of PDX1 as a highly efficient tool to redirect the developmental fate of liver to pancreas [27]. Further studies are needed to investigate the consequences of such a massive transdifferentiation on the function of both liver and pancreas.

Box 1. Recombinant adenoviruses: efficient gene-delivery tools for triggering transdifferentiation

Recombinant adenoviruses are an efficient tool for gene delivery both *in vivo* and *in vitro*. These replication-deficient double-stranded DNA viruses do not integrate into the host genome and are efficient at delivering transgenes to quiescent cells without altering the host genome. Systemic viral administration results in efficient gene expression, mainly in the liver.

FGAD (first generation recombinant adenoviruses) are E1-deleted, replication-deficient recombinant adenoviruses that are immunogenic, possibly because of leakiness of viral gene expression [73]. Therefore, FGAD are eliminated rapidly from the host body and they mediate transient transgene expression. Transgenes delivered by FGAD are expressed for several days to a few weeks [75], and therefore are efficient in triggering a process such as transdifferentiation, but not for sustained ectopic gene expression. Helper-dependent or gutless recombinant adenoviruses (HDAD) have been developed to reduce immunogenicity and increase the life span of the virally delivered genes *in vivo* [66,67]. HDAD lack most of the viral genome, and their replication depends on a helper virus in addition to the packaging cell line. These vectors are typically 50–275-fold more efficient than FGAD at expressing transgenes *in vivo* [66,67]. HDAD result in transgene expression in ~100% of cells in liver, in an essentially irreversible fashion [66,67,76].

Long-term expression of the therapeutic gene is essential in classical gene-therapy approaches. By contrast, during transdifferentiation, ectopic gene expression serves only as a transient trigger that initiates an irreversible process [26,28,73,75].

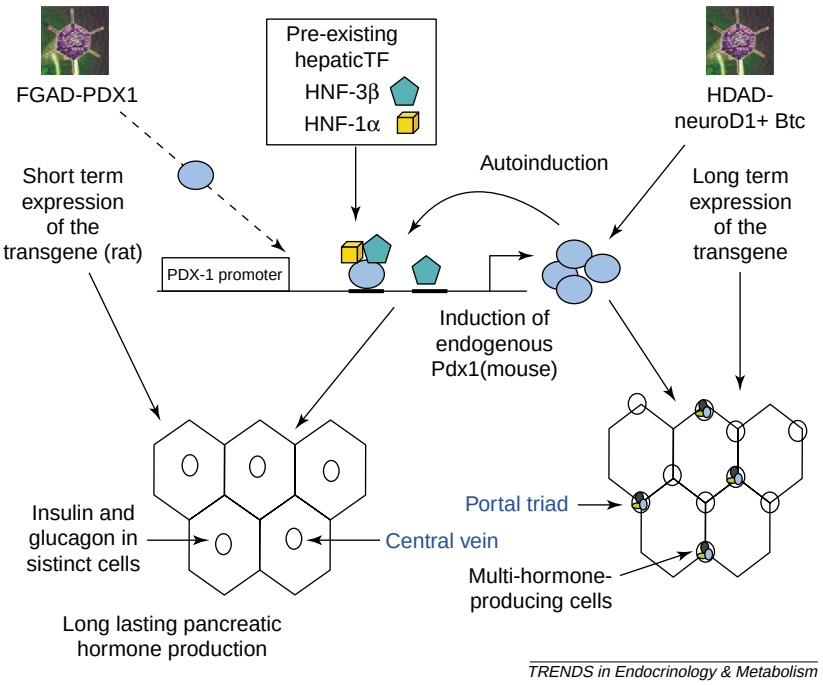

Figure 1. Transient ectopic *Pdx1* expression induces a long-lasting process of transdifferentiation due to autoinduction of *Pdx1* in liver, which is apparent because of the expression of pancreatic genes, and pancreatic phenotype and function. The ectopic *Pdx1* gene (rat) delivered by FGAD, together with pre-existing and induced transcription factors, activates the expression of the endogenous otherwise silent homolog of (mouse) *Pdx1* in liver [73,26]. Ectopic expression of *Pdx1* and *NeuroD1* distinctly affect the developmental redirection of cells in liver: insulin and glucagon are produced by different cells after ectopic expression of *Pdx1* [26], whereas in mice treated with NeuroD1 and Btc, the same cells produce all the pancreatic hormones [28]. PDX1 and NeuroD1 affect distinct zones in liver. PDX1 affects mainly cells that are close to central veins [26], whereas cells that respond to NeuroD1 are close to portal veins and in the liver capsule [28]. Abbreviations: Btc, betacellulin; FGAD, first generation E1-deleted recombinant adenoviruses; HDAD, helper-dependent recombinant adenovirus; HNF, hepatic nuclear factor; NeuroD1, neurogenic differentiation 1; PDX1, pancreatic and duodenal homeobox gene 1; TF, transcription factor.

Functional impact of massive ectopic Pdx-1 levels in mice liver *in vivo:* more is not always better

The effect of massive ectopic expression of *Pdx1* in most of the cells in the liver has been demonstrated recently in mice [28]. Kojima *et al.* [28] used an HDAD to deliver the transgene (Figure 1, Box 1). They demonstrated that, at very high levels, PDX1 is toxic to liver and has no therapeutic effect; the mice become sick, lose weight and die within two weeks. By contrast, as mentioned above, mice treated with FGAD-mediated delivery of *Pdx1* consistently gain weight, have normal hepatic function and the therapeutic benefit of hepatic insulin production is evident even eight months after the initial treatment [26].

The distinct outcome of ectopic expression of *Pdx1* in liver is most likely to be attributable to the increased intracellular levels of the transgene and its wide intrahepatic distribution [26,28]. The use of HDAD vectors in combination with the promoter of *mammalian elongation factor 1α* [28,65] might result in substantially higher expression of *Pdx1* compared with the expression of *Pdx1* achieved with FGAD delivery [26,28]. This difference might range over three orders of magnitude in nearly all the cells in the liver [65–67].

Exaggerated hepatic concentrations of PDX1 could have detrimental effects on both hepatic and 'induced' pancreatic functions. First, although low concentrations of PDX1 do not induce acinar function [26], induction of exocrine functions by high concentrations of PDX1 has yet to be analyzed directly. Second, PDX1 might repress the expression of hepatic genes in mouse liver, as in *Xenopus* [27], which would explain the hepatic dysfunction, weight loss and subsequent death of mice when most of the liver cells permanently express ectopic *Pdx1* [28]. Third, although it has yet to be shown in liver, PDX1 activates insulin gene expression in pancreatic β cells with bell-shaped, dose-dependent characteristics [68,69]. Therefore, substantial overexpression of *Pdx1* might inhibit the insulin gene expression in liver, as does PDX1 in pancreatic β cells [68,69]. Providing the level of *Pdx1* expression can be controlled and restricted to moderate numbers of liver cells, ectopic expression of *Pdx1* could provide an efficient means to activate the pancreatic-gene repertoire in liver without affecting hepatic function.

However, other pancreatic transcription factors might also reprogram liver cells towards a pancreatic phenotype by mechanisms that are distinct to those of PDX1, and with different outcomes.

NeuroD1 and betacellulin-induced liver-to-pancreas developmental switch

Gain-of-function studies can reveal surprising effects of additional transcription factors in the process of the developmental shift from liver to pancreas. Indeed, such a capacity has been demonstrated for NeuroD1. The basic helix-loop-helix transcription factor NeuroD1 [49] is required for morphogenesis of pancreatic islets, and NeuroD1-deficient mice die from severe diabetes [70]. When expressed ectopically in mature liver *in vivo*, *NeuroD1* induces many pancreatic markers including

downstream and upstream transcription factors in the pancreatic transcriptional network, including *Pdx1*. However, unlike *Pdx1*-treated liver, in which glucagon and insulin are produced by different cells [26], individual *NeuroD1*-treated cells produce all pancreatic hormones [28] (Figure 1). Combined ectopic expression of *NeuroD1* and β-cell-stimulating hormone, betacellulin, a member of the epidermal growth hormone family of growth factors [71,72], induced insulin production and ameliorated hyperglycemia in STZ diabetic mice in a glucose-regulated manner. Cells that produce pancreatic hormones are located next to portal veins and in areas that are close to the liver capsule. These two types of pancreatic-hormone-producing cells indicate that the developmental shift occurs in distinct populations of predisposed liver cells, both of which are distinct from those induced by ectopic expression of *Pdx1* [26]. It is not clear what dictates the distinct characteristics of cells induced by NeuroD1 versus PDX1 – is it the distinct transcription factors, or the distinct host cells that are affected in liver, or possibly both (Figure 1)?

The study by Kojima *et al.* [28] is also important in demonstrating the complementary roles of soluble differentiation factors and pancreatic transcription factors in redirecting the development of cells in the liver to a pancreatic phenotype, including the formation of a novel cellular compartment of insulin secretory granules. The physiological and therapeutic impact of coproduction of many pancreatic hormones in liver cells needs to be analyzed further [28]. In addition, it is not clear whether *NeuroD1* alone and *NeuroD1* plus betacellulin induces transdifferentiation, as does *Pdx1* [27], or whether these factors endow liver cells with a pancreatic phenotype and function without turning off the repertoire of hepatic gene expression.

Summary and future studies

Studies in the past 3–4 years demonstrate the surprising ability of pancreatic transcription and growth factors to reprogram cells in the liver and endow them with pancreatic characteristics and function. The ability to convert a small part of the liver into endocrine pancreas that has the capacity to adequately control blood glucose levels represents a revolutionary approach to the treatment of diabetic patients. The vast body of knowledge generated in the past decade about the transcriptional network that controls β-cell differentiation and function could, in theory, be implemented to induce the development of an ectopic pancreas in tissues such as the liver.

To translate this into an efficient and precisely controlled process, we need to address further important questions.

First, most of the studies described here describe a predisposition of some liver cells to the reprogramming process. Identifying markers for predisposed cells might allow these cells to be better targeted *in vivo*. Targeting a specific subpopulation of liver cells should restrict the number of cells affected and preserve normal hepatic function as well as allowing additional endocrine pancreatic function in this organ. Moreover, the heterogeneity of cells in the liver might present distinct intracellular milieu, with possibly adverse effects on the reprogramming process.

Second, several pancreatic transcription factors might be proactive in the process of liver to pancreas transconversion. Each factor might impose a distinct outcome on hepatic cells and the acquired pancreatic function. For example, whereas some pancreatic transcription factors could turn off the expression of host genes, thus impeding the function of the host tissue, other factors might add to the hepatic genes expressed, thus compromising the pancreatic-cell characteristics. Therefore, the optimal cocktail of the relative quantities of differentiation and transcription factors that are effective in promoting the developmental redirection process should be determined.

Third, the effect of soluble differentiation factors on the transconversion process should be analyzed further, alone and in combination with pancreatic transcription factors, for inclusion in the 'transdifferentiation cocktail'.

Fourth, because T1DM is an autoimmune disease, it is important to analyze whether the cells that form the ectopic pancreas in liver acquire 'undesired' β-cell characteristics, such as susceptibility to autoimmune attack. If this is acquired as a part of the transdifferentiation process, further engineering will be needed.

Future gain-of-function studies will bring further insight into the transcription factors that induce pancreas development. This knowledge should be implemented to develop a cell-based-therapy to treat diabetics. The potential to effectively generate new tissues from our

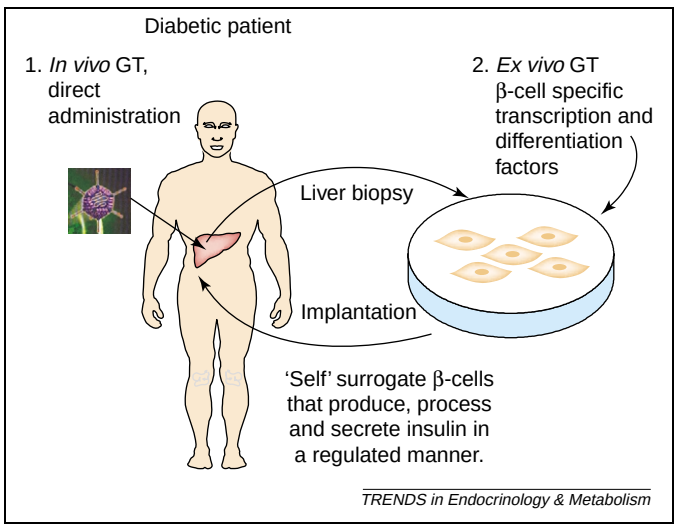

Figure 2. Engineering the liver of a diabetic patient to function as surrogate endocrine pancreas would bypass the shortage of tissue available from cadaveric donors and the need for life-long antirejection treatment. This might be achieved using two approaches. (1) The *in vivo* approach (Box 1). Adenovirus that encodes pancreatic transcription and/or differentiation factors is injected directly into the diabetic patient's liver. It is expected that the delivered gene and the viral genome will be eliminated in a few days, but that 'pancreatic function' induced in liver will provide a therapeutic effect for long periods, possibly indefinitely. (2) The *ex vivo* approach. A liver biopsy from a diabetic patient is treated *in vitro* with adenovirus that encodes pancreatic transcription and/or differentiation factors. The pancreatic function induced in these cells is analyzed *in vitro*, and the 'self' surrogate β cells are implanted in the same patient's liver. A small portion of liver needs to undergo a developmental shift to have a therapeutic effect. The liver is 10–15-fold larger than the pancreas, and pancreatic β cells comprise <2% of the pancreas in adult mammals [74], which indicates that efficient conversion of ~1% of liver cells would produce and secrete enough insulin to continuously control blood glucose levels and, thus, replace pancreatic function [26,28].

own mature organs will overcome both the shortage of available tissue and the need for immunosuppression, and could constitute a breakthrough in the prospects of developing a therapy for diabetes (Figure 2).

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