The diabetes epidemic affects approximately 6–8% of the world population and the number of newly diagnosed patients increases yearly [1]. Although intensive exogenous insulin therapy can achieve the physiologic control of blood glucose and delay the onset of related complications, such treatment is suboptimal. The ultimate goal is to treat diabetes through the replacement of destroyed beta cells by insulin-producing cells capable of restoring glucose homeostasis in the organism.

Pancreas transplantation and islet-cell implantation are efficient in replacing the function of the impaired tissue and thus inducing continuous normoglycemia. These grafts bring freedom from the burden of injections, glucose testing and dietary restrictions; and, importantly, continuous normoglycemia protects against the complications of diabetes [1]. However, these treatments require extensive, lifelong 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 beta cells are found. Since differentiated beta cells cannot be efficiently expanded in vitro [2], alternative approaches are being explored to generate insulin-producing cells, either by genetic engineering of beta cells or by utilizing various potential beta cell precursor cells. The use of embryonic or adult stem cells for generating functional pancreatic endocrine tissue was recently reviewed [3,4]. The present review analyzes the surprising option of using the adult liver as a pancreatic progenitor tissue [5-17]. This approach may result in the generation of custom made "self" surrogate beta cells for the treatment of diabetes, bypassing both the shortage in tissue availability from cadaveric donors and the need for lifelong immunosuppression.

The rationale for using liver as a pancreatic progenitor tissue

Physiologic
Liver is the largest organ in our body with a high level of functional redundancy [18] and, unlike beta cells, liver regenerates efficiently, mainly by the proliferation of mature hepatocytes [19]. Human liver cells can be propagated in vitro for months, and the numbers of cells can be expanded substantially ex vivo. Moreover, since liver has an important role in neutralizing toxins and, in contrast to pancreatic beta cells, hepatocytes have high levels of catalase and dysmutase activities [18], surrogate beta cells derived from liver may resist cellular assaults that beta cells are sensitive to.

Developmental
Trans-conversion between liver and pancreas is conceivable since the liver and pancreas are related developmentally; 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 [20]. Both tissues have many characteristics in common, including responsiveness to glucose and a large group of specific transcription factors mutually expressed in both tissues [21]. Trans-conversion between pancreatic acinar cells and hepatocytes in both rodents and humans has been reported under experimental, pathologic and malignant conditions [22].

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, there...
is no spatial separation between the two organs [23, 24] and the ‘hepato-pancreas’ functions as both liver and pancreas [20].

Molecular

States of developmental commitment are encoded as combinations of transcription factors and the environmental niche that possibly reflects intracellular signals mediated by soluble factors. In certain cases, the determined state is dominated by the presence of just one factor that acts as a sort of master regulator in forcing a particular path of development in the presence of various other possible factors. Examples are the MyoD family of basic helix-loop-helix factors, which can force differentiation of muscle in a variety of cell types in culture [25], or the eyeless/pax6 factor that can force eye development in many of the imaginal discs of Drosophila [26].

The hierarchical role that transcription factors play in pancreatic organogenesis in the embryo, and the role of many soluble factors in promoting β cell maturation have been identified. The basic rationale that underlies the concept of generating functional pancreatic tissue in adult liver is that the sequential or combinatorial use of these factors may be instrumental in inducing developmental redirection along the pancreatic lineage of adult tissues as well. It is assumed that the maximal therapeutic merit of using liver as pancreatic progenitor tissue will be achieved when the optimal combination of pancreatic growth and transcription factors endows a sufficient number of liver cells with mature β cell characteristics and function. Numerous pancreatic transcription and developmental factors have been demonstrated to participate in endowing liver cells with pancreatic characteristics [5-17].

PDX-1 reprograms adult liver cells function

The surprising capacity to activate pancreatic lineage in the liver was first demonstrated in mice by systemic PDX-1 administration using recombinant adenovirus gene delivery [5]. PDX-1 plays a dual and central role in regulating both pancreas organogenesis in embryo and β cell function in adults [27,28]. The capacity of PDX-1 to activate the pancreatic lineage in mature liver has been independently confirmed in numerous studies [5-11,13,14,16,17], and the possible effects of additional pancreatic transcription factors alone or combined with PDX-1 expression on the process has been analyzed [7,10-13,16]. From a developmental point of view, ‘gain of function studies’ using sequential ectopic expression of pancreatic transcription factors in ‘permissive’ extra-pancreatic tissues such as liver may provide valuable information on the role of these factors in normal pancreas organogenesis, in addition to their implemented use in engineering “ectopic pancreas” in liver.

The capacity of PDX-1 to direct pancreas development was demonstrated in mature fully differentiated liver in vivo, both in mice and in Xenopus, possibly via a process called trans-differentiation [5,6,8-11,14]. Trans-differentiation describes an irreversible switch of one type of differentiated cell into another differentiated cell [29].

Transient, ectopic expression of PDX-1 in liver, delivered by systemic administration of recombinant adenovirus, induced a wide repertoire of pancreatic gene expression. Surprisingly, the ectopic PDX-1 expression led to a long-lasting production and secretion of processed, biologically active insulin, despite the short-term expression of PDX-1 transgene in the liver [5,6]. PDX-1 induced its own expression in liver (auto-induction), which in turn explains the irreversible nature of the “liver-to-pancreas” trans-differentiation process.

Hepatic insulin production triggered by PDX-1 administration was functional for long periods; not only did it maintain euglycemia in STZ-induced diabetic mice [5], it also resisted the STZ deleterious effects, even 8 months after the initial treatment [6]. This may suggest that developmentally redirected insulin-producing cells in liver may resist assaults to which normal β cells are sensitive.

PDX-1 induced liver to pancreas trans-differentiation by a recombinant adenovirus delivery system only in predisposed cells in the liver, comprising less than 1% of liver cells and located close to central veins, despite initial random expression of the transgene in the liver [5,6,10,11]. Such a predisposition could be caused by the heterogeneity of cells in the liver [30]. Different cells could distinctly respond to the pancreatic transgene in the sense of ultrastructural modifications in chromatin compaction, the presence of silencing effects or the need for complementing transcription factors that may work in concert with the ectopic transcription factor [31,32]. A fusion protein of PDX-1 with the transcriptional activation domain of VP-16 (a regulatory protein from Herpes simplex virus) was used to overcome some of these barriers, thus increasing the number of ‘responding’ cells in the liver [8-11]. PDX-1/VP16 markedly increases insulin biosynthesis, inducing the expression of various pancreas-related factors in the liver [9-11]. Furthermore, in STZ-induced diabetic mice, PDX-1/VP16 expression was more efficient than the non-activated pancreatic transcription factor in ameliorating diabetes [9-11].

PDX-1/VP16 expression in transgenic tadpoles allowed the conversion of most of the liver into exocrine and endocrine pancreas [8]. The massive transformation of the tadpole’s liver into pancreas suggests that activation of PDX-1 by the VP-16 might have eliminated the requirement for additional co-factors that may work in concert with PDX-1 in activating the pancreatic repertoire of gene expression [33]. Alternately, VP-16 could also affect the chromatin condensation and remodeling of PDX-1 target genes on the host genome [34]. Indeed, the activation of PDX-1 by VP-16 allowed Imai et al. [9] to induce the pancreatic lineage in liver, using a relatively low titer of recombinant adenoviruses for in vivo gene delivery.

Additional pancreatic transcription factors induce pancreatic lineage in liver

Several pancreatic transcription factors were demonstrated to promote the PDX-1 effect on the developmental redirection process. These factors promoted β cell maturation manifested by improved amelioration of hyperglycemia. While NeuroD-1, MafA or Ngn-3 were incapable of individually inducing a functional β cell function in liver, they significantly promoted the PDX-1
effect on the process [10,11]. In an additional study, individual NeuroD-1 expression moderately activated the pancreatic repertoire and function, but its effect was substantially augmented when combined with betacellulin administration [12]. This basic helix-loop-helix transcription factor is required for morphogenesis of pancreatic islets, and NeuroD-1-deficient mice die from severe diabetes [35]. Interestingly, ectopic NeuroD-1 expression in the liver induced both downstream and upstream transcription factors, which are part of the pancreatic transcriptional network, including PDX-1. However, while in PDX-1-treated liver, glucagon and insulin were produced in distinct cells [6], all the pancreatic hormones were co-produced within the same cell in mice liver treated with NeuroD-1 [12]. The appearance of distinct types of pancreatic hormone-producing cells may suggest that the developmental shift upon PDX-1 ectopic expression occurred in distinct populations of predisposed liver cells [6]. It is not clear what dictates the distinct characteristics of the cells induced: the distinct transcription factors, the distinctly affected host cells in liver, or possibly both.

Soluble factors promote the induction of pancreatic lineage in liver

A role for soluble factors in promoting transcription factor-induced liver to pancreas trans-differentiation has been suggested. Betacellulin (β cell-stimulating hormone, a member of the epidermal growth factor family of growth factors) [36] has been implemented in promoting NeuroD-1-induced liver to pancreas trans-differentiation in mice [12]. Combined ectopic expression of NeuroD-1 and betacellulin induced higher levels of insulin production and ameliorated hyperglycemia in STZ diabetic mice in a glucose-regulated manner. Hepatic regeneration using 70% hepatectomy was also demonstrated to improve the effect of PDX-1 in accelerating the trans-differentiation process along the pancreatic lineage [14]. The improved therapeutic outcome of the process could be due to the increased number of proliferating and possibly pluripotent cells in this organ. The invasiveness of the proposed approach could be compromised by co-treating the mice with growth factors such as hepatocyte growth factor or interleukin-6, the levels of which increase during liver regeneration. In addition, hepatocyte growth factor could also promote the pancreatic lineage induced by PDX-1 [37].

Hepatic pluripotent cells may serve as pancreatic progenitors

It is reasonable to assume that immature liver cells may be more susceptible to acquire pancreatic characteristics due to their potential plasticity. Therefore, several groups analyzed the potential of inducing developmental redirection of hepatic pluripotent cells such as oval cells or fetal derived hepatic cell line [15-17]. The bile duct-derived progenitors called oval cells were characterized in rodents, but their human equivalents have not yet been found [19]. These cells were documented previously to possess several developmental options, including hepatocytes, biliary epithelium, intestinal epithelium and pancreatic acinar epithelium [38]. Yang et al. [15] 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. These cells express several genes that encode pancreatic transcription factors, including PDX-1 [15]. The capacity to redirect the developmental fate of immortalized, human fetal liver cells by ectopic expression of PDX-1 was recently demonstrated [16,17]. Cells that ectopically expressed PDX-1 produced, stored and secreted processed insulin in a glucose-regulated manner [17]. Treatment with activin A resulted in enhanced differentiation of the cells, as judged by an increase in insulin content and the expression of a number of β cell genes as well as a decrease in the expression of non-β cell genes [16]. The modulated cells were functional, as they restored and maintained euglycemia in diabetic immunodeicient mice [16]. That study demonstrates the capacity of PDX-1 to shift the developmental route of committed cells in human fetal liver and endow them with many characteristics and functions of pancreatic β cells [16,17]. However, further study showed that primary cultures derived from either adult or fetal human livers were similarly affected by ectopic PDX-1 expression. Thirty percent of fetal and 25% of adult human liver cells activated the insulin promoter in response to ectopic PDX-1 expression, suggesting that PDX-1 activates the pancreatic repertoire in liver regardless of the original state of liver cells differentiation [7].

Converting adult human liver cells into insulin-producing tissue

The capacity of inducing functional redirection of mature human liver cells carries a substantial therapeutic significance, since it may allow autologous cell replacement therapy for diabetics. Indeed, a recent study demonstrated the potential use of primary cultures of adult human liver cells as pancreatic progenitors [7]. Cells isolated from adult human liver were propagated in vitro for many passages, and upon PDX-1 and soluble factors (EGF and nicotinamide), up to 50% of PDX-1-expressing cells activated the ectopic insulin promoter. Insulin in PDX-1-treated adult human liver cells was determined at molecular, cellular and functional levels. The produced hormone was processed, stored in induced secretory granules and secreted upon glucose challenge. Although located in a high position in the transcription factor hierarchy, leading to pancreatic organogenesis, PDX-1 induced neither exocrine gene expression nor function.

The most important ‘pancreatic-like’ function these cells demonstrated was their capacity to ameliorate hyperglycemia upon implantation in diabetic immunodeicient mice in vivo. Human C-peptide secretion and the parallel amelioration of diabetes persisted for the whole duration of the experiment (60 days). Removal of implanted trans-differentiated adult human liver cells by nephrectomy 23, 45 and 60 days after implantation resulted in reversal to hyperglycemia, which was associated with decreased human C-peptide levels. Consistent with the functional properties of trans-differentiated adult human liver cells was the expression of the pancreatic transcription factors, including that of the

EGF = epidermal growth factor
endogenous human PDX-1 [7]. Li and collaborators [13] documented that in PDX-1-treated HepG2 cells as well, the transgene was only a temporary trigger that was no longer required once the pancreatic differentiation program was activated.

Thus, it might not be surprising that over-expression of PDX-1 can lead to the development of insulin-producing cells. It is surprising that over-expression of this single gene in hepatocytes would turn them into pancreatic β cells so efficiently and effectively. Firstly, most work to date has focused on PDX-1 as a regulator of “metabolic” genes in the islet (e.g., insulin, Glut-2, glucokinase, etc.) and less on structural genes required for the development of classical, authentic dense-core neuroendocrine, regulated secretory granules. Sapir and team converted a classical constitutively secreting cell type (the hepatocyte) into a classical neuroendocrine cell type (the beta cell): their new beta cells not only make insulin and other metabolic molecules (e.g., Glut-2 and glucokinase, which together comprise the glucose sensing apparatus), but also contain the key cellular components for regulated neuroendocrine secretion: secretory granules, secretory granule proteins and neuroendocrine hormone-processing enzymes. Moreover, the trans-differentiation appears to be sustained in vivo for up to 60 days. Since the endogenous human PDX-1 gene appears to be activated in the new islets, it suggests an irreversible trans-differentiation process.

**Summary**

The induction of developmental redirection constitutes a novel approach in the field of regenerative medicine, which uses adult tissues for generating new functional organs. The capacity of using liver as a pancreatic progenitor tissue has been demonstrated both in vivo and in vitro in Xenopus mice and humans. At first glance it may seem disappointing that less than 1% of PDX-1-positive cells actually produce, store and secrete insulin, in vivo [5,6]. In vitro the process is more efficient, such that up to 20–50% of PDX-1-expressing cells produce insulin [7]. However, in both approaches hepatic insulin production ameliorates hyperglycemia. Considering that normal pancreatic β cell mass is only about 0.1% of the liver mass, even if each trans-differentiated cell produces only 10% of the insulin produced by a pancreatic β cell, the conversion of 1% of liver cells is expected to compensate for the entire pancreatic insulin production.

**Next steps in the developmental redirection paradigm**

Additional efficacy studies should indicate whether developmentally shifted liver cells become a target for autoimmune attack that characterizes type 1 diabetes? Can insulin really be secreted within 2 or 3 minutes of exposure to glucose, as occurs in normal β cells? Will the developmentally shifted liver cells become a target for autoimmune attack that characterizes type 1 diabetes?

**Conclusion**

The data presented in this review serve as a proof of concept for the basic capacity of directing cells in liver towards the pancreatic lineage and function. A further “fine-tuning” of the combination of transcription and soluble factors is expected to increase the therapeutic merit of this novel regenerative medicine approach. It has long been thought that animal cells once committed to a specific lineage, generally, can no longer change their fate and thus become “terminally differentiated” [39]. We now know that adult liver retains substantial plasticity and can be induced to assume new fates and function upon appropriate molecular manipulation.

The use of adult human liver cells for generating functional insulin-producing tissue may pave the way to autologous implantations, thus allowing the diabetic patient to be the donor of his or her own insulin-producing tissue [Figure 1]. This approach may circumvent the shortage in tissue availability, the need for anti-rejection treatment, and the ethical issues associated with the use of fetal or embryonic stem cells for this purpose.

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