Can We Create New Organs from Our Own Tissues?

Sarah Ferber PhD
Endocrine Institute, Sheba Medical Center, Tel-Hashomer, Israel

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Abstract
The many new technologies of the past few years have set the stage for novel human therapeutic methods. Identification of pluripotent stem cells as being capable of generating various cell types in the body, together with advanced genetic and cell engineering techniques, may enable the design of custom tissues and organs and thus solve the problem of donor organ scarcity and the need for immune compatibility and immunosuppression to avoid graft rejection. One of the most prevalent metabolic disorders that will benefit from such technologies is insulin-dependent diabetes mellitus. The purpose of our study is to review potential future methods of curing metabolic disorders such as diabetes, and analyze the capacity to genetically manipulate the developmental fate of a tissue in vivo using “master regulator” genes. We systematically delivered the homeobox gene Pancreatic and Duodenal Homeobox gene-1 to liver of mice, by recombinant adenovirus technology, and analyzed whether it induces a developmental shift toward a β cell phenotype. We demonstrated that PDX-1 is sufficient to activate the endogenous, otherwise silent, mouse insulin 1 and 2 and pro-insulin convertase gene expression in liver. PDX-1 expression in liver resulted in a 25-fold increase in hepatic immunoreactive insulin content and a threefold increase in plasma immunoreactive insulin levels, as compared to control adenovirus-treated mice. Hepatic immunoreactive insulin, induced by PDX-1, was processed to mature mI-1 and mI-2 and was biologically active; it ameliorated hyperglycemia in streptozotocin-treated diabetic mice. PDX-1 has the capacity to reprogram extra-pancreatic tissue toward a β cell phenotype. The data provide a valuable approach to generate “self” surrogate β cells that are suitable for replacing impaired islet cell function in diabetics.

Current therapeutic methods for diabetes
Since the discovery of insulin in 1921 by Banting and Best, insulin therapy has enabled diabetic patients to survive. Nevertheless, chronic complications evolving years after the onset of diabetes, such as blindness, renal failure, myocardial infarcts and limb amputation, damage the quality of life and impose a multi-billion dollar annual burden on the health care systems in industrialized countries [1]. It is estimated that the prevalence of type II diabetes in western countries is as much as 5% and of insulin-dependent type I diabetes approximately 0.4%. About 50% of diabetics will develop chronic diabetic complications [2].

Several clinical trials have demonstrated that strict glycemic control can slow or even prevent the progression of diabetic complications [3]. However, intensive insulin therapy increases the incidence of hypoglycemic episodes threefold and is suitable only for selected patients [4]. Pancreas transplantation and islet cell implantation, although efficient in inducing normoglycemia, require extensive and life-long suppression of the immune system and are restricted by limited tissue supply. For decades the inability of insulin therapy to physiologically control glycemia in type I diabetic patients has motivated the search for insulin-delivery grafts. These grafts are expected to both sense the circulating levels of glucose and respond appropriately by releasing insulin at the right dose and tempo. Its availability would eliminate the need for injections, glucose testing and dietary restrictions, and even more importantly, provide protection from the dreaded complications of the disease [5].

The use of transformed or conditionally transformed ‘surrogate β cells,’ engineered to mimic pancreatic islet function, has been suggested as a means to overcome the severe shortage of donor tissue for transplantation [6–9]. However, since such engineered cells are derived from rodent cells, besides the potential risk they carry of inducing zoonosis in humans, their use will still require immunosuppression or immuno-isolation [6]. New hope for replacing malfunctioning organs lies in the advances in tissue engineering and stem cell biology.

Potential application of stem cells to replace damaged tissues
Most of our organs will probably prove to contain cells capable of repopulating the organs, either during normal life or at least under circumstances of tissue repair [10].
Stem cells are units of biological organization; they are responsible for the development and regeneration of tissue and organ systems, having both the capacity of self-renewal and multi-lineage differentiation [11]. The earliest stem cells in ontogeny are totipotent, extending from the zygote to the inner cell mass of the blastocyst. These cells are attracting increasing interest for two reasons: the first is the successful cultivation of human embryonic stem cells, and the second is their potential to spontaneously give rise to developmentally unrelated cell types [12]. Developing capacities to direct these cells’ differentiation into a desired tissue requires a better understanding of the permissive, instructive and selective factors and signals that allow this process to occur [13]. In diabetic mice, the transfer of insulin-secreting cells derived by selection pressure from mouse embryonic stem cells was shown to normalize glycemia [14]. The properties of human embryonic stem cells have been described, and the possibility of obtaining them from cloned human embryos discussed [15].

Unlike the totipotent cells derived from the blastocyst, mature tissues contain developmentally committed stem cells. However, adult stem cells possess an intrinsic plasticity; for example, neural stem cells can give rise to hematopoietic stem cells, hematopoietic stem cells can differentiate into liver oval cells and muscle cells, and conversely muscle stem cells can generate blood cells [16].

Two main factors may direct pluripotent cells in the choice of the specific developmental route: the microenvironment or niche that surrounds them, and the profile of the genes they express [17]. An important question addressed in the present study is whether we can influence predisposed cells to take an alternate developmental route by ectopic expression of tissue-specific master regulator genes.

**Master regulator genes and their role in modulating cell fate**

Although the genetic information in each of the somatic cells is identical, the transcription profile of each tissue is distinct, being endowed with unique characteristics and function. For example, insulin gene expression is restricted to pancreatic islets β cells, while in every other tissue the insulin gene is transcriptionally silent due to specific control mediated in part by specific transcription factors [18]. On the other hand, transcription factors, acting as “master regulators,” were demonstrated to possess instructive roles in modulating a given tissue fate. MyoD was suggested to be a master regulatory gene for myogenesis. Ectopic MyoD leads to the expression of muscle-specific proteins in primary fibroblasts and in differentiated melanoma, neuroblastoma, liver and various derived cell lines in vitro [19]. Ectopic expression of NeuroD in *Xenopus* embryos caused premature differentiation of neuronal precursors. NeuroD seemed competent to bypass the normal inhibitory influences that usually prevent neurogenesis in ventral and lateral ectoderm and was capable of converting most of the embryonic ectoderm into neurons [20].

Master regulators, though not the sole “decision makers,” could play instructive roles in dictating developmental avenues of pluripotent cell differentiation and are suggested as being a major factor in tissue development.

**The regenerative capacity of pancreatic islets**

It has been proposed that pancreatic duct cells are a source of pluripotent cells from which mature endocrine tissue regenerates upon normal physiological conditions [21]. However, in most cases, the regenerative capacity of pancreatic duct cells is insufficient to compensate for massive loss of mature β cell function in pathophysiological states, such as autoimmune attack that occurs in IDDM [21]. Ramiya et al. [22] reported that large numbers of islet cells could be derived from pancreatic ductal epithelial cells removed from pre-diabetic non-obese diabetic mice. These pancreatic ductal cells, expanded in culture, had proven efficient in correcting hyperglycemia in another model of IDDM that was chemically induced by streptozotocin treatment [22]. The fact that ductal cells may develop into mature pancreatic β cells is proven in the STZ model of IDDM. However, these cells’ inability to prevent hyperglycemia in the NOD model of IDDM (autoimmune attack), from which they were derived, may indicate that their proliferation or maturation capacities could be ablated by the NOD in vivo environment, which in many ways resembles IDDM in humans. The availability of such cells, our capacity to isolate them from pre-diabetic patients, and the capacity of the cells to survive, differentiate and function in type I diabetics therefore remains uncertain.

**Possible novel directions to generate pancreatic β cells**

The possible role of master regulator genes in directing cell fate, together with the documented plasticity of some of the mature tissues and pluripotent cells, motivated us to analyze whether we can induce a β cell phenotype in liver by ectopic expression of islet cell-specific “master regulator” genes.

Studies performed by multiple groups suggest that the homeodomain protein PDX-1 plays a central role in regulating pancreas development and islet cell function [23]. PDX-1 regulates insulin gene expression and is involved in islet cell-specific expression of various genes [24–27]. A role for PDX-1 in islet cell differentiation and function has been demonstrated mainly by ‘loss-of-function’ studies [28–31], and by ectopic expression in pancreatic transformed cell lines [32].

*IDDM = insulin-dependent diabetes mellitus*

*STZ = streptozotocin*

*NOD = non-obese diabetic*
Liver, unlike β cells, regenerates very efficiently, indicating a relatively high percentage of functional stem cells [33–35]. When hepatocyte-damaging agents impair the regenerative ability of surviving hepatocytes, a potential stem cell system of biliary origin is activated to generate new hepatocytes. The bile duct-derived progenitors, called oval cells, possess several developmental options including hepatocytes, biliary epithelium, intestinal epithelium and acinar epithelium [35].

Since the liver and pancreas are both of endodermal origin, derived from appendages of the upper primitive foregut, the possibility of interconversion between these two types of tissues is conceivable. Indeed, the conversion of pancreatic acinar cells into hepatocytes in certain conditions has been reported [36].

**PDX-1 induces extended β cell phenotype in liver in vivo**

Using an in vivo ‘gain-of-function’ study, we tested whether PDX-1 could endow liver cells with pancreatic β cell characteristics (a schematic presentation of our approach and its outcome is presented in Figure 1, adopted with permission and slight modifications from: Axel Kahn, *Nature Medicine* 2000;6:505–6, which reviews our study).

We delivered *AdCMV-PDX-1* recombinant adenovirus (encoding the rat homolog of PDX-1 [32]) to 11–14 week old male Balb/c and C57BL/6 mice. Reverse transcription-polymerase chain reaction analysis of total RNA revealed that *AdCMV-PDX-1* administration resulted in PDX-1 expression mainly in the liver. PDX-1 induced the expression of the endogenous mI-1 and mI-2 genes in the liver [37].

To analyze whether hepatic insulin mRNA is effectively translated into protein, immunoreactive insulin content was tested in extracts derived from hepatic tissue by radioimmunoassay. Livers from PDX-1 treated mice that tested positive for insulin gene expression by RT-PCR [37] contained about 25-fold more IRI than livers of mice treated by a control virus [Table 1]. Mean IRI levels in extracts derived from PDX-1 treated livers was 20.7±3.97 µU/mg protein, while in control livers (from mice treated by the same number of *AdCMV-β-gal* recombinant adenoviruses), IRI was only 0.78±0.25 µU/mg protein. While IR1 detected in PDX-1 treated liver extracts was <1% of the levels detected in pancreatic extracts [Table 1], serum IRI levels in PDX-1 treated mice were almost threefold higher compared to controls (323±48.4 vs. 118.2±23.7 µU/ml, respectively [Table 1]), indicating that insulin was being synthesized and a large portion of it secreted into the bloodstream. Mice treated by *AdCMV-PDX-1* were not hypoglycemic, but their blood glucose level was slightly lower than that of mice treated by *AdCMV-β-gal* [Table 1]. Since the experiment described in Table 1 was performed in mice with intact pancreatic islets, extreme alterations in blood glucose levels were not expected. However, the normoglycemia associated with a significant elevation of serum IRI levels suggests that the hormone produced and secreted from the liver may be partially unprocessed.

High performance liquid chromatography analysis of hepatic IRI content from PDX-1 treated mice revealed that it contained 59±7% (n=3) of fully processed mI-1

![Figure 1](image)

**Figure 1.** Systemic delivery of PDX-1 via recombinant adenoviruses results in PDX-1 protein expression in 50% of liver cell nuclei. The endogenous, otherwise silent, insulin genes are expressed, and result in a threefold increase in blood immunoreactive insulin levels (IRI) and a 25-fold increase in hepatic IRI content. PDX-1 induces also the expression of pro-insulin convertase (PC1/3), such that the hepatic content of insulin was processed into a mature biologically active form of the hormone.

**Table 1. Blood glucose and immunoreactive insulin levels in serum and liver extracts**

<table>
<thead>
<tr>
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<th>Control virus-treated mice</th>
<th><em>AdCMV-PDX-1</em> treated mice</th>
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<tr>
<td>Blood glucose (mg/dl)</td>
<td>228±15.74 (n=18)</td>
<td>197±11.2 (n=40)</td>
</tr>
<tr>
<td>Serum IRI (µU/ml)</td>
<td>118.2±23.7 (n=14)</td>
<td>323±48.4 (n=26)</td>
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<tr>
<td>Liver extracts IRI (µU/mg protein)</td>
<td>0.78±0.25 (n=10)</td>
<td>20.7±3.97 (n=12)</td>
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<tr>
<td>Pancreas extracts IRI (µU/mg protein)</td>
<td>2627±24 (n=6)</td>
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Statistical analysis was performed with Sigma-Stat software, using two-way ANOVA, Mann-Whitney rank sum test. Blood glucose in PDX-1 treated mice was significantly lower in control treated mice (*P*<0.0098). Serum IRI in PDX-1 treated mice was significantly higher than in control treated mice (*P*<0.0023). IRI content in PDX-1 treated mice was significantly higher than in control treated mice (*P*<0.05, tested by Kruskal-Wallis one-way analysis of variance on ranks, Dunn’s method). (Ferber et al., *Nature Medicine* 2000;6:568–72, with permission).

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RT-PCR = reverse transcription-polymerase chain reaction  
IRI = immunoreactive insulin
and mI-2. In comparison, pancreatic extracts contained 85±5% (n=3) mature insulin [37], whereas ectopic expression of human insulin (AdCMV-hIns) did not result in retention of IRI in the liver cells except for one mouse liver in which most of the extracted IRI was immature insulin. This is in line with previous observations in FAO cells where there was no retention of transfected insulin genes and the insulin gene product was secreted by the constitutive secretory pathway. Moreover, only livers from animals treated by PDX-1 exhibited the induction of PC1/2 expression, a Kexin family protease, the expression of which is restricted to endocrine and neuroendocrine cells with regulated secretory pathway [38].

To analyze whether PDX-1 induced hepatic insulin production is capable of controlling blood glucose levels in diabetic mice, C57BL/6 mice were rendered hyperglycemic (>600 mg/dl glucose) with ketonures 24 hours after STZ administration (220 mg/kg). At 24–48 hours after the injection, mice were systemically treated by either AdCMV-PDX-1 or by AdCMV-β-gal (control) recombinant adenoviruses. In control AdCMV-β-gal treated mice, hyperglycemia persisted and was associated with ketonuria, resulting in an increased rate of mortality. Of the 22 controls, 12 AdCMV-β-gal treated diabetic mice died 2–3 days after administration, and none of the mice survived beyond 8 days after STZ treatment. In contrast, AdCMV-PDX-1 treated diabetic mice survived the whole duration of the experiment. Moreover, they exhibited a gradual decrease in blood glucose levels starting 2 days after recombinant adenoviral treatment. Blood glucose levels in PDX-1 treated mice declined from 600 to about 200 mg/dl one week after viral administration [Figure 2].

Our data show that expression of PDX-1 is sufficient to induce mature and biologically active insulin production in liver. In pancreatic islets, PDX-1 functions in concert with additional transcription factors regulating expression of insulin and additional islet-specific genes [18,30,39]. It is possible that naturally occurring transcription factors in the liver, ubiquitous as well as tissue-specific, act in concert with the ectopic PDX-1 to induce and regulate insulin gene expression in this organ. Indeed, pancreatic and liver tissues share common expression of several transcription factors such as HNF1α, CEBP/β, and E47 [40]. Alternatively, PDX-1 may promote expression of additional β cell-specific transcription factors in liver, similar to the induction of muscle-specific transcription factors by MyoD in non-muscle tissue [19], although this remains to be demonstrated.

It is not yet known which subpopulation of liver cells supports the developmental shift. In vitro transduction of primary culture of hepatocytes (which consists of 98% mature hepatocytes) did not result in induction of the endogenous insulin genes [37]. This may suggest that the trans-differentiation process induced by ectopic PDX-1 expression could have occurred in a pluripotent population of progenitor liver cells present in in vivo culture of mature hepatocytes.

Recombinant adenovirus infection used in our studies as a gene transfer vehicle could be recognized by the liver (in vivo) as a cellular assault, leading to an increase in the number of progenitor cells [35]. In turn, these cells could be more permissive than mature hepatocytes to a PDX-1 mediated developmental shift. It should be emphasized, however, that viral infection per se could not be responsible for the developmental modulation, since insulin gene expression was not apparent upon control AdCMV-β-gal treatment.

**Summary and Conclusions**

This study presents a novel approach for extending the β cell phenotype to liver, in vivo, using ectopic expression of "master regulated" genes. The newly formed tissue mimics pancreatic islet function; it expresses the endogenous otherwise "silent" insulin genes, produces the hormone and processes it into mature, biologically active insulin. Insulin produced and secreted by liver ameliorates hyperglycemia in diabetic mice. Hence, the trans-differentiated liver cells compensate for the inadequate β cell function, and may, in a future therapeutic approach, circumvent the need for transplantation and immunosuppression.

The broad and basic developmental issues raised may establish the basis for stem cell therapy for a large spectrum of metabolic disorders in which a given tissue phenotype will be induced by the ectopic expression of transcription factors.

PC1/2 = pro-insulin convertase

**Figure 2.** Ectopic PDX-1 expression in mice livers ameliorates STZ-induced hyperglycemia. C57BL/6 males at 12-13 weeks were treated by 220 mg/kg STZ in citrate buffer. At 36-48 hours after STZ treatment mice were injected by AdCMVPDX-1 (n=15 mice), or as control by AdCMV-β-gal (n=22); however, 12 died 3–5 days after STZ treatment and an additional 3 mice 6-7 days after STZ treatment. No mortality occurred upon AdCMVPDX-1 treatment. Each treatment included systemic injection of 2x10⁹ plaque-forming units of recombinant adenovirus in 200 µl saline. Glucose levels were determined in blood samples drawn from the ocular vein. (Ferber et al., *Nature Medicine* 2000:6:568-72, with permission).
Although considerable efforts will be needed to fully characterize the events triggered in the liver by expression of PDX-1, these results could constitute a breakthrough in the prospects of therapy for type I diabetes. We have demonstrated that therapeutic strategies for diabetes should focus not only on transplantation approaches, but also on the induction of trans-differentiation of “self” liver cells into an insulin-secreting cell type.

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References


Correspondence: Dr. S. Ferber, Endocrine Institute, Sheba Medical Center, Tel-Hashomer 52621, Israel. Tel: (972-3) 530 3152; Fax: (972-3) 530 2083; email: sferber@netvision.net.il.