Functional, Persistent, and Extended Liver to Pancreas Transdifferentiation*

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Pancreatic and duodenal homeobox gene-1 (PDX-1) regulates pancreas development during embryogenesis, whereas in the adult it controls β-cell function. Here we analyze whether PDX-1 functions as a pancreatic differentia-
tion factor and a bona fide master regulator when ectopically expressed in mature fully differentiated liver in vivo. By ectopic and transient PDX-1 expression in liver in vivo, using the first generation recombinant adenoviruses, we demonstrate that PDX-1 induces in liver a wide repertoire of both exocrine and endocrine pancreatic gene expression. Moreover, PDX-1 induces its own expression (auto-induction), which in turn may explain the long lasting nature of the “liver to pancreas” transdifferentiation. Insulin as well glucagon-producing cells are mainly located in the proximity of hepatic central veins, possibly allowing direct hormone release into the bloodstream, without affecting normal hepatic function. Importantly, we demonstrate that hepatic insulin production triggered by Ad-CMV-PDX-1 recombinant adenovirus administration is functional and prevents streptozotocin-induced hyperglycemia in Balb/c mice even 8 months after the initial treatment. We conclude that PDX-1 plays an important instructive role in pancreas differentiation, not only from primitive gut endoderm but also from mature liver. Transconversion of liver to pancreas may serve as a novel approach for generating endocrine-pancreatic tissue that can replace malfunctioning β-cells in diabetics.

The possible role of “master regulator” transcription factors in directing cell fate, taken together with the documented plasticity of many mature tissues and pluripotent cells, motivated us to analyze whether it is possible to redirect the developmental route of cells in liver toward a pancreatic β-cell phenotype. The strategy used for this purpose is ectopic expression of a specific pancreatic master regulator gene. Our hypothesis was that such a developmental redirection was most likely to occur between tissues that are developmentally related, such as liver and pancreas (1–4). Transdifferentiation of pancreas to liver has been described in both experimental models and human pathology (5–10).

Pancreatic and duodenal homebox gene-1 (PDX-1,1 also known as IDX-1, IPF-1, STF-1, or IUF-1) possesses a dual role in the pancreas; it is essential for normal pancreas development during embryogenesis and for maintenance of β-cell function in the adult (11, 12). During organogenesis PDX-1 is expressed in all cells differentiating toward the exocrine and endocrine components of the pancreas (11–13). In the adult, its expression is restricted to β-cells and to 20% of somatostatin producing δ-cells. PDX-1 is neither expressed in glucagon producing α-cells nor in mature exocrine pancreas (11, 12, 14).

We demonstrated previously that transdifferentiation of liver to pancreas could be induced by ectopic expression of PDX-1; expression of PDX-1 in liver induced expression of the otherwise silent endogenous insulin genes and was sufficient to direct the production and secretion of mature, biologically active insulin from a restricted population of cells in the liver in vivo. Furthermore, insulin secreted from liver of PDX-1-treated mice ameliorated streptozotocin (STZ)-induced diabetes (1).

In the present study PDX-1 cDNA is delivered in vivo by systemic administration of recombinant adenoviruses that carry the rat PDX-1 gene under the cytomegalovirus promoter (Ad-CMV-PDX-1). This gene delivery system results in efficient transgene expression, mainly in the liver (15). Because genes delivered in vivo by recombinant adenoviruses do not integrate into the host genome, ectopic expression is transient, lasting from days to a few weeks (15–17). Therefore, our previous study monitored the effects of the ectopic PDX-1 over a short period (up to 2 weeks), to parallel the predicted time frame of the PDX-1 transgene expression (1).

In order to determine whether PDX-1 fulfills the role of a differentiation factor in mature hepatic tissues, we sought to determine whether it induces, in addition to insulin, a broad repertoire of pancreatic markers, especially those that are not considered its immediate targets. Moreover, if indeed PDX-1 acts as a differentiation factor also in mature liver and not only in the primitive gut endoderm, then it may trigger a cascade of events that do not require continuous expression of the transgene. Therefore, we sought to determine whether the transient ectopic PDX-1 induces a long lasting process of liver to pancreas transconversion. Moreover, bona fide master regulators such as MyoD have the capacity to activate their own expres-

1 The abbreviations used are: PDX-1, pancreatic and duodenal homebox gene-1; Ad-CMV-PDX-1, replication-deficient recombinant adenovirus that carries expression of PDX-1 cDNA under the control of the cytomegalovirus promoter; STZ, streptozotocin; RT, reverse transcriptase; RIA, radioimmunoassay.

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sion (18). This has been proven also for PDX-1 but only in pancreatic β-cells (19, 20). Because additional transcription factors such as hepatic nuclear factor 1α and 3β that are known to control PDX-1 expression in pancreatic β-cells are also expressed in liver and could cooperate with the PDX-1 transgene, we sought to analyze whether the endogenous otherwise silent homologue of PDX-1 is induced also in liver in response to the transgene expression, thus in turn directing a long lasting process of liver to pancreas transdifferentiation, also when the transgene is no longer expressed.

The present study analyzes PDX-1 capacity to function as a pancreatic differentiation factor in mature liver and investigates part of the mechanism of liver to pancreas transconversion. Moreover, it examines whether the PDX-1-induced liver to pancreas transdifferentiation process carries a therapeutic benefit long after the initial ectopic PDX-1 gene expression decays.

**MATERIALS AND METHODS**

**Recombinant Adenoviruses—**Ad-CMV-PDX-1 was constructed as described previously (1, 21). It contains the STF-1 cDNA, the rat homologue of PDX-1 ligated into BamHI site of pACCMVpLpA vector. Ad-CMV-β-galactosidase (generously contributed by C. B. Newgard) serves as a control for nonspecific effect of adenovirus-mediated gene delivery.

**Cell Culture—**The mouse pancreatic derived cell lines β-TC-1 and α-TC-1 and the rat pancreatic cell line RIN1046-38 were cultured according to conditions published previously (21, 22).

**Animals and Recombinant Adenoviruses—**Mice were housed in an air-conditioned environment, under a 12-h light/dark cycle, and handled according to institutional animal welfare regulations. 8- to 9-week-old (18–19 g) BALB/c mice were treated with 1–5 × 10⁸ plaque-forming units of the indicated recombinant adenoviruses by systemic injection into the tail vein (in a volume of 200–300 µl of physiological saline). Blood was drawn from the tail, for determination of glucose concentration (Accutrend® GC, Roche Applied Science). Liver was harvested for immunohistochemical staining (fixed in 4% formaldehyde and embedded in paraffin), for analysis of gene expression (total RNA), and for determination of pancreatic hormone content in liver. For the last two analyses, hepatic tissues were immediately frozen in liquid nitrogen and stored at −70 °C.

**RNA Isolation and RT-PCR Analysis—**Total RNA was isolated from frozen tissues using Tri-Reagent (Molecular Research Center). RNA samples were treated by 10 units of RQ1 RNase-free DNase I (Promega) for 60 min. cDNA was prepared by reverse transcription (native avian myeloblastosis virus reverse transcriptase, Chimerx) by using 4 µg of DNA-free total RNA and 0.5 µg of oligo(dT)₁₆. PCR was performed using T3 Thermocycler (Biometra, Göttingen, Germany), and products were separated on 1.8% agarose gels and visualized with ethidium bromide. The sequence of the primers used for PCR and reactions conditions were as listed in Table I. Note that in order to discriminate between expression of the endogenous mouse PDX-1 and the ectopic rat homologue, two sets of specific oligonucleotide primers were designed (see Table I). RNA isolated from the β-TC-1 cell line was used as a control for insulin and somatostatin genes expression, and the α-TC-1 cell line was used for glucagon.

**Quantitative Analysis for Pancreatic Gene Expression Using Real Time PCR—**RT-PCR was performed on the LightCycler (Roche Applied Science) using SYBR-Green I dye.

**Amplification conditions included initial denaturation at 95 °C for 10 min, followed by 55 cycles for both mouse and rat PDX-1, or 30 cycles for β-actin. For both PDX-1 homologues, each cycle included denaturation at 95 °C for 15 s, annealing at 59 °C, and extension at 72 °C for 15 s.**

**TABLE I**

<table>
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<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>CDNA Product</th>
<th>°C</th>
<th>Cycles</th>
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<tr>
<td>β-Actin</td>
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<tr>
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<td>628</td>
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<td></td>
<td>1</td>
<td>253</td>
<td>60</td>
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</table>

Denaturation was at 94 °C for 1 min; annealing was as presented in the table and extension was at 72 °C for 1 min.

The sequence of the primers used for PCR and reactions conditions were as listed in Table I. Note that in order to discriminate between expression of the endogenous mouse PDX-1 and the ectopic rat homologue, two sets of specific oligonucleotide primers were designed (see Table I). RNA isolated from the β-TC-1 cell line was used as a control for insulin and somatostatin genes expression, and the α-TC-1 cell line was used for glucagon.

**Pancreatic Hormones Analysis by Immunohistochemistry—**Slides of 4-µm paraffin-embedded sections were deparaffinized and incubated in 3% H₂O₂ and in blocking solution (for both insulin and glucagon detection), using the commercially available Histomouse™-SP Kit (Zymed Laboratories Inc., South San Francisco, CA). Sections were then incubated for 1 h at 37 °C with monoclonal antibodies against human insulin and against human glucagon (Sigma), both at a dilution of 1:200. Because both insulin and glucagon are detected within the same tissue, at the same time using the same dilution of distinct monoclonal antibodies, they are considered extraneous to each other. Slides were exposed to the secondary biotinylated IgG for 30 min at room temperature and then incubated in streptavidin-peroxidase followed by a chromogen peroxide solution. A control using only secondary antibody without primary antibodies followed by streptavidin-peroxidase and a chromogen peroxide solution was performed to rule out possible background of the system.

**Radioimmunoassay (RIA) of Pancreatic Hormones—**Frozen tissues
were homogenized in 0.18 x HCl, 35% ethanol. The homogenates were extracted overnight at 4 °C with continuous stirring, and the super-
natants were lyophilized. Samples were dissolved in 0.8 ml of RIA 
Assay Buffer, supplemented by a mixture of protease inhibitors (Siga-
mas). Hepatic insulin and glucagon levels were determined using rat 
radioimmunooassay (RIA, catalog numbers SRI-13K and GL-32K, 
Linco, MO, and Coat-A-Count, Diagnostic Products Corp.). Soma-
tostatin concentrations were determined by RIA (Euro-Diagnostica, 
Sweden). Hepatic content of pancreatic hormones was normalized to 
the wet weight of extracted tissues.

**Statistical Analysis**—Statistical analysis was preformed using Two-
sample Assuming Unequal Variances t test.

**RESULTS**

Both Pancreatic Endocrine and Exocrine Markers Are Induced in Liver by Ectopic PDX-1 Expression—Fig. 1 shows that ectopic PDX-1 expression in mature liver in vivo activates a wide repertoire of pancreatic genes. Both endocrine and exo-
crine markers including the exocrine pancreas transcription factor p48 were uniquely expressed in response to ectopic 
PDX-1 expression in liver (Fig. 1). Control treated mice were mostly negative to pancreatic gene expression. Although insu-
lin gene expression was induced in close to 100% of mice treated by ectopic PDX-1, it was expressed at very low levels that were not translated into protein also in 20–30% of the control treated mice.

In the developing pancreas PDX-1 serves as an early molec-
ular marker that temporally correlates with pancreatic com-
mitment (13, 23, 24). Our data suggest that PDX-1 recapitu-
lates to some extent its role in pancreas organogenesis, also in 
a mature fully differentiated tissue, such as liver.

PDX-1 Triggers a Long Lasting Process of Liver to Pancreas 
Developmental Shift—In order to explore the mechanism un-
derlying the PDX-1-induced developmental shift, and to specif-
ically determine whether cells in liver that ectopically express 
PDX-1 exhibit several pancreatic developmental options that 
persist for long periods of time, we carefully followed insulin, 
glucagon, and somatostatin gene expression and protein pro-
duction for 6 months after the initial, single adenovirus medi-
ated PDX-1 administration to mice livers in vivo.
that these hormones do not co-localize within the same cell. Liver cells present in areas close to the central vein in liver are known to correspond to mature cells (25).

Quantitative analysis of hepatic insulin stored in the liver of PDX-1-treated mice indicates that even 4–6 months after treatment, hepatic insulin content is about 30–75 ng/g tissue, compared with 1–9 ng/g tissue in age-matched control livers (Fig. 4A). Although this represents a substantial increase compared with control liver, it is about 1.3–3% of pancreatic content (51280 ng/g tissue), even after we take in consideration that liver is about 20-fold larger than pancreas. A significant 2-fold increase in hepatic proglucagon and somatostatin content was observed up to at least 4 months after initial Ad-CMV-PDX-1 administration (Fig. 4, B and C).

Despite hepatic insulin production, serum insulin and glucose levels in PDX-1-treated mice bearing normal pancreatic function were normal throughout the duration of the experiment (insulin, 1.0 ± 0.5 versus 0.9 ± 0.4 ng/ml, and glucagon, 0.16 ± 0.08 versus 0.12 ± 0.05 ng/ml in PDX-1-treated compared with controls, respectively).

The persistent production of pancreatic hormones in liver suggests that ectopic PDX-1 triggers a cascade of events that may not require the continuous presence of the PDX-1 transgene.

Ectopic PDX-1 Triggers the Expression of the Endogenous, Otherwise Silent PDX-1 Gene in Liver; a Possible Mechanism of Sustained Liver to Pancreas Transdifferentiation—In order to explain the sustained developmental shift in liver, triggered by the transient ectopic PDX-1 expression, we analyzed whether the transgene induces the expression of otherwise silent pancreatic transcription factors, which in turn dictate the continuous expression of pancreatic genes. An obvious candidate is the endogenous PDX-1 gene itself.

To analyze the induction of the endogenous and otherwise silent PDX-1 gene in liver by the ectopic gene, we treated mice by systemic delivery of recombinant adenovirus that directs expression of the rat PDX-1 homologue, and we used specific oligonucleotide primers to distinguish between the ectopic PDX-1 transgene (rat) mRNA (cDNA) and the endogenous mouse mRNA, by RT-PCR.

PCR analysis of DNA samples isolated from liver of Ad-CMV-PDX-1-treated mice confirms that the virally encoded transgene disappears between 30 and 56 days after adenovirus injection (Fig. 5A).

Fig. 5B demonstrates that the ectopic rat PDX-1 expression parallels the observed presence of delivered viral DNA in liver and also extinguishes after 1 month (Fig. 5A). The only homologue of PDX-1 expressed in treated livers for the whole duration of the experiment is the endogenous and otherwise silent mouse homologue (Fig. 5B). Endogenous PDX-1 expression was exclusive to mice that received the rat PDX-1 transgene, was evident in 75% of ectopic PDX-1-treated mice (21 of 28 mice), and in none of the 25 control treated livers analyzed. By using real time PCR, we further analyzed the identity and quantities of the relative levels of mouse versus rat PDX-1 gene expression in liver as a function of time after the initial treatment, using identical conditions (but different primers), and we normalized it to β-actin within the same samples.

As demonstrated in Fig. 5C, the mRNA encoding the ectopic rat PDX-1 is maximal at 5 days, drops by 85% at day 30, and disappears thereafter. By contrast, the endogenous mouse PDX-1 is expressed at substantial levels for the whole duration of the experiment. Unfortunately, due to the high level of homology between the two nuclear proteins, no specific antibody could distinguish between the rat and the mouse homologues of PDX-1, although it seems highly unlikely that the nuclear protein detected only in mouse livers 2–6 months after the initial adenovirus delivery corresponds to the ectopic transiently expressed transgene (data not presented). These data taken together suggest an auto-induction of the endogenous and otherwise silent PDX-1 in liver, which in turn may suggest a mechanistic explanation for the long lasting mode of liver to pancreas transconversion process.

Insulin Produced in Liver in Response to PDX-1 Transgene Expression Is Functional and Prevents STZ-induced Hyperglycemia—In order to determine whether PDX-1 gene delivery
Our data demonstrate that PDX-1 recapitulates its role in pancreas organogenesis when ectopically expressed in liver. Thus, we show that PDX-1 expression in liver induces a wide array of endocrine and exocrine pancreatic genes. PDX-1 induces its own expression in liver (auto-induction), which in turn explains the prolonged nature of the "liver to pancreas" transdifferentiation process embodied by pancreatic hormone production in liver of adenovirus-treated mice that persists

\[ \text{FIG. 5. Ectopic rat PDX-1 delivered by Ad-CMV-PDX-1 induces expression of the endogenous mouse PDX-1 gene in liver. A, PCR analysis of Ad-CMV-PDX-1 DNA. DNA was extracted from mice livers at indicated time points and analyzed for the systemic delivered recombinant adenovirus infection, using specific primers (see Table I). Primers used for the PCR analysis were designed to amplify a DNA sequence that spans part of the CMV promoter and part of the PDX-1 cDNA and is therefore unique to the ectopically delivered transgene and the specific recombinant adenovirus, Ad-CMV-PDX-1. B, RT-PCR analysis of rat PDX-1 (rPDX-1), mouse PDX-1 (mPDX-1), and β-actin gene expression. Total RNA was extracted from livers of mice treated by Ad-CMV-PDX-1 (lanes 1–7) or untreated (lane 8), representative sample of 25 controls analyzed and all tested negative to endogenous PDX-1. β-actin (lane 9) and RIN-38 (lane 10) are controls for mouse and rat PDX-1, respectively. Right margin, molecular sizes in bp. Both A and B represent demonstrate representative results out of at least four mice livers at each indicated time point. C, quantiation of ectopic (rat) versus endogenous (mouse) PDX-1 expression as a function of time after initial Ad-CMV-PDX-1 treatment using real time PCR. Mouse PDX-1 and rat PDX-1 expression was analyzed at different time points by LightCycler. The calculation is performed relative to β-actin expression in each individual sample. Data presented are mean values ± S.E. of samples presented in B, each analyzed at least twice on LightCycler PCR.} \]

\[ \text{FIG. 6. Hepatic insulin production protects mice from STZ-induced hyperglycemia 8 months after the initial Ad-CMV-PDX-1 treatment. Eight months after Ad-CMV-PDX-1 administration, BALB/c mice and age-matched control group were treated by 220 mg/kg STZ. In both groups pancreatic immunoreactive insulin (IRI) content dropped to 95 ± 1% that in normal pancreas. Four of the five PDX-1-treated mice remained normoglycemic (n = 4; 124 ± 14.8 mg % despite pancreas destruction) and 6 of 10 age-matched control mice developed hyperglycemia (n = 6; 332 ± 70 mg %). The figure demonstrates the ratio of hepatic to pancreatic insulin content as a function of PDX-1 treatment.} \]
of evidence suggest that glucagon gene expression is indirectly repressed by PDX-1 (26). The fact that ectopic PDX-1 expression leads to activation of glucagon as well as acinar gene expression in liver suggests that PDX-1 acts in this tissue as a differentiation factor, mediating its effect via downstream induced or pre-existing transcription factors in liver (26, 27). The development of distinct cell types characteristic of endocrine pancreas in mature liver could also be explained by the formation of a gradient of PDX-1 protein levels that may differentially affect the endocrine pancreatic lineage identity in liver (21, 28). For example, transient PDX-1 expression followed by a decrease in PDX-1 levels could promote the activation of glucagon gene expression, as suggested for INS-1 cells (26).

The capacity of PDX-1 to induce its own expression in an extra-pancreatic tissue, such as liver, strengthens the view that it functions as a bona fide master regulator (18). Several transcription factors including PDX-1 itself (29, 30) have been suggested as regulators of PDX-1 gene expression in pancreatic β-cells, most also present in liver (19, 20, 30). Indeed, liver may possess a unique predisposition to activate endogenous PDX-1 gene expression due to pre-existing hepatocyte nuclear factor 3β and hepatocyte nuclear factor 1α in this tissue.

Our data are consistent with the notion that only a subpopulation of cells in the liver are predisposed to undergo a long lasting and comprehensive PDX-1-induced developmental shift. Despite the initial and relatively abundant ectopic PDX-1 expression in liver, uniformly spread among 30–50% of the cells, insulin- and glucagon-positive cells represent less than 1% of the total cells in liver. Immunohistochemical analysis localizes pancreatic hormone-producing cells mainly to the proximity of central veins (Fig. 3) (1). This localization is not attributable to blood circulation because the blood supply that originally brings the recombinant adenoviruses into the liver enters via the hepatic artery and drains through the central veins (31). Therefore, we suggest that liver cells located in the proximity of central veins may possess a certain predisposition that allows them to undergo PDX-1-induced developmental shift. Hepatocytes located in the proximity of central veins are definitely mature and were reported to express high levels of glutamine synthetase (25). A metabolic zonation in liver was suggested by many researchers, and oxygen pressure was suggested to develop and maintain gradients of gene expression in liver (25, 32–35). Therefore, the different populations of cells in liver may consist of slightly distinct levels of certain hepatic transcription factors expression that in turn may affect the ability of these cells to undergo a PDX-1-induced developmental shift (33). An additional possible reason for preferred central vein localization of PDX-1-induced developmentally shifted cells could be the proximity to signals released from endothelial cells around blood vessels. These were documented to induce essential steps in organ formation such as morphogenesis and cell differentiation of both liver and pancreas (36, 37). These cells may harbor signals that instruct PDX-1-expressing cells in liver epithelium to adapt pancreatic cell fate (36, 37). However, we do not know whether signals released from endothelial cells that surround central veins are different from these released from endothelial cells that surround other blood vessels. Additional, yet unknown factors may be involved in the process of liver to pancreas developmental shift that occurs in response to ectopic PDX-1 expression. The location of insulin-producing cells in the proximity of central veins may have a substantial functional advantage in allowing direct release of the produced hormones into the bloodstream, thus preventing intra-hepatic accumulation of pancreatic hormones that could adversely affect carbohydrate metabolism and glycogen storage.

Fig. 7. Body weight of in PDX-1-treated versus control treated mice. 8-week-old mice were systemically injected by Ad-CMV-PDX-1 (□) as well as by control virus (▲) as described under “Materials and Methods.” Body weight was monitored once a month after 4 h of fasting. n = 6 at every time point analyzed; * indicates statistical significant increase in body weight upon PDX-1 treatment; p < 0.05.
Whereas PDX-1 expression alone in our study is sufficient to induce pancreatic hormones in mice liver, studies performed in transgenic Xenopus tadpoles suggest that PDX-1 should be further activated by VP-16 to exert profound effects and convert most of the liver into pancreas (38). This study confirms the pivotal role of PDX-1 in controlling the process of liver to pancreas fate redirection; however, the functional consequences of converting most of the liver into pancreas are in question and are possibly demonstrated in the study by Köjima et al. (39).

Additional pancreatic transcription factors (40–42) may possess the capacity to induce pancreatic repertoire of gene expression in liver. Recently Köjima et al. (39) demonstrated the capacity of NeuroD to induce pancreatic markers. Interestingly, ectopic NeuroD combined with ectopic betacellulin expression in liver induced both downstream and upstream transcription factors that are part of the pancreatic transcriptional network including the PDX-1, and corrected hyperglycemia in STZ diabetic mice (39). In addition, the same study suggests that ectopic PDX-1 expression is toxic to liver due to the induction of the pancreatic acinar function. Hepatic function analyses performed in our model do not demonstrate similar effects; serum amylose levels in PDX-1-treated mice were normal, and mice consistently gained weight (Tables II and III and Fig. 7). The discrepancy between our approach and the approach by Köjima and co-workers is likely to be caused by the distinct levels and the longer time frame of the ectopic PDX-1 expression. The use of helper-dependent adenovirus combined with the potent mammalian elongation factor-1 promoter used in the study by Köjima et al. (39) may result in substantial in vivo expression of PDX-1 transgene, the triggered developmental shift remains functional for the whole duration of the experiment, which is at least 8 months, and possibly forever.

Moreover, our data suggest that the developmentally shifted cells in liver may resist the deleterious effects of the β-cell-specific toxins. The distinct STZ effects on liver versus pancreatic β-cells may stem from the fact that β-cells unlike liver cells possess a restricted capacity to metabolize hydroxyl radicals and hydrogen peroxide. By contrast, liver cells are equipped with high enzymatic activities of peroxidase, catalase, and superoxide dismutase, which together prevent accumulation of free radicals, thus rendering the cells resistant to many toxins and cytokines (46, 47). The exact mechanism of the therapeutic effect and the possible resistance of developmentally shifted liver cells to β-cell toxins should be further analyzed.

Additional studies are needed to fully understand the mechanism of PDX-1 and possibly additional pancreatic transcription and growth factors, mediated liver to pancreas transdifferentiation process. Markers of predisposed cells should be identified, and transdifferentiated cells should be better characterized in order to determine to what extent they mimic normal pancreatic β-cell functions.

Islet cell implantation as a treatment for diabetic patients will be widely available only when new sources of islets or β-cells are found. Our study suggests that liver could serve as an important source of tissue for generating functional insulin-producing cells to be used in replacing malfunctioning β-cells in patients with diabetes.

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