Pancreatic and Duodenal Homeobox Gene 1 Induces Hepatic Dedifferentiation by Suppressing the Expression of CCAAT/Enhancer-Binding Protein β

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It is believed that adult tissues in mammals lack the plasticity needed to assume new developmental fates because of the absence of efficient pathways of dedifferentiation. However, the well-documented ability of the transcription factor pancreatic and duodenal homeobox gene 1 (*PDX-1*) to activate pancreatic lineage development and insulin production following ectopic expression in liver suggests a surprising degree of residual plasticity in adult liver cells. This study seeks a mechanistic explanation for the capacity of *PDX-1* to endow liver cells with pancreatic characteristics and function. We demonstrate that *PDX-1*, previously shown to play an essential role in normal pancreatic organogenesis and pancreatic β-cell function and to possess the potential to activate multiple pancreatic markers in liver, can also direct hepatic dedifferentiation. *PDX-1* represses the adult hepatic repertoire of gene expression and activates the expression of the immature hepatic marker α-fetoprotein. We present evidence indicating that *PDX-1* triggers hepatic dedifferentiation by repressing the key hepatic transcription factor CCAAT/enhancer-binding protein β. Hepatic dedifferentiation is necessary though not sufficient for the activation of the mature pancreatic repertoire in liver. *Conclusion:* Our study suggests a dual role for *PDX-1* in liver: inducing hepatic dedifferentiation and activating the pancreatic lineage. The identification of dedifferentiation signals may promote the capacity to endow mature tissues in mammals with the plasticity needed for acquiring novel developmental fates and functions to be implemented in the field of regenerative medicine. (HEPATOLOGY 2007;46:898-905.)

Recent studies have demonstrated that pancreatic-specific transcription factors, previously known to control organ differentiation in the embryo, also possess instructive roles in diverting the developmental fate of adult liver cells along the pancreatic lineage.[1-11] This process represents a novel and yet unexplained pathway of regeneration capacity in mammals. The first studied pancreatic transcription factor possessing such capacities is the pancreatic and duodenal homeobox gene 1 (*PDX-1*; also known as insulin-promoting factor 1[1]). *PDX-1* plays a central role in pancreatic development in the embryo[12,13] and in β-cell function in the adult pancreas.[14] The role of *PDX-1* in inducing a developmental redirection of the liver along the pancreatic lineage has been demonstrated both *in vivo* and *in vitro*, in *Xenopus*,[5] mouse[1,2,6-10] and human.[4,11] It has been suggested that liver cells that ectopically express *PDX-1* may undergo a transdifferentiation process that results in an irreversible switch of one differentiated cell type to another.[3,5,11] However, the mechanism by which *PDX-1* modifies the hepatic phenotype and function is unknown.

Here we demonstrate that to acquire the alternate functional pancreatic lineage, adult liver cells are obliged to undergo a dedifferentiation process manifested by a loss of adult markers and the expression of the immature hepatic marker α-fetoprotein (AFP). *PDX-1*, but none of

Abbreviations: AAT, alpha-1 antitrypsin; Ad-INS, replication-deficient recombinant adenovirus that encodes human proinsulin complementary DNA under the control of the cytomegalovirus promoter; Ad-PDX-1, replication-deficient recombinant adenovirus that encodes rat PDX-1 complementary DNA under the control of the cytomegalovirus promoter; ADH1, alcohol dehydrogenase-1; ADH1B, alcohol dehydrogenase-1β; AFP, α-fetoprotein; ALB, albumin; C/EBPβ, CCAAT/enhancer-binding protein β; FOXA2, forkhead box A2; G6PC, glucose 6-phosphatase; GLUL, glutamate synthase; HNF, hepatocyte nuclear factor; LAP, liver-enriched activating protein; LIP, liver-enriched inhibitory protein; NGN3, neurogenin 3; PDX-1, pancreatic and duodenal homeobox gene 1; RT-PCR, reverse-transcription polymerase chain reaction.

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Received April 11, 2006; accepted April 9, 2007.

Supported in part by a Juvenile Diabetes Research Foundation grant (1-2006-221), an Israel Science Foundation grant (ISF-99-05), and a European Foundation for the Study of Diabetes/Lilly award (2005), all to S.F.

This work was performed in partial fulfillment of the requirements for the Ph.D. degree for T.S., S.G.-H., and V.A.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.21766

Potential conflict of interest: Nothing to report.

Supplementary material for this article can be found on the HEPATOLOGY Web site (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).

several other pancreatic transcription factors analyzed, induced hepatic dedifferentiation. The capacity of *PDX-1* to affect a wide array of hepatic gene expression can be explained, at least in part, by its capacity to repress specifically the expression of the key hepatic transcription factor CCAAT/enhancer-binding protein β (*C/EBPβ*). It has been previously demonstrated that *C/EBPβ* induces pancreas-to-liver transdifferentiation.[15-17] Our findings document the mechanistic characterization of the reverse process, which activates pancreatic lineage in liver and identifies proactive roles for *PDX-1* and *C/EBPβ* as key components in this process.

Materials and Methods

Human Liver Cells. Adult human liver tissues were obtained from 3 different liver transplantation surgeries from children 4-10 years old and 8 individuals over 40 years old. Liver tissues were used with approval from the Committee on Clinical Investigations (the institutional review board).

The isolation of human liver cells was performed as described.[4,18] The cells were cultured in Dulbecco's minimal essential medium (1 g/l of glucose) supplemented with 10% fetal calf serum (20 ng/ml; Cytolab, Ltd., Israel) and nicotinamide (10 mM; Sigma) and kept at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Viral Infection. Liver cells were infected with recombinant adenoviruses at 500 moi (multiplicity of infection) for 5 days.

The adenoviruses used in this study were as follows: *Ad-CMV-PDX-1*, *Ad-CMV-INS*, *Ad-RIP-β-GAL*,[19] and *Ad-CMV-GFP* (Clontech, BD Biosciences, United States); *Ad-CMV-NEUROD1* (a generous gift from M. Walker and G. Ridner, Weizmann Institute, Israel); *Ad-CMV-NKX6.1* (a generous gift from C.B. Newgard, Duke University); *Ad-CMV-NGN3* (a generous gift from M.S. German, University of California at San Francisco); and *Ad-CMV-LIP* and *Ad-CMV-LAP* (a generous gift from H. Sakaue and M. Kasuga, Kobe University, Japan).

Animals. Balb/c mice (8-9 weeks old, 18-19 g) were housed and treated with recombinant adenoviruses as described.[1,2]

RNA Isolation and Real-Time Reverse-Transcription Polymerase Chain Reaction (RT-PCR). The total RNA was isolated, and complementary DNA was prepared and amplified as described.[2,4] The primer pairs and annealing temperatures are listed in Supplementary Data 1. The TaqMan fluorogenic probes and the Assay-On-Demand (Applied Biosystems, Foster City, CA) used in this study are listed in Supplementary Data 2.

C-Peptide Detection. C-peptide secretion was measured in primary cultures of adult liver cells 3-5 days after the initial exposure to the viral treatment as described.[4] C-peptide secretion to the medium was measured by a radioimmunoassay with a human C-peptide radioimmunoassay kit (Linco Research, St. Charles, MO; <4% cross-reactivity to human proinsulin). C-peptide secretion was normalized to the total cellular protein measured by a Bio-Rad protein assay kit.

Immunofluorescence. Human liver cells treated by *Ad-PDX-1* for 5 days were plated on glass cover slides in 6-well culture plates. Forty-eight hours later, the cells were fixed and stained as described.[4] The antibodies used in this study are listed in Supplementary Data 3. The slides were analyzed with a laser scanning confocal microscope (LSM-1024, Bio-Rad).

Western Blot Analyses. Protein extraction, separation, and western blot analyses were conducted as described.[19] For protein immunoprecipitation, 500 mg of whole-cell extracts was incubated with 5 mg of rabbit polyclonal anti–rat C/EBPβ antibodies (Santa Cruz Biotechnology) at 4°C for 16 hours. Protein A–Sepharose CL-4B (Amersham Bioscience, Sweden) was added to the samples, which were incubated for an additional 2 hours at 4°C. Agarose beads were collected through 1 minute of centrifugation at 12,000*g* and washed 3 times with a lysis buffer. The samples were separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted with mouse monoclonal anti–human C/EBPβ antibodies (1:1000; Santa Cruz Biotechnology).

Histology and Staining. Livers were fixed and stained as described.[1,2]

Flow Cytometry. Cells (5×10^5) were collected, washed twice in phosphate-buffered saline (5 minutes, 1000*g*), and resuspended in 400 μl of phosphate-buffered saline containing 1 mg of ribonuclease per milliliter and 20 μl of a propidium iodide solution (2 mg/ml). Flow cytometry was performed (FACSCalibur, Becton Dickinson, Heidelberg, Germany) with the CellQuest program.

Statistical Analyses. Statistical analyses were performed with a 2-sample Student *t* test assuming unequal variances.

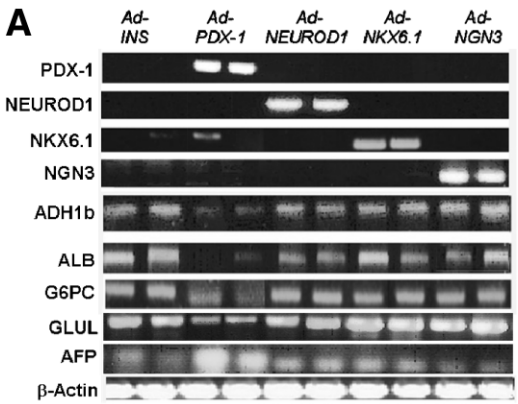
Results

PDX-1 Suppresses the Expression of Adult Hepatic Genes. The ability of *PDX-1* and other pancreatic transcription factors to activate pancreatic markers in the liver has been extensively documented.[1-11] Because different transcription factors may have distinct effects on this process, we analyzed the effects of pancreatic transcription factors on the hepatic repertoire. Using recombinant adenovirus–mediated gene delivery, we expressed *PDX-1*,

neurogenic differentiation 1 (*NEUROD1*), NK6 transcription factor related, locus 1 (*NKX6.1*), or neurogenin 3 (*NGN3*; also known as *NEUROG3*) in primary cultures of adult human liver cells and evaluated their effects on the characteristic markers of differentiated liver cells. RT-PCR analyses revealed that *Ad-PDX-1* reduced the expression of the mature liver cell markers; albumin (*ALB*), alcohol dehydrogenase-1β (*ADH1B*), glucose 6-phosphatase (*G6PC*), and glutamate synthase (*GLUL*) genes, but activated the expression of the immature hepatic marker *AFP*. None of the other pancreatic transcription factors tested had significant effects on hepatic markers (Fig. 1A,B). These effects of *PDX-1* were confirmed at the protein level through western blot analysis (Fig. 1C). A clear inhibitory effect of *PDX-1* on the host repertoire of markers was manifested with double immunofluorescence analyses (Fig. 2A,B); each of the PDX-1–expressing cells exhibited a reduction in adult hepatic markers, whereas PDX-1–negative cells remained unaffected (Fig. 2A,B). In agreement with results obtained by RT-PCR and immunoblotting, *PDX-1*, but none of the other pancreatic transcription factors, increased the staining observed for the immature hepatic marker *AFP* (Figs. 1 and 2C,D).

Importantly, *PDX-1*–induced hepatic dedifferentiation was not associated with increased cell proliferation (Fig. 2E,F). *PDX-1* neither increased the number of cells present in the S phase nor increased the number of apoptotic cells in comparison with control *Ad-INS*–treated cells (Fig. 2I). These data strongly suggest that the *PDX-1*–mediated activation of the pancreatic repertoire in the liver represents a bona fide liver-to-pancreas transdifferentiation process.

PDX-1 Suppresses Adult Hepatic Markers In Vivo. The capacity of *PDX-1* to activate the pancreatic lineage in the liver was demonstrated originally *in vivo*.[1,2] Moreover, several studies have suggested that prolonged ectopic expression of *PDX-1*[6,7] but not *NEUROD1* in most cells in the liver may cause hepatic dysfunction.[6] To analyze whether some of the *PDX-1* effects on hepatic function are attributable to its capacity to repress the hepatic repertoire of gene expression, we examined the effect of *PDX-1* on mature hepatic markers in mice. As previously reported, ectopic *PDX-1* expression delivered by a first-generation recombinant adenovirus is transient and peaks at day 5.[1,2] Indeed, the *PDX-1* effect on the expression of hepatic markers displayed similar temporal characteristics, with maximal repression of adult hepatic markers on the same day (Fig. 3A). However, 30 days after the *PDX-1* treatment, as the levels of the transgene decreased, hepatic gene expression was restored, and the expression of *ALB*, alcohol dehydrogenase-1 (*ADH1*), and alpha-1 antitrypsin (*AAT*) genes was comparable to that in age-matched control adenovirus-treated mice (Fig. 3A). This time course suggests that the effect of *PDX-1* on the hepatic repertoire depends on its continuous expression in the liver. Double immunohistochemistry analyses of liver sections, 5 days after *Ad-PDX-1* administration, revealed an inverse correlation between PDX-1 and ALB; all ALB-positive cells were negative to *PDX-1* nuclear staining, whereas PDX-1–positive cells had very low ALB levels (Fig. 3B). As in the primary culture *in vitro*, *PDX-1* ex-



B

	Ad-INS	Ad-PDX-1	Ad-NEUROD1	Ad-NKX6.1	Ad-NGN3
ADH1b	1±0.26	0.35±0.27**	3.09±1.38	1.67±1.13	2.16±2.12
ALB	1±0.22	0.46±0.43*	2.26±1.10	1.20±1.06	2.54±1.17
G6PC	1±0.42	0.12±0.15**	1.73±1.25	1.31±0.39	1.08±0.19
GLUL	1±0.52	0.40±0.15**	1.28±1.23	1.66±0.54	1.7±0.45
AFP	1±0.45	13.50±5.1**	1.95±0.74	1.20±1.00	2.16±0.69

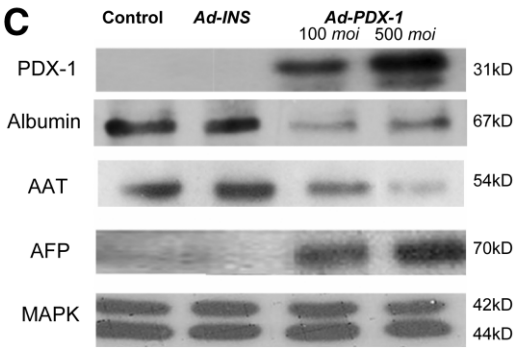


Fig. 1. *PDX-1* suppresses adult hepatic marker gene expression in human liver cells *in vitro*. RT-PCR analyses of human liver cells treated with *Ad-PDX-1*, *Ad-NEUROD1*, *Ad-NKX6.1*, or *Ad-NGN3* for *ALB*, *ADH1B*, *G6PC*, *GLUL*, and *AFP* gene expression. *Ad-INS*–infected cells serve as both the viral infection and the produced proinsulin control. (A) Representative results of ethidium bromide staining of agarose-separated polymerase chain reaction products. (B) Quantitative real-time RT-PCR analyses presented as the relative levels of the mean ± standard deviation versus *Ad-INS*–treated liver cells ($n \geq 8$ in 4 different experiments; $^*P < 0.005$, $^{**}P < 0.01$). (C) Western blot analyses of ALB, AAT, AFP, and PDX-1 proteins in control untreated (lane 1), *Ad-INS*–treated (lane 2), and *Ad-PDX-1*–treated (100 and 500 *moi*, lanes 3 and 4, respectively) cells. Mitogen-activated protein kinase served as the protein load control. Representative results ($n = 4$).

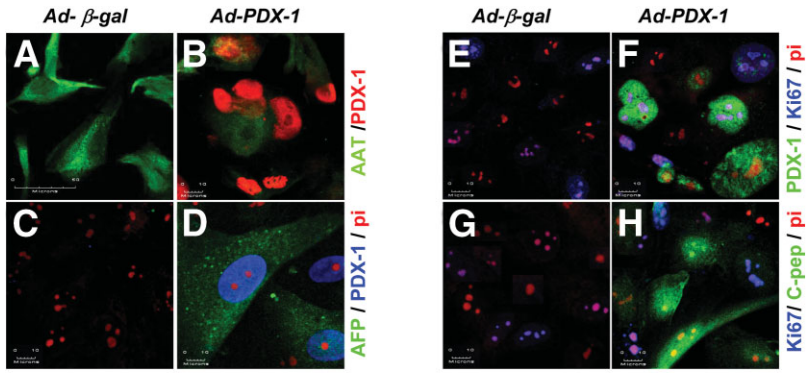


Fig. 2. *PDX-1* represses hepatic markers in adult human liver cells without inducing cell proliferation. Double immunofluorescence analyses for (A,B) hepatic AAT (green) and PDX-1 (red), (C,D) AFP (green) and PDX-1(blue), (E,F) PDX-1 (green) and Ki67 (blue), and (G,H) C-peptide (green) and Ki67 (blue). Parts A, C, E, and G present control treated liver cells, and parts B, D, F, and H present *Ad-PDX-1*–treated cells. Cell nucleoli were stained by propidium iodide (red). The scale bar is 10 μm. (I) Cell cycle analyses at 2, 4, and 5 days after the *Ad-PDX-1* treatment in adult human liver cells versus control *Ad-INS*–treated cells. The data are presented as the mean ± standard deviation (n ≥ 4 in 2 different experiments).

I

Day	Ad-INS				Ad-PDX-1			
	G1	S	G2	Apoptosis	G1	S	G2	Apoptosis
2	55.9±5.3	26.5±4.5	17.6±0.8	0.4±0.2	62.8±7	20.1±5.8	17.1±1.4	0.93±0.6
4	72.7±2.3	15.2±3.1	12±0.9	1±0.3	74.9±2.6	11.5±2.1	13.6±0.5	0.7±0.3
5	79.1±4.8	6.75±2.3	14.2±3.1	1.8±0.6	76.5±4.7	7.2±2.4	16.38±1.4	0.37±0.3

pression *in vivo* did not induce accelerated cell proliferation, as determined by Ki67 staining (data not presented).

These data suggest that *PDX-1* has the capacity to repress the adult hepatic repertoire in mice *in vivo*, as it does in human liver cells *in vitro*.

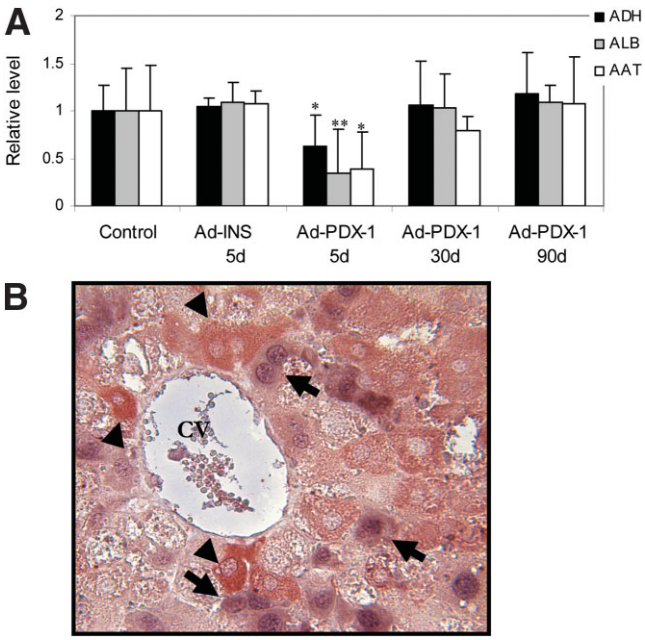


Fig. 3. *PDX-1* suppresses adult hepatic markers in mice livers, *in vivo*. (A) Quantitative real-time RT-PCR analyses for *ADH1*, *ALB*, and *AAT* gene expression levels. *Ad-INS* or *Ad-β-gal* served as viral infection controls. The results are presented as the relative levels of the mean ± standard deviation versus untreated control liver cells (n ≥ 6; $*P < 0.005$, $**P < 0.05$). (B) Double immunohistochemical analysis for PDX-1 (nuclei, arrow) and ALB (cytoplasm, headless arrow) in mice liver sections 5 days after *Ad-PDX-1* administration.

PDX-1 Suppresses C/EBPβ Expression in Liver Cells. It seems unlikely that *PDX-1* could directly repress the expression of multiple genes in the liver through a direct effect.[20] Therefore, we tested whether hepatic transcription factors could mediate the wide effects of *PDX-1* on hepatic markers. *PDX-1*, but none of the other pancreatic transcription factors, decreased only the *C/EBPβ* expression (Fig. 4A; the data are not presented for the rest). The *C/EPBβ* transcript is a single 1.4-kilobase messenger RNA with 4 distinct translation initiation sites. However, at the protein level, multiple C/EBPβ isoforms have been reported. The full-length isoform (38/40 kDa) C/EBPβ and the 34-kDa isoform liver-enriched activating protein (LAP) are identical in their activity, whereas the liver-enriched inhibitory protein (LIP), which encodes a 14-kDa protein, lacks the trans-activation domain, and serves as a dominant negative inhibitor of C/EBPβ/LAP activity.[21,22]

Ectopic *PDX-1* expression in adult human liver cells resulted in diminished *C/EBPβ* and *LAP* levels (Fig. 4). *PDX-1* reduced the protein levels of C/EBPβ and LAP in a dose-dependent manner (Fig. 4B). Moreover, double immunofluorescence for PDX-1 and C/EBPβ revealed that although all control liver cells exhibited strong nuclear C/EBPβ staining in culture, PDX-1–expressing cells exhibited diminished C/EBPβ levels(Fig. 4C).

This raises the possibility that the effect of *PDX-1* on hepatic dedifferentiation could be mediated by its capacity to decrease *C/EBPβ* levels.

Hepatic Dedifferentiation Is Obligatory for PDX-1–Induced Activation of the Pancreatic Lineage in the Liver. To further analyze the possible role of *C/EBPβ* activity in mediating the effects of *PDX-1* on the transdif-

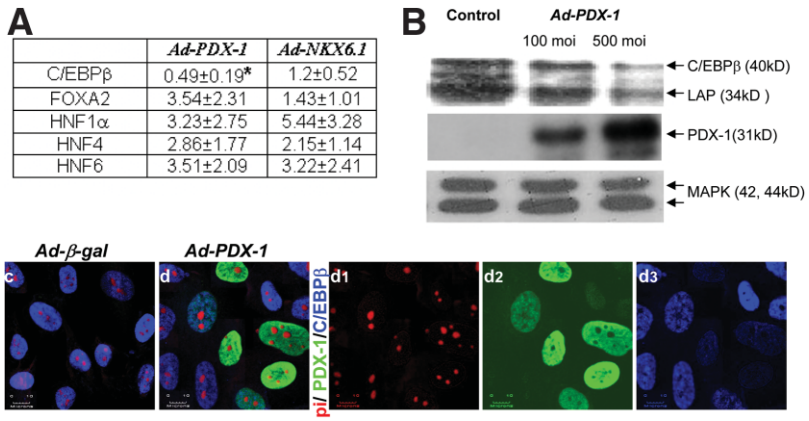


Fig. 4. *PDX-1* inhibits *C/EBPβ* without affecting additional hepatic transcription factor gene expression. (A) Quantitative real-time RT-PCR of hepatic transcription factors: *C/EBPβ*, *C/EBPα*, *FOXA2*, *HNF1α*, *HNF4*, and *HNF6* gene expression in *Ad-PDX*-1–treated or *Ad-NKX6.1*–treated adult human liver cells. The results are presented as the relative levels of the mean ± standard deviation versus *Ad-GFP*–treated liver cells ($n \geq 8$ in 4 different experiments; $^*P < 0.01$). (B) Western blot analyses for C/EBPβ (45 kDa) and its variant LAP (35 kDa) and PDX-1 in control untreated (lane 1) and *Ad-PDX-1*–treated cells (100 and 500 *moi*, lanes 2 and 3, respectively). Mitogen-activated protein kinase served as the protein load control. Double immunofluorescence analyses for C/EBPβ (blue) and PDX-1 (green) in (C) control treated liver cells and (D) *Ad-PDX-1*–treated cells. Part D is presented by an RGB filter split: (D1) red, (D2) green, and (D3) blue. Cell nucleoli were stained by propidium iodide (red). The scale bar is 10 μm.

ferentiation process, we manipulated the *C/EBPβ* activity in adult human liver cells in a *PDX-1*–independent manner, using ectopic expression of *LAP* and *LIP*. Indeed, *LIP*, the dominant negative inhibitor of C/EBPβ activity, repressed the expression of adult hepatic markers, whereas *LAP*, which mimics C/EBPβ function, restored adult liver characteristics, as manifested by decreased levels of *AFP* and increased levels of adult hepatic markers, including that of *C/EBPα* (Fig. 5 and Supplementary Fig. 1).

To examine whether *CEBPβ* and the restoration of adult hepatic markers prevent the activation of the pancreatic lineage, we ectopically expressed *LAP* in a primary culture of adult human liver cells in combination with *PDX-1*. *LAP* prevented the effects of *PDX-1* on both the hepatic dedifferentiation and the activation of the pancreatic lineage in liver cells (Figs. 5A and 6). These data suggest an inhibitory role of C/EBPβ activity on *PDX-1*–induced activation of the pancreatic lineage and function in the liver.

Diminished C/EBPβ Activity Per Se Is Insufficient for Inducing Mature Pancreatic Phenotype in the Liver. We further examined whether decreased C/EBPβ activity per se is sufficient for inducing the pancreatic phenotype and function in the liver. A *PDX*-independent decrease in the hepatic markers was induced with the ectopic expression of *LIP*. The inhibition of C/EBPβ activity by ectopic *LIP* expression modestly activated the expression of the *INS* gene in adult human liver cells (Fig. 7A). The activation of *INS* gene expression by *LIP* was substantially lower than that induced by *PDX-1*; however, combined *PDX-1* and *LIP* expression resulted in a marked stimulation of the expression of the *INS* gene. The pronounced effect of *LIP* on *INS* gene expression is

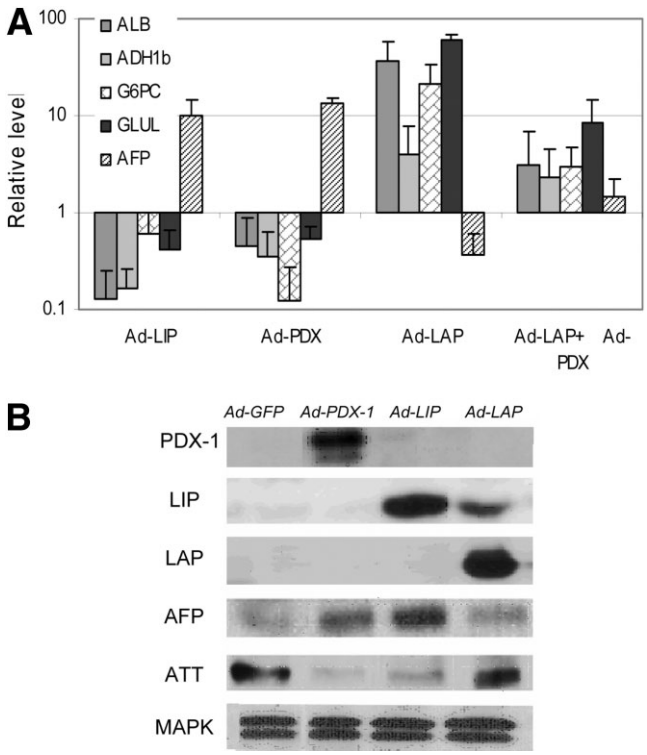


Fig. 5. *CEBPβ* prevents the effect of *PDX-1* on the hepatic phenotype. (A) Quantitative real-time RT-PCR analyses for *ALB*, *ADH1B*, *G6PC*, *GLUL*, and *AFP* gene expression of human liver cells treated with *Ad-LIP*, *Ad-LAP*, and/or *Ad-PDX-1*. The results are presented as the relative levels of the mean ± standard deviation versus control liver cells ($n \geq 7$ in 4 different experiments; $^*P < 0.05$ for all). (B) Western blot analyses for PDX-1, C/EBPβ (LIP and LAP), AFP, and AAT proteins in control untreated cells (lane 1) and cells treated with *Ad-PDX-1* (lane 2), *Ad-LIP* (lane 3), and *Ad-LAP* (lane 4). Mitogen-activated protein kinase served as the protein load control. Representative results ($n = 5$).

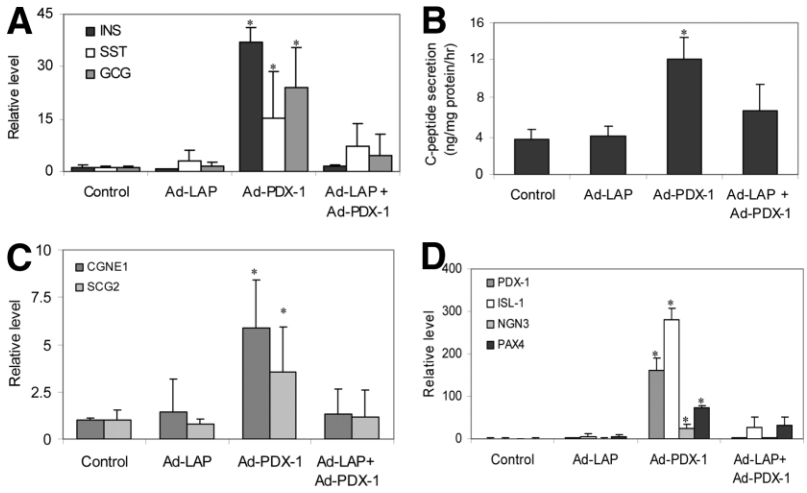


Fig. 6. Hepatic dedifferentiation is obligatory for the activation of the pancreatic lineage. Quantitative real-time RT-PCR analyses for (A) the pancreatic hormone, (C) neuroendocrine secretory granule protein, and (D) endogenous pancreatic transcription factor gene expression. The results are presented as the relative levels of the mean ± standard deviation versus untreated control liver cells. (B) C-peptide secretion at KRB media containing 17.5 mM glucose in response to *Ad-PDX-1* and/or *Ad-LAP* treatments ($n \geq 7$ in 4 different experiments; $^*P < 0.05$).

in agreement with the documented inhibitory effect of *C/EBPβ* on the activation of *INS* gene expression in β-cells.[23] Unlike ectopic *PDX-1* expression,[4] *LIP* expression alone did not induce differentiated characteristics specific to β-cells, such as glucose-stimulated C-peptide secretion (Fig. 7B) or the expression of pancreatic transcription factors that characterizes mature pancreatic endocrine differentiation. *LIP* induced the expression of *NGN3* alone (Fig. 7C). *NGN3* expression marks immature endocrine progenitor cells in the pancreas and is not expressed in adult pancreatic islets.[24] Interestingly, the coexpression of *PDX-1* and *LIP* resulted in a specific increase in the expression pancreatic specific transcription factors, including the expression of the endogenous *PDX-1* gene (Fig. 7D).

These data suggest that the loss of hepatic differentiation per se, mediated by a decrease in *C/EBPβ* activity, may be sufficient only for inducing an immature pancreatic phenotype in liver cells and further strengthen the notion that the pancreatic lineage could

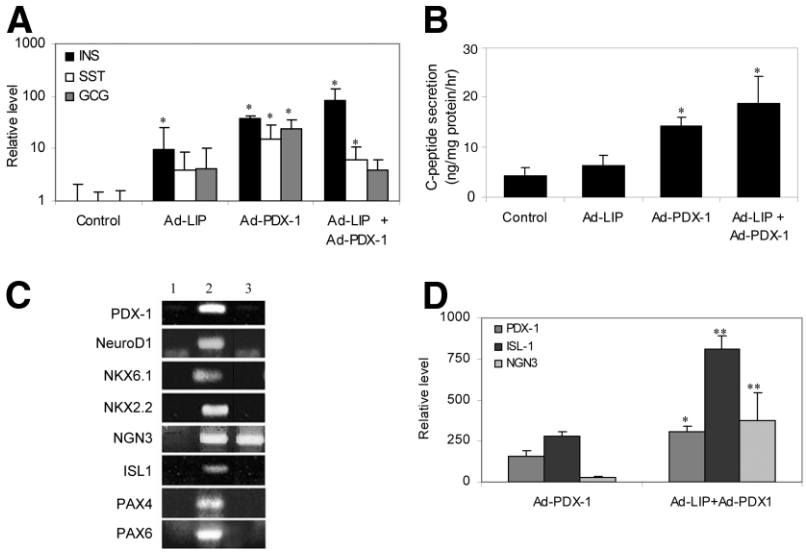


Fig. 7. Hepatic dedifferentiation promotes the *PDX-1* effect on *INS* gene expression and C-peptide secretion but is insufficient to induce a mature pancreatic phenotype in liver. (A) Quantitative real-time RT-PCR analyses for the pancreatic hormone gene expression. The results are presented as the relative levels of the mean ± standard deviation versus untreated control liver cells. (B) C-peptide secretion at KRB media containing 17.5 mM glucose in response to *Ad-PDX-1* and/or *Ad-LIP* treatments ($n \geq 7$ in 4 different experiments; $^*P < 0.05$, $^{**}P < 0.01$). (C) RT-PCR analyses for the pancreatic transcription factors in adult control liver cells (lane 1), in *Ad-PDX-1*–treated liver cells (lane 2), and in *Ad-LIP*–treated liver cells (lane 3). Representative results of ethidium bromide staining of agarose-separated polymerase chain reaction products ($n = 8$). (D) Quantitative real-time RT-PCR analyses for endogenous *PDX-1*, *ISL-1*, and *NGN3* gene expression. The results are presented as the relative levels of the mean ± standard deviation versus untreated control liver cells ($n \geq 8$ in 4 different experiments; $^*P < 0.05$, $^{**}P < 0.01$ between *Ad-PDX-1* and combined treatment with both *Ad-PDX-1* and *Ad-LIP*).

be a default developmental option of the hepatic program.[25]

Discussion

This study suggests a role for hepatic dedifferentiation in the activation of the pancreatic lineage in the adult liver and identifies *PDX-1* as a hepatic dedifferentiation factor.

PDX-1, previously shown to be essential for controlling both pancreas organogenesis and mature β-cell function,[26,27] can, upon ectopic expression in adult liver cells, repress adult hepatic markers and activate *AFP* expression without inducing cell proliferation (Figs. 1-3 and the supplementary material). The effects of *PDX-1* on the hepatic state of differentiation and function are accompanied by and appear to be mediated in part by its capacity to suppress the expression of the transcription factor *C/EBPβ*. Hepatic dedifferentiation and *C/EBPβ* repression were induced by *PDX-1*, but not by several other pancreatic transcription factors analyzed. However, the capacity of *NKX 6.1* and *NEUROD1* to activate the pancreatic lineage in the liver was augmented upon *C/EBPβ* repression by *LIP*, and this further strengthened the notion that hepatic dedifferentiation is necessary for the activation of the alternate pancreatic repertoire in the liver (Supplementary Fig. 2). Considering this along with its capacity to activate the pancreatic repertoire and function in the liver,[1-11] we suggest that *PDX-1* may possess the unique capacity to induce liver-to-pancreas transdifferentiation.

The effects of *PDX-1* on the activation of the pancreatic lineage were temporally and spatially distinct from those on hepatic dedifferentiation. Although irreversibly activating the pancreatic lineage in predisposed liver cells,[1,2,4] *PDX-1* transiently suppresses adult hepatic markers in each liver cell (Figs. 2 and 3).

PDX-1–induced *C/EBPβ* suppression in the liver may have a wide physiological impact on this organ's differentiation and function as the C/EBP family of proteins plays important roles in liver development in the embryo and in normal hepatic function in adults.[28,29] Indeed, several publications have demonstrated that sustained high levels of ectopic *PDX-1* expression in the liver *in vivo* cause hepatic dysmorphogenesis and ablated function.[6,7] However, these undesirable effects of prolonged *PDX-1* expression on hepatic function do not invalidate its use in generating pancreatic function in the liver. Rather, they emphasize the importance of limiting both the duration and extent of PDX1 expression and its location to those cells capable of activating the pancreatic lineage.[1,2,4]

Previous studies have demonstrated that a single allele of either *C/EBPβ* or *C/EBPα* is sufficient for normal liver development.[28,29] In contrast, our data indicate that a 50% decrease in *C/EBPβ* levels induces a profound decline in the adult hepatic repertoire. There are several possible explanations for this apparent discrepancy; it is anticipated that the actual *C/EBPβ* repression in each *PDX-1*–positive liver cell is more substantial than that demonstrated at messenger RNA and protein levels (Figs. 1B,C and 4) because the transgene is expressed in only 50%-70% of the cells and does not affect the remaining 30%-50% of the cells in the culture.[4] Moreover, in our experimental system, *C/EBPα* cannot compensate for the reduced expression of *C/EBPβ* because of its initial low levels (Supplementary Fig. 1). Therefore, cells expressing high levels of *PDX-1* may completely lack both *C/EBPα* and *C/EBPβ* and therefore display dramatic reductions in hepatic markers.

Although it has been previously suggested that *C/EBPβ–PDX-1* interplay may affect the activation of insulin gene expression in pancreatic tissues,[5,23,30] our data raise the possibility that these proteins have a much wider reciprocal impact on the cellular phenotype. Although *PDX-1* inhibits hepatic differentiation through *C/EBPβ* suppression, *C/EBPβ* not only inhibits insulin gene expression but also restricts pancreatic differentiation in the liver. Ectopic *C/EBPβ* (*LAP*) expression inhibits the *PDX-1* effects on the activation of pancreatic hormone, pancreatic adult marker, and pancreatic transcription factor expression (Fig. 6). Thus, *PDX-1* and *C/EBPβ* control the pancreatic and liver function in part through their reciprocal function. These data further suggest a role of these transcription factors in the separation between the hepatic and pancreatic repertoires and functions. Further studies are needed to determine whether *PDX-1* and *C/EBPβ* directly interact with each other.

PDX-1 did not affect the expression of other liver-enriched nuclear factors, such as hepatocyte nuclear factor 1α (*HNF1α*), hepatocyte nuclear factor 4 (*HNF4*), hepatocyte nuclear factor 6 (*HNF6*), and forkhead box A2 (*FOXA2*). These factors are essential for both liver and pancreas development and function and, therefore, may not possess proactive roles in the interconversion between the two tissues. The distinct effects of these factors in the liver versus the pancreas could be manifested at posttranscriptional levels. Indeed, the expression of these factors remained unaltered also in the opposite process of activating the hepatic lineage in the pancreatic cell line AR42J-B13. However, it was noticed that hepatocyte nuclear factor 4α may increase *CEBPβ* activity once translocated into the nucleus upon dexamethasone treatment.[30]

Our data suggest a fundamental role for transcription factors in continuously controlling mature tissue differentiation and proper function in mammals. *C/EBPβ* and *PDX-1* may play important roles in the developmental and functional separation of the liver from the pancreas,

both demonstrating dominant roles in marking these tissues spatially, not only during embryonic development but also through adult life.[16,31] Furthermore, our study suggests that the developmental decisions made during embryonic organogenesis in mammals could be altered in differentiated cells through ectopic expression of dedifferentiation-inducing factors. The capacity of dominant transcription factors in instructing adult tissues to assume new fates may have important implications for regenerative medicine.

Acknowledgment: We thank M. D. Walker for fruitful discussions and for a critical review of the manuscript. C. V. E. Wright is acknowledged for the generous supply of anti–PDX-1 antibodies. M. D. Walker (Weizmann Institute), C. B. Newgard (Duke University), M. S. German and R. Gasa (University of California at San Francisco), and H. Sakaue and M. Kasuga (Kobe University, Japan) are acknowledged for their generous contributions of recombinant adenoviruses. We thank B. A. Sela (Institute for Chemical Pathology) and A. Schlosberg (Pathology Institute, Sheba Medical Center) for their fruitful help.

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