A Sox9/Fgf feed-forward loop maintains pancreatic organ identity

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SUMMARY
All mature pancreatic cell types arise from organ-specific multipotent progenitor cells. Although previous studies have identified cell-intrinsic and -extrinsic cues for progenitor cell expansion, it is unclear how these cues are integrated within the niche of the developing organ. Here, we present genetic evidence in mice that the transcription factor Sox9 forms the centerpiece of a gene regulatory network that is crucial for proper organ growth and maintenance of organ identity. We show that pancreatic progenitor-specific ablation of Sox9 during early pancreas development causes pancreas-to-liver cell fate conversion. Sox9 deficiency results in cell-autonomous loss of the fibroblast growth factor receptor (Fgfr) 2b, which is required for transducing mesenchymal Fgf10 signals. Likewise, Fgf10 is required to maintain expression of Sox9 and Fgfr2 in epithelial progenitors, showing that Sox9, Fgfr2 and Fgf10 form a feed-forward expression loop in the early pancreatic organ niche. Mirroring Sox9 deficiency, perturbation of Fgfr signaling in pancreatic explants or genetic inactivation of Sox9 also result in hepatic cell fate conversion. Combined with previous findings that Fgfr2b or Fgf10 are necessary for pancreatic progenitor cell proliferation, our results demonstrate that organ fate commitment and progenitor cell expansion are coordinately controlled by the activity of a Sox9/Fgf10/Fgfr2b feed-forward loop in the pancreatic niche. This self-promoting Sox9/Fgf10/Fgfr2b loop may regulate cell identity and organ size in a broad spectrum of developmental and regenerative contexts.

KEY WORDS: Pancreas, Sox9, Fgf10, Fgfr2b, Mesenchyme, Progenitor, Liver, Mouse

INTRODUCTION
In mammals, the dorsal and ventral pancreas, as well as the liver, hepatobiliary system, stomach and duodenum, all arise from foregut endoderm cells. Their lineage segregation into organ-committed progenitors is governed by both cell-intrinsic regulators and extrinsic mesodermally derived signals. For example, ventral pancreas and liver arise from the same bi-potential population of ventral foregut endoderm: while ventral pancreas forms by default, cardiac mesoderm-derived Fgf signaling diverts nearby endoderm cells to an hepatic fate (Bort et al., 2004; Deutsch et al., 2001; Jung et al., 1999). Similarly, the transcription factors Ptf1a and Sox17 have been shown to govern lineage segregation of foregut endoderm between ventral pancreas and duodenum (Kawaguchi et al., 2002) or ventral pancreas and biliary system (Spence et al., 2009), respectively. It remains unclear, however, whether, once allocated to a particular organ, progenitors are solely restricted to organ-specific cell fates or whether programming is still flexible and dependent on inductive signals.

Pancreas development in mice is first morphologically detectable at embryonic day (E) 8.75-9.0, when dorsal and ventral pancreatic buds emerge on opposing sides of the foregut endoderm, fusing by E12.5 to form the unified organ. Until around E12.5, buds predominantly comprise Pdx1+, Ptf1a+ and Sox9+ pancreas-specific multipotent progenitor cells, shown by lineage tracing at the population level (Gu et al., 2002; Kawaguchi et al., 2002; Kopp et al., 2011), to give rise to all pancreatic cell types: endocrine cells and the exocrine acinar and ductal cells. As initial size of the progenitor cell pool dictates final organ size (Stanger et al., 2007), maintenance of appropriate progenitor cell numbers is vital for normal pancreas morphogenesis. Progenitor-intrinsic cues, such as Notch target genes (Apelevist et al., 1999; Jensen et al., 2000) and the transcription factors Sox9 (Seymour et al., 2007), Pdx1 (Ahlgren et al., 1996; Offield et al., 1996) and Ptf1a (Kawaguchi et al., 2002; Krapp et al., 1998), act to maintain appropriate progenitor cell numbers and hence are crucial for pancreatic growth. Pancreas-specific Sox9 deletion in mice causes organ hypoplasia owing to reduced proliferation of progenitors (Seymour et al., 2007), and similarly, early pancreatic growth arrest in mice lacking either Pdx1 (Ahlgren et al., 1996; Offield et al., 1996), Ptf1a (Kawaguchi et al., 2002; Krapp et al., 1998), or both (Burlison et al., 2008), reveals roles for Pdx1 and Ptf1a in pancreatic epithelial expansion.

In addition to these progenitor-intrinsic cues, extrinsic cues from the surrounding pancreatic mesenchyme also promote progenitor cell proliferation. The importance of mesenchymal signals for pancreatic growth was first revealed through pancreatic transplant and transplantation experiments, demonstrating reduced epithelial expansion following mesenchyme removal (Gittes et al., 1996; Golosow and Grobstein, 1962; Wessells and Cohen, 1967). As co-culture with heterotopic organ mesenchymes restored the growth of explanted pancreatic epithelia (Wessells and Cohen, 1967), it was concluded that signal(s) expressed in different types of mesenchyme stimulate pancreatic progenitor cell expansion. Subsequent studies have identified roles for several signaling molecules in pancreatic growth, including Wnt (Jonkheere et al., 2008; Landsman et al., 2011), Bmp (Ahnfelt-Ronne et al., 2010) and, most notably, Fgf10 (Blushan et al., 2001), which is expressed...
in mesenchyme surrounding the ventral and dorsal pancreatic buds between E9.5 and E11.5. How such extrinsic mesenchymal cues are integrated with intrinsic epithelial cues at the level of the pancreatic progenitor cell, however, remains unknown.

While the liver and ventral pancreatic bud emerge in close proximity to one another from the ventral foregut, the dorsal pancreatic bud arises independently of the liver bud from the dorsal-posterior foregut region. Explant studies have shown that hepatic competence is not limited to the ventral foregut region, but that dorsal gut endoderm, which normally does not give rise to the liver, also possesses the ability to activate hepatic programs (Bossard and Zaret, 1998; Bossard and Zaret, 2000; Gualdi et al., 1996). When dorsal gut endoderm is isolated at E11.5 and cultured in the absence of gut tube mesoderm, the early hepatic marker albumin is ectopically activated (Bossard and Zaret, 1998; Gualdi et al., 1996). The observation that albumin expression is not induced in dorsal endoderm isolated at E13.5 and beyond (Bossard and Zaret, 2000) has led to the proposal that cues from the mesoderm actively repress hepatogenic gene activity in dorsal gut endoderm up until E13.5. Studies in zebrafish further suggest that hepatic competence of the fish posterior endoderm, which is thought to correspond to the dorsal gut endoderm in mice, is negatively regulated by Fgf10 signaling (Dong et al., 2007; Shin et al., 2011). To date, the identity of the mesenchymal signal(s) that suppress liver-specific genes in dorsal gut endoderm in mice has remained elusive. Moreover, it is unclear how progenitors gain competence to interpret specific mesenchymal cues and how stable relay mechanisms between epithelial progenitors and adjacent mesenchyme are established to reinforce fate commitment of dorsal gut endoderm-derived organs, such as the pancreas.

In this study, we show that in epithelial pancreatic progenitors, Sox9 cell-autonomously controls expression of the Fgf receptor, Fgfr2b, which is required for transducing mesenchymal Fgf10 signals. In turn, Fgf10 is required to maintain progenitor expression of Sox9 and Fgfr2, showing that Sox9, Fgfr2, and Fgf10 form a feed-forward loop to repress liver-specific gene expression programs.

MATERIALS AND METHODS

Mouse strains

All mouse strains have been previously described: Sox9lox/lox (Kist et al., 2002), Sox9-eGFP (Gong et al., 2003), Pdx1-Cre (Gu et al., 2002), Ptf1aCre (Kawaguchi et al., 2002), Rosa26-CreER (Voorjis et al., 2001), Pdx1lox/lox (Offield et al., 1996), Fgf10loxP (Min et al., 1998), Rosa26R (Soriano, 1990) and Z/EGR (Novak et al., 2000). Tamoxifen (Sigma, St Louis, MO, USA) was dissolved at 20 mg/ml in corn oil (Sigma) and a single dose of 6 mg/40 g body weight administered by intraperitoneal injection. Midday on the day of vaginal plug appearance was considered E0.5. All animal experiments described herein were approved by the University of California Irvine and University of California San Diego Institutional Animal Care and Use Committees.

Histology and immunohistochemistry

Dissected E9.0-E12.5 embryos and E15.5 gut preparations were fixed in 4% paraformaldehyde in PBS, cryoembedded in Tissue-Tek OCT (Sakura Finetek USA, Torrance, CA, USA) and cut into 10 µm frozen sections. For immunofluorescence analysis, antigen retrieval was conducted in citrate buffer (pH 6.0) followed by permeabilization in 0.15% Triton X-100 in PBS. Sections were blocked in 1% normal donkey serum in PBS with 0.1% Tween-20 then incubated overnight at 4°C with primary antibodies diluted in the same buffer. The following primary antibodies were used at the given dilutions: rat anti-E-Cadherin (Sigma), 1:1000; rabbit anti-phosphohistone H3 (Upstate Biotechnology, Lake Placid, NY, USA), 1:300; rabbit anti-Sox9 (Chemicon, Temecula, CA, USA), 1:1000; goat anti-Sox9 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1:200; guinea-pig anti-Pdx1 (kindly provided by C. Wright, Vanderbilt University, Nashville, TN, USA), 1:10,000; rabbit anti-Ptf1a (kindly provided by B. Bréant, Université Pierre et Marie Curie-Paris 6, Paris, France), 1:1000; rabbit anti-Ptf1a (NIH Beta Cell Biology Consortium), 1:1000; mouse anti-glucagon (Sigma), 1:500; rat anti-GFP (kindly provided by C. Kioussi, Oregon State University, Corvallis, OR, USA), 1:500; rabbit anti-AFP (DAKO USA, Carpenteria, CA, USA), 1:1000; goat anti-albumin (Sigma), 1:3000; rabbit anti-α1-antitrypsin (Sigma), 1:1000; goat anti-Hnf4α (Santa Cruz), 1:1000; and rabbit anti-Bek (Fgf10) (Santa Cruz), 1:1000.

Staining for glucagon was conducted using the M.O.M. Kit in conjunction with AMCA Avidin D (both Vector Laboratories, Burlingame, CA, USA). Goat anti-Sox9 was detected using biotinylated horse anti-goat (1:2000) in combination with Fluorescein Avidin D (both Vector Laboratories). Simultaneous detection of AFP and either α1-antitrypsin, Bek/Fgfr2 or phosphohistone H3 with two rabbit-raised antibodies was performed with a Zenon Rabbit IgG Labeling Kit (Invitrogen, Carlsbad, CA, USA). TUNEL was conducted using an ApoTag Fluorescein Apoptosis Detection Kit (Merck Millipore, Billerica, MA, USA). When necessary, nuclei were counterstained with Hoechst 33342 (Invitrogen).

Primary antibodies were detected with donkey-raised secondary antibodies conjugated to Cy3, Cy5, DyLight488 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) or Alexa488 (Invitrogen) at 1:1500 dilution (1:500 for Cy5).

ApoTome images were captured on a Zeiss Axio Observer Z1 microscope with Zeiss AxioVision 4.8 (both Carl Zeiss, Thornwood, NY, USA) and figures prepared with Adobe Photoshop/Illustrator CS4 (Adobe Systems, San Jose, CA, USA). Where necessary, the Cy5 channel was pseudo-colored blue.

Whole-mount X-Gal staining of embryos was performed as described previously (Seymour et al., 2004); images were acquired on a Zeiss Stemi 2000C with a Zeiss AxioCam digital camera driven by Zeiss AxioVision 3.1.

Gut explants

The foregut-midgut region was dissected from 24-25 somite (E9.5) Sox9-eGFP embryos and cultured on human fibronectin-coated eight-well culture slides (BD Biosciences, San Jose, CA, USA) and figures prepared with Adobe Photoshop/Illustrator CS4 (Adobe Systems, San Jose, CA, USA). Where necessary, the Cy5 channel was pseudo-colored blue.

Whole-mount X-Gal staining of embryos was performed as described previously (Seymour et al., 2004); images were acquired on a Zeiss Stemi 2000C with a Zeiss AxioCam digital camera driven by Zeiss AxioVision 3.1.

Cell quantification

Cell counting was performed on every fifth section through pancreata from a minimum of three mutants and three control somite-matched littermates for embryos. For gut explants, all cells were counted in the dorsal pancreatic bud from three SU5402-treated and three control DMSO vehicle-treated explants for each of the three time-windows examined.

mRNA quantification

Total RNA was isolated and pooled from E12.5 dorsal pancreatic epithelia of Sox9fl/fl; Pdx1-Cre and Sox9fl/f+ littermates. Each individual RNA sample was prepared from four pancreata as per the manufacturer’s instructions (RNeasy Micro Kit, Qiagen, Valencia, CA, USA). RNA quality was assessed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Approximately 250 ng of total RNA was amplified and labeled with Cy3 using the QuickAmp Labeling Kit (Agilent Technologies). Four independent samples were hybridized to Agilent Whole Mouse Genome Oligo Microarray G4122A chips. Microarray data have been deposited in Gene Expression Omnibus under accession number GSE28669.

For qRT-PCR analyses, cDNA was synthesized (Superscript III cDNA Kit; Invitrogen) from total RNA pooled from dorsal pancreatic epithelium from six E12.5 Sox9fl/fl, Pdx1-Cre and Sox9fl/f+ littermates. qRT-PCRs were
performed in triplicate using SYBR Green (Applied Biosystems, Foster City, CA, USA); for primer sequences, see supplementary material Table S1.

**Statistical analyses**

All data are displayed as mean±s.e.m. Statistical significance was determined using the two-tailed Student’s t-test (Minitab 15, Minitab, PA, USA).

**RESULTS**

**Liver differentiation in Sox9-deleted pancreas**

In Sox9<sup>fl/fl</sup>; Pdx1-Cre mice, Pdx1-Cre activation in dorsal and ventral pancreatic endoderm by E9.5 (supplementary material Fig. S1A) efficiently recombines the Sox9<sup>fl/fl</sup> allele in dorsal and ventral pancreatic cells by E10.5 (Fig. 2D’.F’). Although significantly hypoplastic by E11.5, dorsal and ventral pancreatic remnants arise in these mice (Fig. 1F’,F’).

To identify Sox9-regulated pathways during early pancreas morphogenesis, we compared the transcriptional profiles of dorsal pancreatic epithelium in E12.5 Sox9<sup>fl/fl</sup>; Pdx1-Cre mice with those of control (Sox9<sup>fl/fl</sup>) littermates. Microarray analysis confirmed a significant 8.1-fold downregulation of Sox9 in mutant pancreata (Fig. 1A). Surprisingly, however, we observed elevated expression of a number of genes associated with hepatic differentiation in Sox9-deficient pancreas, most notably α-fetoprotein (AFP) and albumin (Fig. 1A). Upregulation of these, as well as of additional liver markers, was verified by qRT-PCR analysis (Fig. 1B). Thus, Sox9 ablation in the pancreas appears to aberrantly activate a liver developmental program.

To temporally and spatially dissect the onset of hepatic differentiation in Sox9-deficient pancreas, co-immunofluorescence analysis was performed for Pdx1 and the liver markers AFP and albumin. Immediately after Sox9 ablation at E10.5, Pdx1 expression was maintained in Sox9-deficient progenitors and neither AFP nor albumin were detected in control or Sox9-deficient pancreata (Fig. 1C–D’). At E11.5, rare AFP<sup>+</sup> cells (0.4±0.1% of total pancreatic cells; n=3) were seen in the epithelium of control dorsal, but never ventral, pancreas (Fig. 1E,E’). By contrast, AFP<sup>+</sup> cells were abundant (24.0±2.0% of total pancreatic cells; n=5) in the Sox9-deficient hypoplastic dorsal pancreas in all mutant embryos examined (n=10; Fig. 1F,I). Paralleling AFP expression, while only scarce cells expressing albumin were evident in control pancreas (0.7±0.3% of total pancreatic cells; n=3), albumin<sup>+</sup> cells were readily detected in the Sox9-deficient dorsal pancreas (17.1±2.9% of total pancreatic cells; n=3) (Fig. 1F,G’,H,I). Both AFP and albumin were, however, undetectable in the Sox9-ablated ventral bud at E11.5 (Fig. 1F’,H’), which could be explained by the ~24-hour developmental delay of the ventral compared with the dorsal pancreas (Spooner et al., 1970). Severe hypoplasia of the Sox9-deficient ventral pancreas precluded its analysis in E11.5 Sox9<sup>fl/fl</sup>; Pdx1-Cre dorsal pancrea. (D) AFP+ or Alb+ cells as a percentage of total pancreatic cells in E11.5 Sox9<sup>fl/fl</sup>; Pdx1-Cre dorsal (F,H), but not ventral (F’,H’), pancreas. Arrowheads in E indicate two rare AFP+ cells in control dorsal pancreas. (I) AFP+ or Alb+ cells as a percentage of total pancreatic cells in E11.5 Sox9<sup>fl/fl</sup>; Pdx1-Cre dorsal (n=5), AFP or 3, Alb) and control Sox9<sup>fl/fl</sup> (n=3) dorsal pancrea. (J) Phosphohistone H3 (pHH3) staining in Pdx1<sup>+</sup> progenitors and AFP<sup>+</sup> cells of dorsal pancreas (I) or AFP<sup>+</sup> hepatoblasts in liver (K) of E11.5 Sox9<sup>fl/fl</sup>; Pdx1-Cre embryos. (L–M) AFP+ cells are observed in dorsal (M) and ventral (M’) pancreas of Sox9<sup>fl/fl</sup>; Ptf1a-Cre but not control (L,L’) embryos at E12.5. Transf., transferrin; G-6-P, glucose-6-phosphatase; dp, dorsal pancreas; vp, ventral pancreas; duo, duodenum; li, liver; FDR, false discovery rate. Error bars represent s.e.m.; *P<0.05; **P<0.001. Scale bars: 20μm in C–H’,I,K; 50μm in L–M’.

E11.5 Sox9<sup>fl/fl</sup>; Pdx1-Cre dorsal pancrea (Fig. 1J) revealed similar rates of proliferation between the pancreatic AFP<sup>+</sup> cell population (3.2±2.3% pHH3<sup>+</sup> cells of total AFP<sup>+</sup> cells; n=62 cells) and the
Pdx1+ (AFP+) pancreatic progenitor population (2.9±1.4% pHH3+ cells of total Pdx1+ cells; n=933 cells). AFP+ cells were extremely rare in control pancreata (four to seven cells per dorsal pancreas) and displayed proliferation rates similar to AFP+ cells in rare endogenous pancreatic AFP+ cells as a result of downregulation. Although conceivable that increased AFP+ cell numbers might manifest from reduced apoptosis in Sox9-deficient and control embryos at E11.5 (data not shown). Taken together, this evidence strongly supports the notion that the increased numbers of AFP+ hepatic-like cells in Sox9-deficient pancreas is much reduced in comparison with the mitotic index of endogenous AFP+ hepatic bud cells in the same mutant embryos (9.5±2.1% pHH3+ cells of total AFP+ cells; n=777 cells; Fig. 1K). Thus, their relatively low proliferative index argues against increased AFP+ cell numbers in Sox9-deficient pancreatic progenitors in the ventral bud are competent to adopt hepatic fates, although AFP and glucagon expression were almost wholly exclusive (supplementary material Fig. S4).

To define the competence window through which Sox9-deleted pancreatic progenitors are able to adopt hepatic fates, Sox9 was ablated using Ptf1a-Cre, which is activated in dorsal and ventral pancreatic endoderm at E10.5 (supplementary material Fig. S1B,C) (Kawaguchi et al., 2002), 1 day after Pdx1-Cre. Recapitulating the results of Sox9 inactivation using Pdx1-Cre, Ptf1a-Cre-driven Sox9 ablation induced a pancreatic-to-hepatic fate switch, as evidenced by AFP expression in both dorsal and ventral pancreata at E12.5 (Fig. 1M, M'). Thus, like their dorsal counterparts, Sox9-deficient progenitors in the ventral bud are competent to adopt hepatic fates, albeit following some temporal delay, most probably reflecting the aforementioned developmental lag of the ventral pancreas (Spooner et al., 1970).

To test the competency of later pancreatic progenitors to adopt an hepatic fate, Sox9 was inducibly deleted via tamoxifen administration at E12.5 in Sox9fl/+ mice carrying the Rosa26-CreER allele (supplementary material Fig. S5A). Although Sox9 was robustly downregulated at both mRNA and protein levels in E15.5 pancreatic epithelium (Dubois et al., 2011), no expression of AFP or albumin was evident (supplementary material Fig. S5B,C). As such, the competence window for Sox9-deleted pancreatic progenitors to adopt hepatic fates closes by E12.5. Together, these experiments reveal that, even after allocation to the organ niche, pancreatic progenitors remain labile in their organ fate decision.

**Sox9 maintains Fgfr2b expression in pancreatic progenitors**

To elucidate the mechanism underlying hepatic reprogramming of Sox9-ablated pancreatic progenitor cells, we re-examined the transcriptional profile of E12.5 Sox9-deleted pancreatic epithelium to identify potential downstream targets of Sox9. We found that Sox9 ablation resulted in significant downregulation of both Fgfr2 and Fgfr4 (Fig. 2A), suggesting that Sox9 is required for maintaining their pancreatic expression. Of the two Fgfr2 splice variants, Fgfr2b is expressed in early mouse pancreatic epithelium, whereas Fgfr2c expression is confined to the pancreatic mesenchyme (Elghazi et al., 2002; Sylvesteren et al., 2011). Concordantly, qRT-PCR analysis confirmed downregulation of Fgfr4 and Fgfr2b in Sox9-ablated dorsal pancreatic epithelium, whereas Fgfr2c expression was unchanged (Fig. 2B). Significantly, Fgfr2b is the cognate receptor for Fgf10 (Omitz et al., 1996), which is expressed by dorsal and ventral pancreatic mesenchyme between E9.5-E11.5 and is required for pancreatic progenitor cell proliferation (Bhushan et al., 2001). Moreover, Fgf10 ablation manifests in formation of a hypoplastic pancreas, closely phenocopying Sox9 deficiency (Bhushan et al., 2001). Likewise, Fgfr2b−/− mice exhibit pancreatic hypoplasia owing to reduced epithelial proliferation (Pulkkinen et al., 2003; Revest et al., 2001). Together, these findings indicate that Sox9 acts in the same pathway as the Fgf10-Fgfr2b axis to maintain pancreatic progenitor cell proliferation.

To dissect the genetic link between Sox9 and Fgf10-Fgfr2b signaling, we characterized the pancreatic expression pattern of Fgfr2b with respect to that of Sox9. From E9.0 until E10.5, Pdx1+ pancreatic cells expressing high levels of Sox9 exhibited nuclear Fgfr2b expression (Fig. 2C,C',E,E'; Fig. 3A-D). At E10.5, concerted program of liver differentiation in Sox9-ablated pancreas. The AFP+ cells occurred in small clusters predominantly at the periphery of the dorsal pancreas, in a pattern reminiscent of the first-wave glucagon+ endocrine cells although AFP and glucagon expression were almost wholly exclusive (supplementary material Fig. S4).
93.9±0.472% of Sox9+ cells in the dorsal bud (n=491 cells) and 91.1±2.99% of Sox9+ cells in the ventral bud (n=243 cells) were Fgfr2+; conversely, all Fgfr2+ cells in both dorsal (n=460 cells) and ventral (n=221 cells) buds expressed Sox9. Notably, evidence from previous studies has suggested such Fgf receptor nuclear localization to be associated with active Fgf signaling (Reilly and Maher, 2001). Furthermore, such close concordance between Sox9 and nuclear Fgfr2b expression has been reported previously in Sertoli cell precursors during testis development in response to active Fgf signaling (Bagheri-Fam et al., 2008; Schmahl et al., 2004). Concomitant with loss of the mesenchymal Fgfr10 signaling source by E12.5 (Bhushan et al., 2001), Fgfr2b became excluded from the nucleus between E11.5 and E12.5 (Fig. 3E-H). Throughout development, Fgfr2b expression remained restricted to the Sox9+ domain (Fig. 3I,J).

To temporally dissect Fgfr2b downregulation following Sox9 ablation, we assayed for Fgfr2b in Sox9null; Pdx1-Cre pancreata. Fgfr2b expression was almost completely extinguished in both dorsal and ventral pancreas by E10.5 (Fig. 2D,D′,F,F′). To further determine whether Sox9 controls Fgfr2b expression cell-autonomously, we analyzed Sox9 and Fgfr2 expression in Sox9null; Ptf1a-Cre pancreata, which show mosaic deletion of Sox9 (Fig. 4; 69.8±5.5% of E-cadherin+ pancreatic cells expressed Sox9 in control (n=576 cells) versus 16.9±6.2% in Sox9null; Ptf1a-Cre (n=368 cells) embryos). Progenitors retaining Sox9 expression were Fgfr2+ (yellow arrowheads in Fig. 4B,B′,D,D′), while Sox9-deficient progenitors were devoid of Fgfr2b (white arrowheads in Fig. 4B′,D,D′). In Sox9null; Ptf1a-Cre pancreata, 73.1±9.4% of Sox9+ cells were also Fgfr2+; conversely, all Fgfr2+ cells in both dorsal (n=560 cells) expressed Sox9 (note, Sox9low cells also exhibited an Fgfr2low state). This strongly suggests that loss of pancreatic Sox9 – while AFP was largely excluded from Pdx1– mice manifests in adoption of hepatic fate, we assayed for AFP in E11.5 Pdx1-null pancreas. This revealed a significant upregulation of AFP+ cells (Fig. 5D,E), most of which were Sox9− while AFP was largely excluded from Pdx1− cells that retained Sox9 expression (Fig. 5D). These findings

**Sox9 is dominant over Pdx1 in pancreatic fate maintenance**

Given previous reports of reduced Fgfr2b expression in Pdx1-nulligous pancreas (Svensson et al., 2007), we tested whether this decrease was Sox9 dependent. Although Sox9 is expressed in E10.5 Pdx1-deficient pancreas, varying degrees of Sox9 downregulation are evident (Fig. 5B, Ba, Bb, D). In wild-type embryos, 65.5±1.65% of pancreatic epithelial cells (n=572 cells) displayed Sox9high, 17.5±5.70% Sox9low and 17.0±5.27% no Sox9 immunoreactivity. By contrast, a Sox9high state was observed in only 23.3±3.29% of Pdx1− deficient epithelial cells (n=749 cells); 51.5±3.51% exhibited a Sox9low state and 25.2±0.804% showed no Sox9 immunostaining. Consistent with Sox9-dependent regulation, Fgfr2b was downregulated in Pdx1− deficient progenitors in a pattern mirroring the loss of Sox9 (Fig. 5B-Bb). In Pdx1-null pancreatic, 89.8±1.07% of Sox9+ cells (n=749 cells) expressed Fgfr2b, whereas all Fgfr2+ cells (n=503 cells) expressed Sox9 (note, Sox9low cells also exhibited an Fgfr2low state). This strongly suggests that loss of pancreatic Fgfr2b in Pdx1-null mice is Sox9 dependent. To examine whether decreased pancreatic expression of Sox9 and, thus, Fgfr2b in Pdx1− mice manifests in adoption of hepatic fate, we assayed for AFP in E11.5 Pdx1-deficient pancreas. This revealed a significant upregulation of AFP+ cells (Fig. 5D,E), most of which were Sox9+ while AFP was largely excluded from Pdx1+ cells that retained Sox9 expression (Fig. 5D). These findings
indicate that both loss of Fgfr2b expression and hepatic cell fate conversion of Pdx1-deficient progenitors are Sox9 dependent, inferring Sox9 to be dominant over Pdx1 in maintaining pancreatic fate. As Ptf1a is also co-expressed with Sox9 in pancreatic progenitors and governs early pancreas organogenesis (Kawaguchi et al., 2002), we assayed for both Fgfr2b and AFP in Ptf1a-nullizygous embryos. In contrast to Pdx1-/- mice, pancreatic progenitors in Ptf1a-null mutants retained Fgfr2b, consistent with persistence of Sox9 expression (supplementary material Fig. S6C,F,H,J). Concordantly, at E11.5, when AFP was evident in pancrea lacking either Sox9 or Pdx1, AFP was undetectable in Ptf1a-deficient cells (supplementary material Fig. S6L). Taken together, these observations are consistent with Sox9 being dominant over Pdx1, as well as Ptf1a in maintaining Fgfr2b expression and pancreatic identity in pancreatic progenitors.

Fgf receptor signaling maintains pancreatic fate
Consistent with Sox9 maintaining Fgfr2b expression in pancreatic progenitors, it is predicted that pharmacological perturbation of Fgfr signaling will induce their hepatic reprogramming. To test this, we assayed for hepatic fate induction in early pancreatic explants following Fgfr inhibition. Caudal foregut and midgut endoderm was microdissected from 24- to 25-somite (E9.5) Sox9-eGFP embryos (Fig. 6A) and grown in culture on a two-dimensional fibronectin substrate for up to 5 days. In control (DMSO vehicle-treated) explants, eGFP+ dorsal pancreatic endoderm budded out of the gut tube, expanded rapidly and branched, giving rise to a readily distinguishable dorsal pancreatic bud (Fig. 6B,D,F,H,J). By days four (d4) and five (d5), the dorsal pancreas in explants closely resembled in situ E12.5 and E13.5 dorsal pancreata, respectively (Fig. 6B,H,J). Through this five-day window, the pancreatic endoderm principally comprised Sox9+/Sox9-eGFP+ Pdx1+ cells, as well as a few glucagon+ endocrine cell clusters; as in vivo, scarce AFP+ cells were also evident (Fig. 6D,F,H,J). This culture system therefore closely recapitulates the in situ program, albeit with some developmental delay (~1 day per 4 days of culture), as previously reported for E11.5 dorsal pancreas explants (Percival and Slack, 1999). Inhibition of Fgfr receptor activity with SU5402 for 72 hours immediately after gut dissection (d0-d3) manifested in downregulation of dorsal bud Sox9 expression, although Sox9-eGFP was retained (Fig. 6E) owing to eGFP perdurance (Corish and Tyler-Smith, 1999). Recapitulating its effects on pancreatic Sox9 expression, Fgfr abrogation for 72 hours also produced a reduction in Pdx1 expression in the dorsal pancreas (Fig. 6G). Moreover, blockade of Fgf signaling resulted in a significant induction of AFP+ cells in the dorsal pancreas (Fig. 6E,G,L). These observations are consistent with a model whereby Fgfr-transduced Fgfr signaling maintains Sox9 expression in pancreatic progenitors, which in turn maintains their Fgfr2b expression and, thus, Fgf receptivity. This signaling cascade therefore maintains the identity of pancreatic progenitors by blocking their adoption of an alternative hepatic program.

Fig. 5. Loss of Fgfr2 and hepatic fate conversion in Pdx1-deficient pancreas is Sox9 dependent. (A-D) While control E10.5 dorsal pancreatic progenitors show robust nuclear Fgfr2 expression (A,A’), in Pdx1-deficient dorsal progenitors, Fgfr2 is downregulated in a pattern following that of Sox9 (B-B’; arrows indicate Sox9+ Fgfr2+ cells), consistent with AFP expression in Sox9-deficient progenitors in E11.5 Pdx1+/− (D), compared with wild-type (WT) (C) mice. Immunofluorescence staining for the truncated Pdx1 protein in Pdx1+/− dorsal pancreas is shown in Ba and Bb as a reference. (E) AFP+ cells as a percentage of total pancreatic cells (E) in E11.5 Pdx1+/− and wild-type (WT) (n=4) dorsal pancreata. dp, dorsal pancreas; duo, duodenum; li, liver. Error bars represent s.e.m.; **P<0.01. Scale bars: 20 μm.
We next sought to define the competence window through which Fgf-deprived pancreatic progenitors are able to adopt a liver fate. Explants were therefore grown in the presence of SU5402 for additional overlapping 72-hour windows: either d1-d4 or d2-d5 (Fig. 6C). Mirroring our initial results, Fgf receptor inhibition for 72 hours from d1-d4 manifested in disrupted branching morphogenesis and significantly increased numbers of AFP+ cells (Fig. 6H,I,L). By contrast, after SU5402 treatment over d2-d5, AFP+ cells were not increased in number (Fig. 6L-L). Thus, for around 2 days after E9.5, pancreatic progenitors are competent to adopt hepatic fates when deprived of extrinsic Fgf signaling. This temporal requirement for Fgf/Fgfr signaling in pancreatic fate maintenance is concurrent with the E9.5-E11.5 expression of Fgf10 in pancreatic mesenchyme in vivo (Bhushan et al., 2001) and also corresponds to the competence period during which removal of gut mesoderm results in spontaneous activation of liver genes in the dorsal gut endoderm (Bossard and Zaret, 1998; Bossard and Zaret, 2000; Gualdi et al., 1996). Furthermore, this time-window matches with that through which pancreatic Sox9 ablation induces hepatic reprogramming.

Fgf10 maintains Sox9 and Fgfr2b expression in pancreatic progenitors

Pancreatic Sox9 downregulation in gut explants following pharmacological Fgfr inhibition strongly suggests that Fgf signaling is required for maintaining Sox9 and, thus, Fgfr2b expression in pancreatic progenitors. To test this hypothesis, we assayed for Sox9 and Fgfr2b in the early dorsal pancreas of 25-26 somite (E9.5) Fgf10-deficient mice. Our analysis revealed downregulation of both Sox9 and Fgfr2b in a corresponding pattern within Pdx1+ dorsal pancreatic epithelial cells of Fgf10-/- embryos (Fig. 7A,B). Thus, while not required for Sox9 induction, mesenchymal Fgf10 is crucially required for maintaining the expression of Sox9 and, thus, Fgfr2b in pancreatic progenitors. This is consistent with pancreatic Fgf10 misexpression inducing Sox9 expression (Seymour et al., 2007). Therefore, in the early pancreas, Fgf10 participates in a feed-forward loop with Sox9 and Fgfr2b to ensure that progenitor cells in the epithelium remain receptive to Fgf10 signaling from the pancreatic mesenchyme.

An Fgf10/Fgfr2b/Sox9 feed-forward loop maintains pancreatic fate

The finding that Sox9 and Fgfr2b are downregulated in Fgf10-deficient pancreata would predict that pancreatic progenitors undergo hepatic reprogramming as in Sox9-deficient or Fgfr-inhibited pancreata. Thus, to test whether Sox9 acts in the same pathway as Fgf10 and Fgfr2b to maintain pancreatic fate, we assayed for pancreatic AFP in Fgf10-/- mice. In E11.5 Fgf10-nullizygous mice, both dorsal and ventral pancreatic buds were severely hypoplastic (Fig. 7C,E; data not shown) as reported previously (Bhushan et al., 2001). While Pdx1 was partly maintained in Fgf10-deficient dorsal pancreas, Sox9 was almost entirely extinguished; concordantly, AFP+ cells were significantly upregulated (Fig. 7E,F). Intriguingly, several AFP+ Sox9+ cells were also observed in the dorsal pancreas of E11.5 Fgf10-heterozygous mutant embryos with apparently greater prevalence than the scarce AFP+ cells observed in E11.5 wild-type pancreas (Fig. 7D,F). Thus, the prevalence of pancreatic AFP+ cells increases with decreasing Fgf10 gene dose (Fig. 7C-F). Taken together, our data demonstrate that pancreatic progenitors depend on an Fgf10/Fgfr2b/Sox9 feed-forward loop in the pancreatic niche to maintain their organ identity (Fig. 7G).
and Hep1-deficient mice, respectively (Bort et al., 2006; Kawaguchi et al., 2002; Sumazaki et al., 2004). Reassignment of pancreatic progenitors to an intestinal fate has also been observed after exposure of pancreatic epithelium to splenic mesenchyme, suggesting that extrinsic signals can reverse progenitor fate commitment even after their allocation to the pancreatic anlage (Asayesh et al., 2006). Our study demonstrates that extrinsic cues are not only capable of reversing cell fate commitment, but that Fgf signals in the pancreatic niche are required to maintain organ identity by suppressing hepatic gene expression programs. It remains to be seen whether the niche micro-environment is similarly required to reorganize- or tissue-specific fate commitment of stem or progenitor cells in other developing organs or stem cell niches.

This pro-pancreatic role of Fgf signaling in pancreatic progenitors appears to contradict its previously identified role as an inducer of hepatic fate in mice and zebrafish (Calmont et al., 2006; Deutsch et al., 2001; Jung et al., 1999; Shin et al., 2007). Notably, the early function of Fgf as an activator of hepatic genes temporally precedes the herein-described requirement for Fgf signaling in repressing hepatic programs in dorsal and ventral pancreatic progenitors. Hepatic induction occurs in the ventral foregut around E8.25 and during this time requires Fgf signaling from cardiac mesenchyme (Jung et al., 1999). We show that Fgf signaling exerts its liver-repressive role significantly later, as evidenced by pancreas-to-liver cell fate conversion following perturbation of the Sox9/Fgf10/Fgfr2b loop between E9.5 and E11.5. Paralleling this changing influence of Fgf signaling on hepatopancreatic specification, Bmp signaling has similarly been shown to first induce hepatic fate, then, hours later, to promote adoption of pancreatic fate (Wandzioch and Zaret, 2009). Together, these studies exemplify the dynamism of cellular competence in response to extracellular signals. Our finding that Sox9 controls the expression of Fgf2b and, thus, receptivity of epithelial progenitors to Fgf10 signaling identifies Sox9 as an important progenitor-intrinsic cue for regulating cellular competence in the developing gut tube. As Sox9 does not become expressed in the foregut endoderm until the onset of pancreatic cell specification (Seymour et al., 2007), Fgfr2-mediated signaling responses can only be elicited after Sox9 expression has been initiated. The finding that Sox9 ablation also results in Fgfr2 loss in the developing testes (Bagheri-Fam et al., 2008) and ventral prostate (Thomsen et al., 2007) suggests that Sox9 might have a more global role in enabling cells to respond to Fgf signaling.

Similar to the role of Fgf10 in mice described here, Fgf10 is required for proper specification of the zebrafish hepatopancreatic system. Fgf10 mutant zebrafish exhibit ectopic hepatic cells in the proximal pancreas and ectopic pancreatic cells in the liver (Dong et al., 2007). Whereas Fgf10 is also expressed in mesenchyme surrounding the hepatic diverticulum in mouse embryos from E9.5 to E12.5 (Berg et al., 2007), Fgfr2 is not detected in hepatic progenitors during this time (data not shown). Thus, during the time-window required for repressing hepatic fate in pancreatic progenitors, the Fgf10-Fgfr2 signaling axis does not appear to have a direct influence on hepatic progenitors in mouse embryos. However, the finding that Fgf10 signaling negatively regulates hepatic competence of non-hepatic endoderm in zebrafish embryos (Shin et al., 2011) points to a conserved role for Fgf10 in repressing hepatic programs during gut development. Although the role of Sox9 and its potential regulation of Fgf receptors in the zebrafish gut tube remain to be explored, studies in other tissues or organs suggest conserved genetic interactions between Sox9 and Fgf.

In addition to maintaining pancreatic identity, this Fgf10/Fgfr2b/Sox9 loop between epithelial progenitors and pancreatic mesenchyme also ensures proper pancreatic growth and morphogenesis.

**DISCUSSION**

Our findings reveal that organ commitment of progenitors in the early pancreas is not stably programmed, but instead requires extrinsic cues from the progenitor niche (Fig. 7G). We show that maintenance of pancreatic identity depends on mesenchymal Fgf10 signaling, transduced via Fgfr2b to maintain Sox9 expression in progenitors. In turn, Sox9 is required for Fgfr2b expression and, thus, for receptivity to Fgf10. Therefore, Sox9 and Fgf10-Fgfr2b form a feed-forward loop in the pancreatic progenitor cell niche that maintains pancreatic organ identity. Pancreatic progenitors require activity of this loop to suppress their adoption of an alternative hepatic program. Our finding that Sox9 deletion in pancreatic progenitors causes their conversion to a liver fate provides additional evidence for a previously recognized developmental plasticity of early pancreatic, liver and gut progenitors. Examples for cell fate interconversions upon loss of a single transcription factor include pancreatic-to-intestinal, liver-to-intestinal and biliary-to-pancreatic conversions in Ptf1α−, Hhex−
signaling. For example, Fgf receptor-transduced signaling promotes Sox9 expression in embryonic zebrafish hindbrain (Esain et al., 2010) and mouse chondrocytes (Murakami et al., 2006), and Fgf2 ablation diminishes Sox9 expression in both developing testes (Bagheri-Fam et al., 2008) and lung (Abler et al., 2009). Our findings now pave the way for future studies addressing whether Sox9, Fgf2b and Fgf10 also operate in a feed-forward loop in other tissues and organs, and whether this loop acts as a paradigm for integrating cell-extrinsic and -intrinsic cues to expand progenitors and reinforce their fate commitment in multiple developmental and regenerative contexts.

Our discovery that Sox9, Fgf2b and Fgf10 form a regulatory loop in the early pancreas explains why deletion of each of these genes similarly impairs progenitor proliferation and causes pancreatic hypoplasia (Bhushan et al., 2001; Pulkkinen et al., 2003; Revest et al., 2001; Seymour et al., 2007). However, the more subtle pancreatic hypoplasia of mice lacking Fgfr2b (Pulkkinen et al., 2003; Revest et al., 2001) compared with that in mice deficient for Fgf10 (Bhushan et al., 2001) suggests that Fgf10 signaling might not be transduced exclusively through Fgfr2b during early pancreas organogenesis. Although Fgf10 has not been formally required for normal pancreas development (Bhushan et al., 2001; Pulkkinen et al., 2003; Revest et al., 2001; Seymour et al., 2007). However, the more subtle pancreatic hypoplasia of mice lacking Fgfr2b (Pulkkinen et al., 2003; Revest et al., 2001) compared with that in mice deficient for Fgf10 (Bhushan et al., 2001) suggests that Fgf10 signaling might not be transduced exclusively through Fgfr2b during early pancreas organogenesis. Although Fgf10 has not been formally shown to signal through Fgfr4, our observation that Sox9 also regulates Fgfr4 expression suggests that Fgfr2 and Fgfr4 might serve partially redundant functions in the pancreatic epithelium.

Notably, not all pancreatic progenitors convert to an hepatic fate upon perturbations of the Sox9/Fgf10/Fgfr2b loop, but pancreatic and hepatic differentiation is initiated in parallel within the organ. This observation raises the issue of why some progenitors undergo hepatic reprogramming whereas others follow a pancreatic differentiation path. Susceptibility to hepatic fate conversion may be limited to a subset of progenitors and could depend on cell cycle phasing and cell growth, as shown for cells in the Drosophila imaginal disc (Sustar and Schubiger, 2005). In this respect, it is interesting that hepatic cells were preferentially found in the outer layer tip cells of the developing pancreas (Fig. 7E; supplementary material Fig. S4), which have higher proliferation rates than centrally located progenitors (Zhou et al., 2007). Later in development, when tip cells have committed to an acinar fate (Zhou et al., 2007), disruption of the Sox9/Fgf2b/Fgf10 pathway no longer causes pancreas-to-liver conversion, suggesting that developmental plasticity of pancreatic progenitors is limited to an early time window, when progenitors are still multipotent and highly proliferative. Comparative analysis of early and late pancreatic progenitors could provide insight into the transcriptional and epigenetic mechanisms that underlie their developmental plasticity.

Finally, our results have important implications for stably inducing pancreatic programs in directed differentiations of embryonic stem cells towards pancreas. The simultaneous induction of pancreas- and liver-specific programs in current directed differentiation protocols has hampered progress in generating insulin-producing cells (D’Amour et al., 2006; Nostro et al., 2011). Although previous strategies to prevent induction of hepatic programs have largely focused on inhibiting Bmp signaling (D’Amour et al., 2006; Nostro et al., 2011), our findings suggest that stimulating Fgf signaling in embryonic stem cell cultures beyond the time of pancreas induction could stabilize pancreatic fate commitment.

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Competing interests statement
The authors declare no competing financial interests.

Supplementary material
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References


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**Fig. S1.** Pdx1-Cre and Ptf1a-Cre are active in the pancreas by E9.5 and E10.5, respectively. (A-C) X-Gal staining for β-gal activity from the recombined Rosa26R reporter allele reveals Pdx1-Cre to be active in the dorsal (dp) and ventral (vp) pancreatic buds by E9.5 (A) while Ptf1a-Cre is not active until E10.5 (B,C). Scale bars: 250 µm.

**Fig. S2.** AFP+ cells arise from Pdx1+ progenitors in Sox9-deficient pancreata. (A-F) eGFP induced by Pdx1-Cre-driven recombination of the Z/EG reporter allele (A) labels Pdx1+ cells and their progeny in the dorsal pancreas (dp) (A,F). AFP+ cells (B), including AFP+ Pdx1+ cells (arrows in A-F), are eGFP+, showing their derivation from Pdx1-expressing pancreatic progenitors in Sox9-deleted pancreata (E,F). Most first-wave glucagon+ endocrine cells (D) are also eGFP+ and are thus derived from Sox9-deleted pancreatic progenitors (F). Broken line indicates the boundary between dorsal pancreas and duodenum (duo). Scale bar: 20 µm.
Fig. S3. Sox9-deleted pancreatic progenitors activate a bona fide hepatic program. (A,B) Immunodetection reveals almost complete absence of AFP or α₁-antitrypsin (α₁-AT) in the E11.5 control Sox9<sup>fl/fl</sup> dorsal pancreas (dp) (A), but abundant expression in Sox9<sup>fl/fl; Pdx1-Cre</sup> pancreas (B). (C,D) Although low-level Hnf4α nuclear expression is seen in Pdx1<sup>+</sup> pancreatic progenitors in E11.5 control pancreas (C), high-level Hnf4α expression is evident in AFP<sup>+</sup> cells of Sox9<sup>fl/fl; Pdx1-Cre</sup> pancreas (D), similar to that seen in the liver (li) (C,D). Scale bars: 20 μm.
Fig. S4. Variable ratios of mutually exclusive AFP+ and glucagon+ populations. (A-L) Immunodetection on 12 sequentially ordered (rostral to caudal) serial sections through a single E11.5 Sox9<sup>fl/fl</sup>; Pdx1-Cre dorsal pancreas (dp) shows both glucagon+ cells and AFP+ cells to occur in clusters. Glucagon and AFP expression are mutually exclusive. The ratio of glucagon+ cells:AFP+ cells:Pdx1+ cells varies greatly between different sections of the same Sox9<sup>fl/fl</sup>; Pdx1-Cre pancreas. Scale bar: 20 µm.

Fig. S5. Competence window for Sox9-deleted pancreatic progenitors to adopt hepatic fates closes by E12.5. (A-C) AFP+ or albumin+ cells are not detected in the E15.5 Sox9<sup>fl/fl</sup>; Rosa26-CreER pancreatic epithelium (indicated by broken lines in B,C) after intraperitoneal (i.p.) tamoxifen-induced Sox9 deletion at E12.5. li, liver. Scale bar: 50 µm.
Fig. S6. *Ptf1a*-deficient pancreatic progenitors retain pancreatic identity. (Aa-Cd) Pancreatic progenitors in E10.5 wild-type (WT) dorsal (Aa-Ad) and ventral (Ba-Bd) pancreas express Pdx1, Sox9 and Ptf1a; both Pdx1 and Sox9 are expressed in dorsal pancreas of *Ptf1a*−/− mice (Ca-Cd). (Da-Fd) Like Pdx1 and Sox9, Fgfr2 expression persists in the *Ptf1a*−/− dorsal pancreas (Fa-Fd) as in dorsal (Da-Dd) and ventral (Ea-Ed) wild-type pancreata. (G-J) At E11.5, both Sox9 and Pdx1 are maintained in *Ptf1a*−/− dorsal pancreas (H,J), as in wild-type dorsal (G,I) and ventral (G′,J′) pancreata. AFP is not detected in E11.5 wild-type dorsal (I) and ventral (I′) pancreata or dorsal pancreas of *Ptf1a*−/− mice (J). *Ptf1a*−/− ventral pancreas is not shown as it is histologically undetectable by E11.5. Broken line demarcates the boundary between the dorsal pancreas and duodenum (Aa-Ad,Ca-Cd,Da-DD,Fa-Fd). dp, dorsal pancreas; vp, ventral pancreas; duo, duodenum; cbd, common bile duct; li, liver. Scale bars: 20 μm.
**Table S1. Sequences of primers used for mRNA quantification by qRT-PCR**

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<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
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<td>Sox9</td>
<td>5'-GAGCCGGATCTGAAGAGGGA-3'</td>
<td>5'-GCTTGACGTGTGGCTTGTTTC-3'</td>
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<tr>
<td>AFP</td>
<td>5'-CTTCCCTCATCCTCTGCTAC-3'</td>
<td>5'-ACAAACTGGGTTAAAGGTGATGG-3'</td>
</tr>
<tr>
<td>Albumin</td>
<td>5'-TGCTTTTTCCAGGGGTGTGTT-3'</td>
<td>5'-TTACTTTCTGCACATTATTTGCA-3'</td>
</tr>
<tr>
<td>Transferrin</td>
<td>5'-TGGGGGGTTGGGTACGAT-3'</td>
<td>5'-AGCGTAGTAGTAGGTCTGTG-3'</td>
</tr>
<tr>
<td>G-6-P</td>
<td>5'-CGACTCGCTATCTCCAAGTGA-3'</td>
<td>5'-GTTGAACCAGTCTCCGACCA-3'</td>
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<tr>
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