Persistent expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation

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The transcription factor pancreatic and duodenal homeobox factor 1 (PDX-1) is expressed in pancreatic progenitor cells. In exocrine pancreas, PDX-1 is down-regulated during late development, while up-regulation of PDX-1 has been reported in pancreatic cancer and pancreatitis. To determine whether sustained expression of PDX-1 could affect pancreas development, PDX-1 was constitutively expressed in all pancreatic lineages by transgenic approaches. The transgenic pancreas was markedly small with the replacement of acinar cells by duct-like structures, accompanied by activated Stat3. Genetic ablation of Stat3 in the transgenic pancreas profoundly suppressed the metaplastic phenotype. These results provide a mechanism of pancreatic metaplasia by which persistent PDX-1 expression cell-autonomously induces acinar-to-ductal transition through Stat3 activation.

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Pancreatic and duodenal homeobox factor 1 [PDX-1], a member of the homeodomain-containing transcription factor family, has been demonstrated to play a crucial role throughout pancreas development and differentiation (Jonsson et al. 1994; Offield et al. 1996; Sander and German 1997; Edlund 2002, Fujitani et al. 2006). PDX-1 is initially expressed in the gut region when the foregut endoderm becomes committed to common pancreatic precursor cells, and PDX-1-expressing cells give rise to all three types of pancreatic tissue: endocrine, exocrine, and duct (Öhllson et al. 1993; Guz et al. 1995; Gu et al. 2002). In exocrine and ductal cells, PDX-1 gene expression is down-regulated after late embryonic development, and in the postnatal pancreas it is observed only in a restricted subset of exocrine and duct cells (Wu et al. 1997; Stoffers et al. 1999). In addition, PDX-1 is known to participate in the activation of the promoter of at least one acinar-specific gene, elastase-1 (Swift et al. 1998). On the other hand, re-up-regulation of PDX-1 has been reported in human patients and several mouse models with pancreatic cancer and pancreatitis (Song et al. 1999; Koizumi et al. 2003; Jensen et al. 2005). PDX-1 was also shown to be induced in ductal cells isolated from acinar components in vitro (Rooman et al. 2000). Thus, while induction of PDX-1 expression is observed under certain physiological conditions, it remains to be elucidated whether re-expression of PDX-1 is a causative factor or simply a consequence of these metaplastic changes.

We previously generated a transgenic line, the “CAG-CAT-PDX1” mouse, which can induce the exogenous expression of PDX-1 using the Cre-loxP system (Miyatsuka et al. 2003). To address the effect of the continuous expression of PDX-1 in all three pancreatic lineages, we crossed the CAG-CAT-PDX1 mice with Ptf1a-Cre mice, which express Cre recombinase driven by the Ptf1a [PTF1-p48] gene promoter in the precursors of all three pancreatic cell types (Kawaguchi et al. 2002). In this study, we show that persistent expression of PDX-1 is sufficient to induce acinar-to-ductal metaplasia in a cell-autonomous manner. In metaplastic duct-like cells, signal transducer and activator of transcription 3 (Stat3) was activated, which has been reported to be induced in other mouse models of pancreatic metaplasia (Greten et al. 2002). Furthermore, conditional knockout of Stat3, by crossing Ptf1a-Cre; CAG-CAT-PDX1 mice with floxed-Stat3 mice, revealed that activation of Stat3 is essential for the formation of duct-like structure. These findings suggest that up-regulation of PDX-1 and consequent activation of Stat3 play a primary role in the pathogenesis of tubular complex formation, which could lead to the initiation of pancreatic neoplasia.

Results and Discussion

In the transgenic CAG-CAT-PDX1 mouse strain, the transgene-derived PDX-1 is not expressed until a loxP-flanked stop cassette encoding chloramphenicol acetyltransferase (CAT) is excised by Cre-mediated recombination. Once recombined, the PDX-1 gene is constitutively expressed under the control of the chicken β-actin gene promoter with the CMV enhancer [CAG], which has potent promoter activity in various tissues (Fig. 1A). To induce the persistent expression of PDX-1 in all three lineages from the early embryonic pancreas, CAG-CAT-PDX1 mice were crossed with Ptf1a-Cre knock-in mice, which have been shown to express the Cre recombinase in Ptf1a-positive progenitor cells (Kawaguchi et al. 2002).

[Keywords: PDX-1, acinar-to-ductal transition, metaplasia, pancreas differentiation, Stat3]
80% of cells in acini and ducts (arrows) in addition to islet cells at E18.5. In the transgenic pancreas, PDX-1 is strongly expressed in control Ptf1a-Cre (E18.5) and Ptf1a-Cre; CAG-CAT-PDX1 (F) mouse at PDX-1; ROSA26-lacZ mouse was found to be dramatically smaller than the nontransgenic pancreas (Fig. 2A,B). Transgenic pancreata weighted ~60% less than those of control littermates at 3 wk of age (Supplementary Fig. 1A). Because the exocrine portion of the normal pancreas contributes to >95% of its mass, it was speculated that hypoplasia of the transgenic pancreata would be accompanied by abnormalities in exocrine tissue. We therefore examined pancreatic exocrine function in the transgenic mice. Quantitative biochemical analyses confirmed that the transgenic mice showed a >50-fold reduction in lipase and amylase activity as compared with littermate controls (Supplementary Fig. 1B,C). Consistent with the findings that exocrine enzymes of the transgenic pancreas are dramatically reduced, Oil Red O staining revealed abundant lipid droplets in stool smears from the Ptf1a-Cre; CAG-CAT-PDX1 mice (Supplementary Fig. 1D,E).

The microscopic morphology of the transgenic pancreas exhibited no apparent abnormalities before the age of 1 wk (Figs. 1E,F, 3A,B). Around the age of 10 d, however, acinar areas with normal morphology substantially disappeared and were replaced by abnormal compact cells with high nucleocytoplasmic ratio, and by 2–3 wk after birth, substantial numbers of cells with duct-like morphology were observed (Fig. 3C–F). The morphology of transgenic islets and interlobular ducts had no apparent abnormalities compared with control littermates. Immunoreactive insulin levels detected in whole pancreas extracts from Ptf1a-Cre; CAG-CAT-PDX1 mice were slightly higher than those from control littermates (1.26 vs. 0.90 µg/g body weight, respectively; p < 0.05), although the cell proliferation rate in islets, as assessed by Ki67 and BrdU staining, was not altered in the 3-wk-old transgenic pancreas (data not shown). The glucagon levels from whole-pancreas extracts were unaffected in the transgenic mice.

To further evaluate the characteristics of these cells

Flag-positive exogenous PDX-1 was detected only in the pancreatic bud (Fig. 1B–D), which is consistent with the pancreas-specific expression of the Ptf1a gene within the foregut region (Kawaguchi et al. 2002). At E18.5, while PDX-1 was expressed more strongly within islets than in ductal epithelia and acinar cells in Ptf1a-Cre mice, the pancreata of Ptf1a-Cre; CAG-CAT-PDX1 mice exhibited strong expression of PDX-1 in all three types of cells: islets, acini, and ducts (Fig. 1E,F).

The appearance of transgenic pancreata had no apparent abnormalities before 1 wk of age. After ~2 wk, however, the whole pancreas of the Ptf1a-Cre; CAG-CAT-PDX1; ROSA26-lacZ mouse was found to be dramatically smaller than the nontransgenic pancreas (Fig. 2A,B). Transgenic pancreata weighted ~60% less than those of control littermates at 3 wk of age [Supplementary Fig. 1A]. Because the exocrine portion of the normal pancreas contributes to >95% of its mass, it was speculated that hypoplasia of the transgenic pancreata would be accompanied by abnormalities in exocrine tissue. We therefore examined pancreatic exocrine function in the transgenic mice. Quantitative biochemical analyses confirmed that the transgenic mice showed a >50-fold reduction in lipase and amylase activity as compared with littermate controls [Supplementary Fig. 1B,C]. Consistent with the findings that exocrine enzymes of the transgenic pancreas are dramatically reduced, Oil Red O staining revealed abundant lipid droplets in stool smears from the Ptf1a-Cre; CAG-CAT-PDX1 mice [Supplementary Fig. 1D,E].

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Figure 1. Cre-mediated expression of exogenous PDX-1. (A) Schematic representation of the transgenes of CAG-CAT-PDX1 and ROSA26 reporter locus and their recombination by Cre recombinase. Before recombination, the transcription of PDX-1 and lacZ is blocked by the floxed STOP cassette. When the mice are mated with Cre-expressing mice, the floxed sequence is removed by Cre recombinase, and the CAG promoter and cell type-independent ROSA26 promoter can activate the expression of PDX-1 and β-galactosidase, respectively. [A] Polyadenylation signal. (B–D) Immunofluorescence for PDX-1 (red) and the Flag-tagged peptide (green) in a Ptf1a-Cre; CAG-CAT-PDX1 mouse at E10.5. The merged image demonstrates that exogenous PDX-1 is expressed only in dorsal pancreas (dp, surrounded by a dashed line), and not in duodenum (duo). Note that the red and green spots located outside the dorsal pancreas and in the vein (v) are considered to be autofluorescent signals from erythrocytes. (E,F) Immunostaining for PDX-1 in the pancreas of a control Ptf1a-Cre (E) and Ptf1a-Cre; CAG-CAT-PDX1 (F) mouse at E18.5. In the transgenic pancreas, PDX-1 is strongly expressed in >80% of cells in acini and ducts (arrows) in addition to islet cells (surrounded by a broken line).

By an additional crossing, we generated Ptf1a-Cre; CAG-CAT-PDX1 mice with the ROSA26-lacZ reporter allele, which carries a modified β-galactosidase gene under the control of the cell type-independent ROSA26 promoter, enabling us to perform cell lineage tracing of cells expressing exogenous PDX-1 [Soriano 1999]. In the pancreatic progenitor cells of Ptf1a-Cre; CAG-CAT-PDX1; ROSA26-lacZ trigenic mice, Ptf1a-driven expression of Cre recombinase excises the stop cassettes of both CAG-CAT-PDX1 and ROSA26-lacZ, and both exogenous PDX-1 and β-galactosidase are constitutively expressed in their progeny, even if Cre expression is subsequently lost from the cells. It is noted that the Flag-tagged sequence is attached to the transgene, so that exogenous PDX-1 can be distinguished from endogenous PDX-1. First, to examine whether exogenous PDX-1 protein is indeed expressed in the pancreatic progenitor cells of transgenic mice, we performed double immunostaining against PDX-1 and the Flag-tagged epitope at early stages of development. At embryonic day 10.5 (E10.5), the PDX-1 protein was detected in both pancreatic buds and duodenum of Ptf1a-Cre; CAG-CAT-PDX1 mice, whereas

Figure 2. Pancreatic hypoplasia in Ptf1a-Cre; CAG-CAT-PDX1 mice. [A,B] Macroscopic phenotypes of 3-wk-old pancreata of Ptf1a-Cre; ROSA26-lacZ mice (A) and the trigenic Ptf1a-Cre; CAG-CAT-PDX1; ROSA26-lacZ mice (B) stained with X-gal. Note the overall reduction in size of the trigenic pancreas.
magnifications in Educt-like cells were positive for cytokeratin. Staining for PDX-1 (green) and cytokeratin (red). PDX-1-positive cells were positive for carboxypeptidase A. (J) Double immunostaining for PDX-1 (green) and carboxypeptidase A (red). Small PDX-1 positive rowheads are spread throughout the acinar area of Ptf1a-Cre; CAG-CAT-PDX1 mice. The boxed areas in D and F are shown at higher magnifications in E and F, respectively. (K) Abnormally shaped cells with duct-like morphology (arrows) and small acinar-like cells (arrowheads) are spread throughout the acinar area of Ptf1a-Cre; CAG-CAT-PDX1 mice. Bar, 100 µm. (L) Double immunostaining for PDX-1 [green] and carboxypeptidase A [red]. Small PDX-1-positive cells were positive for carboxypeptidase A. (L) Double immunostaining for PDX-1 [green] and cytokeratin [red]. PDX-1-positive duct-like cells were positive for cytokeratin.

Figure 3. Loss of normal acinar character in Ptf1a-Cre; CAG-CAT-PDX1 mice. Hematoxylin and cosin (HE) staining of control and transgenic pancreata. There is no apparent difference in the morphology of 1-wk-old pancreata between Ptf1a-Cre (A) and Ptf1a-Cre; CAG-CAT-PDX1 (B) mice. Bar, 50 µm. (C–F) HE-stained sections of 3-wk-old pancreata of Ptf1a-Cre (C, F) and Ptf1a-Cre; CAG-CAT-PDX1 (D, F) mice. The boxed areas in C and D are shown at higher magnifications in E and F, respectively. (F) Abnormally shaped cells with duct-like morphology (arrows) and small acinar-like cells (arrowheads) are spread throughout the acinar area of Ptf1a-Cre; CAG-CAT-PDX1 mice. Bar, 100 µm. (G–I) Double immunostaining for PDX-1 [green] and carboxypeptidase A [red]. Small PDX-1-positive cells were positive for carboxypeptidase A. (I) Double immunostaining for PDX-1 [green] and cytokeratin [red]. PDX-1-positive duct-like cells were positive for cytokeratin.

expressing exogenous PDX-1, double immunostaining was performed for PDX-1 and carboxypeptidase A, which is a pancreatic exopeptidase expressed in acinar cells, and for PDX-1 and cytokeratin, which is a marker of duct cells [Fig. 3G–L]. In the 3-wk-old transgenic pancreas, most compact cells with reduced cytoplasm were positive for both PDX-1 and carboxypeptidase A [Fig. 3G–I], suggesting that these cells are derived from acinar cells and that exogenous PDX-1 causes dysmorphogenesis in the exocrine lineage. On the other hand, most duct-like cells expressing high levels of PDX-1 were positive for cytokeratin [Fig. 3J–L], suggesting that these cells have at least partially adopted the character of duct cells. These duct-like cells and compact cells in the transgenic pancreas had a larger number of TUNEL-positive cells compared with the acinar cells in control mice (32.9 vs. 3.56 cells/mm³, respectively; n = 4, p < 0.001). These data suggest that hypoplasia of the transgenic pancreas may be a result of both replacement of normal acinar cells with compact cells and the increase of apoptotic cells.

Next, to evaluate whether or not these morphological changes were the direct effect of exogenous PDX-1, immunostaining against the Flag tag was performed in the transgenic pancreas at the age of 3 wk [Fig. 4A,B]. Whereas many Flag-negative cells appeared to have maintained the morphology of normal acinar cells, Flag-positive cells expressing exogenous PDX-1 were severely atrophic [Fig. 4A] or had abnormal duct-like morphology [Fig. 4B]. These cells were found distributed all over the acinar areas. These findings suggest that the phenotypes in the transgenic pancreas are caused by a cell-autonomous effect of PDX-1 without damaging neighboring cells, which are negative for PDX-1. Thus, we assume that persistent up-regulation of PDX-1 directly inhibits the formation and/or maintenance of the differentiated state of acinar cells and that programmed down-regulation of PDX-1 might be required for normal maintenance of exocrine differentiation. It has been recently reported that PDX-1 is required during midpancreatic development for the formation of exocrine pancreas as well as during earlier development of pancreatic buds (Hale et al. 2005). The reason our transgenic mice exhibited no apparent abnormalities before the age of 1 wk might be associated with this requirement of PDX-1 expression for the formation of exocrine pancreas.

As shown in Figures 3 (D, F, J–K) and 4B, substantial numbers of duct-like cells were observed in the pancreas of Ptf1a-Cre; CAG-CAT-PDX1 mice. These duct-like...
cells have two potential origins: 1) They are derived from acinar cells, and acinar-to-ductal transdifferentiation occurred. 2) They are derived from duct cells themselves, and expansion of duct cells occurred. To test these possibilities, we performed a cell lineage study. To induce exogenous expression of PDX-1 selectively in the exocrine lineage, the CAG-CAT-PDX1 mouse was crossed with a transgenic Elastase-Cre mouse, which expresses Cre recombinase under the control of the rat elastase promoter [Grippo et al. 2002]. Concomitantly, the resulting mice were crossed with the ROSA26-lacZ reporter mouse. In the pancreas of the Ptf1a-Cre; ROSA-lacZ mouse, Cre-mediated recombination was observed in all three lineages, marked as blue β-galactosidase-positive cells [Fig. 4C], whereas in the pancreas of the Elastase-Cre; ROSA-lacZ mouse, recombination was observed primarily in the exocrine lineage, although some terminal ductal and centroacinar cells were also marked [Fig. 4D]. Therefore, in the pancreas of Elastase-Cre; CAG-CAT-PDX1 mice, exogenous expression of PDX-1 should be induced mainly in the exocrine lineage. Similar to the pancreas of Ptf1a-Cre; CAG-CAT-PDX1; ROSA26-lacZ trigenic mice [Fig. 4E], substantial numbers of duct-like cells, marked as blue cells, were observed in the pancreas of Elastase-Cre; CAG-CAT-PDX1; ROSA26-lacZ trigenic mice [Fig. 4F], while before the age of 1 wk the morphology of the trigenic pancreas exhibited no apparent abnormality. Most non-blue-stained cells in the acinar regions [Fig. 4E,F] seemed to maintain normal acinar morphology in both trigenic mice. These findings suggest that exogenous expression of PDX-1 cell-autonomously induces acinar-to-ductal transdifferentiation, although there is a possibility that exogenous expression of PDX-1 in intercalated or centroacinar cells induces the proliferation of these cells. To rule out this possibility, cell proliferation in these cells was investigated using immunostaining for BrdU and Ki67 at the ages of 1 and 2 wk. Since the number of proliferating cells in terminal ductal epithelia and centroacinar cells did not differ between Ptf1a-Cre; CAG-CAT-PDX1 and control Ptf1a-Cre mice [data not shown], we conclude that duct-like cells in the Ptf1a-Cre; CAG-CAT-PDX1 pancreas are most likely derived from acinar cells.

To verify the molecular mechanism by which PDX-1 induces acinar-to-ductal differentiation, we investigated the expression and activation of signal transducer and activator of transcription 3 [Stat3], which has been shown to be activated in cells within ductal structures in human pancreatic cancer as well as in a mouse model [Greten et al. 2002; Scholz et al. 2003]. Immunostaining for Stat3 phosphorylated at Tyr 705, which is responsible for Stat3 activation, revealed that a number of metaplastic cells were positive for phosphorylated Stat3 in the transgenic Ptf1a-Cre; CAG-CAT-PDX1 pancreas, whereas none of the acinar and duct cells in the control pancreas were positive for phosphorylated Stat3 [Fig. 5A,B]. In addition, suppressor of cytokine signaling-3 (SOCS3), a downstream mediator of Stat3, was overexpressed in the transgenic pancreas [Fig. 5C], further providing evidence for Stat3 activation, although low levels of SOCS3 expression were detected in control pancreata at a higher number of PCR cycles. To address the physiopathological significance of Stat3 activation in inducing metaplastic duct-like cells, Ptf1a-Cre; CAG-CAT-PDX1 mice were crossed with floxed-Stat3 mice [Takeda et al. 1998]. Similar to the pancreata of Ptf1a-Cre; CAG-CAT-PDX1; Stat3flox/flox mice [Fig. 3D,F], a substantial number of duct-like cells were observed in the pancreas of Ptf1a-Cre; CAG-CAT-PDX1; Stat3flox/+ mice (Fig. 5D). However, in the pancreata of Ptf1a-Cre; CAG-CAT-PDX1; Stat3flox/+; ROSA26-lacZ (F) and Ptf1a-Cre; CAG-CAT-PDX1; Stat3flox/flox ROSA26-lacZ (G) mice, metaplastic duct-like cells were rarely observed in the acinar area, although some compact cells still existed [Fig. 5E]. In accordance with this microscopic phenotype, the pancreatic hypoplasia seen in PDX-1-overexpressing pancreata (Figs. 2B, 5F) was dramatically restored to >70% of wild-type pancreatic weight in the pancreata of Ptf1a-Cre; CAG-CAT-PDX1; Stat3flox/+ mice (Fig. 5G). These findings show that Stat3 activation is essential for the formation of tubular complexes induced by sustained PDX-1 expression.

It has been reported that duct-like cells or tubular complexes appear in both pancreatitis and pancreatic neoplasia in humans and rodent models [Parsa et al. 1985; Bockman 1997; Wagner et al. 1998; Song et al. 1999, Means et al. 2003]. Song et al. [1999] have observed the up-regulation of PDX-1 expression in the metaplastic ductal epithelium of transgenic mice overexpressing
transforming growth factor-α (TGF-α). In addition, re-expression of PDX-1 has been reported in human patients and several mouse models of pancreatic cancer (Hingorani et al. 2003; Koizumi et al. 2003). Moreover, activation of Stat3 has been reported in a mouse model expressing TGF-α, human patients with pancreatic cancer, and human pancreatic carcinoma cell lines (Greten et al. 2002; Scholz et al. 2003). These findings, together with our data, indicate that the persistent up-regulation of PDX-1 and consequent activation of Stat3 can lead to acinar-to-ductal metaplasia. This novel mechanism could explain the pathogenesis of ductal metaplasia observed in various conditions, such as pancreatic cancer and pancreatitis. On the other hand, ectopic expression of PDX-1 has also been observed in gastric epithelial lesions in gastric carcinomas and Ménétrier’s disease, as well as in pancreatic metastatic lesions (Sakai et al. 2004; Nomura et al. 2005). Since metaplasia has been implicated in the progression to neoplasia, further studies of the involvement of PDX-1 in metaplasia might lead to a better understanding of the molecular mechanisms of epithelial carcinogenesis.

On the other hand, no apparent duct-like cells were reported in the transgenic mice expressing exogenous PDX-1 under the control of the rat elastase-1 promoter [Elastase-PDX1 mice], although these mice exhibit marked dysmophogenesis of the exocrine pancreas [Heller et al. 2001]. The most important difference between the Elastase-PDX1 mice and our CAG-CAT-PDX1 mice is the promoter that regulates the exogenous expression of PDX-1. The expression of the transgene in Elastase-PDX1 mice is driven by the exocrine-specific elastase-1 promoter, and therefore the expression levels of PDX-1 are expected to decrease as PDX-1-expressing acinar cells differentiate into other types of cells and elastase-1 promoter activity is down-regulated. In contrast, since our CAG-CAT-PDX1 mice express exogenous PDX-1 driven by the CAG promoter, in which the CMV-IE enhancer is linked to the chicken β-actin gene promoter [Niwa et al. 1991], exogenous PDX-1 should be constitutively active in all types of cells independent of the differentiated state. Therefore, it appears that the persistent, but not transient, expression of PDX-1 is essential to induce acinar-to-ductal metaplasia.

In conclusion, our in vivo conditional expression and inactivation system revealed that the persistent up-regulation of PDX-1 alone is sufficient to induce acinar-to-ductal metaplasia in the exocrine lineage and that Stat3 activation is essential for the formation of this metaplasia. Metaplastic conversion is considered to result from a variety of cellular mechanisms, such as trans-determination [or reprogramming] of tissue-specific stem cells and the actual transdifferentiation of one differentiated cell type to another [Tosh and Slack 2002]. Therefore, the next important step in this study would be to elucidate the developmental stage at which the exocrine cells can transdifferentiate into duct-like complexes. In other words, we need to examine whether the same metaplastic change is observed when exogenous PDX-1 is induced only in a specific cell type, such as fully differentiated exocrine cells. To address this question, it would be useful to cross our CAG-CAT-PDX1 mice with other transgenic mice carrying inducible Cre recombinase, such as the transgenic mouse expressing tamoxifen-inducible Cre driven by the elastase promoter (Means et al. 2005).

Materials and methods

Transgenic and knockout mice
Ptf1a-Cre, Elastase-Cre, CAG-CAT-PDX1, ROSA26-lacZ, and floxed-Stat3 mice were generated as described (Takeda et al. 1998; Soriano 1999; Grippo et al. 2002; Kawaguchi et al. 2002; Miyatsuka et al. 2003). Age- and sex-matched wild-type or single-transgenic littermates were used as control mice throughout the study. All animal procedures were approved by the Ethics Review Committee for Animal Experimentation of Osaka University Graduate School of Medicine.

Histology
For histology and immunostaining, tissues were fixed, sectioned, and stained as previously described [Miyatsuka et al. 2003]. Immunostaining for PDX-1, the Flag epitope, carboxypeptidase A, cytokertatin, and tyrosine-phosphorylated Stat3 was performed at the following dilutions: rabbit and guinea pig antibody against PDX-1 [Hingorani et al. 2003]; 1:1000 and 1:500, respectively, rabbit antibody against Flag epitope tag [Affinity BioReagents]; 1:500, rabbit antibody against carboxypeptidase A [Biogenesis Ltd.]; 1:1000, rabbit monoclonal antibody against Stat3 phosphorylated at Tyr 705 [Cell Signaling]; 1:100, mouse antibody against cytokeratin [Sigma]; 1:1000. For the detection of PDX-1, the Flag epitope, and cytokeratin, mounted sections were microwaved at 95°C for 20 min in citrate buffer (pH 6.0) for antigen retrieval before being incubated with blocking serum.

Additional information is provided in the Supplemental Material.

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