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Stabilization of β-catenin impacts pancreas growth

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A recent study has shown that deletion of β-catenin within the pancreatic epithelium results in a loss of pancreas mass. Here, we show that ectopic stabilization of β-catenin within mouse pancreatic epithelium can have divergent effects on both organ formation and growth. Robust stabilization of β-catenin during early organogenesis drives changes in hedgehog and Fgf10 signaling and induces a loss of Pdx1 expression in early pancreatic progenitor cells. Together, these perturbations in early pancreatic specification culminate in a severe reduction of pancreas mass and postnatal lethality. By contrast, inducing the stabilized form of β-catenin at a later time point in pancreas development causes enhanced proliferation that results in a dramatic increase in pancreas organ size. Taken together, these data suggest a previously unappreciated temporal/spatial role for β-catenin signaling in the regulation of pancreas organ growth.

KEY WORDS: β-Catenin, FGF, Hedgehog, Organ size, Pancreas development, Pdx1, Wnt, Mouse, Pancreatomegaly

INTRODUCTION

β-Catenin function is essential for the canonical arm of the Wnt signaling pathway (reviewed by Willert and Nusse, 1998). In the absence of Wnt ligand, cytoplasmic pools of β-catenin are highly unstable because of multiple phosphorylations in the N terminus of the protein that target the protein for degradation. However, when the Wnt co-receptors Lrp and frizzled are engaged by ligand, the assembly of proteins responsible for this phosphorylation state is inhibited. Consequently, the unphosphorylated form of β-catenin accumulates in the cytoplasm, making it available for entry into the nucleus. Once in the nucleus, β-catenin interacts with Tcf/Lef transcriptional co-activators to promote the expression of target genes (Nelson and Nusse, 2004).

Previous work has established that the third exon of the β-catenin gene encodes the N-terminal phosphorylation sites necessary for degradation of the protein via ubiquitylation (Harada et al., 1999). Therefore, the removal of this exon in transgenic mice using Cre/loxP technology results in a constitutively stabilized, or activated, form of the β-catenin protein (Harada et al., 1999). These *β-cat^active^* mice have proven to be useful in probing the effects of β-catenin signaling on embryonic stem cell differentiation, progenitor cell expansion in the nervous system, epithelial-mesenchymal transition in the epiblast and other phenomena (Kemler et al., 2004; Kielman et al., 2002; Zechner et al., 2003).

Previous studies have demonstrated that Wnt signaling components are dynamically expressed within the developing pancreas, suggesting that canonical Wnt signaling may be involved in pancreas organogenesis (Dessimoz et al., 2005; Heller et al., 2003; Murtaugh et al., 2005; Papadopoulou and Edlund, 2005). Two independent laboratories recently reported divergent phenotypes resulting from the conditional deletion of β-catenin within the pancreatic epithelium. In one instance, loss of β-catenin resulted in a reduction in pancreatic endocrine cell numbers, whereas the gross morphology of the organ appeared normal at birth (Dessimoz et al., 2005). However, a separate report demonstrated that loss of β-catenin did not affect pancreatic endocrine cell mass, despite the almost complete loss of the exocrine compartment. Here, we have used the *β-cat^{active}* mouse to help clarify how β-catenin stability affects pancreas development and organ maturation.

In mice, pancreas morphogenesis begins by 9.5 days post coitum (E9.5) when epithelial tissue fated to become the dorsal pancreas buds from the gut endoderm within a mesenchymal cap (Kim and Hebrok, 2001). Emergence of two distinct ventral pancreatic buds occurs slightly later, by E10.25-10.5. Signaling by the mesenchyme is essential for epithelial proliferation and branching. The epithelium eventually gives rise to two distinct tissue compartments: exocrine cells that produce digestive enzymes and endocrine cells that produce hormones essential for regulating blood glucose levels.

Pdx1, a homeobox transcription factor, is one of the earliest genes to be expressed within the developing pancreatic epithelium and is essential for normal organ formation. Moreover, Pdx1-expressing pancreatic progenitor cells have been shown to give rise to all three types of pancreatic tissue: endocrine, exocrine and duct (Gu et al., 2002). A number of independent lines of transgenic mice that express Cre recombinase under the control of *Pdx1* promoter fragments have been generated (Gannon et al., 2000; Gu et al., 2002; Herrera, 2000). Our characterization of two of these strains indicated that the temporal and spatial activity of Cre-recombinase differed. This allowed us to determine how β-catenin stabilization in these distinct temporal/spatial domains of the pancreatic epithelium affected organogenesis and adult organ function. Interestingly, we observed significantly different pancreatic phenotypes depending on the *Cre* strain employed. Using the *PdxCre* mice generated in the laboratory of D. Melton that displayed early and robust Cre recombinase activity (*PdxCre^{early}*), we observed a nearly complete loss of pancreatic tissue (Gu et al., 2002). Conversely, slightly delayed and more mosaic Cre recombinase expression in Pdx mice generated in one of our laboratories (*PdxCre^{late}*) drives outgrowth of pancreatic tissue, resulting in a grossly enlarged pancreas (Gannon et al., 2000; Herrera, 2000). Thus, in one instance, β-catenin stabilization drives tissue loss, and in the other culminates in an increase in organ size relative to body mass. Therefore, ectopic stabilization of β-catenin blocks/deregulates the normal mechanisms that control embryonic pancreas formation and postnatal organ growth.

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DEVELOPMENT

MATERIALS AND METHODS

Mice

Noon of the day when vaginal plugs are detected is treated as E0.5 day post coitum. Mice carrying the floxed exon 3 allele of β-catenin (*β-cat*^{active}) (Harada et al., 1999) were crossed with strains expressing Cre-recombinase under the control of the *Pdx1* promoter (*PdxCre*^{early}, *PdxCre*^{late}, *PdxCre*^{ER}) (Gannon et al., 2000; Gu et al., 2002; Herrera, 2000), all of which were maintained in a mixed background. In all experiments presented in this work, the floxed exon 3 allele of β-catenin was maintained as a heterozygote to insure that the level of β-catenin signaling pathway activation was consistent. Thus, the *β-cat*^{active} nomenclature used refers to mice containing one wild-type and one floxed exon 3 allele of β-catenin. To verify the expression pattern of Cre-recombinase, a *lacZ* reporter line was used (*R26R*) (Soriano, 1999).

Tamoxifen preparation and injection

Tamoxifen (10 mg/ml) (Sigma, T5648) was dissolved in corn oil (Sigma, C8267) following 30 minutes of incubation at 37°C and vigorous vortexing. Intraperitoneal injections (100 μl, 1 mg/mouse) were made into the pregnant female at the indicated developmental timepoint using a 21-gauge needle.

Tissue preparation, immunohistochemistry, and microscopy

Embryonic tissues were fixed and paraffin wax imbedded as previously described (Kawahira et al., 2003). Hematoxylin/Eosin staining, immunohistochemical and immunofluorescence analyses were performed as previously described (Kim et al., 1997). The primary antibodies used in this study are listed in Table 1. For immunohistochemistry, a biotinylated anti-goat (Vector; BA-9500) was used at a dilution of 1:200. Staining for diaminobenzidine (DAB) was performed with the ABC Elite immuoperoxidase system (Vector). The Alexa series of secondary antibodies from Molecular Probes was used for the immunofluorescent analysis performed in this study. However, in order to amplify the signal from the β-galactosidase antibody, we found it necessary to use the TSA Plus Fluorescence system (Perkin Elmer, fluorescein NEL741). Slides were mounted with Vectashield mounting media containing the nuclear stain, DAPI (Vector). Bright-field images were acquired using a Zeiss Axio Imager D1 scope; fluorescent images were captured using a Leica DMIRE2 SP2 confocal microscope.

Staining for β-galactosidase activity and whole mount in situ hybridization

Fixed *PdxCre*^{early} or *PdxCre*^{late} *R26R* were incubated overnight at room temperature in phosphate-buffered saline (PBS) supplemented with 5-bromo-4-chloro-3-indolyl-D-galactopyranoside (X-gal: 1 mg/ml), 0.02% Triton X-100, 5 mM potassium ferricyanide, and 5 mM potassium ferrocyanide. For whole-mount in situ hybridization, the gastrointestinal cavity of intact embryos was opened to allow greater penetration of fix and probe, and then incubated in 4% paraformaldehyde overnight at 4°C. Whole-mount in situ hybridization with digoxigenin-labeled *Fgf10* riboprobe was performed as previously described (Chuang et al., 2003). Stained tissues were photographed with a Leica MZ FL3 dissecting microscope equipped with a Leica IM500 system.

Morphometric quantification of proliferation and cell density

Pancreatic paraffin wax-embedded sections (6 μm) were cut from both the dorsal and ventral pancreas. Following immunofluorescent staining for the proliferation marker phospho-histone H3, positive cells were then scored from 20 non-overlapping fields at 20× magnification from the dorsal and ventral sections of four control and four *PdxCre*^{late} *β-cat*^{active} mice. The average number of cells per field was then normalized against the control. In order to determine endocrine and exocrine cell density, Hematoxylin/Eosin-stained tissues were used to count cell nuclei from 20 non-overlapping fields at 40× magnification isolated from four control and four *PdxCre*^{late} *β-cat*^{active} mice. The average number of nuclei present in the field was then normalized against control.

For P0 pancreata, the whole pancreas was sectioned and aliquoted as described previously (Kawahira et al., 2003), in order to obtain representative results. Islet area was assayed at P0 as described previously (Hebrok et al., 2000; Perez et al., 2005). Error bars represent s.e.m., and confidence intervals were determined using a Student's *t*-test analysis.

Glucose tolerance testing

Six control and six *PdxCre*^{late} *β-cat*^{active} mice were fasted for 14 hours before intraperitoneal injection of a 20% glucose (w/v) solution at a dose of 2 g per kg body mass. Venous blood glucose readings were then taken at the indicated intervals using a Bayer Ascensia Elite XL to analyze samples collected from tail nicks. Error bars represent standard error of the mean.

RESULTS

Temporal and spatial difference in Cre recombinase activity in the *PdxCre*^{early} and *PdxCre*^{late} transgenic lines

The *PdxCre*^{late} transgenic mouse was generated using a 4.5 kb promoter fragment, while the *PdxCre*^{early} transgenic mouse used a 5.5 kb fragment of the promoter. Because the size of the promoter fragment used, the site of integration, and other factors that can affect the expression of the transgene, we directly compared the activity of the Cre-recombinase by crossing both *PdxCre* strains to the reporter line *R26R*, which carries a β-galactosidase gene whose transcription is activated after Cre-mediated recombination (Soriano, 1999). In this manner, we were able to map the timing and expression of pancreatic

Table 1. Antibodies used in this study

Antigen	Species	Source	Dilution
Amylase	Rabbit	Sigma, A8273	1:800
β-Catenin	Mouse	BD, 610154	1:200
β-Galactosidase	Rabbit	ICN, 55976, lot 03660	1:200
E-cadherin	Mouse	BD, 610181	1:200
Glucagon	Rabbit	Linco, 4030-01f	1:400
Glut2	Rabbit	Chemicon, AB1342	1:100
Insulin	Guinea pig	Linco, 4011-01	1:400
Mucin 1	Hamster	NeoMarkers, HM-1630	1:500
Nkx6.1	Rabbit	Gift from Michael German (UCSF)	1:1,000
Pancreatic polypeptide	Guinea pig	Linco, 4041-01	1:400
Pdx1	Rabbit	Gift from Michael German (UCSF)	1:1,000
Phospho-histone H3	Rabbit	Upstate Laboratories 65-570	1:200
Ptc	Goat	Santa Cruz, SC6149, lot E142	1:100
Somatostatin	Rabbit	DAKO, A0566	1:200
Sonic hedgehog*	Goat	Santa Cruz, SC1194, lot F262	1:100

Antibodies were used at the stated dilution on paraffin wax-embedded tissues.
*This particular antibody recognizes multiple hedgehog ligands. Therefore, in the legend to Fig. 4, we have denoted the staining as hedgehog ligand, rather than claiming specificity for sonic hedgehog.

DEVELOPMENT

Cre-recombinase. By E10.5, robust *lacZ* staining was detected in the pancreatic epithelium of *PdxCre^early R26R* animals at the gross and histological levels (Fig. 1C,F). Moreover, expression was detectable in the majority of Pdx1+ cells observed (Fig. 1F). Interestingly, no *lacZ* staining was observed in the *PdxCre^late R26R* animals at this time point (Fig. 1B,E). By E11.5, a few *lacZ*-positive cells were found in the pancreatic epithelium of *PdxCre^late R26R*, a delay of ~24 hours (data not shown) from the onset of expression in the *PdxCre^early*. Furthermore, comparison of lacZ/Pdx1 co-stained pancreatic tissue revealed that even at E12.5, the *Cre* expression in the *PdxCre^late* strain was more mosaic than in the *PdxCre^early* (Fig. 1H,I).

Analysis of *lacZ* stained pancreas sections in adult *PdxCre^late R26R* mice suggested that *Cre* expression within the terminally differentiated exocrine and endocrine cells remained mosaic in the *PdxCre^late* strain (Fig. 1K). Interestingly, β-galactosidase can rarely be detected within the adult pancreatic ducts in *PdxCre^late R26R* mice (Fig. 1N). By comparison, the majority of endocrine, exocrine and ductal cells in *PdxCre^early R26R* mice exhibit β-galactosidase activity (Fig. 1L,O). The higher number of *lacZ*+ cells in the adult *PdxCre^early R26R* mice strongly indicates that the onset of *Cre* expression was not only earlier in timing, but also targeted a greater portion of the pancreatic epithelium. Control tissue was stained for each of the time points assayed to demonstrate the specificity of the *lacZ* staining reaction/immunofluorescence (Fig. 1A,D,G,J,M). Therefore, the *PdxCre^late* and *PdxCre^early* mice were used in this study as tools to probe the effect of increased β-catenin signaling on distinct temporal/spatial populations of cells in the early embryonic pancreas.

Stabilization of β-catenin results in disruption of pancreas formation in *PdxCre^early β-cat^active*

In order to assess the consequences of increased β-catenin signaling on pancreas organogenesis, we crossed *β-cat^active* animals with the *PdxCre^late* or *PdxCre^early* mice. Because previous studies have shown that the loss of the third exon of one allele of β-catenin is sufficient to drive strong increases in Wnt pathway activity, all experiments were carried out using mice that were heterozygous for the floxed β-catenin allele (Harada et al., 1999; Kemler et al., 2004; Zechner et al., 2003). Analysis of gross morphology and pancreas architecture at E18.5 in the *PdxCre^late β-cat^active* mice did not reveal any overt changes compared with control littermates (Fig. 2A,B,D,E). By contrast, the *PdxCre^early β-cat^active* animals displayed near total pancreas agenesis, and the pancreatic remnant contained multiple large cysts (Fig. 2C,F). Histological examination revealed a significant reduction in the epithelial derived exocrine and endocrine tissues. Consequently, *PdxCre^early β-cat^active* survive on average only 7 days after birth, whereas *PdxCre^late β-cat^active* are viable and reproductively active.

Nuclear β-catenin localization was abundant and easily detected by confocal microscopy in both *PdxCre^late β-cat^active* and *PdxCre^early β-cat^active* pancreata at E18.5 (Fig. 2H,I). In control samples, β-catenin was detected only at the plasma membrane (Fig. 2G). Therefore, as has been shown in other tissues (Jamora et al., 2003; Miller and Moon, 1997; Tolwinski and Wieschaus, 2004), stabilization of β-catenin leads to increased nuclear β-catenin signaling and, presumably, hyperactivation of the canonical Wnt signaling pathway in the pancreas.

In addition to its role in the canonical Wnt signaling pathway, the β-catenin protein also participates in cell adhesion at adherens junctions. β-Catenin links the cytoplasmic domain of transmembrane cadherins to the actin cytoskeleton via its association with the adaptor protein α-catenin (reviewed by Bienz, 2005). Therefore, β-catenin stabilization may also impact cell adhesion. However, despite clear evidence of nuclear localization of β-catenin in *PdxCre^late β-cat^active*

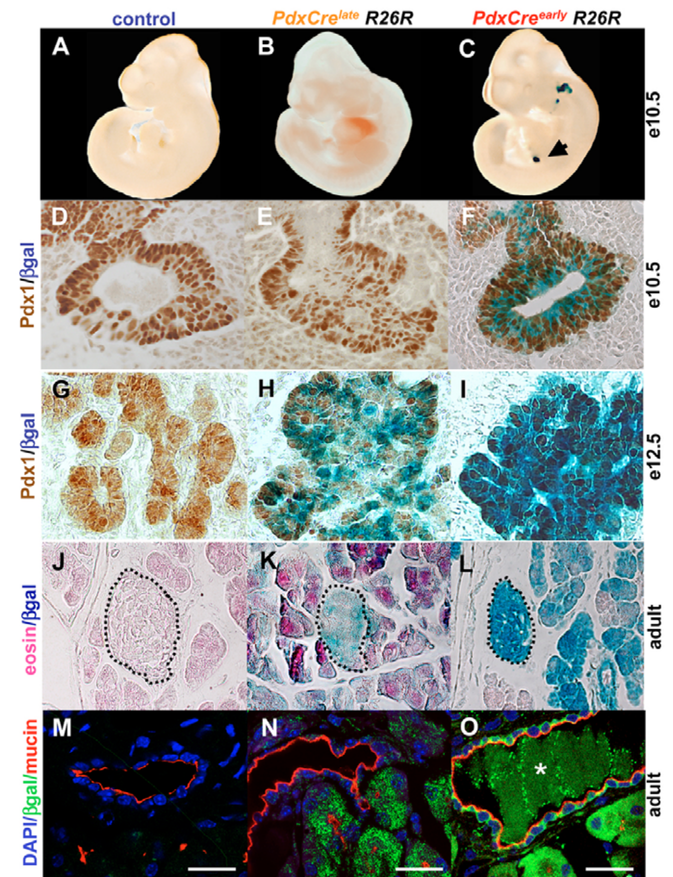

Fig. 1. Temporal and spatial differences in Cre recombinase activity in the *PdxCre^early* and *PdxCre^late* transgenic lines. Staining for *lacZ* expression marks cells that have undergone Cre-mediated recombination in *PdxCre^late* and *PdxCre^early R26R* mice. (**A**) Control E10.5 embryo. (**B**) No β-galactosidase staining is detectable within the *PdxCre^late R26R* embryo at E10.5. (**C**) Strong β-galacotosidase staining is present within the pancreas (arrow) of a *PdxCre^early R26R* E10.5 embryo. (**D-F**) Histological examination of pancreas sections immunostained for Pdx1 (brown) and enzymatically stained for β-galactosidase activity (blue). (F) The majority of Pdx1$^+$ cells in the *PdxCre^early R26R* animal, are also positive for *lacZ* at E10.5. (**G-I**) β-Galactosidase (blue) and Pdx1 (brown) staining at E12.5. β-Galactosidase staining is detectable within a subset of the Pdx1$^+$ cells in the *PdxCre^late R26R* animals (H). (I) Equivalent pancreas sections of a *PdxCre^early R26R* animal show robust β-galactosidase staining in the majority of Pdx1$^+$ cells. (**J-L**) Adult animals stained with Eosin (red) and for β-galactosidase (blue). Islets are outlined in black. (K) In the adult *PdxCre^late R26R* animal, only a subset of islet cells exhibits β-galactosidase staining. Cre expression within the exocrine tissue is also mosaic in these animals. (L) A greater percentage of islet cells and exocrine cells exhibit strong β-galactosidase staining in the adult *PdxCre^early R26R* pancreas. (**M-O**) Adult pancreatic ducts. (O)The majority of pancreatic ducts (stained with an antibody against mucin 1, red) are also β-galactosidase $^+$ (green) in the *PdxCre^early R26R* pancreas (non-specific staining sometimes encountered within the center of ducts indicated with an asterisk). (N) β-Galactosidase$^+$ cells are seldom encountered within the *PdxCre^late R26R* pancreas. No β-galactosidase staining is observed in control animals (A,D,G,J,M) at any of the time points characterized. Scale bars: 25 μm.

and *PdxCre^early β-cat^active* mice, E-cadherin remained properly localized to the plasma membrane (Fig. 2K,L inset), suggesting that adhesion has not been disrupted. Similarly, other studies using the *β-*

DEVELOPMENT

cat^{active} mouse strain have not found adhesion defects in cells expressing the stabilized form of β-catenin (Gounari et al., 2002; Harada et al., 1999). Using E-cadherin as a marker of epithelial cells, immunohistochemical analysis revealed the extensive loss of epithelial tissue mass in the *PdxCre^{early} β-cat^{active}* organ remnant. As expected, the developing cysts are E-cadherin positive, indicating that the cysts were epithelial in origin (Fig. 2L).

By E18.5, endocrine islets in mice start to form a stereotypical structure consisting of a core of insulin producing β-cells surrounded by glucagon producing α-cells (Fig. 2M). Although the *PdxCre^{late} β-cat^{active}* mutants displayed islet architecture (Fig. 2N) and islet area (data not shown) equivalent to control, *PdxCre^{early} β-cat^{active}* mutants had few insulin^{+} or glucagon^{+} cells (Fig. 1O). In addition, these endocrine cells were found scattered throughout the remaining organ, rather than organized into discrete islet structures (Fig. 2O).

Thus, early and widespread upregulation of the β-catenin signaling pathway in the *PdxCre^{early} β-cat^{active}* mouse strain prevents normal formation of the exocrine and endocrine compartments of the pancreas. However, the delayed and more mosaic upregulation of the β-catenin signaling pathway in the *PdxCre^{late} β-cat^{active}* mutants appears to be well tolerated and does not result in any obvious developmental defect.

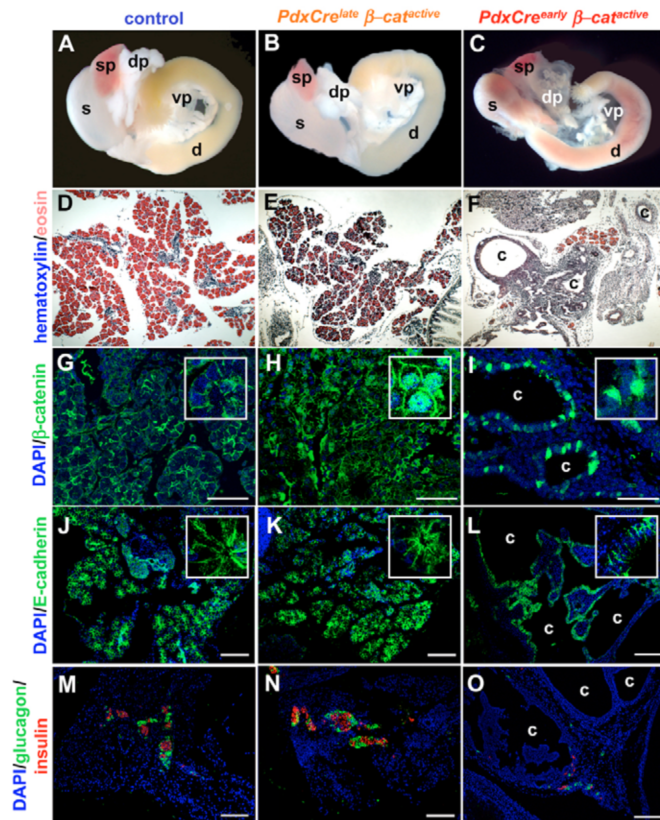

Fig. 2. Stabilization of β-catenin results in disruption of pancreas formation in *PdxCre^{early} β-cat^{active}* mice. (**A-C**) Examination of pancreas gross morphology. The majority of the dorsal pancreas (dp) and ventral pancreas (vp) has been lost in *PdxCre^{early} β-cat^{active}* mice (C) at E18.5. By contrast, *PdxCre^{late} β-cat^{active}* pancreas morphology (B) appears equivalent to control (A) at E18.5. s, stomach; sp, spleen; d, duodenum. (**D-F**) Hematoxylin and Eosin staining was performed on paraffin sections from E18.5 tissue from either control (D), *PdxCre^{late} β-cat^{active}* (E) or *PdxCre^{early} β-cat^{active}* (F) animals. Whereas *PdxCre^{late} β-cat^{active}* pancreas structure (E) is normal, the *PdxCre^{early} β-cat^{active}* pancreatic remnant (F) contains very little exocrine tissue and exhibits multiple pronounced cyst structures (c). The majority of the remaining tissue appears mesenchymal in *PdxCre^{early} β-cat^{active}* mutants. (**G**) Staining of pancreas sections for β-catenin (green) and the nuclear co-stain DAPI (blue) reveals that β-catenin is localized exclusively to the membrane in control animals. (**H**,**I**) Significant levels of nuclear β-catenin can be detected in both the *PdxCre^{late} β-cat^{active}* and *PdxCre^{early} β-cat^{active}* mutants. Insets show increased magnification of equivalent fields in each image. Scale bars: 50 μm. (**J-L**) Staining for the epithelial marker E-cadherin (green) indicates the dramatic loss of epithelial tissue in the *PdxCre^{early}* β-*cat^{active}* mutant (L) when compared with both the *PdxCre^{late} β-cat^{active}* (K) and control (J). The *PdxCre^{early} β-cat^{active}* cysts are lined by E-cadherin-positive cells. Insets show increased magnification of equivalent fields in each image to show detailed plasma membrane localization of E-cadherin in both *PdxCre^{late} β-cat^{active}* and *PdxCre^{early} β-cat^{active}* mutants. Scale bars: 100 μm. (**M-O**) Staining for glucagon (green), insulin (red) and the nuclear marker DAPI (blue), reveals islet structure in the *PdxCre^{late} β-cat^{active}* mutant (N) that is equivalent to control (M). By contrast, insulin^{+} and glucagon $^{+}$ cells are scattered within the walls of the cysts and surrounding mesenchyme in the *PdxCre^{early} β-cat^{active}* mutant tissue (O). Scale bars: 100 μm.

Stabilization of β-catenin at E11.5, but not E13.5, results in pancreas hypoplasia

Other differences between the *PdxCre^{early}* and *PdxCre^{late}* mouse strains, beyond the delay in onset of Cre expression, might account for the difference in phenotypes observed. For example, differences in the specific subset of cells targeted by each Cre strain after E12.5 when both lines are active might be responsible for driving the pancreas hypoplasia observed. Therefore, we directly assessed the temporal dependence of the phenotype seen in the *PdxCre^{early} β-cat^{active}* mice by crossing the *β-cat^{active}* mouse to an inducible *PdxCre* strain (*PdxCre^{ER}*) (Gu et al., 2002) containing the same 5.5 kb promoter fragment as the *PdxCre^{early}* mouse. In this system, the *Cre* recombinase is expressed as a fusion protein with the estrogen receptor, and remains inactive in the cytoplasm in the absence of tamoxifen ligand. However, once bound to tamoxifen, the Cre recombinase enters the nucleus where it can catalyze recombination (Gu et al., 2002).

Injection of tamoxifen at E11.5 induced accumulation of β-catenin in the nucleus of a large number of pancreatic epithelial cells in *PdxCre^{ER} β-cat^{active}* mice (Fig. 3J), resulting in a severe reduction of pancreas mass in all mutants observed (n=9, Fig. 3B). Nuclear localization of β-catenin was not observed in control pancreata (Fig. 3I). Moreover, cystic structures similar to those seen in *PdxCre^{early} β-cat^{active}* are apparent in histological sections of the pancreas (Fig. 3F, indicated by arrows).

Conversely, injection of tamoxifen at E12.5 induced more variable phenotypes. The majority of *PdxCre^{ER} β-cat^{active}* mutants exhibited an intermediate phenotype with some reduction of the ventral and dorsal pancreas obvious in examination of the gross morphology of the organ (Fig. 3C). The pancreas histology of these intermediate *PdxCre^{ER} β-cat^{active}* mutants (Fig. 3G) appeared equivalent to control (Fig. 3E). However, a smaller number of *PdxCre^{ER} β-cat^{active}* mutants from litters injected with tamoxifen at E12.5 displayed either pancreas hypoplasia similar to those in the E11.5 injection group (Fig. 3B) or appeared unaffected (exact proportion of mutants in each category summarized in Fig. 3M). Despite the variability in phenotype, significant numbers of cells with nuclear β-catenin localization were present in the pancreatic epithelium in all mutants analyzed (Fig. 3K). Injection of tamoxifen at E13.5 did not disrupt pancreas formation in any of the eleven *PdxCre^{ER} β-cat^{active}* mutants observed (Fig. 3D,H), despite clear nuclear localization of β-catenin in a large number of pancreatic cells (Fig. 3L).

DEVELOPMENT

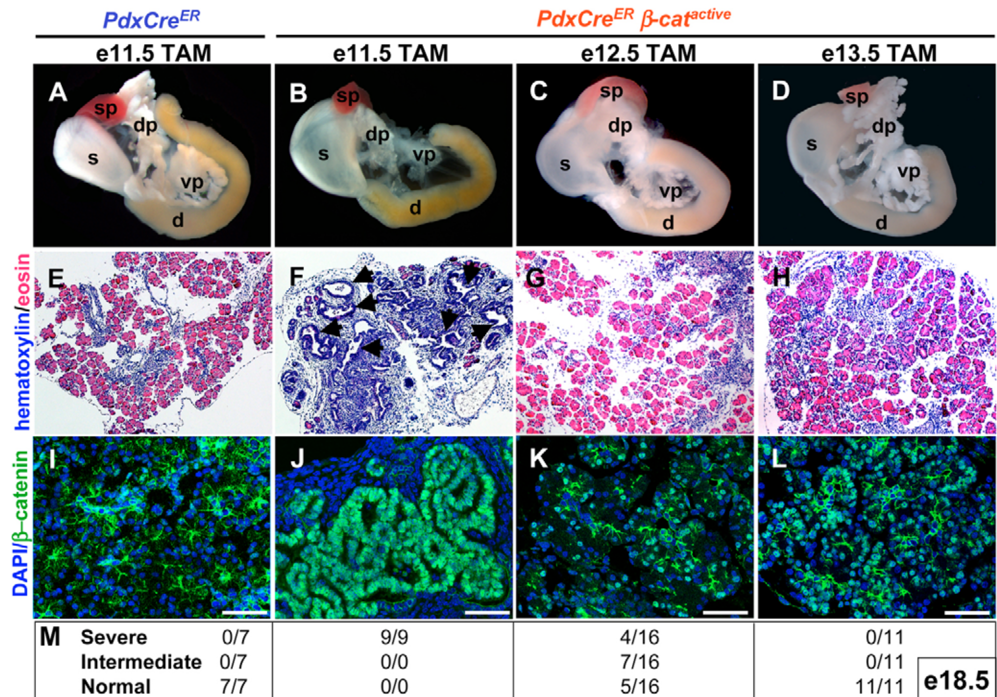

Fig. 3. Stabilization of β-catenin at E11.5, but not E13.5, results in pancreas hypoplasia. All images are from E18.5 embryos. (**A-D**) Whole-mount views of control (A) and *PdxCre^{ER} β-cat^{active}* mutant (B-D) tamoxifen-injected embryos. (B) After injection at E11.5, mutants display a dramatic loss of pancreas mass. (C) When injected at E12.5, the dorsal and ventral pancreas appear moderately reduced in size in the majority of cases, but after E13.5 injection (D), gross pancreatic morphology appears normal. (**E-H**) Hematoxylin and Eosin-stained control (E) and mutant (F-H) pancreata after tamoxifen treatment. Mutants display the formation of large cysts (F, cysts indicated with black arrows) after injection at E11.5, but appear normal when injected at E12.5 (G) and E13.5 (H). (**I-L**) Pancreatic epithelia in control (I) and *PdxCre^{ER} β-cat^{active}* mutant (J-L) tamoxifen-injected embryos. (J) Tamoxifen injection at E11.5 effectively targets a large number of cells within the *PdxCre^{ER} β-cat^{active}* pancreatic epithelium, indicated by the accumulation of nuclear β-catenin (compare with I). (K) After injection at E12.5, β-catenin can be detected within the nuclei of a significant number of cells within the mutant pancreatic epithelium. (L) A large number of cells are present that exhibit nuclear β-catenin within the pancreatic epithelium after injection at E13.5 (compare with morphology and histology in D and H). (**M**) The number of mutants seen at each tamoxifen injection time point, and the severity of the pancreas phenotype observed. Scale bars: 50 μm. s, stomach; sp, spleen; d, duodenum; dp, dorsal pancreas; vp, ventral pancreas.

Taken together, these results indicate that β-catenin stabilization can adversely affect pancreas development only during the earlier stages of organ formation. After E12.5, the increased levels of canonical Wnt signaling that the stabilized β-catenin presumably induces are well tolerated. Therefore, it appears likely that the pancreas phenotype seen in the *PdxCre^{early} β-cat^{active}* is dependent on the earlier onset of *Cre* recombinase expression seen when compared with the *PdxCre^{late}* mouse strain.

Pancreatic defects in *PdxCre^{early} β-cat^{active}* correlate with changes in FGF and hedgehog signaling, and loss of Pdx1+ progenitor cells

In order to analyze the defects in *PdxCre^{early} β-cat^{active}* mice in more detail, we performed a series of histological and molecular assays aimed at characterizing the early progression of the phenotype and identifying the underlying molecular mechanisms involved. By E12.5, stabilization of β-catenin within the pancreas of *PdxCre^{early} β-cat^{active}* mice caused the pancreatic epithelium in some mutants to exhibit abnormal dilation not seen in control tissues, representing the earliest histological change that we could detect (data not shown). By E15.5, the rudimentary clusters of exocrine cells seen in control embryos are almost completely absent in the *PdxCre^{early} β-cat^{active}* (data not shown). In addition, abnormally enlarged pancreatic ducts are frequently observed at this time point in *PdxCre^{early} β-cat^{active}* pancreatic tissues and it is likely that these dilated ducts later form the cystic structures seen at E18.5 (data not shown).

Numerous studies have shown the importance of mesenchymal-epithelial interactions for proper pancreas formation. One of the mesenchymal molecules known to regulate expansion of pancreatic epithelial cells is fibroblast growth factor 10 (Fgf10) (Bhushan et al., 2001). *Fgf10* expression is normally detectable within the mesenchyme surrounding the pancreatic bud beginning at E9.5 and peaking at E10.5. By E12.5, *Fgf10* expression can no longer be detected. Loss of Fgf10 in mice has been shown to disrupt pancreas formation (Bhushan et al., 2001), a phenotype similar to the one we observed in *PdxCre^{early} β-cat^{active}* mice. Therefore, we asked whether stabilization of β-catenin resulted in changes in mesenchymal *Fgf10* expression. Whole-mount in situ hybridization at E10.5 revealed that *Fgf10* expression is decreased in the pancreatic mesenchyme of *PdxCre^{early} β-cat^{active}* animals (Fig. 4C) when compared with control (Fig. 4A). *Fgf10* expression appeared normal in the *PdxCre^{late} β-cat^{active}* mutants (Fig. 4B). This observation supports our *R26R* expression analysis, which indicated that *Cre* recombinase is not active in *PdxCre^{late} β-cat^{active}* mice at this time point. Thus, β-catenin stabilization in pancreas epithelium before E11.5 disrupts an important component of the signaling exchange that occurs between the mesenchyme and epithelium, which at least partially explains the dramatic defects in organogenesis observed.

Wnt signaling has also been implicated in maintaining the expression of hedgehog (Hh) ligands in both *Drosophila* wing discs and mouse limb buds (Parr and McMahon, 1995; Pinson et al., 2000; Tabata and Kornberg, 1994). In addition, ectopic Wnt signaling in the epidermis has been shown to upregulate the expression of Hh signaling pathway components (Silva-Vargas et al., 2005). Hedgehog signaling, in turn, stabilizes Wnt expression in *Drosophila* wing (Tabata and Kornberg, 1994), indicating that both pathways can crossregulate the activity of one another. A previous study has shown that overexpression of the *Hh* ligand, Shh, under the *Pdx1* promotor results in pancreas agenesis (Apelqvist et al., 1997), a phenotype similar to that observed in the *PdxCre^{early} β-cat^{active}* mice. To determine whether stabilization of β-catenin activates Hh signaling

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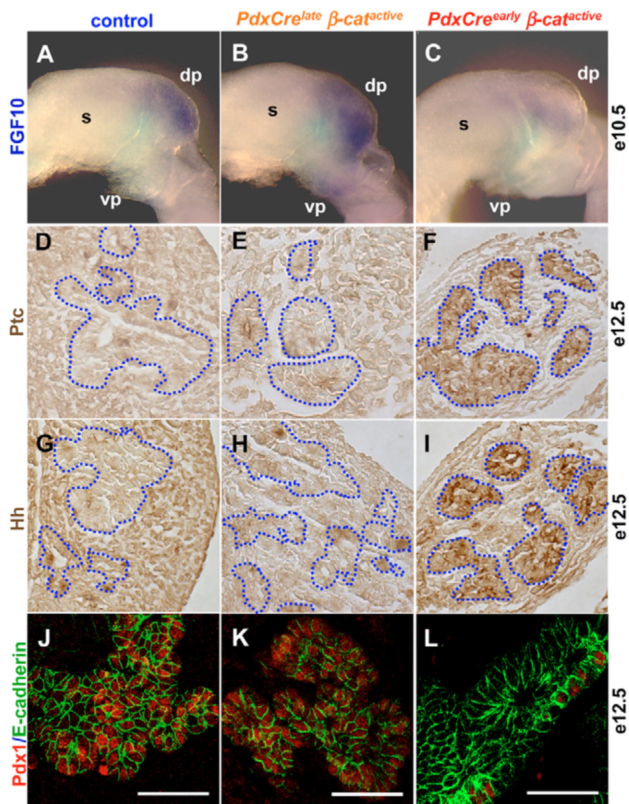

Fig. 4. Pancreatic defects in *PdxCre^{early} β-cat^{active}* correlate with changes in FGF and hedgehog signaling, and loss of Pdx1$^+$ progenitor cells. (**A-C**) Whole-mount in situ hybridization. *Fgf10* expression is downregulated in the dorsal pancreatic (dp) and ventral pancreatic (vp) mesenchyme of the *PdxCre^{early} β-cat^{active}* mutants (C) at E10.5. *Fgf10* expression within the pancreatic mesenchyme of *PdxCre^{late} β-cat^{active}* mutants (B) is equivalent to control (A). s, stomach. (**D-F**) Immunohistochemical staining. Pancreatic sections at E12.5 stained for the hedgehog (Hh) receptor Ptch1 reveal increased levels of Ptch1 within the pancreatic epithelium (circled in blue) of the *PdxCre^{early} β-cat^{active}* mutants (F) when compared with control pancreas (D). Ptch1 staining in *PdxCre^{late} β-cat^{active}* (E) pancreatic sections is equivalent to control (D). (**G-I**) Pancreatic sections at E12.5 stained for Hh. The *PdxCre^{early} β-cat^{active}* pancreatic epithelium (circled in blue, I) also exhibits increased levels of Hh when compared with control (G) and *PdxCre^{late} β-cat^{active}* pancreas tissue (H). (**J-L**) Staining of E12.5 pancreata with E-cadherin (green) to mark the pancreatic epithelium and Pdx1 (red) reveals a dramatic loss of Pdx1+ progenitor cells in the *PdxCre^{early} β-cat^{active}* mutants (L). By contrast, the *PdxCre^{late} β-cat^{active}* (K) display Pdx1$^+$ cell numbers that are equivalent to control (J). Scale bars: 50 μm in J-L.

in pancreatic tissue, we analyzed E12.5 pancreas tissue from wild-type, *PdxCre^{late} β-cat^{active}* and *PdxCre^{early} β-cat^{active}* mice. By immunohistochemistry, protein levels of the Hh receptor Ptch1, a direct transcriptional target of Hh signaling (Goodrich et al., 1996), are significantly upregulated within the pancreatic epithelium of the *PdxCre^{early} β-cat^{active}* mice (Fig. 4F) when compared with control or *PdxCre^{late} β-cat^{active}* (Fig. 4D,E). Protein levels of Hh ligand, are also increased in the *PdxCre^{early} β-cat^{active}* (Fig. 4I) when compared with control or *PdxCre^{late} β-cat^{active}* mice (Fig. 4G,H). Therefore, stabilization of β-catenin in the *PdxCre^{early} β-cat^{active}* mice results in upregulation of the Hh signaling pathway within the early pancreatic epithelium, which may also partially account for the dramatic changes observed during organ formation/morphogenesis.

A previous study has indicated that *Fgf10$^{-/-}$* mice exhibit a marked loss of the Pdx1$^+$ progenitor cells by E12.5-E13.5 (Bhushan et al., 2001). Additionally, loss of Pdx1 in mice has been shown to cause pancreas agenesis (Offield et al., 1996; Jonsson et al., 1994). Therefore, we examined Pdx1 expression within the E12.5 pancreatic epithelium. Interestingly, *PdxCre^{early} β-cat^{active}* mice exhibited a 76% reduction in Pdx1$^+$ progenitor cells (Fig. 4L). By contrast, *PdxCre^{late} β-cat^{active}* mice had numbers of Pdx1$^+$ progenitor cells (Fig. 4K) that were equivalent to control (Fig. 4J). Moreover, only cells that did not display elevated levels of β-catenin retained Pdx1 expression (data not shown). Presumably, these cells escaped recombination. Cells with a clear increase in cytoplasmic/nuclear β-catenin were rarely detected in the *PdxCre^{late} β-cat^{active}* mutant pancreatic epithelium at E12.5 (data not shown).

Thus, the pancreatic hypoplasia and cyst formation in the *PdxCre^{early} β-cat^{active}* appears to be mediated by loss of Fgf10 signaling, concomitant with an increase in Hh signaling. Together, these perturbations in early pancreas specification may contribute to the loss of Pdx1$^+$ pancreatic progenitors.

Stabilization of β-catenin causes increased pancreas organ size in *PdxCre^{late} β-cat^{active}* mice

While the morphology and histological architecture of pancreata dissected from *PdxCre^{late} β-cat^{active}* appeared normal at the end of development (Fig. 2B,E), pancreas mass was not. Despite the fact that mutant pups were equivalent in body mass to control littermates, pancreas mass at day 0 was increased by 53% (Fig. 5B). Pancreas mass continued to increase independent of animal mass after birth, resulting in a grossly enlarged organ (Fig. 5A) that was 2.7-fold greater than control pancreata at 98 days postnatally (Fig. 5B). In transgenic mice older than 1 year, the pancreas had grown 4.6-fold larger than those found in control littermates (Fig. 5B). Strong nuclear β-catenin staining could be detected within the adult exocrine pancreas, suggesting that increased Wnt activity in the *PdxCre^{late} β-cat^{active}* mice was responsible for the increase in organ size after birth. In support of this hypothesis, we found a 2.5 fold increase in the number of proliferating adult exocrine cells in the *PdxCre^{late} β-cat^{active}* (Fig. 5I), as revealed by staining for the mitotic marker phosphohistone H3 (Fig. 5E,F).

Interestingly, *PdxCre^{ER} β-cat^{active}* mice injected with tamoxifen 4 weeks after birth, also showed a twofold increase in pancreas mass by 1 year of age (n=8, data not shown). Injection of *PdxCre^{ER} R26R* mice with tamoxifen results in clear Cre recombinase activity (as indicated by *lacZ* staining) in a significant proportion of the exocrine pancreas at this stage (data not shown). Thus, as has been shown for other tissues (Bierie et al., 2003; Huelsken and Birchmeier, 2001), β-catenin signaling, as a part of the canonical Wnt signaling pathway, can act as a proliferative signal in mature exocrine pancreas. In addition, the density of cells within the exocrine pancreas, as determined by counting the number of nuclei present in a defined histological field, is also increased 1.8-fold in *PdxCre^{late} β-cat^{active}* when compared with controls (Fig. 5G,H,J). By contrast, the density of endocrine cells within the mutant is normal. Although these data suggest that exocrine cell size is reduced in *PdxCre^{late} β-cat^{active}* mutants, the mechanism driving this phenomenon is unclear. Stabilization of β-catenin within the adult exocrine tissue may directly deregulate cell size. However, it is also possible that the rapidly proliferating exocrine cells do not need to reach their normal size before dividing again. Electron microscopy will be used in future studies to further characterize how this mutation affects pancreatic cellular ultrastructure.

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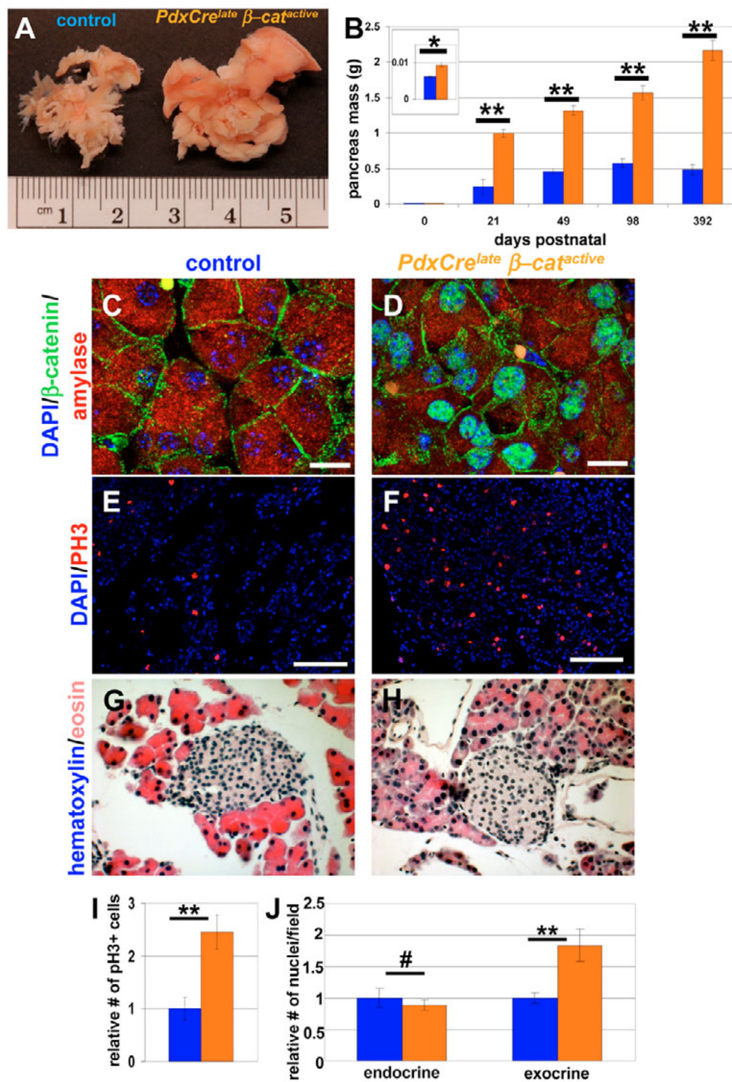

Fig. 5. Stabilization of β-catenin causes increased pancreas organ size in *PdxCre^{late} β-cat^{active}* mice. (**A**) Gross morphology of pancreata from a control (left) and *PdxCre^{late} β-cat^{active}* mutant (right). (**B**) Quantitative measurements revealed a 4.6-fold increase in pancreas mass in *PdxCre^{late} β-cat^{active}* mutants between birth and 1 year ($n \geqslant 7$ for each time point analyzed; control, blue; *PdxCre^{late} β-cat^{active}*, orange). (**C**,**D**) High levels of β-catenin (green) can be detected in the DAPI stained (blue) nuclei of adult *PdxCre^{late} β-cat^{active}* exocrine cells (D), identified by amylase staining (red). By contrast, β-catenin is localized exclusively to the plasma membrane of control cells (C). Scale bars: 10 μm. (**E**,**F**) Staining of pancreatic sections for the proliferation marker, phospho-histone H3 (PH3, red) and co-stained for DAPI (blue) reveals an increase in the number of proliferating cells in the *PdxCre^{late} β-cat^{active}* mutant (F) when compared with control (E). Scale bars: 100 μm. (**G**,**H**) Hematoxylin and Eosin staining of adult pancreas sections from *PdxCre^{late} β-cat^{active}* mice (H) reveals an increase in cell density within the exocrine pancreas, but not the endocrine islet (at center) when compared with control (G). (**I**) Quantification of PH3 positive cells (n=4 per genotype analyzed) indicates that the relative number of proliferating cells in the *PdxCre^{late} β-cat^{active}* mutant (orange) is increased 2.5-fold over control (blue) 21 days postnatally. (**J**) Quantification of the relative number of nuclei per field confirms that the density of cells within the exocrine pancreas of *PdxCre^{late} β-cat^{active}* mice (orange) is increased 1.8-fold over control (blue), while the endocrine cell density is normal (n=4). Confidence intervals calculated using Student's t-test. #, not significant; *, $P<0.05$; **, $P<0.01$. Error bars represent s.e.m.

Despite the dramatic increase in exocrine cell proliferation and the resultant increase in organ mass, pancreatic tumors have not been observed in any of the *PdxCre^{late} β-cat^{active}* mutants, up to 1 year of age. The mice are viable and fertile, and appear healthy.

β-Cell differentiation and islet function appear normal in the *PdxCre^{late} β-cat^{active}* mouse at 3 months

As shown in Fig. 1K, islets within the *PdxCre^{late}* strain were mosaic for Cre activity. Therefore, only a subset of β-cells were targeted for stabilization of β-catenin in the *PdxCre^{late} β-cat^{active}* mouse. Immunofluorescence staining indicated that *PdxCre^{late} β-cat^{active}* adult mutants exhibit stereotypical murine islet architecture, consisting of a central core of insulin producing β-cells and a periphery of glucagon-producing α-cells (Fig. 6A,B). Despite the increase in overall organ size at birth, the total cross sectional area of endocrine islets remained equivalent to control (data not shown), suggesting that the ratio of endocrine content to animal mass was not perturbed. In addition, analysis of relative hormone content in pancreas homogenates at three months of age indicated that the total amount of insulin and glucagon present in the *PdxCre^{late} β-cat^{active}* is equivalent to control (data not shown). Thus, in contrast to the vast expansion of the exocrine compartment of the pancreas, the size of the endocrine compartment remains largely unaffected.

As expected from the *R26R* expression data, the majority of β-cells in adult *PdxCre^{late} β-cat^{active}* mutants exhibited plasma membrane localization of β-catenin that appeared equivalent to control (Fig. 6C,D, higher magnifications are shown in Fig. 6F,G) and only a subset of β-cells was marked by upregulated β-catenin levels. However, unlike the exocrine compartment (Fig. 5D), confocal analysis revealed that Cre-mediated excision in β-cells did not result in a significant increase in nuclear β-catenin, but instead in an increase in cytoplasmic β-catenin levels (Fig. 6E, higher magnification can be found in Fig. 6H).

Within the β-cells marked by high levels of cytoplasmic β-catenin protein, expression of insulin (Fig. 6H), adult β-cell transcription factors Pdx1 (Fig. 6J) and Nkx6.1 (data not shown), and the glucose transporter Glut2 (data not shown) appear equivalent to control (Fig. 6F,I). These results demonstrate that the β-cells are fully differentiated, despite the dramatic increase in cytoplasmic β-catenin. Additionally, fasting glucose tolerance is normal in *PdxCre^{late} β-cat^{active}* animals (Fig. 6J), suggesting that either β-cell function is normal in these mutants or that the number of β-cells expressing the stabilized form of β-catenin is insufficient to affect

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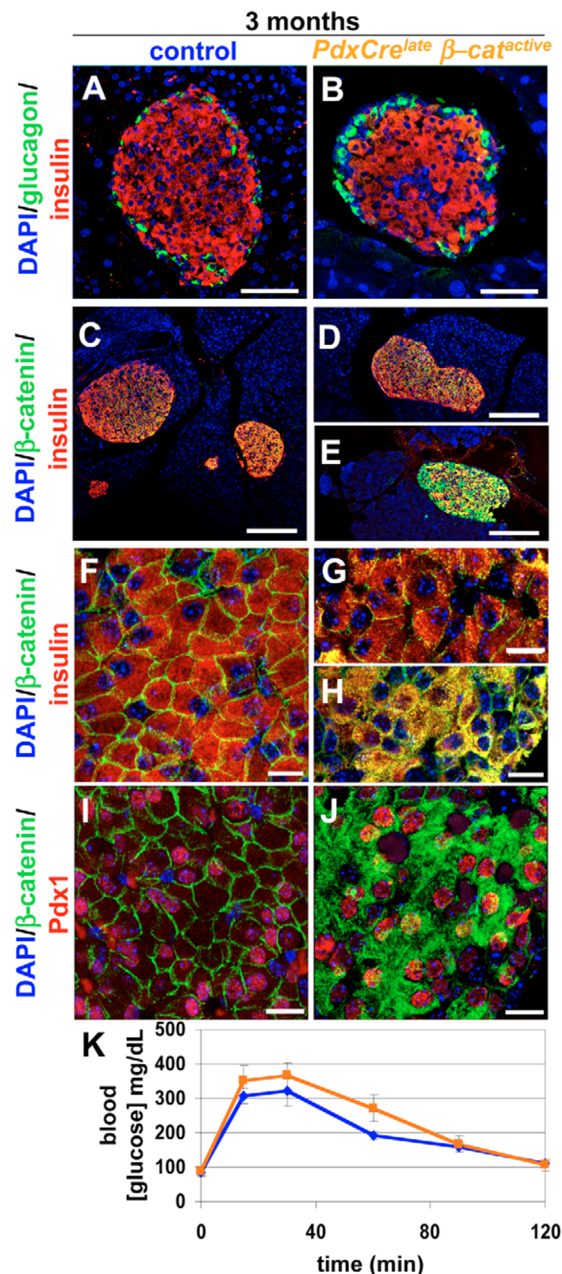

Fig. 6. Pancreas insulin content and islet function appear normal in the *PdxCre^{late} β-cat^{active}* mouse. (**A**,**B**) The islet architecture of adult *PdxCre^{late} β-cat^{active}* mutant mice (B) is comparable with control (A) as revealed by glucagon (green) and insulin (red) staining of pancreas sections. DAPI stained nuclei are indicated in blue. Scale bars: 50 μm. (**C-H**) The majority of β-cells, identified by insulin staining (red), in the adult *PdxCre^{late} β-cat^{active}* mutant (D; at higher magnification in G) display localization of β-catenin (green) to the plasma membrane that is equivalent to control (C; at higher magnification in F). However, a subset of β-cells in the *PdxCre^{late} β-cat^{active}* mutants (E; at higher magnification in H) exhibit high levels of cytoplasmic β-catenin staining. Scale bars: 100 μm in C-E; 15 μm in F-H. (**I**,**J**) The β-cells that exhibit strong cytoplasmic β-catenin localization (green) in the *PdxCre^{late} β-cat^{active}* mutant (J) still stain positive for the adult β-cell transcription factor Pdx1 (red), a characteristic of properly differentiated β-cells. β-Cells present in equivalent adult pancreas sections from control animals are shown stained for Pdx1 (I). (**K**) Resolution of a fasting glucose challenge is equivalent in control (blue) and *PdxCre^{late} β-cat^{active}* (orange) animals (*n*=6 for each genotype, error bars represent s.e.m.).

this physiological assay. Future experiments using cultured islets are necessary to determine what biochemical mechanisms might protect pancreatic endocrine cells from accumulating nuclear β-catenin.

Abnormal cells with nuclear localization of β-catenin are present in a subset of islets in 1-year-old *PdxCre^{late} β-cat^{active}* mice

Surprisingly, analysis of β-catenin expression patterns in 1-year-old mice revealed the presence of cells within a small subset of islets that exhibited nuclear localization of β-catenin (Fig. 7B, higher magnification is in Fig. 7D), a phenomenon not seen in younger mice. Despite their location in the center of islets, where β-cells typically are found, these cells do not express insulin (Fig. 7B, higher magnification is in Fig. 7D). These cells also lack expression of β-cell markers, including Pdx1 (Fig. 7F, higher magnification is in Fig. 7H), Nkx6.1 and Glut2, or markers of other endocrine cell populations (glucagon, somatostatin, PP; data not shown). Moreover, these cells do not express the pancreatic duct marker mucin 1 or the exocrine marker, amylase (data not shown). Fasting glucose tolerance is also normal in *PdxCre^{late} β-cat^{active}* mutants at 1 year of age (data not shown), indicating that the presence of these abnormal cells in a subset of islets has not impacted this physiological assay. Given the lack of expression of pancreatic endocrine, exocrine or ductal markers, these cells potentially represent a subpopulation of cells that have dedifferentiated as a result of the accumulation of nuclear β-catenin. Further genetic experimentation is necessary to identify the lineage of this cell population.

DISCUSSION

Our studies underscore the fact that important differences in Cre-activity can be found in mouse Cre-lines that use similar promoter elements of the same gene. Differences in the site of integration, the length of the promoter fragment used, and gene silencing can all exert significant influence over the transgenic expression of Cre recombinase. Here, we show how differences in the temporal/spatial stabilization of the β-catenin protein within the developing pancreas result in dramatically different phenotypes: organ ablation versus organ enlargement. The *PdxCre^{early}* mouse strain efficiently targets all three pancreatic lineages – endocrine, exocrine and duct – and is expressed early in pancreatic development. By contrast, the *PdxCre^{late}* strain targets a subset of the endocrine and exocrine cell lineages, but does not appear to be expressed in a significant number of duct cells.

Wnt, Hh and FGF interaction during pancreas development

Our observation that stabilization of β-catenin in *PdxCre^{early} βcat^{active}* mice results in upregulation of Hh signaling components concomitant with a loss of Fgf10 suggests that the activities of these three major embryonic signaling pathways are intimately linked during this stage of pancreas development. Although we cannot exclude the possibility that Wnt signaling in the epithelium directly affects the expression of mensenchymal Fgf10, it is interesting to note that upregulation of Hh signaling resulting from loss of the Hh inhibitor *Hhip* is known to cause a delay in *Fgf10* expression in the pancreatic mesenchyme (Kawahira et al., 2003). Therefore, our results are consistent with the idea that at least some of the defects observed in epithelium and mesenchyme are mediated via increased Hh activity. A reduction of pancreas size has also been observed in transgenic mice expressing Wnt1 under the control of the Pdx1 promoter (Heller et al., 2003). The phenotype observed in the

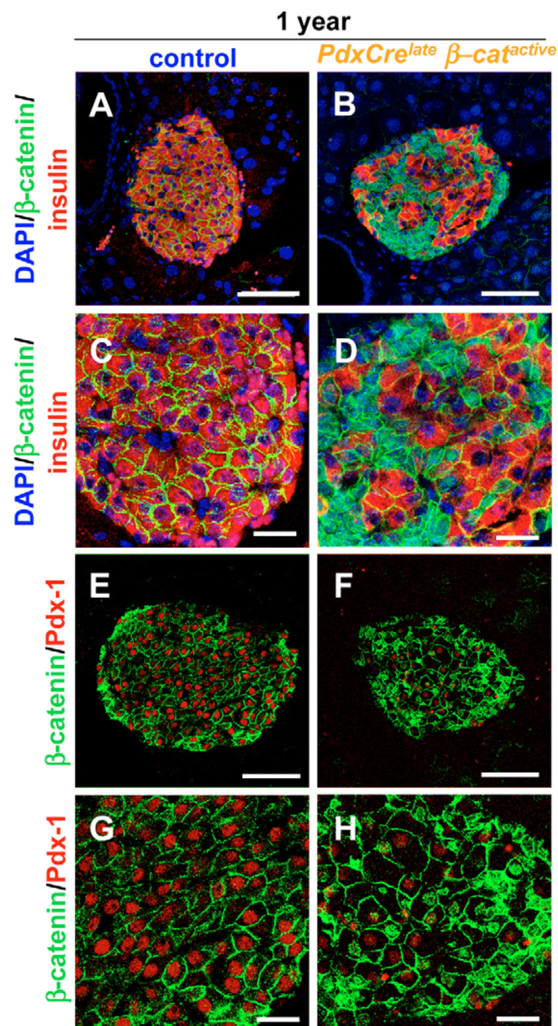

Fig. 7. Abnormal cells with nuclear localization of β-catenin are present in a subset of islets in 1-year-old *PdxCre*^{late} *β-cat*^{active} mice. (**A-H**) Nuclear localization of β-catenin in control (A,C,E,G) and mutant (B,D,F,H). Cells within a small subset of islets in 1-year-old *PdxCre*^{late} *β-cat*^{active} mutant pancreata (B,D,F,H) exhibit nuclear localization of β-catenin. These cells do not express insulin or Pdx1 (B,F; at higher magnification in D,H) seen in control islets (A,E; at higher magnification C,G). Scale bars: 50 μm in A,B,E,F; 25 μm in C,D,G,H.

PdxCre^{early} *βcat*^{active} mice results from upregulation of β-catenin signaling in a cell-autonomous fashion within the epithelium. However, transgenic expression of the soluble Wnt1 ligand presumably exerts a direct effect on both the pancreatic mesenchyme and epithelium. Therefore, it would be interesting to compare whether Hh and FGF signaling are also affected in the Pdx-Wnt1 mice. Moreover, future studies are necessary to determine the precise signaling hierarchy between the Wnt, Hh and FGF signaling pathways during normal pancreas specification and organogenesis.

β-Catenin activation influences pancreas morphogenesis and growth

Despite the 4.6-fold increase in mass, the pancreas of *PdxCre*^{late} *β-cat*^{active} mutant remains functional. *PdxCre*^{late} *β-cat*^{active} animals have a body mass equivalent to control littermates, suggesting that the duodenum still receives the pancreatic enzymes necessary for digestion, and their glucose tolerance profile is normal, indicating that the endocrine function of the pancreas is intact. Moreover, the shape of the organ is also conserved; it is simply bigger. Despite the increased proliferation, the *PdxCre*^{late} *β-cat*^{active} mice do not appear to develop tumors.

Interestingly, although the pancreas becomes grossly enlarged, the exocrine cells that comprise it appear smaller. Modulation of Wnt signaling has been implicated in increasing the size of skeletal muscle cells after mechanical overload (Armstrong and Esser, 2005). In addition, Wnt activation via overexpression of a canonical Wnt ligand has been shown to result in chondrocyte hypertrophy (Day et al., 2005). Although the opposite was observed in the *PdxCre*^{late} *β-cat*^{active} mice, it is possible that deregulation of Wnt signaling in pancreatic exocrine cells may directly impact cell size. However, exocrine cells contain a large number of granules whose digestive enzyme content is emptied into the gut in response to feeding. Because the *PdxCre*^{late} *β-cat*^{active} mutant pancreas contains significantly more exocrine cells than are present in the wild type, a feedback mechanism might exist that causes a reduction in the number of granules in each cell, thereby reducing cell size. Ultrastructural analysis is required to precisely determine how β-catenin stabilization affects pancreatic exocrine cell morphology.

β-Catenin activation and adult β-cell function

The absence of a proliferative response or enlargement of the β-cell compartment, within *PdxCre*^{late} *β-cat*^{active} mutants may reflect differences in the way in which exocrine and endocrine cells respond to activation of β-catenin. Alternatively, β-cells may have a mechanism that allows for the active exclusion of stabilized β-catenin from the nucleus, which then prevents downstream activation of the canonical Wnt signaling pathway. A recent study involving β-catenin stabilization in preimplantation embryos also observed similar cytoplasmic localization of β-catenin (Kemler et al., 2004). The authors propose several mechanisms that might prevent localization of the stabilized form of β-catenin in the blastomere nuclei. Among these are the presence of an alternative ubiquitylation pathway that can degrade even the stabilized form of β-catenin or the activity of endogenous inhibitors that might prevent β-catenin from exerting its nuclear activity. Endocrine cells may lack, or have low levels of, nuclear transport proteins required for normal translocation and retention of β-catenin in the nucleus. Experiments using cultured islets and known inhibitors of nuclear transport might prove instructive in uncovering the mechanism underlying the resistance of the pancreatic endocrine cell to nuclear accumulation of β-catenin.

One of the truly puzzling findings of our study is that a low number of cells with clear nuclear localization of β-catenin can be found only in the islets of one year old *PdxCre*^{late} *β-cat*^{active} animals. Thus, it is possible that pancreatic endocrine cells possess some kind of innate mechanism to prevent nuclear localization of β-catenin that was gradually circumvented over time. Alternatively, it might be a matter of the cellular dose of the stabilized β-catenin; it might take time for enough of the protein to accumulate to affect cell function.

Moreover, the location of these cells within the center of the islet raises the intriguing possibility that these are β-cells that have undergone de-differentiation, after accumulating sufficient levels of nuclear β-catenin. Genetic cell lineage tracing experiments would need to be performed to confirm this hypothesis. The absence of Pdx1 expression in these cells is consistent with the fact that the pancreatic epithelial cells with nuclear β-catenin accumulation in the *PdxCre*^{early} *β-cat*^{active} also display a loss of Pdx1 expression at

E12.5. However, the absence of normal pancreatic cell lineage markers makes it impossible to conclude what these cells were/are without further experimentation.

Given the fact that fewer than 30% of β-cells are marked by cytoplasmic β-catenin staining, it is also possible that potential β-cell defects in β-catenin-activated cells are masked by the remaining wild-type cells. Therefore, although the results presented here are intriguing, future studies using a transgenic strain that expresses Cre in a higher percentage of β-cells are necessary to clarify these findings regarding the effect of β-catenin stabilization on β-cell differentiation, expansion and function.

Conclusion

Our findings further illustrate the dual nature of β-catenin signaling. In one context, β-catenin activation prevents proper differentiation and expansion of early pancreatic progenitor cells; in another context, β-catenin activation acts as a proliferative cue that induces gross enlargement of the exocrine pancreas. Conversely, deletion of β-catenin has been shown to cause loss of exocrine cell mass (Murtaugh et al., 2005) and, in a different context, reduction of endocrine islet mass (Dessimoz et al., 2005). Taken together, these results highlight the complex roles β-catenin signaling plays in pancreas organ growth and the determination of the exocrine:endocrine cell ratio.

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