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Pancreatic Duct Glands Are Distinct Ductal Compartments That React to Chronic Injury and Mediate Shh-Induced Metaplasia

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See editorial on page 823.

BACKGROUND & AIMS: Pancreatic intraepithelial neoplasia (PanIN) are pancreatic cancer precursor lesions of unclear origin and significance. PanIN aberrantly express sonic hedgehog (Shh), an initiator of pancreatic cancer, and gastrointestinal mucins. A majority of PanIN are thought to arise from ducts. We identified a novel ductal compartment that is gathered in gland-like outpouches (pancreatic duct glands [PDG]) of major ducts and characterized its role in injury and metaplasia.

METHODS: The ductal system was analyzed in normal pancrea and chronic pancreatitis in humans and mice. Anatomy was assessed by serial hematoxylin and eosin sections and scanning electron microscopy of corrosion casts. Expression of mucins and developmental genes and proliferation were assessed by immunohistochemistry or real-time quantitative polymerase chain reaction. Effects of Shh on ductal cells were investigated by exposure to Shh in vitro and transgenic misexpression in vivo.

RESULTS: Three-dimensional analysis revealed blind-ending outpouches of ducts in murine and human pancreata. These PDG are morphologically and molecularly distinct from normal ducts; even in normal pancrea they display PanIN and metaplastic features, such as expression of Shh and gastric mucins. They express other developmental genes, such as Pdx-1 and Hes-1.

In injury, Shh is up-regulated along with gastric mucins. Expansion of the PDG compartment results in a mucinous metaplasia. Shh promotes this transformation in vitro and in vivo.

CONCLUSIONS: PDG are distinct gland-like mucinous compartments with a distinct molecular signature. In response to injury, PDG undergo an Shh-mediated mucinous gastrointestinal metaplasia with PanIN-like features. PDG may provide a link between Shh, mucinous metaplasia, and neoplasia.

Keywords: Pancreatic Duct Glands; Sonic Hedgehog; PanIN; GI Metaplasia.

Chronic injury is linked to carcinogenesis in several tumors, including pancreatic cancer. Chronic pancreatitis (CP) and pancreatic cancer share the presence of pancreatic intraepithelial neoplasia (PanIN), believed to be cancer precursors.1 Although the morphology and molecular signature of PanIN and cancer make a ductal origin likely, their cellular origin is still under debate; recent studies suggest that at least a part of the lesions is of acinar origin.2–3 The ductal epithelium itself is poorly characterized and often discussed as a single population of cells. Better characterization of ductal compartments may advance our understanding of diseases such as CP and pancreatic cancer. One main characteristic defining PanIN is an increased aberrant mucin expression, suggesting mucinous metaplasia as an early step in carcinogenesis. Little is known about the origin, onset, and underlying molecular events of this metaplasia.

The same genes involved in embryonic development appear to play a role in regeneration and, through inappropriate regulation, in metaplasia and ultimately neoplasia.6,7 Pdx-1 is important for early pancreatic development and is involved in regeneration.6,8–10 Notch signaling is involved in pancreatic development, regeneration, and neoplasia; its effector Hes-1 is discussed as a marker of progenitor cells.6,11–13 Sonic hedgehog (Shh) inhibits pancreatic development and directs the foregut mesoderm toward a gastrointestinal (GI) fate.14 Shh is aberrantly expressed in PanIN and pancreatic adenocarcinoma and contributes to carcinogenesis.15 In contrast to Pdx-1 and Hes-1, Shh has not been previously identified in the normal pancreas.1,14,15

Identification of Shh and gastric mucins called our attention to small outpouches of major ducts in normal pancrea. Here we identify and characterize this novel ductal compartment, which is gathered in outpouches, i.e., pancreatic duct glands (PDG). PDG have a unique molecular signature distinct from the pancreatic epithelium: they retain expression of gastric mucins and Shh. Additionally,
PDG express other developmental genes, such as Pdx-1 and Hes-1, which are also expressed in other regions of the pancreas. In response to chronic injury, PDG up-regulate developmental genes and proliferative rates and undergo an Shh-mediated mucinous metaplasia. This response suggests PDG may play a role in pancreatic duct protection, renewal, metaplasia, and possibly neoplasia.

**Materials and Methods**

**Human Samples**

Human samples were collected and analyzed in accordance with Institutional Review Board approval. Histologically normal control pancreata (n = 8) were obtained from 6 organ donors (21–48 years) or from patients who underwent pancreatectoduodenectomy for extrapancreatic disease (n = 2, one duodenal cancer and one bile duct cancer). Histologically confirmed human CP samples were obtained from pancreatectoduodenectomy specimens at the Massachusetts General Hospital (n = 10).

**Mouse Samples**

All experiments were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care. Healthy CD-1 mice (Charles River) served as controls (n = 10). Acute pancreatitis was induced in CD-1 mice of either sex by 6 hourly intraperitoneal injections of 50 μg/kg cerulein (Sigma, St Louis, MO). Chronic pancreatic injury was induced by 3 series of injections per week for periods of 3 (n = 10), 6 (n = 4), 10 (n = 3), and 18 (n = 2) weeks in CD-1 mice and Pch1-LacZ reporter mice. Pancreata were harvested 72 hours after the last injection using a microsurgical microscope (Codman Microsystems, Randolph, MA; magnification 15–45×). Proliferation was assessed by immuno-staining after in vivo pulse labeling by intraperitoneal injection of bromodeoxyuridine (BrdU; Sigma). Specimens were prefixed for 75–90 minutes in 4% paraformaldehyde at 4°C, washed in buffer, and incubated in X-Gal solution containing protease inhibitors at room temperature for 24 hours. Specimens were postfixed in 4% paraformaldehyde for 4 hours, dehydrated, paraffin-embedded, sectioned, and counterstained with nuclear-fast red. Age-matched wild-type littermates were negative controls.

**Casts of the Ductal System**

Corrosion casts of the ductal system of 5 murine pancreata and 1 human pancreas from organ donation were obtained by intraductal infusion of the casting medium (Mercox Resin; Ladd Research Industries, Burlington, VT; diluted with 10% methyl methacrylate monomer). The resin-filled tissue was immersed in hot water (60°C) for 1 hour for resin curing. Tissue was then removed by maceration in alternating rinses of 5%–10% KOH and hot water, cleaned in formic acid, washed in distilled water, and lyophilized. Casts were imaged by light microscopy and then sputter-coated (Hummer V; Anatech, Springfield, VA) with gold/platinum for scanning electron microscopy (SEM) with a LEO 1450VP scanning electron microscope (Carl Zeiss, Thornwood, NY) at 15 kV.

**Quantitative Real-Time Polymerase Chain Reaction**

RNA was extracted (RNA Isolation Kit; Ambion, Austin, TX) from tissues stored at −20°C in RNAlater (Ambion). One-step multiplex TaqMan real-time quantitative polymerase chain reaction (RT-qPCR) was performed using an ABI 7700 Sequence Detector system. Expression of SHH, IHH, DHH, PTCH, SMO, GLI1, and GLI2 was evaluated using 18S RNA as internal control. Probes and primers were designed to span exon–exon junctions to avoid amplification of genomic DNA. The thermal cycler conditions were: reverse transcription for 30 minutes at 48°C, initial activation step for 10 minutes at 95°C, followed by 40 cycles of denaturation for 15 seconds at 95°C, and annealing/extension for 60 seconds at 60°C. Fluorescence data were collected during annealing and analyzed using SDS1.7 software (Applied Biosystems). Relative gene expression was determined based on corresponding Ct (threshold cycle) values.

**In Vitro SHH Experiments**

Human pancreatic ductal epithelial (HPDE) cells were cultured in keratinocyte serum-free media supplemented with bovine pituitary extract and human epidermal growth factor (Invitrogen). Cells were exposed to 0, 5, 10, or 20 nM recombinant human SHH (R&D Substrate (Invitrogen, Carlsbad, CA). Slides were counterstained with hematoxylin.

Pch1 expression was identified in Pch1-LacZ animals by staining with the LacZ substrate 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) (Sigma). Specimens were prefixed for 75–90 minutes in 4% paraformaldehyde at 4°C, washed in buffer, and incubated in X-Gal solution containing protease inhibitors at room temperature for 24 hours. Specimens were postfixed in 4% paraformaldehyde for 4 hours, dehydrated, paraffin-embedded, sectioned, and counterstained with nuclear-fast red. Age-matched wild-type littermates were negative controls.
Systems, Minneapolis, MN) in culture media. Media was changed every 48 hours. After 8 days (192 hours), cells were stained for PAS and total RNA was harvested for analysis by RT-qPCR.

**Statistics**

Statistical analysis was performed using GraphPad Prism4 software (San Diego, CA). Proliferation in PDG vs main ducts was compared using 2-way contingency table analyses and χ² and Fisher exact tests. Quantitative gene expression is given as fold expression over mean expression in normal pancreata or control cells. Wilcoxon matched pairs test was used for comparison of main duct vs parenchymal tissue. P values are 2-tailed.

**Results**

**Pancreatic Ducts Have Blind-Ending Outpouches: PDG**

H&E stains of serial sections were used to morphologically assess the normal pancreatic ductal system in healthy mice and normal human pancreata. In both mice and humans, an epithelial compartment morphologically distinct from the normal ductal epithelium was identified. This unique compartment appears as either gland-like outpouches or coiled structures residing within the mesenchyme surrounding larger ducts (Figure 1). The epithelial lining of these structures is composed of cells with basally located nuclei and abundant supranuclear cytoplasm. Comparison of proximal (near the ampullary region) and peripheral ducts revealed that these branches/outpouches are more frequent in proximal ducts. Histologic evaluation of 3-μm serial sections suggested that these structures are blind-ending outpouches rather than branches (Supplementary Figures 1 and 2).

To reliably distinguish blind-ending “glands” from small branch ducts based on 3-dimensional information, we used SEM of ductal corrosion casts. We obtained detailed, high-resolution casts of the entire ductal system of 5 mice and 1 human (Figure 2A and B). Ramification in both the human and rodent pancreas is hierarchical: large interlobular ducts branch off the main duct and into smaller interlobular ducts, which then branch again into smaller ducts (Figure 2C and D). Multiple small branches of large ducts do not exist. SEM directly confirmed the presence of outpouches in the ductal system of mice and humans (Figure 2E). The distribution of these outpouches is not uniform: in mice, outpouches are frequently found in the biliopancreatic duct, in the main duct draining the left part of the pancreas, and in proximal interlobular ducts. Outpouches are less frequently observed in small pancreatic ducts. Interestingly, outpouches are often located near ductal branching sites (Figure 2E). Overall, this distribution results in a proximal pattern with a decreasing frequency from large toward small ducts. The distribution of outpouches in humans is similar, but they are observed less frequently.

![Figure 1. Morphologically distinct ductal compartment. Normal mouse (A) and human (B) pancreas. Cells with abundant supranuclear cytoplasm and basally located nuclei (arrowheads) are located in outpouches/small branches (arrowheads) within the ductal wall mesenchyme. This compartment is morphologically distinct from the epithelial lining of the adjacent main lumen (asterisk) and peripheral ducts of comparable size (lower panels). (C) Serial sections reveal that the novel compartment has unique molecular features, such as expression of Muc6 (brown).](image-url)
than in rodents. Outpouches can have a single lumen or display a complex arrangement of several sac-like dilations (lower panels in Figure 2).

These SEM data confirm the existence of a unique gland-like ductal compartment that we will henceforth call PDG. The existence of specialized duct-associated glands raises the question of their function and their role in pancreatic disease.

**PDG Undergo Hyperplasia in Response to Chronic Injury**

The entire ductal system was assessed for morphologic changes induced by a well-established murine model of chronic pancreatic injury.2,16 Chronic injury for 6 or more weeks results in severe changes in both ductal epithelium and mesenchyme;
PDG change in both frequency and morphology (Figure 3). Controls have a thin mesenchymal wall surrounding the proximal ducts, with few PDG. PDG are small, often have a single lumen, and only rarely display several glandular units (Figure 3A). In response to chronic injury, the ductal mesenchyme thickens. PDG increase in both number and size and display an often complex architecture, with branching and folds, as well as features of atypia, such as papillations and nuclear pseudostratification (Figure 3B). The epithelium takes on a more mucinous appearance. These PanIN-like features are more frequently found in PDG of larger ducts, but can be detected scattered in peripheral ducts.

Thus, in response to chronic injury, PDG appear to undergo hyperplasia and develop a mucinous metaplasia with PanIN-like features.

**PDG Retain GI Mucins and Are a Source of GI Metaplasia**

Metaplastic ductal lesions, including PanIN and cancer, frequently express GI markers. Normal pancreatic ducts express PAS⁺, but not Alcian-Blue⁺ mucins. In contrast, metaplastic lesions frequently express Alcian Blue⁺ mucins and gastric Muc5ac, which is considered an early sign of metaplasia. To determine whether PDG are a possible source, their mucin-expression patterns were characterized in normal and injured pancreata and compared to normal pancreatic and GI epithelium.

Results in mice (Figure 4) closely mirror results in humans (Supplementary Figure 3). PAS⁺ mucins and Muc1 are present at the luminal membrane in the entire ductal system (Figure 4A). However, only PDG exhibit intense cytoplasmic PAS staining, suggesting a function in mucin secretion (Figure 4B). Moreover, PDG express Alcian Blue⁺ mucins, including mucins that are normally found in the deep part of the gastric pyloric glands, namely Muc6 (Figure 4B and C). Thus, PDG in the normal pancreas express mucins, which so far have been considered metaplastic. In response to chronic injury, PDG appear hyperplastic and exhibit not only an up-regulation and expansion of Muc6, but also de novo expression of Muc5ac, resulting in a mucinous metaplasia (Figure 4C and D). Whereas the gastric Muc5ac is frequently found in PDG in response to injury, de novo expression of the intestinal Muc2 is observed only rarely.

Mucin-expression patterns confirm that PDG are distinct from the rest of the pancreatic ductal epithelium and undergo a progressive mucinous metaplasia that closely recapitulates features of the gastric pyloric epithelium.

**PDG Retain and Up-Regulate Developmental Pathways, Including Shh**

The pancreatic ductal epithelium has a low turnover rate, and little is known about its regeneration. In many tissues, regeneration and formation of metaplasia are controlled by the same genes that direct tissue differentiation during development. Our next step, therefore, was to analyze PDG for expression of developmental genes known to play a key role in pancreatic development and disease. Expression of Hh pathway genes as well as Pdx-1 and Hes-1 was analyzed in controls and in response to chronic injury in the same mice used previously. Ad-
Additionally, proliferative activity was analyzed by Ki-67 expression or BrdU incorporation.

**Normal pancreas.** In contrast to previous studies, which had not identified Shh in normal pancreas, Shh was identified specifically and only in PDG (Figure 5A). In Ptch1-LacZ animals, the Hh receptor Ptch1 was identified in the ductal mesenchyme surrounding PDG. The Ptch2 receptor is coexpressed with Shh in the epithelium of PDG and in single cells of the normal ductal epithelium. Pdx-1 is expressed predominantly in PDG, but can also be found in the epithelial lining of the main lumen. Hes-1, which is known to be expressed in peripheral ducts and centroacinar cells, is predominantly expressed in PDG in larger ducts (Figure 5A).

**Chronic injury.** In response to chronic injury, all 3 pathways appear up-regulated with different patterns (Figure 5B and C). Shh appears to be up-regulated, but remains specific to mucinous metaplastic PDG. Additionally, Shh was focally observed in mucinous lesions located in the periphery. Ptch1 expression was expanded in the fibrotic ductal mesenchyme, and Ptch2 expression in PDG and in the main ductal epithelium. Expression of both Pdx-1 and Hes-1 was expanded throughout the ductal epithelium, but Pdx-1 maintained a more proximal and Hes-1 a more peripheral predominance. All developmental genes remained expressed in severely metaplastic PDG (Figure 5C).

**Proliferation.** In contrast to the GI epithelium, the pancreatic ductal epithelium has a low turnover rate. In controls, proliferation is only occasionally detected in the ductal epithelium, including PDG (Figure 5D). In response to injury, proliferation was predominantly localized to PDG. In ducts with pronounced metaplasia, Ki-67 demonstrated proliferative activity predominantly in PDG and in the deep portion of mucosal folds and was lost toward the surface. In response to injury, BrdU^+ or Ki-67^+ nuclei were significantly more frequent in PDG compared to the ductal lining (P < .001; odds ratio = 6.6 [95% confidence interval: 3.9–11.0] for acute injury and 14.0 [95% confidence interval: 8.2–24.1] for chronic injury).

Results in human pancreata (Supplementary Figure 4) reflect the observations made in the mouse.

**Evidence for Peripheral PDG**

To search for peripheral PDG, the markers specific for the PDG compartment, Shh, Alcian Blue, and gastric mucins, were assessed in peripheral ducts of con-
control mice and mice with chronic injury. None of these markers could be identified in small pancreatic ducts in controls; but outpouches coexpressing these markers could be identified in the thickened mesenchyme of both small interlobular (Figure 6A) and intralobular ducts (Figure 6B) in response to chronic injury, suggesting that their presence is likely too small to be identified in a nonhyperplastic state.

Figure 5. PDG are a preferential site of expression of developmental factors and of proliferation. (A) In the normal state, Shh is expressed at a low level specifically in PDG (arrowheads), but not in the luminal epithelium. The receptor Ptch2 is coexpressed with Shh in PDG and additionally in some cells of the ductal surface epithelium (arrowheads); Ptch1 is expressed in the mesenchyme around PDG. Pdx-1 is expressed predominantly in PDG (arrowheads) but can be identified in the nonhyperplastic state.

(B) In response to chronic injury, all these markers appear up-regulated. Shh remains specific to mucinous cells within PDG. Ptch2 is expressed in PDG but expanded in surface epithelium (arrowheads); Ptch1 is generalized in the ductal mesenchyme. Expression of Pdx-1 and Hes-1 is expanded or generalized in the ductal epithelium. (C) In severe metaplasia and atypia these changes are more pronounced. Shh remains specific to mucinous cells within PDG. Ptch2 is expressed in PDG but expanded in surface epithelium (arrowheads); Ptch1 is generalized in the ductal mesenchyme. Expression of Pdx-1 and Hes-1 is expanded or generalized in the ductal epithelium. (D) In response to injury, proliferation (BrdU incorporation) in large ducts is predominantly located to PDG (arrows). In chronic injury and metaplasia, expression of the proliferation marker Ki-67 is identified predominantly in PDG and the apex of mucosal folds. MD, main duct epithelial lining.
Shh Misdirects Pancreatic Ducts Toward a GI Mucinous Metaplasia

In development, Shh directs the foregut mesoderm toward a GI fate; in the adult, Shh appears to promote gastric gland differentiation, and has been identified as an early mediator of pancreatic carcinogenesis. The strict colocalization of Shh and gastric-type mucinous metaplasia in PDG led us to hypothesize that inappropriate up-regulation of Shh may be responsible for the metaplasia seen in chronic injury by directing the pancreatic ductal epithelium toward a gastric fate. HPDE cells transfected with the Hh effector Gli1 up-regulate foregut markers. We aimed to determine whether the pancreatic ductal epithelium is directly responsive to the ligand Shh and whether increased levels of Shh can drive a gastric mucinous metaplasia.

HPDE cells were exposed to recombinant Shh and analyzed for Ptch and mucin expression. HPDE exposed to recombinant SHH for 8 days showed a phenotypical change with enhanced mucin expression compared to control cells (Figure 7A). RT-qPCR revealed that HPDE cells express the Hh receptor and downstream gene PTCH2 and up-regulate this expression in response to Shh exposure, demonstrating Hh pathway activation. Moreover, HPDE cells up-regulate expression of the gastric mucins MUC6 and especially MUC5ac (Figure 7A). Thus, HPDE cells are responsive to the ligand Shh and undergo gastric mucinous transformation after Hh pathway activation in vitro.

To analyze whether Shh has similar effects in vivo, we evaluated the effects of engineered Shh misexpression on phenotype and mucin expression of pancreatic ducts. Analysis of pancreata from Pdx-Shh transgenic mice demonstrated that the ductal epithelium first has a non-mucinous appearance and secondarily develops a mucinous metaplasia. Again, this Shh-mediated metaplasia is characterized by expression of gastric mucins, such as Muc5ac (Figure 7B).

In vitro and in vivo results demonstrate that the adult pancreatic ductal epithelium is responsive to Shh signaling and that enhanced levels of Shh are sufficient to drive a GI mucinous metaplasia. Thus, PDG-derived Shh may drive mucinous metaplasia and PanIN-like formation in pancreatic disease.

PDG Display SHH Up-Regulation and Metaplasia in Human CP

To assess the relevance of our findings to human disease, we analyzed Shh expression and metaplastic changes in human pancreata.

SHH was not detected in peripheral pancreatic ducts, acini, or islets of normal human pancreata (n = 8). However, SHH is expressed in PDG (Figure 8A). In CP (n = 6), SHH appears up-regulated. In all pancreata evaluated, SHH was aberrantly expressed at a much higher level in an expanded, morphologically hyperplastic epithelium exhibiting increased mucin expression and cellular atypia, features of PanIN (Figure 8A). Comparison of proximal (Figure 8A) to peripheral ducts (Figure 8B) revealed that these changes are present focally throughout the ductal epithelium, but have a proximal predominance. SHH expression in peripheral ducts was always colocalized with mucinous metaplasia or atypia.

RT-qPCR was performed for the Hh pathway (Figure 8C). In normal pancreata (n = 6), all Hh transcripts were detected at a low level. In CP (n = 10), the entire pathway, including ligands, receptors, and transcription factors, was up-regulated. Although all 3 ligands were identified, SHH had the highest up-regulation. Expression of Shh and its pathway members is found at much higher levels in biopsies of proximal ducts compared to matched peripheral biopsies. Whereas by immunostaining, the enhanced expression of Shh appears to be localized to the metaplastic epithelium, up-regulation of the downstream genes may also involve the mesenchyme.

Together immunostaining and RT-qPCR confirm that SHH remains expressed in the PDG compartment in human pancreata and suggest that in the human SHH has effects similar to those shown in mice.

Conclusions

The pancreatic ductal epithelium is a heterogeneous group of cells. We present evidence that this epithelium harbors a specialized compartment, pancreatic
duct glands. In its normal state this compartment retains expression of developmental markers, including Shh, and is a site of production of gastric-type mucins. In response to chronic injury, the compartment is a source of a Shh-mediated gastric mucinous metaplasia.

Specialized mucin-producing glands in the pancreas were first noted in the 1960s. In the 1980s, Bock et al investigated secretory cells within pancreatic ducts by electron microscopy and by their histochemical staining pattern. They found that mucin-producing cells were gathered in mucosal folds, forming endoepithelial glands, which they called “duct glands.” However, their anatomic character as blind-ending structures could not be conclusively elucidated. This compartment and its role in injury have not been further analyzed until now.

In the present study, SEM of ductal corrosion casts allowed us to directly demonstrate in both rodents and humans that these structures are blind-ending outpouches. PDG have a greater prevalence in rodents than in humans, consistent with interspecies variability in the frequency of mucin-producing cells. The predominant cells in these glands are mucin-producing and have basally located nuclei and abundant supranuclear cytoplasm. This morphology resembles PanIN-1A. Because PanIN-1A may be seen in otherwise normal pancreata, the original PanIN classification acknowledges the possible existence of non-neoplastic “lesions” in normal pancreata, referred to as PanIN/L-1A. Our data suggest that PDG may account for at least a subset of such early PanIN and may be the origin of later PanIN.

The existence of PDG raises the question of their function. Clearly, they are a specific site of mucin production that serves for mucosal protection. Because ductal cell turnover in the normal pancreas is low, studies investigating its...
renewal are difficult and little is known about renewal of the pancreatic ductal epithelium. In a study of the ductal epithelium in human CP, expression patterns of growth factors identified a specialized epithelium, which buds off the main lumen and was suggested to play a role in repair. In rodent models of pancreatic injury, Pdx-1 is induced in the main duct and “small evaginations.” PDG express and up-regulate several of the genes that mark progenitor cells in intestinal crypts, Shh, Pdx-1, and Hes-1. Although the proliferation data and the expression of developmental markers show that, unlike intestinal crypts, PDG are not the exclusive site of cells likely to be involved in epithelial renewal, their predominance in PDG suggests that PDG contribute to epithelial renewal and repair.

Dysregulation of mechanisms involved in regeneration and repair can lead to metaplasia and, ultimately, neoplasia. In the pancreas, the Hh pathway is involved in regeneration. Its ligand Shh has been identified as an initiator of neoplasia. Most recently, Shh has been identified in pancreatic cancer stem cells. The finding that...
Shh remains expressed in PDG that exhibit features of “metaplasia” may explain prior observations of Shh and GI marker expression in early PanIN, even in the absence of other signs of atypia or alterations of other cancer-related genes. The finding that the PDG compartment is particularly prone to Shh-mediated GI metaplasia in response to injury suggests both a source and mechanism of metaplasia in the diseased pancreas. Interestingly, injured human and mouse pancreata exhibit a highly organized gastric-type metaplasia that closely mirrors the mucin-expression pattern of the pylorus. Although this is speculative, we may propose that Shh-mediated metaplasia in the pancreas may play a similar role to that described for CDX-1—mediated intestinal metaplasia in the stomach and esophagus, and may, like Barrett’s metaplasia,33,34 be associated with the risk for cancer.

Recent attention has focused on the importance of paracrine Hh signaling for desmplasia and pancreatic cancer progression.33,34 In a recent study by Tian et al, Hh ligands were expressed in the epithelium, but only the stromal compartment was found to be able to transduce downstream pathway activity during pancreatic carcinogenesis.37 Similar to our study, Tian et al used the Ptc1-LacZ mouse and—mirroring our results—Hh pathway activity was reported exclusively in the mesenchyme.37 During development, Ptc1 is preferentially expressed in the mesenchyme, whereas the Ptc2 homologue is coexpressed with Shh in the epithelium.38 Our data suggest that this differential expression of Ptc receptors is maintained into adulthood. In response to chronic injury, we found enhanced coexpression of Ptc2 along with Shh in PDG and in the ductal surface epithelium, whereas Ptc1 was expressed in the fibrotic stroma. Ptc2 expression and up-regulation in the surface epithelium in vivo and in HPDE cells in vitro demonstrate that PDG-derived Shh can influence responsive target cells not only in PDG, but in the entire ductal epithelium, similarly as suggested for growth factor signaling.28 Whereas we focused on the epithelial effects of Hh signaling and their role in epithelial metaplasia and early carcinogenesis, paracrine stromal Hh activation35-37 via the Ptc1 receptor may represent a parallel and even predominant mechanism of Hh activation within the entire tissue. At present, the exact role of Hh signaling in the complexity of epithelial—mesenchymal interactions remains to be understood.

Although much work must still be done to confirm the role of PDG in protection, regeneration, and cancer, it is clear that they represent a distinct gland-like compartment, principally located in the proximal ductal system, which has a distinct molecular signature and is different from the normal pancreatic ductal epithelium. PDG may thus provide a link between injury, Shh activation, mucinous metaplasia, and neoplasia and represent a possible cell of origin.

### Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2009.12.005.

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Conflicts of interest
The authors disclose no conflicts.

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