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Review

Hedgehog signaling in pancreas development and disease

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Abstract. Since its discovery, numerous studies have shown that the Hedgehog (Hh) signaling pathway plays an instrumental role during diverse processes of cell differentiation and organ development. More recently, it has become evident that Hh signaling is not restricted to developmental events, but retains some of its activity during adult life. In mature tissues, Hh signaling has been implicated in the maintenance of stem cell niches in the brain, renewal of the gut epithelium and differentiation of hematopoietic cells. In addition to the basal function in adult tissue, deregulated signaling has been implicated in a variety of cancers, including basal cell carcinoma, glioma and small cell lung cancer. Here, we will focus on the role of Hh signaling in pancreas development and pancreatic diseases, including diabetes mellitus, chronic pancreatitis and pancreatic cancer.

Key words. Hedgehog; Indian Hedgehog; Desert Hedgehog; pancreas development; pancreatic cancer; chronic pancreatitis, islets of Langerhans.

Hedgehog signaling pathway

In 1980 Nüsslein-Volhard and Wieschaus [1] first described Hedgehog (Hh) mutants in the fruitfly *Drosophila melanogaster*. In this work, Nüsslein-Volhard and Wieschaus found that certain genetic mutations caused flies to look like little hedgehogs with continuous spiky outer skins. Once isolated, the gene mutated in these flies was named *hedgehog (hh)*. During the following decade, homologues of the Drosophila *hh* gene have been isolated and identified in many vertebrates, including mice and humans. As in Drosophila, the vertebrate *Hh* genes have indispensable functions during embryonic development, differentiation and morphogenesis. Here, we will briefly discuss what is currently known about Hh signaling but refer readers to the following papers for more specific details of the pathway [2–7].

Although there is only one *hh* gene in Drosophila, three known Hh genes exist in mammals, *Sonic hh (Shh), Indian hh (Ihh)* and *Desert hh (Dhh)*. *Shh* is the best studied of mammalian *Hhs* with the broadest expression pattern, including in the developing nervous system, limb buds, skin and gut [3, 7–9]. *Ihh* expression is restricted to the developing bone and cartilage, gut and pancreas [3, 7, 10–13], whereas *Dhh* expression is found primarily in the gonads and testes, with some expression also in peripheral nerves and pancreas [3, 7, 11, 14]. While their binding affinities are slightly different, all three ligands have been shown to bind the same receptors, and to elicit similar responses in target cells [5]. Thus, individual ligand activities are mainly regulated via their distinct expression patterns; however, independent ligand functions have not been entirely excluded.

Hh processing

Despite their distinct expression patterns, all Hh molecules appear to share the same posttranslational modifications [3, 7]. Processing of Hh molecules requires that

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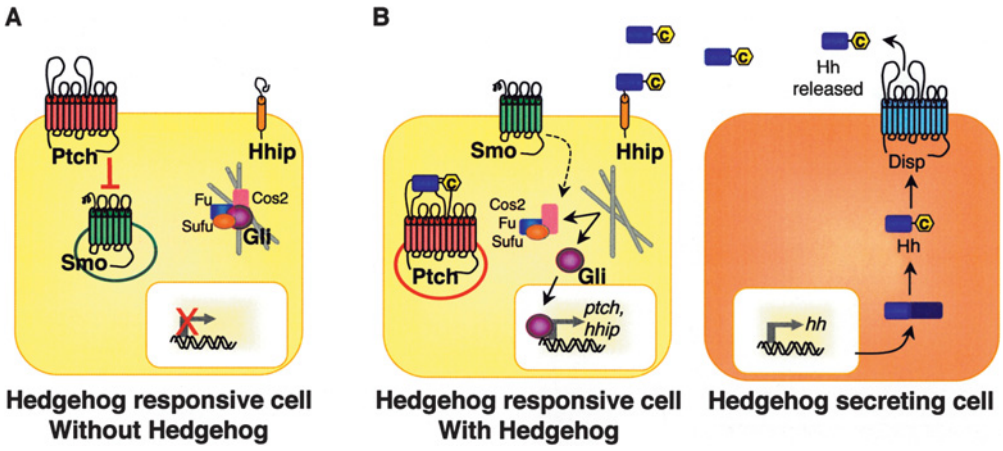

Figure 1. Hedgehog signaling pathway. (*A*) Hh responsive cell without Hh ligand. In the absence of Hh ligand, Patched (Ptch) receptor is localized to the cell membrane, where it inhibits Smo vesicle relocalization to the cell surface. Gli transcription factors are bound to the cytoskeleton by a complex of proteins that include Fused (Fu), Suppressor of Fused (Sufu) and Costal2 (Cos2). There, Gli proteins may be cleaved into transcriptional repressors so they can later inhibit Hh target gene transcription in the nucleus. Hh Interacting Protein (Hhip) remains on the cell surface. (*B*) Hh secreting cells transcribe and process cholesterol-modified Hh ligands. Release of processed Hh ligands occurs through Dispatched (Disp). In the presence of Hh ligand, secreted Hh binds its receptor Ptch, causing Ptch internalization, and Smo vesicle relocalization to the cell surface. There, Smo initiates downstream events that result in Gli release from the cytoskeleton and its translocation into the nucleus where it may act as a transcriptional activator of Hh target gene transcription. Hhip may bind excess Hh ligand on the cell surface.

nascent pre-proteins undergo an autocatalytic cleavage event resulting in an N-terminal fragment that carries all known signaling activities (see fig. 1). During cleavage, a cholesterol group is covalently attached to the C-terminus of the signaling fragment. Although many proteins are lipidated, sterol modifications of secreted molecules are quite unusual and may be unique to Hh ligands. Consequently, cholesterol modification has been extensively analyzed, and results from these studies point to its importance in Hh molecule retention to cell membranes and range of diffusion [3, 5, 15–17]. In addition to cholesterol modification, processing of Hh molecules requires palmitoylation of the N-terminus to yield a fully processed Hh protein [15, 17, 18].

Hh signaling

Two transmembrane receptors, Patched1 (Ptch1) and Patched2 (Ptch2), have been identified as receptors for processed Hh ligands. *Ptch1* expression is more prominent, and its function has been analyzed in detail [3, 7, 19]. In the absence of ligand, Ptch1 represses the activity of Smoothened (Smo), a G-protein coupled-like receptor (see fig. 1). Repression of Smo halts all downstream signaling events. Upon ligand binding, Ptch1 repression of Smo is alleviated, and Smo initiates a signaling cascade that results in the translocation of Gli transcription factors into the nucleus [3, 7]. In mammals, there are three known Gli transcription factors, Gli1, Gli2 and Gli3. Depending on the tissue-specific context, Gli1 seems to act as a transcriptional activator [20]. Gli2 usually functions as a transcriptional activator but retains some repressor function, while Gli3 mainly works as a transcriptional repressor [20–22]. Furthermore, both *Gli1* and *Gli3* are known Hh transcriptional target genes [7]. Additional known target genes for Hh signaling include *Ptch1* and Hedgehog Interacting Protein (*Hhip*) (see below.)

Regulating Hh signaling

Hh signaling has been shown to regulate cell differentiation in a concentration-dependent fashion. Given the fact that small changes in signaling activity can result in dramatic changes in responding cells, tight regulation of the pathway activity is essential. In the context of Hh signaling, this is accomplished by negative feedback loops in which transcriptional targets curb the activity of the pathway. One example is Ptch1, the Hh receptor that blocks activity by interfering with Smo function, in the absence of ligands. *Ptch1* is a downstream target gene whose expression is upregulated when the pathway is active [7]. In return, the increased level of Ptch proteins at the surface of cells active in Hh signaling decreases pathway activity by blocking Smo activity. Moreover, Ptch removes soluble Hh proteins through internalization of the receptor-ligand complexes. This particular function limits the diffusion range of Hh ligands and thereby the area of Hh signaling in a given tissue. The level of available ligands is also controlled by the expression of Hedgehog Interacting Protein (*Hhip*) [23]. Hhip is a Hh receptor that lacks a cytoplasmic signaling domain. Therefore, Hhip acts as a sink by binding to Hh ligand without transmitting any downstream signaling events. Additionally, *Hhip* is a Hh target gene, and pathway activation results in in-

creased levels of *Hhip* that can bind more ligand and prevent ligand diffusion. Thus, the presence of endogenous Hh inhibitors as integral parts of the signaling cascade prevents increased pathway activity.

The pancreas and its development

The adult pancreas is a heterogeneous organ formed by two primary tissues, the exocrine compartment consisting of acinar and ductal cells, and the endocrine compartment consisting of cells localized within discrete structures known as islets of Langerhans. The exocrine acinar cells make up the majority of the mature organ and produce digestive enzymes that are collected by ductal tissue that drain into the intestinal tract. Embedded within the exocrine tissue are the endocrine islets of Langerhans. Islets consist of four distinct cell types, insulin-producing β-cells, glucagon-producing α-cells, somatostatin-producing δ-cells and pancreatic polypeptide-producing PP-cells, which produce key hormones that regulate blood glucose levels. Thus, pancreatic functions include production of enzymes that aid in digestion of nutrients and hormones that regulate glucose homeostasis.

During embryogenesis, the pancreas is specified in the anterior midgut region of the endoderm epithelium before embryonic day 8.0 (e8.0) in mice [24]. (If not noted otherwise all ages refer to stages during mouse development). By e8.5, the expression of pancreatic duodenal homeobox 1 gene (*pdx1*), a transcription factor essential for proper pancreas development and β-cell function, marks the endodermal area destined to give rise to the dorsal and ventral pancreas buds [24–27]. By e9.0, the first morphological signs of pancreas formation are seen when the dorsal pancreatic bud evaginates from the endodermal epithelium, just caudal to the stomach anlage. Subsequently, two ventral pancreatic buds form next to the liver diverticulum. Eventually, one of the ventral buds

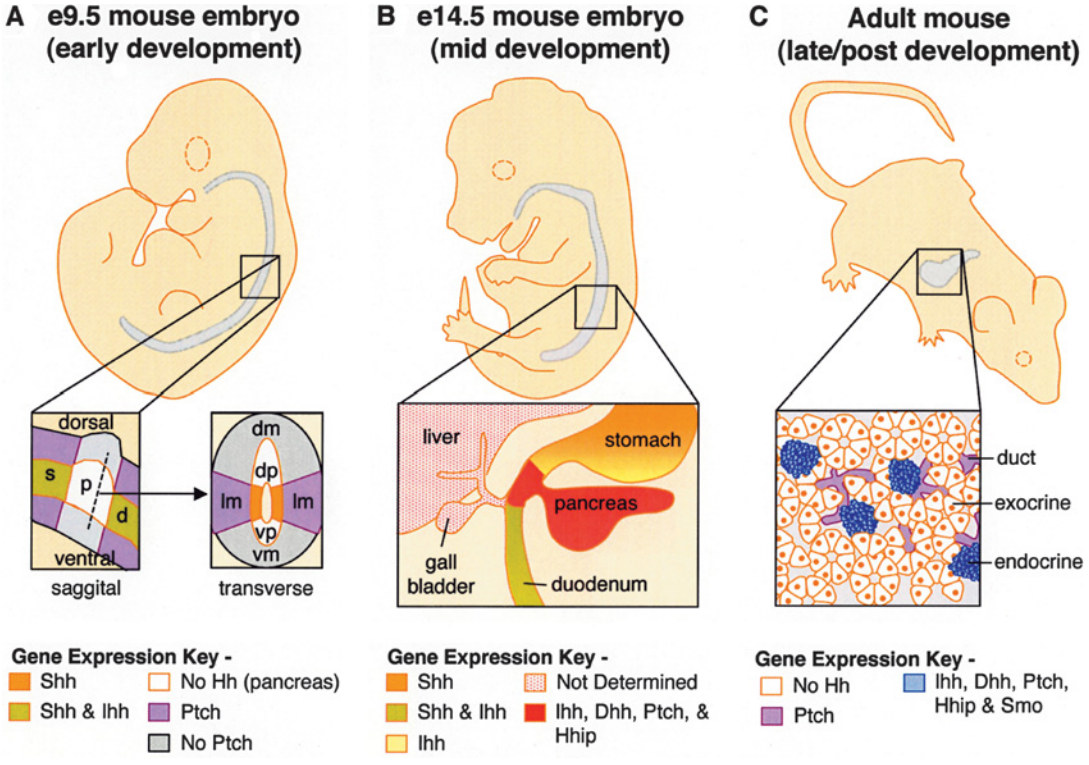

Figure 2. Hedgehog signaling during mouse pancreas development. (*A*) e9.5 mouse embryo (early development). In the sagittal section, *Shh* is expressed in the specified stomach (s) endoderm, anterior to the specified pancreatic (p) endoderm. *Shh* and *Ihh* are expressed in the specified duodenal (d) endoderm, posterior to the pancreatic endoderm. *Ptch* expression surrounds Hh expressing areas. In the corresponding transverse section through the pancreas, *Shh* is expressed along the lateral epithelium, and *Ptch* is found within the reciprocal lateral mesenchyme (lm). There is no expression of *Shh* in the dorsal (dp) or ventral (vp) pancreatic epithelium, and there is no *Ptch* expression in the neighboring dorsal (dm) and ventral (vm) pancreatic mesenchyme. (*B*) e14.5 mouse embryo (mid development). In the stomach, *Shh* is expressed along a gradient with highest expression in the anterior stomach. Conversely, *Ihh* is expressed along a gradient with highest expression in the posterior compartment. Both *Shh* and *Ihh* are expressed in the duodenum, and *Ihh, Dhh, Ptch* and *Hhip* are detected in the pancreas. Expression of Hh signaling components has not been determined in developing liver or gall bladder at e14.5. (*C*) Adult mouse (late/post development). *Ihh, Dhh, Ptch, Hhip* and *Smo* are expressed in islet endocrine cells. *Ptch* expression has been detected in pancreatic ducts. Hh signaling components are not normally expressed in exocrine cells.

is lost while the other fuses with the dorsal bud during gut and stomach rotation. Ultimately, a unified pancreatic organ forms with a ventral domain nestled in the duodenal loop and a dorsal domain next to the spleen and stomach. Thus, the adult pancreas is composed of tissue derived from both the dorsal and ventral endoderm.

It is important to note that all endocrine and exocrine cell types form from the endoderm epithelium. As early as e11.5, the expanding pancreatic epithelium branches into the surrounding mesenchyme, and differentiation of endocrine and exocrine cells commences [24]. Endocrine cells delaminate from the pancreatic epithelium and migrate into the mesenchyme. Concomitantly, exocrine cells organize into acini with duct cells lining the central epithelium. From e15.5 until birth, the pancreatic epithelium continues to grow, and endocrine cells aggregate to organize themselves into functional islets that are embedded within the exocrine matrix. Differentiation and development continue on past the gestational period, and it is not until several weeks after birth that the pancreas fully matures to aid in digestion and nutrient storage/utilization.

Hedgehog signaling in pancreas development

Hh signaling appears to play multiple roles during mouse embryonic pancreas development. During early stages of gut formation, expression of both *Shh* and *Ihh* is found throughout the endoderm epithelium [8–10, 28] (see fig. 2A). In contrast, both genes are absent from the early endodermal area specified to become pancreas [29–31]. Similarly, *in situ* hybridization studies on e9.5 pancreas tissue have shown that *Ptch1* expression is found in the mesenchyme adjacent to, but missing from, the pancreas anlage [29]. However, by e13.5 the developing pancreas expresses several Hh components (including *Ihh, Dhh, Hhip* and *Ptch1* [11, 32, 33] and unpublished results) (see fig. 2B). Within the mature pancreas, expression of *Ihh, Dhh, Hhip, Ptch1,* and *Smo* has also been observed in islet and ductal cells [11, 32, 33] (see fig. 2C). Thus, both ligand and receptor expression patterns indicate that Hh signaling is present and active during later stages of pancreas development and in the mature organ. While these results might indicate that a certain level of Hh signaling is required for proper organ formation and operation, gain of function studies have demonstrated that deregulation of Hh pathway activity results in severe changes of pancreas morphogenesis and function.

Gain of Hh function studies in the pancreas

Several studies have been performed to assess the effects of increased Hh signaling during pancreas formation [29, 33, 34]. Ectopic expression of *Shh* at the onset of pancreas formation under the control of the *Pdx-1* promoter results in almost complete organ ablation [29]. Similarly, ectopic expression of either *Shh* or *Ihh* under the control of the human *PAX4* promoter midway through pancreas formation also severely disrupts pancreas morphogenesis [34]. Interestingly, these results suggest that it is the overall level of Hh signaling rather than individual ligand expression that is important during pancreas development. These findings, and the fact that Hh components are absent from early pancreatic endoderm but present in tissue immediately adjacent to the pancreas area, suggest that Hh signaling acts during early pancreatic organogenesis to establish organ boundaries by regulating the size of the pancreas domain. In fact, ectopic overexpression of Hh ligands instructs the overlying mesenchyme to adopt a duodenal fate. As a consequence, the reciprocating mesenchymal signals that are received by the pancreas epithelium are incompatible with proper pancreas organogenesis. An explanation for this finding may be found in studies that demonstrate how increased Hh activity at early stages of pancreas formation in *Hhip* mutant mice results in reduction of mesenchymal *fgf10* expression [33]. FGF10 activity between e9.5 and e11.5 has been shown to promote proliferation of Pdx-1-positive pancreas progenitor cells [35]. Thus, increased Hh activity perturbs mesenchymal-epithelial signaling required for proper organogenesis. However, *Ptch* expression is also found in pancreatic epithelial cells [11, 32], and it is possible that Hh ligands may have a direct effect on epithelial cells during the later stages of pancreas development. Experiments designed to specifically block epithelial Hh signaling will reveal whether these cells are actively responding to primary Hh signals or to secondary epithelial-mesenchymal interactions during development.

Dosage-dependent effects of increased Hh signaling in the pancreas have also been assessed. As seen in other gain of function studies, loss of *Hhip*, an inhibitor of the pathway, results in increased formation of pancreatic mesenchyme at a cost to the developing pancreatic epithelium [33]. However, the additional loss of one *Ptch1* allele in *Hhip* mutant mice leads to a more profound loss of epithelial cells, indicating that pancreatic cells can respond to different levels of Hh activity [33]. Therefore, uncontrolled Hh signaling interferes with normal pancreas formation.

Loss of Hh function studies in the pancreas

In addition to gain of function studies, loss of function experiments have revealed pertinent information about the role of Hh signaling during pancreas development. Gain of function experiments indicate that Hh activity needs to be contained to permit proper pancreas development. Conversely, loss of Hh signaling, at least in some model systems, results in increased pancreas formation. For ex-

ample, inhibition of Hh activity in chick embryos by treatment with cyclopamine, a natural plant-derived steroid alkaloid that binds to and blocks the activity of Smoothened, results in enlarged islets in the dorsal pancreas [36]. Expansion of *Pdx-1* expression into the posterior stomach and proximal duodenum is also observed [36]. Moreover, carboxypeptidase A, glucagon and insulin producing cells were found in cyclopamine-treated stomach explants, revealing an expansion of both pancreatic exocrine and endocrine cells upon Hh inhibition [36]. Thus, loss of Hh signaling can increase the relative pancreas area in chick explant studies.

So far, complete ablation of Hh signaling in pancreatic tissue has not been achieved in transgenic mice due to the early embryonic lethality associated with Hh depletion. In contrast, embryos marked by the loss of individual Hh ligands develop at least until birth and can be analyzed for pancreatic defects. Loss of Shh signaling in $Shh^{-/-}$ mice leads to a relative increase in both pancreas weight and number of endocrine cells [11]. However, the increase of pancreas mass was only noted relative to the significant decrease in body size in *Shh* mutant embryos. In other words, despite the fact that *Shh* mutant embryos are severely reduced in body weight, the actual weight of the pancreas in these mutant embryos was equivalent to the pancreas weight measured in control littermates. Thus, these data indicate that in contrast to other tissues, pancreas development does not depend on Shh signaling. However, both *Ihh* and *Dhh* are expressed in pancreatic cells during development as well as in adult islets [11, 32]. Therefore, to eliminate pancreatic Hh signaling, *Ihh/Dhh* double mutants would have to be generated and analyzed for defects. Alternatively, tissue-specific elimination of *Smoothened* in pancreatic cells should allow us to determine Hh signaling requirements during pancreas formation.

Zebrafish: the exception to the rule?

Current assessments for Hh signaling requirements during mammalian pancreas development indicate that increased Hh activity blocks pancreas morphogenesis [29]. In contrast to this inhibitory role, studies on zebrafish embryos have revealed a different, instructive role of Shh during pancreas organogenesis [37]. Inhibition of Hh signaling at early stages of gastrulation, when the mesodermal and endodermal germ layers are formed, results in loss of insulin-expressing cells [37]. Similar phenotypes are seen both in zebrafish embryos mutant for either *Shh (syu)* or *smoothened* (*smu*). These results indicate that Hh signaling during early gastrulation is required for specification of pancreatic endocrine cells. As noted above, similar findings have not been obtained from studies in conventional knockout mice due to the fact that complete elimination of Hh signaling results in embryonic lethality shortly after gastrulation, before pancreas morphogenesis is initiated. Interestingly, treatment of zebrafish embryos with cyclopamine shortly after gastrulation leads to

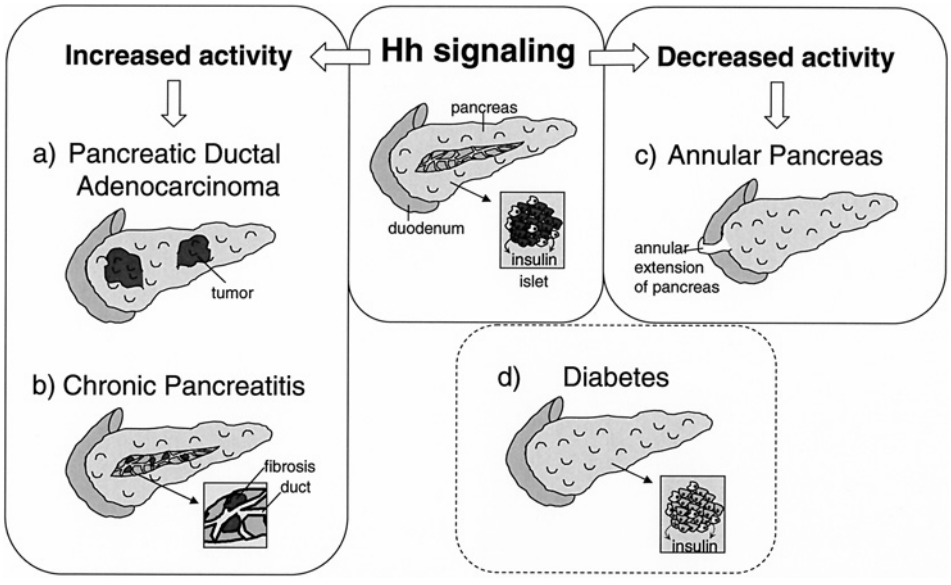

Figure 3. Hh signaling in pancreas disease. Normal pancreas has islets along a ductal system that secrete insulin. (Dark grey areas, areas with increased/high Hh signaling.) (*A*) Pancreatic ductal adenocarcinoma: increased Hh activity in ductal cells results in tumor foci (dark areas) and participates in sustained tumor growth and progression. (*B*) Chronic pancreatitis: increased Hh activity in degenerating exocrine acini and proliferating ductal cells participates in progression of fibrotic lesions along pancreatic ducts. (*C*) Annular pancreas: decreased Hh activity during pancreas development results in annular extensions (white area) of ventral pancreatic tissue that encircle and may constrict the duodenum. (*D*) Diabetes mellitus: altered Hh activity may result in deregulated insulin production and secretion, and loss of glucose homeostasis.

the formation of multiple clusters of insulin producing cells [38]. Conversely, both *vhnf1* zebrafish and *HNF1β* mouse mutants are marked by an expansion of *shh* expression and a reduction of *pdx1* expression in the posterior gut [39, 40]. Thus, similar to its role in mice, Hh signaling has a secondary, inhibitory role during pancreas formation in zebrafish as well. However, it should be noted that pancreas formation in zebrafish differs from mammals. The pancreatic endoderm does not arise from a bud along a specified endodermal gut tube, but rather from cells found alongside the embryonic midline that coalesce and condense to form pancreatic buds [41, 42]. Therefore, it is possible that these fundamental differences in pancreas formation may consequently result in distinct tissue-specific interactions that affect Hh activity in developing zebrafish embryos. Future studies will be required to clarify the exact nature of the temporal and spatial roles of the pathway in both model systems.

Hh signaling in adult pancreas

Traditionally, more emphasis has been placed on the role of Hh signaling during embryogenesis, and only recently have efforts been undertaken to address its role in adult tissue maintenance and/or function. Increasing data now support the notion that developmental signaling pathways remain active in a subset of cells in adult tissues [43–48]. Although Hh signaling is present in differentiated pancreatic tissue [11, 32, 33], little is known of its role in the mature organ. In adult mouse pancreas, expression of Hh components seems to be restricted to the adult islets and ducts [11, 32, 33]. So far, the most compelling evidence for a functional requirement of the pathway in adult endocrine cells comes from cell culture studies performed by Thomas et al. in 2000 and 2001. These studies showed that ectopic expression of *Shh* in the rat insulinoma cell line INS-1 promotes insulin production and secretion at the transcriptional level [32]. Moreover, this response is blocked by the addition of the Hh inhibitor cyclopamine. Further analysis demonstrated that *Pdx1*, a strong regulator of insulin transcription, has Hh responsive elements within its promoter region, and that Hh ligand levels modulate the expression of *Pdx1* in INS-1 cells [49]. These results support the notion that Hh signaling positively regulates insulin production and secretion in normal adult β-cells at least in part through regulation of *Pdx1* expression. In contrast, adult *Ptch1* heterozygous mice have increased levels of Hh signaling but display glucose intolerance phenotypes [11], a finding that contradicts the positive effects of Hh signaling on insulin production and secretion obtained in insulinoma cells. A potential explanation may lie within the apparent temporal differences of Hh activity in developing and fully differentiated β-cells. Hedgehog signaling is increased throughout pancreas formation in *Ptch1* heterozygous mutants, suggesting that β-cell developing in this milieu might acquire defects that hinder proper function in differentiated cells. In contrast, these inhibitory effects might be missing in adult β-cells. Additional studies that might include temporal tissue-specific activation and inactivation of Hh signaling in mature β-cells will be required to address these discrepancies.

Hh signaling and pancreatic diseases

During pancreas morphogenesis, deregulation of Hh signaling results in severe defects. Recent studies suggest that perturbation in Hh activity is also implicated in congenital disorders and adult pancreatic diseases. Here, we review the known role of the pathway in some common human pancreatic disorders, including annular pancreas, diabetes mellitus, chronic pancreatitis, and pancreatic cancer.

Annular pancreas

Reduced Hh signaling has been associated with several congenital malformations throughout the intestinal tract in mice and humans [28, 44, 50–53]. Within the pancreas, a number of distinct genetic disorders, including annular pancreas, have been attributed to deregulated Hh activity. Annular pancreas is a rare condition characterized by an extension of pancreatic tissue that encircles the duodenum [54]. In the most severe cases, extensions form a ring that can constrict the passage of nutrients through the intestine, a serious condition that requires surgery to alleviate this blockage. Although the causes of this defect in humans are unknown, studies in transgenic mice suggest that loss of Hh signaling may contribute to the development of this disorder. Depending on the genetic background of the strains analyzed, ventral pancreatic extensions have been found in *Ihh* and *Shh* mutants with a 42% incidence in *Ihh*–/– and *Shh*–/– mutants [11, 28]. However, these defects have been observed in conventional knockout mutants where Hh ligands were eliminated in all cells of the body rather than specifically in pancreatic cells. Therefore, it cannot be excluded that annular pancreas may be caused by defects in gut rotation rather than due to changes that directly affect pancreas formation. Nonetheless, the reduction of Hh signaling must be considered a prime candidate for this disorder in humans.

Diabetes mellitus

Diabetes mellitus is a pleiotropic disease that causes deregulation of blood glucose levels. According to the World Health Organization report in 2004, more than 170 million people suffer from diabetes worldwide, a number that is projected to rise to 370 million by 2030. (The dia-

betes Program 2004, http://www.who.int/diabetes/en/). In the US, it is estimated that 18.2 million people, or 6.3% of the population, suffer from diabetes and its long-term complications, which include heart disease, stroke, renal failure, neuropathy, blindness and amputations. Two main types of diabetes with distinct etiologies can be distinguished: type 1 diabetes, caused by the autoimmune destruction of insulin-producing β-cells, and type 2 diabetes, the most common form that is caused by β-cell defects resulting in insufficient insulin production or secretion, or insulin resistance in peripheral tissues. So far, Hh signaling has not been implicated in the autoimmune response that leads to type 1 diabetes.

Type 2 diabetes

Recent evidence may implicate deregulated Hh signaling as a potential risk factor for type 2 diabetes. As previously described, Thomas et al. demonstrated that Hh signaling regulates insulin production and secretion, in part via transcriptional activation of the *Pdx1* gene [32, 49]. Inhibition of Hh activity with cyclopamine in cultured insulinoma cells resulted in decreased transcriptional activity of the *Pdx1* promoter. Previous results had shown that reductions in Pdx1 levels result in adult onset of hyperglycemia in mice and a special form of type 2 diabetes in humans, maturity-onset diabetes of the young (MODY) [55, 56]. MODY is a form of dominantly inherited type 2 diabetes mellitus characterized by pancreatic β-cell dysfunction with an onset age of 25 years or younger. Six MODY gene mutations have been identified [57–63], and one of these is caused by mutations in the *Pdx1* gene locus (MODY4, [58]). Thus, it is possible that loss of Hh signaling may reduce *Pdx1* expression, resulting in the progressive development of a MODY4-like type 2 diabetes. By contrast, results from *Ptch1* heterozygous mice (*Ptch1*+/–) also suggest that increased Hh signaling results in defective glucose tolerance [11]. As previously discussed, a possible explanation for this discrepancy is that in contrast to cultured INS-1 insulinoma cells, β-cells in *Ptch1* heterozygous mice have been exposed to increased Hh signaling throughout embryonic development. While it cannot be excluded that altered levels of Hh signaling during β-cell development can lead to defects in mature β-cell function, changes in Hh signaling have not been identified as the cause of diabetes human patients. Future studies will need to clarify whether a relation between disrupted Hh signaling and development of type 2 diabetes exists.

Chronic pancreatitis

Chronic pancreatitis is a progressive disease characterized by an irreversible destruction of exocrine tissue, extension of ductal structures and widespread fibrosis [64–66]. During the early stages of acinar cell destruction, the islets of Langerhans are well preserved. However, morphological changes and a reduction in islet number are found in advanced stages of chronic pancreatitis. In addition to alcohol, the most common cause of this disease, many other factors contribute to its pathology, including heredity, autoimmunity and tropical factors [67]. Recent studies by Kayed et al. have shown that increased Hh signaling is associated with chronic pancreatitis [68, 69]. Unlike rodents, in which analysis of transgenic mice that express β-galactosidase under the control of the *Ptch1* promoter marks adult ductal cells [11], expression of *PTCH* and other Hh components, including *IHH, HHIP* and *SMO,* is not found by immunohistochemistry in normal adult human exocrine or ductal cells [68, 69]. However, these data do not exclude that Hh signaling components are expressed in normal pancreatic tissues at levels below detection by immunohistochemistry. In contrast, increased expression of Hh pathway components within degenerating acini and proliferating ductular complexes has been noted in fibrotic tissues of patients with chronic pancreatitis. Thus, elevated Hh signaling marks the progression of normal to fibrotic tissue in chronic pancreatitis. In addition, treatment of non-transformed pancreatic duct cells with Hh agonists induces cell proliferation and provides initial evidence for a requirement of Hh signaling in the progression of chronic pancreatitis [68]. Nevertheless, proof that deregulation of Hh signaling is essential during initiation or progression of this disease would require the genetic ablation of the pathway in mouse models for chronic pancreatitis. If these results would prove positive, novel Hh inhibitors that are currently developed could eventually be used for therapeutic treatment of patients suffering from this disease.

Pancreatic cancer

Chronic pancreatitis is one of the risk factors for pancreatic adenocarcinoma, the most prominent form of all pancreatic cancers. Pancreatic cancer currently ranks as the fourth leading cause of cancer death in the United States, with a peak incidence in the 65–75 year age group (American Cancer Society, 2004). Adenocarcinomas of the pancreatic duct are rarely curable, as diagnosis usually occurs at a time when the tumor has spread to adjacent organs and surgical removal of the tumor, the only effective intervention, has become impractical. Even for those few patients with localized disease and small cancers (< 2 cm), the 5-year survival rate after complete surgical resection is a dismal 18% [70–72]. Despite the high mortality rate associated with pancreatic cancer, the causes of pancreatic cancer have not been identified. It is likely that treatment of this fatal disease will required the detailed understanding of the molecular biology of pancreatic cancer.

Uncontrolled activation of Hh signaling has been linked to the development of distinct cancers in brain, muscle, skin and digestive system [73–75]. Similarly, uncontrolled activation of Hh signaling has been implicated in the progression and maintenance of pancreatic adenocarcinomas [68, 69, 76–79]. Pancreatic duct cells are believed to be the progenitor cells for pancreatic adenocarcinoma. The current progression model for pancreatic adenocarcinoma proposes that pancreatic duct cells develop into pancreatic intraepithelial lesions (PanIN) that have been classified into distinct stages by the appearance of morphological markers [80, 81]. PanIN-1A lesions are characterized by hyperplasia, PanIN-1B/2 by dysplasia, and PanIN-3 by ductal carcinoma in situ, the final stage before metastasis occurs. Analysis of human pancreatic sections revealed that the level of Hh signaling increases dramatically during the progression from PanIN lesions to metastatic tumors. Functional evidence supporting the notion that increased Hh signaling is sufficient to induce pancreatic cancer formation comes from studies in transgenic mice. Ectopic expression of the Hh ligand *Shh* under control of the *Pdx1* promoter leads to morphological changes that resemble human PanIN lesions [78]. Pancreatic lesions in these mice are marked by overexpression of molecular markers, including HER/neu and mutated K-ras, which are commonly associated with early-stage human pancreatic cancer [78]. In addition to this early role during the formation of the disease, studies on pancreatic cancer cell lines have shown that Hh signaling is involved in maintaining the tumor phenotype [77, 78]. Analysis of a large number of these cell lines showed active Hh signaling in all lines tested. Inhibition of pathway activity with cyclopamine resulted in increased tumor cell apoptosis and decreased proliferation and growth in cultured cells and xenotransplantated tumors. Interestingly, the majority of pancreatic cancer cell lines assayed also express Hh ligands, indicating that autocrine signaling keeps the pathway in an active state [77]. Providing evidence to support this hypothesis, Berman and colleagues used Hh antibodies to block Hh ligand activity in these cell lines [77]. This treatment prevented growth of pancreatic tumor cells, demonstrating that these cells require ligand stimulation for proliferation. Thus, uncontrolled activation of Hh signaling is sufficient to maintain tumor growth and progression, and may play a critical role in pancreatic tumor ontogeny.

More recently, studies analyzing the expression of HHIP, an endogenous inhibitor of the Hh pathway, present increasing evidence that upregulation of Hh signaling is linked to pancreatic cancers. Initially, growth inhibition in some pancreatic cancer lines was observed upon ectopic expression of recombinant mouse Hhip [69], suggesting that reduction of Hh antagonist activity is an important mechanism during cancer progression. Furthermore, silencing of HHIP by DNA methylation is common in pancreatic cancer cell lines [76]. In addition, complementary DNA (cDNA) array assays showed that several distinct foregut markers are upregulated in PanIN lesions, suggesting that increased Hh signaling might convert pancreatic duct cells into gastric cells [79]. These results indicate that elevated Hh signaling may mediate PanIN progression by changing the differentiation potential of pancreatic cells towards a gastric epithelial differentiation pathway.

Cumulatively, the current data implicate a participatory role for Hh signaling in pancreatic cancer. However cancer is a highly complex disorder usually caused by multiple factors. For example, mutations in the *K-RAS* gene are found in 99% of all human pancreatic adenocarcinomas [82, 83], and forced expression of the mutated gene in mouse pancreatic cells results in tumors that display all the hallmarks of the human disease. Introduction of signature mutations in the *p53* tumor suppressor gene can increase tumor progression in these mice. Interestingly, mice carrying both *K-ras* and *p53* mutations in pancreatic cells display increased levels of Hh signaling in the forming tumor lesions [84]. Given the observation that forced expression of *Shh* in pancreatic cells is sufficient to induce the most common mutation in the *K-ras* gene, it will be important to determine whether *K-ras* induced tumors can form when Hh signaling is ablated in transgenic mice. These studies will shed light on the question of whether pancreatic adenocarcinoma requires both K-ras and Hh deregulation for tumor progression, and could lead to novel insights into clinical treatments for this dreaded disease.

Future directions

As our understanding of Hh signaling in pancreas grows, a number of questions remain unanswered. In addition to maintaining proper organ boundaries, could Hh signaling regulate differentiation states or maintain the appropriate number of differentiated cells within the developing and adult pancreas? Examination of pancreatic diseases with associations to deregulated Hh signaling suggests that this may be so. If so, are these effects mediated through direct or indirect interactions of Hh signaling on the epithelium? Future studies assessing tissue-specific loss of Hh signaling will clarify some of these questions. Little is known about the normal function of Hh signaling in adult tissues. Does the pathway function to modulate appropriate insulin production and secretion in β-cells as suggested by cell culture studies? In humans, is Hh signaling active in a small subset of adult duct cells where it might control cell proliferation? The advent of transgenic regulation of pathway activity in adult tissues will allow us to study these questions in the future.

Another unresolved question concerns the mechanisms by which Hh signaling causes pancreatic disease. Is altered Hh activity linked to the deregulation of other developmental signaling pathways such as Wnt and Notch signaling? Indeed, Hh, Wnt and Notch signaling have independently been implicated in tumor progression of multiple organs, including the blood, brain, skin, colon and pancreas [78, 85–87]. Moreover, evidence that these pathways influence each other during tumor progression is accumulating [44, 88–90]. While interactions among these pathways in pancreatic diseases are currently missing, future experiments that address changes in Hh signaling in the context of altered Wnt or Notch signaling may shed further light on the molecular processes that underlie formation and progression of pancreatic diseases such as pancreatic cancer. More importantly, these studies may reveal requirements of key molecules for tumor survival and thus could lead to novel therapies for treatment of pancreatic cancer patients.

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