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The Role of Sonic Hedgehog as a Regulator of Gastric Function and Differentiation

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Abstract

The Hedgehog (Hh) genes play a key role in the regulation of embryonic development and govern processes such as cell differentiation, cell proliferation, and tissue patterning. In vertebrate embryos, Hh gene expression regulates correct formation of limbs, skeleton, muscles, and organs including stomach. In the adult, the Hh pathway functions in tissue repair and regeneration, along with maintenance of stem cells. Sonic Hedgehog (Shh) signaling has been extensively studied for its role in developmental and cancer biology. Recent advances in the field of gastroenterology show that in the stomach, Shh is responsible for proper differentiation of the gastric glands. The aberrant activity of the Shh signaling pathway leads to an altered gastric differentiation program and loss of gastric acid secretion that is the predominant function of the stomach. In this chapter, we review the most recent findings that reveal the role of Shh as a regulator of gastric function and differentiation and how this signaling is dysregulated during the development of gastric cancer in response bacterial infection.

I. Introduction

In 1980, using a mutagenesis screen in *Drosophila*, Nüsslein-Volhard and Wieschaus first discovered the Hedgehog (Hh) gene (Nüsslein-Volhard and Wieschaus, 1980). In these experiments, it was demonstrated that one of these mutants alters the patterning within the segment in the larva. In the wild-type *Drosophila*, each segment contained an anterior half covered with the presence of a band of denticles and a posterior half that was smooth. In the mutant, each segment was depleted of the smooth posterior half with only the anterior half present, causing the entire larva to be covered by denticles that resembled a “Hedgehog,” and thus the origin of this funny name (Nüsslein-Volhard and Wieschaus, 1980). Later, the corresponding Hh gene was described in *Drosophila*, followed by the identification of three Hh homologs that are highly conserved from the fruit fly to humans. The three homologs were named Sonic Hedgehog (Shh), named after a Sega game character, Desert Hedgehog (Dhh), based on an Egyptian species of Hh and Indian Hedgehog (Ihh), a species of Hh that resides in Pakistan. Although little is known about how the transcription of these genes is controlled, a quite comprehensive recognition about the protein products has been achieved by intensive studies, such as the protein secretion, the signaling induction, and the physiological function. In the stomach, Hh is known to control cellular functions including proliferation, differentiation, and acid secretion. Therefore, identifying the role of Shh as a

regulator of adult stomach physiological function and differentiation is important for understanding the pathogenesis of gastric disease.

II. Hh in the Adult Stomach

A. Shh expression and processing

Shh is synthesized as a 45kDa precursor protein, containing a 19kDa amino-terminal fragment and a 26kDa carboxy-terminal fragment. The 26kDa fragment is the known enzymatic component that cleaves the precursor protein into two fragments and catalyzes the transfer of a cholesterol moiety to the carboxy terminus of the 19kDa fragment. The cholesterol-modified 19kDa fragment contains all signaling activities but requires further modification by a palmitate group at its amino-terminal end (Martí *et al.*, 1995; Lee *et al.*, 1994; Porter *et al.*, 1995). This palmitoylation is catalyzed by a membrane-bound *O*-acyltransferase that is commonly known as Skinny Hedgehog (Ski) and is essential to the biological activity of the processed Hh proteins (Chen *et al.*, 2004).

Although most of the initial studies of Hh processing were performed in invertebrate systems, our understanding of Hh processing in the mammalian system has been advanced by Zavros *et al.* (2007). By using primary parietal cell cultures and tissue extracts from mouse and human stomachs, Zavros *et al.* (2007) demonstrated that Shh is processed by an acid- and protease-dependent mechanism. Pepsin A processing is acid dependent where acid facilitates the conversion from the inactive pre-enzyme pepsinogen A to the active enzyme pepsin. The investigators explain that the absence of processed Shh in human gastric cancer tissue may be a consequence of hypochlorhydria present due to the parietal cell atrophy (Zavros *et al.*, 2007). Interestingly, recently, it is revealed that the unprocessed full-length Shh precursor protein is also biologically active (Tokhunts *et al.*, 2010). It is shown that full-length Shh also binds to Patched (Ptch) and induces the similar target gene expression in the developing chick neural tube and rescues the eye developmental defect in *Drosophila* Hh mutants (Tokhunts *et al.*, 2010). Whether the full-length and processed Shh ligands play the same role in the adult tissues is unknown but may have significant implications in the role of Shh in the proliferation of cancer stem cells.

Shh processing is also shown to be regulated by gastrin (El-Zaatari *et al.*, 2010; Zavros *et al.*, 2007). Studies using a mouse model of gastrin deficiency show that there is a significant decrease in Shh protein expression, and the reinfusion of gastrin successfully restored Shh expression, indicating the important regulatory role of gastrin (Zavros *et al.*, 2007). In addition, recent studies by Xiao *et al.* using a mouse model expressing a parietal cell-specific deletion of Shh (HKCre/Shh^{KO}) demonstrated that these mice developed severe hypergastrinemia reflective of increased circulating gastrin concentration (Xiao *et al.*, 2010a,b). Xiao *et al.* also showed that with loss of Shh and accompanied hypergastrinemia, mice expressed increased Ihh within the surface mucous pit epithelium. Somatostatin is a known physiological inhibitor of gastrin and when HKCre/Shh^{KO} mice were treated with the somatostatin analogue octreotide, hypergastrinemia was normalized and Ihh expression decreased to levels similar to controls groups (Xiao *et al.*, 2010a,b). Such data suggested that gastrin not only regulates Shh processing but also has the potential to also regulate Ihh.

Using isolated canine parietal cell and mouse organ cultures to identify the precise mechanism that gastrin regulates Shh, El-Zaatari *et al.* recently demonstrated that intracellular calcium release and protein kinase C (PKC) activation stimulate Shh gene expression (El-Zaatari *et al.*, 2010). Based on previous reports that intracellular calcium release stimulates acid secretion in parietal cells, gadolinium-, thapsigargin-, and carbachol-mediated release of intracellular calcium induced Shh expression (El-Zaatari *et al.*, 2010).

The role of the gastric hormone gastrin in acid secretion of the stomach was first recognized by Edkins (1905). The gastrin gene located on chromosomes 17q21 is expressed by a variety of cell in the body. Among them, gastrin is predominantly manufactured by the antral mucosa of the stomach, where G-cells secrete this peptide hormone for the purpose of stimulating gastric acid secretion (McGuigan, 1968; Taylor *et al.*, 1982). The actions of gastrin are mediated by G-protein-coupled receptors, cholecystokinin (CCK)/gastrin receptors. Two types of CCK receptors, CCK₁-R and CCK₂-R (formerly CCKAR and CCKBR), have been identified. CCK₁-R has high affinity for CCK (reviewed in Noble and Roques, 1999), another gastrointestinal hormone regulating digestion. The CCK₂-R, originally defined in central nervous system, has a high affinity for both CCK and gastrin (Dufresne *et al.*, 2006a,b).

In stomach, CCK₂-R is mainly located on parietal cells (Kopin *et al.*, 1992) and enterochromaffin-like (ECL) cells within gastric mucosa, where it mediates action of gastrin in acid secretion (reviewed in Dockray *et al.*, 2005). The precise cellular localization of the CCK₂-R by *in situ* hybridization and immunohistochemistry demonstrates that expression is also detected in gastric precursor cells (Kulaksiz *et al.*, 2000). Presence of CCK₂-R on precursor cells supports a number of previously unrecognized roles for gastrin as a regulator of cellular proliferation, migration, and differentiation.

Like typical G-protein-coupled receptor, gastrin binding with CCK₂-R activates classical signaling pathway, such as phospholipase C (PLC)- β /diacylglycerol (DAG)/Ca²⁺/PKC cascade or the adenylyl cyclase (AC) pathway (reviewed in Dufresne *et al.*, 2006a,b). Binding of gastrin activates Gq-coupled receptor, which subsequently activates the PLC family (principally PLC- β isoforms). PLC then hydrolyzed plasma membrane-bound phosphatidyl inositol 4,5-bisphosphate (PIP₂) into the second messengers inositol trisphosphate (IP₃) and DAG. IP₃ binds to a ligand-gated Ca²⁺ channels in ER and release Ca²⁺ from intracellular stores, while DAG activates PKC (Roche *et al.*, 1990; Yassin and Murthy, 1991). PKC and/or Ca²⁺ mobilization, in turn, activate a cascade of mitogen-activated protein kinases (MAPKs), including extracellular signal-related kinase (ERK) and c-jun NH₂-terminal kinase (JNK) in several studied cell models (Hocker, 2004; Höcker *et al.*, 1997). Activation of ERK-dependent transcription factors has been shown to be involved in cell growth, differentiation, survival, and apoptosis (Daulhac *et al.*, 1999; Dockray *et al.*, 2005). CCK₂-R also induces several other signaling pathways that are activated by tyrosine kinase receptors (Dabrowski *et al.*, 1997; Stepan *et al.*, 1999). In addition to the signaling pathways described above that mediate acid secretion, cellular proliferation, and migration, the transcription factor NF- κ B activated by CCK₂-R leads to the expression of interleukin-8 and other proinflammatory gene products (Hiraoka *et al.*, 2001). Through signaling, gastrin increases Ca²⁺ release in target cells (Tsunoda *et al.*, 1988). Thus, the increase of intracellular Ca²⁺ by

gastrin may also be a plausible mechanism regulating *Ihh* gene expression but requires further investigation (Fig. 21.1).

B. Hh signaling in the adult stomach

In vertebrates, the Hh signal is triggered by Hh proteins and transmitted by Smoothened (Smo) that is a seven-span transmembrane receptor. However, Hh proteins do not activate Smo through a direct interaction. Instead, they regulate the activity of Smo via a Ptch-mediated mechanism. Ptch is a 12-span transmembrane protein receptor for Hh. In the absence of Hh, Ptch inhibits Smo (Bijlsma *et al.*, 2006). Binding of Hh to Ptch terminates the inhibitory restraint on Smo and enables Smo to relay the signaling cascade. Activated Smo prevents the processing of Hh transcription factor—Glioblastoma (Gli) family proteins into their repressor forms (Gli-R), causing the accumulation of the transcriptional activators (Gli-A). Gli-A then induces the transcription of Hh-responsive target genes including some Hh pathway components themselves, such as Ptch and Gli1 (reviewed in van den Brink, 2007).

Recent studies have provided emerging evidence for the existence of a “noncanonical” pathway that may be Gli- and Smo independent. Studies of nervous system development in chick embryos showed that Shh expression is crucial for normal neural cell migration while Smo antagonist cyclopamine or interference RNA targeting Smo did not alter this process, providing evidence for the involvement of Shh in a noncanonical and Smo-independent pathway (Bourikas *et al.*, 2005; Jarov *et al.*, 2003; Testaz *et al.*, 2001). In a Gli1 null mutant mouse model, injection of Shh-expressing retroviral vectors into the embryonic central nervous system induces medulloblastoma, suggesting Gli1 is not required for Shh to initiate tumorigenesis in the brain (Weiner *et al.*, 2002). Further support for the existence of such a noncanonical Hh signaling pathway comes from a recent *in vitro* study using C3H10T1/2 mouse fibroblast cells treated with soluble Shh protein. Shh-treated mouse fibroblast cells exhibited actin cytoskeletal reorganization within 10min, a time frame much shorter than required to induce the expression of Hh-responsive target genes after treatment (Bijlsma *et al.*, 2007). Another *in vitro* study using 293T cells identifies a physiological interaction between Ptch1 and CyclinB1, a critical cell division regulator whose phosphorylation and nuclear entry promote G2/M phase cell cycle progression. In this study, the Ptch1-CyclinB1 complex exhibits an antiproliferative effect on 293T cells. Exposure of these cells to Shh dissociates this complex, relocalizes CyclinB1 into the nucleus, and reverses the cell proliferation, suggesting Shh and Ptch1 participate in G2/M phase checkpoint through regulation of the cellular distribution of CyclinB1 (Barnes *et al.*, 2001). The interaction between Ptch and CyclinB1 has been further confirmed in Madin-Darby canine kidney (MDCK) cells where Ptch1 is colocalized with endogenous CyclinB1 at the apical surface. Treatment of MDCK cells with Shh causes degradation of Ptch1 accompanied by cytoplasmic and nuclear relocalization of CyclinB1 (Jenkins, 2009). In an *in vivo*, Ptch1^{-/-} mouse model, ablation of Ptch1 results in increased number in basal cells expressing nuclear CyclinB1 and formation of skin tumors arising from Ptch1-ablated cells, further supporting the role of Ptch in G2/M phase checkpoint (Adolphe *et al.*, 2006). Collectively, these data suggest the existence of a noncanonical Hh signaling pathway that regulates cell proliferation in a Smo- and Gli-independent and CyclinB1-mediated mechanism.

C. Hh as a regulator of gastric epithelial cell function and differentiation

Several studies indicate that Shh functions as a morphogen in the adult stomach to guide gastric epithelial cell differentiation and function. The first of these studies treated mice with the Hh inhibitor cyclopamine demonstrating enhanced gastric epithelial cell proliferation and diminished protein expression of BMP4, islet-1, and Hnf3 β , all of which are transcription targets of Shh signaling participating in tissue-specific differentiation (van den Brink *et al.*, 2001). Subsequent studies further indicated that down-regulating Shh expression is a mechanism by which *Helicobacter pylori* infection triggers gastric cancer, since the consequence of *H. pylori* infection is parietal cell atrophy and gastric hypochlorhydria. Shh positive cells are dramatically reduced in the intestinal metaplastic areas of *H. pylori*-positive human stomach (Shiotani *et al.*, 2005a,b). Other studies suggest that *H. pylori* infection may be the direct cause of downregulation of Shh in human gastric mucosa and that the early eradication restores the expression of Shh and the differentiation of the stomach (Shiotani *et al.*, 2006, 2007). The causative relationship between *H. pylori* infection and decreased Shh expression has been further confirmed by a study in Mongolian gerbils (Suzuki *et al.*, 2005), an established animal model of *H. pylori* infection recapitulating the histopathological changes observed in humans (Hirayama *et al.*, 1996; Watanabe *et al.*, 1998). Recent work by Waghray *et al.* (2010) using a mouse model of *H. pylori* infection, it was demonstrated that Shh gene expression in parietal cells is suppressed and precedes parietal cell atrophy. In the study, the inhibitory effect of *H. pylori* on Shh expression was mediated by the proinflammatory cytokine interleukin-1 β via a reduction in acid secretion and intracellular calcium release (Waghray *et al.*, 2010). Together, these studies support the hypothesis of the *H. pylori* infection results in loss of Shh that subsequently contributes to preneoplastic transformation and eventually the development of gastric cancer. Nevertheless, all above evidence are built on the observations or studies that are associated with *H. pylori* infection. *H. pylori* infection induces not only repressed Shh expression but also complicated immune and inflammatory response, thus it is hard to verify the weight of loss of Shh *per se* in contributing to the development of gastric cancer.

The development of a mouse model expressing a parietal cell-specific deletion of Shh (HKCre/Shh^{KO} mice) has allowed us to assay changes in gastric epithelial cell differentiation and function independent of factors such as inflammation induced by *H. pylori* infection (Xiao *et al.*, 2010a,b). Compared to the normal gastric pathology of the control animals in these studies, every HKCre/Shh^{KO} mouse demonstrated an age-dependent increase in the number of surface pit mucous cells that was reminiscent of foveolar hyperplasia. Interestingly, the phenotype observed in the HKCre/Shh^{KO} mouse was similar to that observed in the overexpressing TGF α transgenic mice (Bockman *et al.*, 1995; Goldenring *et al.*, 1996; Nomura *et al.*, 2005) and in patients with Menetrier's disease (Larsen *et al.*, 1987; Wolfsen *et al.*, 1993). However, unlike both Menetrier's disease and *H. pylori*-infected patients, the HKCre/Shh^{KO} mouse model did not develop atrophy. Given that HKCre/Shh^{KO} mice lacked inflammation, additional factors are required, such as inflammatory cytokines, for parietal cell atrophy to occur (Xiao *et al.*, 2010a,b).

Emerging evidence also suggests that regulating the formation of cell junctions may be one of the mechanisms by which Hh signaling controls the homeostasis of gastric epithelium.

The maintenance of functional adherens- and tight junctions is essential for epithelial morphology, physiological function, and differentiation. A study in rat kidney epithelial cells (RK3E) shows that Snail, a suppressor of E-cadherin, is a transcriptional target of Hh signaling (Li *et al.*, 2006), suggesting Hh signaling is involved in the regulation of E-cadherin expression. E-cadherin is a transmembrane protein with its intracellular segment directly binding to β -catenin, which, in turn, binds to actin filament binding protein α -catenin, to form the adherens junction complex. The adherens junction cadherin/ β -catenin/ α -catenin protein complex is morphologically associated with actin filaments. Loss of E-cadherin expression has been involved in epithelial-to-mesenchymal transition (EMT). EMT is characterized by loss of connections between cells, expression of mesenchymal markers within epithelial cells, and increased cell motility. This molecular event is necessary for embryonic organogenesis, inflammatory response, and normal wound healing; however, under certain pathological condition such as cancer, EMT contributes to tumor invasion and metastasis. In immortalized human pancreatic ductal epithelial cells, Gli1 overexpression causes almost a complete loss of E-cadherin and an invasive phenotype of transformation. In contrast, in invasive pancreatic cancer cell lines, treatment of cyclopamine represses Snail and restores E-cadherin expression, thus inhibiting EMT and invasive capacity *in vitro* (Feldmann *et al.*, 2007). Loss of membrane-bound E-cadherin and abnormal β -catenin localization is also observed in gastric cancer with a correlation with the risk of early submucosal invasion and lymphoid node metastasis (Cheng *et al.*, 2005; Tanaka *et al.*, 2002).

The integrity of adherens junctions is a crucial factor determining epithelial morphology, physiological function, and differentiation (Braga, 2002; Braga *et al.*, 1997; Hopkins *et al.*, 2003; Samarin *et al.*, 2007). The HKCre/Shh^{KO} mice were later used to demonstrate that Shh signaling regulates cell-cell adhesion (Xiao *et al.*, 2010a,b). Recent studies from our laboratory using the HKCre/Shh^{KO} demonstrated that the loss of Shh triggers a number of molecular changes (Xiao *et al.*, 2010a,b) that were consistent with EMT of gastric epithelial cells (Ikenouchi *et al.*, 2003; Li *et al.*, 2006). Such molecular changes included loss of E-cadherin expression and translocation of β -catenin to the nucleus (Xiao *et al.*, 2010a,b). Nuclear translocation of β -catenin binds to the DNA-binding proteins Tcf/Lef1 that subsequently regulates target genes including CyclinD1 (Nelson and Nusse, 2004) important in proliferation (Luo *et al.*, 2008). Consistent with our published data, the HKCre/Shh^{KO} mice also exhibited hyperproliferation of the surface mucous cells (Xiao *et al.*, 2010a,b). Hyperproliferation of surface mucous cells often disrupts the differentiation of other cell lineages such as the zymogen cells (Bredemeyer *et al.*, 2009). The HKCre/Shh^{KO} mice have a delay in the differentiation of zymogen cells from mucous neck cells, whereby zymogen cells located in the base of the gland frequently coexpressed mucous neck cell markers (Xiao *et al.*, 2010a,b). Intriguingly, disruption of the Hh signaling pathway in the transduced IMGE-5^{ski} cells resulted in the loss of zymogen cell marker pepsinogen and expression of mucous neck cell marker MUC6 when compared to the IMGE-5^{scram} controls. Expression of MUC6 in IMGE-5^{ski} cells was accompanied by an increase in cell proliferation and dissociation of the E-cadherin/ β -catenin complex (Xiao *et al.*, 2010a,b). In support of our findings, Bredemeyer *et al.* (2009) discovered that in a model knocking out the cytoskeleton-regulating gene *Cd2ap* in *Mist1*^{-/-} mice, zymogen cells reorganize their

cytoskeleton as they differentiate to form a polarized epithelium. Our data support findings that show that aberrant E-cadherin expression causes changes in zymogenic cell morphology and differentiation (Bredemeyer *et al.*, 2009).

Our recent studies demonstrate that adherens junction protein E-cadherin may be a downstream target of the Hh signaling pathway. Shh regulates critical pathways in epithelial cell differentiation but we are only beginning to understand the mechanism (Shiotani *et al.*, 2005a,b; Stepan *et al.*, 2005; Suzuki *et al.*, 2005; van den Brink *et al.*, 2002; Xiao *et al.*, 2010a). The integrity of adherens junctions is a crucial factor determining not only epithelial morphology, physiological function, and differentiation (Braga, 2002; Braga *et al.*, 1997; Hopkins *et al.*, 2003; Samarin *et al.*, 2007) but also tumor growth, invasiveness, and metastasis (reviewed in Birchmeier *et al.*, 1993; Yilmaz and Christofori, 2009). Given the crucial role of Shh during gastric homeostasis and disease, understanding the role of Hh signaling as a regulator of the adherens junctions advances our knowledge by which Shh may regulate gastric epithelial cell physiology and pathophysiology.

III. The Potential Role of Sonic Hh as a Gastric Hormone

The predominant gastric hormone gastrin has two major roles in the gastrointestinal tract, the first of which is the major role in stimulation of gastric acid secretion in the stomach. Gastrin released from G-cells in the gastric antrum acts upon ECL cells, stimulating the release of histamine, again dependent on increased intracellular Ca^{2+} both from stores and from activation of calcium channels (Prinz *et al.*, 1993; Zeng *et al.*, 1996). Histamine then interacts with parietal cells, stimulating the upregulation of apical H^+/K^+ proton pumps and thus the secretion of acid. Gastrin and Ach also directly bind with the receptor on parietal cells, increasing acid secretion. Therefore, the role of gastrin in acid secretion is either direct or indirect by increasing histamine release. As acid increases, D endocrine cells release somatostatin, which acts as an inhibitory regulator on parietal cells to inhibit acid secretion and on ECL cells to inhibit histamine secretion (Allen *et al.*, 1986). As a result, acid secretion is regulated by this negative feedback control mechanism.

Gastrin also stimulates and maintains the proliferation of the gastric epithelium (reviewed in Dockray, 1999). Hypertrophic gastric mucosa is observed in patients with gastrin-producing tumors and also in rats infused continuously with gastrin (Ryberg *et al.*, 1990). In contrast, hypotrophic or atrophic mucosa results from fasting in rats whose serum gastrin levels become low (Solomon, 1986). Gastrin does this by upregulating its own receptor expressing on ECL cells and on mucous neck cells in the gastric mucosa, which can further differentiate into other cells types, including parietal cells and surface pit cells. The acceptance of gastrin as a growth factor for the gastric mucosa is also proven by trophic effect of gastrin and its receptor $\text{CCK}_2\text{-R}$ during gastric regeneration. CCK_2 receptors are expressed and localized to the regenerative mucosa in the ulcer margin. Binding with the receptors, gastrin shows significant trophic effects on ulcer margin, enhancing wound healing (Ashurst *et al.*, 2008; Schmassmann and Reubi, 2002).

Another significant characteristic of the $\text{HKCre/Shh}^{\text{KO}}$ mice was the lack of acid secretion in response to histamine stimulation. Ultrastructural analysis showed altered secretory

canalicular membranes that were distorted and disorganized in the HKCre/Shh^{KO} parietal cells. Similar changes have recently been reported in the Huntington-interacting protein 1-related 1 (Hip1r)-deficient mouse model (Jain *et al.*, 2008). The Hip1r protein has been shown to be crucial for the normal dynamics of the secretory canalicular membrane of the parietal cells (Jain *et al.*, 2008). In addition, similar parietal cell morphological changes were observed between the HKCre/Shh^{KO} mouse and the basolateral Ae2 Cl^-/HCO_3^- exchanger (Slc4a2)-deficient, Atp4a(−/−) and NHE4 mice (Recalde *et al.*, 2006). Collectively, such findings suggest that Shh may be involved in the development of the secretory membrane dynamics that is involved in parietal cell function.

The HKCre/Shh^{KO} mice lacked the ability to secrete acid that was accompanied by severe hypergastrinemia and decreased somatostatin expression (Xiao *et al.*, 2010a,b). Hypergastrinemia has been reported previously in a pharmacological model of Hh signaling inhibition, using cyclopamine (El-Zaatari *et al.*, 2008). In fact, treatment of HKCre/Shh^{KO} mice, with the somatostatin analogue octreotide, significantly suppressed hypergastrinemia and subsequently hyperproliferation. Hypergastrinemia was associated with increased Ihh consistent with observations made in human stomach where Ihh was predominantly expressed in the pit cells where it induces pit cell differentiation in primary mouse gastric cells, while Shh in the gland/neck region (Fukaya *et al.*, 2006). Octreotide treatment of the HKCre/Shh^{KO} mice resulted in decreased Ihh expression similar to levels measured in the control group (Xiao *et al.*, 2010a,b). We propose that the increased Ihh gene expression may be a consequence of hypergastrinemia. Further studies using the gastrin-deficient mouse model and the current HKCre/Shh^{KO} mice will be necessary to test the role of gastrin as a direct regulator of Ihh and hyperproliferation. Thus, the phenotype observed with loss of Shh may be attributed to increases in circulating gastrin concentrations due to loss of somatostatin. Our current understanding of hypergastrinemia is now extended because, besides the proposed role as a morphogen for the gastric epithelium, Shh may also be a fundamental regulator of the gastrin–gastric acid negative feedback mechanism (Fig. 21.2).

The potential role of Shh to act as a gastric hormone would suggest that the protein would be secreted from the parietal cell via the basolateral membrane. The first suggestion for the secretion of Shh from the basolateral membrane came from our earlier studies using isolated rabbit gastric glands demonstrating that Shh expressed not only in parietal cells and associated with the H⁺,K⁺-ATPase-containing tubulovesicles and secretory membranes but also colocalized with the gamma-actin at the basolateral membrane (Zavros *et al.*, 2008). Moreover, in a polarized Shh- and H⁺,K⁺-ATPase-expressing human gastric cancer cell line (23132/87) (Dimmler *et al.*, 2003), histamine treatment induces secretion of the processed 19kDa Shh protein from both the apical and basolateral membranes (Zavros *et al.*, 2008). Interestingly, recent investigations during development also show the trafficking of Hh to the basolateral membrane to form a morphogenetic gradient in the *Drosophila* wing imaginal disk epithelium (Callejo *et al.*, 2011). Functional analysis of the 12-transmembrane protein Dispatched, the glypican Dally-like protein (Dlp), and the Ig-like and FNIII domains of protein, Interference Hh (Ihog) revealed that Dispatched may be involved in the regulation of vesicular trafficking for basolateral release of Hh, Dlp, and Ihog (Callejo *et al.*, 2011). This important study also revealed that during development, Dlp is needed in the Hh-

producing cells to allow for protein release. Ihog, a Hh coreceptor, then anchors Hh to the basolateral part of the disk epithelium (Callejo *et al.*, 2011). Whether a similar mechanism regulates the release of basolateral Shh from the gastric parietal cell remains to be determined. However, collectively these findings support the potential role of Shh as gastric hormone in particular as a regulator of acid secretion.

IV. Conclusions and Future Directions

Shh is a parietal cell-secreted protein regulating gastric cell proliferation, differentiation, and function. Recent studies reveal that Shh may be secreted basolaterally into the circulation not only during development (Callejo *et al.*, 2011) but also during gastric acid secretion (Zavros *et al.*, 2008). These exciting findings advance our understanding of the role of Shh as a potential hormone and chemoattractant. Dunaeva *et al.* shows that Shh activates classical intracellular signal transduction pathways related to human monocytes recruitment *in vitro* (Dunaeva *et al.*, 2010). An intriguing question that remains is whether Shh acts as a chemokine for monocyte recruitment during *H. pylori* infection. Another question that remains unanswered is the identity of the major organ producing Shh. What is the role of the stomach as a Shh-producing organ? Given the emerging role of Shh as a regulator of the immune response, adult tissue function and homeostasis and gastric disease, this appears to be a promising area of research and will certainly broaden our understanding of this developmental morphogen in adult stomach.

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Figure 21.1.

Proposed mechanism for the role of Ihh as a regulator of gastrin-induced proliferation. (A) Gastrin binding with Gq-associated CCKBR activates phospholipase C (PLC)-β which may regulate calcium mobilization by producing inositol trisphosphate (IP₃) and diacylglycerol (DAG). Ihh may be induced by gastrin in PKC/Ca²⁺-dependent mechanism. (B) Ihh secreted from the surface pit epithelium targets Gli positive mesenchymal cells, inducing activation of Wnt pathway. (C) Activation of the Wnt pathway causes translocation of β-catenin from cytoplasm to nucleus and production of CyclinD1. Wnt-mediated CyclinD1 is a key regulator of cell proliferation.

Figure 21.2.

Shh as a regulator of the gastrin–gastric acid negative feedback. (A) During normal gastric function, gastrin induces acid secretion that is accompanied by Shh processing and release from the parietal cell. As a regulator of gastric acid secretion, Shh may induce somatostatin (SOM) secretion, which, in turn, inhibits the gastrin production from G-cells and acid secretion from the parietal cells. (B) Loss of gastric Shh results in diminished production of SOM and the subsequent increase in serum gastrin concentrations (hypergastrinemia) and thus an overall disruption in the gastrin–gastric acid negative feedback mechanism.