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Sonic Hedgehog in Gastric Physiology and Neoplastic Transformation: Friend or Foe?

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Abstract

Purpose of review—To understand the role of sonic hedgehog (Shh) in normal gastric physiology and neoplastic transformation.

Recent Findings—Emerging evidence shows that gastric epithelial cells produce Shh ligand, which subsequently targets the mesenchyme. This paracrine signaling event is recapitulated by Shh-producing tumors that signal to the supporting stroma to encourage growth. Primary cilia contain components of the hedgehog (Hh) signaling apparatus, and thus are typically found on responding stromal cells.

Summary—In the stomach, Shh is produced in epithelial cells and received by responding cells in the mesenchyme. *In vitro*, Shh enhances gastric acid secretion and induces mucin expression. It remains to be determined whether the canonical signaling pathway mediates the observed epithelial effects. Shh expression and signaling is reduced in chronic gastritis, and $Shh^{-/-}$ embryos exhibit hyperplasia and metaplastic changes in the gastric mucosa. After its loss in the corpus, Shh is re-expressed in some gastric carcinomas typically arising in the distal stomach or antrum suggesting that Shh promotes tumor growth.

Keywords

paracrine; hypochlorhydria; inflammation; metaplasia; stomach cancer

Introduction

Pelayo Correa first described the chronology of histologic events in stomach transformation in which chronic inflammation (gastritis) sequentially leads to glandular atrophy, metaplasia then carcinoma [1]. Investigators considered the contribution of Shh to gastric carcinogenesis after observing that the $Shh^{-/-}$ mouse embryos exhibit preneoplastic changes, e.g., intestinal metaplasia, a lesion reminiscent of the changes occurring in human stomach [2,3]. Additional studies further implicated Shh in gastric carcinogenesis [2,4–6]. In this report, we will review the physiological role of Shh in the stomach, the effect of inflammation and hypochlorhydria on Shh gene expression and the impact of elevated Shh levels on neoplastic transformation.

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Role of Shh in the Normal Stomach

Although there are three hedgehog (Hh) family members in the mammalian gastric epithelium (Sonic, Indian, Desert), Sonic hedgehog (Shh) is the most frequently studied due to the gastric phenotype observed in the $Shh^{-/-}$ mice. Although Indian hedgehog (Ihh) is highly expressed, the Ihh knockout mice die at or before birth and do not have a gastric phenotype [2]. Similarly, the $Dhh^{-/-}$ mice are not known to have a stomach phenotype [7]. Shh is a peptide morphogen that is produced in gastric epithelial cells, e.g., pit, parietal, and zymogenic cells [8–11], but is relatively absent from the antrum. Although stromal cells express some Shh, unlike the gastric epithelium, these cells also robustly express the signal transduction components comprised of the 12-transmembrane receptor Patched-1 (Ptc1), Smoothened (Smo), a 7-transmembrane receptor and the Gli transcription factor family (Gli 1–3) [12]. There are no reports fully characterizing the nature of Shh-responsive cells in the stomach. However, detection of the Shh transcriptional target, Gli-1, in stromal cells at the base of the gastric mucosa [13] indicates that responding cells reside in the mesenchyme as reported for other tissues, e.g., the small intestine [14]. However, recent evidence has emerged showing that there are non-canonical pathways capable of activating Gli1, e.g., TGFβ and RAS, implicating activation of downstream Hh target genes by other ligands [15,16].

Recent studies have indicated that Shh regulates the proliferation and specification of epithelial cell types. In particular, Shh induces H^+/K^+-ATPase gene expression in parietal cells [17], and mucin gene expression in pit cells [11]. One study also showed that blocking Shh signaling with cyclopamine leads to increased gastric proliferation [10], while another study suggested that proliferation might be indirect, and occur in response to hypergastrinemia [9]. The hypergastrinemia that results from cyclopamine treatment might be due to the hypochlorhydria (low acid) triggered by Shh inhibition and reduced H/K-ATPase levels [17,18]. Thus, it remains to be determined whether Shh directly regulates proliferation in the gastric mucosa. Studies in $Shh^{-/-}$ embryos have shown that Shh increases epithelial cell survival by reducing the apoptotic rate in the stomach, but has no effect on the rate of proliferation [2,3]. Consistent with the latter result, Ma et al. showed that the Shh pathway induces gastric epithelial cell survival by inhibiting apoptotic activity [6]. In order to elucidate discrepancies concerning the physiological role of Shh in the stomach *in vivo*, it will be important to study mice that are conditionally null for Shh in the stomach [18].

Association between Shh and gastric acid

Shh expression and processing in the stomach is regulated by acid pH [19,20]. However, it is unclear how gastric acidity affects Shh transcription. Shh has been shown to induce H^+/K^+-ATPase gene expression and by this mechanism enhance secretagogue-stimulated acid secretion [17]. Moreover, Shh co-localizes with the H^+/K^+-ATPase enzyme [8–10]. Upon secretagogue stimulation, both H^+/K^+-ATPase and Shh translocate from the basolateral to the apical plasma membrane of parietal cells [8]. We confirmed that Shh overlaps with H/K-ATPase by observing movement of both molecules to the apical membrane with histamine (acid secretagogue) using both confocal microscopy and subcellular fractionation (Figure 1). Moreover, Shh is processed to its active 19 kDa form in the presence of acid, indicating that a substantial portion of the processing occurs after translocation to the apical surface [8]. Collectively, these results suggest that Shh is being secreted into the gastric lumen. Recent *in vitro* studies have revealed that Shh ectopically expressed in a polarized kidney epithelial cell line is secreted both apically and basolaterally with histamine treatment [8]. Nevertheless, how apical Shh activates responding cells in the mesenchyme remains speculative.

Signal transduction from epithelium to mesenchyme

Studies on *Drosophila* Hedgehog (Hh) have suggested that it can be carried by lipoprotein particles that migrate far from the site of synthesis to form a gradient of Hh molecules [21]. While lipoprotein molecules traverse cellular layers through passive diffusion, further studies in mammalian cells have suggested that the lipoprotein receptor-related protein (LRP-2), called Megalin, is involved in transcytosis of Shh across epithelial cells [22]. Another transport model proposed states that Shh molecules form multimeric complexes, analogous to micelles, with lipid embedded in the core of such complexes [23]. The common feature of both models is that lipid has the ability to transport Shh through the epithelium either through passive diffusion or energy-requiring transcytosis. These models could explain how Shh moves from the lumen through the epithelial cell to the mesenchyme. Mesenchymal cells respond to Shh by inducing target gene expression, e.g., Gli1, while epithelial cells show no effect [24]. In fact, the mechanisms by which the canonical Hh pathway operates were initially analyzed in a fibroblast cell line COS-7 and subsequently in other mesenchymal-derived cell lines, e.g., NIH3T3 and C3H10T1/2, but were not studied in epithelial-derived cells [25]. Thus, canonical Hh signaling might only apply to the mesenchyme or stroma and, not to the epithelium.

Recently, the subcellular localization of Hh signaling components has been detected in primary cilia. Therefore several reports have linked Shh signaling to this organelle [26–28]. Primary cilia are solitary organelles that protrude from the plasma membrane, and are comprised of microtubules organized in a 9+0 array of the dynein arms. These structures once believed to be insignificant vestigial remnants of flagella are now thought to be important in receiving information from the extracellular environment [29]. For example, primary cilia can sense environmental cues such as hydrostatic and osmotic pressure [30], or in the brain, chemosensory signals that activate through the somatostatin receptor 3 on the surface of neurons [31]. In current models, Ptc1 localized on primary cilia prevents Smo from accumulating in the cilia. Once the Shh ligand engages the Ptc receptor, Smo accumulates in the cilia, Hh signaling is initiated and Gli factors also detected within the cilia are released from a multiprotein complex to translocate to the nucleus [26,28,32]. These observations have led some to conclude that Smo and Gli must interact within cilia to initiate signaling [26,28]. Assuming that canonical Shh signaling also operates in the stomach, the importance of primary cilia or alternative means of sensing Hh ligands in the environment have yet to be investigated in this tissue. The presence of cilia has previously been reported in the stomachs of human patients with ciliated metaplasia [33], and in mice lacking the H^+/K^+-ATPase alpha-subunit [34], but whether these gastric cilia are associated with Shh signaling has not been examined.

Inflammation and Hh signal transduction

The sequence of events leading to gastric carcinoma begins with inflammation, generally initiated by *Helicobacter pylori* (*H. pylori*) and thus has been designated a class 1 (definitive) carcinogen [1,35]. Experiments in Mongolian gerbils have shown that Shh expression is down-regulated in response to *Helicobacter*-induced metaplasia in the stomach [36]. Since the *H. pylori*–infected stomach can also induce hypochlorhydria, it remains to be determined whether it is the lack of acid or presence of inflammation that is responsible for the changes in Shh expression.

Chronic inflammation in the stomach, usually triggered by *H. pylori* infection, consists of T helper type 1 cells (Th1), neutrophils and macrophages. Th1 cells mediate the innate immune response by producing interferon gamma (IFNγ), interleukin-2 (IL-2), and tumor necrosis factor alpha (TNFα), while macrophages secrete IL-1β, IL-8 and TGFβ cytokines. Not many studies have examined the effect of inflammation on Shh expression. One report showed that Shh expression increased with gastritis [37], and another showed that IL-8 induces Shh

expression in gastric cancer epithelial cells [13]. Both Shh and KC, the mouse homolog of IL-8 were up-regulated in gastric tumors of the insulin-gastrin (INS-Gas) mouse model infected with *H. felis* [13]. There are no other reports on the effects of inflammation on Shh in the stomach, but inferences can be drawn from other tissue types. For example in the cerebellum, overexpression of the Th1 cytokine IFNγ in astrocytes was sufficient to induce Shh expression and promote medulloblastoma development [38]. Another study on pancreatic cancer showed that the activation of nuclear factor kappa beta (NFκB) by IL-1β and TNFα, induced activation of the Hh signaling pathway, which was essential for tumor cell proliferation [39].

The cytokine TGFβ induces Hh signaling by regulating Gli-1 and Gli-2 in a variety of transformed cell lines from lung fibroblasts, keratinocytes, breast, and pancreas. However this activation was independent of Shh ligand suggesting non-canonical signal transduction playing a significant role in promoting a cancer phenotype [40]. In contrast, TGFβ activation of the canonical Hh signaling is observed in human embryonic stem cells [41]. Since Th1 cytokines are released during *H. pylori*-induced gastritis, these cytokines might also be direct regulators of the Shh pathway in the stomach, as observed for the cerebellum. Yet, it remains to be elucidated whether the loss of Shh during chronic gastritis and atrophy occurs in direct response to suppression by cytokines, or an indirect response to sustained hypochlorhydria triggered by *H. pylori* infection. In either case, the regulation of Shh influences the development of preneoplastic lesions and thus might contribute to gastric carcinogenesis.

Shh and neoplastic transformation in the stomach

The Shh pathway has been shown to be necessary and sufficient for tumor growth in several tissues, e.g., medulloblastomas [42], basal cell carcinoma [43], malignant cartilage chondrosarcomas [44], prostate [45], non-small cell lung [46], hepatocellular [47], and colorectal carcinomas [48]. Medulloblastomas and basal cell carcinomas are examples of malignant tumors that develop due to a mutation in the Ptc1 receptor [49]. Using a transgenic mouse model heterozygous for inactivating mutations of Ptc1, overactive Hh signaling was found to be sufficient for medulloblastoma development [50]. Inhibition of the Shh pathway reduced the proliferation of this type of pediatric brain tumor [51] and caused the regression of medulloblastoma allografts *in vivo* [52]. Inducible expression of Shh revealed that Hh signaling was required for tumor initiation, but not progression [53]. By contrast, other Hh-dependent tumors produce high levels of ligand, which in turn are thought to support tumor growth through activation of target genes in the mesenchyme [49]. In both instances, therapies are directed towards blocking Hh signaling [4,24,54].

Gastric cancers tend to exhibit high levels of Shh ligand and not exhibit mutations in the Ptc1 receptor. Shh mRNA has been detected in a large number of gastric carcinoma cell lines including SNU1, SNU16, NCI-N87, MKN1, MKN7, MKN45, MKN74, 23132/87 and SIIA [4–6,19]. In one report, Shh was overexpressed in epithelial cells of gastric tumors; whereas the target genes Ptc1 and Gli1 were expressed in the supporting stromal cells [13]. These observations suggest that Shh signaling might be paracrine in at least a subset of gastric cancers [13] as observed in xenograft tumor models [24]. However, the effect of Shh on gastric tumor growth has not been well documented. In particular, inhibitors of Hh signaling have only been examined *in vitro* on gastric epithelial cell lines [4–6], with no reports demonstrating that Shh is required for gastric tumor development *in vivo*. Therefore, our understanding of Shh in gastric tumor development is incomplete, and is primarily based upon correlating elevated tissue levels with the presence of malignant histology [5,6,13]. In addition, several human and rodent studies have correlated the presence of gastritis with reduced Shh expression in the corpus [36,55, 56]. These observations raise questions as to the significance of reduced Shh expression observed in the oxyntic gland as an etiologic event for neoplastic transformation in the distal stomach. The distal stomach or antrum typically expresses significantly lower levels of Shh

compared to the normal corpus. Therefore, when gastric cancers arise in the distal stomach, Shh expression increases compared to the normal antrum. Future studies incorporating transgenic modulation of Shh expression (increased or decreased) will be essential to clarifying the role Shh plays in these two gastric compartments in normal physiology and during carcinogenesis [8].

Conclusion

The role of Shh in normal gastric physiology is poorly understood, despite clear evidence that gastric acidity appears to play a critical role in its transcriptional expression and processing. Targeted deletion of Shh in cells of the gastric mucosa should eventually further our understanding of its specific role in normal physiology once gastric cell specific promoters are better characterized to generate tissue specific knockouts. In cancer, numerous studies in non-gastric tissues have established the Hh signaling pathway as a therapeutic target. As such, several inhibitors of the pathway are being tested in clinical trials. Due to the differential expression of Shh in the corpus versus the antrum, the use of general Hh inhibitors for gastric cancer will need to be carefully tested in vivo and cautiously pursued if used in clinical trials.

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Figure 1A

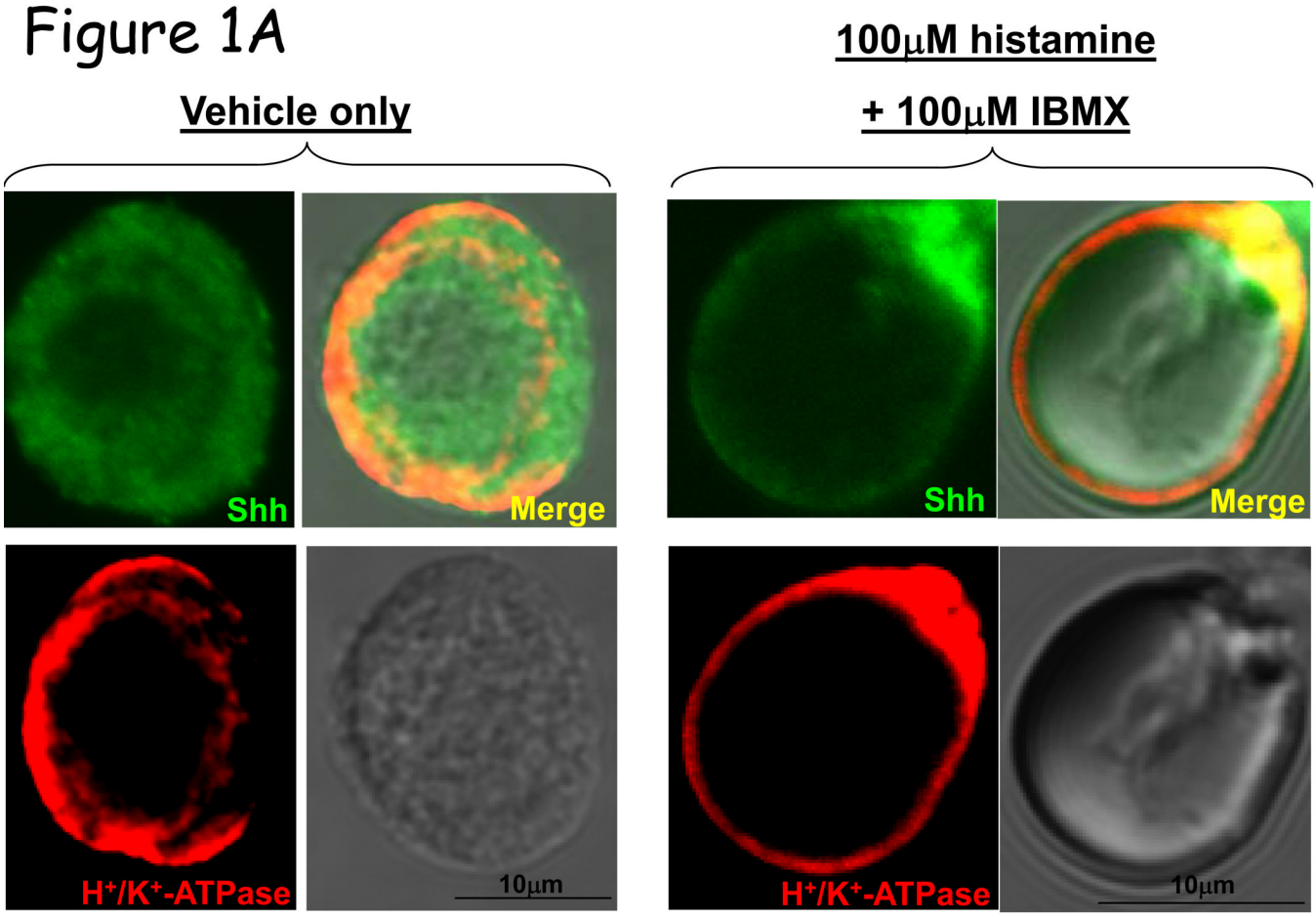

Figure 1B

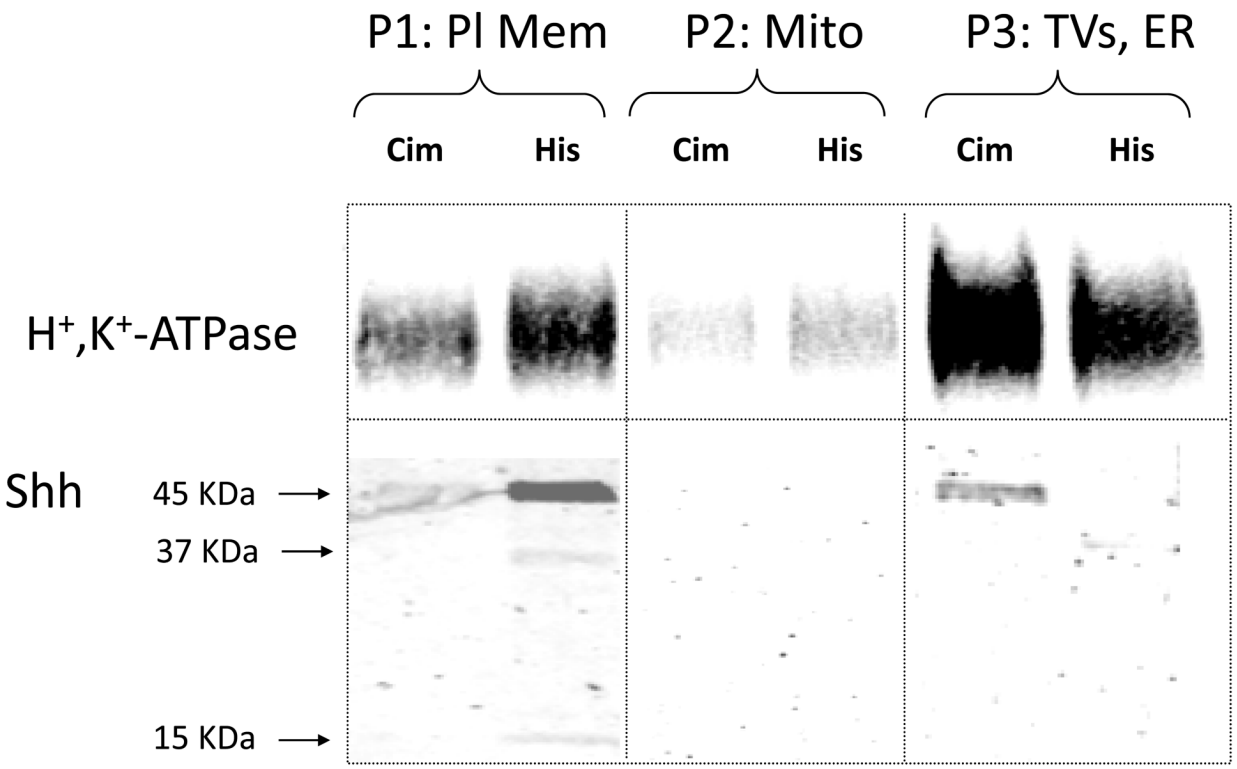

Figure 1. Shh and H^+/K^+-ATPase co-translocate to the apical membrane of parietal cells upon stimulation of acid secretion

A). Co-translocation of Shh (green) and H^+/K^+-ATPase (red) to the apical membrane of parietal cells upon stimulation of gastric acid secretion. Mouse parietal cells were identified in gastric primary cultures by H^+/K^+-ATPase staining, and compared with and without stimulation by 100μM histamine and 100μM isobutylmethylxanthine (IBMX). Fluorescent staining was visualized by confocal microscopy. Top left panel: Shh protein (green); bottom left panel: H^+/K^+-ATPase protein (red); top right panel: merge (yellow); bottom right panel: differential interference contrast (DIC) microscopy.

B). Western blot of tubulovesicle (TV), plasma membrane (Pl Mem), and mitochondrial (Mito) fractions demonstrating co-translocation of H^+/K^+-ATPase and Shh proteins upon stimulation with histamine (His) versus cimetidine (Cim) in canine parietal cells. H^+/K^+-ATPase and Shh proteins decrease in P3 (TV fraction) but increase in P1 (plasma membrane fraction) upon stimulation with histamine. Different sizes of the processed Shh protein (45kDa, 37kDa and 15kDa) are annotated. The plasma membrane and tubulovesicle fractions were obtained by centrifugation through a 12% – 18% Ficoll 400 density step gradient and a 40% - 35% -10% sucrose gradient respectively.