The Adventures of Sonic Hedgehog in Development and Repair.

IV. Sonic hedgehog processing, secretion, and function in the stomach

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Sonic Hedgehog (Shh) is synthesized as a full-length precursor protein that migrates with a relative mass of ~45 kDa. It has been shown previously that the precursor protein undergoes an autocatalytic cleavage reaction to generate a biologically active amino-terminal polypeptide corresponding to an approximate 19-kDa fragment. The amino-terminal domain (ShhN) is the active signaling peptide. During the cleavage process, a cholesterol moiety is added to the carboxy terminus of ShhN (ShhNp, 26 kDa), which then remains membrane tethered. A multimeric form of ShhN is secreted, soluble, and freely diffusible (reviewed in Ref. 6) (Fig. 1). Either the cell-retained or secreted forms of Shh are able to bind to the hedgehog receptor patched (Ptc) to activate Hh signaling (reviewed in Ref. 6). Studies demonstrating autocatalysis of Shh protein have been performed using either Drosophila or zebrafish models (13). In both models, hedgehog proteins undergo proteolytic processing in the absence of microsomal membranes (13). In contrast, processing of mammalian Shh protein translated in vitro fails unless microsomal membranes are added (1). Interestingly, in vitro experiments using a gastric cancer cell line, having similar characteristics to parietal cells (23132/87 cells), showed that Shh transcription is induced by low-pH conditions in culture media (3). This evidence suggests that, at least in mammalian systems, processing of Shh protein requires specific cellular factors.

Recently, we have found that in the stomach Shh processing occurs via a hormonally regulated and acid-dependent mechanism (24). In addition to regulating acid secretion, gastrin acts as a growth factor for the gastric mucosa. An infusion of gastrin for 14 days resulted in a significant stimulation of both Shh expression and processing. Moreover, in vitro studies revealed that processing of the 45-kDa Shh precursor protein occurred under acidic conditions and was cleaved by the acid-activated protease pepsin A (24) (Fig. 1). Consistent with our findings was a recent publication by El-Zaatari et al. (4) demonstrating that during hypergastrinemia and elevated acid secretion there is a concomitant increase in Shh expression. Thus evidence from these studies shows for the first time that production of the biologically active Shh protein depends on stimulation of gastric acidity.

Sonic Hedgehog Expression and Secretion from the Gastric Mucosa

The expression of Shh within the gastric mucosa is controversial. Although it has been shown by a number of laboratories that Shh is expressed and secreted from parietal cells (18, 21, 24), protein expression in the pit cells is unclear. One study has detected Shh expression in the pit region of Mongolian gerbils by using in situ hybridization (19), but in the mouse...
Although there is report of an absence of Shh expression in pit cells of the mouse gastric mucosa (21), another study confirms the Shh expression in this region (4). The discrepancy has been attributed to differences between mouse strains, specificity of antibodies, and sensitivity of methods used to detect Shh expression. The reason for the discrepancy remains unclear.

Despite the discrepancy of Shh expression within the pit cells of the gastric mucosa, it is clear that Shh is expressed and secreted from the acid-producing parietal cells (18, 24). Emerging evidence from our laboratory suggests that both processing and secretion occur at the apical membrane of parietal cells (Y. Zavros, M. Orr, D. H. Malinowska, unpublished observations). In support of apical expression of Shh, green fluorescent protein fused with the COOH terminus of the human Shh protein transfected into polarized 293T cells revealed that expression of Shh protein was concentrated at the lateral and apical surfaces of the plasma membrane, areas that are enriched in cholesterol and the likely site of posttranslational modification (22). Interestingly, the apical secretory membrane of the parietal cell also contains a high lipid content that is critical for selective permeability of water and ions, but not urea or ammonia. One working hypothesis is that the Shh 45-kDa precursor may be translocated to the apical membrane during tubulovesicular fusion and processed at the canaliculal membrane where activated H⁺-K⁺-ATPase has been recruited, acid is secreted, and pepsinogen A is converted to pepsin A (Fig. 2).

**Sonic Hedgehog Function in the Adult Stomach**

Shh is believed to regulate epithelial cell differentiation in the adult stomach, but its role as a morphogen is based on evidence that correlates the loss of Shh with neoplastic transformation of the gastric mucosa (17, 21). Although Shh-null mice have given us insight into the importance of this protein in the development of the gastric epithelium, deletion of the Shh gene is embryonic lethal (9, 15), making it impossible to study the function of Shh in the adult stomach under physio-

![Fig. 1. Schematic diagram of Sonic hedgehog (Shh) processing: autocatalytic versus protease-dependent mechanisms. The cleavage of the 45-kDa full-length/nascent peptide generates the signal peptide (39 kDa). Autocatalytic cleavage at the Gly-Cys residues yields a processed and secreted 19-kDa peptide that is lipid modified (ShhNp). In the parietal cell Shh undergoes processing by an acid- and protease-dependent mechanism. Processing via the protease dependent mechanism generates a 19-kDa freely secreted protein (ShhN). The predicted site of pepsin A cleavage is at the Cys-Phe residues. G, Gly; C, Cys; F, Phe.](http://ajpgi.physiology.org/)
logical conditions. Moreover, data from the gastrin-deficient mice demonstrate that gastrin may be a trophic factor for the gastric epithelium through its activation of Shh secretion (24), but the direct effect of Shh cannot be tested in this model.

Certainly, secretion of Shh across the apical membrane of parietal cells would suggest that Shh binds to its receptor Ptc in primary cilia. Primary cilia are projections found on almost all vertebrate cell surfaces that extend into the extracellular environment and function as sensors for various signals, including changes in Shh levels (16). From these findings we may hypothesize that ciliated cells may be capable of sensing changes in luminal or apically secreted Shh.

Besides its proposed role as a growth factor for the gastric epithelium, Shh may also be a fundamental regulator of the physiological regulation of acid secretion. We know that Shh regulates H^+-K^+-ATPase expression in canine parietal cells (18). Moreover, treatment of mice with the hedgehog signaling inhibitor cycloamine results in elevated circulating gastrin levels, suggesting that Shh may be an important factor in the regulation of gastrin secretion (4). Gastrin, histamine, and acetylcholine are the major secretagogues of gastric acid secretion. Increased acidity then stimulates chemoreceptors on the D cells to secrete somatostatin and block further release of gastrin and gastric acid. Thus gastric acid secretion is regulated by a negative feedback mechanism. Certainly, the hypergastrinemia observed with cycloamine treatment (4) may suggest that blockade of Shh activity results in the removal of the somatostatin-inhibitory effect on gastrin. The possible role of Shh as a mediator of such a crucial regulatory mechanism of acid secretion in the stomach is an area that requires further investigation.

Pepsinogen A and Sonic Hedgehog: Markers of Parietal Cell Atrophy

The stomach is not only the site of acid secretion, but also the major source of factors, including TGF-β, Wnt, FGFs, and hedgehog proteins, that are responsible for regulating the differentiation of the gastric epithelium. Thus it is not surprising that gastric atrophy and disruption of normal cell proliferation and differentiation triggers a cascade that develops into neoplasia (2). Prior to the development of dysplasia, the gastric mucosa is typically atrophic, with the acid-producing parietal cells drastically reduced or absent. Analysis of human stomachs has shown that the presence of gastric atrophy is a reliable indicator of preneoplastic changes in the stomach (2). Loss of mature parietal cells from the body of the stomach by either genetic or pharmacological methods results in severe abnormalities in the differentiation and development of gastric cell lineages characterized by the appearance of intestinal or gastric metaplastic cells (8). Interestingly, changes in a number of proteins or physiological functions have been reported as indicative or as markers of atrophy. These include changes in serological pepsinogen A-to-C ratio, loss of acid secretion, hypergastrinemia (elevated plasma gastric concentrations), suppressed somatostatin expression, loss of E-cadherin (protein involved in cell-adhesion) (10), and more recently loss of Shh (17).

It is widely accepted that inflammation that is caused by *Helicobacter pylori* infection is a trigger for the development of gastric cancer (2). In fact, exogenous infusion of only IFN-γ into mice is sufficient to induce significant mucous gland metaplasia and hypergastrinemia (25). However, the question of the mechanism by which inflammatory cytokines induce mucosal damage remains unanswered. Given that loss of Shh may be predicted to be an initial marker for the development of gastric cancer, there is surprisingly little information regarding the regulation of Shh by proinflammatory cytokines. In a Mongolian gerbil model of *H. pylori* infection, it has been shown that bacterial colonization leads to downregulation of Shh expression (19), and in humans loss of Shh may be an early change that occurs in the mucosa prior to neoplastic transformation (17). Therefore, loss of Shh expression has been correlated with intestinal metaplasia and may play a role in carcinogenesis. However, the mechanism that results in loss of Shh in the infected gastric mucosa is unexplored. If gastric acidity is reduced, because of inhibition of acid secretion by inflammatory cytokines, then pepsinogen A is not activated and we would hypothesize that there would be loss of Shh function due to either loss of processing or secretion. This notion is consistent with the observation that gastric atrophy correlates with reduced pepsinogen A-to-C ratios in stomachs of patients with increased risk of cancer development (17).

Numerous studies have evaluated the ratio of pepsinogen A to pepsinogen C in the assessment of a patient’s increased risk for developing gastric cancer (10). Low levels of pepsinogen A are predictive of chronic atrophic gastritis, which is the most important predictor of gastric transformation (10). Certainly, our data demonstrate that in tumor tissue from human stomach, pepsinogen A expression is significantly lower compared with normal gastric mucosa. Moreover, in tumor tissue Shh processing is lost similar to that of normal extracts immunodepleted of pepsinogen A (24). Collectively, these findings underscore the importance of Shh in the maintenance of normal gastric mucosal differentiation in the corpus.

Given that an acid environment is translatable to other cells, the processing mechanism for Shh also gives us new insight into the Shh expression in other systems. For example, a number of studies show that another member of the aspartic protease family, cathepsin D, is involved in osteoclastogenesis (23). Certainly, this setting suggests a favorable environment for the activation of the aspartic protease family that includes pepsin A and C and cathepsins, but their roles as processing enzymes for Shh during development are unknown. Generally, members of the aspartic protease family are also increased in the tumor tissues that are highly acidic and intriguingly rich in Shh protein (14). In both the setting of either tissue development or cancer, the ubiquitous expression of this family of proteases in different tissues suggests...
that regulation of Shh processing may not be limited to the stomach.

**Sonic Hedgehog and Gastric Carcinogenesis**

The role of Hh signaling in the development of gastric cancer has become evident from studies of both various gastrointestinal cell lines and analysis of patient gastric samples (17, 20). A study by Berman et al. (reviewed in Ref. 20) clearly demonstrated that elevated Hh signaling activity, supported by increased Ptc receptor expression, occurred in gastric carcinoma. The overactivity of the Hh pathway during carcinoma was explained by overexpression of the Hh ligand that was blocked by both cyclopamine (hedgehog signaling antagonist) and a Hh blocking antibody (Berman et al., 2003, reviewed in Ref. 20). Conversely, when patients with evidence of precancerous lesions such as atrophy and intestinal metaplasia are studied, there is a strong loss of Shh protein expression (17). Interestingly, a recent study showed that during pseudopyloric metaplasia, a lesion induced by *Helicobacter* infection, resulted in the reactivation of Shh (5). It may be that such conflicting data indicates that Hh signaling is active in the later stages of gastric carcinogenesis in response to inflammatory cytokines. There may also be differences in the expression of Shh and tumor subtype. Testing Hh pathway activation in a cohort of patients with gastric cancer would address the role of Hh ligand production and expression in tumors. This also underscores the need for genetically modified mice to study the role of Hh signaling in tumorigenesis.

**Summary**

Recent studies have shown that processing of Shh within the adult stomach is dependent on acid secretion from parietal cells. In the stomach Shh plays a crucial role in parietal cell function and possibly the regulation of epithelial cell differentiation. Thus loss of Shh during atrophic gastritis, a known precursor lesion to gastric cancer, is clinically significant. Unfortunately, our understanding of Shh in the adult stomach is dependent on acid secretion from parietal cells. In the stomach Shh plays a crucial role in parietal cell function and possibly the regulation of epithelial cell differentiation. Thus loss of Shh during atrophic gastritis, a known precursor lesion to gastric cancer, is clinically significant. Unfortunately, our understanding of Shh in the adult stomach has been disadvantaged by the embryonic lethality of the hedgehog signaling mutant models. The use of cell-specific inducible hedgehog signaling mutants will be critical to revealing the mechanisms by which Shh regulates epithelial cell homeostasis in the stomach.

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**REFERENCES**