The Role of Sonic Hedgehog Reemergence During Gastric Cancer

Jason Martin,
Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, 231 Albert B. Sabin Way, Room 1113, MSB, Cincinnati, OH 45267-0576, USA

Jessica M. Donnelly,
Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, 231 Albert B. Sabin Way, Room 1113, MSB, Cincinnati, OH 45267-0576, USA

JeanMarie Houghton, and
Division of Gastroenterology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01635, USA

Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01635, USA

Yana Zavros
Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, 231 Albert B. Sabin Way, Room 1113, MSB, Cincinnati, OH 45267-0576, USA

Yana Zavros: yana.zavros@uc.edu

Abstract

Sonic Hedgehog (Shh) signaling has been extensively studied for its role in developmental biology and cancer biology. The association between Shh and cancer development in general is well established but the functional role of Shh in the development and progression of gastric cancer specifically is largely unknown. Bone marrow-derived stem cells, specifically mesenchymal stem cells (MSCs) infiltrate and engraft into the gastric mucosa in response to the chronic inflammatory environment of *Helicobacter* infection. In this review, MSC infiltration and changes in the cytokine and cellular profiles of later-stage chronic environments will be tied into their interactions with the Shh pathway. We will discuss how these changes shape tumorigenesis and tumor progression in the gastric mucosa. The current review focuses on the Shh signaling pathway and its role in the development of gastric cancer, specifically in response to *Helicobacter pylori* infection. We follow with an in-depth discussion of the regulation of the Hedgehog pathway during acute and chronic gastric inflammation with a focus on signaling within the MSC compartment.

Keywords

Gastric cancer; Sonic Hedgehog; Bone-marrow-derived cells; Cancer development

Correspondence to: Yana Zavros, yana.zavros@uc.edu.

Introduction

Hedgehog (Hh) was discovered by Nüsslein-Volhard and Wieschaus in a saturation mutagenesis screen performed to study the effect of mutations on the patterning of segmented *Drosophila* embryos [1]. As a result of the mutagenesis screen, they identified a group of mutants in which the *Drosophila* embryo remained covered entirely with denticles [1]. The inspiration for the name Hedgehog came from the "spiny" phenotype of the embryos, which resembled a hedgehog. Since the identification of the Hedgehog mutant, three vertebrate Hh homologs have been identified. These homologs include Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh). Of the Hedgehog homologs, Shh has been the most studied in terms of the Hedgehog signaling pathway in vertebrates.

It is well established that Shh plays a crucial role in the development of the stomach. Evidence for this is documented from the Shh null mice that develop an intestinal rather than gastric-type mucosa, loss of normal gastric cytodifferentiation, and gut malrotation [2]. Shh is also highly expressed in the adult stomach. However, it is only recently that investigators have begun to elucidate the role of Hedgehog in the adult stomach [3–5]. Pharmacological inhibition of Shh signaling in the adult stomach results in a loss of parietal cells and disruption of glandular differentiation [4]. While, in a mouse model expressing a parietal cell-specific deletion of Shh shows that Shh regulates the maintenance of gastric epithelial cell differentiation and parietal cell function [5]. We are only starting to understand the exact mechanisms through which Shh functions in the adult stomach. What is clear, however, is that investigating these mechanisms is increasingly important as dysregulated Shh expression plays a role in promoting gastric carcinogenesis [6, 7]. The current review focuses on the Shh signaling pathway and its role in the development of gastric cancer.

Autocatalytic Processing of Hedgehog and the Shh Signaling Pathway

Most of what we know about Shh processing is from work done in *Drosophila* or zebrafish models [8]. Sonic hedgehog (Shh) is synthesized as a 45-kDa precursor protein. The full-length precursor subsequently undergoes an autocatalytic cleavage to yield a 26-kDa carboxy-terminal fragment (ShhC) and a 19-kDa amino-terminal fragment (ShhN). ShhC is responsible for catalyzing cleavage of the 45-kDa precursor protein while ShhN is the active signaling peptide. Concomitant with cleavage, ShhC acts as a cholesterol transferase covalently linking a cholesterol moiety to the carboxy-terminus of the 19-kDa fragment (ShhN) [9]. The 19-kDa fragment (ShhN) is further modified by a membrane bound O-acyltransferase commonly known as Skinny hedgehog (Ski), which covalently links a molecule of palmitate to the 19-kDa fragment (ShhNp) [10, 11] (Fig. 1). The finding that the phenotypes of drosophila lacking Ski resemble those of drosophila with Shh loss of function mutations demonstrates the importance of Palmitoylation in Shh signaling [12]. Following processing, ShhNp can remain anchored to the cell membrane or form multimeric units that are secreted, soluble and freely diffusible [9]. Both the cell-retained and secreted Shh protein forms are able to activate hedgehog signaling through the hedgehog receptor Patched (Ptc) [9].

Shh signaling in vertebrates is relayed via the seven-span transmembrane receptor Smoothened (Smo) [13, 14]. Shh does not directly bind to Smo, but instead indirectly controls the activity of Smo through binding to a second receptor Ptc [13, 14]. Ptc is a 12-span transmembrane receptor that catalytically inhibits signaling through Smo in the absence of Hedgehog [13, 14] (Fig. 2a). Binding of Hh to Ptc results in loss of Ptc activity, consequently activating Smo, which then transduces the Hh signal into the cytoplasm [13, 14]. Transduction of the Hedgehog signal into the cytoplasm leads to activation of the Glioblastoma (Gli) family of transcription factors [15] (Fig. 2b). Experiments using rat kidney epithelial cells (RK3E cells) show that Gli induces transcription of signaling targets such as Wnt [16] and Snail [17] as a mechanism of regulating cell proliferation. In isolated canine parietal cells, Gli induces H^+,K^+-ATPase expression [18]. Overall, the series of molecular events that couples signaling through Smo to the activation of Gli transcription factors is inadequately established in vertebrates and Hedgehog signaling targets in the vertebrate stomach are poorly understood.

Protease- and Acid-Mediated Processing and Secretion of Sonic Hedgehog Protein Within the Stomach

Processing of Shh in mammalian cells is largely unclear, however, the gastric mucosa appears to have diverged from the autocatalytic processing originally elucidated in *Drosophila* and zebrafish models [8, 19]. In the mammalian stomach, Shh processing is found to be a hormonally regulated and acid-dependent process [19]. Gastrin regulates Shh expression and processing via acid secretion [19]. In particular, gastrin has been shown to mediate Shh processing through increased acid secretion in parietal cells facilitating an increased conversion of pepsinogen A to the active aspartic protease pepsin A [19]. Within acidic environments, pepsin A acts to cleave the 45-kDa precursor protein into the active 19-kDa protein [19] (Fig. 1). These studies indicate that although autocatalytic processing of Shh may occur in the gastric mucosa, the predominate processing pathway for the 45-kDa Shh precursor protein requires acidic conditions and the acid-activated protease pepsin A.

A study using the Shh-LacZ reporter mice demonstrates that all major cell lineages of the stomach corpus including surface pit, mucous neck, zymogenic and parietal cells express Shh [20]. Studies using parietal cells isolated from mouse, rabbit, and canine models demonstrate that Shh is secreted from the parietal cells [18, 19, 21]. Our group has localized Shh expression and secretion within resting and stimulated parietal cells [21]. In resting parietal cells, the 45-kDa precursor Shh protein co-localizes with the H^+-K^+-ATPase and F-actin located on intracellular membranes [21], suggesting that in resting parietal cells, the precursor Shh protein is localized to the H^+-K^+-ATPase-containing tubulovesicles abundant in the cytoplasm of parietal cells [21]. In histamine-stimulated parietal cells, Shh protein remains co-localized with both the H^+-K^+-ATPase and F-actin [21]. However, in contrast to resting parietal cells, Shh protein is localized to the expanded canalicular membranes of activated parietal cells [21]. Furthermore, 19-kDa Shh protein localized to the expanded canalicular membrane [21]. As stated earlier, proper processing of the mammalian 45-kDa precursor Shh protein requires cholesterol, acidic conditions, and pepsin A [9, 19]. Apical membranes, such as the canalicular membrane of parietal cells, are often enriched in

cholesterol and serve as sites for posttranslational modification of proteins [22]. The lipid-enriched canalicular membrane, along with the acidic pH, and proximity to pepsinogen-A secreting chief cells make the environment at the apical membrane of activated parietal cells ideal for processing Shh protein. Thus, based on this in vitro study, our working hypothesis is that the 45-kDa precursor Shh protein is translocated to the canalicular membrane during tubulovesicular fusion and is processed by pepsin A at the lipid rich and acidic environment of the canalicular membrane [21] (Fig. 3a). Investigation at the molecular level will be required to confirm or reject this hypothesis and determine the steps underlying the trafficking, processing, and secretion of Shh in parietal cells.

The acid-dependent and pepsin A-mediated processing of Shh in the mammalian gastric mucosa is currently a novel pathway, however, processing of Hedgehog proteins by aspartic proteinases may exist in other mammalian systems. The osteoclast system may be another example of this processing mechanism. Pepstatin A, a known inhibitor of aspartic proteinases, suppresses receptor activator of nuclear factor-κB ligand (RANKL) induced differentiation of osteoclasts [23]. Suppression of RANKL induced differentiation by pepstatin A is mediated through blockade of extracellular signal-regulated kinase (ERK) phosphorylation and inhibition of nuclear factor of activated T-cell cytoplasmic calcineurin-dependent 1 (NFATc1) expression [23]. Both the phosphorylation of ERK and expression of NFATc1 are dependent upon increases in intracellular calcium concentrations [23, 24]. One can speculate that Pepstatin A-mediated reduction of ERK phosphorylation and NFATc1 expression in osteoclasts could be a consequence of reduced intracellular calcium levels caused by a decrease in hedgehog activation by aspartic proteinases. This assumption is based on evidence that Shh can increase the phosphorylation of ERK in mammalian gastric cells by increasing intracellular calcium concentrations [24]. Currently, very little is known about Hedgehog processing, localization, and signaling within osteoclasts; however, the abundance of aspartic proteinases such as cathepsins D and E in the acidic environments maintained by osteoclasts parallel the abundance of pepsin A in the acidic environment of the gastric mucosa [23, 25]. There is a remarkable similarity between the ruffled border of osteoclasts and the apical membrane of gastric parietal cells (Fig. 3). Based on these similarities, propose a similar mechanism for the expression and processing of Shh at the ruffled border of osteoclasts (see model Fig. 3b). Further research into the localization and processing of Hedgehog proteins in osteoclasts would provide a better understanding of the interplay between Hedgehog proteins, acidic environments, and aspartic proteinases, and strengthen the known ties between the bone marrow environment and the gastric mucosal environment [26].

Sonic Hedgehog in Homeostasis of the Adult Stomach

Deletion of the Shh gene is embryonic lethal limiting the use of Shh-null models to developmental studies [2, 27]. As a result, the direct examination of Shh function under physiological conditions in the adult stomach has proven impractical until recently with the development of a mouse model expressing a parietal cell-specific Shh deletion [5]. Work using genetic and disease models of the gastric mucosa indicate that Shh regulates epithelial cell differentiation in the adult stomach [3–5, 28]. Evidence indicating Shh as a morphogen comes from disease models that correlate the loss of Shh with neoplastic transformation of

the gastric mucosa [3, 4, 28]. A recent study examining gastric differentiation subsequent to loss of Shh acts as a morphogen by directing the differentiation of zymogenic cells from the mucous neck cell lineage [5].

Aside from its role as a regulator of gastric epithelial cell differentiation, Shh may also act to regulate the physiological secretion of acid from the parietal cells. In response to EGF, parietal cells express Shh which positively regulates the expression of the H^+,K^+-ATPase [18]. The interplay between gastrin and Shh also identifies Shh as an important regulator of acid secretion. Emerging studies using mouse models in which Shh signaling or expression have been pharmacologically or genetically inhibited suggest that Shh may directly and/or indirectly act as part of the negative feedback mechanism that controls gastrin and acid secretion [5, 29, 30]. Treatment of mice with cyclopamine, an inhibitor of Hedgehog signaling, results in elevated circulating gastrin levels [29]. We propose that a loss of Shh impairs acid secretion by decreasing the activity and/or expression of the H^+-K^+-ATPase on parietal cells. This reduction in acid secretion would reduce somatostatin release from D-cells of the stomach, thus removing the somatostatin-inhibitory effect on gastrin secretion. In support of this proposal, lack of acid secretion in our parietal cell-specific Shh deletion mouse model (HKCre/Shh^{KO}) was accompanied by significant hypergastrinemia. Treatment of HKCre/Shh^{KO} mice, with the somatostatin analogue octreotide, significantly suppressed hypergastrinemia and subsequently restored differentiation of the zymogen cell lineage and parietal cell function [5]. As gastrin is believed to promote the growth of gastric adenocarcinoma, the role of Shh as a regulator of gastrin secretion has important implications for the study of gastric carcinogenesis.

The Role of Sonic Hedgehog in *Helicobacter pylori*-Induced Atrophic Gastritis

Helicobacter pylori (*H. pylori, Hp*) infects approximately half of the world's population. *Hp* is a chronic inflammatory infection that persists in the absence of specific antibiotics. Chronic inflammation caused by persistent *Hp* infection is directly linked to the development of gastric cancer [31, 32]. The gastric mucosal changes of chronic Hp infection begin with chronic inflammation followed by hyperproliferation, atrophy, metaplastic cell lineage changes including spasmolytic polypeptide-expressing metaplasia (SPEM), intestinal metaplasia, and antralization of glands. These changes then proceed to dysplasia and eventually cancer [33, 34]. While all patients infected with Hp develop chronic inflammation, not all patients progress through all of the mucosal changes outlined. Only a small minority of patients develop gastric cancer. Loss of mature parietal cells from the gastric glands of the stomach plays a central role in the progression of gastric alterations. Loss of the acid-secreting parietal cells (atrophy) leads to alterations in the cell lineages with the expansion of metaplastic mucous cells. Since the stomach secretes numerous factors such as TGFβ, Wnt, FGFs and Hedgehog proteins, which are known to be responsible for the differentiation of the gastric epithelium, one favored explanation linking inflammation and progression to cancer is the loss of these factors (reviewed in [35]).

As stated previously, Shh acts as a morphogen in the adult stomach. In conditions such as gastric atrophy and intestinal metaplasia, where gastric morphogenesis is reduced or absent, Shh is reduced or lost [3, 4, 28]. Such is the case in Mongolian gerbils, which show a loss of Shh expression in response to *Hp* colonization of the stomach [28]. Moreover, this loss correlates with the loss of parietal cells, impaired maturation of the zymogenic chief cells in gastric glands, and intestinal metaplasia [28, 36]. In human gastric mucosa, Ptc is predominately expressed on gastric chief cells [36]. Loss of Shh signaling through Ptc in chief cells could explain the impaired maturation of chief cells associated with *Hp*-induced gastritis. Furthermore, loss of Shh is associated with upregulation of caudal-type homeobox transcription factor 2 (CDX2), a known promoter of intestinal metaplasia in the stomach [36]. Therefore, loss of Shh signaling may underlie the impairment of chief cell differentiation and the development of intestinal metaplasia found in late-stage *Hp*-associated gastritis.

Only recently has the mechanism responsible for the loss of Shh expression during *Hp* infection been elucidated in vivo [20, 37]. Appropriate acid secretion maintains Shh expression and morphogenic function in gastric glands [19–21, 37]. In vivo experiments of parietal cell dysfunction such as the histamine H(2) receptor-knockout mice [37] and ex vivo isolated rabbit gastric glands and canine parietal cells treated with H^+,K^+-ATPase blocker omeprazole [19, 21], confirm that in the absence of acid secretion Shh expression is significantly reduced supporting the notion that hypoacidity induces the loss of Shh and gastric morphogenesis found in *Hp* infection. However, other potential culprits are the inflammatory cytokines released in response to *Hp* colonization. While it has been proven that exogenous infusion of interferon-γ (IFN-γ) alone is sufficient to induce hypergastrinemia and metaplasia in mice, very little is known about the regulation of Shh by pro-inflammatory cytokines [38]. IL-1β, another abundant cytokine released during *Hp* infection, correlates with gastric atrophy and gastric cancer [39, 40] and is a potent inhibitor of gastric acid secretion [40]. A recent study using Shh-LacZ reporter mice demonstrates that IL-1β produced during *Helicobacter* infection inhibited gastric acid and Shh expression through IL-1 receptor activation [20]. The investigators concluded from this study that that proinflammatory cytokine IL-1β reduces Shh expression and function in the gastric mucosa by reducing acid secretion from parietal cells [20]. Shh induces H^+,K^+-ATPase gene expression in isolated canine parietal cells [18], therefore, the investigators rationalized that chronically suppressed levels of Shh may eventually reduce enzyme expression that is sufficient to induce gastric atrophy [41] and thus, inhibiting Shh expression in parietal cells is likely to promote atrophy [20]. Our recent study using a mouse model expressing a parietal cell-specific deletion of Shh demonstrates that in the absence of inflammation, while Shh induces foveolar hyperplasia and hypochlorhydria it was not sufficiently to induce parietal cell atrophy [5]. These data suggest that there may be a requirement for additional factors such as inflammatory cytokines, for parietal cell atrophy to occur.

Sonic Hedgehog Over-Expression Plays a Crucial Role in *H. Pylori* (*Hp*)-Induced Carcinogenesis

One of the more fascinating aspects of *Hp*-associated gastric carcinogenesis is the increase in Shh production associated with gastric tumor biopsies [6, 42]. This increase in Shh production during tumorigenesis contrasts the loss of Shh correlated with atrophy and metaplasia in *Hp*-associated atrophic gastritis. A subset of tumor types exhibit an increase in Shh production during tumorigenesis [6, 7, 42], indeed Ptc expression and elevated activity of the Shh signaling pathway has been demonstrated in several gastric cancer cell lines [7]. Increased activity of the Shh pathway is due to over-expression of the Hedgehog ligand and is blocked by Shh pathway antagonists [7]. Loss of Shh signaling using Smo antagonist cyclopamine or Hedgehog neutralizing antibody 5E1 causes a decrease in cell growth in vitro and regression of xenograft tumors in vivo [7]. Indicating that while loss of Shh is essential to the formation of atrophy and metaplasia earlier on in *Hp* gastritis, increased Shh signaling is important in promoting tumor growth and proliferation at later stages. Identification of the mechanism(s) responsible for the reemergence of Shh production in malignant lesions remains controversial.

Several mechanisms have been proposed to explain the reemergence of Shh. One mechanism is the reactivation of Shh production by NF-κβ during pseudopyloric metaplasia arising from *Hp* infection [30]. Upregulation of bcl-2, Gli-1, and Ptc are observed in the stroma surrounding *Hp*-induced metaplastic lesions [30]. The upregulation of bcl-2 and Gli-1 in the stroma has important implications for tumorigenesis as Gli-1 is known to promote cell proliferation, and both Gli-1 and bcl-2 have anti-apoptotic activity [30]. Reemergence of Shh production may be the result of NF-κβ activation by *Hp*. Both NF-κβ and IL-8, one of the transcriptional targets of NF-κβ, have been shown to induce Shh expression in gastric tumors [43, 44]. NF-κβ-binding sites have been identified in the human Shh promoter region, indicating that Shh is a transcriptional target of NF-κβ [45]. While these studies indicate that inflammatory cytokines could be responsible for the reemergence of Shh, they do not identify which cells are expressing Shh. Promising candidates include transformed epithelial cells, infiltrating immune cells and other bone marrow-derived cells (BMDCs).

Summary and Future Directions

The cancer stem cell theory proposes that many tumors originate from a small population of stem cells, which act much like normal tissue stem cells in that they self-renew and give rise to all the differentiated cells of the tumor [26]. Cancer stem cells possess unique properties that allow them to metastasize, resist apoptosis, self-renew indefinitely, and avoid destruction by conventional therapies [26]. During chronic *Hp* infection, bone-marrow-derived cells home to and repopulate the gastric mucosa [26]. Subsequently, BMDCs progress through metaplasia and dysplasia before progressing to intraepithelial cancer [26]. BMDCs are not recruited to the gastric mucosa in response to acute inflammation or transient parietal cell loss, but appear to require long-standing inflammation and damage to repopulate the epithelium [26]. Data from our laboratory shows that the MSC population express and secrete Shh protein in response to inflammatory cytokine IFNγ (unpublished

findings, Donnelly and Zavros). Hedgehog signaling is an important factor for the maintenance of cancer stem cells and the proliferation of tissue progenitor cells in several cancers [46–48]. Therefore, investigations into the response of BMDCs to Shh and the possibility of BMDCs secreting Shh in response to NF-κβ or other inflammatory cytokines have great implications for determining the role of Shh in gastric tumorigenesis and the mechanism(s) behind Shh reemergence in malignant lesions (Fig. 4).

Several groups have implicated the CXCR4/stromal-derived factor-1 (SDF-1) axis in the recruitment of mesenchymal stem cells to sites of injury as well as to areas of developing carcinoma/tumor stroma [49–51]. The gastric mucosa isolated from *Hp*-infected mice with chronic gastritis exhibit increased levels of SDF-1 [26]. Recruitment of bone marrow cells is found to be coincident with increased expression of SDF-1 in both the stomach and serum of *Helicobacter*-infected animals [52]. What emerges from this body of work is a plausible mechanism for the recruitment of bone-marrow-derived MSCs to the site of developing carcinoma. Tracking of BMDC, specifically MSC into the gastric mucosa in response to inflammation and injury introduces a new cell type to the gastric mucosal environment. This MSC introduces altered Shh regulation and signaling from that found in the atrophic gastric mucosa. We propose that up-regulation of serum SDF-1 α during chronic inflammation acts as a further mechanism for the recruitment of MSCs to the gastric epithelium. Figure 4 is an illustration of this proposed mechanism.

Emerging studies indicate that Shh plays a crucial role in parietal cell function and regulation of gastric epithelial cell differentiation [3–5, 18, 28]. Loss of Shh is associated cytokine-induced inhibition of acid secretion which is correlated to impaired gastric function and eventually parietal cell atrophy, both of which precede gastric carcinogenesis [20]. The reemergence of Shh expression in gastric tumors is correlated with increased tumor growth [7]. The mechanism(s) behind Shh reemergence in malignant lesions remains unknown; however, several mechanisms are plausible. First, *Hp*-induced inflammation has been shown to promote the over-expression of Shh in pseudopyloric metaplasia [30]. Second, we propose the recruited MSCs expressing Shh to an environment rich in inflammatory cytokines including NF-κB and IFN-γ are able to repopulate the damaged gastric epithelium. The specific signals within the Hedgehog pathway necessary for trafficking and differentiation of cancer stem cells may be manipulated and used in a clinical setting for the treatment of a number of illnesses including cancer [53, 54]. Technology such as short interfering RNA (siRNA) may be potentially effective in suppressing or inducing genes within the Hedgehog pathway to treat or prevent cancer.

Acknowledgments

Abbreviations

Shh	Sonic Hedgehog
Ihh	Indian Hedgehog
Dhh	Desert Hedgehog
Smo	Smoothened
Ptch	Patched
Gli1	Glioma-associated Oncogene homolog 1
Hh	Hedgehog
H. pylori	*Helicobacter pylori*
Hp	*H. pylori*
BMDC	Bone-marrow-derived cells
MSCs	Bone-marrow-derived mesenchymal stem cells
CDX2	Caudal-type homeobox transcription factor 2
SDF-1	Stromal-derived factor-1
MDSC	Myeloid-derived suppressor cells

References

1. Nüsslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in drosophila. Nature. 1980; 287:795–801. [PubMed: 6776413]

2. Ramalho-Santos M, Melton DA, McMahon AP. Hedgehog signals regulate multiple aspects of gastrointestinal development. Development. 2000; 127:2763–2772. [PubMed: 10821773]

3. Shiotani A, Iishi H, Uedo N, et al. Evidence that loss of sonic hedgehog is an indicator of *Helicobater pylori*-induced atrophic gastritis progressing to gastric cancer. Am J Gastroenterol. 2005; 100:581–587. [PubMed: 15743355]

4. van den Brink GR, Hardwick JC, Nielsen C, et al. Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. Gut. 2002; 51:628–633. [PubMed: 12377798]

5. Xiao C, Ogle SA, Schumacher MA, et al. Loss of parietal cell expression of sonic hedgehog induces hypergastrinemia and hyperproliferation of surface mucous cells. Gastroenterology. 2010; 138:550–561. [PubMed: 19909751]

6. Ma X, Chen K, Huang S, et al. Frequent activation of the hedgehog pathway in advanced gastric adenocarcinomas. Carcinogenesis. 2005; 26:1698–1705. [PubMed: 15905200]

7. Berman DM, Karhadkar SS, Maitra A, et al. Widespread requirement for hedgehog ligand stimulation in growth of digestive tract tumours. Nature. 2003; 425:846–851. [PubMed: 14520411]

8. Porter JA, von Kessler DP, Ekker SC, et al. The product of hedgehog autoproteolytic cleavage active in local and long-range signalling. Nature. 1995; 374:363–366. [PubMed: 7885476]

9. Goetz JA, Singh S, Suber LM, Kull FJ, Robbins DJ. A highly conserved amino-terminal region of sonic hedgehog is required for the formation of its freely diffusible multimeric form. J Biol Chem. 2006; 281:4087–4093. [PubMed: 16339763]

10. Torroja C, Gorfinkiel N, Guerrero I. Mechanisms of hedgehog gradient formation and interpretation. J Neurobiol. 2005; 64:334–356. [PubMed: 16041759]

11. Mann RK, Beachy P. Novel lipid modifications of secreted protein signals. Ann Rev Biochem. 2004; 73:891–923. [PubMed: 15189162]

12. Pepinsky RB, Zeng C, Wen D, et al. Identification of a palmitic acid-modified form of human sonic hedgehog. J Biol Chem. 1998; 273:14037–14045. [PubMed: 9593755]

13. Goodrich LV, Johnson RL, Milenkovic L, McMahon JA, Scott MP. Conservation of the hedgehog/ patched signaling pathway from flies to mice: induction of a mouse patched gene by hedgehog. Genes Dev. 1996; 10:301–312. [PubMed: 8595881]

14. Taipale J, Cooper MK, Maiti T, Beachy PA. Patched acts catalytically to suppress the activity of smoothened. Nature. 2002; 418:892–896. [PubMed: 12192414]

15. Hui CC, Slusarski D, Platt KA, Holmgren R, Joyner AL. Expression of three mouse homologs of the drosophila segment polarity gene cubitus interruptus, gli, gli-2, and gli-3, in ectoderm- and mesoderm-derived tissues suggests multiple roles during postimplantation development. Dev Biol. 1994; 162:402–413. [PubMed: 8150204]

16. DW, LiX; Lobo-Ruppert, SM.; Ruppert, JM. Gli1 acts through snail and E-cadherin to promote nuclear signaling by beta-catenin. Oncogene. 2007; 26:4489–4498. [PubMed: 17297467]

17. Li X, Deng W, Nail CD, et al. Snail induction is an early response to gli1 that determines the efficiency of epithelial transformation. Oncogene. 2006; 25:609–621. [PubMed: 16158046]

18. Stepan V, Ramamoorthy S, Nitsche H, Zavros Y, Merchant JL, Todisco A. Regulation and function of the sonic hedgehog signal transduction pathway in isolated gastric parietal cells. J Biol Chem. 2005; 280:15700–15708. [PubMed: 15691835]

19. Zavros Y, Waghray M, Tessier A, et al. Reduced pepsin a processing of sonic hedgehog in parietal cells precedes gastric atrophy and transformation. J Biol Chem. 2007; 282:33265–33274. [PubMed: 17872943]

20. Waghray M, Zavros Y, Saqui-Salces M, et al. Interleukin-1beta promotes gastric atrophy through suppression of sonic hedgehog. Gastrotenterology. 2010; 138:562–572.

21. Zavros Y, Orr M, Xiao C, Malinowska DH. Sonic hedgehog is associated with h +, k + -ATPase-containing membranes in gastric parietal cells and secreted with histamine stimulation. Am J Physiol. 2008; 295:G99–G111.

22. Vincent S, Thomas A, Brasher B, Benson JD. Targeting of proteins to membranes through hedgehog auto-processing. Nat Biotechnol. 2003; 21:936–940. [PubMed: 12858181]

23. Yoshida H, Okamoto K, Iwamoto T, et al. Pepstatin A, an aspartic proteinase inhibitor, suppresses rankl-induced osteoclast differentiation. J Biochem. 2006; 139:583–590. [PubMed: 16567424]

24. Osawa H, Ohnishi H, Takano K, et al. Sonic hedgehog stimulates the proliferation of rat gastric mucosal cells through erk activation by elevating intracellular calcium concentration. Biochem Biophys Res Commun. 2006; 344:680–687. [PubMed: 16630542]

25. Yoshimine Y, Tsukuba T, Isobe R, et al. Specific immunocytochemical localization of cathepsin E at the ruffled border membrane of active osteoclasts. Cell Tissue Res. 1995; 281:85–91. [PubMed: 7621529]

26. Houghton J, Stoicov C, Nomura S, et al. Gastric cancer originating from bone marrow-derived cells. Science. 2004; 306:1568–1571. [PubMed: 15567866]

27. Kim JH, Huang Z, Mo R. Gli3 null mice display glandular overgrowth of the developing stomach. Dev Dyn. 2005; 234:984–991. [PubMed: 16247775]

28. Suzuki H, Minegishi Y, Nomoto Y, et al. Down-regulation of a morphogen (Sonic hedgehog) gradient in the gastric epithelium of *Helicobacter pylori*-infected Mongolian gerbils. J Pathol. 2005; 206:186–197. [PubMed: 15818572]

29. El-Zaatari M, Grabowska A, McKenzie AJ, Powe DG, Scotting PJ, Watson SA. Cyclopamine inhibition of the sonic hedgehog pathway in the stomach requires concomitant acid inhibition. Regul Pept. 2008; 146:131–139. [PubMed: 17996964]

30. El-Zaatari M, Tobias A, Grabowska AM, et al. De-regulation of the sonic hedgehog pathway in the insgas mouse model of gastric carcinogenesis. Br J Cancer. 2007; 96:1855–1861. [PubMed: 17505514]

31. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001; 345:784–789. [PubMed: 11556297]

32. Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M. A model for gastric cancer epidemiology. Lancet. 1975; 2:58–60. [PubMed: 49653]

33. Correa P, Houghton J. Carcinogenesis of *Helicobacter pylori*. Gastroenterology. 2007; 133:659–672. [PubMed: 17681184]

34. Goldenring JR, Nomura S. Differentiation of the gastric mucosa III. Animal models of oxyntic atrophy and metaplasia. Am J Gastroenterol. 2006; 291:G999–G1004.

35. Katoh Y, Katoh M. Hedgehog signaling pathway and gastrointestinal stem cell signaling network (review). Int J Mol Med. 2006; 18:1019–1023. [PubMed: 17089004]

36. Dimmler A, Brabletz T, Hlubek F, et al. Transcription of sonic hedgehog, a potential factor for gastric morphogenesis and gastric mucosa maintenance, is up-regulated in acidic conditions. Lab Invest. 2003; 83:1829–1837. [PubMed: 14691301]

37. Minegishi Y, Suzuki H, Arakawa M, et al. Reduced shh expression in tff2-overexpressing lesions of the gastric fundus under hypochlorhydric conditions. J Pathol. 2007; 213:161–169. [PubMed: 17763396]

38. Zavros Y, Rathinavelu S, Kao JY, et al. Treatment of *Helicobacter gastritis* with il-4 requires somatostatin. Proc Natl Acad Sci U S A. 2003; 100:12944–12949. [PubMed: 14555768]

39. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000; 404:398–402. [PubMed: 10746728]

40. El-Omar EM. The importance of interleukin 1beta in *Helicobacter pylori*-associated disease. Gut. 2001; 48:743–747. [PubMed: 11358884]

41. Franic TV, Judd L, Robinson D, et al. Regulation of gastric epithelial cell development revealed in h(+)/k(+)-ATPase beta-subunit- and gastrin-deficient mice. Am J Physiol. 2001; 281:G1502–G1511.

42. Ohta M, Tateishi K, Kanai F, et al. P53-independent negative regulation of p21/cyclin-dependent kinase-interacting protein 1 by the sonic hedgehog-glioma-associated oncogene 1 pathway in gastric carcinoma cells. Cancer Res. 2005; 65:10822–10829. [PubMed: 16322228]

43. Aihara M, Tsuchimoto D, Takizawa H, et al. Mechanisms involved in *Helicobacter pylori*-induced interleukin-8 production by a gastric cancer cell line, mkn45. Infect Immun. 1997; 65:3218–3224. [PubMed: 9234778]

44. Chu SH, Kim H, Seo JY, Lim JW, Mukaida N, Kim KH. Role of NF-kappab and ap-1 on *Helicobater pylori*-induced il-8 expression in ags cells. Dig Dis Sci. 2003; 48:257–265. [PubMed: 12643600]

45. Kasperczyk H, Baumann B, Debatin KM, Fulda S. Characterization of sonic hedgehog as a novel NF-kappab target gene that promotes NF-kappab-mediated apoptosis resistance and tumor growth in vivo. FASEB J. 2009; 23:21–33. [PubMed: 18772349]

46. Watkins DN, Peacock C. Hedgehog signaling in foregut malignancy. Biochem Pharmacol. 2004; 68:1055–1060. [PubMed: 15313401]

47. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature. 2003; 422:313–317. [PubMed: 12629553]

48. Karhadkar SS, Bova G, Abdallah N, et al. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature. 2004; 431:707–712. [PubMed: 15361885]

49. Kitaori T, Ito H, Schwarz EM, et al. Stromal cell-derived factor 1/cxcr4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum. 2009; 60:813–823. [PubMed: 19248097]

50. Kyriakou C, Rabin N, Pizzey A, Nathwani A, Yong K. Factors that influence short-term homing of human bone marrow-derived mesenchymal stem cells in a xenogeneic animal model. Haematologica. 2008; 93:1457–1465. [PubMed: 18728032]

51. Haider, HKh; Jiang, S.; Idris, NM.; Ashraf, M. Igf-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of sdf-1alpha/cxcr4 signaling to promote myocardial repair. Circ Res. 2008; 103:1300–1308. [PubMed: 18948617]

52. Tu S, Bhagat G, Cui G, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. Cancer Cell. 2008; 14:408–419. [PubMed: 18977329]

53. Yagi N, Manabe I, Tottori T, et al. A nanoparticle system specifically designed to deliver short interfering RNA inhibits tumor growth in vivo. Cancer Res. 2009; 69:6531–6538. [PubMed: 19654315]

54. Schugar RC, Robbins P, Deasy BM. Small molecules in stem cell self-renewal and differentiation. Gene Ther. 2008; 15:126–135. [PubMed: 17989703]
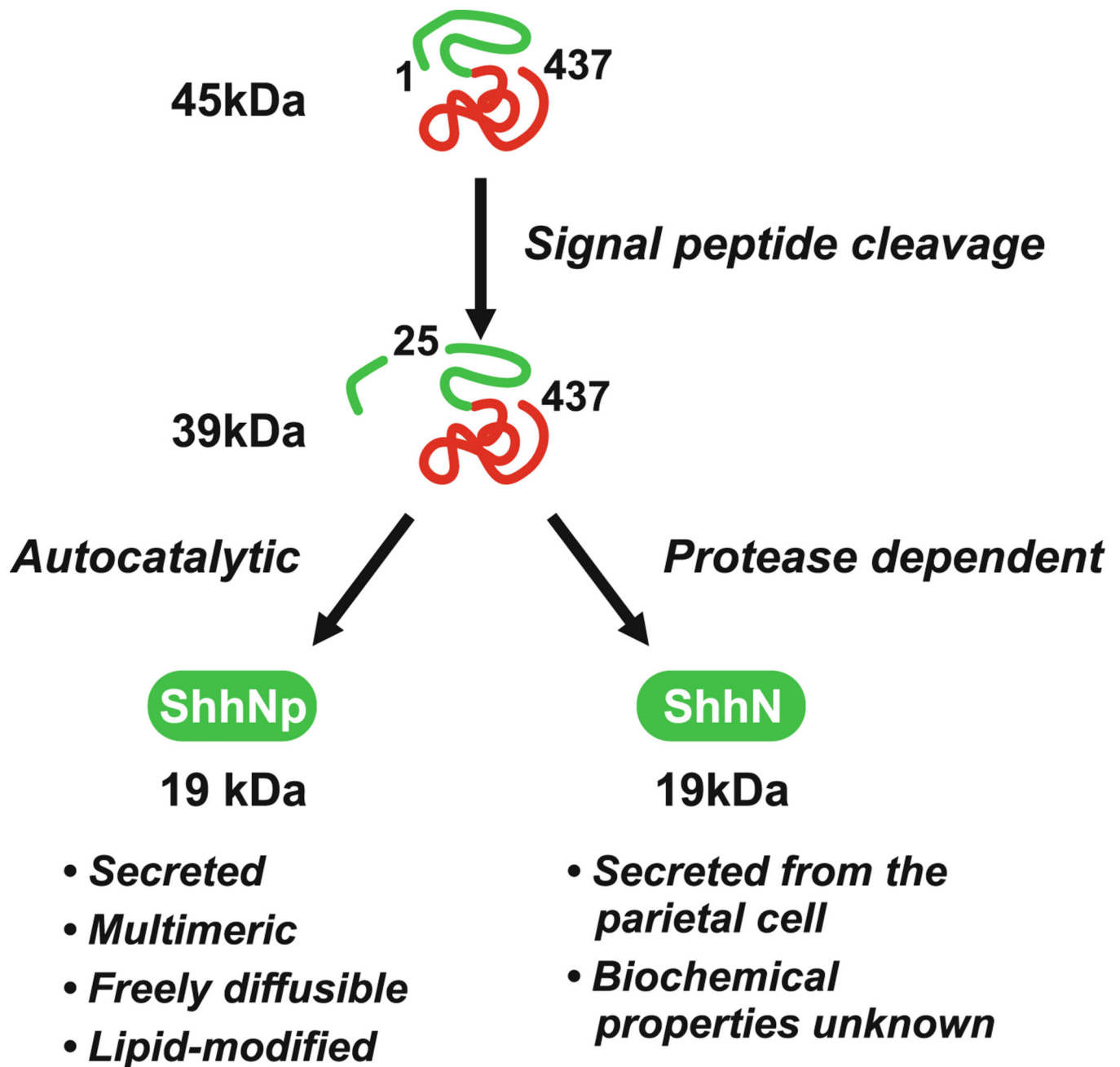
Fig. 1.
Schematic diagram of Shh processing: Autocatalytic versus protease-dependent mechanisms. The cleavage of the 45-kDa full length/nascent peptide generates the signal peptide (39 kDa). Autocatalytic cleavage yields a secreted 19-kDa peptide and a 26-kDa cholesterol-modified cell-bound protein (ShhNp). 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) in which the biochemical properties are largely unknown
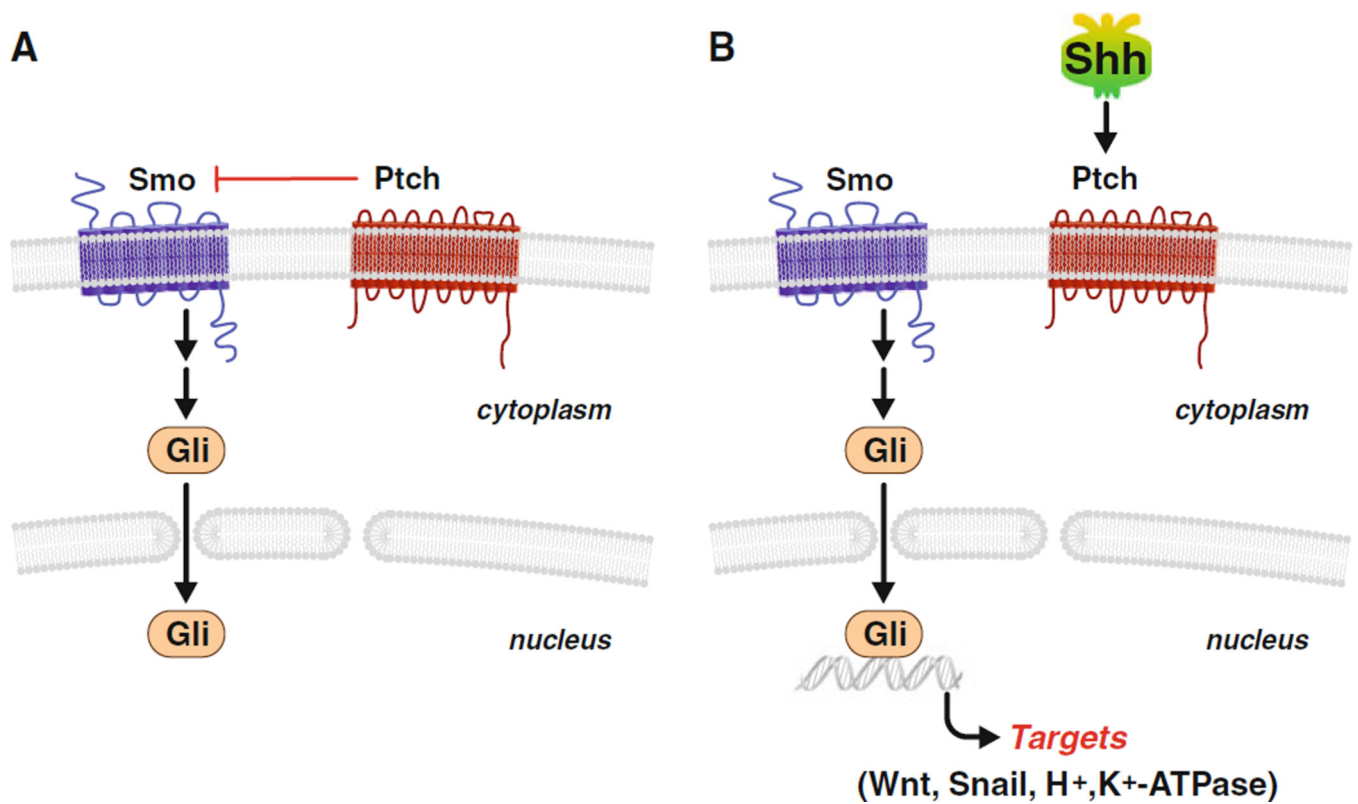
Fig. 2.

Schematic diagram of the Hedgehog signaling pathway. **a** In the absence of Shh or unstimulated cells, the activity of the transmembrane protein Smo is suppressed by the Hedgehog receptor Ptch. **b** Binding of Shh to Ptch results in the removal of the inhibitory effect on Smo and consequently activating Smo. Smo then transduces the Shh signal into the cytoplasm. Transduction of the Shh signal into the cytoplasm leads to activation of the Glioblastoma (Gli) family of transcription factors and activation of downstream targets such as Wnt, Snail, and H^+,K^+-ATPase
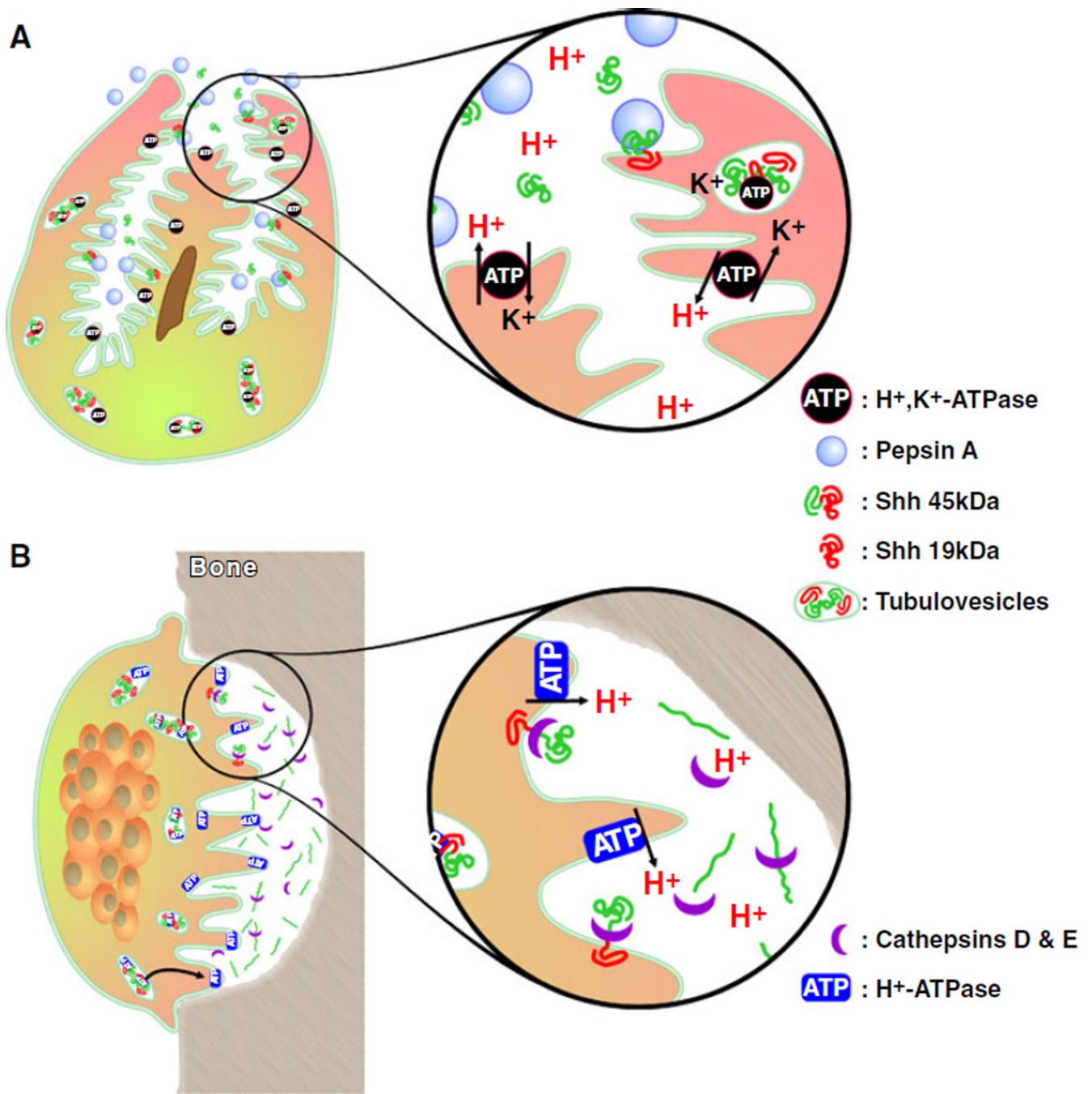
Fig. 3.

A theoretical model for the expression and processing of Sonic Hedgehog at the apical membrane of the parietal cell and the ruffled border of osteoclasts. **a** Processing of Shh may occur at the apical membrane of the parietal cell after stimulation. Shh protein present within tubulovesicles of resting parietal cells traffic to the apical membrane upon stimulation of the parietal cell and acid secretion. The protein is then accessible to the active pepsin A enzyme where Shh is processed and secreted across the apical membrane. **b** Similar to the environment at the apical membrane of parietal cells, the environment at the ruffled border

of osteoclasts shows an abundance of aspartic proteinases and an acidic environment that is maintained by the trafficking of vesicular ATP-dependent proton pumps. On the basis of these similarities, Shh processing in osteoclasts may occur at the ruffled border in an acid-dependent and aspartic proteinase mediated manner similar to Shh processing at the apical membrane of parietal cells. Shh protein present within tubulovesicles of resting parietal cells traffics to the ruffled border upon stimulation of acid secretion. The protein is then accessible to aspartic proteinases such as cathepsins D and E that process Shh at the ruffled border region of osteoclasts
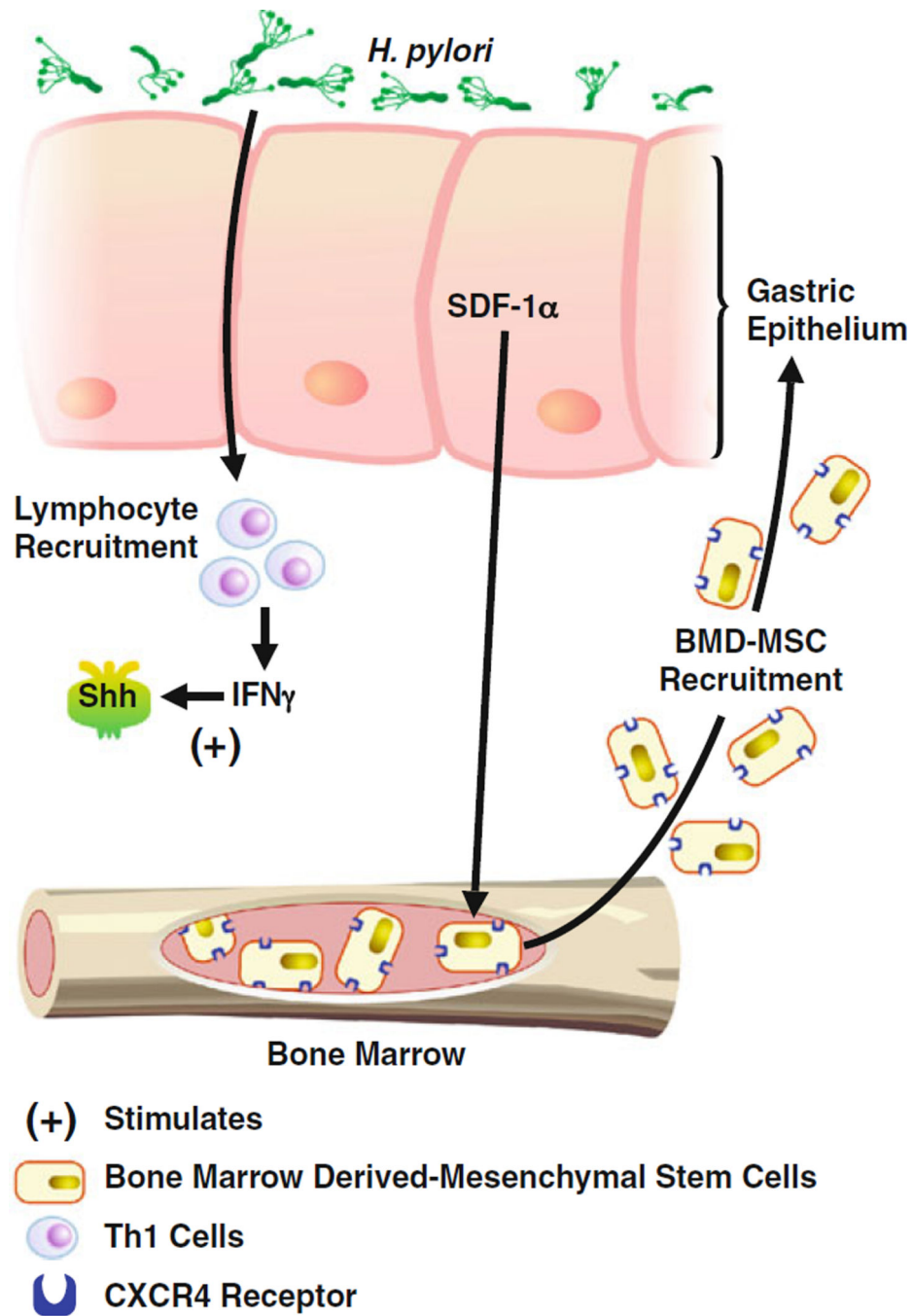
Fig. 4.
An SDF-1α gradient serves to recruit bone-marrow-derived mesenchymal stem cells to the gastric mucosa in response to inflammation. *Helicobacter pylori* infection induces a Th1 immune response that is characterized by increased IFNγ cytokine production. We hypothesize that Shh signaling mediates IFNγ recruitment of mesenchymal stem cells (*MSCs*). The increase in production of SDF-1α either by myeloid cells or the gastric

epithelium itself may then generate a gradient that allows migration of MSCs to the gastric epithelium and the progression of gastric cancer