

TOPICAL REVIEW

Hedgehog signalling in gut development, physiology and cancer

Juanita L. Merchant

Departments of Internal Medicine and Molecular and Integrative Physiology, Division of Gastroenterology, University of Michigan, Ann Arbor, MI, USA

Abstract The Hedgehog pathway is one of the most common signal transduction pathways used by mammalian cells. Most studies have focused on its role during development, primarily of the nervous system, skin, bone and pancreas. Due to the activation of this pathway during proliferation and neoplastic transformation, more recent studies have examined its role in adult tissues. Significant levels of sonic hedgehog are expressed in the gastric mucosa, which has served to direct analysis of its role during organogenesis, gastric acid secretion and neoplastic transformation. Therefore the goal of this review is to apply current knowledge of this pathway to further our understanding of gastrointestinal physiology and neoplasia, using the stomach as a prototype.

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Corresponding author J. L. Merchant: University of Michigan, Internal Medicine, 109 Zina Pitcher PL, BSRB, 2051, University of Michigan, Ann Arbor, MI 48105-2200, USA. Email: merchanj@umich.edu

Introduction

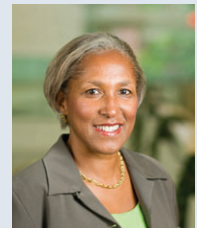
This review summarizes the basic components of the Hedgehog (Hh) signalling pathway and considers our current understanding of this pathway in the luminal gastrointestinal tract with a focus on gastric physiology and tumorigenesis. Hh signalling in the pancreas and hepatobiliary systems has been recently reviewed elsewhere (Omenetti & Diehl, 2008; Teglund & Toftgard, 2010; Omenetti *et al.* 2011).

Overview of hedgehog pathway

The Hh signalling pathway was initially identified during a mutagenesis screen conducted by the fly geneticists Eric Wieschaus and Christiane Nusslein-Volhard in 1978 (Nusslein-Volhard & Wieschaus, 1980). The mutated gene corresponding to abnormal denticle formation (hair-like projections) resulted in flies that had the appearance of a hedgehog. They ultimately received the Nobel prize in 1995 for discovering several signalling pathways, including the Hh pathway, which control the segmentation patterning in *Drosophila* (Roush, 1995). In flies, the pathway is initiated by a 471-residue protein called Hedgehog (hh). However, its prototypical mammalian counterpart was named Sonic Hedgehog

(Shh) after the Sega video game character. Eventually, three mammalian *hh* genes activating the same signalling pathway were identified (Katoh & Katoh, 2005; Merchant *et al.* 2010b). The other two gene products in addition to Shh were named Indian (Ihh) and Desert (Dhh) Hedgehog (Adolphe *et al.* 2004) (Shimeld, 1999; Ramalho-Santos

Juanita L. Merchant holds faculty appointments in the Departments of Internal Medicine and Integrative & Molecular Physiology at the University of Michigan. She received her BS in Biology from Stanford University and received both her MD and PhD (Cell Biology) from Yale University. She completed Internal Medicine residency and a research fellowship in Gastroenterology at the Massachusetts General Hospital in Boston and her clinical fellowship in Gastroenterology at UCLA. Her primary research interests include transcriptional control mechanisms regulating cell growth and differentiation and microbial–host interactions in the luminal GI tract and their application to cancer pathogenesis. She is known for her work on how regulatory hormones in the stomach, e.g. gastrin and Shh, are regulated by the immune system. Her studies combine a number of modalities including molecular and cell biology, mouse genetics and translation to human biology.



et al. 2000). Since the major genes expressed in the luminal gastrointestinal tract are *Shh* and *Ihh*, this review will primarily refer to these two ligands. In general, the three ligands bind the same receptor Patched (Ptc1 and 2) and activate a signal transduction pathway resulting in the proteolytic processing of glioma-associated oncogene (Gli) transcription factor family members (Carpenter *et al.* 1998). Ultimately, the processed Gli proteins bind the same DNA consensus binding site (5'-GACCACCCA-3') (Kinzler & Vogelstein, 1990; Winklmayr *et al.* 2010).

Although there are distinct differences in the tissue specific expression of the mammalian ligands, all three bind the Ptc receptors with the same affinity and activate identical signalling cascades. Therefore the difference between the three ligands appears to be their tissue specificity and the level of expression in each tissue (Pathi *et al.* 2001). For example, *Shh* expression is highest in the developing organs of the foregut, which include lung, liver, pancreas, oesophagus and the proximal stomach (corpus) (Litingtung *et al.* 1998; Motoyama *et al.* 1998). By contrast, *Ihh* expression emerges in hindgut-derived tissues, specifically the distal stomach (antrum), intestine and colon (Kolterud *et al.* 2009; van Dop *et al.* 2009; Zacharias *et al.* 2010).

Hh ligand processing

Like many signal transduction pathways, there are multiple steps prior to activation of specific target genes. Both *Shh* and *Ihh* undergo proteolytic processing from the ~45 kDa precursor to the ~19 kDa cleaved ligand (Roelink *et al.* 1995; Valentini *et al.* 1997). Since the steps for processing *Shh* have been the most intensively studied, generation of this ligand will be described in greater detail. Initially, the 24-amino acid signal peptide is cleaved from the amino terminus of the nascent precursor polypeptide (Lee *et al.* 1994; Bumcrot *et al.* 1995; Hammerschmidt *et al.* 1997; Goetz *et al.* 2002; Wendler *et al.* 2006) then further processed within the endoplasmic reticulum (Chen *et al.* 2011). The peptide undergoes cholesterol modification at cysteine 199 followed by autocatalysis mediated by serine protease-like activity residing within the C-terminal domain (Porter *et al.* 1995, 1996*a,b*). The resulting 19 kDa-processed peptide is palmitoylated at cysteine 25 (cysteine 24 in human *Shh*) (Pepinsky *et al.* 1998; Buglino & Resh, 2010) (Fig. 1). Recent studies show that the palmitoylation step is required for the peptide to be tethered to the plasma membrane in position to be clipped by a member of the ADAM protease family (Dierker *et al.* 2009; Ohlig *et al.* 2011) (Fig. 1). The cholesterol-modified truncated *Shh* ligand then forms micelles with the protein Dispatched rendering the micelles capable of diffusion away from the cell of origin (Caspary *et al.* 2002; Callejo *et al.* 2011).

By contrast, we have observed in primary parietal cells that *Shh* processing is acid dependent (Zavros *et al.* 2007) (Fig. 1). Specifically, the consensus site for the aspartic protease pepsin A is GCFP residing at residues 198–201 within the *Shh* polypeptide. Thus pepsin cleavage is expected to occur on the C-terminal side of the phenylalanine residue (Kageyama, 2002). Since spontaneous processing of the endogenous ligand to the 19 kDa form was not observed in primary gastric cell cultures, we concluded that *Shh* processing in the normal stomach requires acid to generate pepsin from its zymogen pepsinogen. It has not been determined whether the N-terminal form generated by gastric parietal cells is lipid-modified or forms micelles. Pepsin is a member of the aspartic protease family that includes both intracellular and transmembrane proteases activated at low pH, e.g. cathepsins and β -secretases, respectively (Vassar *et al.* 1999; Ivanova *et al.* 2008). Thus it is possible that *Shh* might undergo proteolysis in the vesicular compartments of other cell types, e.g. neurons (Beug *et al.* 2011). At present, it is difficult to reconcile whether autocatalytic processing of Hh ligands occurs in the mature gut epithelium. The studies establishing the autocatalytic mechanism for processing *Drosophila* hh and *Shh* were performed using transgenes transfected into *Drosophila* cells, embryos or mesenchymally derived mammalian cell lines, e.g. COS, BOSC, CHO, NIH3T3, HEK293, which typically do not express Hh ligands (Lee *et al.* 1994; Bumcrot *et al.* 1995; Porter *et al.* 1995; Chen *et al.* 2011). Recent studies using *in situ* hybridization and *LacZ* reporter mice have established that both *Shh* and *Ihh* mRNAs are expressed exclusively in the intestinal and colonic epithelium and not in the mesenchyme (Kolterud *et al.* 2009; Varnat *et al.* 2010). However, studies are needed to compare the endogenous peptide forms generated by other epithelium outside of the stomach.

One might speculate that the two different mechanisms of processing *Shh* ligand reflect tissue specific differences, i.e. one mesenchymal and the other epithelial. Since gastric acid secretion occurs at the apical surface, pepsin-mediated processing implies that *Shh* produced by the parietal cell could be secreted lumenally or basolaterally creating a diffusion gradient. The role of *Shh* ligand in the lumen has yet to be defined since the Hh target cell typically resides on the basolateral surface in the mesenchyme. Mesenchymal cells express both the receptor Ptc and the Hh signalling apparatus, e.g. Gli1 (Kolterud *et al.* 2009; Varnat *et al.* 2010). However, without direct evidence, one cannot exclude the possibility that *Shh* ligands secreted apically move via transcellular or paracellular mechanisms to the basolateral surface (Rogers & Schier, 2011). Therefore, the current dogma based on these observations is that Hh signalling is unidirectional (paracrine) and proceeds from the gut epithelium to the mesenchyme.

Table 1. Shh concentration gradient and target genes

	Shh ^{High}	Shh ^{Med}	Shh ^{Low}
Gene A	On	Off	Off
Gene B	Off	On	On
Gene C	Off	On	Off
Neural tube	Ventral	→	Dorsal
Limb buds	5th digit	3rd digit	Thumb

Shh gradients and gene targets

As a morphogen, Shh forms a gradient such that the concentration of the active ligand inversely correlates with its diffusion distance (Rogers & Schier, 2011). It is well established during embryonic development of the neural tube and limb bud that different subgroups of genes become activated or repressed depending on the concentration of Shh binding to the cell (Table 1). Thus a different group of genes becomes activated or repressed by high concentrations of Shh (close to the cell of origin) compared to those activated or repressed by low concentrations of Shh (far from the cell of origin)

(Table 1). For example in the developing neural tube, the Shh gradient determines polarity along the ventral–dorsal axis, whereas in the developing limb bud, the Hh gradient determines digit formation (Wong & Reiter, 2008). In this way, distinct gene clusters and subsequent cell fate can be regulated by the morphogen in a time-dependent and concentration-dependent manner (Rogers & Schier, 2011).

Hh signalling and primary cilia

The *Drosophila* gene encoding the transcription factor targeted by Hh signalling is called Cubitus interruptus (*Ci*) (Hui & Angers, 2011) (Fig. 2). However in mammals, there are three *Ci* isoforms designated Gli1, Gli2 and Gli3 that exhibit different functions (Kinzler & Vogelstein, 1990; Zhu & Lo, 2010) (Fig. 2). The N-terminus contains a repressor domain and binding site for a component of the repressor complex Suppressor of Fused (SuFu); while the C-terminus contains a transcription activation domain (TAD). The five C₂–H₂ zinc finger DNA binding domain (Znf) lies adjacent to the N-terminal repressor domain (Sasaki *et al.* 1999). Centrally located nuclear localization

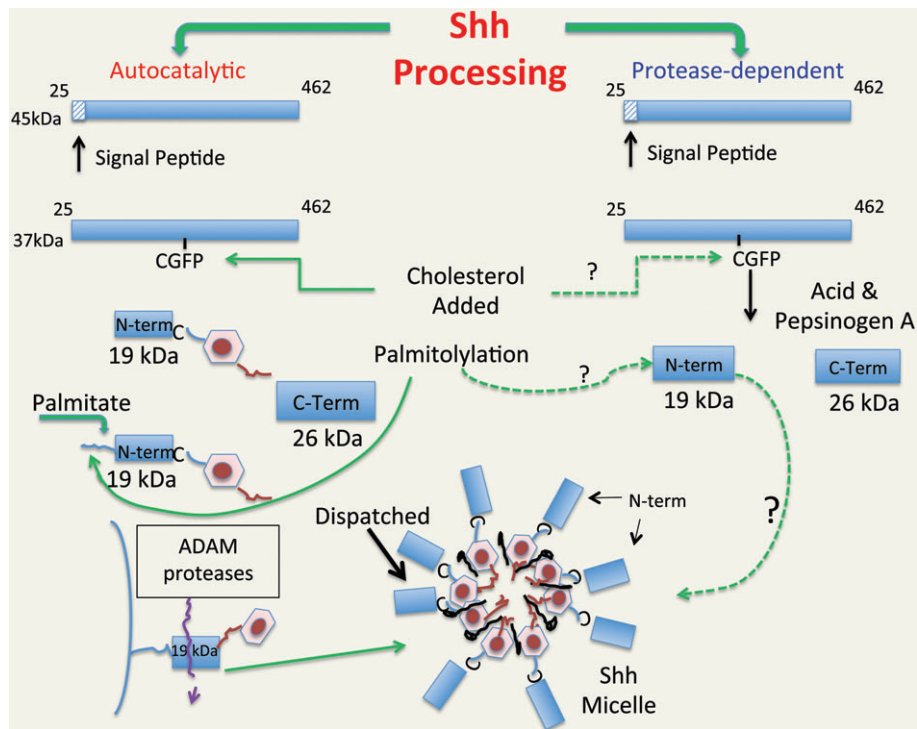


Figure 1. Sonic Hedgehog processing

Two mechanisms of Shh processing have been reported. The best known is the autocatalytic mechanism of Shh processing in which the C-terminus functions as a cholesterol esterase adding the sterol to cysteine 199 followed by palmitoylation at residue 25. The fatty acid permits Shh to be tethered to the plasma membrane until it is cleaved by an ADAM protease. The cleaved Shh molecules form micelles in the presence of the protein Dispatched (Disp). This mechanism has been described for *Drosophila* cells and cells derived from mesenchyme. By contrast, gastric acid producing parietal cells cleave Shh in a protease (pepsin)-dependent manner. It is not known whether Shh produced from the parietal cell is post-translationally modified with cholesterol or a fatty acid.

and export motifs between the zinc finger and C-terminal domains direct the cellular location of the Gli factors (Sheng *et al.* 2006). However, only Gli 2 and 3 possess an N-terminal repressor and C-terminal activator domain, whereas Gli1 does not contain a repressor domain (Hui & Angers, 2011). Therefore masking or unmasking these two domains by proteolysis (via the cleavage domain) regulates whether these two transcription factors activate or repress Hh target genes (Wong & Reiter, 2008).

Components of the Hh signalling pathway accumulate within specific organelles called primary cilia (Pazour & Witman, 2003; Haycraft *et al.* 2005; Kim *et al.* 2009). Initially identified in *Chlamydomonas*, these plasma membrane protrusions serve both motile and sensory functions. The Hh transmembrane transducer Smoothed (Smo) accumulates in primary cilia upon ligand binding to Ptch1 (Kim *et al.* 2009). There is only one primary cilium per cell and it is composed of acetylated α -tubulin condensed into nine microtubule arms arranged around the internal perimeter of the cilium. Since there is no central microtubule, the primary cilia are described as forming a 9 + 0 configuration (Pedersen *et al.* 2008).

In the unstimulated state Smo, a 7-pass transmembrane receptor related to the G-protein coupled receptor (GPCR) family, resides in vesicles just below the plasma membrane surface. The repressor complex composed of the serine/threonine kinase Fused, adapter SuFu and kinesin-like Kif7 sequesters Gli 2 and 3 in the cilium tip (Fig. 3A). Sequential phosphorylation of Gli2 and 3

by protein kinase A (PKA), glycogen 3b (GSK3b) and casein kinase 1 (CK1) create a phosphopeptide motif on Gli 2 and 3 that is recognized by β -TrCP (β -transducin repeat-containing protein). β -TrCP binding subsequently recruits the Skip/Cullin1/F-box (SCF) E3 ubiquitin ligase. Addition of ubiquitin then directs the Gli proteins to the proteasome for limited (Gli3) or complete (Gli2) degradation by the proteasome. This in turn regulates the abundance of the Gli2 and Gli3 repressor forms that ultimately inhibit Hh target genes in the nucleus. In the nucleus, the Gli3 repressor inhibits Hh target genes by forming complexes with co-repressors such as Sin3A-HDAC or Ski-HDAC (Fernandez-Zapico, 2008) (Fig. 3A).

Upon Hh ligand binding to its 12-pass transmembrane receptor Ptch, the Smo-containing vesicles fuse with the plasma membrane (Fig. 3B). 'Cargo' proteins such as the intraflagellar proteins (IFTs) latch onto the microtubular tracks and move protein (e.g. Smo) up to the cilium tip where it encounters the Hh repressor complex (Haycraft *et al.* 2005; Milenkovic & Scott, 2010). Once Smo interacts with the suppressor complex, Fu phosphorylates SuFu releasing the Gli2 and Gli3 transcription factors. This initiates retrograde migration from the tip of primary cilia to the cytoplasm where phosphopeptide formation and ubiquitination are blocked, preventing complete (Gli2) and limited (Gli3) degradation by the proteasome (Zhu & Lo, 2010) (Fig. 3B). Another scenario suggests that the absence of sequential phosphorylation results in limited

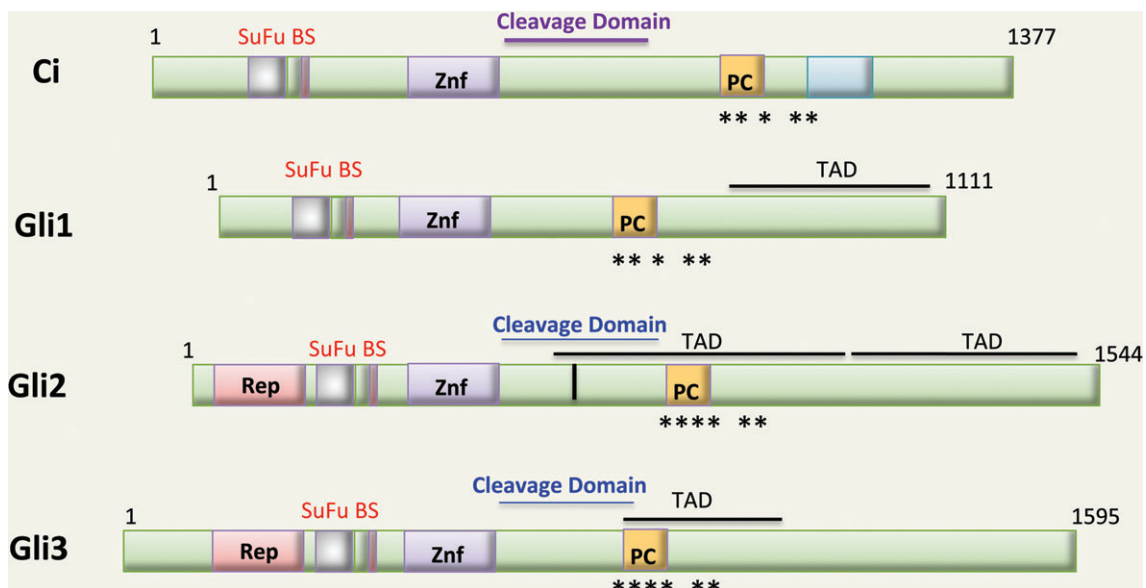


Figure 2. Schematic diagram of Gli transcription factors

Gli1, Gli2 and Gli3 are zinc finger homologues of *Drosophila* Cubitus interruptus (Ci). The 4 transcription factors are aligned to demonstrate the homology between the various isoforms. Various functional domains of these proteins are indicated. ZnF, zinc finger domain; TAD, transcription activation domain; Rep, repressor domain; SuFu BS, suppressor of fused binding site; PC, phosphorylation cluster; *protein kinase A phosphorylation site; cleavage domain: site of cleavage by proteasome. Modified from Hui & Angers, 2011 with permission.

degradation of Gli2 that removes the N-terminal repressor domain completely unmasking the activator domains (Hui & Angers, 2011). The resulting ratio of activator to repressor Gli forms then shuttle to the nucleus to bind specific gene targets. Thus, it is the ratio of the Gli 2>3 activator to Gli 3>2 repressor proteins that regulates whether the cell response will be transcriptional induction or repression (Wong & Reiter, 2008) (Fig. 3B).

Since Ptch1, Hedgehog interacting protein (Hip1) and Gli1 are all transcriptional targets of Hh signalling, the pathway is capable of activating negative (Ptch1, Hip1) or positive (Gli1) feedback mechanisms that further modify the intensity and duration of the signal (Stecca & Ruiz, 2010). Hh targets include genes regulating proliferation and differentiation, e.g. *cyclin D1*, *D2*, *N-myc*, *Wnts*, *Pdgfr α* , *Igf2*, *FoxM1*, *Hes1*; cell survival (*Bcl2*); self-renewal (*Bmi1*, *Nanog*); angiogenesis (*Vegf*); and epithelial-mesenchymal transition (*Snail1*, *Sip1*, *Elk1*, *Msx2*). Moreover, crosstalk between the TGF β -Smad3 (Johnson *et al.* 2011) and receptor tyrosine kinase-Ras-Erk pathways through phosphorylation or protein interactions with Gli proteins (Lauth & Toftgard, 2007; Dennler *et al.* 2009; Chang *et al.* 2010; Fan *et al.* 2010) implicate these two signalling pathways in Hh ligand-independent regulation.

Hedgehog signalling in gastric physiology

Ramalho-Santos and colleagues reported abnormal stomachs in day old *Shh* null mice (Ramalho-Santos *et al.* 2000). Since that fortuitous observation, there has been a flurry of interest in understanding the role of Hh signalling in the luminal gastrointestinal tract (van den Brink, 2007). The gut tube forms at embryonic day 9.5. During development, *Shh* gene expression remains high in the anterior gut (destined eventually to become oesophagus and gastric corpus) until embryonic day 15.5. At the pylorus, which demarcates the stomach from the duodenum, *Shh* expression is repressed and *Ihh* becomes the predominant ligand in the small and large intestine (Kolterud *et al.* 2009). Reports on *Shh* expression in the adult mouse colon have varied according to the detection method. Normal human colon expresses Hh ligand and signalling components and expression increases with transformation (Oniscu *et al.* 2004). Using *in situ* hybridization, van Dop *et al.* (2009) showed that *Ihh* mRNA is the predominant ligand in the adult colon and *Shh* expression was not detectable. Other reports indicate that *Shh* expression level is higher than observed in the small intestine, but is still significantly lower than observed in the stomach (Saqui-Salces & Merchant, 2010; Varnat *et al.* 2010). Interestingly, Ptch1 and Gli1 (indicators of Hh signalling) overlap with the epithelial expression of *Shh* until E18.5 when these two Hh target genes become

restricted to the mesenchyme. Thus during the early stages of embryonic development, Hh signalling is potentially autocrine, but switches to the epithelial to mesenchymal paracrine pattern just prior to birth.

In the adult corpus (anterior stomach), the stem cell gives rise to four major cell lineages, the acid-secreting parietal cell, mucous producing neck and surface pit cells (Fig. 4), and the endocrine lineages (not shown) (Karam & Leblond, 1992). The mucous neck lineage then gives rise to the zymogenic lineage as it migrates to the base of the gland (Ramsey *et al.* 2007). Recent studies show that the TFF2-positive metaplasia that develops with gastritis arises from the zymogenic lineage via trans-differentiation (Nozaki *et al.* 2008; Nam *et al.* 2010). *Shh* expression occurs in all gastric epithelial cells (Waghray *et al.* 2010). Stimulation of acid secretion, e.g. with gastrin or histamine, induces *Shh* gene expression (Zavros *et al.* 2007). Todisco and coworkers demonstrated that *Shh* stimulates H⁺,K⁺-ATPase gene expression in primary parietal cells (Stepan *et al.* 2005). Moreover, we have shown that acid is required to process the 45 kDa *Shh* precursor in the stomach (Zavros *et al.* 2007). Agents that inhibit acid, e.g. omeprazole, IL-1 β and TNF α , block parietal cell *Shh* expression in primary cell cultures (Waghray *et al.* 2010). Thus clearly Hh signalling is essential to the physiology of the stomach and specifically parietal cell acid secretion. The latter point was recently underscored by Zavros and coworkers who conditionally deleted the *Shh* locus in parietal cells (H⁺,K⁺-ATPase Cre mice bred to *Shh*^{FL/FL} mice) (Xiao *et al.* 2010). These mice were hypochlorhydric, hypergastrinaemic and developed foveolar hyperplasia (expansion of the surface mucous pit cell layer). This result suggests that the high levels of *Shh* produced by the parietal cells restrict expansion of the surface pit compartment (Fig. 4). We also found that transgenic expression of the transcriptional target and Hh ligand inhibitor called *Hip1* from the H⁺,K⁺-ATPase promoter inhibits acid secretion and increases plasma gastrin levels in 2-month-old mice (El-Zaatari *et al.* 2010).

Under normal conditions, the mucous neck population appears to function as the precursor cell population that gives rise to the pepsinogen producing zymogenic cell lineage (Ramsey *et al.* 2007). Thus the high levels of *Shh* produced by the parietal cells in the central portion of the gland appear to support this transition from mucous neck to zymogenic lineage (Fig. 4). However, there are no transgenic studies demonstrating a role for *Shh* on other gastric epithelial cell populations. Nevertheless, the cells at the gland base are likely to be exposed to lower concentrations of *Shh* secreted from the parietal cells, raising the possibility that it is a gradient from high to low concentrations of *Shh* that correlates with the lineage shift (Fig. 4). In gradient scenario no. 1, *Shh* diffuses from the central portion of the gland populated by parietal and mucous neck cells to the luminal surface (with acid).

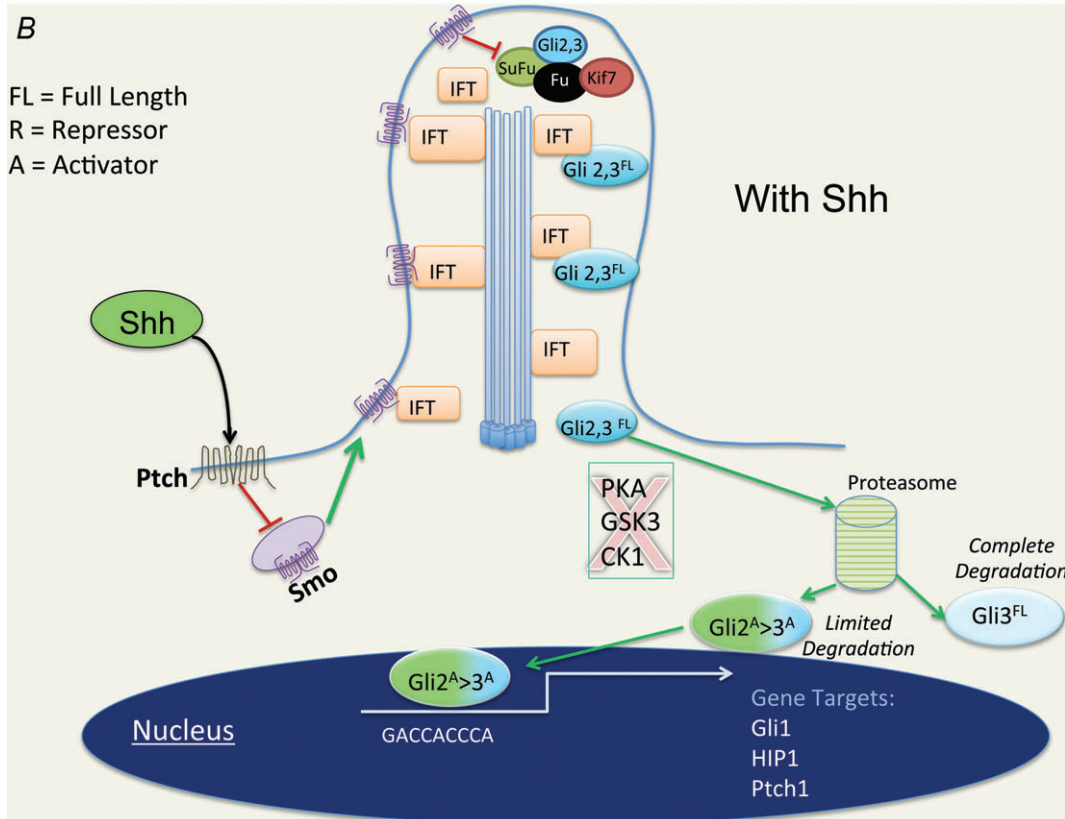
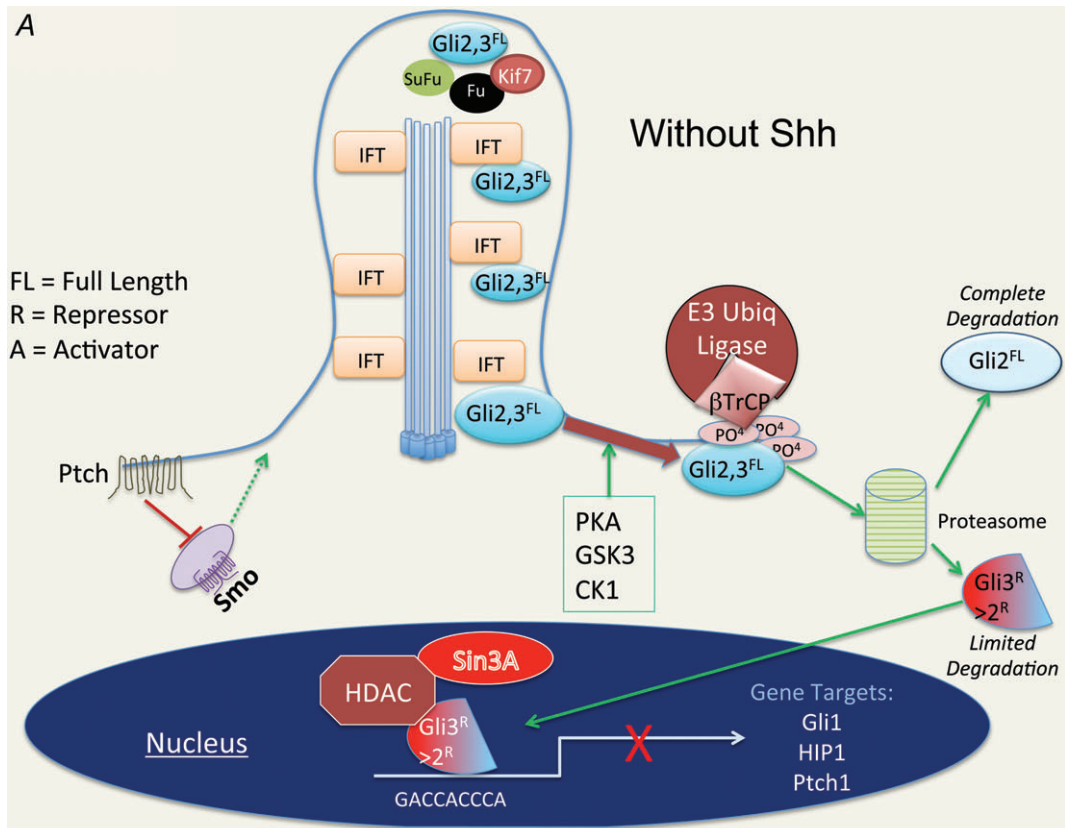


Figure 4. Gastric Shh inversely correlated with mucous cell compartment expansion

A significant amount of Shh in the stomach is generated by the acid-producing parietal cells. Conditionally null Shh mice expand their surface pit mucous cell compartment (foveolar hyperplasia). Shh processing in stomach requires acid secretion and is stimulated by gastrin. Shh is known to form a concentration gradient and in turn exert a differential effect on genes as a result. Thus there is a sliding scale of Shh concentrations and subsequent effects on gene expression. Therefore gastric Shh might form a gradient with increasing Shh concentration towards the gastric gland lumen (scenario no. 1) or maintain a gradient in which the concentrations are elevated near the mid-gastric gland then diffuse toward the lumen and gland base (scenario no. 2). Examples of potential gene targets expressed by specific cell types are listed on the right.

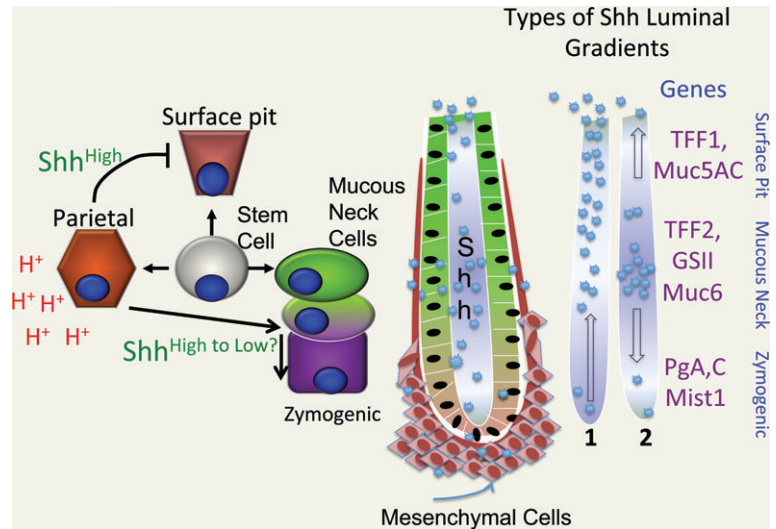


Table 2. Shh gradients and gut development

	Shh ^{High}	Shh ^{Med}	Shh ^{Low}
H ⁺ ,K ⁺ -ATPase	On	On	Off
Gastrin	Off	On	Off
Sucrase	Off	Off	Off
<i>Gut polarity</i>	<i>Corpus</i>	<i>Antrum</i>	<i>Intestine/Colon</i>

Table 3. Model for Shh gradients and gastric cell fate

	Shh ^{High}	Shh ^{Med}	Shh ^{Low}
TFF1	Off	Off	On
TFF2	On	Off	Off
Mist1	Off	On	On
<i>Gastric lineages</i>	<i>Mucous neck</i>	<i>Mucous neck to zymogenic</i>	<i>Zymogenic surface pit</i>

By contrast in gradient scenario no. 2, there is bidirectional diffusion of Shh to both the gastric lumen and gland base. Typical lineage markers for these cell types are indicated to infer that the Shh concentration gradient established might affect these gene targets indirectly once canonical gene targets become activated in the mesenchyme (Fig. 4). Examples of gene targets influenced by a Shh gradient are shown in Table 2 for the longitudinal gut axis and in Table 3 predicted for the gastric corpus gland. Therefore Shh ligand secreted specifically from parietal cells is required to maintain parietal cell homeostasis and secretory function *in vivo*.

An important exception to the paracrine Hh signalling route is the response of myofibroblasts to tissue injury of which there are several examples that will be highlighted. Normally, the adult liver does not produce

Hh ligands. However, the hepatic stellate cell initiates a tissue repair programme in which this myofibroblastic cell type both produces and responds to Shh by expressing collagen (Syn *et al.* 2009; Choi *et al.* 2010). Persistent tissue injury produces liver fibrosis (cirrhosis) a preneoplastic change that can ultimately lead to hepatocellular cancer (Omenetti & Diehl, 2008). Recently, it has been shown that infiltrating mesenchymal stem cells (MSCs) from the bone marrow will produce Shh once recruited to the stomach during *Helicobacter*-induced chronic gastritis (Quante *et al.* 2011). This example suggests that bone marrow-derived cells recruited to the stomach exhibit a Hh ligand-signal transduction loop in response to tissue injury and inflammation. Finally in a recent study, it has been shown that

Figure 3. Hedgehog signalling in primary cilia

A, in the absence of Shh ligand, Smo-laden vesicles reside in the cytoplasm. Full-length (FL) Gli2 and Gli3 reside at the cilia tip with repressor complex proteins Fused, SuFu and Kif7. The intraflagellar protein (IFT) moves into the cilium with cargo proteins. If Gli2 and 3 are phosphorylated sequentially by PKA, CK or GSK3 β , the docking protein β TrCP recognizes the phosphopeptide motif and recruits an E3 ligase to ubiquitinate (Ub) the protein. Ub Gli proteins undergo degradation by the proteasome. The repressor Gli2 protein moves to the nucleus and inhibits genes with co-repressor complexes. B, Hh signalling triggers Smo-laden vesicles to fuse with the plasma membrane once relieved from the inhibitory effects of the receptor Ptch. IFTs carry Smo antegrade to the tip of the cilium. Fused phosphorylates SuFu, Gli2 and Gli3 are released to the cytoplasm, phosphorylation is inhibited and Gli3 repressor is degraded. Gli2 migrates to the nucleus to bind Hh target genes.

Hh signalling is required for haematopoietic stem cell (HSC) proliferation and differentiation (Merchant *et al.* 2010a). In that study, Gli1 null mice (deficient Hh signalling) showed defective myeloid differentiation and stress-induced haematopoiesis but no effect on T or B cell development. Thus the bone marrow-derived lineages appear to be especially sensitive to deficient Hh signalling.

The best-known example of regulated Shh expression in the setting of tissue injury and neoplastic transformation occurs in the development of cerebellar tumours (medulloblastoma). Specifically, the pro-inflammatory cytokine interferon γ (IFN γ) induces Shh gene expression in cerebellar granule neurons through a STAT1-dependent mechanism. A common theme among the examples in which Hh signalling exhibits an autocrine loop is that all four cell types (hepatic stellate, MSC, HSC, cerebellar granule cell) are a type of stroma-derived progenitor or stem cell (Silbereis *et al.* 2010; Quante *et al.* 2011; Reister *et al.* 2011). We have confirmed that pro-inflammatory cytokines, e.g. IFN γ , stimulate Shh gene expression in primary parietal cell cultures (El-Zaatari *et al.* 2010). Thus a plausible scenario to consider and further examine is the ability of pro-inflammatory cytokines to initiate carcinogenesis through chronic stimulation of Shh ligand production in stromal cells triggering their proliferation (fibrosis) and subsequent secretion of factors (Wnts, TGF β) that stimulate specific epithelial cell populations, e.g. gastric mucous cell populations (Fig. 5).

In summary, Hh ligands typically produced by the epithelium target mesenchymal cells in the gut stroma (Kolterud *et al.* 2009). This has been described as the classic 'paracrine' Hh pathway observed in multiple tissues. By contrast, other ligand-dependent mechanisms observed in cancers include 'autocrine' and 'reverse' paracrine in which the ligand is generated in stroma in

addition to ligand-independent mechanisms (cell autonomous) (Scales & de Sauvage, 2009; Marini *et al.* 2011). In addition, regulation and tissue specific differences in Hh ligand processing might complicate our understanding of Hh signalling, but this will require analysis of the native protein in other epithelia. During chronic inflammation, pro-inflammatory cytokines create an environment supporting autocrine regulation of Hh signalling in MSCs (Quante *et al.* 2011). This mechanism would imply a type of feedforward pathway initiated by inflammation. In the gut, infiltrating MSCs responding to cell autonomous Hh signalling might be an important contributor to gastrointestinal tumorigenesis.

Hedgehog signalling in gastrointestinal cancer

There are two schools of thought with respect to the role of the Hh pathway in gastric tumorigenesis. The first scenario is related to immunohistochemical staining of Hh pathway components in human gastric cancer showing Shh ligand and signalling components expressed in supporting mesenchymal cells (Fukaya *et al.* 2006; Teglund & Toftgard, 2010). This theory would suggest that Hh mediates autocrine signalling in the mesenchyme that either initiates or supports neoplastic transformation of the epithelium. However, it is difficult if not impossible to conclude causation by Hh signalling without functional testing by knocking down various components of the pathway in cell lines. The second scenario is based on emerging transgenic mouse studies in which conditional deletion of Shh or Hh signal transduction components (Ptch1, Smo, Gli1) would prevent transformation. As previously discussed, Xiao *et al.* (2010) performed conditional deletion of Shh from the parietal cells and observed hyperplastic changes. The proliferative phenotype was

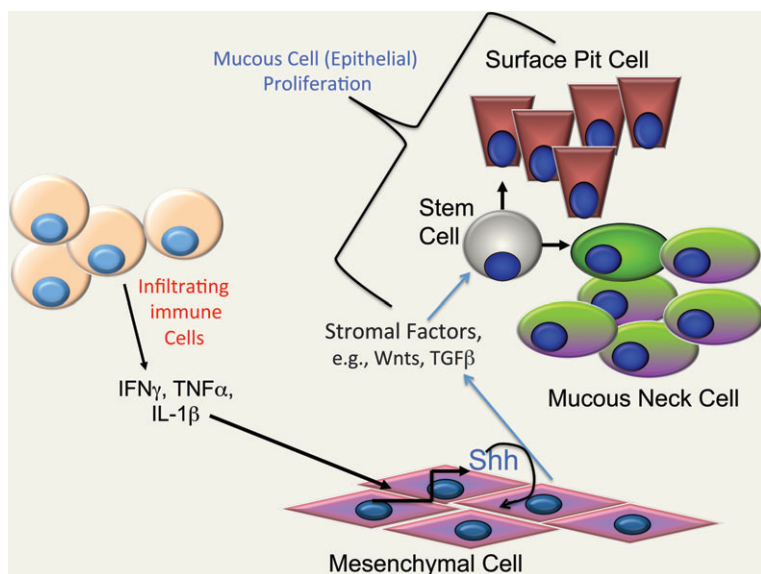


Figure 5. Pro-inflammatory cytokine initiation of Shh feedforward loop

During chronic gastric inflammation, pro-inflammatory cytokines (Th1) are able to stimulate Shh gene expression in stromal cells. Inflammation in the stomach inhibits parietal cell acid secretion and eventually triggers loss of the parietal and zymogenic cell lineages (atrophy). This potentially results in expansion of the mesenchymal cell population and production of pro-proliferative stromal factors, e.g. Wnts, TGF β . Wnt signalling is then available to induce mucous cell proliferation (metaplasia) and subsequently dysplasia/cancer.

attributed to activation of epithelial to mesenchymal transition regulators, e.g. Snail.

In the small intestine, inhibition of Hh signalling by transgenic expression of *Hip1* results in expansion of the mesenchyme and abnormal villus formation (Madison *et al.* 2005). Overexpression of *Ihh* from the intestinal epithelium stimulates proliferation of the smooth muscle cells and over time epithelial expansion of the crypt compartment (Zacharias *et al.* 2011). By contrast, Kaestner and co-workers found using a cell line based approach that mesenchymal targets of the Hh pathway FoxF1 and FoxL1 suppress Wnt signalling (Madison *et al.* 2009). Thus when Hh ligands are deleted, specific Fox homeobox genes are not activated and Wnt signalling becomes de-repressed. This result suggests that Hh signalling initiated from the epithelium exerts an anti-proliferative effect on targets in the mesenchyme. Thus loss of Hh ligand from the intestinal epithelium appears to produce similar outcomes. In the stomach, loss of Shh with parietal cell atrophy appears to create an environment of epithelial hyperplasia whereas in the small intestine, there is smooth muscle atrophy (mesenchyme) but subsequent epithelial hyperplasia with loss of *Ihh*. The stronger mesenchymal response in the intestine might reflect subtle differences between Shh and *Ihh*.

Several studies have examined the expression of Shh and Hh signalling proteins in human colon cancer by immunohistochemistry (Oniscu *et al.* 2004; Alinger *et al.* 2009; Yoshikawa *et al.* 2009; Saqui-Salces & Merchant, 2010), but few have demonstrated a causative role. Deletion of the Hh receptor Ptch1 increases the accumulation of myofibroblasts but reduced epithelial proliferation (van Dop *et al.* 2009). However, Varnat *et al.* (2010) showed strong expression of the Shh promoter in normal mouse colon by crossing ShhCre mice to the LacZ indicator mouse line ROSA26. In addition, the group showed that conditional deletion of Smo (decreased Hh signalling) decreases polyp formation in APC mutant mice (APC^{Fl/Fl} × CreERT2). Collectively, these initial genetic studies suggest that Hh signalling in the intestine appears to be required for epithelial proliferation and neoplastic transformation.

Future directions

In summary, understanding Hh signalling in the luminal GI tract is still in its infancy but is poised to be of critical importance due to the high levels of expression in the normal adult stomach and to a lesser extent in the colon. Hh signalling is clearly important in the mesenchyme, but might be driven by ligand-independent mechanisms. In this review, the role of Hh signalling in normal gastric physiology, specifically its requirement for the stomach to secrete acid was highlighted due to a number of functional studies that currently exists. Although Shh levels have been

strongly implicated in a number of epithelial cancers, e.g. skin, brain, pancreas, the direct role of Hh ligands in gastrointestinal cancers is not yet certain. The current evidence is most convincing for Hh signalling (Ptch1, Smo) inducing transformation, but this might be due to ligand-independent mechanisms. These issues will likely be sorted out as more genetic reagents become available for the luminal GI tract.

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