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REVIEW

Hedgehog Signaling Links Chronic Inflammation to Gastric Cancer Precursor Lesions

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SUMMARY

Hedgehog signaling plays an essential role in gastric development, homeostasis, and neoplastic transformation. This article reviews the evidence for its role in the initiation of gastric inflammation due to Helicobacter infection but then chronically polarizes myeloid cells into myeloid-derived suppressor cells creating a microenvironment favoring cancer development.

Since its initial discovery in *Drosophila*, Hedgehog (HH) signaling has long been associated with foregut development. The mammalian genome expresses 3 HH ligands, with sonic hedgehog (SHH) levels highest in the mucosa of the embryonic foregut. More recently, interest in the pathway has shifted to improving our understanding of its role in gastrointestinal cancers. The use of reporter mice proved instrumental in our ability to probe the expression pattern of SHH ligand and the cell types responding to canonical HH signaling during homeostasis, inflammation, and neoplastic transformation. SHH is highly expressed in parietal cells and is required for these cells to produce gastric acid. Furthermore, myofibroblasts are the predominant cell type responding to HH ligand in the uninfected stomach. Chronic infection caused by *Helicobacter pylori* and associated inflammation induces parietal cell atrophy and the expansion of metaplastic cell types, a precursor to gastric cancer in human subjects. During *Helicobacter* infection in mice, canonical HH signaling is required for inflammatory cells to be recruited from the bone marrow to the stomach and for metaplastic development. Specifically, polarization of the invading myeloid cells to myeloid-derived suppressor cells requires the HH-regulated transcription factor GLI1, thereby creating a microenvironment favoring wound healing and neoplastic transformation. In mice, GLI1 mediates the phenotypic shift to gastric myeloid-derived suppressor cells by directly inducing *Schlafen 4* (*slfn4*). However, the human homologs of SLFN4, designated SLFN5 and SLFN12L, also correlate with intestinal metaplasia and could be used as biomarkers to predict the subset of individuals who might progress to gastric cancer and benefit from treatment with HH antagonists. *(Cell Mol Gastroenterol Hepatol 2017;3:201–210; http://dx.doi.org/10.1016/j.jcmgh.2017.01.004)*

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Hedgehog (HH) signaling initiates cancer in several organ systems,[1,2] but a clear etiologic role has not been shown for this pathway in gastric cancer. Because HH inhibitors currently are undergoing clinical trials for different types of cancer, understanding the role of HH signaling in regulating the tumor microenvironment becomes an important target to consider.[3] Based on prior mouse studies of increased HH signaling in preneoplastic lesions,[4–6] we have suggested that the use of HH inhibitors in human subjects chronically infected with *Helicobacter* might prevent progression of chronic atrophic gastritis to mucous gland metaplasias, a sentinel lesion that increases the likelihood of gastric cancer.[7–10] Thus, the focus of the current review is to understand the basis for HH signaling in normal adult stomach and how this developmental pathway might play a role in neoplastic transformation. Because our current understanding of HH signaling in the stomach arises from transgenic mouse models, the information presented refers to the mouse except when information from human studies exists.

Role of Hedgehog Signaling in Gastric Homeostasis

To date, there are 3 known mammalian genes encoding the hedgehog ligands: Sonic hedgehog (SHH), Indian hedgehog, and Desert hedgehog.[11–13] During embryonic development, SHH is expressed throughout the gut and in other foregut-derived organs (eg, lung, pancreas).[14–16] Although its function in mature gastric epithelium was not initially studied in adult mammals, it became apparent that SHH remains highly expressed in the stomach once expression in

Abbreviations used in this paper: ATPase, adenosine triphosphatase; DAMP, damage-associated molecular pattern; GLI, glioma-associated protein; Gr-MDSC, granulocytic myeloid-derived suppressor cell; HH, hedgehog; HHIP, hedgehog-interacting protein; IFN, interferon; IL, interleukin; MDSC, myeloid-derived suppressor cell; Mo-MDSC, monocytic myeloid-derived suppressor cell; mRNA, messenger RNA; PTCH, Patched; SHH, sonic hedgehog; SLFN4, Schlafen 4; SMO, Smoothened; SP, spasmolytic polypeptide; SPEM, spasmolytic polypeptide–expressing mucosa; SST, somatostatin; TLR, Toll-like receptor.

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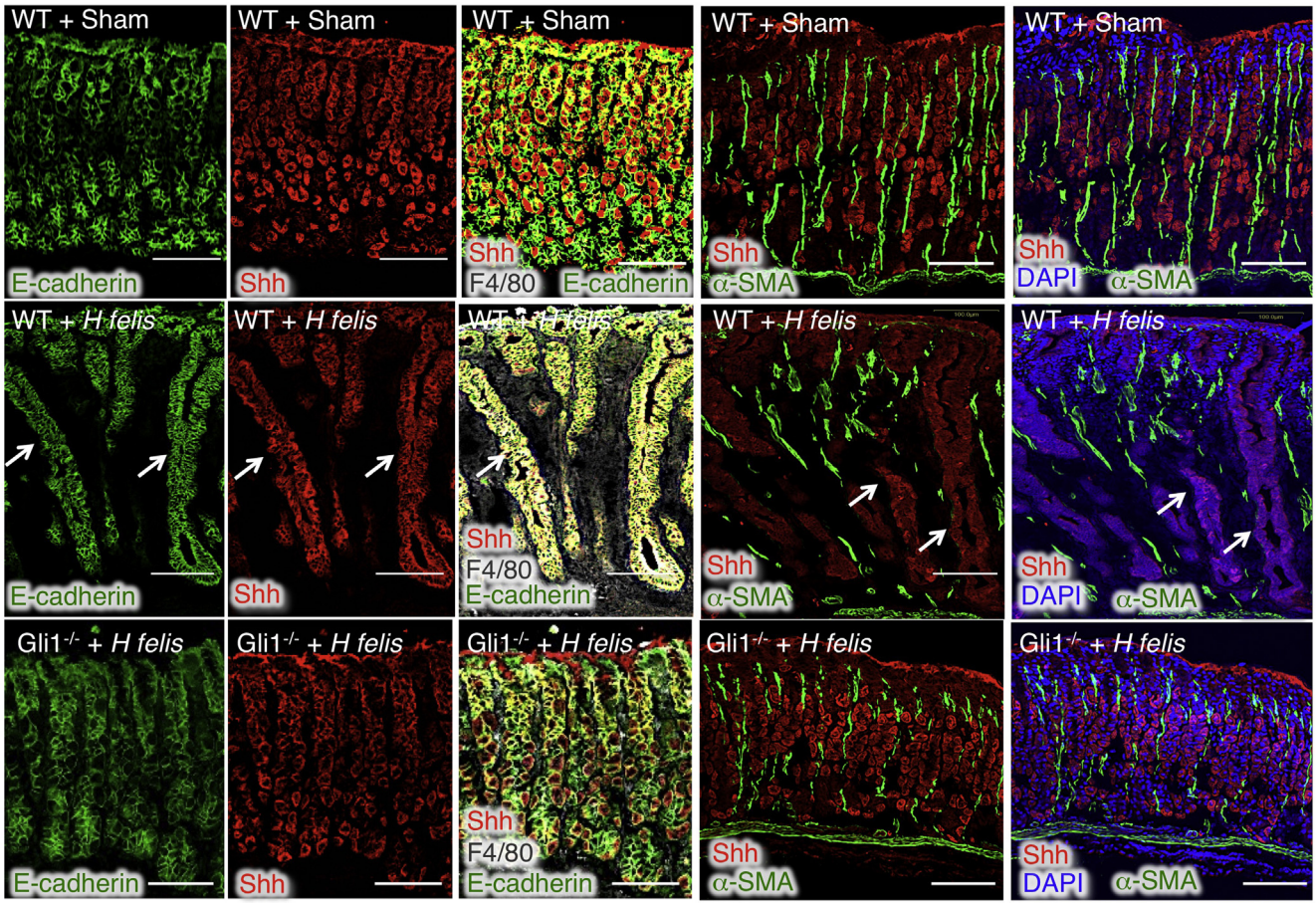

Figure 1. SHH expression in the stomach corpus of wild-type (WT) and *Gli1*$^{-/-}$ mice. Shown is the co-localization of SHH with E-cadherin, F4/80 (macrophage/myeloid marker), or α-smooth muscle actin (SMA) (myofibroblasts) protein markers in the absence or presence of *Helicobacter felis* infection. 4′,6-diamidino-2-phenylindole (DAPI) indicates cell nuclei. *Arrows* indicate the presence of SPEM. Reprinted with permission from El-Zaatari et al.[4] Scale bars = 100 μm.

the intestine diminishes.[17,18] Subsequently, it was reported that SHH regulates epithelial cell maturation and differentiation in the adult stomach.[19,20] Normally, SHH is expressed in mature acid-secreting glands of the adult mouse and human stomachs, primarily within parietal cells[19,21–23] (Figure 1). During progression from the inflamed stomach to gastric cancer, the acid-producing parietal cells fail to produce acid and eventually are replaced by mucous-secreting cells that express spasmolytic polypeptide (SP) or trefoil factor 2.[7,24] Mostly in mice, but also in human subjects, SP-expressing mucosa (SPEM) is a type of oxyntic gland atrophy.[25,26] In concert with parietal cell atrophy, SHH expression in these acid-producing cells also is lost.[23,27] Although SHH expression diminishes along with loss of parietal cells, the expanding mucous cell compartment or SPEM continues to produce SHH in both human subjects[20,23] and rodents,[4,27] but remains unprocessed, maintaining the full-length 45-kilodalton form[28] (Figure 1). Surprisingly, even unprocessed Hedgehog protein (*Drosophila*) shows activity where it traffics to the cell membrane to participate in cell–cell signaling.[29] This result suggests that aberrant HH signaling in cancer might function as an autocrine or paracrine regulator, especially in the stem cell niche.[30–32]

Processing of SHH to its active form (19 kilodaltons) in parietal cells becomes compromised in the absence of gastric acid.[28] Atrophy of parietal and zymogenic (chief cell) lineages result in hypochlorhydria and reduced serum pepsinogen I (A) levels compared with pepsinogen II (C).[33–39] These zymogens are proteins encoded by different gene loci that are used clinically to indicate preneoplastic changes in the stomach.[38,39] Pepsinogens A and C are converted to the enzymatically active aspartic proteinases, pepsin A and pepsin C, through intramolecular self-cleavage.[39,40] We showed previously that pepsinogen A is produced primarily in the mouse corpus by parietal cells, whereas pepsinogen C is produced primarily by both mucous neck and chief cells throughout the stomach.[28] This result is consistent with the exclusive expression of pepsinogen A in the human corpus and not the antrum, whereas pepsinogen C marks mucous cells of both the antrum and corpus (www.proteinatlas.org). Pepsin A prefers to cleave proteins at hydrophobic and aromatic residues, particularly at phenylalanine (F) when the pH is less than 2. By contrast, pepsin C recognizes a broader consensus site and uses a wider pH spectrum than pepsin A.[40,41] Specifically, we showed using site-directed