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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Regulation of Gastric Lgr5+ve Cell Homeostasis by Bone Morphogenetic Protein (BMP) Signaling and Inflammatory Stimuli

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SUMMARY

This study reports the novel observation that inflammation and inhibition of bone morphogenetic protein signaling activate Leucine-rich repeat-containing G-protein-coupled receptor 5 +ve cells located on the lesser curvature of the oxyntic mucosa that give rise to spasmolytic polypeptide expressing metaplasia as well as dysplastic, proliferating lineages. These findings offer new insights into the mechanisms that lead to gastric metaplasia and dysplasia.

BACKGROUND & AIMS: Gastric Leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) cells exert important functions during injury and homeostasis. Bone morphogenetic protein (BMP) signaling regulates gastric inflammation and epithelial homeostasis. We investigated if BMP signaling controls the fate of Lgr5^{+ve} cells during inflammation.

METHODS: The *H^+/K^+-adenosine triphosphatase β-subunit* promoter was used to express the BMP inhibitor noggin (*Nog*) in the stomach (*H^+/K^+-Nog* mice). Inhibition of BMP signaling in Lgr5 cells was achieved by crossing *Lgr5-EGFP-ires-CreERT2* (*Lgr5-Cre*) mice to mice with floxed alleles of BMP receptor 1A (*Lgr5-Cre;Bmpr1a$^{flox/flox}$* mice). Lgr5/GFP^{+ve} cells were isolated using flow cytometry. Lineage tracing studies were conducted by crossing *Lgr5-Cre* mice to mice that express *Nog* and *tdTomato* (*Lgr5-Cre;H^+/K^+-Nog;Rosa26-tdTom*). Infection with *Helicobacter felis* was used to induce inflammation. Morphology of the mucosa was analyzed by H&E staining. Distribution of H^+/K^+-adenosine triphosphatase-, IF-, Ki67-, CD44-, CD44v9-, and bromodeoxyuridine-positive cells was analyzed by immunostaining. Expression of neck and pit cell mucins was determined by staining with the lectins Griffonia (Bandeiraea) simplicifolia lectin II and Ulex europaeus agglutinin 1, respectively. *Id1*, *Bmpr1a*, *Lgr5*, *c-Myc*, and *Cd44* messenger RNAs were measured by quantitative reverse-transcription polymerase chain reaction.

RESULTS: *Lgr5-Cre;Bmpr1a$^{flox/flox}$* mice showed diminished expression of *Bmpr1a* in Lgr5/GFP^{+ve} cells. Infection of *Lgr5-Cre;Bmpr1a$^{flox/flox}$* mice with *H felis* led to enhanced inflammation, increased cell proliferation, parietal cell loss, and to the development of metaplasia and dysplasia. Infected *Lgr5-Cre;H^+/K^+-Nog;Rosa26-tdTom* mice, but not control mice, showed the presence of tomato^{+ve} glands lining the lesser curvature that stained positively with Griffonia (Bandeiraea) simplicifolia lectin II and Ulex europaeus agglutinin 1, and with anti-IF, -CD44, -CD44v9, and -bromodeoxyuridine antibodies.

CONCLUSIONS: Inflammation and inhibition of BMP signaling activate Lgr5^{+ve} cells, which give rise to metaplastic, dysplastic, proliferating lineages that express markers of mucus neck and zymogenic cell differentiation. *(Cell Mol Gastroenterol Hepatol 2018;5:523–538; https://doi.org/10.1016/j.jcmgh.2018.01.007)*

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Chronic inflammation has been recognized as an important causative factor for the development of gastric metaplasia, dysplasia, and neoplasia. Numerous experimental models have confirmed that exposure of the gastric epithelium to chronic inflammatory stimuli such as infection with *Helicobacter* organisms can lead to significant aberrations in gastric epithelial homeostasis.[1–5]

The mechanisms involved in the pathogenesis of metaplasia, dysplasia, and neoplasia in the context of chronic inflammation have been only partially characterized. One current hypothesis is that chronic inflammation and mucosal injury can cause aberrations in the normal biological functions of gastric epithelial cells, leading to the development of metaplastic and dysplastic changes of the gastric mucosa and, ultimately, to neoplasias.[1–6] Indeed, both intestinal

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Abbreviations used in this paper: ATPase, adenosine triphosphatase; BMP, bone morphogenetic protein; BrdU, bromodeoxyuridine; EGFP, enhanced green fluorescent protein; ERK, extracellular signal–regulated kinase; GFP, green fluorescent protein; GSII, Griffonia (Bandeiraea) simplicifolia lectin II; HBSS, Hank's balanced salt solution; H/K-nog, H/K-noggin; IF, intrinsic factor; mRNA, messenger RNA; QRT-PCR, quantitative reverse-transcription polymerase chain reaction; SPEM, spasmolytic polypeptide expressing metaplasia; TFF2, Trefoil factor 2.

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metaplasia and spasmolytic polypeptide expressing metaplasia (SPEM), which is characterized by the aberrant expression of Trefoil factor 2 (TFF2), and of mucins that bind the lectin Griffonia (Bandeiraea) simplicifolia lectin II (GSII) at the base of glands of the oxyntic mucosa, have been associated with inflammation-induced gastric neoplasms.[2,5,7–9] It has been suggested that SPEM might derive from reprograming of zymogenic cells during situations of inflammation and injury such as those triggered by infection with *Helicobacters*.[10]

Several studies have identified, on the basis of expression of molecular markers, such as the Wnt target gene *Lgr5*,[11] populations of gastric epithelial cells that can self-renew and that can show multilineage differentiation capacity. In addition to *Lgr5*, other potential markers for gastric stem/progenitor cells have been investigated recently, including *villin*, *Prom1/Cd133*, *Cd44*, *Dckl1/ Dcamkl1*, *Troy*, *Mist-1, Sox-2*, *Tff2*, and *Runx1*.[11–17] It has been suggested that genetic and epigenetic alterations of these cells might lead to their transformation and to the initiation of tumor growth.[14]

Leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) cells, in particular, have been shown to play an important role in gastrointestinal tissue homeostasis.[11,13,14] In the stomach, cells with *Lgr5* transcriptional activity initially were identified at the base of antral glands and, to a lesser degree, along the lesser curvature of the oxyntic mucosa, an area known to be one of the most common locations for the development of gastric carcinomas.[11,18,19] Recent studies, however, which have taken advantage of a different mouse model, have shown that Lgr5 cells are widely distributed in glands of the corpus where they represent a subpopulation of chief cells.[20] It also has been suggested that in the corpus these cells do not show stem cell characteristics during homeostasis but that after injury they acquire stem cell functions to promote epithelial repair.[20]

An increased number of *Lgr5*-expressing cells has been detected in patients with gastritis and intestinal metaplasia and in gastric carcinomas.[20–22] In support of these findings, a recent study showed that *Lgr5*-expressing chief cells can be a major cell-of origin of gastric neoplasms.[20] Reports also have shown that inflammatory stimuli such as *Helicobacter* organisms can adhere to *Lgr5*-expressing cells, leading to their proliferation and activation.[23]

The bone morphogenetic proteins (BMPs) are regulatory peptides that exert important effects on the growth and differentiation of gastrointestinal tissues. The actions of these proteins can be blocked by secreted inhibitory molecules, such as noggin, gremlin, and chordin, which are expressed in vivo to modulate the actions of the BMPs.[24,25] It has been shown that loss of BMP signaling in the stomach leads to perturbations of the normal homeostatic mechanisms of the gastric mucosa, leading to the development of metaplasia, dysplasia, and neoplasia.[25–27] In support of these observations studies have shown that BMP receptors such as BMPR1A and the BMP signal transducing proteins Smad1, 5, and 8 are widely expressed in the glandular stomach.[26,28]

In a series of published studies[26] we showed that transgenic expression in the corpus of the mouse stomach of the BMP inhibitor *noggin* leads to the development of SPEM, parietal cell loss, and to increased cell proliferation. We also reported that expression of noggin enhances *H felis*– *and Helicobacter pylori*–induced inflammation and gastric epithelial cell proliferation, leading to the accelerated development of dysplasia, and to increased expression and activation of the oncogenic proteins activation-induced cytidine deaminase and signal transducer and activator of transcription (STAT)3.[27]

The mechanisms that regulate the number and function of $Lgr5^{+ve}$ cells during gastric inflammation, and those involved in the development of gastric metaplasia and dysplasia, have been only partially characterized. Accordingly, we sought to investigate the role of BMP signaling in the regulation of Lgr5 cell homeostasis during gastric inflammation. In particular, we tested the hypothesis that inflammation and inhibition/ loss of BMP signaling induce the activation of $Lgr5^{+ve}$ cells, located in the oxyntic mucosa of the lesser curvature, that lead to the development of metaplastic and dysplastic epithelial cell lineages.

Materials and Methods

Mice

The *H/K-noggin* (*H/K-nog*) transgenic mice were generated in our laboratory and they were described previously.[26] Pathogen-free C57BL/6 mice, *Lgr5-enhanced green fluorescent protein (EGFP)-ires-CreERT2* (*Lgr5-Cre*) mice, in which the expression of both *Cre* and green fluorescent protein (*GFP*) is driven by endogenous *Lgr5* regulatory sequences,[11] and *Rosa26-tdTom* (*Rosa26-Tom*) mice aged 4–10 weeks were purchased from Jackson Laboratory (Bar Harbor, ME). $Bmpr1a^{flox/flox}$[29] mice were received from the laboratory of Dr Yuji Mishina (University of Michigan). Mouse genotyping was described elsewhere.[26]

Specific inhibition of BMP signaling in Lgr5 cells was achieved by crossing *Lgr5-Cre* mice to $Bmpr1a^{flox\text{-}flox}$ mice to generate *Lgr5-Cre;*$Bmpr1a^{flox\text{-}flox}$ mice. To conduct lineage tracing in the presence of Noggin, H^+/K^+*-nog* mice were crossed to *Rosa26-Tom* reporter mice to generate H^+/K^+*-nog;Rosa26-Tom* mice. *Lgr5-Cre* mice then were crossed to both H^+/K^+*-Nog;Rosa26-Tom* and *Rosa26-Tom* mice to generate *Lgr5-Cre;*H^+/K^+*-Nog;Rosa26-Tom* and *Lgr5-Cre;Rosa26-Tom* mice. Cre was activated by 1 intraperitoneal injection of tamoxifen (0.1 mg/g body weight). All mice, including *Lgr5-Cre*, *Lgr5-Cre;*$Bmpr1a^{flox\text{-}flox}$, *Lgr5-Cre;*$H^+/K^+$*-Nog;Rosa26-Tom*, and *Lgr5-Cre;Rosa26-Tom* mice received tamoxifen injections. The mice were maintained on a C57BL/6 background. In some experiments, mice were injected with 200 μL of a 10 mg/mL solution of bromodeoxyuridine (BrdU, BD Biosciences, San Jose, CA) 2 hours before tissue collection. In all experiments, animals were fasted overnight with free access to water before tissue collection. Mice were housed under specific pathogen-free conditions in automated watered and ventilated cages on a 12-hour light/ dark cycle in the animal maintenance facility at the University of Michigan. All animal experiments were approved by the University of Michigan Animal Care and Use Committee.

H felis *Culture and Infection*

Two- to 3-month-old *Lgr5-Cre* mice, *Lgr5-Cre;*$Bmpr1a^{flox/flox}$mice, *Lgr5-Cre;Rosa26-Tom* mice, and