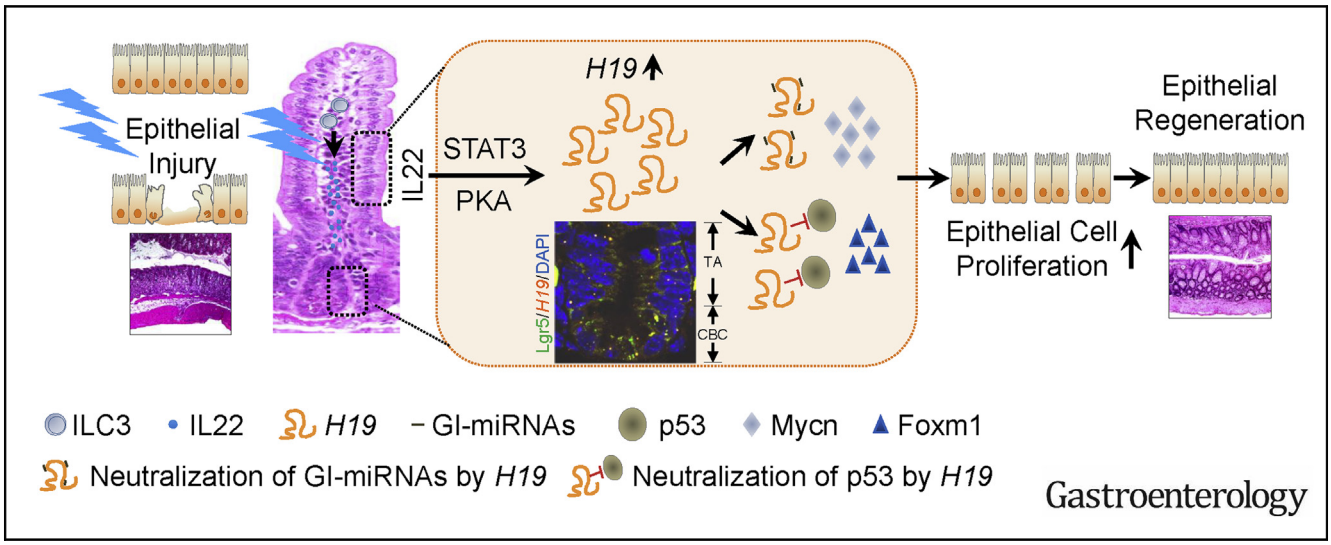
Gastroenterology 2018;155:144–155

In Inflamed Intestinal Tissues and Epithelial Cells, Interleukin 22 Signaling Increases Expression of *H19* Long Noncoding RNA, Which Promotes Mucosal Regeneration

Hua Geng,[1,2] Heng-Fu Bu,[1,2] Fangyi Liu,[1,2,3] Longtao Wu,[4,5] Karl Pfeifer,[6] Pauline M. Chou,[7] Xiao Wang,[1,2] Jiaren Sun,[8] Lu Lu,[9] Ashutosh Pandey,[9] Marisa S. Bartolomei,[10] Isabelle G. De Plaen,[1,2] Peng Wang,[1,2] Jindan Yu,[4] Jiaming Qian,[3] and Xiao-Di Tan[1,2,7]

[1]Center for Intestinal and Liver Inflammation Research, Stanley Manne Children's Research Institute, Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois; [2]Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; [3]Department of Gastroenterology, Peking Union Medical College Hospital, Peking Union Medical College, Beijing, People's Republic of China; [4]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; [5]Department of Surgery, University of Chicago, Chicago, Illinois; [6]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland; [7]Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; [8]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas; [9]Department of Genetics, Genomics and Informatics, University of Tennessee Health Science Center, Memphis, Tennessee; and [10]Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania



BACKGROUND & AIMS: Inflammation affects regeneration of the intestinal epithelia; long noncoding RNAs (lncRNAs) regulate cell functions, such as proliferation, differentiation, and migration. We investigated the mechanisms by which the lncRNA *H19*, imprinted maternally expressed transcript (*H19*) regulates regeneration of intestinal epithelium using cell cultures and mouse models of inflammation. **METHODS:** We performed RNA-sequencing transcriptome analyses of intestinal tissues from mice with lipopolysaccharide (LPS)-induced sepsis to identify lncRNAs associated with inflammation; findings were confirmed by quantitative real-time polymerase chain reaction and in situ hybridization analyses of intestinal tissues from mice with sepsis or dextran sulfate sodium (DSS)–induced mucosal wound healing and patients with ulcerative colitis compared to healthy individuals (controls). We screened cytokines for their ability to induce expression of *H19* in HT-29 cells and intestinal epithelial cells (IECs), and confirmed findings in crypt epithelial organoids derived from mouse small intestine. IECs were incubated with different signal transduction inhibitors and effects on *H19* lncRNA levels were measured. We assessed intestinal epithelial proliferation or regeneration in $H19^{\Delta Ex1/+}$ mice given LPS or DSS vs wild-type littermates (control mice). *H19* was overexpressed in IECs using lentiviral vectors and cell proliferation was measured. We performed RNA antisense purification, RNA immunoprecipitation, and luciferase reporter assays to study functions of *H19* in IECs. **RESULTS:** In RNA-sequencing transcriptome analysis of lncRNA expression in intestinal tissues from mice, we found that levels of *H19* lncRNA changed significantly with LPS exposure. Levels of *H19* lncRNA increased in intestinal tissues of patients with ulcerative colitis, mice with LPS-induced and polymicrobial sepsis, or mice with DSS-induced colitis, compared with controls. Increased *H19* lncRNA localized to epithelial cells in the intestine, regardless of *Lgr5* messenger RNA expression. Exposure of IECs to interleukin 22 (IL22) increased levels of *H19* lncRNA with time and dose, which required STAT3 and protein kinase A activity. IL22 induced

expression of *H19* in mouse intestinal epithelial organoids within 6 hours. Exposure to IL22 increased growth of intestinal epithelial organoids derived from control mice, but not $H19^{\Delta Ex1/+}$ mice. Overexpression of *H19* in HT-29 cells increased their proliferation. Intestinal mucosa healed more slowly after withdrawal of DSS from $H19^{\Delta Ex1/+}$ mice vs control mice. Crypt epithelial cells from $H19^{\Delta Ex1/+}$ mice proliferated more slowly than those from control mice after exposure to LPS. *H19* lncRNA bound to p53 and microRNAs that inhibit cell proliferation, including microRNA 34a and let-7; *H19* lncRNA binding blocked their function, leading to increased expression of genes that promote regeneration of the epithelium. **CONCLUSIONS:** The level of lncRNA *H19* is increased in inflamed intestinal tissues from mice and patients. The inflammatory cytokine IL22 induces expression of *H19* in IECs, which is required for intestinal epithelial proliferation and mucosal healing. *H19* lncRNA appears to inhibit p53 protein and microRNA 34a and let-7 to promote proliferation of IECs and epithelial regeneration.

Keywords: Gene Regulation; Mouse Model; Ulcerative Colitis; Tissue Repair.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Intestinal epithelial regeneration plays an important role in sustaining intestinal epithelial homeostasis under inflammatory conditions. Little is known about how inflammatory signals regulate this process.

NEW FINDINGS

The researchers identified an IL22/*H19* lncRNA signaling axis in intestinal epithelial cells. Induction of *H19* by the proinflammatory cytokine IL22 inhibited the activity of negative regulators of cell proliferation (including p53, MIR34a, and let-7) to promote intestinal epithelial regeneration.

LIMITATIONS

This study did not fully investigate the mechanism by which *H19* lncRNA attenuates the activities of negative regulators of intestinal epithelial cell proliferation.

IMPACT

The IL22/*H19* lncRNA signaling axis represents an novel target for therapeutic interventions to promote gut mucosal wound healing.

Long noncoding RNAs (lncRNAs) represent a large and diverse class of non-protein coding transcripts longer than 200 nucleotides.[1] High-throughput sequencing analysis of the whole mammalian genome and transcriptome revealed a vast number of lncRNAs, and growing evidence shows that several lncRNAs have biological roles in regulating gene expression, controlling protein function, and organizing multiprotein complex assembly.[1] The lncRNAs act as intracellular signals, decoys, guides, and scaffolds via DNA, RNA, and protein interactions. Several lncRNAs have also been proposed to participate in cell signaling, thereby impacting cellular functions and homeostasis in vivo.[2,3] Mounting studies support a role of lncRNAs in the pathogenesis of diseases.[1]

The intestinal epithelium is a single layer of columnar cells lining the luminal surface of the intestinal mucosa that is regenerated throughout adult life. It provides a critical barrier to harmful intraluminal entities, including foreign antigens and micro-organisms and their toxins. In the normal physiological state, renewal of the intestinal epithelium is governed by canonical Wnt signaling.[4] Intestinal epithelial injury often results from inflammatory bowel disease, which causes exacerbated inflammation in the intestinal mucosa. It also can occur in several critical conditions, such as sepsis, severe burn injury, and gastrointestinal radiation injury, during which bacteria or bacterial products are permitted to translocate across the intestinal epithelial barrier and into the bloodstream. Epithelial regeneration is a critical step for wound healing of the intestinal mucosa and plays an important role in sustaining intestinal epithelial integrity in response to inflammation and in critically ill patients. Previous studies suggest that interleukin (IL) 6 and IL22 promote intestinal epithelial regeneration in pathological conditions through distinctive receptor-mediated signal pathways,[5–7] suggesting a role for inflammatory cytokine-associated signaling. In addition to inflammatory cytokine-associated signaling pathways, studies have shown that intestinal epithelial cells (IECs) express a number of negative regulators to control regeneration of intestinal epithelium, for example, p53 protein.[8] Recently, microRNAs (miRNAs), such as the let-7 family members have been identified as negative regulators of IEC proliferation.[9] It remains unknown whether and how lncRNAs participate in regulation of intestinal epithelial regeneration and homeostasis.

In this report, we provided evidence that *H19*, an evolutionarily conserved and maternally expressed imprinted lncRNA,[10] is induced by inflammation in IECs. We defined the effects of inflammatory mediators on expression of *H19* in IECs, investigated the role of *H19* lncRNA in intestinal epithelial wound healing, and elucidated the underlying molecular mechanisms by which *H19* lncRNA promotes re-establishment and sustains homeostasis of intestinal epithelium. Our study revealed that *H19* lncRNA is an inflammatory lncRNA induced by IL22 that antagonizes negative regulators of intestinal epithelial proliferation and plays an important role in sustaining intestinal epithelial regeneration under inflammatory conditions.

Materials and Methods

Detailed protocols are provided in the Supplementary Materials and Methods.

Abbreviations used in this paper: **cAMP, adenosine 3′,5′-cyclic monophosphate; DSS, dextran sulfate sodium; GI, growth inhibitory; IL, interleukin; lncRNA, long noncoding RNA; LPS, lipopolysaccharide; MIR675, microRNA 675; miRNA, microRNA; PKA, protein kinase A; RNA-seq, RNA sequencing; TA, transit-amplifying.**

Most current article

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2018.03.058