Krüppel-Like Factor 5 Mediates Transmissible Murine Colonic Hyperplasia Caused by *Citrobacter rodentium* Infection

BETH B. MCCONNELL,* JAN–MICHAEL A. KLAPPROTH,* MAIKO SASAKI,* MANDAYAM O. NANDAN,* and VINCENT W. YANG*,‡

*Division of Digestive Diseases, Department of Medicine, ‡Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia

Background & Aims: **Krüppel-like factor 5 (KLF5) is a transcription factor that is highly expressed in proliferating crypt cells of the intestinal epithelium. KLF5 has a pro-proliferative effect in vitro and is induced by mitogenic and stress stimuli. To determine whether KLF5 is involved in mediating proliferative responses to intestinal stressors in vivo, we examined its function in a mouse model of transmissible murine colonic hyperplasia triggered by colonization of the mouse colon by the bacteria *Citrobacter rodentium*.** ***Methods:*** **Heterozygous *Klf5* knockout (*Klf5*$^{+/-}$) mice were generated from embryonic stem cells carrying an insertional disruption of the *Klf5* gene. *Klf5*$^{+/-}$ mice or wild-type (WT) littermates were infected with *C rodentium* by oral gavage. At various time points postinfection, mice were killed and distal colons were harvested. Colonic crypt heights were determined morphometrically from sections stained with H&E. Frozen tissues were stained by immunofluorescence using antibodies against Klf5 and the proliferation marker, Ki67, to determine Klf5 expression and numbers of proliferating cells per crypt.** ***Results:*** **Infection of WT mice with *C rodentium* resulted in a 2-fold increase in colonic crypt heights at 14 days postinfection and was accompanied by a 1.7-fold increase in Klf5 expression. Infection of *Klf5*$^{+/-}$ mice showed an attenuated induction of Klf5 expression, and hyperproliferative responses to *C rodentium* were reduced in the *Klf5*$^{+/-}$ animals as compared with WT littermates.** ***Conclusion:*** **Our study shows that Klf5 is a key mediator of crypt cell proliferation in the colon in response to pathogenic bacterial infection.**

The mammalian gut epithelium is a dynamic tissue that plays an active role in maintaining tissue homeostasis in the face of rapid cell turnover and constant changes in the bacterial milieu. Maintaining the status quo requires the stringent regulation of pathways involving proliferation, differentiation, apoptosis, and inflammation. This balance becomes even more critical in instances of inflammatory bowel disease (IBD), in which dysregulation of these pathways can result in excessive tissue injury, inadequate tissue regeneration, and increased risk of developing cancer. Although the primary pathway involved in the normal regeneration of the intestinal epithelium has been relatively well characterized, little is known about the molecular events that regulate proliferation during pathogenic bacterial infection or exposure to other stressors.

Krüppel-like factor 5 (KLF5) is a member of a family of zinc finger-containing transcription factors that function in the regulation of diverse cellular processes, including development, proliferation, and differentiation.[1–6] KLF5 is highly expressed in the intestinal epithelium and is found predominantly in the proliferating cells of the crypt,[6–8] suggesting that it has a positive growth regulatory role in the intestinal tissue. Indeed, several in vitro studies support a pro-proliferative role for KLF5 in nontransformed cultured epithelial cells.[9–11] In addition, ectopic expression of KLF5 in NIH3T3 cells has been shown to promote proliferation.[10] Moreover, KLF5 has been shown to mediate the transforming effects of oncogenic H-RAS.[12,13] Transcriptional targets of KLF5 include a number of genes that encode pro-proliferative components of the cell-cycle machinery, including cyclin D1, cyclin B1, and Cdc2.[12,13]

Various external stimuli have been reported to activate KLF5 expression, including addition of fetal bovine serum to serum-deprived cells[10] and treatment of cultured cells with basic fibroblast growth factor[14] and phorbol 12-myristate 13-acetate.[14,15] Furthermore, in vivo studies conducted in mouse vascular tissue have shown an increase in KLF5 expression in response to physical stress caused by injury.[16] Induction of KLF5 has been shown to be downstream of activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK)1/2 pathway, with KLF5 expression being driven by the early response gene, early growth response factor 1.[14,17] Recently, our laboratory reported that KLF5 expression is induced in IEC-6 rat intestinal epithelial cells after exposure to the bacterial component lipopolysac-

Abbreviations used in this paper: KLF5, Krüppel-like factor 5; PCR, polymerase chain reaction; pi, postinfection; RT, reverse-transcription; WT, wild-type.

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charide.[18] Thus, given the pro-proliferative activity of KLF5 in vitro and its activation in response to various mitogenic and stress stimuli, we hypothesize that KLF5 may play a role in hyperproliferative responses to external stressors in intestinal tissues.

To address this hypothesis, the current study uses a mouse model of hyperproliferation known as transmissible murine colonic hyperplasia to examine the involvement of KLF5 in proliferative changes induced by enteric bacterial pathogenic infection. Transmissible murine colonic hyperplasia is caused by infection with *Citrobacter rodentium*, a gram-negative, noninvasive bacterial pathogen that colonizes the distal colon of mice by forming attaching and effacing lesions.[19] Infection is characterized by dramatic elongation of colonic crypts, hyperproliferation of epithelial cells, goblet cell depletion, and mucosal thickening.[20] Over a 2-week period, the crypts in the distal colon double in height, reaching a maximum between 14 and 21 days postinfection (pi). Inflammatory responses with *C rodentium* infection are minimal, making this model an excellent tool for examining hyperproliferative changes in the colon.

To determine the role of KLF5 in proliferative responses of the mouse colon to *C rodentium* infection, we have generated mice with heterozygous knockout of the *Klf5* gene ($Klf5^{+/-}$). Homozygous deletions of *Klf5* are embryonic lethal; however, heterozygous *Klf5* mouse models have been used in other studies to show key roles for Klf5 in cardiovascular remodeling and adipocyte differentiation.[16,21] In this study, we compared hyperproliferative responses to *C rodentium* infection in wild-type (WT) and $Klf5^{+/-}$ mice. Results show that Klf5 in the colonic crypts is induced in response to *C rodentium* infection and that hyperproliferative responses are suppressed in the colons of $Klf5^{+/-}$ animals. These findings suggest that induction of KLF5 is a key event that contributes to epithelial cell hyperproliferation after infection with *C rodentium* and shows that KLF5 is an important mediator for colonic hyperproliferation in response to in vivo stressors.

Materials and Methods

Animals

C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice strains were bred and housed in the Whitehead Animal Research Facility at Emory University. Animal care and in vivo research complied with all relevant Emory University institutional policies and federal guidelines.

Generation of $Klf5^{+/-}$ Knockout Mice

Mouse embryonic stem cells containing a disrupted allele for *Klf5* ($Klf5^{+/-}$), clone XB751, were obtained from BayGenomics, Inc (San Francisco, CA). The disrupted allele was generated by insertion of a gene trap vector into the first intron of the *Klf5* gene. The insertional mutation created a fusion transcript containing exon 1 of *Klf5* joined to a β-galactosidase/neomycin cassette and followed by a translational stop codon. Chimeras for expression of the disrupted *Klf5* allele were generated by the Emory Transgenic Facility by injecting blastocysts with $Klf5^{+/-}$ embryonic stem cells and implanting the embryos into pseudopregnant females. Chimeric progeny were bred to C57BL/6 animals to produce $Klf5^{+/-}$ founder mice. Male $Klf5^{+/-}$ mice were backcrossed with C57BL/6 females (WT) for 4 generations to produce $Klf5^{+/-}$ mice on a homogeneous C57BL/6 background. $Klf5^{+/-}$ mice used for the described experiments were F5 generation mice.

Genotyping

A 0.5-cm section of tail was removed and used to prepare genomic DNA with the Extract-N-Amp Tissue polymerase chain reaction (PCR) kit (Sigma Aldrich, St Louis, MO) according to the manufacturer's instructions. To identify $Klf5^{+/-}$ mice, PCR analysis was conducted to amplify a portion of the β-galactosidase gene that was part of the insertional mutation. Primers used were as follows: forward: 5′-TTATCGATGAGCGTGGTGGTTATGC-3′ and reverse: 5′-GCGCGTACATCGGGCAAATAATATC-3′.

Transmissible Murine Colonic Hyperplasia

The *C rodentium* strain, *C rodentium* deposited under the name *C freundii* (Braak) Werkman and Gillen, was obtained from the American Type Culture Collection. Six-week-old C57BL/6 WT or $Klf5^{+/-}$ littermates were infected with 100 μL of phosphate-buffered saline (PBS) containing 5×10^8 colony-forming units of *C rodentium* by oral gavage. Uninfected controls were given PBS alone. Animals were provided unlimited access to food and water throughout the experiment. Mice were weighed before infection to determine baseline weights, and mice were weighed and observed daily. At various time points pi, mice were euthanized by carbon dioxide asphyxiation and the distal colon was removed. Transmissible murine colonic hyperplasia was apparent by the presence of a significantly thickened distal colon and loose stool.

Histology

Sections of distal colon were isolated from the region 3 cm proximal to the anal verge, fixed in 10% formalin solution for 48 hours, and processed for embedding in paraffin. Transverse sections (5 μm) were stained with H&E for morphologic evaluation. Photomicrographs were taken using a Zeiss Axioskop2 plus microscope, and crypt height measurements were determined with AxioVision 4.5 software (Carl Zeiss, Inc, Maple Grove, MN). Crypt measurements were conducted in a blinded fashion, taking 3 separate measurements per tissue sample on well-oriented crypts.