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ORIGINAL RESEARCH

GATA4 Is Sufficient to Establish Jejunal Versus Ileal Identity in the Small Intestine

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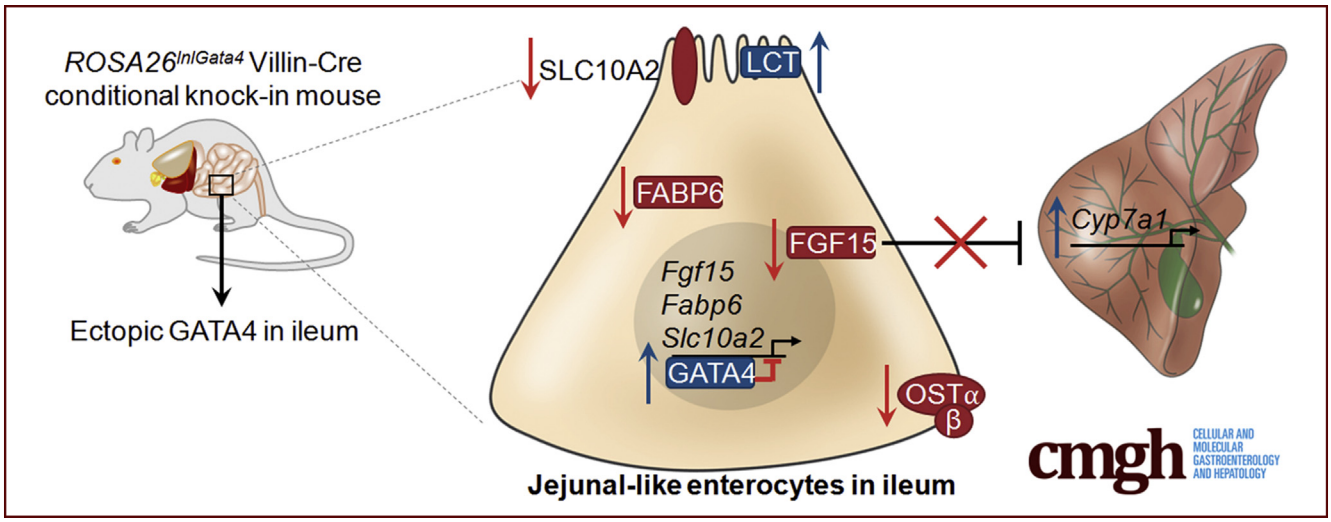

SUMMARY

GATA binding protein 4 (GATA4) establishes jejunal enterocyte identity and represses ileal enterocyte identity in the intestine, likely through direct activation and repression of expression of key regional-specifying genes. One important GATA4 target is fibroblast growth factor 15, a key regulator of enterohepatic bile acid cycling.

BACKGROUND & AIMS: Patterning of the small intestinal epithelium along its cephalocaudal axis establishes three functionally distinct regions: duodenum, jejunum, and ileum. Efficient nutrient assimilation and growth depend on the proper spatial patterning of specialized digestive and absorptive functions performed by duodenal, jejunal, and ileal enterocytes. When enterocyte function is disrupted by disease or injury, intestinal failure can occur. One approach to alleviate intestinal failure would be to restore lost enterocyte functions. The molecular mechanisms determining regionally defined enterocyte functions, however, are poorly delineated. We previously showed that GATA binding protein 4 (GATA4) is essential to define jejunal enterocytes. The goal of this study was to test the hypothesis that GATA4 is sufficient to confer jejunal identity within the intestinal epithelium.

METHODS: To test this hypothesis, we generated a novel *Gata4* conditional knock-in mouse line and expressed GATA4 in the ileum, where it is absent.

RESULTS: We found that GATA4-expressing ileum lost ileal identity. The global gene expression profile of GATA4-expressing ileal epithelium aligned more closely with jejunum and duodenum rather than ileum. Focusing on jejunal vs ileal identity, we defined sets of jejunal and ileal genes likely to be regulated directly by GATA4 to suppress ileal identity and promote jejunal identity. Furthermore, our study implicates GATA4 as a transcriptional repressor of *fibroblast growth factor 15 (Fgf15)*, which encodes an enterokine that has been implicated in an increasing number of human diseases.

CONCLUSIONS: Overall, this study refines our understanding of an important GATA4-dependent molecular mechanism to pattern the intestinal epithelium along its cephalocaudal axis by elaborating on GATA4's function as a crucial dominant molecular determinant of jejunal enterocyte identity. Microarray data from this study have been deposited into NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) and are accessible through GEO series accession number GSE75870. *(Cell Mol Gastroenterol Hepatol 2017;3:422–446; http://dx.doi.org/10.1016/j.jcmgh.2016.12.009)*

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The small intestine is composed of duodenum, jejunum, and ileum. Enterocytes within each perform specialized functions dictated by their position along the cephalocaudal axis to mediate digestion and absorption of nutrients, vitamins, and fluids to sustain growth, hydration, and electrolyte balance. Duodenal enterocytes are specialized to complete the digestive process.[1] Enzymes secreted from the pancreas as well as bile synthesized by liver and stored by the gall bladder enter the duodenum and combine with enzymes secreted by the duodenal enterocytes to facilitate digestion. Jejunal enterocytes accomplish the bulk of nutrient uptake by absorbing digestive products, namely lipid–bile acid emulsions, sugars, and oligopeptides/amino acids.[1,2] In addition to absorbing vitamin B12, ileal enterocytes play a critical role in maintaining the enterohepatic circulation of bile acids and in regulating bile acid metabolism.[1,2] Ileal enterocytes absorb bile acids from the intestinal lumen, and bile acids travel via the portal circulation from the intestine to the liver, where they are taken up and re-secreted into bile. Uptake of bile acids by ileal enterocytes activates farnesoid X receptor (FXR)-mediated transactivation of genes encoding proteins required for enterocyte bile acid transport including *fatty acid binding protein 6* (*Fabp6*), *solute carrier 51a (Slc51a)*, *Slc51b* and the secreted enterokine *fibroblast growth factor 15/19 (Fgf15/19)*.[3–6] Binding of FGF15/19 to its receptors on hepatocytes represses expression of *cytochrome P450 family 7 subfamily A member 1* (*Cyp7a1*), which encodes the rate-limiting enzyme in conversion of cholesterol to bile acids, to control hepatic bile acid synthesis.[4,5] Moreover, bile acids and FGF15/19 may function in the regulation of energy expenditure and lipid and carbohydrate metabolism.[7–9] As such, the importance of bile acid enterohepatic cycling and homeostasis extends beyond intestinal function, and defects in enterohepatic FGF15/19 signaling have been linked to human diseases including cholestatic liver disease, nonalcoholic fatty liver disease, type 2 diabetes, metabolic syndrome, Crohn's disease, bile acid malabsorption, and bile acid diarrhea.[7–11]

Disruption of enterocyte functions caused by Crohn's disease and other inflammatory bowel diseases, intestinal tumors, trauma, necrotizing enterocolitis, and congenital defects, along with surgical interventions used to treat these disorders, can result in intestinal failure or short-bowel syndrome (SBS).[12,13] High morbidity and mortality are associated with SBS, and the economic and quality-of-life costs for SBS patients are high.[12,14] Better SBS therapies are needed, particularly interventions that restore lost function to remaining intestinal tissue, as well as novel tissue engineering approaches to overcome small-bowel organ shortages for transplant. To make these advances a reality, it will be necessary to understand how duodenal, jejunal, and ileal epithelial identities are patterned along the cephalocaudal axis of the small intestine. Currently, the molecular mechanisms underlying establishment and maintenance of enterocyte populations with regionally defined functions are delineated poorly. Extrinsic cues such as luminal contents and hormones can influence expression of regional-specific enterocyte markers, but tissue transplantation and isograft experiments show that regionalized gene expression programs are intrinsic to the epithelium.[15–17] More recently, using a long-term organoid culture model, Middendorp et al[18] showed that adult small intestinal stem cells maintain regional identity and regionally defined gene expression programs in a cell autonomous manner. We propose that the repertoire of transcription factors expressed in duodenal, jejunal, and ileal enterocytes drives patterning by activating and repressing expression of the set of downstream targets defining each region. GATA binding protein 4 (GATA4), a zinc-finger–containing transcription factor with a spatially restricted expression pattern along the cephalocaudal axis of the small intestine, represents one such factor.

GATA4 is expressed in enterocytes of the duodenal and jejunal epithelium but absent from enterocytes of the ileal epithelium.[19–21] Studies of mouse intestinal development show that GATA4 initially is expressed throughout the developing intestinal epithelium and that it becomes excluded from the ileal domain relatively early at embryonic day (E)12.5–13.5.[22] GATA4 plays a role in early intestinal development, regulating intestinal epithelial cell proliferation.[23] Specifically, GATA4 deletion in the intestinal epithelium via Sonic hedgehog–Cre transiently reduces cellular proliferation in the intestinal epithelium (E10.5–E11.5), resulting in a shorter intestine with decreased epithelial girth. Furthermore, the onset of villus morphogenesis is delayed in the intestine of *Gata4 Sonic hedgehog–Cre* conditional knockout embryos, perhaps because of reduced epithelial cell proliferation, and villus structure is abnormal. In adult mice, GATA4 is essential for jejunal function.[19,21] Elimination of GATA4 from the intestinal epithelium using Villin-Cre causes a global shift in regional identity within the jejunum.[21] In the absence of jejunal GATA4, expression of a wide array of jejunal-specific genes is lost, including expression of genes encoding proteins with important roles in uptake, transport, and processing of cholesterol and lipids.[21] Moreover, expression of numerous

Abbreviations used in this paper: **bio-ChIP-seq, biotin-mediated chromatin immunoprecipitation with high-throughput sequencing; bp, base pair; cDNA, complementary DNA; cKI, conditional knock-in; cKO, conditional knockout; Cyp7a1, cytochrome P450 family 7 subfamily A member 1; dATP, deoxyadenosine triphosphate; E, embryonic day; EMSA, electrophoretic mobility shift assay; Fabp6, fatty acid binding protein 6; Fgf, fibroblast growth factor; FXR, farnesoid X receptor; lnl, *loxP*-flanked PGK-Neo-3xSV40 polyadenylation sequence; mRNA, messenger RNA; OSTα/β, organic solute transporter α/β; pA, polyadenylation; PCR, polymerase chain reaction; qRT, quantitative reverse-transcription; SBS, short-bowel syndrome; Slc, solute carrier; TSS, transcription start site; xiFABP, *Xenopus* I-FABP.**

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