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GATA4 and GATA6 regulate intestinal epithelial cytodifferentiation during development ☆

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ABSTRACT

The intestinal epithelium performs vital roles in organ function by absorbing nutrients and providing a protective barrier. The zinc-finger containing transcription factors GATA4 and GATA6 regulate enterocyte gene expression and control regional epithelial cell identity in the adult intestinal epithelium. Although GATA4 and GATA6 are expressed in the developing intestine, loss of either factor alone during the period of epithelial morphogenesis and cytodifferentiation fails to disrupt these processes. Therefore, we tested the hypothesis that GATA4 and GATA6 function redundantly to control these aspects of intestinal development. We used Villin-Cre, which deletes specifically in the intestinal epithelium during the period of villus development and epithelial cytodifferentiation, to generate *Gata4Gata6* double conditional knockout embryos. Mice lacking GATA4 and GATA6 in the intestinal epithelium died within 24 h of birth. At E18.5, intestinal villus architecture and epithelial cell populations were altered. Enterocytes were lost, and goblet cells were increased. Proliferation was also increased in GATA4-GATA6 deficient intestinal epithelium. Although villus morphology appeared normal at E16.5, the first time at which both *Gata4* and *Gata6* were efficiently reduced, changes in expression of markers of enterocytes, goblet cells, and proliferative cells were detected. Moreover, goblet cell number was increased at E16.5. Expression of the Notch ligand *Dll1* and the Notch target *Olfm4* were reduced in mutant tissue indicating decreased Notch signaling. Finally, we found that GATA4 occupies chromatin near the *Dll1* transcription start site suggesting direct regulation of *Dll1* by GATA4. We demonstrate that GATA4 and GATA6 play an essential role in maintaining proper intestinal epithelial structure and in regulating intestinal epithelial cytodifferentiation. Our data highlight a novel role for GATA factors in fine tuning Notch signaling during intestinal epithelial development to repress goblet cell differentiation.

Introduction

The intestinal epithelium plays a central role in orchestrating organ function through nutrient absorption and by providing a barrier between the environment and underlying tissues. During embryonic development, epithelial morphogenesis and cytodifferentiation in midgut endoderm produce a precisely structured epithelium composed of specialized cell types that perform these functions (Spence et al., 2011). In mouse, between embryonic day 14 (E14) and birth, the immature pseudostratified epithelium of the gut converts to a simple columnar epithelium covering mucosal projections known as villi (Grosse et al., 2011). Coincident with epithelial morphogenesis, progenitor cells differentiate into absorptive or secretory cell types. As the epithelium remodels, proliferative progenitor cells become restricted to intervillus regions, which mark the future sites of crypts where intestinal stem cells and secretory Paneth cells will reside (Spence et al., 2011).

One family of factors implicated in enterocyte development is the GATA family of zinc-finger DNA binding transcription factors, specifically GATA4 and GATA6. Both GATA4 and GATA6 are expressed in midgut endoderm during development and continue to be expressed in the small intestinal epithelium throughout adulthood although in differing patterns (Koutsourakis et al., 1999; Bosse et al., 2006; Bosse et al., 2007; Watt et al., 2007; Battle et al., 2008; Beuling et al., 2011). Epithelial cells of duodenum and jejunum express GATA4, whereas those of the ileum lack GATA4 (Bosse et al., 2006; Battle et al., 2008). GATA6, however, is expressed in all regions of the small intestinal epithelium (Fang et al., 2006). Because *Gata4*−/− and *Gata6*−/− mice die during embryogenesis prior to organ development (Kuo et al., 1997;

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Molkentin et al., 1997; Morrisey et al., 1998; Koutsourakis et al., 1999), it was necessary to use a conditional knockout approach to study their function in the small intestine. Using Villin-Cre, which directs robust intestinal epithelium-specific recombination during development beginning at the time of villus morphogenesis and epithelial cytodifferentiation (Madison et al., 2002), we previously eliminated GATA4 from the intestine. We found that although GATA4 is dispensable for proper intestinal development when deleted during the period of epithelial morphogenesis and cytodifferentiation with Villin-Cre, it is essential for jejunal function (Battle et al., 2008). Mice lacking GATA4 in the jejunal epithelium displayed severe defects in fat and cholesterol absorption. Moreover, mutant jejunum lost expression of many jejunal-specific genes and gained expression of many ileal-specific genes suggesting that GATA4 plays a key role in determining jejunal enterocyte identity. Conditional knockout of GATA4 in adult mouse small intestine using tamoxifen-inducible Villin-Cre resulted in a similar phenotype (Bosse et al., 2006). Loss of GATA6 from the intestinal epithelium using Villin-Cre also fails to disrupt embryonic intestinal development (Battle lab, unpublished data). Conditional knockout of *Gata6* in adult mouse small intestinal epithelium using tamoxifen-inducible Villin-Cre alters ileal epithelial cell populations including a reduction of proliferative, enteroendocrine, and Paneth cells and an increase in goblet cells (Beuling et al., 2011). Loss of *Gata6* in the ileum also causes changes in the ileal enterocyte-specific gene expression pattern, shifting it toward a more distal colon-like pattern (Beuling et al., 2011).

The finding that GATA4 and GATA6 are expressed in the developing intestine, yet loss of either factor alone during the period of epithelial morphogenesis and cytodifferentiation fails to disrupt intestinal development, suggests that these factors function redundantly during this period of small intestinal development. Redundancy in GATA4-GATA6 function has been demonstrated during development of other organs. For example, loss of either GATA4 or GATA6 in the heart results in subtle phenotypes whereas loss of both factors causes acardia (Zhao et al., 2008). Studies examining GATA4 and GATA6 in pancreatic development confirm a similar model of GATA function. Loss of either GATA4 or GATA6 causes minor defects, whereas elimination of both results in pancreatic agenesis (Carrasco et al., 2012; Xuan et al., 2012). Further supporting redundant function of GATA factors in the small intestinal epithelium, conditional knockout of *Gata4* and *Gata6* in adult mouse small intestinal epithelium using tamoxifen-inducible Villin-Cre causes changes in the duodenum and jejunum similar to those seen in GATA6-deficient ileum including a reduction of proliferative, enteroendocrine, and Paneth cells and an increase in goblet cells (Beuling et al., 2011). The impact of loss of both GATA4 and GATA6 on intestinal development, however, is unknown. Therefore, to test the hypothesis that GATA4 and GATA6 regulate a common set of genes to control intestinal development, *Gata4Gata6* double conditional knockout (*G4G6* dcKO) mice were generated using Villin-Cre. We found that unlike single *Gata4* or *Gata6* Villin-Cre cKO mice or mice with deletion of both *Gata4* and *Gata6* in adult intestine, elimination of both *Gata4* and *Gata6* during development resulted in death within 24 hours of birth. Both epithelial architecture and cell type allocation were affected in GATA4-GATA6 deficient intestine. Although villi emerged normally in mutants, villus structure was abnormal at E18.5 with mutant tissue containing scarce short, broad villi. We observed a decrease in enterocytes and an increase in proliferating cells and goblet cells. Reduced expression of the Notch ligand *Dll1* (Pellegrinet et al., 2011) and the Notch downstream target *Olfm4* (VanDussen et al., 2012) suggested defective Notch signaling, and we found that GATA4 occupied binding sites within the *Dll1* gene in the intestinal epithelium. We conclude that GATA4 and GATA6 play an essential role in regulating intestinal epithelial structure and cytodifferentiation. Moreover, the data we present suggest a role for GATA factors in intestinal epithelial cell fate decisions by modulating Notch signaling through regulation of *Dll1*.

Materials and methods

Animals

Gata4^{loxP}(*Gata4*$^{tm1.1Sad}$), *Gata6*^{loxP}(*Gata6*$^{tm2.1Sad}$), *Gata6*$^{-}$(*Gata6*$^{tm2.2Sad}$), *Villin-Cre(Tg(Vil-cre)997Gum)*, *Gata4*^{flbio}(*Gata4*$^{tm3.1Wtp}$) and *Rosa26*^{BirA}(*Gt(ROSA)26Sor*$^{tm1(birA)Mejr}$) mice were used (Madison et al., 2002; Watt et al., 2004; Driegen et al., 2005; Sodhi et al., 2006; He et al., 2012). Embryonic mice were generated by timed mating considering noon on the day of a vaginal plug as E0.5. Genotypes were determined by PCR of tail tip or ear punch DNA following a standard protocol. Primers are listed in Supplemental Table 1. For proliferation studies, 200 μg 5-ethynyl-2'deoxyuridine (EdU) was administered by intraperitoneal injection 3 h prior to euthanizing animals. The Medical College of Wisconsin's Animal Care Committee approved all animal procedures.

Intestinal epithelial cell isolation

Whole small intestine harvested from control and *G4G6* dcKO E16.5, E17.5, or E18.5 embryos was cut along its longitudinal axis and incubated in cell dissociation buffer (BD Biosciences, San Jose, CA) for 6 h at 4 °C with gentle agitation to release epithelial cells (Madison et al., 2005; Li et al., 2007). Total RNA prepared from epithelial cells was used for gene array and qRT-PCR analyses.

Reverse transcription polymerase chain reaction

cDNA was generated from DNase treated RNA harvested from intestinal epithelial cells isolated from the whole small intestine (E16.5-E18.5) or from intact jejunal tissue harvested from the midpoint of small intestine (E15.5) as previously described (Duncan et al., 1997; Bondow et al., 2012). Supplemental Table 1 and 2 contain primer sequences and TaqMan assay identifiers, respectively. qRT-PCR data were analyzed using DataAssist software (Applied Biosystems, Carlsbad, CA). *Gapdh* was used for normalization. Each gene was assayed in at least three independent experiments using cDNA from three control and three mutant intestines. Error bars represent standard error of the mean (SEM).

Oligonucleotide array analysis

Mouse Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA) were used to determine gene expression changes between intestinal epithelial cells harvested from three control and three *G4G6* dcKO embryos at E18.5 as previously described (Bondow et al., 2012). To be considered significantly changed between groups, we required an expression change of $\geq$ 2.0-fold, $p \leq 0.05$ (Supplemental Table 3).

Histochemistry, Immunohistochemistry, and Immunofluorescence

Histochemistry, immunohistochemistry, and immunofluorescence were performed as previously described using jejunal tissue harvested from the midpoint of each embryonic small intestine (Duncan et al., 1997; Bondow et al., 2012). For each stain, four to six sections from three controls and three mutant animals were used. See Supplemental Table 4 for antibody details.