Foxl1-deficient mice exhibit aberrant epithelial cell positioning resulting from dysregulated EphB/EphrinB expression in the small intestine

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Foxl1-deficient mice exhibit aberrant epithelial cell positioning resulting from dysregulated EphB/EphrinB expression in the small intestine. Am J Physiol Gastrointest Liver Physiol 290: G163–G170, 2006. First published February 9, 2006; doi:10.1152/ajpgi.00019.2006.—The winged helix transcription factor Foxl1, expressed in the gut mesenchyme, regulates epithelial cell proliferation and differentiation through the Wnt/β-catenin pathway. To better understand the role of Foxl1 in epithelial morphogenesis, we examined the tissue structure and positioning of epithelial cells in the small intestine of Foxl1-deficient mice. The small intestine of Foxl1-deficient mice manifested aberrant crypt structure, including widely distributed Paneth cells, which coincided with the ectopic and increased expression of EphB2 and EphB3, which are key regulators of epithelial cell positioning. Furthermore, real-time quantitative PCR indicated that a subset of Wnt family genes was highly expressed in the gut mesenchyme of Foxl1-deficient mice compared with that of wild-type mice. Such an increase in Wnt expression was remarkable in the mesenchyme, where the aberrant Paneth cell positioning was observed by in situ hybridization. Foxl1 plays an important role in the maintenance of crypt architecture and epithelial cell positioning through the mesenchymal-epithelial interaction in the small intestine. This interaction is essential for the normal regulation of the Wnt/β-catenin pathway and the subsequent EphB/EphrinB expression.

Foxl1; EphB/EphrinB; Wnt; mesenchymal-epithelial interaction; gut

THE GUT MESENCHYME plays an essential role in the proliferation and differentiation of epithelial cells. Although the precise molecular machinery regulating the mesenchymal-epithelial interaction has not been fully elucidated, a number of mesenchymal factors have been reported to influence epithelial homeostasis in the gut (17, 20, 29, 39). Among them, the winged helix transcription factor Foxl1 is considered to be one of the key regulators of epithelial proliferation and differentiation. Foxl1 is expressed in the mesenchyme of developing and adult gastrointestinal tract. The deletion of Foxl1 results in the delayed formation of gastric glands and intestinal villi during fetal development in mice (16). Adult Foxl1-deficient (−/−) mice exhibit hyperplasia of the gastric mucosa and the intestinal villi. Expansion of the crypt compartment is also observed in these mice. Such disorders are associated with the abnormal proliferation of epithelial cells in the gastrointestinal tract (26).

These findings clearly suggest that Foxl1 works as a negative regulator of the proliferation of gut epithelial cells.

Foxl1 target genes remain to be clarified; however, the genes encoding the molecules involved in the Wnt/β-catenin signaling pathway could be among the possible targets of Foxl1. Previous studies have shown that 7 of the 19 Wnt family molecules are expressed in the murine gastrointestinal tract during development and in adulthood (22, 33). The binding of Wnt to the Frizzled receptor expressed on epithelium induces the stabilization and translocation of β-catenin to the nucleus (23, 36). Nuclear β-catenin serves as a transcriptional coactivator for TCF4, and the β-catenin/TCF4 complex promotes the expression of c-myc, leading to epithelial cell proliferation (9, 35). It is therefore supposed that the activity of the β-catenin/TCF4 complex may determine the fate of intestinal epithelial stem cells, namely, whether to proliferate or to differentiate. A previous study has suggested that Foxl1 negatively regulates the β-catenin/TCF4 signaling pathway, because the nuclear translocation of β-catenin is apparently enhanced in the stomach and the small intestine of Foxl1−/− mice (26). Furthermore, Foxl1 deficiency leads to a marked increase in tumor multiplicity in gastric and colorectal cancers induced by a mutated APC tumor suppressor gene (27) that is known to promote the degradation of β-catenin. This indicates that Foxl1 could cooperate with APC in terms of controlling epithelial cell proliferation.

We and others have recently reported that Foxl1−/− mice demonstrate multiple pathological symptoms, including impaired acid secretion by gastric parietal cells, defect in small intestinal uptake of D-glucose, and abnormal formation of gut-associated lymphoid follicles (7, 8, 18, 19, 27). These observations raise the possibility that Foxl1 may function as a multifunctional regulator in the context of the mesenchymal-epithelial interaction. In the present study, we show that Foxl1 deficiency leads to disorder in epithelial cell positioning in the murine small intestine. This phenomenon is mainly explained by the dysregulation of the EphB/EphrinB system, which is critical for epithelial cell positioning (2), because the balanced expression of EphB/EphrinB is dysregulated in the gut of Foxl1−/− mice. Furthermore, the aberrant expression of EphB/EphrinB is coincident with the enhanced expression of Wnt by mesenchymal cells, suggesting that Foxl1 regulates the β-catenin/TCF4 pathway and the subsequent epithelial EphB expres-
AVERT AND METHODS

Mice. Fox11−/− mice were backcrossed onto a BALB/c background 10 times (7). The morning of the appearance of a vaginal plug was considered to be 0.5 day postcoitus (dpc). All animal experiments were carried out in accordance with the guidelines for the care and use of laboratory animals of RIKEN.

Whole mount staining. The small intestine was dissected into the small pieces and fixed in methanol for 1 h at −20 °C. The pieces were rinsed with PBS and immersed in PBS containing FITC-conjugated Ulex europaeus I (UEA-1, 20 μg/ml; Vector Laboratories, Burlingame, CA) and rhodamine-conjugated wheat germ agglutinin (WGA, 25 μg/ml; Vector Laboratories) for 1 h at room temperature. The specimens were examined with an LSM510 confocal microscope (Carl Zeiss, Deutschland, Germany).

Immunohistochemistry. The small intestine was dissected and fixed overnight in 4% paraformaldehyde in PBS at 4 °C, embedded in paraffin wax, and processed into 3-μm sections. The sections were deparaffinized in xylene, rehydrated through a graded series of ethanol, boiled in 0.01 M citrate buffer (pH 6.0) for 20 min, and incubated with 0.5% blocking reagent (TSA Fluorescence System; Perkin-Elmer, Boston, MA) in TBS [0.1 M Tris-HCl (pH 7.5) and 0.15 M sodium chloride] for 30 min. Incubation with primary antibodies against EphB2, EphB3, and EphrinB1 (2.5 μg/ml; R&D Systems, Minneapolis, MN) was performed for 3 h followed by incubation with biotin-conjugated donkey anti-goat IgG antibody (0.5 μg/ml; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h. The sections were quenched with 3% H2O2 in PBS for 10 min. Signal amplification and detection was performed with an Elite ABC kit (Vector Laboratories) using FITC-conjugated tyramide (Perkin-Elmer) as the substrate according to the manufacturer’s instructions. To detect Paneth cells, the sections were incubated with rhodamine-conjugated UEA-1 (20 μg/ml; Vector Laboratories) for 5 min. In some experiments, further incubation was performed after EphB2 staining with rabbit anti-Ki67 antibody (1:1,000; Novocastra Laboratories, Newcastle, UK) for 2 h. For visualization of antibody binding, the sections were incubated with Cy3-conjugated anti-rabbit IgG antibody (1.2 μg/ml; Jackson ImmunoResearch Laboratories) for 1 h.

Separation of epithelial cells and mesenchymal cells. The small pieces of the small intestine were washed with ice-cold calcium- and magnesium-free Hanks’ balanced salt solution (HBSS). The pieces were immersed in HBSS containing 30 mM EDTA for 7 min. The epithelial monolayer was peeled off with 29-gauge needles under monitoring with a transillumination stereomicroscope (MZ12.5; Leica Microsystems, Heerbrugg, Switzerland) to separate epithelial cells from mesenchymal cells (11, 15). Hematoxylin and eosin staining of the sections confirmed that these preparations were almost exclusively composed of either epithelial monolayers or the mesenchymal tissue [Supplemental Fig. 1 (Supplemental data for this article may be found at http://ajpgi.physiology.org/cgi/content/full/00019.2006/DC1)].

Immunoblot analysis. The separated epithelial cells and mesenchymal cells were homogenized with lysis buffer [50 mM HEPES-KOH (pH 7.4), 1% Triton X-100, 150 mM sodium chloride, 5 mM EDTA, 5 mM EGTA, 20 mM sodium fluoride, and 1 mM dithiothreitol]. After centrifugation, the supernatants were obtained, and the protein contents were quantified with BCA protein assay reagents (Pierce, Rockford, IL) following the manufacturer’s instructions. The supernatants were boiled with Laemmli’s buffer, separated on 8% SDS-PAGE, and transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore, Billerica, MA). The membranes were incubated with goat anti-mouse EphB2 antibody, followed by horseradish peroxidase-conjugated donkey anti-goat IgG antibody (0.26 μg/ml; Jackson ImmunoResearch Laboratories). Specific binding was detected using the SuperSignal Detection System (Pierce) according to the manufacturer’s instructions. The blots were stripped and reprobed with goat anti-mouse EphB3 antibody or mouse anti-glyceraldehyde-3-phosphate dehydrogenase antibody (0.25 μg/ml; Chemicon, Temecula, CA).

To detect the nuclear translocation of β-catenin, the isolated epithelial cells were homogenized in sucrose solution [10 mM HEPES-KOH (pH 7.4), 0.25 M sucrose, 5 mM EDTA, 1 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride] using 22-gauge needles. The homogenates were centrifuged at 600 g for 10 min. The nuclear fraction and membrane/cytosolic fraction were prepared from the precipitate and supernatant, respectively, as described previously (21). Aliquots of these fractions containing 20 μg of protein were denatured, separated on 8% SDS-PAGE gels, and transferred to PVDF membranes. The membranes were incubated with mouse anti-β-catenin antibody (1 μg/ml; Zymed Laboratories, San Francisco, CA), followed by goat horseradish peroxidase-conjugated anti-mouse IgG antibody (0.26 μg/ml; Jackson ImmunoResearch Laboratories). Specific binding was detected using the SuperSignal Detection System (Pierce) according to the manufacturer’s instructions.

Real-time quantitative RT-PCR analysis. Total RNAs were isolated from the separated epithelial cells and mesenchymal cells with TRIzol reagent (Invitrogen, Carlsbad, CA), and first-strand cDNAs were synthesized with ReverTra Ace-α (Toyobo, Osaka, Japan). Real-time quantitative PCR was performed using SYBR Green PCR Master Mix and an ABI PRISM 7900 Analyzer (Applied Biosystems, Foster City, CA). Amplification of the expected single products was confirmed using 2% agarose gel and ethidium bromide staining. All procedures were performed in accordance with the manufacturer’s instructions. The amount of actin mRNA in each sample was quantified and used as the internal control for comparison among the different samples. The primer sets are shown in Supplemental Table 1.

In situ hybridization. A 417-bp DNA fragment corresponding to nucleotide positions 685–1101 of mouse Wnt4 cDNA (GenBank accession no.: NM_009523) was subcloned into pGEM-T-Easy vector (Promega, Madison, WI). Digoxigenin-labeled RNA probes were prepared by in vitro transcription with T7 or SP6 RNA polymerase and Digoxigenin RNA Labeling Mix (Roche Diagnostics, Penzberg, Germany). Paraffin-embedded small intestine sections (6 μm) of Fox11−/− or wild-type (WT) mouse were deparaffinized, treated with protease K, and hybridized with 100 ng/ml digoxigenin-labeled RNA probes at 60°C for 16 h. Specific binding was detected by incubating with alkaline phosphate (AP)-conjugated anti-digoxigenin antibody (Roche Diagnostics) for 2 h at room temperature and visualized with purple AP substrate (Roche Diagnostics) overnight. The sections were counterstained with nuclear fast red.

Statistical analysis. Results are expressed as means ± SD. Statistical analysis of differences between two groups was performed using the unpaired Student’s t-test (two-tailed analysis). Differences with P values of <0.05 were considered statistically significant.

RESULTS

Abnormal crypt structure in Fox11−/− mice. To assess the crypt structure in Fox11−/− mice, we performed whole mount staining of the small intestine using lectins UEA-1 and WGA. In the crypt region, UEA-1 has been reported to react with sulfated proteoglycans (HSPGs) constituting the extracellular matrix (25). Because HSPGs are abundantly distributed at the mesenchymal-epithelial interface in the small intestine (12, 32), WGA staining is able to outline the whole crypt structure. Confocal microscopic analysis clearly showed that the size and shape of crypts were uniform throughout the small intestine in...
WT mice (Fig. 1A). In contrast, the crypt structure was irregular in Foxl1−/− mice, where parts of the crypts were enlarged by more than twofold compared with WT crypts (Figs. 1, B and C). Whereas UEA-1+ Paneth cells were restricted to the crypt bottom in WT mice (Figs. 1D and 2E), these cells were often found in the middle of the crypts in Foxl1−/− mice (Figs. 1E and 2F). The abnormalities in the crypt structure were observed throughout the small intestine, particularly in the jejunum.

**Dysregulated expression of EphB/EphrinB in Foxl1−/− mice.** The histochemical analyses presented above and the Ki67 staining shown in Fig. 2, B and D, revealed the abnormal positioning of Paneth cells and proliferative epithelial cells in Foxl1−/− mice. The epithelial cell positioning in the crypt-villus axis is regulated by the repulsive interaction between tyrosine kinase receptors EphB2/EphB3 and their ligand EphrinB1 in the small intestine (2). Because EphB/EphrinB are the target molecules of the β-catenin/TCF4 signaling pathway and this pathway is activated in Foxl1−/− mice (26, 35), we postulated that the aberrant epithelial cell positioning in Foxl1−/− mice may result from the dysregulation of EphB/EphrinB expression. To this end, we first examined the expression pattern of EphB/EphrinB in the jejunum by immunohistochemistry. Although EphB2 was predominantly expressed in Ki67+ proliferative epithelial cells in both WT and Foxl1−/− mice, its distribution was quite different between them. Whereas EphB2 expression was restricted to the lower half of the crypt in WT mice (Fig. 2, A and C), a broader expression of EphB2 was observed throughout the crypt except its base in Foxl1−/− mice (Fig. 2, B and D). Some EphB2+ cells were observed even in the middle of villi (Fig. 2, B and D). EphB3 was expressed in UEA-1+ Paneth cells that resided at the crypt base in WT mice (Fig. 2E), as described in a previous report (2). However, in Foxl1−/− mice, the EphB3+ Paneth cells were widely distributed throughout crypts (Fig. 2F). EphrinB1 was observed at the crypt-villus junction in WT mice (Fig. 2G) in accordance with a previous observation (2). Although the distribution was almost similar, a weaker EphrinB1 expression was sporadically observed in Foxl1−/− mice (Fig. 2H).

To determine the amount of EphB/EphrinB proteins in the jejunal epithelium, we separated the epithelial cell layer from the mesenchyme (Supplemental Fig. 1 and Ref. 11) and performed Western blot analysis. A marked increase in EphB2 protein expression was observed in Foxl1−/− mice compared with WT mice (Fig. 3, A and B), which was consistent with its broader distribution in Foxl1−/− mice as observed by histochemical analysis (Fig. 2, B and D). EphB3 protein expression also tended to be upregulated in Foxl1−/− mice compared with WT mice, although the difference was not statistically significant. Collectively, these data suggest that the epithelial cell positioning in Foxl1−/− mice could be altered because of the ectopic upregulation of EphB2/EphB3 in conjunction with the downregulation of EphrinB1.

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Fig. 1. Abnormal architecture of the small intestinal crypts in Foxl1−/− mice. A and B: whole mount specimens of the jejunum from wild-type (WT) mice (A) and Foxl1−/− mice (B) at 12 wk old were stained with lectins Ulex europaeus I (UEA-1) and wheat germ agglutinin (WGA) and analyzed with a confocal microscope. UEA-1 (green) stains the l-fucose epitope on Paneth cells, and WGA (red) stains the N-acetylglucosamine epitope of the extracellular matrix. Confocal images (x-y plane) orthogonal to the villi-crypt axis are shown. The area encircled by a dotted line in B represents an abnormally large single crypt in Foxl1−/− mice. C: the width of crypts was measured at eight different fields of the jejunum. *P < 0.05. D and E: Periodic acid-Schiff and hematoxylin staining of the jejunal tissue sections from WT (D) and Foxl1−/− mice (E). The localization of Paneth cells was restricted to the crypt bottom in WT mice (arrowheads), whereas these cells were often found in the middle of the crypts (arrows) in addition to the crypt bottom (arrowhead) in Foxl1−/− mice. Scale bars = 50 μm.
Widespread EphB2 expression during crypt formation in Foxl1−/− mice. In the developmental murine small intestine, EphB2/EphB3 and EphrinB1 are expressed in the intervillous region and villi in a mutually exclusive manner. The reciprocal interaction between EphB and EphrinB plays critical roles in the compartmentalization of proliferative cells and differentiated cells and is essential for regular crypt development (2). To examine whether the regulation of EphB2 expression is already disordered during crypt development in Foxl1−/− mice, we analyzed the expression pattern of EphB2 in small intestine at three different perinatal stages: 18.5 dpc, the stage before crypt formation; postnatal day 3 (P3), the early stage of crypt development before significant crypt morphogenesis; and postnatal day 10 (P10), the late stage of crypt formation where the cells constituting crypts settle in their appropriate positions. During these stages, EphB2 expression was limited to the intervillous region (18.5 dpc) and the bottom of crypts (P3 and P10) in control Foxl1+/+ littermates (Fig. 4, A, C, and E). These EphB2+ cells constitutively expressed the Ki67 proliferative marker (data not shown), consistent with a previous report (2). On the other hand, in Foxl1−/− mice, EphB2 was expressed throughout from the intervillous region to the villi at 18.5 dpc (Fig. 4B). At the later stage in Foxl1−/− mice, EphB2+ cells were not restricted to the crypt bottom but also observed in the middle of the villi (Fig. 4D). At P10, EphB2+ cells occasionally formed clusters in the distal part of the villi, apparently isolated from the EphB2+ cells in the crypts (Fig. 4F). In contrast, EphrinB1, whose expression was observed throughout the villi in control littermates (Fig. 4G), was remarkably downregulated in Foxl1−/− mice (Fig. 4H). These data suggest
that the Foxl1 deficiency causes dysregulation of EphB/EphrinB expression, thereby leading to the aberrant positioning of proliferating epithelial cells at the developmental stage of crypt in Foxl1−/− mice. This may disturb the processes necessary for normal crypt formation, eventually resulting in crypt expansion in the adult small intestine.

Increased Wnt4 and Wnt11 expression in the mesenchyme of Foxl1−/− mice. A previous study has demonstrated that Foxl1 is involved in the regulation of the β-catenin signaling pathway (26). We also confirmed that the nuclear β-catenin translocal-
Discussion

Foxl1 in the gut mesenchyme has been considered to control the growth and differentiation of gastrointestinal epithelial cells. Additionally, we described here that Foxl1 could contribute to the sorting of epithelial cells, because Foxl1−/− mice displayed mispositioning of proliferative epithelial cells and Paneth cells in the small intestine. In the adult small intestine, epithelial stem cells are located near the crypt base and produce transit amplifying cells. These cells give rise to terminally differentiated epithelial cell lineages as they migrate upward with the exception of Paneth cells, which downmigrate to the bottom of the crypts (5, 30). Such epithelial cell allocation is most likely controlled by the complementary expression of EphB2/B3 and its ligand EphrinB1 (2). EphB2 and EphB3 are expressed by proliferative epithelial cells and Paneth cells, respectively, whereas EphrinB1 is strongly induced in the differentiated epithelial cells at the crypt-villus junction, and its expression is gradually decreased toward the lower region of crypts. The interaction between EphB and EphrinB generates repulsive cues (14, 38) that allow EphB-expressing cells to keep away from the EphrinB1-expressing domain. Consequently, the localization of proliferative epithelial cells and Paneth cells is compartmentalized to the bottom of crypts in the normal small intestine (2). However, the expression balance between EphB and EphrinB is disturbed in Foxl1−/− mice, where EphB2 and EphB3 expression is upregulated and, inversely, EphrinB1 expression is sporadically decreased. This observation suggests that Foxl1 plays an important role in the expression and regulation of these molecules.

Fig. 6. Increase in mesenchymal Wnt expression in Foxl1−/− mice. Epithelial and mesenchymal tissues were separated from the jejunum of WT (open bars) and Foxl1−/− (filled bars) mice at 12 wk old and subjected to quantitative real-time PCR analysis. The mRNA expression levels of Wnt4 (A) and Wnt11 (B) were normalized to that of β-actin as the internal control, and were described as the degree of change relative to WT epithelium. Mean values ± SD of 3 (WT) or 4 (Foxl1−/−) samples from different mice are shown. *P < 0.05.

Fig. 7. In situ hybridization analysis of Wnt4 expression in the jejunum. The jejunal tissue sections from WT (A-C) or Foxl1−/− (D-F) mice were hybridized with the antisense probe to Wnt4 mRNA (A, B, D, and E) or the control sense probe (C and F). Arrows indicate the enhanced mesenchymal Wnt4 expression frequently observed near the mispositioned Paneth cells (arrowheads) in the upper crypts, which are easily distinguished from other epithelial cells by their characteristic granule-enriched cytoplasm. Scale bars = 25 μm.
The increase in EphB2 expression and the decrease in EphrinB1 expression are also observed at the developmental stage of Foxl1+/− mice. In the embryonic intestine, proliferative epithelial cells segregate in the flat intervillous region that eventually forms the nascent crypt. The newly generated epithelial cells in the nascent crypt begin to migrate upward, and thereby the mature crypt structure is gradually developed in 2 wk (31, 37). The EphB/EphrinB system most likely contributes to the crypt development as well, because Ephb2/B3 double-deficient mice display atrophy of the nascent crypt as well as mispositioned proliferative epithelial cells that intermingle with differentiated epithelial cells in the villi. In contrast, Foxl1−/− mice display the expansion of the nascent crypt, which consequently gives rise to an enlarged crypt compartment in the adult small intestine, as shown here and in a previous report (26). Not only is the crypt elongated but it shows multiple branching as well, which may result from the fusion of nascent crypts and/or incomplete crypt fission during crypt formation (37, 39). Such hyperplastic lesions of the intestinal crypt in Foxl1−/− mice may result from the increase in the number of proliferative cells as reported (16, 26) but also by the extension of the EphB-expressing region during crypt development.

The mode of action by which Foxl1 promotes the mesenchymal-epithelial interaction has been a subject of research interest. The intestinal epithelium of Foxl1−/− mice tends to accumulate more nuclear β-catenin than that of WT mice, which, in turn, forms a complex with TCF4 and acquires transcriptional activity (3, 24, 26, 35). The target genes of the β-catenin/TCF4 complex are mainly the regulators of cellular proliferation and survival (9). Interestingly, however, they also include Paneth cell-specific antimicrobial peptides and matrix metalloproteinase-7/matrilysin (36), implying a role of β-catenin signaling in the maturation of this cell lineage. Furthermore, EphB/EphrinB expression is inversely controlled by β-catenin signaling, because the inhibition of β-catenin/TCF4 with the dominant negative form of TCF4 results in the downregulation of EphB2 and EphB3 and the upregulation of EphrinB1 in a colorectal cancer cell line (2). Taking into account these reports and our observations, the physiological activity of Foxl1 on epithelial cell proliferation and positioning is probably mediated by the modulation of the β-catenin activity. Consistent with this notion, several of the Wnts tested here are upregulated in the intestinal mesenchyme of Foxl1−/− mice. The enhanced expression of Wnt4 is particularly remarkable in the mesenchyme, around which mispositioned Paneth cells are observed. On the other hand, Perreault et al. (26) reported that there were no differences in the Wnt mRNA expression between Foxl1−/− and WT mice using whole intestinal tissues as samples. This discrepancy is likely because of the technical differences in sample preparation. In the present study, we utilized a mesenchymal purification technique that involved removal of the epithelial monolayer from the intestinal tissue, which was expected to improve the sensitivity for detecting mesenchymal Wnt expression. Another possibility that may account for the discrepancy is the distinct genetic background of the mice used in the two studies, i.e., F1 hybrids of 129Sv/Ev and C57BL6 (26) versus BALB/c (the present study). Recent reports have indicated that certain Wnt molecules (Wnt3, -6, and -9a) are specifically expressed in the crypt epithelium (9, 36), suggesting their autocrine/paracrine role in promoting the β-catenin signaling pathway. Consistent with that notion, we also detected Wnt6 mRNA expression in the epithelium but not in the mesenchyme of the small intestine; however, the expression of Wnt6 is unlikely controlled by Foxl1, because there were no significant differences in the Wnt6 expression between WT and Foxl1−/− mice (Supplemental Table 2). It remains to be clarified whether Foxl1 directly regulates mesenchymal Wnt expression.

There might be yet other mechanisms that link Foxl1 and the Wnt/β-catenin signaling pathway. Kaestner et al. (16) showed the downregulation of Bmp2 and Bmp4 in the gastrointestinal tract of Foxl1−/− mice. Bmp4, which is expressed in the intestinal mesenchyme, functions by binding to its receptor Bmpr1a on epithelial cells. According to a recent model, Bmp signaling inhibits β-catenin through phosphatase and tensin homolog deleted on chromosome 10 (PTEN) activity regulation, thereby suppressing the self-renewal of stem cells in the intestinal epithelium (10, 13). These observations suggest that Foxl1 may regulate the β-catenin activity also by controlling Bmp4 expression, although further evidence is required to confirm this suggestion.

In conclusion, the present study indicates that Foxl1 contributes to the regulation of epithelial cell positioning through the regulation of Wnt/β-catenin signaling and the subsequent expression of EphB/EphrinB. These observations reinforce the importance of Foxl1 in promoting the mesenchymal-epithelial interaction that is essential to maintaining intestinal homeostasis.

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