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Yamamoto, Shuji

Kyoto University (京都大学)

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Thesis or Dissertation

ETD
Heparan sulfate on intestinal epithelial cells plays a critical role in intestinal crypt homeostasis via Wnt/β-catenin signaling

Shoji Yamamoto,1,2 Hiroshi Nakase,1 Minoru Matsuura,1 Yusuke Honzawa,1 Kayoko Matsumura,1 Norimitsu Uza,1 Yu Yamaguchi,3 Emiko Mizoguchi,4 and Tsutomu Chiba1

1Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Kyoto; 2Japan Society for the Promotion of Science, Tokyo, Japan; 3Sanford Children’s Health Research Center, Sanford-Burnham Medical Research Institute, La Jolla, California; 4Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

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The small intestinal epithelium comprises a single layer of columnar cells that are organized into villi and crypts. Villi extend into the intestinal lumen and contain three types of terminally differentiated cells: enterocytes, goblet cells, and enteroendocrine cells. Enterocytes absorb nutrients, goblet cells secrete mucin, and enteroendocrine cells release gastrointestinal hormones. Crypts are formed by epithelial invaginations into the connective tissue of the intestine and contain stem cells, their transit-amplifying daughter cells, and Paneth cells, which secrete antibacterial peptides into the crypt lumen (99–13). An intricate cell-replacement process maintains the integrity of the mucosal epithelium of the intestine. Vigorous proliferation occurs in crypt compartments. When the committed transit-amplifying cells reach the crypt-villus junction, they rapidly and irreversibly differentiate. The differentiated cells migrate up to the villus tip, where they are shed. In mouse, the small intestinal epithelium is renewed every 5 days (2).

Several intracellular signaling pathways, such as the Wnt/β-catenin, bone morphogenetic protein, phosphoinositide 3-kinase (PI3K)/Akt, and Notch pathways, have critical roles in crypt-villus homeostasis (2). In addition, several extracellular components have also been shown to regulate intestinal crypt homeostasis (36, 39), but their precise roles remain obscure.

Heparan sulfate (HS) is a linear polysaccharide constituting repeating disaccharide units of glucuronic acid and N-acetylglucosamine. HS is found on the surface of most cells as a constituent of HS proteoglycans (HSPGs), which comprise a core protein with covalently attached HS chains. In the small intestinal epithelium of humans (42) and mice (5), HSPGs are predominantly located on the basolateral surface of the cells. HSPGs bind to various growth factors, including Wnt, Hedgehog, transforming growth factor-β, and FGF and modulate the biologic activities of these molecules (4, 58).

The importance of HSPG in embryological development is established. In Drosophila, HS is required for distribution and signaling of morphogens such as Wingless, Hedgehog, and the Drosophila homologue of bone morphogenetic protein, Decapentaplegic, in the embryonic epidermis and the wing disc (8, 20, 22). HS is also involved in FGF signaling during the migration of mesodermal and tracheal cells (32). In vertebrate, HS influences FGF function in zebrafish limb development (40) and is essential for Wnt11 during zebrafish and Xenopus gastrulation (41). In mice, HS is required for proper Indian Hedgehog distribution during endothondral bone development (30) and Fgf8 function in brain development (26). Despite many evidences demonstrating that HSPGs play pivotal roles in embryogenesis, it remains unclear how HSPGs are involved in intestinal epithelial homeostasis.

In the present study, we used the intestine-specific HS-deficient mouse model (5) to elucidate the role of HS in the small intestine.

MATERIALS AND METHODS

Mice. We used C57Bl/6 mice. Conditional Ext1 allele (Ext1lox) was created as described previously (26). Villin-Cre mice (16) were obtained from the Jackson Laboratory. Mice were maintained on a 12-h:12-h light/dark cycle and fed standard laboratory mouse chow ad libitum in specific pathogen-free conditions. All experiments were performed with 2- to 4-mo-old mice. All animal experiments in this study were approved by the Review Board of Kyoto University and adhered to their institutional ethical guidelines. For survival data, mice were euthanized if moribund or seriously injured (e.g., vocaliz-
body irradiation (TBI) was performed using a 137Cs source emitting at 3.7 GBq/mCi. Cells were counted, diluted to 5 x 10^5 cells/ml in Iscove’s Modified Dulbecco’s Medium with 10% FBS, and plated at 10^4 cells/cm^2. After overnight serum starvation, cells were stimulated with recombinant mouse Wnt3a (R&D Systems), or 60 mM lithium chloride. Cells were then collected and processed for immunoblotting or RNA isolation.

In vivo microcolony assay of crypts. Crypt stem cell survival was determined 3.5 days after TBI based on BrdU incorporation into proliferating crypt cells, as previously reported (59). Each mouse was killed, and marrow cells were harvested from the medullary cavities of the femur by flushing with Hank’s balanced salt solution. Small intestines were placed in PBS with 3% EDTA and 50 mM DTT and stored on ice for 1 h. After washing, the small intestines were cut longitudinally with scissors and ice-cold PBS flushed through the lumen of the intestines using a syringe. The intestines were then cut into 1-cm-long segments and rinsed with cold PBS. The small intestines were placed in PBS with 30 ng/ml Alexa Fluor 488-conjugated Wnt3a for 60 min at 37°C. The cells were filtered through nylon filters (Becton Dickinson, NJ) and fixed in 10% neutral buffered formalin, and embedded in paraffin. Paraffin sections (4 µm) were cut perpendicularly to the long axis of the intestine before immunohistochemical staining.

Quantification of gene expression using real-time PCR. The total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. PCR amplification of cDNA (1 µg/20 µl of PCR reaction) was carried out in the LightCycler (Roche Diagnostics, Tokyo, Japan) with FastStart Universal SYBR Green Master (Roche). Results are expressed as the ratio of each molecule to Gapdh. Primers were as follows: Gapdh: AGCCTTCTCCATGGTG- GTGAAAG (forward), CCGGATCCACGATTGCTGTAT (reverse); Lgr5: CTACCTTGACTTGTAGAAGGAC (forward), AGGA- AAGGCGCAGACTG (reverse); c-Myc: GCTCGCCCAAATCTGTA (forward), AGGAC- TCGGAGGACAAGCA (reverse); Survivin: GCGGAGGTGGTC- TTCA (forward), AAAAAACAGCTGGCAAATCTCA (reverse); Keratin6: TCATCTCTCGGAGACTCC (forward), TCTTCACAC- CACAGCCTTG (reverse); Frizzled6: GACAACCTTCAGGC- TCATC (forward), CCAGGCAAACCAATTCTCCTCAG (reverse); Frizzled7: GTGTTGTATCTCTGTCGCTGTAT (forward), CTCG- GCGCTCTCAGTATG (reverse); Frizzled7: ATATGCCCTA- CAACCAGACACTCC (forward), AAGGAAACGCCAGGAATG (reverse); and Axin2: TCACAGGCTTTGGTTCAAG (forward), GTGATAGCTCTGTAGGCCTAGT (reverse).

Wnt3a binding assay and ex vivo stimulation of primary mouse IECs. Recombinant mouse Wnt3a (R&D Systems, Minneapolis, MN) was labeled with Alexa Fluor 488 dye using the Alexa Fluor 488 Microscale Protein Labeling Kit (Invitrogen) according to the manufacturer’s instructions. Primary mouse IECs (1 x 10^5) were incubated with 30 ng/ml Alexa Fluor 488-conjugated Wnt3a for 60 min at 37°C. The cells were then washed three times with PBS, and mean fluorescence intensity was measured using the FACS Canto II (Becton Dickinson). The data were analyzed using FACS Diva software. The number of surviving crypts per cross-section was scored. A surviving crypt was defined as a crypt with five or more BrdU-labeled epithelial cells.

Isolation of IECs. IECs were isolated as described previously (27). Mice were killed and the small intestine was removed and placed in ice-cold PBS flushed through the lumen of the intestines using a syringe. The intestines were then cut longitudinally with scissors and rinsed with cold PBS. The small intestines were placed in PBS with 3 mM EDTA and 50 mM DTT and stored on ice for 1 h in 50-ml conical tubes. The supernatant was filtered and centrifuged for 5 min at 400 g, and the cell pellet was resuspended in cold PBS. The sample was then centrifuged at 500 g for 5 min. Purity of IECs was assessed by flow cytometric analysis using rat anti-mouse E-Cadherin antibody (Santa Cruz Biotechnology). The remaining cell pellet was resuspended in cold PBS with 30 ng/ml Alexa Fluor 488-conjugated Wnt3a for 60 min at 37°C. The samples were then washed three times with PBS, and mean fluorescence intensity was measured using the FACS Canto II. The data were analyzed using FACS Diva software.

RESULTS

Generation of intestine-specific HS-deficient mice. To examine whether HS on IEC of the small intestine affects crypt homeostasis, we generated mice with intestine-specific conditional Ext1 knockout by crossing Ext1<sup>F/F</sup> mice with Villin-Cre mice as reported previously (5). Ext1 encodes glycosyltransferase, which polymerizes alternating glucuronic acid and N-acetylgalactosamine sugar residues in the HS biosynthetic process (35), and is indispensable for HS synthesis because cells lacking a functional Ext1 allele do not synthesize HS (34,
Intestine of Ext1 from Ext1 PCR products of undetectable in the IEC of mice as previously reported (5), suggesting that HS on IECs Ext1 was defective in intestine-specific Fig. 1. Heparan sulfate (HS) biosynthesis in intestinal epithelial cells (IECs) macroscopic and microscopic findings were normal in revealed that HS was expressed on the basolateral surface of IEC Immunohistochemical study with a mAb to HS (10E4) re-

PCR and immunoblot analyses of IEC separated from the small intestine of Ext1/F/F and Ext1/F/F mice were used as controls. Both mRNA and protein expression of Ext1 were disrupted in Ext1/Δ/Δ mice. C: immunohistochemistry for HS in the small intestine of Ext1/F/F (left) and Ext1/Δ/Δ (right) mice. Scale bar = 200 μm.

37). Intestine-specific Ext1 knockout mice are referred to as Ext1/Δ/Δ mice, and Ext1/F/F mice were used as controls.

Fig. 1. Heparan sulfate (HS) biosynthesis in intestinal epithelial cells (IECs) was defective in intestine-specific Ext1-deficient mice. A and B: Analysis of PCR products of Ext1 (A) and immunoblotting of Ext1 (B) in IECs isolated from Ext1/F/F and Ext1/Δ/Δ mice. Both mRNA and protein expression of Ext1 were disrupted in Ext1/Δ/Δ mice. C: immunohistochemistry for HS in the small intestine of Ext1/F/F (left) and Ext1/Δ/Δ (right) mice. Scale bar = 200 μm.

HS is involved in Wnt/β-catenin signaling in intestinal epithelium. Given that IEC-specific Ext1 deletion results in decreased proliferation of IECs, we investigated the Wnt/β-catenin and the MAPK pathways that are important for regulating IEC proliferation (17, 50). Immunoblot analysis demonstrated no significant difference in the phosphorylation of the MAPKs, including p38 MAPK, Erk1/2, and JNK1/2, in IECs 4 days after TBI between Ext1-deficient and control mice. Given that IEC-specific Ext1 deletion results in decreased proliferation of IECs, we investigated the Wnt/β-catenin and the MAPK pathways that are important for regulating IEC proliferation (17, 50). Immunoblot analysis demonstrated no significant difference in the phosphorylation of the MAPKs, including p38 MAPK, Erk1/2, and JNK1/2, in IECs 4 days after TBI between Ext1-deficient and control mice.
Ext1\(\Delta\Delta\) and Ext1\(^{F/F}\) mice (Fig. 3A). In contrast, the protein levels of total \(\beta\)-catenin were lower in IECs isolated from the small intestine of Ext1\(^{F/F}\) and Ext1\(^{\Delta\Delta}\) mice 4 days after 10 Gy TBI than those of Ext1\(^{F/F}\) mice (Fig. 3B). In addition, nonphosphorylated \(\beta\)-catenin (active \(\beta\)-catenin) was also lower in Ext1\(^{\Delta\Delta}\) mice (Fig. 3C). Moreover, immunofluorescence staining revealed lower levels of \(\beta\)-catenin nuclear localization in crypt cells of Ext1\(^{\Delta\Delta}\) mice 4 days after TBI, compared with those of Ext1\(^{F/F}\) mice (Fig. 3, D and E). These data suggest the decreased protein levels of cytoplasmic \(\beta\)-catenin with attenuated canonical Wnt signaling in IECs of Ext1\(^{\Delta\Delta}\) mice after TBI.

To address whether decreased levels of \(\beta\)-catenin had functional consequences, we examined the expression of Wnt target genes including, cyclin D1, c-Myc, Survivin, and Lgr5, in IECs isolated from Ext1\(^{F/F}\) and Ext1\(^{\Delta\Delta}\) mice. Both the mRNA and protein levels of these genes in IECs with disrupted HS biosynthesis were lower than those in HS-competent IECs after TBI (Fig. 4, A and B). To exclude the possibility that reduced

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**Fig. 3.** The protein levels of total and active \(\beta\)-catenin were decreased in intestine-specific HS-deficient mice after TBI. A: immunoblotting analysis of phosphorylated and total MAPKs. A representative blot from 5 independent experiments is shown. B and C: immunoblotting analysis of total \(\beta\)-catenin (B) and activated nonphosphorylated \(\beta\)-catenin (C). A representative blot is shown, and graph bars are expressed as the ratio of each molecule to \(\beta\)-actin. \((n = 5) \quad **P < 0.05.\)

**Fig. 4.** The mRNA and protein levels of Wnt-target genes were lower in IECs from intestine-specific Ext1 knockout mice than those from Ext1\(^{F/F}\) mice 4 days after 10 Gy TBI. A: The mRNA levels of Wnt-target genes cyclinD1, c-Myc, Survivin, and Lgr5. Results are expressed as the ratio of each molecule to Gapdh. \((n = 4–7) \quad **P < 0.05.\) B: immunoblotting analysis of Wnt-target genes in IECs from Ext1\(^{F/F}\) and Ext1\(^{\Delta\Delta}\) mice. A representative blot is shown, and graph bars are expressed as the ratio of each molecule to \(\beta\)-actin. \((n = 5) \quad **P < 0.05.\) C: mRNA levels of Wnt-target genes normalized by Keratin8. \((n = 4–7) \quad **P < 0.05.\)
numbers of crypt cells reflected lower levels of expression of Wnt target genes in Ext1<sup>Δ/Δ</sup> mice, we used Keratin8, the major intermediate filament proteins in the intestinal epithelia (57), to normalize the data in addition to a housekeeping gene. As expected, the mRNA levels of Wnt target genes were significantly lower in IECs harvested from Ext1<sup>Δ/Δ</sup> mice than those from Ext1<sup>FF</sup> mice even when normalized by Keratin8 (Fig. 4C). These data indicate that disruption of Ext1 in IECs impaired Wnt/β-catenin signaling and that HS plays an important role in regeneration of intestinal crypts following TBI-induced intestinal injury.

In addition, we evaluated the mRNA expression of Frizzled receptors, acting as receptors for Wnt proteins (2, 44, 50), which resulted in similar levels between Ext1<sup>FF</sup> and Ext1<sup>Δ/Δ</sup> mice (Fig. 5, A–C).

Deletion of HS in IECs does not affect the FGF-FGFR axis or the PI3K-Akt pathway. Next, we investigated the mechanism by which intestinal epithelial HS mediates Wnt/β-catenin signaling. It was recently reported that FGFR3 signaling regulates crypt epithelial stem cell expansion and crypt morphogenesis partly through β-catenin/T cell factor-4-dependent pathways (56). In addition, HS binds to both FGFs and FGFRs (43, 49) and is required for FGF signal transduction (32). Therefore, we first evaluated the FGF-FGFR axis in radiation enteritis. Immunohistochemical analysis revealed no significant difference in the phosphorylation of FGFR3 and FGFR1 in crypts 4 days after 10 Gy TBI between Ext1<sup>Δ/Δ</sup> and Ext1<sup>FF</sup> mice (Fig. 6, A and B). Moreover, levels of phosphorylation of the docking-protein FRS2α, which plays a critical role in FGFR-mediated signal transduction pathways (19, 21), were similar in IECs between Ext1<sup>FF</sup> and Ext1<sup>Δ/Δ</sup> mice (Fig. 6C).

Given that the PI3K-Akt pathway activates Wnt/β-catenin signaling by phosphorylating β-catenin at Ser552 (24, 31), we next examined whether PI3K-Akt pathway-mediated β-catenin activation was involved in the disruption of HS in IECs. Phosphorylation of Akt at Ser 308 was a similar level in IECs of Ext1<sup>Δ/Δ</sup> mice and Ext1<sup>FF</sup> mice. In addition, protein levels of phosphorylated β-catenin at Ser552 in IECs from Ext1<sup>Δ/Δ</sup> were comparable with those from Ext1<sup>FF</sup> mice (Fig. 6D).

These data suggest that neither the FGF-FGFR axis nor the PI3K-Akt pathway is involved in HS-dependent Wnt/β-catenin signaling in IECs from HS-deficient mice after irradiation.

HS affects binding affinity of IECs to Wnt3a and enhanced Wnt/β-catenin signaling. Because HS chains are capable of binding Wnt proteins and increases the dose of the ligands on the cell surface, which may result in enhanced Wnt/β-catenin signaling. Therefore, we assessed Wnt3a binding in
IECs harvested from Ext1\(^{+/+}\) and Ext1\(^{+/−}\) mice. Flow cytometric analysis indicated that the binding of fluorescent-labeled Wnt3a to IECs of Ext1\(^{+/−}\) mice was significantly lower than that of Ext1\(^{+/+}\) mice (Fig. 7, A and B). Moreover, immunoblot analysis revealed that stabilization of β-catenin by ex vivo stimulation with Wnt3a was attenuated in IECs from Ext1\(^{+/−}\) mice compared with Ext1\(^{+/+}\) mice, whereas β-catenin stability was not affected by deletion of HS sugar chains when IECs were incubated with LiCl (Fig. 7C), which directly inhibits the activity of glycogen synthase kinase-3 β (GSK-3β) (25, 28). In addition, phosphorylation of Wnt coreceptor LRP6 at Ser 1,490 (2, 44, 50) after ex vivo stimulation with Wnt3a was not affected by deletion of HS sugar chains when IECs were incubated with LiCl (Fig. 7D). The mRNA levels of Wnt target gene Axin2 were lower in IECs of Ext1\(^{+/−}\) mice than those of Ext1\(^{+/+}\) mice after incubation with Wnt3a, and the difference of Axin2 expression between Ext1\(^{+/+}\) and Ext1\(^{+/−}\) mice after stimulation with 100 ng/ml of Wnt3a was augmented, whereas similar levels of Axin2 gene expression were observed between two groups after ex vivo stimulation with LiCl (Fig. 7E). These results suggest that HS on IECs increases cell surface binding affinity of IECs to Wnt ligands, enhances Wnt/β-catenin signaling, and facilitates crypt regeneration after intestinal epithelial injury.

**DISCUSSION**

In this study, we showed that intestine-specific HS-deficient mice were more sensitive to TBI-induced intestinal injury than Ext1\(^{+/+}\) mice and that HS influenced Wnt binding affinity of IECs and subsequent Wnt/β-catenin signaling. Our data suggested that HS plays an important role in Wnt/β-catenin signaling during regeneration of the small intestinal crypts in mice.

First, we assessed the role of epithelial HS in regeneration of small intestine after TBI. The gross intestinal phenotype and histology of intestine-specific HS-deficient mice without TBI appeared normal in our study, being in agreement with the findings by Bode et al. (5). However, our study demonstrated that the survival rate of these mice after TBI was significantly reduced compared with that of Ext1\(^{+/+}\) mice due to severe small intestinal injury. Furthermore, in vivo crypt colony assay, which reflects the capacity of regeneration of intestinal crypts after irradiation (59), the number of surviving crypts in intestine-specific Ext1-deficient mice was significantly lower than that in Ext1\(^{+/+}\) mice. These findings suggest that HS is essential for crypt regeneration after intestinal epithelial injury.

Next, we investigated whether HS plays some roles in MAPK pathways or Wnt/β-catenin signaling in IECs. Several
lines of evidence indicate that MAPK pathways are involved in, not only intestinal cell proliferation, but also intestinal tumorigenesis (17). Our study demonstrated that activation of MAPK pathways in IECs was similar between Ext1F/F and Ext1ΔΔ mice after TBI, suggesting that HS does not play a significant role in MAPK signaling during the regeneration of intestinal crypts after lethal irradiation. In contrast, protein levels of total as well as active form of β-catenin were lower, and β-catenin nuclear localization was reduced in IECs isolated from the small intestine of Ext1ΔΔ mice than Ext1F/F mice. To confirm whether decreased levels of β-catenin had functional consequences, we examined the expression of Wnt target genes in IECs isolated from Ext1F/F and Ext1ΔΔ mice. Expressions of Wnt target genes in IECs in Ext1ΔΔ mice were lower than those in Ext1F/F mice. Thus the Wnt/β-catenin pathway was disturbed, and the expression of Wnt target genes was reduced in HS-deficient IECs. Wnt/β-catenin signaling plays a central role in the regeneration of intestinal epithelium (29, 44). In addition, the role of HSPGs in the regulation of Wnt pathway has been extensively studied in cell signaling during development. In Drosophila, the abrogation of HSPG activity by mutation of the EXT family genes leads to reduced extracellular Wingless levels and loss of Wnt target gene expression (6, 23, 52). In Xenopus, HSPGs have been shown to interact with Wnt11 during gastrulation (41) and axis formation (53). Taken together, our data and previous findings indicate that HS on IECs has an important function in canonical Wnt signaling and is essential for the proliferation of small intestinal epithelium. Notably, our data demonstrated that both mRNA expression and protein levels of c-Myc and Lgr5 were reduced when intestinal epithelial HS was disrupted. c-Myc is essential for accelerating the cell cycle of progenitor cells (38), and Lgr5 is one of the intestinal stem cell markers (3). These findings may suggest that HS on IEC is required for the expansion of intestinal stem cells, as well as for the proliferation of transit-amplifying cells after crypt injury.

Finally, we examined the effect of HS on cell-binding affinity of Wnt proteins, which may result in enhanced Wnt/β-catenin signaling. Our results revealed that Wnt binding affinity of IECs and Wnt/β-catenin signaling in ex vivo stimulation with Wnt3a were clearly reduced by HS deficiency. On the other hand, activation of Wnt/β-catenin signaling following direct inhibition of glycosynthase kinase-3β by LiCl was not affected by HS deficiency although the possibility cannot be denied that other effects of LiCl than inhibition of glycosynthase kinase-3β, such as inducing autophagy by inhibiting inositol monophosphatase (48), are influenced. Furthermore, phosphorylation of LRP6 by stimulation with Wnt3a was reduced in IECs from Ext1ΔΔ mice. These findings strongly suggest that HS on the cell surface enhanced binding of Wnt ligands to IECs and thereby promoted the canonical Wnt pathway in the regeneration of intestinal epithelium.

Modification of HS structures has been reported to be important for binding to Wnt ligands or receptors. The Drosophila mutants of sulfateless, HS N-deacetylase/N-sulfotransferase, are completely deficient in HS sulfation and have disrupted Wingless signaling (33, 54). Reducing 6-O-sulfation of the HS chains results in the reduction of Wnt binding to HS, facilitating the interaction between Wnt ligand and receptor and promotes canonical Wnt signal transduction (1, 18). Because we disrupted the Ext1 gene, which is indispensable for HS synthesis, HS was almost completely eliminated on the surface of IECs in our study. Therefore, specific conformations of HS for binding to Wnt and facilitating ligand-receptor signal transduction in crypt regeneration remain to be elucidated.

Furthermore, in our study, HS synthesis was disturbed irrespective of the family of HSPGs. Cell surface HSPGs are classified into two major families based on their core protein structure, glypicans and syndecans. Glypicans are linked to the plasma membrane by a glycosylphosphatidylinositol linkage and syndecans by a transmembrane domain. Several studies demonstrated that glypicans play an important role in the interaction between HS and Wnt signaling (33, 55), whereas syndecans are important for wound repair (15, 51) and IEC proliferation (14). Further studies are needed to determine which HSPGs are most involved in intestinal crypt regeneration.

Recently, several populations of intestinal stem cells, including Lgr5+ cells (3), Bmi1+ cells (47), and Lrig1+ cells (45), have been identified. Each of these populations has its own distinctive adjacent niches and plays separate but cooperative functions in homeostasis of intestinal crypt (3, 45, 47). In this study, we focused Lgr5 expression in IECs after TBI. At the minimum, our data suggested that gene expression and protein levels of Lgr5 were attenuated in IECs with disrupted HS biosynthesis although there was one limitation that specificity of anti-Lgr5 antibody used in this study was not validated by immunohistochemistry. Thus further investigations on the interaction between HS on IECs and intestinal stem cells would be required.

In conclusion, we elucidated an important role of HS in extracellular regulation of Wnt signaling in crypt regeneration of the small intestine. Further studies on the interactions of HSPGs with Wnt ligands and their receptors will provide new insights into the homeostatic mechanisms of the intestine.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

1. Ai X, Do AT, Lozynska O, Kusche-Gullberg M, Lindahl U, Emerson CP Jr. QSulf1 remodels the 6-O sulfation states of cell surface heparan
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