KLF4 Regulates Goblet Cell Differentiation in BMI1
Reserve Intestinal Stem Cell Lineage during Homeostasis

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Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor, expressed in villus cells of the intestinal epithelium, that promotes cellular differentiation and tissue homeostasis. Previous studies suggest that BMI1+ cells represent secretory progenitors with reserve intestinal stem cell (rISC) activity. However, it has not been elucidated how KLF4 contributes to crypt regeneration originated from BMI1+ rISC lineage during homeostasis. In this study, Bmi1-CreER;Rosa26eYFP (Bmi1Ctrl) and Bmi1-CreER;Rosa26eYFP;Klf4fl/fl (Bmi1ΔKlf4) mice were injected with tamoxifen to label BMI1+ cells and their lineage and to delete Klf4. During homeostasis, MUC2+ goblet cells appeared in the BMI1+ cell lineage 2, 3 and 7 days after tamoxifen administration. After Klf4 deletion in BMI1+ cells, the number of KLF4+ and MUC2+ cells in eYFP+ cells decreased in Bmi1ΔKlf4 mice compared with Bmi1Ctrl mice. Thus, KLF4 was positively correlated with goblet cell differentiation in BMI1+ cell derived lineage. In ex-vivo analysis, organoids derived from single eYFP+ cells of Bmi1Ctrl mice contained MUC2-expressing cells that co-expressed KLF4. On the other hand, organoids derived from Klf4-deleted eYFP+ cells from Bmi1ΔKlf4 mice showed reduced number of MUC2-expressing cells. In conclusion, these results suggest that KLF4 regulates goblet cell differentiation in BMI1+ ISC-derived lineage during homeostasis.

Keywords: Krüppel-like factor 4, Intestinal stem cell, Goblet cell, Mucin 2

Introduction

Intestinal epithelium is a rapidly self-renewing tissue, which consists of a crypt/villus unit. The renewal process is driven by leucine-rich G-protein-coupled receptor 5 (LGR5)+ intestinal stem cells (ISCs) at the crypt bottom (1). ISC has another population of the cells for tissue maintenance, named reserve ISC (rISC) located around +4 position from the bottom of the crypt (2). The rISCs are slowly cycling, injury-resistant and are capable of giving rise to multiple cell types upon injury or LGR5+ ISC loss (2, 3). B-cell-specific Moloney murine leukemia virus integrin site 1 (BMI1) is known as one of the rISC marker (4). Previous studies demonstrated that BMI1+ ISCs contribute to the regenerative response after injury to the small intestinal epithelium while LGR5+ ISCs are ablated (5, 6).
During homeostasis, ISCs produce the progenitors in the transit-amplifying cell zone that are committed to becoming differentiated cells (1). Differentiated cells in the small intestine are classified into two groups, absorptive and secretory cells (7). The absorptive progenitors differentiate into enterocyte. Secretory cells include goblet, Paneth, enteroendocrine and tuft cells. Previous studies suggest that during homeostasis secretory cell lineage originates from BMI1+ ISC, however it remains unclear how secretory cell differentiation from BMI1+ ISC is regulated (6, 8).

The zinc finger transcription factor, Krüppel-like factor 4 (KLF4) is normally expressed in the differentiated epithelial cells of the intestine, and contributes to epithelial homeostasis (9). Previously, we reported that KLF4 modulates the fate of BMI1+ ISCs and contributes to crypt regeneration from the BMI1+ cell-derived lineage by promoting its clonal expansion after radiation-induced injury (10). Furthermore, several studies suggested that goblet cell differentiation is regulated by KLF4 in the small intestine (11-13). However, it has not been elucidated how KLF4 regulates intestinal BMI1+ ISC during homeostasis.

These findings provide definitive evidence that KLF4 is essential in regulating goblet cell differentiation in the BMI1-expressing intestinal stem cells both in vivo and in cultured organoids in vitro.

Materials and Methods

Mouse strains and treatment

Bmi1-CreER,Tα26;Rosa26eYFP (Bmi1CreO) mice and Bmi1-CreER,Tα26;Klf4fl/fl (Bmi1ΔKlf4) mice were used as described previously (10). Three to four months old mice were used in this study. Tamoxifen was dissolved in a corn oil (37.5 mg/ml) and administrated by single intraperitoneal injection (9 mg per 40 g of body weight). All studies and procedures involving animal subjects were approved by the Stony Brook University Institutional Animal Care and Use Committee and conducted strictly in accordance with the approved animal handling protocol.

Immunofluorescence (IF) staining

Proximal parts of small intestines were swiss-rolled as described previously (10), and paraffin embedded blocks were cut into 5 μm thick sections. Tissues were de-paraffinized in xylene, rehydrated in ethanol gradient and incubated in a 10 mM Na-citrate buffer (pH 6.0) at 120°C for 10 min in a pressure cooker in order to retrieve antigen. Sections were then washed with water, incubated for 1h at 37°C in a blocking solution (5% Bovine serum albumin and 0.01% Tween 20 in 1x Tris-based phosphate-buffered saline [PBS]), and incubated with primary antibodies for GFP (AvesLabs), KLF4 (R&D Systems), MUC2 (GeneTex), chromogranin A (Abcam), Lysozyme (Leica biosystems). EdU was administered 3 h prior to euthanasia. EdU labelled cells were stained using Click-IT EdU imaging kit (Thermo Fisher Scientific) according to manufacturer’s instruction. Tissues were also counterstained with Hoechst 33258 to visualize nuclei.

EYFP+ cell isolation and organoid culture

Proximal small intestine was harvested from mice at 48 h after tamoxifen injection. Intestinal epithelial cells were dissociated as previously described (14). EYFP+ cells were sorted by flow cytometry (BD FACSARIA III; BD Biosciences, San Jose, CA) from Bmi1CreO and Bmi1ΔKlf4 mice. Sorted eYFP+ cells were embedded in Matrigel (Corning) and dispersed into 24-well plates as 20~30 μl droplets. Organoid culture medium was prepared using L-WRN cells as previously described (15) and supplemented with 1x N2 suplement (Thermo Fisher Scientific), 1x B27 supplement (Thermo Fisher Scientific), gastrin I (10 nM) (Sigma-Aldrich), recombinant human epidermal growth factor (50 ng/ml) (Thermo Fisher Scientific), transforming growth factor β inhibitor A83-01 (500 nM) (Tocris Bioscience, Bristol, United Kingdom), N-acetylcysteine (1 mM) (Sigma-Aldrich) and antibiotic cocktail Primocin (100 μg/ml) (Thermo Fisher Scientific). GSK3β inhibitor CHIR99021 (10 μM) (Tocris) and ROCK inhibitor Y-27632 (10 μM) (Sigma-Aldrich) were also added during the first 2 days of the culture. The media were changed every 2 days. Live organoids were imaged using inverted microscope (Eclipse Ti2, Nikon).

Intestinal organoid paraffin section preparation

Cultured organoids were washed with PBS. Matrigel was dissolved with Cell Recovery Solution (Corning) and collected organoids were resuspended in iPGell (GenoStaff, Tokyo, Japan). The iPGell-embedded organoids were fixed in 4% paraformaldehyde and embedded in paraffin.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 8.3.0 for Windows (GraphPad). Student t test, Dunn’s multiple comparisons test with Kruskal-Wallis test, Spearman correlation were used. Values of p < 0.05 were considered significant. All data are shown as Mean±SEM.
Results and Discussion

KLF4 is expressed in goblet cells in the crypt of small intestine

As previously reported, KLF4 is expressed mainly in the terminally differentiated epithelial cells lining the intestinal villi with the highest levels of expression within the proximal intestinal epithelium and also in a small population of the cells within the intestinal crypts of Bmi1<sup>Ctrl</sup> mice (Fig. 1A) (12, 16). BMI1<sup>+</sup> ISCs exist predominantly in the crypts of the proximal intestinal epithelium. Therefore, in this study, we focused on the KLF4 function in BMI1<sup>+</sup> ISC lineage within this region. Immunostaining revealed that the goblet cell marker, MUC2 is expressed in 74.1±4.0% of KLF4<sup>+</sup> cells in crypts (Fig. 1B and 1C). We also performed immunofluorescent staining (IF) of KLF4 and chromogranin A (an enteroendocrine cell marker) and lysozyme (a Paneth cell marker) (Fig. 1D and 1E). Co-expression rates of KLF4 in MUC2<sup>+</sup>, chromogranin A<sup>+</sup> and lysozyme<sup>+</sup> cells are 79.0±2.0%, 3.3±1.7% and 0.7±0.7%, respectively (Fig. 1F). Thus, KLF4 is mainly expressed in goblet cells in the crypt of small intestine.

![Fig. 1.](image-url)
KLF4 regulates goblet cell differentiation and proliferation in BMI1⁺-ISC lineage in vivo

Next, we investigated the effect of Klf4 deletion in BMI1⁺ ISC population on goblet cells differentiation. Bmi1<sup>Ctrl</sup> and Bmi1<sup>ΔKlf4</sup> mice were injected with tamoxifen and the proximal small intestinal tissues were collected on days 2, 3, and 7 post-treatment and stained for eYFP (BMI1⁺ cells and their lineage), KLF4 and MUC2 (Fig. 2A). The num-

**Fig. 2.** KLF4 modulates goblet cell differentiation in Bmi1 cell lineage during homeostasis. (A) Immunostaining for eYFP (green), MUC2 (red) and KLF4 (yellow). Representative image of proximal small intestinal crypt at day 2, 3 and 7 following tamoxifen injection. Goblet cell differentiation is involved in Bmi1⁺ cell lineage. Scale bar, 25 μm. (B), (C) and (D) The number of eYFP, MUC2 and KLF4 positive cells per eYFP⁺ including crypt increased as the time following tamoxifen administration in both groups. Data were collected from at least 20 crypts per mouse (n=3 each group). Dunn’s multiple comparisons test with Kruskal-Wallis test. (B) The number of eYFP⁺ cells was significantly higher in Bmi1<sup>ΔKlf4</sup> than in Bmi1<sup>Ctrl</sup> at day 3 following tamoxifen administration. (C) The number of KLF4⁺ cells was significantly higher in Bmi1<sup>Ctrl</sup> than in Bmi1<sup>ΔKlf4</sup> at day 7 following tamoxifen administration (p < 0.001). (D) The number of MUC2⁺ cells was significantly lower in Bmi1<sup>ΔKlf4</sup> than in Bmi1<sup>Ctrl</sup> at day 3 and 7 following tamoxifen administration (p < 0.001). (E) Immunostaining for eYFP (green), KLF4 (yellow) and EdU (red) for the proximal small intestinal crypts at day 3 following tamoxifen administration. Scale bar, 25 μm. (F) At day 3 after tamoxifen injection, EdU positivity in eYFP⁺ cells of Bmi1<sup>ΔKlf4</sup> was significantly higher than Bmi1<sup>Ctrl</sup> (46.7 ± 2.1% and 20.7 ± 0.9%, respectively). Student t test. EYFP⁺ cells were collected from 50 crypts per mouse (n=3 each group). ***p < 0.001.
The number of eYFP$^+$ cells increased in both $Bmi1^{Ctrl}$ and $Bmi1^{Klf4\Delta}$ mice after tamoxifen injection ($Bmi1^{Ctrl}$ vs. $Bmi1^{Klf4\Delta}$, 2.0±0.1 vs. 2.5±0.2, 4.0±0.2 vs. 6.3±0.2, 9.0±0.6 vs. 9.4±0.6 at days 2, 3, and 7, respectively) (Fig. 2B). At 3 days after tamoxifen injection, the number of eYFP$^+$ cells per eYFP crypt in $Bmi1^{Klf4\Delta}$ mice was significantly higher than $Bmi1^{Ctrl}$ mice (6.3±0.2 and 4.0±0.2, respectively; p < 0.001) (Fig. 2A and 2B). Importantly, the number of KLF4$^+$ cells in Bmi1-eYFP$^+$ cells in $Bmi1^{Klf4\Delta}$ mice was significantly lower than $Bmi1^{Ctrl}$ mice on days 3 and 7 after tamoxifen injection (Fig. 2C). The number of goblet cells marked by MUC2 stain per eYFP$^+$ crypt in $Bmi1^{Ctrl}$ mice was significantly higher than in $Bmi1^{Klf4\Delta}$ mice on day 7 (Fig. 2D). Thus, goblet cell differentiation was significantly inhibited in BMI1$^+$ ISC derived lineage after Klf4 deletion in BMI1$^+$ ISC during homeostasis. Deletion of Klf4 within BMI1$^+$ cell population did not affect differentiation of other secretory lineages marked by chromogranin A and lysozyme (data not shown). Three days after tamoxifen administration, the percentage of Edu$^+$ cells in eYFP$^+$ cells in $Bmi1^{Klf4\Delta}$ mice was also significantly higher than in $Bmi1^{Ctrl}$ mice (46.7±2.1% and 20.7±0.9%, respectively; p<0.001) (Fig. 2E and 2F). Taken together these data suggest that Klf4 deletion in BMI1$^+$ ISC leads to increased cell proliferation in BMI1$^+$ ISC derived cell lineages and a reduction of goblet cell differentiation.

**KLF4 is positively correlated with goblet cell differentiation**

To confirm relationship between KLF4 expression and goblet cell differentiation, we counted BMI1-eYFP positive cells from proximal small intestinal crypts of $Bmi1^{Ctrl}$ and $Bmi1^{Klf4\Delta}$ mice 7 days after tamoxifen injection. In $Bmi1^{Ctrl}$ mice 18.0±2.4% of eYFP$^+$ cells co-expressed MUC2 while in $Bmi1^{Klf4\Delta}$ mice only 9.2±1.9% (Fig. 3A). Additionally, in $Bmi1^{Ctrl}$ mice, 60–70% of MUC2 positive cells co-expressed KLF4. The Spearman correlations analysis showed that KLF4 and MUC2 are significantly positively correlated (Spearman correlation=0.9429, p<0.01) (Fig. 3B).

**Goblet cell differentiation from single sorted Bmi1-eYFP$^+$ cell is regulated by KLF4**

To determine the effect of Klf4 deletion from BMI1$^+$
cells on tissue regenerative process, we used organoids derived from fluorescence-activated cell sorting-isolated eYFP+ cells. Cultured organoids obtained from Bmi1^Col^ and Bmi1^ΔKLF4^ mice showed budding formation around culture day 5 (Fig. 4A and 4B) with no significant difference between these two groups. However, on day 7 organoids obtained from Bmi1^ΔKLF4^ mice showed increased in the proliferation in comparison to organoids from Bmi1^Col^ mice. This is in accordance with our previous in vivo observation that demonstrated that Klf4 deletion in BMI1+ cells led to their increased proliferation (10). Immunostaining of cultured organoids demonstrated that MUC2+ cells are found in organoids derived from Bmi1^Col^ mice, and 73.9% of MUC2+ cells co-expressed with KLF4. Scale bars, 50 μm. (D) and (E) Percentage of KLF4+ and MUC2+ cells were significantly higher in Bmi1^Col^ organoid than Bmi1^ΔKLF4^ at culture day 7 (Bmi1^Col^ vs. Bmi1^ΔKLF4^, 11.2±1.1% vs. 1.4±0.5% and 13.4±1.6% vs 2.1±0.8% for KLF4+ and MUC2+ cells, respectively). Cells were analyzed from 10 sections for each group and 10~200 cells were collected from each section. Organoids were pooled from 3 mice for each group. ***p<0.001.

**Fig. 4.** Organoids derived from single FACS sorted eYFP+ cells of Bmi1^Col^ and Bmi1^ΔKLF4^ mice. (A) Representative time course images of cultured organoids derived from Bmi1^Col^ and Bmi1^ΔKLF4^ mice. Upper panels, merged image of bright-field and fluorescent image; middle panel, bright field image; lower panel, fluorescent image. Scale bars, 500 μm. (B) Hematoxylin and eosin staining at culture day 7. Scale bars, 100 μm. (C) Cultured organoids were collected at day 7. Immunostaining for eYFP (green), MUC2 (red), KLF4 (yellow). Left panel merged image for eYFP, MUC2, KLF4 and DAPI (blue); middle panel, merged image for MUC2, KLF4 and DAPI; right panel merged image for MUC2 and KLF4. Upper right panel is magnified image of green square in upper middle panel. In Bmi1^Col^ mice derived organoids, 73.9% of MUC2+ cells were co-expressed with KLF4. Scale bars, 50 μm. (D) and (E) Percentage of KLF4+ and MUC2+ cells were significantly higher in Bmi1^Col^ organoid than Bmi1^ΔKLF4^ at culture day 7 (Bmi1^Col^ vs. Bmi1^ΔKLF4^, 11.2±1.1% vs. 1.4±0.5% and 13.4±1.6% vs 2.1±0.8% for KLF4 and MUC2, respectively). Cells were analyzed from 10 sections for each group and 10~200 cells were collected from each section. Organoids were pooled from 3 mice for each group. ***p<0.001.
In this study, we have shown that goblet cell differentiation is regulated by KLF4 in Bmi1+ ISC lineage during homeostasis. Initially, Bmi1+ ISC was identified as a quiescent and radiation-resistant cell located around +4 position in the proximal small intestinal crypt (4, 6). Later, several studies observed controversial results that Bmi1 was expressed throughout the crypt using mRNA in situ hybridization and Bmi1 expressing cells were located in crypt cells, including goblet, enteroendocrine, Paneth and Lgr5+ cells (8, 17, 18). These data and our results suggest that Bmi1+ ISCs are partially responsible for the intestinal homeostasis not only in injury state but also under homeostatic condition.

Goblet cells are the most abundant intestinal secretory lineage, comprising ∼15% of the small intestinal epithelial cells (2, 19). Although turnover time of MUC2+ goblet cells is slower in the crypt compared to goblet cells along the villi, newly produced MUC2 expressing goblet cell will disappear within 24 h (2, 19). In this study, MUC2 positive cells were detected in Bmi1+ cell lineage at 7 days after tamoxifen injection. Thus, Bmi1+ cells act not only rISC but also active intestinal stem cells (aISC) with self-renewal and multipotency during homeostasis. Previous studies indicated that KLF4 had a critical role in the development of goblet cells and the function of BMI1+ ISC lineage during homeostasis and differentiation. In these studies, the effect of KLF4 deletion was included in both differentiated cells and ISC. Here, we have investigated the direct effect of Bmi1+ ISC specific KLF4 deletion using lineage tracing mouse model from Bmi1 locus and ex vivo organoid model.

In conclusion, we have shown that KLF4 regulates goblet cell differentiation in Bmi1+ intestinal stem cell lineage during homeostasis.

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Potential Conflict of Interest

The authors have no conflicting financial interest.

References

1. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449:1003-1007.
2. Clevers H. The intestinal crypt, a prototype stem cell compartment. Cell 2013;154:274-284.
3. Kim CK, Yang VW, Bialkowska AB. The role of intestinal stem cells in epithelial regeneration following radiation-induced gut injury. Curr Stem Cell Rep 2017;3:320-332.
4. Sangiorgi E, Cepeccchi MR. Bmi1 is expressed in vivo in intestinal stem cells. Nat Genet 2008;40:915-920.
5. Tian H, Bihs B, Warming S, Leong KG, Rangell L, Klein OD, de Sauvage FJ. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature 2011;478:255-259.
6. Yan KS, Chia LA, Li X, Ootani A, Su J, Lee JY, Su N, Luo Y, Heilshorn SC, Amieva MR, Sangiorgi E, Cepeccchi MR, Koo CJ. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proc Natl Acad Sci U S A 2012;109:466-471.
7. Noah TK, Donahue B, Shroyer NF. Intestinal development and differentiation. Exp Cell Res 2011;317:2702-2710.
8. Muñoz J, Stange DE, Schepers AG, van de Wetering M, Koo BK, Itzkovitz S, Volckmann R, Kung KS, Koster J, Radulescu S, Myant K, Versteeg R, Sansom OJ, van Es JH, Barker N, van Oudenaarden A, Mohammed S, Heck AJ, Clevers H. The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent ‘+4’ cell markers. EMBO J 2012;31:3079-3091.
9. Ghaleb AM, Yang VW. Krüppel-like factor 4 (KLF4): what we currently know. Gene 2017;611:27-37.
10. Kuruvilla JG, Kim CK, Ghaleb AM, Bialkowska AB, Koo CJ, Yang VW. Krüppel-like factor 4 modulates development of Bmi1+ intestinal stem cell-derived lineage following γ-radiation-induced gut injury in mice. Stem Cell Reports 2016;6:815-824.
11. Ghaleb AM, Aggarwal G, Bialkowska AB, Nandan MO, Yang VW. Notch inhibits expression of the Krüppel-like factor 4 tumor suppressor in the intestinal epithelium. Mol Cancer Res 2008;6:1920-1927.
12. Ghaleb AM, McConnell BB, Kaestner KH, Yang VW. Altered intestinal epithelial homeostasis in mice with intestine-specific deletion of the Krüppel-like factor 4 gene. Dev Biol 2011;349:310-320.
13. Imaio M, Ebisu M, Nishida E. Dual role of YAP and TAZ in renewal of the intestinal epithelium. Nat Cell Biol 2015;17:7-19.
14. Kim CK, Saxena M, Maharjan K, Song JJ, Shroyer KR, Bialkowska AB, Shivdasani RA, Yang VW. Krüppel-like factor 5 regulates stemness, lineage specification, and regeneration of intestinal epithelial stem cells. Cell Mol Gastroenterol Hepatol 2020;9:587-609.
15. Miyoshi H, Stappenbeck TS. In vitro expansion and genetic engineering of human intestinal epithelial progenitor cells. Nat Biotechnol 2006;24:1247-1254.
modification of gastrointestinal stem cells in spheroid culture. Nat Protoc 2013;8:2471-2482

16. Flandez M, Guilmeau S, Blache P, Augenlicht LH. KLF4 regulation in intestinal epithelial cell maturation. Exp Cell Res 2008;314:3712-3723

17. Itzkovitz S, Lyubimova A, Blat IC, Maynard M, van Es J, Lees J, Jäcks T, Clevers H, van Oudenaarden A. Single-molecule transcript counting of stem-cell markers in the mouse intestine. Nat Cell Biol 2011;14:106-114

18. Yan KS, Gevaert O, Zheng GXY, Anchang B, Probert CS, Larkin KA, Davies PS, Cheng ZF, Kaddis JS, Han A, Roelf K, Calderon RI, Cynn E, Hu X, Mandlewala K, Wilhelmy J, Grimes SM, Corney DC, Boutet SC, Terry JM, Belgrader P, Ziraldo SB, Mikkelsen TS, Wang F, von Furstenberg RJ, Smith NR, Chandraicesan P, May R, Chrissey MAS, Jain R, Cartwright CA, Niland JC, Hong YK, Carrington J, Breault DT, Epstein J, Houchen CW, Lynch JP, Martin MG, Plevritis SK, Curtis C, Ji HP, Li L, Henning SJ, Wong MH, Kuo CJ. Intestinal enteroendocrine lineage cells possess homeostatic and injury-inducible stem cell activity. Cell Stem Cell 2017;21:78-90.e6

19. Schneider H, Pelaseyed T, Svensson F, Johansson MEV. Study of mucin turnover in the small intestine by in vivo labeling. Sci Rep 2018;8:5760

20. Gersemann M, Becker S, Kühler I, Koslowski M, Wang G, Herrlinger KR, Griger J, Fritz P, Fellermann K, Schwab M, Wehkamp J, Stange EF. Differences in goblet cell differentiation between Crohn's disease and ulcerative colitis. Differentiation 2009;77:84-94

21. Katz JP, Perreault N, Goldstein BG, Lee CS, Labosky PA, Yang VW, Kaestner KH. The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. Development 2002;129:2619-2628