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Role of Krüppel-like factor 5 in the maintenance of the stem cell niche in the intestinal crypt

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Abstract

The intestinal epithelium is a tissue that undergoes continuous self-renewal initiated at the bottom of the crypts, which harbor the intestinal stem cell (ISC) pool. The ISC pool is sub-divided into crypt base columnar (CBC) cells at the crypt bottom and label retention cells (LRC) at position +4 from the crypt bottom. CBC cells are marked by Leucine-rich repeat-containing G-protein coupled receptor (Lgr5) while LRC cells are identified by several markers including Bmi1, mTert, Hopx, Lrig1, and Sox9. Krüppel-like factors (KLFs) belong to a family of transcription factors that exert important physiological function in various tissues. In the intestine, KLF4 is predominantly expressed in the terminally differentiated, non-proliferating cells lining the villus. Its deletion in the adult mouse intestine results in perturbed homeostasis. In contrast, KLF5 is expressed in actively proliferating cells of the intestinal crypt, including CBC cells and transit amplifying (TA) cells. We recently investigated the effect of *Klf5* deletion specifically from the Lgr5-expressing CBC cells in adult mouse intestine using an inducible Cre recombinase system. Shortly (3–5 days) after Cre induction, proliferation of both CBC and TA cells ceased, which was accompanied by an increase in apoptosis in the crypt. Beginning at two weeks following Cre induction, both Klf5 expression and proliferation re-appeared but without the re-emergence of Lgr5-positive CBC cells, which were eventually depleted by four months following induction. These findings indicate that KLF5 plays an important role in regulating proliferation and survival of CBC stem cells in the intestine.

Keywords

intestinal epithelium; stem cell; Lgr5; Krüppel-like factors

The intestinal epithelium is divided into two compartments: the crypt and the villus. Intestinal stem cells (ISC) reside in the bottom of each crypt and give rise to daughter

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Conflict of Interests

No conflict of interest for any authors.

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progenitor cells (also called transit amplifying or TA cells) that eventually populate all of the cells in the villus [1, 2]. Historically, cells located at position +4 from the bottom of the crypt [3], through DNA label retention experiments, were found to be slow-cycling or quiescent and thought to be the source of ISC [3, 4]. The +4 cell was later identified to express B lymphoma Mo-MLV insertion region 1 (Bmi1), a component of the polycomb repressor protein complex [5]. Subsequently, +4 cells are found to express several other markers including Hopx, Lrig1, mTert and Sox9 [6–9]. The $Bmi1^+$ quiescent +4 stem cells are resistant to ionizing radiation and contribute to homeostatic regeneration post-irradiation [10–12].

Recently, a second pool of cells, called crypt base columnar (CBC) cells that are situated at the bottom of the intestinal crypt, was found to exhibit stem cell characteristics [13]. These cells express an orphan G protein-coupled receptor called Lgr5, which is part of the Wnt signaling pathway [13]. By employing sophisticated gene-targeting and lineage tracing approaches, Lgr5-expressing CBC cells were found to serve as the precursors to all cell lineages along the crypt-villus axis [13–15]. These observations provide evidence that the rapidly cycling CBC cells represent the pool of active ISC in the intestine.

Our laboratory has a longstanding interest in understanding the mechanisms by which a number of Krüppel-like factors (KLFs) regulate homeostasis of intestinal epithelial cells [16–18]. KLFs belong to a family of 17 transcription factors with homology to the *Drosophila* Krüppel gene product [19, 20]. KLFs are closely related to the Sp1 family of transcription factors in that they contain three highly conserved C_2H_2 Zn finger motifs at the carboxyl terminus [18, 20, 21]. The zinc fingers of KLFs bind to GC-rich or CACCC sequences in the promoters of many genes with which to exert their transcriptional effects [18, 22]. Amino acid sequences outside the zinc finger domains of KLFs are quite diverse and are involved in determining their transcription-regulatory activities [19]. Based on structure-function characteristics, KLFs are divided into 3 groups: Group 1 (KLFs 3, 8 and 12), which is predominantly transcriptional repressors; Group 2 (KLFs 1, 2, 4, 5, 6, and 7); which are predominantly transcriptional activators; and Group 3 (KLFs 9, 10, 11, 13, 14, and 16), which are also transcriptional repressors by interacting with Sin3A. KLFs 15 and 17 are grouped separately [20]. KLFs are expressed in diverse mammalian tissues and regulate fundamentally important biological processes such as adipogenesis [23, 24], proliferation [16], differentiation [25], cancer [26–29], inflammation [30], and apoptosis [31–34]. Among them, KLF4 and KLF5 are differentially expressed in the adult intestinal epithelium and are involved in maintaining epithelial homeostasis [16, 17].

KLF4, also called gut-enriched Krüppel-like factor or GKLF, was initially found to be expressed in the intestine [35] and subsequently in epithelial cells of the skin, therefore also named epithelial zinc finger (EZF) [36]. In the intestine, KLF4 is primarily expressed in the terminally differentiated cells of the intestinal epithelium, where it maintains a quiescent state by negatively regulating the cell cycle [35, 37]. Intestine-specific deletion of *Klf4* in mice results in increased proliferation and altered differentiation [37]. KLF4 expression is also activated by agents causing DNA damage such as ionizing irradiation [33, 38, 39] and a recent study indicates that KLF4 is a radio-protective factor for the intestine following

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ionizing radiation-induced gut injury [34]. These findings point to an important role of KLF4 in maintaining intestinal epithelial homeostasis.

In addition to KLF4, KLF5 is known by its abundant expression in the intestinal epithelium and was initially called intestinal Krüppel-like factor or IKLF [40]. It was later found to be present in many other tissues including other epithelial cells as well as adipocytes, neuronal cells, leukocytes, and vascular smooth muscle cells [41]. KLF5 has important functions during development as its homozygous deletion from mice results in embryonic lethality [42]. In the intestine, Klf5 in mice is primarily expressed in the actively proliferating cells of the intestinal crypts [17]. Mice with intestine-specific deletion of *Klf5* (as directed by villin-Cre recombinase) die in the neonatal period due to failure of the intestine to develop [41]. Those with variegated deletion survived but suffered from stunted growth compared to their littermates with wild-type [41]. Further investigation into the intestine-specific function of Klf5 in adult mice led to the development of an inducible intestine-specific knockout mouse model. Here an estrogen-regulated Cre recombinase driven by the villin promoter is only expressed following treatment with the inducer, tamoxifen [43]. Mice were phenotypically normal in the absence of tamoxifen. Within 3 to 5 days after the administration of tamoxifen, there was a loss of proliferating cells in the intestine [44]. Transcriptome analysis after induction showed an increase in expression of genes in the regenerative pathway including *Reg1A*, *Reg3G*, *Reg3B*, as well as *Sox9* [44]. Importantly, despite the initial loss of the proliferative response due to *Klf5* deletion, there was a robust response to repair and replenish the epithelium later during the regenerative process [44]. The precise mechanism by which the regenerative response is mediated following *Klf5* deletion remains to be determined.

Since KLF5 plays a crucial role in regulating proliferation of intestinal epithelial cells, we sought to determine whether it regulates ISC proliferation. A careful inspection shows that Klf5 is not only expressed in the TA cell population of the intestinal crypt but in CBC cells that express Lgr5 [45]. Using the inducible Cre recombinase driven by the Lgr5 promoter (Lgr5/EGFP-Cre^{ER}), we deleted *Klf5* in adult mice from CBC cells only. Intestinal tissues were sampled at various time points from 3 to 112 days following the initial administration of tamoxifen. During the early phase (between 3 and 11 days) of deletion, both CBC and TA cells in crypts that express the Lgr5/EGFP-Cre^{ER} transgene (which has a variegated penetrance) were no longer proliferating [45]. This was accompanied by an increase in apoptosis in those crypts that express EGFP. By day 14 following the initial administration of tamoxifen, both Klf5 expression and proliferation were reestablished in the TA cells but not in the CBC cells that express EGFP [45]. Eventually by 112 days after the initial administration of tamoxifen, over 90% of intestinal crypts that express EGFP were lost [45]. These results suggest that in the absence of active proliferation, Lgr5-expressing CBC cells have an approximate half-life of 30 to 50 days in the intestine.

The ability of intestinal crypt to replenish itself with proliferating TA cells post-*Klf5* deletion from Lgr5-expressing CBC cells is quite intriguing. Although we currently do not understand how this is accomplished, a number of possibilities exist: (1) stem cells migrated from adjacent crypts (where *Klf5* is not deleted from CBC cells); (2) activation of resident quiescent stem cells (e.g. Bmi1-expressing LRC) following cessation of CBC cell division;

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or (3) in conjunction with the neutral-drift model for competing stem cells [46, 47]. Perhaps a model with a more robust expression of the Lgr5/Cre from which a more complete deletion of *Klf5* can be accomplished would reveal the true physiological function of Klf5 in regulating ISC function.

During the course of our experiments, an independent study reported similar effects as ours upon deletion of *Klf5* from Lgr5-positive CBC cells [48]. That study corroborated our findings but also demonstrated that Klf5 is an obligatory factor, necessary for the survival and transformation of intestinal epithelial cells [48]. The latter conclusion stemmed from the observation that Klf5 is crucial in mediating Wnt/β-catenin-driven intestinal tumorigenesis, a fact previously established by our group [28].

It is now a well-established fact that a high degree of plasticity occurs in ISC. Various cell populations as identified by different markers exhibit diverse behavior and function in various physiological or pathophysiological contexts. The series of studies from our group using gene knockout have shown that KLF5 plays a significant role in regulating proliferation of CBC ISC under homeostatic conditions and perhaps in the subsequent regenerative response following perturbation of ISC homeostasis. Further investigation into how KLF5 is involved in epithelial regeneration may shed additional light on the biochemical characteristics of ISC in pathological states.

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