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Mini Review

The tortoise and the hare?: Two distinct intestinal stem cell populations

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Adult stem cells have the capacity to self-renew and to regenerate tissues long-term, in both homeostasis and wound repair. Genetic lineage tracing studies have identified two principal stem cell populations in the intestine. One population consists of actively cycling *Lgr5*$^+$ cells residing at the crypt base. The other population consists of quiescent *Bmi1*$^+$ cells that largely reside at approximately the +4 cell position directly above the Paneth cells in the crypt. Recent studies demonstrate a functional relationship between these two intestinal stem cell (ISC) populations. This review provides an overview of ISCs and the ISC niche.

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Introduction

Stem cells have the ability to self-renew and to differentiate into multiple cell types that constitute their tissue of origin[1)]. Adult stem cells are critical for replenishing and maintaining tissue homeostasis and for wound repair. The intestinal mucosa is lined by a simple columnar epithelium that undergoes complete regeneration every 5-7 days[2)]. Intestinal stem cells (ISCs) divide to produce transit amplifying cells, which migrate toward the lumen, differentiate into absorptive enterocytes, goblet cells and enteroendocrine cells. Paneth cells migrate and reside at the crypt bottom, but are absent from the colon. Two stem cell populations have been described in distinct positions within the intestinal crypts. *Lgr5*$^+$ ISCs reside at the crypt bottom intercalated with Paneth cells[3)]; *Bmi*$^+$ ISCs are predominantly located just above the Paneth cell compartment and are referred to as "+4 cells", indicating their position from the bottom of the crypt[4)]. Although the relationship and interdependency of these different types of ISC remain a matter of debate, recent studies suggest the functional differences of these two ISC populations[5-7)]. Within the tissue, stem cells need to reside in a particular microenvironment termed "niche" to maintain its "stemness"[8)]. The ISC niche is notable for pericryptal myofibroblasts adjacent to the crypt base, which are believed to elaborate paracrine signals regulating the neighboring ISCs[9)]. In this mini-review, I discuss the current concepts surrounding the identity of the ISC and the microenvironment-derived signals that regulate crypt homeostasis.

Crypt base columnar cells (CBCs) and label retaining cells (LRCs)

Two models regarding the exact identity of the ISCs were formulated: the stem cell zone model and the +4 position model[2]. The existence of ISCs has also been supported by studies with both chimeric and heterozygous mutant mouse strains, which indicated that intestinal crypts were monoclonal in nature[10, 11]. However, the exact location of these cells has remained controversial. In 1974, Cheng and Leblond reported that crypt base columnar cells (CBCs), which are interspersed between Paneth cells at the crypt base, are mitotically active and give rise to multiple cell types by a series of simple lineage-tracing experiments[12-15]. They proposed that the existence of a stem cell zone in the crypt bottom and CBCs are the stem cells[16]. Studies from other stem cell systems, including blood and skin, indicated that adult stem cells were either in a prolonged quiescent state or extremely slow cycling[17, 18]. Potten and the colleagues reported that most of the DNA label-retaining cells (LRCs) were localized at the +4 position from the bottom of the crypt directly above the Paneth cell compartment and proposed that these LRCs are the stem cells[19]. Neither of these two hypotheses on ISC identity, however, was supported by direct evidence for stemness such as lineage tracing and/or transplantation.

Lgr5$^+$ and *Bmi1*$^+$ ISCs

The identification of specific cell-surface markers has allowed for the detection of pluripotential stem cells in a number of tissues, including bone marrow[20], hair follicle[21], and mammary gland[22]. In 2007, a single marker, *Lgr5/Gpr49*, a leucine-rich orphan G protein-coupled receptor, was identified to specifically label stem cells in the mouse small intestine, such as the CBCs between the Paneth cells[3]. Genetic lineage tracing studies have demonstrated that *Lgr5*$^+$ CBCs are multipotent for all mature intestinal epithelial cells, undergo self-renewal, and persist long term, demonstrating that CBCs function as ISCs. Single *Lgr5*$^+$ cells can self-renew and differentiate into all of the epithelial lineages *in vitro* when exposed to the appropriate milieu of key extracellular matrix and signaling factors[23]. Further studies show other specific markers for these cells, such as *Prominin1/CD133*[24], *Olfm4* and *Ascl2*[25]. Around the same time, Sangiorgi and Capecchi characterized the progeny of crypt *Bmi1*$^+$ cells and make the argument in support of the +4 LRCs as a population of stem cells within the small intestine[4]. *Bmi1* encodes a chromatin remodeling protein of the polycomb group that has essential roles in self-renewal of hematopoietic and neural stem cells. Genetic lineage tracing studies have demonstrated that *Bmi1* consistently mark long-lived cell clones populated by all intestinal lineages and serves as a specific marker of a cell population located around the +4 position of the crypt. Furthermore, ablation of *Bmi1*$^+$ cells by targeted expression of the diphtheria toxin depletes the epithelium of the genetically marked crypts. Consistent with their *in vivo* stem cell function, we have confirmed that isolated *Bmi1*$^+$ single ISCs exhibit self-renewing and multi-lineage differentiation *in vitro*[7]. This is further substantiated by lineage tracing studies using both mouse telomerase reverse transcriptase (*mTert*) and an atypical homeobox protein *Hopx* which also mark cells at the +4 position that are long-lived, slowly cycling, and exhibit multi-lineage differentiation[26, 6].

Relationship between active and quiescent ISC populations

Recent advances in purifying ISCs reveal that like the hair follicle stem cell niche, two distinct ISC pools have been demonstrated based on location and cell cycling kinetics[2]. Actively-cycling ISCs express *Lgr5* and are present at the crypt base as CBCs[3]. Slowly-cycling or quiescent ISCs express *Bmi1* and are largely located at the +4 position[4]. A recent report shows that +4 position cells can compensate for the loss of CBCs to maintain homeostasis after experimental ablation of *Lgr5*$^+$ cells[5]. Using a diphtheria toxin receptor gene knocked into the *Lgr5* locus, specific ablation of *Lgr5*$^+$ ISCs does not perturb homeostasis of the intestinal epithelium and instead leads to an increase in the production of *Bmi1*$^+$ ISCs. Lineage tracing experiments demonstrate that *Bmi1*$^+$ ISCs give rise to *Lgr5*$^+$ cells both under normal physiological conditions and after insults that deplete CBCs. Further studies demonstrate that quiescent +4 ISCs express the atypical homeobox gene *Hopx* and give rise to *Lgr5*$^+$ CBCs[6]. Conversely, rapidly cycling CBCs expressing *Lgr5* give rise to +4 cells expressing *Hopx*. These results provide a bidirectional lineage relationship between active and quiescent ISCs in the niche. We have recently found that active cycling *Lgr5*$^+$ ISCs are radiosensitive, whereas quiescent *Bmi1*$^+$ ISCs are resistant to high dose radiation and vigorously proliferate with expansion of downstream progeny into multiple contiguous crypts and villi after irradiation injury[7]. Further, isolated *Bmi1*$^+$ ISCs

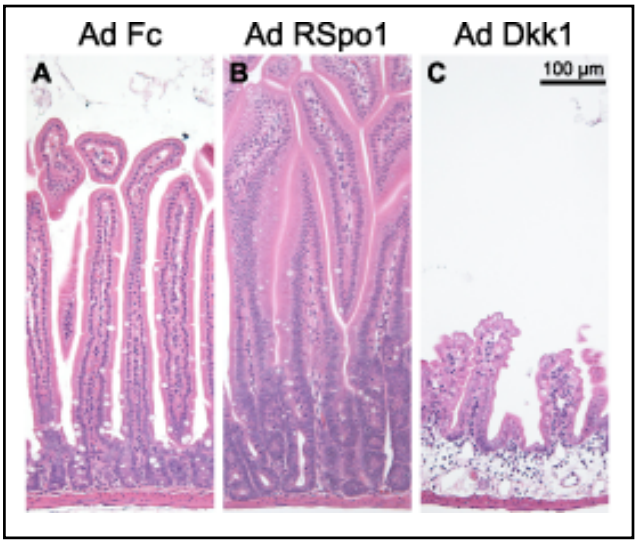

Fig.1 Modulation of extracellular Wnt signaling in adult mouse intestine

(A) Small intestine of vehicle control. (B) Wnt agonist R-spondin1 induces marked expansion of the intestinal crypts with increasing proliferative activities. (C) Wnt inhibitor Dkk1 causes ablation of the intestinal crypts. At day 7 after single i.v. injection of Ad Fc (A), Ad RSpo1-Fc (B), or Ad Dkk1 (C).

can give rise to *Lgr5*$^+$ cells *in vitro*[7]. These findings indicate that *Bmi1*$^+$ ISCs represent both a reserve stem cell pool in case of injury to the small intestinal epithelium and as a source for replenishment of the *Lgr5*$^+$ ISCs under non-pathological conditions. It will be critical to elucidate how different stem cell populations sense the activity of other populations and whether additional subpopulations of stem cells exist.

ISC niche

Epithelial stem cells are generally influenced by a microenvironmental niche, typically comprised of epithelial and mesenchymal cells and extracellular substrates, which instruct the cell to either self-renew or selectively adopt a particular cell lineage[27]. The ISC niche is notable for the presence of myofibroblasts adjacent to the crypt base, which are believed to elaborate paracrine signals regulating the neighboring ISCs[28]. In fact, our ISC culture system demonstrate the importance of mesenchymal myofibroblasts for maintaining ISCs *in vitro*[29]. A wide range of evidence indicates that extracellular Wnt signals have a crucial role in intestinal proliferation and ISC maintenance[9]. We have clearly demonstrated that *Lgr5*$^+$ ISCs exhibits exquisite sensitivity to modulation of canonical Wnt signals resulting in quantitative expansion in response to gain-of-function with R-spondin1 (RSpo1) and ablation with loss-of-function with Dickkopf-1 (Dkk1) (Fig.1)[7, 29, 30]. Interestingly, *Bmi1*$^+$ ISCs are relatively insensitive to Wnt signaling modulation compared to *Lgr5*$^+$ ISCs[7]. A recent report suggests the importance of Paneth cells for the maintenance of *Lgr5*$^+$ ISCs as the niche[31]. However, ablation of Paneth cells can be tolerated without significant structural defects of the epithelium nor disturbance of *Lgr5*$^+$ ISC functions[32-36], implying that Paneth cells are nonessential constituents of the ISC niche. Notch signals are similarly essential, with stimulation amplifying the progenitor pool and inhibition resulting in conversion to post-mitotic goblet cells[37, 38]. Inhibition of BMP signaling by Noggin and Gremlin produced by submucosal tissue below the crypts is another requirement to confer intestinal stemness[39, 40]. The factors that maintain the unique properties of each stem cell population and regulate the interplay between discrete populations of stem cells remain to be characterized.

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Conflicts of interest

The author declares no conflict of interest.

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