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Recent Advances in Intestinal Stem Cells

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Abstract

Purpose of review—The intestine is a dynamic organ with rapid stem cell division generating epithelial cells that mature and apoptose in 3–5 days. Rapid turnover maintains the epithelial barrier and homeostasis. Current insights on intestinal stem cells (ISCs) and their regulation are discussed here.

Recent findings—The Lgr5+ ISCs maintain intestinal homeostasis by dividing asymmetrically, but also divide symmetrically to extinguish or replace ISCs. Following radiation or mucosal injury, reserve BMI1+ ISCs as well as other crypt cells can de-differentiate into Lgr5+ ISCs. ISC niche cells, including Paneth, immune and myofibroblast cells secrete factors that regulate ISC proliferation. Finally, several studies indicate that the microbiome metabolites regulate ISC growth.

Summary—ISC cells can be plastic and integrate a complexity of environmental/niche cues to trigger or suppress proliferation as needed.

Keywords

intestine; stem cell; Wnt; Lgr5+; Bmi1+; CBC

Introduction

The gastrointestinal system is highly dynamic, thereby allowing rapid adaption to environmental and diet changes. Intestinal fluctuations/alterations occur at the level of the microbiome, barrier function, epithelial enzyme and transporter expression, secretion and cell turnover. The latter, cell turnover, is constant and critical for the self-renewal of the crypt-to-villus epithelium and the barrier it forms [1, 2]. Regulation of intestinal stem cell (ISC) proliferation is essential for maintaining intestinal cellular homeostasis. ISCs produce

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Conflict of Interest

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Human and Animal Rights and Informed Consent

This article contains no studies with human or animal subjects performed by any of the authors.

Figure 1

Figure 2

daughter cells that divide, become transit-amplifying progenitors (which undergo an additional 4–5 divisions), and then differentiate into goblet (mucus producing), endocrine, enterocyte (absorptive) or Paneth cells (mucosal defense cells). This differentiation process progresses as cells move toward the villus tip (except for Paneth cells which differentiate as cells move toward the crypt). After 3–5 days, cells that reach the upper villus region express genes to maximize digestion and absorption of dietary nutrients [1](Figure 1). The top of villus is the most exposed to luminal contents which makes the cells at this location vulnerable to degradation and infection with luminal pathogens. Once epithelial cells reach the villus tip they undergo programmed cell death and are sloughed off into the lumen (more than 17 billion cells per day).

Rapid cell turnover in the intestine provides the capacity for adaptation, mucosal repopulation and mucosal healing. It is essential that new epithelial cells (migrating up the villus) are available to replace the sloughed off cells to prevent a barrier break. Thus, intestinal stem cell proliferation must match the need for replacing the dying epithelial cells. There are two types of intestinal stem cells critical for intestinal homeostasis: 1) rapid/active proliferating cells that express leucine-rich G-protein-coupled receptor 5 (Lgr5+) also known as crypt basal columnar (CBC) cells; and 2) slow-cycling cells (located in the +4-cell position in the crypt) which are quiescent and express B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi1+). In addition, the crypt area has a stem cell niche that includes cells that contribute to the regulation of ISCs by releasing factors that can promote ISC quiescence, division or differentiation. This review will focus on the ISCs (active Lgr5+ and quiescent BMI1+), niche cells and the regulatory interactions between these cells.

Crypt Basal Columnar Lgr5+ ISCs

Actively proliferating crypt basal columnar (CBC) stem cells are found in the crypt region interspersed between Paneth cells. CBC ISCs support the continued regeneration of epithelial cells that differentiate along the villus, form the epithelial barrier, and ultimately apoptose. Functional identification of intestinal stem cells has been difficult due to the lack of unique markers. Because Wnt signaling is a critical regulator in the cell biology of intestinal crypts, Barker et al [3] examined specific Wnt target genes in the stem cells. Out of ~80 target genes identified, the authors found the LGR5 gene to be expressed in crypt cells in mouse small intestine. They further discovered that Lgr5 was specifically expressed in the cycling CBC cells, which were found to be interspersed between Paneth cells. Lgr5 belongs to a group of orphan G-protein coupled receptors, and earlier studies [4] have demonstrated a crucial role for Lrg5 in development. Knockout of Lrg5 in mice leads to neonatal lethality with several gastrointestinal malformations and craniofacial defects. Using a LacZ expressing heterozygous Lrg5 knockout mouse, Barker et al found in histological sections about 3.5 blue (LacZ+) cells per crypt [3]. In addition, they found that Lgr5+ CBC cells actively proliferate with an average cycling time of about 1 day and have a unique wedge-like shape [3]. Morphologically they are slender but broad at the base with scant cytoplasm and the nuclei are also wedge-shaped pointing towards the crypt lumen. Although the potential role of CBCs to function as the stem cells was suggested in early 1970s [1],

Barker et al demonstrated this with specific markers and elegant genetic and microscopy approaches.

While ISCs usually divide asymmetrically (into one ISC and one epithelial cell), Lgr5+ cells are also capable of dividing symmetrically into either 2 CBCs or 2 transit amplifying progenitor cells [5, 6]. This means that stem cells can be “extinguished” and replaced. In addition to self-renewal, ISCs can be derived from other cells. Recently it has been shown that the transit amplifying cells and Paneth cells can de-differentiate into LGR5+ cells in response to Wnt3A or irradiation [7]. In addition, a subset of reserve Lgr5+ cells are multipotent, marked by expression of Mex3A (an RNA binding protein), and can convert to fast-dividing ISCs to maintain homeostasis and regenerate the epithelium after chemotherapeutic injury [8]. In contrast to mice, drosophila midgut ISCs do not rapidly repopulate after induced depletion; rather, drosophila ISCs continue to divide asynchronously but enterocyte differentiation slows down to facilitate epithelial homeostasis [9]. It is hypothesized that this effect may be related to the short lifespan of the fly versus the comparatively long life of mice or humans. In the latter species, a responsive ISC pool of cells is actively maintained to undertake epithelial barrier injury recovery as well as removal of defective genetically damaged ISCs [9].

Regulation of ISC proliferation is complex. However, there are a few major signaling pathways that are involved, most notably Wnt signaling. Wnt3 binds to LRP5/6 and frizzles receptors on ISCs causing an increase in β -catenin, TCF/LEF activation and c-myc levels, thereby promoting proliferation. Wnt signaling in the crypt region is further fine-tuned by several factors including Mule, an intracellular ubiquitin ligase, which regulates ISC proliferation and expansion. Mule works by suppressing c-myc and Wnt [10]. At the same time, Mule regulates EphB3 to maintain the crypt-to-villus EphB/Ephrin B gradient to determine cell positioning [10]. R-Spondin 1 also increases Wnt signaling by binding Lgr4/5 and preventing Dkk1/Kremlin mediated internalization of LRP6 which leads to increased levels of LRP6 on the cell membrane and therefore increased Lgr5+ISC Wnt signaling and proliferation [11]. Wnt signaling also promotes Paneth cell maturation [12] and results in mature Paneth cells which secrete Wnts to further increase the level of Wnt ligands locally.

In addition to Wnt signaling, there are several other pathways shown to regulate ISC proliferation. Activation of MAPK, by EGF and other factors, promotes Lgr5+ CBC proliferation. The Notch pathway, activated by Dll1/4, promotes CBC proliferation. Dll1/4 KO mice have decreased ISC proliferation but display increased epithelial cell differentiation [13]. This is because as epithelial cells differentiate, the signals that promote proliferation (Wnt, EGF, Noggin signaling) decrease, while BMP signaling increases. Consistent with this, Noggin supports CBC cell proliferation by binding to BMP4 to inactivate signaling. In addition, mutations in these pathways (ie: Apc, KRAS, SMAD4) lead to gut malformation.

By virtue of being stem cells in the gut, the role of CBCs in a variety of gut pathologies has been examined including cancer. Utilizing the same mouse model described for identification of Lgr5+ CBCs, Barker et al [14] further examined the role of these cells in

intestinal adenomas. Again, using elegant genetic approaches, they showed that deletion of *Apc* in these specific stem cells led to transformation of cells within days, that further led to formation of microadenomas and macroadenomas within a few weeks. Scheepers et al [15] utilized multicolor Cre-reporter mice (R26R-Confetti) to carry out lineage tracing studies and identified subpopulation of adenoma cells that were *Lgr5* positive, that could enhance the growth of established intestinal adenomas.

In addition to tumorigenesis (inappropriate proliferation), much work has been put into understanding ISC responses to radiation injury and inflammation, conditions where rapid regeneration is critical. *Lgr5*⁺ CBCs are distinct from other types of intestinal stem cells, such as *BMI1*⁺ cells (discussed later), by their sensitivity to irradiation [16]. Specifically, Yan et al [16] showed that after 12 Gray (Gy) radiation injury, *Lgr5*⁺ cells are ablated. These cells are thought to be replaced by the *BMI1*⁺ cells, but Asfaha et al [17] discovered that colonic *Krt19*⁺ cells can also give rise to *Lgr5*⁺ CBC cells. They further showed that these *Krt19*⁺ cells are located above the crypt base and are functionally distinct from *Lgr5*⁺ CBCs by their ability to be more radio-resistant compared to *Lgr5*⁺ CBCs. Interestingly, Metcalf et al [18] showed that a combination of less radiation (10 Gy) along with diphtheria toxin receptor-mediated ablation of *Lgr5*⁺ cells leads to remarkable reduction in crypts with stretches of crypts devoid of regeneration. Interestingly however, depletion of *Lgr5*⁺ cells in the context of *Apc*-driven or DSS-induced hyperplasia does not affect intestinal epithelial regeneration. *Lgr5*⁺ cells appear to be important for regeneration of epithelia following low dose radiation induced damage. This may be due to mechanistic differences in DNA repair in *Lgr5*⁺ CBCs compared to other ISCs or that there may be a heterogeneous cell population (*Lgr5*⁺) that may be slow cycling and/or resistant to DNA damage. Roche et al [19] showed that *SOX9* (a transcription factor) is critical for ISC proliferation and epithelial regeneration after high-dose irradiation; thus, *SOX9* knockout mice are highly sensitive to radiation with respect to crypt damage. In another model, the dextran sodium sulphate (DSS) induced chemical injury model, Davidson et al [20] showed that the rapidly cycling *Lgr5*⁺ cells in the colon are susceptible to DSS-induced damage and are completely eliminated in the distal colon early after injury but return after 5 days. This suggests the potential plasticity of ISCs or other ISC sources to replenish the *Lgr5*⁺ cells.

Quiescent/reserve *BMI1*⁺ ISC

Reserve ISCs are thought to be important for facilitating epithelial regeneration after injury. These cells express *BMI1* (part of transcriptional silencing complex) and *mTert*. Reserve stem cells are in the 4⁺ cell position of the crypt (4 cells away from the base of the crypt) and can give rise to actively proliferating ISCs to maintain the stem cell pool [21, 22, 16]. *BMI1*⁺ cells are resistant to high dose of irradiation and are in fact induced to proliferate giving rise to progeny to repopulate for intestinal regeneration. Reserve ISCs are thought to be the injury-inducible reserve intestinal stem cell population whereas *Lgr5*⁺ cells are most important for homeostatic function. In a recent study [22], Tian et al. ablated *Lgr5*-expressing cells in mice using the diphtheria toxin receptor gene and demonstrated that epithelial homeostasis was not affected; this indicated that there was compensation by other stem cells. Those cells were demonstrated to be *BMI1*-expressing cells. Furthermore, lineage tracing studies demonstrate that post-radiation injury, new epithelial cells are derived

from BMI1+ ISC cells rather than LGR5+ ISCs [23]. Consistent with this, Kruppel-like factor 4 (KLF4) has recently been shown to be important regulator of clonal expansion of BMI1+ ISC derived cells following gamma-radiation injury to the gut in mice [24]. Specifically, using lineage tracking it was shown that KLF4 maintains BMI1+ cells in a quiescent state, but upon irradiation KLF4 exerts a pro-proliferative activity on BMI1+ cells, while also reducing intestinal epithelial cell senescence and crypt apoptosis. Sox 9 has also been shown to limit ISC proliferation (including BMI1+) and impart radiation resistance and regenerative capacity [25].

ISC Niche Cells

The ISC niche is a major regulator of ISC homeostasis and comprises several cell types that are found in the crypt region including: Paneth (deep crypt cells in colon), stromal/myofibroblast and immune cells [26, 27] (Figure 2). Paneth cells in the small intestine are capable of secreting signals for ISC health (Wnt3, EGF, Notch receptor ligands). In the colon, these factors are produced by Paneth-like cells including deep crypt secretory cells, which are Reg4 positive (regenerating islet-derived family member 4) [28]. Paneth cells also secrete cyclic ADP ribose to promote SIRT1 levels as well as proliferation in ISCs under caloric-restricted conditions [29]. Paneth cells (and Paneth-like cells in the colon) express intracellular group IIA and X phospholipase A2 (PLA2) which are ISC niche factors that intracellularly restrict Wnt signaling through Yap1; however, during inflammation the phospholipases are secreted and through autocrine and paracrine signaling activate Wnt expression and signaling [30]. Wnt ligands promote both ISC proliferation and Paneth cell differentiation. Furthermore, loss of CD166 (a cell adhesion marker highly expressed by Lgr5+ ISCs and Paneth cells), results in slower LGR5+ growth and reduced differentiation of Paneth cells via altered Wnt signaling [31]. These studies suggest the potential role of cell:cell interactions in the ISC niche (ISC-epithelial and perhaps ISC-stromal) or cell:matrix interactions in regulating ISC proliferation. In addition, CD166 deficient mice display a reduction of differentiated Paneth cells that produce Wnt ligands, and combined with the finding that CD166 itself may be a target for Wnt5a implicates a role for Wnt signaling in regulating ISC proliferation (consistent with previous studies) [10, 32–34].

Myofibroblasts are underlying intestinal stromal cells that also contribute to ISC regulation by producing a variety of key factors such as R-Spondin1 (increases Wnt signals), Noggin (agonist of BMP/Smad), and BMP antagonists [3, 35]. These cells provide factors that can regulate ISCs and may serve as natural feeder cells for intestinal stem cells *in vitro*. In fact, 3-dimensional organoid ISC cultures have been successfully developed through co-culture with sub-epithelial myofibroblasts or supplementation with factors that these cells make: R-Spondin 1 (or 3) and Wnt3a [32–34]; further demonstrating the importance of myofibroblasts for the ISC turnover. The lamina propria also contains immune cells that contribute to the regulation of ISCs. Most notably, immune cells secrete cytokines such as IL-6 and IL-17 which can stimulate ISC proliferation [26]. Recently it has been shown that inhibition of IL-6 signaling (via a neutralizing antibody) can prevent inflammation-induced ISC proliferation [36]. The authors hypothesize that IL-6 affects Paneth cells which then modify Wnt signaling and ISC proliferation [36].

While not in the local niche environment, the microbiome is also a contributor to the regulation of ISCs. Recent *in vitro* ISC studies demonstrate that ISCs can respond to bacterial PAMPS (pathogen-associated molecular patterns). Specifically, butyrate (a byproduct of fiber fermentation) can inhibit ISC proliferation through a Foxo3-dependent mechanism. However, this effect requires butyrate to reach the crypt cells to induce a signal [37]. Luminal butyrate suppresses ISC proliferation only when a) there is mucosal injury that damages the crypt structure or b) in cases where animals do not have a crypt morphology (i.e., zebrafish)[37]. This is consistent with the mammalian crypt structure, protecting colonic ISCs from the luminal contents such as toxins, chemicals, and bacteria. In addition, colonocytes utilize butyrate for energy and therefore further reduce the chances of butyrate reaching the ISCs. Interestingly, it has also been shown that microbiota can influence ISC proliferation through microRNA changes, in particular miR-375 [38]. ISC miRNA levels are thought to be particularly sensitive to the microbiota, relative to differentiating intestinal epithelial cells, and it has been demonstrated that knockdown of miR-375 increases proliferation capacity [38]. Additionally, peptidoglycans derived from commensal and pathogenic bacteria, have been shown to be important for intestinal stem cell homeostasis. These bacterial ligands activate pattern recognition receptors, nucleotide-binding oligomerization domain 1 (NOD1/NRLC1) and NOD2. Both receptors belong to Nod-like receptor family. In the context of stem cells, a recent report by Nigro et al [39], demonstrated that NOD2 recognition and activation by peptidoglycan is essential for the cytoprotective function of NOD2 in intestinal stem cells. When stimulated with NOD2 agonist Lgr5+ stem cells had increased organoid growth *in vitro* and this was shown to be due to cytoprotective effects of NOD2 activation. This was further demonstrated *in vivo* by utilizing doxorubicin to induce oxidative stress and injury to IESCs and further showing that muramyl dipeptide (MDP, a NOD2 ligand) can reverse the effect of doxorubicin. Importantly, in the absence of NOD2 (NOD2 knockout mice) doxorubicin treatment caused cell death in ISCs. Taken together, these studies suggest that the microbiome and their derived products may potentially be a therapeutic target to regulate ISCs under disease or altered crypt architecture conditions.

Conclusions

Regulated ISC proliferation is critical for intestinal homeostasis under normal and disease/injury conditions. Because this population of cells is constantly undergoing division, it makes sense that there are at least two ISCs in place (Lgr5+ and BMI1+) and some cells can transdifferentiate into ISCs. The goal is to maintain proliferation to provide intestinal barrier function that prevents pathogen invasion/inflammation. As this field advances, it is becoming clear that a complex system of regulators controls the proliferation and differentiation of the ISCs. While low turnover can result in diminished barrier function, promoting too much growth can contribute to cancer and/or waste energy on unnecessary cell expansion. Understanding the mechanisms of the ISC regulatory system is important for identifying therapeutic targets to treat mucosal diseases/damage perhaps by modulating ISC niche cells, factors that activate Wnt, Noggin, Notch, and MAPK signaling, and/or the microbiome and its metabolites. This is an exciting area of research that has much more to reveal.

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Figure 1.

Model of the small intestinal cell organization in the crypt and villus region. Key ISC cells are the CBC (green cells in crypt) and the +4 position cells (in purple). TA progenitor cells are tan. Cells of the ISC niche (noted by blue letters) include paneth (orange), immune cells (yellow) and myofibroblasts (blue). The microbiome is also thought to influence the ISC. Note that the colon structure (not shown) does not include villi or Paneth cells, although there are Paneth-like cells present in the colon crypts.

Figure 2.

Model of ISC regulation in the crypt region. A variety of signals regulate stem cell proliferation. Many signals (noted in boxes – green = simulate while red = decrease) are from the ISC niche which is composed of myofibroblasts (blue), paneth cells (orange), and immune cells (yellow). The microbiome is also thought to contribute to ISC regulation. Several signaling pathways promote growth and their suppression is important for differentiation. Similarly, factors that suppress growth are found to be at higher levels in the villus compared with the crypt region. Overall, factors that stimulate ISC proliferation include Wnt, EGF, Noggin, R-spondin. Factors that reduce ISC proliferation include BMP signaling and miR375.