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Intestinal stem cells and inflammation

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The intestinal epithelium is renewed every 3–5 days from at least two principal stem cell pools. Actively cycling crypt based columnar (CBC) *Lgr5*$^{+}$ cells and slower cycling *Bmi1*-expressing or *Krt19*-expressing cells maintain the small intestinal and colonic epithelium in homeostasis and injury. Following acute epithelial damage, Lgr5+ stem cells are susceptible to injury and a reserve stem cell or progenitor pool is responsible for regeneration of the epithelium. Current data suggests that intestinal stem cells respond to inflammatory signals to modulate their expansion during epithelial regeneration. Here, we review how inflammation and injury affect intestinal and colonic stem cells.

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Introduction

The mammalian gastrointestinal tract is lined by a single cell epithelium that serves a variety of important biologic functions including secretion of mucus and defensive proteins, and absorption of nutrients and vitamins. Structurally, the intestinal epithelium is organized into villi and crypts, whereas, the colonic epithelium has crypts, but lacks villi. The gastric epithelium, on the other hand, has a simple columnar epithelium that contains numerous invaginations known as gastric pits that lead into a variety of glandular types (cardiac, fundic and antral).

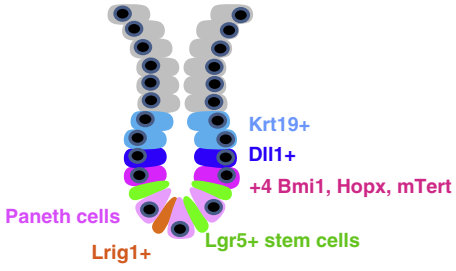
Remarkably, the gastric antral, intestinal and colonic epithelium are rapidly renewed on a continual basis. Indeed, in the small intestine the cells in the crypt quickly migrate along the crypt-villus axis within 3–5 days [1,2]. The human colonic epithelium is similarly estimated to turnover approximately every 3–4 days [3], and this continual replacement of epithelial cells and regeneration upon injury is believed to originate from stem cells that reside at or near the crypt base. The stem cells are distinct from the rest of the epithelium in that they are multipotent and capable of self-renewal.

Evidence of an intestinal stem cell population was initially provided by tracking inheritance patterns of genetic marks introduced at random into crypt cells [4,5]. Similar results were subsequently obtained in adult human crypts by studying the inheritance patterns of spontaneous mitochondrial mutations [6]. However, these initial studies failed to identify or label specific stem cells within the crypt. Surprisingly, the definitive identification of stem cells within the gastrointestinal tract was only demonstrated as recently as 2007. Through an elegant series of genetic lineage tracing studies carried out by Hans Clevers and colleagues, it was shown that the protein leucine rich repeat containing G-protein coupled receptor-5 (Lgr-5) marks a group of actively cycling stem cells known as the crypt base columnar (CBC) cells [7]. Using *Lgr5*-GFP-IRES-CreERT2 knock-in mice, Barker *et al.* (2007) demonstrated that Lgr5 marks long-lived cells that give rise to all the cells within the adult gastric antrum, small intestine and colonic crypts [7,8]. Furthermore, data from clonal lineage tracing of crypt-villus units in combination with mathematical modelling has suggested that Lgr5-positive cells reconstitute all cells of the epithelium via stochastic outcomes of symmetric division [9]. Since the discovery of Lgr5+ cells, several groups have also identified additional stem cell populations in the intestine and colon (Figure 1a) [10–16]. Unfortunately, due to limitations of current lineage tracing technology, it has been difficult to discern whether overlap among these various stem cell markers can explain the observations made to date. Certainly, the findings by Tian *et al.* (2013) that selective ablation of Lgr5+ stem cells does not alter intestinal homeostasis has strongly supported the notion that more than one stem cell or progenitor pool exists within the intestine [17••]. Following Lgr5+ cell ablation, Bmi1+ cells and Krt19+ cells, function as reserve stem cell pools in the intestine and colon, respectively [17••,18•].

Intestinal stem cells and epithelial injury

The identification of intestinal stem cell populations using genetic fate mapping techniques has led to considerable excitement about our ability to understand the epithelial response to injury and carcinogenesis. Indeed, the contribution of Lgr5+ stem cells to carcinogenesis was demonstrated by the formation of intestinal adenomas upon mutation of the APC gene in these cells [19]. Lgr5+ stem cells additionally serve as cancer stem cells within established adenomas [20].

Figure 1



(b)

Promote ISC expansion post injury	Inhibit ISC expansion post injury
Wnt/R-spondin	YAP
Notch	Bmp
JAK/STAT	p53

Current Opinion in Pharmacology

Schematic of intestinal stem cell region and modulating factors or pathways. **(a)** Schematic diagram of the intestinal crypt demonstrating the location of *Lgr5*-expressing stem cells in relation to *Lrig1*+, *Krt19*+ and +4 stem cells (Bmi1+, Hopx+, mTert+) as well as *Dll1*+ secretory progenitors. **(b)** A list of pathways known to modulate intestinal stem cell activity post-epithelial injury.

A number of studies have also examined the effects of epithelial injury or inflammation on ISCs. The majority of these studies have focused on the small intestine rather than the colon, and most of these studies have concentrated on Lgr5+ stem cells rather than cells marked by other stem cell markers. Recognizing these limitations, several studies have attempted to examine the effects of epithelial injury on ISCs, and vice versa, the role of stem cells in the regenerative response. Perhaps the best studied of these injury models is that of radiation injury. Yan *et al.* (2012) first demonstrated that Lgr5+ stem cells are exquisitively sensitive to high dose radiation injury, whereas, +4 Bmi1+ cells dramatically expand to clonally repopulate the epithelium [21••]. More recently, reserve Sox-9+ cells that are also slower-cycling have been shown to respond to epithelial injury following high-dose irradiation [10]. This also appears to be true of the colon where Krt19+ cells located above the crypt base are similarly radioresistant and expand post injury. Nevertheless, the findings of Metcalfe *et al.* (2014) suggest that Lgr5+ cells are indispensable for intestinal regeneration in the immediate post-radiation period [22••]. Thus, Lgr5+ stem cells are radiosensitive, yet remain essential for the rapid regenerative response [23,24]. Both p53, and p53 upregulated modulator of apoptosis (PUMA) are key regulators of apoptotic death of Lgr5+ ISCs after radiation, and may contribute to the radio-sensitivity of these cells [25,26]. The role of these factors within the +4 stem cell pool, however, remains unexplored. Thus, the observations to date would suggest that following radiation, epithelial regeneration requires radioresistant reserve stem cell pools to rapidly regenerate actively cycling Lgr5+ stem cells that then allow for proper epithelial regeneration.

Interestingly, cellular plasticity also appears important in epithelial regeneration in the setting of inflammation. van Es *et al.* (2012) showed that Dll1+ secretory progenitors revert back to an active stem cell state following intestinal radiation injury [27•]. A number of groups have additionally shown that other relatively quiescent cells may revert to a stem cell state following injury in both the small intestine and colon, allowing for the rapid regeneration of actively cycling Lgr5+ stem cells [12,18•,28–30].

During normal homeostasis the gastrointestinal epithelium plays an integral role in the regulation of luminal contents including a diverse microbiota that on occasion can induce epithelial injury and infection. Stem cells within the gastrointestinal tract must therefore, be ready to respond to a variety of antigens, viruses and microbiota. Recent work in this area has revealed Lgr5+ stem cells constitutively express NOD2, an innate immune sensor [31]. NOD2-mediated signaling by microbiota-derived muramyl-dipeptide (MDP) exerts cytoprotection of Lgr5+ stem cells against oxidative stress-mediated cell death [31]. Furthermore, Ly6C+ monocytes suppress stem cell expression and abrogate the response to luminal microbes, suggesting a fascinating relationship between the immune system, microbiota and ISCs [32].

Despite the numerous studies examining stem cell responses to small intestinal injury and more recently the microbiota, there remain few studies examining the effects of inflammation on colonic stem cells. This is particularly important with respect to Inflammatory bowel disease (IBD) where it is believed a multitude of factors ranging from genetics, an altered immune system and the environment (i.e. microbiota) contribute to epithelial injury [33]. Indeed, alterations in the microbiome have been associated with the development of IBD, yet our understanding of how these changes affect ISCs have yet to be defined. The recent ability to culture human intestinal organoids will in the future help us understand the ISC response to inflammation [34]. This culture system provides a novel approach to studying the interaction of bacterial products, host genetic factors and intestinal stem cells [34,35,36•,37]. Intriguingly, Davidson *et al.* (2012) examined the colon of mice post DSS-colitis and reported that ablation of Lgr5+ cells occurs with concurrent loss of crypts [38]. These authors reported mRNA expression of the stem cell markers Ascl2 and Hopx were decreased in the damaged colon, but Lgr5+ stem cells returned back to