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Authors

Palikuqi, Brisa
Rispal, Jérémie
Klein, Ophir

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Good Neighbors: The Niche that Fine Tunes Mammalian Intestinal Regeneration

Brisa Palikuqi,[1,3] Jérémie Rispal,[1,3] and Ophir Klein[1,2]

[1]Program in Craniofacial Biology and Department of Orofacial Sciences; [2]Department of Pediatrics and Institute for Human Genetics, University of California, San Francisco, California 94143, USA

Correspondence: ophir.klein@ucsf.edu

The intestinal epithelium undergoes continuous cellular turnover, making it an attractive model to study tissue renewal and regeneration. Intestinal stem cells (ISCs) can both self-renew and differentiate along all epithelial cell lineages. Decisions about which fate to pursue are controlled by a balance between high Wnt signaling at the crypt bottom, where *Lgr5*$^+$ ISCs reside, and increasing bone morphogenetic protein (BMP) levels toward the villus, where differentiated cells are located. Under stress conditions, epithelial cells in the intestine are quite plastic, with dedifferentiation, the reversal of cell fate from a differentiated cell to a more stem-like cell, allowing for most mature epithelial cell types to acquire stem cell–like properties. The ISC niche, mainly made up of mesenchymal, immune, enteric neuronal, and endothelial cells, plays a central role in maintaining the physiological function of the intestine. Additionally, the immune system and the microbiome play an essential role in regulating intestinal renewal. The development of various mouse models, organoid co-cultures and single-cell technologies has led to advances in understanding signals emanating from the mesenchymal niche. Here, we review how intestinal regeneration is driven by stem cell self-renewal and differentiation, with an emphasis on the niche that fine tunes these processes in both homeostasis and injury conditions.

The intestinal epithelium undergoes continuous physiological renewal and rapidly regenerates under stress conditions, making it an attractive model to investigate tissue regeneration (Weichselbaum and Klein 2018; Trentesaux et al. 2020). The gut is constantly challenged by aggressive agents present in the lumen, such as acidity in the proximal part of the small intestine and microbes along the length of the entire gut, as well as by physical constraints, such as friction caused by peristalsis. In response, the intestinal epithelium normally renews every 3 to 5 days to maintain tissue integrity, and this turnover is driven by intestinal stem cells (ISCs) located at the crypt base that self-renew and give rise to all intestinal epithelial cells (IECs). Crypts are populated, in addition to ISCs, by proliferating progenitor cells (also called transit-amplifying cells) descended from the ISCs, and these continuously divide and produce the two lineages of differentiated cells located in the villus, the absorptive, and secretory cells (Fig. 1; Barker 2014; Haber

[3]These authors contributed equally to this work.

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et al. 2017). The absorptive lineage is composed of enterocytes, which absorb nutrients and are the most abundant cell type in the intestine, whereas the secretory lineage is comprised of four cell types (goblet, Paneth, tuft, and enteroendocrine) that are mainly involved in safeguarding the epithelium. Goblet cells secrete the mucus layer that covers IECs, tuft cells initiate immune response to infection, and enteroendocrine cells secrete a large panel of peptides and hormones. Paneth cells, which are the only differentiated cell type present in the crypts, protect stem and proliferative cells through the secretion of antimicrobial peptides (Gehart and Clevers 2019; Beumer and Clevers 2021).

Another interesting feature of the gut is the regionalization of structures and functions, with major differences between the small intestine and the colon. For example, the colonic epithelium proliferates to a lesser extent than the small intestinal epithelium, and colonic stem cells are less sensitive to radiation-induced apoptosis (Gândara et al. 2012). The epithelial structure, with the lack of villi in the colon, and the composition of IECs, with more goblet cells and lack of Paneth cells in the colon, are also important differences between the different parts of the gut. The cause of this regionalization is not yet fully understood, but the differential activation of signaling pathways during the developmental process and the specific environmental cues, such as the increase of bacterial content along the gut lumen, may explain these differences.

It is clear that the maintenance of tissue integrity requires tight control to allow for the rapid self-renewal while protecting against the emergence of pathologies such as inflammatory bowel disease (IBD) or colorectal cancers. In this review, we highlight mechanisms of intestinal epithelial renewal and regeneration under ho-

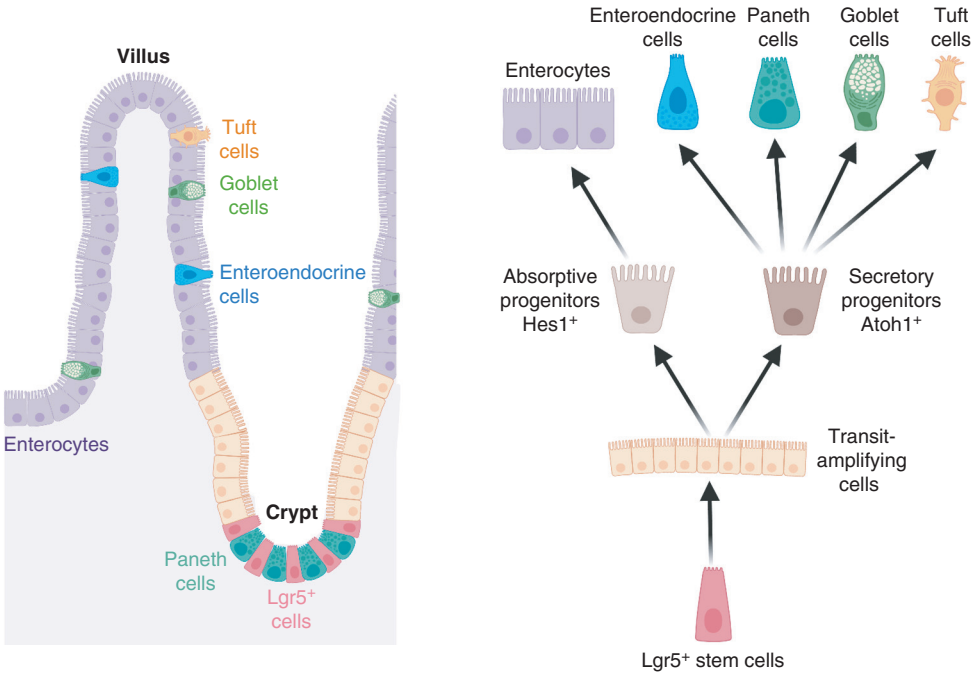

Figure 1. Cell composition in crypt-villus structures in the small intestine. The small intestine is organized in crypt-villus structures with intestinal stem cells located in the crypt and more differentiated cells in the villus. Continuous proliferation of transit-amplifying cells facilitates rapid tissue turnover. There are two major branches of differentiated cells in the intestine, the absorptive lineage and the secretory lineage. Absorptive progenitor cells differentiate into mature enterocytes that are responsible for the absorption of nutrients and water. Secretory progenitor cells give rise to four types of cells: Paneth cells, goblet cells, tuft cells, and enteroendocrine cells.

meostatic and injury conditions and how this renewal is regulated by the microenvironment.

INTESTINAL EPITHELIAL RENEWAL

The presence of ISCs at the bottom of crypts was suggested several decades ago (Cheng and Leblond 1974). However, clear experimental demonstration that the crypt-base columnar cells (CBCs) interspersed between Paneth cells in the small intestine act as the homeostatic stem cells responsible for the renewal of the epithelium occurred several decades after, with the discovery of a marker of these cells, *Lgr5* (Leucine-Rich Repeat-Containing G Protein-Coupled Receptor 5). Using lineage tracing experiments, *Lgr5*$^+$ CBCs were shown to have the two key characteristics of stem cells: the ability to self-renew and to generate all IECs throughout the entire life span of the organism (Barker et al. 2007). Unlike stem cells in some other adult tissues, CBCs actively proliferate, allowing for renewal of IECs but also rendering the stem cells very sensitive to injury. *Lgr5*$^+$ ISCs reside in and are supported by a surrounding cellular milieu called the niche, and ISCs compete in a neutral fashion for occupancy of the niche (Snippert et al. 2010; Ritsma et al. 2014). Furthermore, when *Lgr5*$^+$ ISCs are eliminated, differentiated cells in the intestine can maintain tissue renewal by regaining a stem cell–like identity (Tian et al. 2011). The high plasticity of IECs thus maintains epithelial renewal in injury conditions through dedifferentiation (Fig. 2A). Several studies have shown that the majority of cells present in small intestinal crypts are able to dedifferentiate after the ablation of *Lgr5*$^+$ ISCs, such as secretory or absorptive cells. Indeed, cells capable of dedifferentiation include progenitors of all secretory cells (marked as *Dll1*$^+$ or through the property of label retention) (van Es et al. 2012b; Buczacki et al. 2013), enteroendocrine cells (*Prox1*$^+$ cells) (Yan et al. 2017a), Paneth cells (*Lyz*$^+$ cells) (Schmitt et al. 2018; Yu et al. 2018), or enterocyte lineage cells (*Alpi*$^+$ cells) (Tetteh et al. 2016). In the colon, in contrast to the small intestine (Tetteh et al. 2016), absorptive progenitor cells, which are *Notch1*$^+$, do not seem competent to renew the epithelium under homeostatic or injury conditions (Castillo-Azofeifa et al. 2019). Instead, loss of *Lgr5*$^+$ cells as a result of injuries such as colitis leads to colonic epithelial regeneration driven by dedifferentiation of *Atoh1*$^+$ secretory progenitor cells (Castillo-Azofeifa et al. 2019).

Recently, it was shown that IECs can dedifferentiate to acquire fetal-like features, including a fetal transcriptional signature in vivo and the ability to give rise to spheroids devoid of adult differentiated cells in three-dimensional (3D) culture, after injury in both the small intestine and colon (Fig. 2B; Nusse et al. 2018; Yui et al. 2018). In the small intestine, infection by a parasitic helminth (*Heligmosomoides polygyrus*), which forms granulomas in the submucosa, results in the loss of adult ISC markers such as *Lgr5* in the intestinal crypts overlying the granulomas. Concomitantly, these crypts up-regulate the marker *Sca1* (a gene expressed in fetal but not homeostatic adult intestine) and acquire fetal-like properties (Nusse et al. 2018). Furthermore, these fetal-like cells appear transiently after other types of injury, such as irradiation, *Lgr5*$^+$ ISC genetic ablation (Nusse et al. 2018), or—in the colon—dextran sulfate sodium (DSS)-induced colitis (Yui et al. 2018), demonstrating that this fetal conversion could be a conserved mechanism of epithelial renewal in the absence of *Lgr5*$^+$ ISCs.

SIGNALING PATHWAYS IN THE ISC NICHE

The maintenance of ISCs, and thus of intestinal regeneration, is tightly controlled by activation of specific signaling pathways. The environment that maintains the stem cells is known as the ISC "niche."

Wnt Pathway

The most-studied niche signal is the canonical Wnt pathway, which is essential for stemness and cell proliferation in the gut. The binding of Wnt ligands (such as WNT3) to Frizzled receptors inhibits the protein complex responsible for β-catenin degradation. As a result of active Wnt signaling, β-catenin accumulates and translocates to the nucleus, where it binds to the T-cell

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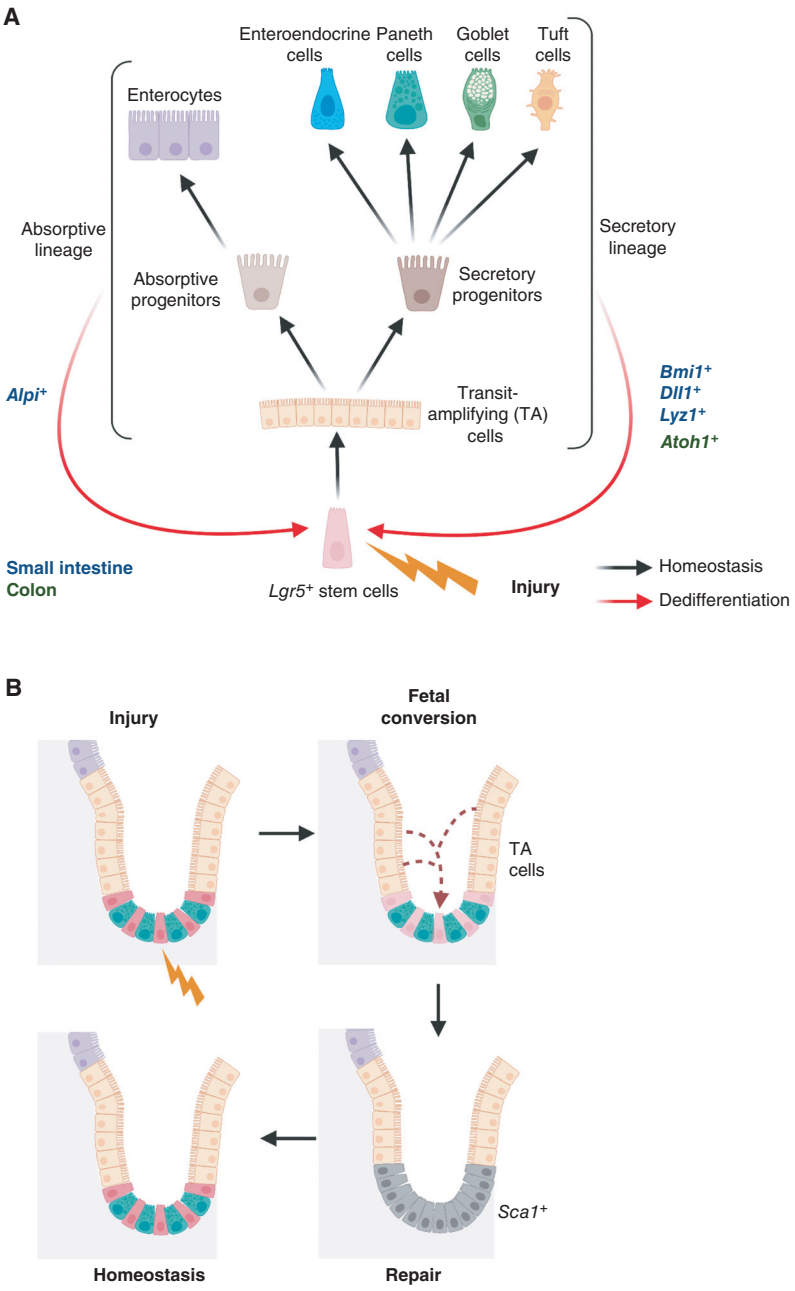

Figure 2. Paths to intestinal dedifferentiation. (*A*) Fully differentiated cells are capable of dedifferentiation. In case of injury, leading to the loss of *Lgr5*$^+$ stem cells, various differentiated cells can dedifferentiate to maintain epithelial function. Several secretory lineage cells such as *Bmi1*$^+$ cells (enteroendocrine precursors), *Dll1*$^+$ cells (secretory progenitors), and *Lyz1*$^+$cells (mature Paneth cells) in the small intestine and *Atoh1*$^+$ (secretory progenitors) in the colon are capable of dedifferentiation. Cells of the absorptive lineage can also dedifferentiate in the small intestine, such as the enterocyte precursors marked by *Alpi*$^+$, whereas the same has not been shown to be the case in the colon. (*B*) Model of a potential dedifferentiation pathway. After the loss of *Lgr5*$^+$ stem cells, transit-amplifying cells undergo an identity conversion to become fetal-like cells (*Sca1*$^+$) that allow for epithelial repair and the restoration of normal homeostasis.

factor (TCF) family of transcription factors (such as TCF7L2) to activate the expression of Wnt target genes (Nusse and Clevers 2017; Rispal et al. 2019). The essential role of this pathway in adult intestinal regeneration was shown, for example, with Wnt inhibition by DKK1 or by the deletion of *Tcf7l2* in intestinal epithelium, leading to the loss of proliferation of crypts in mice and extensive disruption of epithelial renewal in the small intestine and colon (Kuhnert et al. 2004; van Es et al. 2012a). Furthermore, deletion of *Tcf7l2* causes loss of *Lgr5*$^+$ ISCs, showing the indispensability of this pathway for intestinal renewal (van Es et al. 2012a). On the other hand, mutations in *Apc*, a major component of the complex responsible for Wnt inhibition, leads to Wnt overactivation and an increase in proliferation, eventually initiating colorectal tumorigenesis (Vermeulen et al. 2013). This mutation confers a proliferative advantage on the mutant cell compared to its neighbors and also in turn induces the differentiation of wild-type ISCs to rapidly outcompete them (van Neerven et al. 2021).

Additionally, to function fully in the intestine, the Wnt pathway needs to be potentiated by secreted Wnt agonists known as R-spondins (RSPOs). RSPOs bind to LGR receptors (LGR4 and LGR5 in the intestine) to enhance the stability of Frizzled receptors (Glinka et al. 2011), thus making cells competent for Wnt activation. Deletion of *Lgr4/5* or inhibition of the binding of RSPO to LGR receptors result in the same phenotype as Wnt inhibition (i.e., loss of ISCs and IEC renewal) (de Lau et al. 2011; Yan et al. 2017b). Conversely, overexpression of *Rspo1* or *Rspo2* increases the size of the stem cell compartment and favors ISC self-renewal at the expense of differentiation (Yan et al. 2017b). Finally, another essential regulatory mechanism is control over secretion of Wnt ligands. Major events in this process include palmitoylation of Wnt ligands by Porcupine (PORCN) (Takada et al. 2006) and association of Wnt ligands with Wntless (WLS) (Bänziger et al. 2006), both of which are required for Wnt intercellular transport and its subsequent secretion from niche cells. Thus, deletion of *Porcn* or *Wls* can both serve as powerful tools for inhibiting Wnt signaling and dissecting the role of individual intestinal cell types in the production of Wnt ligands.

Notch Pathway

The Notch pathway operates via juxtacrine signaling, whereby one cell communicates with its direct neighboring cell. Moreover, the Notch pathway controls a binary cell fate choice through a process known as lateral inhibition, in which one cell produces a transmembrane ligand, δ-like (DLL), that binds to Notch receptors of a neighboring cell, leading to Notch activation in the receiving cell, which becomes *Hes1*$^+$. In turn, the receiving cell is not capable of activating Notch in the first cell, which becomes *Atoh1*$^+$. This pathway has two roles in the intestine: stem cell maintenance and lineage commitment of progenitor cells (Sancho et al. 2015). Inhibition of the Notch pathway by deletion of *Dll1* and *Dll4* expressed by secretory cells (Paneth and goblet) results in the disruption of epithelial renewal, with ISC loss and decrease of progenitor proliferation (Pellegrinet et al. 2011; van Es et al. 2012b). Conversely, ectopic Notch activation in all IECs increases the number of proliferative cells (Fre et al. 2005). Together, these results show the important role of the Notch pathway in intestinal epithelial renewal.

Use of Organoids to Identify Candidate Niche Signals

The development of ex vivo 3D models of the intestinal epithelium called organoids has facilitated the identification of niche signals essential for the maintenance of IEC renewal. In organoids, ISCs placed in a 3D extracellular matrix (e.g., Matrigel) maintain long-term survival and stemness while giving rise to IECs, thus recapitulating the in vivo intestinal organization. These structures are composed of a spherical domain that undergoes budding to form crypts that extrude into the matrix. The development of organoids has also led to conclusions regarding ISC niche signals consistent with those achieved using in vivo models, such as the indispensable role of epidermal growth factor (EGF) pathway activation and of bone morphogenetic protein

(BMP) pathway inhibition for epithelial regeneration (Sato et al. 2009), in addition to the aforementioned Wnt and Notch pathways. EGF signaling is important for the proliferative capacities of ISCs but not for their maintenance. In organoids, inhibition of the EGF pathway leads to the total loss of ISC proliferation, but *Lgr5* expression is maintained and proliferation and organoid growth are restored upon discontinuation of EGF inhibition (Basak et al. 2017). Similarly, *Lrig1* deletion, which activates the EGF pathway, results in the expansion of ISC number in mouse crypts (Wong et al. 2012). Conversely, the BMP pathway is involved in differentiation of IECs and inhibition of ISC identity. Indeed, disruption of the BMP pathway through deletion of a BMP receptor gene (*Bmpr1a*) causes an increase of proliferative cells and ISC numbers (He et al. 2004; Qi et al. 2017). The BMP pathway seems to play this role through at least two mechanisms: inhibition of the Wnt pathway (He et al. 2004) and direct repression of stem cell genes (Qi et al. 2017). Thus, tight control of BMP activation is essential, with low BMP signaling in the crypts and higher BMP signaling toward the more differentiated cells in the villus. Intestinal epithelial homeostasis is also maintained through expression of Noggin and Gremlin, which inhibit the BMP pathway, in the stem cell niche (Kosinski et al. 2007).

Paneth Cells as Epithelial ISC Niche

The location of Paneth cells sandwiched between ISCs and their production of Wnt, EGF, and Notch ligands made them appear to be an attractive candidate for the ISC niche (Clevers and Bevins 2013). However, there is still a debate regarding the fundamental role of these cells. In vitro, Paneth cells are essential for organoid cultures, which are only composed of epithelial cells (Sato et al. 2011; Farin et al. 2012), pointing to an important role for Paneth cells in the niche. Indeed, cells from mice with epithelial-specific *Wnt3a* or *Porcn* deletion cannot give rise to organoids. Additionally, organoids cannot be grown from *Atoh1* knockout mice, which lack all the secretory lineages, including Paneth cells (Farin et al. 2012; Kabiri et al. 2014). Therefore, it was surprising when a series of studies demonstrated that, in vivo, Paneth cells are dispensable for the survival, proliferation, and function of stem cells in the small intestine (Durand et al. 2012; Farin et al. 2012; Kim et al. 2012). Strikingly, *Atoh1* knockout mice show no change in the *Lgr5*$^+$ cell compartment, with stem cell proliferation potential remaining unperturbed (Durand et al. 2012; Kim et al. 2012). Furthermore, despite loss of all Paneth cells, Wnt target genes and β-catenin signaling remained largely unaffected. Additionally, loss of Wnt secretion from all epithelial cells does not disrupt intestinal tissue morphology, including crypt cell proliferation and differentiation, in vivo (Farin et al. 2012; Kabiri et al. 2014; San Roman et al. 2014). However, under homeostatic conditions, Notch activation in ISCs by Paneth cells results in *Atoh1* inhibition, which is essential to conserve ISC proliferation (Kim and Shivdasani 2011). Thus, *Atoh1* deletion in all IECs mimics Notch activation in ISCs, explaining the maintenance of ISCs in this model of Paneth cell ablation compared to others and suggesting an essential role of Paneth cells in providing Notch signals in ISC niche. Furthermore, deletion of Paneth cells by knocking in diphtheria toxin receptor leads to loss of ISCs (Sato et al. 2011), but if enteroendocrine cells expressing *Dll* are replaced between ISCs, then ISCs are maintained (van Es et al. 2019). Last, in the colon, where ISCs are maintained without Paneth cells, *Reg4*$^+$ cells, which are a subset of goblet cells, are located between ISCs and express Notch ligands (Rothenberg et al. 2012; Sasaki et al. 2016). Together, it can be concluded that in vivo, Paneth cells are essential for the activation of the Notch pathway in ISCs, whereas Wnt and EGF signaling are also modulated by nonepithelial cells.

MESENCHYMAL CELLS AS PART OF THE ISC NICHE

The nonepithelial niche of the intestine is comprised of several cell types, such as mesenchymal, immune, endothelial, and enteric neuronal cells (Fig. 3; McCarthy et al. 2020a; Zhu et al. 2021). As epithelial-derived Wnt signaling is dispensable for in vivo ISC maintenance, and organoids composed of only epithelial cells require the supple-

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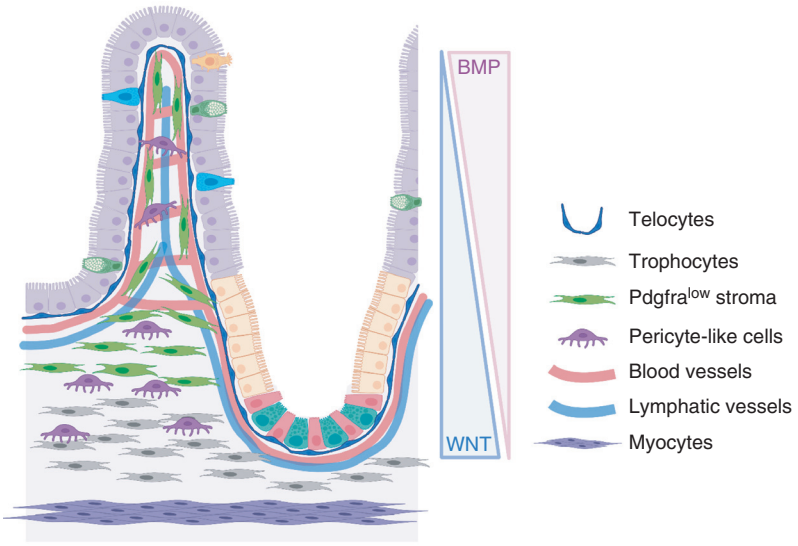

Figure 3. Mesenchymal niche of the small intestine. The mesenchymal niche of the small intestine is comprised of several cell types such as telocytes, trophocytes, Pdgfra^{low} stroma, and pericyte-like cells. The mesenchymal niche plays a key role in maintaining the Wnt and bone morphogenetic protein (BMP) gradients along the crypt-villus structures.

mentation of exogenous growth factors, a major focus has been placed on uncovering the role of the mesenchymal niche in intestinal function. Several mesenchymal populations have been shown to be essential for stem cell maintenance in the small intestine (Kabiri et al. 2014; Valenta et al. 2016; McCarthy et al. 2020a; Zhu et al. 2021). A key population identified in the intestinal stroma of both the small intestine and colon are the mesenchymal cells marked by Podoplanin (a glycoprotein) and CD34 (a phosphoglycoprotein) (Stzepourginski et al. 2017). These crypt stromal cells express low levels of BMP ligands (*Bmp2* and *Bmp4*) and high levels of *Wnt2b*, *Grem1*, and *Rspo1*, and they differ from myofibroblasts, which are aSMA$^+$ CD34$^-$ (Stzepourginski et al. 2017). Another significant mesenchymal cell type are the *Foxl1*$^+$ (a target gene of the Hedgehog pathway) mesenchymal cells, also known as telocytes (Aoki et al. 2016; Shoshkes-Carmel et al. 2018; Kondo and Kaestner 2019). Ablation of *Foxl1*$^+$ telocytes, which also express *Pdgfra* (platelet-derived growth factor A), leads to a significant decrease of proliferation in the intestine, with differentiated cell lineages such as Paneth cells and goblet cells remaining unperturbed (Aoki et al. 2016; Shoshkes-Carmel et al. 2018). Compared to *Foxl1*-negative mesenchymal cells and to epithelial cells in the small intestine, *Foxl1*$^+$ telocytes express high levels of *Wnt2*, *Wnt5a*, and several Bmp family ligands. Interestingly, telocytes display zonation along the crypt to villus axis, expressing higher levels of *Wnt2b* in the crypt compared to the villus tip. Conditional ablation of *Porcn* from *Foxl1*$^+$ telocytes results in rapid stem and progenitor cell loss in both the small intestine and colon (Shoshkes-Carmel et al. 2018).

Pdgfra and *Foxl1* have been used as markers when studying the stroma in the intestine, and recently several groups have tried to further break down the mesenchymal population and define more markers and subpopulations. In one recent study, the intestinal stromal compartment was subdivided into *Pdgfra* low and *Pdgfra* high mesenchymal fractions in the small intestine (McCarthy et al. 2020b). A *Pdgfra* low mesenchymal population that resides in close proximity to ISCs was studied. These cells, which were defined as trophocytes, are also *CD34*$^+$, *Gli1*$^+$, *Grem1*$^+$, and *Wnt2b*$^+$ and express high

levels of all RSPOs. The *Pdgfra* low trophocyte population was further subclustered into two different populations based on their differential expression, among others, of *Grem1*, *CD81*, and *Sfrp*. Ablation of the *Grem1*$^+$ trophocyte population led to loss of *Lgr5*$^+$ cells and *Olfm4*$^+$ cells, but transient-amplifying cells remained stable (McCarthy et al. 2020b).

In another study, a unique stromal cell population was reported in both the antrum (stomach) and the small intestine that was *Ng2*$^+$ (*Cspg4*$^+$) (Kim et al. 2020). Although pericytes are usually *Ng2*$^+$, these cells, unlike pericytes, were not located near vessels, and therefore were called pericyte-like cells. Mesenchymal *Ng2*$^+$ cells are also *Foxl1*$^+$ and high in *Wnt2b* and *Wnt4*. Perturbation of Wnt, by deletion of *Wls* from *Ng2*$^+$ cells, resulted in mild effects, with no change in the overall proliferative compartment in the small intestine, despite the significant loss of *Lgr5*$^+$ and *Olfm4*$^+$ cells. In a series of other elegant studies, activation of Hedgehog signaling in *Ng2*$^+$ cells was shown to result in hyperproliferation in both the antrum and the ileum and an increase in WNT2b secretion (Kim et al. 2020).

In the colon, the ISC niche also includes mesenchymal cells expressing *Gli1* (a transcription factor in the Hedgehog pathway), which are located near the base of the crypts. Single-cell RNA-sequencing (RNA-seq) analysis of colonic *Gli1*$^+$ cells revealed that this is a heterogenous population, with differential expression of *CD34*, *Foxl1*, *Myh11*, and *Sma*, despite similar expression of *Pdgfra* (Degirmenci et al. 2018). Upon deletion of *Wls* from *Gli1*$^+$ mesenchymal cells, the colonic crypt epithelium was completely destroyed, with a loss of *Lgr5*$^+$ stem cells. However, for loss of crypts in the duodenum, deletion of *Wls* from both epithelial and *Gli1*$^+$ mesenchymal cells was required, suggesting that in the small intestine Paneth cells could compensate for loss of *Gli1*$^+$ cells (Degirmenci et al. 2018).

In Vitro Mesenchyme-Organoid Cocultures

As organoid cultures require exogenous growth factors, organoids have been cocultured with various mesenchymal subfractions to try to replace some of these factors in vitro. For instance, when cocultured with small intestinal organoids, mesenchymal CD34$^+$ Podoplanin$^+$ cells give rise to cyst-like structures that are highly enriched in *Lgr5*$^+$ and proliferative cells. This process is hampered in the presence of a neutralizing antibody against GREM1, indicating that this mesenchymal cell type is essential for the BMP inhibition necessary for ISC maintenance. Furthermore, CD34$^+$ crypt stromal cells can replace RSPO1 conditioned media when cocultured with organoids in vitro (Stzepourginski et al. 2017). In the *Pdgfra* stromal populations, *Pdgfra* high cells do not support organoid growth in the absence of external factors. Meanwhile, the *CD81*$^+$ *Pdgfra* low stromal cells can replace exogenous growth factors and support organoid growth (McCarthy et al. 2020b). In the colon mesenchyme, *Gli1*$^+$ stromal cells can support colon organoids in the absence of exogenous WNT3a, and when cocultured with duodenal organoids shift them toward a spheroid phenotype (Degirmenci et al. 2018). These experiments show that mesenchymal cells can replace various external factors that are supplemented in organoid cultures and support stem cell maintenance similarly to in vivo settings.

The Mesenchymal Niche in Injury Conditions

Several studies have suggested the involvement of mesenchymal niche cells in injury conditions as well. For instance, upon DSS-induced colitis, there is an increase in *Gli1*$^+$ mesenchymal cells in the colon, with higher expression of *Rspo3* (Degirmenci et al. 2018). Furthermore, whole-body, irradiation-induced injury in *Ng2-Cre*; *Wls*$^{fl/fl}$ mice results in loss of body weight and loss of *Olfm4*$^+$ and *Cd44*$^+$ stem and progenitor cells (Kim et al. 2020). In humans, biopsies from colons of ulcerative colitis patients also display alterations in the mesenchyme, with enrichment in a stromal population that is high in *IL33, CCL19, TNSF14* (*LIGHT*), and others (Kinchen et al. 2018).

In summary, the mesenchymal niche plays a key role not only during physiological intestinal function, but also in regeneration and injury conditions of the intestinal tissue. Future studies

will be needed to pinpoint the exact mesenchymal cell populations and mesenchyme-derived factors involved in intestinal regeneration.

ROLE OF THE IMMUNE SYSTEM

Immune system activation is also central to intestinal regeneration after injury (Fig. 4), with the innate branch of the immune system of particular relevance to intestinal function and regeneration. Interestingly, innate lymphoid cells (ILCs), which lack a recombined antigen receptor, have been established as key players in the cross talk between the innate immune system and epithelial cells. Type 3 ILCs (ILC3s), which participate in innate defense mechanisms of mucous membranes, have been particularly associated with maintaining intestinal function. IL-22 is one of the best-studied cytokines secreted by tissue-resident ILC3s in response to intestinal damage (Hanash et al. 2012; Aparicio-Domingo et al. 2015; Lindemans et al. 2015). This cytokine, secreted by ILC3s, which are radioresistant, is protective against graft-versus-host disease (GVHD) after bone marrow transplantation. ISCs and epithelial progenitors express the IL-22 receptor and are therefore direct targets of IL-22. IL-22 deficiency results in crypt apoptosis, ISC depletion, and loss of the epithelial barrier (Hanash et al. 2012). Additionally, supplementation with IL-22 enhances growth of small intestine and colon organoids in vitro. IL-22-

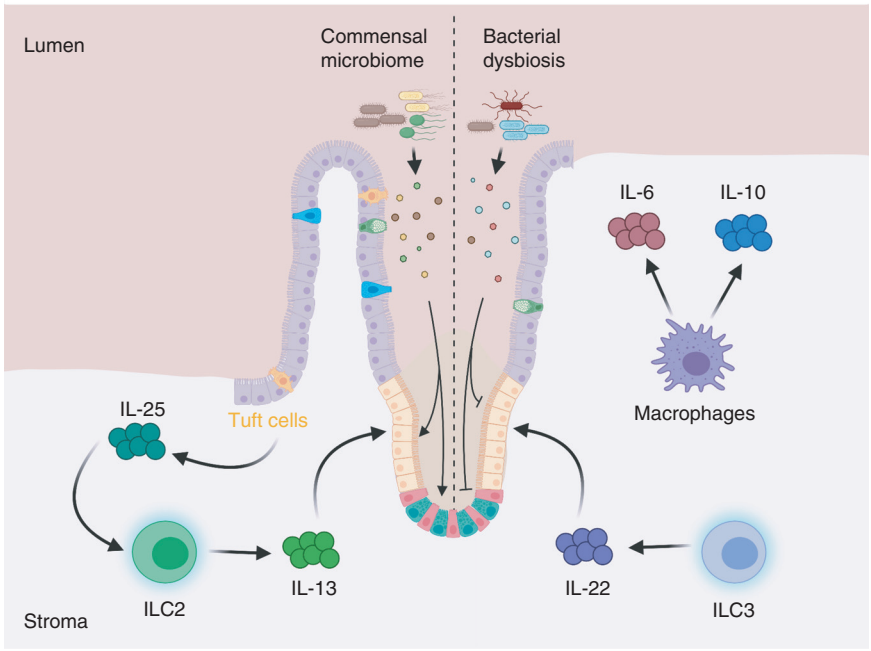

Figure 4. Immune and microbiome systems in the small intestine. Immune cells play a crucial role in intestinal regeneration. In particular, the innate lymphoid cells (ILCs) have been recognized for their contribution to epithelial repair post injury. For instance, ILC3s through the secretion of IL-22 influence the restoration of intestinal function. Tuft cells also directly interact with the immune system through the secretion of IL-25, which stimulates ILC2s to secrete IL-13, which in turn affects the proliferation of transit-amplifying cells. Macrophage-secreted cytokines such as IL-6 and IL-10 have also been associated with intestinal function and regeneration. Luminal cues modulate epithelial regeneration. The commensal microbiome communicates with epithelial cells through the production of metabolites or by the activation of Toll-like receptors (TLRs). Bacterial dysbiosis (bacterial composition change or loss) results in the modification of signals provided by the microbiome and thus affects the renewal capacities of epithelial cells. For instance, mice without an intestinal microbiome (germ-free [GF]) have fewer proliferative cells in intestinal epithelium than mice raised with a commensal microbiome.

mediated intestinal recovery has been linked to activation of phosphorylation of STAT3 in ISCs (Pickert et al. 2009; Lindemans et al. 2015), and recombinant IL-22 injection helps mice recover from GVHD, inducing epithelial regeneration and recovery of ISCs (Lindemans et al. 2015). IL-22 secretion by ILC3s is also essential for intestinal recovery upon chemotherapy-induced damage (Aparicio-Domingo et al. 2015). More recently, a study has highlighted the role of ILC3-driven intestinal repair under injury conditions that is IL-22 independent where ILC3s activate the transcriptional regulator YAP in transit-amplifying cells, leading to mucosal intestinal repair (Romera-Hernández et al. 2020).

Macrophages are also important for an appropriate regenerative response of the intestine upon injury. For example, macrophages support proliferation of progenitor epithelial cells in a DSS injury model (Pull et al. 2005). Biopsy-induced wound healing in the colon correlates with an increase in *Trem2*$^{+}$ macrophage infiltration and an increase in IL-4 and IL-13 cytokines (Seno et al. 2009). In another study, infiltrating macrophages were established to play a key role in wound healing through secretion of IL-10. Macrophage-derived IL-10 results in CREB activation, which in turn causes WISP-1 (connective tissue growth factor) secretion during wound healing (Quiros et al. 2017). Interestingly, WISP-1 is also increased in individuals with chronic colitis (Quiros et al. 2017). IL-6 secreted by macrophages and dendritic cells in the lamina propria is another factor essential for maintaining intestinal integrity. *IL-6* knockout mice are more susceptible to DSS-induced colitis, resulting in a shorter colon, loss of proliferative cells, and an increase in apoptosis; these effects are due to IL-6-mediated activation of STAT3 in epithelial cells (Grivennikov et al. 2009). Macrophage-derived Wnt is also important for intestinal recovery after irradiation injury. Macrophage-restricted *Porcn* deletion results in mice that are hypersensitive to radiation-induced gastrointestinal syndrome (Saha et al. 2016).

Of note, epithelial cells themselves have been linked to activation of the innate immune system. Tuft cells, for instance, secrete higher levels of IL-25 upon helminth infection, thus resulting in activation of ILC2s (von Moltke et al. 2016). In turn, ILC2s feed back to the epithelial cells by producing IL-13, which leads to Tuft and goblet cell hyperplasia. This hyperplasia is due to IL-13 skewing differentiation of epithelial progenitor cells. Accordingly, ablating IL-25 specifically from epithelial cells hinders clearing of the helminth infection (von Moltke et al. 2016).

Some studies have suggested that the adaptive immune system might be dispensable for epithelial regeneration. *Rag1*$^{-/-}$ mice, which lack mature lymphocytes, undergo normal restoration of the crypt compartment after methotrexate-induced injury (Aparicio-Domingo et al. 2015). Additionally, *Rag1*$^{-/-}$ mice recover normally after biopsy-induced wound injury of the colon (Quiros et al. 2017). However, γδT cells, which are a minor T-cell subset in the spleen and lymph nodes but a major subset within the epithelia, are important for recovery from DSS-induced colitis (Tsuchiya et al. 2003). Additionally, in a GVHD model, mice transplanted with bone marrow and T cells show impaired organoid formation compared to mice transplanted with bone marrow only (Takashima et al. 2019). T-cell-derived IFN-γ causes decreases of ISCs both in vitro and in vivo, in part by inducing Jak1-Stat1-dependent toxicity and initiation of apoptosis. Inhibition of IFN-γ, by a neutralizing antibody or lack of the IFN-γ receptor in epithelial cells, rescues organoid formation in vitro and restores ISCs in vivo in GVHD (Takashima et al. 2019). In sum, the immune system plays an essential role in modulating the intestinal regeneration response, especially under injury conditions, which often involves inflammation or infection.

ROLE OF THE MICROBIOME

The intestinal lumen is colonized by trillions of organisms to form the commensal microbiome. The presence of these microorganisms is essential for the host organism's health, and changes in the composition of the microbiome, known as bacterial dysbiosis, are associated with pathologies such as IBD (Fig. 4). Moreover, germ-free (GF) (Shi and Walker 2004) mice raised without microbiota are more sensitive to DSS treatment,

as treatment with high dose DSS (5%) results in the rapid death of GF mice, whereas low-dose (1%) treatment leads to an increase in the severe forms of colitis in GF mice compared to conventionally raised mice (Kitajima et al. 2001). Thus, even if the microbiome composition seems essential in protection against IBD, the exact mechanism linking both is not fully elucidated (Rispal et al. 2020).

The first evidence for the role of the microbiome in IEC renewal was observed with demonstrations that colonization of GF mice with microbiota increases intestinal epithelial turnover (Khoury et al. 1969). This phenotype could be explained by two mechanisms: the decrease of proliferation of progenitor cells in GF mice compared to conventional mice (Yu et al. 2016) and the potential increase of stem cell characteristics (increase of ISC gene expression) observed during the recolonization of GF mice with conventional microbiota (Peck et al. 2017). The remission of IBD requires the repair of sections affected by the inflammation through epithelial wound healing, a process that contains an intense phase of cell proliferation (Okamoto et al. 2009).Thus, the role of the microbiome in IEC proliferation suggests that bacterial dysbiosis observed in IBD can affect wound healing during remission, but this still remains to be studied in detail.

Interestingly, IEC renewal modulated by the microbiota seems to be dependent on the composition of these microorganisms. For instance, there is some evidence showing the beneficial effect of probiotics, which are microorganisms defined based on their potential beneficial effects on gut health. The ingestion of *Lactobacillus reuteri* probiotics in conventional mice leads to an increase in IEC turnover (Preidis et al. 2012). Moreover, this probiotic is also involved in protection of IECs against inflammation by allowing the maintenance of ISCs after tumor necrosis factor (TNF) treatment, in a Wnt-dependent manner (Wu et al. 2020).

The presence of commensal microbiota is also essential for protecting against infection by pathogenic microorganisms, which can have a dramatic effect on IEC renewal. For example, infection by the pathogen *Citrobacter rodentium*, which causes potentially fatal diarrhea, leads to the hyperproliferation of colon crypt cells and to the loss of goblet cells via activation of the Wnt pathway (Papapietro et al. 2013). These results demonstrate how pathogens can hijack the ISC niche to amplify the severity of intestinal infection. To conclude, the gut's bacterial composition is essential for IEC regeneration, and the analysis of molecular mechanisms underlying this phenomenon could open new therapeutic strategies to treat diseases such as IBD.

CONCLUDING REMARKS

Stem and transient-amplifying cells in the intestine continuously give rise to all epithelial cell lineages during homeostasis. It has therefore been surprising to discover that, in the absence of stem cell populations and under injury conditions, regeneration can be achieved through dedifferentiation of epithelial cells to reacquire stem cell–like properties. Whereas more work needs to be done to investigate the mechanisms of dedifferentiation, these findings highlight the remarkable plasticity of the intestinal epithelium. In some cases, dedifferentiation to a fetal-like state was shown to drive epithelial cell plasticity as a response to injury conditions. However, the signals and niche components controlling these processes still remain to be investigated.

The emergence of single-cell RNA-seq technologies has increased our understanding of intestinal mesenchymal niche heterogeneity (McCarthy et al. 2020a). As a whole, these analyses seem to suggest that there is a significant overlap of several ligands and other factors produced by various mesenchymal niches, with no niche factor restricted to a single cell type. As a result of these studies, a coherent picture is emerging of the distinct stromal fractions and the markers that define them. However, a more comprehensive survey is needed to study the plasticity of mesenchymal cells and whether there is a stem cell population or common progenitor that gives rise to all or some of the identified mesenchymal cells during both homeostasis and regeneration after injury. Comparison of the mesenchymal niche in the colon and small intestine is also an area of ongoing investigation.

In vitro, mesenchymal cells can replace cytokines when cocultured with organoids, but this effect lasts for only a few days. New models are being developed to mimic such ISC niche components as mesenchyme, endothelial cells, immune cells, and the enteric nervous system, providing an opportunity for better in vitro models of regeneration and disease (Bar-Ephraim et al. 2020; Palikuqi et al. 2020). While the role of the mesenchymal niche and immune system has been widely studied, evidence regarding the role of other niche components such as endothelial cells and enteric neurons is still limited (Paris et al. 2001; Van Landeghem et al. 2011; Rosenbaum et al. 2016; Ogasawara et al. 2018). Furthermore, there is still a lack of understanding of how these components interact with the microbiome system. It is important to note that most of the conclusions about the intestinal niche have been achieved from studies in the mouse system, and comparisons of human and mouse intestinal niches and their regenerative processes are still scant. Ultimately, the hope is that further resolution of the intestinal regenerative capacity and niche components, in physiological and injury conditions, can be harnessed for better clinical outcomes for patients with gastrointestinal pathologies.

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