Injury-Induced Cellular Plasticity Drives Intestinal Regeneration

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SUMMARY

Intestinal regeneration after damage occurs through the activation of several signaling pathways to promote cellular plasticity and dedifferentiation of remaining cells to a stem cell state. This review highlights many of the key cell types and signaling networks involved in the intestinal regenerative response.

The epithelial lining of the intestine, particularly the stem cell compartment, is affected by harsh conditions in the luminal environment and also is susceptible to genotoxic agents such as radiation and chemotherapy. Therefore, the ability for intestinal epithelial cells to revert to a stem cell state is an important physiological damage response to regenerate the intestinal epithelium at sites of mucosal injury. Many signaling networks involved in maintaining the stem cell niche are activated as part of the damage response to promote cellular plasticity and regeneration. The relative contribution of each cell type and signaling pathway is a critical area of ongoing research, likely dependent on the nature of injury as well as the regional specification within the intestine. Here, we review the current understanding of the multicellular cooperation to restore the intestinal epithelium after damage. (Cell Mol Gastroenterol Hepatol 2022;13:843–856; https://doi.org/10.1016/j.jcmgh.2021.12.005)

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The epithelial lining of the intestine is composed of a single layer of cells that serve many functions, including digestion, nutrient absorption, barrier function, and immunity. In the small intestine, the epithelium is organized into repetitive crypt–villus units that have slightly different architecture and cellular composition along the proximal–distal axis reflecting unique functions of each region (ie, duodenum, jejunum, and ileum). By contrast, the colon has a much simpler organization containing only crypt units that directly merge into surface epithelium. Remarkably, the entire intestinal epithelial lining is replaced approximately every 3–5 days, and this constant renewal is required to maintain intestinal homeostasis and tissue integrity.1 Multipotent leucine-rich repeat-containing G-protein–coupled receptor-5–positive (LGR5+) intestinal stem cells (ISCs) located in the crypt base are responsible for the constant renewal and rapid replenishment of all epithelial cell types lining the crypt–villus axis.2 The intestine must maintain a high rate of renewal under normal homeostasis to withstand a harsh luminal environment. The spatial distribution of ISCs at the base of crypt invaginations is important because it offers some isolation and protection from luminal stressors. However, in the event of damage to the stem cell compartment, the intestinal epithelium has a remarkable ability to regenerate after injury. In this review, we highlight some of the key findings related to the signaling pathways and mechanisms involved in intestinal homeostasis, cellular plasticity, and regeneration after injury.

Cellular Architecture in the Intestine

LGR5+ ISCs give rise to a bipotent progenitor cell population known as transit-amplifying (TA) cells. TA cells rapidly proliferate and eventually undergo lineage commitment by differentiating into either absorptive or secretory progenitors. Ultimately, absorptive progenitors go through a limited number of divisions and upon exiting the crypt differentiate into postmitotic absorptive enterocytes or into rare microfold cells that are associated with lymphoid structures and involved in immune surveillance.3 In contrast, secretory progenitors differentiate directly into several postmitotic secretory cell types including hormone-secreting enteroendocrine cells, mucus-secreting goblet cells, and antimicrobial-secreting Paneth cells.4 Chemosensory tuft cells also have been proposed to be a secretory cell type, although recent studies have raised questions about the cellular origin of distinct tuft cell populations within the gastrointestinal tract.5 Constant proliferation and differentiation in the crypt drives migration of differentiated cells toward the tip of the villus where they eventually undergo anoikis and are shed into the lumen.6 Paneth cells are the exception and are retained at the crypt base where they are intercalated between LGR5+ ISCs and have an extended half-life of approximately 30 days.

Abbreviations used in this paper: ASCL2, Achaete-scute complex homolog 2; ATOH, atonal bHLH transcription factor 1; BMI1, B cell–specific Moloney murine leukemia virus integration site 1; BMP, bone morphogenetic protein; CLU, clusterin; DCLK1, Doublecortin-like kinase 1; DLL, delta like canonical Notch ligand; DSS, dextran sodium sulfate; EGF, epidermal growth factor; FOXL1, forkhead box L1; IL, interleukin; ILC, innate lymphoid cell; ISC, intestinal stem cells; KRAS, Kirsten rat sarcoma viral oncogene homolog; LGR5+, leucine-rich repeat-containing G-protein–coupled receptor-5–positive; LRC, label-retaining cell; PDGF, platelet derived growth factor; RTK, receptor tyrosine kinase; SCF, stem cell factor; TA, transit-amplifying; WNT, wingless-related integration site.
**Cell Fate Specification**

LGR5⁺ ISC s historically have been defined through the use of LacZ or eGFP-IREs-CreERT2 knock-in reporter alleles. However, there is increasing evidence that not all LGR5⁺ stem cells are the same. Data suggest that LGR5<sup>hi</sup> cells that are located deeper in the crypt base intercalated between mature Paneth cells are more likely to be retained and to serve as bona fide ISC s that contribute to ISC renewal and crypt homeostasis. ISC retention is achieved through symmetrical division of LGR5<sup>hi</sup> cells in a process called neutral drift. LGR5⁺ ISC s are actively dividing and have a cell-cycle time of approximately 1 day. A complex signaling system tightly regulates LGR5⁺ stem cell renewal as well as cell fate decisions in the intestine. For example, Notch signaling initially is required for the maintenance and survival of LGR5⁺ ISC s, but also triggers bipotent progenitors to secrete the repressory lineage transcription factor, atonal bHLH transcription factor 1 (ATOH1), and drive absorptive enteroocyte differentiation. On the other hand, Paneth cells are dependent on both Notch signaling for their cell fate specification and wingless-related integration site (WNT) signaling for their functional differentiation. Establishment of these signaling gradients are critical for lineage commitment and terminal differentiation of each of the mature cell types in the intestine.

**Intestinal Stem Cell Niche**

The microenvironment that precisely regulates the maintenance, self-renewal, proliferation, and differentiation of stem cells is referred to as a stem cell niche. In the small intestine the stem cell niche is critically dependent on the crypt architecture, with LGR5⁺ ISC s positioned between Paneth cells at the crypt base and an intricate network of support cells including stromal cells, immune cells, neural cells, and endothelial cells embedded in the extracellular matrix of the lamina propria surrounding the crypt. The physical orientation of the intestinal epithelium and crypt architecture within this microenvironment allows the establishment of spatially restricted growth factor gradients for WNT, epidermal growth factor (EGF), NOTCH, bone morphogenetic protein (BMP), and other signaling molecules that are essential for maintaining a precise balance between stemness and differentiation. Both intrinsic signals derived from intestinal epithelial cells found within the crypt compartment and extrinsic short-range signals generated by surrounding stromal cell populations contribute to the stem cell niche. Additional signals derived from other cell types found within the lamina propria and submucosa (eg, macrophages, dendritic cells, lymphocytes, enteric neurons, glial cells, endothelial cells, pericytes, lacteal cells, myocytes, and so forth) also contribute to maintaining crypt homeostasis. Furthermore, the intestinal stem cell niche continuously monitors and responds to the luminal environment for changes in nutrients, metabolites, and signals from the microbiome to maintain integrity and function (Figure 1).

**Epithelial Compartment**

In the small intestine, Paneth cells are in direct physical contact with LGR5⁺ ISC s and provide both cell-to-cell contact and secreted signals to support the stem cell niche. For example, Paneth cells are essential for initiating Notch signaling, which is critical for LGR5⁺ ISC maintenance and survival. Paneth cells (signal-sending cells) directly communicate with stem cells through the expression of membrane-bound delta like canonical Notch ligands DLL-1 and DLL-4, which bind to NOTCH1 and NOTCH2 receptors expressed on the surface of LGR5⁺ ISC s (signal-receiving cells). Notch ligand binding to the receptor triggers cleavage and release of the intracellular signaling fragment of the receptor (Notch intracellular domain), which is translocated to the nucleus and activates transcription of Notch target genes in ISC s. Because Notch ligand presentation requires cell-to-cell contact, Paneth cells can produce a highly localized Notch gradient at the crypt base. Still, normal crypt homeostasis can be maintained after Paneth cell ablation, at least in the short term, by other secretory cell types including enteroendocrine and tuft cells that gain DLL-1 expression and migrate down to occupy the space left by Paneth cells at the crypt base. Such cellular redundancy highlights the importance of maintaining functional Notch signaling within the intestinal stem cell niche. In the colon, regenerating family member 4 (REG4)-expressing deep crypt secretory cells have been proposed to function as the colon equivalent to Paneth cells and maintain the colon stem cell niche.

In addition to Notch signaling, Paneth cells produce short-range secreted ligands including WNT ligands such as WNT3, WNT6, and WNT9b, and EGF family ligands such as EGF and transforming growth factor α. In vitro studies with murine small intestine organoid cultures supplemented with stem cell niche factors (EGF, Noggin, and R-spondin) indicate that WNT production from Paneth cells is sufficient to support organoid stem cell maintenance and proliferation. However, epithelial-specific WNT ligand production is not required or sufficient to maintain crypt homeostasis in vivo, suggesting redundancy of WNT signaling from the stromal compartment to maintain the intestinal stem cell niche. Similarly, EGF receptor (EGFR) signaling is critical for organoid growth in vitro, but not for normal intestinal homeostasis in vivo, suggesting signaling redundancy with other erb-b2 receptor tyrosine kinase (ERBB) receptors or receptor tyrosine kinases (RTKs). For example, Joosten et al. showed that MET proto-oncogene receptor tyrosine kinase (MET) signaling can compensate for a loss of EGFR signaling in normal and colorectal cancer stem cells. Furthermore, the stromal compartment provides several ligands that also can activate ERBB receptors and other RTKs. Similar to Notch and WNT signaling, this redundancy highlights the importance of RTK signaling in supporting the intestinal stem cell niche. In addition to secreting niche factors, Paneth cells also provide antimicrobial peptides to ensure a sterile crypt environment and important metabolic niche signals to support ISC s.

**Stromal Compartment**

Multiple stromal cell types are embedded in the lamina propria and contribute to a network that establishes a gradient of signaling molecules and supports the stem cell.
of these supporting cell types have long projections that assist in cell-to-cell communication with the epithelial compartment. Recent studies have implicated overlapping populations of stromal cells that are defined by different cell surface markers and transcription factors including platelet derived growth factor receptor alpha (PDGFRα), CD34, glioma-associated oncogene homolog 1 (GLI1), and forkhead box L1 (FOX1), which produce diverse agonistic and antagonistic ligands that regulate WNT/R-spondin and BMP pathways.22–26 Despite marked mesenchymal expression of WNT2B, WNT4, and WNT5A in stromal cells surrounding intestinal crypts, studies blocking WNT secretion in either epithelial cells or myosin heavy chain 11 (MYH11)-positive myofibroblasts had no effect on ISC renewal.27–32 However, ablation of FOXL1+ telocytes (a distinct subset of mesenchymal cells) or blockade of WNT secretion from FOXL1+ cells results in the rapid loss of ISCs and crypt failure, indicating that FOXL1+ cells are an essential source of WNT ligands for ISC renewal. Interestingly, gene expression studies have shown that telocytes located in different regions of the crypt–villus axis have distinct functions. Telocytes located near the crypt base express WNT2B and R-spondin 3 (RSPO3), consistent with the high WNT activity that is required to maintain the intestinal stem cell niche. On the other hand, telocytes located at the tips of villi express WNT antagonists and noncanonical WNT5A, likely to inhibit proliferation and promote differentiation, cellular polarity, and villus formation.33 Similarly, recent analysis of PDGFRα expression has identified 3 distinct stromal populations that establish a BMP signaling gradient in the intestine. Two of the PDGFRα+ populations show low expression of PDGFRα and can be distinguished by the presence of the cell surface marker CD81 and the BMP inhibitor Gremlin1.34 PDGFRα+CD81- cells (also known as trophocytes) that express high levels of Gremlin1 are located primarily surrounding the crypt base and block BMP signaling in the ISC compartment. PDGFRαhi stromal cells that are negative for CD81 and Gremlin1 are situated above and around crypts. Contrastingly, PDGFRαlo telocytes are abundant at the crypt–villus shoulder and express BMP ligands that drive differentiation in epithelial cells exiting the crypt. The stromal compartment also produces other signaling molecules including other EGF-like ligands, insulin-like growth factor 1 and 2 (IGF1/2), and metabolites such as prostaglandin E2 (PGE2) that act directly on the epithelial compartment.35–40

Other supporting cells also can contribute to the intestinal stem cell niche. For example, Zhu et al11 provided evidence that interleukin (IL)13 produced by group 2 innate lymphoid cells (ILCs) promotes the maintenance and self-renewal of LGR5+ ISCs. IL10 secreted by regulatory T cells also help maintain the LGR5+ ISC niche.42 Likewise, Kosinski et al43 showed that myofibroblasts and smooth muscle cells are a source of BMP antagonists (Gremlin1, Gremlin2, and Chordin-like 1) that maintain the stem cell niche at the crypt base.43 Furthermore, each of the lineages that contribute to the intestinal stem cell niche are continuously responding to environmental cues and likely are activated in response to acute or chronic intestinal injury to aid in intestinal regeneration. The crosstalk between these cell populations and their relative contributions to the intestinal stem cell niche and regeneration after injury is an important area of ongoing research.

**Intestinal Injury and Repair**

The epithelium that lines the intestine faces harsh conditions from the luminal environment and is susceptible to a number of damaging events including infection with pathogenic organisms, acute or chronic inflammatory disease, as well as genotoxic stress associated with chemotherapy/radiation treatment. Therefore, numerous model systems have been developed to study intestinal injury and repair. Mice are the most frequently used animal model for intestinal studies because their intestinal development is similar to human beings, and they share many of the same genes and immune responses while remaining small and easy to genetically manipulate. The use of transgenic mouse lines has been imperative for advances in our understanding of cellular plasticity and regenerative responses in the intestine. For instance, knocking-in the DTR gene into the Lgr5 locus allows toxin-induced LGR5+ cell ablation and provides a way to conditionally study the response to specific stem cell loss in the intestine.44 Other common injury models used to investigate the regenerative response in the intestine include treatment with high-dose radiation or doxorubicin (a chemotherapy drug), which results in the rapid loss of LGR5+ ISCs as well as proliferating progenitor cells. Although each of these models affect the stem cell compartment, specific LGR5+ ISC ablation allows proliferating progenitor cells to contribute to regeneration, whereas radiation or chemotherapy destroy most proliferating cells (including progenitors) and limits the pool of cells that contribute to regeneration to postmitotic cells. It also is important to note that there are regional differences in the response to epithelial damage along the proximal-distal axis. For example, it is well documented that the colon is more radiation-resistant than the small intestine.45,46 This highlights the

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**Figure 1.** (See previous page). Diagram of the intestinal structure and the stem cell niche. The intestinal epithelium is a monolayer of cells with a distinct composition along the proximal-distal axis. In both the small intestine and colon, the stem cell compartment is located at the base of the crypt. Stem cells continuously generate transit-amplifying and progenitor populations that further differentiate into 5 main functional cell types: enterocytes, goblet cells, Paneth cells, enteroendocrine cells, and tuft cells. Signaling molecules produced by cells within the epithelial compartment as well as a network of supporting cells establish a gradient to balance between stemness and differentiation in the crypt. WNT, EGF, and Notch signaling are restricted to the base of the crypt and work to maintain intestinal stem cells. BMP antagonists such as Gremlin1/2 also are restricted to the crypt base to establish a BMP signaling gradient from the crypt–villus shoulder to the villus tip and promote differentiation in cells exiting the crypt. BMPRI, bone morphogenetic protein receptor type 1B; BMPRII, bone morphogenetic protein receptor type 2; CSL, CBF-1/Suppressor of Hairless/Lag-1; EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol 3-kinase; RAS, rat sarcoma viral oncogene; RSPO, R-spondin; TCF, transcription factor 4.
need for further investigations that take both the damaging event and the regional specification into consideration.

**Cellular Plasticity**

The intestinal epithelium shows remarkable flexibility upon damage. Acute injury that results in ISC loss activates a regenerative response to restore the stem cell compartment. Several groups have tried to identify the cellular source of intestinal regeneration after damage using genetic labeling and lineage tracing of distinct cell types. These approaches fail to assess the level of contribution from each population in overall epithelial regeneration and cannot exclude the possibility that other cellular sources exist. It appears that a variety of cell types are involved synchronously in damage-induced epithelial regeneration and several different cell types can acquire stem cell characteristics to repair the intestinal epithelium after stress, damage, infection, or disease (Figure 2). To clarify the degree each cell population contributes to overall epithelial regeneration, it is necessary to combine genetic lineage tracing, depletion studies, single-cell gene expression profiling, chromatin structure analysis, and organoid-formation assays.

**Quiescent Stem Cells**

LGR5⁺ cells are considered active multipotent intestinal stem cells because they rapidly divide and give rise to TA cells and all differentiated intestinal cell types. There is also a second population of ISCs, considered reserve ISCs, that are quiescent and resistant to stress but are mobilized upon injury to repopulate the crypt. Reserve intestinal stem cells generally reside in the +4 to +6 cell position from the crypt base. Several markers have been proposed for the reserve

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**Figure 2.** Diagram of cells that show cellular plasticity and dedifferentiate after damage. In an injury-dependent manner, there is hierarchical dedifferentiation and mobilization of epithelial cells that contribute to regeneration. Cells from both the secretory and absorptive lineage branches are capable of dedifferentiating to LGR5⁺ intestinal stem cells to repopulate the crypt after damage.
stem cell population including B cell-specific Moloney murine leukemia virus integration site 1 (BMI1), leucine rich repeats and immunoglobulin like domains (LRIG1) HOXB homeobox (HOPX), and mouse telomerase reverse transcriptase (MTERT). Lineage-tracing studies using Bmi1, Lrig1, Hopx, or mTert drivers show that reserve stem cell populations proliferate in response to irradiation damage. Reserve stem cells appear to share some properties with label-retaining cells (LRCs) originally described by Potten et al, but represent a molecularly and functionally distinct population. LRCs consist of both Paneth cells and non-Paneth cells located in the +4 position of the crypt or above. Importantly, Buczacki et al showed that LRCs are heterogeneous and non-Paneth LRCs are a secretory progenitor that also have reserve stem cell activity. It has been proposed that reserve stem cells are required to protect the niche from stress, and to generate LGR5+ stem cells and/or TA cells in response to damage. Therefore, maintenance of the quiescent stem cell population is considered a critical element of radioreistance. However, there is overlapping gene expression between many of the active and reserve stem markers, highlighting both the plasticity and inter-relation of cells within the stem cell zone, but also raising the question of whether these cells represent discrete stem cell populations.

Progenitor Cells

Several lineage-committed progenitor cells, including ATOH1+ or DLL-1+ secretory progenitors or intestinal alkaline phosphatase (ALPI)-positive enterocyte progenitors, also can act as facultative stem cell populations capable of repopulating the crypt after injury. For example, lineage tracing studies have indicated that all differentiated cell types can be derived from ALPI+ enterocyte progenitors upon LGR5+ ablation. Similarly, DLL1high secretory progenitors retain cellular plasticity and can revert to a stem cell state after radiation damage. It appears that more committed precursor populations also can contribute to regeneration, such as CD69+ CD274+ goblet cell precursors. However, it is important to remember that proliferating progenitor cell populations are more susceptible to radiation or chemotherapy treatment and therefore only are available to contribute to regeneration if they survive the damaging event.

Paneth Cells

Under homeostatic conditions, Paneth cells are postmitotic and support the stem cell niche and host defense. However, recent studies have provided evidence that mature Paneth cells and/or Paneth cell progenitors also can contribute to epithelial regeneration after damage to the intestine. For instance, Yu et al showed that a subset of lysozyme 1+ Paneth cells proliferate and produce differentiated cell types after radiation damage. Likewise, acute injury with doxorubicin treatment triggers defensin a4+ Paneth cells to dedifferentiate into multipotent stem cells in a Notch-dependent manner. On the other hand, Hayakawa et al found that basic helix-loop-helix family member a15 (BHLHA15)-positive Paneth cells and short-lived secretory precursors showed no plasticity after LGR5+ ISC ablation or radiation damage, but did contribute to regeneration after doxorubicin-induced injury. These results suggest that Paneth cell and/or Paneth cell progenitors represent at least one differentiated lineage that is capable of repopulating the crypt after at least some types of damage. However, the minimal regenerative contribution from defensin a6+ Paneth cells after doxorubicin treatment highlights the need for a detailed analysis of Cre-expressing cells in the crypt compartment at a single-cell level.

Enterendocrine Cells

In contrast to the reserve ISC studies, Jadhav et al suggested that BMI1+ cells are not a dedicated population of quiescent stem cell, but instead are mature enterendocrine cells that express Chga and Neurod1. Whether a reserve ISC or enterendocrine cell, it appears that BMI1+ cells do contribute to intestinal regeneration after injury. In fact, Yan et al showed that BMI1+ cells are enriched for other enterendocrine markers, including Prox1. Lineage tracing using a Prox1 driver showed that mature enterendocrine cells can acquire stemness during homeostasis and injury-induced regeneration.

Tuft Cells

Doublecortin-like kinase 1 (DCLK1) also previously was reported as a marker of quiescent stem cells, but it is highly expressed in chemoceptive tuft cells residing in the stomach and intestine. Westphalen et al described a rare subpopulation of long-lived DCLK1+ cells that acquired stem cell properties under homeostatic conditions and played a critical role in regeneration. Ablation of DCLK1+ cells before irradiation or dextran sodium sulfate (DSS) injury in mice diminished epithelial repair and caused crypt dropout. Not only did tuft cells show cellular plasticity during homeostasis and regeneration, but both tuft cells and enterendocrine cells showed cellular plasticity and up-regulated Notch signals to support the stem cell niche upon Paneth cell depletion.

For many of the cell types involved in the regenerative response, reversion to a stem cell state analogous to LGR5+ ISCs is required. Indeed, Metcalfe et al showed that recovery after radiation-induced damage is acutely perturbed in the presence of sustained depletion of LGR5+ ISCs with diphtheria toxin. In this case, regenerating cells must transition to a LGR5+ ISC state before they contribute to repair of the intestinal epithelium. Other studies have suggested that a subpopulation of slow-cycling mex-3 RNA binding family member A (MEX3A) or Keratin 15-expressing LGR5+ ISCs are less radiosensitive, allowing them to contribute to radiation-induced regeneration. On the other hand, it has been suggested that early progeny of LGR5+ and Olfactomedin 4 (OLFM4)-positive cells are available to support regeneration after chemotherapy damage owing to a reduced DNA damage response and lack of apoptosis induction. Sheng et al described a heterogenous population of Musashi RNA Binding Protein 1 (MSI1)-positive cells
that also showed DNA damage resistance. Interestingly, rapid-cycling MS1\textsuperscript{+} cells can contribute to postradiation regeneration in the absence of LGR5 expression, questioning the need to transition to an LGR5-expressing cell population before initiating a regenerative response. Thus, the efficiency and hierarchical contribution of different intestinal cell types likely is dependent on the type and extent of injury and the intestinal region involved. Regardless, it appears that the simultaneous activation of multiple cell lineages to acquire stemness and proliferate is required to combat and overcome many different types of intestinal injury.

**Regulation of Plasticity**

Many of the signaling pathways that support the intestinal stem cell niche also are up-regulated to promote postinjury cellular plasticity and regeneration, including both the WNT and Notch signaling networks.

**Achaete-Scute Complex Homolog 2 Protein**

WNT signaling is one of the most important regulators of adult intestinal stem cells. Under homeostasis, Ascl2 is a WNT target gene that is restricted to LGR5\textsuperscript{+} ISCs. Achaete-scute complex homolog 2 (ASCL2) is a basic helix-loop-helix transcription factor that works together with \(\beta\)-catenin/transcription factor 4 (TCF4) to activate transcription of stem cell genes and coordinate stemness in the intestinal epithelium in a WNT-dependent manner.\textsuperscript{56} Murata et al\textsuperscript{67} showed increased expression of Ascl2 in regenerating cells and showed that ASCL2 is essential for crypt cell dedifferentiation after ISC injury. Therefore, it is likely that the transcription factor ASCL2 can regulate gene expression in both adult ISCs and in the course of cellular plasticity after injury. Murata et al\textsuperscript{67} suggested that Il11ra1 may be a functional target gene of ASCL2, however, further studies are needed to fully understand the mechanistic role of ASCL2 in intestinal regeneration.

**Hippo Pathway: Yes-Associated Protein 1/ Tazazzin Signaling**

The Hippo signaling pathway plays a significant role in intestinal development, regeneration, and cancer.\textsuperscript{68} At the center of this pathway is a kinase cascade that starts with activation of macrophage stimulating 1 (MST) and ends with phosphorylation of transcriptional regulators yes-associated protein (YAP) and tazazzin (TAZ), resulting in their cytoplasmic sequestration and/or degradation. Hippo activity is regulated by several input signals including immune modulators, mechanotransduction, prostaglandins, and WNT signaling. Interestingly, regenerating crypts have increased YAP levels and YAP inactivation severely impairs intestinal regeneration. It appears that, at least in part, the Hippo pathway functions to counterbalance WNT signaling because YAP/TAZ inhibit WNT target genes.\textsuperscript{69,70} In addition, YAP accumulation in the nuclei of regenerating crypt cells activates the transcription of a number of genes that help with wound healing including Areg, Ereg, Il33, Ctfy, and Ccn1. Hyperactivation of YAP, on the other hand, is associated with cancer development.\textsuperscript{71} Thus, the Hippo pathway must be tightly regulated to promote tissue regeneration, but also block tumorigenesis.

**Stem Cell Factor/c-Kit Receptor Signaling**

Stem cell factor (SCF) is a ligand for the receptor tyrosine kinase c-Kit. SCF-mediated activation of c-Kit results in autophosphorylation and initiation of downstream signaling. It has been reported that SCF/c-Kit signaling plays a role in cell survival, proliferation, and migration. Although certain signaling events may be present in all regenerating cells, other pathways may be restricted to specific cell subsets or types of injury. For example, levels of SCF expression are increased with inflammation, as seen with inflammatory bowel disease patients or in DSS mouse models, while the c-Kit receptor is expressed by antimicrobial-secreting Paneth cells. Schmitt et al\textsuperscript{72} showed that the SCF/c-Kit signaling axis contributes to Paneth cell plasticity after inflammation-driven ISC injury. It appears that Paneth cells acquire stem-like features downstream of inflammatory activation of c-Kit through glycosyn synthase kinase 3 beta (GSK3\(\beta\)) inhibition and WNT activation. Several other tyrosine kinases also appear to play a role in intestinal regeneration, including SRC proto-oncogene non-receptor tyrosine kinase (SRC) and MET.\textsuperscript{19,73}

**Notch Signaling Pathway**

Although Notch signaling remains a critical component of the intestinal stem cell niche, it appears that up-regulation of Notch signaling also is associated with aspects of cellular plasticity and regeneration. Yu et al\textsuperscript{74} showed that Paneth cells from irradiated mice that were undergoing dedifferentiation gained ectopic expression of Notch1 and activated the Notch signaling pathway. In addition, forced expression of the Notch intracellular domain in Paneth cells promoted dedifferentiation in the absence of injury. Furthermore, Paneth cell dedifferentiation after doxorubicin-induced injury was inhibited after loss of Notch signaling in Paneth cell progenitors.\textsuperscript{75} Taken together, these results suggest that Paneth cell plasticity after intestinal injury is regulated by Notch activation.

**Fetal-Like Transcriptional Program**

Although the role of WNT, YAP/TAZ, RTKs, and Notch signaling in regeneration is well established, there are other signaling networks that coordinate cellular plasticity in response to damage to the intestinal epithelium. For instance, several groups have reported a fetal-like transcriptional program, distinguished by the expression of Sca-1, in regenerative lineages.\textsuperscript{74–76} In this case, intestinal epithelial cells repurpose aspects of fetal development to promote repair. Interestingly, single-cell transcriptionomics have identified a rare population of quiescent facultative stem cells termed revival stem cells, which are marked by clusterin (CLU) expression. CLU\textsuperscript{+} revival stem cells express stem cell antigen-1 (Sca-1 or Ly6a) after injury, suggesting potential overlap with a fetal gene signature involved in regeneration. CLU\textsuperscript{+} cells do not contribute to homeostasis
but are mobilized in a YAP-dependent manner to produce LGR5\(^+\) ISCs after various mechanisms of intestinal injury including irradiation, LGR5\(^+\) cell ablation, and DSS-induced colitis.\(^{77}\)

**Other Nonepithelial Signals**

In addition to the intrinsic signaling networks that regulate cellular plasticity, stromal and immune cell populations accumulate at sites of damage in the intestine and secrete factors that promote the regenerative process. Jarde et al\(^{23}\) showed that a subset of GLI1\(^+\) mesenchymal cells also contribute to post-DSS treatment recovery by over-expressing RSPO3 to boost WNT signaling. Complementary changes were observed in immune cells, for example, macrophage-derived WNT ligands packaged in extracellular vesicles are critical for recovery after radiation-induced injury.\(^{78}\) Group 3 ILC-derived IL22 also promoted repair of the intestinal epithelium after graft-versus-host disease or chemotherapy-induced damage.\(^{79,80}\) Alternatively, Romero-Hernandez et al\(^{81}\) reported that group 3 ILC-driven tissue regeneration is independent of IL22 and relies on amplification of the Hippo/YAP signaling pathways. Altogether, it is likely that multicellular cooperation and a sophisticated network of connected signaling pathways is required to restore intestinal homeostasis after injury.

**Epigenomic State and Chromatin Remodeling**

Although there is direct evidence that upon damage crypt regeneration is achieved through the mobilization and dedifferentiation of a diverse population of facultative stem cells, the precise role that epigenetic mechanisms play in regulating cell fate decisions during homeostasis and restoring ISC function after injury is poorly understood. Early studies on chromatin status indicated that adult ISCs and differentiated cells generally possess similar DNA methylation as well as enhancer and open chromatin profiles.\(^{82-84}\) Not only does the broadly permissive epigenomic state between ISCs and committed lineages provide a platform for Notch/ATOH1-dependent lateral inhibition and lineage specification, but the lack of chromatin barrier provides a simple explanation for achieving cellular plasticity during regeneration. Therefore, it is likely that transcription factor occupancy plays a major role in the outcome for both cell fate decisions and dedifferentiation, and activation of distinct signaling pathways to induce appropriate transcription factor expression is required. Recent studies have arrived at a conflicting conclusion that suggests a more dynamic chromatin regulation during cell fate specification into differentiated intestinal cell types.\(^{57,85-88}\)

In the case of injury-induced dedifferentiation of committed progenitor populations such as preterminal enteroendocrine cells and maturing goblet cell progenitors, histone methylation remodeling is required to overcome the chromatin barrier and allow dedifferentiation. Although investigations into epigenetic factors that regulate intestinal differentiation are in their infancy, data suggest that different signaling pathways and transcription factors are used based on the region of the intestine and how the epithelium is injured (eg, whole-body irradiation, ISC ablation, chronic inflammation, and so forth). Remodeling a chromatin barrier likely is dependent on the extent of injury, for example, LGR5\(^+\) cell ablation leaves proliferating progenitors available for dedifferentiation, whereas dedifferentiation after radiation-induced injury is dependent on the level of radiation and radiosensitivity in different regions of the intestine. Thus, the signaling pathways involved in driving dedifferentiation in the context of permissive chromatin likely are different from those required to initiate chromatic remodeling.

**Implications for Tumor Initiation**

LGR5\(^+\) stem cells and committed progenitor populations share a similar epigenomic profile, which provides a reasonable mechanism for crypt progenitor dedifferentiation and repopulation of the stem cell compartment after stress or damage. An important area of ongoing research is whether similar mechanisms of cellular plasticity are present during tumor initiation. Two key models for the cellular origin of intestinal tumorigenesis have been proposed: stem vs non–stem cell origin. The bottom up model claims that early adenoma formation originates from stem cells at the bottom of the crypt, while the top down model asserts that tumor initiation occurs at the top of the crypt independent of crypt stem cells.\(^{89,90}\) To test both models, WNT-activating mutations or perturbations in other signaling pathways (such as Kirsten rat sarcoma viral oncogene homolog (KRAS) and nuclear factor-κB) are introduced to specific cell types throughout the crypt–villus axis. For example, loss of adenomatous polyposis coli (APC) in LGR5\(^+\) stem cells triggers adenoma formation, supporting the bottom up model. Likewise, WNT-activating mutations in specific +4 reserve stem cell populations (eg, prominin 1 (PROM1)-positive, or LRIG1\(^+\)) also can initiate tumor formation.\(^{90,91}\) However, because of the overlapping gene expression of +4 reserve stem cell markers in LGR5\(^+\) stem cells, it is uncertain whether adenoma formation in +4 stem cells represents a distinct transforming event that occurs separate of the LGR5\(^+\) ISCs. Dissimilar to stem cell populations, Barker et al\(^{92}\) showed that APC inactivation in short-lived TA cells using an AhCre driver produced foci with limited
expansion that failed to progress to adenomas. In the early stages of tumorigenesis, dysplastic cells often were observed in the upper crypt region with morphologically normal cells at the base in support of the top down model. Furthermore, Metcalfe et al. showed that adenoma initiation occurred in LGR5− cell populations when diphtheria toxin receptor–mediated LGR5+ ISC ablation was combined with APC loss. Although it is clear that tumor initiation can originate outside the LGR5− stem cells, a non–stem cell origin has required constitutive WNT activation in combination with tissue damage, compound mutations, or alterations in the microenvironment to drive tumorigenesis. For instance, WNT-dependent tumor initiation in radiosensitive keratin 19+ cells only occurred after radiation-induced injury and loss of LGR5+ ISCs. Likewise, constitutive WNT activation in X-box binding protein 1 (XBP1)-positive villus cells and Paneth cells failed to produce adenomas until WNT signaling was coupled with activation of oncogenic KRAS or loss of NFKB inhibitor alpha (IκBα). In addition, APC-deficient Paneth cells are insensitive to WNT activation, but upon Notch activation and dedifferentiation into stem cell/progenitors they become WNT responsive and readily are transformed. APC-deficient DCLK1+ tuft cells also readily can form tumors in the presence of chronic inflammation in the colon. Unlike other intestinal cell types, APC or APC/KRAS mutations in ALP1+ enteroendocrine progenitors fail to form tumors. It has been proposed that mutated enterocyte progenitors cannot form adenomas because these cells rapidly migrate toward the villus tips where they are shed into the lumen, not allowing enough time for tumor initiation. Although cellular plasticity is implicated in tumor initiation, cancer cells also harness plasticity to evade and become resistant to therapy. Understanding the role of cellular plasticity and the underlying signaling networks that drive cancer development in the intestine are important to identify preventative approaches, diagnostic tools, and therapeutic strategies.

Concluding Remarks and Future Perspectives

In this review, we have highlighted some of the key regulatory signals and pathways that are required for homeostasis, cellular plasticity, and postinjury regeneration of the intestinal epithelium. Under homeostasis, the intestinal stem cell niche is composed of a multicellular and highly interactive signaling network that continually monitors its surroundings to achieve balance between stemness and differentiation. The epithelium that lines the intestine is highly susceptible to damage from the constant exposure to harsh luminal insults and pathogenic states including infections and inflammation. However, the intestine has a remarkable ability to repair itself. Dedifferentiation of intestinal cells into multipotent stem cells, a process cumulatively termed intestinal cell plasticity, provides a mechanism to maintain barrier integrity and homeostatic stability in the face of persistent injury. Still, one of the major outstanding questions is as follows: how is disruption or loss of the LGR5− stem cell compartment sensed? It is probable that different types of injury produce distinct signals and cellular responses, but this remains an active area of ongoing research. Nevertheless, it appears that many intestinal cell types can undergo dedifferentiation to multipotent ISCs and there is likely a hierarchical mobilization of different cell populations in an injury-dependent manner. For example, the regenerative response after radiation-induced genotoxic injury, which kills LGR5+ ISCs and most proliferating cell progeny, is reliant on different radio-resistant cell populations including more quiescent cells such as secretory cell progenitors or distinct proliferating progenitors that display reduced DNA damage responses. How these different populations contribute to regeneration may depend on the extent of injury and regional differences in sensitivity to injury. For example, the colon is more radiosensitive than the small intestine, likely owing to differences in the composition and regulation of the stem cell niche in each region. It also remains to be seen whether the facultative stem cell populations mobilized by genotoxic insults are the same as those involved in regenerative responses to more pathophysiological stressors such as infection or inflammation. If progenitor cells or other committed precursor lineages withstand the injury, their ability to dedifferentiate and contribute to regeneration also likely is dependent on having the appropriate signaling networks necessary to overcome any chromatin barrier. The chromatin barrier to dedifferentiate is lower in early progenitor cell populations than more mature cell types.

Most of the work in the field of intestinal cellular plasticity and regeneration has combined mouse intestinal injury models with Cre-dependent reporter systems that often lack cell-type specificity and sufficient spatial and temporal control. In the future, it is important to implement more unbiased approaches with single-cell resolution to examine the hierarchy and relative contribution of distinct cell types and signaling networks to overall repair. This will be particularly important for addressing whether similar facultative stem cell populations and mechanisms of regeneration occur in the human intestine. To date, studies of cellular plasticity in the human intestine have been limited. There are likely significant differences between mouse and human intestine stem cell niches. For instance, in human beings, crypt–villus structures are formed during fetal development, unlike mice, in which the crypt–villus axis is established postnatally. In addition, the kinetics of stem cell proliferation and neutral drift are slower in human beings, indicating that the dynamics of regeneration in human beings also may be different. Indeed, organoid cultures suggest the human intestinal stem cell niche is far more complex and requires more stem cell signals than mice. Although it remains unclear if current human intestinal organoids are an appropriate in vitro model to study such regenerative events, the development of more complex multicellular in vitro systems that recapitulate the intestinal stem cell niche is an important avenue for further study.

Recent advances in intestinal regeneration have suggested that cellular plasticity and dedifferentiation are a common feature of injury-induced regenerative events and
in many cases involve up-regulation of intestinal stem cell niche signaling pathways. Understanding the hierarchical nature of these regenerative events may provide new insights and approaches to improved intestinal regeneration and repair during chronic injury such as in inflammatory bowel disease and new therapeutic avenues to address tumor resistance that focus on impacting multiple aspects of cancer stem cells and the tumor microenvironment.

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2022 Injury-Induced Cellular Plasticity 853

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The authors disclose no conflicts.

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