The Role of Intestinal Stem Cells in Epithelial Regeneration Following Radiation-Induced Gut Injury

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Abstract

Purpose of Review Intestinal epithelial cells show remarkable plasticity in regenerating the epithelium following radiation injury. In this review, we explore the regenerative capacity and mechanisms of various populations of intestinal stem cells (ISCs) in response to ionizing radiation.

Recent Findings Ionizing radiation targets mitotic cells that include “active” ISCs and progenitor cells. Lineage-tracing experiments showed that several different cell types identified by a single or combination of markers are capable of regenerating the epithelium, confirming that ISCs exhibit a high degree of plasticity. However, the identities of the contributing cells marked by various markers require further validation.

Summary Following radiation injury, quiescent and/or radioresistant cells become active stem cells to regenerate the epithelium. Looking forward, understanding the mechanisms by which ISCs govern tissue regeneration is crucial to determine therapeutic approaches to promote intestinal epithelial regeneration following injury.

Keywords Intestinal epithelial cells · Stem cells · Irradiation · Regeneration

Abbreviations

ALPI Alkaline phosphatase
ASCL2 Achaete-scute family BHLH transcription factor 2
ATOH1 (MATH1) Atonal BHLH transcription factor 1
BMI1 B lymphoma Mo-MLV insertion region 1 homolog polycomb ring finger proto-oncogene
DCLK1 Doublecortin like kinase 1
DLL1 Delta-like canonical notch ligand 1
DTR Diphtheria toxin receptor
GFP Green fluorescent protein
HOPX HOP homeobox
KLF4 Krüppel-like factor 4
KRT19 Keratin 19
LGR5 Leucine-rich repeat-containing G protein-coupled receptor 5
LRIG1 Leucine-rich repeats and immunoglobulin like domains 1
MEX3A Mex-3 RNA binding family member A
mTERT Telomerase reverse transcriptase
OLFM4 Olfactomedin 4
PROM1 Prominin 1
PUMA p53 Upregulated modulator of apoptosis
RFP Red fluorescent protein
SMOC2 SPARC-related modular calcium binding 2
SOX9 SRY-box 9
STAT5 Signal transducer and activator of transcription 5
Introduction

Radiation therapy (RT) is a common treatment modality for malignant cancers and is used in approximately half of all cancer patients [1]. The major dose-limiting factor of RT is the damage to normal, non-cancerous tissues. Almost all patients undergoing RT to the abdomen, pelvis, or rectum develop acute enteritis [2]. In addition, 5–15% of the patients develop chronic enteritis due to fibrotic epithelium, ulceration, and damaged submucosa [3]. With the increasing cancer survival rate for all types of cancers [4], it is important to address the RT-induced side effects in patients. The major advances in radiation oncology have been in the increased precision of radiation dose delivery to reduce the damages to normal tissues and amplify effects on tumor cells [5]. Such advancements include image-guided radiation therapy and tumor-targeted radiosensitizers. However, currently available treatments for patients experiencing radiation enteritis are symptomatic cares for diarrhea, dehydration, malabsorption, and abdominal or rectal discomfort [1]. Amifostine, an organic thiophosphate that can act as a free radical scavenger, is the only drug used in clinics to protect normal tissues from radiation-induced toxicity [1]. Considering a large number of cancer patients receiving radiotherapy and the critical functions it plays in cancer cures, the adverse effects of RT on the intestinal epithelial physiology warrant investigation.

Intestinal Stem Cells (ISCs)

The mammalian intestinal epithelium is a rapidly self-renewing tissue, with the entire epithelium replaced in approximately 3–5 days. Intestinal stem cells and progenitor cells that reside within the proliferative compartment of intestinal crypts are responsible for the rapid renewal of the tissue [6]. The cells within the crypts divide and differentiate as they migrate up the differentiated compartment of intestinal villi, which are primarily composed of absorptive enterocytes, goblet cells, enteroendocrine cells, and tuft cells [6].

Until now, two populations of ISCs have been identified. Active intestinal stem cells (aISCs), also called crypt base columnar (CBC) cells, rapidly divide to upwardly give rise to progenitor cells within the transit-amplifying (TA) zone [7]. The aISCs are major contributors to epithelial renewal, yet are sensitive to injuries incurred from radiation or chemotheraphy. Markers that identify aISCs include LGR5, ASCL2, OLFM4, SMOC2, PROM1, and SOX9[8–13]. Located at +4 to +6 positions from the bottom of the crypts are quiescent stem cells that are resistant to stress but become activated upon perturbation to aISCs, thus named reserve intestinal stem cells (rISCs). This population was initially proposed by Potten and colleagues as the labeling-retaining cells (LRCs) that are long-living dormant cells but with proliferative potential [14]. Several proposed markers for rISCs include BM11, MTERT, HOPX, LRIG1, SOX9[15–22]. The development of lineage-tracing mouse models using promoter-driven expression of reporter genes, such as GFP, RFP, or LacZ, and inducible Cre recombinase system enormously contributed to identifying ISC markers. These mice showed that both active and reserve ISCs possess two major stem cell characteristics: the capacity to self-renew and to generate differentiated cells. In addition, the crypt TA zone harbors lineage-committed progenitors that express specific cell markers, such as ALPI for enterocyte progenitors [23], DLL1 or ATOH1 (MATH1) for secretory progenitors [24], and KRT19 for TA cells and rISCs [22]. Known ISC markers or those that regulate ISC function are summarized in Appendix Table 1.

Intestinal Epithelial Tissue Response to Radiation Injury

The clinically relevant γ-irradiation doses of 8 to 15 Gy are sufficient to induce intestinal epithelial injury in mice, although these doses are lethal due to bone marrow failure [73]. The mouse intestinal epithelium is capable of regenerating when exposed to total body irradiation (TBI) at doses below 14 Gy, as indicated by the increase in survival with bone marrow transplant or partial bone marrow shielding [73, 74]. However, doses above 15 Gy result in gastrointestinal acute radiation syndrome (GI-ARS) and mortality even with bone marrow transplant [74]. The regenerative responses by the intestinal epithelium can be divided into three phases: apoptotic phase, proliferative phase, and normalization phase (Fig. 1a). The apoptotic phase consists of first 2 days following irradiation. Histology and immunostaining analysis of irradiated mouse intestines show continuous crypt loss, shrinkage in crypt size, and shortening of the villi during this phase [73]. The regenerative phase follows the apoptotic phase between 2 to 4 days post irradiation, where surviving crypt cells regenerate entire crypts. While the overall number of crypts decrease in both small and large intestines, the regenerative crypts enlarge in size due to an approximately twofold increase in the number of proliferating cells. During the normalization phase, the size of the crypts and the length of the villi are restored to “homeostatic” post-irradiation condition.

Intestinal Epithelial Cellular Response to Radiation Injury

Recent studies using lineage-tracing mouse models demonstrated remarkable plasticity within ISCs and progenitor cells in their capability to drive regeneration of the intestinal epithelium upon injury (Fig. 1b). The LGR5+ stem cell population is highly susceptible to ionizing radiation and is diminished upon injury [25]. One prominent source of regenerative...
responses are rISCs, regarded as a radioresistant population owing to its quiescence. When LGR5\(^+\) aISCs are specifically depleted, rISCs enter the cell cycle, repopulate the crypt, and give rise to aISCs [44]. Of note, colonic and small intestine LGR5\(^+\) cells respond differentially to irradiation damage. Under low- to medium-dose radiation, small intestinal LGR5\(^+\) cells tolerate better compared to colonic LGR5\(^+\) cells in terms of DNA damage repair and cell proliferation at 72 h following 0.1-, 1-, or 4-Gy irradiation [75]. On the other hand, colonic LGR5\(^+\) aISCs are more resilient to high-dose irradiation injury compared to small intestine LGR5\(^+\) aISCs and survive better following 19-Gy irradiation with complete DNA double-strand break repair [76].

Multiple studies have reported that rISCs are responsible for the regenerative response following radiation injury using Bmi1, mTert, Lrig1, or Hopx promoters as the lineage-tracing drivers [17, 19, 25, 39] (Appendix Table 1). These studies confirmed that cells expressing these markers were slow-cycling, distinguishably expressed in “position +4 to +6,” and able to regenerate the epithelium by their lineages. Supporting the regenerative functions of non-LGR5-expressing stem cells, KRT19\(^+\)-expressing cells at “position +4” were also determined to have stem cell functions, distinguished from CBCs, and able to regenerate the epithelium following radiation injury in colon [22].

Maintenance of quiescence in rISCs is a crucial mechanism of radioresistance. A subpopulation of BMI1\(^+\) rISCs also express KLF4, a zinc-finger transcription factor that is involved in regulation of the cell cycle by decelerating the rate of cell proliferation [70, 77]. Mice in which Klf4 is deleted from BMI1\(^+\) rISCs failed to regenerate the epithelium following 12-Gy irradiation, suggesting that the quiescence regulation by KLF4 also regulates cellular responses to radiation damage. In addition to KLF4, the transcription factor SOX9 [18] and RNA-binding proteins MSI1/2 [53] are involved in regulation of rISC quiescence via differential expression levels. The intestinal epithelial crypt cells with high levels of Sox9 expression (EGFP/PHI) maintain quiescence through SOX9-mediated WNT signaling suppression [18]. Lineage-tracing experiments using Sox9-CreERT2 showed that under 12-Gy irradiation, where aISCs are supposedly depleted due to their radiosensitivity, SOX9\(^+\) cells were able to regenerate crypts, whereas conditional deletion of Sox9 in the intestinal epithelium impaired this regeneration. On the contrary, MSI proteins...
in rISCs are dispensable in homeostatic condition, but their increased levels are required to exit from quiescence during regeneration following 12-Gy irradiation [53]. Collectively, these studies confirm our understanding that quiescence in stem cell population provides a regenerative mechanism for the intestinal epithelium to restore homeostasis when aISCs are depleted.

In addition to rISCs, contributors to regeneration following radiation injury include the short-lived secretory progenitor cells in the TA zone. Abrogation of LGR5+ aISCs in diphtheria toxin-treated Lgr5-DTR mice did not alter the epithelial structure, indicating that the intestinal epithelial renewal was maintained without LGR5+ aISCs, but the number of enteroendocrine cells in the crypts doubled [44]. The increase in secretory cells suggested that secretory progenitor cells may serve as a source of tissue renewal in the absence of LGR5+ cells. Using markers for lineage-committed secretory or enterocyte progenitor cells, lineage-tracing experiments showed that DLL1+ secretory progenitor cells participate in regeneration of the epithelium when the homeostasis is disrupted with 6-Gy radiation injury [24]. The progenitor cells stop proliferating once they are committed to secretory lineages, and they stimulate NOTCH signaling pathways in neighboring cells by expressing NOTCH ligands to induce their fate to non-secretory lineages [78]. The stalled proliferation may also provide advantages to cell survival following irradiation injury, allowing them to de-differentiate to stem cells.

While multiple studies have reported that aISCs are more susceptible to radiation injury and undergo apoptosis within approximately 24 h following irradiation [22, 25], other studies suggest that LGR5+ aISCs also contribute to tissue regeneration [30, 79]. According to Hua et al., not all LGR5+ cells undergo apoptosis within 24 h following 10- and 12-Gy irradiation, and surviving LGR5+ cells at 48 h are an important source of tissue regeneration [28]. The authors suggest that LGR5+ cells are more efficient in DNA damage repair by homologous recombination compared to progenitor and villi cells, although further studies are necessary to confirm this observation. Supporting the notion that surviving LGR5+ cells are involved in the regenerative response, depletion of LGR5+ cells using the Lgr5-DTR mouse model immediately after 10-Gy irradiation decreased the number of regenerating crypts [30]. This study demonstrates that high-dose irradiation does not completely eliminate LGR5+ cells, allowing surviving LGR5+ cells to replenish the intestinal epithelium.

The contribution of LGR5+ aISCs in the regenerative response is reasonable, considering aISCs have relatively slow proliferation rate of one cell cycle every 21.5 h, whereas progenitor cells divide once every 12 h [80]. It may be that the surviving LGR5+ aISCs were at a cell cycle phase that is less sensitive to DNA damage. Another hypothesis is that there are subpopulations of cells within LGR5+ cells identified in Lgr5-reporter mice that are differentially responsive to radiation injury. Indeed, single-cell transcript profiling of LGR5+ aISCs isolated from Lgr5-EGFP mice showed that cells with high levels of GFP expressions are not exclusively aISCs but also include early progenitors derived from aISCs [27]. Interestingly, these early progenitor cells expressed high levels of cell cycle inhibitors, such as Cdkn1a, compared to “true” aISCs, indicating that these cells proliferate at a relatively slower rate. Recently, a subpopulation of slow-cycling LGR5+ cells was identified, which expresses high levels of the RNA-binding protein Mex3a and is capable of organoid formation in ex vivo culture [56••]. Upon 12-Gy irradiation, LGR5+ Mex3Ahigher cells survived and regenerated crypts. Collectively, these data demonstrate the existence of slow-cycling LGR5+ cell populations that are resistant to irradiation and may play a role during regeneration. Reserve ISCs were first identified as label-retaining cells (LRCs) that incorporated labels in DNA and resided at position +4 [81]. A study using H2B-YFP mice for nuclear labeling showed that LRCs are secretory progenitor cells that ultimately differentiate to Paneth and enteroendocrine lineages, but with retained ability to de-differentiate into stem cells when homeostasis is perturbed [82]. Under 6-Gy irradiation, these LRCs were also capable of regenerating the crypts. Further supporting the notion that rISCs may be secretory progenitor cells, the transcriptome and open chromatin structure analyses of GFP+ cells from transgenic reporter Bmi1-GFP mice showed strong similarities to secretory progenitor cells and enteroendocrine cells, suggesting Bmi1-GFP+ cells are predetermined enteroendocrine cells [45••]. Additionally, Bmi1-GFP+ cells were enriched for enteroendocrine marker messenger RNAs (mRNAs), such as Gip, Pax6, Chga, and Chgb, confirming that these cells are enteroendocrine lineages with the plasticity to convert to regenerative population after radiation injury [45•••, 83•••]. Interestingly, these cells also exhibited open chromatin structures in regions that are lineage-restricted, which potentially explains the high plasticity of these cells in response to aISC depletion. These observations provide first mechanistic explanations of how BMI1+ rISCs are capable of regenerating crypts following irradiation injury.

Beside the secretory progenitors, mRNAs of putative rISC markers, Bmi1, mTert, Hopx, and Lrig1, have been found to be expressed in LGR5+ cells using transcriptome analysis and in situ hybridization [11]. Curiously, the lineage-tracing mouse models using some of these rISC markers as driving promoters did not show tracings that occurred from CBCs or with similar mitotic kinetics. This may be that the lineage tracing of cells expressing these markers does not solely depend on the mRNA or protein levels.

A comparison study using Lgr5-EGFP, Bmi1-CreERT2, and Hopx-CreERT2 transgenic mice with reporter genes and inducible Cre system showed that long-term LRCs are
progenitor cells may contribute to regeneration following radiation injury, absorptive progenitor cells may be more sensitive to radiation injury, absorptive progenitor cells have the highest proliferation rate, which ablation of the niche results in increased proliferation and crypt regeneration following irradiation injury. In addition to MSCs, other cell types involved in the regenerative response following irradiation injury include endothelial cells and immune cells. Collectively, these data indicate that external environments created by supporting cells are crucial to ISC proliferation and regeneration.

Factors Involved in Intestinal Epithelial Regeneration

As previously discussed, global effects of non-epithelial cells play critical functions in the regenerative response following radiation injury. Several factors have been implicated in acceleration of the regenerative process. Such factors include general inducer of proliferation, including R-spondin 1 and Slit guidance ligand 2, secretory proteins that cooperatively promote the WNT/β-catenin signaling, growth factors insulin-like growth factor 1 (IGF-1) and bFGF that promote growth of the regenerative crypts and impair radiation-induced apoptosis through inhibition of PUMA expression, and prostaglandins. Other factors involved in the regenerative response after irradiation injury are Pectin and glucagon-like peptide 2 (GLP-2) that directly stimulates BMI1+ rISCs to promote stem cell proliferation during homeostasis and crypt regeneration following 10-Gy radiation injury. We can postulate that many other external modulatory factors are available to enhance the regeneration of the tissue. These factors may be dietary or secretory proteins contributed by epithelial or non-epithelial cells. It is of great interest to study natural molecules involved in maintenance of tissue homeostasis and regeneration.

Cellular Response to Radiation-Induced DNA Damage

The lethal effect of radiotherapy arises from cellular and molecular responses to DNA damage induced by free radicals causing double-strand breaks. Ionizing radiation induces water radiolysis in cells, which generates reactive oxygen species (ROS) that become the major source of DNA damage. This type of damage is more detrimental to cells in the early DNA synthesis, which undergo apoptosis at a higher incidence than cells in other stages of the cell cycle. In the
intestinal epithelium, aISCs and progenitor cells in the crypts are more radiosensitive. The ultimate fate of these cells can be predicted based on the presence of apoptotic factors and DNA damage repair proteins. Indeed, stem cell populations with large accumulation of DNA damages may be more detrimental to overall health of the tissue, while survival of cells capable of DNA damage repairs and tissue regeneration is crucial.

**P53/P21/PUMA**

The transcription factor P53 is the key regulator of DNA damage response in the intestinal crypt. Following high-dose ionizing radiation, activated P53 upregulates the expressions of P21 and PUMA [100]. The protein P21 is a cyclin-dependent kinase (CDK) inhibitor that blocks the G1-to-S phase transition of the cell cycle and allows DNA repairs in the crypts [101]. On the other hand, activation of PUMA results in aISC and progenitor cell apoptosis following DNA damage, whereas the absence of PUMA leads to improved survival of intestinal stem cells and crypt regeneration [101]. Modulation of p53 activity following irradiation increases the cell survival. KLF4-mediated inhibition of p53 resulted in suppression of apoptosis and proliferation, thus allowing DNA damage repair [102, 103]. In addition to this observation, the pro-survival effects of GSK-3 inhibitor CHIR99021 on in vitro 3D culture of LGR5+ aISCs following radiation was due to inhibition of p53-dependent induction of PUMA [104]. This suggests that inhibition of apoptotic pathways may be a therapeutic target for radiation-induced injuries.

**NOTCH**

The cell-to-cell NOTCH signaling in ISC and progenitor cells has been shown to be important for stem cell functions and lineage commitments in the intestinal epithelium [33]. Deletion of Notch1 increased the number of post-mitotic goblet cells, whereas deletion of Notch2 had no effect on the lineage commitment [33]. However, inhibition of NOTCH with difluorophenacetyl-l-alanyl-S-phenylglycine t-buty1 ester (DAPT) or deletions of Notch1 or Notch2 impaired crypt regeneration following irradiation injury due to absence of proliferation, suggesting that NOTCH receptors play important functions that are beyond cell fate determination, and strong NOTCH signaling is required for tissue regeneration [31, 33, 59]. Further investigations are necessary to elucidate the effect of irradiation on intestinal NOTCH signaling and its potential role in post-IR regeneration.

**PI3K-AKT-mTORC1**

Another important regulatory signaling pathway in intestinal epithelial tissue homeostasis is PI3K-AKT-mTORC1 signaling cascade. PTEN is a negative regulator of the PI3K-AKT-mTORC1 signaling pathway implicated in various cellular functions, such as proliferation and metabolism. PTEN is also implicated in maintenance of quiescence of mTERT+ cells at “position +4” in the crypt, and deletion of Pten resulted in loss of quiescent ISCs [105], potentially due to aberrant proliferation and exhaustion of stem cells. Interestingly, PTEN functions to regulate dormancy of mTERT+ rISCs. The GFP+ cells’ population obtained from the mTert-GFP mouse model showed lack of expression of inactive form of PTEN (P-PTEN) during homeostasis [17]. Yet, when these mice were irradiated with dose of 16 Gy, which is sufficient to eliminate the aISCs, the intestinal epithelium was able to regenerate 96 h later, possibly due to activation of rISCs, whereas Pten-deleted mice showed significantly less regenerative colonies [105]. Of note, the radiation dose used in this study induces GI-ARS, resulting in complete loss of intestinal epithelial regeneration. Thus, it is difficult to conclude that mTERT+PTEN+ rISCs play critical functions in regenerative response after irradiation, unless lower doses are used to study during the actual regenerative process.

The functions of the PI3K-AKT-mTORC1 pathway in the intestinal crypts may be cell type-specific. While the inhibition of this pathway is important for maintenance of quiescence in rISCs, mTOR plays critical functions in lineage generation and intestinal stem cell physiology of OLFM4+ aISCs through mechanisms that are not mediated by WNT activity [34]. During intestinal crypt regeneration after 10-Gy irradiation injury, deletion of mTOR in the intestinal epithelium resulted in impaired regeneration, as well as decreased level of Olfm4. This result is interesting, because the expressions of Ascl2 and mTert, which are WNT target genes and putative aISC markers, increase even with mTOR deletion [34]. This suggests that the putative stem cell markers may be indicators of active signaling pathways, and there is no bona fide single marker for ISCs, just as there are multiple essential signaling pathways involved in the maintenance of stem cell functions.

**Conclusions**

Radiotherapy is an essential part of cancer treatment, although clinical advancements in addressing its adverse effects on non-cancerous tissues have been insufficient. Understanding the mechanisms of intestinal epithelial regenerative processes is a critical step in developing therapeutic approaches for protection of normal tissue and may be beneficial to reestablish homeostasis post injury. The application of lineage-tracing mouse models has greatly contributed to determining cell populations responsible for the tissue homeostasis and regeneration following irradiation (Fig. 1b). However, the attempts to use a single marker to identify a cell population have resulted in an intricate list of cell markers and types. The recent focuses have been placed on delineating ISC or progenitor markers.
using various methods, such as transgenic reporter mouse models, RNA sequencing, in situ hybridization, and chromatin structure analysis. Looking forward, it may be worthwhile to understand the global effects on the intestinal epithelium instead of targeting a cell population that contributes to regeneration, at least in achieving our goal to develop therapeutic approaches to ameliorate the adverse effects on normal tissue by radiotherapy. This will include determining the inter- and intra-cellular key mediators of the tissue regeneration that globally influence the tissue homeostasis and regeneration.

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Compliance with Ethical Standards

Conflict of Interest Chang-Kyung Kim, Vincent W. Yang, and Agnieszka B. Bialkowska declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Appendix

Table 1  Intestinal stem cell markers and functional proteins during homeostasis and post-irradiation regeneration

| Gene name | Name | Expression pattern | Functions during homeostasis and post-IR regeneration | References |
|-----------|------|--------------------|------------------------------------------------------|------------|
| **Active ISC markers (CBCs)** | | | | |
| Lgr5 | Leucine-rich repeat-containing G protein-coupled receptor 5 | alSCs | Lgr5 is a WNT target gene that encodes a receptor for R-spondins that are involved in maintaining the expression of surface frizzled receptors to enhance WNT signaling. Marks rapidly cycling stem cells that maintains the homeostasis of the intestinal epithelium with the capacity for multilineage differentiation and self-renewal. LGR5+ cells are more susceptible to radiation injury and undergo apoptosis within 24 h following irradiation. A subpopulation of LGR5+ cells can survive radiation injury and regenerate crypts. | [8, 25–30] |
| Olfm4 | Olfactomedin-4 | alSCs | Olfm4 encodes secretory glycoprotein olfactomedin 4, where the molecular function of OLFM4 is unknown. Olfm4 gene expression is WNT-independent; however, it is regulated by the NOTCH signaling pathway in CBCs and progenitor cells. Lineage tracing with Olfm4-IRES-eGFPCreERT2 mice showed that OLFM4+ cells are long-lived and multipotent. mTOR signaling is important for OLFM4+ aISC-driven crypt regeneration following irradiation. | [10, 31–34] |
| Smoc2 | SPARC-related modular calcium binding 2 | alSCs | SMOC2 is a matricellular protein involved in matrix assembly. Lineage-tracing with Smoc2-EGFP-ires-CreERT2 mice showed that SMOC2+ cells are long-lived and multipotent. The function of SMOC2+ cells in IR response is not determined. | [11] |
| Ascl2 | Achaete scute-like 2 | alSCs | Ascl2 is a WNT target gene, where its expression is enriched in LGR5+ aISCs. It maintains the stemness of LGR5+ aISCs. Ascl2 gene is expressed in the entire crypt during crypt regeneration, but the function of ASCL2+ cells in IR response is not determined. | [9, 26, 35] |
| Rnf43 and Znrf3 | Ring finger protein 43/zinc and ring finger 3 | alSCs | RNF43/ZNRF3 are RING-type E3 ubiquitin ligases that negatively regulate the WNT signaling pathway by targeting frizzled receptors for degradation. Their roles in IR response are not determined. | [36] |
| TNfsf19 (Troy) | TNF (tumor necrosis factor) receptor superfamily, member 19 | alSCs | TNfsf19 is a WNT target gene that encodes a receptor tyrosine kinase required for cell migration and positioning in the intestinal crypts. Lineage tracing with Troy-CreERT2;Rosa-LacZ mice shows that TROY+ cells are long-lived and multipotent. Troy negatively regulates LGR5-mediated signaling. Its role in IR response is not determined. | [37] |
| **Reserve ISC markers** | | | | |
| Lrig1 | Leucine-rich repeats and immunoglobulin-like domains 1 | qISCs (+4 position) | LRIG1 is a negative inhibitor of EBBB signaling and functions to regulate aberrant proliferation by stem cells. LRIG1+ cells are slowly cycling and long-living. | [19, 38] |
| Gene name | Name | Expression pattern | Functions during homeostasis and post-IR regeneration | References |
|-----------|------|--------------------|------------------------------------------------------|------------|
| Hopx      | Homeodomain-only protein | rISCs (+4 to +7 position) | LRIG1<sup>+</sup> cells are radioresistant and capable of crypt regeneration following 8-Gy irradiation | [11, 20, 39–41] |
|           | | | *Hopx* encodes a homeodomain-only protein, but the function of this protein in ISC's is unknown | |
|           | | | HOPX<sup>+</sup> cells are slow-cycling stem cells found at +4 position and capable of giving rise to rapidly cycling ISCs (CBCs) | |
|           | | | mRNA and protein expressions detected in the CBCs; however, *Hopx*-driven reporter proteins are usually found at +4 to +7 positions | |
|           | | | HOPX<sup>+</sup> cells have differential expressions of CD24/CD44 compared to Lgr5<sup>+</sup> cells | |
|           | | | *HOPX* + ISCs contribute to crypt regeneration following 12-Gy irradiation | |
| mTert     | Mouse telomerase reverse transcriptase | rISCs (+4 position) | *mTert* encodes mouse telomerase reverse transcriptase that regulates telomerase activity | [17, 42] |
|           | | | mTERT<sup>+</sup> cells are slow-cycling and multipotent cells found at +4 position | |
|           | | | mTERT<sup>+</sup> cells are radioresistant and capable of surviving 1- or 10-Gy irradiation and contributing to crypt regeneration | |
| Bmi1      | Polycomb group RING finger protein 4 | Reserve ISCs (+4 position) and predetermined enteroendocrine cells | BMII is a member of polycomb group of transcription repressors that are expressed in slow-cycling stem cells found at +4 position | [11, 25, 26, 39, 43, 44, 45] |
|           | | | BMII<sup>+</sup> cells can replace aSCs when LGR5<sup>+</sup> cells are depleted | |
|           | | | BMII<sup>+</sup> cells are radioresistant and capable of regenerating crypts following 12-Gy irradiation | |
|           | | | Notably, the *Bmi1*-lineage tracing model (Bmi1-CreER<sup>+</sup>) has a penetrance of 10% and is limited to the proximal region of the small intestine | |
|           | | | mRNA expressions detected in LGR5<sup>+</sup> and TA cells; however, *Bmi1*-driven reporter proteins are usually found at supra-Paneth positions (+1 to +6 positions) | |
|           | | | Transcriptome and open chromatin structure analyses showed that *Bmi1<sup>+</sup>* cells may be predetermined enteroendocrine cells | |
| EphB2     | EPH receptor B2 | Highest in active ISCs and gradually decreased as cells differentiate | *EphB2* is a WNT target gene and a receptor tyrosine kinase that is required for cell migration and positioning in the intestinal crypts | [46–49] |
|           | | | EphB2<sup>+</sup> cells express mRNA of ISC-specific markers, such as *Lgr5* and *Ascl2*, capable of forming in vitro organoid | |
|           | | | Its role in IR response is not determined | |
| Prom1 (CD133) | Prominin-1 | Active ISCs and TA cells | Prominin-1 is a transmembrane protein that co-expresses *Lgr5* are capable of self-renewal and multilineage differentiation | [12, 50] |
|           | | | Prom1-CreERT2-IRES-nLacZ-PGK-Neo mice showed that PROM1<sup>+</sup> cells are long-living and multipotent cells | |
|           | | | Its role in IR response is not determined | |
| Msi1      | Musashi-1 | Active ISCs and reserve ISCs (+4 position) | *Msi1* encodes an RNA-binding protein that regulates proliferation through activation of WNT and NOTCH signaling pathways | [51–53] |
|           | | | Using *Msi1*-eGFP mice, GFP<sup>hi</sup> cells co-express mRNA of ISC markers *mTert*, *Hopx*, and *Lrig1*, whereas GFP<sup>lo</sup> cells express high levels of *Lgr5*, *Ascl2*, *Olfm4*, and *Smoc2* | |
|           | | | MSI1 and MSI2 expressions in rISCs are required for effective regeneration following 12-Gy irradiation. | |
| Sox9      | SRY (sex-determining region Y)-box 9 | Sox9<sup>EGFP<sub>LO</sub></sup> (using *Sox9<sup>EGFP</sup>* transgenic mouse) in active ISCs; *Sox9<sup>EGFP</sup>* in +4 to +7 reserve ISCs; also expressed in enteroendocrine cells | SOX9<sup>+</sup> cells are expressed in rapidly cycling stem cells that reside at the crypt base at a relatively low level | [13, 18, 54] |
|           | | | SOX9<sup>+</sup> cells are slow-cycling and are enriched for *Bmi1* and *Hopx* | |
|           | | | Sox9<sup>EGFP</sup> cells are radioresistant, and SOX9 is required for crypt regeneration following 12-Gy irradiation | |
| Mex3a     | Mex-3 RNA-binding family member A | Subpopulation of slow-cycling LGR5<sup>+</sup> cells at +3 to +4 positions | MEX3A is an RNA-binding protein that regulates a transcription factor CDX2 | [55, 56] |
|           | | | LGR5<sup>+</sup>MEX3A<sup>hi</sup> cells are a slow-cycling and multipotent subpopulation of LGR5<sup>+</sup> cells | |
Table 1 (continued)

| Gene name | Name | Expression pattern | Functions during homeostasis and post-IR regeneration | References |
|-----------|------|--------------------|-------------------------------------------------------|------------|
| Dclk1 | Doublecortin and CaM kinase-Like-1 | rISCs (+4 position) and differentiated Tuft cells | LGR5+MEX3A<sup>high</sup> cells are radioresistant and survive at 48 h following 12-Gy irradiation | [21, 26, 57–62] |
| Nkx2.2 | NK2 homeobox 2 | Subset of enteroendocrine cells, BMI1<sup>+</sup> cells, and LGR5<sup>+</sup> cells | Nkx2.2 encodes the transcription factor NK2 homeobox 2 that plays a critical function in enteroendocrine cell fate determination | [63] |
| Krt19 | Keratin-19 | Reserve ISCs and TA cells | Keratin-19 is an intermediate filament that maintains the cytoskeleton | [22] |
| Dll1 | Delta-like 1 | Secretory precursor cells and differentiated secretory cells | DLL1 inhibits neighboring cells become secretory cells by stimulating NOTCH signaling pathways | [64–66] |
| Math1 (Atoh1) | Atonal BHLH transcription factor | Secretory precursor cells | Math1 is a basic helix-loop-helix transcription factor that regulates secretory cell fate determination | [67, 68] |
| Klf4 | Krüppel-like factor 4 | Subpopulation of BMI1<sup>+</sup> rISCs at +4 position and differentiated cells | KLF4 is a transcription factor that regulates cell lineage differentiation and maintains epithelial homeostasis | [69, 70] |
| Stat5 | Signal transducer and activator of transcription 5 | Intestinal epithelial cells, including LGR5<sup>+</sup> cells | STAT5 is transcription factor that plays a functional role in maintenance of intestinal epithelial cell integrity and response to gut injury | [71, 72] |

References

Papers of particular interest, published recently, have been highlighted as:

- Of major importance

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