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Mechanisms modulating the activities of intestinal stem cells upon radiation or chemical agent exposure

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ABSTRACT

Intestinal stem cells (ISCs) are essential for the regeneration of intestinal cells upon radiation or chemical agent damage. As for radiation-induced damage, the expression of AIM2, YAP, TLR3, PUMA or BVES can aggravate ISCs depletion, while the stimulation of TLR5, HGF/MET signaling, *Ass1* gene, Slit/Robo signaling facilitate the radioresistance of ISCs. Upon chemical agent treatment, the activation of TRAIL or p53/PUMA pathway exacerbate injury on ISCs, while the increased levels of IL-22, β-arrestin1 can ease the damage. The transformation between reserve ISCs (rISCs) maintaining quiescent states and active ISCs (aISCs) that are highly proliferative has obtained much attention in recent years, in which ISCs expressing high levels of Hopx, Bmi1, mTert, Krt19 or Lrig1 are resistant to radiation injury, and SOX9, MSI2, clusterin, URI are essential for rISCs maintenance. The differentiated cells like Paneth cells and enteroendocrine cells can also obtain stemness driven by radiation injury mediated by Wnt or Notch signaling. Besides, Mex3a-expressed ISCs can survive and then proliferate into intestinal epithelial cells upon chemical agent damage. In addition, the modulation of symbiotic microbes harboring gastrointestinal (GI) tract is also a promising strategy to protect ISCs against radiation damage. Overall, the strategies targeting mechanisms modulating ISCs activities are conducive to alleviating GI injury of patients receiving chemoradiotherapy or victims of nuclear or chemical accident.

Keywords: intestinal stem cells (ISCs); radiation damage; chemical agent damage; ISCs transformation; gut microbiota balance

INTRODUCTION

In mammalian, the small intestine represents the major digestive organ and the fundamental line of defense against external damage. The multitude of villi which embody epithelial cell layer with enteroendocrine cells, enterocytes and goblet cells surrounding the lamina propria, endow it strong absorption capacity [1]. Small intestine regenerate about once every 3 days as to counteract the hazards induced by external physical, chemical, or biological exposure [2]. The intestinal cells are generated from the crypts composed of stem cells that gradually proliferate into transit-amplifying (TA) cells thereafter differentiating into different intestinal cells [3]. The highly proliferative rate of intestinal stem cells (ISCs) facilitates the restoration of the disrupted barrier caused by damage exposure, which are finely modulated by intrinsic and extrinsic mechanisms. Wnt signaling pathway is critical for the regulation of ISCs activities, and upon the binding of Wnt ligands to Frizzled receptor or Lrp 5/6 transmembrane coreceptor, the accumulation of β-catenin in nucleus induced by degradation complex (composed of Axin 2, adenomatous colonic polyposis complex, creatine kinase-1 α, and GSK 3β) inhibition will promote the proliferation of ISCs [4]. As for the differentiation of ISCs, the activation of the receptors with Notch signaling ligands will induce the cleavage and release of Notch homolog 1 intracellular domain (NICD), and the interaction between NICD and transcription factor Cbf/Su (H)/Lag1 (CSL) then stimulates the transcription of target genes such as Hes1. The negative regulation of Math1 by Hes1 prevents the differentiation into secretory cells thereby maintaining the self-renewal and proliferative state of ISCs [5]. By contrary, bone morphogenetic proteins (BMPs) signaling attenuates ISCs proliferation via the inhibition of Wnt signaling

mediated by the phosphatase PTEN. Besides, microenvironment regulated by the Paneth cells, mesenchymal cells and immune cells that secrete signaling ligands including WNT3a, epidermal growth factor (EGF) and Notch ligand DLL4 is also vital for ISCs maintenance [6]. Nevertheless, the signaling pathways mentioned above are not sufficient for interventions development to ease gastrointestinal (GI) syndrome induced by chemoradiotherapy, accidental or combat exposure [7, 8]. As ISCs in the crypts serve as a source of intestinal regeneration, the elucidation of the underlying mechanisms modulating ISCs activities during radiation or chemical agent damage is essential for the development of alternative strategies to alleviate intestinal injury.

RADIATION DAMAGE

Due to the high mortality and scarce therapeutic measures in clinical practices, GI syndrome caused by high doses of penetrating radiation remains a concern. Victims with GI syndrome suffer from impairment of the villi regeneration and epithelial integrity damage of the entire GI tract, which leads to malabsorption, fluid loss, electrolyte imbalances, sepsis and even death [9, 10]. As ISCs are thought to be a critical determinant in the regenerative process of patients with GI injury, a better understanding of the intrinsic mechanisms modulating the activities of ISCs upon radiation exposure is therefore important.

The sensibilization of ISCs to radiation damage

In recent years, various signaling pathways have been demonstrated to aggravate ISCs damage upon radiation exposure, in which the inhibition of the critical components in these pathways abrogate the injury on intestinal tract. Melanoma defect factor 2 (Absent In Melanoma 2, AIM2) is a member of the interferon-induced HIN-200 family recognizing double-stranded DNA in the cytoplasm through oligonucleotide or oligosaccharide binding sites, and the activation of caspase-1 by targeting apoptosis-associated speck-like protein containing a CARD (ASC) promotes the assembly of inflammatory bodies [11]. Previous study has showed that the knockout of AIM2 or its downstream caspase-1 significantly protected mice against high dose ionizing radiation, in which radiation-induced damage on small intestinal crypts and organoids were alleviated [8, 11]. Yes-associated protein (YAP) is the critical component of Hippo signaling pathway, and the phosphorylation of YAP at serine 127 by Large Tumor Suppressor Kinase 1/2 (LATS1/2) will restrict its nuclear translocation thereby inhibiting cell proliferation [12, 13]. At the early stage of radiation injury, YAP-mediated Wnt signaling pathway restriction by decreasing disheveled protein nuclear translocation inhibit the excessive proliferation of small intestinal cells [14], but the transiently reprograms of ISCs induced by YAP will promote the regeneration at the later phase of injury [15]. Toll Like Receptor 3 (TLR3) is an essential pathogen-related pattern recognition receptor that can recognize viral RNA and innate immunity [16]. Takemura *et al.* have demonstrated that the blockade of TLR3 could alleviate intestinal injury on mice caused by radiation exposure, in which cell apoptosis and death rate in crypts of $TLR3^{-/-}$ mice was much lower than that of their counterparts, and TRIF–RIP1 pathway, p53 and Self-RNA were demonstrated to be involved in the process of TLR3-mediated crypts damage [17]. p53 upregulated modulator of apoptosis (PUMA) is one of the targets of p53 [18, 19], and during the process of radiation damage, apoptosis rates in intestinal progenitor and stem cells were induced by PUMA over-expression, whereas the crypt proliferation and regeneration were significantly restored in PUMA deficiency mice [20]. Interestingly, the alleviation of radiation damage on ISCs of mice with β-arrestin-2 ($\beta arr2$) deficiency was mediated by PUMA suppression [21]. In addition, Vascular epicardial active substance (BVES/Popdc1), which is a transmembrane protein associated with junctional complexes in malignant tumors, was found to negatively modulate the activities of small intestinal crypts cell during radiation injury [22].

In terms of mechanisms mentioned above, the design of related agents targeting these molecules or the related pathways might be the alternative strategies for intestinal radiation injury mitigation. For instance, as a cyclin-dependent kinase 4/6 (CDK4/6) selective inhibitor, the FDA-approved drug PD 0332991 was reported to improve ISCs survival and regenerative rates by targeting p53 that is the regulator of TLR3 and PUMA [23], and auranofin remarkably alleviated radiation-induced enteritis via inhibiting PUMA related apoptosis [24].

Signaling protecting ISCs against radiation damage

The stimulation of some molecules or signaling pathways involved in the pattern recognition family or immune cells was reported to facilitate the regeneration of ISCs upon radiation exposure [25, 26], Toll-like receptor 5 (TLR5) is expressed on the endothelial cells of the small intestine lamina propria, which can recognize flagellin and induce immune responses [27]. Upon radiation injury, the pre-existing inflammation by pretreatment of TLR5 agonists dramatically reduced the level of apoptosis on intestinal crypt cells and promoted crypt stem cells regeneration via NF-κB signaling pathway [28]. Consistently, PUMA suppression by the protracted NF-κB activation in $\beta Arr2$-deficient mice improved the survival rates of ISCs during radiation damage [21].

Saha *et al.* have demonstrated that bone marrow-derived adherent stromal cell transplantation mitigated lethal intestinal injury by restituting the ISCs niche [29], but the depletion of porcupine in macrophage would result in the hypersensitivity of intestine to radiation injury. The radioprotective effects of macrophage was involved in the secretion of Wnt signaling [30]. Wnt signaling pathway is essential in maintaining the homeostasis of ISCs, in which the administration of Rspondin 1 that is an ISC growth factor stimulated the Wnt signaling thereafter triggering ISCs proliferation and regeneration following radiation exposure [31].

As is mentioned above, increased level of apoptosis rate in ISCs by TLR3 or PUMA was associated with p53. Nevertheless, dual effects of p53 during radiation injury has also been demonstrated, in which crypt cell cycle arrest by p53 retards villi destruction and mice lethality [32]. *Argininosuccinate synthase 1* (*Ass1*) encodes the enzyme catalyzing argininosuccinate formation represented the rate-limiting step of arginine synthesis [33], and Miyamoto *et al.* have reported that *Ass1* was transactivated by p53 in response to radiation, while the deficiency of *Ass1* significantly increased the level of apoptosis on small intestinal crypts [34]. Besides, (DNA-dependent protein kinase) DNA-PK mediated DNA repair and p53 mediated cell cycle arrest cooperate to eliminate DNA damage induced by radiation in the ISCs niche [35].

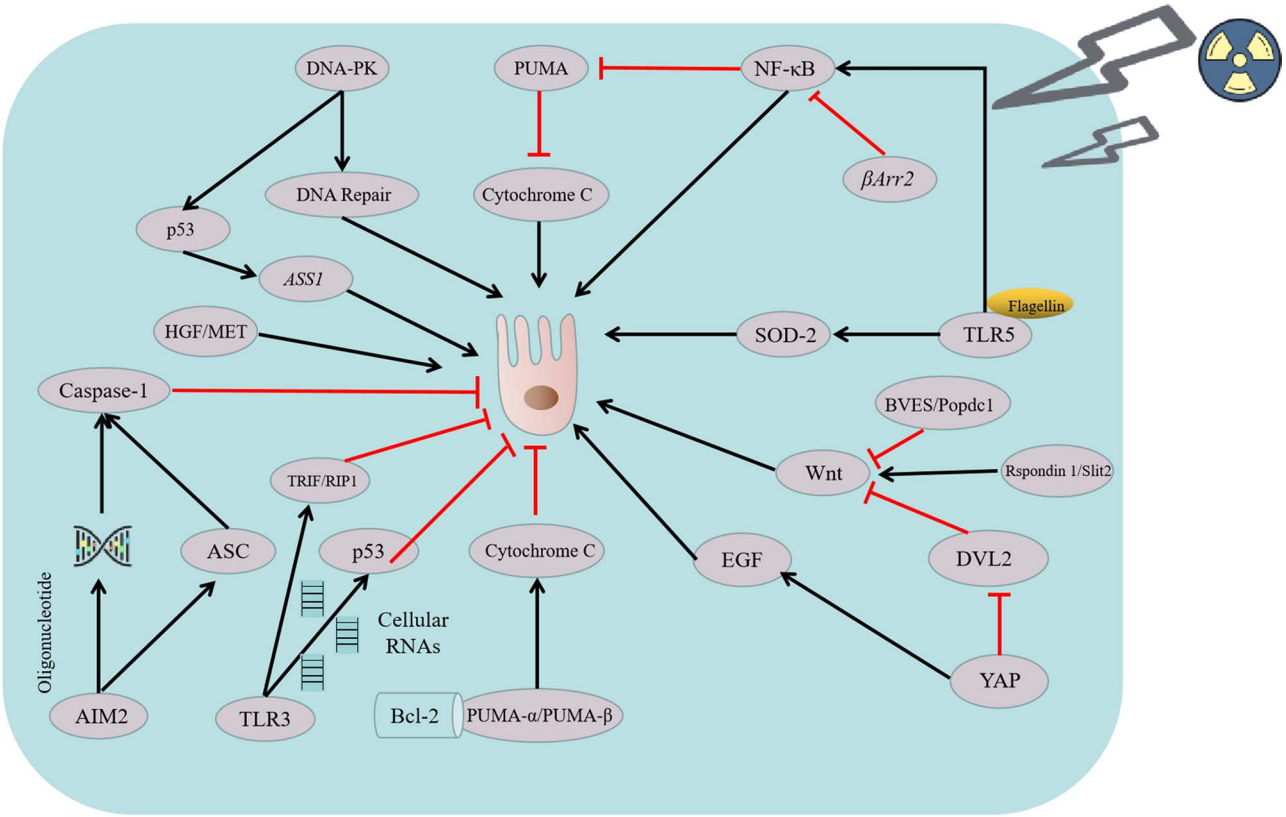

Fig. 1. Signaling pathways modulating radiosensitivity of ISCs.

In addition, as one of the receptors for hepatocyte growth factor (HGF), MET Proto-Oncogene (MET) was reported to support the proliferation and differentiation of ISCs, in which the ablation of MET exacerbated radiation-induced damage on intestine [36]. As the deprivation of MET was reported to trigger the stimulation of p53-p38 signaling and c-Met mediated Wnt/β-catenin pathway [37, 38], whether the radioprotective effect of MET on ISCs was involved in p53 or Wnt signaling remains elucidation.

The interconversion of ISCs during radiation injury

Under homeostatic conditions, crypt base columnar cells (CBCs) which express leucine rich repeat–containing G protein-coupled receptor 5 (Lgr5), can divide and migrate upward to transform into the progenitors or TA cells that will differentiate and then generate the villi [39–41], but the highly proliferative rate of CBCs inevitably accumulate a mass of DNA damage leading to cell death with intense radiation. At the +4–5 position from the base of the crypt, there is a few numbers of reserve intestinal stem cells (rISCs) population that remain slow proliferation and quiescent states [42]. The atypical homeobox protein Hopx [43], polycomb group protein Bmi1 [44], Mouse telomerase reverse transcriptase mTert [45], the smallest known acid keratin Krt19 [46], and the Pan-ErbB negative regulator Lrig1 [47] were found to be the specific markers of rISCs. These kinds of ISCs are radioresistant and can survive after radiation damage thereafter contributing to the regeneration of Lgr5-expressing cells or other intestinal epithelial lineages. For example, Krt19-experssing cells that are normally restricted in the proliferating compartments (including stomach, small intestine, colon, the pancreatic ducts and the hepatobiliary ducts) [48], represent the first kinds of ISCs demonstrated to be interconverted with radiosensitive ISCs ($Lgr5^+$). In the lineage-tracing experiment, although the surviving intestinal cells upon radiation exposure mainly expressed high level of Krt19, $Lgr5^+$ cells could also generate a quiescent stem cell population, such as $Krt19^+$ cells, in certain physiological conditions [46]. Little is known about the mechanisms modulated the interconversion between radioresistant ISCs and their radiosensitive states. Tao *et al.* have demonstrated that ISCs with high Wnt/β-catenin activity are more susceptible to DNA damage caused by γ-irradiation as compared to ISCs located in +4 position with minor Wnt activity [49]. Besides, the quiescent ISCs expressing Bmi1 are more insensitive to Wnt perturbations as compared to the radio-sensitivity ISCs ($Lgr5^+$), in which the gain or loss of Wnt function did not influence the proliferation of $Bmi1^+$ ISCs [44]. Recently, unconventional prefoldin RPB5 interactor (URI) was reported to maintain the radio-resistance of rISCs via inhibiting β-catenin activity [7]. Thus, the Wnt/β-catenin signaling might play an important role in this modulatory process. Consistent with this, SOX9 that is indispensable for the generation and maintenance of radioresistant ISCs was demonstrated to be the negative regulator of Wnt target genes [50, 51]. Nevertheless, there are other kinds of mechanisms modulating the interconversion between ISCs. Musashi (Msi) RNA-binding proteins, which are translational

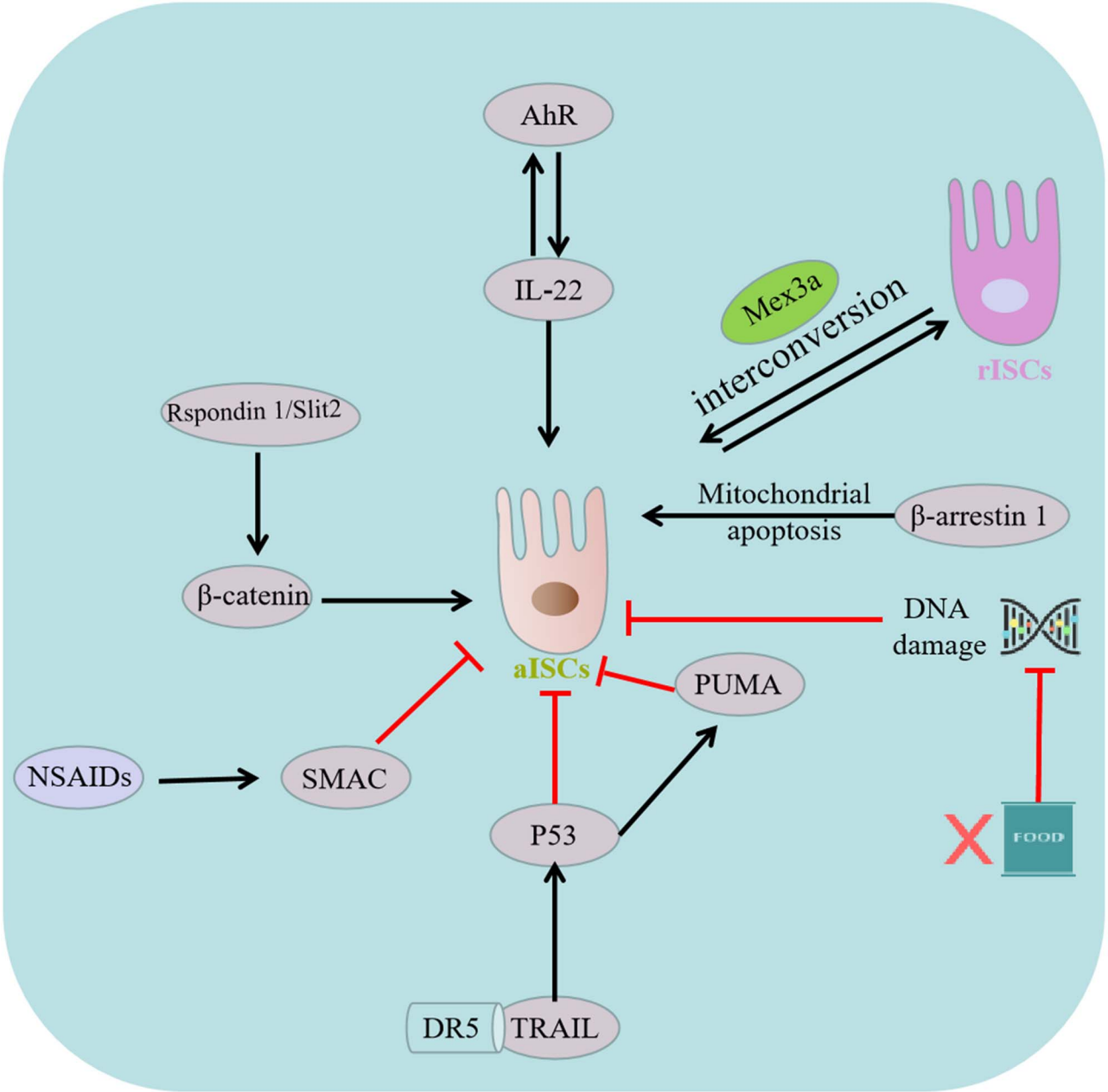

Fig. 2. Signaling pathways modulating susceptibility of ISCs upon chemical agent damage. aISCs, active intestinal stem cells; rISCs, reserve intestinal stem cells.

regulators binding to messenger RNAs, was reported to facilitate the entrance of rISCs into cell cycle in a Wnt-independent manner [41, 52, 53]. A recent study reported that clusterin (CLU) was highly expressed in the revival stem cells that was quiescent and radio-resistant [54], and the stimulation of YAP ultimately induced ectopic CLU^+ cells, which could reconstitute $Lgr5^+$ cells and then stimulated intestinal regeneration.

Differentiated intestinal cells may also imitate the role of quiescent ISCs and give rise to proliferative ISCs and villus epithelial cells upon radiation challenge. Evidence comes from a study showing that the post-mitotic intestinal epithelial cells and paneth cells acquired a stem cell-like transcriptome and differentiated into villus epithelial cells, and this process was forced by the activation of Notch signaling pathway upon radiation injury [55]. $Dll1^+$ secretory progenitors was also demonstrated to revert into stem cells upon radiation damage, and Wnt signals was essential in this physiological process [56]. More efforts are required to clarify the underlying mechanism regulating the quiescent ISCs maintenance and conversion, which is conducive to providing strategy for activating intestinal cells regeneration and radioactive enteritis treatment after radiation damage.

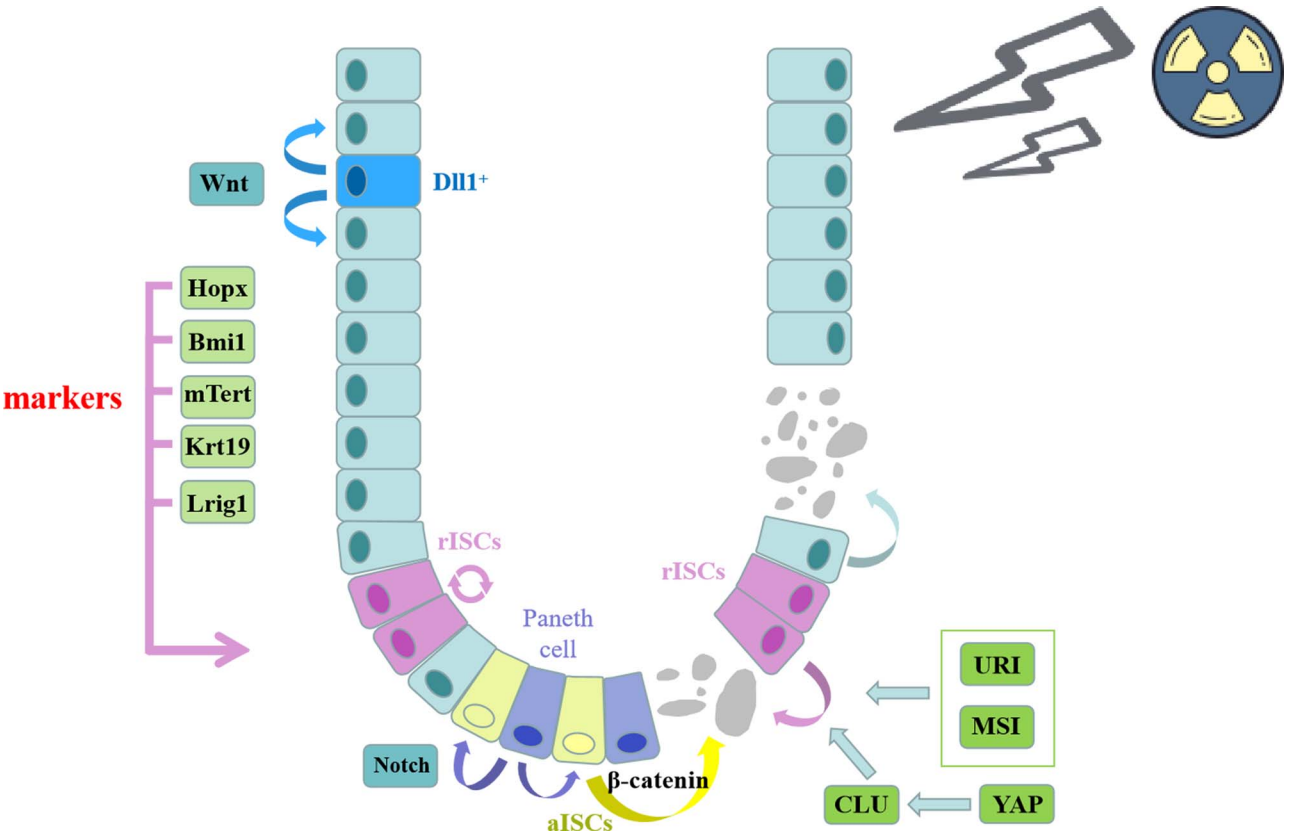

Fig. 3. Schematic diagram about the interconversion between aISCs and rISCs upon radiation exposure. aISCs, active intestinal stem cells; rISCs, reserve intestinal stem cells.

Maintenance of ISCs during radiation injury with gut microbiota

There are trillions of symbiotic microbes harboring mammalian GI tract, and the dysbiosis or imbalance of gut flora caused by radiation is closely linked to the prognosis of victims due to the high diversity among individuals [57]. Correspondingly, transplantation of fecal microbiota from elite-survivors those are radio-resistance, supplement of probiotics or treatment with microbe-derived metabolites significantly mitigate radiation-induced hematopoietic and GI syndromes [57–60]. The radio-protective effect on intestine by manipulation of microbes remains far from elucidation, which might be involved in the restoration of gut bacterial composition structure, inflammatory response, or crypts microenvironment modulation [58, 59, 61, 62], etc. TIPE0 belongs to the tumor necrosis factor-alpha-induced protein 8-like (TNFAIP8-like, or TIPE) family that was reported to regulate PI3K/Akt/β-catenin pathway mediated by commensal microbiome, and *Tipe0*$^{-/-}$ mice were resistant to radiation enteritis via the dysregulation of the revival stem cells program [63]. As for proliferative stem cells, the erythroid differentiation regulator-1 (Erdr1) regulated by early-life microbiota and lactate produced by lactic-acid-producing bacteria (LAB) remarkably improved survival rate of Lgr5^{+} ISCs in a Wnt/β-catenin-dependent manner [62, 64]. Based on this, properly targeting the intestinal flora could alleviate radiation-induced GI tract injury via maintaining ISCs regeneration. For example, the modulation on the balance of gut microbiota by vanillin derivative VND3207 or carbon nanoparticles suspension injection significantly mitigated radiation enteritis via protecting ISCs [65, 66]. Nonetheless, how these compounds stimulate probiotics growth but inhibit harmful bacteria proliferation thereby relieving ISCs injury would favor the development of alternative treatment strategy counteracting radiation enteritis and deserves further investigation.

CHEMICAL AGENT DAMAGE

Damage on GI caused by chemotherapeutic agents seriously influence the life quality and prognosis of patients receiving chemotherapy. For example, as the major choice of chemotherapeutics for colorectal cancer, irinotecan and 5-fluorouracil inevitably induce severe gene damage on GI cells via the accumulation of SN-38 and inhibition of homologous recombination repair, respectively [67] . Besides, dextran sulfate sodium (DSS) is the agent most extensively employed to induce colitis which mimic inflammatory bowel diseases of human [68]. DSS treatment ultimately leads to acute injury including the atrophy of intestinal crypts and even the necrosis of intestinal villus. Thus, the exploration of the underlying mechanism involved in ISCs injury or

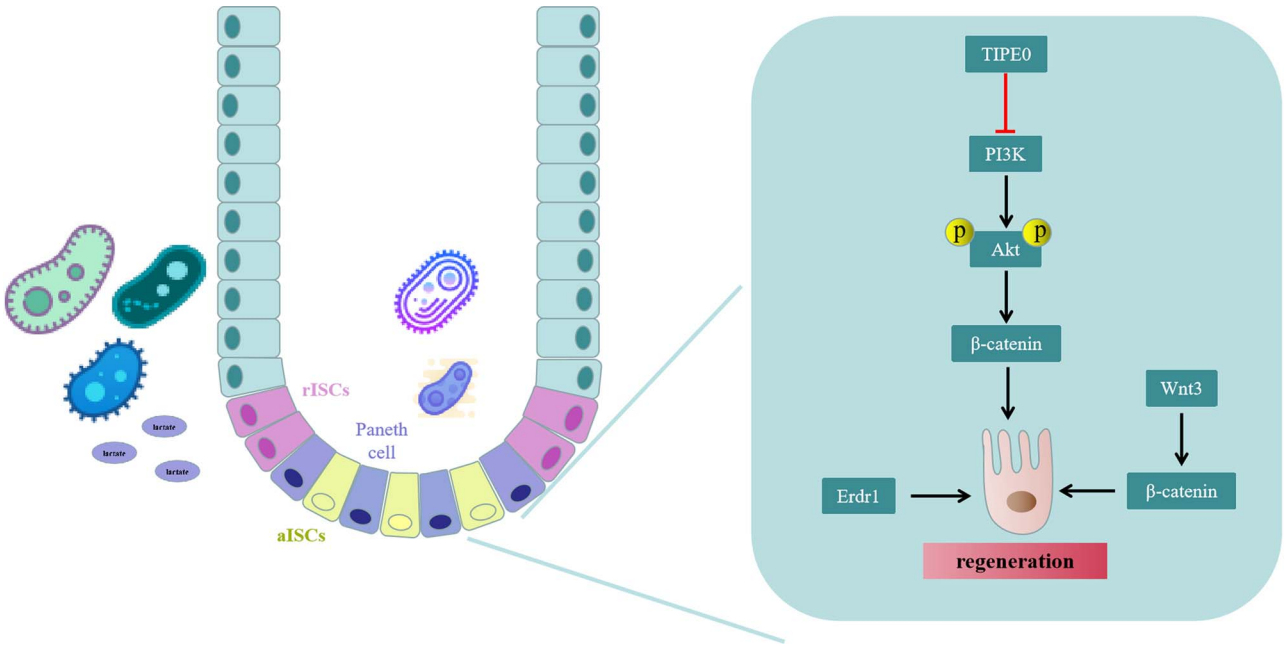

Fig. 4. The influence of gut microbiota on ISCs upon radiation exposure. aISCs, active intestinal stem cells; rISCs, reserve intestinal stem cells.

regeneration upon chemical agent damage might facilitate the development of novel strategy to alleviate GI side effects of patients receiving chemotherapy.

The sensitization of ISCs to chemical agent damage

DNA damage induced by chemical agents is the main mechanism mediating intestinal injury, in which the stimulation of DDR pathway might facilitate the tissue repairment. Based on this theory, Kelsey *et al.* found that the dietary restriction could improve the viability of ISCs thereby restoring intestinal barrier by mitigating DNA damage upon etoposide treatment. Besides, the numbers of ISCs labeled by Lgr5, Bmi1 or HopX were significantly increased after dietary restriction and whether the transformation between different types of ISCs stimulated intestinal repairment remains further investigation [69]. Similar to that of radiation injury, p53 pathway is also essential in mediating ISCs toxicity upon chemical agents treatment, in which the depletion of Lgr5-positive ISCs upon irinotecan exposure was significantly inhibited with PUMA ablation [70]. Thus, the modulation of p53 during chemotherapy is likely to ease the side effects caused by chemotherapeutic drugs. For instance, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor superfamily, and the stimulation of TRAIL will aggravate GI injury induced by chemotherapeutic drugs, in which the massive death of Lgr5-positive ISCs was mediated the p53 pathway [71]. The stimulation of Wnt and Notch signaling pathways might be involved in the adverse effects of p53 pathway on GI, and the evidence comes from the elimination of ISCs induced by hyperphosphorylated β-catenin in $APC^{Min/+}$ mice with non-steroidal anti-inflammatory drugs (NSAIDs) via the second mitochondrial activator of caspases (SMAC)-mediated cell apoptosis [72].

Mechanisms protecting ISCs from chemical agent damage

Interleukin 22 (IL-22) is a cytokine produced by three types of innate lymphocytes as well as $\gamma\delta$ T cells. Gronke *et al.* have demonstrated that IL-22 is essential for DDR pathway regulation in ISCs upon chemical agents exposure, in which the upregulated levels of ISCs apoptosis induced by DSS was remarkably inhibited with IL-22 suppression, and the protective effect of IL-22 on ISCs against genotoxic stress required the expression of aryl hydrocarbon receptor (AhR) in ILC3 and T cells [73]. β-arrestin1 is a member of the G protein-coupled receptor family, which has been demonstrated to be critical for the development of sepsis, cerebral ischemia and asthma. Zhan *et al.* have shown that the knock-down of *β-arrestin1* gene aggravated chemotherapy-induced ISCs apoptosis, and the process was involved in the ER stress-mediated mitochondrial apoptosis signaling pathway [74]. R-spondin 1 and Slit2 that are primarily expressed in ISCs also play an essential role in protecting small intestinal crypt cells against chemical injury, and the over-expressed of Slit2 or pretreatment with recombinant R-spondin 1 or Slit2 protein could significantly improve the survival rate of mice upon 5-fluorouracil injury [31].

The transformation of reserve stem cells into active stem cells also plays a vital role in repairing chemotherapy-induced intestinal injury. Mex3a protein is a member of the Mex family (the other Mex family proteins include Mex3b, Mex3c and Mex3d), and it contains a highly conserved RNA binding sites and C-terminal loop sites of E3 ubiquitin ligase. Upon 5-fluorouracil damage, ISCs expressing high level of Mex3a protein would survive and then promote the regeneration of intestinal cells at the late stage of injury [75, 76]. Therefore, Mex3a might be a promising drug target to improve the tolerance of patients receiving chemotherapy.

CONCLUSION

As the basic resource for intestinal regeneration, ISCs are regulated by precise and elaborate mechanisms to sustain their cell cycles upon external hazards exposure or under homeostatic conditions. As for radiation injury, molecules such as AIM2, YAP, TLR3, PUMA and BVES exacerbate GI syndrome by increasing radiosensitivity of ISCs, while TLR5, HGF/MET signaling pathway, *Ass1* gene and Slit/Robo signaling can facilitate the radio-protection on ISCs (Fig. 1). As for chemical agent damage, the stimulation of TRAIL or p53 aggravates toxicity on ISCs, whereas the overexpression of IL-22 or β-arrestin1 can significantly relieve the damage (Fig. 2). In particular, the stimulation of Wnt signaling pathway with Rspondin 1 could simultaneously alleviate damages induced by radiation and chemical agents on ISCs, which might facilitate the development of drug prophylaxis for patients receiving chemoradiotherapy.

Recently, quiescent ISCs have attracted great attention due to their radiation or chemical resistance, in which the conversion of rISCs into active ISCs (aISCs) upon external damage might be the promising strategy to ease the adverse effects during chemoradiotherapy (Fig. 3). As there are trillions of gut flora harboring mammalian GI tract, the modulation of symbiotic microbes balance has been demonstrated to alleviate GI toxicity upon radiation exposure, but the underlying mechanisms remain further investigation (Fig. 4).

To sum up, various genes or proteins have been confirmed to be the potential targets to reduce intestinal injury via regulating the activities of ISCs, and the designing of the relevant agents will improve the life quality of patients receiving chemoradiotherapy or victims of nuclear or chemical accidents.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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