Radiation-induced intestinal damage: latest molecular and clinical developments

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Practice points

- Patients undergoing radiation therapy for tumors in the pelvis, abdomen and colorectal system generally develop short-term enteritis, but as many as 15% develop chronic intestinal problems.
- Radiation-induced intestinal damage (RIID) leads to a loss of quality of life in patients of a range of cancer types.

The mammalian intestinal epithelium & the roles of intestinal stem cells

- The renewal of the gastrointestinal (GI) mucosal epithelium following injury is dependent on GI resident stem cells (SCs) which undergo self-renewal over extended time-periods.
- Active intestinal SCs are the major cells governing epithelial renewal and rapidly divide to progenitor cells in the transit-amplifying region.
- Reserve intestinal SCs are stress-resistant quiescent SCs with proliferative potential, which are activated in response to perturbations to active intestinal SCs.

GI response to radiation: lessons learned from animal models

- Radiation causes DNA damage, most selectively in rapidly dividing cells that include cancer cells and healthy cells that undergo self-renewal.
- By means of transgenic and knock-out mouse models, the signaling cascades regulating ISC renewal have been characterized, including Wnt, bone morphogenetic protein, Notch, Ephrin, JAK/STAT1, PTEN, AKT and PI3K.
- Endothelial dysfunction mediates the response to radiation in GI tissues.

Recent advances in RIID treatment

- Prophylactic and therapeutic strategies include improved radiotherapy techniques, natural anti-oxidation agents such as vitamins and amino acids, known in vivo protective agents, newly synthesized compounds and biotechnological approaches targeting known RIID pathways.

Future perspective

- Due to the increased lifespan of cancer patients, understanding the mechanisms of GI regeneration following radiotherapy is critical to developing therapeutic approaches that protect ‘healthy’ intestinal tissue from radiation injury.
- Targeting the global effects of radiotherapy on the intestinal epithelium as opposed to individual subsets of intestinal cells and ISCs that contribute to regeneration is most likely to achieve the highest therapeutic efficacy.
- The next challenge is to forward the recently identified anti-RIID compounds into human studies to improve the quality of life of current and future cancer patients.

Aim: To systematically review the prophylactic and therapeutic interventions for reducing the incidence or severity of intestinal symptoms among cancer patients receiving radiotherapy. Materials & methods: A literature search was conducted in the PubMed database using various search terms, including ‘radiation enteritis’, ‘radiation enteropathy’, ‘radiation-induced intestinal disease’, ‘radiation-induced intestinal damage’ and ‘radiation mucositis’. The search was limited to in vivo studies, clinical trials and meta-analyses published in English with no limitation on publication date. Other relevant literature was identified based on the reference lists of selected studies. Results: The pathogenesis of acute and chronic radiation-induced intestinal damage as well as the prevention and treatment approaches were reviewed. Conclusion: There

is inadequate evidence to strongly support the use of a particular strategy to reduce radiation-induced intestinal damage. More high-quality randomized controlled trials are required for interventions with limited evidence suggestive of potential benefits.

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Surgery, radiation and chemotherapy remain the mainstay treatment regimens for cancer [1–3]. With advancements in medicine and related technologies, notable improvements in drug delivery, dosing regimens and treatment combinations have emerged, but the toxicity to normal ‘healthy’ tissue remains an issue [4]. In 2016, there were approximately 10.5 million cancer survivors in USA, among which 3.05 million received radiation therapy and this treatment accounted for 29% of all cancer cures/remissions [5]. With increased knowledge of the molecular and cellular basis of radiation therapy, information on the side effects is urgently required in order to treat and/or prevent healthy tissue damage through the use of protective adjuvant agents [6].

Patients undergoing radiation therapy for tumors in the pelvis, abdomen and colorectal system generally develop short-term enteritis, but a majority develop chronic intestinal problems [7–11]. The intestine is particularly sensitive to ionizing radiation, leading to side effects that include vomiting, weight loss, anorexia, dehydration, diarrhea and infections [12–15]. When the intestinal damage is severe, septic shock-induced death can also occur. Radiation-induced intestinal damage (RIID) affects the treatment, leading to a loss of quality of life in patients of a range of cancer types (Table 1). Tissue damage and disrupted healing due to repetitive radiation exposure over the course of treatment results in alterations of the tissue architecture and functions. RIID can be categorized into acute or chronic type. Acute RIID occurs during or up to 90 days after radiation treatment and is characterized by crypt cell apoptosis that leads to the breakdown of the epithelial barrier and subsequent inflammation [16]. The symptoms of acute radiotoxicity are usually reversible and include nausea, bloating, diarrhea, abdominal pain and fatigue. However, such symptoms may necessitate dosage reduction and treatment interruption, which might compromise the efficacy of the treatment. On the other hand, chronic RIID is delayed and occurs after a latency period of 90 days to several years after radiotherapy [17,18]. This condition leads to a loss of gastrointestinal (GI) function and is associated with vascular sclerosis and fibrosis of the GI wall [19]. A key phenotype of chronic RIID is the loss of intestinal stem and progenitor cells. Since several sections of the GI tract that lie in the radiation beam path can be affected, the symptoms can be associated with more than a single physiological cause. Chronic RIID symptoms are very common, with about 90% of patients experiencing permanent bowel habit changes, and up to 30, 40 and 66% of urological, gynecological and colorectal cancer patients, respectively, reporting chronic GI symptoms [20]. Therefore, this has subsequently emerged as one of the major targets of radiation protective drugs [21,22].

Herein, we will discuss the causes of RIID, our increased understanding of its effects at the molecular level and the very recent developments in the treatment approaches. A variety of different strategies have been proposed to reduce its impact on normal tissues and prevent adverse GI effects. These recent prophylactic and therapeutic strategies include improved radiotherapy techniques, natural anti-oxidation agents such as vitamins and amino acids, known in vivo protective agents, newly synthesized compounds and biotechnological approaches targeting known RIID pathways.

The mammalian intestinal epithelium & the roles of intestinal stem cells

In humans, the columnar epithelium lines almost all organs of the digestive tract. The columnar epithelium of the GI system is single-layered and represents one of the body’s most rapidly renewing tissues [23–25]. The renewal of the GI mucosal epithelium following injury is dependent on GI resident stem cells (SCs) which undergo self-renewal over extended time-periods, serving as an internal repair system with limitless capacity for division [26]. Each adult tissue contains its own unique SC subsets. Generally, SCs originate from the embryo during blastocyst development (embryonic) or are nonembryonic in origin and are therefore termed somatic cells [25]. SCs are distinguished from other cell types by their ability to renew and divide after long periods of inactivity, and their induction into tissues with specialized biological functions in response to well-characterized physiological stimuli [21]. The GI epithelial villus/crypt and the neighbouring pericryptal fibroblasts and mesenchyme constitute a well-characterized anatomical unit that is responsible for the production of four major cell lineages, including absorptive enterocytes,
goblet cells, enteroendocrine cells and tuft cells [27–29]. The crypt encompasses a pocket of epithelial cells at the villus base, at which GI SCs (ISCs) are activated to produce progenitor cells committed to mature cell lineages [27]. The crypt contains undifferentiated cells which divide, differentiate and migrate up the differentiated compartments of the GI villi (Figure 1) [25].

Active ISCs (aISCs) are the major cells governing epithelial renewal [30]. They can be identified through the expression of LGR5, ASCL2, OLFM4, SMOC2, PROM1 and SOX9lo (reviewed in [25]). aISCs termed crypt base columnar cells, rapidly divide to progenitor cells in the transit-amplifying (TA) region [28]. The second type of ISCs are stress-resistant quiescent SCs, in other words, reserve ISCs (rISCs) which are activated in response to perturbations to aISCs [25]. rISCs are persistent dormant cells with proliferative potential [25,26]. The ability to trace the expression of the reporter genes through reporter tagging showed that aISCs and rISCs possess the principal characteristics of SCs, namely self-renewal ability and the capability of producing differentiated cells. The TA zone of the crypt also contains lineage-committed progenitor cells that express either ALPI (enterocyte progenitors), MATH1 (secretory progenitor cells) and KRT19 (rISCs and TA cells).

In the crypt base, the ISC microenvironment is composed of the extracellular matrix (ECM) and the resident cells embedded in the ECM [31]. The ECM comprises a network of proteoglycans and glycoproteins which act as scaffolding to the intestinal architecture [32]. The resident cells include fibroblasts, endothelial cells, immune cells, smooth muscle cells, etc., which secrete various matrix components and growth factors to control ISC proliferation.
and differentiation. Communication between the microenvironment and the ISCs is modulated by the Wnt/β-catenin, Notch, epidermal growth factor, bone morphogenic protein(transforming growth factor-β) and Hedgehog signaling pathways to maintain proliferation and differentiation homeostasis [33]. Perturbations in these pathways or ECM homeostasis due to radiotherapy, toxins, chemotherapy and malnutrition can affect the ISC niche, leading to increased intestinal injury.

GI response to radiation: lessons learned from animal models
The underlying mechanisms for the development of RIID are highly complex. RIID can be divided into two stages: mucosal breakdown and subsequent inflammation [34]. Since the sensitivity of a cell type to ionizing radiation is dependent upon its mitotic rate and the extent of its differentiation, cells lining the GI tract are vulnerable to the radiation-induced damage, with SCs being most radiosensitive. In the chronic phase, fibroblasts stimulated upon radiation exposure induce proliferation and collagen deposition, eventually resulting in tissue fibrosis [35]. During the acute phase, mucosal breakdown due to decreased expression of tight junctions and epithelial cell death results in inflammation [36]. Damaged vascular endothelium stimulates the secretion of cytokines, chemokines and growth factors as well as the expression of adhesion molecules [35]. The presence of polymorphonuclear neutrophils is an indication of acute inflammation and is a hallmark of irradiated tissues [37]. Additional recruitment of monocytes and the activation of resident mast cells stimulate the production of proinflammatory and profibrosing mediators IL-1β, IL-6, TGF-β and TNF-α [34]. Moreover, excessive and sustained reactive oxygen species generation injures healthy cells and is associated with chronic RIID. Altogether, the response of intestinal tissues to radiation is mediated by cell death as well as activation of oxidative and inflammatory components.

By means of transgenic and knock-out mouse models, the signaling cascades regulating ISC renewal have been characterized, including Wnt, bone morphogenic protein, Notch, Ephrin, JAK/STAT1, PTEN, AKT and PI3K [38,39]. More recent studies highlighted the role of miRNAs in both ISC differentiation and proliferation [40,41]. Perturbations of these key pathways alter GI mucosal growth and induce GI damage, and/or GI tumorigenesis. As such, knowledge of these pathways is the key to our ability to reduce or prevent RIID.

Endothelial dysfunction mediates the response to radiation in GI tissues [23,42,43]. Radiation causes DNA damage, most selectively in rapidly dividing cells that include cancer cells and healthy cells that undergo self-renewal. Several major studies assessing the effects of ionizing radiation on the GI tissues have been performed in mice (Figure 2) [12,44–49]. In this regard, Du and colleagues recently identified the optimal radiation dose for GI damage studies in Non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice [45]. Doses
of 5 Gy irradiation in mouse models led to the major symptoms of RIID including decreased body weight, GI mucosa damage and inflammatory cell shedding. The survival of these animals following 5 Gy dosing permitted the assessment of anti-RIID compounds. Clinically relevant γ-irradiation doses of 8 to 15 Gy induce RIID in mice and are eventually lethal due to bone marrow failure [45,46,50]. At doses ≤14 Gy, the mouse intestinal epithelia can regenerate, indicated by animal survival following bone marrow transplant [51]. In contrast, doses ≥15 Gy produce acute RIID, radiation syndrome and mortality, for which bone marrow transplant has no benefits [45]. Knowledge about the optimal radiation doses required for RIID studies is essential for testing various therapeutic strategies.

The intestinal response to RIID consists of apoptotic, proliferative and normalization phases [52–55]. The apoptotic phase in mice undergoing RIID constitutes crypt loss, shrinkage and shortening of the villi [16,56,57]. This is followed by the regenerative phase during which the surviving crypt cells act in unison to regenerate entire crypt [28,58]. The total number of crypts subsequently decrease in the intestine and the crypts undergoing regeneration enlarge and divide more rapidly [59]. During the final normalization phase, crypt size and villi length recover to their pre-RIID conditions. Understanding the mechanisms by which each of these phases is regulated has been crucial to the generation of potential therapeutic approaches to intestinal epithelial regeneration following RIID.

Recent advances in RIID prevention & treatment

With the advent of more efficacious treatments, cancer patients are surviving longer. Despite the increasing occurrence of cancer therapy-related side effects including RIID, only symptomatic treatments for diarrhea, dehydration, malabsorption and abdominal discomfort are available [6,51]. To date, only amifostine (ethiofos, 1, Figure 3) has been used as a cytoprotective adjuvant to reduce radiation-induced symptoms in cancer patients due to its capacity to scavenge free radicals [60–67], but its use as a RIID adjuvant is limited. A few randomized Phase II trials explored the radioprotective effect of amifostine in RIID [68,69]. It also reduces xerostomia in patients undergoing radiotherapy for head and neck cancer [70]. Due to the larger number of cancer patients receiving radiotherapy and its critical importance to cancer cures, therapeutic approaches that can circumvent the undesirable effects of radiation on the intestinal epithelium have attracted immense attention and exciting advances in this area are discussed herein.

Radiotherapy delivery techniques

Intensity-modulated radiotherapy prevents toxicity of normal tissues related to radiotherapy owing to a variety of treatment shapes and steep dose gradients [71]. It uses numerous small beams with nonuniform intensities in the volume of tumour tissue, while conventional radiotherapy employs fewer beams with uniform intensities. Up to 40% decrease in intestinal radiation dose using intensity-modulated radiotherapy has been demonstrated relative to 3D-conformal or conventional whole pelvic radiotherapy [71]. Even though this method seems promising, chronic symptoms can present several years following radiotherapy, thus long-term data needs to be collected. In contrast, image-guided radiotherapy employs imaging for visualization of the radiotherapy target volume and anatomy for accuracy [72]. This technique has the potential to reduce the setup margin for a tumor site, decreasing the radiation exposure to healthy tissue. Four dimensional-adaptive radiotherapy involves a combination of intensity-modulated and image-guided radiotherapies to take into account the 3D-tumor shape over the course of treatment by tracking tumor motion [73]. Since RIID is more common at higher radiation doses, restricting the extent of normal tissues exposed to high doses using dose-volume constraints is essential [74].

The position of a patient during radiotherapy can also affect RIID. A systematic review suggested that radiotherapy to patients in the prone position, and utilizing a positioning device such as belly board, might decrease the volume of intestine irradiated [75]. In addition, radiotherapy in the morning may cause greater RIID since GI cell proliferation follows a circadian rhythm and is the highest in the morning and lowest in the evening [76]. Typically, the total prescribed radiotherapy dose is administered over a period of several weeks in a number of dose fractions. Increasing the fraction sizes (hypofractionation) for each treatment might allow the entire dose to be administered in fewer steps. A number of randomized trials on hypofractionation in prostate cancer have been carried out [77,78]. Moderate fractionation (2.5–4 Gy/fraction) results in chronic RIID similar to conventional fractionation, while extreme fractionation (5–10 Gy/fraction) may cause greater RIID compared with conventional fractionation [79].

Pharmacological interventions

Amino acid & vitamin adjuvant therapies

β-Hydroxy-β-methylbutyric acid (HMB, 2) is naturally produced through the breakdown of leucine in the body and has been used to promote wound healing. Yavas et al. evaluated the effects of a blend of HMB, L-glutamine
Figure 3. Examples of protective adjuvants against radiation-induced intestinal damage.
and L-arginine (5.2, 29.6 and 29.6 g, respectively, orally administered) in RIID rat models induced with 12.5 Gy abdominal irradiation and revealed their ability to diminish intestinal damage through their protective effects on surface epithelium, GI inflammation, cryptitis, crypt distortion and fibrosis [80]. This highlighted the protective properties of amino acids and their metabolic products on rat intestines. However, glutamine failed to induce protection against acute RIID in a double-blind randomized clinical trial [81].

Rebamipide (3) is an amino acid derivative of 2-(1H)-quinolinone that has frequently been shown to promote mucosal protection and the healing of gastroduodenal ulcers. The beneficial effects of rebamipide are mediated through its ability to enhance free radical scavenging and to inhibit COX-2. With this in mind, Sim and co-workers investigated the protective effects of rebamipide in C57BL/6 mice exposed to 13 Gy of radiation [82]. Rebamipide oral administration (200 mg/kg/day) subsequent to RIID promoted intestinal barrier recovery and the expression of the tight junction components damaged by irradiation. It simultaneously reduced the levels of proinflammatory cytokines, matrix metallopeptidase 9 (MMP9) and increased intestinal cell division. It is thus clear that rebamipide holds promise to reverse the impairments of the intestinal barrier in mouse models of RIID.

Vitamins E, A, C and B have been demonstrated as promising GI radioprotectors. Gamma-Tocotrienol (GT3, 4) is a vitamin E that has been demonstrated to confer protection to RIID in vitro [83]. While the benefits of this vitamin have long been recognized, the molecular targets that underlie its protective functions in vivo have only recently been appreciated. For instance, Banerjee and colleagues demonstrated that mice devoid of the CCAAT enhancer binding protein delta (Cebpδ(-/-)), a basic leucine zipper transcription factor, displayed higher RIID-related mortality, confirming that Cebpδ is RIID protective [83]. Following total body irradiation (6 or 8.5 Gy), GT3-pretreated (200 mg/kg, subcutaneously) Cebpδ(-/-) mice demonstrated a recovery of white blood cell numbers relative to GT3-treated Cebpδ(+/+) mice that had fewer GI crypt colonies, elevated inflammatory cytokine levels and increased oxidized glutathione, S-nitrosoglutathione and 3-nitrotyrosine expressions. In addition, just radiation, as well as a combination of radiation and GT3, was shown to augment plasma granulocyte-colony stimulating factor (G-CSF), which is a mediator of the radioprotective actions of GT3. Thus, the potential role of Cebpδ was confirmed in GT3-mediated protection against RIID, independent of G-CSF. Knowledge of the GT3 mechanism of action now increases its potential utility as a co-therapy for RIID with other protective drugs that act through divergent pathways. In another human study, chronic radiation enteritis/proctitis patients treated with a combination of pentoxifylline (a xanthine derivative) and tocophersols showed symptomatic improvement [84].

Pyridoxamine, a form of vitamin B6, has been demonstrated to prevent apoptosis of intestinal epithelial cells in mice exposed to 4 or 8 Gy whole body irradiation [85]. On the other hand, radioprotective activity of vitamin A is unclear. For example, vitamin A could not protect intestinal function in mice exposed to 7–10 Gy whole body irradiation [86]. However, a combination of vitamins A, C and E prior to irradiation protected the intestinal function under identical conditions [87]. Administration of vitamin C prior to irradiation improved survival by reducing free radical metabolites in mice exposed to 7–8 Gy whole-body irradiation [88]. No clinical studies have been conducted so far on the individual effects of vitamins in radiotherapy. In a pilot study, vitamin E and C combination supplementation improved diarrhea and other symptoms in prostate or gynecological cancer patients exposed to radiotherapy [89].

**Repurposing of known in vivo therapeutic agents**

In recent studies, Bhanja and colleagues showed that BCN057 (5), a known antineoplastic agent, induces ISC proliferation and GI repair following RIID in vivo [27]. In the study, C57Bl6 male mice were injected with BCN057 (90 mg/kg, subcutaneously) 24 h after abdominal irradiation of 16 Gy and its ability to recover Lgr5-positive ISCs was established in Lgr5-EGFP-Cre-ERT2 reporter mice. Treatment with BCN057 following the dose of otherwise lethal radiation rescued the ISCs and promoted mice GI regeneration, whereas irradiated mice that did not receive BCN057 suffered 100% mortality. While these data are exciting, BCN057 did not display any radioprotective activity in colon tumor tissues which may limit its efficacy as an anti-RIID monotherapy.

Geranylgeranylacetone (GGA, 6) holds value as a therapy for peptic ulcers, but only recently have its protective effects against RIID been appreciated. Han and colleagues demonstrated that GGA (200 mg/kg, oral administration) could improve intestinal crypt survival, villi lengths and survival times in 12.5 Gy irradiated mice [42]. GGA was administered 24 and 1 h prior to as well as 24, 48 and 72 h following radiation. The effects of GGA were mediated through enhanced VEGF/VEGFR1/eNOS signaling and reduced TNF-α expression in intestinal epithelial cells. Altogether, these results highlighted GGA as a potential therapeutic agent for RIID.
Given the known ability of ionizing radiation to activate NF-κB signaling and increase the levels of inducible nitric oxide synthase and nitric oxide production in GI cells, Kong et al. investigated the protective roles of the NOS inhibitors 2-amino-5,6-dihydro-4H-1,3-thiazine hydrobromide (2-ADT, 7) and 2-acetylamino-5,6-dihydro-4H-1,3-thiazine hydrobromide (2-AADT, 8) toward RIID in mice [44]. Pretreatment with both compounds via intraperitoneal injection enhanced the survival of mice receiving otherwise lethal radiation doses (7.5 Gy whole body irradiation), with 2-ADT treatment (20 mg/kg) achieving the highest protection. The protective effects were due to accelerated hematopoietic recovery, decreased oxidative and nitrosative stress and enhanced antioxidant defenses, that culminated in reduced DNA damage.

The combination of podophyllotoxin (9) and rutin (10), termed G-003M, can induce endogenous cellular antioxidant defenses and inflammatory responses in intestinal cells. Kalita et al. highlighted the protective potential of G-003M (6.5 mg/kg, intramuscular route) through its ability to improve C57BL/6J mouse survival when administered 60 min prior to total body irradiation (9 Gy) [21]. Immunohistochemical analysis showed that G-003M promoted the renewal of Lgr5(+) crypt SCs through enhanced β-catenin nuclear translocation. The failure of G-003M to rescue Wnt knockdown cohorts confirmed that Wnt signaling mediated these anti-RIID effects. Moreover, G-003M-treated mice exhibited superior antitumor responses in comparison to the US FDA-approved amifostine, highlighting its potential as a radiotherapy adjuvant. Other natural compounds that have shown potential to be repurposed as adjuvants to radiotherapy in recent studies include chamomile extracts [90], silibinin (11) [24] and polydatin (resveratrol-3-β-mono-D-glucoside, 12) [23].

Pirfenidone (13), previously used for idiopathic pulmonary fibrosis treatment due to its ability to inhibit intestinal fibroblast proliferation and differentiation, was determined to be protective toward RIID [8]. The PPAR-gamma agonist rosiglitazone (14) was also identified as protective toward RIID in murine models due to its effects on inflammation, fibrosis and apoptosis [91]. These effects are particularly interesting given the fact that rosiglitazone is a licensed antidiabetic drug. Sucralfate creates a physical barrier over damaged mucosal surfaces and facilitates epithelial healing. A recent Cochrane analysis suggested that sucralfate may be used for the treatment of acute radiation-induced rectal bleeding [92].

In patients undergoing radiotherapy, administration of and angiotensin-converting enzyme inhibitors and statins resulted in superior acute and long-term GI symptom scores due to their vasculoprotective properties [93]. Furthermore, in a retrospective nonrandomized cohort trial, it has been demonstrated that the combination of statins and ACE inhibitors ameliorated acute GI symptoms during radical pelvic radiotherapy [94]. However, the efficacy and safety of this combination needs to be further explored in clinical trials.

Novel compounds & biotechnological strategies

Cheng and colleagues designed a novel precursor compound termed XH-105 (15) as a protective agent for RIID in vivo due to its predicted cleavage into polyphenol and aminothiol [95]. To assess its efficacy, C57BL/6J mice were subjected to XH-105 gavage (100 mg/kg) 1 h prior to 9 Gy total body irradiation, which significantly enhanced post-RIID survival rates, reduced intestinal damage, decreased intestinal cell apoptosis and minimized DNA damage [95]. The culmination of these effects led to enhanced crypt proliferation and differentiation. At the cellular level, XH-105 decreased the expressions of Bax and p53 in intestinal epithelial cells suggesting that it circumvents RIID through a reduction of p53-dependent apoptosis [95]. Therefore, this compound now warrants further assessment in human toxicity and RIID trials.

Anti-inflammatory agents such as aminosalicylates (e.g. sulfasalazine) as well as corticosteroids (e.g. dexamethasone) used for the treatment of inflammatory bowel conditions might prevent the acute inflammatory GI injury caused by radiation [96]. For instance, Elshawi and coworkers designed a novel benzopyran-4-one derivative (2E)-2-((4-oxo-4H-chromen-3-yl)methylene amino-4-nitrobenzoic acid (16), a nonsteroidal anti-inflammatory drug, to inhibit COX-2 and NF-κB (known radioprotective targets) in RIID in vivo [97]. The intraperitoneal injection of the compound (20 mg/kg) significantly reduced RIID-mediated inflammatory responses induced by 6 Gy total body irradiation. Hence, this derivative represents a promising NSAID to reduce radiation-induced inflammation through COX-2 inhibition.

R-spondin1 (RSPO1) is a cytokine that has proliferative activity on GI crypt cells. Mesenchymal SCs (MSCs) display multiple RIID-healing effects due to their ability to regulate the immune system and hone in damaged GI epithelia. In novel studies by Chen et al., the anti-RIID activity of MSCs was combined with the mitogenic ability of RSPO1 to ISC through the development of RSPO1-modified C3H10 T1/2 cells that permitted targeted RSPO1 delivery to the sites of RIID in mice [26]. RSPO1-modified C3H10 T1/2 cells (50 μl/mouse) were intravenously
administered into mice after 18 Gy abdominal irradiation. The cells migrated to the sites of injury, enhanced ISC survival, proliferation and differentiation, and repaired the injury [26]. This highlighted how the targeted delivery of RSPO1 by MSCs could ameliorate RIID, representing a novel anti-RIID strategy.

Glucagon-like peptide 2 (GLP-2) is an intestinal growth factor that improves short bowel syndrome and inflammatory bowel disease through its ability to promote intestinal cell growth. Gu and colleagues fabricated a novel degradation-resistant GLP-2 analog termed GLP-2⃞ composed of two modified GLP-2 genes with a linker and confirmed its enhanced stability over GLP-2 [98]. Notably, GLP-2⃞ (200 μg/kg/day, subcutaneous route) after 12 Gy total abdominal irradiation in mice led to the activation of signaling cascades involved in GI epithelial cell survival, highlighting its ability to reduce RIID. Hence, the promising effects of this analog warrant further investigation in clinical applications.

Nonpharmacological interventions

Probiotics
Probiotics contain microbes which alter the host microflora and potentially offer health benefits upon administration in sufficient amounts [99]. They may act by epithelial cell proliferation, stimulating secretion of mucins, inhibiting bacterial translocation and stimulating the immune response [100,101]. Radiotherapy may disturb the indigenous gut microbiome. In an interesting study, Lactobacillus rhamnosus GG ATCC 53103 supplementation in mice prevented epithelial apoptosis and enhanced crypt survival after 12 Gy whole-body radiation [102]. L. plantarum 299v supplementation reduced RIID in rats exposed to 10 Gy lower abdominal irradiation [103]. In clinical trials, a probiotic preparation VSL#3⃞ composed of strains of Lactobacilli, Bifidobacteria and Streptococcus, prevented diarrhea in patients subjected to radiotherapy and improved their quality of life [104,105]. However, cautious supplementation of probiotics is encouraged, particularly in critically ill or immunocompromised patients. In this context, complete safety tests of individual components of probiotic combinations followed by well-designed randomized controlled clinical trials are essential.

Nutritional interventions
Available evidence on dietary interventions to prevent or reduce RIID is of a low certainty. Malnutrition may occur as a consequence of RIID and may also result in the development of GI toxicity. A cochrane review evaluated several nutritional interventions in radiotherapy patients and determined that dietary fat, lactose, and/or nonstarch polysaccharides may reduce diarrhoea after radiotherapy [106]. However, another review demonstrated that there was inadequate research on nutritional interventions to guide clinical practice [101]. In a pilot study of patients subjected to pelvic radiotherapy, supplementation with psyllium hydrophilic fiber concentrate (5–20 ml/day) reduced the incidence of diarrhea [107]. Hence, clinical trials evaluating the effect of a soluble fiber-rich diet are required to determine its benefits for radiotherapy patients.

Future perspective
While cancer therapies continue to improve due to advances in sequencing technologies and precision genetic-based therapy, radiotherapy remains an essential component of cancer treatment. The ability to specifically deliver high doses of radiation to cancer tissue has improved, but studies to prevent its detrimental effects on noncancer tissues have been unfruitful. In particular, in the face of the increased lifespan of cancer patients, understanding the mechanisms of GI regeneration following radiotherapy is critical to developing therapeutic approaches that protect ‘healthy’ intestinal tissue from radiation injury. Lineage-tracing mouse models have greatly advanced our knowledge of the cell populations responsible for intestinal tissue regeneration following RIID as has the determination of the intracellular mediators of the intestinal tissue homeostasis. The recent focus on novel compound synthesis and drug repurposing strategies targeting the pathways perturbed by radiotherapy highlights the fruits of this labor. Going forward, targeting the global effects of radiotherapy on the intestinal epithelium as opposed to individual subsets of intestinal cells and ISCs that contribute to regeneration is most likely to achieve the highest therapeutic efficacy. The next challenge is to forward the recently identified anti-RIID compounds into human studies to improve the quality of life of current and future cancer patients.

Author contributions
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Papers of special note have been highlighted as: • of interest; •• of considerable interest


● Explores the regenerative capacity and mechanisms of various populations of intestinal stem cells in response to ionizing radiation.


● Focuses on what has been observed in the healthy gut and what needs to be done concerning the immunoinflammatory response after localized radiation exposure.


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● Studies the effect of different doses of radiation on intestinal injury, with an attempt to find the optimal radiation dose for establishing intestinal injury models in NOD/SCID mice.


