Directional delivery of RSPO1 by mesenchymal stem cells ameliorates radiation-induced intestinal injury

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Abstract

Radiation-induced intestinal injury (RIII) commonly occurs in patients who received radiotherapy for pelvic or abdominal cancer, or who suffered from whole-body irradiation during a nuclear accident. RIII can lead to intestinal disorders and even death given its integrity damage that results from intestinal stem cell (ISC) loss. Recovery from RIII relies on the intensity of supportive treatment, which can attenuate lethal infection and give surviving stem cells an opportunity to regenerate. It has been reported that RSPO1 is a cytokine with potent and specific proliferative effects on intestinal crypt cells. MSCs have multiple RIII-healing effects, including anti-inflammatory and anti-irradiation injury properties, due to its negative immune regulation and its homing ability to the damaged intestinal epithelia. To combine the comprehensive anti-injury potential of MSCs, and the potent ability of RSPO1 as a mitogenic factor for ISCs, we constructed RSPO1-modified C3H10 T1/2 cells and expected that RSPO1, the ISC-proliferative cytokine, could be delivered to the site of injury in a targeted manner. In this study, we transferred C3H10/RSPO1 intravenously via the retro-orbital sinus into mice suffering from abdominal irradiation at lethal dosages. Our findings demonstrated that C3H10/RSPO1 cells are able to directionally migrate to the injury site; enhance ISC survival, proliferation, and differentiation; and effectively repair the radiation-damaged intestinal epithelial cells. This study suggests that the directional delivery of RSPO1 by MSCs is a promising strategy to ameliorate, and even cure, RIII.

1. Introduction

Radiation-induced intestinal injury (RIII) is a common complication associated with radiotherapy treatment for solid organ malignancies in the abdomen or pelvis [1]. Recovery from RIII relies on the intensity of supportive treatment, which can attenuate lethal infection and give surviving stem cells an opportunity to regenerate [2]. Self-renewing intestinal stem cells (ISCs) that reside at the base of the intestinal crypts generate all types of differentiated cells, including enterocytes, goblet cells, enteroendocrine cells, and Paneth cells, to support the rapid and continuous regeneration of the intestinal epithelium. Loss of the ISCs results in the architectural impairment of the intestinal villi and crypts, and it brakes the absorptive and protective functions of the gut [3]. LGR5⁺ ISCs are mitotically active and sensitive to canonical Wnt modulation; they contribute robustly to homeostatic regeneration of the intestinal epithelia and are quantitatively ablated by irradiation [4]. Hence, protection of LGR5⁺ ISCs and reconstitution of the intestinal villi and crypts are critical in the treatment of RIII.

Mesenchymal stem cells (MSCs) are not only pluripotent progenitor cells that maintain and regenerate various connective tissues, but they also exhibit potent immunomodulatory activities [5]. MSCs can also migrate to wounded sites, owing to their chemotactic abilities [6]. Previous reports have demonstrated the therapeutic effects of MSCs in alleviating the pathological damage of gastrointestinal disorders [7,8]. In an irradiation mouse model, the adoptive transfer of MSCs contributes to reconstituting the ISC niche, and it also induces ISC regeneration by elevating the blood levels of intestinal growth factors and anti-inflammatory cytokines [9]. MSCs are currently among the most promising anti-injury candidate in the treatment of RIII.

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R-spondin1 (RSPO1) is a thrombospondin domain-containing cytokine expressed by the enteroendocrine and epithelial cells in various tissues. It is a potent and specific epithelial mitogen that stimulates the growth of the mucosa in both the small and large intestine [10,11]. It was reported that binding of RSPO1 to the conserved sites on LGR4/LGR5/LGR6, which forms the Wnt receptor and coreceptor complexes, might participate in direct signaling for LGR4/LGR5/LGR6 [12]. As the ligand of LGR5, RSPO1 acts as a Wnt agonist; it contributes to the expansion of LGR5+ damage-induced stem cells, and it also drives LGR5+ ISC proliferation and multilineage differentiation [13–16]. In all, RSPO1 is a potent cytokine that promotes reparation in response to intestinal impairment.

Altogether, we assumed that the directional delivery of RSPO1 by MSCs toward the injured tissues might exert optimal efficacy to ameliorate RIII. In this study, to combine the comprehensive anti-injury potential of MSCs and the potent ability of RSPO1 as a mitogenic factor for ISCs, we constructed C3H10/RSPO1 gene transfected MSCs, named C3H10/RSPO1; these are murine MSC C3H10 T1/2 with stably expressed hRSPO1. The full length of human RSPO1 (hRSPO1) cDNA (consistent with Genbank ID: BC114966) was subcloned into retroviral-expressing vector pEG2-Term (kindly gifted by Prof. Seiling, University of Wurzburg, Wurzburg, Germany). The recombinant plasmid, together with its helper virus vectors pHIT456 and pHIT60, were cotransfected into the packaging cell 293T. The surface epitopes of the cells were analyzed by flow cytometry assay, cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mAbs. For intracellular staining, the cells were fixed with 2% paraformaldehyde, permeabilized with 0.5% saponin, and stained with FITC- or PE-conjugated antibodies.

2. Materials and methods

2.1. Animals

LGR5–EGFP–ires–CreERT2 mice (Jackson Laboratories, Bar Harbor, ME, USA) were maintained under specific pathogen-free conditions in the animal maintenance facility of Soochow University. They were used for the experiments at 8–10 weeks of age. All animal experiments were approved by the Institution Animal Care and Use Committee (IACUC) of Soochow University.

2.2. Cell line and reagents

293T cells and the mouse MSC line, C3H10 T1/2, were originally obtained from the American Tissue Culture Collection (ATCC, Manassas, VA, USA) and maintained in our lab. The G418 and PGE2 enzyme-linked immunosorbent assay (ELISA) Kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The hRSPO1 protein was purchased from R&D Systems (Minneapolis, MN, USA).

2.3. Establishment of a genetically engineered cell line

The full length of human RSPO1 (hRSPO1) cDNA was subcloned into retroviral-expressing vector pEG2-Term (kindly gifted by Prof. Seiling, University of Wurzburg, Wurzburg, Germany). The recombinant plasmid, together with its helper virus vectors pHIT456 and pHIT60, were cotransfected into the packaging cell 293T. The surface epitopes of the cells were analyzed by flow cytometry assay, cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mAbs. For intracellular staining, the cells were fixed with 2% paraformaldehyde, permeabilized with 0.5% saponin, and stained with FITC- or PE-conjugated antibodies.

2.4. Flow cytometry

The surface epitopes of the cells were analyzed by flow cytometry (BD, San Diego, CA, USA) using a series of anti-mouse monoclonal antibodies (mAbs) (eBioscience, San Diego, CA, USA). For the direct immunofluorescence assay, cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mAbs. For intracellular staining, the cells were fixed with 2% paraformaldehyde, permeabilized with 0.5% saponin, and stained with FITC- or PE-conjugated antibodies.

2.5. Cell migration assay

Cell migration assays were performed using a 24-well transwell plate (Corning, NY, USA). Then, 100 μL of C3H10T1/2 cells (5 × 10⁵ cells/mL) were resuspended in Dulbecco’s Modified Eagle’s Medium (DMEM) without serum and placed on the upper chamber (pore size: 8 μm). Subsequently, 600 μL of DMEM containing SDF-1α (R&D Systems) with or without its blocking antibody (R&D Systems) was placed in the lower chamber. Twenty-four hours after incubation at 37°C in 5% CO₂, the upper chambers were taken out. After scrapping and removing the unemigrated cells at the top of the filter of the upper chamber, the upper chamber was fixed with cold phosphate buffered saline (PBS) and 4% paraformaldehyde (PFA) for 30 min; it was then stained with 1% crystal violet in 70% ethanol for 30 min. This was followed by washing with PBS and the chamber was set to air dry. 10 high-power fields (HPF) per chamber were selected randomly for cell counting.

2.6. Crypt isolation

The intestines were opened longitudinally and washed with cold PBS. The tissue was chopped into 5 mm pieces. After washing with cold PBS, the tissue fragments were incubated with a solution of PBS and 2 mmol/L of ethylenediaminetetraacetic acid (EDTA) for 30 min on ice. Following removal of the EDTA solution, the tissue fragments were vigorously pipetted up and down with cold PBS, and the suspension was settled for 5 min. The sediment was vigorously resuspended with cold PBS once again, and it was then centrifuged to harvest a supernatant that enriched the crypts. The crypt fraction was filtered with a 70 μm cell strainer (BD) to remove the residual villous material. The filtrated fluid was centrifuged at 300g for 3 min to remove the single cells. The final fraction consisted of essentially pure crypts and it was subsequently used for culture.

2.7. Culture crypts

A total of 500 isolated crypts were mixed with 50 μL of Matrigel (BD) and seeded in 24-well plates. Following polymerization of the Matrigel, 500 μL of the crypt culture medium was added. Three groups of crypt culture medium were set up as follows: the C3H10/RSPO1 group contained 250 μL of C3H10/RSPO1 culture supernatant and 250 μL of advanced DMEM/F12 (Thermo Fisher Scientific) containing 50 ng/mL of epidermal growth factor (EGF) and 100 ng/mL of Noggin at its final concentration; and the RSPO1 group consisted of 500 μL of advanced DMEM/F12 containing 50 ng/mL of EGF, as well as 100 ng/mL of Noggin at its final concentration; and the RSPO1 group consisted of 500 μL of advanced DMEM/F12 containing 50 ng/mL of EGF, as well as 100 ng/mL of Noggin and 500 ng/mL of hRSPO1 for its final concentration. Crypts were cultured at 37°C in 5% CO₂. Pictures were taken at days 4 and 6.

2.8. Establishment of an abdominal irradiation mouse model

Abdominal irradiation (AIR) was performed on anesthetized mice (which were intraperitoneally injected with 1% pentobarbital
sodium per 10 μL/g body weight) using a medical linear accelerator (Primus 5811; Siemens, Munich, Germany) at a dose rate of 200 MU/minute and at a total dosage of 18 Gys. To avoid injury to the bone marrow and other tissues, the animal’s thorax, head and neck, and extremities were shielded when receiving AIR.

2.9. Adoptive transfer of C3H10/RSP01

C3H10/RSP01 cells were collected and washed twice with PBS and resuspended with PBS at a concentration of 2 × 10^7 cells/mL. Following inhalation of isoflurane anesthesia, 50 μL/mouse of C3H10/RSP01 cells were transferred into those mice that received AIR intravenously via the retro-orbital sinus 2 h post-irradiation and 48 h post-irradiation. C3H10 T1/2, C3H10/mock, and PBS adoptive transferring were set up as the control groups. Survival rates and changes in body weight were observed every day.

2.10. Statistical analysis

GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA) was used to carry out all of the statistical analyses. Student’s t-test was used to compare two samples with unequal variance. The survival rates of the mice were analyzed using Kaplan–Meier estimates. P-values <0.05 were considered statistically significant.

3. Results

3.1. Adoptive transfer of C3H10/RSP01 cells rescue the mice that receive a lethal dosage of AIR

In our previous study of a mouse irradiation model, exposure to 18 Gy of AIR resulted in acute intestinal epithelial injury characterized by bloody stool, weight loss, and death within 5–9 days. To evaluate the radioprotective effects of C3H10/RSP01, we administered 18 Gy of a lethal dosage of AIR to the mice prior to treatment. Two hours and 48 h after irradiation, C3H10/RSP01 cells, C3H10/mock cells, C3H10 T1/2 cells, and PBS were adoptively transferred into 4 different groups of mice. The survival ratios and weight changes among the mice were observed for 30 days. Mice from the PBS group started passing bloody stool and they exhibited a progressive loss in body weight. In contrast, mice receiving the C3H10/RSP01 treatment demonstrated body weight loss within the first week after radiation. As the treatment continued, the body weight loss had halted, and the mice recovered progressively (Fig. 1A).

Within the first 9 days following AIR, 10/10 of the irradiated mice treated with PBS died, and the average survival time was 7.9 ± 1.0 days. Moreover, 1/10 of irradiated mice treated with C3H10 T1/2 cells survived, and the average survival time was 11.1 ± 6.8 days. In contrast, 10/10 of irradiated mice treated with C3H10/mock died within 13 days and the average survival time...
was 10.0 ± 1.7 days. Finally, 5/10 of the irradiated mice from the C3H10/RSPO1-treated group survived, and the average survival time was 20.2 ± 10.4 days.

The survival ratio of the C3H10/RSPO1-treated group was significantly enhanced when compared to that of the C3H10 T1/2, C3H10/mock, and PBS-treated groups (Fig. 1B), and the survival time of the C3H10/RSPO1-treated group was significantly prolonged as well (Fig. 1C). All of the surviving mice appeared to be healthy, and they were scarified at 90 days to terminate the experiment. Fig. 1D showed the recovered mouse (lower panel), which featured the typical sequela of depigmentation of the irradiated area.

Taken together, C3H10/RSPO1 administration significantly improved the survival time of mice exposed to 18 Gy of lethal irradiation.

3.2. C3H10 T1/2 cells exhibit a negative immunoregulatory phenotype

Injury to the radiated intestine could be exacerbated by inflammation and dysregulation of immune homeostasis. MSCs could counteract the inflammation process by expressing a series of inhibitory molecules and cytokines to inhibit the proliferation and activation of radiation-activated immune cells [17]. To evaluate the immunomodulatory activities of the MSC line, C3H10 T1/2, a series of immunoregulatory molecules and cytokines was assayed. The results showed that C3H10T1/2 cells expressed membrane-inhibitory molecules, such as PD-L1, B7-H3, B7-H4, and membrane transforming growth factor (mTGF)-β (Fig. 2A). Intracellular staining showed that the C3H10T1/2 cells could produce the inhibitory molecule interleukin, (IL)-10, and the Th2 cytokine, IL-4 (Fig. 2B). Interestingly, ELISA demonstrated that C3H10T1/2 cells secreted the inhibitory molecule, PGE2 (Fig. 2C).

3.3. RSPO1 is critical for the survival and proliferation of intestinal crypts

RSPO1 is a novel secreted cytokine that enhances the proliferation of intestinal crypt cells by triggering Wnt signaling. To examine the biological effect of RSPO1 on the intestinal crypt’s survival and proliferation, we isolated and enriched the intestinal crypts from LGR5–EGFP–ires–CreERT2 mice. Then, the intestinal crypts were cultured in cocktails in vitro, with or without RSPO1. The intestinal crypts cultured in cocktails (DMEM/F12 containing 50 ng/mL of EGF and 100 ng/mL of Noggin) with the addition of RSPO1 or a supernatant of C3H10/RSPO1 still survived, while those in cocktails with a supernatant of C3H10/mock died at day 4 (Fig. 3A). The intestinal crypts in cocktails with RSPO1 or a supernatant of C3H10/RSPO1 had proliferated (pictures were taken at day 6; upper panel, Fig. 3B) and it still weakly expressed the green fluorescent protein (GFP) that traced LGR5+ ISC (lower panel of Fig. 3B).

![Image](https://via.placeholder.com/150)

**Fig. 2.** C3H10 T1/2 cells exhibit a negative immunoregulatory phenotype. (A) Immunostaining and flow cytometry were performed to analyze the inhibitory membrane molecules PD-L1, B7-H3, B7-H4, and mTGF-β expressed on the C3H10 T1/2 cells. (B) Levels of IL-4, IL-10, and IFN-γ in C3H10 T1/2 cells were evaluated by intracellular staining and flow cytometry. The shaded histogram illustrates antibody staining, while the solid-line histogram showcases the Ig isotype control staining. (C) The PGE2 concentration in the supernatants of C3H10 T1/2 cells was assayed with ELISA.
3.4. C3H10/RSPO1 cells can migrate to the radiation-damaged intestinal epithelium

Homing of infused MSCs to the injured intestine is regarded as a prerequisite to various therapeutic effects. Radiation-damaged tissues might secrete various chemoattractants, such as SDF-1α, to attract stem cells to the site of injury for tissue repairation. To confirm this chemotactic effect, we first analyzed the expression of CXCR4, which is an SDF-1α receptor, located on the surface of C3H10 T1/2 cells (Fig. 4A). Then, a transwell assay revealed that the C3H10 T1/2 cell could be attracted by SDF-1α, and that migration could be inhibited by blocking an antibody against SDF-1α (Fig. 4B).

To combine the comprehensive anti-injury potential of MSCs and the potent ability of RSPO1 as a mitogenic factor for ISCs, we constructed RSPO1-modified C3H10 T1/2 cells and expected that RSPO1, the ISC proliferative cytokine, could be directionally delivered to the site of injury. To demonstrate this directional migration, C3H10/RSPO1 cells were dyed with DIL and then adoptively transferred into abdominal-irradiated mice. Twenty-four hours later, the mice were sacrificed and their intestines were cut and made into slides to track the DIL-positive C3H10/RSPO1 cells. Laser confocal microscopy observation demonstrated that C3H10/RSPO1 cells could migrate to and become enriched in the epithelium of the irradiation-injured intestine (Fig. 4C).

3.5. C3H10/RSPO1 cells promote ISC survival and epithelium regeneration following irradiation

LGR5⁺ cells are actively proliferating ISCs that are responsible for the maintenance of the intestinal epithelium. To evaluate the protective effect of C3H10/RSPO1 cells on ISCs, we prepared a single cell suspension by dissociating the isolated intestinal crypts with TrypLE on day 6 following irradiation. Flow cytometry analysis (Fig. 5A and B) showed that the GFP indicated that LGR5⁺ ISCs had almost disappeared in the mice that were adoptively transferred with PBS (labeled as “PBS”). The LGR5⁺ ISC populations remained in the C3H10/mock (labeled as “C3H10/mock”) and C3H10 T1/2 (labeled as “C3H10 T1/2”) transferred groups, while LGR5⁺ ISCs were well preserved in those mice adoptively transferred with C3H10/RSPO1 cells (labeled as “C3H10/RSPO1”). Laser confocal microscopy observation demonstrated that 6 days after
radiation, almost no LGR5+ ISCs could be detected in the PBS treatment group, while a few of the LGR5+ ISCs were localized at the very base of the crypts in both C3H10 T1/2 and C3H10/mock groups. However, the number of LGR5+ ISCs had significantly increased after being treated with C3H10/RSPO1 cells (Fig. 5C).

Intestinal epithelial cell proliferation served as an indicator of intestinal epithelial regeneration, and this was assessed on histological slides of the small intestine stained with Ki-67. Six days after irradiation, the adoptive transfer of C3H10/RSPO1 cells enhanced the proliferation of the intestinal villi when compared with the PBS, C3H10/mock, and C3H10 T1/2 transfer groups (Fig. 5C and D). Hematoxylin and eosin (HE) staining of the intestinal tissue was performed to observe the impairment and restoration of the intestinal epithelia at day 6 following AIR. The villi of the PBS-treated group were structurally disorganized and they exhibited sloughing; the mean height of the villi was 15.7 ± 6.6 μm. Although the structure still existed in the C3H10/mock and C3H10 T1/2 transfer groups, many villi seemed to be atrophied and even broken, and the villus heights of the two groups were 27.1 ± 7.4 μm and 24.1 ± 2.7 μm, respectively. Damage to the intestinal epithelium of the C3H10/RSPO1 rescue group was obviously ameliorated. The epithelial structure was well organized and the mean height of the villi was 41.0 ± 3.5 μm, which was significantly increased when compared to those of the other groups (Fig. 5E and F).

In all, C3H10/RSPO1 cells confer a significant therapeutic benefit against radiation by directionally delivering RSPO1 to the site of injury, where these cells exert the comprehensive anti-injury potential of both MSCs and RSPO1.

4. Discussion

RIII leads to severe intestinal syndromes resulting from direct and indirect cytotoxic effects on the intestinal crypts and endothelial cells. The intestinal epithelium undergoes rapid and continuous regeneration supported by ISCs in the crypt. Loss of these ISCs interrupts the normally rapid regeneration process and impairs the barrier function of the intestinal epithelium due to a burst of free radicals and, consequently, the surge of inflammatory responses [18]. The LGR5+ ISCs, which are sensitive to irradiation damage, are also mitotically active stem cells that contribute robustly to the homeostatic regeneration of the intestinal epithelium [4]. Therefore, the strategy of effectively protecting and promoting LGR5+ ISC differentiation in different cell types in the intestinal epithelium is critical when treating those who have suffered from RII.

MSCs are pluripotent progenitor cells that are responsible for the maintenance and regeneration of various connective tissues [19,20]. Owing to their immunomodulatory activities and...
migration abilities, MSCs are experimentally transplanted into animal models to ameliorate RIL [21]; thus, these cells provide a promising approach through which to manage radiation enteropathy [2]. Mounting evidence has revealed that MSCs can alleviate inflammation and prevent the adverse effects associated with ionizing radiation by expressing a series of inhibitory molecules [22–24]. Our data demonstrated that the MSC cell line, C3H10 T1/2, preserved its immunomodulatory potential by expressing PD-L1, B7-H3, and B7-H4, as well as mTGF-β and IL-10; it also secreted PGE2. PD-L1, B7-H3, and B7-H4 belong to a class of negative costimulatory molecules [25], which are able to mediate an inhibitory pathway to restrain the stormy inflammatory response associated with RIL. PGE2 could suppress acute inflammatory mediators and regulate various aspects of inflammation and multiple functions of different immune cells [26]. Interestingly, recent reports revealed that treating irradiated mice with PGE2 increased the survival of hematopoietic stem cells [27], and it further regulated hematopoietic stem cell self-renewal by directly interacting with Wnt signaling by cAMP/protein kinase A activation [28,29]. PGE2 and TGF-β1 synergized to attenuate mast cell degranulation, thus alleviating the development of autoimmune diseases [30]. In a mouse model, PGE2 could enhance IL-10 and Treg cell production to reduce the severity of colitis [8]. Meanwhile, C3H10 T1/2 produced notable levels of IL-4 and hardly any interferon (IFN)-γ, ultimately driving the immune response to Th2 and inhibiting the Th1 response; this cascade alleviated the acute inflammatory response. This finding indicated that MSCs can protect LGR5+ ISCs by serving as a powerful anti-inflammatory and anti-injury agent. Consistently, our in vivo data showed that C3H10 T1/2 could preserve LGR5+ ISCs and extend the survival time, to a certain extent, in an irradiation mouse model. This suggested that protecting ISCs is necessary, but insufficient, for treating and rescuing those suffering from RIL, R-spondin 1 (RSPO1) is a novel cytokine that stimulates the growth of the intestinal mucosa. Endogenous RSPO1 protein is localized within the villus epithelium and it is also found in the crypt Paneth cells of the small intestine. In a dextran sulfate sodium (DSS)-induced colitis mouse model, RSPO1 retained the mucosal integrity of the intestine by stimulating crypt cell growth and epithelial regeneration. RSPO1 could reduce DSS-induced inflammation by decreasing myeloperoxidase activity and inhibiting the overproduction of proinflammatory cytokines, including tumor necrosis factor (TNF)-α, IL-1α, IL-6, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in intestinal tissue [10,11]. More importantly, RSPO1 could enhance epithelial regeneration and accelerate its reparation through upregulation of the Wnt/beta-catenin pathway [31].

![Image](image_url)

**Fig. 5.** C3H10/RSPO1 cells enhanced the survival and differentiation of ISCs. (A, B) LGR5+ ISCs (GFP-positive cells) were analyzed by fluorescence-activated cell sorting (FACS). (C) Ki-67 staining (red color) showed proliferation of the intestinal villi. The green color exhibits the LGR5+ ISCs of the crypts. The blue color represents DAPI counterstaining to identify the nuclei. (D) Ki-67+ cells per villus were counted. (E) HE staining showed the epithelia of the different groups. (F) The heights of the villi were measured. *P < 0.05; **P < 0.01.
administration of a recombinant adenovirus expressing human RSPO1 significantly increased the survival ratio in irradiation mouse models [32]. Our in vitro data proved that the RSPO1 expressed by C3H10/RSP01 cells could maintain the survival and stimulate the proliferation of the intestinal crypts just as sufficiently as the commercial RSPO1 protein. Thus, we proposed a strategy to combine the comprehensive anti-injury potential of MSCs with the biological effects of RSPO1 in the treatment of RII.

One intrinsic tissue repairation mechanism is that the wound tissues release chemokines to recruit stem cells that express their corresponding receptors. Our data verified that C3H10 T1/2 expressed CXCR4, which can be attracted by SDF-1α in vitro, and which could also successfully migrate to the radiation-injured intestine in vivo. To ensure the targeted delivery of RSPO1, we constructed RSPO1-modified C3H10 T1/2 cells (C3H10/RSP01). Our data demonstrated that the infusion of C3H10/RSP01 cells effectively protected the ISC; they also simultaneously stimulated the proliferation and regeneration of the intestinal epithelium and conferred a significant survival benefit to the abdominally irradiated mouse model at lethal dosages of irradiation.

However, the potential tumorigenesis that may result following MSC transplantation is the critical obstacle currently facing stem cell therapy [33]. Recent progress has shed light on how to address this limitation by pretreating MSCs with gamma irradiation and preserving their inhibitory function, migration ability, and survival in a hypoxic microenvironment [22]. Hence, inducing MSCs that are pretreated with irradiation and synergized with RSPO1 to treat RII is a promising approach, and it should be discussed more extensively in the future. An alternative way to push forward MSC application is to use the conditioned medium from TNF-α, IL-1β and nitric oxide (NO) pre-activation MSC secreting higher level of immunomodulatory and trafficking molecules, including the pivotal factor, insulin-like growth factor (IGF)-1 [33]. Thus, this promising strategy provides us with a sense of enlightenment, insofar as we can pre-activate the RSPO1-transfected MSCs and acquire the conditioned medium to rescue the radiation-injured intestinal epithelia. Overall, we described a promising strategy that employed the directional delivery of RSPO1-transfected MSCs to ameliorate, and even cure, RII.

Conflict of interest

None.

Acknowledgments

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