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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 RSPO1 gene transfected MSCs, named C3H10/RSPO1; these are murine MSC C3H10 T1/2 cells that stably expressed RSPO1. In this radiation-induced intestinal injury mouse model, C3H10/RSPO1 cells could migrate to the damaged intestinal epithelium, where they enhanced the survival of ISCs, stimulated the proliferation and differentiation of the intestinal epithelium, and thus effectively rescued the mice.

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 (consistent with Genbank ID: BC114966) was subcloned into retroviral-expressing vector pEGZ-Term (kindly gifted by Prof. Sefling, University of Wurzburg, Wurzburg, Germany). The recombinant plasmid, together with its helper virus vectors pHIT456 and pHIT60, were cotransfected into the package cell 293T. C3H10 T1/2 cells, the murine mesenchymal cell line, were infected with the supernatant of the transfected 293T cells, and they were then selected with G418. The G418-resistant C3H10 T1/2 cell line that stably expressed hRSPO1 was named C3H10/RSPO1.

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 rat anti-mouse mAbs. Rat isotype immunoglobulin (Ig)–PE or Ig–FITC served as negative controls.

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 (5×10^5 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% CO_2, the upper chambers were taken out. After scrubbing and removing the unmigrated 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 300*g* 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 (Peprotech, Rocky Hill, NJ, USA) in its final concentration; the C3H10/mock group consisted of 250 μL of C3H10/mock culture supernatant and 250 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% CO_2. 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