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# Effects of human amnion–derived mesenchymal stromal cell transplantation in rats with radiation proctitis

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**Abstract**

*Background aims.* Mesenchymal stromal cells (MSCs) have been reported to be a promising cell source in cell therapy, and large amounts of MSCs can easily be isolated from human amnion. Therapeutic irradiation for intra-pelvic cancer often causes radiation proctitis; however, there is currently no effective treatment. We therefore investigated the effect of transplantation of human amnion–derived MSCs (AMSCs) in rats with radiation proctitis. *Methods.* Amnion was obtained at cesarean delivery, and AMSCs were isolated and expanded. Sprague-Dawley rats were γ-irradiated (5 Gy/d) at the rectum for 5 days. On day 5, AMSCs ($1 \times 10^6$ cells) were intravenously transplanted. Rats were killed on day 8. Histological analyses were performed, and messenger RNA expression of inflammatory mediators was measured. *In vitro*, after γ-irradiation of rat intestinal epithelial cells (IEC-6), the cells were cultured with AMSC-conditioned medium (CM). The effect of AMSC-CM was evaluated by measurement of caspase-3/7 activity, p53 transcription activity and quantitative reverse-transcription–polymerase chain reaction for p53-target genes. *Results.* Histological examination demonstrated that epithelial injury and infiltration of inflammatory cells in the rectum were significantly suppressed by transplantation of AMSCs. *In vitro*, the cell injury in IEC-6 cells induced by γ-irradiation was inhibited by AMSC-CM, which also inhibited the upregulation of p53 transcription activity, caspase-3/7 activity and p21 expression. *Conclusions.* Transplantation of AMSCs improved radiation proctitis, possibly through inhibition of cell injury and inflammatory reactions. AMSC transplantation should be considered as a new treatment for radiation proctitis.

**Key Words:** *amnion, cell injury, mesenchymal stromal cells, radiation proctitis*

## Introduction

Radiation therapy (RT) is frequently used for pelvic malignancies such as prostate and cervical cancer; however, RT also induces acute and chronic injury to the normal tissue. Radiation proctitis is one of the most common side effects in patients receiving RT for intra-pelvic cancer [1,2]. Early reactions occurring in 50% to 78% of the patients include diarrhea, defecation disorders and anal pain; late reactions occurring in approximately 80% of the patients include hemorrhage, with stenosis occurring in approximately 1% and fistula formation occurring in 0.4% of the patients [3–6]. Treatment for early reactions includes suppository, osmotic laxative and enema therapy; treatment for late reactions includes topical application of formalin, hyperbaric oxygen therapy, argon plasma coagulation and surgery [7,8]. Early reactions are generally reversible; however, late reactions are irreversible, and treatments for this pathology remain unsatisfactory [9]. Therefore, development of alternative treatment is needed. Preclinical studies provide encouraging proof-of-concept regarding the therapeutic potential of stem cells for treating the adverse side effects associated with radiotherapy [10].

Mesenchymal stromal cells (MSCs) are multipotent cells that can differentiate into a variety of lineages, including bone, cartilage or fat, and are present in many tissues [11]. At present, MSCs have been investigated as cell sources for regenerative medicine because of their differentiation ability and their potential to improve damaged tissues by secreting a variety of growth factors and

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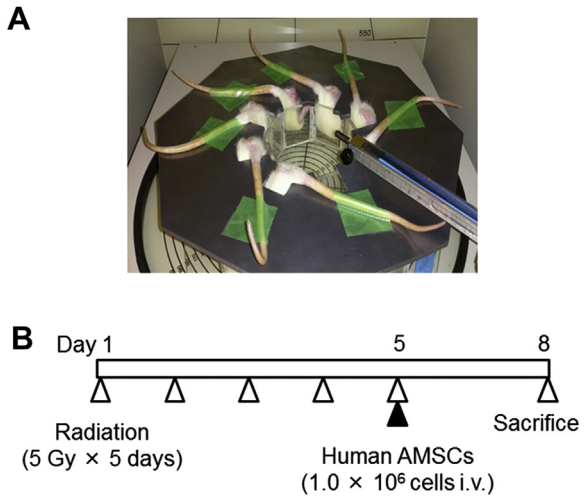


Figure 1. Experimental protocol for the radiation proctitis model. (A) Rats were placed in the supine position under a 6-mm-thick lead shield with a 3 × 4 cm opening around the anus. (B) Rats were γ-irradiated by use of 5 Gy/d for 5 days (day 1 through day 5), and human AMSCs ($1 \times 10^6$ cells) were injected intravenously on day 5. All rats were killed on day 8.

anti-inflammatory molecules [12,13]. The efficacy of autologous and allogeneic MSC transplantation in patients with inflammatory bowel disease has recently been reported [14,15]. Systemic MSC therapy of three patients with refractory pelvic irradiation damage was safe and effective on pain, hemorrhage and inflammation, with a long-term effect in inhibition of chronic inflammation as well as fistulization [16].

The fetal membrane (FM) consists of the amnion and chorion, which envelops the developing fetus. Although the FM is generally discarded as medical waste after delivery, fetal tissues have been found to be rich sources of MSCs [17–20]. We and others have reported that in rats or mice, systemic administration of amnion-derived MSCs (AMSCs) improved hindlimb ischemia [21], myocarditis [22,23], glomerulonephritis [24], ischemia/reperfusion-induced acute kidney injury [25], graft-versus-host disease [26] and severe colitis [27] by inducing angiogenesis and anti-inflammatory effects.

In the present study, we investigated whether the administration of human AMSCs improves radiation proctitis in rats and explored the mechanisms underlying this.

## Methods

### *Isolation and expansion of human AMSCs*

The Medical Ethical Committee of Hokkaido University Graduate School of Medicine, Sapporo, Japan, approved this research, and all pregnant women gave written informed consent. Human FMs were obtained during cesarean deliveries, and the amnion was manually peeled from the chorion. AMSCs were isolated and expanded by digestion with collagenase type III (Worthington Biochemical Corporation), followed by seeding in uncoated plastic dishes with minimal essential medium (MEM)-α (Life Technologies) supplemented with 10% fetal bovine serum (FBS; Life Technologies), 100 U/mL of penicillin and 100 μg/mL of streptomycin (Wako Pure Chemical Industries). Cell cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$. After 3 to 4 days in culture, non-adherent cells were removed and adherent cells were maintained in culture until they reached 80% confluence. The passage was performed with the use of 0.5% trypsin/EDTA (Life Technologies).

Table I. Quantitative RT-PCR primer sequences.

| Gene | Primer sequence |
|---|---|
| CCL2 | F: ATGCAGTTAATGCCCCACTC |
| | R: TTCCTTATTGGGGTCAGCAC |
| CXCL1 | F: AGAACATCCAGAGTTTGAAGGTGAT |
| | R: GTGGCTATGACTTCGGTTTGG |
| IL-6 | F: CCCTTCAGGAACAGCTATGAA |
| | R: ACAACATCAGTCCCAAGAAGG |
| TNF-α | F: AGAACTCCAGCGGTGTCT |
| | R: GAGCCCATTTGGGAACTTCT |
| p21 | F: CACGGCTCAGTGGACCAGAA |
| | R: ACTGGAGCTGCCTGAGGTAGGA |
| Bax | F: TTGCTGATGGCAACTTCAACTG |
| | R: CTTTAGTGCACAGGGCCTTGAG |
| β-Actin | F: CCAACCGTGAAAAGATGACC |
| | R: ACCAGAGGCATACAGGGACA |

F, forward primer; R, reverse primer.

### *Differentiation of AMSCs into adipocytes and osteocytes*

AMSCs were seeded onto six-well plates, and differentiation into adipocytes and osteocytes was induced when the AMSCs were 80% to 90% confluent. To induce differentiation into adipocytes, AMSCs were cultured with human MSC Mesenchymal Stem Cell Adipogenic Differentiation Medium (Lonza), according to the manufacturer's instructions. After 3 weeks of differentiation, cells were stained with 10 μg/mL of BODIPY 493/503 (Life Technologies) and 10 μmol/L Hoechst 33342 (Life Technologies). To induce differentiation into osteocytes, AMSCs were cultured in human MSC Mesenchymal Stem Cell Osteogenic Differentiation Medium (Lonza), according to the manufacturer's instructions. After 2 weeks of differentiation, cells were stained with 10 μg/mL calcein (Dojindo Laboratories). Fluorescent images were obtained with the use of a fluorescent microscope (BZ-9000, Keyence).