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Adipose mesenchymal stromal cells response to ionizing radiation

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Abstract

Background aims. This study evaluates the biological response of adipose tissue–derived mesenchymal stromal cells (aMSCs) to ionizing radiation (IR). *Methods.* Irradiated BALB/c mice aMSCs were characterized for functionality and phenotype. The clonogenic capacity of irradiated aMSCs was assessed and compared with those of metastatic breast cancer cell line (4T1) and normal mouse fibroblasts (NIH3T3-wt). We investigated the IR-induced DNA damage response, apoptosis, changes in cell cycle (CC) dynamics and protein and gene expression. *Results.* Irradiated and non-irradiated aMSCs were able to differentiate into adipocytes, chondrocytes and osteocytes with no significant difference. Irradiated aMSCs maintained the expression of mesenchymal stromal cells (MSCs) surface antigens and, as expected, were negative for hematopoietic stem cells (HSCs) surface antigens when tested up to 7 days after IR for all irradiation doses with no significant difference. Clonogenically, irradiated aMSCs had higher relative survival fraction and plating efficiency than 4T1 and NIH3T3-wt. Irradiated aMSCs expressed higher □H2AX and significantly showed faster and more time-efficient IR-induced DNA damage response evident by up-regulated DNA-PKcs and RAD51. Two hours after IR, most of aMSCs DNA damage/repair-related genes showed up-regulation that disappeared within 6 h after IR. Irradiated aMSCs showed a significant rise and an earlier peak of p-ATM-dependent and -independent (p84/5E10-mediated) G2/M CC arrest compared with 4T1 and NIH3T3-wt. *Conclusions.* After IR exposure, aMSCs showed a robust and time-efficient radiation-induced DNA damage repair response, stable phenotypical characteristics and multi-lineage differentiation potential, suggesting they may be reliable candidates for cell therapy in radiation oncology regenerative medicine.

Key Words: *adipose, cell cycle, DNA damage repair, G2/M arrest, gene expression, ionizing radiation, mesenchymal stromal cells, radiation resistance*

Introduction

Adipose tissue–derived mesenchymal stromal cells (aMSCs) are multi-potent progenitor cells located in the stromal vascular fraction (SVF) of adipose tissue [1]. They are characterized by expressing surface antigens Sca1, CD106, CD105, CD73, CD29 and CD44 and lack the expression of hematopoietic stem cell (HSC) surface antigens (e.g., CD11b and CD45) [1–3]. In addition to their multi-lineage differentiation potential, they have anti-inflammatory/immune-modulatory and paracrine effects. They also have the ability to home to the site of tissue injury after irradiation and inflammation [1,4,5]. aMSCs' osteogenic differentiation potential for instance has been applied in preclinical studies [6]. aMSCs are promising for cellular therapies because of their prominent anti-inflammatory effects, enhancement of interleukin (IL)-10 secretion, ease of isolation, high cell count after expansion and their source abundance [7].

In radiation oncology regenerative medicine applications, aMSC therapy is a rapidly growing domain of cell therapy for radiation-induced normal tissue injury. aMSCs have been investigated in many studies for cutaneous radiation syndrome [8–12] and photoaging [13] where they have shown significant tissue repair. In addition, aMSCs systemic cell therapy has shown significant restoration and improvement of acute salivary gland [14] and intestine injuries [15–18] induced by ionizing radiation (IR). Furthermore,

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aMSCs show a promising potential as a successful cell therapy option in chronic injuries induced by radiotherapy as well [9,19].

Such studies have highlighted the need for characterizing the radiation resistance/sensitivity of aMSCs for their application in radiation oncology regenerative medicine cell therapies because that will allow determination of future behavior and outcome of aMSC therapies before or during fractionated radiotherapy [20,21]. In that context, it was found that, some cell surface antigens found on MSCs (Sca-1, CD29 and CD44) have been linked to cellular radio-resistance [22,23]. In addition, the presence of surface antigen CD105 is important for normal cellular DNA repair [24]. Various mechanisms have been reported explaining such radio-resistance, such as cell cycle (CC) arrest (G2/M arrest) and activation of double-stranded DNA (dsDNA) damage repair—namely, the homologous recombination repair (HRR) and non-homologous end jointing repair (NHEJR) [25–29]. These mechanisms have been implicated in the IR resistance of cancer stem cells (CSC), also known as cancer-initiating cells, which have been linked to cancer disease recurrence and aggression [30–33].

4T1 cells are highly metastatic triple-negative mouse breast cancer cell line expressing mesenchymal antigens. It has been documented that these cells have a considerable subpopulation of CSC that confer proven IR resistance [22,31,34–38]. These two characteristics made these cells a reliable candidate for comparing their IR biological response to that of aMSCs.

In this study, we aimed to evaluate the biological response of aMSCs to IR exposure in comparison to 4T1 cells, as a mesenchymal-like cancer cell model that has considerable IR-resistant CSC subpopulation, with mouse fibroblast (NIH3T3-wt) as a normal cell model.

Methods

Isolation of mouse aMSCs

aMSCs were isolated according to the published methodology [39–42] with minimal modifications. Briefly, white adipose tissue of BALB/c mice from Charles River Laboratories was sterilely collected, washed, minced and digested in 1 × sterile phosphate buffered saline (PBS; Invitrogen), 2% heat-inactivated fetal bovine serum (iFBS; Wisent) and 2 mg/mL collagenase type II (Invitrogen) at 37°C for 15 min. After filtration, cell suspension was spun down, and cell pellet SVF was re-suspended in 0.83% ammonium chloride (NH_4Cl) for erythrocytes lysis. SVF cells were plated in 25-mL flask containing 1 × Dulbecco's Modified Eagle's Media (Invitrogen), 10% iFBS (Wisent), 1% penicillin/streptomycin (Gibco, distributed by Invitrogen Canada) at 37°C and 5% CO_2 after counting with trypan blue for checking the cell viability. Medium was freshly supplemented with 2–20 ng/mL mouse fibroblast growth factor-2 (Sigma-Aldrich) and 5 U/mL sodium-purified Heparin (Sigma-Aldrich).

Determination of cell survival

Cell sensitivity to IR was measured by clonogenic assay (CA) published previously by our lab [43]. Cells were plated in six-well plastic plates at plating densities of 100, 200, 400, 600 and 800 cells/well for IR doses of 0, 2, 4, 6 and 8 Gy, respectively, using 18-MV photons of a Varian 21EX linear accelerator. NIH3T3-wt cells were plated in six-well plates with a feeding layer of 1×10^4 cells/well of NIH3T3-wt cells pre-irradiated with 50 Gy to enhance their platting efficiency. CAs were irradiated 24 h after plating. Colonies were counted 10 days after culture at 37°C and 5% CO_2 incubator.

aMSCs functional differentiation assays

The Mouse Mesenchymal Stem Cell Functional Differentiation Kit (R& D Systems, cat. no. SC010) was used for differentiation of irradiated and non-irradiated aMSCs to adipocytes, osteocytes and chondrocytes according to the manufacture's protocol. IR doses were 2, 4, 6 and 8 Gy. For adipogenesis, cells were seeded until 80% confluence was reached. Medium was then replaced by 0.5 mL adipogenic differentiation media and kept in culture for 10–14 days. For osteogenesis, cells were seeded until reaching 70% confluence. Medium was then replaced by 0.5 mL osteogenesis differentiation medium and kept in culture for 14–21 days. Both newly formed adipocytes and osteocytes were fixed with paraformaldehyde for immunohistochemistry (IHC) staining. For chondrogenesis, a cell pellet of 15×10^3 cells was kept in chondrogenic differentiation medium for 17–21 days. Cell pellet was then fixed with zinc formalin solution overnight, paraffin-embedded and sectioned. Antigen retrieval was done using the Universal Antigen Retrieval Reagent (R&D Systems, Cat. no. CTS015) before IHC.

IHC staining

Cells and sections were washed then blocked with 0.3% Triton X-100, 1% bovine serum albumin (BSA) and 10% normal donkey serum in PBS for 45 min at room temperature. Cells were incubated at 4°C overnight with goat anti-mouse fatty acid binding protein-4 (FABP-4) primary antibody for adipocytes, goat anti-mouse osteopontin antibody for osteocytes, and sheep anti-mouse collagen-II antibody for chondrocytes. Antibodies were purchased from R&D Systems. After three washes, cells were incubated in the dark with diluted (1:200) NL557-conjugated donkey anti-goat secondary