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# Clinical Impact of Radiation-Resistant Mesenchymal Stem Cells in Bone Marrow Deduced from Preclinical Studies

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## Abstract

Mesenchymal stem cells in the bone marrow have attracted great interest over the past decades, not only as a basic scientific subject but also as a novel and advanced clinical tool. More than 100 mesenchymal stem cell-related clinical trials are currently registered in the world. Hematopoietic stem cells in bone marrow are extremely radiation-sensitive, whereas mesenchymal stem cells show considerable radiation-resistance. Intrinsic cellular mechanisms, including highly efficient reactive oxygen species-scavenging ability and active DNA damage response pathways, have been reported to explain the radiation-resistance of mesenchymal stem cells in the bone marrow. The precise interactions between residual host mesenchymal stem cells and donor mesenchymal stem cells at the time of bone marrow transplantation following whole-body irradiation, however, remain unknown. This short review summarizes our current understanding of the clinical impact of the radiation-resistance of endogenous mesenchymal stem cells in the bone marrow.

**Keywords:** Mesenchymal stem cells; Bone marrow; Radiation

## Introduction

Human bone marrow contains at least two distinct types of stem cells [1] in terms of sensitivity to radiation. Hematopoietic Stem Cells (HSCs) are extremely sensitive to radiation [2,3], whereas Mesenchymal Stem Cells (MSCs) are highly resistant to radiation-induced damage [4,5]. Exposure of bone marrow to radiation leads to the rapid depletion of radio-sensitive HSCs and their progenitors, and hematopoietic failure presenting with pancytopenia [3]. Bone marrow transplantation is a useful clinical treatment for this hematopoietic failure. Engrafted donor HSCs may home to the most appropriate sites, created by depletion of the host HSCs and their progenitors, and then reconstruct hematopoiesis. Host MSCs that survive radiation exposure support the regeneration of the donor hematopoietic system [6], although the underlying mechanism is unclear. MSCs are considered key components of the HSC niche, a specialized microenvironment that regulates the maintenance of HSCs and the production and maturation of hematopoietic progenitors [6]. Because MSCs are also present as minor components in transplanted bone marrow cells, donor MSCs are likely to meet and interact with the host MSCs in the patient's bone marrow, as illustrated in figure 1. This short review focuses on our current understanding of the biologic relevance of radiation resistance of host MSCs in bone marrow based on the clinical outcome, while pointing out unresolved questions to facilitate further research in this field.

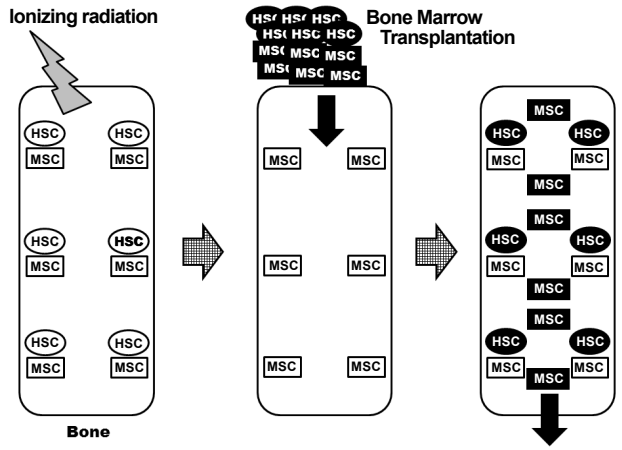


**Figure 1: Replacement of radiation-sensitive HSCs by bone marrow transplantation, while maintaining radiation-resistant MSCs.** Sensitive host HSCs (open circles) were effectively replaced by donor HSCs (closed circles), whereas resistant host MSCs (open boxes) rejected replacement by the donor MSCs (closed boxes).

## MSC Basics

The term "mesenchymal stem cell" has been traditionally used for heterogeneous cell populations with a rather blurred broad definition. MSCs were originally isolated from the bone marrow [7,8], and then considered to be present in virtually all postnatal organs and tissues [9]. The International Society for Cellular Therapy defines that human MSCs must be plastic adherent when maintained *in vitro* and be able to differentiate into osteoblasts, adipocytes, and chondroblasts in standard differentiating cell culture conditions [10]. In addition, the MSC population must express CD73, CD90, and CD105, and lack expression of hematopoietic markers such as CD14, CD34, CD45, and HLA-DR [10]. Thus, the definition of MSCs is not directly applicable to *in vivo* cells. Accordingly, the *in vivo* identity, physiologic function, and biologic properties of MSCs have been investigated mainly by systemic

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transplantation of *in vitro*-cultured cells [11]. Clinical application also requires *ex vivo* cell amplification due to the low content of MSCs in the bone marrow (0.001%-0.01% of total nucleated cells) [12], even though the culture conditions may modify cellular properties, as reported for mouse MSCs [13]. The notion of *in vivo* MSCs, therefore, is rather indirect and hypothetical, leading to gaps in our understanding. Furthermore, unlike human MSCs, mouse bone marrow-derived MSCs have significant limitations in isolation by their adherence to plastic, mostly due to frequent contamination of cultures by hematopoietic cells [14-17]. To overcome this, a methodology was recently developed to selectively isolate a pure population of mouse MSCs directly from bone marrow cells based on surface marker expression [18,19]. An *in vivo* identification method of MSCs still needs to be developed.

## Radiation Resistance of MSCs in Bone Marrow

### Clinical observations

Differences in radiation sensitivity among bone marrow cells were originally noted in several clinical reports [20-25]. In patients surviving a long time after allogeneic bone marrow transplantation following whole body irradiation, the origin of the two stem cells, HSCs and MSCs, in the bone marrow tends to be significantly different. To identify the origin of the two types of stem cells, genetic markers such as a variable number of tandem repeats in the genomic DNA, which can discriminate origin of stem cells, were used for sex-matched cases [26] and Y-chromosome specific nucleotide sequences were used for sex-mismatched cases [27]. HSCs were found to derive mostly from donors, whereas MSCs derived mostly from the host. These observations were assumed to reflect an inherent difference in the radiation sensitivity of each type of stem cell, namely radio-sensitive host HSCs were effectively replaced by donor HSCs, whereas radio-resistant host MSCs rejected replacement by donor MSCs. Interestingly, pediatric patients who receive allogeneic bone marrow transplantation show mixed chimerism, indicating successful engraftment of donor MSCs [28]. The exact mechanism underlying the easier engrafting of donor MSCs in childhood compared with adulthood, however, remains unclear. MSCs in childhood are still actively proliferating to increase their number [29], and this might enhance their radio-sensitivity, leading to easier depletion of host MSCs and higher efficiency in the engraftment of donor MSCs.

### *In vitro* culture study

The radiation resistance of MSCs isolated from human bone marrow has also been demonstrated in *in vitro* culture studies [4]. In these studies, cells showed considerable *in vitro* radiation resistance compared with so-called radiation resistant cell lines, such as A 549 lung cancer cells [4]. MSCs have several cellular mechanisms, such as highly efficient reactive oxygen species-scavenging ability and avoidance of cell death by active DNA damage response pathways, including cell cycle arrest and DNA repair [4,5,30]. Interestingly, MSCs isolated from different anatomic bone marrow sites display variable responses to ionizing radiation treatment [31]. MSCs derived from maxillary and mandibular trabecular bones are more radiation-resistant than those derived from the iliac crest. MSCs isolated from the maxilla and mandibular trabecular bones induce higher p21 expression, which is known to inhibit apoptosis and harborless DNA damage after ionizing radiation exposure. The induction of p21 expression is considered to activate a robust G1 arrest and DNA repair mechanisms. It remains unclear, however, whether or not MSCs isolated from other organs or tissues have similar radiation resistance.

### *In vivo* animal model study

The radiation resistance of MSCs has been also demonstrated in *in vivo* animal model studies. In a pig model, the mandible was subjected to fractionized radiation of 2 × 9 Gy within 1 week [32]. This treatment corresponds with that of a standardized clinical treatment regimen of head and neck cancer patients fractionally-irradiated with 30 × 2 Gy. Isolation of MSCs at different time-points post-irradiation revealed no significant differences regarding the proliferation capacity and osteogenic differentiation potential. These findings imply that MSCs can effectively cope with higher doses of irradiation *in vivo*. In a murine model, the radiation sensitivity of HSCs and MSCs was compared using a flow cytometry-mediated prospective identification method [18]. HSCs were almost undetectable at 10 days after whole-body irradiation at a dose of 10.5 Gy, whereas a significant number of MSCs remained in bone marrow on the same day. Approximately 71% of freshly isolated MSCs of non-irradiated bone marrow were in the $G_0$ phase, which could have protected them from lethal irradiation by escaping the cell cycle-mediated apoptotic program [18]. In another murine model, mouse MSCs isolated from flushed bone marrow aspirates were more radio-sensitive than those isolated from collagenase-digested bone marrow [33]. These findings suggest that MSCs in mouse bone marrow are not uniform, but heterogeneous. Therefore, more attention should be paid to the isolation procedures of MSCs from mouse bone marrow.

## Clinical Relevance of Radiation Resistance of MSCs Deduced from Preclinical Study

### Uncertainty of the functional quality of surviving endogenous MSCs

The detailed properties of radiation-surviving endogenous MSCs are not well documented in human or animal studies. It is quite uncertain whether they can perform usual functions *in vivo* even after exposure to life-threatening ionizing radiation. For example, in an *in vitro* study, MSCs are considered to be sources of tumorigenic cells due to the acquisition of some genetic modifications such as telomere shortening by non-life-threatening low dose radiation exposure, though these findings were not confirmed *in vivo* [34]. The physiologic properties of surviving MSCs after a life-threatening dose of radiation are more likely to differ significantly from those before radiation exposure, despite their having an active DNA damage responding pathway.

### Consequences of functionally depleting endogenous HSC niches in bone marrow

MSCs are considered to be part of the HSC niche in the bone marrow [6]. Although selective depletion of MSC functions in bone marrow has not yet been achieved, an interesting observation was made using diphtheria toxin receptor-mediated selective depletion of other defined HSC niche cells, which are called CXC chemokine ligand 12- abundant reticular (CAR) cells [35]. The CAR cells are primary mesenchymal cells with the ability to differentiate into adipocytes as well as osteoblasts, which may be functionally identical to MSCs. HSCs from CAR cell-depleted mice were reduced in number and cell size, were more quiescent, and had increased expression of early myeloid selector genes. Thus, a niche composed of adipo-osteogenic progenitors is required for the proliferation of HSCs and lymphoid and erythroid progenitors, as well as maintenance of HSCs in an undifferentiated state [35]. Accordingly, radiation-damaged host MSCs may similarly influence the donor HSCs.

### Lack of evidence regarding direct interactions between surviving host MSCs and donor MSCs

Although many reports indicate *in vivo* or *in vitro* interactions of exogenous MSCs with various endogenous surviving cell types, there is no report concerning direct interaction between host MSCs and donor cells, including MSCs. This is probably due to technical difficulties or due to a lack of interest in this subject. It is worthwhile to point out here a current trend in stem cell biology to intensively investigate interactions between host stem cells and donor stem cells. Emerging concepts propose that transplanted-stem cells act to initiate and stimulate host stem cell-based tissue repair, rather than directly participating in the repair processes [36,37].

### Non-hematopoietic antigen-presenting cells are sufficient to induce lethal acute Graft-versus-Host Disease

Graft-versus-Host-Disease (GVHD) is a potentially life-threatening complication and a major limitation of allogeneic hematopoietic stem cell transplantation outcome [38]. This complication is thought to be initiated by the activation of mature donor $CD4^+$ T-cells that are co-infused with the hematopoietic stem cell transplant. $CD4^+$ T-cells recognize target alloantigens presented on major histocompatibility complex molecules expressed on the antigen-presenting cells that reside somewhere within the host tissues [39]. Upon alloantigen recognition, donor $CD4^+$ T-cells become activated, expand, and induce cytotoxic effects on target organs, including the skin, gut, and liver [40]. Although it has been established that recipient dendritic cells are the major antigen-presenting cells expressing allogeneic peptides on their surface [39,41], recent evidence indicates that only non-hematopoietic recipient cells surviving radiation exposure can induce experimental lethal acute GVHD by expressing allogeneic antigens [42]. Because of the properties of MSCs described in previous reports, some bone marrow MSCs surviving after ionizing radiation exposure might represent the GVHD-causing allogeneic antigen presenting cells. Importantly, the immunomodulatory functions of MSCs can be converted to suppressing or promoting functions, depending on the conditions surrounding them [43,44]. Thus, the clinical relevance of surviving endogenous MSCs might be stochastic in bone marrow, as illustrated in figure 2.

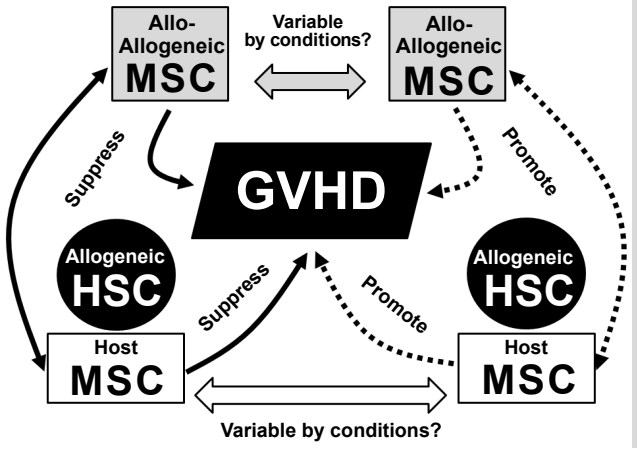


**Figure 2: MSCs might be double-edged swords that suppress or promote GVHD according to conditions surrounding them.** Clinical relevance, particularly the immunomodulatory properties, of surviving host MSCs (open boxes) might be stochastic according to various conditions, including effects of the allo-allogeneic donor MSCs (grayed boxes) in the bone marrow exposed to ionizing radiation. In contrast, the immunomodulating properties of the allo-allogeneic donor MSCs could also be influenced by their culture conditions and by the host MSCs. Allogeneic donor MSCs are omitted due to their low frequency compared to the culture-expanded allo-allogeneic donor MSCs. Closed circles: allogeneic donor HSCs.

## Future Perspectives Regarding the Radiation Resistance of MSCs

MSCs are now considered as advanced therapy medical products by the European Medicines Agency by regulation No. (EC) 1394/2007 of the European Commission [45,46]. Product approval has been granted for the treatment of pediatric GVHD in Canada and New Zealand (Prochymal®; Osiris Therapeutics, Columbia, MD). Clinical-grade large-scale expansion of MSCs is currently available [47-49], supporting more than 100 MSC-related clinical trials registered at http://www.clinicaltrials.gov/. To improve the clinical effectiveness of the MSCs, it will be important to understand the interactions between these therapeutic exogenous MSCs and endogenous MSCs. In particular, the immunomodulatory properties of MSCs are suggested to be a double edged-sword [43,44]. Thus, it might be necessary to carefully monitor the functional status of endogenous MSCs and to precisely control them to achieve maximal therapeutic effects of the exogenous MSCs.

Besides hematopoietic regeneration, the radiation resistance of MSCs might be problematic in cases of radiation therapy. MSCs are also regarded as a key component of the tumor stroma, which promotes not only tumor growth, but also angiogenesis and metastasis [50,51]. Thus, the regulation and control of radio-resistant MSCs in the bone marrow might be a critical issue to be addressed in radiation therapy.

Adult mouse MSCs prospectively purified from bone marrow by flow cytometry were recently demonstrated to be suitable sources for highly efficient generation of high quality induced pluripotent stem (iPS) cells compared to other somatic cells such as fibroblast cells [52]. The iPS cells derived from MSCs appear to be the closest equivalent of the embryonic stem cells based on the gene expression profile and germline-transmission efficiency. Therefore, in the case of severe radiation emergency accidents, especially whole body irradiated cases, radiation-resistant MSCs with high efficiency in the generation of iPS might be the best choice for autonomous regenerative medicine in the future.

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