Successful Mitigation of Radiation Injuries in Mice using Mesenchymal Stem Cells Genetically Modified to Secrete Extracellular Superoxide Dismutase

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Abstract

Oxidative stress is a major determinant for radiation-induced tissue injuries. We present a novel method that harnesses the power of migration of mesenchymal stem cells (MSCs) to radiation injured tissues and adeno-virus-mediated extracellular superoxide dismutase (ECSOD) gene therapy for oxidative stress. This report demonstrates for the first time that intravenous administration of MSCs genetically modified to secrete ECSOD at 24 hours after radiation exposure can improve survival from 10% to 52%, extend lifespan for 207 days, retard cataract formation for 39 days, and prevent carcinogenesis in mice. For proof-of-concept, we further demonstrate for the first time that human MSCs can be genetically modified with adenoviral vector to secrete high levels of biologically active ECSOD. Our findings suggest that mesenchymal stem cell-based antioxidant gene therapy has the potential for mitigation of radiation injuries in humans as a consequence of radiological and nuclear emergencies, space radiation exposure, and cancer radiotherapy toxicities.

Keywords: Gene therapy; Mesenchymal stem cells; Radiation

Introduction

Exposure to high doses of ionizing radiation can lead to radiation injuries such as death, lifespan shortening, cataract formation, or carcinogenesis [1-5]. There is currently no approved drug or therapy for mitigation or therapeutic treatment of radiation injuries. Formation of superoxide anion ($O_2^-$) after ionizing radiation is a major determinant of radiation injuries. Irradiated tissues release $O_2^-$ for days to months after radiation exposure [6]. Extracellular superoxide dismutase (ECSOD), a potent antioxidant enzyme catalyzing the dismutation of $O_2^-$, can alleviate oxidative stress [7]. Mesenchymal stem cells (MSCs), a subset of adult stem cells from bone marrow, have been found to migrate to radiation injured tissues such as bone marrow, gut, skin, and muscle after intravenous administration [8]. Therefore, MSCs hold promise as vehicles for adult stem cell-based gene therapy of radiation injuries.

To test the hypothesis that MSCs genetically modified to secrete ECSOD (ECSOD-MSCs) exert a therapeutic effect on radiation injuries, in our previous study mouse MSCs (mMSCs) were transduced with Ad5CMVECSOD, an adeno-virus carrying human ECSOD gene, and dose-dependent secretion of biologically active ECSOD by Ad5CMVECSOD-transduced mMSCs (ECSOD-mMSCs) was detected. mMSCs were also transduced with Ad5CMVntlacZ, an adeno-virus carrying reporter gene ntlacZ, and dose-dependent expression of β-galactosidase by Ad5CMVntlacZ-transduced mMSCs (ntlacZ-mMSCs) was detected. Mice were then given 9 Gy total body irradiation at a dose rate of 1.23 Gy/min ($LD_{90}$) and 24 hours later they received a tail vein injection of 0.5×10^6 ECSOD-mMSCs, 0.5×10^6 ntlacZ-mMSCs, or phosphate-buffered saline (PBS). Remarkably, 52% of mice in the ECSOD-mMSCs treatment group survived for 35 days, whereas only 9% of mice in the ntlacZ-mMSCs treatment group and 10% of mice in the PBS treatment group survived for 35 days. This finding demonstrates for the first time that intravenous administration of ECSOD-MSCs improves survival in irradiated mice, suggesting its clinical potential for mitigation of potentially lethal complications of acute radiation syndrome [1,9].

To test the hypothesis that ECSOD-MSCs can mitigate delayed effects of acute radiation exposure, we conducted the following study and our data showed for the first time that intravenous administration of ECSOD-MSCs at 24 hours after radiation exposure extended lifespan, retarded cataract formation, and prevented carcinogenesis in mice.

Methods

Ad5CMVECSOD and Ad5CMVntlacZ were purchased from University of Iowa Gene Transfer Vector Core (Iowa City, IA) [10]. mMSCs were isolated from 6-week-old female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) and then ex vivo expanded as previously described [9]. Human MSCs (humMSCs) were isolated from healthy bone marrow donors by their adherence to tissue culture plastic and then ex vivo expanded as previously described [11]. All research involving human participants in this study were approved by the authors’ institutional review board (Spectrum Health IRB #2004-179). MSCs were transduced with Ad5CMVECSOD and the culture supernatant was assayed for the secretion of biologically active ECSOD using a superoxide dismutase activity assay kit (Cayman Chemical Company, Ann Arbor, MI) [9,12,13]. MSCs were transduced with Ad5CMVntlacZ and the cells were analyzed for β-galactosidase activity by X-gal cytochemistry staining [9,12-15]. Persistence of adeno-viral-mediated transgene expression in mMSCs was determined.

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Received May 19, 2015; Accepted June 22, 2015; Published June 24, 2015

Citation: Deng W, Abdel-Mageed AS, Connors RH, Pietryga DW, Senagore AJ, et al. (2015) Successful Mitigation of Radiation Injuries in Mice using Mesenchymal Stem Cells Genetically Modified to Secrete Extracellular Superoxide Dismutase. J Stem Cell Res Ther 5: 288. doi: 10.4172/2157-7633.1000288

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in vitro using Ad5CMVECSOD or Ad5CMVntlacZ at MOI 2,000 and culture medium containing 2% fetal bovine medium [12]. Intravenous administration of ECSOD-mMSCs into irradiated mice through tail vein injection was conducted as previously described [9]. The mice were then monitored for survival, cataract formation, and carcinogenesis over the whole lifespan. Analysis of colony-forming unit of hMSCs in bone marrow mononuclear cells or peripheral blood mononuclear cells was conducted using a previously described method [16]. Flow cytometric analysis for phenotype and spectral karyotyping analysis for cytogenetics were conducted on ex vivo expanded hMSCs using previously described methods [9,17].

Results and Discussion

To study the persistence of ECSOD transgene expression in vitro, mMSCs were transduced with Ad5CMVECSOD and the cells were then cultured for 35 days. As shown in Figure 1A, ECSOD–mMSCs secreted 1.52 ± 0.27 unit/1×10⁶ cells/48 hour (mean ± SEM, n=3) ECSOD at day 0 and 0.32 ± 0.09 unit/1×10⁶ cells/48 hour (mean ± SEM, n=3) ECSOD at day 35. To study the persistence of ntlacZ transgene expression in vitro, mMSCs were transduced with Ad5CMVntlacZ and the cells were then cultured for 35 days. As shown in Figure 1B, the percentage of cells expressing β-galactosidase was 99% ± 1 (mean ± SEM, n=3) at day 0 and 24% ± 5 (mean ± SEM, n=3) at day 35. Therefore, adenoviral-mediated transgene expression in mMSCs can persist for more than 35 days in culture.

To determine whether intravenous administration of ECSOD-mMSCs can mitigate delayed effects of acute radiation exposure such as lifespan shortening, cataractogenesis, and carcinogenesis [2-5], mice that had survived for 35 days were then monitored for survival, cataract formation, and carcinogenesis over their remaining lifespan. Previous
studies have demonstrated that overexpression of superoxide dismutase extends lifespan in Drosophila [3] and prevents cataract formation in rats [4]. In our study, irradiated mice had a shortened lifespan. However, mice in the ECSOD-mMSCs treatment group survived 207 days longer than mice in the PBS or ntlacZ-mMSCs treatment group (Figure 1B and 1C). Mice in the ECSOD-mMSCs treatment group developed cataracts 39 days later than mice in the PBS or ntlacZ-mMSCs treatment group (Figure 1D). No tumor development was observed in mice in the ECSOD-mMSCs treatment group, whereas large abdominal tumor was found in mice in the PBS treatment group (Figure 1E). Therefore, mitigation of both acute radiation syndrome and delayed effects of acute radiation exposure has been successfully achieved by intravenous administration of ECSOD-MSCs at 24 hours after radiation exposure in mice. The mechanism might be that scavenging of $\text{O}_2^-$ in the extracellular space of irradiated tissues by ECSOD secreted from ECSOD-MSCs that have migrated to radiation-injured tissues after intravenous administration can prevent further tissue injuries.

For the first stage in clinical proof-of-concept, human MSCs (hMSCs) were isolated by their adherence to tissue culture plastic from 42 healthy bone marrow donors and ex vivo expanded as previously described [11]. Figure 2A shows colony-forming unit (CFU) of hMSCs in 14-day cultures of human bone marrow mononuclear cells (BM-MNCs). Figure 2B shows quantification of CFU of hMSCs in 14-day culture of $1\times10^5$ human BM-MNCs or PB-MNCs. Figure 2C shows flow cytometric analysis showing typical phenotype of hMSCs. Figure 2D shows spectral karyotyping (SKY) cytogenetic analysis showing hMSCs with a normal diploid pattern of male human origin (46, XY). Figure 2E shows dose-dependent expression of nuclear-targeted $\beta$-galactosidase by ntlacZ-hMSCs. Figure 2F shows dose-dependent, high-level secretion of biologically active ECSOD by ECSOD-hMSCs. Data were expressed as mean ± SEM (n = 3) and analyzed statistically using a one-way analysis of variance (ANOVA) followed by post-hoc analysis with Tukey test. *P < .001 versus MOI 0; **P < .001 versus MOI 0 or 300.
MNCs). No CFU of hMSCs was identified in 14-day cultures of human peripheral blood mononuclear cells (PB-MNCs). Quantification of CFU assay demonstrated that \( n = 42 \) colonies of hMSCs were derived from \( 1 \times 10^5 \) human BM-MNCs (Figure 2B), similar to a previous study by other investigators [16]. The cells were then differentiated into osteoblasts and adipocytes in vitro and cell phenotype was analyzed by flow cytometry. Figure 2C shows that the cells express CD105, CD73, CD90, CD29, and CD44. The cells do not express CD45, CD34, CD14, Lin1, and HLA-DR. Therefore, these cells are typical MSCs [18-20].

To study the efficacy of adenovalinal-mediated transgene expression in hMSCs, hMSCs were transduced with Ad5CMVntlacZ and then analyzed by X-gal staining for nuclear-targeted \( \beta \)-galactosidase activity. As shown in Figure 2E, adenovalinal-mediated ntlacZ transgene expression in Ad5CMVntlacZ-transduced hMSCs (ntlacZ-hMSCs) is dose-dependent. hMSCs were further transduced with Ad5CMVECSOD and the culture supernatant was analyzed for superoxide dismutase activity. Figure 2F demonstrates a dose-dependent, high-level secretion of bioactive ECSOD by Ad5CMVVECSOD-transduced hMSCs (ECSOD-hMSCs). Therefore, hMSCs can be genetically modified with adenovalinal vector to secrete high levels of bioologically active ECSOD. Our findings suggest that mesenchymal stem cell-based antioxidant gene therapy has the potential for mitigation of radiation injuries in humans as a consequence of radiological and nuclear emergencies, space radiation exposure, and cancer radiotherapy toxicity.

Acknowledgements

This work was supported by research funding from Spectrum Health Foundation (Grand Rapids, MI) and Jay and Betty Van Andel Foundation (Grand Rapids, MI). We thank Lisa DeCamp for assistance with animal health observations, Richard West for assistance with flow cytometric assay, and Julie Koeiman for assistance with SKY cytogenetic assay.

Conflict of Interest

A patent application related to the methodology described in the present work has been filed by W.D., A.S. A.-M., and A.J.S. to United States, European Union, China, and India. The authors declare no other competing financial interests.

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