Cytotherapy, 2015; 17: 262–270

International Society for Cellular Therapy
ISCT

Genomic and functional comparison of mesenchymal stromal cells prepared using two isolation methods

SATORU OTSURU[1,2], TED J. HOFMANN[1], PICHAI RAMAN[3], TIMOTHY S. OLSON[1], ADAM J. GUESS[2], MASSIMO DOMINICI[4] & EDWIN M. HORWITZ[1,2,5]

[1]*Division of Oncology/Blood and Marrow Transplantation, The Children's Hospital of Philadelphia and The University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA,* [2]*Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA,* [3]*Center for Biomedical Informatics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA,* [4]*Department of Oncology and Hematology, University-Hospital of Modena and Reggio Emilia, Modena, Italy, and* [5]*Divsion of Hematology/Oncology/BMT, Nationwide Children's Hospital, Columbus, Ohio, USA*

Abstract

Background aims. Mesenchymal stromal cells (MSCs) have been applied to patients in cell therapy for various diseases. Recently, we introduced a novel MSC separation filter device which could yield approximately 2.5-fold more MSCs from bone marrow in a closed system compared with the conventional open density gradient centrifugation method. MSCs isolated with these two methods were phenotypically similar and met the criteria defining human MSC proposed by the International Society for Cellular Therapy. However, these criteria do not reflect the functional capacity of MSCs. It has been shown that the donor, source, isolation method, culture condition and cryopreservation of MSCs have potential to alter their therapeutic efficacy. To determine the equivalency of MSCs isolated by these two methods, we compared their genomic profiles as an index of their biologic potential and evaluated their growth promoting potential as an index of function. *Methods.* The gene expression profiles of human MSCs isolated from 5 healthy donors with two distinct methods were obtained from microarray analyses. The functional activity of freshly expanded/cryopreserved MSCs from these two isolation methods was evaluated using an *in vitro* chondrocyte proliferation assay. *Results.* Freshly expanded MSCs isolated by these two methods were found to exhibit similar gene expression profiles and equivalent therapeutic effects, while freshly thawed, cryopreserved MSCs lacked all measureable therapeutic activity. *Conclusions.* The MSC separation device generates genomically and functionally equivalent MSCs compared with the conventionally isolated MSCs, although freshly thawed, cryopreserved MSCs, isolated by either method, are devoid of activity in our bioassay.

Key Words: *bone marrow MSC, cryopreservation, functional assay, gene expression profile*

Introduction

Mesenchymal stromal cells (MSCs), frequently termed mesenchymal stem cells, are one of the most investigated cell types in cell therapy for various diseases. Accumulating data have demonstrated that MSCs, a heterogeneous population of *ex vivo*–expanded cells [1], exert therapeutic effects by differentiating into target cells, secreting trophic factors which can stimulate or protect endogenous cells, and releasing immunomodulatory molecules which suppress immune cells, even though the detailed mechanism(s) underlying the therapeutic effects has not been fully elucidated [2–5].

The conventional protocol most commonly used in preclinical and clinical studies to isolate MSCs from bone marrow is centrifugation over a density gradient followed by *ex vivo* expansion in culture, which removes hematopoietic cell contamination. However, this conventional procedure is an open system that has risk of bacterial contamination. Moreover, the cell recovery from bone marrow with this method is variable between operators and technical expertise is required to consistently obtain MSCs with high efficiency. We have been using this conventional method to isolate MSCs from bone marrow in our clinical trials of MSC therapy for patients with osteogenesis imperfecta (OI), a genetic bone disorder caused by mutations in type I collagen

Correspondence: **Edwin M. Horwitz**, MD, PhD, Nationwide Children's Hospital, ED555, 700 Children's Drive, Columbus, OH 43205, USA. E-mail: edwin.horwitz@nationwidechildrens.org

(Received 7 July 2014; accepted 30 October 2014)

ISSN 1465-3249 Copyright © 2015, International Society for Cellular Therapy. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcyt.2014.10.013

[6,7]. After MSC infusion, OI patients demonstrated an acute acceleration of bone growth. In these clinical trials, patients received infusions of $1-5 \times 10^6$ MSCs/kg body weight, which requires 50 mL of donor bone marrow to isolate sufficient MSCs by the conventional method without excessive expansion in culture [6,7]. If repeated infusions of MSCs are needed to establish this cell therapy, substantially more bone marrow will be required. Thus, it would be of great benefit for donors to use protocols that allow more efficient MSC isolation from bone marrow compared with the conventional methods.

Recently, we introduced a novel MSC separation filter device that allows bone marrow processing in a closed system without centrifugation [8]. Importantly, we showed that the device yielded approximately 2.5-fold more MSCs at passage 2 than the conventional methods from the same initial volume of bone marrow. The processing time using the device was about 20 minutes; the conventional method typically takes more than 1 hour. MSCs isolated by these two methods expressed CD105, CD73 and CD90 but not CD45, CD34, CD11b, CD19 or HLA-DR. Together with the observation that MSCs isolated from both methods successfully differentiated into osteoblasts, adipocytes and chondrocytes *in vitro*, both cell preparations met the defining criteria proposed by the International Society for Cellular Therapy (ISCT) in 2006 [9], suggesting that the device can be used to isolate MSCs from bone marrow for cell therapy in lieu of the conventional method.

Although these two MSC preparations are indistinguishable according to the ISCT criteria, they do not necessarily exert equivalent therapeutic activity, which is a key benchmark for clinical applications, because it has been shown that differences in donor, source, isolation method, and culture condition of MSCs could affect clinical outcomes [10−19].

Another strategic element that could alter the therapeutic effect of MSC is cryopreservation. With the cell therapy industry supplying Good Manufacturing Practice (GMP)-prepared MSCs, most investigators have utilized cryopreserved MSCs immediately after thawing in their clinical trials [20−24]. The availability of these "off-the-shelf" MSCs is necessary to provide MSC therapy to patients at hospitals without GMP facilities. In contrast to freshly expanded MSCs, however, Francois *et al.* [25−28] reported that cryopreserved MSCs failed to achieve therapeutic effects in acute graft-versus-host disease (GvHD), raising questions about the therapeutic equivalency of freshly expanded and freshly thawed, cryopreserved MSCs.

In this study, we compared gene expression profiles obtained by microarray analysis between MSCs isolated with the MSC separation filter device and the conventional density gradient method to examine the fundamental character and biologic potential of these MSCs. Additionally, we evaluated the therapeutic potential for growth promotion and bone regeneration using our *in vitro* chondrocyte proliferation assay, which we developed in the course of studying the mechanism of MSC-stimulated bone growth in patients with OI [6,7]. Finally, by use of the proliferation assay, we examined whether cryopreserved MSCs have the potential to maintain their therapeutic activity in the treatment of OI.

Methods

Mesenchymal stromal cells

MSCs were isolated and expanded as previously described [8]. Briefly, nucleated cells were isolated from bone marrow of five healthy donors (ages between 22−52 years, sample 3 is from a male donor and other samples are from female donors) through the use of two distinct methods, density centrifugation with lymphocyte separation medium (LSM; MP Biomedicals, LLC, Solon, OH, USA) and the Bone Marrow MSC Separation Device (KANEKA CORPORATION, Tokyo, Japan). Isolated bone marrow nucleated cells were cultured in Dulbecco's modified of Eagle's medium (DMEM; Corning Cellgro, Manassas, VA, USA) supplemented with 10% fetal bovine serum (FBS; Gemini Bio-Products, West Sacramento, CA, USA) to establish MSCs. MSCs at passage 3 were collected and used for RNA isolation and for infusion into mice.

Chondrocyte isolation

Primary chondrocytes were isolated from 2−5 days old neonatal C57BL/6 mice as previously described [7]. Briefly, femoral and humeral heads were dissected under a stereo microscope and treated with 0.25% Trypsin solution (Corning Cellgro) for 20 minutes at 37°C to remove attached soft tissues, followed by digestion with 86.5 U/mL collagenase type 1 (Worthington Biochemical Corporation, Lakewood, NJ, USA) at 37°C overnight. After dissociation by pipetting and passing through a cell strainer, the isolated chondrocytes were used for the chondrocyte proliferation assay.

Chondrocyte proliferation assay

Freshly expanded or cryopreserved MSCs, 1×10^6 in 300 μL of phosphate-buffered saline (PBS), or 300 μL of PBS as a control were infused intravenously into 6- to 8-week-old C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME, USA), and serum was

Download English Version:

https://daneshyari.com/en/article/2171200

Download Persian Version:

https://daneshyari.com/article/2171200

Daneshyari.com