Cytotherapy, 2013; 15: 1062–1072

International Society for Cellular Therapy
ISCT®

ORIGINAL PAPERS

Age-dependent decrease in the chondrogenic potential of human bone marrow mesenchymal stromal cells expanded with fibroblast growth factor-2

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Abstract

Background aims. Human bone marrow mesenchymal stromal cells are useful in regenerative medicine for various diseases, but it remains unclear whether the aging of donors alters the multipotency of these cells. In this study, we examined age-related changes in the chondrogenic, osteogenic and adipogenic potential of mesenchymal stromal cells from 17 donors (25–81 years old), including patients with or without systemic vascular diseases. *Methods.* All stem cell lines were expanded with fibroblast growth factor-2 and then exposed to differentiation induction media. The chondrogenic potential was determined from the glycosaminoglycan content and the *SOX9*, collagen type 2 alpha 1 *(COL2A1)* and aggrecan (*AGG*) messenger RNA levels. The osteogenic potential was determined by monitoring the alkaline phosphatase activity and calcium content, and the adipogenic potential was determined from the glycerol-3-phosphate dehydrogenase activity and oil red O staining. *Results.* Systemic vascular diseases, including arteriosclerosis obliterans and Buerger disease, did not significantly affect the trilineage differentiation potential of the cells. Under these conditions, all chondrocyte markers examined, including the *SOX9* messenger RNA level, showed age-related decline, whereas none of the osteoblast or adipocyte markers showed age-dependent changes. *Conclusions.* The aging of donors from young adult to elderly selectively decreased the chondrogenic potential of mesenchymal stromal cells. This information will be useful in stromal cell–based therapy for cartilage-related diseases.

Key Words: *aging, bone marrow, chondrocytes, differentiation, mesenchymal stromal cells*

Introduction

Mesenchymal stromal cells (MSCs) can differentiate into chondrocytes, osteoblasts and adipocytes and participate in the development and regeneration of skeletal tissues (1–4). MSCs are thought to be promising for regenerative therapy in various diseases, including osteoarthritis, the degeneration of intervertebral disc tissue and the failure of bone fracture healing in the elderly. For example, Wakitani *et al.* (5) have shown that autologous culture–expanded bone marrow MSC transplantation promotes the repair of cartilage defects in osteoarthritic knees. However, there have been conflicting reports on the effects of donor age on the chondrogenic, osteogenic and adipogenic potential of MSCs (6–26).

It is difficult to compare the *in vitro* differentiation potential of various MSC lines because the differentiation activities of *ex vivo*–expanded MSCs are unstable. MSCs gradually lose differentiation potential in culture as the result of culture stress or *in vitro* senescence. Some batches of fetal bovine serum (FBS) markedly enhance culture stress or *in vitro* senescence of MSCs. In contrast, MSCs expanded with fibroblast growth factor-2 (FGF-2) maintain

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(Received 30 January 2013; accepted 31 March 2013)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2013.03.015

their trilineage differentiation potential at high levels throughout many mitotic divisions (27). Furthermore, FGF-2 markedly enhances MSC proliferation (27). FGF-2 may affect the innate properties of MSCs, but MSCs readily lose multipotency in culture without FGF-2. Some other studies also used FGF-2 in MSC cultures (28). FGF-2 or other members of the FGF family may play a role in the maintenance of stemness both *in vitro* and *in vivo*. In the present study, MSCs were expanded in a medium containing the best batch among >10 batches of 10% FBS, and FGF-2 was added to the cultures every other day.

It is also difficult to compare the differentiation potential of many MSC lines isolated individually at the time of surgery because of the large degree of interassay variation. To minimize interassay variation, we cryopreserved all MSC lines at an early passage; all MSC lines were then exposed to each differentiation induction medium concurrently. On the other hand, the systemic condition of donors may affect the differentiation potential. In previous age-related studies, MSC lines were isolated from the bone marrow of patients with trauma, osteoarthritis, osteoporosis and other conditions. In the present study, we used bone marrow MSCs from patients with or without systemic vascular diseases.

The results indicated that glycosaminoglycan (GAG) accumulation and the messenger (mRNA) expression of *SOX9*, type 2 collagen (*COL2A*) and aggrecan (*AGG*) after exposure of MSC cultures to chondrogenesis induction medium decreased with increasing donor age. No such age-dependent changes were observed in alkaline phosphatase (ALPase) activity, calcium content and glycerol-3-phosphate dehydrogenase (GPDH) activity after exposure to osteogenesis induction medium or adipogenesis induction medium. Thus, the chondrogenic potential was the most sensitive to the aging of donors.

Methods

MSC lines

Human iliac bone marrow MSCs were isolated from patients undergoing surgical operations for periodontitis, rebuilding of the mandible or injury after a traffic accident. In addition, iliac bone marrow MSCs were isolated from patients with vascular disease (arteriosclerosis obliterans or Buerger disease) at Hiroshima University Hospital with the approval of the Hiroshima University Ethics Committee (Table I).

Table I. Donor information.

Donor No.	Age, years	Sex	Reasons for surgery	Systemic disorder
1	63	Male	Periodontitis	
2	39	Male	Periodontitis	
3	64	Female	Periodontitis	
4	25	Male	Periodontitis	
5	46	Female	Periodontitis	
6	56	Female	Periodontitis	
7	64	Male	Rebuilding of mandible	
8	59	Male	Arteriosclerosis obliterans	Renal failure
9	55	Male	Arteriosclerosis Obliterans	
10	61	Male	Arteriosclerosis obliterans	Arteriosclerosis
11	53	Male	Buerger disease	
12	81	Male	Arteriosclerosis obliterans	Arteriosclerosis
13	28	Male	Traffic injury	
14	68	Male	Arteriosclerosis obliterans	Diabetic
15	65	Male	Arteriosclerosis obliterans	Arteriosclerosis
16	29	Male	Buerger disease	
17	57	Male	Arteriosclerosis obliterans	Diabetic

Bone marrow aspirates were mixed with Dulbecco modified Eagle's medium (DMEM) (Sigma, St Louis, MO, USA) supplemented with 10% FBS (Hyclone, Logan, UT, USA), 100 U/mL penicillin G (Sigma) and 100 μg/mL streptomycin (Sigma) (growth medium/medium-A) in the presence of 200 units/mL heparin. The cells were centrifuged at 500*g* for 5 min and were then suspended in medium-A without heparin. The cellular elements of the bone marrow were identified with the use of a blood cell counter (Celltac α) (Nihon Kohden, Tokyo, Japan). Suspended cells were seeded at a density of 5×10^5 white blood cells per cm^2 on 10-cm culture dishes (Corning, Nagog Park Acton, MA, USA) to obtain plastic-adherent marrow cells at 37°C in a 5% CO_2 incubator. After 72 h, floating cells were removed, and the medium was replaced by fresh medium-A. Attached cells were fed with medium-A supplemented with 1 ng/mL FGF-2 (Kaken Pharmaceutical, Tokyo, Japan). FGF-2 was added every other day. When cultures approached confluence, cells were harvested with 0.05% trypsin/ethylenediamine tetra-acetic acid (EDTA) (GIBCO Life Technologies Corp, Carlsbad, CA, USA). The isolated cells were seeded at 3000 cells per cm^2 with medium-A supplemented with 1 ng/mL FGF-2. At passage 3, cells were suspended with freezing medium (Cell Bunker I) (Juji Field Inc, Tokyo, Japan) at 1×10^6 cells/tube and cryopreserved in a freezer at −152°C until the differentiation assays. Frozen MSCs were thawed and re-cultured on 10-cm dishes until cultures reached 80% confluence. These cells were passaged once on 10-cm dishes with medium-A supplemented with 1 ng/mL FGF-2 before the differentiation assays. MSCs were cultured for 24–28 d before cryopreservation and for 14 d after thawing before the differentiation assays. The time in culture and expansion before differentiation may affect the differentiation potential of MSCs, but