Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 332 (2005) 297–303
BBRC
www.elsevier.com/locate/ybbrc

Molecular markers distinguish bone marrow mesenchymal stem cells from fibroblasts

Masakazu Ishii [a,1], Chika Koike [a,1], Akira Igarashi [a,b], Katsuyuki Yamanaka [a,b], Haiou Pan [a,b], Yukihito Higashi [c], Hiroyuki Kawaguchi [d], Masaru Sugiyama [e], Nobuyuki Kamata [e], Tomoyuki Iwata [d], Takehiro Matsubara [f], Kozo Nakamura [f], Hidemi Kurihara [d], Koichiro Tsuji [b], Yukio Kato [a,b,*]

[a] *Department of Dental and Medical Biochemistry, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*
[b] *Two Cells Co. Ltd. Hiroshima, Japan*
[c] *Department of Cardiovascular Physiology and Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*
[d] *Department of Periodontal Medicine, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan*
[e] *Department of Oral and Maxillofacial Surgery, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*
[f] *Department of Orthopedic Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*

Received 15 April 2005
Available online 30 April 2005

Abstract

To characterize mesenchymal stem cells (MSC), we compared gene expression profiles in human bone marrow MSC (11 lines) and human fibroblasts (4 lines) by RT-PCR and real time PCR. Messenger RNA levels of MHC-DR-α, MHC-DR-β, MHC-DR-associated protein CD74, tissue factor pathway inhibitor-2, and neuroserpin were much higher in MSC than in fibroblasts, even in the presence of large interindividual variations. Those of adrenomedullin, apolipoprotein D, C-type lectin superfamily member-2, collagen type XV α1, CUG triplet repeat RNA-binding protein, matrix metalloproteinase-1, protein tyrosine kinase-7, and Sam68-like phosphotyrosine protein/T-STAR were lower in MSC than in fibroblasts. FACS analysis showed that cell surface expression of MHC-DR was also higher in MSC than in fibroblasts. MHC-DR expression decreased after osteogenic differentiation, whereas the expression of adrenomedullin—a potent stimulator of osteoblast activity—along with collagen XV α1 and apolipoprotein D increased after osteogenic differentiation. The marker genes identified in this study should be useful for characterization of MSC both in basic and clinical studies.

Keywords: Bone marrow; Marker genes; Mesenchymal stem cell; Regenerative medicine

Bone marrow mesenchymal stem cells (MSC), which are also called plastic-adherent marrow cells or bone marrow stromal cells, can differentiate into osteoblasts, chondrocytes, adipocytes, tenocytes, and muscle cells in vitro and/or in vivo [1–11]. MSC can easily be isolated from adult bone, and can be expanded with serum ex vivo, so these cells are promising for regenerative medicine: they are already being used for treatment of osteogenesis imperfecta or bone/cartilage defects [7,8]. Nonetheless, MSC have not been fully characterized, and thus it is difficult to examine whether ex vivo expanded MSC population is free of fibroblasts. Bone marrow may contain fibroblasts or can be contaminated by fibroblasts during aspiration, and the fibroblasts—together with MSC—could possibly be expanded in the presence of serum ex vivo. However, the appearance

* Corresponding author. Fax: +81 82 257 5629.
E-mail address: ykato@hiroshima-u.ac.jp (Y. Kato).
[1] These authors contributed equally.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.04.118

of MSC is similar to that of fibroblasts. In addition, we found that several cell surface antigens, previously considered to be MSC markers, were also expressed in fibroblasts at similar levels. In other words, molecular markers for MSC remain unknown. To address this issue, we compared gene expression profiles between human bone marrow MSC and connective tissue fibroblasts—using DNA macroarrays, RT-PCR, and real time PCR. We identified 13 genes differentially expressed between these cells. Since contamination of transplantable cells by fibroblasts may delay regeneration, we used these markers to distinguish MSC from fibroblasts before transplantation at Hiroshima University Hospital.

Materials and methods

MSC and fibroblast cultures. MSC were obtained from iliac crest or alveolar/jaw bone according to a protocol approved by Ethical Authorities at Hiroshima University. In addition, human iliac MSC were purchased from Bio-Whittaker (Walkersville, MD). For isolation of alveolar/jaw bone marrow, we selected patients whose bone marrow sites had been opened during oral surgery, and obtained marrow aspirates using routine syringes and needles [12]. Bone marrow cells including erythrocytes were seeded at a density of 0.1 ml aspirate per 35-mm tissue culture dish (Corning) and maintained in 2 ml DMEM supplemented with 10% fetal bovine serum (Hyclone), 100 U/ml penicillin G, and 100 μg/ml streptomycin (medium-A). Three days after seeding, floating cells were removed and the medium was replaced by fresh medium-A. Thereafter, attached cells (plastic-adherent marrow cells) were fed with fresh medium-A supplemented with 1 ng/ml FGF-2. FGF-2 was added every other day. Passages were performed when cells were approaching confluence: the cells were seeded at a density of 5×10^3 cells/cm^2 on 100-mm tissue culture dishes (Corning) and maintained in 10 ml medium-A supplemented with 1 ng/ml FGF-2 [9]. To avoid direct actions of FGF-2 on gene expression, FGF-2 was removed from the culture medium of MSC or fibroblasts 72 h before isolation of RNA. Human skin fibroblasts were purchased from Kurabo (Tokyo, Japan), and human gingival fibroblasts were isolated as described previously [13]. Fibroblasts were also maintained in culture as described above.

Osteogenic differentiation. MSC or fibroblasts at passage fifth to eighth were seeded at 4×10^4 cells per 16-mm well in a 24-well plate, and maintained for 7–28 days in medium-A supplemented with 10 mM β-glycerophosphate, 100 nM dexamethasone, and 50 μg/ml ascorbic acid-2-phosphate [1,9].

Microarray analysis and RT-PCR. Total RNA was isolated using TRIZOL reagent (Invitrogen), when the fifth to eighth passage cultures became confluent. Poly(A)$^+$ RNA was purified using Micro poly(A) purist (Ambion). DNA microarray analysis was performed with 0.5 μg poly(A)$^+$ RNA by Kurabo Life Array analysis service (Incyte Genomics; Lot # KL01081).

For RT-PCR, first-strand cDNA was synthesized with 1 μg of total RNA using SuperScript first-strand synthesis system for RT-PCR (Invitrogen). Using the cDNAs as a template, PCR was carried out under the following conditions: denaturation at 94 °C for 30 s and primer extension at 65 °C for 1.5 min in 28 cycles for adrenomedullin; 30 cycles for matrix metalloproteinase-1 (MMP-1), tissue factor pathway inhibitor-2, apolipoprotein D, collagen type XV α1, CUG triplet repeat RNA-binding protein, serine (or cysteine) proteinase inhibitor clade-1 member-1 (neuroserpin), protein tyrosine kinase-7, Sam68-like protein, MHC-DR-α and MHC-DR-β; 33 cycles for C-type lectin superfamily member-2. The sequences of primers are shown in supplementary Table 1. Obtained PCR products were separated on 1% agarose gels,and stained with ethidium bromide.

Real time PCR. With the above cDNAs (1 μg) as a template, real time quantitative RT-PCR analyses were performed using an ABI Prism 7700 Sequence Detection System instrument and software (PE Applied Biosystems, Foster City, CA). Sequences of the primers and probes are shown in supplementary Table 2. The primers and probes for CD73 (4331182-CD74) and GAPDH (4310884E) were purchased from PE Applied Biosystems. The mRNA level relative to that of GAPDH was calculated.

FACS analysis. Cells at passage fifth to eighth were harvested with trypsin and EDTA, centrifuged at 1500*g* for 5 min, and resuspended at 5×10^6 cells/ml in phosphate-buffered saline (PBS) containing 3% fetal bovine serum. Aliquots containing 10^5 cells were incubated with individual primary antibodies or control IgG for 30 min at room temperature. The cells were washed in PBS containing 3% fetal bovine serum and incubated with a fluorescent conjugated secondary antibody for 30 min at room temperature. Samples were analyzed using a FACSCalibur cytometer (Becton Dickinson), and the data were analyzed using CELLQUEST software (Becton Dickinson). The following monoclonal antibodies (mAbs) were used: fluorescein isothiocyanate (FITC)-conjugated or *R*-phycoerythrin (PE)-conjugated antibodies against HLA-DR (MHC-DR), CD13, CD14, CD29, CD34, CD44, CD49b, CD54, CD56, CD71, CD90, CD105, CD106, CD117, CD124, CD138, CD144, MHC-DR, HLA-ABC, mouse-IgG1, mouse-IgG2a or mouse IgM (Immunotech Coulter Company); antibodies against MHC-DR, CD73, CD74, CD123, CD140b, CD166 or mouse-IgG3 (Pharmingen); antibodies against Flk-1 (Santa Cruz Biotechnology); antibodies against MT-MMP-1 (Sigma); antibodies against STRO-1 (Genzyme); antibodies against RANKL (R&D Systems), and anti-rabbit-IgG (Chemicon International).

Statistical analysis. Student's *t* test was used.

Results

Differential expression of candidate marker genes between MSC and fibroblasts

MSC or fibroblast lines were obtained from alveolar/jaw bone, ilium, gums or skin of young adults of similar age (Table 1). Under the osteogenic conditions, alveolar (MSC-1) and iliac MSC (MSC-9) induced matrix calcification—which was stained with alizarin red—on days 21 and 28 (supplementary Fig. 1). All of the other MSC lines—but none of the fibroblast lines—also induced calcification by day 28 (Table 1).

Gene expression profile was compared between MSC-1 and fibroblast-2 using DNA microarrays (9400 genes): many (~100) genes showed different signals (>2-fold) between these cells. To confirm the different expressions, we performed RT-PCR analysis for these genes with seven MSC and four fibroblast lines: many genes showed large interindividual variations or very low expression levels, so the comparison was difficult. Nonetheless, the following genes appeared to be expressed differently between MSC and fibroblasts: the mRNA levels of MMP-1 (Fig. 1A), adrenomedullin, (B), protein tyrosine kinase-7 (C), collagen type XV α1 (D), Sam68-like phosphotyrosine protein/T-STAR (E), C-type lectin superfamily member-2 (F), CUG triplet repeat RNA-binding protein

Table 1
Fibroblast and MSC lines used in this study

Cell lines	Tissues	Age	Sex	OB
Fibroblast-1	Gums	17	M	–
Fibroblast-2	Gums	18	F	–
Fibroblast-3	Skin	29	F	–
Fibroblast-4	Skin	33	F	–
MSC-1	Alveolar/jaw	26	M	+
MSC-2	Alveolar/jaw	24	F	+
MSC-3	Alveolar/jaw	19	F	+
MSC-4	Alveolar/jaw	23	F	+
MSC-5	Alveolar/jaw	24	F	+
MSC-6	Alveolar/jaw	19	F	+
MSC-7	Alveolar/jaw	36	M	+
MSC-8	Ilium	24	F	+
MSC-9	Ilium	22	F	+
MSC-10	Ilium	19	F	+
MSC-11	Ilium	24	M	+

Note. OB, osteogenic differentiation.

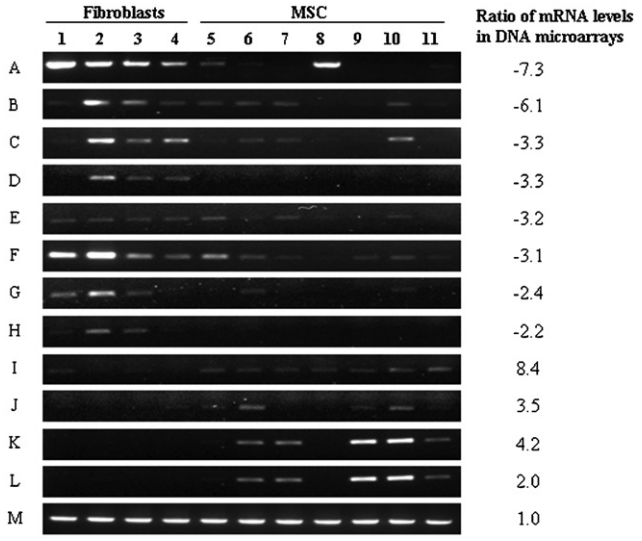

Fig. 1. RT-PCR analysis for genes expressed differently in fibroblasts and MSC. Fibroblast-1, -2, -3, and -4 (lanes 1–4, respectively) and MSC-1, -2, -3, -4, -9, 10, and -11 (lanes 5–11, respectively) were isolated from 11 different donors. The ratio of the mRNA level between MSC and fibroblasts in DNA microarray analysis is shown on the right of the panel. (A) MMP-1; (B) adrenomedullin; (C) protein tyrosine kinase-7; (D) collagen type XV α1; (E) Sam68-like phosphotyrosine protein; (F) C-type lectin superfamily member-2; (G) CUG triplet repeat RNA-binding protein; (H) apolipoprotein D; (I) tissue factor pathway inhibitor-2; (J) neuroserpin; (K) MHC-DR-α; (L) MHC-DR-β; (M) GAPDH.

(G), and apolipoprotein D (H) were lower in MSC than in fibroblasts. In contrast, mRNA levels of tissue factor pathway inhibitor-2 (I), neuroserpin (J), MHC-DR-α (K), and MHC-DR-β (L) were higher in MSC than in fibroblasts. The GAPDH mRNA level in MSC was equal to that in fibroblasts (M).

Real time PCR analysis showed that mRNA levels of these genes, relative to GAPDH, were statistically different ($P < 0.05$) between 11 MSC and 4 fibroblast lines (Fig. 2). Of the 12 genes, MHC-DR-α and -β showed differential expression between iliac and alveolar MSC (see below).

FACS analysis of MHC-DR expression

Since MHC-DR-α and -β mRNA levels were higher in MSC than in fibroblasts, we examined the protein level of MHC-DR by FACS analysis (Fig. 3A): no positive cells were detected with fibroblast lines, whereas MSC lines showed MHC-DR expression at low or moderate levels.

Differential expression of CD74 mRNA between MSC and fibroblasts

Since MHC-DR expression was higher in MSC than in fibroblasts, we compared the expression of MHC-DR-associated protein CD74 in MSC and fibroblasts. The mRNA level of CD74 in iliac MSC was higher than that in fibroblasts, although alveolar/jaw MSC showed CD74 expression at lower levels than did iliac MSC (Fig. 3B).

Changes in marker expressions after osteogenic differentiation

We compared marker gene expressions before and after osteogenic differentiation. The MHC-DR-α and/or -β mRNA levels in five MSC lines decreased after osteogenic differentiation, and the MHC-DR-β mRNA level in alveolar MSC was lower than that in iliac MSC (Fig. 4A). In contrast, apolipoprotein D, adrenomedullin, and collagen type XV α1—which were suppressed both in iliac and alveolar MSC—increased after osteogenic differentiation (Fig. 4B). No changes in mRNA levels of tissue factor pathway inhibitor-2, neuroserpin, C-type lectin superfamily member-2, CUG triplet repeat RNA-binding protein, MMP-1, protein tyrosine kinase-7, or Sam68-like phosphotyrosine protein could be detected after osteogenic differentiation (data not shown). These findings suggest that MHC-DR is a positive marker for undifferentiated MSC, whereas the expression of apolipoprotein D, adrenomedullin, and collagen type XV α1 is temporarily suppressed at the undifferentiated stage.

FACS analysis of cell surface antigens

Some cell surface antigens—in addition to MHC-DR—may be expressed selectively in MSC. SH2 (CD105), SH3 (CD73), ALCAM (activated leukocyte cell adhesion molecule/CD166), CD13, CD29 (integrin β-1), PDGF receptor, CD44 (hyaluronate receptor), and CD90 (Thy-1) were expressed in MSC and/or perichondrium mesenchymal stem cells

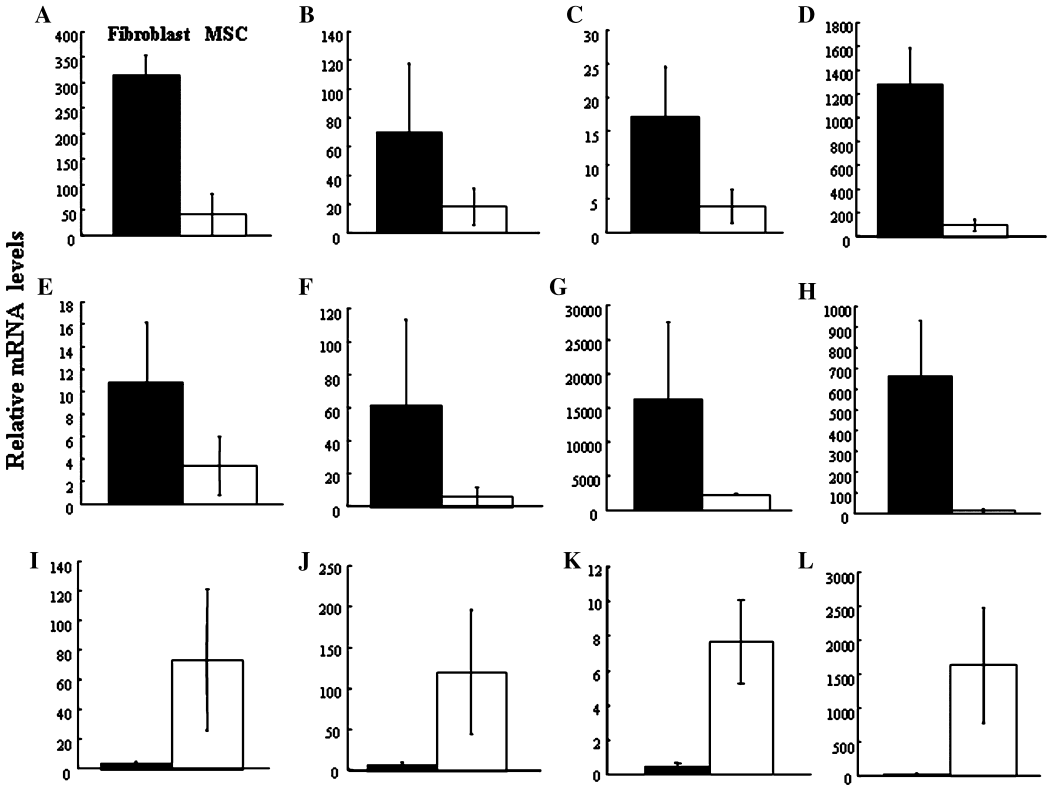

Fig. 2. Real time PCR analysis of candidate genes. Expressions of the candidate genes relative to GAPDH in confluent cultures of fibroblast-1, -2, -3, and -4 (closed columns) and MSC-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, and -11 (open columns) were examined by real time PCR. (A) MMP-1; (B) adrenomedullin; (C) protein tyrosine kinase-7; (D) collagen type XV α1; (E) Sam68-like phosphotyrosine protein; (F) C-type lectin superfamily member-2; (G) CUG triplet repeat RNA-binding protein; (H) apolipoprotein D; (I) tissue factor pathway inhibitor-2; (J) neuroserpin; (K) MHC-DR-α; (L) MHC-DR-β. Values are averages ± SD for 4 or 11 cultures. The mRNA levels of all examined genes were statistically significant between MSC and fibroblast lines ($P < 0.05$).

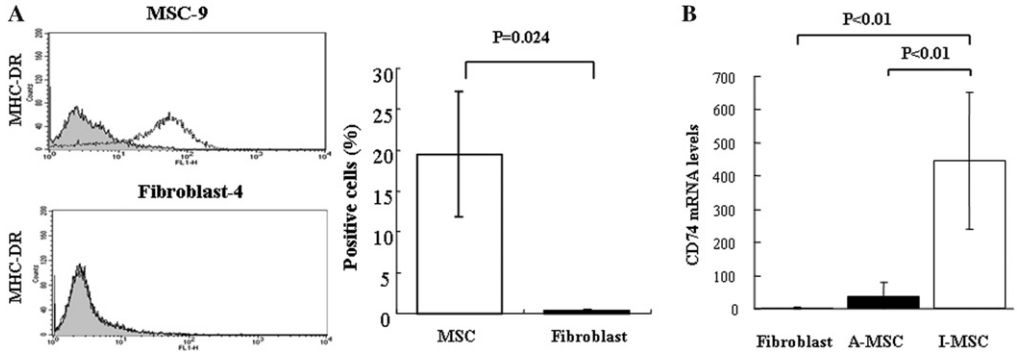

Fig. 3. Expression levels of MHC-DR and CD74 in MSC and fibroblasts. (A) FACS analysis of MHC-DR expression on the cell surface of MSC. MHC-DR expression in fibroblast-2, -3, and -4 (closed columns) and MSC-5, -6, -7, -8, -9, -10, and -11 (open columns) were examined by FACS analysis with anti-MHC-DR antibody (Immunotech Coulter). Values are averages ± SD for three fibroblast or seven MSC lines. (B) The expression of CD74 mRNA in alveolar/jaw MSC (A-MSC), iliac MSC (I-MSC), and fibroblasts. CD74 mRNA expression in fibroblast-1, -2, -3, and -4, alveolar MSC-1, -2, -3, -4, and -5, and iliac MSC-9, 10, and -11 in confluent cultures was examined by real time RT-PCR. Values are averages ± SD for three to five cultures.

[1,14–18]. SH2 and SH3 levels decreased after osteogenic differentiation [14], and ALCAM was expressed selectively in perichondrium mesenchymal cells [15]. However, it is unknown whether these cell surface markers were present or absent in fibroblasts. In this study, we compared the levels of these markers in human MSC and fibroblasts: overall, the surface marker expression levels in MSC in this study were consistent with those reported in the literature [1,14–16,18], but none of the cell surface antigens—including SH2,

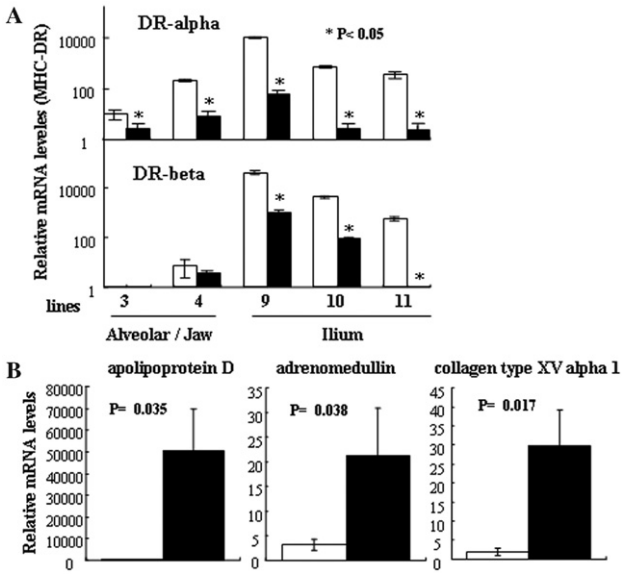

Fig. 4. Effects of osteogenic differentiation on gene expression in MSC. (A) Decrease in MHC-DR mRNA expression after osteogenic differentiation. MSC-3, -4, -9, -10, and -11 were maintained for 28 days in medium-A alone (open column) or in medium-A supplemented with 10 mM β-glycerophosphate, 100 nM dexamethasone, and 50 μg/ml ascorbic acid-2-phosphate in the osteogenic status (closed columns). Values are averages ± SD for three cultures. (B) Increases in mRNA levels of marker genes in MSC after osteogenic differentiation. MSC-3, -4, -9, -10, and -11 were maintained for 28 days in medium-A alone (open columns) or in medium-A supplemented with 10 mM β-glycerophosphate, 100 nM dexamethasone, and 50 μg/ml ascorbic acid-2-phosphate (closed columns). Values are averages ± SD for five cultures.

SH3, ALCAM, and STRO-1—differed between MSC and fibroblasts (supplementary Fig. 2). The levels of these cell surface markers in iliac MSC or fibroblasts were similar to those in alveolar MSC [12]. STRO-1 has been found in freshly isolated MSC [17], but in this study, MSC showed STRO-1 expression at a low level (supplementary Fig. 2). Previous studies have also shown that STRO-1$^+$ cells in human MSC population are only 7 ± 6% [18], so STRO-1 may be progressively lost with time in these cultures. In any case, our findings suggest that STRO-1 is not essential for the differentiation potential of MSC.

Application of marker genes to regenerative medicine

At Hiroshima University Hospital, we have commenced clinical studies on regenerative medicine for periodontal diseases, using autologous MSC. Before transplantation, we compared the marker gene expressions in patients' MSC (iliac MSC) with those in standard iliac MSC and fibroblast lines (Table 2). In the case of patient-1 (63, male), all examined genes in expanded plastic-adherent cells showed a similar expression pattern to that of standard MSC lines. Fibroblast contamination was unlikely, because MMP-1 and collagen type XV α1 mRNA levels in the marrow cells were only 1% of those in the fibroblastic lines (Table 2). In contrast, MHC-DR-α and -β mRNA levels in the marrow cells were 1000-fold greater than those in the fibroblast lines. Similar results were obtained with cells from patient-2 (39, male), patient-3 (64, female), patient-4 (46, female), patient-5 (25, male), patient-6 (56, female), and patient-7 (22, male) (data not shown). After the quality examination, we were able to transplant these cells with abundant self-confidence.

Table 2
Evaluation of ex vivo expanded MSC population before transplantation using marker genes

Genes	Relative mRNA levels		
	Patient-1	Standard MSC	Standard fibroblasts
MMP-1	28	9 ± 10	1206 ± 1097
Adrenomedullin	3	1 ± 1	15 ± 5
CUG triplet repeat, RNA-binding protein-2	7	6 ± 5	148 ± 107
Collagen type XV αl	1	No signal	475 ± 163
Sam68-like phosphotyrosine protein	No signal	No signal	3 ± 2
C-type lectin, superfamily member-2	3	5 ± 4	231 ± 191
Apolipoprotein D	14	3 ± 2	426 ± 326
Tissue factor pathway inhibitor-2	9	23 ± 17	4 ± 4
MHC-DR-β	2121	563 ± 769	2 ± 1
MHC-DR-α	1561	582 ± 402	2 ± 2

Before transplantation, we examined the expression pattern of marker genes in patient's MSC expanded ex vivo, standard MSC, and standard fibroblasts. The patient's MSC were cultured, and total RNA in confluent cultures at passage 3 was isolated as described in Materials and methods. Gene expression profile in the patient's cells was compared with that in RNA samples of MSC-9, MSC-10, MSC-11, fibroblast-1, fibroblast-2, and fibroblast-3 by real time PCR. Values are averages of duplicate determinations (patient-1) or averages ± SD for three cultures (standard lines).

Discussion

We identified several genes differentially expressed between MSC and fibroblasts, and the differential expression was unrelated to age, sex or culture conditions. We used MSC and fibroblasts from donors of similar age and cultured under similar conditions; sex did not affect the gene expressions (data not shown).

In addition, the difference was not due to in vivo location of the cell, since we used iliac and alveolar MSC, and skin and gingival fibroblasts. Alveolar MSC had potent osteogenic potential in vitro and in vivo, although their chondrogenic or adipogenic potential was less than that of iliac MSC [12]. Alveolar and iliac MSC shared many common marker genes, with a few genes (MHC-DR and CD74) being expressed at different levels.

MSC showed a lower level of MMP-1 and a higher level of its inhibitor—tissue factor pathway inhibitor-2—than did fibroblasts. Tissue factor pathway inhibitor-2 suppresses the activity of the collagenases—MMP-1 and MMP-13—as well as the gelatinases—MMP-2 and MMP-9 [19], so MSC may be less active in collagen-matrix breakdown. On the other hand, reduced expression of neuroserpin may increase activities of tissue-type plasminogen activator [20].

Type XV collagen occurs in basement membrane zones of tissues [21], and type XV collagen-derived endostatin has antiangiogenic actions [22]. Adrenomedullin—a member of the calcitonin family—stimulates osteoblastic activity and bone growth in vivo [23,24]. Accordingly, adrenomedullin mRNA expression increased after osteogenic differentiation of MSC. Protein tyrosine kinase-7 is essential for neural tube closure [25] and is involved in tumor metastasis [26]. Sam68-like phosphotyrosine protein (an Src substrate) is involved in cell proliferation [27,28]. Apolipoprotein D increases platelet-derived growth factor actions and synergistically stimulates migration of vascular smooth muscle cells [29]. Roles of the molecules in MSC remain unclear, but higher or lower levels of these molecules are characteristic of MSC. We are investigating the physiological roles of molecules in MSC or fibroblasts.

We also found that the expression of MHC-DR (class II) was higher in MSC than in fibroblasts, whereas MHC (class I)/HLA-ABC was expressed in MSC and fibroblasts at similar levels (supplementary Fig. 2). The expression level of MHC-DR-associated protein CD74 was also higher in MSC than in fibroblasts. CD74 binds to MHC-DR and this interaction involves the peptide-binding groove of MHC-DR [30]: MHC-DR-α and -β molecules are assembled with CD74 protein in the endoplasmic reticulum [31]. The physiological relevance of MHC-DR and CD74 expression in MSC is unknown, but MSC can modulate immune response [32].

Plastic-adherent marrow cells/MSC are often designated as a colony-forming unit-fibroblastic (CFU-F), and some CFU-F colonies do not show any differentiation potentials. Thus, ex vivo expanded adherent cell populations may contain fibroblast-like cells. In addition, bone marrow samples can be contaminated by connective tissue fibroblasts during surgery or aspiration. Contamination of MSC populations by fibroblasts delays regeneration and could be harmful, and thus we need to confirm the absence of fibroblasts before transplantation. Conventionally we have to incubate transplantable cells in differentiation-induction media for 21–28 days to examine the presence of MSC with multi-lineage differentiation potential, but this does not show the presence of fibroblasts directly. In the present study, we developed a method of distinguishing MSC from fibroblasts promptly before transplantation—using marker genes. This quality examination should be crucial to regenerative medicine with MSC. In addition, identification of marker genes will help us characterize MSC and examine their in vivo location.

Acknowledgment

This study was supported by a fund from JST, Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2005.04.118.

References

[1] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[2] S.A. Kuznetsov, M.H. Mankani, S. Gronthos, K. Satomura, P. Bianco, P.G. Robey, Circulating skeletal stem cells, J. Cell Biol. 153 (2001) 1133–1140.
[3] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrck, Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng. 7 (2001) 211–228.
[4] S. Makino, K. Fukuda, S. Miyoshi, F. Konishi, H. Kodama, J. Pan, M. Sono, T. Takahashi, S. Hori, H. Abe, J.-I. Hata, A. Umezawa, S. Ogawa, Cardiomyocytes can be generated from bone marrow stromal cells in vitro, J. Clin. Invest. 103 (1999) 697–705.
[5] R.J. Deans, A.B. Moseley, Mesenchymal stem cells: biology and potential clinical uses, Exp. Hematol. 28 (2000) 875–884.
[6] G.C. Kopen, D.J. Prockop, D.G. Phinney, Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocyte after injection into neonatal mouse brains, Proc. Natl. Acad. Sci. USA 96 (1999) 10711–10716.
[7] E.M. Horwitz, D.J. Prockop, L.A. Fitzpatrick, W.W. Koo, P.L. Gordon, M. Neel, M. Sussman, P. Orchard, J.C. Marx, R.E. Pyeritz, M.K. Brenner, Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta, Nat. Med. 5 (1999) 309–313.
[8] R. Quarto, M. Mastrogiacomo, R. Cancedda, S.M. Kutepov, V. Mukhachev, A. Lavroukov, E. Kon, M. Marcacci, Repair of large bone defects with the use of autologous bone marrow stromal cells, N. Engl. J. Med. 344 (2001) 385–386.
[9] S. Tsutsumi, A. Shimazu, K. Miyazaki, H. Pan, C. Koike, E. Yoshida, K. Takagishi, Y. Kato, Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response of FGF, Biochem. Biopys. Res. Commun. 288 (2001) 413–419.
[10] G. Bianchi, A. Banfi, M. Mastrogiacomo, R. Notaro, L. Luzzatto, R. Cancedda, R. Quarto, Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2, Exp. Cell Res. 287 (2003) 98–105.
[11] T. Matsubara, S. Tsutsumi, H. Pan, H. Hiraoka, R. Oda, M. Nishimura, H. Kawaguchi, K. Nakamura, Y. Kato, A new technique to expand human mesenchymal stem cells using

basement membrane extracellular matrix, Biochem. Biophys. Res. Commun. 313 (2004) 503–508.
[12] T. Matsubara, K. Suardita, M. Ishii, M. Sugiyama, A. Igarashi, R. Oda, M. Nishimura, M. Saito, K. Nakagawa, K. Yamanaka, K. Miyazaki, M. Shimizu, U.K. Bhawal, K. Tsuji, K. Nakamura, Y. Kato, Alveolar bone marrow as a cell source for regenerative medicine: differences between alveolar and iliac bone marrow stromal cells, J. Bone Mineral Res. 20 (2005) 399–409.
[13] K. Kawahara, A. Shimazu, Expression and intracellular localization of progesterone receptors in cultured human gingival fibroblasts, J. Periodontal Res. 38 (2003) 242–246.
[14] S.E. Haynesworth, M.A. Baber, A.I. Caplan, Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies, Bone 13 (1992) 69–80.
[15] F. Arai, O. Ohneda, T. Miyamoto, X.Q. Zhang, T. Suda, Mesenchymal stem cells in perichondrium express activated leukocyte cell adhesion molecule and participate in bone marrow formation, J. Exp. Med. 195 (2002) 1549–1563.
[16] W.J. Rombouts, R.E. Ploemacher, Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture, Leukemia 17 (2003) 160–170.
[17] S. Gronthos, S.E. Graves, S. Ohta, P.J. Simmons, The STRO-1^+ fraction of adult human bone marrow contains the osteogenic precursors, Blood 84 (1994) 4164–4173.
[18] M. Bensidhoum, A. Chapel, S. Francois, C. Demarquay, C. Mazurier, L. Fouillard, S. Bouchet, J.M. Bertho, P. Gourmelon, J. Aigueperse, P. Charbord, N.C. Gorin, D. Thierry, M. Lopez, Homing of in vitro expanded Stro-1^- or Stro-1^+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment, Blood 103 (2004) 3313–3319.
[19] M.P. Herman, G.K. Sukhova, W. Kisiel, D. Foster, M.R. Kehry, P. Libby, U. Schonbeck, Tissue factor pathway inhibitor-2 is a novel inhibitor of matrix metalloproteinases with implications for atherosclerosis, J. Clin. Invest. 107 (2001) 1117–1126.
[20] M. Yepes, D.A. Lawrence, Tissue-type plasminogen activator and neuroserpin: a well-balanced act in the nervous system? Trends Cardiovasc. Med. 14 (2004) 173–180.
[21] L. Eklund, J. Piuhola, J. Komulainen, R. Sormunen, C. Ongvarrasopne, R. Fasslker, A. Muona, M. Ilves, H. Ruskoaho, T.E.S. Takala, Pihlajaniemi, Lack of type XV collagen causes a skeletal myopathy and cardiovascular defects in mice, Proc. Natl. Acad. Sci. USA 98 (2001) 1194–1199.
[22] R. Ramchandran, M. Dhanabal, R. Volk, J.F. Waterman, M. Segal, H. Lu, B. Knebelmann, P.S. Vilkas, Angiogenic activity of restin, NC 10 domain of human collagen XV: comparison to endostatin, Biochem. Biophys. Res. Commun. 255 (1999) 735–739.
[23] S.J. Wimalawansa, Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials, Endocr. Rev. 17 (1996) 533–585.
[24] J. Cornish, K.E. Callon, D.H. Coy, N.Y. Jiang, L. Xiao, G.J. Cooper, I.R. Reid, Adrenomedullin is a potent stimulator of osteoblastic activity in vitro and in vivo, Am. J. Physiol. 273 (1997) E1113–E1120.
[25] X. Lu, A.G. Borchers, C. Jolicoeur, H. Rayburn, J.C. Baker, M. Tessier-Lavigne, PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates, Nature 430 (2004) 93–98.
[26] D.J. Easty, P.J. Mitchell, K. Patel, V.A. Florenes, R.A. Spritz, D.C. Bennett, Loss of expression of receptor tyrosine kinase family genes PTK7 and SEK in metastatic melanoma, Int. J. Cancer 71 (1997) 1061–1065.
[27] J.P. Venables, C. Vernet, S.L. Chew, D.J. Elliott, R.B. Cowmeadow, J. Wu, H.J. Cooke, K. Artzt, I.C. Eperon, T-STAR/ETOILE: a novel relative of SAM68 that interacts with an RNA-binding protein implicated in spermatogenesis, Hum. Mol. Genet. 8 (1999) 959–969.
[28] M. Di Fruscio, T. Chen, S. Richard, Characterization of Sam68-like mammalian proteins SLM-1 and SLM-2: SLM-1 is a Src substrate during mitosis, Proc. Natl. Acad. Sci. USA 96 (1999) 2710–2715.
[29] W.C. Leung, A. Lawrie, S. Demaries, H. Massaeli, A. Burry, S. Yablonsky, J.M. Sarjeant, E. Fera, E. Rassart, J.G. Pickering, M. Rabinovitch, Apolipoprotein D and platelet-derived growth factor-BB synergism mediates vascular smooth muscle cell migration, Circ. Res. 95 (2004) 179–186.
[30] R.J. Cherry, I.E. Morrison, I. Karakikes, R.E. Barber, G. Silkstone, N. Fernandez, Measurements of associations of cell-surface receptors by single-particle fluorescence imaging, Biochem. Soc. Trans. 31 (2003) 1028–1031.
[31] A.J. Sant, J. Miller, MHC class II antigen processing: biology of invariant chain, Curr. Opin. Immunol. 6 (1994) 57–63.
[32] K. Le Blanc, I. Rasmusson, B. Sundberg, C. Gotherstrom, M. Hassan, M. Uzunel, O. Ringden, Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells, Lancet 363 (2004) 1439–1441.