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Gene and Protein Expression Analysis of Mesenchymal Stem Cells Derived From Rat Adipose Tissue and Bone Marrow

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Background: Mesenchymal stem cells (MSC) are multipotent and reside in bone marrow (BM), adipose tissue and many other tissues. However, the molecular foundations underlying the differences in proliferation, differentiation potential and paracrine effects between adipose tissue-derived MSC (ASC) and BM-derived MSC (BM-MSC) are not well-known. Therefore, we investigated differences in the gene and secretory protein expressions of the 2 types of MSC.

Methods and Results: ASC and BM-MSC were obtained from subcutaneous adipose tissue and BM of adult Lewis rats. ASC proliferated as rapidly as BM-MSC, and had expanded 200-fold in approximately 2 weeks. On microarray analysis of 31,099 genes, 571 (1.8%) were more highly (>3-fold) expressed in ASC, and a number of these genes were associated with mitosis and immune response. On the other hand, 571 genes (1.8%) were more highly expressed in BM-MSC, and some of these genes were associated with organ development and morphogenesis. In secretory protein analysis, ASC secreted significantly larger amounts of growth factor and inflammatory cytokines, such as vascular endothelial growth factor, hepatocyte growth factor and interleukin 6, whereas BM-MSC secreted significantly larger amounts of stromal-derived factor-1α.

Conclusions: There are significant differences between ASC and BM-MSC in the cytokine secretome, which may provide clues to the molecule mechanisms associated with tissue regeneration and alternative cell sources. (*Circ J* 2011; **75:** 2260–2268)

Key Words: Cell therapy; Mesenchymal stem cells; Microarray; Secretory protein

Mesenchymal stem cells (MSC) are multipotent cells that reside within various tissues, including bone marrow (BM), adipose tissue and many other tissues,[1,2] and can differentiate into a variety of cell types of mesodermal lineage.[1,3] MSC can be expanded in vitro over the short term, and they are thought to be an attractive tool for cell therapy. It has been demonstrated in animal and human studies of cardiovascular disease that transplanted BM-MSC induce neovascularization and differentiate into functional cells.[4–8] In addition, recent studies suggest that MSC exert tissue regeneration, secreting various kinds of angiogenic and cytoprotective factors.[6,9,10]

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Subcutaneous adipose tissue can be harvested more safely and noninvasively than BM, and ASC have emerged as a possible alternative cell source to BM-MSC.[9,11] We and others have demonstrated that ASC transplantation induces neovascularization in animal models of myocardial infarction and hindlimb ischemia.[12,13] ASC are similar to BM-MSC in terms of morphology and surface marker expression.[14] However, few data exist regarding their differences in biological activity, such as proliferative activity, differentiation potential and productive ability. Using microarray and enzyme-linked immunosorbent

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assay (ELISA), we have performed a comprehensive analysis to evaluate both the differences between ASC and BM-MSC, and their usage as an effective transplanted cell source from the point of view of the gene and protein expression profile of the 2 MSC sources.

Methods

Isolation and Culture of ASC and BM-MSC

All protocols were performed in accordance with the guidelines of the Animal Care Committee of the National Cardiovascular Center Research Institute and Kanazawa University. MSC isolation and culture were performed according to previously described methods.[15] In brief, we harvested BM from male Lewis rats (Japan SLC, Hamamatsu, Japan) weighing 200–250 g by flushing their femoral cavities with phosphate-buffered saline. Subcutaneous adipose tissue was harvested from the inguinal region and minced with scissors, then digested with 0.1% type I collagenase (300 U/ml; Worthington Biochemical, Lakewood, NJ, USA) for 1 h at 37°C in a water-bath shaker. After filtration with 100-μm filter mesh (Cell Strainer; Becton Dickinson, Bedford, MA, USA) and centrifugation at 1,240 g for 5 min, MSC were cultured in complete culture medium: α-minimal essential medium (α-MEM; Invitrogen, Carlsbad, CA, USA), 10% fetal bovine serum (FBS, Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). A small number of cells developed visible symmetric colonies by days 5–7. Nonadherent hematopoietic cells were removed, and the medium was replaced. The adherent, spindle-shaped MSC population expanded to >5×10^7 cells within 3–5 passages after the cells were first plated.

Cell Proliferation

We compared the proliferative activity of ASC and BM-MSC in cell culture, as reported previously.[16] In brief, cells (3×10^5 cells/dish) at passage 1 were cultured in a 10-cm dish with complete culture medium, and harvested at 70–90% confluency at each passage. Cell number was counted with a hemocytometer (n=5).

Differentiation of ASC and BM-MSC Into Adipocytes and Osteoblasts

MSC (1×10^5 cells/well) were seeded onto 12-well plates, and differentiation into adipocytes and osteocytes was induced when MSC were 70–80% confluent. MSC were cultured in α-MEM with MSC osteogenesis supplements (Dainippon Sumitomo Pharma, Osaka, Japan) according to the manufacturer's instructions. After 14–17 days of differentiation, cells were fixed and stained with Alizarin Red S (Sigma-Aldrich, St Louis, MO, USA). To induce differentiation into adipocytes, MSC were cultured with adipocyte differentiation medium: 0.5 mmol/L 3-isobutyl-1-methylxanthine (Wako Pure Chemical Industries, Osaka, Japan), 1 μmol/L dexamethasone (Wako Pure Chemical Industries), 50 μmol/L indomethacin (Wako Pure Chemical Industries), and 10 μg/ml insulin (Sigma-Aldrich) in Dulbecco's modified Eagle medium (DMEM, Invitrogen) containing 10% FBS. After 21 days of differentiation, adipocytes were stained with Oil Red O (Sigma-Aldrich). In order to measure lipid accumulation, isopropyl alcohol was added to the stained culture plate, the extracted dye was immediately collected, and the absorbance was measured spectrophotometrically at 490 nm (Bio-Rad, Hercules, CA, USA).

Microarray Analysis of ASC and BM-MSC

To compare the gene expression of ASC and BM-MSC, microarray analysis was performed according to previously reported methods.[17] Total RNA was extracted from cells using an RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was quantified by spectrometry, and its quality was confirmed by gel electrophoresis. Double-stranded cDNA was synthesized from 10 μg of total RNA, and in-vitro transcription was performed to produce biotin-labeled cRNA using GeneChip One-Cycle Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. After fragmentation, 10 μg of cRNA was hybridized with a GeneChip Rat Genome 230 2.0 Array (Affymetrix) containing 31,099 genes. The GeneChips were then scanned in a GeneChip Scanner 3000 (Affymetrix). Normalization, filtering and Gene Ontology analysis of the data were performed with GeneSpring GX 7.3.1 software (Agilent Technologies, Palo Alto, CA, USA). The raw data from each array were normalized as follows: each CEL file was preprocessed with RMA, and each measurement for each gene was divided by the 80th percentile of all measurements. Genes showing at least a 3-fold change were then selected.

Quantitative Real-Time Reverse-Transcription–Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from cultured BM-MSC and ASC as described, and 5 μg of total RNA was reverse-transcribed into cDNA using a QuantiTect reverse-transcription kit (Qiagen) according to the manufacturer's instructions. PCR amplification was performed in 50 μl containing 1 μl of cDNA and 25 μl of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, amplified from the same samples, served as an internal control. After an initial denaturation at 95°C for 10 min, a 2-step cycle procedure was used (denaturation at 95°C for 15s, annealing and extension at 60°C for 1 min) for 40 cycles in a 7700 sequence detector (Applied Biosystems). Gene expression levels were normalized according to that of GAPDH.

ELISA

To investigate differences in protein secretion between ASC and BM-MSC, we measured the levels of various bioactive proteins, including proliferative and anti-apoptotic factors such as hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and adrenomedullin (AM); chemokines such as stem cell-derived factor-1α (SDF-1α); inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6); and adipokines such as leptin and plasminogen activator inhibitor-1 (PAI-1). Protein levels were measured in conditioned medium 24 h after medium replacement. MSC (1×10^6 cells/dish) were plated in 10-cm dishes and cultured in complete culture medium. After 24 h, conditioned medium (n=6) was collected and centrifuged at 2,000 g for 10 min, and the supernatant was filtered through a 0.22-μm filtration unit (Millipore, Bedford, MA, USA). Angiogenic and growth factors were measured by ELISA according to each of the manufacturer's instructions (VEGF, TNF-α: R&D Systems, Minneapolis, MN, USA; HGF: Institute of Immunology, Tokyo, Japan; AM: Phoenix Pharmaceuticals, Burlingame, CA, USA; IL-6: Pierce, Rockford, IL, USA; adiponectin: AdipoGen, Seoul, Korea; PAI-1, Oxford Biomedica Reseach, Oxford, CT, USA).

Statistical Analysis

Data are expressed as mean±standard error of the mean. Comparisons of parameters among groups were made by 1-way ANOVA, followed by Newman-Keuls' test. Differences were

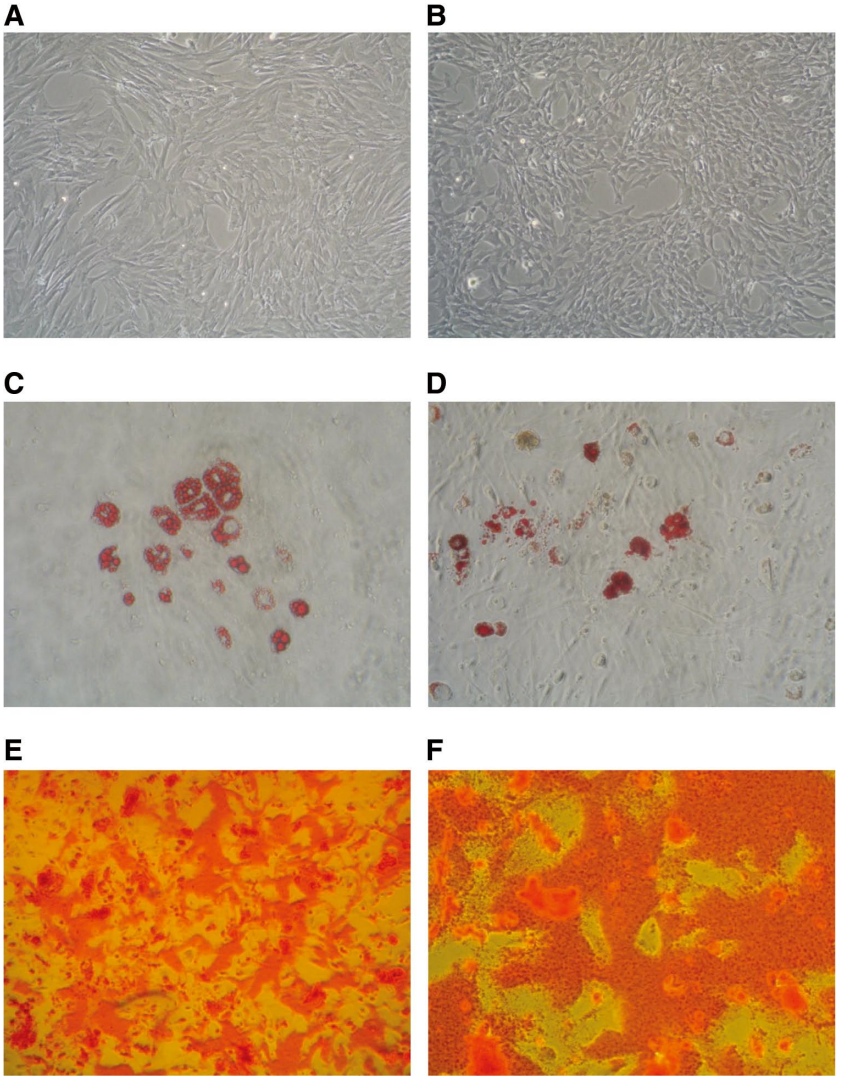

Figure 1. Morphology and differentiation of adipose tissue-derived mesenchymal stem cells (ASC) and bone marrow-derived mesenchymal stem cells (BM-MSC). Cultured ASC (**A**) and BM-MSC (**B**) at passage 2 (×100). Their typical morphology is a similar spindle shape, but ASC are slightly longer than BM-MSC. ASC (**C**) and BM-MSC (**D**) differentiated into adipocytes (×100; Oil Red O). ASC (**E**) and BM-MSC (**F**) differentiated into osteoblasts (×100; Alizarin Red S).

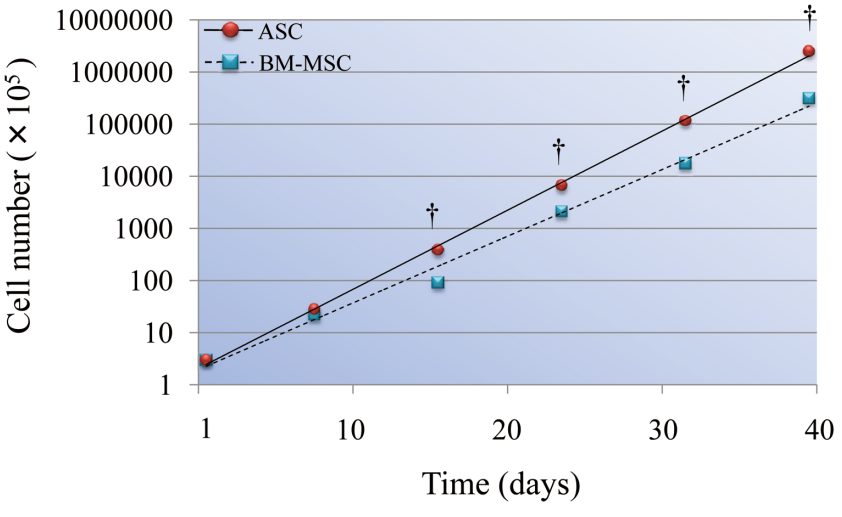

Figure 2. Proliferation of adipose tissue-derived mesenchymal stem cells (ASC) and bone marrow-derived mesenchymal stem cells (BM-MSC) shown as a growth chart. Vertical axis, cell number; horizontal axis, day after first passage. Day 1 is the first day at passage 1. Values are mean±SEM. *P<0.05, †P<0.01 vs. BM-MSC.

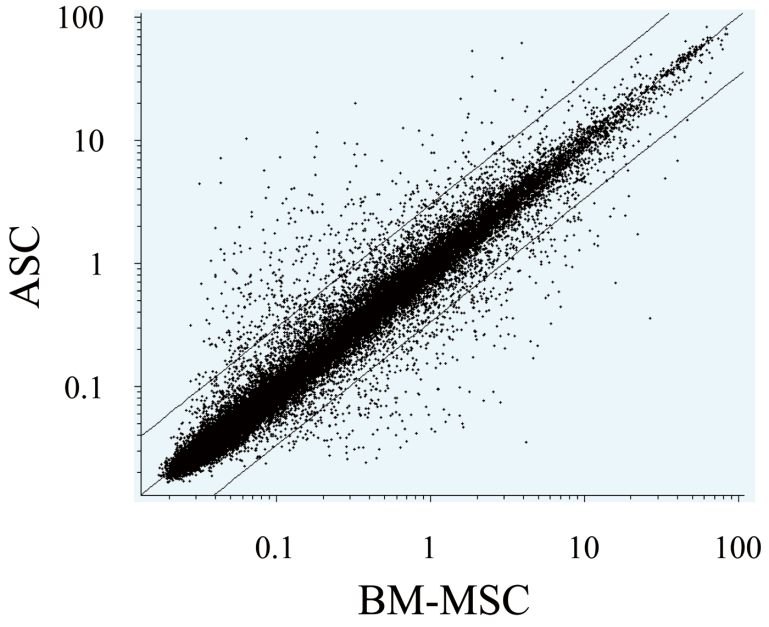

Figure 3. Gene expression profiles of adipose tissue-derived mesenchymal stem cells (ASC, vertical axis) and bone marrow-derived mesenchymal stem cells (BM-MSC, horizontal axis) by microarray. The scatter plot shows normalized microarray datasets of ASC and BM-MSC. All 31,099 gene probes are represented in these plots. The outer lines indicate a 3-fold difference; the central line represents equality.

Table 1. Genes Upregulated in ASC in Comparison With BM-MSC (>10-Fold Upregulation)

Gene name	GenBank Acc. no.	Fold change
Interleukin 1α (Il1a)	NM017019	38.1
Interleukin 1 receptor, type II (Il1r2)	NM053953	21.7
Chemokine (C-X-C motif) ligand 1 (Cxcl1)	NM030845	21.6
Lipocalin 2 (Lcn2)	NM130741	21.5
Fast myosin alkali light chain (Rgd:620885)	NM020104	20.6
Interleukin 6 (Il6)	NM012589	20.5
Chemokine (C-C motif) ligand 20 (Ccl20)	AF053312	17.6
Twist homolog 2 (Twist2)	NM021691	17.5
RAS, dexamethasone-induced 1 (Rasd1)	AF239157	17.1
Complement component 3 (C3)	NM016994	16.9
NADPH oxidase 1 (Nox1)	NM053683	16.3
Matrix metallopeptidase 9 (Mmp9)	NM031055	15.2
Colony-stimulating factor 3 (Csf3)	NM017104	14.5
Prostaglandin E synthase (Ptges)	AB048730	12.8
Adenosine A2B receptor (Adora2b)	NM017161	12.5
Oxidized low-density lipoprotein receptor 1 (Oldlr1)	NM133306	12.4
Uterine sensitization-associated gene 1 protein (Sostdc1)	AA892798	12.1
Chemokine (C-X-C motif) ligand 5 (Cxcl5)	NM022214	11.9
Neuregulin 1 (Nrg1)	U02315	11.8
CD24 antigen (Cd24)	BI285141	11.6
Cathepsin c (Ctsc)	AA858815	11.2
Lymphocyte antigen 68 (C1qr1)	BI282932	11.2
Interleukin 1 receptor antagonist (Il1rn)	NM022194	11.1
Chemokine (C-C motif) ligand 2 (Ccl2)	NM031530	10.8

ASC, adipose tissue-derived mesenchymal stem cells; BM-MSC, bone marrow-derived mesenchymal stem cells.

considered significant at $P<0.05$.

Results

Proliferation and Differentiation of ASC and BM-MSC

Both ASC and BM-MSC could be expanded on a plastic dish, and they exhibited a similar fibroblast-like morphology (Figures 1A,B). To examine the potential of ASC and BM-MSC to differentiate into adipocytes, the cells were cultured in adipogenesis medium for 21 days (Figures 1C,D). Although lipid droplets were not observed in undifferentiated ASC or BM-MSC, ASC and BM-MSC cultured in adipogenesis

Table 2. Genes Upregulated in BM-MSC in Comparison With ASC (>10-Fold Upregulation)

Gene name	GenBank Acc. no.	Fold change
WNT1 inducible signaling pathway protein 2 (Wisp2)	NM031590	202.5
Complement component factor H (Cfh)	NM130409	81.9
Osteomodulin (Omd)	NM031817	67.4
Solute carrier organic anion transporter family, member 2a1 (Slco2a1)	AI407489	65.8
Dynein, cytoplasmic, intermediate chain 1 (Dncic1)	NM019234	64.8
3-α-hydroxysteroid dehydrogenase (RGD:708361)	BF545626	37.7
Preproenkephalin, related sequence (Penk-rs)	NM017139	29.3
Fc receptor, IgG, low affinity Iib (Fcgr2b)	X73371	29.3
Actin, γ 2 (Actg2)	NM012893	25.9
α–2-macroglobulin (A2m)	NM012488	23.2
Lysozyme (Lyz)	L12458	22.2
Jagged 1 (Jag1)	NM019147	19.3
Phospholamban (Pln)	BI290034	17.6
Procollagen, type XI, α 1 (Col11a1)	BM388456	16.2
Gamma sarcoglycan (RGD:1359577)	AA850867	15.3
Pleiomorphic adenoma gene-like 1 (Plagl1)	NM012760	15.0
Matrix metallopeptidase 12 (Mmp12)	NM053963	14.7
Cyclin D2 (Ccnd2)	L09752	14.4
Transforming growth factor, β 2 (Tgfb2)	NM031131	14.3
Solute carrier family 29, member 1 (Slc29a1)	NM031684	14.1
Tissue inhibitor of metalloproteinase 3 (Timp3)	AA893169	13.2
Procollagen, type XI, α 1 (Col11a1)	BM389291	13.1
Down syndrome critical region gene 1-like 1 (Dscr1l1)	AI138048	12.8
Bone morphogenetic protein 4 (Bmp4)	NM012827	12.7
Matrix metallopeptidase 13 (Mmp13)	M60616	11.8
Macrophage galactose N-acetyl-galactosamine specific lectin 1 (Mgl1)	NM022393	11.2
Glycoprotein nmb (Gpnmb)	NM133298	10.7
Aquaporin 1 (Aqp1)	AA891661	10.6
Cadherin 13 (Cdh13)	NM138889	10.5
Selenoprotein P, plasma, 1 (Sepp1)	AA799627	10.5
Secreted frizzled-related protein 4 (Sfrp4)	AF140346	10.4
Cellular retinoic acid binding protein 2 (Crabp2)	U23407	10.2

ASC, adipose tissue-derived mesenchymal stem cells; BM-MSC, bone marrow-derived mesenchymal stem cells.

medium stained positively with Oil Red O in 3 weeks. To quantify lipid accumulation, the absorbance of the extracted cells was measured; however, there was no difference in the absorbance between differentiated ASC and BM-MSC. In addition, both ASC and BM-MSC differentiated identically into osteocytes (Figures 1E,F). ASC proliferated more rapidly than BM-MSC; the number of ASC was approximately 10-fold higher than that of BM-MSC at the 40th day (Figure 2). In approximately 2 weeks, ASC had expanded almost 200-fold, whereas BM-MSC had expanded nearly 30-fold.

Differences in the Gene Expression of ASC and BM-MSC

Of 31,099 genes analyzed, 571 (1.8%) were more highly (>3-fold) expressed in ASC, whereas 571 genes (1.8%) were more highly (>3-fold) expressed in BM-MSC (Figure 3). The genes showing the most enriched expression (>10-fold) in ASC and BM-MSC are listed in Table 1. Of note, the genes that were highly expressed in ASC included various types of molecules involved in inflammation, such as IL-1α and IL-6, and chemotaxis, such as chemokine (C-C motif) ligand 20 and chemokine (C-X-C motif) ligand 5 (Table 1). The genes that were highly expressed in BM-MSC included differentiation-associated genes, such as WNT1-inducible signaling pathway protein 2 (Wisp2), osteomodulin and jagged1 (Table 2). Furthermore, the differential expression patterns of 5 representative genes in ASC and BM-MSC obtained by microarray were confirmed by qRT-PCR, which gave the relative expression of IL-1α as 438.2±560.9 (ratio ASC/BM-MSC, n=5), IL-6 as 54.0±26.6, MMP9 as 3.9±2.2, VEGF 1.8±0.4, and Wisp2 as 7.0±2.2.

To evaluate the genes upregulated in ASC, 571 genes that were more highly expressed in ASC were classified by functional annotation using gene ontology terms (Table 3). The 31 terms listed had a P-value <0.00001, and included mitosis (eg, pituitary tumor-transforming 1, cyclin B1, cyclin-dependent kinase 2), immune response (eg, chemokine (C-C motif) ligand 20, cathepsin C and IL-1α) and response to stress (glutathione peroxidase 2, superoxide dismutase 2 and metallothionein). In BM-MSC, 22 terms were listed for the 571 enriched genes, and included regulation of organ development (eg, Wisp2, osteomodulin and bone morphogenetic protein 4), morphogenesis (cadherin 13, elastin and Neuropillin 2) and cell migration (chemokine (C-X3-C motif) ligand 1 and chemokine (C-X-C motif) receptor 4) (Table 4).

Differences Between ASC and BM-MSC in Secretory Proteins Determined by ELISA

In previous reports, MSC evoked a cell protective effect and induced angiogenesis via secretion of various cytokines, includ-

Table 3. Classification of Highly (>3-Fold) Expressed Genes in ASC According to Gene Ontology Terms

Category	% of genes in category	% of genes in list in category	P value
0007067: Mitosis	1.3	11.4	4.43×10^{-24}
0000279: M phase	1.8	12.4	6.87×10^{-22}
0000278: Mitotic cell cycle	2.2	12.7	2.53×10^{-19}
0007049: Cell cycle	7.0	21.1	3.23×10^{-16}
0007059: Chromosome segregation	0.31	4.14	6.51×10^{-12}
0006260: DNA replication	1.3	7.32	9.94×10^{-12}
0007088: Regulation of mitosis	0.34	3.82	3.10×10^{-10}
0000070: Mitotic sister chromatid segregation	0.15	2.86	3.11×10^{-10}
0051301: Cell division	0.79	5.41	3.30×10^{-10}
0006955: Immune response	5.7	14.9	1.15×10^{-9}
0007017: Microtubule-based process	1.6	7.32	1.60×10^{-9}
0007093: Mitotic checkpoint	0.13	2.54	2.01×10^{-9}
0000074: Regulation of progression through cell cycle	4.5	12.7	2.77×10^{-9}
0006259: DNA metabolism	4.8	13.1	5.12×10^{-9}
0006952: Defense response	6.2	15.2	7.53×10^{-9}
0009613: Response to pest, pathogen or parasite	3.5	10.8	8.31×10^{-9}
0000075: Cell cycle checkpoint	0.44	3.82	9.71×10^{-9}
0009607: Response to biotic stimulus	6.6	15.6	1.32×10^{-8}
0043207: Response to external biotic stimulus	3.7	10.8	1.73×10^{-8}
0006950: Response to stress	9.2	19.1	3.41×10^{-8}
0031577: Spindle checkpoint	0.084	1.91	7.56×10^{-8}
0007018: Microtubule-based movement	0.87	4.77	8.72×10^{-8}
0006954: Inflammatory response	1.6	6.05	9.52×10^{-7}
0009605: Response to external stimulus	5.9	12.7	4.21×10^{-6}
0050896: Response to stimulus	16	25.8	4.24×10^{-6}
0031649: Heat generation	0.046	1.27	4.68×10^{-6}
0007052: Mitotic spindle organization and biogenesis	0.153	1.91	5.28×10^{-6}
0000226: Microtubule cytoskeleton organization and biogenesis	0.649	3.51	5.55×10^{-6}
0007010: Cytoskeleton organization and biogenesis	4.39	10.1	8.14×10^{-6}
0000067: DNA replication and chromosome cycle	0.0993	1.59	8.43×10^{-6}
0007051: Spindle organization and biogenesis	0.168	1.91	9.76×10^{-6}

ASC, adipose tissue-derived mesenchymal stem cells.

ing VEGF, HGF and SDF-1α.[4,5,10] To compare the proteins secreted by cultured ASC and BM-MSC, we used ELISA to investigate the production of several angiogenic and growth factors from ASC and BM-MSC cultures (Figure 4). As compared with BM-MSC, ASC secreted significantly larger amounts of not only HGF and VEGF, which are growth and angiogenic factors, but also PAI-1 and IL-6, which are adipokines. On the other hand, BM-MSC secreted significantly larger amounts of SDF-1α, which is a cell migration-related chemokine, than ASC. There was no significant difference between ASC and BM-MSC for several secreted adipokines, such as adiponectin and TNF-α.

Discussion

In this study, we examined the differences between ASC and BM-MSC in proliferation, differentiation, gene expression and secreted proteins. We showed that (1) ASC are more proliferative than BM-MSC, although there is no difference in differentiation into adipocytes or osteocytes; (2) genes associated with mitosis, inflammation and stress response are highly expressed in ASC; (3) genes associated with regulation of organ development, morphogenesis and cell migration are highly expressed in BM-MSC; and (4) ASC secrete significantly larger amounts of growth factors and inflammatory cytokines than BM-MSC, although BM-MSC secrete significantly larger amounts of chemokine than ASC.

In terms of differentiation, both ASC and BM-MSC differentiated into adipocytes and osteocytes, and there was no difference between them in adipogenesis in our quantitative analysis. A previous report demonstrated that BM-MSC had distinct osteogenic differentiation capability in comparison with ASC,[18] although we did not evaluate difference in osteogenesis between ASC and BM-MSC. Indeed, osteomodulin, which is an osteogenesis-related gene, was upregulated in BM-MSC in comparison with ASC (Table 2). Therefore, BM-MSC might have more osteogenic potential than ASC. These findings suggest that ASC and BM-MSC have multilineage potential and an equivalent potential to differentiate into unfavorable cells. Under these conditions, we found that ASC proliferated more rapidly than BM-MSC, and expanded 4-fold as much BM-MSC in approximately 2 weeks. Lee et al compared the proliferation and gene expression profile of human ASC and BM-MSC,[19] and also demonstrated that ASC differ from BM-MSC in terms of proliferation according to culture medium. A large number of MSC are needed for cell transplantation, so rapid proliferation of ASC ex vivo is thought to be a favorable source of transplanted cells in the acute clinical setting, although there remain prob-

Table 4. Classification of Highly (>3-Fold) Expressed Genes in BM-MSC According to Gene Ontology Terms

Category	% of genes in category	% of genes in list in category	P value
0048513: Organ development	8.86	21.9	5.02×10^{-12}
0008283: Cell proliferation	5.07	15.4	1.77×10^{-11}
0040007: Growth	2.18	9.62	4.23×10^{-11}
0009653: Morphogenesis	8.46	20.6	5.90×10^{-11}
0007275: Development	21.1	37.1	1.64×10^{-10}
0016049: Cell growth	1.53	7.56	6.82×10^{-10}
0016477: Cell migration	1.88	8.24	1.29×10^{-9}
0001558: Regulation of cell growth	1.31	6.52	8.83×10^{-9}
0007155: Cell adhesion	5.82	14.7	1.47×10^{-8}
0001501: Skeletal development	1.73	7.21	3.42×10^{-8}
0000902: Cellular morphogenesis	4.19	11.3	1.92×10^{-7}
0040008: Regulation of growth	1.64	6.52	3.21×10^{-7}
0009887: Organ morphogenesis	3.96	10.6	5.31×10^{-7}
0050678: Regulation of epithelial cell proliferation	0.0687	1.71	6.13×10^{-7}
0051674: Localization of cell	2.87	8.59	1.10×10^{-6}
0007626: Locomotory behavior	3.16	8.93	1.92×10^{-6}
0050673: Epithelial cell proliferation	0.084	1.71	2.17×10^{-6}
0006952: Defense response	6.27	13.7	2.26×10^{-6}
0009607: Response to biotic stimulus	6.59	14.1	3.12×10^{-6}
0045785: Positive regulation of cell adhesion	0.045	1.37	3.46×10^{-6}
0042127: Regulation of cell proliferation	3.32	8.93	4.56×10^{-6}
0050874: Organismal physiological process	16.7	27.1	4.75×10^{-6}

BM-MSC, bone marrow-derived mesenchymal stem cells.

lems concerning tumorigenesis and instability.

In this study, we carried out a comprehensive analysis in rat ASC and BM-MSC using microarrays. Interestingly, there was a considerable difference between the gene profile of our data and that of Lee et al,[19] who demonstrated that highly expressed genes in ASC accounted for less than 1% of all genes, and keratin 18, thrombospondin 1 and heat shock protein were included in the list of genes upregulated in ASC as compared with BM-MSC. Their human study was of 16–84-year-old patients undergoing arthroplasty and abdominoplasty, whereas we used 6-week-old rats. It is possible that differences in species and culture conditions, as well as age, contributed to these differences in gene expression.

We demonstrated that many of the genes that were highly expressed in ASC could be classified into categories such as mitosis, cell cycle and inflammatory cytokines, suggesting that ASC are more proliferative than BM-MSC. Thus, ASC transplant may not be superior to BM-MSC in terms of improvement of cardiac function in acute myocardial infarction, although it might be expected that ASC would contribute more to cell proliferation because of their secretion of VEGF and HGF. Also, ASC might initiate a stronger inflammatory response, because of the significantly increased upregulation of genes associated with inflammation as compared with BM-MSC. On the other hand, many of the genes that were highly expressed in BM-MSC were classified into categories such as organ development and morphogenesis. BM-MSC upregulated the expression of genes associated with cardiogenesis and angiogenesis, such as Wisp2, jagged1 and insulin-like growth factor binding protein 4 (IGFBP4). In particular, jagged1 and IGFBP4 have been reported to induce cardiogenesis and angiogenesis, respectively, via activation of notch signals and inhibition of Wnt signals.[20,21] Indeed, a previous report demonstrated that BM-MSC transplantation into the infarcted heart induces cardiogenesis and angiogenesis.[22–24] On the other hand, ASC are also reported to be able to differentiate into cardiomyocytes.[25] Therefore, ASC and BM-MSC both might improve cardiac function by supplementing cardiomyocytes, as well as in a paracrine manner, although we did not investigate differences in differentiation into cardiomyocytes between them.

BM-derived mononuclear cells and MSC have been used for therapeutic angiogenesis in ischemic disease.[26,27] MSC are thought to be more effective than mononuclear cells as a source of transplanted cells because MSC secrete larger amounts of growth factors.[26] Recent studies suggest that MSC exert tissue regeneration not only by differentiation into specific cell types, but also through paracrine actions, secreting various kinds of angiogenic and cytoprotective factors,[5,10] as shown in the present study. A recent report has shown that the combination of VEGF and MSC can enhance angiogenesis after acute myocardial infarction in rats.[28] Additionally, a previous study demonstrated that BM-MSC activate cardiac progenitor cells, which have the ability to differentiate into cardiomyocytes, in a paracrine manner in vitro and in vivo.[29,30] HGF and SDF-1α improve cardiac function via the activation of cardiac progenitor cells.[31] In our study, both ASC and BM-MSC secreted various cytokines and chemokines that are related to angiogenesis and cardiogenesis.

Although ASC are used as an adequate transplanted cell type for the treatment of ischemic limb disease,[32] ASC secrete larger amounts of not only inflammatory cytokines, such as IL-6, but also PAI-1 which promotes coagulation. In our gene analysis, several genes associated with other inflammatory cytokines and chemokines were upregulated in ASC. Not only the gene analysis but also the ELISA results suggested that ASC evoke more inflammation and thrombogenesis than BM-MSC. Therefore, ASC transplantation might be a more useful

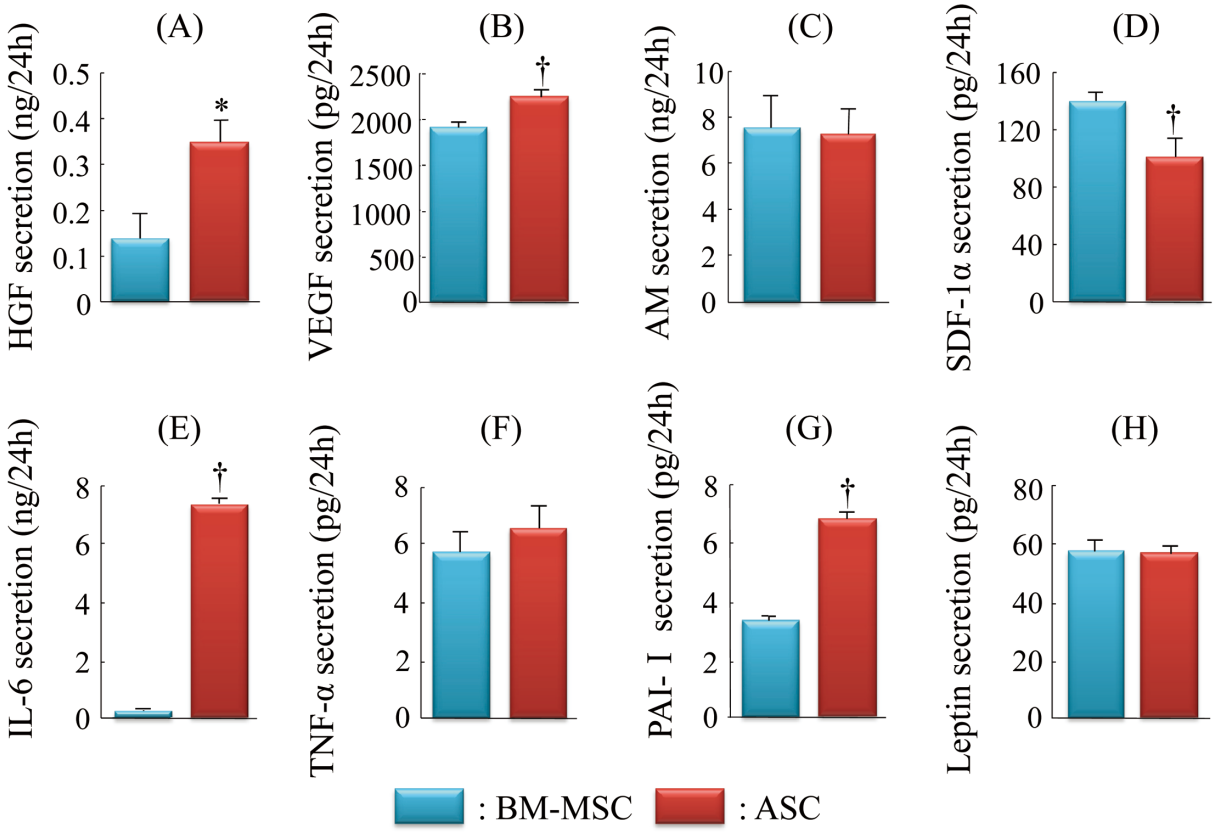

Figure 4. Secretory proteins from adipose tissue-derived mesenchymal stem cells (ASC: red) and bone marrow-derived mesenchymal stem cells (BM-MSC: blue). Conditioned media from ASC and BM-MSC were collected after incubation for 24 h in complete medium. Hepatocyte growth factor (HGF, **A**), vascular endothelial growth factor (VEGF, **B**), adrenomedullin (AM, **C**), stem cell-derived factor-1α (SDF-1α, **D**), interleukin-6 (IL-6, **E**), tumor necrosis factor-α (TNF-α, **F**), plasminogen activator inhibitor-1 (PAI-1, **G**), and leptin (**H**) in conditioned media were measured by enzyme-linked immunosorbent assay. Values are mean±standard error of the mean. *P<0.05, †P<0.01 vs. BM-MSC.

treatment for chronic ischemia without severe inflammation.

In this study, we investigated ASC and BM-MSC obtained from young, 6-week-old rats, and we did not examine differences among various generations of rats. A previous report showed that MSC are subject to molecular genetic changes, such as alterations in p53, HGF and VEGF, during aging.[33] Our results might reflect the character of MSC obtained from young rats, contributing to difference from results in humans.[18] We need to further investigate differences between ASC and BM-MSC not only derived from rats but also derived from humans of various ages.

Conclusion

We have demonstrated difference in proliferation and gene expression between ASC and B-MSC, and accordingly, we suggest the importance of selecting the appropriate cell type for transplantation according to the therapeutic indication.

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