ELSEVIER

CYTOKINE

www.elsevier.com/locate/issn/10434666

Cytokine 31 (2005) 119–126

# Gene expression profile of cytokine and growth factor during differentiation of bone marrow-derived mesenchymal stem cell

Dong Hyun Kim [a,b], Keon Hee Yoo [b], Kyung Suk Choi [b], Jaewon Choi [b], Sang-Yun Choi [a], Sung-Eun Yang [c], Yoon-Sun Yang [c], Ho Joon Im [e], Kye Hyun Kim [d], Hye Lim Jung [b], Ki Woong Sung [b], Hong Hoe Koo [b,*]

[a] *School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea*
[b] *Department of Pediatrics, Samsung Medical Center, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-gu, Seoul 135-710, Republic of Korea*
[c] *Department of Research and Development for Cellular Therapy, Medipost Inc., Seoul, Republic of Korea*
[d] *Department of Obstetrics and Gynecology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 108 Pyung-dong, Jongro-gu, Seoul 110-746, Republic of Korea*
[e] *Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, 388-1 Pungnap-2dong, Songpa-gu, Seoul 9138-736, Republic of Korea*

Received 22 September 2004; received in revised form 31 March 2005; accepted 4 April 2005

**Abstract**

Mesenchymal stem cells (MSCs), which are adherent stromal cells of a nonhematopoietic origin, have the ability to give rise to various differentiated cell types. MSCs regulate localization, self-renewal and differentiation of hematopoietic stem cells (HSCs) due to MSCs' secretion of cytokines and growth factors, the cell-to-cell interactions and the influence of the extracellular matrix proteins. Using RT-PCR analysis, we examined the expression levels of cytokines and growth factors from MSCs and their differentiated cell types, including osteoblasts, adipocytes and endothelial cells. Cytokine and growth factor genes, including IL-6, IL-8, IL-11, IL-12, IL-14, IL-15, LIF, G-CSF, GM-CSF, M-SCF, FL and SCF, were found to be expressed in the MSCs. In contrast, there was no IL-1α, IL-1β, or IL-7 expression observed. The IL-12, IL-14, G-CSF, and GM-CSF mRNA expression levels either disappeared or decreased after the MSCs differentiated into osteoblasts, adipocytes, and endothelial cells. Among the differentiated cells derived from MSCs, osteoblasts, adipocytes, and endothelial cells expressed the osteopontin, $aP_2$, and the VEGFR-2 gene, respectively. These profiles could help determine future clinical applications of MSCs and their derivatives for cell therapy.


*Keywords:* Cytokines; Growth factors; Mesenchymal stem cells; Reverse transcriptase polymerase chain reaction

## 1. Introduction

Bone marrow (BM) contains hematopoietic cells and adherent stromal cells of a nonhematopoietic origin, which together with the extracellular matrix provide supportive tissue known as the BM microenvironment [1]. As a result of cell-to-cell interaction, secretion of cytokines and growth factors, and extracellular matrix proteins, the BM microenvironment provides the ability

*Abbreviations:* BM, bone marrow; HSCs, hematopoietic stem cells; MSCs, mesenchymal stem cells.

* Corresponding author. Tel.: +82 2 3410 3524; fax: +82 2 3410 3649.
*E-mail address:* hhkoo@smc.samsung.co.kr (H.H. Koo).

1043-4666/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2005.04.004

of localization, self-renewal and differentiation of hematopoietic stem cells [1,2]. Mesenchymal stem cells (MSCs), which are pluripotent cells in the BM microenvironment, have the ability to differentiate into various cell types such as adipocytes, osteoblasts, fibroblastic cells, chondrocytes and endothelial precursor cells [2–5]. A recent report has suggested that the cotransplantation of marrow-derived MSCs enhanced the engraftment of the HSCs, corrected mesenchymal disorders and might be used in corrective gene delivery [6].

It is believed that the secretion of various cytokines and growth factors by MSCs plays important roles in hematopoiesis [7] and in the enhancement of HSCs engraftment in stem cell transplantation [8,9]. Several studies have shown that cytokines and growth factors, including interleukin (IL)-1α, IL-1β, IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, leukemia inhibitory factor (LIF), granulocyte colony stimulating factor (G-CSF), granulocyte–macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-SCF), Flt-3 ligand (FL) and stem cell factor (SCF), are secreted by human marrow-derived MSCs [1]. A recent study has suggested that these factors are enriched in stem cells by which the MSCs express several transcripts for various growth factors and genes [10–12]. The main question for this study was how the expression levels of those cytokines and growth factors change during the time when the MSCs are differentiating into otseoblasts, adipocytes and endothelial cells.

This study investigated the differentiation of marrow-derived MSCs into osteoblasts, adipocytes and endothelial cells using specific differentiation media, and we examined the cell surface molecules of the MSCs and the marker genes specific to each differentiated cell type.

## 2. Results

### 2.1. Surface molecules and cellular morphologies of MSCs

By means of FACS analysis, the MSCs were shown as a homogeneous cell population (Fig. 1). It was demonstrated that 96.95% and 98.86% of the MSCs collected from the passage 1 culture were positive for the monoclonal antibodies against MSCs, CD166 and CD105, respectively. In contrast, the MSCs labeled with monoclonal antibodies CD45 and CD33, which recognize leucocytes and monocytes, were demonstrated to be 0.03% and 0.05% positive, respectively. No $CD34^+$ cells were noted from the MSCs collected at passage 1 (Fig. 3B).

After media were exchanged, the differentiated cells showed different morphologies. The osteoblasts displayed calcium deposition as black spots. Granules of adipocytes were stained red with Oil-red O. The endothelial cells were stained with FITC conjugated anti-CD31 (PECAM, platelet endothelial cell adhesion molecule-1) antibodies (Fig. 2).

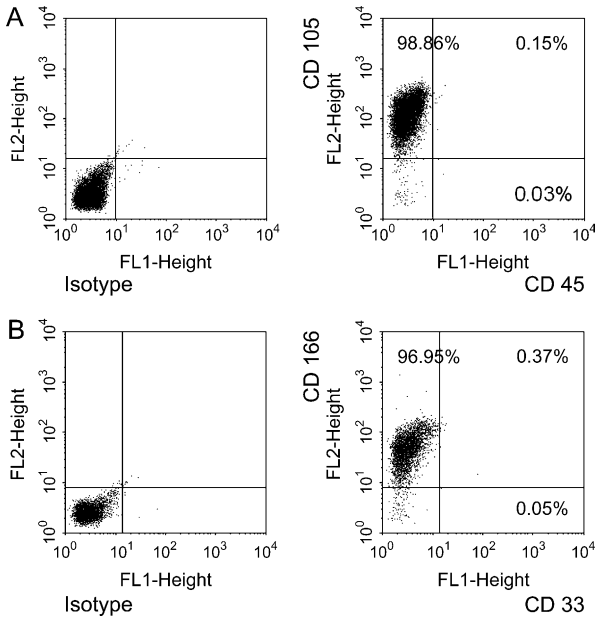


Fig. 1. FACS analysis showed that the MSCs were a CD105 and CD166 positive, homogeneous cell population. (A) In the population of collected MSCs labeled with monoclonal antibodies CD105 (PE) and CD45 (FITC), the cells were 98.86% positive and 0.03% positive, respectively. (B) The percentages of collected MSCs labeled with the monoclonal antibodies CD166 (PE) and CD33 (FITC) were 96.95% and 0.03% positive, respectively. The gated 10,000 cells for each test were analyzed by FACS Vantage. The data are representative of two repetitive experiments.

### 2.2. Specific marker genes

In order to confirm differentiation, this study tested for the osteogenic marker osteopontin, the adipogenic marker $aP_2$ [13], the endothelium marker vascular endothelial growth factor receptor 2 (VEGFR-2) [5], von Willebrand factor (vWF) [14] and vascular endothelial-cadherin (VE-cadherin) [15] by using reverse transcriptase polymerase chain reaction (RT-PCR) with the specific primers (Table 1). The RT-PCR results showed the expression of the specific genes for the different cell types. The level of alkaline phosphatase mRNA, which was used as a stem cell marker, either decreased or disappeared after the differentiations. Osteopontin was specifically expressed in the osteoblasts, $aP_2$ was specifically expressed in the adipocytes and VEGFR-2 was specifically expressed in the endothelial cells. vWF and VE-cadherin were expressed in endothelial cells. However, vWF was also expressed in MSCs. Although undifferentiated MSCs were negative to CD34 antibody in FACS results, the CD34 antigen was expressed by all types of differentiated cells in RT-PCR results (Fig. 3A).

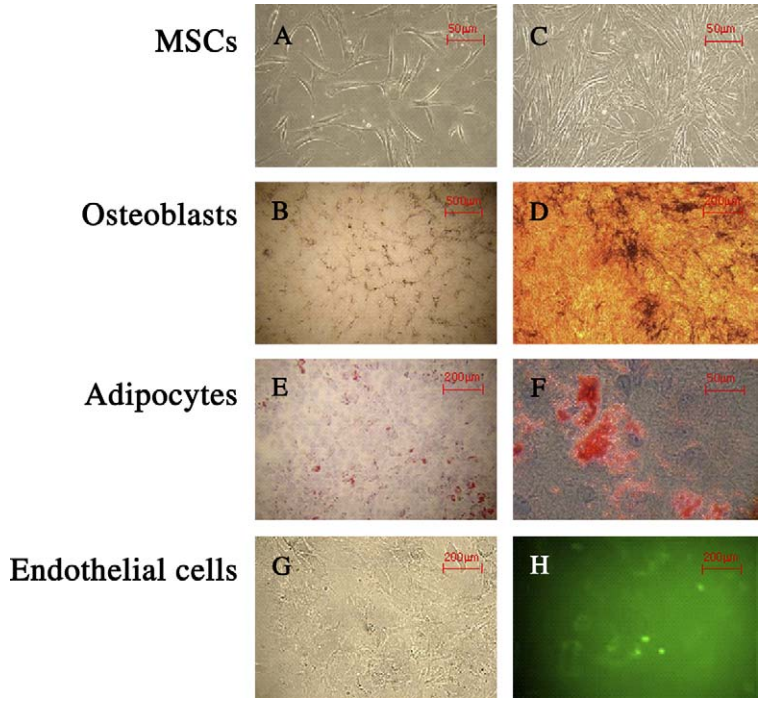


Fig. 2. Morphologies of the differentiated cells from MSCs. The differentiated cells were osteoblasts, adipocytes and endothelial cells cultured for 2 weeks after a medium exchange. Passage 1 MSCs (a) were cultured for 2 weeks after seeding (b). Osteoblasts (c,d) displayed calcium deposition as black spots. Granules of adipocytes (e) were stained red with Oil-red O. The endothelial cells (g) were stained with anti-CD31 FITC antibodies on fluorescence microscopy (h).

### 2.3. Cytokine and growth factor profiles

Although expression levels of cytokines and growth factors in MSCs and differentiated cells were similar in general, it was notable that some of them showed a cell-type-specific regulation in their transcription (Figs. 4 and 5). The MSCs maintained in the normal culture (α-minimal essential medium, α-MEM) constitutively expressed mRNAs for IL-6, IL-8, IL-11, IL-12, IL-14, IL-15, LIF, G-CSF, GM-CSF, M-SCF, FL and SCF but they did not express mRNA for IL-1α, IL-1β and IL-7 (Figs. 4 and 5). However, G-CSF and IL-7 mRNAs were absent on all differentiated cells (Fig. 5). In the endothelial cells, IL-11, IL-12, IL-14 and GM-CSF mRNA expression was not detected, and the M-CSF and FL mRNA expression levels were increased more than 2fold.

## 3. Discussion

The detection of MSC surface markers with the monoclonal antibodies is very important for confirming the identity of the isolated MSCs [16]. Although various surface markers of MSCs are known, SH2, SH3 and

Table 1
Specific oligonucleotide primers for marker genes to detect the differentiated cell types derived from MSCs

| Target cell types | Target genes | Sequences | References |
|---|---|---|---|
| MSCs | Alkaline phosphatase | 5′tggagcttcagaagctcaacacca<br>3′cctgaccatgagtctgttgctcta | Pittenger et al. [2] |
| Osteoblasts | Osteopontin | 5′ctaggcatcacctgtgccatacc<br>3′ctacttagactacttgaccagtgac | Pittenger et al. [2] |
| Adipocytes | $aP_2$ | 5′tggttgattttccatcccat<br>3′tactgggccaggaatttgac | Jaiswal et al. [13] |
| Endothelial cells | VEGFR-2 | 5′gagagttgcccacacctgtt<br>3′atgatctgtggaggggatt | AF063658 |
| | VE-cadherin | 5′aacccccacaggaaaagaat<br>3′tgtgatgttggccgtgttat | X79981 |
| | CD34 antigen | 5′gtctcacagccctgcttacc<br>3′tgggcgtaagagatgtcacc | NM_0017733 |
| | vWF | 5′gctctctctcttacccggatg<br>3′atactccttgccctgatgga | NM_000552 |

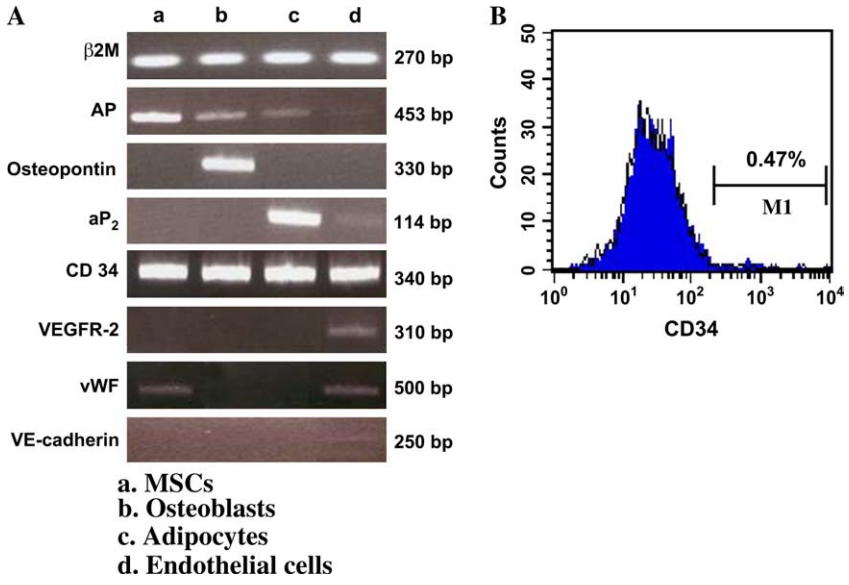


Fig. 3. The RT-PCR results of each differentiated cell types and FACS analysis of MSCs. (A) The RT-PCR results showed the expression of the specific genes for the different cell types. The alkaline phosphatase mRNA level, a stem cell marker, either was lower or had disappeared after differentiations. Osteoblasts, adipocytes and endothelial cells expressed osteopontin, $aP_2$ and VEGFR-2, respectively. (B) MSCs, overlapped isotype and CD 34 (PE) stained cells. Open histogram indicates background signal; shade histogram, positive reactivity.

CD166 are the most effective markers for human MSC staining. The terms 'SH2' and 'SH3' have been replaced with 'CD105' and 'CD73', respectively [17,18]. In our data, a 2-week adherent cell culture yielded highly purified MSCs, and this was confirmed by flow cytometry using the monoclonal antibodies CD105 and CD166. Osteopontin is generally used as a specific marker for osteoblasts, and PPARγ, $aP_2$ and lipopolylipase are used as the markers for adipocytes. PPARγ and $aP_2$ are involved in lipid metabolism and these proteins trigger terminal differentiation of preadipocytes into adipocytes [19]. Our results showed that $aP_2$ was the most appropriate marker for the adipocytes with respect to cell specificity and a sufficient number of gene scripts.

This study showed two major results. One finding was how the expression levels of cytokines and growth factors would be altered during the differentiation of the MSCs. The second important result was what could be

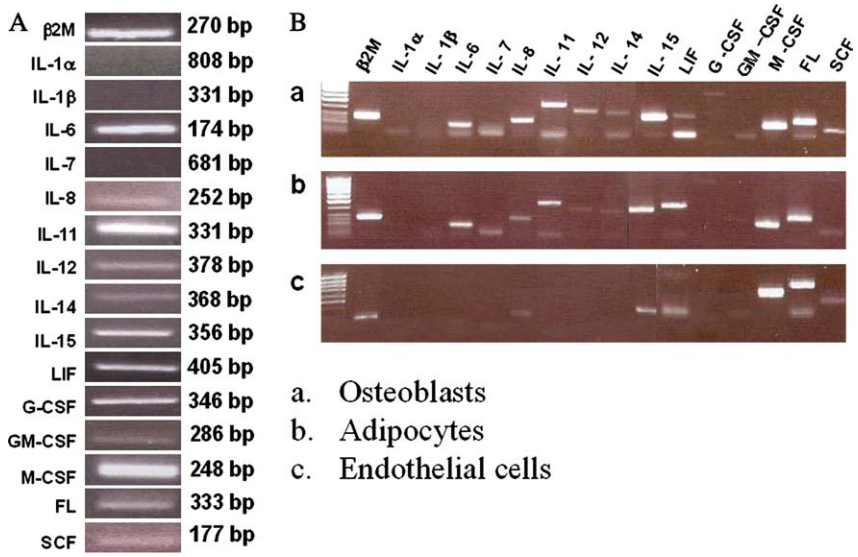


Fig. 4. The RT-PCR results for the cytokines and growth factors. This study compared the mRNA expression levels for cytokines and growth factors at steady-state in MSCs and differentiated cell types by using RT-PCR. (A) RT-PCR products of cytokines and growth factors from the MSCs were IL-6, IL-8, IL-11, IL-12, IL-14, IL-15, LIF, G-CSF, GM-CSF, M-SCF, FL, and SCF. RT-PCR products from the osteoblasts, adipocytes and endothelial cells were similar, but patterns were somewhat different (B). This study used $\beta_2$-microtubulin mRNA to normalize the mRNAs of other genes. The PCR products were analyzed by electrophoresis on 1.5% agarose gel.

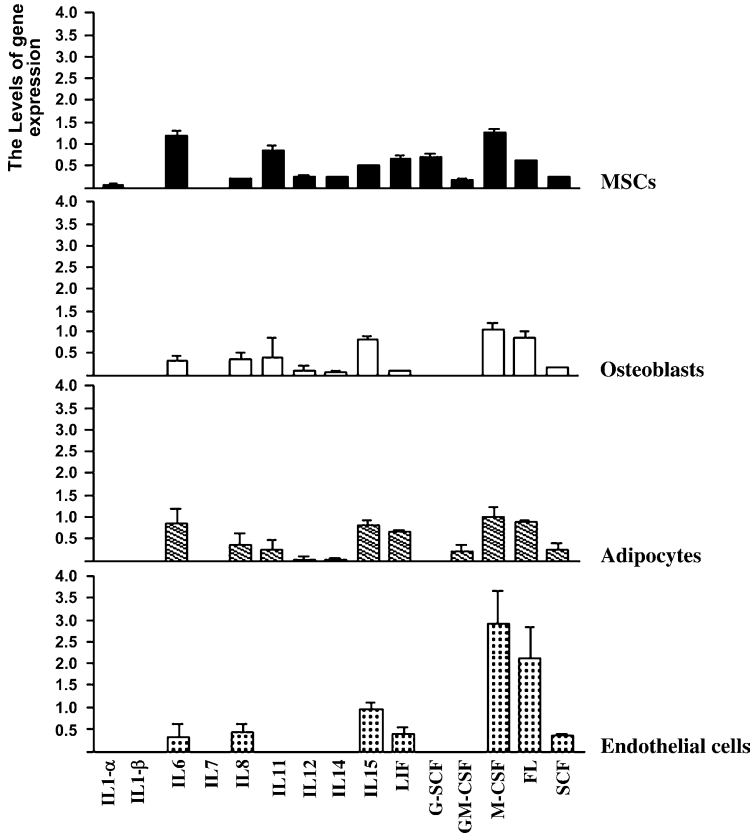


Fig. 5. The expression levels of cytokines and growth factors in MSCs and differentiated cell types. For the quantification of gene expression, the bands of the gel from RT-PCR were analyzed using a GS-800 Calibrated Densitometer (Bio-Rad, CA, USA) and normalized by the $\beta_2$-microtubulin gene from each PCR reaction.

a pertinent specific marker for the endothelial cells using the RT-PCR method.

A previous study reported that the MSCs expressed essential hematopoietic growth factors, including IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, LIF, G-CSF, GM-CSF, M-SCF, FL and SCF [5,12]. We tested the MSC soup to confirm the function of the secreted cytokines and growth factors from MSCs. The $CD34^+$ cells from umbilical cord blood were cultured in the soup of the MSCs for 2 weeks. After seeding, the $CD34^+/CD38^-$ cells had totally differentiated into $CD34^-/CD38^+$ cells in the MSC soup. However, no dividing cells were observed in the soups of the differentiated cell types (data not shown). Accordingly, it was assumed that several cytokines and growth factors secreted from the MSCs could be the major hematopoietic components in the BM because we found that several cytokines and growth factors, including IL-12, IL-14, G-CSF and GM-CSF mRNA, from MSCs either disappeared or decreased to baseline levels after differentiation. However, IL-7 expression was not observed in this study. The reason for this was unknown, but different culture conditions might be possible explanations.

Recent pioneering studies have reported that BM cells could differentiate into endothelial cells of the liver, kidney, lung and GI tract [20,21]. The specific markers of endothelial cells have been known as VEGFR-2, VE-cadherin, E-selctin, PECAM (CD31), vWF, CD34 antigen and so on [5,22,23]. This study tested many candidates, including VEGFR-2, VE-cadherin, CD34 antigen and vWF, and endothelial cells were stained with FITC conjugated CD31 antibody to confirm the type of the cells. The RT-PCR results showed that the CD34 antigen was expressed not only in endothelial cells, but also in all the other cell types (Fig. 3A). Fina and her colleagues suggested that proliferating endothelial cells do not bind CD34 antibodies despite the presence of CD34 mRNA because CD34 protein is down-regulated or processed into a protein, which CD34 antibodies cannot recognize, under proliferation [24]. We found that VEGFR-2, vWF and VE-cadherin were specific to the endothelial cells by RT-PCR analysis. However, VE-cadherin expression was low for confirming endothelial differentiation and vWF was expressed in both MSCs and endothelial cells. Verfaillie and colleagues reported that after treatment with vascular endothelial growth factor (VEGF), MSCs from

mouse BM expressed CD31 and vWF, which is consistent with endothelial differentiation [25]. In contrast, others reported that MSCs from human BM were positive for endothelial cell markers, including VCAM-1 and vWF [26]. We assumed two possibilities to express vWF in undifferentiated MSCs. One is that the vWF level in MSCs could be changed under culture conditions as a sort of serum in media [25,26]. The other is that the contamination of endothelium might cause expression vWF because we used 2-week cultured MSCs from primary BM cells. We supposed that the reason of why VEGFR-2 level was higher than the other gene levels that VEGFR-2 is the critical receptor for transmitting cellular signals for the proliferation, differentiation and migration of endothelial cells [5].

A recent preclinical study has shown that transplantation of marrow-derived endothelial and hematopoietic progenitors can restore tissue vascularization [27]. Marrow-derived endothelial cells could be used as an enhancer for accelerating engraftment in the clinical setting of a cotransplantation of MSCs and hematopoietic stem cells.

Recently, MSC cotransplantation in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice was found to enhance the engrafting of HSCs [8,28]. Therefore, understanding the profiles of the cytokines and growth factors from the MSCs and their derivatives is important for clinical applications of cell therapy in the near future.

## 4. Materials and methods

### 4.1. Isolation of MSCs from bone marrow and cell culture

Bone marrow samples were collected from healthy donors at the Samsung Medical Center, Sungkyunkwan University. We received approval for our study from the institutional review board and we obtained an informed consent from the donors. Ten millilitres of heparinized bone marrow sample was mixed with an equal volume of phosphate-buffered saline (PBS, Bio-Whittaker, Baltimore, MD, USA) and the resuspended cells were layered over 1.077 g/ml Ficoll solution (Lymphoprep, and Oslo, Norway) and centrifuged at 1000 *g* for 30 min at room temperature. The mononuclear cells were recovered at the interface, then the cells were resuspended in an α-minimal essential medium (α-MEM, Bio-Whittaker, Baltimore, MD, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS, Bio-Whittaker, MD, Baltimore, USA) and 1% antibiotic–antimycotic solution (Bio-Whittaker, MD, Baltimore, USA). The cells were plated at a density of $1-5\times10^7$ cells per 100 $cm^2$ dish. The cultures were maintained at 37 °C in a 5% $CO_2$ incubator, and the medium was changed after 48 h and then every 3–4 days. When the MSCs were confluent, the cells were recovered by the addition of 0.25% trypsin–EDTA (Life Technologies, Frederick, MD, USA); they were then replated at a density of $1\times10^6$ cell per 100 $cm^2$ dish.

### 4.2. Differentiation of MSCs into osteoblasts, adipocytes and endothelial cells

For osteogenesis, the recovered MSCs were resuspended in α-MEM medium supplemented with 10% FBS, 1% antibiotic–antimycotic solution, 50 μ/ml ascorbic acid, 5 mM β-glycerophosphate and 0.1 mM dexamethasone (Sigma, St. Louis, MO, USA), then plated at a density of $1-5\times10^6$ cells per 6 well plates (Nunc Inc., Rochester, NY, USA). For adipogenesis, the induction media and the maintenance media were alternately used for 3 days. The induction media consisted of Dulbecco's modified eagle medium (DMEM, High Glucose), 10% FBS, 1% antibiotic–antimycotic solution, 0.5 mM 1-methyl-3-isobutylxanthine (IBMX, Sigma, St. Louis, MO, USA), 200 μM indomethacin (Sigma, St. Louis, MO, USA), 10 μ/ml insulin (Sigma, St. Louis, MO, USA) and 0.1 mM dexamethasone. The maintenance media consisted of DMEM, 10% FBS, 1% antibiotic–antimycotic solution and 10 μ/ml insulin. In order to differentiate the MSCs into endothelial cells, the recovered MSCs were resuspended in an Iscove's Modified Dulbecco's Medium (IMDM, Invitrogen Co., Carlsbad, CA) supplemented with 7.5% FBS, 7.5% horse serum, 1% antibiotic–antimycotic solution, $10^{-12}$ M β-mercaptoethanol (Sigma, St. Louis, MO, USA), 0.1 mM dexamethasone and 200 mM glutamine. The cultures were maintained for 2 weeks at 37 °C in a 5% $CO_2$ incubator, with the medium being changed every 3–4 days.

### 4.3. Flow cytometry

Flow cytometry analyses were performed using the MSCs from the passage 1 culture according to the following procedure. The media were removed from the dishes, and the adherent cells were washed twice with PBS and detached from the dishes. The cells were recovered by centrifugation and washed in 1% bovine serum albumin (BSA, fraction V, Sigma, St. Louis, MO, USA), 2 mM EDTA–PBS, and then they were stained for 30 min at 4 °C with fluorescein isothiocyanate (FITC) conjugated anti-CD45 (BD-Phamingen, Palo Alto, CA, USA), anti-CD33 (BD-Phamingen, Palo Alto, CA, USA), anti-CD31 (BD-Phamingen, Palo Alto, CA, USA), phycoerythrin (PE) conjugated anti-CD34 (BD-Phamingen, Palo Alto, CA, USA), anti-CD105 (Alexis Biochemicals, Lausen, Switzerland), and anti-CD166 (Alexis Biochemicals, Lausen, Switzerland). The cells

stained with the appropriate antibodies were next analyzed using the FACS Vantage (Becton Dickinson, Palo Alto, CA, USA). The data were analyzed using WinMDI 2.8 software.

### 4.4. Histological analysis

To confirm the results of histological differentiation, osteoblasts were stained by the "von Kossa" method as follows. Briefly, osteoblasts were fixed in 1% formaldehyde (Sigma, St. Louis, MO, USA), placed in 1% silver nitrate solution (Sigma) under ultra-violet light for 45 min, treated with 3% sodium thiosulfate (Sigma) for 5 min, counterstained with van Gieson (Sigma) for 5 min, washed in alcohol and mounted with aqueous mounting medium (Biomeda, Foster city, CA, USA). Cells were washed with distilled water in between every step.

Adipocytes were stained with Oil-red O (Sigma) by the following procedure. Briefly, adipocytes were fixed in 1% formaldehyde, washed in Oil-red O for 7 min, rinsed with 85% propylene glycol (Sigma, St. Louis, MO, USA) for 3 min, washed in distilled water with hematoxylin for 1 min, washed with water and mounted with aqueous mounting medium. Endothelial cells were stained with FITC conjugated anti-CD31 (BD-Phamingen, Palo Alto, CA, USA) and observed in fluorescence microscopy. All experiments were performed on three separate bone marrow samples from three donors.

### 4.5. RT-PCR analysis

The mRNA expression levels of the cytokines and growth factors, including IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, LIF, G-CSF, GM-CSF, M-SCF, FL and SCF, were compared at steady-state in the MSCs and in the differentiated cell types by using RT-PCR with the specific primers previously studied [1]. The total RNA from the MSCs, differentiated osteoblasts, adipocytes and endothelial cells were extracted using a QIAmp® RNA Blood mini kit (Qiagen, Germany), as recommended by the manufacturer. cDNA synthesis was performed using Superscript III RNA $H^-$ reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA) with oligo-dT, as suggested by the manufacturer. PCR amplification was performed using the mastermix containing the target primers (Table 1). The samples were incubated for an initial denaturation step at 95 °C for 5 min, followed by 40 cycles each consisting of 95 °C for 30 s, 56 °C for 30 s, and 60 °C for 30 s. The RT-PCR products were analyzed by electrophoresis on a 1.5% agarose gel containing 1 μ/ml EtBr. In order to analyze the gene expression, bands of the gel were analyzed using GS-800 Calibrated Densitometer (Bio-Rad, Hercules, CA, USA) and normalized to the $\beta_2$-microtubulin gene from each PCR reaction. All experiments were repeated with three bone marrow samples from three individual donors.

## Acknowledgements

This study was supported by a grant from Samsung Biomedical Research Institute grant, #SBRI C-A5-203-1 and the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (0405-DB000-0101-0016).

## References

[1] Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. J Cell Physiol 1998;176:57–66.
[2] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
[3] Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 1998;238(1):265–72.
[4] Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. J Cell Biochem 1994;56:283–94.
[5] Rafii S, Meeus S, Dias S, Hattori K, Heissig B, Shmelkov S, et al. Contribution of marrow-derived progenitors to vascular and cardiac regeneration. Semin Cell Dev Biol 2002;13:61–7.
[6] Devine SM, Hoffman R. Role of mesenchymal stem cells in hematopoietic stem cell transplantation. Curr Opin Hematol 2000;7:358–63.
[7] Dorshkind K. Regulation of hemopoiesis by bone marrow stromal cells and their products. Annu Rev Immunol 1990;8:111–37.
[8] Angelopoulou M, Novelli E, Grove JE, Rinder HM, Civin C, Cheng L, et al. Cotransplantation of human mesenchymal stem cells enhances human myelopoiesis and megakaryocytopoiesis in NOD/SCID mice. Exp Hematol 2003;31:413–20.
[9] Erices AA, Allers CI, Conget PA, Rojas CV, Minguell JJ. Human cord blood-derived mesenchymal stem cells home and survive in the marrow of immunodeficient mice after systemic infusion. Cell Transplant 2003;12:555–61.
[10] Silva Jr WA, Covas DT, Panepucci RA, Proto-Siqueira R, Siufi JL, Zanette DL, et al. The profile of gene expression of human marrow mesenchymal stem cells. Stem Cells 2003;21:661–9.
[11] Simone M, Maurizo P, Carlo RR, Pierluigi P, Donato N, Mario L. Use of quantitative real-time PCR to determine immune cell density and cytokine gene profile in the tumor microenvironment. J Immunol Methods 2003;280:1–11.
[12] Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol 1996;166:585–92.
[13] Jaiswal RK, Jaiswal N, Bruder SP, Mbalaviele G, Marshak DR, Pittenger MF. Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. J Biol Chem 2000;275:9645–52.
[14] Conway DS, Pearce LA, Chin BS, Hart RG, Lip GY. Plasma von Willebrand factor and soluble p-selectin as indices of endothelial damage and platelet activation in 1321 patients with nonvalvular

atrial fibrillation: relationship to stroke risk factors. Circulation 2002;106:1962–7.
[15] Stockinger A, Eger A, Wolf J, Beug H, Foisner R. E-cadherin regulates cell growth by modulating proliferation-dependent beta-catenin transcriptional activity. J Cell Biol 2001;154: 1185–96.
[16] Reyes M, Verfaillie CM. Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. Ann N Y Acad Sci 2001;938:231–3 [discussion 3–5].
[17] Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. Bone 1992;13:69–80.
[18] Barry F, Boynton R, Murphy M, Haynesworth S, Zaia J. The SH-3 and SH-4 antibodies recognize distinct epitopes on CD73 from human mesenchymal stem cells. Biochem Biophys Res Commun 2001;289:519–24.
[19] Mandrup S, Lane MD. Regulating adipogenesis. J Biol Chem 1997;272:5367–70.
[20] Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. Blood 2003;102:3483–93.
[21] Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004;22:377–84.
[22] Burger PE, Coetzee S, McKeehan WL, Kan M, Cook P, Fan Y, et al. Fibroblast growth factor receptor-1 is expressed by endothelial progenitor cells. Blood 2002;100:3527–35.
[23] Scholz D, Schaper J. Platelet/endothelial cell adhesion molecule-1 (PECAM-1) is localized over the entire plasma membrane of endothelial cells. Cell Tissue Res 1997;290:623–31.
[24] Fina L, Molgaard HV, Robertson D, Bradley NJ, Monaghan P, Delia D, et al. Expression of the CD34 gene in vascular endothelial cells. Blood 1990;75(12):2417–26.
[25] Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp Hematol 2002;30(8):896–904.
[26] Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 1999;181(1):67–73.
[27] Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med 2003;9:702–12.
[28] Noort WA, Kruisselbrink AB, in't Anker PS, Kruger M, van Bezooijen RL, de Paus RA, et al. Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. Exp Hematol 2002;30:870–8.