

Therapeutic Potential of Umbilical Cord Blood-Derived Mesenchymal Stem Cells in Ischemic Myocardium

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ABSTRACT

Background and Objectives: We designed this study to determine the therapeutic potentials of umbilical cord blood (UCB)-mesenchymal stem cells (MSCs), as compared with bone marrow (BM)-MSCs. **Materials and Methods:** MSCs were isolated from UCB and BM. For the *in vivo* study, myocardial infarction was induced by ligation of the left anterior descending coronary artery (LAD) in rats for 30 min, and this was followed by release; the MSCs were then injected into a designated point around the infarcted area. Echocardiographs were performed two weeks after surgery. For the *in vitro* study, a cDNA microarray and cytokine array were performed to compare the MSCs from UCB and from BM. Cell migration was assessed by a wound scratch assay, and the level of cardiac ankyrin repeat protein (CARP) was determined by reverse transcriptase-polymer chain reaction (RT-PCR) or Western blot analysis. **Results:** For the echocardiograph findings, the fractional shortening (FS) was 43.9% in the UCB-MSCs group and it was 38.6% in the BM-MSC group. The ejection fraction (EF) was 79.8% in the UCB-MSC group and it was 72.4% in the BM-MSC group (control FS: 26.2% and the control EF: 56.6%). CARP was one of the highly expressed genes in the UCB-MSCs on the cDNA microarray. The mRNA and the expressed level of CARP protein in the UCB-MSCs were higher than those in the BM-MSCs. The cell migration of the CARP small interfering ribonucleic acid (siRNA) transfected UCB-MSCs was delayed compared to that of the normal UCB-MSCs ($p < 0.05$). **Conclusion:** Our study directly compared the two types of MSCs from UCB and BM, and we suggest that the CARP molecule might be responsible for the motility of UCB-MSCs. (**Korean Circ J 2008;38:446-454**)

KEY WORDS: Mesenchymal stem cell; Bone marrow; Umbilical cord blood; Myocardial infarction.

Introduction

A leading cause of heart failure is myocardial ischemia, and this causes the dysfunction and death of cardiomyocytes.¹ Adult stem cells have been proposed to be a promising source for the repair and regeneration of the heart and for restoring the heart's function.² Several stem cell types such as skeletal myoblasts, hematopoietic stem cells, endothelial progenitor cells and mesenchymal

stem cells have been applied to damaged hearts;³⁻⁵ however, the optimal cell type remains controversial. Bone marrow (BM) has been widely utilized as a source of autologous stem cells. However, this requires an invasive aspiration procedure to get stem cells from the BM, and the number of cells and their plasticity decreases according to the donors' age or their health condition.⁶ Due to these disadvantages of BM for autologous use, there is a need to identify alternative mesenchymal stem cells (MSCs) sources. Stem cells have recently been identified and isolated from umbilical cord blood (UCB), adipose tissue, amniotic membrane etc.⁷⁻⁹ The use of UCB is especially free from ethical problem. Stem cells from UCB have been derived from cord blood just after birth, and they may represent a more potent type of stem cells than those from BM.

The purpose of the present study was to focus on

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directly comparing the therapeutic potential of MSCs from BM and UCB.

Materials and Methods

Mesenchymal stem cells culture

BM was collected from volunteers and the UCB units were from full term (38-40 weeks' gestation) deliveries; the latter were collected from the unborn placenta with obtaining the mother's informed consent for the use of their material for scientific purposes. MSCs were isolated as described previously with slight modification.⁷⁾ Briefly, to isolate the mononuclear cells (MNC), each BM or UCB unit was diluted 1 : 1 with phosphate-buffered saline (PBS) and then it was carefully loaded onto Ficoll-Hypaque solution (Amersham, Germany). After density gradient centrifugation at 2,500 rpm for 30 minutes at room temperature, the MNCs were collected from interphase and they were washed twice with PBS. The MNCs were seeded at a density 10^6 cells/cm² into 6-well culture plates (Falcon, Becton Dickinson, Heidelberg, Germany) in Dulbecco's modified Eagle's medium (DMEM) (Gibco-Invitrogen Corp., USA) that was supplemented by 10% fetal bovine serum (FBS; selected lots; Gibco-Invitrogen), 100 U/mL penicillin and 100 g/mL streptomycin (Sigma-Aldrich, USA). After overnight incubation at 37°C in a humidified atmosphere containing 5% CO₂, the non-adherent cells were removed and fresh medium was added to the wells. The cultures were maintained, and the remaining non-adherent cells were removed by changing the media every 7 days. The culture wells were continuously screened to obtain the developing colonies of adherent cells. The cells were detached by incubating them for 5 minutes in 0.05% trypsin/EDTA (Gibco-Invitrogen, USA), and then they were harvested and replated at a density of 5,000 cells/cm²; these were then termed as passage 1 cells thereafter. Before transplantation into the rat heart, the MSCs were trypsinized, washed and labeled with 50 µg/mL of 4',6-diamidino-2'-phenylindole (DAPI) (Sigma, USA) for 30 minutes.

Flow cytometry

To analyze the cell-surface expression of typical marker proteins, the MSCs were labeled with anti-human antibodies against HLA-ABC, HLA-DR, CD14, CD29, CD34, CD44, CD45, CD73, CD90, CD105 and CD106 (Becton Dickinson, CA, USA). Mouse isotype antibodies served as the respective controls. Ten thousand labeled cells were acquired and these were analyzed by using a cytomics FC 500 flow cytometer (Beckman Coulter, Krefeld, Germany).

Differentiation studies

Osteogenic differentiation was achieved by culturing

the cells in complete medium along with 0.1 µM dexamethasone, 10 mM β-glycerol phosphate and 50 µM ascorbate. Chondrogenic differentiation was induced by using medium containing 50 µM ascorbic acid, 0.1 µM dexamethasone, 10 ng/mL TGF-β, 40 µg/mL L-proline and 100 µg/mL sodium pyruvate. Adipogenic differentiation was induced by the addition of 0.5 mM isobutylmethylxanthine and 1 µM dexamethasone.

The Ischemia-Reperfusion model and cell transplantation

This study was reviewed and approved by the Chonnam National University Institutional Animal Care and Use Committee.

Male Sprague Dawley rats (200-200 g each, Jung Ang Animals, Korea) were anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Myocardial ischemia-reperfusion injury was induced by temporary ligation of the proximal left anterior descending coronary artery (LAD) for 30 minutes and this was followed by release. The MSCs were directly injected into the peri-infarct areas of the myocardium just before the reperfusion. The sham group rats received PBS (n=15) and the MSC group rats received UCB-MSCs (n=15) or BM-MSCs (n=15) (1×10^6 diluted in 100 µL of PBS).

Assessment of the cardiac function by echocardiography

The left ventricular (LV) function was assessed by performing transthoracic echocardiography at 2 weeks after surgery with a 7.5-MHz sector scan probe. The rats were anesthetized and placed in the supine position and their chests were shaved. M-mode echocardiograms that were guided by two-dimensional long-axis images were obtained. The LV percent fractional shortening (FS) was calculated as $100 \times (\text{LVEDD} - \text{LVESD}) / \text{LV}$, and the LV ejection fraction (EF) was calculated as $100 \times (\text{stroke volume} / \text{diastolic volume})$ where the LVEDD is the LV end diastolic diameter and the LVESD is the LV end systolic diameter.

Confocal immunofluorescence microscopy

After echocardiography, the rats were sacrificed and their hearts were immediately removed and frozen in liquid nitrogen for immunofluorescent study. Immunofluorescent staining was carried out on the 10-µm frozen ventricular sections.

The samples were incubated overnight with antibodies against α-sarcomeric actin (Sigma, USA), CD31 (BD Science), von Willebrand factor (Sigma, USA) and connexin 43 (Sigma, USA). The following secondary antibodies were used: anti-rabbit IgG coupled to Alexa Fluor 488 and anti-mouse IgG coupled to Alexa Fluor 594 (Invitrogen, USA). The images were obtained with using a multitracking laser scanning confocal microscope

(200 \times). The fluorochromes were detected at 488 nm and 543 nm.

cDNA microarray analysis

To compare the gene expression profile of the UCB-MSCs and BM-MSCs, we performed expression analysis by using an Illumina expression chip 24 k (Macrogen, Korea).

Reverse transcriptase-polymerase chain reaction

To compare the mRNA expression levels in cell-injected animals and the control rats, their myocardial specimens were pulverized and then homogenized in Trizol according to the manufacturer's instructions. cDNA was synthesized to perform reverse transcriptase-polymerase chain reaction (RT-PCR). The primers we used for cardiac ankyrin repeat protein (CARP) were F-ACCGCTATAA GATGATCCGA and R-AATGAAGCTCTGCTCACCAG, and the primers we used for β -

actin were, F-GACTACCTCATGAAGATC and R-GATCCACATCTGCTGGAA.

Western blot

The cells were extracted to separate them on 8% to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then they were transferred to PVDF membranes. The blots were incubated with antibodies against CARP (Santa Cruz, USA) and β -actin (Sigma, USA), and the signals were detected by a LAS-3000 (FUJI, Japan).

Wound scratch assay

The BM-MSCs, UCB-MSCs and the CARP or its scrambled control small interfering ribonucleic acid (siRNA)-transfected UCB-MSCs were cultured on 12-well plates. The confluent cell monolayers were scratched using sterilized 200- μ L pipette tips and they were washed with serum free media and then further incubated.

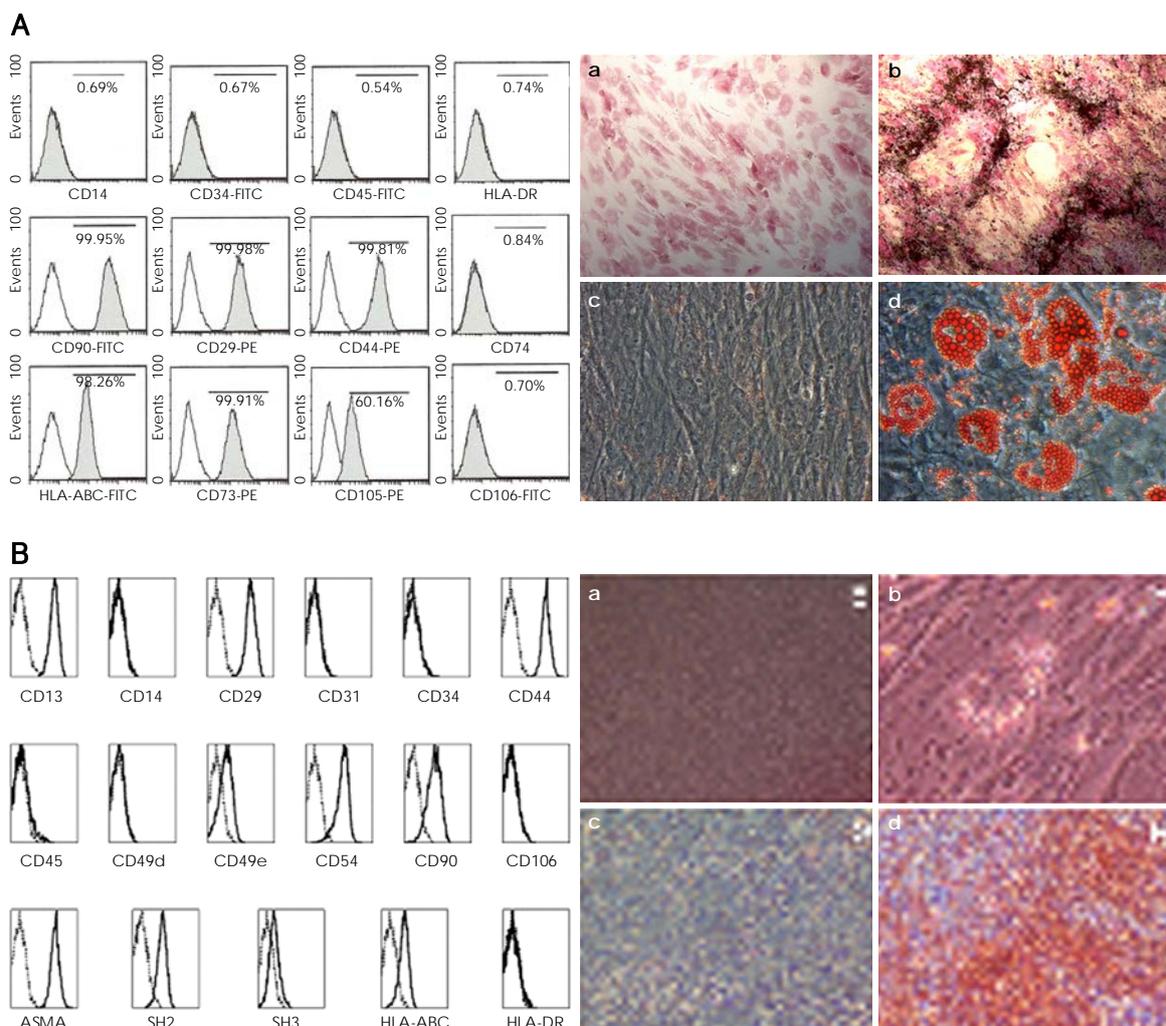


Fig. 1. Mesenchymal stem cells (MSCs) characterization. FACS analysis and a differentiation study were performed on the umbilical cord blood (UCB)-MSCs (A) and the bone marrow (BM)-MSCs (B), and both groups of MSCs expressed the typical MSC markers. Both groups of MSCs (a) were differentiated to osteoblasts (b), chondrocytes (c) and adipocytes (d). FACS: Fluorescence-Activated Cell Sorter.

The images of the cells were captured at 0 hour, 6 hours and 24 hours after the wounding.

Cytokine array

The MSCs were cultured in DMEM supplemented with 1% FBS for 36 hours, and then the cytokine levels in the culture supernatant were detected using the Human Cytokine Antibody Array V (RayBiotech, USA) per the manufacturer's protocol. Briefly, the supernatants were incubated for 2 hours with membranes that were arrayed with antibodies against 79 cytokines. After washing them twice, the membranes were incubated for 2 hours with biotin-conjugated primary anti-cytokine antibodies and then the membranes were washed twice. The membranes were then incubated with horseradish peroxidase-conjugated streptavidin for 1 hour, washed twice and they were placed in the detection buffer for a few minutes. The signals were detected by a LAS-3000 (FUJI, Japan).

Statistical analysis

Each experiment was performed at least three times. The data is presented as means \pm SDs. Differences were analyzed by ANOVA. The statistical significance of the differences was accepted at the level of $p < 0.05$.

Results

The cardiac function after I/R injury was improved by administration of mesenchymal stem cells

The MSCs were characterized by analysis of their surface markers and by the differentiation study. On the cytometric analysis, both groups of MSCs were positive for CD29, CD44, CD90 and HLA-ABC, while they were both negative for CD13, CD34 and CD106 (Fig. 1). Osteogenic, chondrogenic and adipogenic differentiation

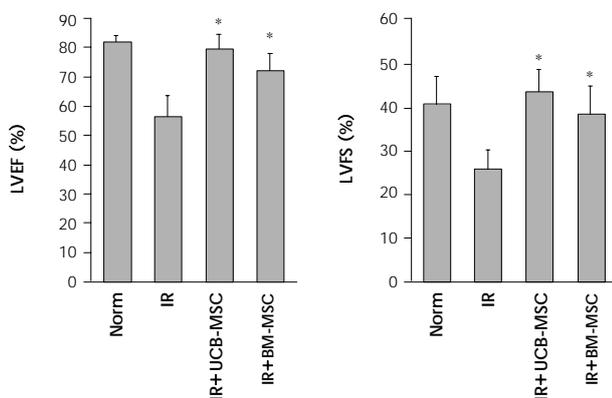


Fig. 2. Echocardiographic parameters. The ejection fraction (EF) and fractional shortening (FS) of the left ventricle in the cardiac ischemia-reperfusion (IR) injured rats that were treated with mesenchymal stem cells. $n=15$ in each group, $*p < 0.05$ vs. the IR control rats. LVEF: left ventricular ejection fraction, LVFS: left ventricular fractional shortening, UCB: umbilical cord blood, MSC: mesenchymal stem cell, BM: bone marrow.

were successfully induced (Fig. 1).

To compare the therapeutic capacity of MSCs for repairing the ischemia/reperfusion damage, we injected MSCs into the IR injured rats. Echocardiography was performed at two weeks after IR injury. As shown in Fig. 2,

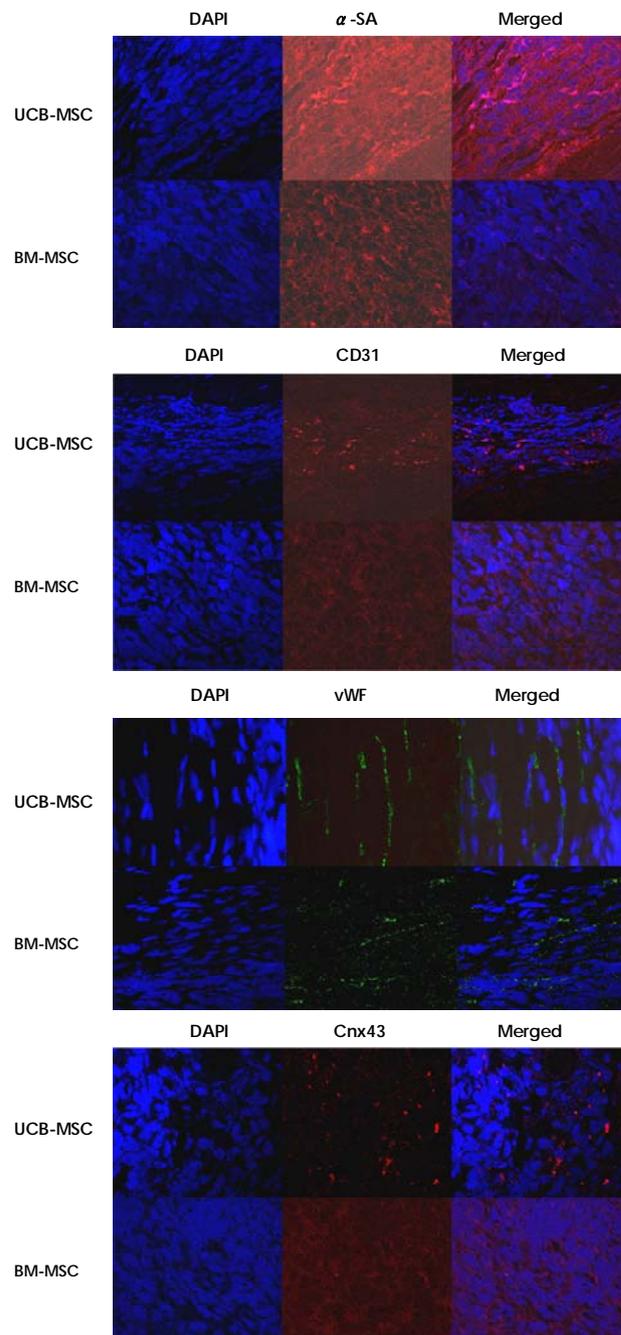


Fig. 3. DAPI-stained mesenchymal stem cells (MSCs) at 14 days. Sections from the engrafted hearts were stained with antibodies against α -sarcomeric actin (α -SA), von Willebrand factor (vWF), CD31 and connexin43 (Cnx43). Immunofluorescence staining was done to examine if the injected MSCs were differentiated. The sections were stained for α -SA, CD31, vWF and Cnx43. The MSCs were stained with DAPI before injection to detect the region of the myocardium where they homed to. UCB: umbilical cord blood, MSC: mesenchymal stem cell, BM: bone marrow, DAPI: 4'-6-diamidino-2-phenylindole.

the EF and FS were significantly increased in the UCB-MSCs group and the BM-MSCs group compared with those values of the IR control group ($p < 0.01$). The EF was $79.8 \pm 4.6\%$ in the UCB-MSCs group and the EF was $76.1 \pm 5.1\%$ in the BM-MSCs group (the IR control: $56.6 \pm 6.8\%$). The FS was $43.9 \pm 5.0\%$ in the UCB-MSCs group and it was $40.6 \pm 6.3\%$ in the BM-MSCs group (IR control: $26.2 \pm 4.2\%$). The echocardiographic findings did not show any statistical difference between the UCB-MSCs and BM-MSCs groups. The myocardial IR significantly increased the end-diastolic and end-systolic dimensions and it significantly reduced their respective FS and EF.

Differentiation of mesenchymal stem cells

DAPI-tagging MSCs were detected in the peri-infarct zone at 2 weeks after injection. Immunofluorescence staining showed positively stained cells for α -sarcomeric actin, CD31, von Willebrand factor and connexin43 in

the UCB-MSCs group. On the other hand, only a few cells or none were stained positively in the BM-MSCs group (Fig. 3).

Gene expression profile

The microarray data was examined and the ranking of the genes is shown in Table 1 and 2, which demonstrates the highly expressed genes. Table 3 shows the genes that were more highly expressed in the UCB-MSCs than in the BM-MSCs. This result provided the putative candidate genes to explore for explaining the UCB-MSCs properties as compared with the BM-MSCs. In Fig. 4, a pie chart represents the distribution of the classifications of complementary deoxyribonucleic acid (cDNAs) that were expressed in the UCB-MSCs (A) and BM-MSCs (B). Among the genes that were highly expressed in the UCB-MSCs, we choose CARP and the CARP mRNA level was determined by RT-PCR in the UCB-MSCs and BM-MSCs.

Table 1. The first 30 most enriched genes in the UCB-MSCs

No.	Gene name	Intensity	Gene bank accession
1	Secreted protein, acidic, cysteine-rich (osteonectin) (SPARC)	5700.9	NM_003118.1
2	Ribosomal protein L41 (RPL41)	4992.1	NM_021104.1
3	Thymosin, beta 10 (TMSB10)	3887.9	NM_021103.2
4	Actin, beta (ACTB)	3885.2	NM_001101.2
5	Ribosomal protein S14 (RPS14)	3714.5	NM_005617.2
6	Fibronectin 1 (FN1), transcript variant 1	3228.9	NM_002026.1
7	Ribosomal protein L35 (RPL35)	3131.2	NM_007209.2
8	Profilin 1 (PFN1)	3074.8	NM_005022.2
9	Connective tissue growth factor (CTGF)	3072.0	NM_001901.1
10	Ribosomal protein L24 (RPL24)	2847.2	NM_000986.2
11	Vimentin (VIM)	2797.5	NM_003380.1
12	Eukaryotic translation elongation factor 1 alpha 1 (EEF1A1)	2794.2	NM_001402.4
13	Ribosomal protein S27 (metallopanstimulin 1) (RPS27)	2655.6	NM_001030.2
14	Filamin A, alpha (actin binding protein 280) (FLNA)	2617.5	NM_001456.1
15	Actin, alpha 2, smooth muscle, aorta (ACTA2)	2610.0	NM_001613.1
16	Ribosomal protein L31 (RPL31)	2571.6	NM_000993.2
17	Metallothionein 2A (MT2A)	2567.2	NM_005953.2
18	Ribosomal protein S2 (RPS2)	2560.3	NM_002952.2
19	Collagen, type I, alpha 2 (COL1A2)	2461.4	NM_000089.2
20	Lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1)	2345.1	NM_002305.2
21	Thymosin-like 3 (TMSL3)	2329.8	NM_183049.1
22	Ribosomal protein S3A (RPS3A)	2302.4	NM_001006.2
23	Transforming growth factor, beta-induced, 68 kDa (TGFBI)	2294.6	NM_000358.1
24	Ferritin, light polypeptide (FTL)	2267.1	NM_000146.2
25	Ribosomal protein S23 (RPS23)	2256.9	NM_001025.2
26	Laminin receptor 1 (ribosomal protein SA, 67 kDa) (LAMR1)	2192.9	NM_002295.2
27	Ribosomal protein L39 (RPL39)	2184.1	NM_001000.2
28	Tubulin, alpha 3 (TUBA3)	2174.2	NM_006009.2
29	Ribosomal protein L32 (RPL32)	2137.1	NM_000994.2
30	NADH dehydrogenase 3 (MTND3)	2125.5	NM_173710.1

UCB-MSCs: umbilical cord blood-mesenchymal stem cells

Table 2. The first 30 most enriched genes in the BM-MSCs

Definition	Intensity	Gene bank accession
1 Ribosomal protein L41 (RPL41)	4554.3	NM_021104.1
2 Secreted protein, acidic, cysteine-rich (osteonectin) (SPARC)	3915.5	NM_003118.1
3 Ribosomal protein S14 (RPS14)	3444.2	NM_005617.2
4 Actin, beta (ACTB)	3277.8	NM_001101.2
5 Tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) (TIMP1)	3048.2	NM_003254.1
6 Vimentin (VIM)	2825.5	NM_003380.1
7 Thymosin, beta 10 (TMSB10)	2745.9	NM_021103.2
8 Insulin-like growth factor binding protein 4 (IGFBP4)	2594.5	NM_001552.1
9 NADH dehydrogenase 3 (MTND3)	2552.7	NM_173710.1
10 Eukaryotic translation elongation factor 1 alpha 1 (EEF1A1)	2484.3	NM_001402.4
11 Ferritin, light polypeptide (FTL)	2464.4	NM_000146.2
12 Fibronectin 1 (FN1), transcript variant 1	2424.1	NM_002026.1
13 Ribosomal protein S2 (RPS2)	2322.9	NM_002952.2
14 Ribosomal protein L35 (RPL35)	2316.2	NM_007209.2
15 Insulin-like growth factor binding protein 3 (IGFBP3)	2251.3	NM_000598.2
16 S100 calcium binding protein A6 (calyculin) (S100A6)	2214.8	NM_014624.2
17 Ribosomal protein S27 (metallopanstimulin 1) (RPS27)	2166.5	NM_001030.2
18 Profilin 1 (PFN1)	2058.5	NM_005022.2
19 Connective tissue growth factor (CTGF)	2031.8	NM_001901.1
20 Ribosomal protein L31 (RPL31)	1989.3	NM_000993.2
21 Ribosomal protein L24 (RPL24)	1968.4	NM_000986.2
22 Filamin A, alpha (actin binding protein 280) (FLNA)	1889.0	NM_001456.1
23 Ribosomal protein S3A (RPS3A)	1844.0	NM_001006.2
24 Collagen, type VI, alpha 2 (COL6A2), transcript variant 2C2	1810.7	NM_001849.2
25 Ribosomal protein L32 (RPL32)	1806.5	NM_000994.2
26 Ribosomal protein S5 (RPS5)	1804.7	NM_001009.2
27 Eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein) (EEF1D), transcript variant 2	1796.3	NM_001960.2
28 Lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1)	1780.7	NM_002305.2
29 Interferon induced transmembrane protein 3 (1-8U) (IFITM3)	1772.0	NM_021034.1
30 Ribosomal protein L18a (RPL18A)	1754.4	NM_000980.2

BM-MSC: bone marrow-mesenchymal stem cells

Effect of cardiac ankyrin repeat protein on cell migration

The wound scratching assay was used for the assay of cell migration and motility. Acceleration of wound closure by the UCB-MSCs was observed, as compared to that by the BM-MSCs. To examine whether CARP, which is highly and exclusively expressed in UCB-MSCs (Fig. 5A), contributes to the UCB-MSCs' motility, the CARP was knocked down by using siRNA transfection to the UCB-MSCs. Knock down of the CARP protein level in the siRNA-transfected UCB-MSCs was confirmed by Western blotting, as compared with the non-treated UCB-MSCs (Fig. 5B). The CARP siRNA-transfected UCB-MSCs showed significantly decreased migration of cells into the wounded area (Fig. 5C).

Cytokine expression profile

The expression levels of angiogenin, insulin-like growth factor binding protein (IGFBP)-6 and osteoprotegerin were lower in the UCB-MSCs, whereas the levels of most

cytokines were similar and that of IGFBP-1 was higher in the UCB-MSCs, as compared to the BM-MSCs (Fig. 6).

Discussion

In this study, we compared the effects of MSCs from UCB and BM on cardiac IR injured rats, and the gene expression profiles and released cytokines in the MSCs from UCB and BM.

The cardiac function after MI was reported to be significantly recovered by the administration of MSCs from various sources.^{11,10} Despite that BM has been widely used for applications of cell therapy, aspiration of BM required invasive procedures and the BM isolated from aged or diseased donors has shown impaired cellular functions.

On the other hand, UCB-derived stem cells have several advantages over other adult stem cells, including the ease of harvesting and storage, and the decreased risk for immune intolerance and transmission of infectious

Table 3. The first 30 most highly expressed genes in the UCB-MSCs

	Definition	Fold	Gene bank accession
1	CD24 antigen (small cell lung carcinoma cluster 4 antigen) (CD24)	10.245	NM_013230.1
2	Ankyrin repeat domain 1 (cardiac muscle) (ANKRD1)	7.397	NM_014391.2
3	Carboxypeptidase E (CPE)	6.916	NM_001873.1
4	A disintegrin and metalloproteinase domain 19 (meltrin beta) (ADAM19), transcript variant 2	6.767	NM_033274.1
5	Chromosome 5 open reading frame 13 (C5orf13)	5.408	NM_004772.1
6	Chromosome 20 open reading frame 100 (C20orf100)	4.993	NM_032883.1
7	Oxytocin receptor (OXTR)	4.939	NM_000916.3
8	Calcium/calmodulin-dependent protein kinase II (CaMKIIAlpha)	4.752	NM_018584.4
9	Calbindin 2, 29 kDa (calretinin) (CALB2), transcript variant CALB2c	4.639	NM_007088.1
10	Matrix metalloproteinase 1 (interstitial collagenase) (MMP1)	4.499	NM_002421.2
11	Erythrocyte membrane protein band 4.1-like 3 (EPB41L3)	4.250	NM_012307.2
12	Cadherin 2, type 1, N-cadherin (neuronal) (CDH2)	4.122	NM_001792.2
13	EphB2 (EPHB2), transcript variant 2	4.039	NM_004442.4
14	Collagen, type IV, alpha 5 (Alport syndrome) (COL4A5), transcript variant 1	3.982	NM_000495.3
15	Retinol binding protein 1, cellular (RBP1)	3.977	NM_002899.2
16	EphB2 (EPHB2), transcript variant 1	3.956	NM_017449.1
17	Regulator of G-protein signalling 4 (RGS4)	3.716	NM_005613.3
18	Brain expressed, X-linked 1 (BEX1)	3.520	NM_018476.2
19	Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)	3.398	NM_006208.1
20	Biglycan (BGN)	3.358	NM_001711.3
21	ALL1-fused gene from chromosome 1q (AF1Q)	3.336	NM_006818.2
22	Kallmann syndrome 1 sequence (KAL1)	3.300	NM_000216.1
23	KIAA0537 gene product (ARK5)	3.277	NM_014840.1
24	TERA protein (TERA)	3.254	NM_021238.1
25	Solute carrier family 38, member 1 (SLC38A1)	3.236	NM_030674.2
26	Protocadherin 10 (PCDH10), transcript variant 1	3.235	NM_032961.1
27	Solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1)	3.166	NM_006516.1
28	Hypothetical protein MGC23947 (MGC23947)	3.148	NM_152278.1
29	Downregulated in ovarian cancer 1 (DOC1), transcript variant 2	3.107	NM_014890.1
30	Cathepsin C (CTSC), transcript variant 2	3.097	NM_148170.1

UCB-MSCs: umbilical cord blood-mesenchymal stem cells

agents.¹¹⁾

Here we report on a comparative study that was performed both *in vitro* and in cardiac injured rats (IR). Both groups of MSCs exerted a protective effect in IR rats. The EF and FS was a bit more preserved in the UCB-MSCs group; however, there was no significant difference between the UCB-MSC group and BM-MSC group in our study.

Due to the lack of direct evidence that could explain the mechanism by which the MSCs protected the ischemic heart, we tried to compare the gene expression profiles and the therapeutic potentials of the two groups of MSCs from UCB and BM. cDNA microarray analysis revealed that CARP was one of the most highly expressed genes in the UCB-MSCs, as compared with those in the BM-MSCs. CARP has been suggested to act as a nuclear transcription co-factor that negatively regulates the cardiac gene expression and it might play a key role in the pathophysiology of heart failure. Furthermore, CARP is up-regulated in response to shear stress *in*

vitro.¹²⁻¹⁴⁾ The CARP expression level increases during human heart failure and also in animal models of cardiac hypertrophy, and it is decreased in cardiomyocytes that are exposed to adriamycin.¹⁵⁾ These reports suggested that the CARP expression might be associated with pathological stress in cardiomyocytes.

To explore the possible role of CARP in UCB-MSCs, strong cell motility, which is one of the prominent characters of UCB-MSCs as compared with that of BM-MSCs, was utilized as a reference for comparison between UCB-MSCs and the CARP-knock down UCB-MSCs. On the wound scratch assay, the CARP knock down UCB-MSCs migrated more slowly than did the control UCB-MSCs; however, the CARP knock down UCB-MSCs were still faster than the BM-MSCs (data not shown). From this result, CARP can be suggested to be partly responsible for the migration of UCB-MSCs. Strong migration may contribute to the homing process into an injured organ site; however, we did not confirm the better homing capacity of UCB-MSCs *in vivo*, and a

long term observation study on this should be conducted in the future.

There is currently debate on whether implanted MSCs

can differentiate into cardiomyocytes or the endothelial phenotype, and if they can enhance cardiac survival and the vascular density after implantation in a rat myocar-

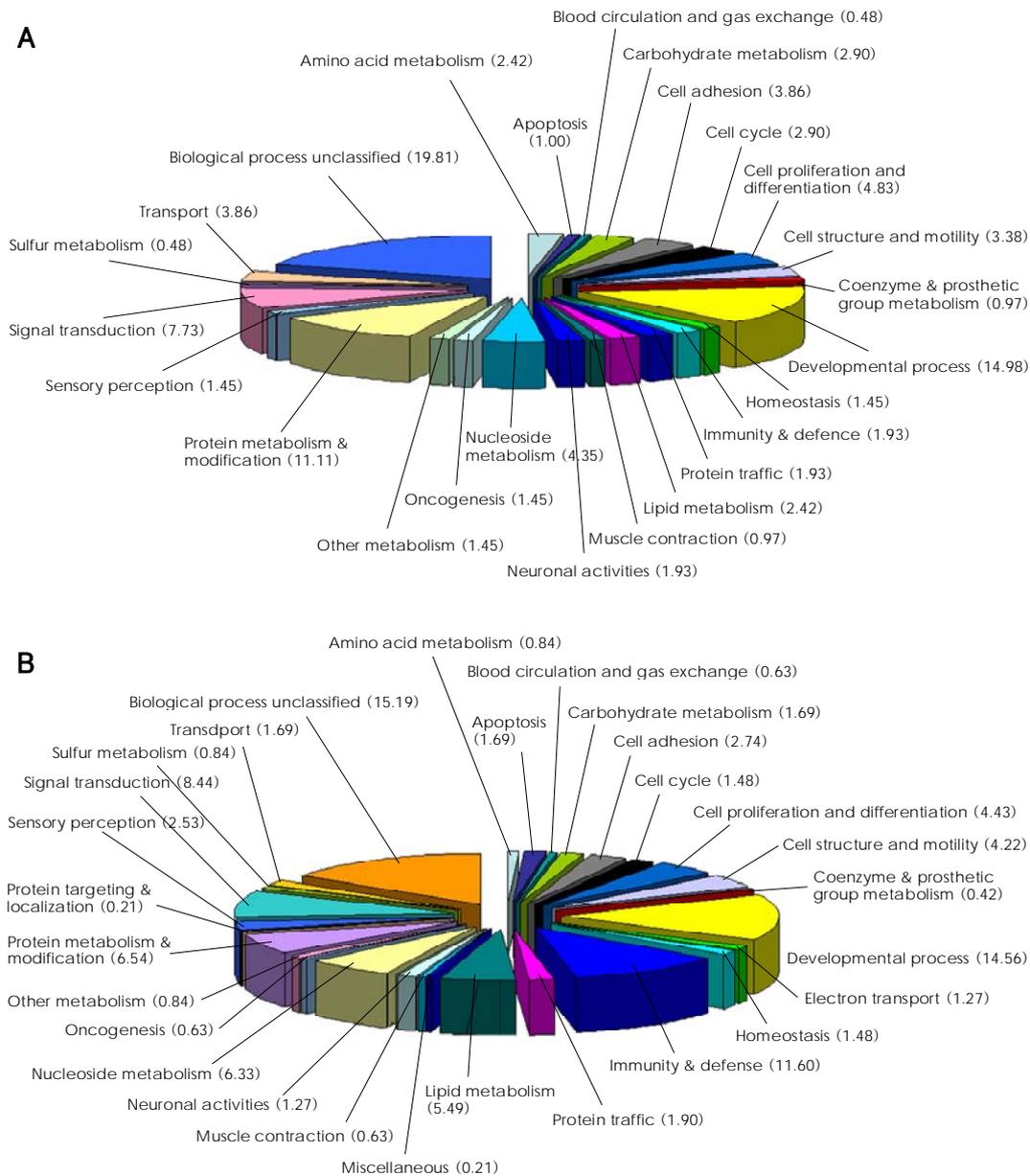


Fig. 4. cDNA microarray profiles of the umbilical cord blood (UCB)-mesenchymal stem cells (MSCs) and the bone marrow (BM)-MSCs. The pie charts show the percentages of the cDNAs that were expressed in the UCB-MSCs (A) and BM-MSCs (B). cDNA: complementary deoxyribonucleic acid.

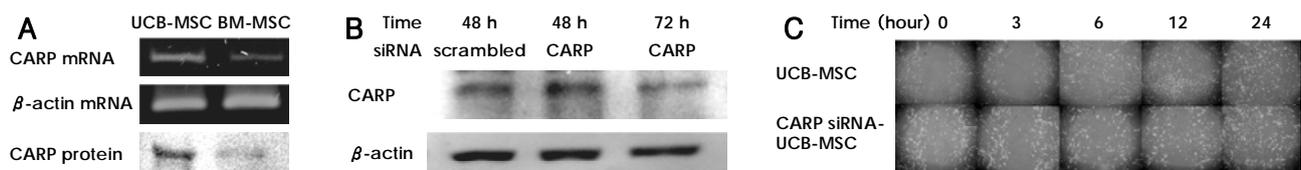


Fig. 5. Expression of CARP and the cell migration assay of the umbilical cord blood (UCB)-mesenchymal stem cells (MSCs). The higher expression level of CARP in the UCB-MSCs was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis (A). To knock down the endogenous CARP in UCB-MSCs, 100 nM of siRNA was transfected and the reduced CARP protein level was shown on Western blotting (B). Cell migration was assayed by the wound scratch assay. UCB-MSCs were transfected with scrambled siRNA or CARP siRNA to examine the effect of CARP on cell motility (C). CARP: cardiac ankyrin repeat protein, siRNA: small interfering ribonucleic acid.

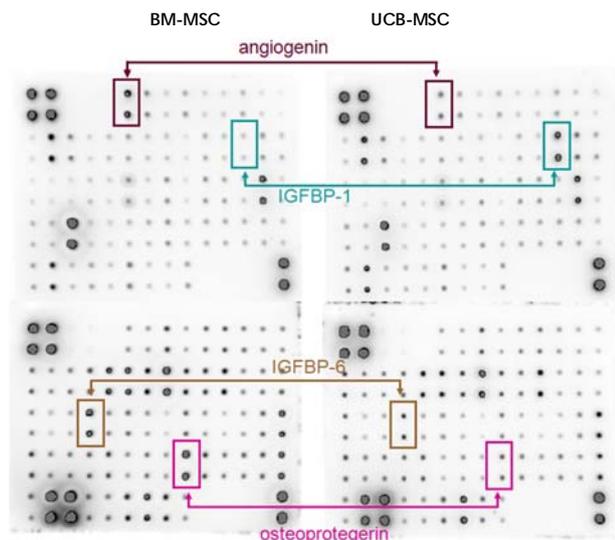


Fig. 6. Cytokine array of the umbilical cord blood (UCB)-mesenchymal stem cells (MSCs) and bone marrow (BM)-MSCs. The different cytokines between the two groups of MSCs are marked on the blots. IGFBP: insulin-like growth factor binding protein.

dial infarction model.^{16,17} More recently, other researchers have reported that grafted stem cells can secrete several important survival factors such as vascular endothelial growth factor, stromal cell-derived factor, basic fibroblast growth factor, hepatocyte growth factor and insulin-like growth factor, and these could protect or salvage endangered ischemic myocardium.¹⁸ This paracrine effect may be as important as the differentiation potential of stem cells for achieving functional improvement. However, a significantly high percentage of the transplanted stem cells was lost within a few days, and their survival rate has been reported to be less than 0.44%.¹⁹⁻²¹ Hence, safe and effective methods to promote stem cell survival need to be developed to perform successful stem cell therapy.

In conclusion, we report here on the differences between UCB-MSCs and BM-MSCs. In these experiments, the UCB-MSCs showed a stronger motility potential, yet there was no significant difference between the UCB-MSCs and BM-MSCs on the *in vivo* study.

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