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Disparate Mesenchyme-Lineage Tendencies in Mesenchymal Stem Cells from Human Bone Marrow and Umbilical Cord Blood

YU-JEN CHANG,[a,b] DANIEL TZU-BI SHIH,[c,d] CHING-PING TSENG,[b] TZU-BOU HSIEH,[a] DON-CHING LEE,[c] SHIAW-MIN HWANG[a]

[a]Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan; [b]Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan; [c]Center for Stem Cell Research and [d]Graduate Institute of Cell and Molecular Biology, Taipei Medical University, Taipei, Taiwan

Key Words. Mesenchymal stem cells • Differentiation • Leptin

ABSTRACT

Bone marrow and umbilical cord blood are reported to be the main sources of mesenchymal stem cells (MSCs), which have been proposed for many clinical applications. This study evaluated and quantitated the differentiation potential of bone marrow–derived MSCs (bmMSCs) and cord blood–derived MSCs (cbMSCs) by in vitro induction. Results indicated that cbMSCs had a significantly stronger osteogenic potential but lower capacity for adipogenic differentiation than bmMSCs. Leptin, an important regulator of mesenchymal differentiation, has a significantly stronger effect of promoting osteogenesis and inhibiting adipogenesis in bmMSCs than in cbMSCs. Moreover, Cbfa1 mRNA expression in bmMSCs and cbMSCs was affected to different degrees by leptin during osteogenesis. In contrast, leptin reduced PPARγ2 mRNA expression to the same level during adipogenesis in both types of MSCs. These results demonstrate the disparate capacities of MSCs from bone marrow and cord blood and suggest that they be used differently in experimental and therapeutic studies. In addition, the disparate differentiation tendencies of MSCs from different sources should be considered in further applications. STEM CELLS *2006;24:679–685*

INTRODUCTION

Mesenchymal stem cells (MSCs) are known to have the capacity for self-renewal and differentiation into mesenchyme-lineage cell types, including osteoblasts, adipocytes, chondrocytes, and myoblasts. The isolation and culture of MSCs, first described in the 1970s, from bone marrow were achieved using adherence to a plastic substratum and in vitro expansion [1]. The selective differentiation of MSCs in vitro depends on specific environmental cues, usually a combination of induction chemicals, growth factors, and cytokines. Currently, MSCs can be isolated from various human sources, including bone marrow, umbilical cord blood, adipose tissue, and muscle [2, 3]. MSCs have become a major cellular source in several areas of developmental research. They are multipotent precursors with low variability from different donors [4]. However, the differences among MSCs isolated from different tissues are still unclear.

Leptin, a 16-kDa peptide hormone discovered in 1994, is an adipocyte-derived signaling molecule that regulates food intake and increases energy expenditure using specific receptors in the hypothalamus [5, 6]. In adults, leptin is secreted mainly by white adipose tissue into the circulation [5]. Moreover, leptin has also been found to be synthesized and secreted from placental trophoblasts into the maternal and fetal circulation in pregnant women [7]. Current evidence suggests that leptin can affect the function of mesenchyme-lineage cells, such as muscle cells [8], chondrocytes [9], osteoblasts [10], and adipocytes [11]. However, it is still unknown if MSCs are the targets of leptin.

Although the immunophenotypic profiles of MSCs from different sources are very similar [2, 3], knowing if these MSCs express quantitative differences in differentiation potentials is important. To investigate the difference in the functional specificity of osteogenesis and adipogenesis between different sources of MSCs, human bone marrow–derived MSCs

Correspondence: Shiaw-Min Hwang, Ph.D., Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan. Telephone: 886-3-522-3191; Fax: 886-3-521-4016; e-mail: hsm@firdi.org.tw Received November 8, 2004; accepted for publication September 12, 2005; first published online in STEM CELLS *EXPRESS* September 22, 2005. ©AlphaMed Press 1066-5099/2006/$20.00/0 doi: 10.1634/stemcells.2004-0308

(bmMSCs) and umbilical cord blood–derived MSCs (cbMSCs) were compared. Differences in differentiation potentials in osteogenesis and adipogenesis were detected under the same induction conditions. Interestingly, cbMSCs exhibit a significantly stronger osteogenic potential but lower adipogenic capacity than bmMSCs. Leptin was found to directly affect the mesenchyme-lineage differentiation potentials of both types of MSCs tested. Data from our study suggest that the diverse functional potentials of MSCs from different sources should play an important role in their further applications.

Materials and Methods

Establishment of MSCs from Human Bone Marrow and Cord Blood

Term umbilical cord blood (UCB) of newborns was harvested into a standard 250-ml blood bag containing citrate-phosphate-dextrose-adenine anticoagulant (Terumo; Tokyo, Japan; http://www.terumomedical.com) with the mother's informed consent. Cord blood samples were processed within 24 hours. Buffy coat was obtained from UCB by centrifugation (700 × g for 20 minutes) and diluted with an equal volume of wash buffer (Dulbecco's phosphate-buffered saline [PBS]; Sigma; St. Louis; http://www.sigmaaldrich.com). The buffy coat cells were then layered onto Ficoll-Paque solution (1.077 g/ml; Amersham; Uppsala, Sweden; http://www.amershambiosciences.com) and centrifuged to deplete the residues of red blood cells, platelets, and plasma (700 × g for 40 minutes). Cord blood mononuclear cells (MNCs) in the interface were collected and washed twice with PBS. Human adult bone marrow MNCs were fresh collected (one male, 19 years old; one female, 37 years old) by Ficoll-Paque density centrifugation or supplied by Cambrex Co. (two males, 19 years and 30 years old; one female, 31 years old) (Walkersville, MD; http://www.cambrex.com). For the frozen MNCs supplied by Cambrex, cells were washed twice with culture medium immediately after being thawed. Both bmMNCs and cbMNCs were seeded at a concentration of 10^6 cells/cm^2 in alpha-modified minimum essential medium (α-MEM; Hyclone; Logan, UT; http://www.hyclone.com), containing 20% fetal bovine serum (FBS; Hyclone), 4 ng/ml basic fibroblast growth factor (R&D Systems; Minneapolis; http://www.rndsystems.com), 100 units/ml penicillin, and 100 μg/ml streptomycin (Sigma), and cultured at 37°C in a humidified atmosphere with 5% CO_2. The nonadherent cells were removed using a medium change after 3 days of seeding, and the medium was changed twice a week thereafter for 2–3 weeks. When well-developed colonies of fibroblast-like cells appeared, cells were washed with PBS, harvested with 0.05% trypsin-EDTA (Sigma) and passed into new T75 flasks. This protocol was approved by the Institutional Review Board of Taipei Medical University, Taiwan.

Immunophenotyping of MSCs

Cells at passage four were trypsinized, washed, and resuspended in PBS at a concentration of about 100,000 cells/ml. After fixing with 1% methanol at 4°C for 10 minutes, cells were blocked in 1% bovine serum albumin (BSA; Sigma) plus 0.1% FBS for 1 hour at room temperature, then washed with three volumes of PBS. The cell pellet was resuspended in 0.5 ml of primary antibody solution containing 1% BSA plus 0.1% FBS for 40 minutes at 4°C, then washed three times in PBS. Cells were immunolabeled with the following mouse anti-human antibodies: CD34, CD45 (Miltenyi Biotech; Auburn, CA; http://www.miltenyibiotec.com), SH2, SH3, and SH4 (culture supernatants obtained from hybridoma cells; American Type Culture Collection; Manassas, VA; http://www.atcc.org). The nonspecific mouse IgG (Vector; Burlingame, CA; http://www.vectorlabs.com) was substituted for the primary antibodies as an isotype control. The secondary antibody, anti-mouse IgG-fluorescein isothiocyanate (FITC) or IgG-phycoerythrin (PE) (Vector), was incubated with cells for 20 minutes at 4°C in the dark. The cells were then washed with PBS and resuspended in 1 ml of PBS for analysis. Data were analyzed using the fluorescence-activated cell sorter (FACS) FACSscan flow cytometer (Becton, Dickinson and Company; San Jose, CA; http://www.bd.com) with a minimum of 30,000 events.

Osteogenic and Adipogenic Differentiation

MSCs established from bone marrow and cord blood were seeded at about 7×10^3 cells/cm^2 into six-well plates and grown to confluence. To induce osteogenic differentiation, cells were incubated in α-MEM supplemented with 10% FBS, 0.1 μM dexamethasone (Sigma), 10 mM β-glycerolphosphate (Sigma), and 50 μM ascorbic acid (Sigma) for 3 weeks. To initiate adipogenic differentiation, cells were incubated in α-MEM supplemented with 10% FBS, 1 μM dexamethasone (Sigma), 0.5 mM methyl-isobutylxanthine (Sigma), 10 μg/ml insulin (GibcoBRL; Carlsbad, CA; http://www.lifetech.com), and 100 μM indomethacin (Sigma) for 3–5 weeks. To assay the effect of leptin on osteogenic and adipogenic differentiation, human recombinant leptin (PeproTech; London, U.K.; http://www.peprotech.com) was added, at concentrations of 0.6 and 1.5 μg/ml, to each differentiation medium. After 3 weeks of culture, cells were analyzed for osteogenic and adipogenic features. Three replicates were tested for each sample.

Quantification and Detection of Osteogenic and Adipogenic Differentiation Potential

Cells were analyzed for alkaline phosphatase (ALPase) activity and Alizarin Red S staining (Sigma) to assess their osteogenic differentiation. ALPase activity was assayed following the manufacturer's instruction (Sigma). Briefly, 1 ml of 0.05 N NaOH in ethanol was added to each well after ALPase activity stain [12]. The extraction was measured spectrophotometrically at 550 nm. The amount of ALPase activity was represented as a ratio compared with that of the undifferentiated cells. To quantify calcium deposition, cells were washed with PBS and incubated with 2% Alizarin Red S solution (preadjusted to a pH of 5.5 with 0.5% NH_4OH) for 5 minutes [12]. After PBS washing, 1 ml phosphate buffer (8 mM Na_2HPO_4 + 1.5 mM KH_2PO_4) containing 10% cetylpyoidimium chloride (Sigma) was added and incubated overnight. The extracted solution of cell-bound Alizarin Red S was measured spectrophotometrically at 550 nm and compared with a standard titration curve [12].

Adipogenic differentiation was assessed by Oil Red O (Sigma) staining for 15 minutes at room temperature. Afterward, cells were washed completely with 60% isopropanol (Sigma), and 1 ml ethanol was added to each well to extract the cell-bound Oil Red O. The amount of Oil Red O released was determined spectrophotometrically at 550 nm with a reference

of 650 nm and compared with an Oil Red O standard titration curve [12]. The extraction of cell-bound Alizarin Red S and Oil Red O was normalized to the average cell number at the time of dye extraction. A panel of wells was trypsinized in parallel to count the number of cells.

Measurement of mRNA Level by Real-Time Polymerase Chain Reaction

Total RNA from each sample was isolated using Trizol reagent (MRC; Cincinnati, OH, http://www.mrcgene.com/tri.htm) following the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1 μg total RNA using ImPro-II reverse transcriptase (Promega; Madison, WI; http://www.promega.com) with oligo-dT primer in 20 μl reaction mixture. The relative expression level of β-actin was used as an internal control to normalize specific gene expression in each sample. Real-time polymerase chain reaction (PCR) using the ABI Prism 7000 Sequence Detection System (Applied Biosystems; Foster City, CA; http://www.appliedbiosystems.com) was performed with 2 μl of the single-stranded cDNA sample with SYBR Green PCR master mix (Applied Biosystems). The relative quantitation of marker genes (Table 1) was performed according to the $\triangle\triangle$Ct method, as described elsewhere [13].

Statistical Analysis

The results of quantification of ALPase activities, the extracted cell-bound Alizarin Red S and Oil Red O, and gene expression by real-time PCR are presented as mean $\pm$ standard deviation (SD). Comparisons between experimental groups and the control were made using the Student's *t*-test. Differences were considered statistically significant at $p < .05$.

RESULTS

Establishment of MSCs from Bone Marrow and Cord Blood

Both bone marrow and cord blood MNCs were seeded at a concentration of 1×10^6 cells/cm^2, and the nonadherent hematopoietic cells were removed by decanting the medium. Two weeks after the first seeding, colonies of adherent cells with elongated fibroblast-like morphology, a characteristic feature of MSCs, were observed in culture. When the cells grew to confluence, they were trypsinized and passaged with a ratio of 1:4 split. Initially, colonies from cord blood established more slowly than those from bone marrow under the same seeding and culture conditions, with the former taking 1–2 weeks longer. The frequency of colony-forming unit-fibroblast (CFU-F) formation in cbMSCs was also markedly lower than that in bmMSCs by about one tenth. However, after MSC culture establishment, cbMSCs had a shorter population doubling time (PD), 28 ± 3 hours ($n = 5$), than bmMSCs, 34 ± 3 hours ($n = 5$). We established five different bmMSC populations (three males and two females), one from each of our three frozen and two fresh bone marrow samples, but only five different cbMSCs (two males and three females) from 10 cord blood samples. After primary culture, MSCs derived from these two sources represented a morphologically homogeneous fibroblast-like population (Fig. 1). FACS analysis was used to characterize cell surface markers on bmMSCs and cbMSCs at passage 4. As shown in Figure 2, two typical profiles from bmMSCs and cbMSCs exhibited similar immunophenotypes, which were negative for CD34 (a hematopoietic stem cell marker) and CD45 (leukocyte common antigen) and positive for the mesenchymal progenitor–specific markers SH2, SH3, and SH4. These data are consistent with previous reports [3, 14].

Osteogenesis and Adipogenesis of bmMSCs and cbMSCs

To assay the differentiation potentials of MSCs along the mesenchyme lineage, confluent cultures of both types of MSC were exposed to osteogenic and adipogenic induction media. In the osteogenic induction medium, both bmMSCs and cbMSCs differentiated into osteoblasts. However, the osteogenic differentiation potential differed significantly between these two types of MSCs. cbMSCs generated a large amount of mineral nodules within 1 week and increased continuously thereafter. More than 60% mineral accumulation was observed after 1 week of osteogenic culture of cbMSCs (Fig. 3A). After 3 weeks in culture, cbMSCs fully differentiated into osteoblasts. Comparatively, bmMSCs showed a relatively lower osteogenic tendency; only about 20% of the bmMSCs produced mineral nodules after 1 week of osteogenic induction and about 60% osteoblasts were found after 3 weeks of culture. The difference in the osteogenic differentiation capacity between cbMSCs and bmMSCs was further examined by Alizarin Red S staining and ALPase activity analysis (Fig. 3A). The amounts of Alizarin Red S and ALPase activity were 6.9-fold and 2.7-fold greater for cbMSCs than for bmMSCs in osteogenic culture, respectively (data not shown).

In contrast, bmMSCs exhibited a significantly stronger capacity for adipogenic induction than cbMSCs. Neutral lipid droplets appeared in the cytoplasm of bmMSCs in the first week, and the amount and volume of lipid droplets increased dramatically. The lipid droplet–containing cells accounted for more than 80% of the induced bmMSC culture at week 3. In contrast, typical lipid droplets were rarely present in the cbMSC adipogenic culture, even when the induction period was extended to 5 weeks. The induction of adipogenesis in cbMSCs resulted in <5% Oil Red O–positive cells found in the culture,

Table 1. Marker genes used in real-time polymerase chain reaction

Gene	Accession number	Primer sequence (5′ → 3′)
β-actin	NM001101	Forward: TGTGGATCAGCAAGCAGGAGTA Reverse: CAAGAAAGGGTGTAACGCAACTAAG
Cbfa1	NM004348	Forward: CATGGCGGGTAACGATGAA Reverse: CGGCCCACAAATCTCAGATC
PPARγ2	NM138712	Forward: CCAGAAAATGACAGACCTCAGACA Reverse: GCAGGAGCGGGTGAAGACT

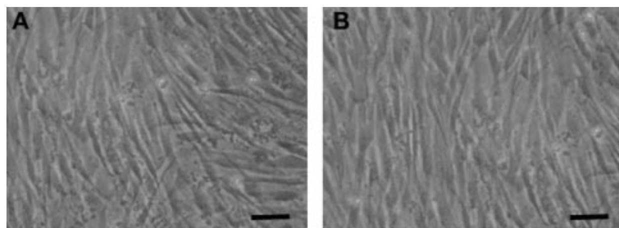

Figure 1. Morphology of cultured human mesenchymal stem cells (MSCs). Typical phase-contrast images of bone marrow–derived MSCs **(A)** and cord blood–derived MSCs **(B)** showed a fibroblast-like homogeneous phenotype. Scale bars = 100 μm.

and the size of lipid droplets was also smaller than that found in the bmMSC adipogenic culture (Fig. 3B).

The adipogenic induction protocol used in this study demonstrated that bmMSCs had a markedly greater adipogenic tendency than cbMSCs. The amount of cell-bound Oil Red O in bmMSC adipogenic cultures was 17.3-fold higher than that found in cbMSC adipogenic cultures (data not shown).

Effects of Leptin on Osteogenesis and Adipogenesis

Leptin has been demonstrated to be an important factor in regulating bone and fat formation in humans [10, 11]. To evaluate whether leptin could affect the differentiation of MSCs in vitro, recombinant human leptin was added in combination with the differentiation media. After 3 weeks, osteogenesis was significantly enhanced by leptin in both bmMSCs and cbMSCs. Mineralization was estimated by the amount of cell-bound Alizarin Red S (Fig. 4A). The effect of leptin in increasing osteogenesis was also confirmed by ALPase activity staining (Fig. 4B). Interestingly, a dose-dependent enhancement of osteogenesis by leptin was only found in bmMSCs. bmMSCs treated with 0.6 μg/ml and 1.5 μg/ml of leptin had mineral accumulation levels that were 73% and 144% greater, respectively. The ALPase activity staining ratios were also 36% and 51% greater, respectively. However, treatment of cbMSCs with 0.6 μg/ml of leptin resulted in only a 25% greater mineral accumulation and a 13% greater ALPase activity staining ratio. Increasing the leptin concentration to 1.5 μg/ml had no effect; the leptin effect was seemingly saturated at the lower level, according to the analysis of mineral accumulation and ALPase activity staining in cbMSCs. These results indicate that leptin had a relatively stronger osteogenesis promoting effect in bmMSCs than in cbMSCs, although the osteogenic capacity of bmMSCs was still much lower than that of cbMSCs under leptin treatment.

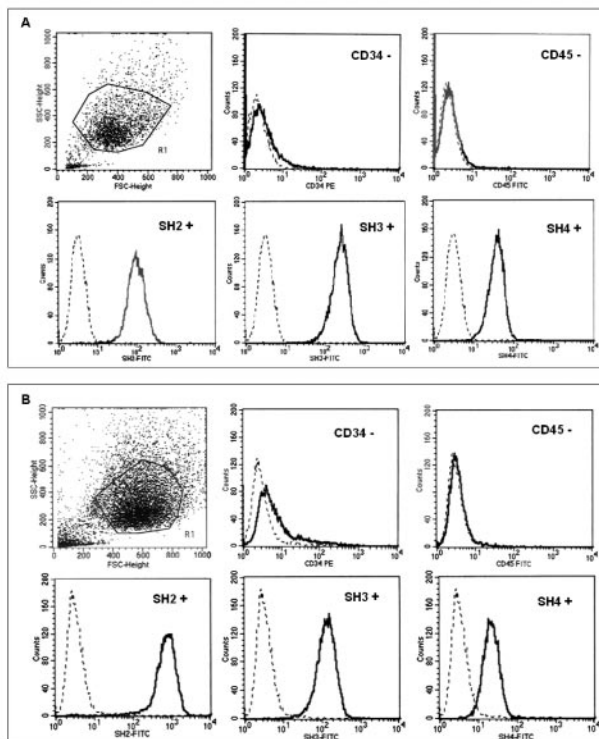

Figure 2. Fluorescence-activated cell sorter (FACS) analysis of undifferentiated human bone marrow–derived mesenchymal stem cells (bmMSCs) **(A)** and cord blood–derived MSCs (cbMSCs) **(B)** at passage 4. A homogeneous confluent monolayer of MSCs was trypsinized and analyzed by staining with various antibodies. Both bmMSCs and cbMSCs lacked the expression of CD34 and CD45 and expressed SH2, SH3, and SH4. The respective isotype control is shown as the dotted line. Abbreviations: FITC, fluorescein isothiocyanate; FSC, forward scatter; PE, phycoerythrin; SSC, side scatter.

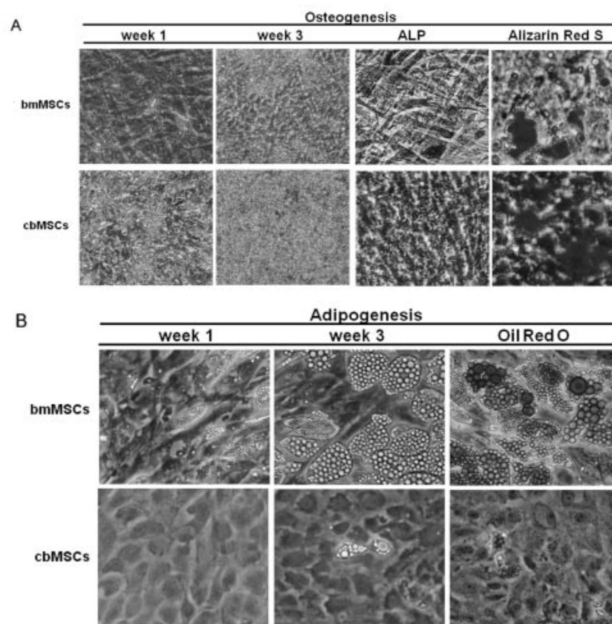

Figure 3. Osteogenic and adipogenic capabilities of bone marrow–derived mesenchymal stem cells (bmMSCs) and cord blood–derived MSCs (cbMSCs). Cells were incubated in osteogenic or adipogenic medium for 3 weeks after confluence. Osteogenic capability was imaged at week 1 and week 3 and characterized by alkaline phosphatase (ALPase) activity and Alizarin Red S staining after 3 weeks' induction **(A)**. Adipogenic capability was imaged at week 1 and week 3, and lipid droplet accumulation was detected by staining with Oil Red O after 3 weeks induction **(B)**. The pictures taken from each culture condition are representative of the entire culture dish.

The adipogenic effect of leptin on both types of MSC was also compared, as shown in Figure 5A. Leptin significantly reduced adipogenesis in both bmMSCs and cbMSCs, as assessed by Oil Red O staining. When leptin was incorporated into the adipogenic media, both types of MSC yielded a lower number of lipid droplets. The accumulation of cell-bound Oil

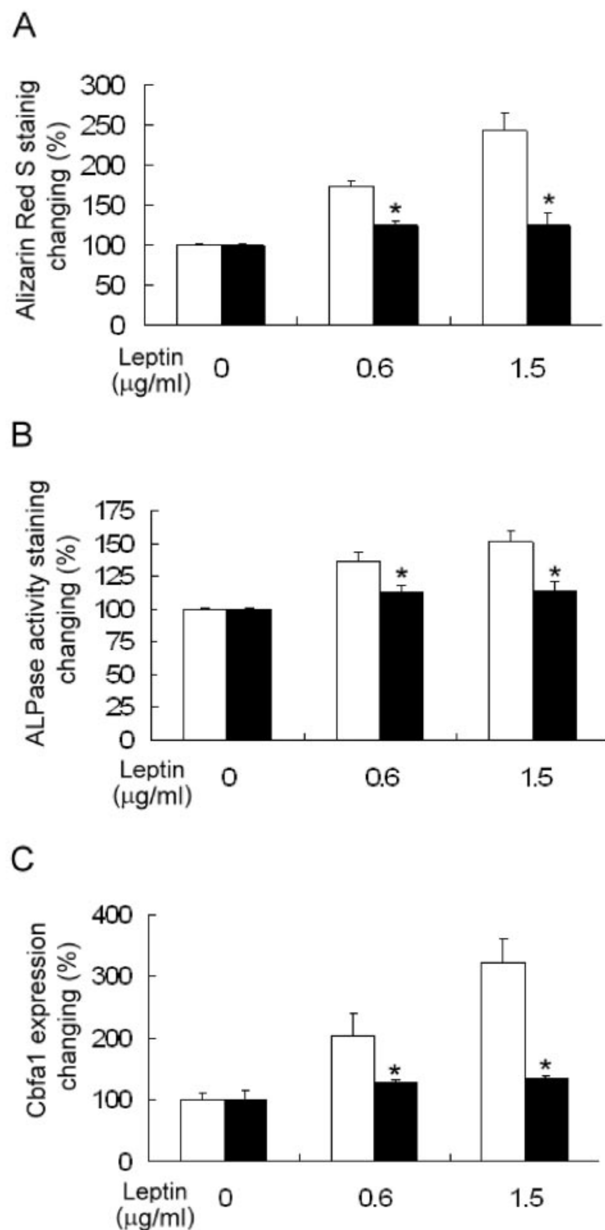

Figure 4. Comparison of leptin effect on osteogenic differentiation of mesenchymal stem cells (MSCs). Bone marrow–derived MSCs (bmMSCs) and cord-blood–derived MSCs (cbMSCs) were exposed to osteogenic medium containing 0, 0.6, or 1.5 μg/ml of leptin. Leptin enhanced osteogenic potential in both types of MSCs as assessed by staining with Alizarin Red S **(A)** and by alkaline phosphatase (ALPase) activity **(B)** after 3 weeks of induction. Expression of the early osteogenic transcription factor Cbfa1 was detected using real-time polymerase chain reaction after 1 week of induction **(C)**. Results are represented as the percentage of the corresponding no leptin treatment and expressed as the mean ± standard deviation of three replicates of bmMSCs (n = 5) and cbMSCs (n = 5). □, bmMSCs; ■, cbMSCs. * indicates statistically significant difference ($p < 0.05$) versus bmMSCs under the same conditions.

Red O was 61% and 68% lower in bmMSCs under treatment with 0.6 μg/ml and 1.5 μg/ml of leptin, respectively, whereas the cell-bound Oil Red O was 41% and 44% lower in cbMSCs under the same conditions. These results indicate that leptin had a relatively stronger suppressive effect on adipogenesis in bmMSCs than in cbMSCs, although the accumulated oil was still much higher in bmMSCs than cbMSCs under leptin treatment.

Leptin Effects on Osteogenic (Cbfa1) and Adipogenic (PPARγ2) Transcription

Because leptin influenced the differentiation potentials of both types of MSC, we further quantitated the gene expression of mesenchyme-lineage transcription factors in osteogenesis and adipogenesis using real-time PCR analysis. Cbfa1 and PPARγ2 are the major transcription factors for early osteogenesis and adipogenesis respectively.

Consistent with the Alizarin Red S staining and ALPase activity assay, leptin produced a dose-dependent increase in Cbfa1 expression during osteogenesis only in bmMSCs. Cbfa1 mRNA expression was 2.0-fold and 3.2-fold greater in bmMSCs under the 0.6 μg/ml and 1.5 μg/ml leptin treatments, respectively, at the first week of osteogenesis, while it was only 1.2-fold and 1.3-fold greater in cbMSCs under the same conditions (Fig. 4C).

During adipogenic induction, PPARγ2 mRNA expression levels in bmMSCs treated with 0.6 μg/ml and 1.5 μg/ml leptin supplementation were only 48% and 41% of the levels observed without leptin treatment, respectively (Fig. 5B). In cbMSCs, the corresponding PPARγ2 mRNA expression levels were 79% and 68% of the level seen without leptin supplementation, respectively. These results confirm that bmMSCs were significantly more sensitive to leptin than cbMSCs in osteogenic promotion and adipogenic suppression.

Discussion

MSCs play a vital role in cell regeneration and the repair of damaged tissues in human growth. The ease of culturing and expanding MSCs ex vivo has recently spurred numerous therapeutic applications and clinical trials [3]. MSCs have also been used as developmental models for osteogenesis and adipogenesis [15, 16]. MSCs isolated by different methods were reported to be similar, without much variation in their differentiation potentials [17]. Currently, the two main sources of human MSCs are bone marrow and umbilical cord blood. Moreover, MSCs from these two different sources possess similar surface marker profiles [2, 3]. Each MSCs sample from bone marrow (n = 5) and cord blood (n = 5) in this study exhibited the typical fibroblast-like morphology and the same immunophenotypes of MSCs—positive for the mesenchymal-specific markers SH2, SH3, and SH4 and negative for the hematopoietic-specific markers CD34 and CD45. We also observed that cbMSCs proliferated faster (PD, 28 ± 3 hours) than bmMSCs (PD, 34 ± 3 hours), which is in agreement with what is known regarding fetuses versus adults. The average telomere length of cbMSCs was significantly longer than that of bmMSCs, 12.0 kb versus 10.0 kb, both at passage 4, respectively (data not shown). Moreover, we didn't find any differences between bmMSCs established from frozen or fresh bone marrow.

At the same developmental stage, however, MSCs isolated from different tissues might exhibit diverse differentiation capacities. For example, MSCs isolated from different fetal tissues during the second trimester showed diverse adipogenic and

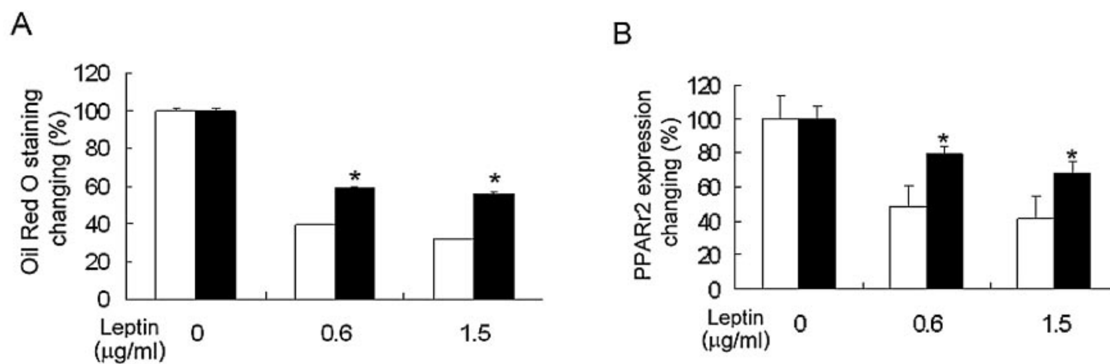

Figure 5. Comparison of the effect of leptin on adipogenic differentiation of mesenchymal stem cells (MSCs). Bone marrow–derived MSCs (bmMSCs) and cord-blood–derived MSCs (cbMSCs) were exposed to adipogenic medium containing 0, 0.6, or 1.5 μg/ml of leptin. Leptin suppressed adipogenesis in both bmMSCs and cbMSCs as assessed by staining Oil Red O staining after 3 weeks of induction **(A)**. Expression of the adipogenic transcription factor PPARγ2 was detected using real-time polymerase chain reaction after 3 weeks of induction **(B)**. Results are represented as the percentage of the corresponding no leptin treatment and expressed as the mean ± standard deviation of three replicates of bmMSCs ($n = 5$) and cbMSCs ($n = 5$). □, bmMSCs; ■, cbMSCs. * indicates statistically significant difference ($p < 0.05$) versus bmMSCs under the same conditions.

osteogenic differentiation potentials [12]. The adipogenic capacity was markedly lower, but the osteogenic capacity was higher, in the MSCs derived from fetal spleen than from bone marrow, liver, or lung [12]. Bieback et al. [18] also reported a higher adipogenic potential in bmMSC clones than cbMSC clones. Thus, do MSCs from adult bone marrow and postnatal cord blood possess different differentiation tendencies? In this study, cbMSCs displayed an apparently higher tendency to undergo osteogenesis than bmMSCs did. During osteogenic differentiation, osteoblasts exhibited strong ALPase activity, followed by matrix maturation and then transition into the matrix mineralization period [19]. cbMSCs accumulated much more mineral nodules and expressed higher ALPase activity than bmMSCs under the same osteogenic induction conditions. In contrast, bmMSCs had more lipid droplet–containing cells than cbMSCs during adipogenesis. These findings indicate that cbMSCs have a stronger osteogenic potential but much lower adipogenic capacity than bmMSCs.

Although leptin has emerged as a potential contributor to bone turnover and fat mass, its effects on bone metabolism remain unclear. Some in vivo evidence has revealed that reducing the serum leptin level or the expression level of functional leptin receptor increases bone mass [20, 21]. Conversely, leptin has also been reported to have many positive effects on osteoblasts, by increasing cell proliferation, differentiation, and bone mineralization [10, 22]. Leptin also acts on muscle cells and chondrocytes in vitro [8, 9]. Not surprisingly, these cells are all derived from mesenchymal stem cells. Thomas et al. [23] reported that leptin could enhance osteogenesis and inhibit adipogenesis in hMS-12 cells, a conditionally immortalized human marrow stromal cell line. Our data demonstrate, for the first time, that leptin can act directly on primary MSCs to regulate mesenchyme-lineage differentiation in vitro, including promoting osteogenesis and suppressing adipogenesis. Moreover, bmMSCs are more sensitive to leptin than cbMSCs in both osteogenesis and adipogenesis. Because of the high leptin expression levels in human bone marrow adipocytes [24], leptin may serve as an endocrine modulator of MSC differentiation. These in vitro studies may be relevant to the clinical observation that obesity is associated with greater bone mineral density [25].

Our real-time PCR data are in agreement with the observation that osteogenesis and adipogenesis are activated by the transcriptional regulators Cbfa1 and PPARγ2, respectively [26]. Leptin consistently showed a dose-dependent upregulation of Cbfa1 expression and downregulation of PPARγ2 expression in bmMSCs during osteogenesis and adipogenesis, while leptin had only a slight effect on cbMSCs.

In conclusion, our data indicate that bmMSCs and cbMSCs possess heterogeneous differentiation potentials and different responses to leptin in vitro. These disparate characteristics of tissue-derived MSCs from different sources should be considered to better apply MSCs in vivo.

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Disclosures

The authors indicate no potential conflicts of interest.

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