Original Article

Cruciate ligament-derived mesenchymal stem cells: a potential cell source for cartilage regeneration

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Background: Mesenchymal stem cells (MSCs) have been widely investigated for potential in tissue regeneration. The aim of this study was to compare the proliferation and induced differentiation potentials of MSCs isolated from bone marrow (BMSCs), adipose (AMSCs) and cruciate ligament (CLMSCs).

Methods: BMSCs, AMSCs and CLMSCs were isolated and identified by flow cytometry with CD markers. Their clonogenicity, proliferation and multi-differentiation capacities were also analyzed. The mRNA expression of adipogenic, osteogenic, and chondrogenic markers at basal state and after multi-lineage inductions was examined using quantitative real-time polymerase chain reaction (qRT-PCR).

Results: BMSCs, AMSCs and CLMSCs showed similar positive expression for CD29, CD90, and negative for CD45. Comparative analyses suggested the superior colony forming and proliferation capacity in CLMSCs. When induced toward adipo-, osteo- and chondro-lineages, BMSCs, AMSCs and CLMSCs could successfully differentiate toward target lineages. At basal state and after multi-lineage inductions, AMSCs expressed the highest level of adipogenic markers [peroxisome proliferator-activated receptor gamma 2 (PPARγ2) and CCAAT enhancer binding protein alpha (c/EBPα)]; BMSCs expressed the highest level of osteogenic markers [Alpl, Bglap, Runx2, bone morphogenetic protein 2 (Bmp2), and Spp1]; CLMSCs expressed the highest level of chondrogenic markers (Col2A1, Acan, and Sox9).

Conclusions: This study demonstrates that CLMSCs and AMSCs exhibit superiority in cell proliferation compared to BMSCs. CLMSCs can be a potential cell source for clinical application in cartilage regeneration.

Keywords: Mesenchymal stem cells (MSCs); cruciate ligament; chondrogenic differentiation; proliferation; tissue regeneration

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Introduction

Tissue engineering based on stem cells is a promising strategy to repair the degenerate or damaged tissues (1-3). Mesenchymal stem cells (MSCs), unlike embryonic stem cells, are considered to be a potential cell source for tissue engineering for less controversy in ethical problems (4). The advantage of MSCs applied in tissue engineering is that these cells can differentiate into targeted tissues, including bone, adipose, cartilage, myoblasts, nucleus pulposus and so on (5-7). The self-renewal and proliferation abilities of MSCs are also robust with phenotype stability (8).

The prevalence of musculoskeletal diseases have motivated researchers to investigate the potential cell

sources of MSCs derived from different tissues for tissue regeneration (9,10). Many tissue engineering researches have been based on the proliferation and differentiation properties of MSCs derived from multiple tissues (11). Bone marrow is a widely investigated source of MSCs, and bone marrow-derived mesenchymal stem cells (BMSCs) have been effectively used in tissue repair and regeneration (12,13). However, BMSCs can only be obtained through bone marrow biopsy, which will cause unnecessary pain. Adipose-derived mesenchymal stem cells (AMSCs) have attracted increasing interest due to easier isolation procedures and relative abundance in adipose tissues, representing a potential alternative to BMSCs (14,15). Recently, a population of multipotent MSCs was isolated from ligament tissues with similar properties of BMSCs (16,17).

Different sources of MSCs may exhibit different characteristics when applied in tissue regeneration (18). How the origin of bone marrow, adipose or cruciate ligament may affect MSCs' ability to differentiate remains controversial and largely unknown. Our aim has been to compare the proliferation and induced differentiation potentials of MSCs isolated from bone marrow, adipose and cruciate ligament.

Methods

Cell isolation and culture

The Animal Research Ethics Committee, the Southeast University approved all experiments. Ten 6-week old male Sprague-Dawley rats weighing 200 to 220 g were used in this study. MSCs isolated were from the same animal. The procedures of the isolation of BMSCs and AMSCs have been previously established (19). Cruciate ligament-derived mesenchymal stem cells (CLMSCs) were isolated by explant culture system based on a study reported with some modifications (17). Briefly, the knee cruciate ligaments were carefully excised from healthy rats overdosed with 2.5% intraperitoneal sodium phenobarbital (1.0 mL/400 g). When opening the knee joint capsule, only the ligaments between the femoral condyle nest but not the meniscus tissues often free form the tibia were collected. The collected cruciate ligaments, including anterior and posterior cruciate ligaments were rinsed twice with sterile phosphate buffered saline (PBS). The possible blood vessels and sheath of the ligaments were carefully scraped and washed by PBS again. The washed tissues were minced into pieces of about 1 mm^3 and placed in a 6-well plates at 37 ℃ for 5 minutes to promote adherence, followed by the addition of 2 mL basal complete culture medium consisting of DMEM supplemented with 10% fetal bovine serum (FBS) (Hyclone), antibiotics (50 IU penicillin/mL and 50 mg streptomycin/mL; Beijing Leagene Biotech, China) for 2 weeks. During this period, cells migrated from the tissue fragments, which formed a population of outgrowth cells. The isolated cells were sub-cultured when they reached 80% to 90% confluence. Medium was changed every 3 days. Third passage cells (P3) were used for all experiments.

Fluorescence-activated cell sorting analysis

BMSCs, AMSCs, and CLMSCs at P3 were utilized for surface marker expression by flow cytometry according to previously established procedures (20). The cultured cells were harvested and washed twice with cold PBS to remove the supernatants. Then the washed cells were resuspended and incubated for 30 min at 4 ℃ in the dark with phycoerythrin (PE) or fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies. Antibodies against CD29 and CD 45 were purchased from BD Biosciences. Antibodies against CD90 were purchased from Santa Cruz Biotech. Nonspecific IgG1 (PE) and IgG1 (FITC) (both from Santa Cruz Biotech) for all fluorochromes were used as comparative controls. Flow cytometry analysis was performed with a FACSAria (BD Biosciences).

Colony-forming assay

BMSCs, AMSCs and CLMSCs were plated at 300 cells per 10-cm^2 dish, 10 dishes in each cell group. Each type of cells was cultured with complete medium for 14 days. Then, cells were washed 3 times by PBS, fixed with 10% methanol for 15 min and stained with 0.5% crystal violet (Sigma, St. Louis, MO, USA) for 15 min to count the number of cell colonies. Colonies smaller than 2 mm in diameter were ignored. The number of colonies in each plate was reported.

Cell proliferation assay

BMSCs, AMSCs and CLMSCs were plated at 5,000 cells/well in a 96-well plate and incubated at 37 ℃, 5% CO_2 for 2 days. Cell proliferation was assessed using the Cell Counting Kit-8 (Qihai-futai Bio Tec, Shanghai, China) according to the manufacturer's instruction. The absorbance value was measured at 370 to 450 nm.

Table 1 Primer sequences for quantitative real-time polymerase chain reaction (qRT-PCR)

Gene	Primer nucleotide sequence	Product size (bp)
PPARγ2	F-CGGTTGATTTCTCCAGCATTTC	103
	R-TCTTGGAGCTTCAGGTCATATTT	
c/EBPα	F-GTGGATAAGAACAGCAACGAGTA	124
	R-TGGTCAACTCCAACACCTTC	
Alpl	F-CATGTTCCTGGGAGATGGTATG	144
	R-GTGTTGTACGTCTTGGAGAGAG	
Bglap	F-CCCTCTCTCTGCTCACTCT	105
	R-GCTTGGACATGAAGGCTTTG	
Runx2	F-TGTTCTCTGAGCGCCTCAGTG	146
	R-CCTGGGATCTGTAATCTGACTCT	
Bmp2	F-CTCCAAGAGACATGTGAGGATTAG	122
	R-CTCGTTTGTGGAGTGGATGT	
Spp1	F-AGGAGTTTCCCTGTTTCTGATG	110
	R-GCAACTGGGATGACCTTGATA	
Acan	F-GGTGTCACTTCCCAACTATCC	101
	R-GCATCACTTCACACCGATAGA	
Col2A1	F-CATCGAGTACCGATCACAGAAG	101
	R-GCCCTATGTCCACACCAAAT	
Sox9	F-GAGCCGGATCTGAAGAAGGA	151
	R-GCTTGACGTGTGGCTTGTTC	
GAPDH	F-GGGAAACCCATCACCATCTT	72
	R-ATACTCAGCACCAGCATCAC	

Expression of osteogenic, adipogenic, and chondrogenic markers at basal state using quantitative real-time polymerase chain reaction (qRT-PCR)

The expression of osteogenic, chondrogenic, and adipogenic markers at basal state was measured using qRT-PCR as previously described (21). Briefly, BMSCs, AMSCs and CLMSCs at P3 were harvested and homogenized for RNA extraction using Trizol (Invitrogen, USA) in triplicate. The mRNA was reverse transcribed to complementary DNA (cDNA) using the Omniscript RT kit (Qiagen, USA). mRNA levels were measured by qRT-PCR (Stepone real-time PCR Applied Biosystems, USA) using the Fast EvaGreen® master mix for quantitative and high-resolution melting PCR (Biotium, USA). The 20-μL reaction contained 1 μL cDNA from each sample mixed with 10 μL 2X Fast EvaGreen® qPCR Master Mix, 2 μL 10X ROX of the assays-on-demand kit (Applied Biosystems), 1 μL primer, and 6 μL RNase/DNase-free water. The PCR conditions were: incubation at 95 ℃ for 10 min followed by 45 cycles at 95 ℃ for 5 s and at 60 ℃ for 1 min. Data was analyzed using the ABI Stepone Sequence Detection Systems software, version 1.0, supplied by Applied Biosystems. The expression of target gene was normalized to that of GAPDH. Relative gene expression was calculated using formula $2^{-\Delta Ct}$. Specific primers for adipogenic markers [peroxisome proliferator-activated receptor gamma 2 (PPARγ2) and CCAAT enhancer binding protein alpha (c/EBPα)], osteogenic markers [Alpl, osteocalcin (Bglap), Runx2, bone morphogenetic protein 2 (Bmp2) and osteopontin (Spp1)], and chondrogenic markers (Col2A1, Acan, and Sox9) were listed in *Table 1*.

Adipogenic differentiation assay

BMSCs, AMSCs and CLMSCs were plated at 5×10^3 cells/cm^2 in a 6-well plate and cultured with complete medium until the cells reached 90–100% confluence. The complete medium was then still cultured with complete medium or adipogenic medium (RASMX-90031, Cyagen Biosciences Inc., Goleta, CA). The medium was replaced every 3 days. At days 21, the oil droplets were assessed using an Oil red O staining assay. Briefly, the cells were washed twice in PBS, fixed in 10% formaldehyde for 10 min at room temperature, and stained with 0.3% filtered Oil red O staining solution (sigma) for 2 hours. The mRNA expression of adipogenic markers PPARγ2 and c/EBPα were assessed at days 21 by qRT-PCR using primers listed in *Table 1*.

Osteogenic differentiation assay

BMSCs, AMSCs and CLMSCs were plated at 4×10^3 cells/cm^2 in a 6-well plate and cultured in basal complete culture medium until the cells reached confluence. They were then incubated in basal complete medium or osteogenic medium (RASMX-90021, Cyagen Biosciences Inc., Goleta, CA, USA). At days 21, the calcium nodule formation was assessed using an Alizarin red staining assay. Briefly, the cell layer was washed with PBS twice, fixed with 10% ethanol, and then washed with pure water twice. For Alizarin red staining, the cells were stained with 0.5% Alizarin red S (pH 4.1, sigma) for 10 min, and washed with pure water twice

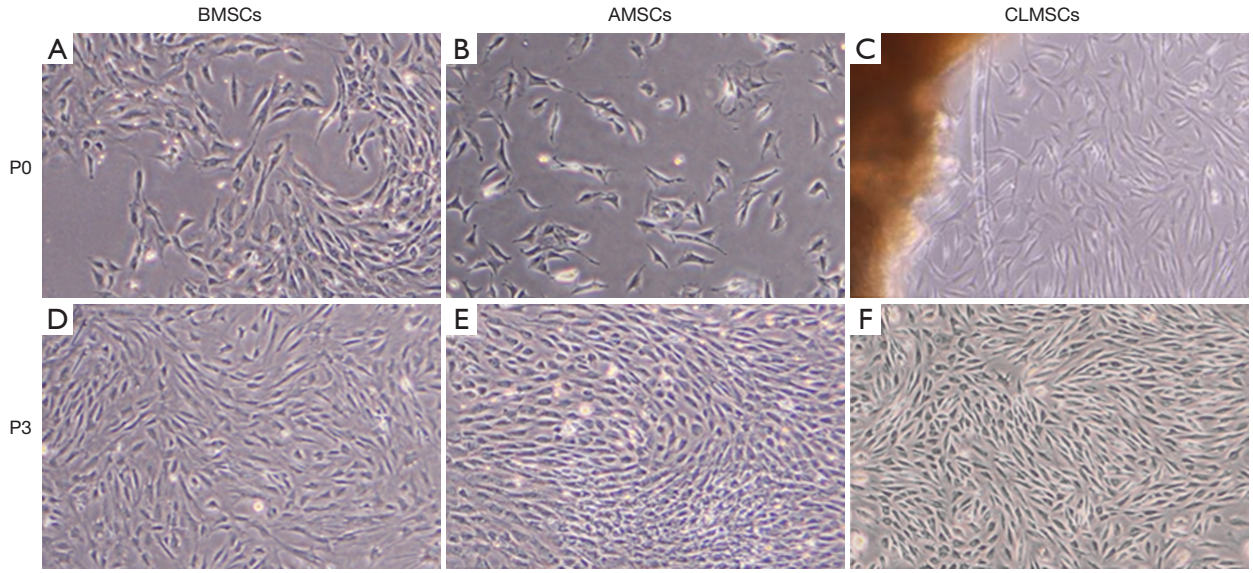

Figure 1 Comparison of cell morphology of BMSCs, AMSCs and CLMSCs. BMSCs at P0 were plastic-adherent with a fibroblast-like spindle-shaped morphology (A) while AMSCs were heterogeneous varying from elongated cells to flat round cells at P0 (B); CLMSCs migrated from cruciate ligament tissues after 5 days of culture (C); rapid increase in cell density were seen after 10 days. At P3, BMSCs and AMSCs demonstrated similar spindle-shaped and fibroblast-like morphology (D,E); At P3, CLMSCs exhibited fibroblast-like morphology (F). Magnification: ×100 (A-F). BMSCs, bone marrow-derived mesenchymal stem cells; AMSCs, adipose-derived mesenchymal stem cells; CLMSCs, cruciate ligament-derived mesenchymal stem cells.

and then air-dried. The mRNA expression of osteogenic markers Alpl, Bglap, Runx2, Bmp2, Spp1 were assessed at days 21 by qRT-PCR using primers listed in *Table 1*.

Chondrogenic differentiation assay

Chondrogenic differentiation was assessed by the pellet culture method as modified recently. A total of 5×10^5 cells were centrifugated at 450 g for 10 min to form a micromass in a 15-mL conical polypropylene tube and cultured in complete or chondrogenic medium (RASMX-90041, Cyagen Biosciences Inc., Goleta, CA, USA) at 37 ℃ 5% CO_2 (R&D Systems, Minneapolis, MN, USA). At days 21, the pellet was fixed for and toluidine blue staining for the examination of glycosaminoglycan deposition. The mRNA expression of Col2A1, Acan, and Sox9 were studied at days 21 by qRT-PCR using primers listed in *Table 1*.

Statistic analysis

Data was presented as mean ± SD and determined by one-way analysis of variance (ANOVA). If there was a significant overall difference between groups, pairwise comparisons were conducted using Scheffe's post hoc test. All data analysis was done using IBM Statistical Package for the Social Sciences, version 19.0 (SPSS Inc.). Values of $P<0.05$ were considered statistically significant.

Results

Cell morphology

BMSCs showed a homogeneous morphology (*Figure 1A*), whereas AMSCs were heterogeneous varying from elongated cells to flat round cells at P0 (*Figure 1B*). CLMSCs migrated from the cut ends of tissue explants after a lag of 5 days' culture. At P0, CLMSCs were spindle and fiber-like (*Figure 1C*). At P3, BMSCs and AMSCs demonstrated similar spindle-shaped and fibroblast-like morphology (*Figure 1D,E)*. At P3, CLMSCs also exhibited fibroblast-like morphology like BMSCs and AMSCs (*Figure 1F*).

Fluorescence-activated cell sorting analysis

Flow cytometry analysis (*Figure 2*) demonstrated that BMSCs, AMSCs and CLMSC s were all positive for CD29 and CD90, while showed negligible expression of CD45.

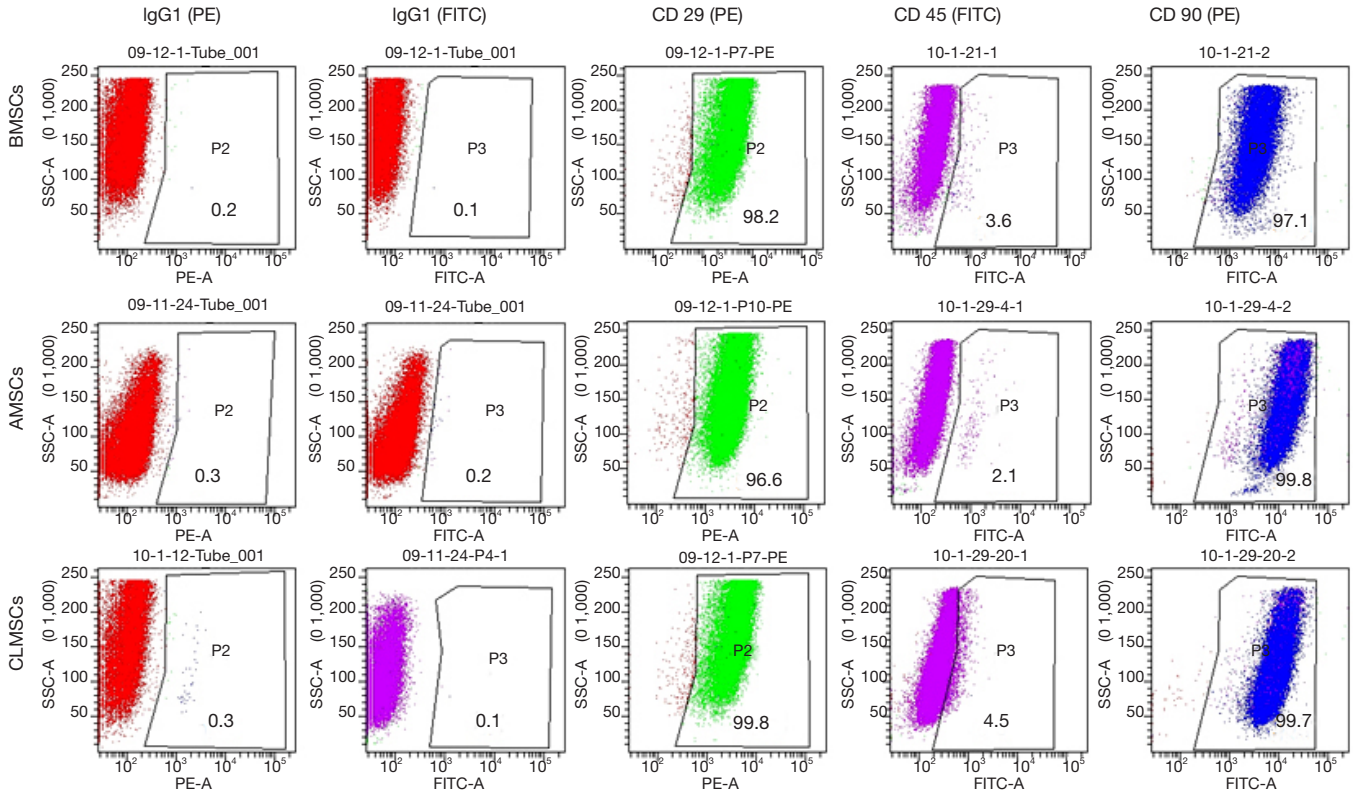

Figure 2 Immunophenotypic characterization of BMSCs, AMSCs and CLMSCs. Nonspecific IgG1 (PE) and IgG1 (FITC) represented negative controls. Percentage of positive cells for each epitope is given within the corresponding panel. PE, phycoerythrin; FITC, fluorescein isothiocyanate; BMSCs, bone marrow-derived mesenchymal stem cells; AMSCs, adipose-derived mesenchymal stem cells; CLMSCs, cruciate ligament-derived mesenchymal stem cells.

Clonogenicity and proliferative potential

AMSCs and CLMSCs formed more colonies (P<0.05) (*Figure 3A,B*) and proliferated faster (P<0.05) (*Figure 3C*) than BMSCs. CLMSCs exhibited superior colony forming and proliferation potentials. However, the colony numbers and absorbance value between CLMSCs and AMSCs were not of statistical significance (P<0.05) (*Figure 3B,C*).

Expression of adipogenic, osteogenic and chondrogenic markers at basal state

AMSCs showed the highest mRNA level of adipogenic (PPARγ2 and c/EBPα) markers among these three types of MSCs in basal complete culture medium (P<0.05) (*Figure 4A,B*). BMSCs expressed the highest mRNA level of osteogenic (Alpl, Bglap, Runx2, Bmp2, Spp1) markers in basal complete culture medium (P<0.05) (*Figure 4C-G*). CLMSCs exhibited the highest mRNA level of chondrogenic (Col2A1, Acan, and Sox9) markers in basal complete culture medium (P<0.05) (*Figure 4H-J*).

Adipogenic differentiation potential

Indistinguishable positive Oil droplets were formed in BMSCs, AMSCs and CLMSCs upon adipogenic induction for 21 days (*Figure 5A-C*). Under the same induction conditions, the expression of adipogenic markers, including PPARγ2 and c/EBPα, was significantly higher in AMSCs than those in BMSCs and CLMSCs (P<0.05, *Figure 5D,E*).

Osteogenic differentiation potential

There was significant increase in matrix mineralization in BMSCs, AMSCs and CLMSCs upon osteogenic induction for 21 days, as demonstrated by positive Alizarin red staining (*Figure 6A-C*). Under the same induction conditions, the expression of osteogenic markers, including Alpl,

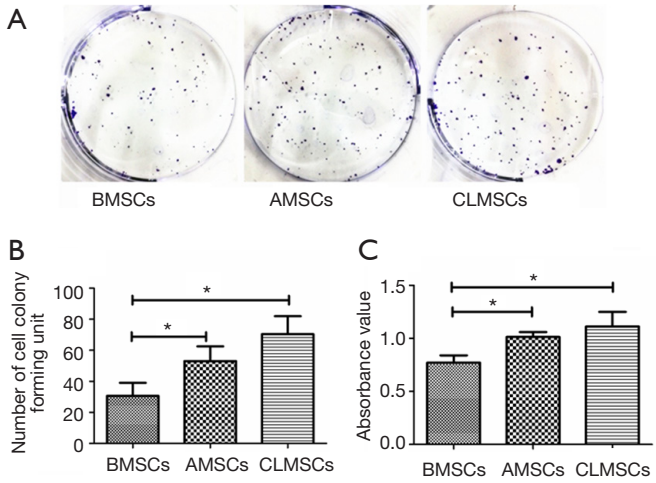

Figure 3 Comparisons of cell clonogenicity and proliferative potential of BMSCs, AMSCs and CLMSCs. Colony forming unit assay on day 14 at P3 (A); comparative analysis of colony forming numbers (B) and absorbance value (C) between groups. *, P<0.05, with a statistical significance between two groups; BMSCs, bone marrow-derived mesenchymal stem cells; AMSCs, adipose-derived mesenchymal stem cells; CLMSCs, cruciate ligament-derived mesenchymal stem cells.

Bglap, Runx2, Bmp2, Spp1, was significantly higher in BMSCs than those in AMSCs and CLMSCs (P<0.05, *Figure 6D-H*).

Chondrogenic differentiation potential

Cell pellets were formed at days 21 upon chondrogenic induction in all cell types. The cartilaginous phenotype of all induced cells was confirmed by positive toluidine blue staining in BMSCs, AMSCs and CLMSCs (*Figure 7A-C*). Magnification: ×200. Under the same induction condition, the expression of chondrogenic markers, including Col2A1, Acan and Sox9, was significantly higher in CLMSCs than those in BMSCs and AMSCs (P<0.05, *Figure 7D-F*).

Discussion

MSCs have been widely investigated and studied for their regenerative capacity. Previous pre-clinical studies have indicated that MSCs isolated from different tissues, including bone marrow, adipose, cruciate ligament and cord blood, can be applied in tissue repair, exhibiting promising therapeutic potentials (22-24). How to choose an appropriate cell source is an important issue when designing an optimal cell therapy, because MSCs isolated from different tissues may have specific proliferation and differentiation properties (25). This study therefore aimed to compare the colony-forming ability, proliferative potential, and multilineage differentiation potentials of BMSCs, AMSCs and CLMSCs.

Microscopic observation during expansion has shown that BMSCs, AMSCs and CLMSCs displayed similar morphology after three passages, which was plastic-adherent with a fibroblast-like spindle-shaped morphology. CLMSCs is a recently isolated MSCs with robust proliferation and differentiation potential (17). According to our best knowledge, the proliferation potential of CLMSCs was not simultaneously analyzed when compared to BMSCs and AMSCs previously. This study suggested that CLMSCs and AMSCs showed superior colony forming and proliferation capacity when compared to BMSCs. In a comparative study of CLMSCs and BMSCs, CLMSCs proliferated faster than BMSCs, which bore a resemblance with our study (26).

MSCs are pluripotent cells identified primarily in bone marrow. Similar cells were reported in multiple tissues, including fat (27), muscle (28), tendon (29), synovium (9) and so on. According to the phenotypic analysis reported, BMSCs and AMSCs are positive for stromal-cell-associated markers, such as CD73, CD29, and CD90 while negative for endothelial markers, including CD45, CD14, CD34 and CD31. CD105, CD13 and CD44 are also expressed by both cell types. Our results showed that BMSCs and AMSCs isolated were positive for CD29, CD90, while negative for CD45, which was consistent with previous studies reported (30,31).

We further showed the highest adipogenic markers in AMSCs, osteogenic markers in BMSCs and chondrogenic markers in CLMSCs at basal state. Our results suggest that AMSCs are more promising cell source for adipose-related tissue regeneration while BMSCs are superior in bone regeneration. This result corroborated with the findings of previous studies (32-34). Interestingly, we noticed that it was CLMSCs, but not BMSCs that exhibited superior chondrogenesis potential. To our best knowledge, it is the first time that BMSCs and CLMSCs were compared at basal state for chondrogenesis-related mRNA expression. Because of higher chondrogenic-relate mRNA expression and proliferation potential, CLMSCs might be a competitive cell source for cartilage regeneration.

When designing a tissue engineered therapy, an *in vitro* pre-induction process for MSCs is often necessary. To confirm the *in vitro* differentiation property of BMSCs, AMSCs and CLMSCs, we induced these three types of

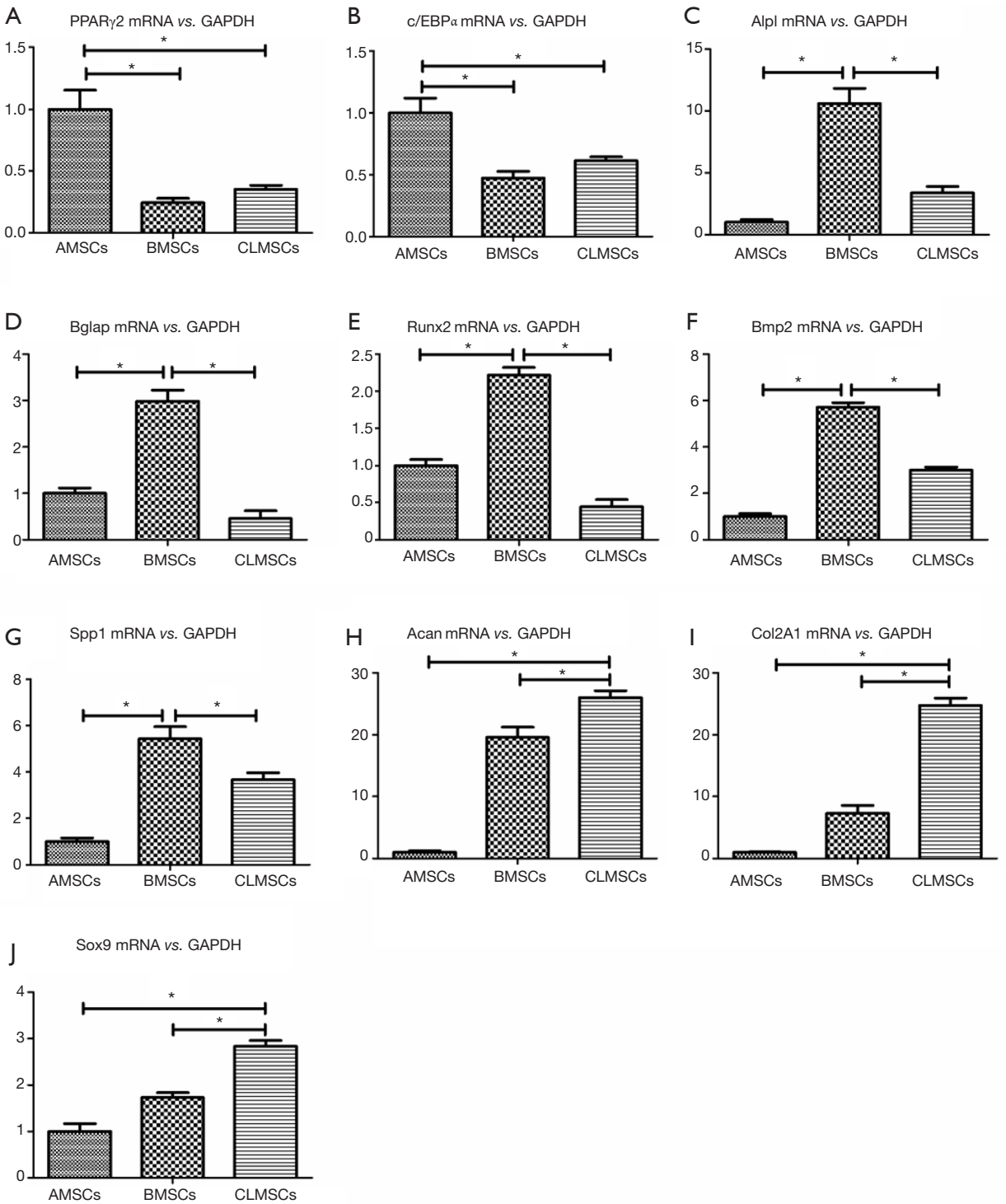

Figure 4 Expression of adipogenic, osteogenic and chondrogenic markers at basal state. mRNA expression of (A) PPARγ2; (B) c/EBPα; (C) Alpl; (D) Bglap; (E) Runx2; (F) Bmp2; (G) Spp1; (H) Acan; (I) Col2A1; (J) Sox9 in BMSCs, AMSCs and CLMSCs in basal complete culture medium. *; P<0.05, with a statistical significance between two groups; AMSCs, adipose-derived mesenchymal stem cells; BMSCs, bone marrow-derived mesenchymal stem cells; CLMSCs, cruciate ligament-derived mesenchymal stem cells.

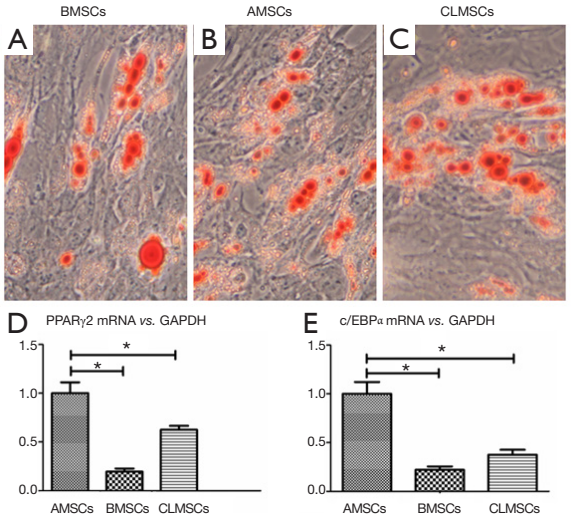

Figure 5 Adipogenic differentiation potential. Photomicrographs showing the indistinguishable positive Oil red O staining of oil droplets in (A) BMSCs; (B) AMSCs; and (C) CLMSCs in adipogenic induction medium after 21 days. Magnification: ×100. mRNA expression of (D) PPARγ2; (E) c/EBPα in AMSCs, BMSCs and CLMSCs in adipogenic medium for 21 days. *, P<0.05, with a statistical significance between two groups; BMSCs, bone marrow-derived mesenchymal stem cells; AMSCs, adipose-derived mesenchymal stem cells; CLMSCs, cruciate ligament-derived mesenchymal stem cells.

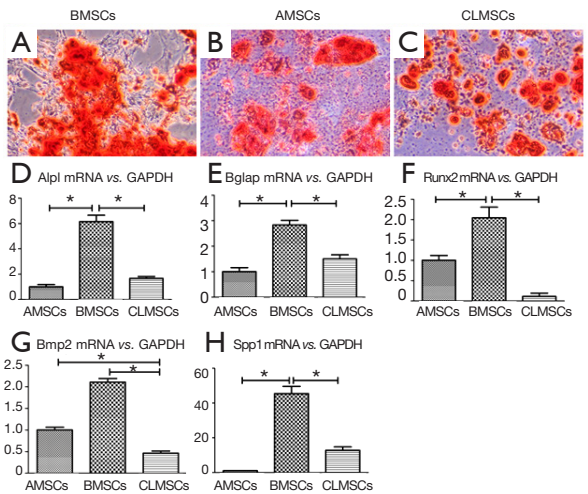

Figure 6 Osteogenic differentiation potential. Photomicrographs showing the indistinguishable positive Alizarin red staining of calcium nodules in (A) BMSCs; (B) AMSCs; and (C) CLMSCs in osteogenic induction medium after 21 days. Magnification: ×100. mRNA expression of (D) Alpl; (E) Bglap; (F), Runx2; (G) Bmp2; (H) Spp1 in BMSCs, AMSCs and CLMSCs in osteogenic medium for 21 days. *, P<0.05, with a statistical significance between two groups; AMSCs, adipose-derived mesenchymal stem cells; BMSCs, bone marrow-derived mesenchymal stem cells; CLMSCs, cruciate ligament-derived mesenchymal stem cells.

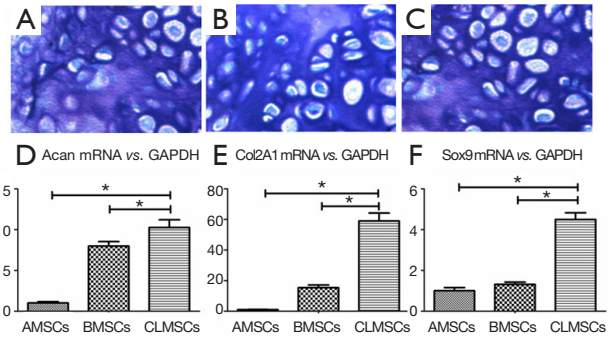

Figure 7 Chondrogenic differentiation potential. Photomicrographs showing indistinguishable positive toluidine blue staining of aggrecan in (A) BMSCs; (B) AMSCs; and (C) CLMSCs in osteogenic induction medium after 21 days. Magnification: ×200. mRNA expression of (D) Acan; (E) Col2A1; and (F) Sox9 in AMSCs, BMSCs and CLMSCs in osteogenic medium for 21 days. *, P<0.05, with a statistical significance between two groups; AMSCs, adipose-derived mesenchymal stem cells; BMSCs, bone marrow-derived mesenchymal stem cells; CLMSCs, cruciate ligament-derived mesenchymal stem cells.

MSCs towards adipogenic, osteogenic and chondrogenic lineages by the same induction agents. Our results showed that after 21 days induction, adipogenic differentiation in BMSCs, AMSCs and CLMSCs were demonstrated by the well-acknowledged Oil red staining. The stainings among these three types of MSCs are indistinguishable and strongly positive. mRNA analysis revealed that under the same induction conditions, AMSCs expressed the highest level of adipogenic markers, including PPARγ2 and c/EBPα. Similar positive Alizarin red staining and toluidine blue staining were observed in each cell group after 21 days induction. However, BMSCs showed the highest osteogenic-related mRNA while CLMSCs expressed the highest chondrogenic-related mRNA. The results are similar to a study of Cheng *et al.* (26), who demonstrated that BMSCs exhibited a higher osteogenic capacity and similar chondrogenic potential compared to CLMSCs. However, studies of different results also exist. Steinert *et al.* (17) reported that BMSCs had a higher chondrogenic potential and similar osteogenic potential compared to CLMSCs. Our investigations provided insights into the cellular characteristics of BMSCs, AMSCs and CLMSCs. However, the present study is limited to the *in vitro* nature. The *in vitro* higher mRNA expression does not necessary guarantee the advantages of the *in vivo* regenerative effect, because gene transfection, cell scaffold and growth factors when co-applied together can also affect the differentiation

process of transplanted MSCs. Thus, further *in vivo* studies are required to clarify their relevance to tissue regeneration of different properties.

The present study compared the biological characteristics of BMSCs, AMSCs and CLMSCs. Our study showed that, despite many similarities among these three types of MSCs, there are differences existing in proliferation and induced differentiation properties, which can offer insights in choosing the most appropriate cell source for tissue engineering strategies. CLMSCs and AMSCs showed superiority in cell proliferation compared to BMSCs. BMSCs is more promising for bone regeneration; AMSCs is superior in adipose tissue applications; CLMSCs might offer a potential cell source in cartilage regeneration.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aoj.2016.05.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Animal Research Ethics Committee, the Southeast University approved all experiments.

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