Molecular Analysis of the Differentiation Potential of Murine Mesenchymal Stem Cells from Tissues of Endodermal or Mesodermal Origin

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Mesenchymal stem cells (MSCs) have received great attention due to their remarkable regenerative, angiogenic, antiapoptotic, and immunosuppressive properties. Although conventionally isolated from the bone marrow, they are known to exist in all tissues and organs, raising the question on whether they are identical cell populations or have important differences at the molecular level. To better understand the relationship between MSCs residing in different tissues, we analyzed the expression of genes related to pluripotency (SOX2 and OCT-4) and to adipogenic (C/EBP and ADIPOR1), osteogenic (OMD and ALP), and chondrogenic (COL10A1 and TRPV4) differentiation in cultures derived from murine endodermal (lung) and mesodermal (adipose) tissue maintained in different conditions. MSCs were isolated from lungs (L-MSCs) and inguinal adipose tissue (A-MSCs) and cultured in normal conditions, in overconfluence or in inductive medium for osteogenic, adipogenic, or chondrogenic differentiation. Cultures were characterized for morphology, immunophenotype, and by quantitative real-time reverse transcription–polymerase chain reaction for expression of pluripotency genes or markers of differentiation. Bone marrow–derived MSCs were also analyzed for comparison of these parameters. L-MSCs and A-MSCs exhibited the typical morphology, immunophenotype, and proliferation and differentiation pattern of MSCs. The analysis of gene expression showed a higher potential of adipose tissue–derived MSCs toward the osteogenic pathway and of lung-derived MSCs to chondrogenic differentiation, representing an important contribution for the definition of the type of cell to be used in clinical trials of cell therapy and tissue engineering.

Introduction

MESENCHYMAL STEM CELLS (MSCs) were first described as bone marrow cells capable of originating fibroblast colonies (colony-forming unit-fibroblasts [CFU-Fs], [1]). The enumeration of CFU-Fs from fresh tissue samples has been considered indicative of the frequency of MSCs, but a direct relationship between the 2 has not been clearly established [2]. In 1985, a relationship between these cells and the bone marrow stroma was proposed by Owen [3], who proposed the existence of stromal stem cells that are able to self-renew and generate mature conjunctive/stromal cell types. The term MSCs was introduced by Caplan in 1991 [4] and is currently used for stem cells with an intrinsic potential to give rise to different mesenchymal cell types such as osteoblasts, chon-

drocytes, adipocytes, tenocytes, and others. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell Therapy has established the minimal criteria to define human MSCs: the capacity to proliferate as adherent cells, a defined surface phenotype (positive for CD105, CD73, and CD90, and negative for CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR), and the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts [5].

Although conventionally isolated from the bone marrow, we and others have shown that MSCs are distributed throughout the whole organism, suggesting that they reside in association with blood vessels [6,7]. We have also suggested that the perivascular location of MSCs, associated to their remarkable immunoregulatory capacity, implies a role in the maintenance of tissue homeostasis: in the case of tissue

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injury, MSCs secrete a panel of cytokines and factors that control the immune response to avoid an autoimmune process [8]. These studies have shown that, although similar in general characteristics, MSCs isolated from different tissues, such as brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus, and pancreas, exhibit particular biological features, raising the question on whether they are identical cell populations or have important differences at the molecular level [9].

Cellular and molecular mechanisms underlying one of the fundamental properties of stem cells, self-renewal, have been the subject of many studies (reviewed in ref. [10]). While these studies provide an acceptable framework for defining MSCs at the molecular level, the presence of a large number of housekeeping genes prevents proper evaluation of their specific genetic message [11].

Gene expression analyses have shown that the differentiation of MSCs into mature cell types is controlled temporally, and that the regulation of the process involves the activity of transcription factors, growth factors, and signaling pathways (reviewed in ref. [12]). Transcription factors, such as Oct3/4 [13,14], Sox2 [15], and Nanog [16], maintain the pluripotency of embryonic stem cells, and may also be expressed in MSCs [17]. Other genes regulate the differentiation of stem cells into specific lineages. For example, the lipoprotein lipase [18], enhancer-binding protein (*C/EBP*) [19,20], and adiponectin receptor 1 (*ADIPOR1*) [19] genes are expressed in the adipogenic differentiation pathway. *COL2A1* [21], collagen type X-alpha 1 (*COL10A1*) [22], and transient receptor potential cation channel, subfamily V, member 4 (*TRPV4*) [23] are highly expressed in the chondrogenic differentiation pathway, while osteomodulin (*OMD*) [20], alkaline phosphatase (*ALP*) [24], and osteocalcin [25] regulate the osteogenic differentiation process.

To better understand the relationship between MSCs residing in different tissues, we analyzed the expression of genes related to pluripotency and to adipogenic, osteogenic, and chondrogenic differentiation in cultures derived from endodermal (lung) and mesodermal (adipose) tissue maintained in different conditions. These variables were also analyzed in cultures isolated from the bone marrow, which represents the typical source of MSCs. These results are also important in the determination of the best source of stem cells to be used in therapeutic applications.

Materials and Methods

Reagents, culture media, and solutions

Complete culture medium (CCM) was composed of Dulbecco's modified Eagle's medium with 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, free acid, 2.5–3.7 g/L) and 10% fetal bovine serum (Cultilab, São Paulo, Brazil). Ca^{2+} - and Mg^{2+} -free Hank's balanced salt solution containing 10 mM sodium HEPES (HB-CMF-HBSS) was used for washing tissues and cells. All reagents were from Sigma Chemical (St. Louis, MO), unless otherwise stated. Plasticware was from BD Falcon (Brazil, São Paulo, Brazil).

Animals

Adult mice (12–16 weeks old) from the C57Bl/6 strain were used in this study. The animals were kept under

standard conditions (12 h light/12 h dark cycle, temperature of $22 \pm 2^\circ\text{C}$, water and food ad libitum) in the animal house at Universidade Federal do Rio Grande do Sul. All animal procedures were conducted in accordance with humane animal care standards, and experimental procedures were performed according to institutional guidelines.

Cell culture

MSCs were isolated and cultured as previously described [26]. Briefly, bone marrow was flushed out of tibias and femurs of mice, washed, and resuspended in CCM. To initiate an MSC culture, cells were plated in 6-well tissue culture dishes at 2×10^5 cells/cm². Lungs and inguinal adipose tissue were collected, rinsed in HB-CMF-HBSS, transferred to a Petri dish, and cut into small pieces. After a further wash with HB-CMF-HBSS, the fragments were digested with collagenase type I (1 mg/mL in CCM) for 30 min at 37°C . After centrifugation in CCM at 400 g for 10 min at room temperature, the pellets were resuspended in 3 mL CCM containing 1% antibiotic solution (GIBCO BRL, Gaithersburg, MD), plated in 6-well dishes at 3 mL/well, and incubated at 37°C in a humidified atmosphere with 5% CO₂. Nonadherent cells were removed after 24 h, and the adherent layer was refed every 3 or 4 days with CCM with antibiotics.

For subculture, the adherent layer was washed with HB-CMF-HBSS and incubated with 0.25% trypsin and 0.01% EDTA. The cultures were split whenever they reached 80% confluence at ratios empirically determined for 2 subcultures a week at most (around 1:4 split ratios). For some experiments, cultures were maintained in overconfluence before analysis.

Cultures were observed with an inverted phase-contrast microscope (Axiovert 25; Zeiss, Hallbergmoos, Germany). Photomicrographs were taken with a digital camera (AxioCam MRc, Zeiss), using the AxioVision 3.1 software (Zeiss). Cultures in passages 6 to 10 were used for all experiments.

Embryo stem cells of the USP1 lineage [27] were used as positive control for the expression of pluripotency genes.

MSC differentiation

Differentiation was induced by cultivating cells in specific media for 1 week, with medium changed every 3 days [6]. For osteogenic differentiation, CCM was supplemented with 10^{-8} M dexamethasone, 5 $\mu\text{g/mL}$ ascorbic acid 2-phosphate, and 10 mM β -glycerophosphate. Calcium deposition in differentiated cultures was revealed by washing once with phosphate-buffered saline (PBS), fixing with 4% paraformaldehyde in PBS for 15–30 min at room temperature, and staining for 5 min at room temperature with Alizarin Red S stain (Vetec, Rio de Janeiro, Brazil) at pH 4.2. For adipogenic differentiation, MSCs were cultured in CCM supplemented with 10^{-8} M dexamethasone, 2.5 $\mu\text{g/mL}$ insulin, 100 μM indomethacin (Merck, Rio de Janeiro, Brazil), and 3.5 μM rosiglitazone (GlaxoSmithKline, Middlesex, UK). Adipocytes were visualized by fixing with 4% paraformaldehyde in PBS for 1 h at room temperature and staining with Oil Red O solution (3 volumes of 3.75% Oil Red O in isopropanol plus 2 volumes of distilled water) for 5 min at room temperature. Chondrogenic differentiation was induced with CCM

complemented with 6.25 µg/mL insulin, 10 ng/mL transforming growth factor-beta1 (Millipore, Billerica, MA), and 50 nM ascorbic acid 2-phosphate. Cultures were analyzed by fixing with 4% paraformaldehyde in PBS for 20 min at room temperature and staining with Alcian Blue (pH 2.5) (Vetec, Rio de Janeiro, Brazil) in 3% acetic acid, for 5 min at room temperature. After staining, the cultures were washed with distilled water and, in some cases, counterstained with Harry's hematoxylin (1 min at room temperature).

To compare the potential of lung- and adipose tissue-derived MSCs to differentiate into chondrogenic and osteogenic lineages, triplicate cultures were maintained for 2 or 4 weeks in media as described previously. After Alcian Blue or Alizarin Red S staining, respectively, 5 fields (original magnification $\times 200$) in each culture were photographed and the stained areas were measured in relation to the total area using ImageJ software (NIH, Bethesda, MD) and quantified in percent.

Immunophenotyping

MSC cultures were analyzed by flow cytometry for determination of surface antigens. Cells were harvested, washed, and incubated for 30 min at 4°C with phycoerythrin- or fluorescein isothiocyanate-conjugated antibodies against murine CD11b, CD29, CD44, CD45, CD49e, and CD90.2 (Pharmingen BD, San Diego, CA). Excess antibody was removed by washing.

The cells were analyzed using an FACScalibur cytometer equipped with 488 nm argon laser (Becton Dickinson, San Diego, CA), with the CellQuest software. At least 5,000 events were collected.

Quantitative real-time reverse transcription-polymerase chain reaction

Cells were harvested and total RNA was extracted with TRIzol reagent (Invitrogen, São Paulo, Brazil). The amount and quality of RNA was assessed using a NanoDrop ND-1000 (Thermo Fisher Scientific Inc., Wilmington, DE). First-strand cDNA synthesis was carried out with 1 µg RNA and oligo-dT primers using the SuperScript III First Strand Synthesis kit (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol, using a thermal cycler T9600 (Tone Derm Genetic, Rio Grande do Sul, Brazil).

The expression of 8 genes was analyzed with quantitative real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR). *C/EBP* and *ADIPOR1* are markers of adipocyte differentiation; *COL10A1* and the *TRPV4*, of chondrogenic differentiation; and *OMD* and *ALP*, of the osteogenic lineage. *Oct-4* and *SOX2* are pluripotency markers, and the ribosomal protein S18 (*RPS18*) gene was used as an endogenous reference gene. The primers were designed using the FastPCR software (Table 1).

A 20-µL volume reaction was used. Each reaction contained 10 µL of the 50-fold diluted cDNA, 200 nM forward primers and reverse primers, 100 µM of 5 mM dNTPs, 1 $\times$ PCR buffer, 3 mM MgCl₂, 1 $\times$ Syber Green I (Invitrogen), 3.45 µL H₂O, and 0.25 U Platinum Taq DNA Polymerase (Invitrogen).

The assays were performed using an automated instrument (Real-Time PCR 7500; Applied Biosystems, Foster City,

TABLE 1. PRIMER SEQUENCES

Gene	Primer
<i>C/EBP</i>	F: 5' CAGAGGATGGTTTCGGGTCGCT R: 5' TAGACGCACCGAGTCCGGCT
<i>ADIPOR1</i>	F: 5' GAAGATCAAGCATGCCCGGTG R: 5' GCAGGTAGTCGTTGTCTTTTCAGCCA
<i>COL10A1</i>	F: 5' CAGTAAGAGGAGAACAAGGCATTCC R: 5' CCGTGGCTGATATTCCTGGTGGT
<i>TRPV4</i>	F: 5' CGCCATGAGATGCTGGCTGT R: 5' CACTGTGGTCCGGTAAGGGT
<i>OMD</i>	F: 5' TGAGCAGAGGAGTACTAACGGTG R: 5' AGTATCTTGCCACTCTTGCATCTCA
<i>ALP</i>	F: 5' CTGGGAAGTCCGTGGGCATTG R: 5' CATGTATTTCCGGCCGCCAC
<i>Oct-4</i>	F: 5' CCAGGACATGAAAGCCCTGCAGAAAG R: 5' ACACATGTTCTTAAGGCTGAGCTGC
<i>SOX2</i>	F: 5' TTAACGGCACACTGCCCTGTC R: 5' TTTCCGAGCTGTCGTTTCG
<i>RPS18</i> (internal control)	F: 5' TTCGGAAGTCTGAGGCCATGAT R: 5' TTTGCTCTGGTCCGTCTTG

CA). Amplifications were performed starting with a 5-min step at 94°C, followed by 40 PCR cycles (94°C for 15 s, 60°C for 10 s, 72°C for 15 s), and 60°C for 35 s for fluorescence measurement. Cycle thresholds (CT) for the individual reactions were obtained and delta Ct (Δ Ct) values were calculated (Δ Ct = experimental Ct - endogenous Ct). All cDNA samples, corresponding to biological triplicates, were amplified in quadruplicates and normalized against the reference gene *RPS18* amplified in the same plate.

Statistical analyses

Data are expressed as mean $\pm$ standard deviation. Data were analyzed using the Prism 5 software (GraphPad Software Inc., San Diego, CA). The significance of differences in the results was evaluated by a one-way analysis of variance followed by a Tukey or Student's *t*-test. A difference of $P < 0.05$ was considered statistically significant.

Results

MSC culture

MSCs isolated from lungs (L-MSCs) and adipose tissue (A-MSCs) exhibited the typical morphology and proliferation pattern of MSCs. Over a period of 5 months, the adherent cells proliferated normally and showed no signs of senescence. When maintained in overconfluence, changes in the morphology were clearly visible under phase-contrast microscopy (Fig. 1A). When cultivated with osteogenic, adipogenic, or chondrogenic media, both types of cultures readily differentiated along these lineages (Fig. 1B). Bone marrow-derived MSCs (M-MSCs) proliferated as adherent cells and were easily induced into the adipogenic and osteogenic differentiation pathways (not shown).

Flow cytometry analyses showed that the cultures were negative for hematopoietic markers (CD11b and CD45), with variable level of expression of CD90.2, and were positive for the remaining surface markers analyzed (Fig. 2).

FIG. 1. Morphology and differentiation potential of adipose-derived (A-MSCs, 2 left columns) and lung-derived (L-MSCs, right columns) mesenchymal stem cells. **Panel (A)** (phase contrast) shows that the morphology of MSCs cultivated under normal conditions is modified by the overconfluent conditions. **Panel (B)** shows that, when cultivated with chondrogenic, adipogenic, or osteogenic media, A-MSCs and L-MSCs show positive staining for Alcian Blue, Oil Red O, and Alizarin Red S, respectively. Nondifferentiated (control) MSCs are not marked with these stains. Original magnifications, 100 $\times$. A-MSCs, adipose-derived mesenchymal stem cells; L-MSCs, lung-derived mesenchymal stem cells.

Gene expression

A-MSCs, L-MSCs, and M-MSCs cultivated under normal conditions did not express SOX2, and low expression of Oct-4 was observed (Fig. 3). In A-MSCs and L-MSCs maintained in overconfluence, despite changes in the morphology, no modifications on the expression profiles of the genes under study were observed (not shown).

When MSCs were induced into the chondrogenic lineage, expression of the chondrocytic marker genes *TRPV4* and *COL10A1* was increased in A-MSCs and L-MSCs, but not in M-MSCs. The *COL10A1* was significantly more expressed in differentiated L-MSCs than in differentiated A-MSCs (Fig. 4). The expression of *TRPV4* was increased in differentiated L-MSCs and A-MSCs when compared with normal cultures (not significant) and with differentiated M-MSCs (Fig. 5).

The induction of adipocyte differentiation resulted in significantly higher expression levels of the *ADIPOR1* genes in A-MSCs and L-MSCs, but without significant differences between themselves; the *C/EBP* gene was more expressed in differentiated L-MSCs (data not shown).

The expression of the osteogenic marker gene *OMD* was higher in nondifferentiated M-MSCs than in the other 2 types of cultures (Fig. 6). In MSCs cultivated with osteogenic medium, the expression of *ALP* was not modified, but *OMD* was significantly more expressed in lung- and adipose

tissue-derived cultures. In A-MSCs, however, the expression of *OMD* was significantly higher than in L-MSCs (Fig. 6).

Quantitative histochemical analysis of differentiation

The potential of L-MSCs and A-MSCs to differentiate into chondrogenic and osteogenic lineages was quantified after 2 and 4 weeks of culture in inducing media. In cultures maintained for the usual period in inducing media (4 weeks), the percentage of stained areas representing differentiated cells was very similar (Table 2). When analyzed after 2 weeks in the inducing media, however, the percentage of Alcian Blue-stained areas in L-MSCs was significantly higher than in A-MSC cultures, whereas the percentage of Alizarin Red S-stained areas was significantly higher in A-MSCs. In 4 weeks, no differences were observed.

Discussion

Although conventionally isolated from bone marrow [28,29], MSCs have been shown to exist in some other tissues [30,31]. The cell populations isolated in the present work from adipose tissue and lung, representing mesoderm and endoderm respectively, showed the immunophenotype and the capacity of prolonged self-renewal and differentiation typical of MSCs. No differences were observed between

FIG. 2. Immunophenotype of adipose tissue-, bone marrow-, and lung-derived MSCs (A-MSCs, M-MSCs, and L-MSCs, respectively). All cultures were negative for CD11b, CD34, CD45, and CD117 and positive for CD29, CD44, and CD49e. The expression of CD90.2 was variable, but mostly negative. Dark lines, control; light lines, marker.

FIG. 3. MSCs isolated from adipose tissue (A-MSCs), lung (L-MSCs), and bone marrow (M-MSCs) express low levels of *Oct-4*. Expression of *Oct-4* is significantly higher in embryonic stem cells (USP1) than in A-MSCs, L-MSCs, or M-MSCs. * $P < 0.05$ compared with MSCs.

lung-, adipose tissue-, and bone marrow-derived cultures regarding these basic characteristics. As previously shown by us [6], these cells also exhibit high proliferative indices, clonal potential, and a large array of surface markers that characterize MSC cultures. Contrary to most studies [5], the expression of CD90.2 has been variable but mostly negative in our MSC cultures derived from different organs and tissues [6].

Although organs and tissues are a mixture of cells with different embryonic origins, the lung is mainly of endodermal origin, whereas adipose tissue is mainly mesodermal. MSCs are currently considered to exist in a perivascular niche [32]. Since blood vessels are exposed to specific conditions according to their surrounding tissue, it is reasonable to assume that some characteristics of their associated MSCs are determined by these tissue-specific conditions. It is of interest, therefore, to investigate in greater detail processes such as the regulation of MSC differentiation, which is of great relevance for the therapeutic application of these cells. Our results of gene expression after induced differentiation

FIG. 4. Lung-derived MSCs show greater potential to differentiate into chondrocytes than adipose-derived MSCs. Expression of *COL10A1* transcript is significantly higher in chondrocyte-differentiated A-MSCs and L-MSCs when compared with nondifferentiated control cultures or with differentiated or nondifferentiated M-MSCs. Differentiated L-MSCs express significantly higher levels of *COL10A1* than differentiated A-MSCs. * $P < 0.05$ compared with control (nondifferentiated) cultures. # $P < 0.05$ compared with differentiated A-MSCs. *COL10A1*, collagen type X-alpha 1.

FIG. 5. The expression of *TRPV4* is increased in differentiated L-MSCs and A-MSCs when compared with normal cultures (not significant) and with differentiated M-MSCs. * $P < 0.05$ compared with differentiated M-MSCs. *TRPV4*, transient receptor potential cation channel, subfamily V, member 4.

of A-MSCs, L-MSCs, and M-MSCs show that all the cultures have similar potential for adipogenic differentiation, but L-MSCs differentiate more readily into the chondrogenic pathway whereas A-MSCs have greater potential for the osteogenic pathway. The analysis of gene expression is important for a clear understanding of the biological variations in different types of stem cells [33]. In our study, we compared MSCs isolated from lung, adipose tissue, and bone marrow for the expression of genes involved with pluripotency or differentiation. A-MSCs, L-MSCs, and M-MSCs were analyzed in different stages: under normal culture conditions in undifferentiated state, and induced to differentiate into the adipogenic, osteogenic, and chondrogenic lineages. A-MSCs and L-MSCs were also analyzed after overconfluent cultivation, a situation in which stem cells may spontaneously differentiate [34].

FIG. 6. Adipose-derived MSCs show greater potential to differentiate into osteoblasts than lung-derived MSCs. Expression of *OMD* transcript is significantly higher in osteoblast-differentiated A-MSCs and L-MSCs when compared with nondifferentiated control cultures, but was not modified in differentiated M-MSCs. Differentiated A-MSCs express significantly higher levels of *OMD* than differentiated L-MSCs. No differences were observed in the expression of *ALP*. *Significantly higher ($P < 0.05$) than in A-MSCs or L-MSCs. #Significantly higher ($P < 0.05$) compared with control (nondifferentiated) cultures. §Significantly higher ($P < 0.05$) compared with differentiated L-MSCs. *OMD*, osteomodulin; *ALP*, alkaline phosphatase.

TABLE 2. QUANTITATIVE HISTOCHEMICAL ANALYSIS OF DIFFERENTIATION IN ADIPOSE TISSUE- AND LUNG-DERIVED MESENCHYMAL STEM CELL: PERCENTAGE OF STAINED AREAS AFTER CULTIVATION WITH CHONDROGENIC- OR OSTEOGENIC-INDUCING MEDIA AND REVEALING WITH ALCIAN BLUE OR ALIZARIN RED S, RESPECTIVELY

Period of cultivation	Chondrogenesis		Osteogenesis	
	A-MSCs	L-MSCs	A-MSCs	L-MSCs
2 weeks	8.3±2.7	17.6±3.5 ^a	25.3±3.7 ^b	11.8±2.7
4 weeks	43.2±5.7	39.6±6.2	72.5±6.3	70.3±5.8

^aSignificantly higher ($P < 0.05$) than A-MSCs.

^bSignificantly higher ($P < 0.05$) than L-MSCs.

A-MSCs, adipose-derived mesenchymal stem cells; L-MSCs, lung-derived mesenchymal stem cells.

We observed the low expression of pluripotency genes in all cultures. *SOX2* was not expressed, and *Oct-4* was expressed at low levels. Conflicting results have been reported for the expression of pluripotency regulators in MSCs. A recent study showed that human bone marrow MSCs express embryonic stem cell markers such as *Oct-4*, *Rex-1*, *FoxD-3*, *SOX2*, and *Nanog* [35], but a similar study showed the presence of *Nanog*, but not *Oct-4* and *SOX2*, in human MSCs [17]. Interestingly, expression of genes related to adipogenic, myogenic, neurogenic, osteogenic, and chondrogenic lineages has also been reported in these cells, even under basal conditions [35].

An increase in expression levels of *ADIPOR1* in all 3 pathways of differentiation was seen in the current study. Similar studies show increased levels of *ADIPOR1* in murine osteoblastic lineages [36] and in adipose tissue [37], and its expression has been shown to have important implications for the control of lipotoxicity [38]. The gene *C/EBP*, also characteristic of adipogenic pathways, was highly expressed in cultures induced to differentiate into adipocytes, similar to findings reported by Liu et al. in 2007 [20]. The *C/EBP* has an important role in the development of the adipose tissue during the early postnatal period and in the regulation of glucose and lipid homeostasis in adults [39], so that its increased expression during adipocyte differentiation is highly expected.

The osteogenic differentiation pathway was marked by expression of *OMD*. Overexpression of this protein was shown to increase osteoblast differentiation features, such as *ALP* activity and in vitro mineralization [40]. The *OMD* expression in bone was reported to be coupled with osteoclast activity, indicating a role as a maturation marker for osteoblasts activated by osteoclasts [41].

Our results showed that chondrogenic differentiation was marked by increased expression of genes well known as markers of this lineage, *TRPV4* and *COL10A1*. *TRPV4* has been shown to contribute to the process of chondrogenesis by regulating the *SOX9* pathway [23], while the *COL10A1* gene is highly activated during the process of chondrocyte maturation that is central to the process of endochondral ossification [42].

The gene expression profile of human MSCs during differentiation toward these 3 mesenchymal lineages (osteogenic, adipogenic, and chondrogenic) has been investigated.

A comparison of the transcriptome profile of human MSCs isolated from bone marrow and adipose tissue and differentiated into the same 3 lineages showed considerable similarities, with expression of a set of common genes during early differentiation, but with important differences in late stages of maturation [20]. This study suggests that the expression of a different set of signature genes explains the higher potential observed in bone marrow-derived MSCs to differentiate more efficiently into bone and cartilage, and of adipose-derived cells into adipocytes. A later study of gene expression analyzed by qPCR described similar capacity of human MSCs isolated from bone marrow, cord blood, and adipose tissue for chondrogenic and osteogenic differentiation, but a lower adipogenic potential of adipose-derived cells [43].

Much less is known for murine MSCs. The transcriptome of murine MSCs isolated from the bone marrow was investigated by serial analysis of gene expression, showing high expression of transcripts encoding connective tissue proteins and factors modulating T-cell proliferation, inflammation, and bone turnover, as well as angiogenesis, cell motility and communication, hematopoiesis, immunity and defense, as well as neural activities [44]. Low expression of *Oct-4* was reported [45].

Genes and pathways have been shown to be conserved across murine and human species, further validating the use of the mouse as a model to study cell-fate decisions and differentiation involved with human diseases [46]. Our results show a higher differentiation potential of adipose tissue-derived MSCs toward the osteogenic pathway and of lung-derived MSCs to the chondrogenic lineage. These results were confirmed by the quantification of histochemical staining of differentiated cells by the use of ImageJ, a useful tool for this kind of analysis [47]. These findings may contribute for the definition of the best source of cells for clinical trials that involve cell therapy or tissue engineering.

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Author Disclosure Statement

No competing financial interests exist.

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