



**HAL**  
open science

## Cell specific differences between human adipose derived- and mesenchymal- stromal cells despite similar differentiation potentials.

Danièle Noël, David Caton, S. Roche, Claire Bony, Sylvain Lehmann, Louis Casteilla, Christian Jorgensen, Béatrice Cousin

### ► To cite this version:

Danièle Noël, David Caton, S. Roche, Claire Bony, Sylvain Lehmann, et al.. Cell specific differences between human adipose derived- and mesenchymal- stromal cells despite similar differentiation potentials.. *Experimental Cell Research*, 2008, 314 (7), pp.1575-1584. 10.1016/j.yexcr.2007.12.022 . hal-00275173

**HAL Id: hal-00275173**

**<https://hal.science/hal-00275173v1>**

Submitted on 19 Dec 2017

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Cell specific differences between human adipose-derived and mesenchymal–stromal cells despite similar differentiation potentials

Danièle Noël<sup>a,b,1</sup>, David Caton<sup>c,1</sup>, Stéphane Roche<sup>d</sup>, Claire Bony<sup>a,b</sup>, Sylvain Lehmann<sup>d</sup>, Louis Casteilla<sup>c</sup>, Christian Jorgensen<sup>a,b</sup>, Béatrice Cousin<sup>c,\*</sup>

<sup>a</sup>Inserm U844, Montpellier, F-34091 France

<sup>b</sup>Université MONTPELLIER1, UFR de Médecine, Montpellier, F-34000 France

<sup>c</sup>UMR5241 CNRS UPS, Toulouse, France

<sup>d</sup>CNRS UPR1142, Montpellier, France

---

## ARTICLE INFORMATION

### Article Chronology:

Received 22 October 2007

Revised version received

17 December 2007

Accepted 19 December 2007

---

### Keywords:

Adipose tissue

Bone Marrow

Transcript pattern

Proteomic pattern

Mesenchymal lineage differentiation

---

## ABSTRACT

Stromal cells from bone marrow and adipose tissue are attractive sources of adult progenitors for cell-based therapy. However, whether those cell populations represent intrinsically different cell types is still largely under debate. The aim of this study was to systematically and quantitatively compare adipose-derived stromal cells (ADSC) and bone marrow-derived multipotent mesenchymal–stromal cells (BM-MS). The quantitative comparison was realized using Taqman Low Density Array, 2D electrophoresis and differentiation functional assays *in vitro*. Furthermore, cells engineered to express TGF $\beta$ 1 were injected into the intra-articular space of mouse knee joints in order to determine whether they were able to form new differentiated tissues *in vivo*. Our data revealed cell specific differences at transcriptional and proteomic levels between both cell types according to their tissue origin as well as functional differences in their differentiation processes towards adipogenic, osteogenic and chondrogenic programs. Nevertheless, *in vitro* as well as *in vivo* ADSC displayed the same ability than MSC to differentiate towards chondrocytes/osteoblasts, comforting the status of both cell sources as promising regenerative cells. In summary, our observations indicate that ADSC and MSC are fundamentally different cell types and differently committed cells.

---

## Introduction

Adipose tissue appears as a suitable reservoir of regenerative cells as this adult tissue is abundant and easy to sample with no ethic limitation. Recent works from independent groups state that adipose tissue hosts cells able to display various differentia-

tion potentials *in vitro* and *in vivo* [1–4]. Such cells can be isolated from the stroma vascular fraction (SVF) obtained after adipose tissue digestion that may be used either freshly prepared or after sub-culture [2,4–7]. In this last case, cultured cells represent a particular cell subpopulation, restricted to the adherent cell fraction of SVF, termed ADSC (adipose-derived stromal cells).

---

\* Corresponding author. IFR31, Institut Louis Bugnard, UMR 5241 CNRS UPS, BP 84225, 31432 Toulouse Cedex 4, France. Tel.: +33 5 61 32 34 98; fax: +33 5 62 17 09 05.

E-mail address: [cousinb@toulouse.inserm.fr](mailto:cousinb@toulouse.inserm.fr) (B. Cousin).

<sup>1</sup> These authors contributed equally to the work.

ADSC are often called adipose-derived mesenchymal stem cells (MSC), as MSC represent an archetype of adult stem cells. MSC are classically isolated from bone marrow (BM) aspirates and are identified as a multipotent cell population in the adult organism able to be induced to express adipogenic, osteogenic and chondrogenic markers [8–11] and especially studied in the context of bone/cartilage regeneration in vivo [12–14]. Although stromal cells from BM and adipose tissue seem to be closely related, notable differences were reported [6,15–19], but no systematic and extensive parallel comparison between these cells has yet been performed. According to the large perspective opened by the easy access to an abundant source of regenerative cells, such study seems requested.

The objective of this study was to realize such comparison on the biological characteristics of ADSC and MSC both in vitro and in vivo. Indeed, two dimensional gel electrophoresis and real-time PCR based Taqman Low Density Array (TLDA) covering differentiation, stemness and signalling pathways, were used to establish, respectively, a proteomic and transcriptional profile of both cell populations. In addition, we analysed their phenotype as well as their differentiation potential in vitro. In vivo, cells were injected into the intra-articular space of mouse knee joints to determine their capacity to differentiate in an in vivo cartilaginous environment.

## Materials and methods

### Cell culture

The SVF was isolated from human adipose tissue as previously described [4]. Human subcutaneous fat was obtained from healthy patients undergoing abdominal dermolipectomy in the department of Plastic Surgery of Toulouse Rangueil Hospital (Toulouse, France). The patients were between 20 and 50 years old and their BMI never over 30. SVF cells were plated overnight in DMEM-F12 (1:1) medium supplemented with 10% FBS, 100 µg/ml pantothenic acid, 100 µM ascorbic acid, 16 µM biotin, 250 µg/ml amphotericin, 5 µg/ml streptomycin and 5 U/ml penicillin (Sigma Aldrich, St. Quentin Fallavier, France). Adherent ADSC growth was pursued until cells reached 75% confluence (passage 0) and used thereafter or at passage 1. Each experiment was reproduced 3 to 5 times with independent ADSC cultures.

MSC cultures were established from four BM aspirates of healthy age-matched donors. Mononuclear cells were plated at the density of  $5 \times 10^4$  cells/cm<sup>2</sup> in  $\alpha$ -MEM, supplemented with 10% FBS, 1 ng/ml basic fibroblast growth factor, 100 U/ml penicillin and 100 µg/ml streptomycin. At sub-confluence, cells were detached with trypsin and plated at the density of 1000 cells/cm<sup>2</sup> to be used at passages 2–4.

### Cell phenotyping

Isolated cells were analysed by flow cytometry using standard protocols [4]. Cells were incubated with FITC, PE and APC-conjugated primary antibodies from BD Biosciences (Le Pont de Claix, France). Cells were then analysed on a fluorescence-activated cell sorter (FACS Calibur, BD, Le Pont de Claix, France). Data acquisition and analysis were performed using the Cell Quest software (BD).

### Whole cell extract and 2D electrophoresis

Cells were washed twice with PBS containing a cocktail of protease inhibitors (Sigma Aldrich) and lysed with 200 µl of lysing buffer (8 M urea, 2 M thiourea, 4% CHAPS, 50 mM dithiothreitol (DTT)). For the first dimension, 30 µg of proteins were added to 250 µl of rehydration buffer (9.8 M urea, 4% zwitterionic detergent CHAPS, 50 mM DTT and 0.5% IPG buffer [3–10]). IPG strips covering a pH range of 3–10 were used. For focalisation, the following voltage/time profile was used: 200 V for 1 h, 1000 V for 1 h, 3000 V for 1 h, a gradient between 3000 V and 8000 V during 2 h and 8000 V for 5 h. For the second dimension, focused strips were equilibrated in 6 M urea, 30% glycerol, 2% SDS, 50 mM Tris pH 8.8, 1% DTT for 30 min and then for an additional 30 min in the same solution except that DTT was replaced by 5% iodoacetamide. After equilibration, proteins were separated by an SDS-PAGE method using 12% acrylamide gel with a ratio of acrylamide/bisacrylamide of 37.5/1.

### Silver staining and protein identification

Gels were stained with a silver nitrate procedure [20] and scanned at 300 dots per inch using the Labscan 3 software (GE Healthcare) after a procedure of calibration using the kaleidoscope LaserSoft Imaging (Kodak, France). Spot detection and quantification were performed with ImageMaster 2D Platinum software (GE Healthcare). The spot detection was performed using 4 as smooth parameter, 10 as minimum area and 75 as cut-off saliency. To take into account experimental variations, gels were normalized to the volume of all spots detected on the 2D gel.

Using ImageMaster Platinum 5, quantification was performed by an integration of grey level of each spot and normalisation with total spot volume. Three to 5 replicates with independent samples were performed to ensure reproducibility of the results. Each gel was matched with others using at least 10 landmarks per pair and a minimal percentage of 70% of matches was obtained between each pair. Reference gel was defined as a MSC gel.

### Real-time RT-PCR and analysis of gene expression

Total RNA was extracted using the RNeasy mini kit (Qiagen S. A., Courtaboeuf, France) and reverse transcribed using MultiScribe reverse transcriptase (Applied Biosystems, Courtaboeuf, France). The amplification was performed using the primer sets (Table 1) and the TaqMan Universal Master Mix.

### TaqMan low density arrays

The 384 wells of each card were preloaded with fluorogenic probes and primers carefully selected from predesigned TaqMan Low Density Arrays (Applied Biosystems). First strand cDNA was synthesized from 1 µg total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems). cDNAs were loaded on the microfluidic cards for thermal cycling on an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems). Expression values for target genes were normalized to the expression of GRCC10. The geNorm and Global Pattern recognition and, the Cluster and TreeView hierarchical clustering

**Table 1 – List of primers used for RT-PCR**

Gene (GenBank code)	Primer sequence	Product size (bp)
GAPDH (NM_002046)	FP: ATGGGGAAGGTGAAGGTCTG RP: GGGGTCATTGATGGCAACAATA	108
Osterix (NM_152860)	FP: ATTCTGTAGATCCGAGCACC RP: GCTCACGTCGCTCATTTCG	81
Alkaline phosphatase (BC021289)	FP: ATGGGATGGGTGTCTCCACA RP: CCACGAAGGGAACTTGTC	108
Osteocalcin (NM_000711)	FP: CACTCCTCGCCCTATTGGC RP: CCCTCCTGCTGGACACAAAG	112
Aggrecan (NM_001135)	FP: CTGCTTCGAGGCATTTCAG RP: CTTGGGTACGATCCACTCC	98
Collagen II (NM_033150)	FP: GCCAGGATGTCCGGAACCAG RP: TCCCTCTGGGTCTTGTTC	106
Collagen X (NM_000493)	FP: GGTATAGCAGTAAGAGGAGAGCA RP: AGGACTTCCGTAGCCTGGTTT	111
LPL (NM_000237)	FP: ACAAGAGAGAACCAGACTCCAA RP: AGGGTAGTTAAACTCCTCCTCC	149
PPAR $\gamma$ (NM_005037)	FP: GTGGCCGCAGAAATGACC RP: CCACGGAGCTGATCCCAA	72

Analysis of gene expression was performed using the ABI Prism 7900 Sequence Detection System software (Applied Biosystems). Content of cDNA samples was normalized and expression of each specific gene was calculated using the formulae  $2^{-(\Delta Ct)}$  and was compared to the value obtained for MSC at J0.

softwares were used. Two filters have been used: one filter aimed at retaining only genes expressed above the median value, and the second filter retained genes for which the difference between the maximum and minimum values was twice the median value.

#### *In vitro adipogenesis assay*

For adipogenic differentiation, cells were plated at the density of  $4-8 \times 10^4$  cells/cm<sup>2</sup>. Adipogenic differentiation was induced in DMEM:F12 supplemented with 10% FBS, 1  $\mu$ M dexamethasone, 0.5 mM isobutyl-methylxanthine and 60  $\mu$ M indomethacin for 3 days. Medium was changed every 2 days and replaced with DMEM:F12 supplemented with 10% FBS. Adipocytes were characterized by oil red O staining. Differentiation was quantified by real-time RT-PCR using specific primers.

#### *In vitro osteogenesis assay*

$1.5 \times 10^4$  cells/cm<sup>2</sup> were cultured in osteogenic differentiation medium consisting in DMEM (high glucose) with 10% FBS, 0.1  $\mu$ M dexamethasone, 3 mM NaH<sub>2</sub>PO<sub>4</sub> and 50  $\mu$ g/ml ascorbic acid. Osteoblasts were characterized by Von Kossa staining for secreted calcified extracellular matrix detection. Osteogenic differentiation was quantified by RT-PCR gene expression analysis.

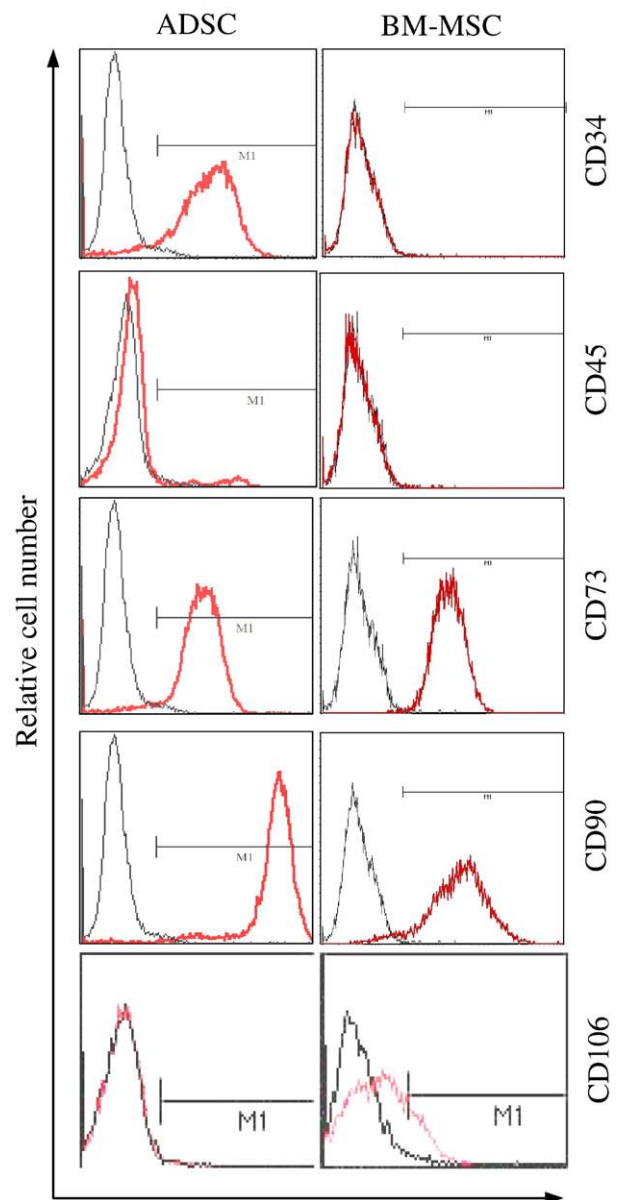
#### *In vitro chondrogenesis assay*

Chondrogenic differentiation was induced by culture of cells in micropellets at the density of  $2.5 \times 10^5$  cells/500  $\mu$ l in DMEM supplemented with 0.1  $\mu$ M dexamethasone, 0.17 mM ascorbate-2 phosphate, 1% insulin-transferrin-sodium selenite

supplement and 100 ng/ml of recombinant bone morphogenetic protein 2 (BMP2) from AbCys (Paris, France). After 3 weeks, the samples were used for RT-PCR gene expression analysis and histological staining with 0.1% Safranin O.

#### *Adenoviral infection and in vivo differentiation assays*

For cell transduction, cells were plated at  $10^6$  cells in 160 mm dishes and infected with a TGF $\beta$ 1-expressing adenoviral vector, kindly provided by Bruce Beutler (La Jolla, CA, USA), at the multiplicity of infection of 100 in 15 ml of serum free RPMI medium overnight. An adenovirus expressing  $\beta$ -galactosidase



**Fig. 1 – Immunophenotype of ADSC (left) and MSC (right). Both cell types (ADSC, P0; BM-MSc, P4) were stained with antibodies against the indicated antigens, and analysed by flow cytometry. Representative histograms are shown as red line and the respective isotype controls are shown as black line.**

enzyme under the control of CMV promoter was also used as a control [21]. The following day, cells were used for injection. CB17-severe combined immunodeficient (SCID)/bg mice were purchased from Harlan (Gannat, France) and cared for according to the Laboratory Animal Care guidelines. For intra-articular injections, mice were anaesthetized and the cell suspensions ( $5 \times 10^5$  cells in  $5 \mu\text{l}$  of PBS) were delivered into the joint cavity using a  $10 \mu\text{l}$  syringe. No injury was performed in this area prior to the injection. At least five mice were included per group of treatment. Knees were dissected from euthanized mice at day 21 and processed for histology.

### Histology and immunohistochemistry

Knee tissues were fixed in 4% formaldehyde solution and decalcified in acid-based DC3 solution (Labonord; Templemars, France) for 24 h. The tissues were then embedded in paraffin and sectioned at  $5 \mu\text{m}$ . Sections were deparaffinized and hydrated before antigen unmasking in a citrate buffer bath at  $97^\circ\text{C}$  for 40 min. The mAb Ab-2 specific for human mitochondrial protein, the anti-aggrecan and the anti-type II collagen mAb, used at the 1:50 dilution, were from Interchim (Montluçon, France). Sections were stained using the AEC kit (DAKO) and counterstained with haematoxylin.

### Statistical analysis

Statistics were performed using the Student's 2-tailed t-test with unequal variances. Transcriptomic and proteomic sta-

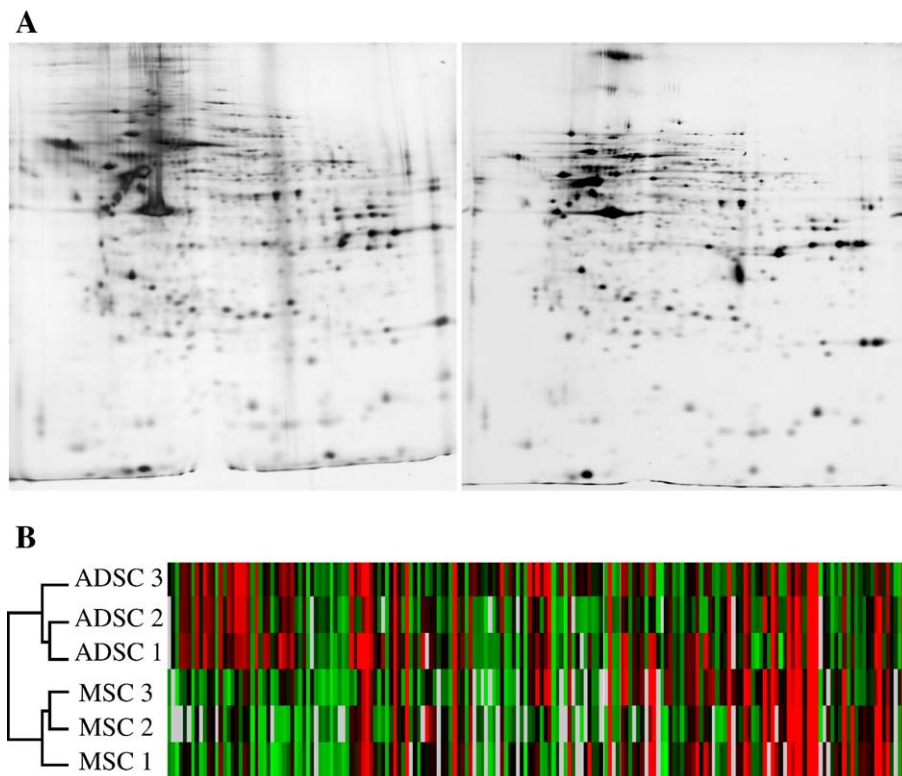
tistical analyses were performed by Hierarchical Clustering Explorer 3.0 software [22] with an average linkage hierarchical clustering algorithm, using the centred Pearson correlation coefficient as the similarity metric.

## Results and discussion

### Phenotypic characterization of ADSC and MSC

After isolation, both MSC and ADSC exhibited fibroblast-like cell morphology. Both cells were positive for known mesenchymal markers such as CD73 and CD90 and negative for the haematopoietic marker CD45 (Fig. 1). As previously described [4,5,9,23,24] CD44 and CD105 were also expressed by both cell types, while CD31 was not detected (data not shown). MSC expressed CD106 (VCAM1), an adhesion molecule found on vascular cells that mediates the binding of haematopoietic progenitors on MSC [25], and did not express CD34 usually detected on haematopoietic progenitors [26] and endothelial cells. By contrast, ADSC were CD34 positive and CD106 negative (Fig. 1). Although we show that CD34 and CD106 are differentially expressed at the surface of both cell types as previously reported [24], CD34 was previously reported to be absent on ADSC and CD106 to be expressed in 30% of ADSC [27]. This discrepancy may be due to cell culture since ADSC between passages 2 and 5 were used by Kern et al. [27], and CD34 expression decreases with passages [28].

Altogether, the panel of surface markers tested revealed a close but distinct phenotypic profile between the two



**Fig. 2 – Differential proteomic analysis of MSC and ADSC. A)** 2D gel electrophoresis was performed using whole cell protein extracts from ADSC (left panel) and MSC (right panel). **B)** Comparison of ADSC and MSC using Hierarchical Clustering Software and Pearson Test. Red: expression above the median; Green: expression below the median; Black: median expression across all samples.



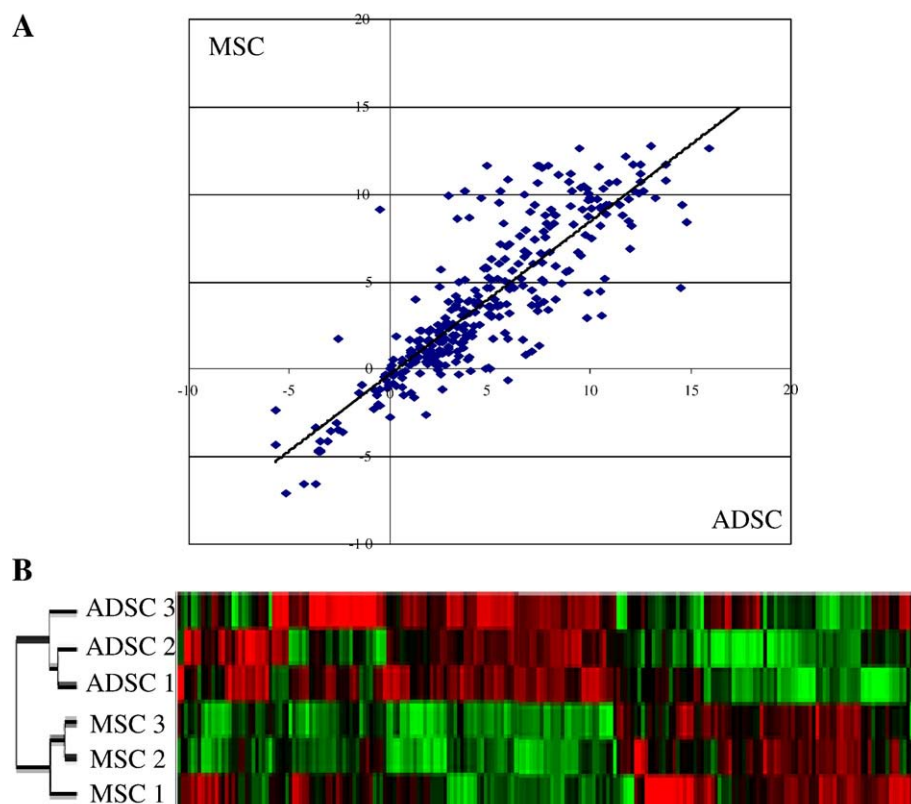
cell types, as already described by other groups [2,3,5,8,17, 24,27].

### Proteomic and genomic characterizations of ADSC and MSC

Proteomic analysis allowed the detection of several protein spots (Supplementary table 1). However, a number of proteins (23%) were expressed specifically in one or the other cell type (Fig. 2A) and 18% of detected spots were found to be differentially expressed between ADSC and MSC. Using the Pearson statistical test, we found that the correlation factor, that represents a statistical analysis of the general expression profile, was around 0.9 between ADSC and MSC. This indicates a good correlation between detected proteins in whole extracts of both cell types (Supplementary table 2). On the other side, the Hierarchical Clustering method allowed to define two different clusters that discriminate ADSC and MSC (Fig. 2B). Thus, despite a general similar proteomic profile between ADSC and MSC, independent samples of ADSC were more related together rather than to MSC.

To confirm these data, genomic analysis was conducted by using Taqman Low Density Array (TLDA). The gene expression panel used was custom-made and composed of 384 different sets of primers covering differentiation pathways, stemness pathway and signaling pathway (for complete list, see [29]). The primers plotted on the TLDA focused on receptors, growth and transcription factors. Such factors are usually localized in

nucleus and/or in membrane compartments that are poorly revealed by 2D electrophoresis approach. On the other side, the protein isolation methods focused on the highly expressed proteins, representing the cellular machinery. Thus, taken together, the proteomic and transcriptomic analyses are most complementary rather than redundant. The expression levels of ADSC transcripts were plotted against the levels of MSC transcripts and a correlation coefficient around 0.7 was found (Fig. 3A and Supplementary table 3). Despite the great similarity between both cells, each sample was clustered with the other samples from the same tissue (Fig. 3B). Most of the genes were highly expressed in ADSC and MSC (13.4% of analyzed genes) and were related to cell adhesion molecules such as collagens, SPARC and fibronectin. Similarly, most of the silent genes were absent in both cell types, representing 8.4% of the analyzed genes (Supplementary table 4). Nevertheless, 3.4% of the analyzed genes were found to be specifically expressed only by one population. Genes expressed only in ADSC are mostly involved in cellular communication (CCL3, FGF9, IL1R2, KDR) and in transcription control (PAX3, SPI1, ZNF45) (Table 1). The genes expressed uniquely in MSC are involved in WNT signalling and differentiation pathways (WNT11, WNT7B, SOX6) (Table 1). Furthermore, 9.7% of the analyzed genes were differentially expressed between ADSC and MSC (Supplementary tables 4 and 5). Taken together this analysis indicates that the differential expression of 13.2% of genes was able to discriminate both populations without being



**Fig. 3 – Differential gene expression between MSC and ADSC. A) Correlation analysis between the levels of gene expression in ADSC and BM-MSC performed using Taqman Low density Array. (B) Hierarchical clustering analysis. The dendrogram visualizes the relationship between different ADSC and MSC preparations. Red: expression above the median; Green: expression below the median; Black: median expression across all samples.**

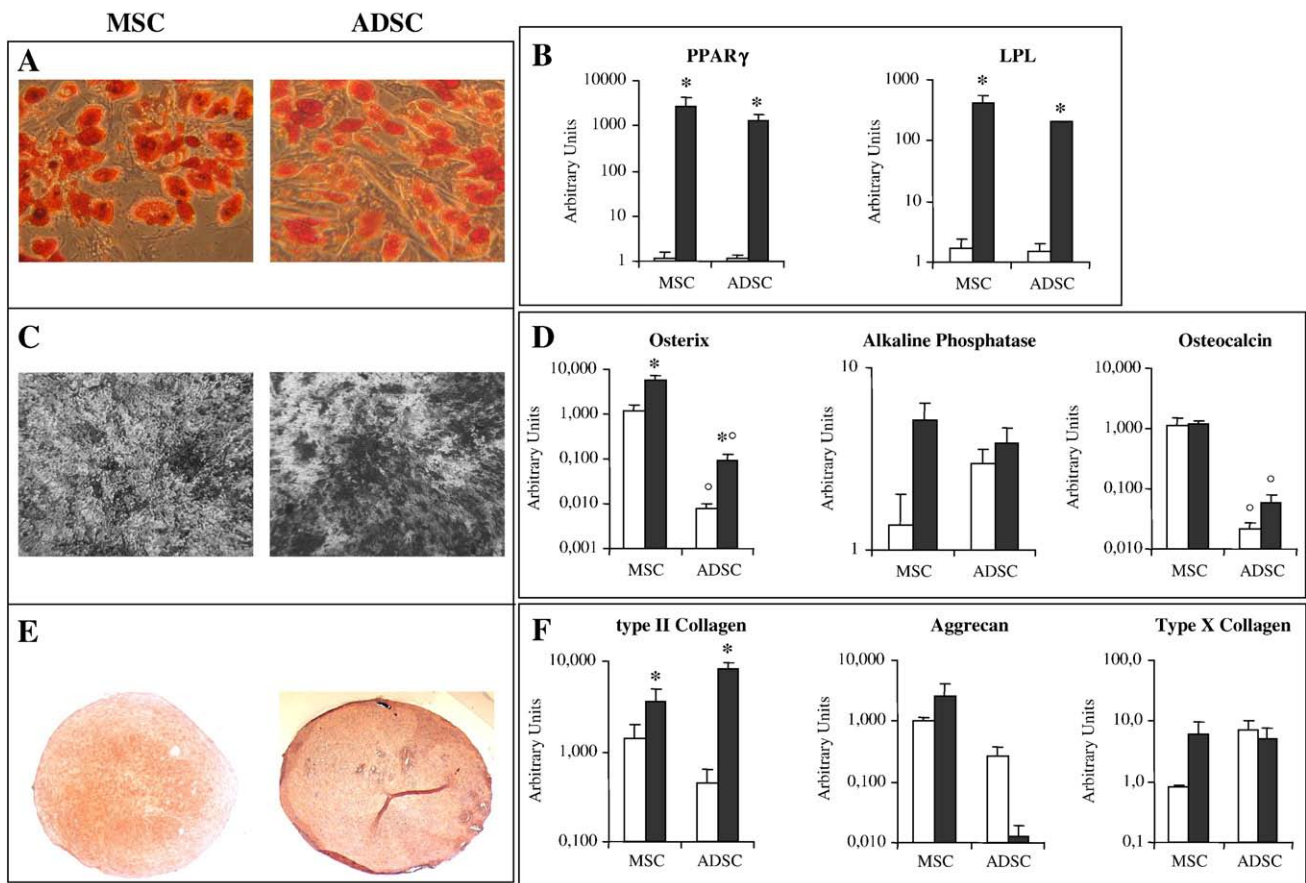
able to define specific markers of each cell population. It has to be noted that both in 2D electrophoresis and in TLDA analyses, independent samples of ADSC were more related together rather than to MSC. This did not exclude but lowered the possibility than the variability observed in the current work reflects variation between individuals rather than between tissue depots of origin. Markers specific for chondro/osteogenesis such as HES-1, DLX-5, TWIST1, osteocalcin, osterix, SOX9, WNT5A, TGF $\beta$ 1 and VEGF were highly expressed in MSC compared to ADSC. To our knowledge, this is the first study comparing adipose- and bone marrow-derived MSC using 2D electrophoresis. One previous study comparing the proteomic profile of BM-MSC cultured under two different expansion media revealed that culture conditions exerted a prominent impact on the proteome [30]. In addition, there is a substantial body of literature examining ADSC or BM-MSC proteome (for reviews, see [31,32]). However, it is difficult to compare the different results described, or to identify a clear proteomic signature for each cell type. One of the reasons is the different paradigms, protein extraction protocols and analytical approaches were used. We also report specific differences at the transcriptomic level between ADSC and MSC. While common differentiation pathways to ADSC and BM-MSC have been previously identified [33], our report is one of the first to

compare undifferentiated MSC isolated from fat and bone marrow [19,34]. In agreement with the study from Liu et al., our data revealed considerable similarities between the two cell populations with a set of genes that can discriminate both populations [33]. It will be of importance to determine whether the differences observed in our study between MSC from various origins may be ascribable to the cell passages, culture conditions or the tissue sources.

#### *In vitro adipogenic, osteogenic and chondrogenic differentiation of ADSC and MSC*

We further investigated whether the differences observed at the transcriptomic and proteomic levels between both cell types could have an impact on the extent of differentiation in vitro. Previous spared functional studies demonstrated that ADSC and MSC display the triple potentials of differentiation [5,8,35] although no strict and quantitative comparison was yet provided.

When cultured in adipogenic medium, both ADSC and MSC displayed a positive staining of cytoplasmic lipid droplets with Oil Red O (Fig. 4A). As expected, no staining was observed in undifferentiated cells (data not shown). To quantify the differentiation capacities of both cell types, real-time RT-PCR was performed to measure the expression of genes specifically



**Fig. 4 – Adipogenic, osteogenic and chondrogenic differentiations of ADSC and MSC.** Oil Red O (A;  $\times 450$ ), Von Kossa (C;  $\times 450$ ) and Safranin O (E;  $\times 100$ ) staining of ADSC and MSC in vitro revealing respectively adipogenic, osteogenic and chondrogenic differentiations. Relative expression of adipogenic (B), osteogenic (D) and chondrogenic (F) markers in ADSC versus MSC before (d0, white bars) or after (d21, black bars) induction of differentiation, \* $p < 0.05$  in differentiated vs undifferentiated cells, <sup>o</sup> $p < 0.05$  in MSC vs ADSC.

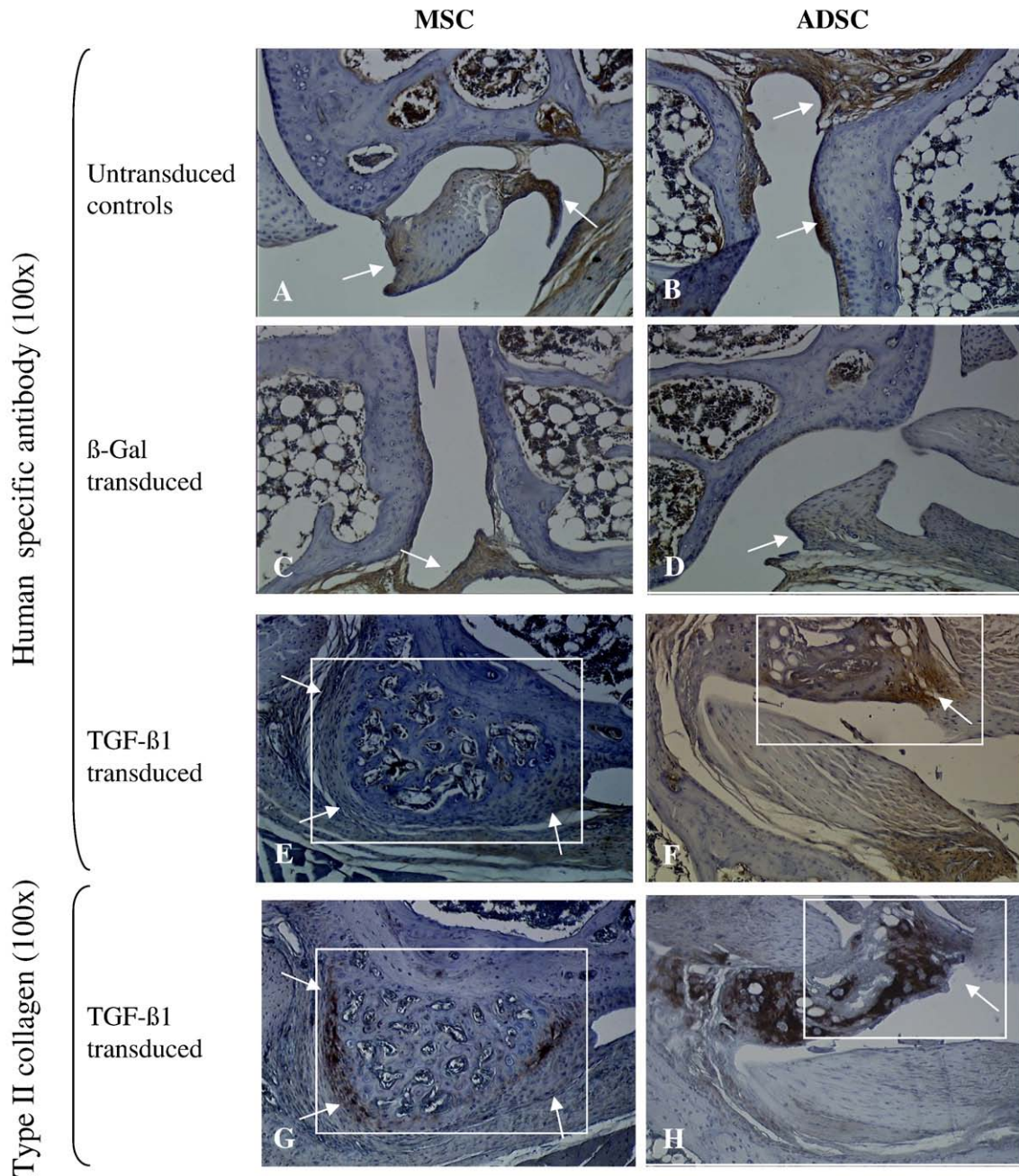


involved in adipocyte differentiation. This analysis revealed similar expression levels for the mRNA coding for PPAR $\gamma$  and LPL in undifferentiated cells (Fig. 4B). These RNA were significantly increased in differentiated cells indicating that the cells underwent adipogenic differentiation to a similar extent.

Induction of osteogenesis in MSC and ADSC cultures resulted in the appearance of mineralised nodular structures (Fig. 4C). To quantify the extent of osteogenic differentiation, the expression of 3 different transcripts was evaluated. Both osterix and osteocalcin transcripts were significantly higher in undifferentiated MSC compared to undifferentiated ADSC (Fig. 4D).

Osterix and alkaline phosphatase mRNAs were stronger up-regulated in differentiated MSC than in differentiated ADSC, in contrast to osteocalcin (Fig. 4D).

After chondrogenic induction [36], the presence of proteoglycans was identified in both cell types (Fig. 4E) but not in non induced control cells (data not shown). The analysis of genes restricted to chondrocyte lineage (collagen type II and aggrecan) revealed a similar level of expression in undifferentiated MSC and ADSC (Fig. 4F). At day 21, a significant increase in collagen type II was detected in both cell types. Aggrecan expression was poorly induced in MSC in our culture conditions and, by



**Fig. 5 - In vivo differentiation of ADSC and MSC in SCID mice. Knee joints of treated animals were analysed by immunostaining. Untransduced and  $\beta$ gal-transduced MSC (A, C) and ADSC (B, D) formed fibrous tissue (arrows). TGF- $\beta$ 1-transduced MSC (E, G) and ADSC (F, H) formed chondrocytes/osteophytes (insets) composed of chondrocytes positive for human specific antibody (E, F arrows) and collagen II (G, H arrows) ( $\times 100$ ).**



contrast, was completely down regulated in ADSC. Finally, collagen type X, a marker associated with hypertrophic chondrocyte, reached a similar level in both cultures (Fig. 4F).

We thus confirm that both cells can undergo the three differentiation programs but the *in vitro* quantitative analysis reveals clear differences. For osteogenesis, osterix and osteocalcin expressions were always more elevated in MSC compared to ADSC, suggesting that cells isolated from BM could be more prone to osteogenic differentiation than those isolated from fat. This is consistent with a previous report [31].

The micropellet culture technique described in other studies [37,38] and BMP-2 supplementation resulted in the condensation of ADSC and MSC into three dimensional aggregates positive for safranin O. A similar expression level of collagen type II and aggrecan was found in undifferentiated cells, although only collagen type II was increased in micropellets obtained from both cell types. A slight increase in aggrecan expression was observed only in MSC under chondrogenic conditions, suggesting that *in vitro* chondrogenesis may be less efficient in ADSC compared to MSC, as already reported [18,31].

Altogether our molecular and functional investigations strongly suggest that MSC already exhibits molecular and functional signs of lineage commitment towards chondrogenic and osteogenic differentiation. Thus the cell lineage commitment could be due to intrinsic differences ascribable to tissue origin. With one exception [27], most of the previous studies comparing the multilineage capacity of MSC isolated from different tissues reported a higher osteogenic and chondrogenic potential of MSC isolated from BM compared with ADSC [16,31,39]. These data, which were essentially based on qualitative histological evaluation, are confirmed here on a quantitative basis.

### *In vivo* chondrogenic differentiation of ADSC and MSC

To go further, we investigated the behaviour of ADSC and MSC after engraftment into a physiological niche [40]). Indeed, although some studies on ADSC and MSC have focused on their ability to generate cartilage at ectopic sites (subcutaneous, intramuscular), no study has investigated their differentiation potential in the intra-articular space, where environmental factors may play a regulatory role. Because MSC do not spontaneously differentiate after ectopic implantation [40], we genetically modified both cell types to express TGF- $\beta$ 1 since TGF- $\beta$ 1 has been shown to induce the differentiation of MSC towards bone/cartilage tissues [41,42].

Injected with untransduced or  $\beta$ gal-transduced cells, none of the naive or  $\beta$ gal expressing cells formed spontaneously cartilage or bone structures (Figs. 5A-D). In joints implanted with TGF- $\beta$ 1-expressing ADSC or MSC (Figs. 5 E-H), the formation of new structures similar to chondrocytes or osteocytes was observed. Importantly, both TGF- $\beta$ 1-transduced ADSC or MSC were detected in histologically proven cartilage region and stained positive for human mitochondrial proteins (Figs. 5 E, F), and collagen II (Figs. 5 G, H), indicating the contribution of both cell types to ectopic cartilage formation. Because the quantification of these structures was difficult to measure, our data suggest that both cell types have a similar ability to form osteo/chondrogenic structures in the cartilage environment. The different behaviour of ADSC *in vitro* and *in vivo* concerning the chondrogenic differentiation may be

ascribable to environmental factors present in the knee joint. The variability between *in vitro* and *in vivo* observation may also be due to overexpression of TGF $\beta$ 1 in ADSC, since *in vitro* the chondrogenic differentiation of adenovirally transduced cells has not been tested.

In conclusion, despite known similarities between ADSC and MSC, our observations revealed cell specific differences at the phenotypic, mRNA, proteomic and functional levels according to their tissue origin. These two cell types have thus to be considered as different even though they can be induced to differentiate towards the three mesenchymal lineages. From an absolute view, and in the perspective of cell therapy, our results favour the use of MSC for osteogenesis and chondrogenesis because it seems that these cells are pre-committed towards these lineages. Nevertheless, the easiness of fat tissue sampling and a better control of ADSC differentiation could overcome this initial disadvantage. Indeed, our results highlight the crucial need for a better identification of the molecular mechanisms involved in the regulation of the stemness and differentiation pathways to specifically optimize the cell specific maintenance/differentiation protocols.

---

## Acknowledgments

Caton D. and Roche S. as well as the study are financially supported by the European Community (Key action LSH 1.2.4-3, Integrated project: "Adult mesenchymal stem cells engineering for connective tissue disorders. From the bench to the bed side", Contract no: 503161), the Cell and Gene Therapy program from Région Midi-Pyrénées (#03011999), and the Etablissement Français du Sang.

---

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.yexcr.2007.12.022](https://doi.org/10.1016/j.yexcr.2007.12.022).

---

## REFERENCES

- [1] P.A. Zuk, M. Zhu, P. Ashjian, D.A. De Ugarte, J.I. Huang, H. Mizuno, Z.C. Alfonso, J.K. Fraser, P. Benhaim, M.H. Hedrick, Human adipose tissue is a source of multipotent stem cells, *Mol. Biol. Cell* 13 (2002) 4279–4295.
- [2] S. Gronthos, A.C. Zannettino, S.J. Hay, S. Shi, S.E. Graves, A. Kortesidis, P.J. Simmons, Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow, *J. Cell Sci.* 116 (2003) 1827–1835.
- [3] M. Ryden, A. Dicker, C. Gotherstrom, G. Astrom, C. Tammik, P. Arner, K. Le Blanc, Functional characterization of human mesenchymal stem cell-derived adipocytes, *Biochem. Biophys. Res. Commun.* 311 (2003) 391–397.
- [4] V. Planat-Benard, J.S. Silvestre, B. Cousin, M. Andre, M. Nibbelink, R. Tamarat, M. Clergue, C. Manneville, C. Saillan-Barreau, M. Duriez, A. Tedgui, B. Levy, L. Penicaud, L. Casteilla, Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives, *Circulation* 109 (2004) 656–663.
- [5] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrick, Multilineage cells from

- human adipose tissue: implications for cell-based therapies, *Tissue Eng.* 7 (2001) 211–228.
- [6] B. Puissant, C. Barreau, P. Bourin, C. Clavel, J. Corre, C. Bousquet, C. Taureau, B. Cousin, M. Abbal, P. Laharrague, L. Penicaud, L. Casteilla, A. Blancher, Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells, *Br. J. Haematol.* 129 (2005) 118–129.
- [7] J.M. Gimble, S. Zvonic, Z.E. Floyd, M. Kassem, M.E. Nuttall, Playing with bone and fat, *J. Cell Biochem.* 98 (2006) 251–266.
- [8] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, *Science* 284 (1999) 143–147.
- [9] J.E. Dennis, P. Charbord, Origin and differentiation of human and murine stroma, *Stem Cells* 20 (2002) 205–214.
- [10] A.I. Caplan, Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics, *Tissue Eng.* 11 (2005) 1198–1211.
- [11] G.Y. Rochefort, B. Delorme, A. Lopez, O. Herault, P. Bonnet, P. Charbord, V. Eder, J. Domenech, Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia, *Stem Cells* 24 (2006) 2202–2208.
- [12] G.R. Erickson, J.M. Gimble, D.M. Franklin, H.E. Rice, H. Awad, F. Guilak, Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo, *Biochem. Biophys. Res. Commun.* 290 (2002) 763–769.
- [13] S. Wakitani, T. Goto, S.J. Pineda, R.G. Young, J.M. Mansour, A.I. Caplan, V.M. Goldberg, Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage, *J. Bone Joint Surg. Am.* 76 (1994) 579–592.
- [14] Y. Lin, E. Luo, X. Chen, L. Liu, J. Qiao, Z. Yan, Z. Li, W. Tang, X. Zheng, W. Tian, Molecular and cellular characterization during chondrogenic differentiation of adipose tissue-derived stromal cells in vitro and cartilage formation in vivo, *J. Cell Mol. Med.* 9 (2005) 929–939.
- [15] G.I. Im, Y.W. Shin, LeeK.B., Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? *Osteoarthr. Cartil.* 13 (2005) 845–853.
- [16] Y. Sakaguchi, I. Sekiya, K. Yagishita, T. Muneta, Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source, *Arthritis Rheum.* 52 (2005) 2521–2529.
- [17] W. Wagner, F. Wein, A. Seckinger, M. Frankhauser, U. Wirkner, U. Krause, J. Blake, C. Schwager, V. Eckstein, W. Ansoerge, A.D. Ho, Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood, *Exp. Hematol.* 33 (2005) 1402–1416.
- [18] T. Hennig, H. Lorenz, A. Thiel, K. Goetzke, A. Dickhut, F. Geiger, W. Richter, Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6, *J. Cell Physiol.* 211 (2007) 682–691.
- [19] B.T. Estes, A.W. Wu, F. Guilak, Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6, *Arthritis Rheum.* 54 (2006) 1222–1232.
- [20] A. Shevchenko, M. Wilm, O. Vorm, M. Mann, Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels, *Anal. Chem.* 68 (1996) 850–858.
- [21] F. Apparailly, D. Noël, V. Millet, A.H. Baker, G. Lisignoli, C. Jacquet, M.J. Kaiser, J. Sany, C. Jorgensen, Paradoxical effects of tissue inhibitor of metalloproteinases 1 gene transfer in collagen-induced arthritis, *Arthritis Rheum.* 44 (2001) 1444–1454.
- [22] J. Seo, M. Bakay, Y.W. Chen, S. Hilmer, B. Shneiderman, E.P. Hoffman, Interactively optimizing signal-to-noise ratios in expression profiling: project-specific algorithm selection and detection p-value weighting in Affymetrix microarrays, *Bioinformatics* 20 (2004) 2534–2544.
- [23] C. Jorgensen, F. Djouad, V. Fritz, F. Apparailly, P. Ponce, D. Noel, Mesenchymal stem cells and rheumatoid arthritis, *Jt. Bone Spine* 70 (2003) 483–485.
- [24] D.A. De Ugarte, Z. Alfonso, P.A. Zuk, A. Elbarbary, M. Zhu, P. Ashjian, P. Benhaim, M.H. Hedrick, J.K. Fraser, Differential expression of stem cell mobilization-associated molecules on multilineage cells from adipose tissue and bone marrow, *Immunol. Lett.* 89 (2003) 267–270.
- [25] P.J. Simmons, B. Masinovskiy, B.M. Longenecker, R. Berenson, B. Torok-Storb, W.M. Gallatin, Vascular cell adhesion molecule-1 expressed by bone marrow stromal cells mediates the binding of hematopoietic progenitor cells, *Blood* 80 (1992) 388–395.
- [26] G.U. Gangenahalli, V.K. Singh, Y.K. Verma, P. Gupta, R.K. Sharma, R. Chandra, P.M. Luthra, Hematopoietic stem cell antigen CD34: role in adhesion or homing, *Stem Cells Dev.* 15 (2006) 305–313.
- [27] S. Kern, H. Eichler, J. Stoeve, H. Kluter, K. Bieback, Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, *Stem Cells* 24 (2006) 1294–1301.
- [28] J.B. Mitchell, K. McIntosh, S. Zvonic, S. Garrett, Z.E. Floyd, A. Kloster, Y. Di Halvorsen, R.W. Storms, B. Goh, G. Kilroy, X. Wu, J.M. Gimble, Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers, *Stem Cells* 24 (2006) 376–385.
- [29] F. Djouad, B. Delorme, M. Maurice, C. Bony, F. Apparailly, P. Louis-Ponce, F. Canovas, P. Charbord, D. Noel, C. Jorgensen, Microenvironmental changes during differentiation of mesenchymal stem cells towards chondrocytes, *Arthritis Res. Ther.* 9 (2007) R33.
- [30] W. Wagner, R.E. Feldmann Jr., A. Seckinger, M.H. Maurer, F. Wein, J. Blake, U. Krause, A. Kalenka, H.F. Burgers, R. Saffrich, P. Wuchter, W. Kuschinsky, A.D. Ho, The heterogeneity of human mesenchymal stem cell preparations—evidence from simultaneous analysis of proteomes and transcriptomes, *Exp. Hematol.* 34 (2006) 536–548.
- [31] H.W. Park, J.S. Shin, C.W. Kim, Proteome of mesenchymal stem cells, *Proteomics* 7 (2007) 2881–2894.
- [32] J.M. Gimble, A.J. Katz, B.A. Bunnell, Adipose derived stem cells for regenerative medicine, *Circ. Res.* 100 (2007) 1249–1260.
- [33] T.M. Liu, M. Martina, D.W. Huttmacher, J. Hoi, P. Hui, E.H. Lee, B. Lim, Identification of common pathways mediating differentiation of bone marrow and adipose tissues derived human mesenchymal stem cells (MSCs) into three mesenchymal lineages, *Stem Cells* 25 (2007) 750–760.
- [34] S. Roche, M. Provansal, L. Tiers, C. Jorgensen, S. Lehmann, Proteomics of primary mesenchymal stem cells, *Regen Med.* 1 (2006) 511–517.
- [35] F. Guilak, H.A. Awad, B. Fermor, H.A. Leddy, J.M. Gimble, Adipose-derived adult stem cells for cartilage tissue engineering, *Biorheology* 41 (2004) 389–399.
- [36] T. Fukumoto, J.W. Sperling, A. Sanyal, J.S. Fitzsimmons, G.G. Reinholz, C.A. Conover, S.W. O'Driscoll, Combined effects of insulin-like growth factor-1 and transforming growth factor-beta1 on periosteal mesenchymal cells during chondrogenesis in vitro, *Osteoarthr. Cartil.* 11 (2003) 55–64.
- [37] B. Johnstone, T.M. Hering, A.I. Caplan, V.M. Goldberg, J.U. Yoo, In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells, *Exp. Cell Res.* 238 (1998) 265–272.
- [38] B. Schmitt, J. Ringe, T. Haupl, M. Notter, R. Manz, G.R. Burmester, M. Sittinger, C. Kaps, BMP2 initiates chondrogenic lineage development of adult human mesenchymal stem cells in high-density culture, *Differentiation* 71 (2003) 567–577.

- 
- [39] H. Afizah, Z. Yang, J.H. Hui, H.W. Ouyang, E.H. Lee, A comparison between the chondrogenic potential of human bone marrow stem cells (BMSCs) and adipose-derived stem cells (ADSCs) taken from the same donors, *Tissue Eng.* 13 (2007) 659–666.
- [40] D. Noel, D. Gazit, C. Bouquet, F. Apparailly, C. Bony, P. Ponce, V. Millet, G. Turgeman, M. Perricaudet, J. Sany, C. Jorgensen, Short-term BMP-2 expression is sufficient for in vivo osteochondral differentiation of mesenchymal stem cells, *Stem Cells* 22 (2004) 74–85.
- [41] H.M. van Beuningen, H.L. Glansbeek, P.M. vdK, W.B. van den Berg, Differential effects of local application of BMP-2 or TGF-beta 1 on both articular cartilage composition and osteophyte formation, *Osteoarthr. Cartil* 6 (1998) 306–317.
- [42] A.C. Bakker, F.A. van de Loo, H.M. van Beuningen, P. Sime, P.L. van Lent, P.M. van der Kraan, C.D. Richards, W.B. van den Berg, Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation, *Osteoarthr. Cartil.* 9 (2001) 128–136.