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Stephane Roche[1, 2], Bruno Delorme[3], Robert A. J. Oostendorp[4*], Romain Barbet[5*], David Caton[6*], Daniele Noel[7, 8], Karim Boumediene[9], Helen A. Papadaki[10], Beatrice Cousin[6], Carole Crozet[1], Ollivier Milhavet[1], Louis Casteilla[6], Jacques Hatzfeld[5], Christian Jorgensen[7, 8], Pierre Charbord[3] and Sylvain Lehmann[1, 2, 8]*

[1] CNRS, Institut de Génétique Humaine, UPR1142, Montpellier, France
[2] CHU Montpellier, Laboratoire de Biochimie, Hôpital St. Eloi, Montpellier, France
[3] Laboratoire d'Hématopoïèse, INSERM/ESPRI EA 3855, Faculté de Médecine de Tours, France
[4] 3rd Department of Internal Medicine, Klinikum rechts der Isar, Technische Universität München, München, Germany
[5] Institut André Lwoff, CNRS, UPR 9045, Villejuif, France
[6] Institut Louis Bugnard, UMR 5241, UPS, CNRS, Toulouse, France
[7] INSERM U844, Montpellier, France
[8] Université Montpellier 1, Montpellier, France
[9] Laboratoire Matrice Extracellulaire et Pathologie, UFR de Médecine, EA3214, Université de Caen Basse-Normandie, Caen, France
[10] Department of Hematology, University of Crete School of Medicine, Heraklion, Crete, Greece

Mesenchymal stem cells (MSC) are adult multipotential progenitors which have a high potential in regenerative medicine. They can be isolated from different tissues throughout the body and their homogeneity in terms of phenotype and differentiation capacities is a real concern. To address this issue, we conducted a 2-DE gel analysis of mesenchymal stem cells isolated from bone marrow (BM), adipose tissue, synovial membrane and umbilical vein wall. We confirmed that BM and adipose tissue derived cells were very similar, which argue for their interchangeable use for cell therapy. We also compared human mesenchymal to embryonic stem cells and showed that umbilical vein wall stem cells, a neo-natal cell type, were closer to BM cells than to embryonic stem cells. Based on these proteomic data, we could propose a panel of proteins which were the basis for the definition of a mesenchymal stem cell proteomic signature.

Keywords:
Adipose tissue / Bone marrow / Embryonic stem cell / Mesenchymal stem cell / Umbilical vein

Correspondence: Professor Sylvain Lehmann, CNRS, Institut de Génétique Humaine, UPR1142, F-34000 Montpellier, France
E-mail: sylvain.lehmann@igh.cnrs.fr
Fax: +33-4-99-61-99-31

Abbreviations: ADSC, adipose derived stem cells; **BM,** bone marrow; **hESC,** human embryonic stem cells; **MSC,** mesenchymal stem cells; **PMF,** peptide mass fingerprint; **SynoSC,** synovial membrane-derived stem cells; **UVSC,** umbilical vein derived somatic cells

1 Introduction

Regeneration takes place in the body in different organs following injury or disease. However, adult tissues such as bone, cartilage, tendons, blood vessels and cardiac muscle have a limited capacity for self-repair which leads to non-functional scar tissue. In this context, mesenchymal stem

* All these authors contributed equally to the work.

cells (MSC) which are adult multipotential progenitors of mesoderm cells (osteoblasts, chondrocytes, adipocytes and stroma cells [1]) represent a major hope for tissue-engineered replacement and regenerative medicine. Cells with MSC differentiation abilities can be isolated from bone marrow (BM-MSC), as well, as from a variety of tissues including adipose derived stem cells (ADSC) [2], synovial membrane-derived stem cells (SynoSC) [3, 4], umbilical cord blood [5], cartilage [6] and umbilical vein derived somatic cells (UVSC) [7]. *In vitro*, culture and differentiation of human MSC before therapeutic use, often require nonphysiological manipulation that might induce some risk for the patients. For example, differentiation of MSC into cardiomyocyte was induced by 5-azacytidine, an anticancer drug [8]. In the field of regenerative medicine, human embryonic stem cells (hESC) represent also an important hope as they can differentiate into cardiomyocytes, endothelial cells, β-pancreatic cells and many other tissue types [9]. Both human MSC and hESC share self-renewal capacity and differentiation into functional tissues. The self-renewal and differentiation potential of human MSC and hESC are however quite different: MSC are multipotent, when hESC are pluripotent. In addition, hESC are isolated from blastocyst and are defined by strict and specific criteria, whereas "MSC like cells" can be isolated from all range of sources and their homogeneity in terms of phenotype and differentiation capacities represent a real issue. To address these differences, the genomic or proteomic approaches could be used to have a general phenotype profile of the cells. The first proteomic analysis of MSC aiming at characterizing subpopulations with various differentiation properties was published in 2001 by Prockop and coworkers [10]. In this study, and in subsequent ones [11, 12], mostly focussing on ADSC and BM-MSC, the conclusion was that these two cell types were very close together with, however, some differences that were difficult to pinpoint [13–17]. As a matter of fact, from the combined analysis of the published human MSC proteomic studies, it has not been possible to identify a clear proteomic signature specific for MSC [18]. One of the reasons is the different paradigms, protein extraction protocols and analytical approaches used in these studies. Moreover, no study involving cells cultured in different laboratories and multi-site comparison was performed. Therefore, combination of these results to generate a common MSC proteomic signature is a task altered by too many biases.

In the present proteomic work, we compared human MSC isolated from different sources and cultured in strict controlled conditions, with respect to their morphology, expansion and multilineage differentiation capacity. We could define a common proteomic profile that distinguished human MSC from hESC. We also concluded a high proteomic similarity between BM-MSC and ADSC, and we observed that UVSC were closer to the other human MSC than to hESC.

2 Materials and methods

2.1 Cell cultures

Human BM-MSC, ADSC, UVSC, SynoSC (all from human tissues) and hESC were cultured in optimal conditions to preserve differentiation potentials (Table 1 of Supporting Information). Cells were cultured in different laboratories as follows: P. Charbord and H. A. Papadaki for BM-MSC, L. Casteilla for ADSC, R. Oostendorp for UVSC, C. Jorgensen for SynoSC, J. Hatzfeld for hESC and K. Boumediene for primary human chondrocyte. The presence of the common CD cell markers was confirmed in each laboratory by FACS analysis (Table 1). Each laboratory collected the cell using the same batch of lysing solution and sent the extracts in dry ice for proteomics analysis as described below.

2.3 Whole cell extract

To improve the reproducibility of the analysis, protein extraction was performed directly in the culture dishes. Briefly, the cells were washed twice with PBS containing a cocktail of protease inhibitors (P8340, Sigma) then lysed directly in the culture dish with 200 μL of lysing buffer (8 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT). The cell lysates were collected and stored at −80°C until use. Protein concentration of the lysates was determined using the Plus-One 2-D Quant Kit (GE Healthcare) following the supplier's recommendations.

2.4 2-DE

Proteins were separated on the bases of two dimensions: p*I* and masses. For the first dimension, proteins were added to 250 μL of rehydration buffer (9.8 M urea 4% CHAPS 50 mM DTT and 0.5% IPG buffer 3–10). IPG strips (13 cm), covering a pH range of 3–10 were rehydrated with this solution during 9 h at 20°C covered by low viscosity paraffin oil. For focalization, the following voltage/time profile was used: 200 V for

Table 1. Phenotypes of the stem cells

	BM-MSC	ADSC	SynoSC	UVSC	hESC
CD90	++	++	++	++	++
CD73	++	++	++	++	−
CD105	++	++	++	++	−
CD44	++	++	++	++	++
CD106	++	−	++	−	−
CD45	−	−	−	−	−
CD34	−	++	−		−
CD56	−	+/−	−	++	−
CD31	−	−	ND	−	−
CD166	++	++	++	++	++

Expression of various markers was tested by flow cytometry (++ strongly positive, + positive, − negative, ND not done).

1 h, 1000 V for 1 h, 3000 V for 1 h, a gradient between 3000 and 8000 V during 2 h and 8000 V for 5 h. A total of 76 000 V·h was used. Focused strips were frozen at −20°C. For the second dimension, strips were equilibrated for 30 min in 6 M urea, 30% glycerol, 2% SDS, 50 mM Tris pH 8.8, 1% DTT and then for an additional 30 min in the same solution except that DTT was replaced by 5% iodoacetamide. After equilibration, proteins were separated in the second dimension by an SDS-PAGE method using 12% acrylamide gel with an acrylamide/bisacrylamide ratio of 37.5:1.

To optimize the 2-DE, different quantities of proteins *per* gel were tested (10, 20 and 30 μg). An amount of 20 μg was selected since it resulted in the best resolution (total number of spots detected) and the best reproducibility.

2.5 Silver staining and scan

Gels were stained with a silver nitrate procedure [19] 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 Ref: R020123). Spot detection and quantitation were performed with ImageMaster 2-DE Platinum software (GE Healthcare) and Progenesis SameSpot (Non Linear). The volume of each spot (integrated OD) was calculated as the product of spot area and spot intensity. To take into account experimental variations, 2-D gels were normalized to the volume of all spots detected on the 2-DE gel.

2.6 Identification of protein expression and statistical test

The quantification was performed using Progenesis SameSpot by an integration of the grey level of each spot and normalization with the total spot volume. In this method, the volume of each spot is divided by the total volume of all of the spots in the gel.

Three to five replicates with independent samples were performed to ensure reproducibility of the results. For comparison, the BM-MSC gel was used as reference.

Statistical analysis was performed using the Hierarchical Clustering Explorer 3.0 software [20], with an average linkage hierarchical clustering algorithm, using the centred Manhattan Distance coefficient as the similarity metric. Differential function analysis was performed using the Ingenuity Software. A fold increase ratio of 1.7 and a *p*-value of 0.05 calculated by Progenesis SameSpot were used to define the limit of significance.

Data were analysed through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). The functional analysis identified the biological functions and/or diseases that were most significant to the dataset. Genes from the dataset that met the differential value cut-off of 1.7 and a *p*-value cut-off of 0.05 and were associated with biological functions and/or diseases in the Ingenuity Pathways Knowledge Base were considered for the analysis.

Fischer's exact test was used to calculate a *p*-value determining the probability that each biological function and/or disease assigned to that data set is due to chance alone.

2.7 Molecular weight and iso-electric point calibration

The experimental molecular weight and pHi were obtained using a 2-DE protein standard (BioRad, Richmond, CA, USA): an additional 2-DE gel was performed with a mixture of 10 μg of whole MSC extract and 5 μL 2-DE standard. Additional spots were positioned and annoted with their respective MW and p*I*. After a step of comparison with this gel, the software calibrates all homologous gels with pHi and MW.

2.8 Protein isolation and identification by MS

Protein spots were excised from coomassie stained 2-DE gels containing 300 μg of proteins. Spots were washed in 15 μL of 100 mM NH_4HCO_3 during 10 min. After addition of 15 μL of ACN for 10 min, supernatants were removed and the procedure repeated. After evaporation to dryness in a benchtop SpeedVac, spots were rehydrated in 10 μL of trypsin solution (15 ng/μL, Promega) and digested overnight at 25°C in 10 μL of 100 mM NH_4HCO_3 5 mM $CaCl_2$ buffer. The tryptic peptides were extracted in a two-step procedure: the first step was composed by an addition of 10 μL of 100 mM NH_4HCO_3 followed by 10 min of 10 μL ACN. This step was repeated twice and supernatants pooled. The second step was a 10 min incubation with 10 μL of 5% formic acid and followed by an addition of 10 μL of ACN for 10 min. This step was repeated twice and the supernatants pooled. Samples were evaporated to dryness and put back in solution in 20% formic acid and desalted on Millipore ZipTip C18 column.

Peptide masses were determined in the positive-ion reflector mode in an Ultraflex mass spectrometer (Bruker). Peptide mass fingerprints (PMFs) were compared to mammalian databases (Swiss-Prot and Trembl) using MASCOT (http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=PMF) and Aldente (http://www.expasy.org/tools/aldente/) algorithms with one missing trypsin cleavage site and a mass deviation smaller than 20 ppm. The probabilistic score of MASCOT/Aldente was required for the identification of an unnamed protein.

3 Results

3.1 CD phenotypes of stem cells

All stem cells were cultured in optimal conditions using adapted media (Table 1 of Supporting Information) to pre-

serve the multipotency of human MSC and the pluripotency of hESC. The ability to differentiate into adipocyte, osteocyte and chondrocyte lineages was experimentally confirmed before proteomic and phenotypic analysis (data not shown). The classical human MSC phenotype (CD90+ CD73+ CD105+ CD44+ CD45−) was validated for BM-MSC, ADSC, SynoSC and UVSC (Table 1). As expected, hESC had a distinct phenotypic pattern with the absence of CD73 and CD105 while some markers were cell specific: ADSC expressed CD34 whereas UVSC lacked CD106.

3.2 Construction of a human MSC 2-DE master gel

Following the optimization of sample preparation and 2-DE procedures (see Section 2), the primary human BM-MSC were used to generate a human MSC master gel (Fig. 1). Since calculated pHi and MW are not reliable parameters following 2-DE, the master gel was calibrated using purified proteins. After silver staining a total of 845 spots was detected (Table 3 of Supporting Information). The pHi distribution of the spots was trimodal, with a maximum around 4.75, 6.25 and 8 while the MW distribution showed that most of the spots were detected between 30 and 40 kDa (Fig. 1 of Supporting Information). Altogether, 231 spots were identified using PMF, representing 164 different proteins, 25% of the total number of spots and 34% of the total volume detected (Table 2 of Supporting Information). Cytoplasmic and cell membrane proteins were identified, as well as proteins from mitochondria and nucleus. Post-translationally modified proteins (which were detected as multiple spots) belonged mainly to cytoplasmic and ER compartments, rather than to the mitochondrion and the nucleus. Identified proteins were involved in a large range of functions including: signal transduction, intracellular trafficking, DNA associated proteins, glycolysis, metabolism, catabolism, folding, biosynthesis and splicing (Table 2 of Supporting Information).

3.3 2-DE of the different stem cells and statistical analysis

Extracts from the different cell lines were used to carry out 2-DE, as illustrated in Fig. 2. Following a thorough analysis using Progenesis SameSpot (Table 3 of Supporting Information) and the Hierarchical Cluster Explorer software, different stem cell proteomic clusters were detected (Fig. 3). Manhattan distance statistical tests indeed revealed the presence of two main clusters: one including BM-MSC, ADSC, SynoSC and UVSC; the second one being composed

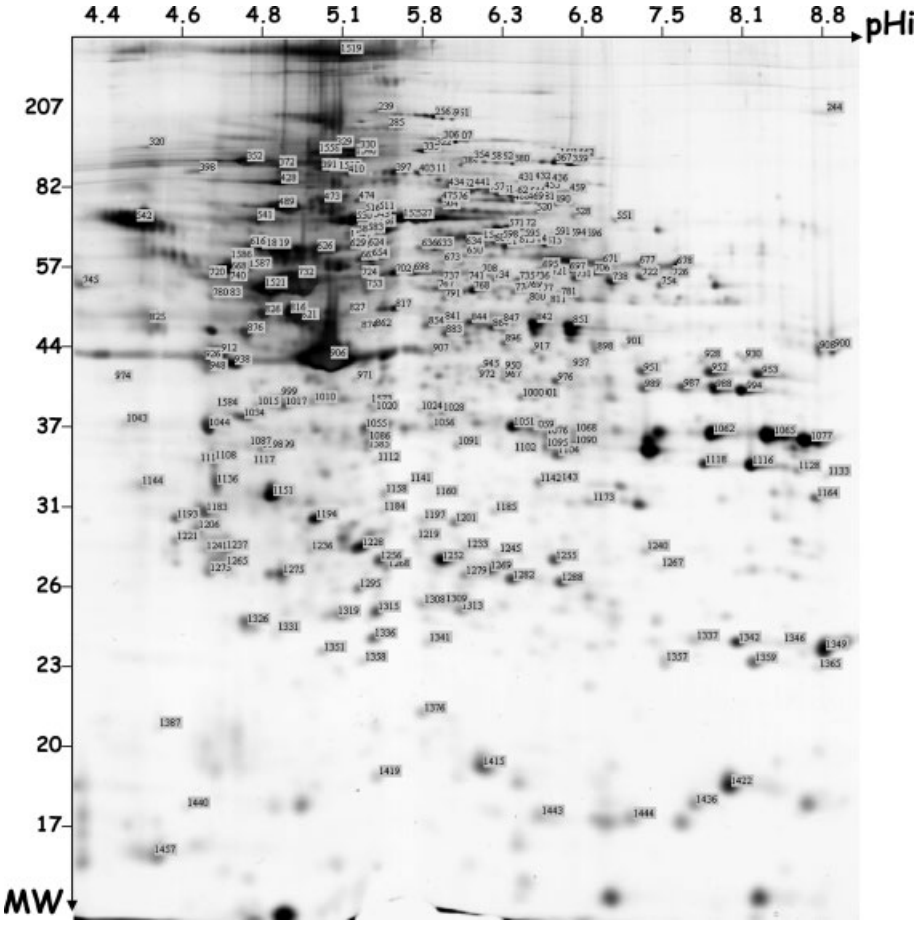

Figure 1. 2-DE master gel of BM-MSC. Human adult primary BM-MSC were separated using 2-DE electrophoresis in a dry strip pH 3–10 for the first dimension, a 12% SDS-PAGE for the second dimension and silver stained. The identification of proteins, noted with their serial number, was performed by PMFs after trypsin digestion and MALDI-TOF on Coomassie-stained spots.

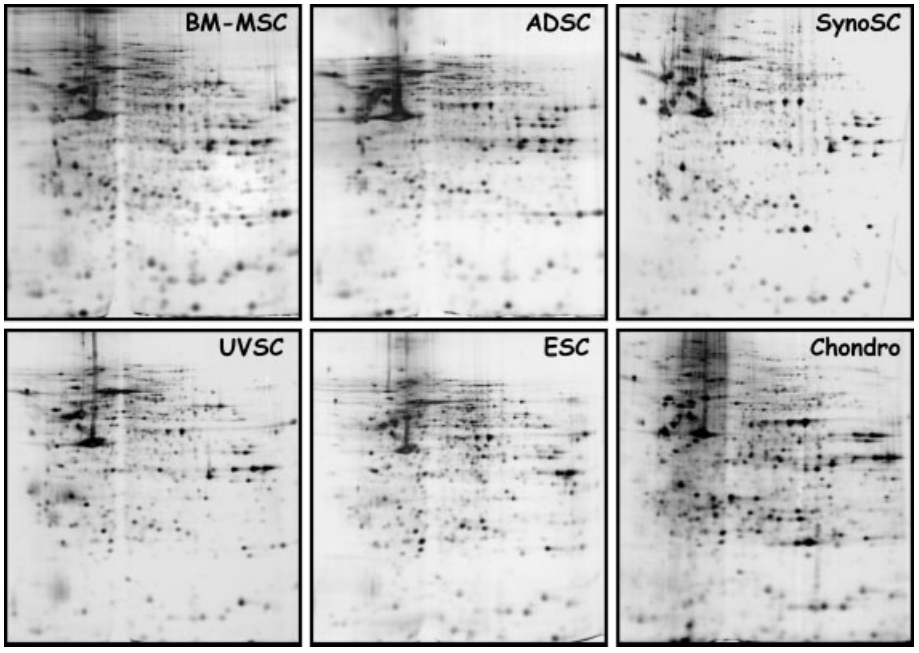

Figure 2. Representative 2-DE gels of the different cultures. Gels were obtained from BM-MSC, ADSC, UVSC, SynoSC, hESC and primary human chondrocyte cultures. Whole cell extracts (30 μg) were separated using 2-DE electrophoresis in a dry strip pH 3–10 for the first dimension and a 12% SDS-PAGE for the second and silver stained. The gel is representative of 3–5 gels from independent cultures samples performed before statistical analysis.

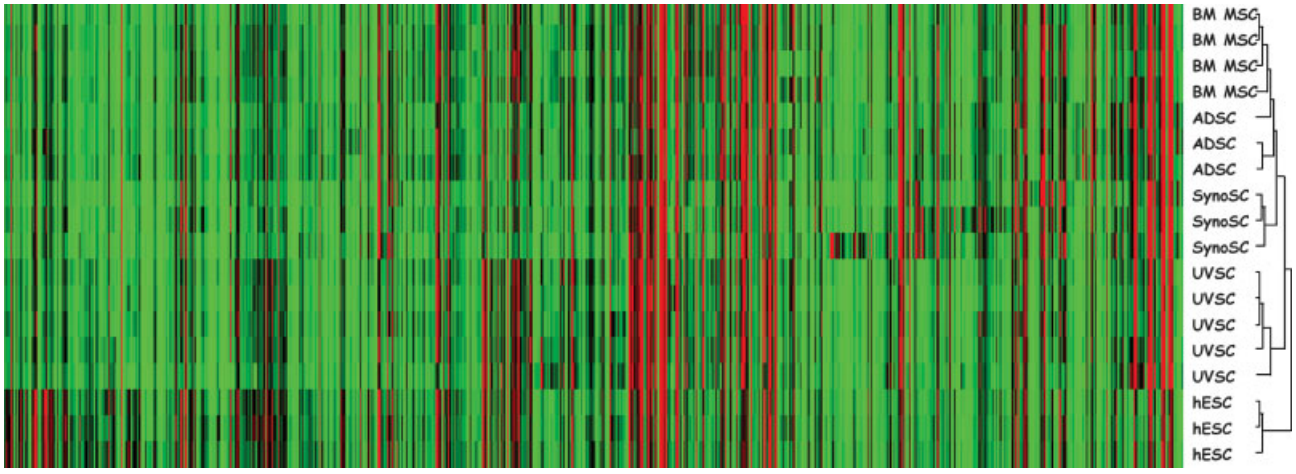

Figure 3. Hierarchical clustering of the 2-DE data. Data from BM-MSC, ADSC, UVSC, SynoSC and hESC have been generated by comparison, quantification and normalization of the different 2-DE gel using Progenesis SameSpot. Clustering was performed using the Hierarchical Clustering Explorer version 3 software with Manhattan Distance as statistical test.

of hESC. Interestingly, BM-MSC and ADSC were very closely related in this analysis while 'adult' stem cells including BM-MSC, ADSC and SynoSC could be segregated from 'foetal' UVSC.

The variability of the spots between replicates for most stem cells was close to 30%, which was satisfactory. Spots with significant modified expression were selected based on an increase or decrease expression by a factor of 1.7 or a *p*-value inferior at 0.05. The *p*-value was calculated by two methods, *t*-test in order to compare each type of cells to BM-MSC and ANOVA to compare all stem cells together. The percentage of spots with a significant modification of expression, when compared to BM-MSC, were 22, 40, 22 and 48 for UVSC, SynoSC, ADSC and hESC, respectively. When limited to identified proteins, the numbers were 16, 25, 14 and 46% showing that the sampling for identification was representative of all the spots detected in the gels.

To illustrate differences in proteomic patterns, the number of common and significantly different spots between BM-MSC, ADSC and SynoSC, as well as, between BM-MSC, UVSC and hESC were computed (Fig. 4). From this general analysis, BM-MSC, ADSC and SynoSC appeared very close with an high percentage of common spots (68.5%) and a low number of cell specific ones (BM-MSC: 5.5%, ADSC: 4%, SynoSC: 14.2%). BM-MSC and ADSC had also more spots in common (98 + 336) than all the other MSC related cells.

When BM-MSC were compared to UVSC and hESC, a high number of spots was present in only one cell type (60 for BM-MSC, 38 for UVSC and 169 for hESC). As expected, hESC were more apart than BM-MSC, ADSC and SynoSC. Importantly, these differences for detected spots were also analysed at the level of identified proteins. It was then possible to group proteins that were specific for MSC-like cells (common to BM-MSC, ADSC and SynoSC) versus hESC (Table 2).

This selection was the basis for a proposed proteomic signature of human MSC (see Section 4). In order to evaluate the specificity of this signature, we compared the 2-D expression of the selected proteins in MSC and in human primary chondrocytes. Proteins which are different between these two types of cells are indicated by a star in the Table 2 and shown in Table 4 of Supporting Information.

3.4 Functional block analysis

To analyse in more details proteomic differences, cell functions were associated to identified proteins using the Swiss-Prot (http://www.expasy.org/) and the Gene Ontology (http://www.geneontology.org/) databases (Table 2 of Supporting Information). The percentage of proteins with a differential expression between BM-MSC and ADSC, SynoSC, UVSC or hESC is plotted in different functional blocks (Fig. 5). hESC were clearly distinguishable from the other cells in particular for function related to 'energy and ROS' and 'metabolism'. In the group 'protein synthesis and proteolysis' that was represented by 61 different proteins, both SynoSC and hESC had a distinct pattern which was in agreement with the clustering analysis (Fig. 3). The Ingenuity software (www.ingenuity.com) which is able to perform a Fisher statistical comparison between functional blocks was also employed. A dataset containing proteins identifiers and corresponding expression values was uploaded into the application. Each protein identifier was mapped to its corresponding object in the Ingenuity Pathways Knowledge Base. A fold increase cut-off of 1.7 and *p*-value cut-off of 0.05 were set to identify genes whose expression was significantly differentially regulated. These proteins, called focus proteins, were overlaid onto a global molecular network developed from information contained in the Ingenuity Pathways Knowledge Base. Networks of these focus proteins were then algorithmically generated based on their connectivity. The analysis identified clear functional differences between the cell types (Fig. 6). SynoSC and hESC appeared the distant from other cells. This was particularly apparent for the 'carbohydrate metabolism', 'amino acid metabolism' and 'cell death' groups for the hESC and for the 'PTM' and the 'protein folding' groups for the SynoSC. In the latter cells the expression of TCP-1 α, β, ε, HSP70 and HSP90 was remarkable.

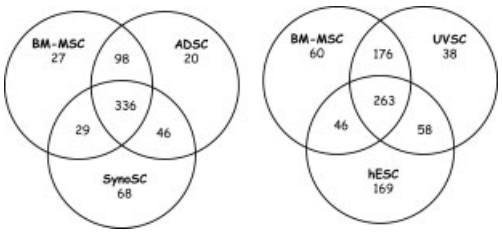

Figure 4. Common and significantly different 2-DE proteins. The numbers correspond to the spots of proteins from the master gel in common or significantly different between BM-MSC, ADSC and ECS (left panel) and BM-MSC, UVSC and SynoSC (right panel).

4 Discussion

In this study, cultured undifferentiated human MSC isolated from BM-MSC, UVSC, ADSC and SynoSC were analysed by 2-DE. The first task was to generate a comprehensive proteomic description of human primary BM-MSC. These cells were then compared with the other 'MSC like' cells and with pluripotent hESC. The main goal of this study was to link the proteomic patterns of the cells to tissue origin and phenotypic properties. This was a way to figure out if all sources of MSC were equivalent, and if a specific human MSC proteomic signature could be generated (Table 2).

While the first proteomic analysis of MSC was performed and published in 2001 by Prockop and coworkers [10], not many studies explored in detail the whole proteome of MSC cells (for review see ref. [18]). Based on the published articles, it was not possible to identify a clear proteomic signature specific for MSC. One of the reasons was the different paradigms, protein extraction protocols and analytical approaches used in the different studies. Through the European Genostem Consortium (www.genostem.org), it was possible to generated proteomic samples from MSC isolated from various tissues using an optimized standard protocol (medium, extraction buffer and procedure). The cells used as reference in this study were human BM-MSC since they were the most commonly used MSC. To facilitate proteomic comparisons, a BM-MSC master gel was constructed with an important number of proteins identified by MS. The later were distributed in a broad range of pHi (Fig. 1 of Supporting Information), belonged to most cellular compartments (cytoplasm, nucleus, mitochondrion, reticulum, golgi and membrane) and represented a wide range of functions (from glycolysis to splicing and signal transduction) (Table 2 of Supporting Information). The list of identified proteins was consistent with previous proteomic investigations with the following common proteins identified: annexin V, TCP1 α, lactate dehydrogenase B, phosphoglycerate kinase 1 [10], programmed cell death 6 interacting protein, peroxiredoxin 2, glucose-6-phosphate 1-dehydrogenase, pyruvate kinase M1/M2 isozyme, annexin A2 [21] or hnRNP A2/B1 [12, 22, 23]. Importantly, these proteins were originally identified following differential studies in subpopulations of MSC, whereas in our case they were expressed in BM-MSC.

Table 2. List of identified proteins with differential expression BM-MSC and ADSC by comparison with hESC

ID	Protein	Accession no.	Fold ratio	*t*-Test (*p*)
505	Adenine phosphoribosyltransferase	P07741	2.5	0.001
623	Adenylyl cyclase-associated 1	Q01518	−1.5	0.008
529	Adenylyl cyclase-associated 1	Q01518	2.2	0.002
758	*Annexin A1	P04083	−1.8	0.005
567	*Annexin A1	P04083	−1.3	0.231
912	*Annexin A2	P07355	−3.5	0.001
606	Annexin A2	P07355	−5.4	<0.001
125	ATP synthase D	O75947	2.3	<0.001
445	*Caldesmon	Q9NYG1	−2.6	0.010
926	*Caldesmon	Q9NYG1	−3.3	0.001
625	*Caldesmon	Q9NYG1	−2.2	0.028
956	*Caldesmon	Q9NYG1	−3.1	0.001
569	Calreticuline	P27797	−2.4	0.007
977	Calumenin	O43852	−2.7	0.003
980	*Chloride intracellular channel protein 1	O00299	−1.4	0.006
160	Chloride intracellular channel protein 4	Q9Y696	−1.9	0.007
976	Cytosol aminopeptidase	P28838	1.9	0.007
561	EF-1-δ	P29692	−2.2	0.001
536	EF-1-δ	P29692	−1.8	0.002
292	EF-1-δ	P29692	−1.0	0.981
297	Elongation factor τ	P49411	2.6	<0.001
626	F-actin capping protein β	P47756	2.0	0.001
953	F-actin capping protein β spot1	P47756	1.4	0.011
80	Fumarate hydratase	P07954	2.3	0.005
332	FUSE-binding protein 1	Q96AE4	2.7	0.002
402	FUSE-binding protein 1	Q96AE4	4.0	<0.001
366	*FUSE-binding protein 2	Q5U4P6	2.9	<0.001
409	FUSE-binding protein 2	Q5U4P6	2.3	0.049
512	FUSE-binding protein 2	Q5U4P6	2.3	0.085
601	Glucose-6-phosphate 1-dehydrogenase	P11413	1.6	0.049
589	Glucose-6-phosphate 1-dehydrogenase	P11413	1.9	<0.001
532	GST P	P09211	2.7	0.001
494	GST P	P09211	1.4	0.005
461	Glutathione transferase ω-1	P78417	1.2	0.135
603	Glutathione transferase ω-1	P78417	−1.1	0.646
866	hn RNP L	P14866	1.5	0.369
503	hn RNP L	P14866	2.4	0.002
538	hn RNP L	P14866	4.7	0.002
885	hnRNP H	P31943	2.3	0.002
562	HSP27 β	P04792	−1.8	0.013
580	*HSP27 β	P04792	−2.6	0.007
519	*Inorganic pyrophosphatase	Q15181	3.3	<0.001
757	Iron-responsive element-binding 1	P21399	1.9	0.114
848	*Isocitrate dehydrogenase [NADP]	O75874	4.2	0.005
616	Lactoylglutathione lyase	Q04760	3.5	<0.001
975	L-lactate dehydrogenase B	P07195	2.0	0.002
386	Microtubule-associated protein 1B	P46821	−1.8	0.047
257	Nucleophosmin	P06748	2.0	<0.001
376	Peroxiredoxin 4	Q13162	−1.1	0.634
476	Peroxiredoxin 6	P30041	1.9	0.002
583	Peroxiredoxin 1	Q06830	2.4	<0.001
802	Peroxiredoxin 2	P32119	2.5	<0.001
57	*Poly(RC) binding protein 1	Q15365	2.6	<0.001
573	Poly(RC) binding protein 1	Q15365	1.5	0.033
314	Poly(RC) binding protein 2	Q68Y55	2.0	0.058
984	Proliferating cell nuclear antigen	P12004	2.1	0.001

Table 2. Continued

ID	Protein	Accession no.	Fold ratio	*t*-Test (*p*)
493	Proteasome subunit α type 1	P25786	1.9	0.010
398	Proteasome subunit α type 3	P25788	1.4	0.027
944	Protein disulphide isomerase A1	P07237	−2.5	<0.001
619	Pyruvate dehydrogenase E1	P11177	1.9	0.004
819	Ras-related protein Rab-11A	P62491	2.6	0.003
400	Stomatin-like protein 2	Q9UJZ1	2.1	0.002

* Indicate proteins with differential expression in human primary chondrocyte.

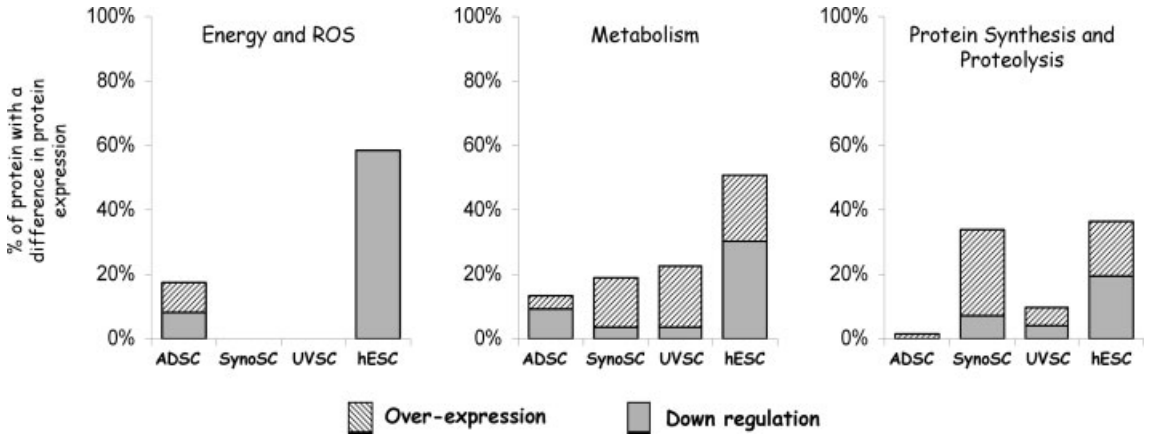

Figure 5. Functional block analysis. Proteins with differential expression (up- or downregulated) were distributed between functional blocks using the Swiss-Prot (http://www.expasy.org/) and the Gene Ontology (http:// www.geneontology.org/) databases. Three main blocks were represented: proteins involved in 'energy and ROS' (left panel, 12 protein spots), in 'metabolism' (middle panel, regrouping 54 proteins involved in 'glycolysis and carbohydrate metabolism', 'metabolism and catabolism' and 'synthesis of metabolites'), and in 'protein synthesis and proteolysis' (right panel, 54 proteins spot, contains also the 'folding and heat shock protein' block).

One important goal of this study was to use proteomics to compare MSC isolated from different tissues. Since the different types of culture were provided by different laboratories, biases were reduced by using in all places the same batches of medium, the same procedures and buffers for the preparation of the proteomic extracts. The heterogeneity related to the fact that the cultures were produced in different laboratories was therefore minimized and almost irrelevant since the cell types (ADSC, UVSC, *etc.*) were anyway generated from different origins and cultured in different conditions (Table 1 of Supporting Information). The different culture methods ensured the preservation of MSC characteristics in terms of differentiation properties (osteoblastic, adipocytic and chondrogenic pathways; data not shown) and expression of major phenotypic markers (Table 1). As an additional control of the impact of the production of the cells in different laboratories, BM-MCS produced in different participating laboratories were indistinguishable (data not shown and [24]).

Although BM-MSC, ADSC, SynoSC and UVSC share common capacities of differentiation into osteoblast, chondrocyte and adipocyte, several studies have already demonstrated differences between these cells in terms of proliferating rate, senescence or differentiation capability in specific pathways [4, 13]. Importantly, while CD expression patterns (Table 1) were not sufficient to differentiate them, our global proteomic approach could clearly achieve this goal (Fig. 3). Our data also suggested that among MSC related cells, ADSC were the closest match to BM-MSC, while the UVSC were the more distant. This conclusion was not only based on the clustering experiments, but also on the functional block analysis and on the number of proteins with differential expression among cells (Figs. 4–6). Our result gives additional basis for the use of ADSC as an alternative to BM-MSC for regenerative medicine, these two types of cells being very close in comparison with the others. It appeared also in the functional analysis (Fig. 6), that SynoSC were quite distant to BM-MSC which was also a conclusion of the genomic analysis of these cells [4].

We also compared MSC cells to hESC. The latter were cultured in a specific medium without the use of feeder cells to facilitate the proteomic analysis (Table 1 of Supporting Information). As mentioned above, differences in culture media could be responsible for proteomic differences, but cannot be avoided to compare cells with various physiologi-

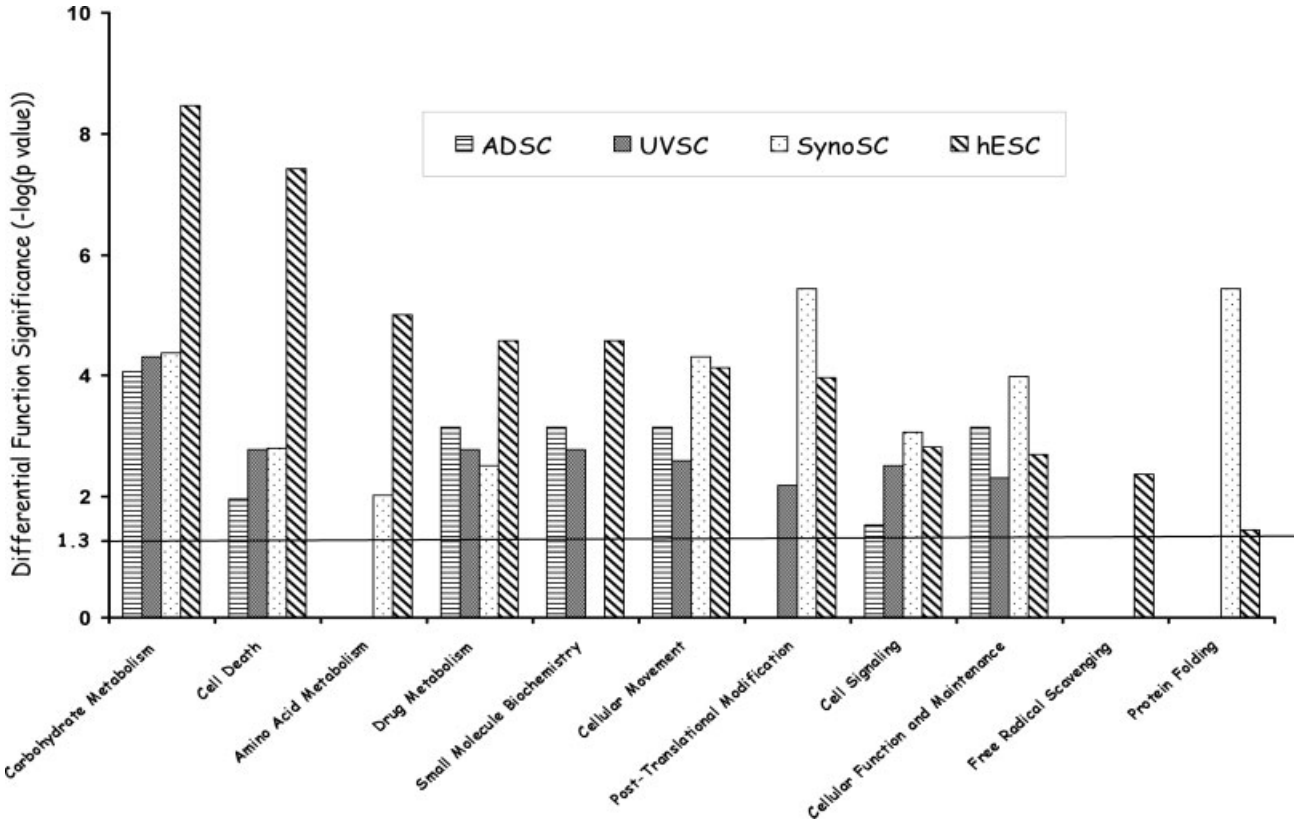

Figure 6. Statistical analysis of differential function. The Ingenuity Software was used to identify differential functions between the various stem cells. In the *y*-axis are plotted the statistical values (as minus log of *p* value) obtained from the comparison with the BM-MSC reference. In the *x*-axis are indicated the different functional networks defined by the Ingenuity analysis.

cal characteristics and phenotypes. Global or functional analysis (Figs. 3–6) revealed that hESC were clearly distinct from BM-MSC, and from the other cell lines studied. BM-MSC, ADSC, SynoSC and UVSC could therefore be regrouped in an 'MSC-like group' with the UVSC being the closest to hESC; this might be related to their foetal origin. The Ingenuity software analysis, which represents an original approach to construct networks of function and calculate a *p*-value related to their modification, confirmed this analysis pointing out differences in particular in the 'carbohydrate metabolism', 'amino acid metabolism' and 'cell death' functional blocks (Fig. 6). The latter group was interesting since it is in relation with stem cell maintenance, self renewal and differentiation during the development [25]. Some individual proteins in this pathways (Table 2) have already been pointed out in others differential proteomic studies. CLIC4 a mitochondrion chloride channel involved in myc induced apoptosis [26], was already identified as having a lower expression in hESC and in TERT-modified MSC [23]. The same observation might be done for annexin A1, A2 which were downregulated in TERT-modified MSC [23] and in our results compared with hESC. In addition, elongation factor TU, peroxiredoxin 1 and 6 were upregulated in both TERT-modified MSC [23] and in hESC. This ingenuity-based analysis renders possible the participation of proteins to different functional blocks which makes sense knowing the multiple relations and functions of proteins.

To progress towards the definition of a human MSC signature, proteins whose expression was similar in BM-MSC, ADSC and SynoSC, but significantly different in hESC, were selected (Table 2, and Tables 2–4 of Supporting Information). Proteins from most functional blocks were present in this selection which suggested that differences between stem cells were more global than focussed. In this list, we selected several proteins which could define a small panel of proteins specific for MSC and hESC. Importantly, we could validate this list by selecting the proteins (Table 2, indicated by a star) which were with differential expression in human primary chondrocyte. Overall, we could select as markers of MSC: annexin A1 and A2 (ANXA1 and ANXA2) and HSP27 β (HSPB1). For hESC, elongation factor Tu (TUFM), isocitrate dehydrogenase (IDH1) and the peroxiredoxin 1, 2, and 6 (PRDX1, PRDX2, PRDX6) were the most interesting ones. These proteins are likely to be involved in the different phenotypes and capacities for differentiation.

In conclusion, the proteomic investigation of human mesenchymal and embryonic stem cells realized in this study is complementary to previous proteomics and geno-

mics works [18]. The challenge in these broad types of approaches is to identify, in the huge amount of data generated, relevant differences and similarities that will help characterizing stem cells and linked phenotypes with molecular markers. The generation of a master 2-D gel from human BM-MSC, combined with the analysis of the other stem cells adds a lot to the general proteomic knowledge of MSC. In this work, we demonstrated that a proteomic 'MSC-like' profile can be defined. Interestingly, umbilical vein wall stem cells, a neo-natal cell type, belonged to this group, and were closer to MSC than to hESC. In addition, we confirmed that BM and adipose tissue derived cells are very similar which argue for their interchangeable use in therapy. Finally, we proposed a panel of proteins which is the basis for the definition of a human MSC proteomic signature that would need further validation on additional cell types.

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