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Similar proteomic profiles of human mesenchymal stromal cells from different donors

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Background aims

Bone marrow (BM) stromal cells, also referred to as mesenchymal stromal cells (MSC), can be expanded ex vivo and are able to differentiate along multiple lineages, including chondrocytes, osteoblasts and adipocytes. MSC are known to secrete a number of cytokines and regulatory molecules implicated in different aspects of hematopoiesis, and seem to modulate the immune system. MSC appear to be promising candidates for cellular therapy associated with BM transplantation (BMT).

Methods

We compared protein expression profiles of MSC cultures derived from different BM donors using two-dimensional (2-D) gel electrophoresis and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) tandem mass spectrometry (MS/MS), and compared mixed lymphocyte reaction (MLR) assays in the absence and presence of third-party human (h) MSC derived from different donors during the same culture passage.

Results

In a window of observation (pH 4–7, molecular weight 10–220 kDa), about 172 protein spots were obtained in each 2-D gel, corresponding to 84 distinct proteins. A comparative analysis demonstrated a very similar proteomic profile of cells of the first passage derived from different donors, suggesting that these cells have the same expression pattern. Additionally, cells derived from different donors were equally able to inhibit lymphocyte proliferation.

Conclusions

These results encourage the use of third-party MSC in cellular therapies, as cells derived from different individuals seem to have the same proteomic pattern and exhibit functionally similar properties.

Keywords

Bone marrow MSC, bone marrow transplantation, cellular therapy, mesenchymal stromal cells, proteomic analysis, third-party MSC.

Introduction

Bone marrow (BM) stromal cells contain a subset of heterogeneous mesodermal progenitor cells called mesenchymal stromal cells (MSC) that are difficult to isolate and characterize as unmanipulated cells *ex vivo* [1]. In contrast, they can be easily expanded in culture from small aspirates of BM, and are able to differentiate along multiple lineages such as chondrocytes, osteoblasts and adipocytes [2]. In the BM microenvironment, these cells supply an appropriate scaffold for hematopoiesis [3]. Several studies have demonstrated that MSC are immunoprivileged and seem to modulate the immune system [1,4,5].

MSC are promising candidates for cellular therapy associated with BM transplantation (BMT). Both fetal and adult human (h) MSC promote hematopoietic engraftment in immunodeficient mice [6–8] and fetal sheep [9,10]. Several authors have tried to exploit MSC to facilitate the engraftment of hematopoietic stem cells (HSC) and reduce graft-versus-host disease (GvHD) [11–15]. These studies have demonstrated that introducing culture-expanded MSC together with allogeneic stem cell transplantation (ASCT) is a safe procedure and that MSC hold promise for the enhancement of hematopoietic engraftment with a low probability of severe acute GvHD and infections [15].

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A relevant question is third-party use of hMSC in therapeutic protocols. The immunosuppressive function of third-party hMSC has been demonstrated to be more effective than autologous MSC, when both alloantigen-induced lymphocyte proliferation and alloantigen-specific cytotoxic activity were analyzed [16]. This finding encourages the use of third-party MSC for the prevention of immune complications related to HSC transplantation. Pre-clinical data also suggest that unrelated, human BM-derived, culture-expanded hMSC may improve the outcome of allogeneic transplantation by promoting hematopoietic engraftment and limiting GvHD [17]. However, neither the putative molecular variations nor the immunomodulatory capacity of hMSC derived from different individuals have been characterized.

Recently, genomic and proteomic studies have emerged as a powerful method for performing quantitative and qualitative analysis of the molecular profile of hMSC cultures. Several reports have employed proteomic strategies to characterize hMSC and their differentiated progenies [18–22]. Taken together, these studies have contributed to our knowledge about the proteome of hMSC populations in culture, and represent a useful inventory. This inventory facilitates the identification of the normal proteomic pattern, as well as changes in activated or suppressed pathways [23].

Because of the interest in the use of third-party hMSC in BM transplants to reduce GvHD, a relevant issue in this field is whether hMSC cultures derived from different donors, which are able to inhibit lymphocyte proliferation *in vitro*, have the same proteomic profile. Therefore, to improve the characterization of hMSC and compare cells derived from different BM donors, we performed a comparative proteomic analysis of hMSC cultures from first passages. In this approach, we used two-dimensional (2-D) electrophoresis (2-DE) and mass spectrometry analysis (MS/MS) in a specific window of observation (pH 4–7 and molecular weight 10–220 kDa).

We obtained about 172 protein spots in each 2-D gel, corresponding to 84 distinct, identified proteins. Forty of them were described for the first time in proteomic analysis, increasing the current hMSC protein profile. Our results showed a very similar proteomic profile in cells derived from different individuals, and these cells were able to inhibit lymphocyte proliferation in a similar manner. The results reinforce the possibility of using these cells in third-party cellular therapy associated with BM transplants.

Methods

Isolation and culture of human BM-derived MSC

All BM samples were obtained from healthy donors registered at the Bone Marrow Transplantation Unit, National Cancer Institute (Rio de Janeiro, Brazil), following the guidelines of the local ethics committee and the Helsinki declaration. BM aspirates were obtained from 37 male donors (mean age 40.3, range 30–59) and 13 female donors (mean age 40.4, range 32–47). Mononuclear cells (MNC) were isolated from BM samples (5–10 mL) by density-gradient centrifugation (Ficoll 1.077 g/mL; GE, Sweden). They were then plated in non-coated 75-cm^2 polystyrene culture flasks (TPP, Switzerland) at a density of 500 000 cells/cm^2, i.e. 1.5×10^5 cells, in low-glucose Dulbecco's modified Eagle medium (DMEM; Invitrogen, USA) supplemented with 15% fetal calf serum (FCS; Hyclone, USA), 100 IU/mL penicillin, 100 μg/mL streptomycin (Invitrogen) and 2 mM L-glutamine (Invitrogen). Adherent MNC were cultured for 10 days, removed from the plates by 0.05% trypsin (Invitrogen) treatment for 5 min at 37°C, and then replated in another culture flask at a density of 1000 cells/cm^2 (passage 1). After 7 days in culture (when the cells reached 80% confluency, i.e. 5×10^5 cells/150-cm^2 flask), the cells were trypsinized to be characterized by different assays.

Confirmation of multipotentiality of MSC

The ability of hMSC to differentiate into adipocytes and osteoblasts was tested. To induce adipogenic differentiation, hMSC were cultured with 10^{-8} M dexamethasone (Sigma-Aldrich, USA) and 5 μg/mL insulin (Sigma-Aldrich), and Oil Red O (Sigma-Aldrich) was used to detect lipid accumulation. Osteogenic differentiation was induced by 10^{-8} M dexamethasone (Sigma-Aldrich), 10 mM β-glycerol-phosphate (Sigma-Aldrich) and 50 μg/mL ascorbic acid (Sigma-Aldrich). Osteoblasts were identified by Alizarine Red S (Isofar, Brazil) staining.

Mixed lymphocyte reaction

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized samples of unrelated healthy volunteers by density-gradient centrifugation. Mixed lymphocyte reaction (MLR) was performed by incubating 5×10^5 carboxy fluorescein diacetate succinimidyl ester (CFSE)-labeled responder PBMC (R) and 5×10^5 irradiated (2500 cGy) stimulator PBMC (S)/well, in 24-well flat-bottomed tissue-culture plates containing RPMI-1640 (Invitrogen)

with 2 mM L-glutamine (Invitrogen), 100 IU/mL penicillin, 100 μg/mL streptomycin (Invitrogen) and 10% FCS (Hyclone) in the absence or presence of 5×10^4 third-party hMSC at 37°C in a humidified 5% CO_2 atmosphere. After 7 days, cultures were harvested and studied by flow cytometry in a FACScan instrument (Becton Dickinson, USA). Data were analyzed with CELLQuest software (Becton Dickinson), and Student's *t*-test *P*-values < 0.05 were considered statistically significant. Statistical analyzes and graphical representations were performed using GraphPad Prism™ software (GraphPad, USA).

2-D gel electrophoresis

To prepare the protein extracts, cells from the first passage were removed from the culture medium by trypsin treatment, washed three times in phosphate-buffered saline (PBS), centrifuged and resuspended in cold lysis buffer containing 50 mM Tris, pH 7.5, 5 mM ethylene diamine tetraacetic acid (EDTA), 10 mm ethylene glycol tetraacetic acid (EGTA), 50 mM sodium fluoride, 20 mM β-glycerol-phosphate, 250 mM sodium chloride, 0.1% Triton X-100, 20 mM sodium orthovanadate and protease inhibitor mix [2.4 μg/mL 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, 0.1 μg/mL bestatin, 0.1 μg/mL pepstatin, 0.1 μg/mL leupeptin, 1.8 μg/mL E-64; Calbiochem, USA]. After 30 min on ice, the lysates were spun at 12 000 r.p.m. in a centrifuge at 4°C for 30 min. The total protein concentration obtained was determined by Bradford assay and varied from 700 μg to 1000 μg. Seven hundred micrograms of total cell protein extracts were precipitated with a 2-D clean-up kit (GE Healthcare, Sweden) and resuspended in 200 μL of reswelling buffer containing 6 M urea, 2 M thiourea, 15 mM dithiothreitol (DTT), 2% (w/v) ASB14, 0.5% IPG buffer pH3–10L (GE Healthcare) and traces of bromophenol blue. Proteins were rehydrated at 40 V into individual strip holders (GE Healthcare). The first dimensional gel was carried out with 11 cm Immobiline DryStrip pH 4–7 (GE Healthcare) on an Ettan IPGphorIII Electrophoresis Unit (GE Healthcare) for a total of 30550 VhT. IPG strips were equilibrated prior to the second dimension for 15 min in equilibration buffer [6 M urea, 30% (w/v) glycerol, 2% sodium dodecyl sulfate (SDS) in 0.05 M Tris–HCl buffer, pH 8.8, and traces of bromophenol blue] containing 100 mg DTT, and incubated for an additional 15 min in equilibration buffer containing 250 mg iodoacetamide.

IPG strips were run in ExcelGel SDS 8–18% according to manufacturer's protocols (GE Healthcare). ExcelGels were stained with colloidal Coomassie Blue G-250 (Sigma-Aldrich). All gels were scanned with an image scanner with LabScan software (GE Healthcare) and Image Master 2-D Platinum 6.0 software (GE Healthcare), followed by an additional visual analysis. The isoelectrical point (pI) was determined using a linear 4–7 distribution, and molecular weight (MW) determination was based on a Benchmark protein standard (Invitrogen), using a logarithmic curve. Gels were analyzed and averaged separately. For all visualized spots in each gel, the normalized spot volumes were compared and averaged. The intensity of each spot was quantified by calculation of the spot volume after normalization of the image using the total spot volume normalization method multiplied by the total area of all the spots. Comparison reports of qualitative and quantitative differences between the samples were generated. To examine the reliability of the data, only spots showing a greater than 2.0-fold difference between samples were considered differentially expressed.

Mass spectrometry identification

The protein spots were cut from the gel and processed for mass spectrometry according to the following protocol. The trimmed gels were transferred to new 1500-μL capped microcentrifuge tubes and washed three times, 15 min each in 50% acetonitrinile (ACN)/25 mm ammonium bicarbonate (NH_4HCO_3), pH 8.0. They were then soaked in 100% ACN and dried in a Hetovac concentrator (HetoLab Equipment, Denmark) for 30 min. After washing and drying, the gel pieces were rehydrated at 4°C in digestion buffer (25 mM NH_4HCO_3, pH 8.0) containing 15 ng/μL trypsin (sequence grade modified trypsin, porcine; Promega, USA) and then incubated overnight at 37°C. The peptides were subsequently extracted from the gel with 50% ACN/5% trifluoroacetic acid (TFA). All digested extracts were collected and dried in a Hetovac concentrator (HetoLab Equipment). Each dried digest was redissolved in 3 μL 50% ACN/1% TFA. For MALDI MS/MS analysis, 0.5 μL of the redissolved peptide was mixed with fresh α-cyano matrix (Sigma-Aldrich) on a MALDI plate. Mass spectra for peptide mass fingerprinting and confirmatory fragmentation analysis (MS/MS) were acquired using a MALDI-TOF instrument 4700 (Applied Biosystems, USA) in reflector positive ion mode. Peptides in the range of 800–4000 Da were analyzed. Spectra were acquired after plate

calibration with calibration mixture 1 or 2 (Sequazyme peptide mass standard kit; Perseptive Biosystems, USA). Proteins were identified using the Mascot Daemon searching engine (Matrix Science, UK) against the International Protein Index protein sequence database (IPI; version 3.12). Search parameters for MS and MS/MS were 0.2 Da. The criterion for positive protein identification was a minimum of two peptides with a Mascot peptide score of ≥ 20.

Results

In vitro differentiation of hMSC

To confirm that our expanded hMSC cultures maintained the multipotent differentiation characteristic, hMSC at passage 1 were tested for differentiation into adipogenic and osteogenic cells. hMSC cultured in adipogenic medium for 3 weeks showed lipid droplets by Oil Red O staining (Figure 1A), characteristic of adipogenic cells. Osteogenic differentiation was demonstrated by calcium deposition, which was stained by Alizarin Red S (Figure 1B). Undifferentiated hMSC were used as a control (Figure 1C).

In vitro T-cell inhibition mediated by a third-Party MSC

To test the ability of hMSC cultures to inhibit proliferation of lymphocytes driven by alloantigen stimulation, we performed MLR assays in the absence and presence of third-party hMSC. hMSC were added to MLR on day 0 and cells harvested on day 7. As shown in Figure 2A, third-party hMSC from five different donors inhibited the proliferation of lymphocytes in response to alloantigens in 12 independent experiments ($P < 0.0001$). One representative result of this assay is shown in Figure 2B. Analyzes of the results indicated a *c.* 6-fold decrease in cell proliferation when hMSC were added as the third party. These results confirmed the *in vitro* immunomodulatory properties of hMSC cultures derived from different donors.

Proteomic profile similarity of the hMSC population

As our hMSC cultures derived from different donors did present immunomodulation activity, we proceeded to comparative proteomic analysis with the aim of verifying

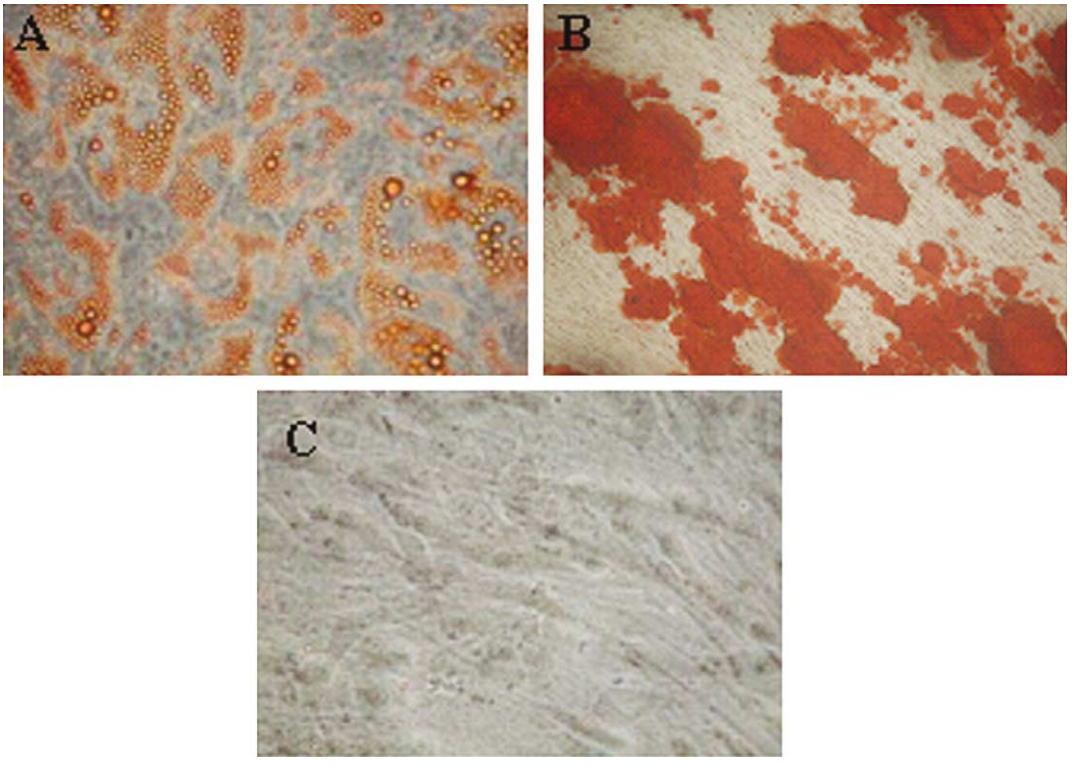

Figure 1*. MSC capacity for differentiation. (A) Adipogenic differentiation was indicated by the accumulation of neutral lipid vacuoles stained with Oil Red O; (B) osteogenic differentiation was indicated by calcium deposition stained with Alizarine Red; (C) undifferentiated hMSC (100 × magnification).*

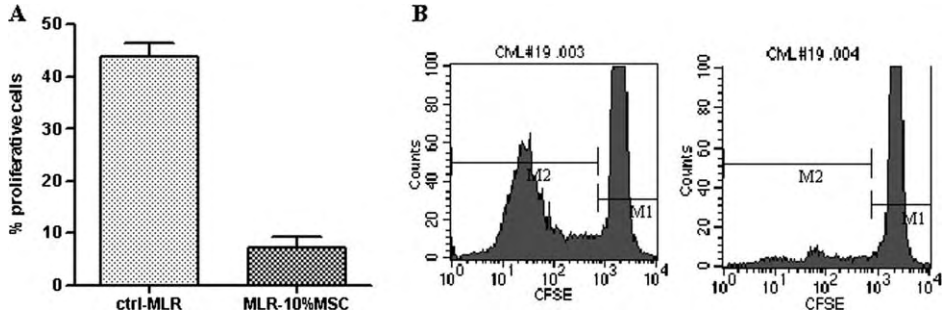

Figure 2. *(A) Inhibition of MLR by third-party MSC. Lymphocyte proliferation was assessed in MLR and performed in the absence (ctrl-MLR) or presence of 5×10^4 third-party MSC/well (MLR-10% MSC). Results are expressed as mean $\pm$ SD for 12 independent experiments ($P < 0.0001$). (B) Representative assay showing MLR in the absence (left panel) or presence of 10% MSC/well (right panel). The histograms show a parental peak (M1) and multiple generations of proliferated cells (M2).*

the hMSC proteomic profiles from first passages. Fifty cultures were established from 50 different donors and 18 of them analyzed and compared in 2-DE gels. One representative gel from two donors is shown in Figure 3. After image analysis was performed, all gels presented 181 ± 10 protein spots on average, showing 94.4% matching. One-hundred and seventy-two protein spots were excised from each 2-D gel and analyzed in the MALDI-TOF instrument. We identified 132 spots (76% identification), corresponding to 84 distinct proteins. The lack of identification observed in this kind of analysis is normally due to post-translational modification, which is frequent in human protein samples, and when insufficient protein concentrations are observed in the 2-D gel. Table I provides a list of the proteins identified, with their respective spot ID, ratio, database number, protein name, theoretical MW/pI, mascot score and percentage of sequence coverage.

The protein identification provided in this work revealed 40 new hMSC-related proteins, contributing to the expansion of the known hMSC proteomic profile. These proteins have been distributed into different classes related to their biologic function (Table I).

In the gel analysis, we observed very high similarity and gel reproducibility, as shown in the scatter plot in Figure 4. Only three proteins were found with an altered abundance ratio greater than 2.0-fold, indicating change in expression (Table I). These results suggested that the protein profile of hMSC cultures derived from different donors is equivalent in this experimental range.

Discussion

During GvHD, the most important side-effect associated with BMT, immunocompetent donor cells attack host tissues and organs [24]. hMSC have been shown to have immunosuppressive properties both *in vitro* and *in vivo* [5], producing cytokines that can support hematopoiesis and potentially enhance marrow recovery [11,12,15,25–27]. On

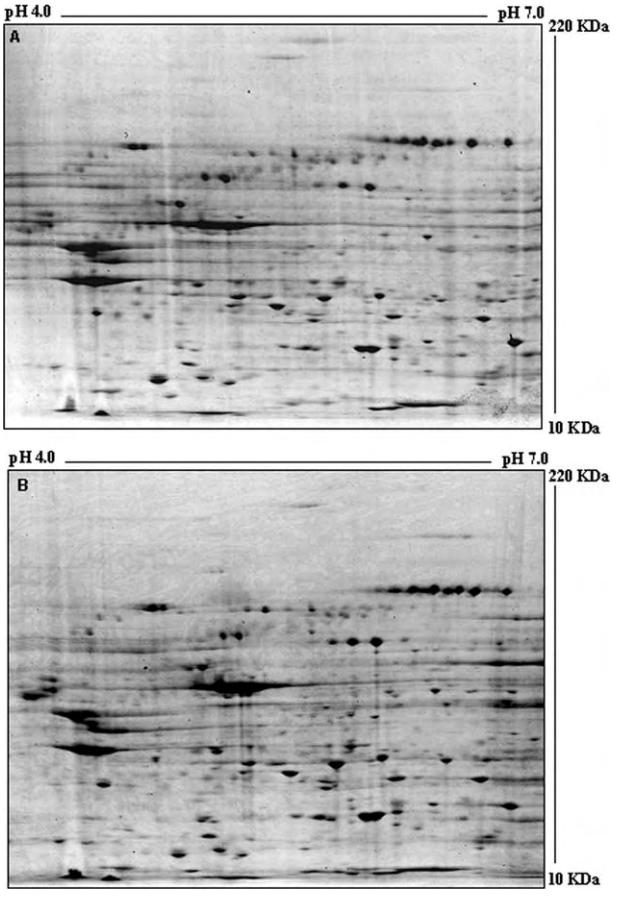

Figure 3. *hMSC proteomic profile obtained with cells on passage 1. (A) 2-DE gel from donor 1 and (B) 2-DE gel from donor 2. Horizontal axes are designated as the pI and vertical axes as the MW.*

Table I. Protein identification

Spot	Ratio*	IPI	Database number, protein name	MW	pI	Score	Coverage (%)
1	1.053 ± 0.026	IPI00013769	ENO1B Alpha-enolase, lung specific	49 845	5.78	36	2
2	1.067 ± 0.032	IPI00465248	ENO1 Isoform alpha-enolase of alpha-enolase	47 481	7.01	196	15
3	1.121 ± 0.057	IPI00216171	ENO2 Gamma-enolase	47 581	4.91	112	7
4	1.060 ± 0.029	IPI00451401	TPI1 Isoform 2 of triosephosphate isomerase	27 451	8.48	218	22
5	1.41 ± 0.12	IPI00465439	ALDOA Fructose-bisphosphate aldolase A	39 851	8.30	100	12
6	1.41 ± 0.12	IPI00418262	ALDOC Fructose-bisphosphate aldolase C	39 830	6.41	68	6
7**	2.320 ± 0.397	IPI00291175	VCL Isoform 1 of vinculin	117 220	5.83	36	3
8	1.372 ± 0.157	IPI00218918	ANXA1 Annexin A1	38 918	6.57	138	11
9	1.689 ± 0.256	IPI00418169	ANXA2 Annexin A2 isoform 1	40 671	8.53	122	16
10	1.458 ± 0.186	IPI00178083	TPM3 29 kDa protein	29 346	4.75	94	10
11	1.364 ± 0.154	IPI00010779	TPM4 Isoform 1 of tropomyosin alpha-4 chain	28 619	4.67	246	14
12	1.066 ± 0.032	IPI00000230	TPM1 tropomyosin 1 alpha chain isoform 2	32 715	4.70	178	14
13	1.036 ± 0.017	IPI00021439	ACTB Actin, cytoplasmic 1	42 052	5.29	245	20
14	1502 ± 0.160	IPI00216691	PFN1 Profilin-1	15 216	8.44	38	20
15	1.072 ± 0.034	IPI00179589	MTPN Myotrophin	13 058	5.27	154	32
16	1341 ± 0.054	IPI00008603	ACTA2 Actin, aortic smooth muscle	42 381	5.23	74	10
17	1.215 ± 0.097	IPI00217236	TBCA Tubulin-specific chaperone A	12 904	5.25	93	10
18	1.032 ± 0.015	IPI00003362	HSPA5 HSPA5 protein	72 492	5.07	221	11
19	1.438 ± 0.179	IPI00479946	STIP1 STIP1 protein	68 687	7.81	38	3
20	1.088 ± 0.042	IPI00003865	HSPA8 Isoform 1 of heat-shock cognate 71 kDa protein	71 082	5.37	88	6
21	1.109 ± 0.052	IPI00007765	HSPA9 Stress-70 protein, mitochondrial precursor	73 920	5.87	67	7
22	1.463 ± 0.187	IPI00472102	HSPD1 61 kDa protein	61 346	5.70	230	15
23	1.022 ± 0.011	IPI00297779	CCT2 T-complex protein 1 subunit beta	57 794	6.01	40	7
24	1.289 ± 0.126	IPI00025512	HSPB1 Heat-shock protein beta-1	22 826	5.98	256	20
25	1.326 ± 0.140	IPI00022433	HSPB6 Heat-shock protein beta-6	18 886	7.90	92	21
26	1.088 ± 0.014	IPI00020599	CALR Calreticulin precursor	48 283	4.29	38	2
27	1.061 ± 0.029	IPI00011229	CTSD Cathepsin D precursor	45 037	6.10	175	13
28	1.192 ± 0.087	IPI00024502	UBQLN4 Ubiquilin-4	63 869	5.14	43	2
29	1.077 ± 0.037	IPI00018352	UCHL1 Ubiquitin carboxyl-terminal hydrolase isozyme L1	25 151	5.33	131	26
30	1.119 ± 0.045	IPI00003949	UBE2N Ubiquitin-conjugating enzyme E2 N	17 184	6.13	43	2
31	1.403 ± 0.167	IPI00071180	UBQLN1 Isoform 2 of ubiquilin-1	59 183	5.01	46	9
32**	2.241 ± 0.382	IPI00376005	EIF5A Isoform 2 of eukaryotic translation initiation factor 5A-1	20 442	6.52	118	25
33	1.738 ± 0.269	IPI00419585	PPIA;PPIAL3;LOC654188 Peptidyl-prolyl cis-trans isomerase A	18 229	7.68	66	20
34	1.225 ± 0.12	IPI00219757	GSTP1 Glutathione S-transferase P	23 569	5.43	82	12
35	1.136 ± 0.063	IPI00027350	PRDX2 Peroxiredoxin-2	22 049	5.66	192	14
36	1.293 ± 0.128	IPI00646689	TXNL5 Thioredoxin-like protein 5	14 217	5.40	37	11
37	1.101 ± 0.047	IPI00023788	PRSS7 Enteropeptidase precursor	114 905	4.90	35	1
38	1.669 ± 0.250	IPI00216298	TXN Thioredoxin	12 015	4.82	98	34
39	0.094 ± 0.366	IPI00013895	S100A11 Protein S100-A11	11 847	6.56	100	23
40	1.429 ± 0.176	IPI00010896	CLIC1 Chloride intracellular channel protein 1	27 248	5.09	47	14
41	1.129 ± 0.060	IPI00009943	TPT1 Tumor protein, translationally controlled 1	21 626	5.34	107	19
42	1.225 ± 0.101	IPI00329801	ANXA5 Annexin A5	35 971	4.94	130	8
43	1.119 ± 0.056	IPI00412792	BTF3L4 Transcription factor BTF3 homolog 4	17 260	5.95	57	5
44	1.036 ± 0.017	IPI00003269	DKFZp686D0972 hypothetical protein LOC345651	42 318	5.39	153	9

Table I. (*Continued*)

Protein binding							
45	1.850 ± 0.298	IPI00008223	RAD23B UV excision repair protein RAD23 homolog B	43 202	4.79	43	5
46	1.412 ± 0.170	IPI00013881	HNRPH1 Heterogeneous nuclear ribonucleoprotein H	49 484	5.89	136	15
47	1.011 ± 0.005	IPI00027341	CAPG Macrophage-capping protein	38 779	5.88	134	12
48	1.482 ± 0.194	IPI00148063	HEBP1 Heme-binding protein 1	21 198	5.71	58	8
49	0.354 ± 0.144	IPI00024993	ECHS1 Enoyl-CoA hydratase, mitochondrial precursor	31 823	8.34	77	11
50	1.926 ± 0.316	IPI00745465	Similar to checkpoint protein HUS1	15 655	8.96	65	54
Regulation of transcription							
51	1.044 ± 0.021	IPI00015361	PFDN5 Prefoldin subunit 5	17 374	5.93	181	33
SH2/SH3 adaptor activity							
52	1.293 ± 0.128	IPI00025318	SH3BGRL SH3 domain-binding glutamic acid-rich-like protein	12 766	5.22	72	8
53	1.853 ± 0.299	IPI00010402	SH3BGRL3 Hypothetical protein	24 108	9.27	125	12
Transport							
54	1.055 ± 0.026	IPI00303882	M6PRBP1 Iso BofMannose-6-phosphate receptor-binding protein 1	47 189	5.30	87	7
55	1.474 ± 0.191	IPI00171664	NUP43 48 kDa protein	49 082	5.75	34	2
56	1.262 ± 0.116	IPI00024911	ERP29 Endoplasmic reticulum protein ERp29 precursor	29 032	6.77	125	25
Cell motility							
57	1.188 ± 0.086	IPI00219301	MARCKS Myristoylated alanine-rich C-kinase substrate	31 707	4.47	36	15
58	1.159 ± 0.073	IPI00014516	CALD1 Isoform 1 of Caldesmon	93 251	5.63	132	7
Signal transduction							
59	1.648 ± 0.244	IPI00025252	PDIA3 Protein disulfide-isomerase A3 precursor	57 146	5.98	292	14
60	1.082 ± 0.039	IPI00003815	ARHGDIA Rho GDP-dissociation inhibitor 1	23 250	5.02	79	14
61	1.194 ± 0.088	IPI00298547	PARK7 Protein DJ-1	20 050	6.33	141	26
62	1.061 ± 0.030	IPI00012011	CFL1 Cofilin-1	18 719	8.22	90	18
63	1.000 ± 0.001	IPI00412987	GMFB Glia maturation factor beta	16 874	5.19	60	21
Calcium ion binding							
64	1.087 ± 0.032	IPI00014537	CALU Isoform 1 of calumenin precursor	37 198	4.47	155	20
65	1.468 ± 0.189	IPI00015842	RCN1 Reticulocalbin-1 precursor	38 866	4.86	119	16
66**	4.495 ± 0.636	IPI00101037	RCN3 Reticulocalbin-3 precursor	37 470	4.74	139	9
67	1.139 ± 0.050	IPI00075248	CALM1;CALM3;CALM2 Calmodulin	16 827	4.09	97	20
68	1.619 ± 0.196	IPI00335168	MYL6 Isoform non-muscle of myosin light polypeptide 6	17 090	4.56	92	25
Metabolic processes							
69	1.850 ± 0.298	IPI00027487	CKM Creatine kinase M-type	43 302	6.77	106	11
70	1.709 ± 0.261	IPI00335280	RPE Isoform 1 of Ribulose-phosphate 3-epimerase	25 139	5.33	44	8
71	1.069 ± 0.033	IPI00029997	PGLS 6-phosphogluconolactonase	27 815	5.70	91	22
72	1.306 ± 0.132	IPI00016832	PSMA1 Isoform short of proteasome subunit alpha type 1	29 822	6.15	63	16
73	1.131 ± 0.077	IPI00219018	GAPDH Glyceraldehyde-3-phosphate dehydrogenase	36 201	8.57	105	8
74	1.225 ± 0.12	IPI00219953	CMPK cytidylate kinase	26 180	8.14	51	9

Table I. (*Continued*)

Apoptosis							
75	1.751 ± 0.273	IPI00000760	DDAH2 NG,NG-dimethylarginine dimethylaminohydrolase 2	29 911	5.66	68	12
76	1.072 ± 0.035	IPI00012048	NME1 Nucleoside diphosphate kinase A	17 309	5.83	108	31
77	1.026 ± 0.013	IPI00218733	SOD1 16 kDa protein	16 340	5.87	86	8
78	1.651 ± 0.245	IPI00219219	LGALS1 Galectin-1	15 048	5.34	286	37
79	1.128 ± 0.060	IPI00022434	ALB Serum albumin	73 753	6.33	68	3
80	1.332 ± 0.142	IPI00014850	PEA15 Astrocytic phosphoprotein PEA-15	15 088	4.93	83	22
Cell cycle							
81	1.055 ± 0.026	IPI00220503	DCTN2 Dynactin 2	44 906	5.06	38	7
82	1.738 ± 0.269	IPI00006052	PFDN2 Prefoldin subunit 2	16 695	6.20	72	9
83	1.486 ± 0.195	IPI00000051	PFDN1 Prefoldin subunit 1	14 202	6.32	45	9
84	1.009 ± 0.004	IPI00479997	STMN1 Stathmin	17 292	5.76	90	23

**Ratio: average normalized volumes.*
***Differentially expressed protein (altered abundance ratio greater than 2.0-fold change in expression).*

this basis, hMSC have been used in association with allogeneic BMT to facilitate the engraftment of HSC and lessen GvHD [11,12,15,27].

All these studies have evaluated the use of third-party MSC infusions, where the BM and hMSC donors were different. Furthermore, Le Blanc *et al.* [28], using MLR, have demonstrated that the hMSC suppressive effect is independent of HLA.

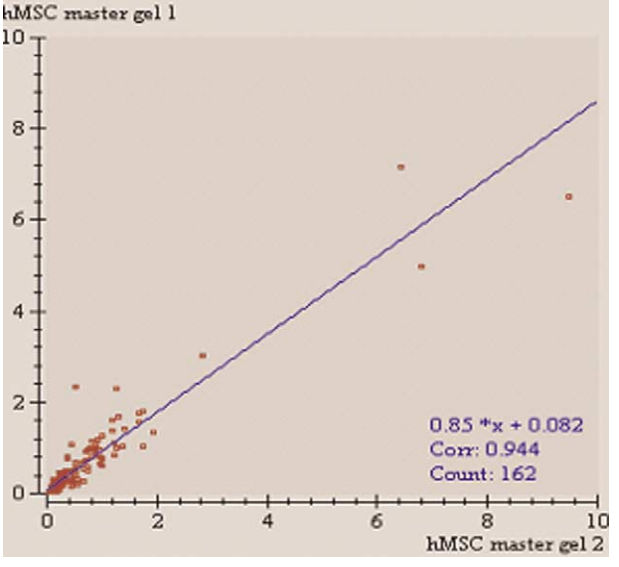

Figure 4. *2-D gel analysis in Image Master 2-D Platinum 6.0. Scatter plot from the analyzed gels shows the relationship between spots values (% volume).*

In this study, we compared *in vitro* mixed reactions and showed an inhibition of lymphocyte proliferation by third-party hMSC. We observed similar results in experiments performed with hMSC from the first passage derived from different individuals, suggesting that immunomodulatory properties are equivalent in different hMSC cultures maintained under standard conditions. This result also provides evidence that even cells in this early passage are able to modulate T-cell proliferation. To compare further cultures derived from different individuals, we proceeded with a comparative proteomic analysis, using first-passage hMSC derived from different BM donors, and demonstrated that these cells have a very similar protein expression pattern.

In the hMSC field, several proteomic approaches have been applied in order to identify their regulatory molecules and potential biomarkers. Several studies have identified the characteristic proteome of MSC [23]. However, none of the identified proteins appears to be specific for hMSC, indicating a lack of unique markers for hMSC. These studies have compared hMSC derived from different human tissues [29], hMSC submitted to different culture conditions [18,20] and hMSC induced to differentiate along multiple lineages [21,30], but none of them has focused on variations observed in cells derived from different individuals.

Park *et al.* [23] reviewed the already published proteomic data of hMSC and described approximately 264 distinct proteins present in hMSC cultures. A wide range

of technologies, including 2-DE, capillary/nano-high pressure liquid chromatography (LC), MS, bioinformatics and protein microarrays were employed in these analyzes.

In this study, 40 new proteins were identified beyond the previously described proteins [23], probably because we used different experimental parameters to perform this 2-DE analysis, specifically 2-D gels covering proteins in the ranges of pH 4–7 and MW 10–220 kDa. Interestingly, we identified galectin-1 (Gal-1) for the first time in proteomic analysis of hMSC, although it has been associated previously with hMSC [31]. This protein, as well as the great majority of proteins identified, was expressed at almost identical levels in all the hMSC analyzed. In an animal model of GvHD, Gal-1 treatment efficiently prevents the development of GvHD [32], indicating it could be a putative biomarker for follow-up of third-party therapy quality.

The similarity of the protein profile in early hMSC culture passages is also an important issue, although several authors have shown huge variability in the proteomic profiles of MNC from BM, peripheral blood and cord blood cells [33,34]. The culture, even in only one passage period, was able to maintain a similar proteomic profile. A question that remains to be investigated is for how long this uniformity is maintained.

In conclusion, this study is the first to demonstrate the similarity in proteomic profile of hMSC derived from different individuals, even in cells from the first culture passage. These results encourage the use of third-party MSC in cellular therapies, because cells derived from different individuals have the same proteomic pattern. Furthermore, the analysis performed in this work has added 40 new proteins to the hMSC proteome, some of them with potential roles in hMSC in cellular therapies.

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