Research Article

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Regenerative Medicine

An insight into the whole transcriptome profile of four tissue-specific human mesenchymal stem cells

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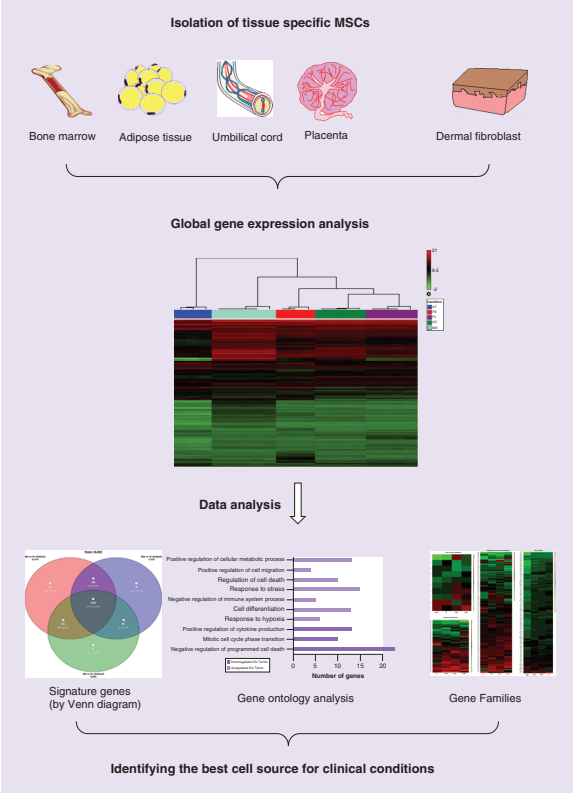
Aim: Variations in the clinical outcomes using mesenchymal stem cells (MSCs) treatments exist, reflecting different origins and niches. To date, there is no consensus on the best source of MSCs most suitable to treat a specific disease. **Methods:** Total transcriptome analysis of human MSCs was performed. MSCs were isolated from two adult sources bone marrow, adipose tissue and two perinatal sources umbilical cord and placenta. **Results:** Each MSCs type possessed a unique expression pattern that reflects an advantage in terms of their potential therapeutic use. Advantages in immune modulation, neurogenesis and other aspects were found. **Discussion:** This study is a milestone for evidence-based choice of the type of MSCs used in the treatment of diseases.



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Mesenchymal stem cells (MSCs) are multipotent stromal cells with self-renewal capacity and multilineage differentiation potential [1]. In recent years, there has been an increasing interest in MSCs. This is mainly due to their exciting properties including long-term proliferation, multilineage differentiation potential and immunomodulatory properties [1].

MSCs have been isolated from different tissues such as, adipose tissue (AT), umbilical cord (UC) and amniotic fluid [2–4]. For the last 40 years, MSCs from bone marrow (BM) were the most studied MSC subtype. Nevertheless, during the last decade the research is focusing on finding new alternative sources of MSC, from which cells can be obtained easily, noninvasively and in abundant quantity. This includes the isolation of MSCs from perinatal tissues such as UC and placenta (PL), and from AT.

It has been argued that MSCs from different tissues exhibit distinct properties, which may influence their potential clinical applications [5]. Studies have compared the expression of certain important genes in MSCs from different sources. While some groups have investigated the expression level of certain embryonic stem cells (ESCs) markers and pluripotency genes [6–8], other groups investigated the expression of genes with functional importance, for example, genes involved in bone [9] and cardiovascular development [7]. In addition, global gene expression profiling for different types of MSCs was performed to get a deeper understanding of the biology and ontogeny of MSCs [2,10–12]. However, these comparative studies were performed with MSCs isolated from different sources, under different culturing conditions, and different gene expression analysis platforms were used. Moreover, distinct data-filtering strategies and various degrees of statistical stringency were applied. All of these differences have introduced many variations in the analysis of the whole gene expression profile leading sometimes to conflicting results.

In an attempt to get a deeper insight into the differences and similarities between MSCs from different origins this study analyzed and compared the transcriptome profile of MSCs isolated from four different tissues (BM, AT, UC and PL). The MSCs transcriptome profile was additionally compared with the transcriptome profile of dermal fibroblasts (DF). All MSCs were cultured and processed under similar conditions. Platelet lysate was used instead of fetal bovine serum (FBS), as a serum supplement.

Materials & methods

Isolation & characterization of human MSCs

The study was approved by the institutional review board at the Cell Therapy Center/The University of Jordan. All procedures performed in this study were in accordance with the Declaration of Helsinki. Signed informed consent was obtained prior to sample collection. All donors were unaffected by congenital or acquired pathologies and were free of any systemic or infectious disease.

MSCs were isolated from BM aspirate, lipoaspirates, UC and PL based on their plastic adherent properties. Detailed description of the isolation procedure is provided in the Supplementary Material 1. Characterization of isolated MSCs was performed in accordance with the International Society for Cellular Therapy (ISCT) recommendations including differentiation potential and surface marker assessments.

Differentiation potential

Assessment of the differentiation potential for MSCs isolated from all four sources was performed using StemPro® Adipogenesis and Osteogenesis Differentiation Kit (GIBCO, NY, USA) according to the manufacturer's instructions. Cells at passages 3–5 were used in differentiation experiments. To detect adipogenic and osteogenic differentiation, oil red O stain and alizarin red S were used, respectively [13]. Due to the 3D culture setting required, chondrogenic differentiation was not performed for all MSCs type (Supplementary Material 1).

Flow cytometry analysis

Surface markers characterization for MSCs isolated from all four sources was performed using BD StemflowTM hMSC Analysis Kit (BD Biosciences, CA, USA) according to the manufacturer's instructions. Cells were stained with antibodies against CD73, CD90, CD105, CD44, CD34, CD11b, CD19, CD45 and HLA-DR. Canto BD

II flow cytometer instrument (BD Biosciences) was used for running samples. The percentage of expressed cell surface markers was calculated from 10,000 gated cells [13].

Isolation & culturing of dermal fibroblasts

DFs were isolated from neonatal foreskin samples as described previously [14]. Detailed procedure for the isolation of DF is provided in Supplementary Material 1.

Total RNA isolation & quality assessment

For RNA isolation, MSCs from all different sources at passage 3 were cultured in six-well plates. RNA was extracted using the mixed Trizol-Qiagen® RNeasy Mini kit (Qiagen, CA, USA) [15].

The total RNA yield and purity of all RNA samples used were checked using NanoDrop 2000c spectrophotometer system (ThermoFisher Scientific, DE, USA). RNA integrity was evaluated using Agilent 2100 Bioanalyzer instrument and Agilent RNA 6000 Nano Kit according to the manufacturer's instructions (Agilent Technologies, CA, USA).

Whole transcriptome analysis: expression microarray

Affymetrix human transcriptome analysis 2 assay

Gene expression profiling of total RNA was performed using Affymetrix® Microarray instrument and GeneChip® Human Transcriptome Array 2 (HTA 2; Affymetrix, CA, USA). A 100-ng aliquot of total RNA from each sample was amplified using GeneChip® WT PLUS Reagent Kit (Affymetrix) according to the manufacturer's instructions. A 5.5 µg of amplified cDNA from each sample was fragmented, labeled and hybridized into the GeneChip HTA 2 arrays. The chips were subsequently laser scanned with Affymetrix GeneChip Scanner 3000 7G. The microarray data can be found at The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (accession number GSE108511).

Controls used in the HTA assay

All controls recommended by Affymetrix protocol were used. This includes HeLa cell line poly-A RNA controls, and BioB, BioC, BioD and CreX.

Real-time PCR validation

Real-time PCR analysis was performed on selected differentially expressed genes obtained from the signature gene list of uniquely upregulated genes in each MSCs type (Table 1). Analysis included; *CASP1, GATA4, TIMP4, MMP3, NCAM1, DKK2* and *HOXD10* (Supplementary Material 2, Supplementary Table 1 & Supplementary Figure 1). The expression levels were normalized to *cyclophilin A*.

Analysis of the HTA array results

The expression data were generated using Affymetrix Expression Console software version 3.1 (Affymetrix). The Signal Space Transformation-robust multiarray analysis algorithm implemented through the Affymetrix Expression Console software was used to normalize the data and for quality control check. A statistical one-way analysis of variance (ANOVA) was performed on the Signal Space Transformation-robust multiarray analysis expression values to minimize the effect of probe-specific affinity differences, in order to increase sensitivity to small changes between MSC groups samples and to minimize variance across the dynamic range. A multiple-testing correction was applied to the p-values of the F-statistics to adjust the false discovery rate (FDR) [16]. Genes with adjusted p-values of <0.05 were analyzed using the Affymetrix Transcriptome Analysis Console Software.

Additionally, gene ontology (GO) term enrichment analysis was performed using Partek® Genomics Suite® Ingenuity Pathway Analysis software (Ingenuity Systems, CA, USA) was used for pathway and network analyses.

Results

Isolation & characterization of MSCs

MSCs isolated from BM, UC, AT and PL showed typical fibroblast-like cell morphology (Figure 1A). Upon induction of differentiation, MSCs from all sources were able to differentiate into adipocytes and osteocytes thus confirming their multipotent potential (Figure 1B). Analysis of surface markers expression by flow cytometer

Table 1. Top eight signature genes upregulated uniquely in each mesenchymal stem cells type.

Signature genes in BM					
Gene symbol	Description	Fold change			FDR F-test
		BM vs AT fold change	BM vs PL fold change	BM vs UC fold change	
MIR3975	MiRNA 3975	603.66	122.13	81.92	0.002
CNTNAP3B	Contactin-associated protein-like 3B	96.18	111.04	30.5	0.009
PLA2R1	Phospholipase A2 receptor 1	26.24	46.83	38.74	0.02
SNORA14A	Small nucleolar RNA, H/ACA box 14A	109.89	45.82	18.95	0.01
FBXO32	F-box protein 32	28.89	26.77	39.65	0.01
CSGALNACT1	Chondroitin sulfate N-acetylgalactosaminyltransferase 1	39.83	52.45	15.39	0.01
SNORD56B	Small nucleolar RNA, C/D box 56B	151.33	11.42	10.53	0.0005
FOS	FBJ murine osteosarcoma viral oncogene homolog	17.19	18.03	25.16	0.01
Signature genes in AT					
Gene symbol	Description	Fold change			FDR F-test
		AT vs BM fold change	AT vs PL fold change	AT vs UC fold change	
MMP3	Matrix metallopeptidase 3	192.05	218.35	212.45	0.01
EBF2	Early B-cell factor 2	147.55	115.76	115.68	0.002
ABCC9	ATP binding cassette subfamily C member 9	18	42.43	32.6	0.003
LRRC15	Leucine-rich repeat containing 15	20.8	39.37	29.84	0.004
SLC6A15	Solute carrier family 6 (neutral amino acid transporter), member 15	9.54	12.67	6.75	0.01
TIMP4	TIMP metallopeptidase inhibitor 4	3.64	4.72	6.29	0.004
STAC	SH3 and cysteine-rich domain	5.26	9.7	7.25	0.01
PRDM1	PR domain containing 1, with ZNF domain	3.94	3.65	3.37	0.03
Signature genes in UC					
Gene symbol	Description	Fold change			FDR F-test
		UC vs BM fold change	UC vs AT fold change	UC vs PL fold change	
HOXD10	Homeobox D10	11.17	5.98	5.86	0.001
TMSB15A	Thymosin β 15a	9.27	4.41	-6.2	0.003
NCAM1	Neural cell adhesion molecule 1	5.41	6.89	-5.91	0.002
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	3.9	8.61	-3.45	0.002
DKK2	Dickkopf WNT signaling pathway inhibitor 2	3.09	5.48	-7.82	0.01
DSC2	Desmocollin 2	12.49	4.12	-3.93	0.003
ADAMTS3	ADAM metallopeptidase with thrombospondin type 1 motif 3	2.8	3.79	2.51	0.01
BCHE	Butyrylcholinesterase	9.41	4.27	3.48	0.01
Signature genes in PL					
Gene symbol	Description	Fold change			FDR F-test
		PL vs UC fold change	PL vs AT fold change	PL vs BM fold change	
CASP1	Caspase 1	3.65	3.13	3.14	0.008
CSNK1A1P1	Casein kinase 1, α 1 pseudogene 1	3.9	3.84	3.93	0.008
FIRRE	Firre intergenic repeating RNA element	3.48	3.85	2.83	0.005
PPP1R14A	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	2.39	2.05	2.53	0.05
VTN; SEBOX	Vitronectin; SEBOX homeobox	2.38	2.11	2.75	0.007
GATA4	GATA binding protein 4	5.54	5.17	5.72	0.008
HOXA13	Homeobox A13	2.17	2.71	6.54	0.02
LINC00184	Long Intergenic Non-Protein Coding RNA 184	12.79	9.73	11.6	0.003
LINC00194	Long Intergenic Non-Protein Coding RNA 194	3.07	5.82	2.59	0.002

AT: Adipose tissue; BM: Bone marrow; FDR: False discovery rate; PL: Placenta; UC: Umbilical cord.

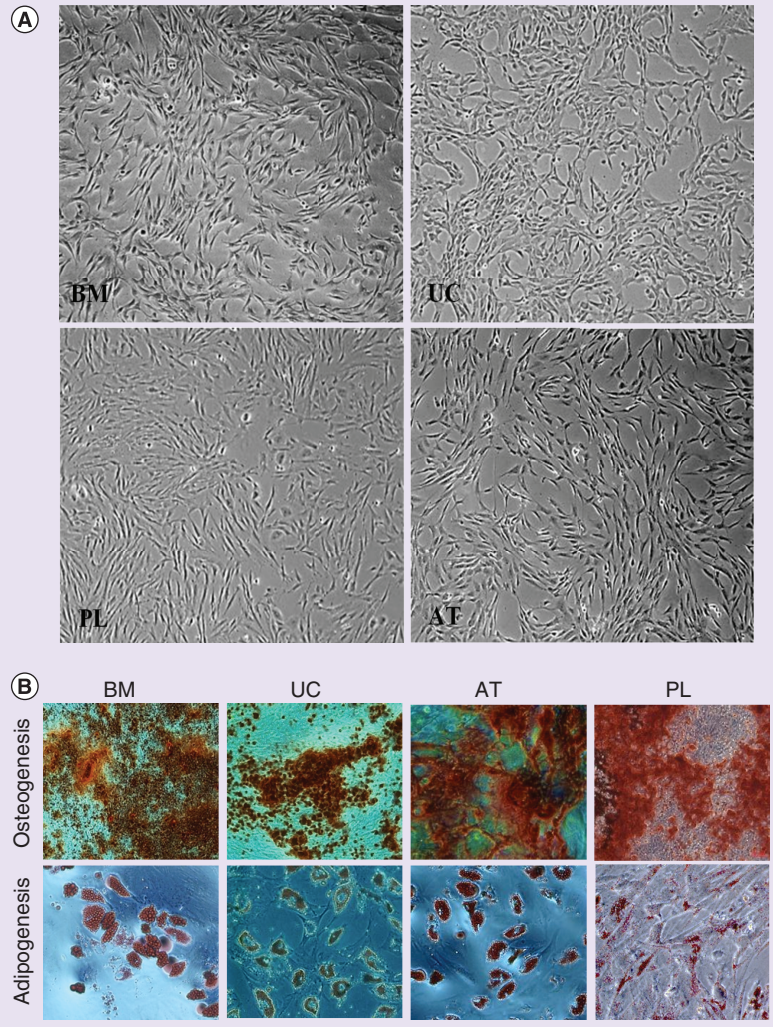

Figure 1. Characterization of mesenchymal stem cells isolated from bone marrow, umbilical cord, placenta and adipose tissue. (A) Morphology of isolated mesenchymal stem cells (MSCs); all cells displayed a fibroblast-like morphology. Phase contrast magnification 5×. **(B)** Representative samples of bone marrow, umbilical cord, placenta and adipose tissue-MSCs trilineage differentiation. In MSCs from all four sources, osteogenesis was detected by Alizarin red stain of calcium mineralization; adipogenesis was evaluated by oil red O staining of lipid droplets; and chondrogenesis was evaluated by alcian blue staining of micromass culture. **(C)** Representative samples for flow cytometric analysis of bone marrow, umbilical cord, placenta and adipose tissue-MSCs surface markers. Gray peaks correspond to the isotype control and pink peaks to the antibody of interest. The conjugated fluorescent dyes are: CD90-FITC, CD105-perCP-Cy5.5, CD73-APC and CD44-PE.
AT: Adipose tissue; BM: Bone marrow; PL: Placenta; UC: Umbilical cord.

revealed that MSCs from all sources were positive for MSCs signature markers; CD90, CD105, CD73 and CD44, and were negative for CD34, CD45, CD11b, CD19 and HLA-DR (Figure 1C).

Whole transcriptome analysis

A list of differentially expressed genes in for DF, BM-, UC-, AT- and PL-MSCs was generated by examining their expression profiles using Transcriptome Analysis Console 4 Software (Affymetrix). The algorithm option of one-way between-subject ANOVA (eBayes), and the filtering criteria of fold change (linear) <-2 or >2 and a p-value <0.05 were used. The total number of genes analyzed in the GeneChip HTA 2 array used for all cell types is 67,528; this includes 44,699 coding genes and 22,829 noncoding genes. A summary of the total differentially expressed genes in the different cell types is represented in Figure 2A, while the top ten differentially expressed

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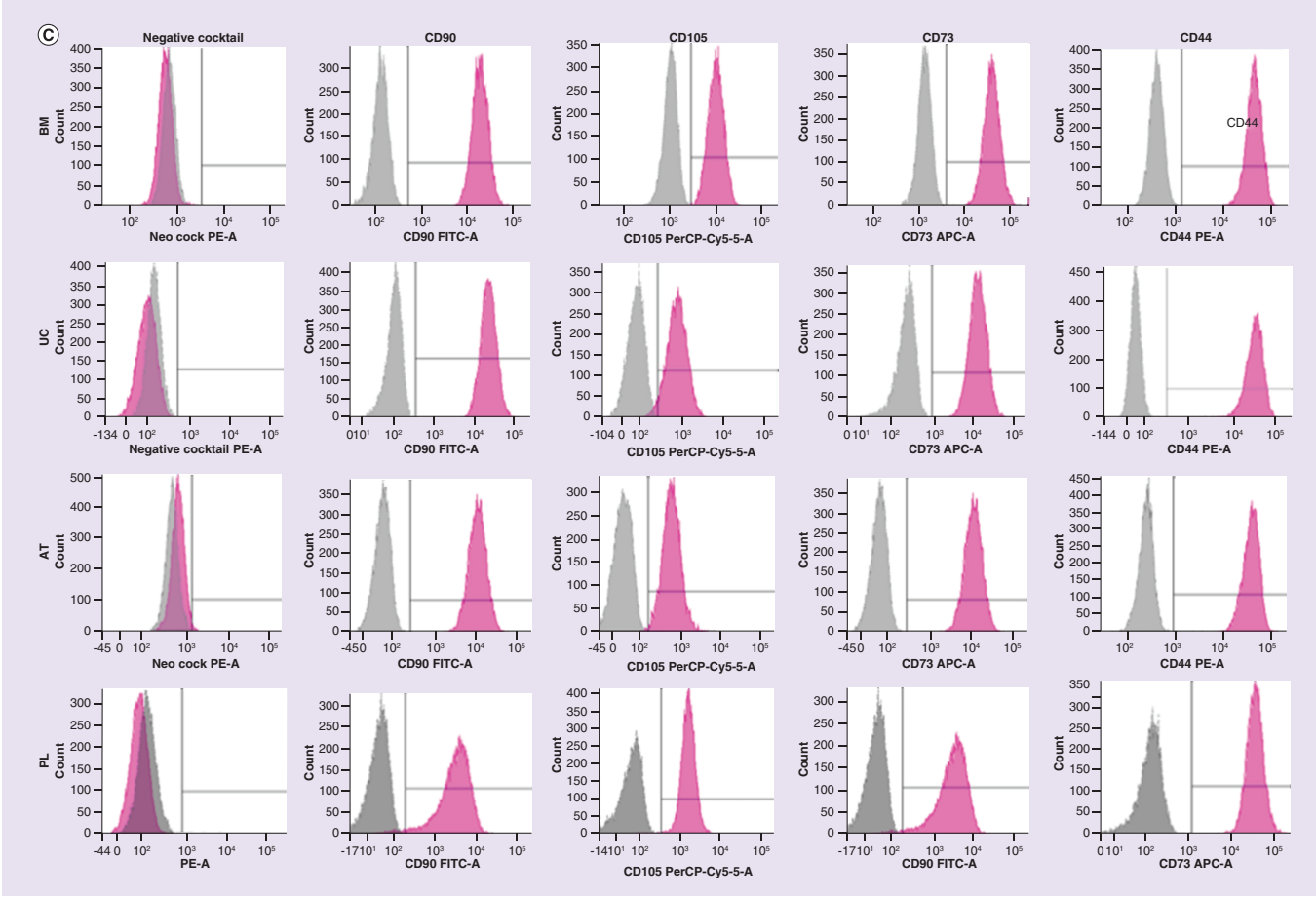

Figure 1. Characterization of mesenchymal stem cells isolated from bone marrow, umbilical cord, placenta and adipose tissue (cont.). **(A)** Morphology of isolated mesenchymal stem cells (MSCs); all cells displayed a fibroblast-like morphology. Phase contrast magnification 5×. **(B)** Representative samples of bone marrow, umbilical cord, placenta and adipose tissue-MSCs trilineage differentiation. In MSCs from all four sources, osteogenesis was detected by Alizarin red stain of calcium mineralization; adipogenesis was evaluated by oil red O staining of lipid droplets; and chondrogenesis was evaluated by alcian blue staining of micromass culture. **(C)** Representative samples for flow cytometric analysis of bone marrow, umbilical cord, placenta and adipose tissue-MSCs surface markers. Gray peaks correspond to the isotype control and pink peaks to the antibody of interest. The conjugated fluorescent dyes are: CD90-FITC, CD105-perCP-Cy5.5, CD73-APC and CD44-PE.
AT: Adipose tissue; BM: Bone marrow; PL: Placenta; UC: Umbilical cord.

genes are listed in Supplementary Table 2.

Hierarchical clustering analysis, with a more stringent criterion of FDR-value ≤0.01%, clearly indicates a close association (stronger correlation) between UC- and PL-MSCs. In addition, a closer association was found between UC- and PL-MSCs with BM-MSCs, compared with AT-MSCs with BM-MSCs while the AT-MSCs clustered the furthest from DF and the rest of the MSCs types (Figure 2B).

Signature genes & gene ontology

Each of the four types of MSCs is analyzed separately for the unique genes that are either up- or downregulated compared with all other stem cell types. To identify these genes for each type of MSCs, three-way Venn diagram was drawn from up- or downregulated genes (fold change ≥2 or ≤-2, FDR ≤0.05) of one MSC type versus the other types (Figure 3). The highest number of genes was found in the BM-MSCs with 642 and 341 unique up- and downregulated genes, respectively. While AT-MSCs were found to have 191 uniquely upregulated genes, whereas 663 genes were found downregulated in AT-MSCs compared with other types of MSCs. Interestingly, only 78 and 62 upregulated unique genes were found in UC-MSCs and PL-MSCs, respectively. In addition, 47 and 43 downregulated unique genes were found in UC- and PL-MSCs, respectively.

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Number of differentially expressed genes

	BM-MSCs vs AT-MSCs	BM-MSCs vs UC-MSCs	BM-MSCs vs PL-MSCs	BM-MSCs vs DF	AT-MSCs vs UC-MSCs	AT-MSCs vs PL-MSCs	AT-MSCs vs DF	UC-MSCs vs PL-MSCs	UC-MSCs vs DF	PL-MSCs vs DF
Upregulated genes	2020	1488	1348	1393	977	781	969	569	683	712
Downregulated genes	1682	1242	1029	1104	1345	1338	1380	498	612	843
Total	3702	2730	2377	2497	2322	2119	2349	1067	1295	1555

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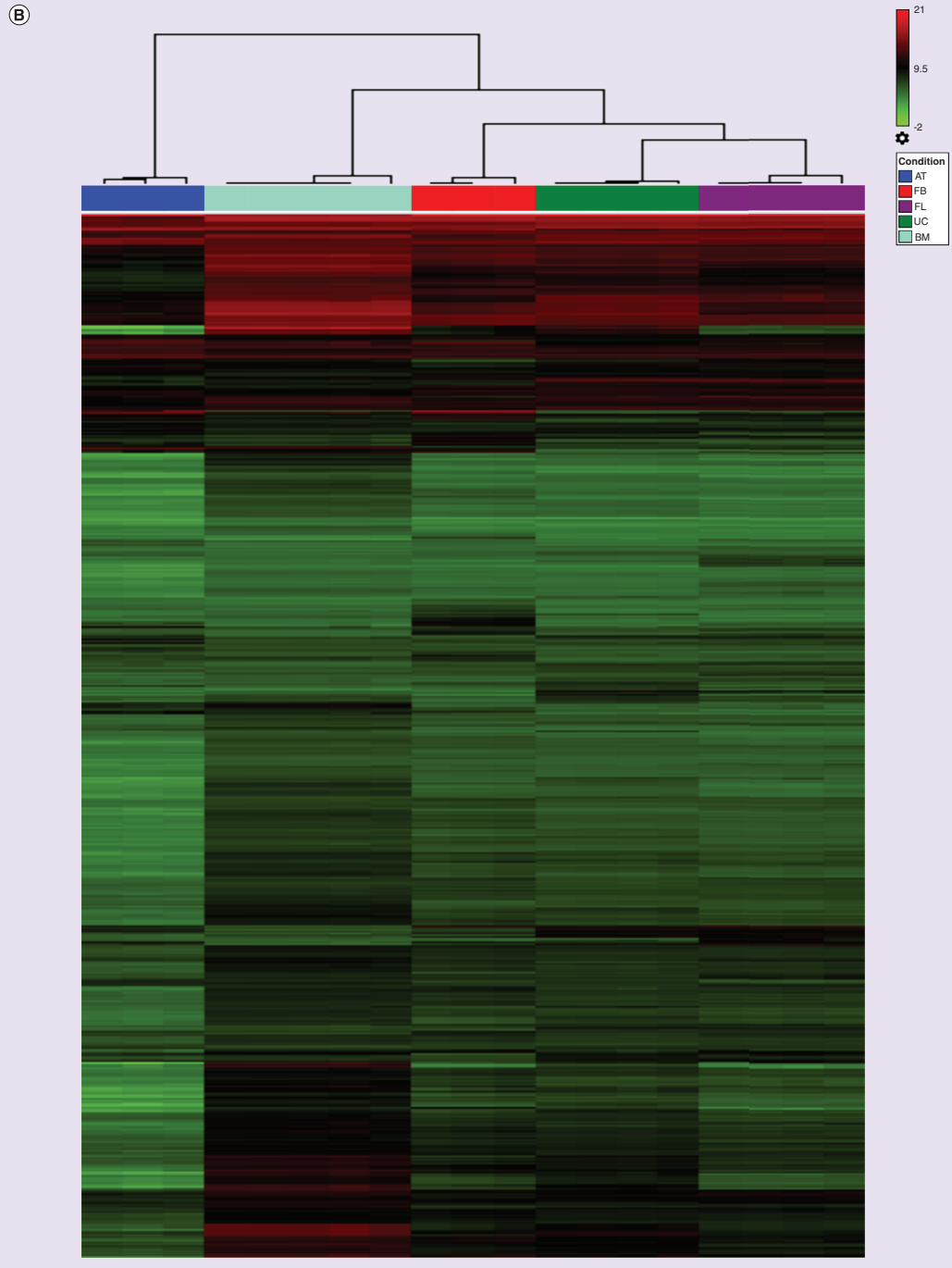


Figure 2. Microarray analysis of mesenchymal stem cells and dermal fibroblast. (A) Total number of differentially expressed genes when comparing one mesenchymal stem cells type versus other. **(B)** Hierarchical clustering showing the relationship of bone marrow, umbilical cord, adipose tissue, placenta-mesenchymal stem cells and dermal fibroblasts.
AT: Adipose tissue; BM: Bone marrow; DF: Dermal fibroblasts; MSC: Mesenchymal stem cell; PL: Placenta; UC: Umbilical cord.

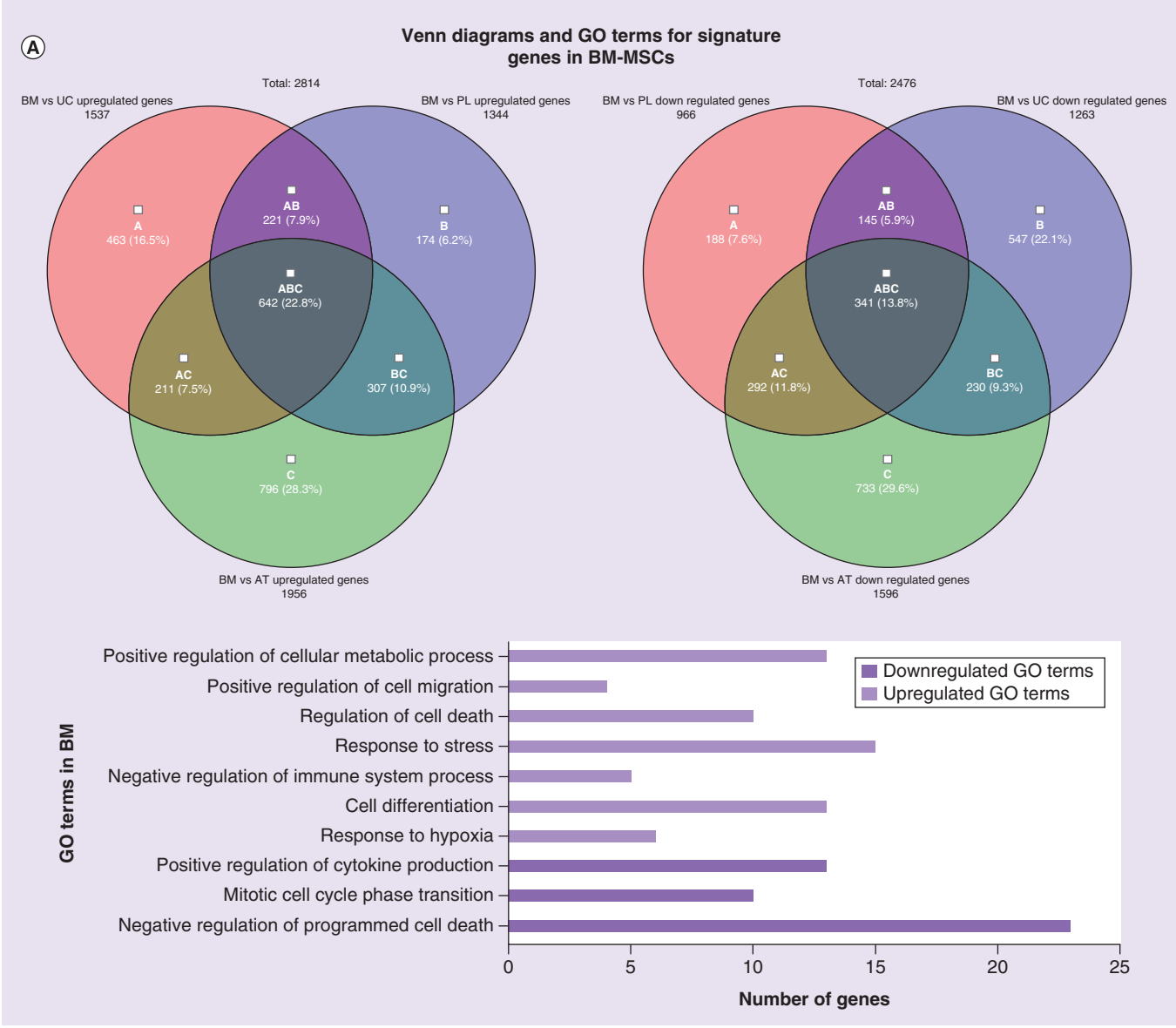
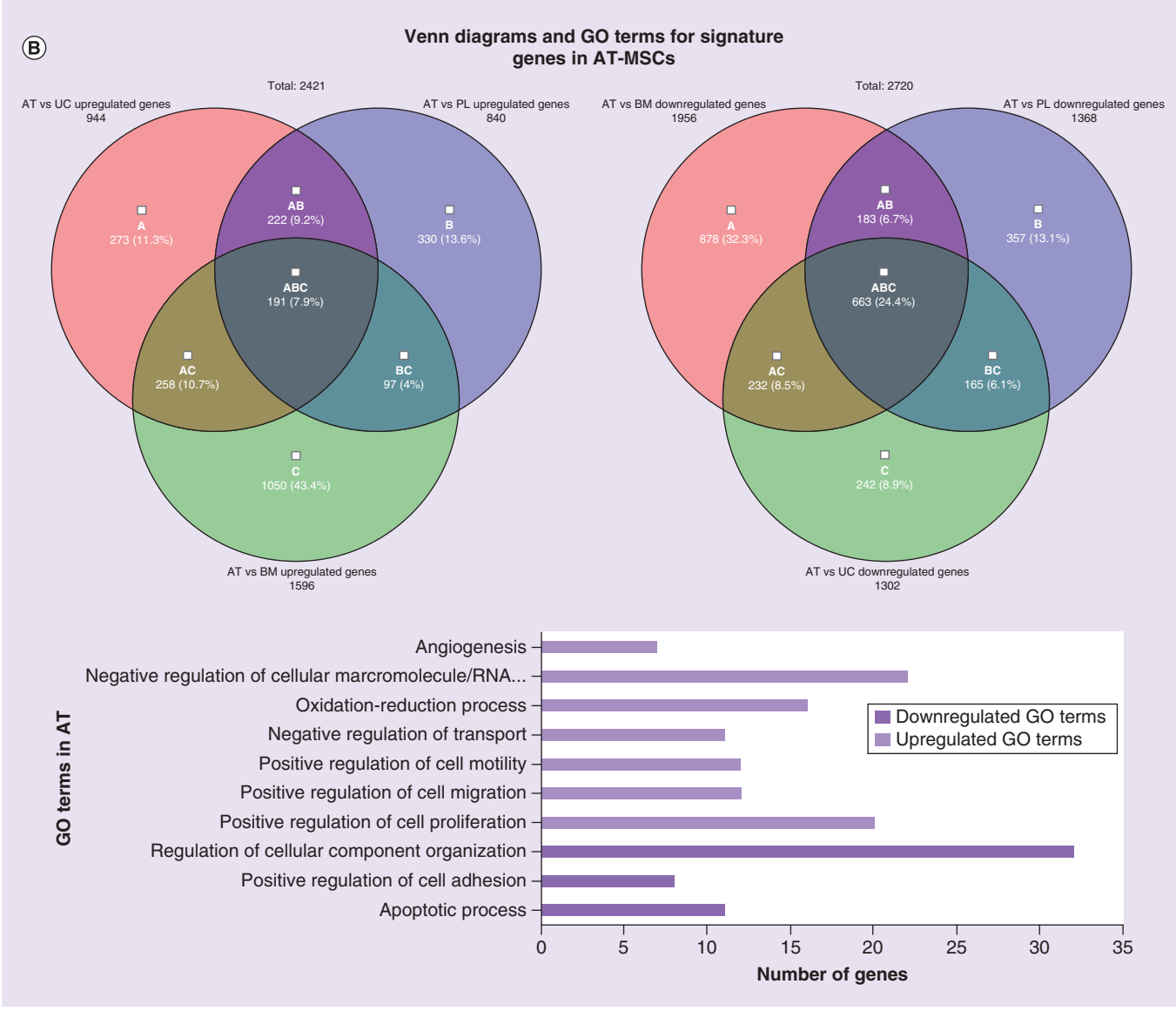


Figure 3. Signature genes & gene ontology. Venn diagrams and gene ontology terms for specifically regulated genes in **(A)** bone marrow-MSCs, **(B)** adipose tissue-MSCs, **(C)** umbilical cord-MSCs, **(D)** placenta-MSCs. Three-way Venn diagram was drawn from upregulated or downregulated genes (fold change ≥2 or ≤-2, false discovery rate ≤0.05) of one MSC type versus the other types. Unique gene lists for each type of MSCs were obtained from the Venn diagrams. These lists were used to perform a gene ontology analysis using Partek Genomics Suite® 7.0 software (enrichment p-value < 0.05).
BM: Bone marrow; GO: Gene ontology; PL: Placenta; UC: Umbilical cord; MSC: Mesenchymal stem cell.

The unique gene lists obtained from Venn diagrams were used to perform a GO analysis using Partek Genomics Suite® 7.0 software. Only GO terms that were significantly enriched (enrichment p-value <0.05) were considered. In BM-MSCs, GO terms related to hypoxia, stress, regulation of cell death, cell differentiation and, cell motility and migration were upregulated. While in AT-MSCs upregulated GO terms were found to be involved in migration and motility in addition to regulation of cell proliferation, negative regulation of biosynthetic process and transportation. Whereas positive regulation of neurogenesis and nervous system development were GO terms, found to be unique to the UC-MSCs unique genes, compared with all other types of MSCs examined. As for PL-MSCs when compared with other MSCs, cell differentiation and growth, response to hypoxia and stress, regulation of cellular metabolic process and protein transport surfaced in the GO term analysis (Figure 3).



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BM: Bone marrow; GO: Gene ontology; PL: Placenta; UC: Umbilical cord; MSC: Mesenchymal stem cell.

From the unique gene list we identified signature genes that are specifically expressed only in one type of MSCs. This was obtained by examining the average log2 value with a cut-off value of 6. Top eight upregulated signature genes in each cell type along with their fold change are presented in Table 1.

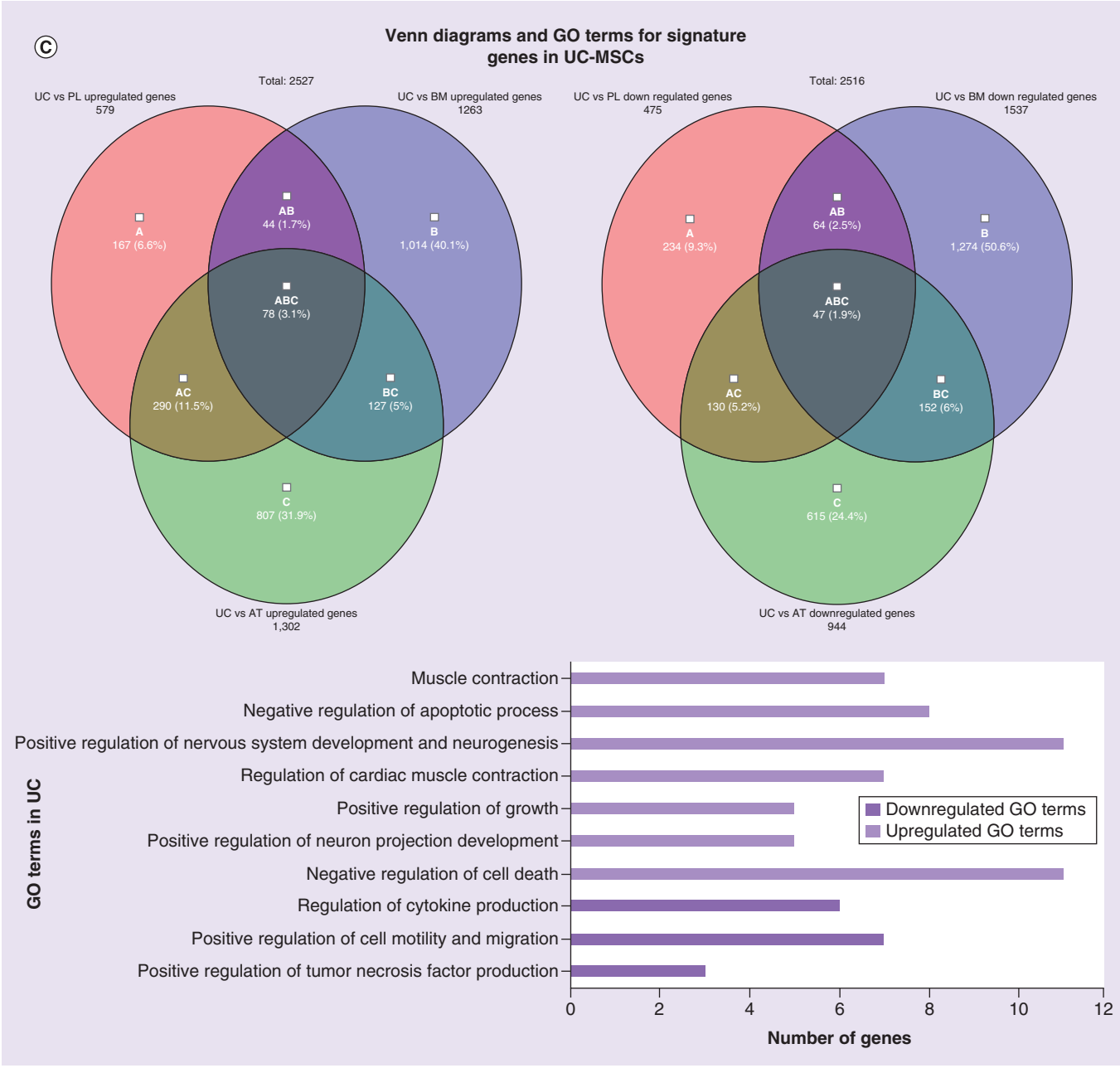
Stem cell-related gene families

The differential expression of selected gene sets relevant in terms of stem cell-inherent characteristics or immunemodulation and differentiation potential was examined in all cell types using BM-MSCs or DF as a baseline. The Molecular Signatures Database (MSigDB) Gene Families [17] was used to identify 11 gene sets while the ESCs and cell surface markers gene sets were compiled by Kolle *et al.* and Gedye *et al.*, respectively [18,19]. A less stringent fold change of 1.5 was used in this analysis with a corrected F ≤0.05. A summary of the differentially expressed genes for each gene family is listed in Table 2 and a heat map was generated displaying the specific genes involved

Table 2. Number of differentially expressed genes in different mesenchymal stem cells types for the studied gene families.

Term	Number of genes differentially expressed compared with BM-MSCs							
	AT-MSCs		UC-MSCs		PL-MSCs		DF	
	No. of upregulated genes	No. of downregulated genes	No. of upregulated genes	No. of downregulated genes	No. of upregulated genes	No. of downregulated genes	No. of upregulated genes	No. of downregulated genes
Cytokines/chemokines and growth factors	15	17	13	17	13	19	8	21
Immune tolerance	23	12	19	16	20	13	15	15
Oncogenes	20	14	26	11	25	13	24	14
Tumor suppressor	10	7	13	3	8	2	11	6
Embryonic stem cell markers	79	43	83	35	68	41	58	49
miRNA	13	127	9	106	15	102	6	97
Cardiogenesis	50	41	44	56	43	51	46	48
Immune modulation	68	42	68	42	67	40	61	46
Neuronal repair and regeneration	89	73	106	67	83	77	89	76
Osteogenesis	42	29	38	33	34	36	33	36
Chondrogenesis	14	9	9	15	12	16	11	21
Term	**Number of genes differentially expressed compared with DF**							
	AT-MSCs		**UC-MSCs**		**PL-MSCs**		**BM-MSCs**	
	No. of upregulated genes	**No. of downregulated genes**	**No. of upregulated genes**	**No. of downregulated genes**	**No. of upregulated genes**	**No. of downregulated genes**	**No. of upregulated genes**	**No. of downregulated genes**
Cell surface markers	2	2	7	3	7	2	5	1
Stemness (stem cell markers)	14	6	13	3	10	10	6	22

AT: Adipose tissue; BM: Bone marrow; DF: Dermal fibroblast; FDR: False discovery rate; MSC: Mesenchymal stem cell; PL: Placenta; UC: Umbilical cord.



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BM: Bone marrow; GO: Gene ontology; PL: Placenta; UC: Umbilical cord; MSC: Mesenchymal stem cell.

in each set (Figure 4).

Cytokines and growth factors: from the 19 downregulated cytokines and growth factors, 13 were found to be commonly downregulated in the MSCs types compared with BM-MSCs. Among them were *ANGPTL5, SEMA6D, CXCL16, IL7* and *CXCL16* in addition to *IL-26*, which had the highest fold change of -13.54 in the UC-MSCs. On the other hand, *CXCL5, SEMA3D, TXLNA, HDGFRP3, BMP4* and *CMTM3* genes were the commonly upregulated cytokines. Interestingly, *CXCL5* was expressed 260-fold higher in AT-MSCs, and *SEMA3D* was expressed 490- and 71-folds higher in PL- and UC-MSCs, respectively. Among the uniquely differentially

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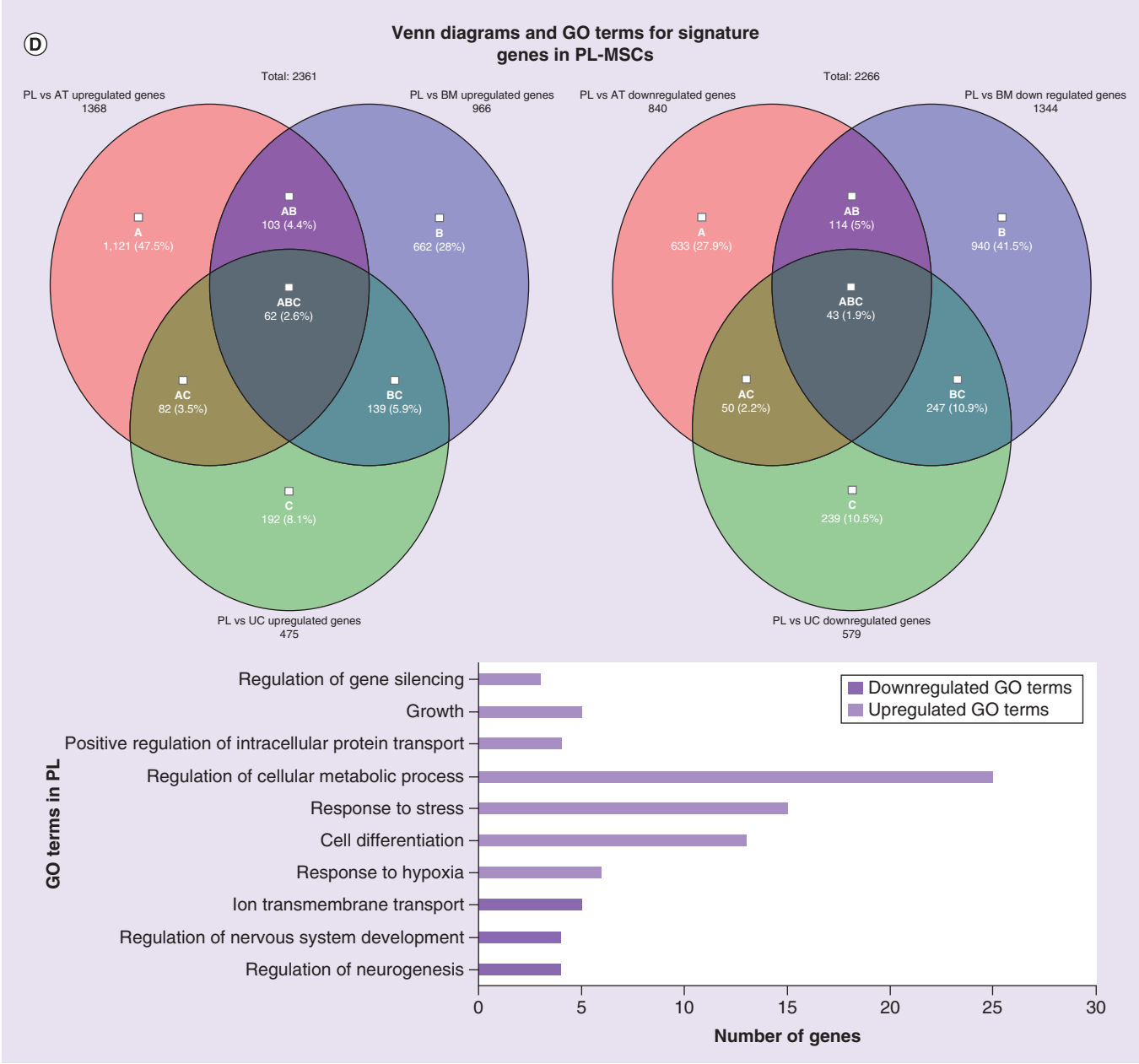

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expressed genes, *HGF* was downregulated in UC-MSCs by 52-folds while it was upregulated in PL-MSCs by twofolds (Figure 4A).

Onco- and tumor suppressor genes: out of the 26 differentially expressed oncogenes 12 were commonly upregulated while a small number of oncogenes were uniquely upregulated in PL-, UC- and AT-MSCs, respectively. Regarding tumor suppressor genes, UC-MSCs expressed the highest number of upregulated genes while AT-MSCs had the highest number of downregulated genes. Five tumor-suppressing genes were commonly upregulated in-

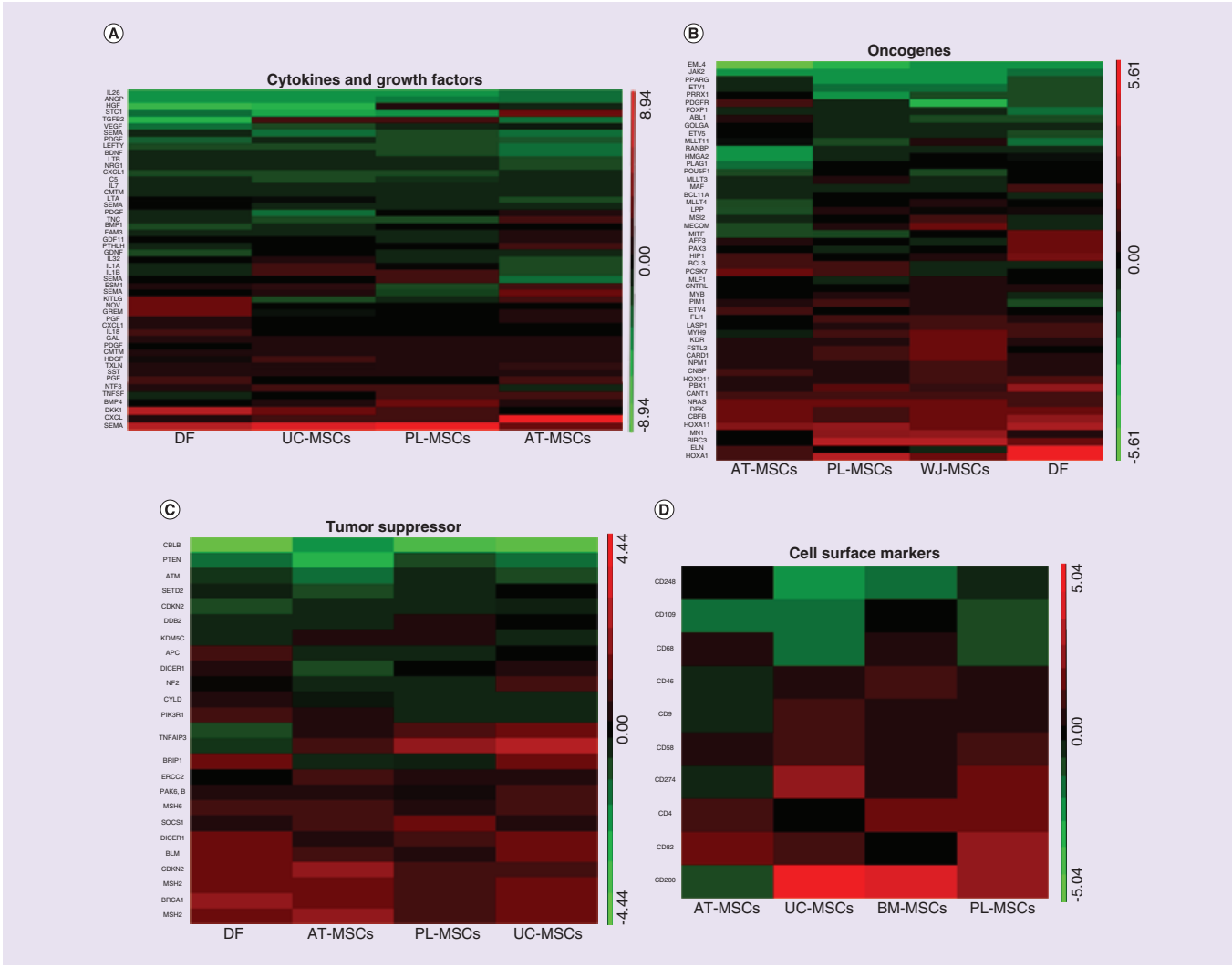

Figure 4. Heat maps for the differential expression level of selected gene families in umbilical cord-, placenta-, adipose tissue-, bone marrow-mesenchymal stem cells and dermal fibroblast. Colors in the heat maps represent log1.5-fold change values, corrected F ≤0.05. AT: Adipose tissue; BM: Bone marrow; DF: Dermal fibroblast; MSC: Mesenchymal stem cell; PL: Placenta; UC: Umbilical cord.

cluding *BRCA1, CDKN2C, MSH2, MSH2* and *TNFAIP3*; whereas *PTEN* and *CBLB* genes were commonly downregulated (Figure 4B & C).

Cell surface markers analyses did not reveal any common marker in MSCs compared with DF. However, *CD4* was found to be upregulated in all MSCs except UC-MSCs compared with DF. *CD82* and *CD4* were the only two markers that were found to be significantly upregulated in AT-MSCs compared with DF. *CD274* was found to be uniquely upregulated in perinatal sources of MSCs. Interestingly, *CD200* was upregulated in BM-, PL- and UC-MSCs with the highest fold change of 32 in UC-MSCs (Figure 4D). In addition, genes related to cell adhesion were compared between MSCs and DF. A total of 12 genes were found to be commonly upregulated in MSCs compared with DF including *ADAMTS12, COL3A1, VACAM1, FOXP1* and *VEGFA, CDH13* and *NRP2*. While a total of 13 genes were found to be upregulated in DF compared with the different MSCs types including *SMAD6, ACVRL1, GPNMB* and *NOV*.

ESC markers: a total of 13 genes were commonly upregulated in all MSCs types compared with DF such as *SLC1A1* and *GJA1*. Counter-intuitively, the perinatal sources of MSCs 'UC- and PL-MSCs' were not found to possess a unique expression pattern for known ESCs markers (Figure 4E).

MiRNA markers: >100 miRNA were differentially expressed, the majority of which were downregulated compared with BM-MSCs. *MIR3975* and *MIR421* were the most downregulated with fold changes of 744 and

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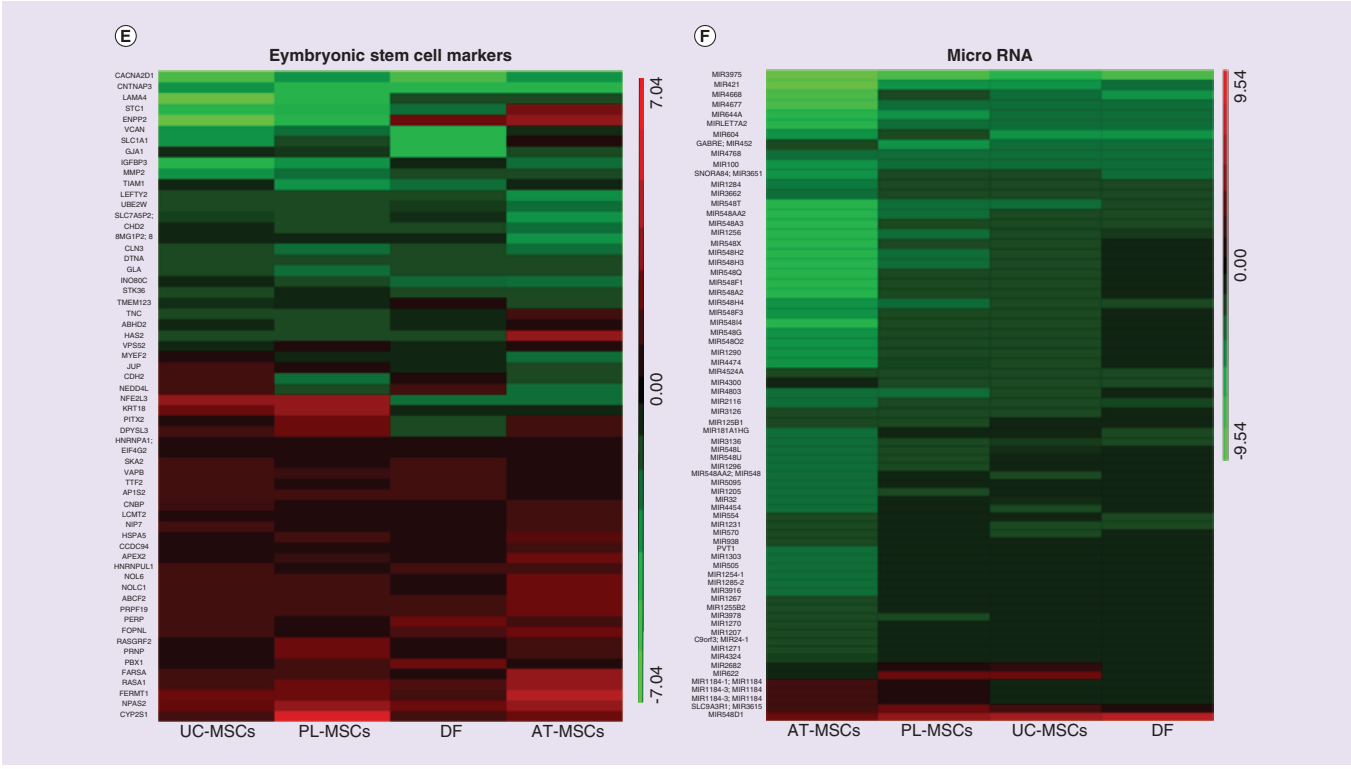

Figure 4. Heat maps for the differential expression level of selected gene families in umbilical cord-, placenta-, adipose tissue-, bone marrow-mesenchymal stem cells and dermal fibroblast (cont.). Colors in the heat maps represent log1.5-fold change values, corrected F ≤0.05.
AT: Adipose tissue; BM: Bone marrow; DF: Dermal fibroblast; MSC: Mesenchymal stem cell; PL: Placenta; UC: Umbilical cord.

265, respectively in AT-MSCs. *MIR548D1* and *MIR3615* were the only commonly upregulated miRNAs in the different MSCs types compared with BM (Figure 4F).

Immune modulation and tolerance: from the 1400 interrogated immune modulation-related genes, 35 genes were upregulated in all types of MSCs compared with BM-MSCs including *CXCL5, GPRC5B* and *TNIK*. Out of the 42 downregulated genes 17 were commonly downregulated, among them were *SOCS5, TLR4, IL7* and *C5*. In addition, a total of 13 immune tolerance-related genes were found to be commonly upregulated compared with BM-MSCs including *C1QBP* and *TNFAIP3*, while seven genes were commonly downregulated including *HLA-DRB1* and *HLA-C* (Figure 4H & I).

Cardiogenesis: among the 876 investigated genes, 24 genes were found to be commonly upregulated compared with BM-MSCs including *ABLIM3, TBX3,TBX5* and *DSG2*, the later was found to have the highest expression in UC-MSCs with a fold change of 158. Among the MSCs type-specific upregulated genes, *HAS2*, *PRICKLE1, ADAMTS1* and *TBX2* were found to be upregulated in AT-MSCs while downregulated in PL- and UC-MSCs (Figure 4M).

Neuronal repair and regeneration: of the 254 differentially expressed neurogenesis-related genes, 45 were commonly upregulated including *SEMA3D, GPRC5B* and *TNIK* with the highest fold change of 491 for *SEMA3D* expression in PL-MSCs. A number of genes were specifically up- or downregulated in each MSC type. For example, *EPB41L3* was upregulated only in AT- and UC-MSCs with fold changes of 52 and 2, respectively. Other genes were uniquely upregulated in UC-MSCs including *NCAM1, CAMK1D, NEDD4L, CDH2, ENC1* and *NEDD4L*. Interestingly, *BDNF* and *GDNF* were both found to be downregulated in PL-MSCs and AT-MSCs while not differentially expressed in UC-MSCs compared with BM-MSCs (Figure 4G).

Pathway enrichment analysis

Ingenuity pathway software (Ingenuity Pathway Analysis) was used to identify top canonical pathways based on the significance and predicted activation or inhibition. The significance of the pathway reflects the number of

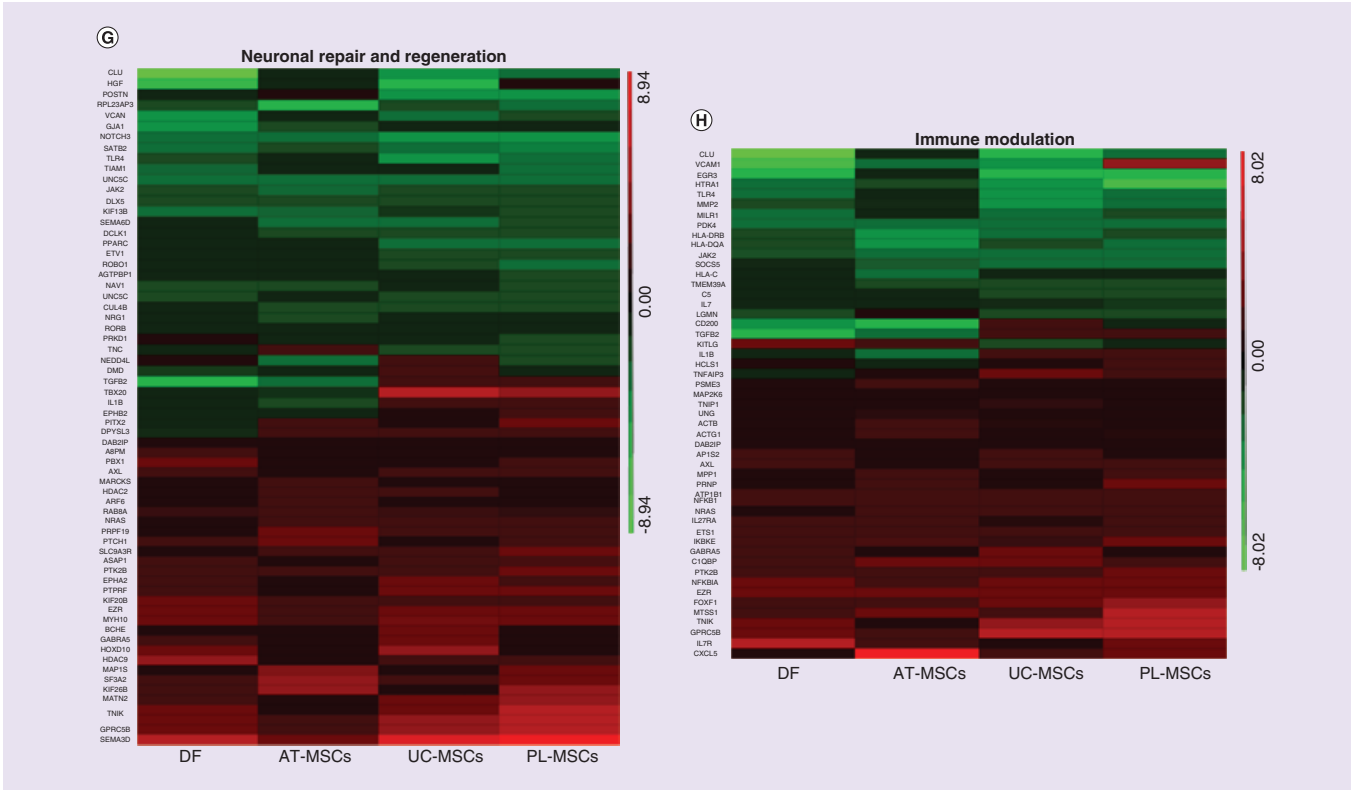

Figure 4. Heat maps for the differential expression level of selected gene families in umbilical cord-, placenta-, adipose tissue-, bone marrow-mesenchymal stem cells and dermal fibroblast (cont.). Colors in the heat maps represent log1.5-fold change values, corrected F $\leq$0.05.
AT: Adipose tissue; BM: Bone marrow; DF: Dermal fibroblast; MSC: Mesenchymal stem cell; PL: Placenta; UC: Umbilical cord.

genes differentially expressed in a pathway compared with the total number of genes known to have a role in that particular pathway. The p-value of <0.05 using right-tailed fisher exact test means a significant number of genes in a pathway were differentially expressed in a type of MSCs compared with BM-MSCs. For the predicted functional activity of a pathway, a z-score of $\geq$2 predicts activation while $\leq$-2 predicts inhibition of the specific pathway.

Based on the significance (p-value $\leq$0.05), top canonical pathways for each MSCs type are listed in Table 3. A complete list of all significantly enriched pathways is presented in Supplementary Figure 2.

Activated and inhibited pathways for each MSCs type compared with BM-MSCs are listed in Table 3 based on the z-score. PI3k/AKT signaling pathway was activated in all MSCs types compared with BM-MSCs. IL-1 signaling pathway was activated in PL- and UC-MSCs, while significantly enriched in AT-MSCs. Type I diabetes mellitus signaling pathway was activated in UC-MSCs, while significantly enriched in AT- and PL-MSCs. BMP signaling pathway was activated specifically in AT-MSCs.

Discussion

MSCs and their potential clinical applications are attracting major attention. Although BM-MSCs have been considered as the gold standard in MSCs research and clinical trials, MSCs isolated from a variety of tissues have been used in clinical trials for the treatment of a multitude of diseases. It is still unclear whether these MSCs have the same biological characteristics or if they are dissimilar in ways that will affect their clinical applications. Many groups attempted to answer this question comparing two or more types of MSCs at a time, providing much insight on the matter [12,20,21]. To the best of our knowledge this study is the first to compare the whole expression profile of two perinatal sources of MSCs (UC and PL) and two adult sources (BM and AT) alongside DF, which share cellular properties with MSCs [22,23].

Human platelet lysate has been established as a suitable alternative to FBS as culture medium supplement that enables efficient expansion of different MSCs types under animal-free conditions [24–26]. Although the percentage

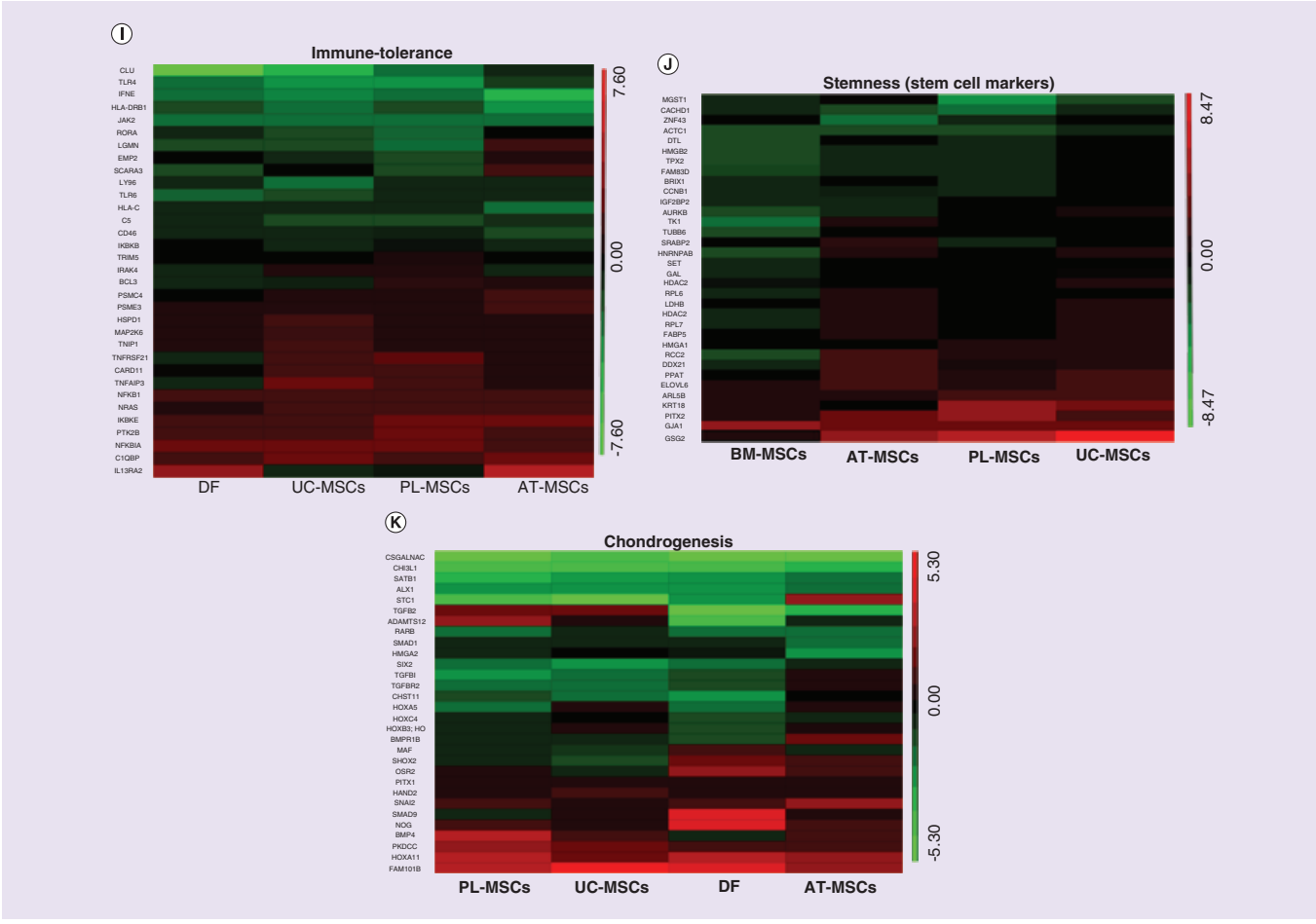

Figure 4. Heat maps for the differential expression level of selected gene families in umbilical cord-, placenta-, adipose tissue-, bone marrow-mesenchymal stem cells and dermal fibroblast (cont.). Colors in the heat maps represent log1.5-fold change values, corrected F $\leq$0.05.
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of platelet lysate in the media has varied between different groups, Becherucci *et al.* provided evidence that 5% platelet lysate could replace FBS for the clinical expansion of MSCs [27]. Moreover, our data clearly demonstrate that 5% platelet lysate allows a clinical grade expansion of MSCs with no differences, compared with FBS, in term of morphology, differentiation potential and immunological properties of MSCs [Unpublished Data]. As different supplement concentration may influence the expression profile, in this study all MSCs types were cultured using 5% platelet lysate to reduce the source of variation and to be in accordance with clinical manufacturing settings.

In the present study, the high principal component analysis (PCA) values and clustering of MSCs from the same source, indicated no evidence of the presence of other contaminating cell types (Supplementary Figure 3). Additionally, our hierarchical clustering data indicate a closer correlation between UC- and PL-MSCs, which might reflect the tissue origin. BM-MSCs clustered closer to the perinatal MSCs source as compared with the AT-MSCs. The microenvironment or the niche of MSCs coming from different anatomical regions might be the most important factor leading to this differential gene expression and clustering of MSCs taken from the different sources. In addition, the age of the tissue used to harvest MSCs might influence the gene expression [28]. This was observed in this study where AT-MSCs donor mean was higher than BM-MSCs. The developmental origin and the heterogeneous composition of MSCs in each source used to harvest MSCs could also play a role. Where, for instance, MSCs with ectodermal origin might be present in a different proportion among the MSCs from mesodermal origin in each source [29].

With the aim of identifying specific markers for each stem cell type, potential signature genes in each MSCs type were investigated. Those were identified as uniquely expressed genes in only one MSCs type (Table 1).

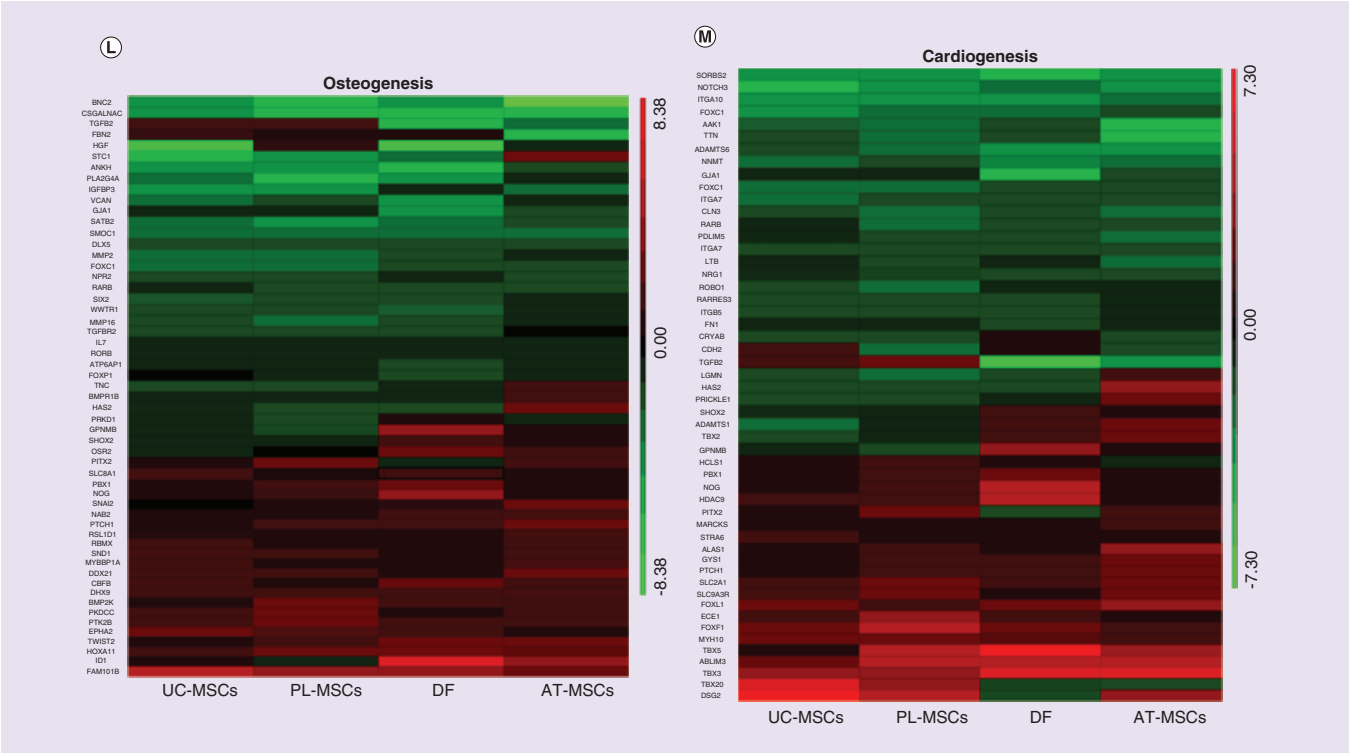

Figure 4. Heat maps for the differential expression level of selected gene families in umbilical cord-, placenta-, adipose tissue-, bone marrow-mesenchymal stem cells and dermal fibroblast (cont.). Colors in the heat maps represent log1.5-fold change values, corrected F ≤0.05.
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Among the genes that were uniquely expressed in UC-MSCs are *HOXD10* and *NCAM1*. HOXD10 is a sequence-specific transcription factor important for stem cells fate determination [30]. NCAM1 or CD56 is a cell surface glycoprotein, that is known to regulate neuron–neuron adhesion, neurite outgrowth and synaptic plasticity in the nervous system [31]; while *Sema3D*, *F3* and *SDPR* were among the uniquely expressed genes in PL-MSCs. Furthermore, among the genes that were specifically expressed in AT-MSCs is *MMP3*. This gene was found to be among the MMPs implicated in adipogenic and osteogenic differentiation [32]. In addition, it plays an important role in the migration and proliferation potential of MSCs [33]. As for BM-MSCs, uniquely expressed genes include *CNTNAP3B, PLA2R1, FOS* and *FBXO32* in addition to several small nucleolar RNAs and miRNAs (Table 1).

When examining previously published findings comparing the transcriptome of two or more MSCs types at a time, discrepancies among the studies and with our study were observed. For instance a much higher differentially expressed genes (DEGs) number was found here compared with similar work using RNA Seq technology comparing AT- to BM-MSCs [34]. Consequently, more GO terms involving the DEGs were annotated in this study providing a deeper insight on the biology of AT-MSCs. On the other hand, we did not find any surface marker unique to AT-MSCs. Those inconsistencies can be due to different culturing conditions, higher passage of MSCs used and different number of biological replicas or a probable higher stringency in Affymetrix data generation compared with the other platforms and different analysis tools.

Counterintuitively, our transcriptome analysis did not find a difference in the expression levels between the adult sources of MSCs and the perinatal sources of the known ESCs markers such as *Sox2, Nanog, Lin28* and *POU5F1* (*OCT4*). On the other hand, PL- and UC-MSCs could be segregated based on the *KRT8* and its conjugate *KRT18* ESC markers with a higher expression than both BM- and AT-MSCs. KRT8 and KRT 18 are intermediate filaments with important roles in cellular structural integrity as well as hepatocyte differentiation [29]. Conversely, certain embryonic stemness markers such as *MGST1* were expressed at a higher level in BM- and AT-MSCs. The higher expression in adult tissue-derived MSCs compared with perinatal MSCs is an interesting finding as *MGST1* has been reported to have an increased expression in $CD3^+$ hematopoietic stem cells with increased age [35]. DSG2

Table 3. Significantly enriched and activated canonical pathways.

Top canonical pathways based on significance	p-value	No. of genes
AT-MSCs		
April-mediated signaling	3.22E-05	11
B-cell-activating factor signaling	5.36E-05	11
OX40 signaling pathway	3.02E-04	11
Type I diabetes mellitus signaling	3.43E-04	17
Regulation of IL-2 expression in activated and anergic T lymphocytes	4.36E-04	14
UC-MSCs		
TWEAK signaling	1.47E-04	9
IL-8 signaling	2.19E-04	26
TNFR2 signaling	3.16E-04	8
iNOS signaling	3.28E-04	10
ILK signaling	4.46E-04	25
PL-MSCs		
TNFR2 signaling	2.07E-04	8
PTEN signaling	5.77E-04	17
Tight junction signaling	8.23E-04	21
IL-8 signaling	1.17E-03	23
TWEAK signaling	2.75E-03	7
Top canonical pathways based on z-score	**z-score**	**No. of genes**
AT-MSCs		
B-cell receptor signaling	2.558	22
Hypoxia signaling in the cardiovascular system	2.449	10
BMP signaling pathway	2.333	10
PI3K/AKT signaling	2.496	14
UC-MSCs		
Type I diabetes mellitus signaling	2.714	15
IL-1 signaling	2.111	13
PI3K/AKT	2.309	13
PL-MSCs		
IL-1 signaling	2.333	12
B-cell receptor	2.183	18
PI3K/AKT signaling	2.309	13

AT: Adipose tissue; MSC: Mesenchymal stem cell; PL: Placenta; UC: Umbilical cord.

is a common stemness marker expressed by ESC cell lines, reported earlier in MSCs with a higher expression in UC-MSCs [36]. Here we report its upregulation in all MSCs types compared with BM-MSCs with a highest expression in UC-MSCs (335-folds).

Immune tolerance and survival mechanisms are important properties of MSCs, and essential for the success of stem cell transplantations in the treatment of diseases. HLA-DR and HLA-DQB2 class II major histocompatibility complex (MHC), and HLA-C class I MHC showed the lowest expression in AT-MSCs and the highest in BM-MSCs with a similar expression pattern in UC- and PL-MSCs. This means that AT-, UC-, PL-MSCs are less likely to elicit an adaptive immune response. Moreover, AT-MSCs have higher expression than BM-MSCs of *IL-13r*, which is implicated in IL-13 cellular sequestration and apoptotic escape mechanism [37]. Our pathway analysis results showed an advantage of the MSCs types studied over BM-MSCs in the activation of PI3K and its downstream target Akt/PKB pathway, involved in the control of cell proliferation and apoptosis. [38]. In this regard, we assessed the proliferation potential of UC- and PL-MSCs compared with BM-MSCs (Supplementary Material 3). Our results clearly indicate a higher proliferation potential for UC and PL-MSCs represented by a higher cumulative population doubling. Other groups have studied the proliferative potential of different MSCs as an indicator reflecting their potential use in cell therapy [34,39,40]. Li *et al.* observed significant differences in

the proliferative potential among the four populations of MSCs, with UC-MSCs possessing the highest potential followed by AT- and PL-MSCs compared with BM-MSCs [41].

The immune modulatory properties of MSCs are well established. However, our aim was to investigate any potential difference for the MSCs types ability to modulate an immune response. For this purpose, around 1400 genes related to immune modulation were studied. Among the differentially expressed genes, *TLR-4* was found to be the highest in BM and the lowest in UC- and PL-MSCs. Although its traditional function has been solely in the activation of innate immune cells against pathogen attacks, novel roles of TLR-4 activation in MSCs include facilitating the interaction with the surrounding environment [42]. More importantly, TLR-4 induces Treg cells, thus modulating the immune response to counteract the inflammatory facet of several diseases [43]. This important immune modulatory trend in BM-MSCs is emphasized with the IL-7 increased expression in BM-MSCs. As IL-7 has been found to inhibit effector T-cell proliferation while enhancing the $CD4^+$ T-cell population, which includes Tregs [44]. Another immune player *CD200*, was found to be increased in both BM- and UC-MSCs compared with other MSCs. In addition to its immune tolerance roles, CD200 has been found to inhibit the maturation of myeloid progenitors into inflammatory cells as well as to suppress the secretion of the proinflammatory TNF-α in stimulated macrophages [45–47]. In addition, *CD274*, which is a known immune modulatory protein that plays a negative role in immune modulation was found to be uniquely upregulated in perinatal sources of MSCs. Cho *et al.* also reported the positive expression of this surface marker in palatine tonsils MSCs compared with BM- and AT-MSCs [48]. In accordance with this, several proinflammatory pathways were found to be activated in the other MSCs types compared with BM-MSCs such as IL-8 signaling, diabetes mellitus signaling and TWEAK signaling. Our transcriptome analysis provides evidence of a possible higher immune modulation potential of BM-MSCs.

Cell adhesion of MSCs is essential for MSC-dependent tissue regeneration *in vivo* as it is implicated in the migration to the injured tissues [49]. Comparing MSCs to DF revealed that all MSCs types have a high expression level of genes related to positive regulation of cell migration such as *FOXP1, NRP2, VEGFA* and *CDH13*, and cell–matrix adhesion such as *ADAMTS12, COL3A1* and *VACAM1*. Some of those have been described earlier in MSCs including VACAM1, important for the adhesion of MSCs to the endothelium [50]. On the other hand, DF has a high expression level of genes related to negative regulation of cell adhesion including, *ACVRL1, PLXNC1*, which points to a clear advantage of MSCs in the homing property.

The issue of using MSCs safely has been demonstrated by several groups and long-terms follow-up after their administration showed no malignancies in humans. However, this has been counteracted with oncology studies of the role they may play in the endothelial–mesenchymal transition. Although BM-MSCs seem to express fewer oncogenes while UC-MSCs have the highest number of tumor suppressor genes. It is difficult to make any conclusion due to the lack of comprehensive studies on the balance of oncogenes and tumor suppressor genes in the context of MSCs biology and their *in vivo* effects.

The principle goal of our study was to analyze the differential expression level of gene families associated with therapeutic outcomes. One of the most successful regenerative applications for MSCs have been cartilage and bone repair. In this regard, we studied the expression of genes, related to osteogenesis. Out of the 96 genes investigated, 13 and ten genes were upregulated specifically in BM-MSCs and AT-MSCs, respectively. Whereas one and five genes upregulated in UC-MSCs and PL-MSCs, respectively. *FOXC1* and *DLX5* were among the upregulated genes in BM-MSCs. *FOXC1* is an important regulator of the initial steps in osteoblast differentiation [51]. It was demonstrated that *FOXC1* directly regulates expression of *Msx2*, a key regulator of early osteogenic events [52]. Moreover, DLX5 transcription factor that acts later in osteogenic differentiation events and regulated by BMP2 signaling was also upregulated in BM-MSCs. On the other hand, AT-MSCs showed upregulation in *BMPR1B* gene; this gene is capable of binding to BMP ligands and transduce BMP signaling [53]. Taken together, these results suggest that both BM- and AT-MSCs have the ability to differentiate into osteoblast in a higher capacity as compared with PL-MSCs and UC-MSC.

Forty-three genes related to chondrogenesis were studied, BM- and AT-MSCs had the highest number of upregulated genes. Among the differentially expressed genes in BM-MSCs, *CSGalNAcT-1, CHI3L1* and *SMAD1* were specifically upregulated in BM-MSCs. CSGalNAcT-1 has been shown to be crucial for the initiation of cartilage chondroitin sulfate biosynthesis [54]. Differential expression of CHI3L1 is accompanied with proliferation and differentiation in osteogenic and chondrogenic cell lineages during fetal development as well as MSC differentiation [55,56]. SMAD1 is a key regulator of Smad-dependent signal transduction pathways, which is essential for the initiation of chondrogenic differentiation [57]. On the other hand, *TGFBI, TGFBR2* and *SHOX2* were downregulated in all MSCs compared with AT-MSCs. TGFB proteins are the most potent activators of chondro-

genesis in human-derived mesenchymal stem cells (hMSCs) [58]. Oka *et al*. have demonstrated that inactivation of TGFBR2 in neural crest cells resulted in aberrant formation of Meckel's cartilage and altered the development of mandible [59]. SHOX2 has been shown to control chondrocyte maturation by regulating the expression of *RUNX* genes, through BMP4 [60]. Therefore, our results propose that BM and AT-MSCs have higher chondrogenic differentiation potential than PL and UC-MSCs. The characteristic trilineage differentiation potential of MSCs includes, beside chondrogenic and osteogenic, adipogenic differentiation potential. However, the adipogenic differentiation potential was not investigated here due to the rare incidence of genetic and acquired lipodystrophies. The US FDA-approved drug has been launched recently to treat these conditions. Moreover, liposuction, instead of MSCs adipogenic differentiation, makes adipocytes readily available for regenerative and cosmetic purposes [61].

Neuronal repair and regeneration are an active area of research, where stem cells provide therapeutic hope for many incurable diseases. Here, 254 genes involved in the different aspects of nervous system maintenance and repair have been found to be differentially expressed. The UC-MSCs was found to have unique GO terms-enriched genes for neurogenesis where genes are members of the BDNF signaling pathway, neural crest differentiation and one gene *ALDH1A1* is involved in the specification step of dopaminergic neurogenesis. These genes are either uniquely expressed such as *NCAM1*; important in neural development and neurite outgrowth or are highly differentially expressed such as *GDNF*, which enhances neural regeneration and decreases apoptosis in cerebral hemorrhage. In addition, UC-MSCs have a unique expression of *HOXD10*, a transcription factor implicated in spinal cord motor neuron cell fate specification and skeletal muscle development.

In terms of the main neurotrophic factors implicated in stem cell neural repair, the expression varied between the MSCs. The BDNF, important for neuronal survival and plasticity has been found highest in BM- and UC-MSCs followed by PL- and AT-MSCs, respectively. It is worth mentioning that UC-MSCs have in addition an increased expression of 11 downstream genes in the BDNF pathway including *IGF2BP1*. The neuroprotective factor, GDNF was found higher in UC- and PL-MSCs compared with BM-MSCs. On the other hand, HGF was 50-times less in UC-MSCs and twofolds higher in PL-MSCs than both BM- and AT-MSCs. The direct administration of HGF or its overexpressing MSCs have been investigated and found beneficial in the treatment of most neurological diseases [62]. It is also worth mentioning that the level of *LIF*, an IL-6 family cytokine, is highly expressed in all MSCs but is twice as high in PL-MSCs compared with BM-MSCs. LIF induces neuronal differentiation and has been suggested as a treatment for multiple sclerosis (MS) and other neurological diseases [63]. Taken together, depending on the neuropathology, the different types of MSCs can be more suitable for therapy with an apparent advantage of UC- and PL-MSCs, followed by BM- and AT-MSCs.

In addition, UC-MSCs also appear to have the highest potential for treating muscle dystrophies and cardiac muscle regeneration. Two genes, *DMD* and *FAM101B* expression were found many folds higher in UC-MSCs compared with other MSCs types. In accordance with our result, DMD has been reported to be higher in UC-MSCs when compared with Dental pulp MSCs [64]. In addition to its positive role in neuron differentiation, DMD is also implicated in the regulation of heart rate, cardiac and muscle development [65,66]. FAM101b (Cfm1) on the other hand is important for the skeletal system morphogenesis [67].

Furthermore, angiogenesis GO term was specifically upregulated in AT-MSCs. Among the related genes was the *MMP3* discussed above. Our findings might offer AT-MSCs as a promising candidate for treatment of ischemia-related disorders. Another related finding is the differential expression of Sema3D, which is >400-folds higher in PL-MSCs than all other types of MSCs. This semaphorin group member is an antiangiogenic factor, and has been suggested as an anticancer therapy, with efficacy against glioblastoma [68]. Sema 3D has been described earlier in the limbal stem cells when compared with BM-MSCs [69]. However, *Sema3D* was not found in a previous expression analysis comparing PL-MSCs to BM-MSCs and AT-MSCs [20,70].

Furthermore, our analysis provided an insight on the miRNAs differential expressions, which are considered master regulators of diverse biological processes of different cells including MSCs [71,72]. Our transcriptome data revealed a unique expression pattern of miRNAs in BM-MSCs, represented in a high number of upregulated miRNA (120) compared with the other types of MSCs studied. The significance of which is yet to be elucidated. One of those is *miRNA3975*, which is among the signature miRNA expressed exclusively in BM-MSCs with no function attributed to it yet. While other miRNAs such as *miRNA466* have been described recently and seem to play roles in inhibiting cell survival and aggressiveness of cancer cells [73,74]. Another interesting finding worth further investigation is the upregulation of 24 members of the miRNA-548 family in BM-MSCs compared with other MSCs [75]. Among those: miR-548q has been found to downregulated HLA-G mRNA and thus increase natural killer (NK)-cell-mediated cytotoxicity [76]. A role not detected by our analysis as HLA-G was not differentially

expressed in the MSCs studied. On the other hand, *miRNA 548d-1, miRNA 3615, miRNA 4683, miRNA 548f-5* and *miRNA 3960* are among the miRNAs with a higher expression in all MSCs compared with BM-MSCs with no reported function so far. While miRNA-548f was reported to inhibit α-SMA expression in human aortic smooth muscle cells *in vitro* [77]. The complete microarray analysis of miRNA, however, would be better analyzed using GeneChip miRNA 4.0 affymetrix.

The current analysis will pave the road for further in-depth analysis, which might reveal other important aspects of MSCs biology. Future-related studies might include a larger number of samples to depict more differences between the MSCs of different origins. Using the RNAseq along the microarray bioanalysis would also be of use. Additionally, functional studies on the different therapeutic potential areas of MSCs would validate the results presented herein. The limitations of this conclusion are that it is not based on any *in vivo* study and has not been validated by other *in vitro* methods. However, the microarray chips used rely on robust hybridization technology and powerful statistical methods, and several GO and enrichment in silicon tools were used for analysis. Thus, our findings are of great interest for different groups to conduct validation studies and tailor clinical trials use of MSC from the suitable origin accordingly.

Conclusion

The current study provides an exciting overview of the transcriptome of four MSCs types cultured in the same xeno-free conditions along with DF. It can be concluded that UC-, PL- and AT-MSCs are more suitable than BM-MSCs for allogeneic administration due to their lower expression of MHC molecules while BM-MSCs seem to be more potent immune modulator with enhanced antimicrobial activity. No common MSCs marker was found in comparison to DF. On the other hand, the surface marker *CD274* was uniquely expressed in the perinatal stem cells sources compared with the adult source. Regarding MSCs plasticity and therapeutic potential, BM- and AT-MSCs might be more suitable for bone and cartilage repair. While PL- and UC-MSCs have been found to secrete larger amounts of neurotrophic factors important in treating the different neurological ailments. UC-MSCs expressed dystrophin in higher amounts suggesting its increased potential in the treatment of muscle dystrophies. AT-MSCs on the other hand, seem to be the best source of MSCs for angiogenesis and the treatment of peripheral arterial disease. More such studies are needed to provide guidance on the best source of MSCs, prior to the translational and clinical studies to treat different inveterate clinical conditions.

Future perspective

We believe that future clinical trials should take into consideration the biologic properties of MSC, which differs according to the tissue of origin. MSC should not be considered exchangeable for the various potential indications despite of their common morphology and surface markers. This work sheds some light on possible options to be considered for future clinical applications, especially in the allogeneic setting.

Our work suggests that using MSCs of adipose tissue are more potentially beneficial than other sources if we aim at osteo- and chondro-regeneration. While MSCs of BM origin are potentially more beneficial for immune suppression, MSCs of perinatal origin are best suited for regeneration of cardiac- and neuro-regeneration.

The limitation of this conclusion is that it is not based on any *in vivo* study and remains an open point for discussion. However, our findings are of great interest to tailor clinical trials with MSC based on it.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: https://www.futuremedicine.com/doi/suppl/10.2217/rme-2018-0137

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval (Cell Therapy Center/The University of Jordan) and have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations.

Summary points

- To date there is no consensus on the best source of mesenchymal stem cell (MSCs) most suitable to treat a specific disease in clinical or preclinical studies.
- Microarray genes interrogating 67,000 transcripts spanning the whole human transcriptome were used to analyze MSCs from different origins.
- The transcriptome of two adult sources, bone marrow (BM) and adipose tissue along with two perinatal sources, umbilical cord and placenta were studied.
- Analyses were performed using the gold standard BM-MSCs or dermal fibroblasts.
- Our results showed that MSCs types studied are more suitable than BM-MSCs for allogeneic administration.
- BM-MSCs appear to have an advantage regarding immune-modulation and antimicrobial activities as well as bone and cartilage tissue repair.
- Whereas the positive regulation of neurogenesis was found to be a specific gene ontology term for umbilical cord-MSCs followed in potency by placenta- and BM-MSCs.
- Adipose tissue-MSCs have an advantage for treating ischemia-related disorders in addition to bone and cartilage repair.

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