MEDICAL SCIENCE MONITOR BASIC RESEARCH

LABORATORY RESEARCH

eISSN 2325-4416
© Med Sci Monit Basic Res, 2017; 23: 87-96
DOI: 10.12659/MSMBR.903518

Received: 2017.01.24
Accepted: 2017.02.14
Published: 2017.03.24

Identification and Evaluation of New Immunoregulatory Genes in Mesenchymal Stromal Cells of Different Origins: Comparison of Normal and Inflammatory Conditions

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 **Mohammad Fayyad-Kazan***
ABCDEFG 2 **Mehdi Najar***
ABCDEF 3 **Hussein Fayyad-Kazan**
ABDF 2 **Gordana Raicevic**
ABCDEFG 2 **Laurence Lagneaux**

1 Institute of Molecular Biology and Medicine, Université Libre de Bruxelles (ULB), Gosselies, Belgium
2 Laboratory of Clinical Cell Therapy, Université Libre de Bruxelles (ULB), Institut Jules Bordet, Brussels, Belgium
3 Laboratory of Cancer Biology and Molecular Immunology, Faculty of Sciences I, Lebanese University, Hadath, Lebanon

* Both authors contributed equally to this work
Corresponding Author: Hussein Fayyad-Kazan, e-mail: hfayyadk@gmail.com
Source of support: This work is supported by "Le Fonds National de la Recherche Scientifique-FNRS"

Background: Mesenchymal stromal cells (MSCs) possess potent immunomodulatory properties that increase their value as a cell-based therapeutic tool for managing various immune-based disorders. Over the past years, accumulated results from trials using MSCs-based therapy have shown substantial contradictions. Although the reasons underlying these discrepancies are still not completely understood, it is well known that the immunomodulatory activities mediated by distinct MSCs differ in a manner dependent on their tissue origin and adequate response to inflammation priming. Thus, characterization of new molecular pathway(s) through which distinct MSC populations can exert their immunomodulatory effects, particularly during inflammation, will undoubtedly enhance their therapeutic potential.

Material/Methods: After confirming their compliance with ISCT criteria, quantitative real time-PCR (qRT-PCR) was used to screen new immunoregulatory genes in MSCs, derived from adipose tissue, foreskin, Wharton's jelly or the bone-marrow, after being cultivated under normal and inflammatory conditions.

Results: *FGL2*, *GAL*, *SEMA4D*, *SEMA7A*, and *IDO1* genes appeared to be differentially transcribed in the different MSC populations. Moreover, these genes were not similarly modulated following MSCs-exposure to inflammatory signals.

Conclusions: Our observations suggest that these identified immunoregulatory genes may be considered as potential candidates to be targeted in order to enhance the immunomodulatory properties of MSCs towards more efficient clinical use.

MeSH Keywords: Immunomodulation • Inflammation • Mesenchymal Stromal Cells

Full-text PDF: http://www.basic.medscimonit.com/abstract/index/idArt/903518

3134 2 4 42

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Mesenchymal stromal cells (MSCs) are adult multipotent cells capable of differentiating into different tissue lineages [1,2], thus representing an attractive candidate for tissue regeneration and repair. Besides their stem/progenitor characteristics, MSCs possess robust immunomodulatory capacities that enable them to modulate most immune cells, particularly T lymphocytes [3,4]. In fact, MSCs can inhibit the activation and proliferation of T cells, thus rendering them in a state of anergy [3,4]. Due to their immunosuppressive potential, MSCs have been used in both animal models and clinical trials to treat various immune disorders, including graft versus host disease [5], systemic lupus erythematosus [6], and rheumatoid arthritis [7]. The immunomodulatory capacity of MSCs is reported to be dependent on the surrounding inflammatory microenvironment. Indeed, MSCs act like biosensors that detect disease specific inflammatory fingerprints and after migration to the site of injury, they deliver the most beneficial response to the host, thereby, lowering the chances of systemic side effects [8]. Alteration of MSCs' immunomodulatory potency by external stimuli, in particular inflammatory cytokines, is reported to be accompanied with an increase in MSCs-mediated secretion of immune regulatory factors [8]. Intriguingly, the *in vitro* preactivation of MSCs (priming) holds promise as an interesting process for improving MSCs-immunomodulatory effects, thus conferring therapeutic advantages. However, a high diversity in the outcomes of clinical trials, using MSCs for treating different diseases, has been reported [9]. This variation remains vague since a complete mechanistic understanding of MSCs-mediated immunomodulation is still lacking. Indeed, MSCs can mediate their immunomodulatory effects either via a complex system of soluble factors, such as transforming growth factor-β (TFG-β), hepatocyte growth factor (HGF), and interlukin-10 (IL-10), which ensure a dynamic crosstalk between MSCs and immune cells, or through direct cellular contacts [10]. Besides the complexity of these signaling systems, MSCs can be isolated from various adult and fetal tissues, among which are the adipose tissue (AT), foreskin (FSK), and Wharton's jelly (WJ) of the umbilical cord, that are considered as potential alternatives to bone-marrow (BM) [11,12]. Despite their common phenotypic and functional characteristics, tissue source-dependent differences in MSCs properties, especially in their immunomodulatory capacities, have recently emerged and led to different clinical applications [13,14].

A comprehensive understanding of the mechanisms regulating the immune responses in MSCs' field remains a major challenge towards improving their clinical applications. In this work, we aimed at identifying new immunoregulatory genes in different MSCs and characterizing their sensitivities to inflammatory signals. Accordingly, we assessed the transcription profiles, within MSCs derived from BM, WJ, AT, or FSK, of a set of genes known to serve important immunomodulatory roles. Our results identified *FGL2*, *GAL*, *SEMA4D*, *SEMA7A*, and *IDO1* as new candidate genes that could be involved in MSCs-mediated immunomodulation. The transcription of these genes was dependent on both the MSCs-tissue source and the surrounding inflammatory status. Our data could help in better understanding the molecular mechanisms employed by the different MSCs to respond to the surrounding inflammatory environment. Moreover, these observations might serve in developing strategies to manipulate MSCs-immunomodulatory properties by adequately targeting these immunological genes.

Material and Methods

Ethical guidelines

This study was conducted in accordance with the Declaration of Helsinki (1964) and approved by the local ethics committee of the Institut Jules Bordet (Belguim). All samples were collected from healthy donors after giving informed written consent.

Isolation, culture, characterization and priming of human MSCs

Once obtained, BM, WJ, FSK, and AT were processed according to our previous protocols [15,16]. Cell cultures were incubated at 37°C in a 5% CO2 humidified atmosphere. After 48 hours, non-adherent cells were removed by washing, and the medium was changed twice a week. When sub-confluency (80–90%) was achieved, adherent cells were harvested by TrypLE Select (Lonza) and replated at a lower density (1,000 cells/cm^2). MSCs of different origins were characterized for their morphology, phenotype and multi-lineage potential according to our previous work [17]. MSCs were cultivated under basic or inflammatory conditions. As previously described [18], inflammation priming was performed upon treating MSCs, for overnight, with a cocktail of pro-inflammatory cytokines, specifically IL-1β (Peprotech, Rocky Hill, NJ, USA) (25 ng/mL), TNF-α (50 ng/mL), IFN-α (10 ng/mL), and IFN-γ (50 ng/mL) (all from Prospec Inc., Rehovot, Israel).

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cord blood mononuclear cells (CBMC), adult purified monocytes (CD14$^+$ selection, Mylteniyi Biotech), human umbilical vein endothelial cells (HUVEC, PromoCell, Heidelberg, Germany), and peripheral blood mononuclear cells (PBMC), and each MSC type, and was extracted in a single step using TriPure Isolation Reagent (Roche Applied Science, Vilvoorde, Belgium). For human placenta tissue, human brain tissue, and human umbilical cord, RNA was directly provided by Stratagene Europe. We performed the reverse transcription

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

reaction with 1 mg RNA using qScript cDNA SuperMix (Quanta Biosciences). Transcripts were quantified by qRT-PCR using 20 ng of cDNA, SYBR Green PCR Master Mix (Applied Biosystems, Lennik, Belgium) and 0.32 mM forward and reverse primers. The primers were designed with Primer Express 2.0 software (Applied Biosystems) or ProbeFinder online software (Roche) and are available in Supplementary Table 1. To control variations in input RNA amounts, the *GAPDH* gene was used as a housekeeping gene to quantify and normalize the results. The reactions were carried out using the ABI Prism 7900 HT system (Applied Biosystems). In all cases, dissociation curves were generated and the specificity of the PCR reactions was confirmed. The comparative $\Delta\Delta$Ct method was used for data analysis. To evaluate the fold change, data were normalized with the *GAPDH* gene to obtain the ΔCt and were then calibrated with the geometric mean of the *GAPDH* ΔCt to generate the $\Delta\Delta$Ct. Fold changes were then calculated as fold change=$2^{-\Delta\Delta Ct}$.

Statistical analysis

Data are presented as means ±SEM of three independent experiments and statistical significance of gene transcription between control and treated cells was determined according to unpaired Mann-Whitney U test. *P*-values <0.05 (*), <0.01 (**), <0.001 (***) were considered significant.

Results

Identification and characterization of immunoregulatory genes in different MSCs

Learning how to control the plasticity of MSCs' immunoregulatory function, both endogenously and exogenously, may provide an important new modality for better therapeutic applications of MSCs, as well as improved understanding of MSCs' role in different stages of disease. Accordingly, a transcription profiling was carried out to identify key immunoregulatory genes in distinct MSCs. It is noteworthy that before starting this screen, we confirmed that all populations of MSCs tested in this study were compliant with ISCT criteria (morphology, phenotype, multi-lineage potential). Thus, cells in culture were adherent, presenting as typical fibroblast-like cells and were capable of providing colony forming unit-fibroblasts (CFU-F), a specific character of MSCs. The phenotype of these cells was established by flow cytometry using a panel of surface markers. Accordingly, representative flow cytometry histograms (Supplementary Figure 1) demonstrate the positivity of the cells for MSC-markers CD73, CD90, and CD105 as well as their negativity for CD45, CD34, CD14, CD19, and HLA-Dr hematopoietic markers. CD146 was specifically expressed, even at different levels, in the distinct MSCs populations and thus considered a marker distinguishing MSCs from fibroblasts [19]. We have also confirmed the multi-lineage potential of these cells by inducing adipogenesis, osteogenesis, and chondrogenesis using specific media and highlighting these cell differentiations by using specific staining techniques. Representative images of adipocytes (lipid vacuoles stained by Oil Red O), osteoblasts (calcium deposit stained by Alizarin red) and chondrogenic pellets (proteoglycans synthesis stained by Alician blue) are provided in Supplementary Figure 2.

In a second step, and upon using individual quantitative Real-Time qPCR (qRT-PCR), the transcription status of a predefined set of immunomodulatory genes (*ARG1*, *CD163*, *FGL2*, *GAL*, *IDO1*, *IDO2*, *SEMA3A*, *SEMA4A*, *SEMA7A*, *SEMA4D*, and *SEMA6D*) was characterized in MSCs, derived from BM, WJ, AT, and FSK, and being cultivated under basic (non-inflammatory) conditions (Figure 1). *FGL2* appeared to be strongly transcribed in AT-MSCs but only weakly in FSK-MSCs (Figure 1A). On the other hand, BM- and WJ-MSCs showed only minimal *FGL2* mRNA levels (Figure 1A). *GAL* gene was transcribed in both AT- and FSK-MSCs, with the latter showing higher transcription levels (Figure 1B). On the contrary, only minimal *GAL* transcription was detected in BM- and WJ-MSCs (Figure 1B). *SEMA4D* and *IDO1* genes exhibited minimal transcription in the different tested MSCs being grown under basic conditions (Figure 1C, 1E, respectively). *SEMA7A* appeared to be differentially transcribed in the distinct MSCs studied, with BM-MSCs showing highest transcription levels followed by FSK- and AT-MSCs and finally WJ-MSCs being characterized by minimal *SEMA7A* mRNA levels (Figure 1D). No significant transcription was detected in the case of *ARG1*, *CD163*, *IDO2*, *SEMA3A*, *SEMA4A*, and *SEMA6D* genes in any of the four mentioned MSCs types (data not shown).

In a next step, we aimed at examining the effect of inflammatory signals on the transcriptional profiles of the mentioned genes. Interestingly, the high *FGL2* mRNA levels exhibited by AT-MSCs were clearly reduced, to less than half, upon cultivating these cells in an inflammatory milieu (Figure 1A). On the contrary, the inflammatory signals positively affected *FGL2* transcription in FSK-MSCs, mainly, and to a lesser extent in in BM- and WJ-MSCs (Figure 1A). Following inflammation-priming, *GAL* transcription was significantly reduced in AT-MSCs but strikingly induced in FSK-MSCs (Figure 1B). On the other hand, no significant alterations in *GAL* transcriptional profiles were detected following inflammation-priming of BM- or WJ-MSCs (Figure 1B). The minimal *SEMA4D* and *IDO1* transcription levels were strongly induced following inflammation-priming of AT-MSCs and FSK-MSCs, with the former showing much higher levels (Figure 1C, 1E). On the other hand, only a slight induction of *SEMA4D* and *IDO1* mRNA levels was detected in inflammation-primed BM- and WJ-MSCs (Figure 1C, 1E). Following inflammation-priming, *SEMA7A* transcription levels were strongly enhanced in BM-MSCs, moderately elevated in AT-MSCs, slightly increased in WJ-MSCs but partly reduced in FSK-MSCs

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

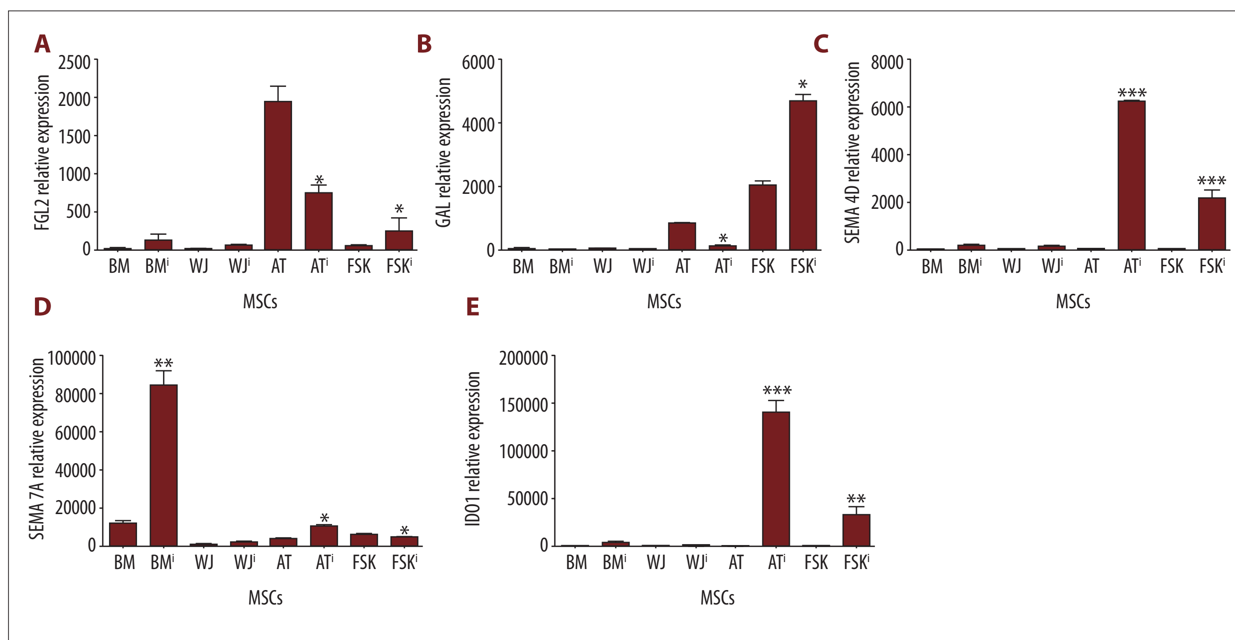


Figure 1. Characterization of *FGL2*, *GAL*, *SEMA4D*, *SEMA7A* and *IDO1* transcription levels in MSCs cultivated under basic or inflammatory conditions. Total RNA was prepared from BM-, WJ-, AT- and FSK-MSCs cultivated in the absence or presence of inflammatory cocktail. *GAPDH*-normalized *FGL2* (**A**), *GAL* (**B**), *SEMA4D* (**C**), *SEMA7A* (**D**) and *IDO1* (**E**) mRNA levels were determined using qRT-PCR. When cultivated under inflammatory conditions, cells were indicated as BM^i, WJ^i, AT^i and FSK^i. Values reported represent the averages of three independent experiments ±SEM. The statistical significance was determined using Mann-Whitney U- test (* p<0.05; ** p<0.01 versus untreated control cells).

(Figure 1D). It is noteworthy that *ARG1*, *CD163*, *IDO2*, *SEMA3A*, *SEMA4A*, and *SEMA6D* genes showed no detectable transcription levels following inflammation-priming of the different tested MSCs. This led us to ask whether the used primers were not efficient in terms of gene-product amplification and thus resulted in false negative results. In order to test this possibility, validation of the primers' efficiency was performed upon assaying the transcription levels of all tested genes using total RNA extracts prepared from seven other cell types (CBMC, PBMC, HUVECs -with and w/o inflammation-priming-, monocytes as well as brain-, placental- and umbilical cord-cells). *CD163* was differentially transcribed in these cells with monocytes showing highest mRNA levels followed by placental cells (Supplementary Figure 3, Supplementary Table 2). Lower *CD163* transcription levels were detected in umbilical cord- and brain-cells as wells as in CBMCs and PBMCs. However, no transcription was detected in HUVECs (Supplementary Figure 3, Supplementary Table 2). *ARG1* appeared to be highly transcribed in CBMCs but modestly in PBMCs (Supplementary Figure 3, Supplementary Table 2). Lower *ARG1* mRNA levels were also observed in monocytes, umbilical cord- and placental-cells (Supplementary Figure 3, Supplementary Table 2). *FGL2* appeared to be transcribed in the different tested cells, with monocytes showing the highest transcription levels (Supplementary Figure 3, Supplementary Table 2). *GAL* gene was strongly transcribed in HUVECs, being cultivated under basic or inflammatory conditions, but only weakly in brain- and placental-cells (Supplementary Figure 3, Supplementary Table 2). Except for CBMCs and monocytes, *SEMA3A* mRNA levels were detected in the different tested cells (Supplementary Figure 3, Supplementary Table 2). Except for HUVECs, all tested cells exhibited *SEMA4A* and *4D* transcription (Supplementary Figure 3, Supplementary Table 2). Except for monocytes, CBMCs and PBMCs, *SEMA6D* was differentially transcribed in the distinct tested cells (Supplementary Figure 3, Supplementary Table 2). *SEMA7A* transcription was detected in all tested cells with HUVECs showing minimal mRNA levels (Supplementary Figure 3, Supplementary Table 2). *IDO1* transcription was observed mainly in inflammation-primed HUVECs, whilst *IDO2* mRNA levels were detected mostly in umbilical cord cells (Supplementary Figure 3, Supplementary Table 2). Altogether these observations indicate that the utilized primers were able to drive the transcription of each of the tested genes within at least one out of the seven examined cells types, indicating that the absence of gene transcription observed for *ARG1*, *CD163*, *IDO2*, *SEMA3A*, *SEMA4A*, and *SEMA6D* genes in BM-, WJ-, FSK- and AT-MSCs was not due to primer inefficiency.

Discussion

Although the immunomodulatory capacities of MSCs are widely described, the nature and role of the immunoregulatory

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

factors involved in mediating these effects are, yet, not clearly demonstrated. Accordingly, profiling MSCs' transcriptome is necessary to identify key immunoregulatory genes that can be targeted to improve MSCs-immunomodulatory potential. In this context, murine MSCs were screened, in a previous work, for new genes with immuno-therapeutic potential [20]. However, in this study, we screened different populations of human MSCs for their transcription profiles of a defined set of immunomodulatory genes. Moreover, we examined the effect of inflammatory signals on those transcription patterns. Besides identifying *FGL2*, *GAL*, *SEMA4D*, *SEMA7A*, and *IDO1* as novel immunoregulatory genes being differentially transcribed in various MSCs, our data revealed that: (1) inflammation-priming of AT-MSCs results in reduced *FGL2* and *GAL* mRNA levels but enhanced *SEMA4D*, *SEMA7A* and *IDO1* transcription; (2) inflammation-primed BM-MSCs exhibit increased transcription of *FGL2*, *SEMA4D*, *SEMA7A*, and *IDO1*; (3) Exposure of FSK-MSCs to inflammation enhances the transcription of *FGL2*, *GAL*, *SEMA4D*, and *IDO1*; (4) Transcription of *FGL2*, *SEMA4D*, *SEMA7A*, and *IDO1* was slightly increased upon cultivating WJ-MSCs in inflammatory microenvironment. The distinct transcription profiles of these immunoregulatory genes among the different tested MSCs as well as the different inflammation-sensitivities exhibited by each of these genes in a manner dependent on the MSC-tissue source clearly highlights the substantial differences in the mechanisms controlling the immunoregulatory roles mediated by different MSCs of distinct tissue origins.

In fact, literature mining revealed that our identified genes were described to be involved in different important immunologic roles. For instance, fibrinogen-like protein 2 (FGL2), a member of the fibrinogen-like protein family, possesses prothrombinase activity and potent immune regulatory functions. FGL2 acts as a Treg effector molecule by suppressing T-cell activities in a FoxP3-dependent manner [21] and also suppresses dendritic cell- (DC) and B cell-functions by binding to FcγRIIB [22]. Moreover, by inducing multiple immune-suppression mechanisms, FGL2 may promote glioblastoma multiforme (GBM) progression via increasing the expression levels of PD-1 and CD39, thus expanding the frequency of tumor-supportive M2 macrophages via the FcγRIIB pathway, and enhancing the number of MDSCs and CD39^{+} regulatory T cells [23]. Collectively, these observations highlight FGL2 as a key immune-suppressive modulator where blocking FGL2 has therapeutic promise for cancer immunotherapy [23]. In our results, a high *FGL2* transcription was observed only in AT-MSCs presenting these cells as an important source for this immunosuppressive molecule. However, inflammation-priming exerted a negative effect on AT-MSCs-mediated *FGL2* transcription, highlighting the importance of *in vitro* inflammation-priming of MSCs before introducing them into patients.

The galanin (*GAL*) gene codes for a neuropeptide within the central and peripheral nervous systems [24]. Galanin is considered an inhibitory peptide with regulatory functions on inflammation [25] and on immune responses via exerting antiproliferative effect [26]. Although, in a previous report using a murine model, galanin was shown to be highly expressed in BM-MSCs [27], in this study, no significant *GAL* transcription was detected in human BM-MSCs. In fact, human and murine cells have important biological differences that may underline this discrepancy in *GAL* transcription profiles. Interestingly, we show that *GAL* gene is mainly transcribed in FSK-MSCs and to a lesser extent in AT-MSCs. External inflammatory signals reinforce its transcription in FSK-MSCs but impair it in AT-MSCs. Such observations could point for an anti-inflammatory role of FSK-MSCs via a mechanism dependent on *GAL* gene. On the other hand, if any anti-inflammatory role is played by AT-MSCs, it does not seem to depend on induced levels of *GAL* gene.

Semaphorins (SEMAs) are newcomers to the growing collection of immunoregulatory proteins. The SEMAs, originally identified as guidance cues in axonal growth, possess critical functions in the immune system [28]. The "immune semaphorins" (*SEMA3A*, *4A*, *4D*, *6D*, and *7A*) have been shown to have various immune regulatory functions, in terms of immune cell-cell contacts and immune cell trafficking. *SEMA4D/CD100* is a transmembrane protein which belongs to the class IV semaphorin subfamily and is the first semaphorin molecule identified with a functional role in the immune system [29,30]. *SEMA4D* is constitutively expressed on T cells and is able to suppress the inhibitory CD72 signaling during B cell and DC activation [31]. Despite a weak expression in B cells, *SEMA4D* is induced by inflammatory stimuli such as anti-CD40 [32]. Consistently, *SEMA4D* transcription was enhanced, even to different extents, in inflammation-primed BM-, WJ-, AT- and FSK-MSCs, suggesting an important role for this gene in mediating MSCs-responses to inflammatory signals. On the other hand, *SEMA7A*, the only GPI-linked protein in semaphorin family [33], being expressed in diverse immune cells (T cells, natural killer cells and monocytes) and acting as a negative regulator of T cell responses [34,35] but as a monocyte stimulator [34], showed enhanced transcription in inflammation-primed BM-MSCs, mainly, and to a lesser extent in inflammation-primed AT- and WJ-MSCs. These observations suggest immunomodulatory roles for BM-, AT- and WJ-MSCs via *SEMA7A*-dependent mechanism(s).The *IDO1* gene encodes the indoleamine 2,3-dioxygenase (IDO) heme enzyme that catalyzes the first and rate-limiting step in tryptophan catabolism to kynurenine [36,37]. In fact, immunosuppression is believed to result from the depletion of tryptophan and the accumulation of tryptophan metabolites locally [38]. Targeting and manipulating the IDO pathway has been described as a promising strategy to achieve clinical therapeutic benefits [39]. Recent observations indicated that *IDO* plays a crucial role in mediating human MSCs-immunomodulatory

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

functions via suppressing T cell proliferation, modulating differentiation of monocytes and inhibiting natural killer-cells [40–42]. According to our observations, *IDO* transcription profile was strongly comparable to that exhibited by *SEMA4D*, i.e., *IDO1* transcription was increased in the four tested MSCs populations following inflammation-priming, with a striking transcription being observed in inflammation-primed AT- and FSK-MSCs, thus highlighting a potential involvement for this gene in mediating MSCs-immunoregulatory roles.

Conclusions

In conclusion, our work identified certain immunoregulatory genes to be differentially transcribed in distinct MSCs in a manner dependent on both MSCs-tissue origin and the surrounding inflammatory status. Intriguingly, given that MSCs-immunoregulatory capacities do not occur spontaneously but are acquired in response to a cocktail of pro-inflammatory cytokines (IFN-γ, TNF-α, and IL-1β), our observations regarding the *in vitro* pre-activation of MSCs (priming) holds promise as an interesting approach to improve MSCs-immunomodulatory capabilities and thus improving MSCs-based therapies upon using engineered or induced types of MSCs to treat immune disorders. In a future work, the protein expression profiles of these identified genes will be further determined. Their functional roles during immunomodulation process will be also investigated upon blocking their expression by siRNAs or by neutralizing antibodies. This will be of great importance towards better understanding their mechanistic role in regulating immune responses, thus paving the way to reach safer and more efficient clinical applications of MSCs.

Acknowledgements

Mehdi Najar and Mohammad Fayyad-Kazan are awardee of a Televie post-doctoral fellowship.

Conflict of interest

All authors declare no conflict of interest.

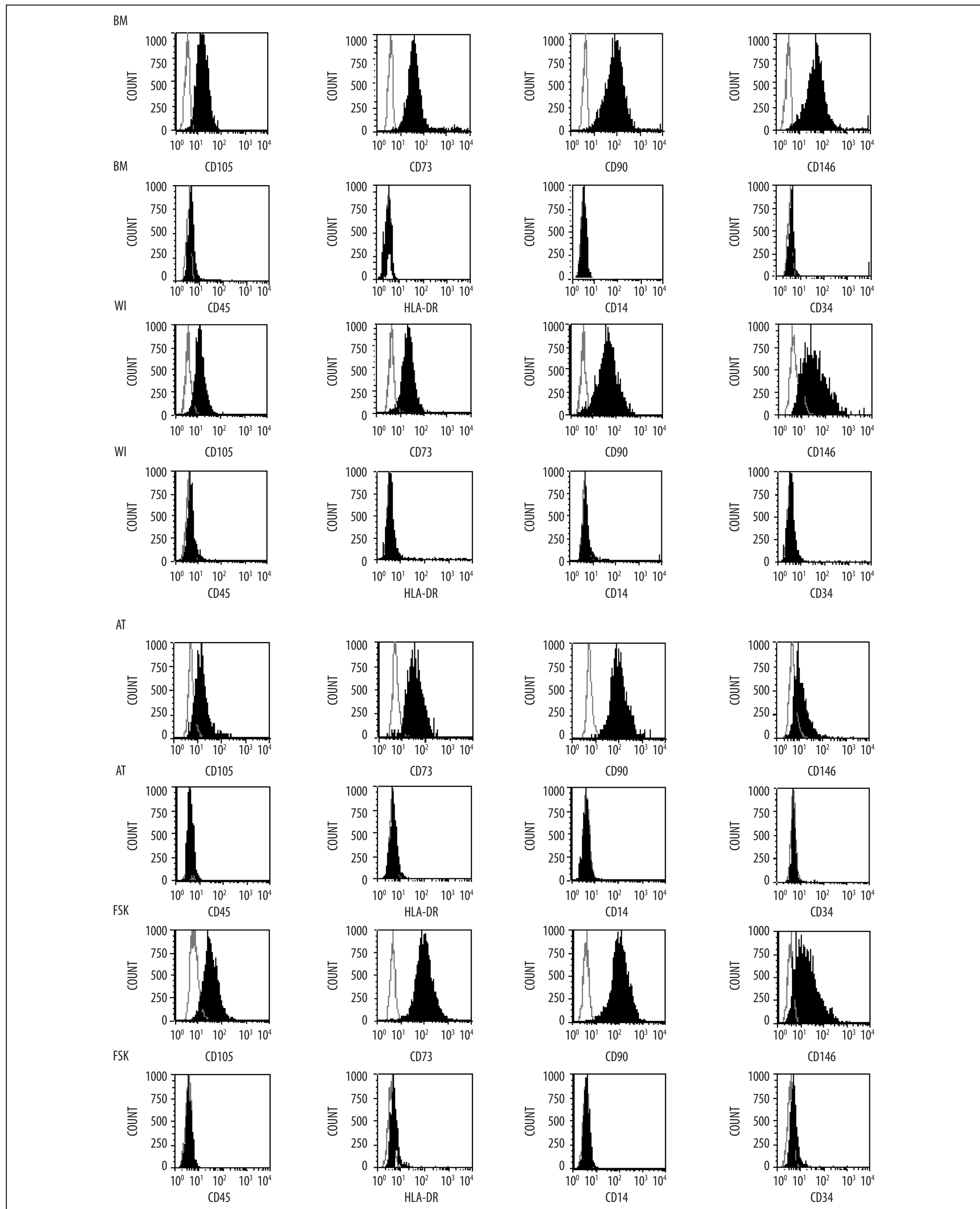
Supplementary Data

Supplementary Table 1. qRT-PCR primers.

Transcripts	Forward	Reverse
CD163	ACATAGATCATGCATCTGTCATTTG	CATTCTCCTTGGAATCTCACTTCTA
ARG1	CAGAGCATGAGCGCCAAGT	TCGTGGCTGTCCCTTTGAG
FGL2	TTTGGATGGCAAATGTTCAAAGT	TTAGATGTTGAACTGGACGTGACTGT
SEMA3A	ACCGACTGCTATTTCAGCAA	CCCAGCCGTTGAACCAATATA
SEMA4A	AATGTGCCTTTAAGAAGAAGAGCAAT	GTAAGAAACCAGGACACGGATGA
SEMA4D	TTGACCCAGCACACAGCTACA	AATTATACGACGTCCCCGAATAAA
SEMA6D	GAACCCCTTAATACTGTCGACTATCAC	GCCTGAAGGGCGTCCTCTA
SEMA7A	GGCCGCTGCATCTCCAT	GTGTGGCTCGGCTGGATT
IDO1	TTCAGTGCTTTGACGTCCTG	TGGAGGAACTGAGCAGCAT
IDO2	TGCTTCATGCCTTTGATGAG	GAAGGCCTTATGGGAAGGAG
GAL	CCCTCAATAGTGCTGGCTACCT	TTCCGTCTTGGTCACCCATT
GAPDH	AATCCCATCACCATCTTCCA	TGGACTCCACGACGTACTCA

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

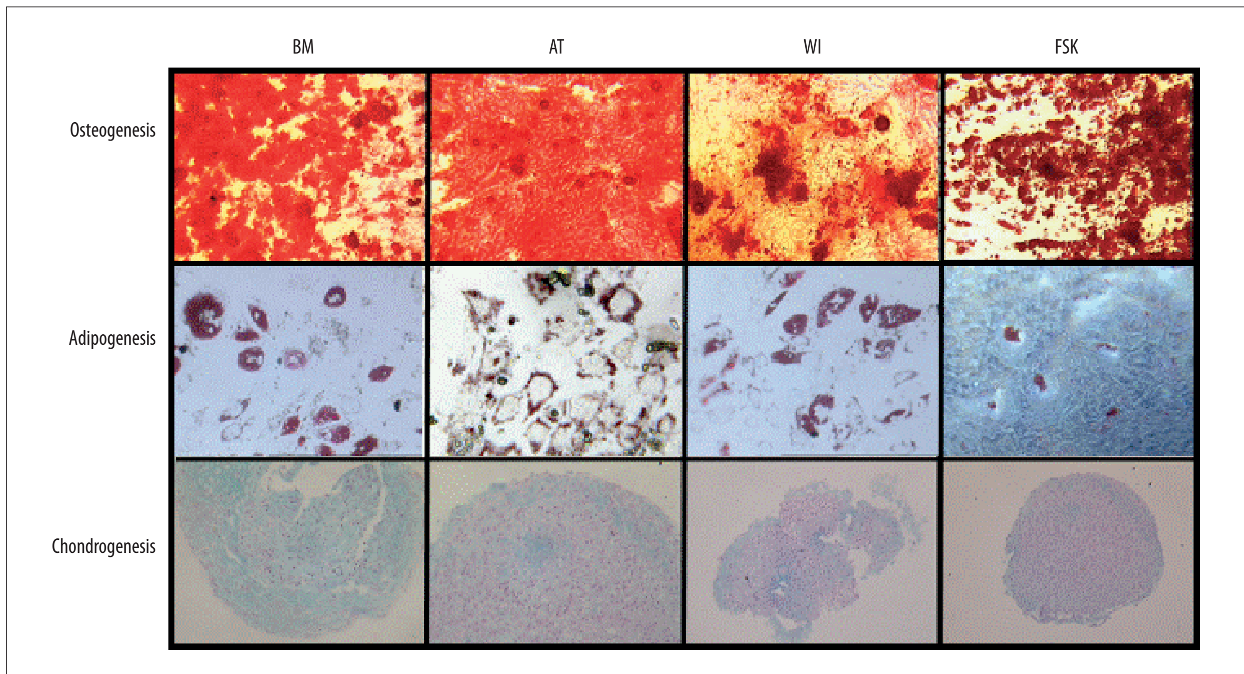
Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]



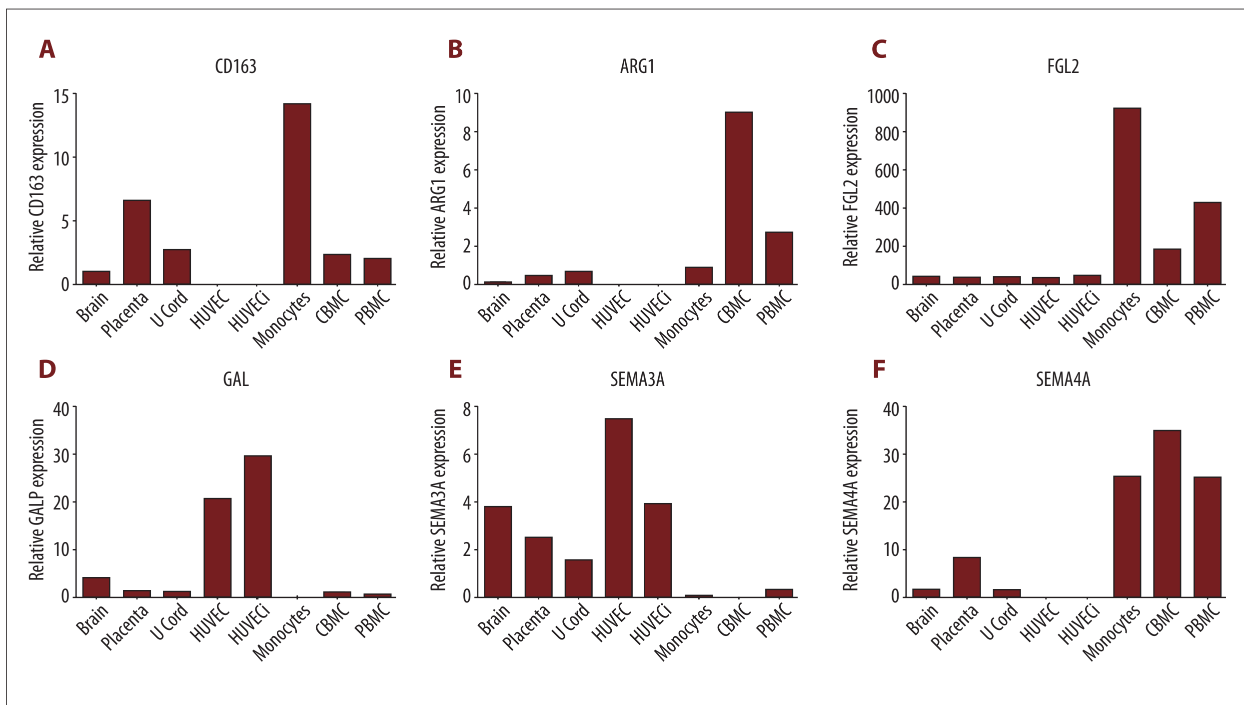
Supplementary Figure 1. The phenotype of the different types of MSCs was established by flow cytometry according to ISCT criteria. Using a panel fluorochrome labelled monoclonal antibodies, various surface marker expression profiles were determined. Representative flow cytometry histograms are presented (solid gray histogram" isotype fluorescence; black line: antibody-specific fluorescence).

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

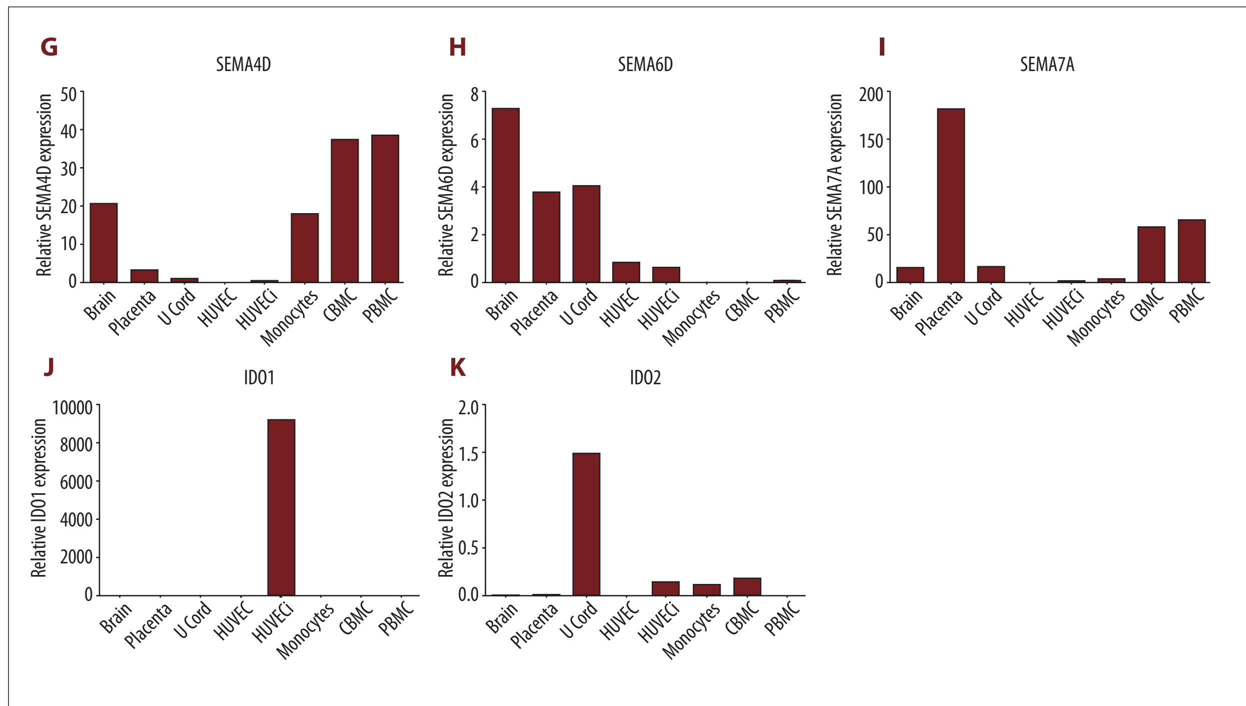


Supplementary Figure 2. The multilineage potential of the different types of MSCs was determined using both specific lineage induction media and staining techniques. Representstive images of the tri-lineage differentaition capacites of MSCs are presented.



This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]



Supplementary Figure 3. Characterization of the transcription profiles of (**A**) *CD163*, (**B**) *ARG1*, (**C**) *FGL2*, (**D**) *GAL*, (**E**) *SEMA3A*, (**F**) *SEMA4A*, (**G**) *SEMA4D*, (**H**) *SEMA6D*, (**I**) *SEMA7A*, (**J**) *IDO1* and (**K**) *IDO2* in different control cells. GAPDH-normalized levels of each of the indicated genes were assayed in brain-, placental-, umblical cord-cells as well as in monocytes, CBMCs and inflammation-primed or not HUVECs. The negative control contained water instead of cDNA.

Supplementary Table 2. Genes' mRNA expression levels in different types of cells.

Genes	Cells								
	Brain	Placenta	U cord	HUVEC	HUVECi	Monocytes	CBMC	PBMC	Negative control
CD163	1.0482	6.5868	2.6739	0	0	14.0568	2.3310	1.9615	0
ARG1	0.04826	0.3815	0.5946	0.0034	0.00312	0.8584	8.9817	2.6647	0
FGL2	38.0948	19.7761	34.8962	24.5161	44.2811	917.8809	171.7302	427.861	0
GAL	3.847710	0.8463	0.3893	20.4482	29.3645	0.0145	0.6736	0.03537	0
SEMA3A	3.7606	2.4933	1.5724	7.4360	3.9122	0.0540	0	0.3188	0
SEMA4A	1.5083	8.5392	1.9493	0.0262	0.0227	25.6638	35.1146	25.5809	0
SEMA4D	20.5775	3.5484	1.0139	0.0176	0.2130	17.9509	37.4008	38.3725	0
SEMA6D	7.315721	3.7995	4.0138	0.80104	0.5851	0.00105	0	0.0691	0
SEMA7A	15.7142	182.012	16.1112	0.16201	0.6935	4.2031	58.04103	64.7137	0
IDO1	0.0029	0.0065	7.0177	0.0029	9146.477	0.067	0.0925	0.3683	0
IDO2	0.0038	0.002	1.4834	0	0.1428	0.1175	0.1787	0	0

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

References:

1. Friedenstein AJ, Deriglasova UF, Kulagina NN et al: Precursors for fibroblasts in different populations of hematopoietic cells as detected by the *in vitro* colony assay method. Exp Hematol, 1974; 2: 83–92
2. Pittenger MF, Mackay AM, Beck SC et al: Multilineage potential of adult human mesenchymal stem cells. Science, 1999; 284: 143–47
3. Bartholomew A, Sturgeon C, Siatskas M et al: Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. Exp Hematol, 2002; 30: 42–48
4. Zappia E, Casazza S, Pedemonte E et al: Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood, 2005; 106: 1755–61
5. Le Blanc K, Rasmusson I, Sundberg B et al: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet (London, England), 2004; 363: 1439–41
6. Carrion FA, Figueroa FE: Mesenchymal stem cells for the treatment of systemic lupus erythematosus: Is the cure for connective tissue diseases within connective tissue? Stem Cell Res, Ther. 2011;2: 23
7. Papadopoulou A, Yiangou M, Athanasiou E et al: Mesenchymal stem cells are conditionally therapeutic in preclinical models of rheumatoid arthritis. Ann Rheum Dis, 2012; 71: 1733–40
8. Wang Y, Chen X, Cao W, Shi Y: Plasticity of mesenchymal stem cells in immunomodulation: Pathological and therapeutic implications. Nat Immunol, 2014; 15: 1009–16
9. Kim N, Cho S-G: Overcoming immunoregulatory plasticity of mesenchymal stem cells for accelerated clinical applications. Int J Hematol, 2016; 103: 129–37
10. Shi Y, Su J, Roberts AI et al: How mesenchymal stem cells interact with tissue immune responses. Trends Immunol, 2012; 33: 136–43
11. Zuk PA, Zhu M, Mizuno H et al: Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng, 2001; 7: 211–28
12. Campagnoli C, Roberts IA, Kumar S et al: Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood, 2001; 98: 2396–402
13. Najar M, Raicevic G, Fayyad-Kazan H et al: Immune-related antigens, surface molecules and regulatory factors in human-derived mesenchymal stromal cells: The expression and impact of inflammatory priming. Stem Cell Rev, 2012; 8: 1188–98
14. El Omar R, Beroud J, Stoltz J-F et al: Umbilical cord mesenchymal stem cells: The new gold standard for mesenchymal stem cell-based therapies? Tissue Eng Part B Rev, 2014; 20: 523–44
15. Najar M, Raicevic G, Fayyad-Kazan H et al: Impact of different mesenchymal stromal cell types on T-cell activation, proliferation and migration. Int. Immunopharmacol, 2013; 15: 693–702
16. Najar M, Raicevic G, André T et al: Mesenchymal stromal cells from the foreskin: Tissue isolation, cell characterization and immunobiological properties. Cytotherapy, 2016; 18: 320–35
17. Fayyad-Kazan H, Faour WH, Badran B et al: The immunomodulatory properties of human bone marrow-derived mesenchymal stromal cells are defined according to multiple immunobiological criteria. Inflamm Res, 2016; 65: 501–10
18. Raicevic G, Rouas R, Najar M et al: Inflammation modifies the pattern and the function of Toll-like receptors expressed by human mesenchymal stromal cells. Hum Immunol, 2010; 71: 235–44
19. Halfon S, Abramov N, Grinblat B, Ginis I: Markers distinguishing mesenchymal stem cells from fibroblasts are downregulated with passaging. Stem Cells Dev, 2011; 20: 53–66
20. Pedemonte E, Benvenuto F, Casazza S et al: The molecular signature of therapeutic mesenchymal stem cells exposes the architecture of the hematopoietic stem cell niche synapse. BMC Genomics, 2007; 8: 65
21. Wang J, Vuitton DA, Müller N et al: Deletion of fibrinogen-like protein 2 (FGL-2), a novel CD4+ CD25+ treg effector molecule, leads to improved control of *Echinococcus multilocularis* infection in mice. PLoS Negl Trop Dis, 2015; 9: e0003755
22. Chan CWY, Kay LS, Khadaroo RG et al: Soluble fibrinogen-like protein 2/fibroleukin exhibits immunosuppressive properties: Suppressing T cell proliferation and inhibiting maturation of bone marrow-derived dendritic cells. J Immunol, 2003; 170: 4036–44
23. Yan J, Kong L-Y, Hu J et al: FGL2 as a multimodality regulator of tumor-mediated immune suppression and therapeutic target in gliomas. J Natl Cancer Inst, 2015; 107(8) pii: djv137
24. Evans H, Baumgartner M, Shine J, Herzog H: Genomic organization and localization of the gene encoding human preprogalanin. Genomics, 1993; 18: 473–77
25. Heppelmann B, Just S, Pawlak M: Galanin influences the mechanosensitivity of sensory endings in the rat knee joint. Eur J Neurosci, 2000; 12: 1567–72
26. Trejter M, Brelinska R, Warchol JB et al: Effects of galanin on proliferation and apoptosis of immature rat thymocytes. Int J Mol Med, 2002; 10: 183–86
27. Louridas M, Letourneau S, Lautatzis M-E, Vrontakis M: Galanin is highly expressed in bone marrow mesenchymal stem cells and facilitates migration of cells both *in vitro* and *in vivo*. Biochem Biophys Res Commun, 2009; 390: 867–71
28. Yazdani U, Terman JR: The semaphorins. Genome Biol, 2006; 7: 211
29. Delaire S, Billard C, Tordjman R et al: Biological activity of soluble CD100. II. Soluble CD100, similarly to H-SemaIII, inhibits immune cell migration. J Immunol, 2001; 166: 4348–54
30. Delaire S, Elhabazi A, Bensussan A, Boumsell L: CD100 is a leukocyte semaphorin. Cell Mol Life Sci, 1998; 54: 1265–76
31. Kumanogoh A, Kikutani H: Biological functions and signaling of a transmembrane semaphorin, CD100/Sema4D. Cell Mol Life Sci, 2004; 61: 292–300
32. Kumanogoh A, Watanabe C, Lee I et al: Identification of CD72 as a lymphocyte receptor for the Class IV Semaphorin CD100. Immunity, 2000; 13: 621–31
33. Angelisová P, Drbal K, Cerný J et al: Characterization of the human leukocyte GPI-anchored glycoprotein CDw108 and its relation to other similar molecules. Immunobiology, 1999; 200: 234–45
34. Holmes S, Downs AM, Fosberry A et al: Sema7A is a potent monocyte stimulator. Scand J Immunol, 2002; 56: 270–75
35. Czopik AK, Bynoe MS, Palm N et al: Semaphorin 7A is a negative regulator of T cell responses. Immunity, 2006; 24: 591–600
36. Dai W, Gupta SL: Molecular cloning, sequencing and expression of human interferon-gamma-inducible indoleamine 2,3-dioxygenase cDNA. Biochem Biophys Res Commun, 1990; 168: 1–8
37. Najfeld V, Menninger J, Muhleman D et al: Localization of indoleamine 2,3-dioxygenase gene (INDO) to chromosome 8p12 – >p11 by fluorescent *in situ* hybridization. Cytogenet Cell Genet, 1993; 64: 231–32
38. Mellor AL, Munn DH: Ido expression by dendritic cells: Tolerance and tryptophan catabolism. Nat Rev Immunol, 2004; 4: 762–74
39. Johnson BA, Baban B, Mellor AL: Targeting the immunoregulatory indoleamine 2,3 dioxygenase pathway in immunotherapy. Immunotherapy, 2009; 1: 645–61
40. François M, Romieu-Mourez R, Li M, Galipeau J: Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther, 2012; 20: 187–95
41. Meisel R, Zibert A, Laryea M et al: Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood, 2004; 103: 4619–21
42. Spaggiari GM, Capobianco A, Abdelrazik H et al: Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: Role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood, 2008; 111: 1327–33

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]