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

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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

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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 energy [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 pre-activation 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 (TGF-β), hepatocyte growth factor (HGF), and interleukin-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 FGIL2, 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 (Belgium). 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/cm²). MSCs of different origins were characterized for their morphology, phenotype and multi-lineage potential according to our previous protocols [15,16]. Cell cultures were incubated in supplemented α-MEM medium with 10% FBS, and 100 U mL⁻¹ penicillin streptomycin (Life Technologies, Grand Island, NY). The medium was changed every 3 to 4 days. Upon reaching 80–90% confluence, MSCs were separated into different tissues and further characterized for their immunomodulatory capacities.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cord blood mononuclear cells (CBMC), adult purified monocytes (CD14⁺ selection, Myltenyi 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 Trizol 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
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 ΔΔCt 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 ΔΔCt. Fold changes were then calculated as fold change=2^ΔΔCt.

**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 complied 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, SEMA4D, SEMA6A, and SEMA6D) was characterized in MSCs, derived from BM, WI, 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 WI-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 WI-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 WI-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 BM- and WI-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 WI-MSCs (Figure 1B). The minimal SEMA4D and IDO1 transcription levels were strongly induced following inflammation-priming of AT- 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 WI-MSCs (Figure 1C, 1E). Following inflammation-priming, SEMA7A transcription levels were strongly enhanced in BM-MSCs, moderately elevated in AT-MSCs, slightly increased in WI-MSCs but partly reduced in FSK-MSCs...
Differential immunoregulatory gene transcription in MSCs

(Figure 1D). It is noteworthy that \( \text{ARG1}, \text{CD163}, \text{IDO2}, \text{SEMA3A}, \text{SEMA4A}, \text{SEMA4D}, \text{SEMA6D}, \text{SEMA7A} \) and \( \text{IDO1} \) mRNA levels were detected mostly in umbilical cord cells (Supplementary Figure 3, Supplementary Table 2). \( \text{FGL2} \) appeared to be transcribed in the different tested cells, with monocytes showing the highest transcription levels (Supplementary Figure 3, Supplementary Table 2). \( \text{CD163} \) was differentially transcribed in these cells with monocytes showing highest mRNA levels followed by placental cells (Supplementary Figure 3, Supplementary Table 2). Lower \( \text{CD163} \) transcription levels were detected in umbilical cord- and brain-cells as well as in CBMCs and PBMCs. However, no transcription was detected in HUVECs (Supplementary Figure 3, Supplementary Table 2). \( \text{ARG1} \) appeared to be highly transcribed in CBMCs but modestly in PBMCs (Supplementary Figure 3, Supplementary Table 2). Lower \( \text{ARG1} \) mRNA levels were also observed in monocytes, umbilical cord- and placental-cells (Supplementary Figure 3, Supplementary Table 2). \( \text{FGL2} \) appeared to be transcribed in the different tested cells, with monocytes showing the highest transcription levels (Supplementary Figure 3, Supplementary Table 2). \( \text{GAL} \) 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, \( \text{SEMA4A} \) mRNA levels were detected in the different tested cells (Supplementary Figure 3, Supplementary Table 2). Except for HUVECs, all tested cells exhibited \( \text{SEMA4A} \) and \( \text{4D} \) transcription (Supplementary Figure 3, Supplementary Table 2). Except for monocytes, CBMCs and PBMCs, \( \text{SEMA6D} \) was differentially transcribed in the distinct tested cells (Supplementary Figure 3, Supplementary Table 2). \( \text{SEMA7A} \) transcription was detected in all tested cells with HUVECs showing minimal mRNA levels (Supplementary Figure 3, Supplementary Table 2). \( \text{IDO1} \) transcription was observed mainly in inflammation-primed HUVECs, whilst \( \text{IDO2} \) 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 \( \text{ARG1}, \text{CD163}, \text{IDO2}, \text{SEMA3A}, \text{SEMA4A}, \text{SEMA6D} \) genes in BM-, WI-, 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
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 WI-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 immunological 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 anti-proliferative 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 re-inforce 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 (SEMs) are newcomers to the growing collection of immunoregulatory proteins. The SEMs, 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-, WI-, 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 WI-MSCs. These observations suggest immunomodulatory roles for BM-, AT- and WI-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
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.

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Conflict of interest

All authors declare no conflict of interest.

Supplementary Data

Supplementary Table 1. qRT-PCR primers.

| Transcripts | Forward | Reverse |
|-------------|---------|---------|
| CD163       | ACATAGATCATGACATCGTCATGGTATG    | GATTCCTGCGAACCTGCCTCTCTA |
| ARG1        | CAGACGAGGTAGAAGCCGAGTGTG    | ACCATCGTGCTCCCTTTGAG |
| FGL2        | TTTGGATGGAAATGTTCAAAGT    | CTTAGATGTTGAACCTGAGCTGCTG |
| SEMA3A      | ACGACGACTGTATTTCAGCAA    | CCCAGCCGTTGAACCAATATA |
| SEMA4A      | AAATGTCCTTTTTAAGAAGAAGACAT    | GTAAGAAACAGGCAACGGATGA |
| SEMA4D      | TTGACCCCGCAGACACAGCTACA    | AATATACGACTCCGGCAGAATAG |
| SEMA6D      | GAACCCTTAAATACGTGCACTATCACA    | GCCGATGAGCGGTGCCTCCTA |
| SEMA7A      | GGGCTGCTGATCCCTG    | GTGCGCTGCTGCTGTTGGAG |
| IDO1        | TTGAGATGATTGGACATGGTCTG    | TGGAGACTGACGTAGGAG |
| IDO2        | TTGCTTCATGCTGCTTGA    | GAAGCCCTTATGCGGCCGAG |
| GAL         | CCCTCATTATCTGCTGACTACCT    | TTCCTGCTTCGCAAGCATT |
| GAPDH       | AATTCCTACCTCACCTTCCA    | TGGAACCTGACGAGCTCCTA |
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).
Supplementary Figure 2. The multilineage potential of the different types of MSCs was determined using both specific lineage induction media and staining techniques. Representative images of the tri-lineage differentiation capacities of MSCs are presented.
**Supplementary Table 2.** Genes’ mRNA expression levels in different types of cells.

| Genes     | 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| 0.0353| 0                |
| SEMA3A    | 3.7606   | 2.4933   | 1.5724 | 7.4360| 3.9122 | 0.0540     | 0.0029| 0.0065| 0                |
| SEMA4A    | 1.5083   | 8.5392   | 1.9493 | 0.0262| 0.0227 | 25.6638    | 35.1146| 25.5809| 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   | 1.4834 | 0     | 0.1428 | 0.1175     | 0.1787| 0 | 0                |
| IDO2      | 0.0038   | 0.002    | 1.4834 | 0     | 0.1428 | 0.1175     | 0.1787| 0 | 0                |
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