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Influence of Umbilical Cord Blood Biochemical Parameters and Disease Condition on the Expression of the *TSG-6* Gene in Umbilical Mesenchymal Stem Cells

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Conflict of interest: None declared

Background: Mesenchymal stem cells (MSCs) are capable of secreting different substances, including the anti-inflammatory protein TSG-6, which can be useful in the treatment of diseases with inflammatory reactions. The main aim of this study was to evaluate the expression of the *TSG-6* gene in MSCs derived from the umbilical cord. For better understanding of the anti-inflammatory properties of MSCs, we additionally assessed the expression of some interleukins (ILs).

Material/Methods: The study group included 45 patients after delivery, aged from 21 to 46 years; the average patient age was 33 years. MSCs were isolated enzymatically from umbilical cord Wharton's jelly, in vitro cultured, and characterized using flow cytometry; qPCR was performed to assess expression of the studied genes. The expression of genes of a number of pro-inflammatory ILs in MSCs was investigated in relation to the health of patients (co-existence of hypertension), the level of leukocytes, pCO2, and hemoglobin in the blood.

Results: Our research showed that the expression of the *TSG-6* gene in MSCs depends on coexisting diseases in the patient and the biochemical parameters of umbilical cord blood, including the important role of cord blood pH. We found that the levels of *IL2* and *IL6* expression were correlated with pCO2, and *IL6* expression were correlated with pO2.

Conclusions: Our study suggests that maternal health status and cord blood biochemical parame-ters could affect the anti-inflammatory properties of MSCs; however, this needs to be confirmed in a future study.

Keywords: Gene Expression • Mesenchymal Stem Cells • MSC 127 • TNFAIP6 Protein, Human

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Background

Mesenchymal stem cells (MSCs) are multipotential cells. According to the International Society for Cell and Gene Therapy, the correct name is "multipotent mesenchymal stromal cells". The term "mesenchymal cells" is synonymous with embryonic connective tissue, which, as the name suggests, only occur in the dividing embryo [1]. MSCs are derived from the third germ layer, the mesoderm, which makes the production of the cells that ultimately make up the connective tissue in the body possible [1].

Clinically, human MSCs are derived from adult bone marrow, adipose tissue, dental pulp, and peripheral blood. The MSCs extracted from perinatal tissues (such as the placenta, umbilical cord, or amnion) in newborns have characteristics that make them superior to cells obtained from adults. These characteristics include proliferative capacity, capability for differentiation, cell longevity (and growth), and a reduced capacity to provoke an immune response (lower immunogenicity) [2,3].

The features that are particularly related to MSCs are a high differentiation potential, especially toward bone tissue, cartilage tissue, and adipose tissue, and the ability of the MSCs to renew themselves. Visually, they are fusiform in shape and resemble fibroblasts. In vitro they show adhesion to plastic, and in regards to human phenotype, they also possess human surface antigens, which are also MSC markers: CD73, CD90, and CD105. Negative MSC markers, which do not show expression in these cells, are CD11b, CD14, CD19, CD34, CD45, and CD79a. It is significant that there are no class II histocompatibility antigens on the MSC surface [1,4,5].

Apart from differentiation potential, MSCs have many other properties. MSCs are capable of secreting substances such as growth factors, angiogenesis stimulation factors, and anti-apoptotic factors [6]. Moreover, MSCs show anti-inflammatory effects, which are mainly caused by the tumor necrosis factor (TNF)-stimulated gene-6 (TSG-6) protein secreted by these cells [7].

TSG-6 is a multifunctional protein with anti-inflammatory and tissue protective properties that mediate the beneficial effects of MSCs. In recent years, the potential of TSG-6 as a therapeutic agent has been emphasized in a wide range of indications [8].

Expression of the *TSG-6* gene is stimulated as a result of pro-inflammatory cytokines and results in the increase of the TSG-6 protein level. This protein stimulates transforming pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages [9], inhibits NF-κB factor [10], and limits the activity of the body's immune cells [11-12], which as a whole translates into a strong anti-inflammatory effect.

It was observed that TSG-6 can be used in the therapy of inflammatory diseases. It was shown that TSG-6 has a positive effect on neural system inflammation [13], reduces swelling in arthritis [14], and also limits the size of a myocardial infarction [7]. Moreover, the latest studies report the possible use of TSG-6 in the diagnosis of acute ischemic stroke [15] and rheumatoid arthritis [16]. It has been shown that the use of MSCs to induce neuroprotection and the lasting alleviation of neuropathic pain is dependent on the secretion of TSG-6 by these cells [13]. Additionally, Li et al indicated the potential use of MSCs in clinical recurrent miscarriages is possible due to the TSG-6-dependent mechanisms [17].

Because numerous studies have shown that the immunomodulatory properties of MSCs are dependent on TSG-6, we speculated that the level of *TSG-6* expression in MSCs will influence their clinical utility and therapeutic potential. Owing to the significant role of the TSG-6 protein in suppressing the inflammatory reaction and potential use of MSCs in therapy, the aim of our study was to evaluate the expression of the *TSG-6* gene in MSCs derived from the Wharton's jelly of the umbilical cord. The study assessed the level of expression depending on the health of the mother, type of delivery, use of oxytocin, and cord blood parameters.

The anti-inflammatory activity of MSCs mediated by the TSG-6 protein has already been proven, but no studies indicate which sources of MSCs are the most optimal in this regard. There is no information of laboratory parameters affecting expression of that gene. To the best of our knowledge, the present study is the first to evaluate the level of *TSG-6* expression in MSCs obtained from the umbilical cord in relation to the biochemical parameters of cord blood and health status of the cell donor.

Our study will provide new information about TSG-6, and therefore about the anti-inflammatory properties of MSCs, what may be crucial for further use of MSCs and TSG-6 in therapy.

Material and Methods

Sample Collection and Ethics Approval

The material used for the research was the umbilical cord, which was collected shortly after delivery from 45 patients hospitalized at the Department of Obstetrics and Pathology of Pregnancy of the Independent Public Clinical Hospital No. 1 in Lublin. The research was conducted with the consent of the Bioethics Committee at the Medical University of Lublin (KE-0254/128/2014).

The age of the patients ranged from 21 to 46 years; the average patient age was 33 years. A total of 6 patients (13%) had

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Table 1. Characteristics of the study group (n=45) in terms of: mother's age, number of pregnancies, number of deliveries, weeks of gestation, newborn's weight, and cord blood parameters.

Parameter	n	Mean	Median	Minimum	Maximum	SD	SE
Mother's age		33.11	33.00	21.00	46.00	5.26	0.78
Number of pregnancies		2.02	2.00	1.00	5.00	1.06	0.16
Number of deliveries		1.73	2.00	1.00	5.00	0.91	0.14
Weeks of gestation		38.29	38.00	34.00	41.00	1.42	0.21
Newborn's weight		3230.67	3240.00	2460.00	4060.00	387.48	57.76
Cord blood parameters							
pH		7.33	7.34	7.03	7.45	0.07	0.01
PO_2		29.16	29.30	16.00	52.90	8.06	1.20
pCO_2	45	44.73	41.90	30.40	77.60	8.96	1.34
WBC		10.65	9.89	5.14	18.73	2.81	0.42
RBC		4.05	4.03	2.79	4.83	0.42	0.06
HGB		12.14	12.30	9.40	14.10	1.08	0.16
HCT		35.48	35.80	27.50	41.30	2.93	0.44
MCV		87.90	89.10	77.40	98.60	4.95	0.74
MCH		30.09	30.60	25.40	34.40	2.23	0.33
MCHC		34.20	34.30	31.70	36.60	1.07	0.16
PLT		205.44	199.00	121.00	324.00	46.44	6.92

pH – potential of hydrogen; pO_2 – partial pressure of oxygen; pCO_2 – partial pressure of carbon dioxide; WBC – white blood cell count; RBC – red blood cell count; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelet count; SD – standard deviation; SE – standard error.

a vaginal delivery, while 37 patients had a cesarean section. Ten patients (22%) out of 45 patients were treated with oxytocin during labor.

Eleven patients gave birth before 37 weeks of pregnancy, and 22 patients gave birth for the first time (18 of them were pregnant for the first time). The women gave birth to 25 boys and 20 girls. Gestational diabetes was diagnosed in 13 patients, hypertension in 8 patients, and hypothyroidism in 14 patients; 3 patients had diabetes and hypertension at the same time, and 4 patients had diabetes and hypothyroidism at the same time. A total of 17 patients had no comorbidities and had healthy babies born full term (control group). Patient characteristics are shown in **Tables 1 and 2**.

Isolation of MSCs from Umbilical Cord

MSCs were isolated using an enzymatic digestion method. Immediately after delivery, the umbilical cord (10-cm piece) was placed in a culture medium containing Dulbecco's Modified Eagle Medium (DMEM) with L-glutamine (584 mg/L) and glucose (4.5 g/L; Corning, Manassas, VA, USA) with 10% fetal bovine serum (FBS; ATCC, Teddington, UK) and 1% addition of antibiotic (penicillin-streptomycin, 10000 U and 10 mg/mL, respectively; Sigma-Aldrich, Israel). The umbilical cord was then rinsed several times in sterile phosphate buffered saline (PBS; Biomed Lublin, Poland) containing the antibiotic and cut into smaller pieces with a scalpel under aseptic conditions. Fragments of umbilical cord (2-5 mm) were digested in a sterile type I collagenase solution (1 mg/mL; Gibco by Life Technologies, Grand Island, NY, USA) for 2 to 3 h, while maintaining a temperature of 37°C at 300 rpm (Eppendorf Thermomixer comfort). After this, the sample was washed twice with warm PBS buffer (21°C, 128 g; Eppendorf Centrifuge 5810 R). The last step was to filter the sample after it was rinsed through a 100-µm sieve to clean the mixture for further testing. After the cells were filtered, they were rinsed in warm PBS with 1% antibiotic, the collagenase was stopped by adding FBS, the sample was centrifuged with warm PBS buffer (21°C, 128 g, 10 min Eppendorf Centrifuge 5810R), and the cell pellet was transferred to a culture vessel. The procedure of cell isolation and cell culture was done as described in a previous study [18].

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Table 2. Characteristics of the control group (n=17), patients with gestational diabetes (n=13), patients with hypertension (n=8), and patients with hypothyroidism (n=14) in terms of mother's age, number of pregnancies, number of deliveries, weeks of gestation, newborn's weight, and cord blood parameters.

Parameter	n	Mean	Median	Minimum	Maximum	SD	SE
Control group							
Mother's age	17	32.412	32.000	23.000	39.000	4.388	1.064
Number of pregnancies		2.059	2.000	1.000	4.000	0.966	0.234
Number of deliveries		1.824	2.000	1.000	4.000	0.883	0.214
Weeks of gestation		38.471	39.000	34.000	41.000	1.700	0.412
Newborn's weight		3337.059	3300.000	2660.000	4060.000	396.701	96.214
Cord blood parameters							
pH		7.308	7.340	7.030	7.380	0.097	0.024
pCO_2		46.847	42.200	35.300	77.600	11.601	2.814
pO_2		28.035	29.100	18.000	42.100	7.713	1.871
$cHCO_3$		22.300	22.600	17.000	24.600	2.004	0.486
WBC		10.763	10.460	5.140	16.930	2.992	0.726
RBC		4.089	4.020	3.580	4.830	0.348	0.084
HCT		35.482	35.300	29.800	41.300	3.058	0.742
HGB		12.153	12.100	10.000	14.100	1.203	0.292
MCV		86.818	87.100	79.800	92.400	3.450	0.837
MCH		29.735	30.000	25.800	31.800	1.771	0.429
MCHC		34.241	34.400	31.700	36.100	1.157	0.281
PLT		194.000	195.000	121.000	268.000	41.195	9.991
Gestational diabetes							
Mother's age	13	36.462	36.000	29.000	46.000	4.630	1.284
Number of pregnancies		2.462	2.000	1.000	5.000	1.198	0.332
Number of deliveries		2.000	2.000	1.000	5.000	1.155	0.320
Weeks of gestation		37.846	38.000	37.000	40.000	0.899	0.249
Newborn's weight		3140.000	2910.000	2460.000	3950.000	470.266	130.428
Cord blood parameters							
pH		7.344	7.340	7.300	7.390	0.028	0.008
pCO_2		42.500	41.900	36.700	50.700	3.711	1.029
pO_2		31.777	31.000	21.600	52.900	9.270	2.571
$cHCO_3$		22.615	22.200	19.700	24.900	1.332	0.369
WBC		9.719	9.390	5.970	18.730	3.152	0.874
RBC		3.876	3.850	2.790	4.690	0.568	0.157
HCT		34.431	35.700	27.500	38.200	3.591	0.996
HGB		11.738	12.100	9.400	13.200	1.194	0.331
MCV		89.454	90.500	79.300	98.600	5.950	1.650
MCH		30.554	30.700	25.400	34.400	2.540	0.704
MCHC		34.115	34.200	32.000	35.400	0.958	0.266
PLT		206.077	209.000	126.000	283.000	50.454	13.993

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Table 2 continued. Characteristics of the control group (n=17), patients with gestational diabetes (n=13), patients with hypertension (n=8), and patients with hypothyroidism (n=14) in terms of mother's age, number of pregnancies, number of deliveries, weeks of gestation, newborn's weight, and cord blood parameters.

Parameter	n	Mean	Median	Minimum	Maximum	SD	SE
Hypertension							
Mother's age	8	32.400	33.000	24.000	39.000	6.066	2.713
Number of pregnancies		2.400	2.000	1.000	5.000	1.517	0.678
Number of deliveries		2.400	2.000	1.000	5.000	1.517	0.678
Weeks of gestation		38.400	38.000	37.000	40.000	1.140	0.510
Newborn's weight		3092.000	3240.000	2460.000	3410.000	372.250	166.475
Cord blood parameters							
pH		7.320	7.310	7.300	7.370	0.028	0.013
pCO_2		46.844	47.700	38.900	50.820	4.875	2.180
pO_2		28.040	26.600	21.800	38.800	6.722	3.006
$cHCO_3$		23.500	23.400	22.000	25.000	1.428	0.639
WBC		10.138	10.070	5.970	13.720	3.422	1.530
RBC		4.406	4.500	3.970	4.740	0.346	0.155
HCT		36.280	36.400	35.000	37.500	0.968	0.433
HGB		12.300	12.400	12.000	12.400	0.173	0.077
MCV		82.640	83.300	77.400	89.900	5.281	2.362
MCH		28.060	27.600	25.500	31.200	2.522	1.128
MCHC		33.920	33.400	33.000	35.400	1.071	0.479
PLT		222.000	196.000	172.000	324.000	62.221	27.826
Hypothyroidism							
Mother's age	14	32.429	33.000	21.000	41.000	5.667	1.514
Number of pregnancies		2.071	2.000	1.000	4.000	1.141	0.305
Number of deliveries		1.571	1.000	1.000	3.000	0.756	0.202
Weeks of gestation		38.286	39.000	34.000	40.000	1.437	0.384
Newborn's weight		3125.714	3110.000	2590.000	3500.000	309.260	82.653
Cord blood parameters							
pH		7.359	7.355	7.240	7.450	0.056	0.015
pCO_2		42.921	41.150	30.400	63.400	8.374	2.238
pO_2		30.343	31.200	16.000	41.200	8.306	2.220
$cHCO_3$		23.279	22.950	20.700	26.200	1.524	0.407
WBC		10.623	9.835	8.130	14.820	2.030	0.542
RBC		4.044	4.100	3.460	4.690	0.333	0.089
HCT		35.864	36.500	31.600	38.900	2.499	0.668
HGB		12.221	12.300	10.800	13.600	0.928	0.248
MCV		88.857	90.800	79.300	92.600	4.111	1.099
MCH		30.336	30.700	25.400	32.800	2.228	0.595
MCHC		34.079	33.900	32.000	36.600	1.260	0.337
PLT		221.357	211.500	158.000	286.000	47.020	12.567

pH – potential of hydrogen; pO_2 – partial pressure of oxygen; pCO_2 – partial pressure of carbon dioxide; WBC – white blood cell count; RBC – red blood cell count; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelet count; SD – standard deviation; SE – standard error.

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Cell Culture

The isolated umbilical cord cells were cultured in vitro for up to 14 days in adherent conditions in a tissue incubator (New Brunswick Galaxy 170 R). Incubation conditions were as follows: temperature 37ºC, O_2 concentration 15%, CO_2 concentration 5%, and moisture 95%. The culture medium (10 mL) consisted of DMEM medium with L-glutamine and glucose, with 10% FBS and a 1% addition of antibiotic (penicillin-streptomycin). The bottle for cell culture had bottom surface area of 25 cm^2 (TC Flask T 25, Cell+; Sarsted, Germany). The cultures were observed every few days, and the medium was changed every 3 days. After successful in vitro culture, the isolated MSCs were detached from the surface of the culture vessel using a sterile cell scraper (Corning). Finally, these cells were washed in PBS buffer. Cells after culture were divided and some were used for cytometric analysis, some for RNA isolation.

Cytometric Analysis

Cytometric analysis was performed using a Navios flow cytometer (Beckman Coulter) to assess cell phenotype. The analysis was performed according to protocols presented in previous studies [19-21]. In vitro cultured umbilical cord MSCs, at a concentration of about 10^6/mL, were taken and suspended in a volume of 100 µL and added to a DuraClone SC Mesenchymal Tube antibodies panel (Beckman Coulter, Bangalore, Karnataka, India), then shaken for 6 s. Subsequently, the cells were incubated in the dark for 15 min at room temperature. The following DuraClone MSCs containing a lyophilisate set of fluorescently labeled monoclonal antibodies against MSC surface antigens were used: CD45-APC, CD73-PE, CD90-FITC, CD105-PC7, CD146-PC 5.5, CD31-PBE, CD14/CD19-Krome Orange, dedicated to the determination of characteristic MSC antigens. After incubation, 2 mL of PBS without calcium and magnesium ions (Biomed, Lublin, Poland) was added. Then, they were centrifuged at 200 g for 5 min. After centrifugation, the cell pellet was dissolved in 400 µL PBS without calcium and magnesium ions. The labeled cells were placed on a Navios flow cytometer carousel and subjected to cytometric analysis. Typically, an analysis of 10 000 events was recorded for the assays. Cells without any staining were used as a negative control, and an isotype control was performed. Single-stained samples were used for compensation. Fluorescence minus one (FMO) samples were used to determine the gating.

RNA Isolation

Total cellular RNA of the obtained umbilical cord MSCs was isolated by the modified method of Chomczyński and Sacchi [22], using TRIzol (Thermo Fisher Scientific, USA), chloroform (Sigma), isopropanol (Sigma), and 75% ethanol (Poch).

After culture, the cell sample was homogenized in 0.5 mL of TRIzol, then incubated for 5 min at room temperature; 0.1 mL of chloroform was successively added, and the sample was shaken for 15 s, incubated for 15 min at room temperature, then centrifuged for 15 min at 4°C, at a speed of 12000 g (Eppendorf Centrifuge 5415 R). After centrifugation, the aqueous phase was withdrawn into a new tube. An amount of 0.25 mL of isopropanol was added to the aqueous phase, incubated for 20 min at room temperature, and then centrifuged for 20 min at 4°C, at 12 000 g (Eppendorf Centrifuge 5415 R). After centrifugation, the supernatant was discarded and the RNA pellet was purified in 75% ethanol, then dissolved in ultrapure water, free of RNase and DNase. Before the next stages of research with the use of isolated RNA, qualitative and quantitative analysis of these isolates was performed using the spectrophotometric method (Nanodrop 2000 c) at wavelengths 260 nm and 280 nm.

Reverse Transcription

The cDNA synthesis was performed using the High-Capacity cDNA Transcription Kit from Applied Biosystems (Thermo Fisher Scientific, Lithuania) and 1 µg of test RNA isolated in the previous step, according to the manufacturer's protocol.

The reverse transcription reaction was carried out in a volume of 20 µL, consisting of: 1 µL (RNase 40 U/µL); 1 µL (reverse transcriptase 50 U/µL); 2 µL (10xRT Buffer); 3.2 µL (Ultrapure water); 0.8 µL (10 xdNTPs (100 mM); 2 µL (10 xRT Random Primer); and 10 µL (1 µg RNA dissolved in 10 µL ultrapure water). The reaction was conducted in a Verit Thermal Cycler (Life Technologies); the reaction mixture was incubated at 25°C for 10 min, then at 37°C for 2 h, and then at 95°C for 5 min. The obtained cDNA was used for real-time PCR.

Evaluation of the Expression Level of the Studied Genes

To assess the expression level of the studied genes, *IL1A*, *IL1B*, *IL1R*, *IL2*, *IL6*, *IL6R,* and *TSG-6*, the real-time PCR method was used. Real-time PCR reactions were performed in 0.1-mL 96-well plates (Applied Biosystems, USA) using cDNA reverse transcription as a template, in a volume of 10 µL/well consisting of 0.5 µL gene-specific primers and probe, 4.5 µL cDNA synthesized by reverse transcription with ultrapure RNAse- and DNAse-free water, and 5 µL TaqMan Gene Expression Master Mix (Applied Biosystems, USA). The reaction, after an initial 10-min denaturation at 95°C, was conducted for 40 cycles according to the following scheme: 15 s at 95°C, then 60 s at 60°C. mRNA expression was detected using the StepOnePlus System (Applied Biosystems, USA). The level of relative expression (RQ) was assessed according to the formula proposed by Livak, where $RQ=2^{-ddCt}$ [23]. To normalize the expression of the tested genes, the cycle threshhold value was determined for each sample against the reference gene that showed the

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greatest stability in the tested material, namely, the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene. In the study, *B2M* and *ACTB* were also considered as endogenous controls, but *GAPDH* turned out to be the best control. Final gene expression was determined relative to the sample used to calibrate the entire experiment. The expression of the studied genes was analyzed using ExpressionSuite Software v.1.0.3. (Life Technologies). The following TaqMan primers and probes were used for the genes tested: (for *TSG-6*: NM_007115.3 and Hs00200180_m1, for *IL6*: NM_000600.4 and Hs00174131_m1, for *ILR6*: NM_000565.3 and Hs00169842_m1, for *Il1A*: NM_000575.4 and Hs00174092_m1, for *IL1B*: NM_000576.2 and Hs00174097_m1, for *Il1R*: NM_000877.3 and Hs00168392_m1, for *Il2*: NM_000586.3 and Hs00174114_m1). The reference gene for endogenous control was the *GAPDH* gene (NM_001289746.1 and Hs99999905_m1). Three technical repetitions were performed on each test sample. The procedure for RNA isolation and evaluation of gene expression is described in the work of Gil-Kulik et al [24,25].

Statistical Analysis

The results of statistical analyzes were obtained with the Statistica v.13 program, using the Mann-Whitney U test and the Spearman's rank correlation coefficient. Statistical significance was established at the level of $P<0.05$.

Evaluation of Clinical Data

In taking the medical history of patients, doctors of the Department of Obstetrics and Pathology of Pregnancy assessed the age and comorbidities of the patients, weeks of gestation, weight of the newborn, sex of the newborn, and patient's past illnesses. The biophysical parameters of umbilical cord blood were assessed in the Department of Obstetrics just after delivery on an ABL90 FLEX gas analyzer (Radiometer, Denmark).

Results

Cytometric analysis and cell culture confirmed that MSCs were isolated from the umbilical cord. The ability of adherence to the plastic walls and the fibroblast-like shape of cells were confirmed (**Figure 1**), and antigens characteristic for MSCs, such as CD73, CD90, CD105, and CD146, and lack of CD45, CD31, CD19, and CD14 were demonstrated on the surface of the cells (**Figure 2**). High expression of *SOX2, POU5F,1* and *NANOG* genes, which are the main factors of stem cell pluripotency, was observed in the examined cells (expression results of *POU5F1, NANOG,* and *SOX2* were not published in this report). Researchers are also looking to the potential of multilineage differentiation to characterize MSCs; however, in this study, we did not test the differentiation potential.

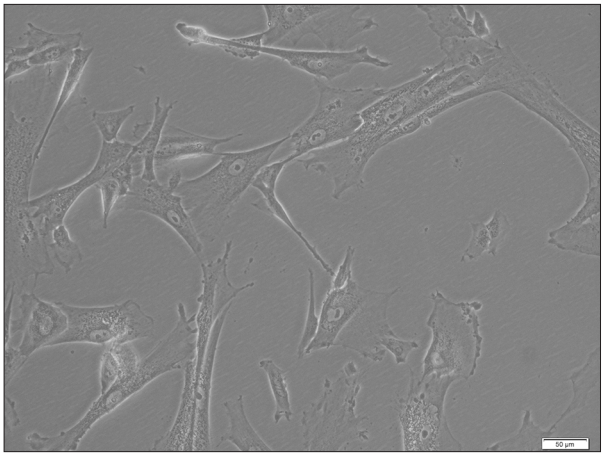

Figure 1. Mesenchymal stem cells from a 5-day culture. Bright field microscopy (BF), 200× magnification, using Xcellence RT system with an IX81 inverted microscope (Olympus).

Molecular analysis showed the presence at the mRNA level of all tested genes in umbilical cord MSCs. We assessed the dependence of the expression of tested genes (*TSG-6*, *IL1A*, *IL1B*, *IL2*, *IL6*, *IL6R*) on patient age, comorbidities, gestation period, newborn's weight, sex of the newborn, and the following biophysical parameters of umbilical cord blood: partial pressure of hydrogen (pH), partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT).

Influence of pH

Analyzing the expression of genes tested from umbilical cord blood pH, we observed that the expression of the *TSG-6* gene in MSCs was statistically significantly nearly 5 times higher ($P=0.032$) at umbilical cord blood pH greater than or equal to 7.35, compared with at lower pH values (**Figure 3A**). Spearman's rank correlation analysis showed that the level of *TSG-6* gene expression in MSCs was statistically significantly positively correlated with cord blood pH (r=0.402, $P<0.05$; **Figure 3B**, **Table 3**). Moreover, the analysis of the correlation of the expression of the studied genes with the cord blood pH in MSCs collected from healthy women showed a negative correlation with the expression of *IL1A* (r=-0.625, $P<0.05$) and with the expression of *IL6* (r=-0.638, $P<0.05$; **Table 4**). There was no significant correlation between the expression levels of *IL1B*, *IL1R*, *IL2,* and *IL6R* genes with cord blood pH.

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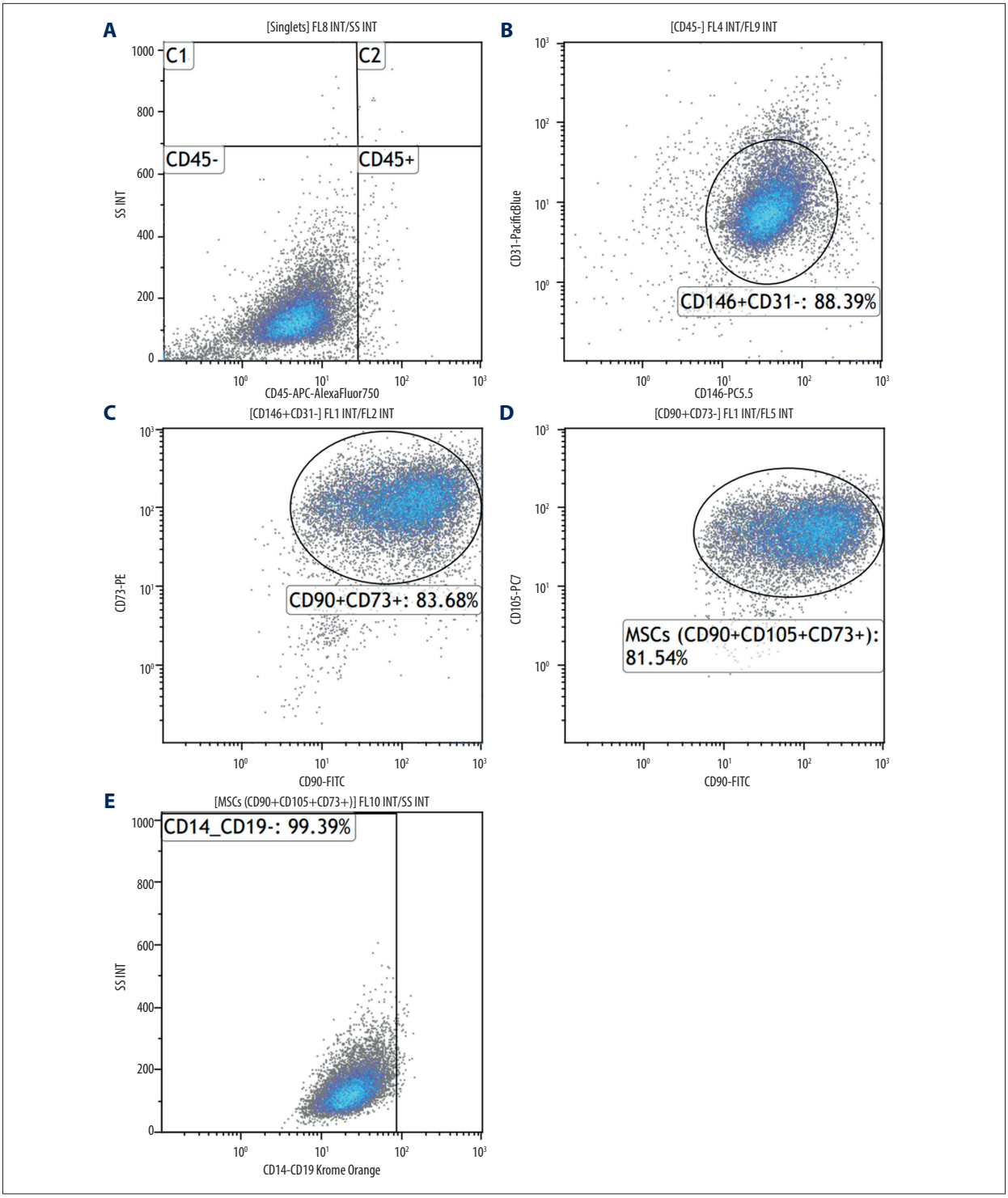

Figure 2. Cytometric evaluation of the expression of CD146, CD73, CD90, and CD105 surface antigens on the tested cells. DuraClone SC Mesenchymal Tube antibodies, Navios cytometer (Beckman Coulter). Sample immunophenotype analysis of umbilical cord-derived mesenchymal stem cells (MSCs) after in vitro culture. (**A**) Cytogram: cell population negative for CD45 antigen (CD45-). (**B**) Cytogram: percentage of CD31-negative and CD146-positive cells (CD146+CD31-). (**C**) Cytogram: percentage of cell population expressing CD90 antigen and CD73 antigen (CD90+CD73+). (**D**) Cytogram: percentage of umbilical cord MSCs positive for CD90, CD105, CD73 (CD90+CD105+CD73+). (**E**) MSC positive for CD90, CD105, CD73 and negative for CD14 and CD19 (CD90+CD105+CD73+CD14-CD19-).

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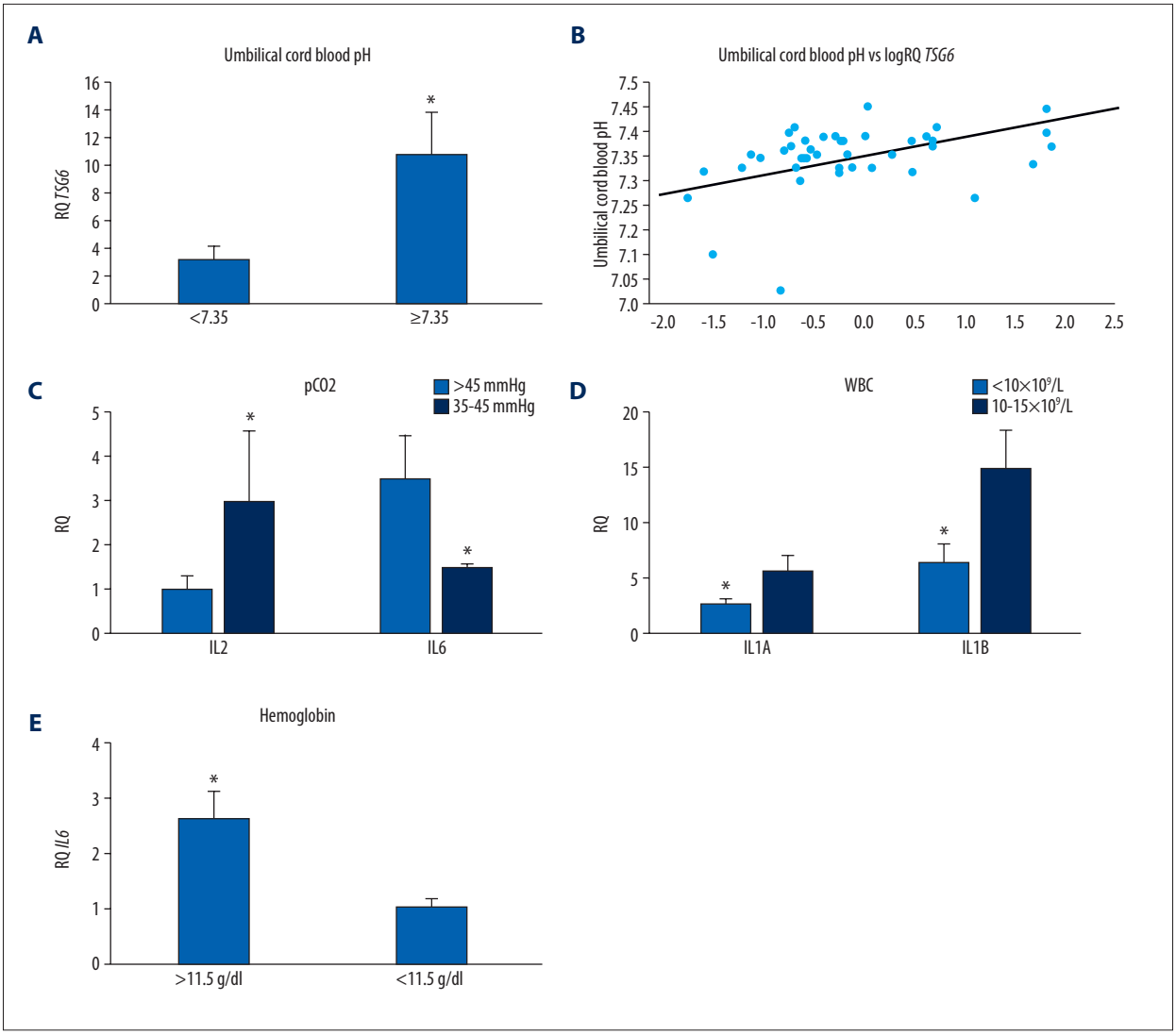

Figure 3. (**A**) Mean *TSG-6* gene expression (relative level of expression±standard error [RQ±SE]) in tested mesenchymal stem cells (MSCs) depending on umbilical cord blood pH. * *P*<0.05 Mann-Whitney U test. (**B**) Graph of distribution *TSG-6* gene expression in tested MSCs and umbilical cord blood pH. Spearman's rank factor r=0.402, *P*<0.05. (**C**) Mean *IL2* and *IL6* gene expression (relative level of expression±standard error [RQ±SE]) in tested MSCs, based on the umbilical cord blood pCO2. * *P*<0.05 Mann-Whitney U test. (**D**) Mean *IL1A* and *IL1B* gene expression (RQ±SE) in tested MSCs depending on umbilical cord blood white blood cell count. * *P*<0.05 Mann-Whitney U test. (**E**) Mean *IL6* gene expression (RQ±SE) in tested MSCs, based on umbilical cord blood hemoglobin level. * *P*<0.05 Mann-Whitney U test.

Influence of pCO_2

The analysis of the dependence of the expression of the studied genes on the pCO_2 level in the umbilical blood, including the entire study group, showed that the level of *IL2* expression was significantly negatively correlated with pCO_2 (r=-0.494 *P*<0.05), while the level of *IL6* expression was significantly positively correlated with pCO_2 (r= 0.470 *P*<0.05; **Table 3**). Moreover, it was observed that with a pCO_2 in the umbilical blood above 45 mm Hg, the expression of *IL2* was statistically significantly 22 times lower (*P*=0.024) than the pCO_2 in the range of 35 to 45 mm Hg. On the other hand, the level of *IL6* expression at pCO_2 above 45 mm Hg was statistically significantly more than 2.5 times higher (*P*=0.016) than the range of 35 to 45 mm Hg (**Figure 3C**). The analysis in the group of healthy women showed a positive correlation of pCO_2 with the level of *IL6* (r=0.628, *P*<0.05) and the level of *IL1A* (r=0.594 *P*<.05; **Table 4**). There was no significant dependence of *TSG-6*, *IL1B*, *IL1R*, and *IL6R* gene expression on the pCO_2 in the umbilical cord blood.

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Table 3. Correlations between the parameters studied in the entire study group (n=45).

Parameter	RQ *IL1A*	RQ *IL1B*	RQ *IL1R1*	RQ *IL2*	RQ *IL6*	RQ *IL6R*	RQ *TSG-6*
RQ *IL1A*	1.000	**0.635***	**0.328***	**-0.585***	**0.606***	-0.280	0.235
RQ *IL1B*	**0.635***	1.000	0.074	-0.190	**0.473**	-0.291	**0.514***
RQ *IL1R1*	**0.328***	0.074	1.000	-0.194	**0.396**	**0.370***	**0.511***
RQ *IL2*	**-0.585***	-0.190	-0.194	1.000	-0.286	0.260	0.149
RQ *IL6*	**0.606***	**0.473***	**0.396***	-0.286	1.000	**-0.337***	0.305
RQ *IL6R*	-0.280	-0.291	**0.370***	0.260	**-0.337***	1.000	0.034
RQ *TSG-6*	0.235	**0.514***	**0.511***	0.149	0.305	0.034	1.000
Mother's age	**0.366***	0.256	-0.008	-0.265	0.233	**-0.322***	0.183
Number of pregnancies	-0.211	0.102	-0.151	0.184	-0.165	-0.078	0.015
Number of deliveries	-0.161	0.124	-0.075	0.224	-0.058	-0.179	0.183
Weeks of gestation	0.114	0.153	-0.119	-0.117	0.168	-0.280	-0.141
Newborn weight	0.073	0.143	-0.010	-0.023	0.183	-0.243	-0.167
pH	-0.045	-0.086	0.123	0.362	-0.054	0.084	**0.402***
pCO_2	0.139	0.116	-0.062	**-0.494***	**0.470***	-0.119	-0.280
PO_2	-0.096	0.065	-0.165	0.207	**-0.441***	0.072	0.151
$cHCO_3$	0.166	0.027	0.118	-0.008	0.248	-0.208	0.176
WBC	0.163	0.123	-0.084	0.032	0.026	-0.013	0.002
RBC	-0.005	0.142	-0.188	-0.130	0.012	-0.076	-0.242
HGB	0.285	0.152	0.233	-0.262	**0.361***	-0.134	0.132
HCT	0.076	0.150	0.100	-0.178	0.178	-0.056	-0.022
MCV	0.197	-0.009	0.314	-0.265	0.219	-0.048	0.260
MCH	0.277	0.015	0.277	-0.179	**0.317***	**-0.344***	**0.323***
MCHC	**0.359***	0.005	**0.320***	0.042	0.287	**-0.327***	**0.308***
PLT	0.126	0.226	-0.077	**0.454***	-0.138	**0.451***	0.363

* $P<0.05$ Spearman's rank factor. pH – potential of hydrogen; pO_2 – partial pressure of oxygen; pCO_2 – partial pressure of carbon dioxide; WBC – white blood cell count; RBC – red blood cell count; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelet count; RQ – level of relative expression; SD – standard deviation; SE – standard error.

Influence of pO_2

The analysis of the dependence of the expression of studied genes in MSCs on pO_2 showed a statistically significant negative correlation with the level of *IL6* expression (r=-0.441, $P<0.05$) (**Table 3**). In the group of healthy women, the level of *IL1R* was negatively correlated with pO_2 (r=-0.676, $P<0.05$; **Table 4**). There was no significant relationship between the expression of *TSG-6*, *IL1A*, *IL1B*, *IL2,* and *IL6R* genes in the MSCs from umbilical cord blood pO_2.

Influence of the Parameters of Cord Blood Morphology

Considering the parameters of cord blood morphology, it was noted that the expression of *IL1A* ($P=0.044$) and *IL1B* ($P=0.04$) in MSCs was more than 2 times higher when the level of WBC in cord blood was in the range of 10 to 15×10^9/L, compared with levels of WBC lower than 10×10^9/L (**Figure 3D**), and this result was statistically significant. There were no relationships between the other genes tested and the level of WBC in the umbilical blood.

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Table 4. Correlations between clinical parameters and expression of genes in the group of healthy women (n=17), patients with gestational diabetes (n=13), patients with hypertension (n=8), and patients with hypothyroidism (n=14).

Parameter	RQ *IL1A*	RQ *IL1B*	RQ *IL1R1*	RQ *IL2*	RQ *IL6*	RQ *IL6R*	RQ *TSG-6*
Control group							
RQ *IL1A*	1.000	**0.940***	0.009	**-0.636***	**0.848***	-0.420	0.090
RQ *IL1B*	**0.940***	1.000	-0.081	-0.269	**0.632***	-0.390	**0.859***
RQ *IL1R1*	0.009	-0.081	1.000	0.392	0.364	**0.784***	-0.087
RQ *IL2*	**-0.636***	-0.269	0.392	1.000	-0.336	**0.898***	0.019
RQ *IL6*	**0.848***	**0.632***	0.364	-0.336	1.000	-0.019	0.031
RQ *IL6R*	-0.420	-0.390	**0.784***	**0.898***	-0.019	1.000	-0.162
RQ *TSG-6*	0.090	**0.859***	-0.087	0.019	0.031	-0.162	1.000
Mother's age	0.010	0.074	-0.316	-0.367	-0.056	-0.502	-0.066
Number of pregnancies	-0.436	0.147	0.052	0.035	-0.289	0.089	0.322
Number of deliveries	-0.356	0.338	-0.026	0.120	-0.267	0.071	**0.527***
Weeks of gestation	0.210	0.118	0.066	-0.314	0.188	-0.231	-0.027
Newborn's weight	0.270	0.079	0.318	-0.135	0.200	0.091	-0.049
pH	**-0.625***	-0.104	-0.028	0.466	**-0.638***	0.186	0.012
pCO_2	**0.594***	0.104	0.104	-0.458	**0.628***	-0.116	0.037
PO_2	-0.226	0.154	**-0.676***	0.026	-0.538	-0.364	0.198
$cHCO_3$	-0.512	0.117	0.318	0.352	-0.463	0.366	0.267
WBC	0.104	0.058	-0.124	0.303	-0.010	0.003	0.033
RBC	0.493	-0.071	-0.040	-0.081	**0.590***	-0.146	-0.226
HGB	0.402	0.055	0.094	0.001	**0.599***	-0.029	-0.109
HCT	0.492	0.080	0.032	-0.199	**0.627***	-0.170	-0.087
MCH	0.025	0.205	0.209	0.067	0.129	0.128	0.150
MCV	0.066	0.334	0.156	-0.277	0.156	-0.079	0.305
MCHC	-0.027	-0.034	0.209	0.491	0.057	0.353	-0.100
PLT	0.312	0.603*	-0.514	0.027	0.058	**-0.593***	**0.767***
Gestational diabetes							
RQ *IL1A*	1.000	0.143	0.390	**-0.762***	0.533	0.000	0.467
RQ *IL1B*	0.143	1.000	-0.302	0.214	0.242	-0.214	-0.192
RQ *IL1R1*	0.390	-0.302	1.000	**-0.714***	0.236	0.363	**0.582***
RQ *IL2*	**-0.762***	0.214	**-0.714***	1.000	-0.548	-0.048	-0.190
RQ *IL6*	0.533	0.242	0.236	-0.548	1.000	-0.505	0.231
RQ *IL6R*	0.000	-0.214	0.363	-0.048	-0.505	1.000	-0.011
RQ *TSG-6*	0.467	-0.192	**0.582***	-0.190	0.231	-0.011	1.000
Mother's age	0.468	0.108	-0.158	-0.470	0.119	-0.376	0.177

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Table 4 continued. Correlations between clinical parameters and expression of genes in the group of healthy women (n=17), patients with gestational diabetes (n=13), patients with hypertension (n=8), and patients with hypothyroidism (n=14).

Parameter	RQ *IL1A*	RQ *IL1B*	RQ *IL1R1*	RQ *IL2*	RQ *IL6*	RQ *IL6R*	RQ *TSG-6*
Number of pregnancies	-0.162	0.102	-0.045	0.218	-0.142	-0.171	0.102
Number of deliveries	-0.085	0.295	-0.160	0.075	0.096	-0.475	0.111
Weeks of gestation	-0.182	0.367	-0.098	-0.282	-0.283	0.042	-0.498
Newborn's weight	-0.099	0.060	-0.121	0.310	-0.099	-0.269	-0.363
pH	-0.463	0.064	-0.105	**0.764***	-0.225	-0.155	-0.022
pCO_2	**0.676***	0.038	0.236	-0.667	0.253	-0.011	0.368
PO_2	-0.388	0.187	-0.061	0.262	-0.325	0.294	-0.025
$cHCO_3$	**0.633***	0.052	0.305	-0.503	0.319	-0.157	**0.575***
WBC	-0.159	0.005	-0.236	-0.405	-0.181	0.104	-0.440
RBC	0.137	0.374	-0.110	0.048	0.033	0.088	-0.088
HGB	0.171	0.011	0.094	-0.415	0.017	-0.182	0.039
HCT	-0.044	0.231	-0.170	-0.095	-0.066	0.000	-0.242
MCH	-0.165	-0.527	0.170	-0.429	-0.170	-0.038	-0.209
MCV	-0.154	-0.437	0.052	-0.357	-0.223	-0.135	-0.275
MCHC	0.270	-0.278	0.190	-0.119	-0.094	-0.253	0.273
PLT	-0.170	0.313	0.181	0.476	-0.176	0.379	0.038
Hypertension							
RQ *IL1A*	1.000	0.392	0.413	0.231	-0.030	0.467	0.298
RQ *IL1B*	0.392	1.000	-0.123	-0.766	-0.005	**0.982***	0.046
RQ *IL1R1*	0.413	-0.123	1.000	0.142	0.841	0.067	**0.962***
RQ *IL2*	0.231	-0.766	0.142	1.000	-0.296	-0.748	-0.129
RQ *IL6*	-0.030	-0.005	0.841	-0.296	1.000	0.159	0.942
RQ *IL6R*	0.467	**0.982***	0.067	-0.748	0.159	1.000	0.232
RQ *TSG-6*	0.298	0.046	**0.962***	-0.129	0.942	0.232	1.000
Mother's age	0.628	0.658	0.666	-0.451	0.620	0.788	0.779
Number of pregnancies	0.035	-0.109	0.904	-0.148	**0.988***	0.067	**0.892***
Number of deliveries	0.035	-0.109	0.904	-0.148	**0.988***	0.067	**0.892***
Weeks of gestation	-0.555	-0.234	-0.936	0.124	-0.815	-0.414	**-0.934***
Newborn's weight	-0.261	**-0.982***	0.075	0.867	-0.131	**-0.974***	-0.344
pH	0.619	-0.434	0.250	0.906	-0.296	-0.396	0.086
pCO_2	-0.750	0.022	0.068	-0.654	0.590	0.043	-0.015
PO_2	0.871	0.130	0.058	0.538	-0.463	0.135	-0.055
$cHCO_3$	-0.652	-0.441	0.420	-0.169	0.749	-0.357	0.056
WBC	0.825	0.159	-0.063	0.509	-0.562	0.140	-0.452

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Table 4 continued. Correlations between clinical parameters and expression of genes in the group of healthy women (n=17), patients with gestational diabetes (n=13), patients with hypertension (n=8), and patients with hypothyroidism (n=14).

Parameter	RQ *IL1A*	RQ *IL1B*	RQ *IL1R1*	RQ *IL2*	RQ *IL6*	RQ *IL6R*	RQ *TSG-6*
RBC	-0.693	0.223	-0.864	-0.525	-0.456	0.063	-0.776
HGB	0.813	0.429	0.787	-0.084	0.546	0.579	0.665
HCT	0.883	0.002	0.722	0.473	0.253	0.135	0.685
MCH	0.705	-0.211	0.859	0.524	0.445	-0.052	0.785
MCV	0.171	-0.563	0.889	0.477	0.698	-0.396	0.832
MCHC	-0.064	0.676	-0.815	-0.542	-0.637	0.523	-0.626
PLT	0.433	-0.557	0.054	**0.959***	-0.451	-0.556	-0.549
Hypothyroidism							
RQ *IL1A*	1.000	0.302	0.547	-1.000	**0.555***	-0.423	0.385
RQ *IL1B*	0.302	1.000	0.154	0.600	0.108	-0.007	-0.020
RQ *IL1R1*	0.547	0.154	1.000	-0.800	0.392	0.110	**0.618***
RQ *IL2*	-1.000	0.600	-0.800	1.000	-0.700	0.600	-0.500
RQ *IL6*	**0.555***	0.108	0.392	-0.700	1.000	**-0.587***	0.433
RQ *IL6R*	-0.423	-0.007	0.110	0.600	**-0.587***	1.000	-0.134
RQ *TSG-6*	0.385	-0.020	**0.618***	-0.500	0.433	-0.134	1.000
Mother's age	0.342	0.139	0.126	-0.500	0.220	**-0.678***	0.097
Number of pregnancies	-0.361	0.282	-0.333	0.527	-0.370	-0.155	-0.356
Number of deliveries	-0.377	0.148	-0.275	0.632	-0.119	-0.237	-0.089
Weeks of gestation	**0.740***	0.176	0.161	-0.707	0.464	-0.338	0.169
Newborn's weight	0.275	0.059	-0.250	-0.616	0.400	**-0.570***	-0.310
pH	0.006	-0.228	0.323	0.205	0.104	0.299	**0.675***
pCO_2	0.044	0.086	-0.238	-0.300	-0.033	-0.336	**-0.578***
PO_2	-0.110	0.160	0.319	0.300	-0.407	**0.635***	0.314
$cHCO_3$	0.377	0.004	-0.117	-0.300	0.363	**-0.705***	-0.101
WBC	0.033	-0.112	-0.176	0.500	0.130	-0.029	0.169
RBC	-0.503	0.042	-0.439	0.700	-0.414	0.137	-0.344
HGB	0.152	0.214	0.119	-0.500	0.293	-0.284	0.247
HCT	-0.154	0.187	0.070	-0.200	0.024	-0.037	0.073
MCH	0.501	0.113	**0.684***	**-0.900***	0.325	-0.102	0.356
MCV	**0.619***	0.145	0.483	**-0.975***	**0.535***	-0.334	0.433
MCHC	**0.634***	0.143	0.471	**-0.900***	0.491	-0.337	0.372
PLT	-0.082	0.345	-0.062	0.700	**-0.609***	0.459	-0.002

* $P<0.05$ Spearman's rank factor. pH – potential of hydrogen; pO_2 – partial pressure of oxygen; pCO_2 – partial pressure of carbon dioxide; WBC – white blood cell count; RBC – red blood cell count; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelet count; RQ – level of relative expression; SD – standard deviation; SE – standard error.

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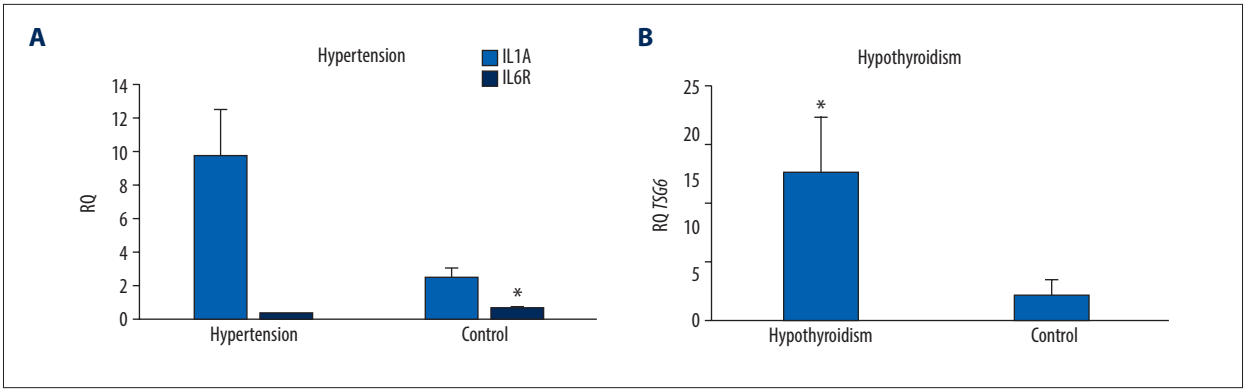

Figure 4. (**A**) Mean *IL1A* and *IL6R* gene expression (relative level of expression±standard error [RQ±SE]) in tested mesenchymal stem cells (MSCs) depending on hypertension in patients. (**B**) Mean *TSG-6* gene expression (RQ±SE) in tested MSCs depending on the hypothyroidism in patient. * *P*<0.05 Mann-Whitney U test.

A number of correlations between the expression of genes under study and the RBC parameters of umbilical blood have been demonstrated. Among other things, we observed that the level of *IL6* gene expression depended on the hemoglobin level. When HGB values exceeded 11.5 g/dL, the expression of *IL6* in the MSCs was statistically significantly 2.5 times higher (*P*=0.041) than that of lower hemoglobin levels (**Figure 3E**). Correlation analysis in the entire study group showed a positive relationship between the level of *IL6* expression and the level of hemoglobin (r=0.361, *P*<0.05; **Table 3**). Correlation analysis in the group of healthy women also showed a significant positive relationship between the level of *IL6* expression and HGB (r=0.599, *P*<0.05). Moreover, it was noted that *IL6* was positively correlated with RBC (r=0.590, *P*<0.05) and HCT (r=0.627, *P*<0.05; **Table 4**).

The relationship between the expression of the tested genes and the number of platelets was also noted. During the correlation analysis of the whole study group, we found the level of *IL2* expression (r=0.545, *P*<0.05) and *IL6R* (r=0.451, *P*<0.05) were positively correlated with PLT (**Table 3**). During the analysis, in the group of healthy women, a positive relationship between *TSG-6* expression and PLT (r=0.767, *P*<0.05) and *IL1B* with PLT (r=0.603, *P*<0.05) and a negative relationship between the expression of *IL6R* and PLT (r=-0.593, *P*<0.05) were observed (**Table 4**).

Influence of Patients' Diseases

The analysis of the dependence of the expression of genes in the examined patients with comorbidities showed that the expression of *IL1A* in the MSCs of the umbilical cord obtained from patients with hypertension was statistically significantly 4 times higher (*P*=0.004) than that in healthy patients. In turn, the expression of the gene encoding *IL6R* in MSCs obtained from healthy patients was statistically significantly 3.5 times higher (*P*=0.037) than in patients with hypertension (**Figure 4A**). The other tested genes did not differ significantly depending on the presence of hypertension.

The expression of the *TSG-6* gene was statistically significantly 5 times higher (*P*=0.026) in the umbilical cord MSCs obtained from women with hypothyroidism (**Figure 4B**). In the case of other tested genes, no significant relationships with the occurrence of hypothyroidism were found.

The analysis of the correlation between the studied genes in the whole study group showed a significant positive relationship between the expression of the *TSG-6* gene and *IL1B* (r=0.514, *P*<0.05) and between *TSG-6* and *IL1R* (r=0.511, *P*<0.05). Also, the level of *IL1A* expression in MSCs was positively correlated with patient age (r=0.366, *P*<0.05), while the level of *IL6R* in MSCs was negatively correlated with patient age (r=-0.322, *P*<0.05; **Table 3**).

It was also shown that the expression of the *TSG-6* gene in a group of healthy women was positively correlated with the number of deliveries (r=0.527, *P*<0.05; **Table 4**).

Analysis of the correlation between the expression of the studied genes and clinical parameters in MSCs from patients with hypertension showed the existence of several strong relationships: *IL1B* expression was positively correlated with *IL6R* expression (r=0.982, *P*<0.05), *IL1R* expression was positively correlated with *TSG-6* expression level (r=0.962, *P*<0.05), *IL6* expression was positively correlated with the number of pregnancies and deliveries (r=0.988, *P*<0.05), *TSG-6* expression in hypertensive patients was positively correlated with the number of pregnancies and deliveries (r=0.892, *P*<0.05), *TSG-6* expression was negatively correlated with the gestational week of delivery (r=-0.934, *P*<0.05), *IL1B* expression was negatively correlated with newborn weight (r=-0.982, *P*<0.05), *IL6R* expression was negatively correlated with newborn weight (r=-0.974, *P*<0.05), and a positive relationship between *IL2* expression

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and PLT was noted (r=0.959, *P*<0.05) (**Table 4**). It should be mentioned here that the study group with hypertension consisted of only 8 patients; moreover, 3 of them also had diabetes, and therefore the obtained results need to be confirmed in a larger group of patients.

The evaluation of the correlation between the tested genes and clinical data in MSCs collected from patients with hypothyroidism showed the following: the level of *IL6* expression was positively correlated with the level of *IL1A* expression (r=0.555, *P*<0.05), the level of *IL6* expression was negatively correlated with *IL6R* expression (r=-0.587, *P*<0.05), *IL1R1* expression was positively correlated with the level of *TSG-6* expression (r=0.618, *P*<0.05), *IL1A* expression was positively correlated with the gestational week in which delivery occurred (r=0.740, *P*<0.05), *TSG-6* expression was positively correlated with the pH of umbilical cord blood (r=0.675, *P*<0.05), and *TSG-6* expression level was negatively correlated with pCO2 (r=-0.578, *P*<0.05). In addition, relationships between *IL1R, IL1A, IL2,* and *IL6* with the parameters MCV, MCH, and MCHC were noted (**Table 4**).

In MSCs from patients with gestational diabetes, the following relationships were observed: *IL1A* expression was negatively correlated with *IL2* expression (r=-0.762, *P*<0.05), *IL1R* expression was negatively correlated with *IL2* expression (r=-0.714 *P*<0.05), *IL2* expression was positively correlated with cord blood pH (r=0.764, *P*<0.05), *IL1A* expression was positively correlated with cord blood pCO2 (r=0.676 *P*<0.05), *IL1A* expression was positively correlated with cHCO3 (r=0.633, *P*<0.05), *TSG-6* expression level was positively correlated with *IL1R* expression (r=0.582, *P*<0.05), and *TSG-6* expression level was positively correlated with cHCO3 (r=0.575, *P*<0.05).

Moreover, in this study, we showed a certain tendency regarding the influence of vaginal delivery and the administration of oxytocin during labor on *TSG-6* expression in the umbilical cord MSCs; however, these results were not statistically significant (*P*=0.06 and *P*=0.09). There is the need for further studies in a larger group to assess the effect of the use of oxytocin and vaginal delivery on the level of *TSG-6* in MSCs.

Discussion

The results of the present study have shown that MSCs express the *TSG-6* gene, which is consistent with the results of other studies [26]; however, to date, there are no data in the literature that consider the correlation of the expression of the *TSG-6* gene in umbilical cord MSCs with the clinical condition of the patient.

TSG-6 is a hyaluronan-binding protein. It is one of the mechanisms responsible for the anti-inflammatory effect of this protein. It has been observed that the affinity of TSG-6 for hyaluronan is pH-dependent [27-29]. This indicates that the anti-inflammatory effect of TSG-6 will depend on the pH value, which is confirmed by the results of our research, in which we have shown that the expression of *TSG-6* in MSCs increases with increasing of pH in umbilical cord blood. No data has been found in the literature that would indicate the influence of cord blood pH on the level of *TSG-6* expression in the umbilical cord MSCs; to the best of our knowledge, our study is the first. This is a very important aspect that influences the decision to collect stem cells in specific cases and supports their potential clinical usefulness.

In addition to the effect of cord blood acid-base balance parameters on the level of *TSG-6* expression in MSCs, we also noticed that patients with hypothyroidism showed higher levels of TSG-6 than did healthy patients. Hypothyroidism is associated with chronic inflammation, usually with elevated levels of cytokines. The elevation of TSG-6 in the MSCs of patients with hypothyroidism may suggest an increase associated with inflammation. We also noted a positive relationship between the level of *TSG-6* expression and the expression of the *IL1* receptor in these patients.

Moreover, arteritis has been shown to worsen after suppression of TSH, compared with the state of hypothyroidism [30]. This is consistent with our study, in which it was observed that *TSG-6* expression was statistically significantly higher in MSCs obtained from patients with hypothyroidism. This suggests that higher levels of *TSG-6* in people with hypothyroidism may be responsible for suppressing arteritis.

We also observed that the expression of *TSG-6* in healthy women depends on the number of deliveries. The more deliveries, the higher the levels of *TSG-6* were observed in healthy women. There are no data on the dependence of *TSG-6* expression in MSC on the number of pregnancies and deliveries in the literature. Perhaps the adaptive changes taking place in the body of women who give birth again affect the level of *TSG-6* expression in the MSCs of the umbilical cord; however, this requires further research.

It has been shown that in inflammation caused by trauma, the platelet lysate activates NF-κB in MSCs, promoting the release of pro-inflammatory cytokines. TSG-6, which also acts through the NF-κB pathway in an inhibitory manner, increases in this situation [31]. Regarding our study, which also showed a significant, positive correlation of *TSG-6* expression with the number of platelets, it can be concluded that TSG-6 may participate in the suppression of inflammation caused by injuries.

Our results showed that there was a tendency to different values of *TSG-6* expression in the umbilical cord MSCs in women

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who received oxytocin while giving birth. This suggests that oxytocin likely influences the anti-inflammatory properties of MSCs. A decrease of pro-inflammatory cytokine levels was observed in earlier studies, with an increase of anti-inflammatory cytokine levels after the administration of oxytocin in lung damage [32] and after microglia activation [33]. The anti-inflammatory effect of oxytocin has also been demonstrated in atherosclerosis, enteritis, and urinary tract inflammation [34]. TSG-6 works in the same way, also by regulation of inflammatory cytokines [35]. Considering the similar mechanisms of TSG-6 and oxytocin action and the results of our research, this topic is worth a closer look.

We also observed that there is a tendency for different values of *TSG-6* gene expression in vaginal delivery compared to cesarean delivery; this situation may be caused by the involvement of oxytocin. These speculations can be explained by the influence of oxytocin on many mechanisms that contribute to oxidative stress and inflammation, thus protecting cells from damage. Studies by other authors have shown that oxytocin suppresses oxidative stress and inhibits the inflammatory process in the acute stages of labor, while protecting against postpartum hypoxia [36]. It should be noted, however, that in our study group only 6 patients out of 45 had vaginal delivery, and only 10 patients had oxytocin-supported labor; therefore, the results obtained should be repeated on a larger number of participants, to really assess whether the use of oxytocin, and natural delivery, have a significant impact on the level of *TSG-6* in the MSC and thus the clinical utility of these cells.

In our study, apart from the evaluation of *TSG-6* expression in umbilical cord MSCs, we focused on the evaluation of selected pro-inflammatory cytokines and the analysis of the relationship between *TSG-6* and these cytokines.

Treatment with TSG-6 has already been shown to reduce IL1B and IL6 levels [37]. Similar effects were observed in another study, in which cells cultured with IL1 and IL6 were shown to have lower TSG-6 production [38]. The aim of our study was to assess whether similar relationships occur in MSCs at the level of gene expression.

Liu et al tested the effect of transplantation of MSCs isolated from the human umbilical cord on the healing of post-burn wounds. MSCs reduced the amount of infiltrating inflammatory cells, lowered the concentration of pro-inflammatory interleukins IL1 and IL6, and increased the concentration of anti-inflammatory factors, such as TSG-6 [39]. During our research, we decided to check whether *TSG-6* inhibited the synthesis of pro-inflammatory interleukins, namely *IL1* and *IL6*, at the mRNA level.

Despite the lack of satisfactory effects of the potential positive impact of MSC on the course and symptoms of toxic shock syndrome presented in the publication by Kim et al [40], our team was inspired to check the relationship between the expression level of pro-inflammatory interleukins, namely *IL1* and *IL6*, and the presence of *TSG-6* in MSCs. Our aim was to determine whether MSCs express mRNA of the mentioned pro-inflammatory mediators or whether this expression may be related to the expression level of *TSG-6* mRNA.

One study has shown that MSC has a positive effect on reducing the concentration of pro-inflammatory cytokines in chronic exposure to cigarette smoke. It turns out that after endotracheal administration of MSC, the concentration of IL1B and TSG-6 are reduced. We were interested in whether there is an effect of the anti-inflammatory TSG-6 in MSCs on the production of IL1B and other pro-inflammatory interleukins [41].

Data in the literature indicate a significant relationship between concentration of IL1A and arterial hypertension. The cytokine IL1A is a predictor of high systolic and diastolic blood pressure [42]. IL6, on the other hand, has multidirectional effects, such as inter alia initiating chronic inflammation. The presence of IL6R on the MSC surface has also been demonstrated [43]. The results of the reports are consistent with the results of our study concerning significantly higher *IL1A* expression in the MSC in hypertensive patients compared with that in healthy patients, and significantly lower *IL6R* expression in MSCs in healthy patients compared with that in hypertensive patients. This may indicate that the increase of pro-inflammatory cytokines is associated with the presence of arterial hypertension. This hypothesis is supported by the results of other studies, in which it was shown that pregnant women with hypertension exhibit higher levels of pro-inflammatory cytokines and acute phase proteins than do healthy women [44]. In addition, in our study, we also showed that in MSCs taken from hypertensive patients, the expression of *TSG-6* was positively correlated with the expression of *IL1R1*. It seems that the increase in the expression of pro-inflammatory cytokines in MSCs of hypertensive patients is also accompanied by a compensatory increase in *TSG-6*. We also observed that *TSG-6* was negatively correlated with gestational week of delivery, and *IL1B* and *IL6R* were negatively correlated with neonatal weight. It has been shown that inflammation, and in particular an imbalance of pro- and anti-inflammatory mediators, is recognized as a key factor in the pathophysiology underlying preterm birth as well as neonatal disease [45].

Reduction of blood oxygen saturation is associated with an increase of IL6 level in serum [46]. Reports on this subject are consistent with the results we obtained, according to which *IL6* in MSCs shows a positive expression, with an increase of pCO_2 and negative expression with an increase of pO_2. On this basis, it can be hypothesized that IL6-induced inflammation worsens with increasing pCO_2.

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Moreover, IL6, apart from its role in the immune response, is responsible for the regulation of hemopoiesis in the organism [47]. Hemoglobin constitutes the majority of intracellular proteins of RBCs [48]. Our study showed an increase in the level of *IL6* expression in patients with hemoglobin levels >11.5 g/dL, which suggests that the increase of hemopoiesis is accompanied by an increase of *IL6*. In our study, we also observed that the expression level of *IL6* in MSCs taken from healthy women was positively correlated with the number of RBC, HGB, and HCT, and we showed that *IL6* expression was positively correlated with pCO_2 and negatively correlated with umbilical cord blood pH.

This is supported by other studies which show that maternal anemia has an effect on the increase in umbilical cord pO_2 [49], which is further negatively correlated with *IL6* expression. This supports that there is a relationship between levels of hemoglobin and *IL6*.

One of MSC's functions is inhibition of the development of an excessive inflammatory response. By negative feedback, pro-inflammatory cytokines stimulate MSCs to secrete TSG-6, which then reduces signaling of NF-κB in macrophages and thus models the pro-inflammatory cytokine cascade [50]. This is consistent with our results, in which we obtained a positive correlation between *TSG-6* expression and *IL1R* and *IL1B* in the entire study group.

Conclusions

Our research has shown that the expression of the *TSG-6* gene in MSCs derived from Wharton's jelly from the umbilical cord probably depends on coexisting diseases in the patient (hypothyroidism) and the biochemical parameters of umbilical cord blood, including a very important role of cord blood pH. We also noted that the type and course of delivery (oxytocin-assisted delivery) is probably important in the levels of *TSG-6* expression in MSCs, but this has to be confirmed in further studies. Based on the above results, we conclude that MSCs obtained from births in which the pH of the umbilical blood is higher probably have a greater therapeutic potential, and considering the function of the TSG-6 protein, this may contribute to an even wider application of these cells in medicine. However, the anti-inflammatory activity of these cells has not been tested, and this needs to be confirmed in the next tests that we plan to do. Considering the subject matter of our research, it is important that a positive correlation was demonstrated between the expression of the *TSG-6* gene and the level of *IL1R* and *IL1B* gene expression in the analyzed study group.

Ethics Statement

The research was conducted with the consent of the Bioethics Committee at the Medical University of Lublin, no. KE-0254/128/2014. All methods were conducted in accordance with relevant guidelines and regulations. Informed consent was obtained from all participants and/or their legal guardian(s).

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

References:

1. Andrzejewska A, Lukomska B, Janowski M. Concise review: Mesenchymal stem cells: From roots to boost. Stem Cells. 2019;37(7):855-64
2. Gil-Kulik P, Świstowska M, Kondracka A, et al. Increased expression of BIRC2, BIRC3, and BIRC5 from the IAP family in mesenchymal stem cells of the umbilical cord Wharton's Jelly (WJSC) in younger women giving birth naturally. Oxid Med Cell Longev. 2020;2020:9084730
3. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif. 2020;53(1):e12712
4. Pojda Z, Machaj E, Kurzyk A, et al. Mezenchymalne komórki macierzyste. Postępy Biochemii 2013;59(2):187-97 [in Polish]
5. Rodríguez-Fuentes DE, Fernández-Garza LE, Samia-Meza JA, et al. Mesenchymal stem cells current clinical applications: A systematic review. Arch Med Res. 2021;52(1):93-101
6. Matsuzaka Y, Yashiro R. Therapeutic strategy of mesenchymal-stem-cell-derived extracellular vesicles as regenerative medicine. Int J Mol Sci. 2022;23(12):6480
7. Lee RH, Pulin AA, Seo MJ, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009;5(1):54-63
8. Day AJ, Milner CM. TSG-6: A multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biol. 2019;78-79:60-83
9. Wang M, Zhang M, Fu L, et al. Liver-targeted delivery of TSG-6 by calcium phosphate nanoparticles for the management of liver fibrosis. Theranostics. 2020;10(1):36-49
10. Li Q, Song WJ, Ryu MO, et al. TSG-6 secreted by human adipose tissue-derived mesenchymal stem cells ameliorates severe acute pancreatitis via ER stress downregulation in mice. Stem Cell Res Ther. 2018;9(1):255
11. Kota DJ, Wiggins LL, Yoon N, Lee RH. TSG-6 produced by hMSCs delays the onset of autoimmune diabetes by suppressing Th1 development and enhancing tolerogenicity. Diabetes. 2013;62(6):2048-58
12. Dyer DP, Thomson JM, Hermant A, et al. TSG-6 inhibits neutrophil migration via direct interaction with the chemokine CXCL8. J Immunol. 2014;192(5):2177-85
13. Yang, H, Wu L, Deng H, et al. Anti-inflammatory protein TSG-6 secreted by bone marrow mesenchymal stem cells attenuates neuropathic pain by inhibiting the TLR2/MyD88/NF-κB signaling pathway in spinal microglia. J Neuroinflammation. 2020;17(1):154
14. Bárdos T, Kamath RV, Mikecz K, Glant TT. Anti-inflammatory and chondroprotective effect of TSG-6 (tumor necrosis factor-alpha-stimulated gene-6) in murine models of experimental arthritis. Am J Pathol. 2001;159(5):1711-21
15. Qu Y, Yang F, Meng F, et al. Plasma concentration of tumor necrosis factor-stimulated gene-6 as a novel diagnostic and 3-month prognostic indicator in non-cardioembolic acute ischemic stroke. Front Immunol. 2022;13:713379

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

16. Hu T, Liu Y, Li X, et al. Tumor necrosis factor-alpha stimulated gene-6: A biomarker reflecting disease activity in rheumatoid arthritis. J Clin Lab Anal. 2022;36(5):e24395
17. Li Y, Zhang D, Xu L, et al. Cell-cell contact with proinflammatory macrophages enhances the immunotherapeutic effect of mesenchymal stem cells in two abortion models. Cell Mol Immunol. 2019;16(12):908-20
18. Świstowska M, Gil-Kulik, P, Krzyżanowski A, et al. Potential effect of SOX2 on the cell cycle of Wharton's Jelly stem cells (WJSCs). Oxid Med Cell Longev. 2019;2019:5084689
19. Walecka I, Gil-Kulik P, Krzyżanowski A, et al. Phenotypic characterization of adherent cells population CD34+ CD90+ CD105+ derived from Wharton's Jelly. Med Sci Monit. 2017;23:1886-95
20. Gil-Kulik P, Świstowska M, Krzyżanowski, A, et al. Evaluation of the impact of pregnancy-associated factors on the quality of Wharton's Jelly-derived stem cells using SOX2 gene expression as a marker. Int J Mol Sci. 2022;23(14):7630
21. Gil-Kulik P, Leśniewski M, Bieńko K, et al. Influence of perinatal factors on gene expression of IAPs family and main factors of pluripotency: OCT4 and SOX2 in human breast milk stem cells – a preliminary report. Int J Mol Sci. 2023;24(3):2476
22. Chomczyński P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162(1):156-59
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402-8
24. Gil-Kulik P, Krzyżanowski A, Dudzińska E, et al. Potential involvement of BIRC5 in maintaining pluripotency and cell differentiation of human stem cells. Oxid Med Cell Longev. 2019;2019:8727925
25. Gil-Kulik P, Chomik P, Krzyżanowski A, et al. Influence of the type of delivery, use of oxytocin, and maternal age on POU5F1 gene expression in stem cells derived from Wharton's Jelly within the umbilical cord. Oxid Med Cell Longev. 2019;2019:1027106
26. Liu L, Song H, Duan H, et al. TSG-6 secreted by human umbilical cord-MSCs attenuates severe burn-induced excessive inflammation via inhibiting activations of P38 and JNK signaling. Sci Rep. 2016;6:30121
27. Parkar AA, Kahmann JD, Howat SL, et al. TSG-6 interacts with hyaluronan and aggrecan in a pH-dependent manner via a common functional element: implications for its regulation in inflamed cartilage. FEBS Lett. 1998;428(3):171-76
28. Blundell CD, Mahoney DJ, Cordell MR, et al. Determining the molecular basis for the pH-dependent interaction between the link module of human TSG-6 and hyaluronan. J Biol Chem. 2007;282(17):12976-88
29. Milner CM, Higman VA, Day AJ. TSG-6: A pluripotent inflammatory mediator? Biochem Soc Trans. 2006;34(Pt 3):446-50
30. Boswijk E, Sanders KJC, Broeders EPM, et al. TSH suppression aggravates arterial inflammation – an 18F-FDG PET study in thyroid carcinoma patients. Eur J Nucl Med Mol Imaging. 2019;46(7):1428-38
31. Xu C, Fu F, Li X, Zhang S. Mesenchymal stem cells maintain the microenvironment of central nervous system by regulating the polarization of macrophages/microglia after traumatic brain injury. Int J Neurosci. 2017;127(12):1124-35
32. An X, Sun X, Hou Y, et al. Protective effect of oxytocin on LPS-induced acute lung injury in mice. Sci Rep. 2019;9(1):2836
33. Inoue T, Yamakage H, Tanaka M, et al. Oxytocin suppresses inflammatory responses induced by lipopolysaccharide through inhibition of the eIF-2-ATF4 pathway in mouse microglia. Cells. 2019;8(6):527
34. Mehdi SF, Pusapati S, Khenhrani RR, et al. Oxytocin and related peptide hormones: Candidate anti-inflammatory therapy in early stages of sepsis. Front Immunol. 2022;13:1-19
35. Yang H, Wu L, Deng H, et al. Anti-inflammatory protein TSG-6 secreted by bone marrow mesenchymal stem cells attenuates neuropathic pain by inhibiting the TLR2/MyD88/NF-κB signaling pathway in spinal microglia. J Neuroinflammation. 2020;17(1):1-21
36. Carter CS, Kingsbury MA. Oxytocin and oxygen: The evolution of a solution to the 'stress of life'. Philos Trans R Soc Lond B Biol Sci. 2022;377(1858):20210054
37. Beltran SR, Svoboda KKH, Kerns DG, et al. Anti-inflammatory protein tumor necrosis factor-α – stimulated protein 6 (TSG-6) promotes early gingival wound healing: An in vivo study. J Periodontol. 2015;86(1):62-71
38. Mahoney DJ, Swales C, Athanasou NA, et al. TSG-6 inhibits osteoclast activity via an autocrine mechanism and is functionally synergistic with osteoprotegerin. Arthritis Rheum. 2011;63(4):1034-43
39. Liu L, Yu Y, Hou Y, et al. Human umbilical cord mesenchymal stem cells transplantation promotes cutaneous wound healing of severe burned rats. PLoS One. 2014;9(2):e88348
40. Kim H, Darwish I, Monroy MF, et al. Mesenchymal stromal (stem) cells suppress pro-inflammatory cytokine production but fail to improve survival in experimental staphylococcal toxic shock syndrome. BMC Immunol. 2014;15(1):1
41. Arreola-Ramírez JL, Vargas MH, Carbajal V, et al. Mesenchymal stem cells attenuate the proinflammatory cytokine pattern in a guinea pig model of chronic cigarette smoke exposure. Cytokine. 2023;162:156104
42. Mirhafez SR, Mohebati M, Disfani MF, et al. An imbalance in serum concentrations of inflammatory and anti-inflammatory cytokines in hypertension. J Am Soc Hypertens. 2014;8(9):614-23
43. Deng W, Chen H, Su H, et al. IL6 receptor facilitates adipogenesis differentiation of human mesenchymal stem cells through activating P38 pathway. Int J Stem Cells. 2020;13(1):142-50
44. Catarino C, Santos-Silva A, Belo L, et al. Inflammatory disturbances in preeclampsia: Relationship between maternal and umbilical cord blood. J Pregnancy. 2012;2012:684384
45. Green EA, Garrick SP, Peterson B, et al. The role of the interleukin-1 family in complications of prematurity. Int J Mol Sci. 2023;24(3):2795
46. Trucco F, Carruthers E, Davies JC, et al. Inflammation in children with neuromuscular disorders and sleep disordered breathing. Sleep Med. 2020;72:118-21
47. Kang S, Tanaka T, Kishimoto T. Therapeutic uses of anti-interleukin-6 receptor antibody. Int Immunol. 2015;27(1):21-29
48. Żak A, Lachert E, Antoniewicz-Papis J, et al. [Leukodepleted red blood cell concentrates frozen and thawed in ACP 215 system; Evaluation study.] J Transf Med. 2011;4(1):32-44 [in Polish]
49. Watkins VY, Frolova AI, Stout MJ, et al. The relationship between maternal anemia and umbilical cord oxygen content at delivery. Am J Obstet Gynecol MFM. 2021;3(1):100270
50. Prockop DJ, Oh JY. Mesenchymal stem/stromal cells (MSCs): Role as guardians of inflammation. Mol Ther. 2012;20(1):14-20

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