The Role of Extracellular Vesicle- and Tunneling Nanotube-Mediated Intercellular Cross-Talk Between Mesenchymal Stem Cells and Human Peripheral T Cells

Summary of the PhD thesis

Zsolt Matula

Semmelweis University

Pathological Sciences Doctoral School

Supervisor: Dr. Veronika Urbán, Ph.D., college professor
Dr. Péter Tátrai, Ph.D., senior researcher

Official opponents: Dr. György Nagy, DSc, associate professor
Dr. Kinga Molnár, Ph.D, senior lecturer

Head of the PhD Examination Committee:
Dr. Károly Nagy, Ph.D., professor

Members of the Doctoral Examination Committee:
Dr. Sára Tóth, Ph.D., associate professor
Dr. Lajos László, Ph.D., associate professor

Budapest
2017

Introduction

Mesenchymal stem cells (MSCs) are tissue-derived stem or predecessor cells that are already partially committed to the developmental pathway of a particular tissue type, so they can produce only certain cell types. As tissue stem cells they are obviously capable of self-renewal through asymmetric cell divisions, but in addition to creating specialized daughter cells for specified functions, they are also able to produce undifferentiated progeny or even proper stem cells.

Mesenchymal stem cells are primarily capable of producing cell types typical to mesodermal tissues; thus, they readily differentiate into bone, cartilage, fat, smooth muscle, and tendon [1]. According to some literature sources, MSCs can be differentiated into ectodermal and endodermal tissues as well under special culturing conditions, although this ability, commonly referred to as unorthodox plasticity, is highly controversial [2]. It is widely accepted that MSCs can be isolated from almost any kind of human tissue and organ [3]. Beside the bone marrow, MSCs can be isolated easily from adipose tissue, the Wharton jelly of the umbilical cord, but also from the thymus, liver, lung, kidney, spleen, muscle, and many other tissues including the aorta/vena cava wall or the brain [4,5].

MSCs are also notable for their prominent immunosuppressive and anti-inflammatory potential. This property of MSCs is highly relevant to therapy [6], and can be exploited to treat severe disorders characterised by dysregulated and increased immune response such as graft-versus-host disease, Crohn's disease, multiple sclerosis, osteoarthritis, diabetes mellitus type I, or systemic lupus erythematosus [7-10]. MSCs have a strong inhibitory effect on the function of all cell types of the natural and adaptive immune system [11-13].

With particular relevance to my field of research, MSCs have a very strong inhibitory effect on T cell functions. MSCs reduce the proliferation of activated T cells, promote regulatory T cell formation, and change the cytokine profile of different T cell subsets [14,15]. While much is known about the inhibitory action of cytokines and soluble factors produced by MSCs, and the strong immunosuppressive activity MSCs exert in co-cultures, partly due to direct cell-cell contact, is well characterised, the role of MSC-derived extracellular vesicles (EVs) in the functional inhibition of T lymphocytes is still poorly understood. Until now, only a few studies have examined the immunomodulatory effect of MSCs on T lymphocyte function, and the results are rather controversial with regard to whether the efficacy of EVs in *in vitro* experiments approximates that of the direct presence of MSCs [16-19].

Recently, the role of EVs in the directed transfer of membrane proteins and cytoplasmic components between different cell types has been extensively investigated, and this field has grown into a prominent scientific research area [20,21]. EVs play a very important role in the complex information transfer between different cell types, since they are capable of targeting a variety of compounds and proteins at high concentrations and also provide protection for a variety of molecules – e.g. ribonucleic acids such as miRNAs, mRNAs – against degradation [22]. EVs also play a pivotal role in the support and spatial extension of the donor cell function, such as the delivery of antigen presenting complex (MHCII-antigen complex) or the promotion of tumor cell proliferation.

Of all EV varieties, microvesicles (MVs) and exosomes (EXOs) have the greatest scientific interest. These two EV subpopulations are classified based on their size, but the mechanism of vesicle formation is completely different as well. Exosomes measure in the range of 50–100 nm and are generated by the spontaneous or stimulus-evoked exocytosis of multivesicular bodies [23,24]. In contrast, microvesicles are in the range of 100–1,000 nm and are released directly from the plasma membrane [25]. Both exosomes and microvesicles carry on their surface a plethora of membrane-associated proteins – including many membrane receptors –, but they contain only few nuclear proteins. Similarly, both EV subtypes carry a multitude of miRNAs and mRNAs in addition to non-coding RNA molecules, and coding RNA molecules have been shown to be translated in the recipient cells following incorporation of the EVs. The cargo of EVs is also responsible for the post-transcriptional regulation of miRNAs, and it is involved in epigenetic changes as well. Furthermore, exosomes play an important role in antigen presentation by delivering MHCII-peptide complexes, and these particles are also involved in non-classical protein secretion pathways, such as leaderless secretion whereby the exported protein has no N-terminal signal sequences [26,27].

During my own research I have found that the plasticity of MSCs becomes radically reduced within a short period of time, until senescence finally ensues. This is a common and significant practical problem; therefore, it is a general expectation in *in vitro* experimental work that, as much as possible, MSCs of low passage number should be used. For experiments of extended duration that require standardization and optimization, such as those utilizing MSC-derived extracellular vesicles, an immortalized mesenchymal stem cell line can be of huge benefit, of course only as long as these cells possess those properties of primary cells that are relevant to the experimental work. While it would be imperative to validate any result obtained with such immortalized cells on primary cultures, the use of immortalized cell lines would assuredly facilitate the work, and the risk of continued use of precious primary

cultures could be eliminated. In addition to the phenomenon of replicative senescence, high heterogeneity of primary cultures is another significant problem. Reproducibility of *in vitro* works using primary MSCs is highly dubious since experiments usually use primary cells originated from different donors, and these cells have a very different life span. The situation is further aggravated, among others, by the fact that bone marrow-derived MSC cultures isolated from healthy donors show remarkable heterogeneity in growth and differentiation capability, but this heterogeneity can not be related to the age or gender of donors [28]. The problem of heterogeneity could also be eliminated by creating immortal MSC lines from primary MSC cultures.

Aims

1. Our primary aim was to investigate the transport processes between T lymphocytes and adipose tissue-derived mesenchymal stem cells, in which the transport processes of membrane and cytoplasmic components were also monitored. We investigated the role of extracellular vesicle populations, microvesicles and exosomes, and compared the results with those seen in co-cultures. The cell and EV membrane was labeled with PKH67 fluorescence membrane labeling dye and the transport of cytoplasm was monitored by calcein assay.

2. In addition to the human T-cells, the efficacy of membrane and cytoplasmic calcein transfer was also tested between mouse thymocytes, human Jurkat lymphoma cells, and adipose tissue-derived mesenchymal stem cells. AD-MSCs derived from C57Bl6 mice were utilized as well; thus, beside the allogeneic models, we have also tested xenogeneic experimental models.

3. To clarify the precise mechanism of transferring cytoplasmic components, we have also examined the significance of microvesicles and direct cell-cell contacts. The transfer of fluorescent calcein between T lymphocytes and human MSCs was assessed by confocal microscopic observation of co-cultures where the membrane was labeled DiI and the cytoplasm was labeled with calcein.

4. The immunosuppressive activity of mesenchymal stem cells on T lymphocytes was investigated after receptor-specific or mitogen activation of T cells. In addition to the

inhibition of T cell proliferation, for which two different assays were used (resazurin and CFSE cell proliferation assay), the inhibition of interferon gamma production of cytotoxic and helper T cells was also studied. The dose-dependent effect of the human AD-MSC-derived microvesicles or exosomes on T cells was investigated separately. The effect of the MSC-conditioned medium (MSC-CM) depleted of extracellular vesicles was tested in serial dilutions. Finally, the inhibition of T cell proliferation and IFN-γ production was determined in co-cultures. In these experiments, an allogeneic experimental model was used, and in co-culture experiments different cell ratios were tested. The inhibition of cell proliferation was also studied using Jurkat cells by examining the functional effect of MSCs in co-cultures, MSC-derived MVs or EXOs *per se*, and MSC-CM.

5. In another line of work, we also aimed to immortalize Ad-MSCs from human donors using a lentiviral gene delivery system. Different immortal cell lines were created by delivering different genes or gene combinations: hTERT, Bmi-1, hTERT + Bmi-1, hTERT + SV40T. Our further aim was to investigate whether these cell lines retained their multipotent differentiation ability and maintained their genomic stability during long-term cultivation while continuing to meet the defining criteria of mesenchymal stem cells.

Methods

- Microvesicle and exosome preparations were extracted from the cell culture supernatants by the combination of differential centrifugation and gravitational filtration.

- The size distribution and concentration of extracellular vesicles were determined using the Dynamic Light Scattering Analysis (DLS) and the Izon qNano device based on the resistive pulse sensing phenomenon.

- Morphological studies of extracellular vesicle subsets were performed using transmission electron microscopy (TEM).

- Surface antigen pattern of the cells and extracellular vesicles was determined by flow cytometry after antibody labeling.

- To study the transfer of membrane vesicles or cytoplasmic materials, PKH67 and DiI fluorescence membrane labeling dyes or calcein assay were applied. The efficiency of the transfer was determined by flow cytometric measurements, but we also examined the interactions in co-cultures by using confocal laser scanning microscopy.

- To investigate the immunosuppressive effects of mesenchymal stem cells, MSC-derived extracellular vesicles and MSC-conditioned medium, resazurin cell proliferation assay, carboxyfluorescein succinimidyl ester cell proliferation assay (CFSE), and intracellular interferon gamma (IFN-γ) immunoassay were used.

- The functional effect of activated T cell-derived extracellular vesicles on mesenchymal stem cells was demonstrated by prostaglandin E2 immunoassay.

- The immortalized mesenchymal stem cell lines were established using a lentiviral gene delivery system.

- Quantitative real-time PCR (RT-qPCR) reactions were performed to determine the copy number of the provirus as well as to quantify gene expression.

- Effective expression of the different immortalizing genes was demonstrated by telomerase enzyme activity measurements (TRAPEZE XL Telomerase Detection Kit) as well as immunocytochemical staining of the generated gene products.

- For cellular proliferation and cellular senescence investigations, resazurin cell proliferation assay and senescence-associated β-galactosidase staining were used.

- Primary mesenchymal stem cells and successfully immortalized stem cell lines have also been tested for multilineage differentiation ability by using the following methods:
    - *Immunocytochemical studies*
    - *Detection of ALP activity by the addition of chromogenic substrate*
    - *Detection of extracellular calcium with Alizarin red staining*
    - *Detection of Intracellular lipid droplets with Oil Red O staining*

- To demonstrate and confirm the supposed transformation event, karyotype determination and *in vivo* tumor-formation assay were performed in NOD/SCID gamma mice. The

associated cell cycle assays were performed using flow cytometer after labeling the DNA content with propidium iodide.

Results

1. Neither murine thymocytes, nor human T lymphocytes or Jurkat cells are able to incorporate significant amounts of membrane components from the different EV subpopulations when AD-MSCs were used as the donor cells, regardless of whether allogeneic or xenogeneic experimental set-up was used. The same was observed in co-culture conditions where no membrane transfer was detected.

2. In line with these data, neither vesicle fraction had any effect in our functional tests when the immunosuppressive effects of AD-MSC-derived EVs were assessed. Neither MVs nor EXOs reduced the proliferation rate or the IFN-g production of the activated T lymphocytes, even when EVs were added in huge excess of the number of cells.

3. In contrast, AD-MSCs incorporated large amounts of membrane components independent of the source of origin. The results of the co-culture models were successfully reproduced by the addition of sufficient amounts of MV or EXO particles; hence we proved that extracellular vesicles are responsible for the delivery of the membrane components.

4. In our subsequent functional assay where MSCs were treated with MVs or EXOs derived from activated T cells, a robust increase in secreted prostaglandin E2 was observed in a dose-dependent manner. Thus, we have successfully demonstrated that T lymphocytes can directly influence the immunosuppressive activity of MSCs through extracellular vesicles.

5. We found that neither mouse thymocytes nor Jurkat lymphoma cells are able to incorporate MSC-derived cytoplasmic calcein, but a very intensive bidirectional cytoplasmic transport process exists between MSCs and T cells. In order to transmit the cytoplasmic calcein for the acceptor cells, it is essential to establish close cell-cell interactions between MSCs and T cells, and the role of EVs cannot be demonstrated in this process. In our investigations we have proved the pivotal role of a so far unknown

interaction, where intensive transfer of cytoplasm between the two cell types occurs through tunneling nanotubes.

6. While the soluble mediators of AD-MSCs collected from healthy individuals have pronounced suppressive effect on both the proliferation rate and IFN-γ production of activated T cells, co-culturing of the two cell types is needed to achieve maximal suppression of the T cell function. Accordingly, the intensive cytoplasmic transport process between the MSCs and T cells mediated by the tunneling nanotubes has been shown to have a determining effect on the function of T cells, and it is likely to influence the phenotype of MSCs as well.

7. In our experiments aimed to immortalize the MSCs, the transfer of the Bmi-1 gene alone has been shown to significantly prolong the population's lifespan; however, by the 60th population doubling (PD) the entire population showed senescence. The transfer of the hTERT gene in itself resulted in transformation of certain cells, which later gained a significant selection advantage (after PD83).

8. With the combined introduction of hTERT and Bmi-1 genes, we succeeded in establishing a stable immortal AD-MSC cell line whose basic properties, including differentiation potential, did not change compared to the primary cells. Also, in the case of this cell line no transformation event was found.

Conclusions

Despite recent efforts to elucidate the physiological mechanisms responsible for the suppressive effect of MSCs on T cell function [29,30], data on contact-dependent or membrane mediated signaling between these two cell types are incomplete. The latest findings confirmed the role of membrane-transported bioactive molecules in modulating the immune response, and these molecules have been shown to be carried by EVs [31-33].

According to our results, neither murine thymocytes, which are mostly immature T cells, nor human T lymphocytes or Jurkat cells are able to incorporate considerable amounts of membrane components from the different EV subpopulations when AD-MSCs are used as the donor cells, irrespective of whether allogeneic or xenogeneic experimental setup is used. In line with these data, neither vesicle fraction had any effect in our functional tests when the

immunosuppressive effects of AD-MSC-derived EVs were assessed. Neither MVs nor EXOs reduced the proliferation rate or the IFN-γ production of the activated T lymphocytes. Interestingly, using a reverse experimental setup, AD-MSCs incorporated large amounts of membrane components independent of the source of origin, and no significant differences could be observed between the allogeneic and xenogeneic models. In our subsequent functional assay where MSCs were treated with various doses of EVs derived from activated T cells, a robust increase of secreted prostaglandin E2 was observed in a dose-dependent manner upon both MV and EXO treatment.

On assessing the potential transfer of cytoplasmic components between T cells and MSCs, we found that neither mouse thymocytes nor Jurkat cells incorporated calcein from AD-MSC donors; only human peripheral T lymphocytes were able to contact the cytoplasm of MSCs and this cytoplasmic material transfer was bidirectional. Still, a significant difference could be observed between the allogeneic and xenogeneic models, suggesting an active recognition of a cell surface component on MSCs by human T lymphocytes. Close cell–cell contacts are required for a significant cytoplasmic material transfer between AD-MSCs and T cells, and the role of extracellular vesicles cannot be demonstrated in this process. To further investigate the mechanism responsible for the calcein transfer between MSCs and T lymphocytes, co-cultures were analyzed with confocal laser scanning microscopy. Plenty of tunneling nanotubes could be observed between these cells, which were originated from the human T cells. Interestingly, nanotubes are able to form between Jurkat cells, too; however, we could not observe these structures between human MSCs and Jurkat lymphoblastoid cells.

In summary, based on our results we can conclude that an active communication exists between MSCs and T cells through T cell-dependent mechanisms, which include vesicular and tunneling nanotube-mediated transfer of T cell components to MSCs. Receiving microvesicles, exosomes and cytoplasmic components originated from T cells profoundly alters the immunomodulatory activity of MSCs, shifting them toward a more suppressive state and inducing the production of soluble factors such as prostaglandin E2. Our results also show that nanotube-mediated transfer of cytoplasmic components is bidirectional between these cells; therefore, the MSC-derived cytoplasmic components may modulate the function of T cells as well.

In the second major part of our work, human primary AD-MSCs were immortalized using a lentiviral gene delivery system. Both crucial problems that usually occur when primary mesenchymal stem cells are applied, namely, high heterogeneity of samples and replicative senescence, can be eliminated by the derivation of immortal cell lines from primary MSC

cultures. The hTERT gene was introduced in combination with the Bmi-1 and SV40T genes via lentiviral transduction. We also tested whether the introduction of hTERT or Bmi-1 genes alone is capable of immortalizing the AD-MSCs, or the immortalization can only successfully accomplished by coexpression of these genes.

In our experience, the Bmi-1-only population was unable to evade senescence and eventually ceased to proliferate at PD 60, although the increase of mean proviral copy number over time suggested that clones overexpressing Bmi-1 might have enjoyed selective advantage.

When introducing the hTERT gene alone, we noted that the unlimited replication capability maintained by hTERT provides a fertile ground for obtaining potentially transforming mutations. After PD 83, one or mores clone in the population underwent transformation and gained a selective advantage. The resulting clonal cell line proliferated much more intensely when compared to the original population, did not exhibit senescence-associated β-galactosidase staining, and showed complete lack of contact inhibition.

However, with the combined introduction of hTERT and Bmi-1 genes, we succeeded in creating an immortal AD-MSC cell line that did not show any transformation events and whose differentiation potential did not change compared to the primary cells.

References

1. Pittenger MF, AM Mackay, SC Beck, RK Jaiswal, R Douglas, JD Mosca, MA Moorman, DW Simonetti, S Craig and DR Marshak. (1999). Multilineage potential of adult human mesenchymal stem cells. Science 284:143-147.
2. Caplan AI and SP Bruder. (2001). Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. Trends in Molecular Medicine 7:259-264.
3. Abu Kasim NH, V Govindasamy, N Gnanasegaran, S Musa, PJ Pradeep, TC Srijaya and ZACA Aziz. (2012). Unique molecular signatures influencing the biological function and fate of post-natal stem cells isolated from different sources. Journal of Tissue Engineering and Regenerative Medicine 9:E252–E266.
4. Meirelles LDS, PC Chagastelles and NB Nardi. (2006). Mesenchymal stem cells reside in virtually all post-natal organs and tissues. Journal of Cell Science 119:2204-2213.

5. Hass R, C Kasper, S Bohm and R Jacobs. (2011). Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Communication and Signaling 9:12.
6. Wang Y, X Chen, W Cao and Y Shi. (2014). Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nature Immunology 15:1009-1016.
7. Sun LY, DD Wang, J Liang, HY Zhang, XB Feng, H Wang, BZ Hua, BJ Liu, SQ Ye, XA Hu, WR Xu, XF Zeng, YY Hou, GS Gilkeson, RM Silver, LW Lu and ST Shi. (2010). Umbilical Cord Mesenchymal Stem Cell Transplantation in Severe and Refractory Systemic Lupus Erythematosus. Arthritis and Rheumatism 62:2467-2475.
8. Gao F, SM Chiu, DA Motan, Z Zhang, L Chen, HL Ji, HF Tse, QL Fu and Q Lian. (2016). Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis 7:e2062.
9. Bai L, DP Lennon, AI Caplan, A DeChant, J Hecker, J Kranso, A Zaremba and RH Miller. (2012). Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nature Neuroscience 15:862-U86.
10. Gordon D, G Pavlovska, JB Uney, DC Wraith and NJ Scolding. (2010). Human Mesenchymal Stem Cells Infiltrate the Spinal Cord, Reduce Demyelination, and Localize to White Matter Lesions in Experimental Autoimmune Encephalomyelitis. Journal of Neuropathology and Experimental Neurology 69:1087-1095.
11. Gebler A, O Zabel and B Seliger. (2012). The immunomodulatory capacity of mesenchymal stem cells. Trends in Molecular Medicine 18:128-134.
12. Najar M, G Raicevic, H Fayyad-Kazan, D Bron, M Toungouz and L Lagneaux. (2016). Mesenchymal stromal cells and immunomodulation: A gathering of regulatory immune cells. Cytotherapy 18:160-171.
13. Tyndall A. (2014). Mesenchymal stem cell treatments in rheumatology-a glass half full? Nature Reviews Rheumatology 10:117-124.
14. Castro-Manrreza ME and JJ Montesinos. (2015). Immunoregulation by Mesenchymal Stem Cells: Biological Aspects and Clinical Applications. Journal of Immunology Research 2015:394917.
15. Aggarwal S and MF Pittenger. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815-1822.
16. Blazquez R, FM Sanchez-Margallol, O de la Rose, W Dalemans, V Alvarez, R Tarazone and JG Casadol. (2014). Immunomodulatory potential of human adipose

mesenchymal stem cells derived exosomes on in vitro stimulated T cells. Frontiers in Immunology 5:556.

17. Conforti A, M Scarsella, N Starc, E Giorda, S Biagini, A Proia, R Carsetti, F Locatelli and ME Bernardo. (2014). Microvescicles Derived from Mesenchymal Stromal Cells Are Not as Effective as Their Cellular Counterpart in the Ability to Modulate Immune Responses In Vitro. Stem Cells and Development 23:2591-2599.
18. Zhang B, Y Yin, RC Lai, SS Tan, ABH Choo and SK Lim. (2014). Mesenchymal Stem Cells Secrete Immunologically Active Exosomes. Stem Cells and Development 23:1233-1244.
19. Di Trapani M, G Bassi, M Midolo, A Gatti, PT Kamga, A Cassaro, R Carusone, A Adamo and M Krampera. (2016). Differential and transferable modulatory effects of mesenchymal stromal cell-derived extracellular vesicles on T, B and NK cell functions. Scientific Reports 6.
20. Simons M and G Raposo. (2009). Exosomes--vesicular carriers for intercellular communication. Curr Opin Cell Biol 21:575-81.
21. Greening DW, SK Gopal, R Xu, RJ Simpson and W Chen. (2015). Exosomes and their roles in immune regulation and cancer. Semin Cell Dev Biol 40:72-81.
22. Raposo G and W Stoorvogel. (2013). Extracellular vesicles: Exosomes, microvesicles, and friends. Journal of Cell Biology 200:373-383.
23. Blanchard N, D Lankar, F Faure, A Regnault, C Dumont, G Raposo and C Hivroz. (2002). TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. Journal of Immunology 168:3235-3241.
24. Wahlgren J, TDL Karlson, P Glader, E Telemo and H Valadi. (2012). Activated Human T Cells Secrete Exosomes That Participate in IL-2 Mediated Immune Response Signaling. Plos One 7:e49723.
25. Osteikoetxea X, A Balogh, K Szabo-Taylor, A Nemeth, TG Szabo, K Paloczi, B Sodar, A Kittel, B Gyoergy, E Pallinger, J Matko and EI Buzas. (2015). Improved Characterization of EV Preparations Based on Protein to Lipid Ratio and Lipid Properties. Plos One 10: UNSP e0121184
26. Yanez-Mo M, PRM Siljander, Z Andreu, AB Zavec, FE Borras, EI Buzas, K Buzas, E Casal, F Cappello, J Carvalho, E Colas, A Cordeiro-da Silva, S Fais, JM Falcon-Perez, IM Ghobrial, B Giebel, M Gimona, M Graner, I Gursel, M Gursel, NHH Heegaard, A Hendrix, P Kierulf, K Kokubun, M Kosanovic, V Kralj-Iglic, EM Kramer-Albers, S Laitinen, C Lasser, T Lener, E Ligeti, A Line, G Lipps, A Llorente, J Lotvall, M

Mancek-Keber, A Marcilla, M Mittelbrunn, I Nazarenko, ENM Nolte-t' Hoen, TA Nyman, L O'Driscoll, M Olivan, C Oliveira, E Pallinger, HA del Portillo, J Reventos, M Rigau, E Rohde, M Sammar, F Sanchez-Madrid, N Santarem, K Schallmoser, MS Ostenfeld, W Stoorvogel, R Stukelj, SG Van der Grein, MH Vasconcelos, MHM Wauben and O De Wever. (2015). Biological properties of extracellular vesicles and their physiological functions. Journal of Extracellular Vesicles 4: 27066

27. El Andaloussi S, I Maeger, XO Breakefield and MJA Wood. (2013). Extracellular vesicles: biology and emerging therapeutic opportunities. Nature Reviews Drug Discovery 12:348-358.
28. Phinney DG, G Kopen, W Righter, S Webster, N Tremain and DJ Prockop. (1999). Donor variation in the growth properties and osteogenic potential of human marrow stromal cells. Journal of Cellular Biochemistry 75:424-436.
29. Ma S, N Xie, W Li, B Yuan, Y Shi and Y Wang. (2014). Immunobiology of mesenchymal stem cells. Cell Death and Differentiation 21:216-225.
30. Bernardo ME and WE Fibbe. (2013). Mesenchymal Stromal Cells: Sensors and Switchers of Inflammation. Cell Stem Cell 13:392-402.
31. de Candia P, V De Rosa, M Casiraghi and G Matarese. (2016). Extracellular RNAs: a secret arm of immune system regulation. J Biol Chem 291:7221-7228.
32. Gyorgy B, TG Szabo, M Pasztoi, Z Pal, P Misjak, B Aradi, V Laszlo, E Pallinger, E Pap, A Kittel, G Nagy, A Falus and EI Buzas. (2011). Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cellular and Molecular Life Sciences 68:2667-2688.
33. Katsuda T, N Kosaka, F Takeshita and T Ochiya. (2013). The therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. Proteomics 13:1637-1653.

Publications connected to the PhD thesis

Zsolt Matula, Andrea Németh, Péter Lőrincz, Áron Szepesi, Anna Brózik, Edit Irén Buzás, Péter Lőw, Katalin Német, Ferenc Uher, Veronika S. Urbán: *The Role of Extracellular Vesicle and Tunneling Nanotube-Mediated Intercellular Cross-Talk Between Mesenchymal Stem Cells and Human Peripheral T Cells.* Stem Cells and Development; 25:1818-1832 (2016) **(IF: 3,562)**

Péter Tátrai, Áron Szepesi, **Zsolt Matula**, Anna Szigeti, Gyöngyi Buchan, András Mádi, Ferenc Uher, Katalin Német: *Combined introduction of Bmi-1 and hTERT immortalizes human adipose tissue-derived stromal cells with low risk of transformation.* Biochemical and Biophysical Research Communications; 422:28-35 (2012) **(IF: 2,406)**

Áron Szepesi, **Zsolt Matula**, Anna Szigeti, György Várady, József Szalma, Gyula Szabó, Ferenc Uher, Balázs Sarkadi, Katalin Német: *In Vitro Characterization of Human Mesenchymal Stem Cells Isolated from Different Tissues with a Potential to Promote Complex Bone Regeneration.* Stem Cells International; 2016:3595941 (2016) **(IF: 3,54)**

Other publications

Dóra Mihály, **Zsolt Matula**, Yi-Che Changchien, Gergő Papp, Péter Tátrai, Zoltán Sápi
First cloned human immortalized adipose derived mesenchymal stem-cell line with chimeric SS18-SSX1 gene (SS-iASC).Cancer Genetics; 216-217: pp. 52-60. (2017) **(IF: 1,93)**

Zsuzsanna Nerada, Zoltán Hegyi, Áron Szepesi, Szilárd Tóth, Csilla Hegedüs, György Várady, **Zsolt Matula**, László Homolya, Balázs Sarkadi, Ágnes Telbisz: *Application of fluorescent dye substrates for functional characterization of ABC multidrug transporters at a single cell level.* Cytometry Part A; 89:826-34 (2016) **(IF: 3,222)**

Zsolt Matula, Gyöngyi Kudlik, Veronika S. Urbán, Ferenc Uher: Quo vadis hematology? Orvosi Hetilap; 157:1819-1829 (2016) **(IF:0,349)**

Gyöngyi Kudlik, **Zsolt Matula**, Tamás Kovács, Veronika S. Urbán, Ferenc Uher: *At the border of pluri- and multipotency: the neural crest stem cells.* Orvosi Hetilap; 156:1683-94 (2015) **(IF:0,291)**

Áron Szepesi, **Zsolt Matula**, Anna Szigeti, György Várady, Gyula Szabó, Ferenc Uher, Balázs Sarkadi, Katalin Német: *ABCG2 is a selectable marker for enhanced multilineage differentiation potential in periodontal ligament stem cells.* Stem Cells and Development; 24:244-52 (2014) **(IF: 3,777)**

Veronika Kovács- Haász, Bettina Dulka, Zita Pöstényi, András Polyák, **Zsolt Matula**, Anna Szigeti, Ana Ivanovska, Julianna Thuróczy, Ferenc Uher, Katalin Német, Lajos Balogh: *A mesenchymalis őssejtek felhasználásának lehetőségei az állatorvosi kutatásokban és gyógyításban 1. rész: Irodalmi áttekintés.* Magyar Állatorvosok Lapja; 138:(6) pp. 349-360. (2016) **(IF: 1,89)**

Veronika Kovács- Haász, Bettina Dulka, Zita Pöstényi, András Polyák, **Zsolt Matula**, Anna Szigeti, Ana Ivanovska, Julianna Thuróczy, Ferenc Uher, Katalin Német, Lajos Balogh: *A mesenchymalis őssejtek felhasználásának lehetőségei az állatorvosi kutatásokban és gyógyításban 2. rész: Saját vizsgálatok.*Magyar Állatorvosok Lapja; 138:(7) pp. 401-412. (2016) **(IF: 1,89)**

Gyöngyi Kudlik, **Zsolt Matula**, Veronika S Urbán, Ferenc Uher: A makrofágpolarizáció szerepe a sebgyógyulásban és a szövetregenerációban. Immunológiai szemle; 7:(1) pp. 27-37. (2015) **(IF: 0)**