MESENCHYMAL STEM CELLS IMMUNE PROPERTIES AS SENSORS OF MICROENVIRONMENT

TIDU Federico[1], JOSE S. Shyam[1], FRIČ Jan[1]

International Clinical Research Center (ICRC), St. Anne's University Hospital Brno

Abstract

Tissue inflammation leads to the recruitment and activation of several cell types, which cooperate to create a particular microenvironment that determines the regression of the pathology or its perpetuation (chronic inflammation). Mesenchymal stem cells (MSCs) are multipotent cells present in the stromal fraction of many tissues (i.e umbilical cord blood, placenta, adipose tissue). Several studies are now showing MSCs potential in tissue engineering, especially because of their ability to differentiate onto nanostructure surface. However the effect of nanostructure on MSCs immune properties is not properly characterized. The aim of the project is to develop MSCs reporter lines for transcription factors associated to inflammation, in order to monitor their activity once the cells are seeded onto nanostructure. This will be helpful to understand, which materials are more suitable in term of tissue regeneration.

Keywords: Nanostructure, mesenchymal stem cells, immune properties and reporter lines

1. INTRODUCTION

Mesenchymal stem cells (MSCs) are essential cells for maintenance of tissue homeostasis, able to differentiate into the three mesodermal lineages: adipocytes, chondrocytes and osteoblasts. Recent studies have shown their capacity to migrate and home into damaged tissue where they play important role in immunologic and tissue regenerative processes [1]. So far most of the studies focused on the effects of nanostructures and biopolymers on MSCs differentiation potential; however a clear insight on MSCs immune properties when seeded onto the nanostructures has been overlooked, even though is crucial to understand their beneficial effects and estimate the risks of the tissue engineering approach [2].

The role of mesenchymal stem cells (MSCs) in inflammation is driven through cytokines and more importantly through direct sensing of microenvironment by Toll-like Receptors (TLRs). TLR signaling cascade can be activated by pathogen associated molecular patterns (PAMPs) or endogenous molecules associated to damage (DAMPs). Majority of TLR triggers leads to NF-κB nuclear translocation and transcription of pro-inflammatory cytokines, influencing the crosstalk with immune cells, regulating inflammatory processes and overall the immune response.

MSCs can be polarized into two different phenotypes, pro-inflammatory (MSC1) or anti-inflammatory (MSC2) phenotype, depending on the type and concentration of stimuli to which they are exposed [3]. MSCs have a high self-renewal capability and expansive potential *ex vivo*. They are present in large numbers in adults, and are also relatively easy to isolate and culture features, which make them suitable for tissue regeneration and cell-based therapy. Because mesenchymal stem cells of multiple adult vertebrate species originate from extra embryonic mesoderm, their capacity to differentiate into adipogenic, chondrogenic, and osteogenic lineages as well as into myogenic and fibroblastic lineages has been extensively studied [4]

The aim of the project is to establish MSCs reporter line for different transcription factors associated to inflammation and use them to investigate whether different nanostructure can lead to MSCs pro-inflammatory phenotype.

2. RESULTS

To investigate MSCs sensing of extracellular environment, we performed analysis of two transcription factors associated to inflammation: NF-kB and NFAT. Our data (**Figure 1**) revealed that TLR2 stimulation with zymosan leads to NFAT nuclear internalization (active form), as shown in the colocalization profile. On the other NF-kB nuclear localization is higher when cells are treated with TLR4 activator, as we demonstrated with ELISA assay on nuclear extracts of mesenchymal stem cells. Altogether these findings show the importance of NF-kB and NFAT in MSCs sensing of microenvironment.

To further describe signaling during inflammation and effects of different nanostructures on MSCs immune properties, we developed MSCs reporter lines for NFAT and NF-kB using Cignal Lenti Reporter Kits.

Once developed NFAT luciferase stable reporter line were triggered cells with an activation cocktail (ionomycin + zymosan) and measure the activity of luciferase in cell lysates using a luminometer (**Figure 3**).

With the same procedure we established NF-kB reporter line and monitor the activation of the pathway both in cell lysates using luminometer (**Figure 4**) and in fixed cells stained with anti-firefly luciferase antibody using confocal microscopy (**Figure 5**). Luminescence assay and confocal imaging show that reporter lines established are suitable to investigate activation of inflammatory pathways in mesenchymal stem cells.

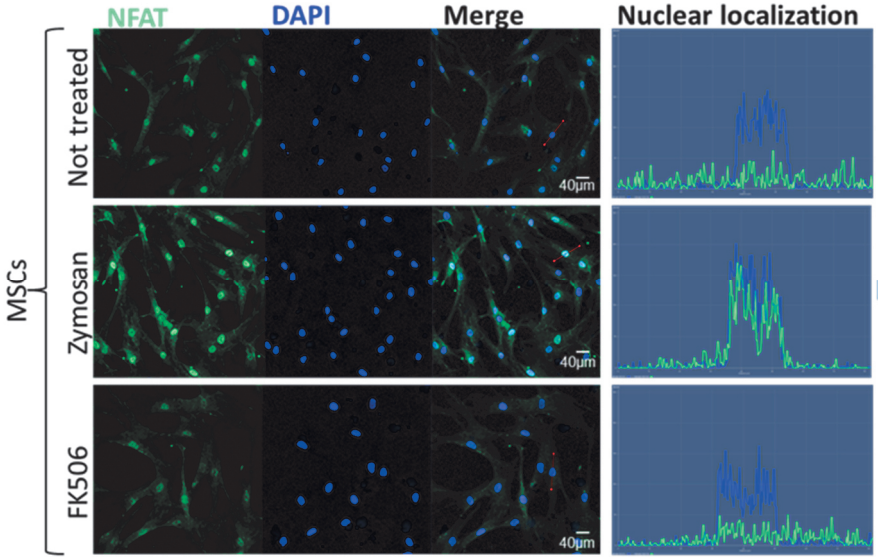

Figure 1 Comparison of NFAT expression and translocation in MSCs differently stimulated. Cultured MSCs were treated with TLR2 and Dectin-1 ligand (zymosan, 10ug/ml, 10 min), NFAT inhibitor (FK 506, 200 ng/mL, 30 min) fixed and stained with DAPI, phalloidin TRITC and NFATc1 monoclonal antibody (coupled to Alexa Fluor 488). Imaging was performed using Zeiss LSM 780 confocal microscope

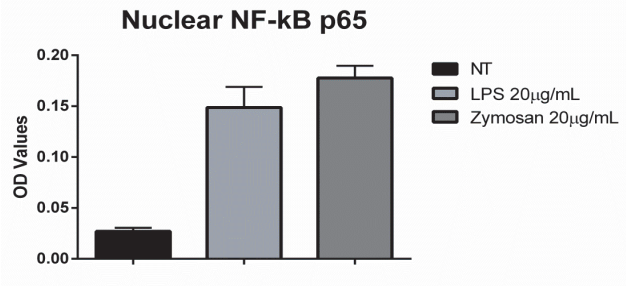

Figure 2 ELISA assay on MSCs nuclear extracts. Cultured MSCs were treated with TLR2 ligand (zymosan, 10ug/ml, 10 min) and TLR4 ligand (LPS, 10ug/ml, 10 min). Nuclear extracts were collected and used for ELISA assay

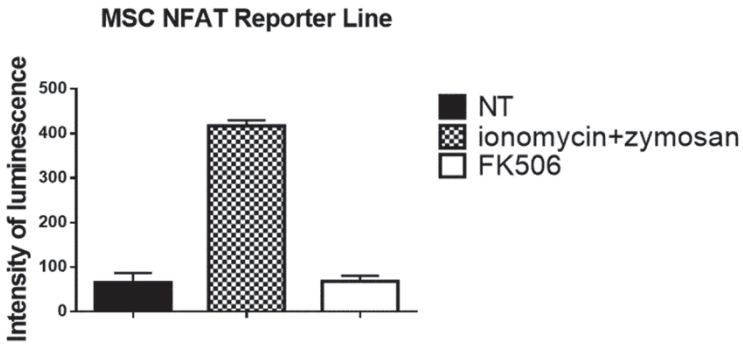

Figure 3 Cultured MSCs NFAT reporter were treated with NFAT stimulation cocktail (zymosan, 10ug/ml + ionomycin 1µg/ml, 6h) and inhibitory molecule (FK506, 0.2ug/ml, 6h). Firefly luciferase activity was measured using luminescence reader

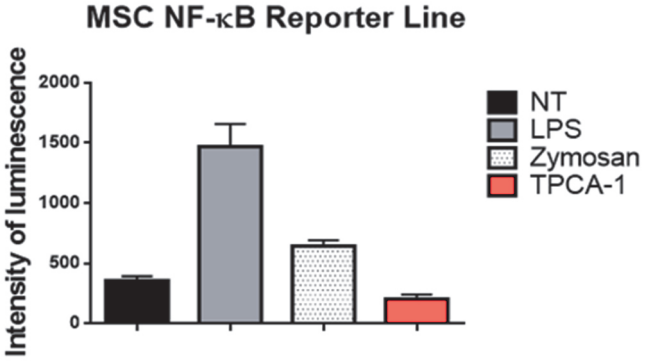

Figure 4 Cultured MSCs NF-κB reporter were treated with TLR2 ligand (zymosan, 10ug/ml, 6h), TLR4 ligand (LPS, 10ug/ml, 6h) and inhibitory molecule (TPCA-1, 0.1ug/ml, 6h). Firefly luciferase activity was measured using luminescence reader

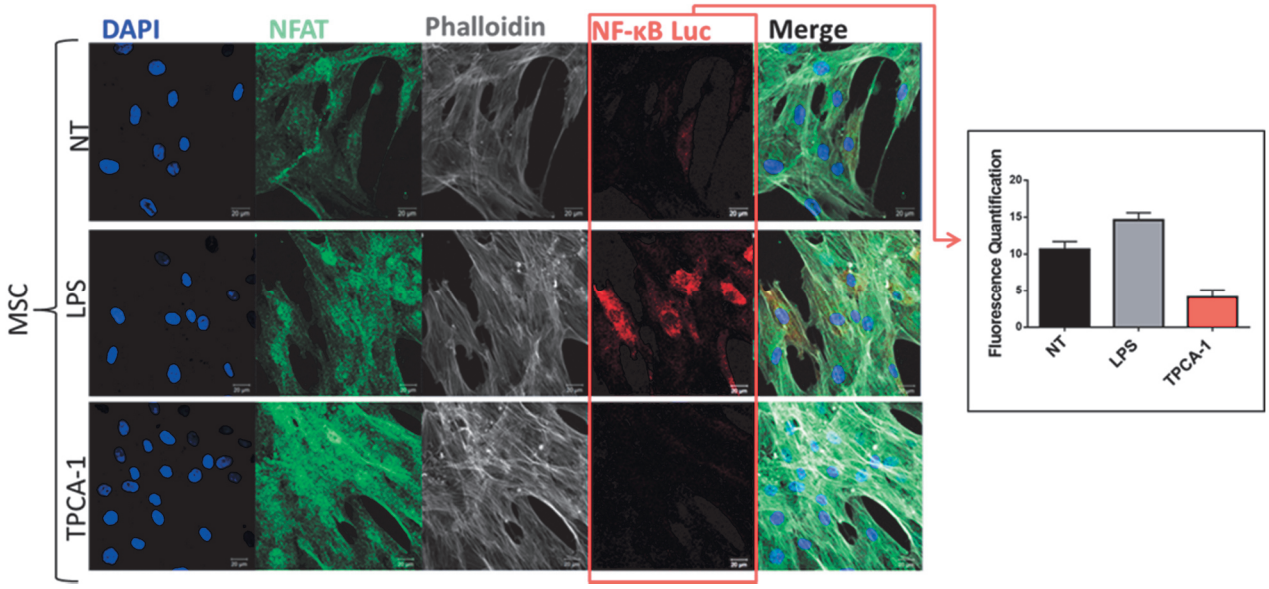

Figure 5 Confocal imaging of Ad-MSCs NF-κB reporter line. Cultured MSCs NF-κB reporter were treated with TLR2 ligand (zymosan, 10ug/ml, 6h), TLR4 ligand (LPS, 10ug/ml, 6h) and inhibitory molecule (TPCA-1, 0.1ug/ml, 6h), fixed and stained using NFAT cell signaling antibody and Anti firefly luciferase Chipgrade antibody. Quantification of the signal was measured using imagej software

3. METHODS

Immunofluorescence: Cells were fixed using PFA 4% (SantaCruz) for 20 minutes, wash three times with PBS and treated overnight using primary antibodies anti-NFAT2 from Cell Signaling, anti-firefly luciferase from Abcam. Cells were washed three times with PBS and stained using Phalloidin TRITC, AlexaFluor 488 and DAPI. Imaging was performed using Zeiss LSM 780 confocal microscope.

ELISA assay: the assay was performed using TransAM NF-κB Family kits from Active Motif and following the manufacturer instructions.

Establish of stable luciferase reporter lines: Cells were seeded in 24 wells plate with 600µl of DMEM without Pen/Strep per well. Cells were transfected adding 2µl of Surentry and 12uL of particles in the media. After 6h transfection media was discarded and complete DMEM was added. After 1 day cells were put in selection (puromycin 0.5µg/ml) in order to obtain a homogeneous population who integrated the vector.

Luciferase assay: Cells were lysed using one-glo from Promega (lysis and luciferase substrate). After 5 minutes the intensity of fluorescence was measured using luminometer

4. DISCUSSION AND COCLUSIONS

Understanding the effects of different materials and structures on MSCs biology will provide new tools to address effective therapeutic strategies [5]. For this reason we challenged MSCs with different TLR ligands [6] and we identify NF-κB and NFAT as two of the main transcription factors involved in MSCs immune response. We also established stable reporter lines for NF-κB and NFAT that allow us to monitor MSCs immune properties.

The aim of this work is shedding light on MSC role during inflammation. Developed reporter lines can be used to analyse the response of MSCs to different material and can be important tool to timely testing of nanostructure properties, which are used for MSCs cultures [4].

The reporter lines established can help to investigate the field and learn more about how MSCs change their immune properties when they are seeded onto different nanostructures.

ACKNOWLEDGEMENTS

Supported by the project no. LQ1605 from the National Program of Sustainability II (MEYS CR).

REFERENCES

[1] YAGI H., Mesenchymal Stem Cells: Mechanisms of Immunomodulation and Homing, *Cell Transplant*. 2010, vol. 19, no. 6, pp 667-79. doi: 10.3727/096368910X508762. Epub 2010.

[2] FU X., Improved osteogenesis and upregulated immunogenicity in human placenta-derived mesenchymal stem cells primed with osteogenic induction medium, *Stem Cell Res Ther.,* 2016, vol. 7, no. 1, pp. 138. doi: 10.1186/s13287-016-0400-6.

[3] WATERMAN et Al., A New Mesenchymal Stem Cell (MSC) Paradigm: Polarization into a Pro-Inflammatory MSC1 or an Immunosuppressive MSC2 Phenotype, *PLoS One*, 2010, vol. 26, no. 5(4), e10088. doi: 10.1371/journal.pone.0010088.

[4] ILIE I., Influence of nanomaterials on stem cell differentiation: designing an appropriate nanobiointerface, *Int J Nanomedicine.* 2012, vol. 7, pp 2211-25. doi: 10.2147/IJN.S29975. Epub 2012 Apr 27.

[5] LI-TZU WANG et Al., Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: review of current clinical trials, J Biomed Sci. 2016; doi: 10.1186/s12929-016-0289-5

[6] SANGIORGI P. et Al., Modulation of Immunoregulatory Properties of Mesenchymal Stromal Cells by Toll-Like Receptors: Potential Applications on GVHD, Stem Cells Int., 2016;2016:9434250. Epub 2016 Sep 21. doi:10.1155/2016/9434250