Title: **Role of mechanotransduction in regulation of immunomodulatory properties of human mesenchymal stem cells (WJ-MSC) and neural stem cells derived from induced pluripotent stem cells (hiPSC-NSC)**

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Abstract: Therapeutic functions of stem cells can result from their dual properties. On the one hand, supporting regeneration or therapy due to releasing of modulating factors, which change immunologic response in the injured tissue and supporting of endogenous regeneration, on the other hand, through replacement and reconstruction, i.e. structural and functional incorporation of stem cells into damaged tissue. Stem cells in vivo, in an endogenous environment, function in a three-dimensional flexible tissue structure, while in vitro the standard type of culture is two-dimensional culture - adhered to hard plastic or culture glass. The presented project sought to answer the question how the spatial conditions of the microenvironment and the physicochemical properties of the environment influence the production of immunomodulatory factors by stem cells and whether it depends on the type of stem cells. The aim of the project was to determine the role of mechanotransduction in this process by the localization and inhibition of YAP/TAZ proteins.

Materials&Methods: Two different human stem cell populations were investigated in this research: mesenchymal stem cells isolated from Wharton's jelly of umbilical cord (WJ-MSC) and neural stem cells derived from induced pluripotent stem cells (hiPSC-NSC). Both cell populations were cultured in standard 2D conditions and in 3D biomimetic conditions in the form of hydrogels – fibrin hydrogel for WJ-MSC and hydrogel made of basement membrane matrix (Geltrex) for hiPSC-NSC, in control groups and with addition of YAP/TAZ inhibitory protein – verteporfin. Cells were then fixed for immunostaining, cell culture supernatants were collected for secretome analysis.

Results: The localization of the YAP / TAZ complex in both populations of stem cells cultured under different spatial conditions revealed a very strong expression of this complex at the nuclear location in two-dimensional cultures. The presence of the YAP/TAZ inhibitor verteporfin resulted in a partial translocation of the YAP/TAZ complex into the cytoplasm of cells grown in 2D. Both WJ-MSC and hiPSC cells grown in hydrogels revealed mainly cytoplasmic location of the YAP / TAZ complex. The presence of verteporfin did not change this localization. Analysis of the secretory profile of the studied cell populations shown that verteporfin decrease the level of almost all investigated proteins (TGFβ, IL-11, TSG-6 and COX2) in both WJ-MSC and hiPSC-NSC, cultured either in 2D or in hydrogels. The level of interleukin 10 was at the limit of detection for both cell populations in all observed culture variants.

Conclusions: The study showed the relationship between the secretion profile of immunomodulatory trophic factors, the elasticity of the culture medium and the location of the YAP / TAZ complex in the cells. Moreover, the cells' response to the microenvironmental stimuli depends on the type of stem cell population.

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