THE INTRACELLULAR CALCIUM CONCENTRATION IN HUMAN MESENCHYMAL STEM CELLS AS A POTENTIAL TARGET FOR IMPROVEMENT OF CHONDROGENIC DIFFERENTIATION

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Human articular cartilage has a low ability to regenerate in case of damage, which is essential for development of such illnesses as osteoarthritis (OA). OA is a very common progressive disease, annually found in almost 10% of human population of which 60% are women [1]. Human mesenchymal stem cells (hMSCs) have attracted attention due to their potential application for the treatment of OA. These cells could be isolated from different types of tissues, where the classical source remain bone marrow (BM). However, due to low amounts of cells and difficulties in receiving healthy bone marrow samples, other sources, such as menstrual blood-derived hMSCs (MenMSCs), seems an attractive alternative. Intracellular calcium (iCa^{2+}) is known to have influence in regulating hMSCs differentiation, however, the basic iCa^{2+} levels in different types of hMSCs and chondrocytes and their role in stem cell chondrogenic differentiation are not elucidated [2].

The aim of this study was to evaluate association of intracellular calcium levels in human BMMSCs, MenMSCs and chondrocytes with chondrogenic differentiation capacity.

All experiments were performed on three different human cell types – MenMSCs (n=5), BMMSCs (n=5) and chondrocytes (n=5). MSCs were characterized by the expression of typical MSC surface markers (flow cytometry) as well as adipogenic and osteogenic differentiation capacity (Oil-Red O and Alizarin S staining). The cells were incubated with different types of calcium channel antagonists/agonists, including L-type voltage-operated calcium channel (VOCC) regulators and endoplasmic reticulum iCa^{2+} inhibitor for 1 and 24 hours to determine iCa^{2+} levels using Cal-520 dye (flow cytometry, fluorescent microscopy). Additionally, VOCC subunit CaV1.2 was analyzed immunocytochemically after treatment of the cells with VOCC regulators. The effects of the same calcium channel regulators on 21 day of chondrogenic differentiation of all three cell types were evaluated by Safranin-O, Collagen II antibody staining and by SOX9, Collagen II and Aggrecan gene expression (RT-PCR).

Similar stem cell properties were observed in both types of MSCs, as determined by surface marker expression and adipogenic and osteogenic differentiation. In MenMSCs and chondrocytes iCa^{2+} levels are significantly higher, which was associated with lower levels of chondrogenic differentiation capacity in those cells, as compared to BMMSCs. Stimulation with L-type calcium channel regulators resulted in improved chondrogenic differentiation potential in all cell types. In addition, iCa^{2+} channel regulators used in this study showed different effects on iCa^{2+} levels in both cell types, suggesting cell-specific regulation.

Taken together, our results demonstrate that MenMSCs exhibit similar stem cell properties to BMMSCs, however their iCa^{2+} levels are significantly higher, which may play a role in regulation of chondrogenic differentiation.

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