THE EXPRESSION OF L-TYPE VOLTAGE-OPERATED CALCIUM CHANNEL SUBUNIT CAV1.2 IN HUMAN MESENCHYMAL STEM CELL CHONDROGENIC DIFFERENTIATION

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Human mesenchymal stem cells (hMSC) have an ability to differentiate into diverse types of cells – osteoblasts, myocytes, adipocytes and chondrocytes. These cells are found in different tissues, such as bone marrow and less studied source – menstrual blood. The field of tissue engineering have used these cells to repair and regenerate nearly all kinds of human tissues. One of the most common applications is repair of cartilage, especially in patients with osteoarthritis.

Osteoarthritis is a degenerative disease which results in the loss of joint structure and deterioration of cartilage, it is also associated with obesity and aging. Moreover, there is evidence that osteoarthritis is often co-diagnosed with hypertension [1]. Such patients are usually prescribed cardiovascular drugs containing nifedipine (chemical name: 3,5-dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine3,5-dicarboxylate) which is a well-known L-type voltage-operated calcium channel (VOCC) inhibitor.

VOCC are important for intracellular intake of ions, regulating such processes as contraction, secretion, neurotransmission and gene expression in many different human tissues. CaV1.2 is one of the VOCCs subunits representing major channel's pore parts, which enable Ca^{2+} flow [2]. Alterations of calcium intake using antihypertensive drugs may influence pathogenesis of osteoarthritis, as intracellular levels of Ca^{2+} are important for chondrogenic differentiation.

The aim of this study was to evaluate the effects of VOCCs antagonist nifedipine on hMSC chondrogenic differentiation potential and expression of CaV1.2, and comparison to chondrocytes. hMSC were isolated from bone marrow (BM) or menstrual blood (Men). Human chondrocytes were isolated from articular cartilage samples. Cells were treated with nifedipine (10 µM) or VOCC agonist BayK8644 (10 µM) for 1 or 3 days and cell migration capacity was analyzed using scratch method. CaV1.2 was evaluated by staining the cells with CaV1.2 fluorescent antibodies (immunocytochemistry) and by its gene expression levels (RT-PCR). Cell metabolism (mitochondrial respiration) was measured after the treatment of the cells with nifedipine (10 µM) for 24 hours, using Agilent Seahorse. Chondrogenic differentiation was stimulated for 21 day, and the effects of nifedipine (10 µM) and BayK8644 (10 µM) were evaluated by Safranin and Collagen II antibody staining (histology) and expression profile of CaV1.2, SOX9, Collagen II genes (RT-PCR).

The levels of CaV1.2 subunit varied in different cell types. The highest expression of CaV1.2 gene (CACNAC1) was observed in chondrocytes, whereas BMMSCs and MenMSCs had similar, lower amounts of CACNAC1. Nifedipine downregulated mitochondrial respiration in all cell types, as well as their migration capacity. Different expression levels were observed during chondrogenesis in three cell types, where nifedipine reduced CaV1.2 gene expression in all cell types, however increased extracellular matrix formation, according to histological analysis.

Taken together, the results of this study might help in understanding the regulation of Ca^{2+} flow through VOCCs CaV1.2 pore during chondrogenic differentiation of hMSCs of different origins, which may help to further comprehend mechanisms of cartilage repair and lead to the development of therapies for patients with osteoarthritis.

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