QUANTITATIVE PROTEOMICS ANALYSIS OF CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS BY SILAC

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The chondrogenic potencial of mesenchymal stem cells (MSCs) makes them an appealing source for cartilage repair; however the molecular mechanisms that participate in chondrogenesis remain still unknown. We employed stable isotope labeling by amino acids in cell culture (SILAC) technique to dig deeper in the chondrogenic differentiation process of human MSCs. Bone marrow cells isolated from osteoarthritic (OA) patients were grown in an expansion medium supplemented with glucose (4,5g/L), dialyzed fetal bovine serum (10%) and antibiotics (1%) but lacking arginine and lysine. Light medium was supplemented with standard amino acids, while isotope-labeled L-lys and L-arg were employed in the heavy medium. Then, chondrogenic differentiation was induced by micromass (3D) culture under a commercial medium for 14 days. Proteins were extracted from the micromasses at day 2 and day 14 of differentiation. Heavy and light samples were mixed 1:1 and in solution digested with trypsin. Separation of the resulting peptides mixtures was performed by nanoscale LC-MS/MS. The identification and quantification of proteins was carried out with Protein Pilot 2.0 software.

A total of 190 MSCs proteins were identified. Although most of them could be quantified, only 20 proteins resulted significantly altered. We found collagen type VI, one of the main component and hallmark of mature chondrocytes, up-regulated at day 14 of chondrogenesis. Moreover, a relevant increase in several biological functions was detected. We encountered up-regulated proteins involved in different cellular processes like transcription regulation (histones), mRNA processing and cytoskeleton remodelling (TUB, VIM, TNC).

We applied SILAC technique to identify proteins differentially expressed in an *in vitro* model of chondrogenesis. These preliminary results provided novel information about proteomic profile of differentiated MSCs in 3D culture and also highlighted that this approach is suitable for quantitative proteomics studies related to chondrogenesis in OA patients.