PROTEOMICS APPROACH FOR THE SEARCH OF BIOMARKERS IN BIOLOGICAL FLUIDS AND CARTILAGE

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Multidimensional liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has become a powerful method for the sensitive detection, quantification and identification of proteins in different samples. Osteoarthritis.(OA) is the most common rheumatic pathology, characterized mainly by cartilage degradation. Despite its high prevalence, the diagnosis methods currently available are limited and lacked of sensitivity. In this work we set up a proteomic methodology for the large-scale identification and quantisation of proteins in serum from OA patients and their comparison with healthy donors. Serum samples were obtained from OA patients at different stages of the disease (grade II, grade IV and control donors) and were grouped in pools to reduce interindividual variability. The top twenty most abundant proteins in crude serum fluids were removed by affinity chromatography using the immunodepletion column ProteoPrep® 20. Proteins from each pool were quantified, digested with trypsin and differentially labelled with isobaric tags using iTRAQ methodology. Peptides from the three conditions were mixed, separated and analyzed by two dimensional LC coupled to MALDI-TOF/TOF mass spectrometry. The peptide mixture was separated by off line reversed phased chromatography at basic pH and each peptide fraction was subjected to nLC chromatographic using reversed phase chromatography at acid pH. Identification and relative quantification of the proteins were performed using ProteinPilot 2.0 software. 349 different proteins were identified and the measurement of the different iTRAQ tags intensities allowed the relative quantification of proteins under the three conditions of the study. 29 proteins were found to be modified in the serum of OA patients (19 up-regulated and 10 down-regulated when compared to healthy donors) with a p-value < 0.05. Identification of some of these proteins in synovial fluid and cartilage from OA patients indicate that these proteins can be potential biomarkers for OA pathology.