

QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF EXOSOMES SECRETED BY RAT HEPATOCYTES

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Exosomes are nanometer-sized vesicles (30-150 nm) that form multivesicular bodies (MVBs) by means of inward budding of the limiting membrane. Upon fusion of the MVBs with the plasma membrane, exosomes are released to the extracellular medium, where they have been shown to play different roles in cell-to-cell communication functions, such as antigen presentation or induction of antitumor responses. Given the specific tissue signatures, exosomes might harbour diagnostic markers. Two well-known cytotoxic compounds, namely galactosamine and lypopolisaccharide, have been chosen to discern diagnostic markers of hepatic diseases in the exosomal proteome. In this study, we have characterized at the protein expression, microscopic and molecular level, exosomes secreted from primary cultured rat hepatocytes treated and untreated with these hepatotoxic agents.

Exosomal proteins were extracted, digested and quantified by a label-free LC-MS approach. Data were acquired and quantified with a data-independent LC-MS^E scanning method, utilizing a NanoAcquity LC reverse phase chromatography system directly interfaced to a QToF Premier mass spectrometer (Waters Corporation). Raw data were processed and submitted to database search and quantified using ProteinLynx Global Server v2.4 and Expression^E software (Waters Corporation). Additionally, 2D-LC-MS^E experiments were performed in order to further mine the exosomal rat proteome. This resulted in a 43% increase in the number of proteins identified. From the total of 600 proteins identified, with a protein-level FDR smaller than 1%, 50% were quantified. 27 proteins were found to be commonly up-regulated and 64 down-regulated after treatments by both methods and exhibiting a fold change greater than 1.3 fold and a standard deviation smaller than 10%. Among these, proteins directly implicated in the metabolism of xenobiotics by P450 or known biomarkers of liver cancer have been identified. To the best of our knowledge, this is the first time that a label-free quantitation approach has been used to study protein expression in exosomes.