

PROTEOMIC ANALYSIS OF SUBPOPULATIONS CD73+ ADULT AND FETAL HUMAN MESENCHYMAL STEM CELLS AND HEPATIC PERICYTES

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Introduction: Mesenchymal stem cells (MSCs) are present in a variety of adult and fetal tissues. These cells have a wide range of differentiation potentials and a complex relationship with hematopoietic stem cells (HSCs) and endothelial cells. Pericytes, also referred to as periendothelial cells, are also closely related to MSCs. Indeed, when activated during injury, pericytes acquire several characteristics of MSC. Using SAGE analysis we similarities at the transcriptional level among pericytes and MSCs from different sources. The identification of the molecular mechanisms shared by these cells as well the differences can have a significant impact cell-based therapy.

Objective: To identify out proteomic similarities and differences among CD73+ MSCs (adult and fetal) and hepatic pericytes using in-depth analysis by LC-MS/MS.

Methods: MSCs were isolated from the mononuclear fraction of bone marrow (BM) from normal adults by adherence to plastic and from fetal liver (FL), of gestational age between 18 and 32 weeks, using collagenase treatment. Hepatic pericytes (HP) were the liver cell line LX2. MSCs and pericytes were purified from cultures by positive selection (FACS) based on the antibody to CD73. The CD73+ cells were expanded, characterized morphologically, immunophenotypically and functionally, by the capacity of cellular differentiation (morphologic and gene expression). Cell protein extracts were obtained by sonication and labeled with acrylamide isotopes. Equal amounts of labeled protein extracts from MSC-FL and HP and MSC-BM and HP were mixed for quantitative proteomic analysis. Samples were fractionated by SDS-PAGE and each gel lane was cut into 9 slices for subsequent LC-MS/MS protein identification and relative quantification by mass spectrometric analysis.

Results: We identified 1829, 1772 and 2319 proteins in MSC-BM, MSC-FL and HP cells respectively. Overall, we detected 2934 unique proteins among the three cell populations. Of those, 1225 proteins were quantified on the basis of acrylamide isotope labeling. The great majority of proteins (>80%) did not present differences in abundance greater than 2-fold among the two MSCs and pericytes. However, approximately 108 proteins were consistently detected above a 2-fold threshold when comparing MSC-FL vs. HP and MSC-BM vs. HP. Among these proteins, we were able to identify molecular functions associated with specific cell types, such as lipid metabolism for HP and angiogenesis for MSCs.

Conclusions: This proteomic study was able identify a subset of proteins that could distinguish mesenchymal stem cells from hepatic pericytes cells. These proteins can be used as markers for a more detailed comparison of these cells as well as contribute to elucidation of the specific biological mechanisms that are relevant to HP and MSCs.