14.4

Identification of Proteins Differentially Expressed by Circulating Human Monocytes in Acute Coronary Syndromes

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Acute coronary syndromes (ACS) are the main cause of mortality due to ischemic heart disease as a result of the occlusion of a coronary artery due to formation of a thrombus on an atherosclerotic plaque or a consequence of left ventricular dysfunction.

Plasma of patients with ACS contain elevated levels of several proinflammatory mediators (TNFa, Il6, CRP) which can affect to circulating cells. Thus, we have investigated whether circulating monocytes from ACS patients express specific proteins that could define a characteristic profile.

We obtained blood samples from patients with myocardial infarction (MI, n = "28") at day 0,4 and 2 and 6 months, as well from patients with stable coronary disease (n = "10") and matched healthy control (n = "12"). Monocytes were obtained with high purity (>95%), lysed and the proteins analysed by two dimensional electrophoresis (2-DE). Differentially expressed proteins were identified by mass spectrometry (MS).

More than forty protein spots have their expression altered in monocytes of ACS patients relative to controls. One of the most relevant features observed is the absence of expression of a set of proteins implicated in extracellular functions, structural functions, proteases etc. The identified proteins in human circulating monocytes may contribute to the ACS and may prove suitable as novel therapeutic targets.

14.5

Standardization and Evaluation of Magnetic Bead-based Proteome Profiling with MALDI-TOF-MS for the Detection of New Biomarkers in Human Blood

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Objective: Magnetic bead purification for the analysis of low-abundance proteins in body fluids facilitates the identification of potential new biomarkers with MALDI-TOF-MS. The aim of our study was to establish this technique for the analysis of human serum and plasma and to validate a standardized blood sampling, processing, and storage procedure for the proteomic pattern analysis.

Methods: We used magnetic bead separation for proteome profiling of human blood by MALDI-TOF-MS and studied the effects on the quality and reproducibility of the proteome analysis of anticoagulants, blood clotting, time and temperature of sample storage, and the number of freeze-thaw cycles. Furthermore, we investigated the variation of the proteomic pattern using different types of blood collection tubes and effects caused by addition of protease inhibitors or promoters.

Results: The obtained proteome profiles showed time-dependent dynamic changes before and after centrifugation of the blood samples. In particular we could observe an influence of platelet content on the plasma proteome pattern. Serum as well as plasma mass patterns differed between native samples and samples frozen once. Also the application of different blood collection tubes has influence on signal amount and intensities in proteome spectra.

Conclusion: Application of the standardized preanalytical blood sampling and storage procedure combined with magnetic bead-based sample preparation decreases the variability of proteome patterns in human body fluids assessed by MALDI-TOF-MS and enables an excellent platform for biomarker profiling. Currently, several large-scale epidemiological studies in patients with cardiovascular or tumor diseases are under way to identify possible disease related biomarkers in human blood.