GENERATION AND CHARACTERIZATION OF AN ANALYTE LIBRARY REPRESENTING THE HUMAN PLASMA PROTEOME: APPLICATION FOR THE IDENTIFICATION OF COGNATE ANTIGENS CORRESPONDING TO MONOCLONAL ANTIBODIES

András Kovács[1,2],
Zoltán Patai[2], Edit Sperling[2], József Lázár[1,3], Attila Balogh[3], Ákos Szekrényes[2], János Kádas[3], András Guttman[2,5], István Kurucz[3], László Takács[1,3,4]
[1] BioDiagnostica Kft., Debrecen
[2] Horváth Laboratory of Bioseparation Sciences, Research Centre for Molecular Medicine, University of Debrecen, Debrecen
[3] BioSystems International Kft., Debrecen
[4] Monoclonal Antibody Proteomics Laboratory, Department of Human Genetics, University of Debrecen, Debrecen
[5] MTA-PE Translational Glycomics Group, MUKKI, University of Pannonia, Veszprém

Introduction: Human plasma contains a wide concentration range of proteins (12 orders of magnitude) with diverse biological functions. Due to its high complexity, the characterization and identification of medium or low abundant proteins often represent a difficult and time-consuming task and require a large amount of sample. Monoclonal antibody proteomics uses a large number of monoclonal antibodies of unknown antigen specificity raised against complex biological samples. The antigen identification process includes the reaction of biological samples with antibodies, immunoprecipitation, SDS-PAGE analysis, Western blot validation and mass spectrometric identification. The aim of this work was to generate and characterize an Analyte Library that contained hundreds of fractions of native plasma proteins, and to use the Library for antigen identification in conjunction with mAb proteomics.
Methods: First the albumin and immunoglobulins were depleted from 500 mL normal human plasma followed by pre-fractionation by ammonium sulphate precipitation steps. Each precipitate was then further fractionated by size exclusion chromatography, followed by cation and anion exchange chromatography and hydrophobic interaction chromatography. The comprehensive screening of the Analyte Library with mAbs of known abundant antigens of biomarker potential was also part of the study to reveal the distribution of these proteins and to demonstrate the applicability of the Library for mAb proteomics. The identification of antigens corresponding to the mAb-s was conducted by the workflow of monoclonal antibody proteomics.
Results: A 783-fraction containing Analyte Library comprising of native protein fractions was generated. The individual fractions contained some 10 or 100 proteins. As a result of the characterization, the Library was considered as applicable for monoclonal antibody proteomics-based antigen identification; and the antigens corresponding to eight monoclonal antibodies have been successfully identified.
Conclusion: The Analyte Library and the developed method were applicable for monoclonal antibody proteomics-based antigen identification, and suggest the potential of the developed workflow in biomarker and protein identification research.