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LC-MS based discovery of novel protein biomarkers and their quantitation in HCV fibrosis and NASH diseased human serum

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We propose a mass spectrometry and liquid chromatography (LC-MS) based approach for protein biomarker discovery and their quantification in human serum sample from patients with hepatitis-C virus (HCV) mediated liver fibrosis and non-alcoholic steatohepatitis (NASH).

Various isotope labelling approaches like ICAT, iTRAQ and TMT have been used for protein discovery and quantification. We aim to use TMT 10-plex labelling which allows up to 10 samples to be compared at one time, resulting into higher through put sample analysis. In order to look for low abundant serum protein biomarkers we are depleting high abundant serum proteins such as albumin, IgG etc by immunoprecipitation. The depleted serum samples would be subjected to tryptic digestion and labelled with TMT 10-plex which is then analysed by LC-MS/MS to detect and find significantly changed proteins in all 10 samples.

An in-solution trypsin digestion protocol was developed for undepleted serum to quantify one of our protein biomarker (lipid transfer inhibitor protein, LTIP) for HCV fibrosis in both selective reaction monitoring (SRM) and parallel reaction monitoring (PRM). In both SRM and PRM, initial studies were performed using recombinant LTIP in order to find most suitable peptides. These selected peptides and their transitions were also analysed in trypsin digested plasma samples. Using SRM, three peptides were found to be most optimal and considered for quantification. Using PRM, four peptides were selected and LOQ was found to be 308-25,000pg/uL. This study requires further validation to establish a complete method.

Biography

Abhinav Kumar holds a B.Sc in Pharmacy, M.Sc in Analytical Chemistry (University of Strathclyde, Glasgow, UK) and a Ph.D. in Bioanalytical Science (UWE, Bristol, UK). He received an award for the best poster by the European Society for Separation Science (EuSSC) for his HILIC application on polar biomarkers analysis and discovery for biomedical applications (ISC 2012, Sept 2012, Torun, Poland). He has worked on untargeted metabolomics and many SRM method developments for the absolute quantification of compounds in biological samples. Presently he is working on proteomics research at the Department of Biochemistry, University of Oxford and is involved in LC-MS based protein biomarker discovery and SRM/PRM method development for absolute quantification.

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