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Proteomic profiling of lipid loading in human hepatocytes

C. Spanos[1], M. E. Weeks[2] and J. B. Moore[1]
[1]*Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, UK and* [2]*Veterinary Laboratories Agency, New Haw KT15 3NB, UK*

Non-alcoholic fatty liver disease (NAFLD) is now considered to be the most common liver disease worldwide[(1)]. Found strongly associated with features of the metabolic syndrome, NAFLD can progress from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and potentially hepatocellular carcinoma. Accurate diagnosis and staging of the disease dictates treatment and is a primary clinical concern. Given the limitations and invasiveness of liver biopsy the diagnostic 'gold standard' for NASH, non-invasive biomarkers capable of diagnosing and staging NAFLD are urgently needed.

The objectives of these experiments were to develop a robust quantitative proteomic strategy for biomarker discovery using an *in vitro* model of progressive steatosis. Human HuH7 hepatocytes cultured with increasing concentrations of palmitic acid (PA) for 24 h showed a dose-dependent accumulation of intracellular lipid. Using covalent isobaric tags (iTRAQ, Applied Biosystems) protein alterations due to progressive lipid accumulation were assessed by MS: first in a duplex experiment where total protein extracts from cells cultured with either vehicle (DMSO-FAF/BSA) or 200 µM PA were compared (n 3); and secondly in a multiplex experiment where protein extracts from cells treated with vehicle, 50, 100 or 150 µM PA were compared (n 3). Following digestion and iTRAQ labelling, peptides were fractionated by strong cation exchange chromatography and subjected to MS/MS with peak lists generated by the Mass Hunter software (Agilent).

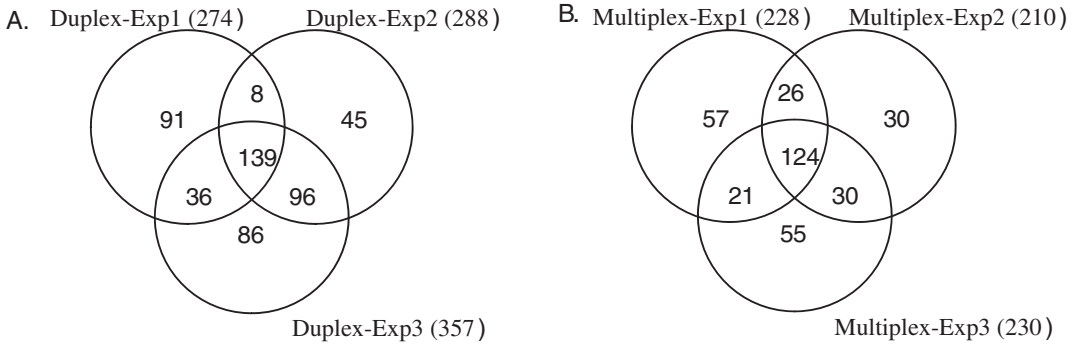

Fig. 1. Venn analysis of proteins identified (≥ 2 peptides) by Spectrum Mill software in three replicate experiments. (a) In the duplex experiment 361 non-redundant proteins were identified in total; 139 of which were common to all three experiments. (b) In the multiplex experiment 266 non-redundant proteins were identified with 124 in common to three replicate experiments.

Initial data analysis performed using Spectrum Mill software identified more than 450 proteins in the duplex experiment (>274 by 99% CI) and more than 340 proteins (>210 by 99% CI) in the multiplex experiment. Venn analysis of proteins identified with 99% CI (≥2 peptides) showed 139 and 124 proteins in common to replicates of the duplex and multiplex experiments (n 3).

Ongoing investigation using the same datasets involves the use and comparison of additional data analysis platforms such as VEMS 5.0 and MASCOT for protein quantification and identification of candidate biomarkers for NAFLD.

1. Moore JB (2010) *Proc Nutr Soc* **69**, 211–220.