Proteomic Analysis of Liver Membranes through an Alternative Shotgun Methodology

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Abstract

The aim of this thesis was to develop a proteomics methodology that improves the identification of membrane proteomes from mammalian liver. Shotgun proteomics is a method that allows the analysis of proteins from cells, tissues and organs and provides comprehensive characterisation of proteomes of interest. The method developed in this thesis uses separation of peptides from trypsin digested membrane proteins by immobilised pH gradient isoelectric focusing (IPG-IEF) as the first dimension of two dimensional shotgun proteomics. In this thesis, peptide IPG-IEF was shown to be a highly reproducible, high resolution analytical separation that provided the identification of over 4,000 individual protein identifications from rat liver membrane samples. Furthermore, this shotgun proteomics strategy provided the identification of approximately 1,100 integral membrane proteins from the rat liver. The advantages of using peptide IPG-IEF as a shotgun proteomics separation dimension in conjunction with label-free quantification was applied to a biological question: namely, does the presence of a spatially unrelated benign tumor affect the abundance of mouse liver proteins. IPG-IEF shotgun proteomics provided comprehensive coverage of the mouse liver membrane proteome with 1,569 quantified proteins. In addition, the presence of an Englebreth-Holm-Swarm sarcoma induced changes in abundance of proteins in the mouse liver, including many integral membrane proteins. Changes in the abundance of liver proteins was observed in key liver metabolic processes such as fatty acid metabolism, fatty acid transport, xenobiotic metabolism and clearance. These results provide compelling evidence that the developed shotgun proteomics methodology allows for the comprehensive analysis of mammalian liver membrane proteins and detailed some of the underlying changes in liver metabolism induced by the presence of a tumor. This model may reflect changes that could occur in the livers of cancer patients and has implications for drug treatments.

Declaration

I certify that the work in this thesis entitled "Proteomic Analysis of Liver Membrane Proteins through an Alternative Shotgun Methodology" has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University. I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Publications Contributing to the Production of the Thesis

1. Chick, J. M.; Haynes, P. A.; Molloy, M. P.; Bjellqvist, B.; Baker, M. S.; Len, A. C., Characterisation of the Rat Liver Membrane Proteome Using Peptide Immobilised pH Gradient Isoelectric Focusing. *J Proteome Res* **2008,** 7, (3), 1036-1045.
2. Chick, J. M.; Haynes, P. A.; Bjellqvist, B.; Baker, M. S., A Combination of pH Gradients Improves Membrane Proteomics, *J Proteome Res* **2008,** 7, (11), 4974-4981.
3. Chick, J. M.; Haynes, P. A.; Baker, M. S.; Robertson, G.; Affects of tumor-induced inflammation on mouse liver membrane protein abundance. Publication in preparation.

Abbreviations

2D	Two-dimensional
2D-GE	Two-dimensional gel electrophoresis
3D	Three Dimensional
ABC	ATP-binding Cassette
Acaa	Acetyl-CoA acetyltransferase
Acadm	acyl-CoA dehydrogenase medium chain
Adh	Alcohol Dehydrogenase
Aldh	Aldehyde Dehydrogenase
Apo	Apolipoprotein
B3galnt	Beta-1,3-Galactosyltransferase
Bcrp	Breast Cancer Resistance Protein
BR	Broad Range
Bsep	Bile Salt Export Pump
C1Galt	Core 1 Synthase, Glycoprotein-N-acetylgalactosamine 3-beta-Galactosyltransferase, 1
cIEF	Capillary Isoelectric Focusing
Cpt	Carnitine O-palmitoyltransferase
CRP	C-Reactive Protein
CSF1R	Colony Stimulating factor Receptor 1
Cyp	Cytochrome p450
E-Fabp	Epidermal Fatty Acid Binding Protein
Ehhadh	Enoyl-CoA, hydratase/3- hydroxyacyl-CoA dehydrogenas
EHS	Englebreth-Holm-Swarm
Fabp	Fatty Acid Binding Protein
FT MS	Fourier Transform Tandem Mass Spectrometry
GPI	Glycosyl phosphatidylinositol
GPM	Global Protein Machine
Gst	Glutathione S-Tranferase
ICAM	Intracellular adhesion molecule
ICAT	Isotope Coded Affinity Tags
IL-6	Interluekin-6
IMP	Integral Membrane Proteins
IPG-IEF	Immobilised pH Gradient Isoelectric Focusing
i-TRAQ	Isobaric Tag for Relative and Absolute Quantification
LACS	Acyl CoA Synthase Long Chain
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography - Tandem Mass Spectrometry
Ldlr	Low-Density Lipoprotein Receptor
Lrp	Low-Density Lipoprotein Associated Protein
Man	Mannose
Manea	Glycoprotein Endo-Alpha-1,2- Mannosidase
Mdr2	Multidrug Resistannce Proten
Mgat	Mannoside Acetylglucosaminyltransferase
Mgst	Microsomal Glutathione S-Transferase
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MudPIT	Multidimensional Protein Identification Technology
NR	Narrow Range
NSAF	Normalised Spectral Abundance Factors
NTCP	Na+/Taurocholate cotransporting polypeptide
OAT	Organic Anion Transporter
Pcca	Propionyl-CoA carboxylase α

Pccb	Propionyl-CoA carboxylase β
pepFDR	Peptide False Discovery Rate
protFDR	Protein False Discovery Rate
PTM	Post-Translational Modification
RT	Room Temperature
SAP	Serum Acute Protein
SCX	Strong Cation Exchange
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SILAC	Stable Isotope Labelling of Amin Acids in Cell Culture
Slc	Solute Carrier
St3Gal	ST3 beta-galactoside alpha-2,3-Sialyltransferase
TM	Transmembrane
TMHMM	Transmembrane Hidden Markov Model
VDAC	Voltage Dependent Anion Channel
(v/v)	Volume/volume