11TH EAST MIDLANDS PROTEOMICS WORKSHOP

Wednesday 28th November 2012

Sir Denis Rooke building, Holywell Park, Loughborough University

11th East Midlands Proteomics Workshop

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11th East Midlands Proteomics Workshop
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The East Midlands Proteomics Workshop (http://www.empw.co.uk) was established to create a network of investigators active in the proteomics area with a view to enhancing awareness of facilities available in the East Midlands, disseminating specialist knowledge and information relating to proteomics technologies, highlighting examples of proteomics research and providing a forum to discuss research interactions.

The meeting will focus on how proteomics-based approaches are being used in biological and medical research. Keynote presentations will be given by invited external speakers describing the latest advances.

The Workshop is supported by the British Society for Proteome Research (http://www.bspr.org) and the British Mass Spectrometry Society (http://www.bmss.org.uk).

Location
The meeting will be held in the Sir Denis Rooke building on the Loughborough University, Holywell Park Campus. Further information about Holywell Park can be found at: http://www.welcometoimago.com/delegates/holywell-park

Directions and Parking
Directions to Holywell Park (post code LE11 3GR) are available at the location http://www.welcometoimago.com/contact/location/maps. There is ample parking in the car park adjacent to the Sir Denis Rooke building.

Registration
Registration opens at 8:30 in the foyer of the Sir Denis Rooke building.

Posters
There will be a display of posters in the exhibition area of the Sir Denis Rooke Building. Presenting authors are asked to attend their posters between 13:00 and 14:00.

Trade Exhibition and Great Exhibition Prize Draw!
There will be a trade exhibition by sponsoring companies. Please visit the trade exhibition at the coffee and lunch breaks, take along the prize draw form received at registration. **Prize Draw:** There is a £50 cash prize for the first completed form picked at random. The winner **must be present** at the end of the meeting to collect the prize.

The organizers wish to thank the following companies for their generous sponsorship:

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Dr Sharad Mistry (University of Leicester)
Dr Susan Liddell (The University of Nottingham)

11th East Midlands Proteomics Workshop
Wednesday 28th November 2012, Loughborough University

8:30 -9:20 **Registration**

Session 1: Chair: Don Jones

9:20 Welcome Don Jones, chair of EMPW2012

9:30 **Keynote Presentation**
Approaches to analyse the microenvironments of membrane proteins
Kathryn Lilley, Cambridge Centre for Proteomics, University of Cambridge

10:10 *Bioorthogonal reagents for MS-based quantitative screening of protein lipidation in biological systems*
Remigiusz Serwa, Department of Chemistry, Imperial College London

10:30 *High Resolution/Accurate Mass Measurements in both Discovery and Targeted Peptide Analysis: You can have it all*
Martin Hornshaw, Thermo Scientific

10:50 **Coffee / tea / Exhibition**

Session 2: Chair: David Boocock

11:20 *Defining molecular species involved in the prevention of colorectal cancer following administration of rice bran fibre*
Don Jones, Department of Cancer Studies & Molecular Medicine, University of Leicester

11:50 *Identification of substrates for the essential malaria protein kinase, PfPKG, using comparative global phospho-proteome analysis*
Mahmood Alam, *Toxicology Unit, University of Leicester*

12:10 *Increased Throughput for 2D LC in the Analysis of Human Placental Samples*
Robert Tonge, Waters Corporation

12.30 Lunch / Exhibition

13:00 to 14:00 Poster Session

Session 3: Chair: Rob Layfield

14:00 *Chemoproteomics in the Ubiquitin system and drug discovery*
Benedikt Kessler, Centre for Molecular and Cellular Physiology, The University of Oxford

14:30 TBA
Dave Tooth, School of Biomedical Sciences, The University of Nottingham

14:50 *Analysis of proteins on surfaces using ambient mass spectrometry*
Dave Barrett, School of Pharmacy, The University of Nottingham

15.20 Coffee / tea / Exhibition

Session 4: Chair: Colin Creaser

15:50 *Development of a sensitive high throughput UHPLC-MS/MS (SRM) method for the quantitation of Glucagon from human plasma*
James Howard, Quotient Bioresearch/Loughborough University

16:10 *Matrix Assisted Laser Desorption Ionisation Ion Mobility Separation Mass Spectrometry Imaging*
Malcolm Clench, Biomedical Research Centre, Sheffield Hallam University

16:40 Announcement of prizes Don Jones

16:50 End of meeting

11th East Midlands Proteomics Workshop: Oral Presentation Abstracts

Approaches to analyse the microenvironments of membrane proteins

Kathryn S. Lilley

Cambridge Centre for Proteomics, Department of Biochemistry,
University of Cambridge, United Kingdom, CB2 1QR

To increase their functional diversity, many proteins exist within multi-protein complexes which may have variable role-dependent compositions. Additionally proteins maximise their range of functions by existing in unique sub-cellular niches crucial for creating the controlled microenvironments which promote diverse biological functions. Knowledge of both these environments and membership of multi-protein complexes is thus of fundamental importance in the analysis of protein function.

Many protocols start with cell lysis, which destroys sub-cellular compartmentalisation, and also employ reagents, which do not distinguish between different protein isoforms that may relate to different sub-cellular compartments and interaction partners. Furthermore, many protein localization approaches are dependent upon the purification of the sub-cellular niche of interest, catalogue the protein content of only a few sub-cellular structures, or require the use of large panels of immuno-reactive reagents.

In this presentation, I will briefly describe the tools that we have developed which allow simultaneous and accurate protein localization of membrane proteins across multiple subcellular locations involving semi-supervised machine learning approaches for data analysis. I will also introduce a novel method for defining the local environments of proteins within membranes. I will discuss how we have applied these to a variety of sample types including yeast, plant and vertebrate cell culture, and whole organisms.

This research was supported by BBSRC grants BB/HO24247/1and BB/D526088/1 and the 7th Framework Programme of the European Union (Contract no. 262067- PRIME-XS)

Bioorthogonal reagents for MS-based quantitative screening of protein lipidation in biological systems

Remigiusz Serwa

Department of Chemistry, Imperial College London

Protein lipidation is the post-translational covalent attachment of hydrophobic moieties to proteins. The process is catalysed by protein lipid transferases and affects localisation and function of modified proteins. Protein lipidation facilitates association with membranes, mediate protein trafficking, and regulate protein stability. To date, misregulation of lipid metabolism and protein lipidation events have been associated with a number of human pathologies. However, the number of in vivo validated protein substrates of protein lipid transferases is only modest. Historically, protein lipidation events were studied utilising radioisotopically labelled lipids. In the 21st century these radioactive probes have been replaced by non-hazardous molecules equipped directly with fluorophores (visualisation), affinity handles (enrichment), or with bio-orthogonal tags for incorporation of these attributes via a highly specific chemical reaction. Our group synthesizes novel lipid probes suitable for in vivo and in vitro applications and utilizes them to identify and quantify proteins lipidated in various biological systems in SILAC based proteomic experiments. Presented will be our recent insights into protein lipidation in human cancer cell lines, in Plasmodium falciparum (parasite that causes Malaria), and in epithelial cells infected with Herpes Simplex Virus.

High Resolution/Accurate Mass Measurements in both Discovery and Targeted Peptide Analysis: You can have it all

Martin Hornshaw

Thermo Scientific, Stafford House, Boundary Way, Hemel Hempstead

The orbitrap mass analyzer, from its first commercial incarnation in 2005 as the hybrid LTQ Orbitrap, has become a widely accepted and utilized mass spectrometry tool in shotgun proteomics. Its development has been rapid with several instrumental generations following, with improvements in acquisition speed, sensitivity, resolution, additional scan modes, dissociation techniques, ionization sources, data dependent workflows and so on. In 2011 a new type of hybrid orbitrap based mass spectrometer was launched, the Q Exactive, a quadrupole-orbitrap mass spectrometer. This geometry allows for novel types of scans and applications compared to previous systems. In addition to the now fairly standard experiment of shotgun proteomics the Q Exactive shows capability in targeted, quantitative peptide (and small molecule) assays analogous to selected reaction monitoring in triple stage quadrupole mass spectrometers. The general benefits of a high resolution/accurate mass (HRAM) approach to targeted quantitation will be discussed. Types of MS scan that have particular utility in targeted HRAM quantitation are full scan MS, selected ion monitoring (SIM) and targeted MS/MS. SIM and MS/MS scans are capable of being multiplexed. Mass spectral performance and examples of shotgun proteomics with and without sample fractionation and targeted quantitation of peptides will be discussed.

Defining molecular species involved in the prevention of colorectal cancer following administration of rice bran fibre.

***Donald JL Jones*[1]*, Leong Ng*[2]*, Leonie Norris*[1]*, Emma Horner-Glister*[1]*, Aditya Malkar*[3]*, Colin Creaser*[3] *and Stewart Sale*[1]

[1]*Cancer Studies and Molecular Medicine (CSMM)*
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[3]*Centre for Analytical Science, Department of Chemistry, Loughborough University,* LE11 3TU, *UK*

Epidemiological evidence suggests that human consumption of whole-grain foods may be associated with a low incidence of cancer, especially in the colorectum. In 2006 the Leicester group published in the British Journal of Cancer that rice bran at 30% in the diet interfered with adenoma development in a preclinical model of colorectal cancer (Verschoyle et al 2006). We have looked to identify molecular differences using both metabonomic (Loughborough University) and proteomic (University of Leicester) approaches on Waters Synapt instruments. For both sets of analyses ion mobility was incorporated and the effect of ion mobility will be shown. Combination of informatics packages led to candidate molecules that could putatively be used as markers of efficacy in fibre intervention trials. This combined approach can have significant advantages to confidence of molecular assignment but the process is not trivial. Pitfalls of the study and work to be done will be discussed.

Identification of substrates for the essential malaria protein kinase, PfPKG, using comparative global phospho-proteome analysis

Mahmood Alam*, Lev Solyakov, Andrew Tobin*

Medical Research Council, Toxicology Unit, University of Leicester

The human malaria parasite, *Plasmodium falciparum*, infection kills 1-2 million people each year. As *P. falciparum* is gaining resistance against the available anti-malarial drugs in different parts of the world, there is need for better understanding of parasite biology so that appropriate drugs can be developed. Like other eukaryotes, signalling mechanism in *P. falciparum* is controlled by set of kinases. cGMP dependent protein kinase (PfPKG) play essential roles during blood stage development and gametocyte development of the parasite. A Specific inhibitor to PfPKG (compound 2) has been developed. This compound was seen to block exit, egress, of the parasite from the red blood cells. A comparative global phospho-proteomics analysis was developed and used for identification of PfPKG substrates. Compound 2 inhibited phosphorylation of 141 sites which can be direct or indirect substrates for PfPKG. Among the substrates identified was PfMyoA which was phosphorylated at serine 19 (S19). Phosphorylation of PfMyoA at S19 by PfPKG was confirmed by *in vitro* kinase assay. This study shows that PfPKG may be involved in controlling actin-myosin motor which can regulate egress of parasite and/or invasion of red blood cell.

Increased Throughput for 2D LC in the Analysis of Human Placental Samples

Robert Tonge[1], *Martha Stapels*[2], *Keith Fadgen*[2], *J. Will Thompson*[3], *M. Arthur Moseley*[3], *James Langridge*[1]

[1]*Waters Corporation, Manchester, UK;* [2]*Waters Corporation, Milford, MA, USA;* [3]*Duke University School of Medicine, Durham, NC, USA*

Many proteome samples have a high complexity and when enzymatically digested, generate peptides with similar distributions of hydrophobicity and mass, irrespective of their originating species (1). Because of this high complexity, orthogonal methods are required to separate all peptides in a sample before they can be efficiently identified and quantified using data-independent MS methods which yield reproducible fragmentation and peak area information for all detectable peptides (2). The use of ion mobility during this analysis inserts an additional orthogonal separation in the gas phase between chromatographic and mass spectral analyses and enhances the deconvolution of highly complex data signals. In this study, 2D chromatography is combined with ion mobility to resolve peptides in multiple dimensions in a high-throughput manner.

Proteins were extracted from human placenta samples into two solubility fractions using TRIzol reagent with sonication. Proteins were then reduced, alkylated, and digested in-solution with trypsin. Samples were injected in triplicate onto a nanoflow liquid chromatography system and analyzed with a data-independent method using alternating low and elevated collision energy on a quadrupole time of flight instrument with ion mobility. Multidimensional chromatographic methods were employed using high-low pH RP-RP (3) with discontinuous step gradients.

A comparison was made between a traditional 2D-LC method and a faster technique that utilized simultaneous gradients in both dimensions. The faster technique took 68% of the time of the traditional 3-fraction method. The percent savings in time will increase as the number of desired fractions increases. Use of the faster method allowed for a 70% increase in the number of ions detected per minute, a 54% increase in the number of peptides identified per minute, and a 46% increase in the number of proteins identified per minute. Incorporation of ion mobility into the analysis yielded an increase in peak capacity of at least another order of magnitude.

1*. Geromanos S.J., et. al, Proteomics. 2011 (11):1189-211.* ***2****. Geromanos, S.J., et. al. Proteomics. 2009 (6):1683-95.* ***3****. Gilar M. et. al, J. Sep. Sci. 2005 (28):1694-1703.*

Chemoproteomics in the Ubiquitin system and drug discovery

Benedikt Kessler

Centre for Molecular and Cellular Physiology, The University of Oxford

Converting lead compounds into drug candidates is a crucial step in drug development, requiring early assessment of potency, selectivity and off-target effects. We have utilized activity-based chemical proteomics to determine the potency and selectivity of deubiquitylating enzyme (DUB) inhibitors in cancer cell culture models. This approach has now been combined with the development of novel ubiquitin-based active site probes that allowed detection of endogenous deubiquitinating enzyme (DUB) activities in cell extracts by direct in-gel imaging-based quantiation and mass spectrometry. We demonstrate that both approaches allow the examination of DUB cleavage specificity and the selectivity properties of promising DUB inhibitor candidates in cells. This information may accelerate the selection of novel lead compounds for anti-cancer drug development.

Analysis of proteins on surfaces using ambient mass spectrometry

Dave Barrett

School of Pharmacy, The University of Nottingham

Identification and imaging of unknown proteins adsorbed to surfaces, 'surface proteomics' is challenging, especially where protein concentrations are small and there is a need for ambient analysis. The recent introduction of ambient surface mass spectrometry technologies has opened up new opportunities in this area. Initial work is presented exploring the potential of the ambient MS techniques of desorption electrospray ionisation (DESI), plasma-assisted desorption ionisation (PADI) and liquid extraction surface analysis (LESA) for measuring and identifying proteins on biologically relevant surfaces. Methods for achieving discrimination between mixtures of proteins will be presented including imaging multivariate data analysis and in situ digest approaches. Examples of analysis of protein mixtures on model surfaces will be given to show potential for high-throughput applications in cell biology and food science.

Development of a sensitive high throughput UHPLC-MS/MS (SRM) method for the quantitation of Glucagon from human plasma

James Howard

Quotient Bioresearch and Loughborough University

Glucagon is a 29 amino acid peptide hormone which acts with insulin to regulate blood sugar levels, and is also a biomarker for pathologies such as diabetes or pancreatic cancer. It is routinely measured using Radio Immunoassay (RIA) approaches, however these assays can be time consuming to perform (up to 3 days), the kits have limited lifetimes (1 month), and there is potential for cross reactivity with similar compounds leading to inaccurate quantitation. The radioactive nature of such assays also necessitates additional precautions.

To circumvent these issues we are developing a sensitive high throughput UHPLC-MS/MS (SRM) based approach on the AB SCIEX QTRAP 5500 for the quantitation of glucagon from human plasma. Due to its low endogenous levels (45- 85 pg/mL) careful assay optimisation was necessary. Various protein precipitation and sold phase extraction (SPE) methods were investigated, both separately and in combination to assess orthogonal extraction techniques.

Quantitation was initially performed using an external plasma calibration line and a standard addition approach, which gave endogenous levels of 67.6 pg/mol, in good agreement with literature values. We are currently investigating whether adding analogue internal standards and the use of surrogate matrix or surrogate analyte based approaches increase assay performance.

Matrix Assisted Laser Desorption Ionisation Ion Mobility Separation Mass Spectrometry Imaging

Malcolm Clench

Biomedical Research Centre, Sheffield Hallam University

Since its first use for the study of biological tissue in 1997 matrix assisted laser desorption mass spectrometry imaging (MALDI-MSI) has been widely applied. Here an overview of the MALDI-MSI work carried out in Sheffield is given. This has been particularly concerned with the combination of ion mobility separation with MALDI-MSI and with the correlation of treatment/exposure and biological response. Data from studies of the distribution of a number of drugs will be presented i.e. vinblastine in whole animal sections, AQ4N and DMXAA in xenografts and drugs used in the treatment of asthma and COPD.

The current University of Sheffield/Sheffield Hallam University CR-UK and EPSRC, MRC and DoH programme grant is concerned with the development of tumour vascular disrupting agents such as the combretastatins. Here we show how a mass spectrometric strategy combining conventional solution based proteomics with MALDI-MSI can be used to follow protein induction in mouse fibrosarcoma models after treatment.

Future work combining MALDI-MSI with MRI to create 3D images of the distribution of multiple compounds in is discussed along with quantification strategies and methods for the validation of MALDI-MSI data.

11th East Midlands Proteomics Workshop: Poster Presentation Abstracts

1. Sample classification and identification of putative biomarkers from mass spectrometry datasets using machine learning

A. L. Swan[1], K. L. Hillier[1], J. R. Smith[2], D. Allaway[3], S. Liddell[1], A. Mobasheri[1], J. Bacardit[1]

[1]University of Nottingham, UK; [2]Bruker UK Limited, Coventry, UK; [3]WALTHAM® Centre for Pet Nutrition, Waltham-on-the-Wolds, UK

The identification of biomarkers is important across a range of biological functions and diseases. Proteomics data generated by high throughput mass spectrometry are suitable for developing machine learning methods, as they provide a semi-quantitative analysis of a diverse range of proteins in samples. A method was developed to classify MS/MS data from secretome samples of an in vitro explant model of synovial joint inflammation, using canine articular cartilage explants, from animals euthanased for reasons other than research. Mascot was used to identify proteins, which were verified by ProteinProphet, and to quantify proteins using emPAI. The rule-based machine learning system BioHEL was used to identify possible biomarkers by classifying data from four treatment classes. To reduce the number of proteins for consideration as potential inflammatory biomarkers an iterative process of protein attribute removal was performed; proteins were returned to the dataset only if, without them, the classification accuracy decreased. In this way the dataset was reduced to a small number of proteins, vital for an accurate classification and therefore may be suitable for use as putative biomarkers. To determine their suitability as biomarkers any proteins identified in this way require further investigation using bioinformatics and other in vitro and in vivo models.

2. Application of HDMSE for the discovery of prognostic biomarkers in Acute Heart Failure

J Auluck[1], LL Ng[2] and DJL Jones[1]

[1]Dept of Cancer Studies and Molecular Medicine and [2]Dept of Cardiovascular Sciences, Leicester Royal Infirmary, University of Leicester, LE2 7LX

Background: Heart failure is a predominant disease of the Western world and is associated with high rates of morbidity and mortality. Current biomarkers suffer from poor levels of accuracy and specificity. Therefore, accurate, reproducible and reliable biomarkers are needed.
In this study we have profiled 100 age and sex matched acute heart failure patients, 50 survivors vs. 50 major adverse events to discern any changes in the protein profiles. Bioinformatic analysis has been undertaken to identify potential prognostic markers of disease.
Method: Plasma protein profiles from 100 acute heart failure patients (50 survivors vs. 50 major adverse events) were obtained using a Waters Synapt G2 HDMS mass spectrometer post plasma ProteominerTM enrichment (Bio-Rad) and 2D-RP-RP fractionation. Statistical comparisons of the protein profiles were made to identify potential candidates for biomarkers.
Results: Individual samples were analysed using PLGS 2.5.2 to identify and quantify the proteins in the samples. Progenesis LC MS was used for statistical analysis and data visualisation. Using a label-free 2D LC-HDMSE experiment we have found that differences in protein expression of acute heart failure patients. Proteins identified have been shown to be physiologically relevant.
Conclusions: Candidate biomarkers will be further verified using LC-MS/MS-SRM strategies on a Waters Xevo triple quadrupole mass spectrometer which is aided by the use of VerifyE and Skyline.

3. LC MS/MS analysis of proteolytic peptides obtained from dried blood spots through automated liquid extraction surface analysis and tryptic digestion.

Nicholas J. Martin[1], Josephine Bunch[2] and Helen J. Cooper[1]

[1]School of Biosciences, [2]School of Chemistry, University of Birmingham, Birmingham, United Kingdom

Analysis of dried blood spots by mass spectrometry is commonplace in both newborn screening programmes and drug development studies in pharmaceutical research. To date, the majority of analytes under routine investigation are metabolites and small molecule organic drugs. In contrast there are very few studies that have attempted to analyse the proteins in dried blood spots.
Here we describe a method for identifying large numbers of proteins from tryptic peptides derived from dried blood spots. The method involves liquid extraction surface analysis based direct surface sampling followed by a fully automated trypsin digestion using an Advion Triversa Nanomate. LC MS/MS analysis was performed using a Thermo Fisher LTQ Orbitrap Velos ETD mass spectrometer coupled to a Dionex Ultimate 3000 nano flow HPLC unit.

4 High throughput proteomic analysis of the cartilage secretome for identification of inflammatory biomarkers

A. Williams[1], J.R. Smith[2], D. Allaway[3], P. Harris[3], S. Liddell[1], A. Mobasheri[1]

[1]University of Nottingham, Sutton Bonington, UK, [2]Bruker UK Limited, Coventry, UK, [3]WALTHAM® Centre for Pet Nutrition, Waltham-on-the-Wolds, UK

Biomarkers of cartilage inflammation and degradation can provide insights into the pathogenesis of arthritic diseases, allowing earlier and improved diagnostic monitoring. Utilizing high throughput proteomics approaches, this study aimed to identify proteins responding to inflammatory and anti-inflammatory stimuli in the secretome of articular cartilage.

Explant cultures were treated as follows: untreated (control) media alone, pro-inflammatory cytokine IL-1β (10 ng/ml), the COX-2 specific non-steroidal anti-inflammatory drug carprofen (100 µg/ml) or IL-1β + carprofen. After 6 days incubation (37°C, 5% CO2) media was collected. Following SDS-PAGE and silver staining analysis, selected samples were analyzed by nanoLC-MS/MS on an amaZon speed ETD (Bruker).

High throughput mass spectrometry analysis identified extracellular matrix proteins (proteoglycans, COMP, collagen type II), secreted proteins related to cartilage maintenance and turnover (MMP-1, -3 and -13, TIMP-1), along with inflammatory stress/molecular chaperone related proteins (clusterin). Qualitative differences observed between untreated and cytokine treated explant secretomes included, PCPE-2, frizzled related protein, heat shock protein 70kDa, MMP-1, -3 and -13. Western blots provided quantitative analysis confirming carprofen treatment significantly reduced IL-1β stimulated release of MMP-1, -3 and -13. Proteins differentially released by inflammatory stimulation within this explant model may enable screening of drugs and natural compounds with anti-inflammatory properties.

5. Multiplexed protein analysis for doping control in horseracing using liquid-chromatography-tandem mass spectrometry

P. Taylor, J. Scarth, P. Teale, P. Brown

HFL Sport Science, LGC Ltd, Newmarket Road, Fordham, Cambridgeshire, CB7 5WW

Protein therapeutics represent a major class of doping agents with the potential for abuse in the horseracing industry. The traditional approach to the control of doping with large molecules has been the use of multiple immunoassays to provide initial screening for exogenous agents, such as the various recombinant human erythropoietins (EPOs). Whilst an effective screening tool, immunoassays are relatively expensive, particularly so when multiple assays are required to achieve maximum analyte coverage. In recent years, significant advances have been made in the application of mass spectrometry to the analysis of proteins in biological matrices. Continuing advances in such technologies are making the application of multiplexed liquid chromatography-mass spectrometry (LC-MS) screens for protein based doping agents a feasible concept.

Currently, research into the potential for a multiplexed LC-MS protein/peptide screening method is being undertaken by HFL Sport Science. Initially, a single LC-MS/MS method for a wide range of proteins and peptides was developed. The current focus is the development of extraction methods for the target analytes in equine biological matrices, which is anticipated to include a solid phase extraction (SPE) approach for the smaller peptides and an immunoaffinity extraction followed by tryptic digestion for the larger protein analytes of interest. Multiplexing will then be achieved by merging individual methods into as few screening streams as possible without significant compromise to the detection of each target molecule.

6. THE IDENTIFICATION OF PROSTATE CANCER ASSOCIATED ANTIGENS AND BIOMARKERS

Abiye Yvonne Dede, David Boocock, Amanda Miles, Robert Rees

The John van Geest Cancer Research Centre, Nottingham Trent University, Clifton, Nottingham NG11 8NS

PROTEOMEX, a method of tumour associated antigen (TAA) identification, combines separation of proteins by 2D gel electrophoresis (2DE) with screening using serum antibodies. Proteins specifically reacting with serum antibodies from patients, may serve as potential TAA candidates following additional investigation.

The proximity of the bladder to the prostate gland increases the likelihood that TAAs in prostate cancer patients will be shed into the urine. Furthermore, urine is considered an ideal biomarker source because it is non-invasive and easily obtained from most patients. This project aims to identify TAA for PCa in urine samples.

In pilot experiments, a small number of urine proteins were common to both PCa and the control group when probed with matched PCa serum. However, differentially expressed proteins unique to PCa urine were observed when probed with the same serum. These proteins were digested and identified by LC/MSMS. One of the proteins identified in PCa urine samples and verified by Western Blotting was an existing PCa biomarker - human Prostatic Acid Phosphatase (hPAP), indicating that hPAP may induce an immune response in PCa, and thus be a valid biomarker for PCa. Current work is on-going to optimise the separation of urinary proteins on 2DE before probing with serum from PCa patients; to identify additional PCa associated TAAs.

7. Identification of candidate markers associated with the tumorigenic potential of the prostate cancer cell line (OPCT1) and derived sub-populations using LC-MALDI- TOF Mass Spectrometry

Divya Nagarajan, Sumanjeet Malhi, David Boocock, Baharak Vafadar-Isfahani, Amanda Miles, Robert Rees

The John van Geest Cancer Research Centre, Nottingham Trent University, Clifton, Nottingham NG11 8NS

Prostate cancer (PCa) is the second most common type of cancer found in men and good prognostic and diagnostic biomarkers are very limited. This research focused on biomarker discovery in the OPCT1 (PCa cell line) and three OPCT-1 derived sub-populations (T21 high/CD44 high, T21 high/CD44 low, T21 low/CD44 high), identified by immunofluorescence, that show differential CD44 and T21 protein expression. Previous studies on both the role of CD44 expression in prostate cancer and T21 as a novel prostate cancer antigen has suggested their role in tumour progression and metastasis (CD44) and prostate cancer stage (T21). Here, we aimed to identify proteins capable of differentiating between these cell populations by generating protein profiles using LC-MALDI-MS technique. Protein extracts from cells were tryptically digested and peptides fractionated using reversed phase LC-MALDI-MS with targeted MS/MS. Following bioinformatics analysis 14 differentially expressed proteins were identified.
These 4 cell populations were separately injected into NOD/SCID mice and their tumourigenicity was observed. The most tumourigenic was found to be the T21 high/CD44 high (F1) population. A number of interesting proteins implicated in the actin cytoskeleton were identified. Several studies have indicated that the expressions of molecules linked with dynamic actin cytoskeleton are up-regulated in metastatic and invasive tumour cells.

8. DETECTING DELLA: PROTEOMIC ANALYSIS OF THE GA REGULATORY PATHWAY

Richard D. Elms[1], Malcolm J. Bennett[1], Markus R. Owen[2], Neil J. Oldham[3]

[1]Division of Plant and Crop Sciences, School of Biosciences, [2]School of Mathematical Sciences, and [3]School of Chemistry, University of Nottingham, UK

Gibberellins (GA) are key regulators of plant growth and development, their biosynthetic and signal transduction pathways have been well characterised in Arabidopsis. GA growth and signalling is regulated by repressor proteins; DELLA proteins. DELLAs are the functional mechanism of GA signal repression. The data presented will look to parameterise a recently published multi-scale model describing GA action, perception and biosynthesis (Middleton et al., 2012) using advanced and classical proteomic techniques. The method utilised in this study allows quantification of the levels of DELLA and closely associated proteins in roots. Using recombinantly expressed isotopically-labelled standard proteins, chromatographic separation coupled with a high resolution mass spectrometer allowed the identification of proteins from a complex total protein extraction.

9. THE INTERACTOME OF TRANSGLUTAMINASE-2 (TG2) IN KIDNEY MEMBRANES

Alessandra Scarpellini[1], Linghong Huang[2], Baharak Vafadar-Isfahani[3], David Boocock[3], Timothy S Johnson[2] and **Elisabetta AM Verderio**[1]

[1]Biomedical, Life and Health Sciences Research Centre and [3]John van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK,
[2] Academic Nephrology Unit & Sheffield Kidney Institute, University of Sheffield, Sheffield

Introduction: Chronic overexpression and cellular secretion of TG2 has been implicated in the pathogenesis of kidney scarring. Building the TG2 interactome in kidney cell membranes is not only a prerequisite to delineate the molecular mechanisms underlying the physiopathological role of extracellular TG2, but also to form a hypothesis on its export pathway in kidney.
Methods: We have combined TG2 immunoprecipitation (IP) with isobaric Tag for Relative and Absolute Quantitation (iTRAQ) and LC−MALDI-TOF/TOF. TG2-associated proteins were isolated by IP of crude kidney membranes from either wild-type (WT) or control TG2-knock-out (KO) mice. Both immunoprecipitates were digested, differentially labelled with isobaric tags and analysed via nanoLC-MS/MS combined with Mascot mouse database search.
Results: 24 proteins were identified as specifically associated to TG2. The presence of the heparan sulphate (HS) proteoglycan Perlecan in the TG2 network is consistent with our prior data that TG2 extracellular trafficking depends on binding to HS chains. Collagen XVIII/Endostatin, the basement membrane glycoproteins Nidogen-1/2 and Clathrin, involved in vesicle-mediated TG2 trafficking, also emerged as consistent TG2 partners.
Conclusions: We have identified novel molecular partners of TG2 in kidney membranes which may be responsible for TG2 extracellular trafficking/membrane targeting in kidney.

Acknowledgements: Work supported by Wellcome Trust project [087163]

10. Investigation of pluripotency-related protein complexes in embryonic environments

Choulia Mola[1], Susan Liddell[1], Andrew Johnson[2], and Ramiro Alberio[1]

School of Biosciences[1], and School of Biology[2], University of Nottingham, UK

Cell differentiation occurs through modifications on the surface of the genome that lead into differential gene regulation, which gives rise to multiple cell lineages. Differentiated cells can be induced to leave the differentiated state and enter pluripotency. There are molecules associated with pluripotency that can act both as regulators and indicators of pluripotency. Nanog, Oct4 and Sox2 are the most studied pluripotency factors and appear to act synergistically in order to promote the pluripotent phenotype. The precise network and mechanism of the pluripotency factors system remains to be unveiled.

The present project aims to identify molecules participating in the pluripotency network, test their biological activity in cell cultures and distinguish factors to be used in the iPS technology. Nanog is used as bait for the co-immunoprecipitation and identification, through Mass spectrometry, of Nanog-related factors in embryonic cell lysates. Chromatographic fractions of the lysate will be assayed for their ability to induce the pluripotent character, in order to isolate and identify the factors responsible for reprogramming. The gene sequences corresponding to the candidate molecules will be used for cell transductions, for the improvement of the present iPS system.