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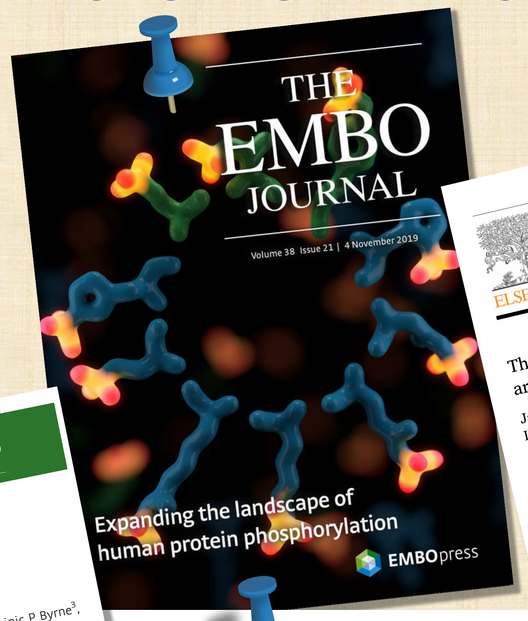

Resource

Strong anion exchange-mediated phosphoproteomics reveals extensive human non-canonical phosphorylation

Gemma Hardman[1,†], Simon Perkins[2,†], Philip J Brownridge[1], Christopher J Clarke[2], Dominic P Byrne[3], Amy E Campbell[2], Anton Kalyuzhnyy[2], Ashleigh Myall[2], Patrick A Eyers[3], Andrew R Jones[2] &, Claire E Eyers[1,*]

Abstract

Phosphorylation is a key regulator of protein function under (patho)physiological conditions, and defining site-specific phosphorylation is essential to understand basic and disease biology. In vertebrates, the investigative focus has primarily been on serine, threonine and tyrosine phosphorylation, but mounting evidence suggests that phosphorylation of other "non-canonical" amino acids also regulates critical aspects of cell biology. However, standard methods of phosphoproteomic characterisation are largely unsuitable for the analysis of non-canonical phosphorylation due to their relative instability under acidic conditions and/or elevated temperature. Consequently, the complete landscape of phosphorylation remains unexplored. Here, we report an unbiased phosphopeptide enrichment strategy based on strong anion exchange (SAX) chromatography (UPAX), which permits identification of histidine (His), arginine (Arg), lysine (Lys), aspartate (Asp), glutamate (Glu) and cysteine (Cys) phosphorylation sites on human proteins by mass spectrometry-based phosphoproteomics. Remarkably, under basal conditions, and having accounted for false site localisation probabilities, the number of unique non-canonical phosphosites is approximately one-third of the number of observed canonical phosphosites. Our resource reveals the previously unappreciated diversity of protein phosphorylation in human cells, and opens up avenues for high-throughput exploration of non-canonical phosphorylation in all organisms.

Keywords mass spectrometry; non-canonical phosphorylation; phosphosite; phosphoproteomics; strong anion exchange chromatography
Subject Categories Methods & Resources; Post-translational Modifications & Proteolysis
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The synovial fluid proteome differentiates between septic and nonseptic articular pathologies

James R. Anderson[a], Aibek Smagul[b], Deborah Simpson[b], Peter D. Clegg[a], Luis M. Rubio-Martinez[c,1], Mandy J. Peffers[a,*,1]
[a] Institute of Ageing and Chronic Disease, William Henry Duncan Building, 6 West Derby Street, Liverpool L7 8TX, UK
[b] Centre for Proteome Research, Institute of Integrative Biology, Biosciences Building, Crown Street, University of Liverpool, Liverpool L69 7ZB, UK
[c] Department of Equine Clinical Studies, Institute of Veterinary Science, Chester High Road, Neston CH64 7TE, UK

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Keywords:
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ABSTRACT

Articular conditions are common in horses and can result in loss of function, chronic pain and/or inability to work. Common conditions include osteoarthritis, osteochondrosis and synovial sepsis, which can be life-threatening, but despite the high clinical prevalence of these conditions, rapid and specific diagnosis, monitoring and prognostication remains a challenge for practicing veterinarians. Synovial fluid from a range of arthropathies was enriched for low abundance proteins using combinatorial peptide ligand ProteoMiner™ beads and analysed via liquid chromatography-tandem mass spectrometry. Changes in protein abundances were analysed using label-free quantification. Principle component analysis of differentially expressed proteins identified groupings associated with joint pathology. Findings were validated using ELISA. Lactotransferrin (LTF) abundance was increased in sepsis compared to all other groups and insulin-like growth factor-binding protein 6 (IGFBP6) abundance decreased in sepsis compared to other disease groups. Pathway analysis identified upregulation of the complement system in synovial joint sepsis and OC compared to the healthy group. Overall, we have identified a catalogue of proteins which we propose to be involved in osteoarthritis, osteochondrosis and synovial sepsis pathogenesis.
Significance: Osteoarthritis, osteochondrosis and synovial sepsis, which can be life-threatening, are common articular conditions in which rapid and specific diagnosis, monitoring and prognostication remains a challenge for practicing veterinarians. This study has identified that the equine synovial fluid proteome exhibits distinctive profile changes between osteoarthritis, osteochondrosis, synovial sepsis and healthy joints. Elevated synovial abundance of lactotransferrin and decreased insulin-like growth factor-binding protein 6 were both found to distinguish synovial sepsis from all other study groups. Thus, these protein markers may have a future role in clinical practice to enable an earlier and reliable diagnosis of synovial sepsis.

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METHODOLOGY ARTICLE — Open Access — BMC Biology

Rapid identification of species, sex and maturity by mass spectrometric analysis of animal faeces

Nicola B. Davidson[1†], Natalie I. Koch[2†], Joscelyn Sarsby[2†], Emrys Jones[3], Jane L. Hurst[1*] and Robert J. Beynon[2*]

Abstract

Background: We describe a new approach to the recovery of information from faecal samples, based on the analysis of the molecular signature generated by rapid evaporative ionisation mass spectrometry (REIMS).
Results: Faecal pellets from five different rodent species were analysed by REIMS, and complex mass spectra were acquired rapidly (typically a few seconds per sample). The uninterpreted mass spectra (signatures) were then used to seed linear discriminant analysis and classification models based on random forests. It was possible to classify each species of origin with a high rate of accuracy, whether faeces were from animals maintained under standard laboratory conditions or wild-caught. REIMS signatures were stable to prior storage of the faecal material under a range of different conditions and were not altered rapidly or radically by changes in diet. Further, within species, REIMS signatures could be used to discriminate faeces from adult versus juvenile mice, male versus female mice and those from three different laboratory strains.
Conclusions: REIMS offers a completely novel method for the rapid analysis of faecal samples, extending faecal analysis (previously focused on DNA) to an assessment of phenotype, and has considerable potential as a new tool in the armamentarium of the field biologist.

Keywords: Faecal analysis, Faeces, Mass spectrometry, Species identification, Rodents

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Quantitative Proteomics of Enriched Esophageal and Gut Tissues from the Human Blood Fluke *Schistosoma mansoni* Pinpoints Secreted Proteins for Vaccine Development

Leandro X. Neves,[†] R. Alan Wilson,[‡] Philip Brownridge,[§] Victoria M. Harman,[§] Stephen W. Holman,[§] Robert J. Beynon,[§] Claire E. Eyers,[§] Ricardo DeMarco,[∥] and William Castro-Borges*[,†]

[†]Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto, Campus Morro do Cruzeiro, Ouro Preto 35400-000, Minas Gerais, Brazil
[‡]Centre for Immunology and Infection, Department of Biology, University of York, Heslington, York YO10 5DD, United Kingdom
[§]Centre for Proteome Research, Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom
[∥]Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos 13566-590, Brazil

Supporting Information

ABSTRACT: Schistosomes are blood-dwelling helminth parasites that cause schistosomiasis, a debilitating disease resulting in inflammation and, in extreme cases, multiple organ damage. Major challenges to control the transmission persist, and the discovery of protective antigens remains of critical importance for vaccine development. Rhesus macaques can self-cure following schistosome infection, generating antibodies that target proteins from the tegument, gut, and esophagus, the last of which is the least investigated. We developed a dissection technique that permitted increased sensitivity in a comparative proteomics profiling of schistosome esophagus and gut. Proteome analysis of the male schistosome esophagus identified 13 proteins encoded by microexon genes (MEGs), 11 of which were uniquely located in the esophageal glands. Based on this and transcriptome information, a QconCAT was designed for the absolute quantification of selected targets. MEGs 12, 4.2, and 4.1 and venom allergen-like protein 7 were the most abundant, spanning over 245 million to 6 million copies per cell, while aspartyl protease, palmitoyl thioesterase, and galactosyl transferase were present at <1 million copies. Antigenic variation by alternative splicing of MEG proteins was confirmed together with a specialized machinery for protein glycosylation/secretion in the esophagus. Moreover, some gastrodermal secretions were highly enriched in the gut, while others were more uniformly distributed throughout the parasite, potentially indicating lysosomal activity. Collectively, our findings provide a more rational, better-oriented selection of schistosome vaccine candidates in the context of a proven model of protective immunity.

KEYWORDS: *Schistosoma mansoni, esophageal gland, gastrodermis, microexon genes, quantitative proteomics, QconCAT*

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Research Article

Glycolytic flux in *Saccharomyces cerevisiae* is dependent on RNA polymerase III and its negative regulator Maf1

Roza Szatkowska[1], Manuel Garcia-Albornoz[2,*], Katarzyna Roszkowska[1], Stephen W. Holman[3,†], Emil Furmanek[1], Simon J. Hubbard[2], Robert J. Beynon[3] and Malgorzata Adamczyk[1]
[1]Chair of Drug and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland; [2]Division of Evolution & Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, U.K.; [3]Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, Liverpool, U.K.
Correspondence: Malgorzata Adamczyk (madamczyk@ch.pw.edu.pl)

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Protein biosynthesis is energetically costly, is tightly regulated and is coupled to stress conditions including glucose deprivation. RNA polymerase III (RNAP III)-driven transcription of tDNA genes for production of tRNAs is a key element in efficient protein biosynthesis. Here we present an analysis of the effects of altered RNAP III activity on the *Saccharomyces cerevisiae* proteome and metabolism under glucose-rich conditions. We show for the first time that RNAP III is tightly coupled to the glycolytic system at the molecular systems level. Decreased RNAP III activity or the absence of the RNAP III negative regulator, Maf1 elicit broad changes in the abundance profiles of enzymes engaged in fundamental metabolism in *S. cerevisiae*. In a mutant compromised in RNAP III activity, there is a repartitioning towards amino acids synthesis *de novo* at the expense of glycolytic throughput. Conversely, cells lacking Maf1 protein have greater potential for glycolytic flux.

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RESEARCH ARTICLE

The impact of postsynaptic density 95 blocking peptide (Tat-NR2B9c) and an iNOS inhibitor (1400W) on proteomic profile of the hippocampus in C57BL/6J mouse model of kainate-induced epileptogenesis

Karen Tse[1,2] | Dean Hammond[3] | Deborah Simpson[4] | Robert J Beynon[4] | Edward Beamer[1] | Michael Tymianski[5] | Michael W Salter[5] | Graeme J Sills[2] | Thimmasettappa Thippeswamy[1]

[1]Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK
[2]Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK
[3]Department of Molecular and Cellular Physiology, Institute of Integrative Biology, University of Liverpool, Liverpool, UK
[4]Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, Liverpool, UK
[5]Department of Physiology and Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada

Correspondence
Graeme J Sills, School of Life Sciences, University of Glasgow, Sir James Black Building, Glasgow G12 8QQ, UK.
Email: graeme.sills@glasgow.ac.uk

Thimmasettappa Thippeswamy, Epilepsy Research Laboratory, Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IW 50011-1250.
Email: tswamy@iastate.edu

Present address
Graeme J Sills, School of Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK
Thimmasettappa Thippeswamy, Epilepsy Research Laboratory, Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IW 50011-1250
Karen Tse, Drug Safety & Metabolism, AstraZeneca, Babraham Institute, c/o Darwin Building, 310 Cambridge Science Park, Milton Road, Cambridge, CB4 0WG, UK
Edward Beamer, Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, 123 St Stephen's Green, Dublin 2, Ireland

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Chapter 18

Myoblast Phosphoproteomics as a Tool to Investigate Global Signaling Events During Myogenesis

Fiona K. Jones, Gemma E. Hardman, Samantha Ferries, Claire E. Eyers, and Addolorata Pisconti

Abstract

Protein phosphorylation is a universal covalent chemical modification of amino acids involved in a large number of biological processes including cell signaling, metabolism, proliferation, differentiation, survival/death, ageing, and many more. Regulation of protein phosphorylation is essential in myogenesis and indeed, when the enzymatic activity of protein kinases is disrupted in myoblasts, myogenesis is affected. In this chapter we describe a method to profile the phosphoproteome of myoblasts using mass spectrometry. Phosphate groups are labile and easily lost during the processing of samples for mass spectrometry. Thus, effective methods to enrich for phosphopeptides from protein extracts have been developed. Here, we discuss and present in detail two such methods that we routinely employ. These methods are based on a sample enrichment step performed on titanium dioxide matrices followed by label-free tandem mass spectrometry and semi-quantitation.

Key words Myogenesis, Phosphoproteomics, Myoblasts, Muscle stem cells, Label-free proteomics, Phosphorylation

PLOS ONE

RESEARCH ARTICLE

The heparin-binding proteome in normal pancreas and murine experimental acute pancreatitis

Quentin M. Nunes[1☯*], Dunhao Su[1,2☯], Philip J. Brownridge[2,3], Deborah M. Simpson[2,3], Changye Sun[2,4], Yong Li[2,5], Thao P. Bui[2], Xiaoying Zhang[1], Wei Huang[1,6], Daniel J. Rigden[2], Robert J. Beynon[2,3], Robert Sutton[1], David G. Fernig[1,2]

1 Liverpool Pancreatitis Research Group, Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom, **2** Department of Biochemistry, Institute of Integrative Biology, Biosciences Building, University of Liverpool, Liverpool, United Kingdom, **3** Centre for Proteome Research, Institute of Integrative Biology, Biosciences Building, University of Liverpool, Liverpool, United Kingdom, **4** Henan Key Laboratory of Medical Tissue Regeneration, Xinxiang Medical University, Xinxiang, Henan, China, **5** College of Life and Environmental Science, Wen Zhou University, Wenzhou, China, **6** Department of Integrated Traditional Chinese and Western Medicine, Sichuan Provincial Pancreatitis Centre and West China-Liverpool Biomedical Research Centre, West China Hospital, Sichuan University, Chengdu, China

☯ These authors contributed equally to this work.
* Quentin.Nunes@liverpool.ac.uk

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Data Availability Statement: The mass spectrometry proteomics data are available to the public and have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (http://www.ebi.ac.uk/pride/archive/) with the following dataset identifiers / accession numbers: 1. Pancreas HBPs PXD001950 https://www.ebi.ac.uk/pride/archive/projects/PXD001950; 2. Plasma HBPs PXD012039 https://www.ebi.ac.uk/pride/archive/projects/PXD012039; jsessionid=E93041199B7ED72C461249E49018AC43.

Abstract

Acute pancreatitis (AP) is acute inflammation of the pancreas, mainly caused by gallstones and alcohol, driven by changes in communication between cells. Heparin-binding proteins (HBPs) play a central role in health and diseases. Therefore, we used heparin affinity proteomics to identify extracellular HBPs in pancreas and plasma of normal mice and in a caerulein mouse model of AP. Many new extracellular HBPs (360) were discovered in the pancreas, taking the total number of HBPs known to 786. Extracellular pancreas HBPs form highly interconnected protein-protein interaction networks in both normal pancreas (NP) and AP. Thus, HBPs represent an important set of extracellular proteins with significant regulatory potential in the pancreas. HBPs in NP are associated with biological functions such as molecular transport and cellular movement that underlie pancreatic homeostasis. However, in AP HBPs are associated with additional inflammatory processes such as acute phase response signalling, complement activation and mitochondrial dysfunction, which has a central role in the development of AP. Plasma HBPs in AP included known AP biomarkers such as serum amyloid A, as well as emerging targets such as histone H2A. Other HBPs such as alpha 2-HS glycoprotein (AHSG) and histidine-rich glycoprotein (HRG) need further investigation for potential applications in the management of AP. Pancreas HBPs are extracellular and so easily accessible and are potential drug targets in AP, whereas plasma HBPs represent potential biomarkers for AP. Thus, their identification paves the way to determine which HBPs may have potential applications in the management of AP.

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Research Paper

Chemerin acts via CMKLR1 and GPR1 to stimulate migration and invasion of gastric cancer cells: putative role of decreased TIMP-1 and TIMP-2

J. Dinesh Kumar[1], Iman Aolymat[1], Laszlo Tiszlavicz[2], Zita Reisz[2], Hanan M. Garalla[1], Rob Beynon[3], Deborah Simpson[3], Graham J. Dockray[1] and Andrea Varro[1]

[1]Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK
[2]Department of Pathology, University of Szeged, Szeged, Hungary
[3]Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, Liverpool, UK
Correspondence to: *Andrea Varro,* ***email:*** *avarro@liverpool.ac.uk*

Keywords: *chemerin; gastric cancer; myofibroblasts; proteomic*
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ABSTRACT

The chemokine-like peptide, chemerin, stimulates chemotaxis in several cell types. In this study we examined the expression of putative chemerin receptors in gastric cancer and the action of chemerin on cancer cell migration and invasion. Immunohistochemical studies of gastric tumors identified expression of two putative receptors, chemokine-like receptor-1 (CMKLR1) and G-protein coupled receptor 1(GPR1), in cancer cells; there was also some expression in stromal myofibroblasts although generally at a lower intensity. The expression of both receptors was detected in a gastric cancer cell line, AGS; chemerin itself was expressed in cultured gastric cancer myofibroblasts but not AGS cells. Chemerin stimulated (a) morphological transformation of AGS cells characterized by extension of processes and cell scattering, (b) migration in scratch wound assays and (c) both migration and invasion in Boyden chamber chemotaxis assays. These responses were inhibited by two putative receptor antagonists CCX832 and α-NETA. Inhibition of receptor expression by siRNA selectively reduced CMKLR1 or GPR1 and inhibited the action of chemerin indicating that both receptors contributed to the functional response. Using a proteomic approach employing stable isotope dynamic labeling of secretomes (SIDLS) to selectively label secreted proteins, we identified down regulation of tissue inhibitors of metalloproteinase (TIMP)1 and TIMP2 in media in response to chemerin. When cells were treated with chemerin and TIMP1 or TIMP2 the migration response to chemerin was reduced. The data suggest a role for chemerin in promoting the invasion of gastric cancer cells via CMKLR1 and GPR1at least partly by reducing TIMP1 and TIMP2 expression. Chemerin receptor antagonists have potential in inhibiting gastric cancer progression.

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Advancing Solutions to the Carbohydrate Sequencing Challenge

Christopher J. Gray,[†] Lukasz G. Migas,[†] Perdita E. Barran,[†] Kevin Pagel,[‡] Peter H. Seeberger,[§] Claire E. Eyers,[∥] Geert-Jan Boons,[⊥] Nicola L. B. Pohl,[#] Isabelle Compagnon,[⊗,▽] Göran Widmalm,[×] and Sabine L. Flitsch*[,†]

[†]School of Chemistry & Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, U.K.
[‡]Institute for Chemistry and Biochemistry, Freie Universität Berlin, Takustraße 3, 14195 Berlin, Germany
[§]Biomolecular Systems Department, Max Planck Institute for Colloids and Interfaces, Am Muehlenberg 1, 14476 Potsdam, Germany
[∥]Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, U.K.
[⊥]Department of Chemistry, Indiana University, Bloomington, Indiana 47405, United States
[#]Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30602, United States
[⊗]Institut Lumière Matière, UMR5306 Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne Cedex, France
[▽]Institut Universitaire de France IUF, 103 Blvd St Michel, 75005 Paris, France
[×]Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

Supporting Information

ABSTRACT: Carbohydrates possess a variety of distinct features with stereochemistry playing a particularly important role in distinguishing their structure and function. Monosaccharide building blocks are defined by a high density of chiral centers. Additionally, the anomericity and regiochemistry of the glycosidic linkages carry important biological information. Any carbohydrate-sequencing method needs to be precise in determining all aspects of this stereodiversity. Recently, several advances have been made in developing fast and precise analytical techniques that have the potential to address the stereochemical complexity of carbohydrates. This perspective seeks to provide an overview of some of these emerging techniques, focusing on those that are based on NMR and MS-hybridized technologies including ion mobility spectrometry and IR spectroscopy.

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Molecular complexity of the major urinary protein system of the Norway rat, *Rattus norvegicus*

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Guadalupe Gómez-Baena[1], Stuart D. Armstrong[1], Josiah O. Halstead[2], Mark Prescott[1], Sarah A. Roberts[2], Lynn McLean[1], Jonathan M. Mudge[3], Jane L. Hurst[2] & Robert J. Beynon[1]

Major urinary proteins (MUP) are the major component of the urinary protein fraction in house mice (*Mus* spp.) and rats (*Rattus* spp.). The structure, polymorphism and functions of these lipocalins have been well described in the western European house mouse (*Mus musculus domesticus*), clarifying their role in semiochemical communication. The complexity of these roles in the mouse raises the question of similar functions in other rodents, including the Norway rat, *Rattus norvegicus*. Norway rats express MUPs in urine but information about specific MUP isoform sequences and functions is limited. In this study, we present a detailed molecular characterization of the MUP proteoforms expressed in the urine of two laboratory strains, Wistar Han and Brown Norway, and wild caught animals, using a combination of manual gene annotation, intact protein mass spectrometry and bottom-up mass spectrometry-based proteomic approaches. Cluster analysis shows the existence of only 10 predicted *mup* genes. Further, detailed sequencing of the urinary MUP isoforms reveals a less complex pattern of primary sequence polymorphism in the rat than the mouse. However, unlike the mouse, rat MUPs exhibit added complexity in the form of post-translational modifications, including the phosphorylation of Ser4 in some isoforms, and exoproteolytic trimming of specific isoforms. Our results raise the possibility that urinary MUPs may have different roles in rat chemical communication than those they play in the house mouse. Shotgun proteomics data are available via ProteomExchange with identifier PXD013986.

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Caught in a Trap? Proteomic Analysis of Neutrophil Extracellular Traps in Rheumatoid Arthritis and Systemic Lupus Erythematosus

Elinor A. Chapman[1], Max Lyon[1], Deborah Simpson[2,3], David Mason[2,4], Robert J. Beynon[2,3], Robert J. Moots[1,5] and Helen L. Wright[1]

[1]Department of Musculoskeletal Biology I, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom, [2]Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom, [3]Centre for Proteome Research, University of Liverpool, Liverpool, United Kingdom, [4]Centre for Cell Imaging, University of Liverpool, Liverpool, United Kingdom, [5]University of Liverpool and Aintree University Hospital, Members of Liverpool Health Partners, Liverpool, United Kingdom

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Research Paper

Covalent Aurora A regulation by the metabolic integrator coenzyme A

Yugo Tsuchiya[a,#], Dominic P. Byrne[b,#], Selena G. Burgess[c,#], Jenny Bormann[d], Jovana Baković[a], Yueyang Huang[a], Alexander Zhyvoloup[a], Bess Yi Kun Yu[a], Sew Peak-Chew[e], Trang Tran[f], Fiona Bellany[f], Alethea B. Tabor[f], AW Edith Chan[g], Lalitha Guruprasad[h], Oleg Garifulin[i], Valeriy Filonenko[i], Matthias Vonderach[j], Samantha Ferries[j], Claire E. Eyers[b,j], John Carroll[d,1], Mark Skehel[e], Richard Bayliss[c,**], Patrick A. Eyers[b,***], Ivan Gout[a,i,*]

[a] Department of Structural and Molecular Biology, University College London, London, WC1E 6BT, UK
[b] Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, L69 7ZB, UK
[c] School of Molecular and Cellular Biology, Astbury Centre for Structural and Molecular Biology, University of Leeds, Leeds, LS2 9JT, UK
[d] Department of Cell and Developmental Biology, University College London, London, WC1E 6BT, UK
[e] MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge, CB2 0QH, UK
[f] Department of Chemistry, University College London, London, WC1E 6BT, UK
[g] Wolfson Institute for Biomedical Research, University College London, London, WC1E 6BT, UK
[h] School of Chemistry, University of Hyderabad, Hyderabad, 500 046, India
[i] Department of Cell Signaling, Institute of Molecular Biology and Genetics, Kyiv 143, Ukraine
[j] Centre for Proteome Research, Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, L69 7ZB, UK

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Identification and Characterization of Canine Ligament Progenitor Cells and Their Extracellular Matrix Niche

Katie J Lee,[†] Eithne J Comerford,[†,‡] Deborah M Simpson,[§] Peter D Clegg,[†,‡,∥] and Elizabeth G Canty-Laird*[,†,∥]

[†]Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, William Henry Duncan Building, 6 West Derby Street, Liverpool, L7 8TX, United Kingdom
[‡]School of Veterinary Science, Leahurst Campus, University of Liverpool, Chester High Road, Neston, CH64 7TE, United Kingdom
[§]Centre for Proteome Research, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom
[∥]The MRC-Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA), Liverpool L7 8TX, United Kingdom

Supporting Information

ABSTRACT: Ligaments are prone to injury and degeneration in humans and animals, however the healing potential of ligament is poor and current treatment options ineffective. Stem cell-based therapies hold potential for treatment of ligament injuries. This study aimed to characterize a ligament progenitor cell (LPC) population and to identify specific niche components which could promote the survival and function of LPCs. LPCs were isolated from canine cranial cruciate ligament and characterized for clonogenicity, multipotency and marker expression. The extracellular matrix (ECM) composition was characterized by the novel application of a metabolic labeling and mass spectrometry technique. LPCs demonstrated different clonogenicity, multipotency, and stem cell marker expression. A number of different collagens, glycoproteins, and proteoglycans were identified in the LPC niche using proteomics. Metabolic labeling of cells demonstrated unique turnover profiles for distinct ECM protein groups, indicating the importance of certain niche components for LPC survival and function. The newly synthesized niche components identified in this study could be exploited to aid identification of LPCs and to promote their survival and function for potential ligament repair strategies.

KEYWORDS: *ligament, matrix, proteomics, turnover, stem cells*

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Landscape of heart proteome changes in a diet-induced obesity model

Danielle F. Vileigas[1*], Victoria M. Harman[2], Paula P. Freire[3], Cecília L. C. Marciano[1], Paula G. Sant'Ana[2], Sérgio L. B. de Souza[1], Gustavo A. F. Mota[1], Vitor L. da Silva[1], Dijon H. S. Campos[2], Carlos R. Padovani[4], Katashi Okoshi[2], Robert J. Beynon[2], Lucilene D. Santos[3] & Antonio C. Cicogna[1*]

Obesity is a pandemic associated with a high incidence of cardiovascular disease; however, the mechanisms are not fully elucidated. Proteomics may provide a more in-depth understanding of the pathophysiological mechanisms and contribute to the identification of potential therapeutic targets. Thus, our study evaluated myocardial protein expression in healthy and obese rats, employing two proteomic approaches. Male *Wistar* rats were established in two groups (n = 13/group): control diet and Western diet fed for 41 weeks. Obesity was determined by the adipose index, and cardiac function was evaluated *in vivo* by echocardiogram and *in vitro* by isolated papillary muscle analysis. Proteomics was based on two-dimensional gel electrophoresis (2-DE) along with mass spectrometry identification, and shotgun proteomics with label-free quantification. The Western diet was efficient in triggering obesity and impaired contractile function *in vitro*; however, no cardiac dysfunction was observed *in vivo*. The combination of two proteomic approaches was able to increase the cardiac proteomic map and to identify 82 differentially expressed proteins involved in different biological processes, mainly metabolism. Furthermore, the data also indicated a cardiac alteration in fatty acids transport, antioxidant defence, cytoskeleton, and proteasome complex, which have not previously been associated with obesity. Thus, we define a robust alteration in the myocardial proteome of diet-induced obese rats, even before functional impairment could be detected *in vivo* by echocardiogram.

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