SPS PhD Students' Symposium

December 1st & 2nd, 2010

Biozentrum, Basel, Switzerland

Contact
Email:
symposium@swissproteomicsociety.org
Website:
http://www.swissproteomicsociety.org/symposium/2010.html

Organizing Committee
Prasad Kolla (Basel)
Domitille Schvartz (Geneva)
Natalia Tiberti (Geneva)
Hung Viet Trinh (Zurich)

Swiss Proteomics Society SPS

SPS PhD Students' Symposium

1st of DECEMBER 2010			
12h30-13h20	REGISTRATION - POSTER INSTALLATION - **WELCOME COFFEE**		
	PTMS and Quantitative proteomics Chair: Prasad Kolla	13h20-14h00	**Invited Speaker Prof. Erich Nigg** - Cell cycle control of chromosome segretation
		14h00-14h20	**Tarun Chopra** - *Analysis of metabolic regulatory mechanisms operating in mycobacteria*
		14h20-14h40	**Alessio Cremonesi** - *Characterization of the Yeast Rapamycin-sensitive Phosphoproteome*
		14h40-15h00	**Maria Ramirez Boo** - *Human hemolysate glycated proteome*
		15h00-15h20	**Jovan Simicevic** - *Novel proteomic approach for the absolute quantification of transcription factors controlling adipogenesis*
15h20-15h50	**COFFEE BREAK**		
	Quantitative proteomics and systems biology Chairs: Domitille Schvartz and Natalia Tiberti	15h50-16h30	**Invited Speaker Dr. Paola Picotti** - Quantitative targeted proteomics for the analysis of cellular networks
		16h30-16h50	**Aurelie Gueho** - *Characterisation of M. marinum niches : establishment of an isolation procedure*
		16h50-17h10	**Dev Sriranganadane** - *Comparison of proteolytic system secreted in Dermatophytes and Aspergillus fumigatus used as a reference*
		17h10-17h30	**Domitille Schvartz** - *Neuronal Pentraxin 1, A new pancreatic beta-cell protein, is a potential mediator of glucotoxicity induced apoptosis*
		17h30-17h50	**Hung Trinh** - *Quantitative Proteomics Analysis of Human Adenovirus Infections by iTRAQ-8plex*
18h00-19h30	**POSTER SESSION + APERO**		
20h00 -	**DINER and SOCIAL EVENT**		

2nd of DECEMBER 2010			
	Biomarkers discovery and clinical application Chair: Hung Trinh	9h00-9h40	**Invited Speaker Prof. Albert Heck** -The human phosphoproteome: promises, deliverables and challenges
		9h40-10h00	**Anne Zufferey** - *High on-treatment platelet reactivity in aspirin-treated cardiovascular patients - A first step to a system biology approach*
		10h00-10h20	**Olivier Rubin** - *Blood microparticles in erythrocyte concentrates*
10h20-10h50	**COFFEE BREAK**		
	Biomarkers discovery and clinical application Chair: Hung Trinh	10h50-11h10	**Thomas Wiedl** - *Activity-based proteomics: biomarker identification in human lung adenocarcinoma*
		11h10-11h30	**Christian Webhofer** - *Proteomic and metabolomic profiling of antidepressant drug action*
		11h30-11h50	**Virginie Licker** - *Proteomic analysis of the substantia nigra in patients with Parkinson's disease*
11h50-13h00	**LUNCH**		
	Bioinformatics Chairs: Natalia Tiberti and Domitille Schvartz	13h00-13h20	**Dominique Koua** - *Extensive conopeptide classification and prediction by bioinformatic approaches: HMM and PSSM complementarity*
		13h20-13h40	**Xavier Robin** - *How to combine biomarkers?*
		13h40-14h00	**Samuel Bader** - *Genome re-annotation reveals novel proteins in a minimal organism*
14h00-15h30	**CAREER SESSION** Chair: Hans Voshol		**Dr. Hans Voshol** (Novartis) **Dr. Paul Cutler** (Roche) **Dr. Michael Affolter** (Nestlé)
15h30-16h00	Price Award - Departure		

Invited Speaker

CELL CYCLE CONTROL OF CHROMOSOME SEGREGATION

E.A. Nigg

Biozentrum, University of Basel, CH-4056 Basel, Switzerland

The error-free segregation of duplicated chromosomes during cell division is crucial to the development and health of all organisms. In humans, extra or missing copies of chromosomes (aneuploidies) are common causes of genetic disorders and birth defects, while chromosomal instability (defined as chromosomal aberrations that change over time) is typical of many cancers and generally correlated with increased malignancy. Chromosomal instability is likely to favor not only tumor development but also the emergence of resistance to anti-cancer therapy. Many chromosome aberrations in tumor cells are thought to result from deregulation of the molecular machinery that controls chromosome segregation during cell division. Central to chromosome segregation is a highly dynamic microtubule-based structure (the mitotic spindle) whose bipolarity depends on correct numbers of microtubule organizing centers (centrosomes). Furthermore, the accurate timing of chromosome segregation depends on a surveillance mechanism, (known as spindle assembly checkpoint) that monitors the correct bipolar attachment of all chromosomes to the spindle apparatus. To understand the multiprotein complexes and regulatory circuits that control chromosome segregation in time and space, we consider the human spindle apparatus as a complex biological system and dissect this system using both comprehensive (proteomics-based) approaches, as well as in-depth analyses of individual components. Our particular emphasis is on the spindle assembly checkpoint and the centrosome duplication cycle. In both research fields, we focus on cell cycle-regulatory protein kinases, many of which are considered as potential targets for the development of novel anti-cancer therapeutics. In my talk, I will review basic aspects of the regulation of cell division and chromosome segregation, with a particular emphasis on the use of phosphor-proteomics for studying mitotic kinases and their substrates. In addition, I will briefly summarize our current understanding of the centrosome duplication cycle and the relevance of centrosome aberrations to human disease.

Invited Speaker

QUANTITATIVE TARGETED PROTEOMICS FOR THE ANALYSIS OF CELLULAR NETWORKS

Paola Picotti

ETH, Zurich

To study and model the properties of cellular networks -e.g. metabolic or signalling networks- it is crucial to measure all the elements that constitute them, which are often associated to a wide range of molecular properties and cellular abundances. However, comprehensive measurements are still technically difficult, even in a simple organism such as yeast and especially at the proteome level. To overcome the limitations of classical approaches we applied a targeted proteomic workflow based on selected reaction monitoring (SRM) to the analysis of yeast cellular networks. First, we tested the depth and sensitivity of the SRM-based approach. We demonstrated that proteins spanning the whole range of abundance, between 1.3E6 copies/cell and <50 copies/cell could be detected by SRM in yeast proteome digests. Then we applied the approach to the analysis of a yeast metabolic network. Proteins in the network were quantified by SRM in yeast grown under a series of conditions inducing radically different metabolic setups and in a growth time-course of yeast cells transiting through a series of metabolic phases. The quantitative dataset generated highlighted how yeast metabolism adapts to changing conditions of supply and demand of nutrients. It indicated that S. cerevisiae expresses superfluous proteins, not necessarily used in a particular metabolic condition and allowed to suggest differential functionality for several metabolic isoenzymes. All the SRM assays developed were deposited to the web-accesible MRMAtlas database, which supports the collection and dissemination of the assays. Finally, to overcome the bottlenecks of SRM assay development, we introduced a method based on the use of unpurified synthetic peptide libraries, that allows for the high-throughput and low-cost optimization and validation of SRM assays (>100 SRM assays/hour) for any set of proteins or proteome of interest. The approach was used to develop a complete set of SRM assays for the ~6,600 proteins that constitute the proteome of S. cerevisiae and to a first set of about 8,500 human proteins. The power and the bottlenecks of this approach will be discussed.

Invited Speaker

THE HUMAN PHOSPHOPROTEOME: PROMISES, DELIVERABLES AND CHALLENGES

Albert J.R. Heck [1,2]

[1] Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands
[2] Netherlands Proteomics Centre and Centre for Biomedical Genetics
Email: a.j.r.heck@uu.nl

At a given moment there are likely more than 500,000 phosphorylation sites in a complete cellular proteome phosphorylated. This dynamic phosphorylation is under tight control of a wide range of kinases and phosphatases. In recent years significant progress has been made in the large-scale analysis of protein phosphorylation, enabling the simultaneous temporal monitoring of thousands of phosphorylation events. Major advances in global phosphoproteomics analyses originate from technological advances, whereby possibly the largest contributions arise from more selective phosphopeptide affinity enrichment technologies and from faster, and more sensitive mass spectrometers. In this lecture I will describe the strength of, and challenges in, current state-of-the-art phosphoproteomics technologies. I will highlight and compare a several new enrichment strategies, and describe how the use of multiple proteases and electron transfer induced dissociation may enlarge the coverage of the human phosphoproteome. Finally, the targeted quantitative phosphoproteomic analyses of phosphotyrosine signaling will be described.

O1

ANALYSIS OF METABOLIC REGULATORY MECHANISMS OPERATING IN MYCOBACTERIA

Tarun Chopra and John D. Mckinney

Global Health Institute, Swiss Federal Institute of Technology (EPFL), 1015 Lausanne, Switzerland

Mycobacterium tuberculosis is the etiological agent of the disease tuberculosis. Transmission of the bacterium occurs primarily through the aerosol route, leading to its uptake by the alveolar macrophages in the lungs. A proinflammatory response is induced by the host macrophages, which results in recruitment of various immune cells and onsets the formation of granulomous structures. Within the host, the organism has a remarkable ability to stay in a dormant state and stay undetected by the host immune system. During immune compromised conditions, the bacilli are reactivated and resurgence of the disease is seen. Several studies have focused on understanding the nutritional state of the organism during infection and it is suggested that fatty acids could be the dominant carbon source utilized by the bacterium in the host. In order to survive these nutritional changes, the organism needs to adapt by re-wiring its metabolic networks. Post-translational modification of proteins is believed to be one of the important mechanisms that enable the bacterium to switch on (and switch off) certain pathways and regulate the overall metabolic flux. We investigated if this could be one of the mechanisms that promote adaptation of Mycobacterium tuberculosis and the non-pathogenic Mycobacterium smegmatis to fatty acids as a carbon source. Two dimensional gel electrophoresis and immuno-blotting studies clearly indicate that this adaptation could indeed be mediated by post-translational modification of various proteins in both bacteria. Analysis of specific metabolic pathways using biochemical and genetic studies further corroborates these findings. It is also interesting to note that in the human pathogen, Salmonella enterica, acetylation plays a key role in regulating metabolic pathways (Science 327, 1004–1007, 2010). These studies highlight the importance of post-translational modifications in dictating the overall metabolic flux and thus the survival of various pathogenic organisms in the host.

O2

CHARACTERIZATION OF THE YEAST RAPAMYCIN-SENSITIVE PHOSPHOPROTEOME

Alessio Cremonesi[1], Alexandre Soulard[1], Suzette Moes[1], Michael N. Hall[1] and Paul Jenö[1]

[1]*Biozentrum, University of Basel, Basel*

The Target of Rapamycin (TOR) is a conserved Ser/Thr kinase that controls and coordinates cell growth and metabolism in response to nutrients and cellular energy status. In yeast there are two TOR proteins, TOR1 and TOR2, which are localized to two functionally and structurally distinct complexes called TORC1 and TORC2. Whereas TORC1 can be inhibited by rapamycin, a bacterial macrocyclic lactone, TORC2 is insensitive to it. Since only few direct and indirect targets of TORC1 are known, we have developed a MS-based approach to identify novel TORC1 substrates in S. cerevisiae.

The approach took advantage of Stable Isotope Labeling with Amino acids in Cell culture (SILAC) to quantitatively analyze the yeast rapamycin-dependent phosphoproteome. Briefly, after labeling proteins in vivo with different isotopes of arginine and lysine, the proteome was fractionated by preparative SDS-PAGE. The generated tryptic peptides were subsequently enriched via IMAC and analyzed on a LTQ-Orbitrap. The data were then analyzed with MaxQuant and the results were statistically evaluated. In total four biological replicates were done.

In this way we were able to identify and quantify 2,444 different phosphorylation sites from around 1,000 different phosphoproteins covering a wide variety of cellular processes and spanning the entire range of known protein abundances. Among them, we found 55 down-regulated and 78 up-regulated phosphoproteins after rapamycin treatment. For some proteins the observed phosphorylation change has been already described previously, confirming the validity of the approach, while for other phosphoproteins further biochemical analysis to unravel their role in the context of TORC1 signaling will be presented.

To identify the protein kinases that bring about the observed changes in phosphorylation after TORC1 inhibition, the sequences surrounding the sites of phosphorylation were searched for known kinase motifs with Motif-X. For those sites whose phosphorylation was decreased by rapamycin, the PKA consensus site (RRXpS) was predominant, while it was absent in the sites that were up-regulated by rapamycin. This suggests that the TORC1 and PKA pathways are interconnected and that TORC1 modulates to a certain extent PKA activity. In vivo and in vitro data supporting this hypothesis will be presented.

O3

HUMAN HEMOLYSATE GLYCATED PROTEOME

Feliciano Priego-Capote[1], Maria Ramirez-Boo[1], Christine Hoogland[1.2], Alexander Scherl[1], Markus Müller[2], Frédérique Lisacek[2], Jean-Charles Sanchez[1]

[1]Biomedical Proteomics Research Group, Department of Structural Biology and Bioinformatics, University of Geneva, 1211 Geneva 4, Switzerland. [2]Proteome Informatics Group, CMU, 1211 Geneva 4, Switzerland.

Aim The extent and duration of hyperglycaemia are significant factors of the onset and severity of health problems frequently observed in diabetes. These disorders are often observed only several years after the development of the illness so, the glycaemic control, if not started at a very early stage of the disease, is not efficient enough to completely reduce complications. Despite significant recent advances in hyperglycaemia treatment and blood glucose monitoring, markers of glycaemic control remain in most diabetic patients a critical challenge. An important change has been the glycated haemoglobin (HbA1c) test, which evaluates the long-term glycaemic control and the risk for the development of complications in diabetes from the assessment of HbA1c in blood. Nevertheless, other measurements indicative of short to medium-term glucose perturbation are needed to understand the potential biological effect of the glucotoxicity. The aim of this work is to develop a method for the identification and quantification of glycated proteins as well as for prediction of new potential targets under different glucose conditions in blood.

Materials and methods A set of innovative approaches for analysis of glycated proteins is here presented by application to reference human hemolysates with 5, 8.3, 11.2 and 13.4% of HbA1c. Qualitative analysis was carried out by tandem mass spectrometry (MS) after endoproteinase Glu-C digestion and boronate affinity chromatography for isolation of glycated peptides. For this purpose, two MS operational modes were used: HCD-MS2 and CID-MS3 by neutral loss scan. Quantitative analysis was based on the labelling of proteins with 13C6-glucose incubation in order to evaluate the native glycated proteins labelled with 12C6-glucose. This approach enabled to differentiate glycated peptides labelled with both isotopic forms resulting from enzymatic digestion by MS (6 Da mass shift per glycation site).

Results A list of glycated proteins were detected in euglycaemic hemolysate sample. Most of identifications were targeted at the different haemoglobin chains (subunits α, β and δ). Other minor glycated proteins were detected as well (bisphosphoglycerate mutase, peroxiredoxin 6 and serum albumin, among others). In hemolysates representing poor glycaemic control, new glycated proteins were detected (taking as reference the protein list before) as well as new glycation sites (e.g. catalase (K92) and peroxiredoxin-2 (R6)). In addition, quantitative analysis resulted in a calibration curve for the preferred glycation site of haemoglobin. Similarly, other haemoglobin peptides as well as other proteins such as carbonyc anhydrase-1 and peroxidexins were monitored to study their behaviour at the different calibration glycaemic levels.

Conclusions The approach combining MS and GIL with 13C-reducing sugars here reported for determination of glycated proteins in hemolysates is a real alternative to standardized methods used by reference organisms.

Innovative aspects From a clinical point of view, this approach would be a potential method to predict the initial stage of glucotoxicity-related diseases.

O4

NOVEL PROTEOMIC APPROACH FOR THE ABSOLUTE QUANTIFICATION OF TRANSCRIPTION FACTORS CONTROLLING ADIPOGENESIS

Jovan Simicevic[1], Erik Ahrné[2], Markus Mueller[2], Sunil Kumar Raghav[1], Carine Gubelmann[1], Florence Armand[3], Diego Chiappe[3], Romain Hamelin[3], Marc Moniatte[3], Frédérique Lisacek[2] and Bart Deplancke[1]

1 Laboratory of Systems Biology and Genetics, Dept. of Bioengineering, EPFL, Lausanne, Switzerland, jovan.simicevic@epfl.ch; bart.deplancke@epfl.ch
2 Swiss Institute of Bioinformatics (SIB) Geneva, Switzerland
3 Proteomics Core Facilty, EPFL, Lausanne, Switzerland

Keywords: adipogenesis, gene regulatory network, transcription factor, SRM, absolute quantification

Most biological processes such as development, cell cycle and cell differentiation are mediated by differential gene expression, which is orchestrated by gene regulatory networks (GRNs). These GRNs consist of physical and functional interactions between transcription factors (TFs) and their target genes. The ultimate function of these regulatory networks is to coordinate and fine-tune the implementation of distinct transcription regulatory states, both in space and time. Unfortunately most models of GRNs are incomplete and their players far from completely characterized. Adipogenesis is a process by which immature pre-adipocytes develop into mature adipocytes through a cascade of gene expression events. In this context, understanding how each TF contributes to the expression output of its respective target gene during this differentiation process will help us understand how the adipogenic regulatory network behaves under different physiological or pathological conditions. Specifically, it would be of great interest to identify and quantify adipogenic TFs in absolute amounts, bridging their abundance to their regulatory capabilities. In recent years, a new technology termed Selected Reaction Monitoring (SRM) has gained popularity due to the targeted nature of its approach that allows the detection and quantification of proteins in complex samples with unmatched sensitivity and specificity. Here we present a method that focuses on the use of spectral libraries of TF peptide libraries, based on “in vitro” protein expression proteomic parameter selection, for the high-throughput development of SRM assays in biological processes of interest such as adipogenesis. This approach is particularly well suited for targeting low abundant proteins such as TFs, which are difficult to identify in a standard shotgun LC-MS experiment. The information obtained with such assays will be used to create a quantitative model of adipogenic gene regulation at the terminal stage of differentiation, providing a link between absolute TF amounts in cells and regulatory mechanisms.

O5

CHARACTERISATION OF *M. MARINUM* NICHES: ESTABLISHMENT OF AN ISOLATION PROCEDURE

Aurelie Gueho[1] and Thierry Soldati[1]

1 Thierry Soldati Laboratory, Biochemistry department, Science II, 30 Quai Ernest Ansermet, 1201 Geneva, Switzerland

Tuberculosis remains a public health issue and still kills 2 million people each year. Mycobacterium tuberculosis, the agent responsible for this disease, is able to manipulate the phagocytes of the innate immune system of its host. After uptake by phagocytosis, it stops the maturation of the phagosome where it resides and establishes a niche where it can proliferate. Mycobacterium marinum, a fish pathogen and a close cousin to M. tuberculosis, is able to infect the social amoeba Dictyostelium discoideum and arrest phagosome maturation. Dictyostelium normally uses phagocytosis for nutrition and its phagocytic pathway is very similar to the macrophage one. Therefore, we make use of this host-pathogen system to characterise the virulence mechanisms and the manipulation of the phagocytic pathway. In particular, we want to study the impact of M. marinum on the composition of the compartment that evolves into a proliferation niche.

For that, we have established a protocole to isolate pure fractions of phagosomes containing the pathogenic strain M. marinum or the non-pathogenic strain M. smegmatis or the avirulent strain M. marinum-L1D. Our protocole is based on the low density of latex. We have optimised the fixation of latex beads on different mycobacteria strains to form bacteria+beads complexes (BBCs). After verification that Dictyostelium is able to phagocytose those BBCs, we have observed that the M. marinum present in the BBCs are still infectious and that the fate of the BBC in a Dictyostelium is determined by the mycobacterium strain incorporated in the BBC. After homogeneisation of the infected cells, we recover the BBCs-containing phagosomes by flotation on a sucrose gradient.

The characterisation of isolated niches at 1hpi by immunofluorescence and western blotting shows the presence of both early (VatA) and late endosomal markers (p80, LmpA). We will also monitor the purity of the isolated niches by immunofluorescence using mitochondrial and ER markers. Using anti-mycobacteria antibodies, we observed that mycobacterial cell wall components are detected on the beads of the BBCs suggesting that they are shed by the mycobacteria. Those cell wall components at the beads surface allow the BBCs to be recognised as mycobacteria by the Dictyostelium cells. By performing immunofluorescence on infected cells, we also observed that those cell wall components are shed by the mycobacteria during infection.

Using isobaric labelling and mass spectrometry, we will study and compare the proteomic composition of those isolated phagosomes in order to identify host factors that potentially modulate resistance or susceptibility to infection. We also want to identify mycobacterial proteins expressed intraphagosomally and involved in the manipulation of phagosome maturation.

O6

COMPARISON OF PROTEOLYTIC SYSTEM SECRETED IN DERMATOPHYTES AND ASPERGILLUS FUMIGATUS USED AS A REFERENCE

Dev Sriranganadane[1], Patrice Waridel[2], Karine Salamin[1], Utz Reichard[3], Eric Grouzmann[4], Jean-Marc Neuhaus[5], Manfredo Quadroni[2], Michel Monod[1*]

[1] Department of Dermatology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland. [2] Protein Analysis Facility, Center for Integrative Genomics, University of Lausanne, Switzerland. [3] Department of Medical Microbiology and National Reference Center for Systemic Mycoses, University Hospital of Göttingen, Germany.
[4] Division de Pharmacologie et Toxicologie Cliniques, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.[5] Laboratory of Molecular and Cellular Biology, University of Neuchâtel, Neuchâtel, Switzerland.

To understand how dermatophytes (filamentous fungus) which lead to cutaneous diseases (usually find in both human and animal), degrade Keratin substrate, we decided to study another fungi, Aspergillus fumigatus. Indeed, those two fungus are really closed phylogeneticaly and Aspergillus are well known than dermatophytes. Dermatophytes and Aspergillii secrete approximately the same proteolytic enzymes. The aim of this project is to build a proteolytic mechanism model with Aspergillus and to validate dermatophytes genome predictions.

The A. fumigatus genome encodes a total of 111 proteases and 26 non-peptidases homologues (among a see MEROPS peptidase database for the A. fumigatus genome) total of 10,000 proteins (1%). Detailed analyses of all recorded proteases reveal that 46 possess a signal sequence, indicating that they could potentially be secreted.

Analysis of in vitro secreted proteases from Aspergilus fumigatus and dermatophytes reveals that there are on one side, alkaline or neutral proteases, and on the other side acidic proteases. SDS-PAGE gel shows two different secretomes according to growth medium pH. Therefore, the existence of two different pathways of protein degradation is suspected. A Shot gun proteomics analysis reveals different profiles of secreted proteases. According to growth medium pH, we discover two sets of proteases, one at low pH and another at neutral pH (data are treated by Scaffold Software). Several enzymes belonging to A. fumigatus and dermatophytes are produced in Pichia pastoris to be characterized and to understand their role in the proteolytic activity.

We conclude that A. fumigatus possesses two different pH dependent proteolytic machineries in order to assimilate short peptides and amino acids via membrane transporters during growth on protein media.

Among those proteases, we decide to focus on a S28 family protease, called AfuS28 and G1 family protease, called AfuGpA. We are interested on AfuS28 because it is suspected to be a prolyl endoprotease and is also a key enzyme for the two proteolytic machineries (acidic and neutral environment). Proline constituting a “stop” to sequential protein hydrolysis by exoproteases, AfuS28 is able to cut after this amino acid. Thus, at acidic pH, synergism of Sedolysin and AfuS28 allow N-terminal sequential degradation of large peptides generated by endoproteolysis into little peptides.

AfuGpA is interesting because he supplies the activity of another endoprotease, Pep1, an aspartic endoprotease. We show that either A. fumigatus Pep1 or AfuGpA is necessary for fungal growth in protein medium at acidic pH.

Dermatophytes secretomes are in the same way, analysed. We have shown that dermatophytes can grow at acidic pH, and secreted proteases which were different from those secreted at neutral pH. Our investigation revealed new proteases and predicts other pathways of protein degradation in dermatophytes. Genome inspection reveals many new genes encoding putative proteases with a

signal sequence. Several predicted amino acid sequences are homologues to acid proteases of the pepsin and the sedolisin families which were previously characterized in Aspergillus spp.. In addition, five secreted proteases of the deuterolysin family (M35) and new prolyl peptidases of the S28 family were predicted, but all of them are also hypothetical.

O7

NEURONAL PENTRAXIN 1, A NEW PANCREATIC BETA-CELL PROTEIN, IS A POTENTIAL MEDIATOR OF GLUCOTOXICITY INDUCED APOPTOSIS

Domitille Schvartz[1], Yannick Brunner[2], Yohann Couté[3], Claes Wollheim[4] and Jean-Charles Sanchez[1]

[1]Biomedical Proteomics Research Group, Département de Biologie Structurale et Bioinformatique, Centre Médical Universitaire, Geneva, Switzerland.
[2] Institut de Bio-Ingiénerie, Hepia, Geneva, Switzerland.
[3] Laboratoire d'Etude de la Dynamique des Protéomes, INSERM, U880, Grenoble, France.
[4] Department of cell physiology and metabolism, Centre Médical Universitaire, Geneva, Switzerland.

Keywords: Type II diabetes, Glucotoxicity, SILAC, Neuronal Pentraxin 1

Introduction

Type 2 diabetes is a metabolic disorder characterized by a defect in insulin secretion and a decrease of peripheral insulin sensitivity. Glucotoxicity, the detrimental effect of glucose, is involved in this disease progression, and has been shown to induce impairment of insulin secretion by beta-cells. We applied proteomic strategies on whole beta-cells, as well as sub-cellular fractions, to detect proteins which expression is modulated by chronic high glucose concentration.

Experimental

SILAC (with Lysine and Leucine labelled amino acids) was applied to rat INS-1E beta-cells cultivated either at medium (11mM, n=2) or high (30mM, n=2) glucose levels. Whole cell extract as well as Insulin Secretory Granules (ISGs), mitochondria, and nuclei were prepared. Proteins were separated on SDS-PAGE, digested with trypsin, and peptides were analysed by LC-MS/MS. Proteins were identified by Phenyx and quantified with MALDIPepQuant, a home-made software, or MaxQuant. Validation of the protein localization and level of expression were performed by qRT-PCR, western blots, immunofluorescence and electron microscopy (EM).

Results

About 2500 proteins were identified in the sub-cellular beta-cell fractions. Among them, 29 displayed an expression affected by high glucose concentration (≥ 1.5 fold). These proteins are mainly related to fatty acid metabolism (7 proteins of whom the Fatty Acid Synthase), proliferation (9 proteins of whom the whole Mini-Chromosome Complex), vesicle biogenesis and trafficking (such as gamma-SNAP), and apoptosis (such as Neuronal Pentraxin 1 – NP1). As it is the first time that NP1 is shown to be present in pancreatic beta-cells, we validated its localization in Insulin Secretory Granules. We also confirmed that NP1 expression is up-regulated after 24h of 30mM of glucose stimulation, at the protein and mRNA levels.

Conclusions and discussion

NP1 is a new beta-cell protein found up-regulated in ISGs of INS-1E cells stimulated with high glucose concentration for 24h. This protein was only described in neurons before, as a mediator of neuronal apoptosis induced by hypoxia-ischemia. Its presence, localization, and modulation were confirmed in INS-1E beta-cells, and we made the hypothesis that NP1 might be a mediator of beta-cells glucotoxicity induced apoptosis, through the AKT/GSK3 pathway. Actually, inhibition of GSK3 is already a target for beta-cell protection and survival, but its action is too broad regarding to its multiple biological roles. As a downstream intermediate in the AKT/GSK3 pathway, NP1 might therefore be a better target for preservation of beta-cell mass and function.

O8

QUANTITATIVE PROTEOMICS ANALYSIS OF HUMAN ADENOVIRUS INFECTIONS BY ITRAQ-8PLEX

Trinh H.V[1], Gehrig P[2], Baetschmann H[2], Grossmann J[2], Schlapbach R[2], Greber U[1], Hemmi S[1*]

[1]Institute of Molecular Life Sciences, [2]Functional Genomics Center Zurich, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Introduction

Adenoviruses (Ads) are self-organizing and the full complexity of Ads is unfolded as soon as they interact with host cells. They associate with respiratory and gastrointestinal tract infections. In addition, they are the most common viral vectors in clinical gene transfer trials. Although studies of Ad-host interactions have emerged as a key driving force in the research of infectious diseases, our understanding of the systems properties of Ad infections has remained incomplete. To address the unsolved issues, we anticipate our quantitative proteomics and known network interactions together with mathematical models to find out deep insights into Ad-host complex interactions. These may lead to the identification of key viral and host genes driving the infectious Ad life cycle.

Methods

Human lung carcinoma cells A549 cells were infected with Ads using a multiplicity of infection of 200. Subsequently, cell lysates were harvested along different time points of viral infection and digested peptides were labeled by iTRAQ-8plex followed by LC-MS/MS analyses with MALDI-TOF/TOF (ABI-4800). Next, Mascot, ProteinPilot search engines and Scaffold Q+ were used for protein identification and quantification. A decoy approach was implemented in database search step to estimate the false discover rate (FDR). Statistical analysis was done by using R packages with t-test followed by network interaction databases of Metacore and STRING. Finally, wet-lab experiments based on Western blot and surface plasmon resonance (SPR) were further employed to validate expression changes and interaction mechanisms.

Preliminary data

Here we employ a comprehensive functional genomics approach to obtain a global picture of the infection dynamics of human Ads in cultured cells. In detail, we targeted multi-time series in both early and late phases of Ad infections. For each infected and non-infected conditions, we included 3 to 4 biological replicates, which end up in total 10 iTRAQ-8plex experiments. As results, each iTRAQ experiment achieved quantification from 1200 to 1800 proteins with FDR below 2.5%. For further analysis, we first compared the protein quantitation obtained from different search engines/softwares, Mascot, Scaffold Q+ and ProteinPilot. A large set of significantly regulated proteins is found with all search engines/softwares. Secondly, a subset of up- and down-regulated proteins is currently applied in the network interactions by hypothesis-driven experimentation. Thirdly, we further analyzed two host proteins, which were strongly and reproducibly downregulated in early to late phases of Ad infections. Parallel transcriptome analyses of infected cells indicated that the mRNA levels for these proteins were apparently not affected in the course of infection. In addition, these proteins are found to interact with Ad receptors by SPR. Further experimentations towards elucidating the anti-viral mechanism of these proteins are ongoing.

Novel aspect

System-wide protein profiling of human Ad infected cells reveals novel host proteins with antiviral properties.

O9

HIGH ON-TREATMENT PLATELET REACTIVITY IN ASPIRIN-TREATED CARDIOVASCULAR PATIENTS - A FIRST STEP TO A SYSTEM BIOLOGY APPROACH

A. Zufferey[1,2], J.-L. Reny[3], S. Nolli[1], C. Combescure[4], J. Mascarini[1], P. de Moerloose[1], J.-C. Sanchez[2], P. Fontana[1].

[1]Division of Angiology and Haemostasis, [2]Biomedical Proteomics Research Group, Structural Biology and Bioinformatics Department, [3]Division of General Internal Medicine, [4]Division of Clinical Epidemiology, Geneva University Hospital and Faculty of Medicine, Switzerland.

Background Atherosclerosis or atherothrombosis is a widespread disorder involving various arterial territories. Platelets play a key role in atherothrombosis progression as well as in acute thrombus formation. Consequently, aspirin, by targeting the platelet-derived thromboxane (Tx) A2 production is a cornerstone in the treatment and prevention of ischemic vascular events in atherosclerotic patients by inhibiting platelet function, in particular platelet secretion. However, despite strong inhibition of TxA2 production by aspirin, a substantial proportion of patients (approximately 30%) display a preserved platelet function as assessed by global platelet function tests, defining “high on-treatment platelet reactivity”. Such patients have a higher risk for the recurrence of ischemic events. Platelet reactivity is a global phenomenon – as opposed to agonist specific – and a subgroup with platelet hyperreactivity to several agonists can be reliably identified in healthy subjects with epinephrine-induced platelet aggregation (EIPA). The characteristics and determinants of platelet reactivity in aspirin-treated cardiovascular patients are unknown. Platelet proteomics is a promising approach to identify molecular basis of platelet reactivity.

Aim To identify the characteristics and the determinants of platelet reactivity in aspirin-treated cardiovascular patients.

Methods Platelet function with various agonists was assessed twice, two weeks apart in 110 stable cardiovascular patients treated with aspirin 100 mg/d. Factorial analysis was performed to determine whether the different platelet function tests could be summarized in one single axis. Multivariate regression analysis was performed to explore determinants of the constructed “platelet reactivity” variable. A quantitative proteomic workflow focusing on platelet secretory pathway has been set up in this context, including 3 sample preparations based on subcellular fractionation.

Results EIPA was associated with aggregation results obtained with various agonists. Factorial analysis showed that the platelet function tests could be summarized in a single variable (“platelet reactivity”) that contained 60% of the information. Results obtained at the first visit were consistent with those obtained at the second visit ($r=0.78$, $P<0.01$). Regression analysis that included more than 25 relevant parameters did not identify any clinical or biological variables associated with platelet reactivity. The proteomic workflow allowed the identification of more than 1000 platelet proteins and of potential candidates of interest differentially expressed in the two groups of patients.

Conclusion Similarly to what has been demonstrated in healthy subjects, platelet reactivity is a global phenomenon in aspirin-treated stable cardiovascular patients. The variability of platelet reactivity is not explained by several biological and clinical parameters pointing out genetic factors at the forefront to regulate platelet reactivity in cardiovascular patients. A platelet proteome analysis targeting patients with extreme hyper- and hypo-reactivity is underway to better understand this phenomenon.

O10

BLOOD MICROPARTICLES IN ERYTHROCYTE CONCENTRATES

Olivier Rubin, Julien Delobel, Michel Prudent, David Crettaz, Jean-Daniel Tissot and Niels Lion

Service Régional Vaudois de Transfusion Sanguine, Lausanne, Switzerland

Introduction

Microparticles (MPs) are small phospholipid vesicles of less than 1.5 micrometer, shed in blood flow by various cell types. These MPs are involved in several biological processes such as hemostasis, thrombosis or inflammation. If presence of MPs in blood is common in healthy individual, an increase in the concentration of MPs in plasma has been demonstrated under various pathological conditions such as thrombocytopenic disorder, diabetes or sepsis.

Our work is focused on MPs in blood products with the aim to obtain qualitative and quantitative data on red cell MPs from erythrocyte concentrates in blood banking conditions. Indeed, the potential deleterious effect of those MPs in transfusion medicine is unknown.

In our previous work, counting method by flow cytometry was developed in order to count erythrocyte MPs either directly in a whole blood sample or in red cell concentrates. Now, our goal is to collect qualitative data on MPs studying their protein content using various proteomic methods as 1D-GE or 2D-GE, western blotting or mass spectrometry.

Results

During storage of erythrocyte concentrates in blood bank conditions, the number of MPs increased from around 3300 ± 1200 MPs/ µl at day 5-6 of storage up to 64'000 ± 37'000 MPs/ µl after 50 days of storage.

Erythrocyte membrane and erythrocyte MPs samples were compared by 1D-GE, allowing us to detect slight pattern differences, particularly in the 25 to 35 kDa region. After gel staining and analysis, bands of interest were thus excised in order to identify the corresponding proteins by MS. Different proteins were identified in both samples, such as RhD or Band 3. However, another level of information, complementary to the MS-based identification, is provided by SDS-PAGE. Thanks to markers of known molecular weight, masses of the identified protein can indeed be deduced from its position on the gel. Migration mass sometimes appears to differ from theoretical computationally-deduced mass, which can be relevant of some protein modification.

Western blot analysis allowed to thoroughly quantify the differences in specific protein content in both sample. Thus, by comparing erythrocyte membranes and MPs, a clear reduction of actin and an accumulation of stomatin were observed on MPs. The staining of CD235a was similar in both gels. Interestingly, stomatin is a major lipid raft component of erythrocytes.

Perspective

After 1D-GE, we started 2D-GE on MPs samples. If 1D-GE reduce sample complexity, band extracts still contain several proteins that can be crosslinked. On the contrary, 2D-GE presents the advantage to lead to individualized proteins. However, due to the fact that MPs are enriched in membrane proteins, results until now are still poor, even following adapted 2D-GE protocols for hydrophobic proteins. New methods have to be developed combining various approaches in order to understand the exact role of MPs in order to improve blood products quality and transfusion safety.

O11

ACTIVITY-BASED PROTEOMICS: BIOMARKER IDENTIFICATION IN HUMAN LUNG ADENOCARCINOMA

T. Wiedl[1*], B. Roschitzki[2], J. Grossmann[2], S. Arni[1], A. Soltermann[3], S. Hillinger[1], R. Aebersold[4,5] and W. Weder[1]

[1]Division of Thoracic Surgery, University Hospital Zurich; [2]Functional Genomics Center Zurich (FGCZ); [3]Department of Pathology, University Hospital Zurich; [4]Department of Biology, Institute of Molecular Systems Biology (IMSB), Swiss Federal Institute of Technology Zurich (ETH Zurich); [5]Faculty of Science, University of Zurich; [*]Raemistrasse 100, CH-8091 Zurich, Switzerland (Phone: +41 (0)44 255 9035 , or Phone: +41 (0)44 255 88 02; Email: thomas.wiedl@usz.ch)

Background

In biomarker discovery studies mRNA or protein abundances are typically compared in normal versus disease states. However, crucial changes in enzymatic activities remain undetected. Based on the work of Prof. Cravatt and others, activity-based proteomics has become a promising option to circumvent this limitation. This study aims to establish a robust and high throughput activity-based proteomics platform and to investigate the role of serine hydrolase activity profiles as prognostic biomarkers in lung cancer.

Methods

Activity profiles of human lung adenocarcinoma biopsies (tumor cell content:>70%, TNM-stage: I-IV) and corresponding normal lung tissues were compared. A fluorophosphate derivate (CAS-Number: 353754-93-5) was used to covalently target active enzymes. Tagged proteins were subsequently affinity purified and analyzed using a directed mass spectrometry based approach (LTQ-FTMS, Thermo Finnigan). Data were qualitatively analyzed using the Mascot 2.2 search engine and Progenesis LC-MS version 2.5 (Nonlinear Dynamics) was used for relative quantification.

Results

The strategy described above allows qualitative and quantitative analysis of serine hydrolase activities simultaneously, thereby representing a valid alternative to activity-based proteomics approaches described so far. Internal standards proved the system to be stable for up to one week and analysis time of 1.5 hours per sample makes the approach feasible even for large number of samples. On average, 30 serine hydrolases were identified per investigated proteome.

Analysis of a first cohort (N=8) of human lung adenocarcinoma biopsies and corresponding normal tissues revealed significant ($p<0.05$) elevated activities of two enzymes that have previously not been associated with lung cancer in 5 out of 8 cases.

Conclusion

The implemented activity-based proteomics platform represents a robust and fast approach for the investigation of serine hydrolase activity profiles. In our next steps we will increase the number of biopsies analyzed to further investigate the role of serine hydrolase activity profiles as prognostic biomarkers in lung adenocarcinoma.

Liu, Y., Patricelli, M.P. & Cravatt, B.F. Activity-based protein profiling: the serine hydrolases. Proceedings of the National Academy of Sciences of the United States of America 96, 14694-14699 (1999).

Jessani, N., *et al.* A streamlined platform for high-content functional proteomics of primary human specimens. *Nature methods* 2, 691-697 (2005).

O12

PROTEOMIC AND METABOLOMIC PROFILING OF ANTIDEPRESSANT DRUG ACTION

Webhofer C[1], Reckow S[1], Gormanns P[1], Tolstikov V[2], Maccarrone G[1], Sillaber I[3], Zieglgänsberger W[1], Turck CW[1]

[1] *Max Planck Institute of Psychiatry, Proteomics and Biomarkers, Munich, Germany*
[2] *University of California Davis Genome Center, Metabolomics Core, Davis, CA, USA*
[3] *Affectis Pharmaceuticals AG, Martinsried, Germany*

Introduction An improved understanding of the molecular pathways targeted by the currently available antidepressants holds great promise for novel drug development for improved treatment efficiency and specificity. *Selective Serotonin Reuptake Inhibitors (*SSRIs*)* and *Selective Noradrenaline Reuptake Inhibitors* (SNRIs) exert their function by elevating monoamine levels in the synaptic cleft. This class of antidepressants, however, exhibits several drawbacks including a delayed onset of clinical action, adverse side effects and only a subset of patients responding to a particular drug.

Aim of the project In order to elucidate affected biochemical pathways and networks downstream of monoamine reuptake inhibition we analyzed proteomic as well as metabolomic profiles in DBA/2Ola mice after chronic *paroxetine* (SSRI) treatment.

Material and methods Quantitative changes in hippocampal protein expression levels were analyzed using ^{15}N metabolic labeling. ^{15}N labeled proteins were used as internal standards for the indirect comparison of *paroxetine vs. vehicle* treated mice. Proteins were separated by SDS gel electrophoresis, in-gel digested and analyzed by LC-ESI-MS/MS. Over 2,500 proteins were quantified in five biological replicates.

Hippocampal metabolomic changes were investigated in six biological replicates using GC-MS profiling. We also identified metabolomic changes in plasma that due to its availability is the preferred specimen for a clinical biomarker assay.

Results Combined protein and metabolite pathway analysis revealed alterations in cellular processes including synaptic transmission, neurogenesis, neuronal death, protein synthesis, energy metabolism and oxidative stress.

Altered metabolites in plasma included glycerol, malic acid, alpha ketoglutaric acid and pelargonic acid.

Conclusion In summary, *paroxetine* affected several downstream pathways in the hippocampal and plasma metabolome and proteome of DBA/2Ola mice. The identified pathways are the source for novel depression biomarker candidates and hold promise for the targeted development of novel antidepressants.

Innovative aspects

- Relative protein quantitation using ^{15}N metabolic labeling applied to the analysis of antidepressant treatment effects.
- First metabolomic analysis of antidepressant treatment effects in mice.
- Combined proteomic and metabolomic based pathway analysis for the elucidation of downstream antidepressant drug targets.

O13

PROTEOMIC ANALYSIS OF THE SUBSTANTIA NIGRA IN PATIENTS WITH PARKINSON'S DISEASE

Licker V. [1], Turck N.[2], Côte M.[1], Rodrigo N.[1], Hochstrasser D.F. [3,4], Sanchez J-C.[2], Burkhard P. R.[1, 5]

[1]Neuroproteomics Group, [2]Biomedical Proteomics Group, [3]Clinical Proteomics Group, Department of Structural Biology and Bioinformatics, Faculty of Medicine, Geneva University, [4]Laboratory Medicine, Department of Genetics and Laboratory Medicine, [5]Neurology Department, Department of Clinical Neurosciences, Geneva University Hospitals, Geneva, Switzerland.

Parkinson's disease (PD) is a common neurodegenerative disorder whose prevalence will further increase along with the ageing population. Neuropathological hallmarks of PD involve the massive degeneration of dopaminergic neurons in the substantia nigra (SN) coupled with the occurrence of protein aggregates called Lewy bodies and neurites in the surviving neurons. The resulting dramatic deficit of dopamine (DA) neurotransmitter in the striatum accounts for the major PD characteristic motor symptoms. Despite years of research, the specific cascade of cellular, biochemical and molecular events ultimately leading to dopaminergic neuron demise is still to be elucidated. Because PD can be viewed as a disorder of brain protein handling, proteomics-based studies of relevant brain samples can offer an instantaneous view of a constantly evolving disease to detect some pathological changes. The present study aimed to gain new insights in PD pathogenesis by comparing the nigral proteome of patients with PD to aged-matched controls.

Human SN tissues of PD patients (n=3) and aged matched controls (n=3) were obtained at autopsy (post-mortem delay < 24h). Proteins were analysed by two-dimensional gel electrophoresis (2-DE) and identified by tandem mass spectrometry.

About 150 proteins were identified on 2-DE gels. Their functional classification reveals that many of them are involved in cellular processes thought to be critical to PD pathogenesis (intracellular signaling, protein folding, protein degradation and oxidative stress). Interestingly, our data show that expression patterns of a selective number of proteins are altered in PD versus control cases (Mann-Whitney U test, $p<0.05$). The overexpression of cytosolic non specific dipeptidase 2 (CNDP2) was immunovalidated. CNDP2 belongs to metalloprotease M20 family and is implicated in the degradation of various dipeptides with antioxidant properties (i.e. carnosine, anserine etc), suggesting a role in PD pathogenesis.

To elucidate the biological function of CNDP2, further analyses will be conducted using an experimental cellular model of PD and siRNA. Ultimately, this work could provide new insights on the underlying molecular mechanisms of neurodegeneration in PD that might foster the development of new therapeutic tools aimed to detect, prevent and control PD's progression.

O14

EXTENSIVE CONOPEPTIDE CLASSIFICATION AND PREDICTION BY BIOINFORMATIC APPROACHES: HMM AND PSSM COMPLEMENTARITY

Dominique KOUA [1,3,*], Silja LAHT [2], Lauris KAPLINSKI [2], Philippe FAVREAU [1], Maido REMM [2], Frederique LISACEK [3], Reto STÖCKLIN [1].

[1] Atheris Laboratories, case postale 314, CH-1233 Bernex-Geneva, Switzerland, [2] Estonian Biocentre, Tartu, Estonia and [3] Swiss Institute of Bioinformatics, Proteome Informatic Group, Geneva, Switzerland.

* corresponding author, dominique.koua@atheris.ch.

Background

Divided in numerous superfamilies and families, conopeptides are the main components of cone snail venoms that attract much interest in the pharmacological field and in drug discovery. The criteria for conopeptide superfamily classification focus on the signal sequence of propeptides. The number of conopeptide sequences is constantly increasing and will rapidly exceed hundreds of thousands of sequences due to venom diversity studies and sequencing techniques. To face this challenging amount of data, specific models have to be designed to allow fast and accurate superfamily assignment.

Results

We have constructed 48 Hidden Markov Models (HMM) and 48 Position-Specific Scoring Matrix (PSSM) generalized profiles that classify conopeptide sequences in their superfamily. The profiles were independently built from signal peptides, propeptides and mature peptides of the 16 known conopeptide superfamilies. Proteins are classified according to their scores for a combination of either HMMs and/or generalized profiles. These models allowed extensive and reliable classification even for superfamilies with mature peptides sharing high sequence similarities. The models provided a good evidence of complementarities between signal peptides, propeptides and mature peptides for superfamily determination, as well as complementarities between HMMs and generalized profiles.

Conclusion

Each model built in this study demonstrates very good discriminative abilities, with high sensitivity and selectivity. Applying the model-based classification to Uniprot databank established that the proposed models can be used either for the classification of known conopeptides or for the identification of new conopeptides.

CONCO, the cone snail genome project for health, is funded by the European Commission: LIFESCIHEALTH-6 Integrated Project LSHB-CT-2007-037592. We are grateful to the governments of New Caledonia and French Polynesia. http://www.conco.eu

O15

HOW TO COMBINE BIOMARKERS?

Xavier Robin[1§], Natacha Turck[1], Alexandre Hainard[1], Natalia Tiberti[1], Frédérique Lisacek[2], Markus Müller[2], Jean-Charles Sanchez[1]

[1]*Biomedical Proteomics Research Group, Department of Human Protein Sciences, Medical University Centre, Geneva, Switzerland.*
[2]*Swiss Institute of Bioinformatics, Medical University Centre, Geneva, Switzerland*
§ Contact author: Xavier.Robin@unige.ch

Background

For over ten years, the translation of proteomics biomarker discovery into clinical practice has been limited. Several explanations can be proposed, such as the difficult verification and validation of findings, the insufficient performance of the considered biomarkers or incorrect analysis. To solve these limitations, multiple biomarkers but also clinical parameters can be combined in what is called a *panel* of biomarkers. Decision trees, logistic regression or support vector machines are commonly employed machine learning techniques to establish combination rules.

Here we present a multiplexing approach with decision based on protein thresholds. Examples of stratification of aneurysmal subarachnoid hemorrhage (aSAH) patients are presented. Performance comparisons with proper ROC analysis and validation strategies are described as well.

Methods

Two cohorts of 28 and 113 aSAH patients were tested for 5 biomarkers and 7 clinical parameters. Panels were determined with six methods: threshold-based method, where optimal thresholds were learned using an exhaustive search (RIL); support vector machines (SVM); linear models (LM), generalized linear models (GLM), partial least square (PLS) and weighted k-nearest neighbour (KKNN). Ten-fold cross-validation was performed to assess the performance and stability of the methods. Panel and biomarker efficiencies were assessed by receiver-operating characteristic with partial area under the curve (pAUC). To compute the ROC curves with AUC and pAUC and perform the statistical comparisons, we developed *pROC*, a package for R and S+ that contains a set of tools displaying, analyzing, smoothing and comparing ROC curves in a user-friendly, object-oriented and flexible interface.

Results

In cross-validation, the RIL method slightly outperformed SVM (pAUC = 4.0 and 3.1 respectively, p=0.44). A RIL panel of 6 biomarkers was determined on the 28-patient cohort (selection set) and confirmed on the 113-patient cohort (verification set). With a pAUC of 7.1% on the training set it significantly out-performed panels containing only clinical (pAUC = 3.8, P = 0.05) or laboratory variables (pAUC = 4.3, P = 0.0004).

Conclusion

Threshold-based RIL is a simple and efficient method to combine biomarkers to improve the accuracy over single predictors.

O16

GENOME RE-ANNOTATION REVEALS NOVEL PROTEINS IN A MINIMAL ORGANISM

Samuel Bader[1], Jan Seebacher[1], Martina O'Flaherty[2], Vera van Noort[1], Shabaz Mohammed[2], Peer Bork[1], Albert Heck[2] and Anne-Claude Gavin[1]

1 Structural and Computational Unit, European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany

2 Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

During the past decades the genomic sequences for over 1000 prokaryotes have been determined. To annotate the genes encoded in those genomes, a series of computational tools have been adopted and applied. However different algorithms can lead to differences in gene annotations and exact gene start site. Furthermore the predicted genes are generally rarely directly validated using large scale proteomic data. High-throughput mass spectrometry can be used as an unbiased approach to identify the proteins. In addition to the identification of the proteins, the correct start of the proteins can be determined and sequencing ambiguities can be validated by mapping the identified peptide back to the genomic region that encodes for the peptide. *Mycoplasma pneumoniae*, a human pathogen causing atypical pneumonia, is one of the smallest self-replicating organisms known. Analysis of the *M. pneumoniae* proteome by high resolution mass spectrometry reveals new open reading frames, instances of alternative amino-terminal, and frame shift mutations leading to carboxy-terminal extension. The respective transcripts for the newly identified sequences were detected by strand specific RNA deep sequencing [1]. Also a majority of these new sequences are conserved in at least one other *Mycoplasma* species, further supporting their biological relevance. These examples show that even in the case of a simple organism such as *M. pneumoniae* unbiased identification of peptides using mass spectrometry can give valuable information about the encoded genes

Acknowledgements

We would like to thank Prof. emeritus Richard Herrmann for fruitful discussions and Dr. Kenji Maeda for helpful comments on the abstract

References

[1] M. Güell, V. van Noort, E. Yus, W.H. Chen, J. Leigh-Bell, K. Michalodimitrakis, T. Yamada, M. Arumugam, T. Doerks, S. Kühner, M. Rode, M. Suyama, S Schmidt, A.C. Gavin, P. Bork, L. Serrano, *Science,* **326,** 1268-1271 (2009).

P1

THE DYNAMIC RANGE OF MS/MS-BASES LABEL-FREE QUANTIFICATION IN ION TRAP INSTRUMENTS

HuiSong Pak, Carla Pasquarello and Alexander Scherl

Proteomics Core Facility, Faculty of Medecine, Geneva University, Geneva, Switzerland

Introduction

We have recently shown that data-independent (DI) precursor-ion selection combined with extensive gas-phase fractionation outperforms data-dependent (DD) ion selection in terms of peptide identification and dynamic range (Scherl *et al.,* ASMS 2008, Scherl *et al.,* ASMS 2009, Panchaud *et al.,* Anal. Chem. 2009). With the DI operation mode, multiple tandem mass spectra are acquired over the entire chromatographic elution profile for a given peptide. We investigated if the so-generated fragment ion signals could be used for direct label-free quantification.

Method

Human plasma was reduced, alkylated and digested with trypsin. Standard proteins (beta-lactoglobulin and trypsin inhibitor protein) were also reduced, alkylated and digested. Plasma digests were normalized to an albumin concentration of 200 fmol/µl and spiked into the standard protein digests at concentrations varying over 6 orders of magnitude (0.2 amol/µl to 200 fmol/µl). A total amount of 5 µl was then injected in a linear ion trap-Orbitrap mass spectrometer (*LTQ Orbitrap velos* from ThermoFischer) and analyzed by either data-dependent MS/MS or targeted MS/MS. Extracted precursor and fragment ion chromatograms were derived from the raw data. The chromatographic peak areas were used for label-free quantification.

Results

Our results showed that label-free quantification can be performed on fragment-ion signals over 5 orders of magnitude. Moreover, a good correlation was observed between the protein concentration and the extracted fragment ion signals (log-Log R2 > 0.98). This demonstrated that accurate label-free protein quantification could be performed in linear ion trap instruments on tandem mass spectrometry data acquired in the DI mode. This method could therefore be applied for the direct quantification of complex protein mixtures, without previous liquid-based fractionation.

P2

PROTEOMIC ANALYSIS TO IDENTIFY POTENTIAL BIOMARKERS OF MALIGNANT PANCREATIC CYSTS

Aurora Cuoghi [1,2], Annarita Farina [1], Kaspar Z'graggen [3], Jean-Marc Dumonceau [4], Aldo Tomasi [2], Denis F. Hochstrasser [1,5], Pierre Lescuyer [1,5], Jean-Louis Frossard [4].

1 Biomedical Proteomics Research Group, Department of Bioinformatics and Structural Biology, Faculty of Medicine, Geneva University, Geneva, Switzerland.
2 Department of Laboratory Medicine, Medical Faculty, University Hospital of Modena and Reggio Emilia, Modena, Italy
3 Klinik Beau-Site, Hirslanden Hospital, Bern, Switzerland.
4 Division of Gastroenterology and Hepatology, Geneva University Hospitals, Geneva, Switzerland.
5 Clinical Proteomics Laboratory, Department of Genetic and Laboratory Medicine, Geneva University Hospitals, Geneva, Switzerland

Background Pancreatic cysts represent approximately 10-15% of primary masses of the pancreas. Pancreatic cysts have been recognized with an increasing frequency in these last twenty years due to the use of advanced imaging modalities in clinical practice.

Accurate differentiation among cysts is an important challenge for clinicians, because of the heterogeneity of the lesions and the potential of malignant transformation. Unfortunately, diagnosis of pancreatic cysts remains unsatisfactory because of poor diagnostic performance at present .

Aim This study involves proteomic analysis to discover differentially expressed proteins in several types of pancreatic cyst fluids. The goal is to show that this approach could help identifying new cancer biomarkers to diagnose, before surgery, potentially malignant neoplasms.

Materials and methods Cystic fluids were collected by direct puncture during open surgery. CEA, CA-19-9 and amylase concentrations were measured using specific immunoassays. After immunodepletion and fractionation on a SDS PAGE gel, proteins were digested with trypsin. Peptides were then analyzed by LC-MS/MS on an LTQ-Orbitrap mass spectrometer (Thermo scientific). Protein identification was performed using Phenyx software (Genebio). Several proteins identified only in potentially malignant cysts were already known as a cancer-related in pancreatic tumour. Western-blot analyses were performed to validate proteins differentially expressed.

Results Fluids from different types of pancreatic cysts are characterized by a specific protein pattern. Western-blot was used to validate proteomic data and evaluate the potential usefulness of selected proteins as biomarkers of malignant pancreatic cysts. The potentially malignant cyst fluids (Mucinous cystadenoma and IPMN) showed an intense expression for olfactomedin-4 (OLFM4), whereas pancreatic neuroendocrine tumor indicated a positive expression of mucin 18 (MelCAM). These markers were not detected in serous cystadenoma and in chronic pancreatitis-associated cysts.

Conclusions Proteomics appears a promising approach for the identification of biomarkers in malignant pancreatic cysts. These proteins could be assessed as reliable candidate for developing a panel of new diagnostic biomarkers. (Controlled-trials.com, Number ISRCTN10626053, www.controlled-trials.com).

Innovative aspects Proteomic approach was successfully used for the first time on pancreatic cystic fluids to reveal differentially expressed proteins.

P3

ANALYSIS OF PROTEOMIC PATTERNS OF CERVICAL CANCER CELL LINES

Higareda-Almaraz, J. C., Enriquez, M. R., Hernandez, M., Encarnación, S.[1]

Center for Genomical Sciences, UNAM, Morelos Campus.

Cervical cancer is an excellent model for the study of cancer progression and maintenance because in the 90.7% of cases it has a common etiology factor: HPV. The oncogenic potential of this virus stems from the interactions between viral-oncoproteins E6 and E7 and two of the most commonly-altered cellular tumor suppressor proteins, p53 and pRb. In this work we set out to identify common patterns in cervical cancer, shared by both HPV positive and HPV negative cell lines, and which contrast with the non-tumourgenic cell line HaCat. Our objectives were to identify cellular events participating in cancer progression and maintenance as well as establishing a pipeline to work with proteomic-derived results. We analyzed by means of 2D SDS-PAGE and MALDI-TOF mass spectrometry the protein extracts of six cervical cancer cell lines from which we obtained a consensus pattern of 66 identified proteins. Starting from this core set of proteins we acquired a protein-protein interaction (PPI) network that pointed, through topological analysis, to some proteins that may well be playing a central role in the neoplastic process, such as 14-3-3z. *In silico* reconstruction of signaling pathways and search for transcription factors pointed to the over expression C-myc and E2F1 as the transcription factors responsible of orchestrating the neoplastic phenotype.

P4

MITOCHONDRIAL DRUG TOXICITY

Andrea Felser, Peter Lindinger, Stephan Krähenbühl

Clinical Pharmacology and Toxicology, Department Biomedicine, University Hospital Basel, Hebelstrasse 20, 4031 Basel

The development of mitochondrial drug toxicity can have severe impact on patients under drug exposure. The reasons for this idiosyncratic drug toxicity are not fully understood. For example, there are no animal models to predict the probability or eventuality of this toxicity. At present, several risk factors have been identified: This includes certain HLA constellations, polymorphisms of enzymes or drug transporters and mitochondrial diseases. Mitochondrial impairment can be caused by genetic mutations or other defects such as defect degradation of reactive oxygen species (ROS). Drugs being metabolized can cause accumulation of ROS, which impairs mitochondria (e.g.,valproic acid). In addition, mitochondrial damage can be caused by the formation of DNA adducts upon drug administration. Thus, molecules bind irreversibly to DNA and lead to strand breaks, which can result in genetic mutations by improper repair. Finally, drugs can directly bind proteins, which can inhibit mitochondrial function such as impairing electron transfer chain or depolarizing the organelle (tolcapone).

In this PhD project, the mechanisms of various drugs associated with hepatoxicity are investigated with a special focus on mitochondria. In order to better describe and understand drug toxicity, cellular models mimicking patients will be established. This includes silencing of ROS degradation or introduction of mutations of the mitochondrial genome.

Finally, the analysis of drug-mediated toxicity is based on two cornerstones, namely (1) a functional characterisation of cells and mitochondria and (2) on a proteomics-based analysis. Therefore, we hope to be able to link proteomics data with functional characteristics observed. Thus, the relevance of the findings should be improved.

The investigation of toxicity and cellular functionality includes toxicity and apoptosis assays as well as approaches to measure oxidative capacities (oxygen consumption), ATP generation rates (ATP and ADP content), membrane potential, mitochondrial density and morphology (confocal microscopy and electron microscopy) and enzymatic assays to measure key enzymes of the citric acid cycle, the electron transfer chain and mitochondrial transporters.

In addition, proteomics tools will be applied to study drug-related mitochondrial impairment. On the one hand, individual complexes of the respiratory chain will be co-immunoprecipitated in order to visualize possible alterations in protein stoichiometries. On the other hand, a “capture compound mass spectrometry”-like approach is of interest. A known examples is tolcapone, which binds to some proteins of the electron transfer chain, leading to depolarization of mitochondria. A strategy like this would allow the identification of drug/protein interactions and help to better characterize possible mechanisms of drug-associated toxicity.

This study was initiated in September 2010. First, the cellular models will be established. Most of the required assays are already available in the laboratory, while only few assays have to be designed in autumn/winter 2010. In 2011, the effects of a broad range of drugs on function and proteome will be investigated by applying our cell models and isolated mitochondria. Later, the analysis will be extended to in vivo settings, such as investigation of rat or mouse liver cells and mitochondria.

P5

CHARACTERIZATION AND COMPARISON OF DIFFERENT MILK PRODUCTS

Flurina Schwander, Katrin Bolanz, Charlotte Egger, Reto Portmann

ALP

Intake of milk and milk products is recommended due to their high concentrations in essential nutrients such as proteins, calcium and vitamin D. In addition, it has been shown that milk products might have anti-inflammatory properties. The components and mechanisms underlying this anti-inflammatory response have not yet been described. Therefore, in the first part of the project different milk products will be characterized by a proteomic approach. The methods used for the protein analysis are 1D and 2D gel-electrophoresis which are performed after different pre-fractionations such as acid precipitation, rennet treatment and sucrose gradient (depending on the food product). Protein bands and spots that are cut out of the gels are digested with trypsin and analyzed with LC-MS. The results are collected in a protein atlas providing an interactive platform to visualize the analyzed samples and allowing direct comparisons between different milk products. In the second part of the project, the milk products will be digested and screened for their inflammatory response in a transwell cell culture system mimicking intestinal absorption.

P6

AGING AND STORAGE OF ERYTHROCYTE CONCENTRATES

Julien Delobel, Olivier Rubin, Michel Prudent, David Crettaz, Aurélia Chambaz, Jean-Daniel Tissot and Niels Lion

Service Régional Vaudois de Transfusion Sanguine, Route de la Corniche 2, CH-1066 Epalinges, Switzerland

Introduction Red Blood Cell (RBC) concentrate is the main labile blood product used in transfusion medicine. Numerous efforts are made to ensure quality and security of blood products from collection to transfusion. Non-physiological cold-storage provokes RBC alterations, well described in the literature as "RBC storage lesions". Those lesions includes morphological, biomechanical and biochemical alterations. How theses lesions may affect transfusion efficiency is not yet clearly understood. Among the different storage lesions, RBCs are subjected to oxidative stress, which leads to the oxidation of part of their protein content. We focus on how oxidative lesions are involved in RBC in vitro aging and in-vivo clearance.

Materials and Methods Erythrocyte concentrates are obtained from voluntary blood donation from Lausanne and Bern blood transfusion centers. Briefly, whole blood donations are collected into anticoagulant solution. Plasma, platelets and leukocytes are removes and RBC concentrates are stored in additive solution for up to 49 days at 4°C. Only RBC concentrates stored in SAGM and refused for transfusion medicine were used here.

Cytoplasmic content is extracted by hypotonic lysis with or without previous density-based fractionation of RBCs populations through Percoll centrifugation. High-density fraction is thought to be enriched in old erythrocytes and low-density fraction in young ones.

Carbonylation content is here spectrophotometrically investigated. The assay is based on carbonylated proteins derivatization by 2,4-dinitrophenylhydrazine. This reaction leads to formation of protein-hydrazone complexes of which optical density at 375 nm allows quantitaion of protein carbonylation.

Since hemoglobin has absorption peak at the wavelength used for this assay, cytoplasmic extracts are previously hemoglobin-depleted by nickel-based IMAC column.

Results Preliminary results show a storage time-related increase of the carbonylated protein content that seems to be constant from day one to day 14, then presents an important increase at day 14 (from 1.7nmol per mg of protein to 3.2nmol/mg), with a maximum at day 22 (3.7nmol/mg), and seems to stay relatively constant with a little decrease until day 42 (2.7nmol/mg).

The age-related fractionation reveals that the supposed old-population enriched fraction presents lower protein carbonylation content than the young-population enriched one. It's however interesting to note that the ratio between lower-density and higher-density fractions is constant at around 1 from day 1 to day 16, then increases until the end of the storage period.

Conclusions RBC cytoplasmic carbonylation content shows an increasing profile from day 14, and a decrease in the end of the storage. Contrary to expected, results show that population lower density population (supposed to be younger RBCs) have higher carbonylation content. In our hands, it appears that number of RBC microparticles increases during storage. A possible explanation could be that microparticulation allows harmful components elimination, of which carbonylated proteins possibly belong. Further experiments on microparticles and oxidation will be conduct to support these results.

P7

INDIMAX: TOWARDS ACCURATE CANDIDATE PEPTIDE INDEXING FOR TANDEM MASS SPECTROMETRY DATA

Kuzyakiv R. (1), Muller M. (1), Beekkof F. (2), Nikitin F. (1), Lisacek F. (1), Voloshynovskiy S. (2)

(1) Protein Informatics Group, Swiss Institute of Bioinformatics, Geneva, Switzerland.
(2) Stochastic Information Processing Group, University of Geneva, Geneva, Switzerland.

Many algorithms for protein and peptide identification from Tandem Mass Spectrometry (MS/MS) data have been published. Nevertheless, the development of reliable candidate peptide indexing and ranking techniques for accelerated pre-processing of large datasets still remains a challenge.

The present work takes up this challenge and proposes a method based on a low-complexity hierarchical search approach suitable for differently charged experimental peptides. The proposed technique efficiently removes noise from the query MS/MS spectra and reasonably utilizes the information on m/z values and intensities of the remaining experimental peaks. It avoids a time consuming one-to-all-database comparison suggesting a short list of ranked candidate indices. The scoring mechanism provided with the algorithm is much more robust to peak misalignments and overcomes the fundamental restrictions of cross-correlation based methods. It also efficiently simplifies the interpretation of the obtained results.

We have used two experimental datasets of annotated, quadrupole ion trap, MS/MS spectra of unmodified human blood peptides. **The first dataset**: 580 spectra of 116 peptides (9 +1, 71 +2, 23 +3, 13 +4 charged). 95-MS/MS have been used for training. **The second dataset**: 1550 spectra of 1550 peptides (214 +1, 901 +2, 364 +3, 44 +4 charged). 280 MS/MS spectra have been used for training. **The Theoretical database**: 43397 theoretically fragmented human peptides**. The Decoy database**: reversed 43397 peptide of the original database.

The developed indexing technique makes it possible to obtain the average list size of theoretical candidate indices to be 4.93 (1st Dataset, 10 the most intensive peaks, calculation time-7sec) and 3.28 (2nd Dataset, 16 the most intensive peaks, calculation time-19sec.) with overall probability of miss of 0.

For the 2nd Dataset, the developed approach obtained 5096 ranked theoretical indices with the average score of the theoretical index to be 3.97 and the average score of the correctly matching theoretical index to be 8.9 out of 17. The average position of the correctly matching theoretical index on the finale list was calculated as 1.0065.

For the 2nd Dataset the algorithm has identified and ranked 4236 decoy candidate indices in 17 seconds with the average list size to be 2.73 and the average score of the decoy index to be 1.8 out of 17. The False Discovery Rate (FDR) for a particular threshold has been calculated from the formula: FDR=FP(Decoy DB) / TP(Original DB) + FP(Decoy DB).

The obtained results have shown the efficiency of the information on m/z values and intensities of the experimental peaks for fast and reliable peptide indexing. Experimentally calculated low values of the average list size and the FDR have proven the soundness of the idea of probabilistic calculations of Precursor Ion Mass and m/z positions of highly intensive experimental peaks.

P8

MOLECULAR APPROACHES AND FUNCTIONAL ANALYSIS OF POTENTIAL DRUG TARGET PROTEINS IN *GIARDIA LAMBLIA*

Dorothea Nillius, Andrew Hemphill, Joachim Müller, Norbert Müller*

Institute of Parasitology, University of Berne, Länggass-Str. 122, CH-3012 Berne, Switzerland

** E-mail corresponding author: dorothea.nillius@ipa.unibe.ch (Dorothea Nillius)*

Introduction

The intestinal protozoan parasite *Giardia lamblia* causes diarrhea in humans and animals worldwide. To date, a limited number of drugs such as metronidazole (MTZ) and nitazoxanide (NTZ) are used for chemotherapy against human giardiasis. However, chemotherapy of this disease has frequently been associated with recurrence of symptoms and formation of drug resistance, which was identified as one of the main reasons for treatment failure. Only little is known with respect to the mechanisms that lead to this important phenomenon.

Methods

In a study focused on the mode of action of NTZ, we identified nitroreductase 1 (NR1) as a NTZ-binding protein in *G. lamblia* trophozoites using affinity chromatography. Subsequently, we overexpressed NR1 in trophozoites to elucidate its possible role in resistance formation. The overexpression was confirmed on the RNA level by RT-PCR and on the protein level by Western blotting. Now, transgenic parasites will be examinated regarding susceptibility/resistance to NTZ and MTZ. Furthermore, we are investigating via differential 2D-gel electrophoresis other drug target proteins covalently binding to either NTZ or metabolites emerging from treatment with this drug.

Key Results

G. lamblia NR1 revealed to be a NTZ-binding protein. Several protein shifts in the 2D gel exemplify the effect of NTZ or its metabolites on the metabolism of the parasite. Using mass spectrometry, several of the shifted proteins were identified: we found that drug treatment resulted in modification of proteins crucial for energy supply (e.g. ornithine carbamoyl transferase), detoxification of oxygen (e.g. NADH oxidase), and protein biosynthesis (e.g. elongation factor).

Discussion

The mode of action of NTZ is assumed to involve NR1 but its role will have to be elucidated by closer examination in transgenic parasites. Moreover, we hypothesize that protein modifications caused by NTZ or its metabolites inactivate essential cellular functions and thus contribute to the parasitocidal effect of NTZ.

P9

IDENTIFICATION OF PATHOGENICITY FACTORS OF *NAEGLERIA FOWLERI*

Denise Burri[1,2], Norbert Müller[2], Beatrice Zumkehr[2], Bruno Gottstein[2], Nadja Schürch[1], Matthias Wittwer[1]

[1]*Spiez Laboratory, Federal Office for Civil Protection, Austrasse, 3700 Spiez, Switzerland*
[2]*Institute of Parasitology, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland*
burri_denise13@students.unibe.ch

Background *Naegleria* species are free-living amoebae found in soil and warm fresh waters throughout the whole world. Some 30 species have been recognized, but *Naegleria fowleri (N. fowleri)* is the only one that causes primary amoebic meningoencephalitis (PAM) in immunocompetent children and young adults. PAM is an acute, fast progressing and mostly fatal disease affecting the central nervous system. Most of the patients die within several days after infection due to a cardio-respiratory failure. Thus, *N. fowleri* is very problematic on the strength of the rapid onset and destructive nature of the disease rather than to the number of cases worldwide.

In a laboratory setting, the pathogenicity of *N. fowleri* can be influenced by different culture conditions: While *Naegleria* cultivated in axenic medium do not kill mice, the 2 fold xenic passage over L929 mouse fibroblasts produces pathogenic *Naegleria* that kill mice within five to ten days.

According to recent findings, the neurotoxic mechanisms of *N. fowleri* include pore-forming polypeptides (naegleriapores A and B) as well as cell and extracellular matrix degrading proteases and phospholipases.

However, presently the cellular events leading to the pathogenicity switch of *N. fowleri* are poorly understood and the interactions of the proposed virulence mechanisms are unknown.

Aim Thus, the aim of this study is the identification of cellular factors and pathways which govern the pathogenicity of *N. fowleri* on a broad scale genomic and proteomic level.

Materials and Methods

Non-pathogenic *Naegleria* are cultivated *in vitro* in modified PYNFH medium at 37°C in a BL3 lab. Pathogenic cultures are produced by xenic cultivation in the presence of L929 mouse fibroblasts.

In a first comparative proteomic approach, protein fractions from pathogenic and non-pathogenic *N. fowleri* are separated by 2D gel electrophoresis. The gels are then searched for differing protein spots, and the corresponding proteins are identified by MALDI-TOF mass spectrometry.

In a second transcriptomic approach, differentially expressed proteins are confirmed by real time PCR.

The precondition for the identification of unknown pathogenic factors by MALDI-TOF mass spectrometry as well as for the design of corresponding primers for real time PCR is the knowledge of the genome sequence of the parasite. Therefore, the whole genome sequencing of *N. fowleri* is a central approach of this work..

Preliminary results Preliminary real time PCR results showed that the pore-forming polypeptide naegleriapore B is up-regulated in pathogenic compared to non-pathogenic *N. fowleri.*

Conclusion and Relevance The switch from non-pathogenic to pathogenic *N. fowleri* is of high relevance regarding the response of cellular regulatory networks to changing environmental conditions also found in other organisms. Therefore, the assessment of these mechanisms with a genomic and proteomic apporoach may also shed light on other fundamental aspects of eukaryotic biology.

P10

MASS SPECTROMETRY CHARACTERIZATION OF AB PEPTIDES FROM ENGINEERED CELL CULTURE OVEREXPRESSING APP AND Γ-SECRETASE

Mitko Dimitrov[1#], Luca Fornelli[2#], Yury O. Tsybin[2] and Patrick C. Fraering[1]

[1] *Laboratory for Molecular and Cellular Biology of Alzheimer's Disease, EPFL, Lausanne, Switzerland*
[2] *Biomolecular Mass Spectrometry Laboratory, EPFL, Lausanne, Switzerland*

[#] These authors contributed equally to this work

Aim of the project

Alzheimer's disease (AD) is neuropathologically characterized by the cerebral accumulation and deposition of fibrillar amyloid-beta (Aβ) peptides into Aβ amyloid plaques. Aβ peptides are derived from the sequential cleavage of the amyloid precursor protein (APP) by β- and γ -secretase. So far the most studied isoforms of Aβ have been the peptides containing 40 and 42 amino acids (Aβ1-40 and Aβ1-42) as these are concentrated in AD plaques and show high propensity to form insoluble fibrils *in vitro*. Recently, shorter Aβ peptides, namely pyroglutamyl-containing and deamidated Aβ species (including Aβ11-40 and Aβ11-42), or even the longer Aβ1-43 were identified in both amyloid plaques and cerebrospinal fluid (CSF) of Alzheimeric patients and were proposed to be implicated in AD pathology. In the present study we have used mass spectrometric approaches to provide a thorough characterization of Aβ species secreted from engineered cell line overexpressing both γ-secretase and its substrate APP.

Materials and methods

We have taken advantage of the CHO-based cell line overexpressing the γ-secretase complex and APP (S-20) [1]. Aβ peptides were collected by TCA precipitation or by immunoprecipitation (IP) of the cell media using two different commercially available anti-Aβ antibodies, namely 4G8 and 6E10. The different Aβ profiles were first assessed by MALDI-TOF MS and further characterized by high resolution ESI FT-ICR tandem mass spectrometry, after both direct infusion and on-line chromatographic separation.

Results

Our results demonstrate the presence of various C- and N-terminally truncated Aβ species, with Aβ42:Aβ40 in physiologically relevant ratio. Notably, we have been able to detect high concentrations of Aβ11-40 in our cell line, akin to its presence in human plaques and CSF of AD patients. The FT-ICR analysis first allowed us to confirm the identity of the truncated Aβ species, including Aβ11-40. It further allowed us to compare in detail the profiles of synthetic and *in vivo* generated Aβ peptides.

Conclusions and innovative aspects

Mass spectrometry techniques have been proven to be a robust method for peptide identification and relative quantification. We have applied both MALDI TOF MS and high-resolution FT-ICR technology to unambiguously identify and compare Aβ species from biological samples and synthetic preparations. By using a combination of engineered cell line, immunoprecipitation and mass spectrometry, our methodology thus provides unique tool to systematically analyze human Aβ peptides in terms of their modifications and relative abundances. Finally, our approach will undoubtedly allow to evaluate the effect of pharmacologically active compounds on the generation of different pathologically relevant Aβ species, such as Aβ11-40/42 and Aβ1-40/42.

P11

DIFFERENT APPLICATIONS OF MASS SPECTROMETRY AND SILAC TO STUDY HISTONE MODIFICATIONS

Soldi[1] M., Bonaldi[1] T.

[1] *Department of Experimental Oncology, European Institute of Oncology, IFOM-IEO Campus, Via Adamello 16, 20139 Milano, Italy*

Histone proteins, which form the proteinaceous core of nucleosomes, are the prototype of multi-site modified proteins. Their *N*-terminal tails are subjected to a variety of posttranslational modifications (PTMs), occurring at multiple but specific residues. Combinations of histone modifications are involved in the establishment of global chromatin environments and in the orchestration of DNA-based biological processes, by recruiting specific effector proteins that further modify chromatin. As a consequence, an aberrant establishment of patterns of histone PTMs (hPTMs) often correlates with diseases, among them the onset of several tumors.

Antibodies are commonly used to study hPTMs. They are sensitive and, in experiments of chromatin immuno-precipitation (ChIP) followed by DNA microarray or sequencing, can provide a genome-wide mapping of modifications at the resolution of just a few mono-nucleosomes. However, the antibody-base approaches are hampered by limitations regarding the specificity in the detection of closely related epitopes (e.g. di- *vs* tri-methylation) and/or combinations of adjacent modifications.

Mass spectrometry (MS) can provide an ideal tool to overcome these limitations. Due to the fact that mass spectrometers measure masses of molecules, certain modifications can be directly detected as a specific “Delta Mass” and complex patterns of PTMs on histones can be dissected without *a priori* knowledge of modifications. Furthermore, the possibility to employ high-resolution mass spectrometers offers a significant improvement in the analysis of hPTMs, with the opportunity to discriminate with high confidence PTMs with similar masses (e.g. acetylation *vs* tri-methylation). Finally, the development of quantitative methods in proteomics, such as metabolic labeling using nonradioactive isotope-containing amino acids (SILAC), allows quantification of changes in abundance of hPTMs in an accurate and linear way.

We apply high-resolution MS combined with SILAC strategy to identify and quantitatively profile the hPTMs changes in a panel of breast cancer cell lines, in comparison with normal epithelial breast cells, and in a promyelocytic cell line upon retinoic acid (RA) treatment. Our analysis focuses on Lysine acetylation and methylation on fourteen distinct sites of Histone H3 and H4. We observed significant changes in the abundance of several modifications in cancer cells.

In my project I’m also interested in the development of a MS-based proteomics approach to study specific functional sub-domains of chromatin, enriched from bulk preparation. My aim is to characterize both the co-associated PTMs and in parallel to annotate a “chromatin interactome”, corresponding to all the non-histonic proteins co-purifying within the same chromatin domains. In particular, I envisage to study the hetero- *vs* euchromatin domains, utilizing specific modifications as a bait for preparative ChIP. By combining the information on the histone modifications and the co-enriched “readers”, we aim to identify novel and specific proteins involved in epigenetic processes orchestrated by specific histone modifications.

P12

PROTEOMIC ANALYSES OF POST-TRANSLATIONAL MODIFICATIONS OF HISTONE PROTEINS IN THE ADULT MOUSE BRAIN

Andrea M. Brunner, Ry Tweedie-Cullen, Jonas Grossmann and Isabelle M. Mansuy

Brain Research Institute, University of Zürich/ETH Zürich, Switzerland Functional Genomics Center, University of Zürich/ETH Zürich, Switzerland.

The formation of long-term memory is a complex process that requires gene transcription and the synthesis of new proteins. The molecular mechanisms that initiate and regulate gene transcription during this process are, however, still poorly understood. Recent evidence has implicated epigenetic processes, in particular, post-translational modifications (PTMs) of histone proteins. Here, we describe a novel proteomic approach based on electron transfer dissociation and collision induced dissociation tandem mass spectrometry that aims at characterizing histone PTMs that occur in the adult mouse brain both qualitatively and quantitatively. Using nuclear isolation, acid extraction and high-pressure liquid chromatography fractionation, we identified a total of 14’000 peptides from histones H1, H2A, H2B, H3 and H4. 10% of the identified peptides were post-translationally modified. On these modified peptides, we detected 231 unique PTM sites, 125 of which were novel. The most prevalent modification was methylation, followed by acetylation and phosphorylation. In order to characterise the crosstalk between identified PTMs, PTMs occurring on the same peptide were analysed using an eclat algorithm to determine the frequency of co-occurrence of different histone PTMs, and derive rules for their distinct patterns and combinations. Overall, the present data demonstrate the abundance of histone PTMs and the complexity of their crosstalk in the mouse brain. Current work is now devoted to quantify histone PTMs in brain regions involved in learning and memory using a SILAC (Stable Isotope Labeling with Amino Acids in Cell Culture) based method, in which SILAC-labeled histones from Neuro2A cells are spiked into the histone samples derived from mouse brain tissue as standards. This novel method will allow the MS-based quantification of histones and their PTMs in the mouse brain and, thus, the establishment of a correlation between the identified histone PTMs and cognitive performance in mice.

P13

PROTEOMICS DISCOVERY AND VALIDATION OF BETA-2-MICROGLOBULIN AS A POWERFUL STAGING MARKER FOR SLEEPING SICKNESS

Natalia Tiberti[1], Alexandre Hainard[1], Veerle Lejon[2], Xavier Robin[1], Dieudonné Mumba Ngoyi[3], Enock Matovu[4], John Enyaru[5], Bertrand Courtioux[6], Philippe Büscher[2], Krister Kristensson[7], Natacha Turck[1], Sylvie Bisser[6], Joseph Mathu Ndung'u[8], Jean-Charles Sanchez[1]

[1]Biomedical Proteomics Research Group, Medical University Centre, Geneva, Switzerland; [2]Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium; [3]Institut National de Recherche Biomédicale, Kinshasa, D.R.Congo; [4]Department of Veterinary Parasitology and Microbiology, Makerere University, Kampala, Uganda; [5]Department of Biochemistry, Makerere University, Kampala, Uganda; [6]Institut de Neurologie Tropicale, Université de Limoges, France; [7]Department of Neuroscience, Karolinska Institutet,Stockholm, Sweden; [8]Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland.

Introduction Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is a disease endemic in sub-Saharan Africa caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense* parasites. After infection, during the first stage (S1), parasites proliferate in patients' bloodstream and lymphatic system. With disease progression, parasites penetrate the blood-brain barrier (BBB) and reach the central nervous system (CNS), leading to the second stage (S2). HAT staging is a critical step in the diagnostic workflow, as treatment choice depends on it. Current staging methods, based on white blood cells (WBC) counting and trypanosome detection in CSF, lack in specificity and/or sensitivity, so that there is an urgent need of new staging tools. The aim of the present study was first to investigate by proteomic strategies the CSF of HAT patients in order to discover new staging biomarkers, and then to validate the potential candidates on a large multi-centric population.

Methods Cerebrospinal fluid (CSF), collected before treatment, originated from parasitologically confirmed HAT patients. The stage of disease was determined on the basis of the number of white blood cells and presence of parasites in CSF, according to WHO guidelines. Samples were first analysed by 2-DE (n=9) and tandem mass tag (TMT) isobaric labelling quantitative mass spectrometry (n=6). Three proteins found over-expressed in S2 samples compared to S1, i.e. complement factor H (CFH), osteopontin (OPN), and beta-2-microglobulin (B2MG), were then verified by Western blot and ELISA on a small cohort (n=58) of patients. The best promising marker, beta-2-microglobulin (B2MG), was finally validated on a large multi-centric cohort (n=600) encompassing patients coming from 5 African-affected countries and infected by *T. b. gambiense* or *T. b. rhodesiense* forms of parasite. B2MG staging performances as well as its correlation with the presence of parasites in CSF and patients' neurological signs were evaluated and compared to WBC, the actual staging method.

Results The investigation of S1 and S2 CSF samples by classical and quantitative proteomics highlighted 73 proteins over-expressed in S2 patients. Among the three verified proteins, B2MG showed most promising staging ability, being able to accurately ($p<0.0001$) distinguish S1 and S2 with AUC of 91%. Its further validation on a large multi-centric cohort highlighted B2MG as a powerful staging marker for sleeping sickness discriminating between S1 and S2 patients with 83% sensitivity and 93% specificity, and AUC of 93%. If the presence of parasites in CSF and the presence of neurological signs were used to classify patients, B2MG resulted to be able to significantly ($p<0.0001$) distinguish the two groups with AUCs higher than 85% in both cases, similar to WBC performances.

Conclusion This study highlighted the value of B2MG, discovered by proteomic approaches, as a powerful biomarker for staging HAT patients. This protein can discriminate between stage 1 and stage 2 with high sensitivity and specificity, for patients infected either by *T. b. gambiense* or *rhodesiense* parasites. Potential use of B2MG as a point-of-care test and as a test of cure will be evaluated on patients infected by other endemic diseases and HAT patients that underwent a treatment failure. This final validation will potentially confirm the ability of B2MG to complement actual methods for stage determination under field conditions.

P14

IDENTIFICATION OF SCREENING BIOMARKERS FOR CHROMOSOMAL ANOMALIES AND PREGNANCY-RELATED DISORDER USING QUANTITATIVE PLASMA PROTEOMICS

Varaprasad Kolla

Laboratory for Prenatal Medicine
University Women's Hospital / Department of Biomedicine

A current major obstacle is that no reliable screening markers exist to detect pregnancies at risk for preeclampsia. Quantitative proteomic analysis employing isobaric labelling (iTRAQ) has been suggested to be suitable for the detection of potential plasma biomarkers, a feature we recently verified in analysis of pregnancies with Down syndrome foetuses. We have now examined whether this approach could yield biomarkers to screen pregnancies at risk for preeclampsia. In our study, we used maternal plasma samples obtained at 12 weeks of gestation, six from women who subsequently developed preeclampsia and six with uncomplicated deliveries.

In our analysis, we observed elevations in 10 proteins in the preeclampsia study group when compared to the healthy control group. These proteins included clusterin, fibrinogen, fibronectin, angiotensinogen, increased levels of which are known to be associated with preeclampsia. An elevation in the immune-modulatory molecule, galectin 3 binding protein, was also noted. Our pilot study, therefore, indicates that quantitative proteomic iTRAQ analysis could be a useful tool for the detection of new preeclampsia screening markers.

Key words: iTRAQ (Isobaric Tags for Relative and Absolute Quantitation), preeclampsia, maternal plasma, biomarker, screening.

P15

COMPARATIVE PROTEOME ANALYSIS OF MILNESIUM TARDIGRADUM IN EARLY EMBRYONIC STATE VERSUS ADULTS IN ACTIVE AND ANHYDROBIOTIC STATE

Elham Schokraie[1], Uwe Warnken[1], Agnes Hotz-Wagenblatt[1], Ralph O. Schill[3], Marcus Frohme[2], Thomas Dandekar[4], and Martina Schnölzer[1*]

[1]Functional Proteome Analysis, German Cancer Research Center, Heidelberg, Germany
[2]Department of Molecular Biology and Functional Genomics, University of Applied Sciences Wildau, Wildau, Germany
[3]Department of Zoology, University of Stuttgart, Stuttgart, Germany
[4]Department of Bioinformatics, University of Würzburg, Würzburg, Germany

Tardigrades have fascinated researchers for more than 300 years because of their extraordinary capability to undergo cryptobiosis and survive extreme environmental conditions. However, the survival mechanisms of tardigrades are still poorly understood mainly due to the absence of detailed knowledge about the proteome and genome of these organisms. High-throughput, high-accuracy proteomics in combination with a newly developed tardigrade specific protein database lead to the identification of more than 3000 unique proteins in three different life states, namely early embryonic state and active and anhydrobiotic state of adult animals. All proteins were further investigated in a semi-quantitative way by calculating their exponentially modified protein abundance index which classifies proteins in major and minor components thereby allowing a comparative analysis of differentially expressed protein families in the different life states of tardigrades.

This comprehensive study is intended to serve as a basis for the identification and functional characterization of important proteins, which might play important roles in early embryonic development as well as in the anhydrobiotic state. Moreover, the comparative analysis of different life states of tardigrades on the protein level will help us to understand survival mechanisms in anhydrobiotic organisms and eventually to develop new methods for preservation of biological materials.

LIST OF PARTICIPANTS

Last Name	First Name	e-mail address	Presentation
Bader	Samuel	samuel.bader@embl.de	O16
Brunner	Andrea	brunner@hifo.uzh.ch	P12
Burri	Denise	burri_denise13@students.unibe.ch	P9
Chopra	Tarun	tarun.chopra@epfl.ch	O1
Cremonesi	Alessio	alessio.cremonesi@stud.unibas.ch	O2
Cuoghi	Aurora	aurora.cuoghi@gmail.com	P2
Delobel	Julien	julien.delobel@mavietonsang.ch	P6
Dimitrov	Mitko	mitko.dimitrov@epfl.ch	P10
Felser	Andrea	andrea.felser@unibas.ch	P4
Fornelli	Luca	luca.fornelli@epfl.ch	P10
Gueho	Aurelie	Aurelie.Gueho@unige.ch	O5
Gutnik	Silvia	silvia.gutnik@gmx.at	/
Higareda Almarez	Juan Carlos	ixca@ccg.unam.mx	P3
Kolla	Prasad	pkolla@uhbs.ch	P14
Koua	Dominique	Dominique.Koua@atheris.ch	O14
Kuzyakiv	Rostyslav	Rostyslav.Kuzyakiv@isb-sib.ch	P7
Licker	Virginie	virginie.licker@unige.ch	O13
Nillius	Dorothea	dorothea.nillius@ipa.unibe.ch	P8
Pak	HuiSong	Hui.pak@unige.ch	P1
Petruzziello	Filomena	filomena_petruzziello@hotmail.it	/
Ramirez Boo	Maria	maria.ramirezboo@unige.ch	O3
Rezaei	Mina	mina.rezaei@dkf.unibe.ch	/
Robin	Xavier	Xavier.Robin@unige.ch	O15
Rothschild	Sacha	sacha.rothschild@dkf.unibe.ch	/
Rubin	Olivier	olivier.rubin@unil.ch	O10
Schmitz	Andrea	andrea.schmitz@vpi.unibe.ch	/
Schokraie	Elham	e.schokraie@gmail.com	P15
Schvartz	Domitille	domitille.schvartz@unige.ch	O7
Schwander	Flurina	flurina.schwander@alp.admin.ch	P5
Simicevic	Jovan	jovan.simicevic@epfl.ch	O4
Soldi	Monica	monica.soldi@ifom-ieo.campus.it	P11
Sriranganadane	Dev	Dev.Sriranganadane@chuv.ch	O6
Tiberti	Natalia	Natalia.Tiberti@unige.ch	P13
Trinh	Hung	hung.viet-trinh@imls.uzh.ch	O8
Webhofer	Christian	webhofer@mpipsykl.mpg.de	O12
Wiedl	Thomas	thomas.wiedl@usz.ch	O11
Zufferey	Anne	Anne.Zufferey@unige.ch	O9

NOTES

NOTES

NOTES