CRG Centre de Regulació Genòmica

ABSTRACTS
(in order of appearance in the program

Annual Proteomics Symposium: "Unveiling the complexity of the cell proteome"

10 November 2017
PRBB Auditorium, Barcelona

Oscar YANES
University of Rovira i Virgili, Reus ES

"Proteomics meets metabolomics: a novel data analysis workflow to study metabolic alterations in diabetic retinopathy"

Protein expression and enzymatic activity are coordinated processes involved in the regulation of metabolic networks, and therefore, cell metabolism. This knowledge, however, is often neglected when statistically evaluating quantitative proteomics data, since protein values are treated as individual variables as if the enzymes in a metabolic network functioned independently. Herein, we present a novel data analysis workflow that uses the topology of the human metabolic network "Recon 2" to interrogate alterations in metabolites by testing the proteins associated to their metabolic reactions (i.e., synthesis and consumption) as a whole, instead of individually. To develop and validate our novel approach we obtained quantitative proteomics data using tandem mass tags, and metabolomics data using LC-MS, GC-MS and NMR in an in vitro model of diabetic retinopathy using retinal pigment epithelium cells. The results were further validated in vitreous humour samples from 28 individuals at different stages of diabetic retinopathy and non-diabetic controls.

CRG Centre de Regulació Genòmica

ABSTRACTS

Simone LEMEER

Biomolecular Mass Spectrometry and Proteomics, Utrecht University NL

"Drug resistance assessed by mass spectrometry based omics technologies"

Targeted therapies against oncogenic receptor tyrosine kinases are showing promising results in the clinic. However, despite the initial response, most patients become resistant. How are cells able to survive initial treatment? A multi-omics approach was used, including mass spectrometry based proteomics, phospho-proteomics, kinomics and metabolomics, to gain more insight in regulated processes during early TKI treatment. Our findings demonstrate how this multi-omics approach contributes to a better understanding of the molecular pathways underlying immediate drug tolerance and elucidates new potential targets that can be co-inhibited to prevent resistance development. In addition to this, I will present an universal and optimized phosphoproteomic workflow that enables comprehensive analysis of signaling pathways, yielding 60% gain in identifications without additional analysis time compared to previous workflows.

Irene CAMPOY

Vall d'Hebron Research Institute (VHIR), Barcelona ES

“Protein signature for endometrial cancer diagnosis in exosome-like vesicles of uterine aspirates”

Irene Campoy1, Eva Borràs2, Silvia Cabrera3, Xavier Matias-Guiu4, Marc Hirschfeld5,6, Jasmin Asberger5, Jaume Reventos2, Antonio Gil-Moreno2,3*, Eva Colás2*, Eduard Sabidó1*

1Biomedical Research Group in Gynecology, Vall Hebron Research Institute (VHIR), Barcelona, Spain. CIBERONC

2 Proteomics Unit, Universitat Pompeu Fabra (UPF) and Centre de Regulació Genòmica (CRG), Barcelona, Spain

3 Department of Gynecological Oncology, Vall Hebron University Hospital, Barcelona, Spain

4 Pathological Oncology Group and Pathology Department, University Hospital Arnau de Vilanova, and University Hospital Bellvitge, IRBLLEIDA and Idibell, University of Lleida. CIBERONC

5 Department of Obstetrics and Gynecology, University Medical Center, Albert-Ludwigs-University, Freiburg, Germany

6 Institute of Veterinary Medicine, Georg-August-University Goettingen, Germany

Background

Endometrial cancer (EC) accounts for more than 10,000 deaths per year in the US alone. EC is divided into the more common estrogen dependent type 1 and the less common but more aggressive estrogen independent type2. There is an urgent need to develop non-invasive tests that can provide early detection of EC and that can discriminate EC subtypes. This study focuses on the identification of protein markers in exosome-like vesicles (ELVs) isolated from uterine aspirates. Uterine aspirates are collected by a minimally invasive procedure and it represents the ideal body fluid since it is the closest to the neoplasic endometrium cells.

Methods

Protein extracts from purified ELVs were obtained following ultracentrifugation of UAs from age-matched groups of control, type1 and type2 EC patients (10 patients/group). The quality of isolated ELVs was monitored by Nanoparticle Tracking Analysis, and immunoblots. To profile protein abundance across different groups, we develop a super-SILAC approach where ELV proteins from 3 different EC cell lines grown in heavy Lys and Arg amino acids were combined with ELV protein extracts of each patient. Proteins were separated by SDS-PAGE and gel-isolated bands were digested with trypsin and analyzed by Mass Spectrometry. We generated a list of 54 protein candidates that was further validated by selected reaction monitoring (SRM) in an independent cohort of 107 patients including 3 age-matched groups: type 1 EC (n=45) EC, type 2 EC (n=21) and healthy individuals (n=41). A total of 86 unique peptides matching the proteins of interest were monitored. Isotopically-labelled peptides were spiked in each sample as peptide standards, and protein quantitation was performed using a QTRAP 5500 Sciex instrument.

Results

Our targeted mass spectrometry approach confirmed that ELVs from uterine aspirates contain proteins that can discriminate between cancer patients and healthy individuals. More importantly, a 2-protein signature improves this capacity to discriminate healthy from EC patients (ROC AUC=0.935). This protein signature can detect endometrial cancer independently of the cancer type. In addition, we also report a new protein signature that can differentiate type1 versus type2 EC (ROC AUC=0.932). This study has important implications in early detection of EC and in patient stratification.

Conclusion

A targeted mass spectrometry approach defines protein signatures for endometrial cancer diagnosis in uterine aspirates.

Gerard SEINSENBACHER

Pompeu Fabra University, Barcelona ES

“Protein-peroxiredoxination in S. cerevisiae”

Post-translational modifications (PTMs) increase the functional diversity of the proteome. PTMs range from e.g. a single phosphate groups up to the attachment of regulatory proteins such as ubiquitin. While regulated PTMs of proteins are important components of cell signaling, unspecific modifications such as protein oxidation impair proper cellular function. Peroxiredoxins are small ubiquitous proteins that have an important role as cellular antioxidants. Using mass spectrometry we show that a plethora of proteins get peroxiredoxinated in vivo. Our data suggest that peroxiredoxin gets covalently attached to target proteins via its peroxidatic cysteine. Here we investigate the potential role of peroxiredoxination as a PTM to regulate protein function in response to environmental changes.

Karl MECHTLER
Vienna Biocenter Core Facilities, Vienna AT

“The dark matter of the proteome”

In shotgun proteomics, proteins are enzymatically digested into peptides that are fed into a highly automated pipeline for chromatography and mass spectrometry (MS) analysis.
When applied to complex mixtures of proteins from cells or tissues, the technique yields tens thousands of peptide sequences, only a minority of which can be mapped to their proteins of origin.
Despite the efforts of many researchers, the identity and origin of the unassigned peptides have remained a mystery.

Most dark proteins are short, rarely interact with other proteins, are:
- frequently excreted and only have a small number of evolutionary relatives
- Sample preparation (e.g. non-soluble membrane proteins
- Unexpected post-translational modifications
- Protein of interest is not in database (long noncoding RNAs, SNPs,..)

Fragmentation behavior of peptides and their modifications – more than 50 % of “dark” MS/MS spectra
On of this unexpected post-translational modification is glycosylation. More than half of all human proteins are glycosylated. This covalent attachment of carbohydrates can alter protein activity. However, existing methods for studying the glycoproteome typically requires laborious sample processing and proprietary software. They also often require enzymatic removal of the glycan, which makes it difficult to identify attachment sites. Recently we developed a proteomic method to identify intact glycopeptides in proteomics data. Focusing on the intact fragments allows both the structure of the glycan and the attachment site in the associated protein to be identified.

Marco FAINI
ETH Zurich CH

“Revealing the organisation and function of cellular modules by structural proteomics”

Protein complexes represent the functional modules of the cell. Studying their assembly and structure in a physiological manner is fundamental to understand cellular processes and their dynamics. Complex interactions have been studied by affinity purification mass spectrometry (AP-MS) that provides bait interactors but requires multiple experiments to attribute high interaction confidence. Conversely, cross-linking mass spectrometry (XL-MS) can pinpoint protein neighbourhood and structural restraints between single amino-acid residues but requires large sample amounts and high purity.
We developed quantitative affinity cross-linking MS (qAXL-MS), combining affinity purification of native protein complexes from human cells with a highly specific cross-link quantitation workflow. We can now stimulate cells or lysates and map the differential abundance of distance restraints onto available structures, determining the structural and compositional dynamics of protein complexes upon perturbation.
First, we applied qAXL-MS to the human chaperonin TRiC. We validated the method by mapping the cross-links on the human TriC structure and measuring their expected distances. Then, we confirmed the conformational changes between open and closed states previously obtained by large-scale purifications. Finally we used qAXL-MS to show complex disassembly in Cul4a complexes upon Nedd8 inhibition.

Brandon INVERGO

European Bioinformatics Institute, EMBL-EBI UK

”Sub-minute phosphoregulation of cell-cycle systems during Plasmodium gamete formation”

Brandon M. Invergo1,2*, Mathieu Brochet2,3*, Lu Yu2, Jyoti Choudhary2,
Pedro Beltrao1, Oliver Billker2

1: European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom

2: Wellcome Trust Sanger Institute, Hinxton, United Kingdom

3: Department of Microbiology & Molecular Medicine, University of Geneva, Switzerland

*: These authors contributed equally to this work.

Malaria parasites are protists of the genus Plasmodium, whose transmission to mosquitoes is initiated by the production of gametes. Male gametogenesis is an extremely rapid process that is tightly controlled to produce eight flagellated microgametes from a single haploid gametocyte within 10 minutes after ingestion by a mosquito. Regulation of the cell cycle is poorly understood in divergent eukaryotes like Plasmodium, where the highly synchronous response of gametocytes to defined chemical and physical stimuli from the mosquito has proved to be a powerful model to identify specific phosphorylation events critical for cell-cycle progression. To reveal the wider network of phosphorylation signalling in a systematic and unbiased manner, we have measured a high-resolution time course of the phosphoproteome of P. berghei gametocytes during the first minute of gametogenesis. The data show an extremely broad response in which distinct cell-cycle events such as initiation of DNA replication and mitosis are rapidly induced and simultaneously regulated. We identify several protein kinases and phosphatases that are likely central in the gametogenesis signalling pathway and validate our analysis by investigating the phosphoproteomes of mutants in two of them, CDPK4 and SRPK1. We show these protein kinases to have distinct influences over the phosphorylation of similar downstream targets that are consistent with their distinct cellular functions, which is revealed by a detailed phenotypic analysis of an SRPK1 mutant. Together, the results show that key cell-cycle systems in Plasmodium undergo simultaneous and rapid phosphoregulation. We demonstrate how a highly resolved time-course of dynamic phosphorylation events can generate deep insights into the unusual cell biology of a divergent eukaryote, which serves as a model for an important group of human pathogens.

CRG Centre de Regulació Genòmica

ABSTRACTS

Enrico CAPPELLINI

Natural History Museum of Denmark, University of Copenhagen DK

“Palaeoproteomics: state of the art and perspectives”

The revolutionary impact of ancient DNA analysis in many different research areas cannot be understated. Nevertheless, despite the exceptional efforts thrown thrown into methodological development, so far, the most ancient DNA sequences recovered from temperate and high-latitude environments are dated at ~0.5 and ~0.8 Ma respectively. However, growing experimental evidence and theoretical models agree in indicating that ancient protein residues can be retrieved from epochs and geographic areas at the moment unsuitable for aDNA recovery. This is not the only edge ancient proteins present over ancient DNA. Being the product of gene expression, protein sets, i.e. proteomes, vary in different tissues, organs or developmental stages. Ancient proteomes can also represent a direct evidence of physiological and pathological processes occurred in the past. On the other hand, ancient proteins are admittedly less informative than aDNA as a source of genetic information and as such they cannot be considered the evidence of choice for reconstruction of ancient population dynamics. In the last few years, investigation of ancient proteomes, i.e. palaeoproteomics, demonstrated the value of this approach in palaeontology and human evolution studies. In the future, palaeoproteomics has the potential to enable access to genetic evidence from epochs and geographic areas incompatible with ancient DNA preservation, and facilitate investigation into deep time evolution, which has so far been intractable for molecular phylogenetics.