CRG Centre de Regulació Genòmica

ABSTRACTS

Annual Proteomics Symposium: "Applying proteomics to life sciences"

11 November 2016
PRBB Auditorium, Barcelona

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"The proteome in context"

Recent advances in various bottom-up proteomic techniques have resulted in significant advances towards the confident and routine identification and quantification of proteins. Data dependent acquisition (DDA) has resulted in saturation coverage of proteomes of a number of species and the generation of near complete spectral libraries paved the way to reliably identify essentially any protein of a number of proteomes by targeted analysis via SRM or SWATH-MS. In both, DDA and targeted strategies each polypeptide is considered as an independent query unit. However, this is not how proteins function in the cell. Most polypeptides carry out their biological function in the context of macromolecular assemblies, including protein complexes, protein-nucleic acid complexes and functional interaction networks.

In this presentation we will discuss emerging computational and laboratory techniques to determine functional context of proteins in the cell. These include the direct measurement of molecular interfaces in macromolecular complexes by chemical cross-linking MS (XL-MS), the integration of XL-MS data and structural data generated by cryo-EM single particle analysis to obtain structural models and correlative analyses of proteins precisely quantified by SWATH-MS across large sample cohorts to determine changes in complex stoichiometry.

We will use selected examples to illustrate the biological significance of measuring or inferring proteins in the context of functional modules

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Olivia BELBIN

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"Combining shotgun and targeted proteomics to identify novel biomarkers for Alzheimer's disease"

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A biomarker capable of detecting synapse loss, which occurs early in Alzheimer's disease (AD) pathophysiology, would greatly assist in pre-symptomatic diagnosis, when treatment would most likely be effective. The objective of this study was to identify, quantify and evaluate synaptic protein levels in cerebrospinal fluid (CSF) as biomarkers of synaptic damage in AD. To determine the proteins that are detectable in the CSF, shotgun liquid chromatography mass spectrometry was performed on CSF samples from 50 cognitively healthy controls. A total of 2,136 proteins (1+ unique peptide) were identified and subsequently filtered for those that are physically or functionally related to the synapse by curation of published proteomic studies of synapse-enriched brain fractions and online databases. A panel of 10 synaptic CSF proteins was selected for evaluation and 22 corresponding peptides were monitored by targeted mass spectrometry (Selected Reaction Monitoring) in CSF samples from our exploratory clinical dementia cohort (n=95). Peptide levels in patients from each dementia stage were compared with those in cognitively healthy controls using the MSstats package in R. All peptides were increased in mild cognitive decline and dementia patients but not in pre-symptomatic subjects. However, when the synaptic protein levels were normalised for neuronal protein loss using an axonal degeneration biomarker, a reduction in synaptic proteins in the CSF was evident even at the pre-symptomatic stage. By combining shotgun and targeted proteomics, we have identified a panel of novel biomarkers that can be used to detect and monitor reduced synapse density in the brain of subjects on the AD continuum, which, if validated in an independent cohort, could be invaluable stage biomarkers for AD.

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Cristina CHIVA

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"Unsupervised quality control system for next generation proteomics"

Proteomics technologies have evolved a lot in the last years. The field is moving towards clinical applications either for diagnosis, prognosis or class classification of patients. The analytical challenge of these new applications has increased substantially and new approaches to warranty the quality of the proteomics experiments are needed.

We believe that the proteomics researchers should agree in quality procedures that will give us an unprecedented power as a community to demonstrate the quality of our technologies, to do inter-lab comparisons, to evaluate new instruments and also to facilitate the interaction with the manufactures and the technical service. We propose an automatic quality control workflow that allow the proteomics researchers to easily monitor their instruments performance and to detect low quality experiments. This tool will improve the reliability of the proteomics results.

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Ileana CRISTEA

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"A Systems Approach to Define Oraganelle Dynamics during Viral Infection"

Viruses and cell hosts have established complex, dynamic interactions that function either in promoting virus replication and dissemination or in host defense against invading pathogens. Thus, viral infection triggers a drastic transformation in intracellular proteomes, including the reorganization of organelles. An important example is human cytomegalovirus (HCMV), a beta-herpesvirus that infects a large majority of the adult population worldwide, leading to life-threatening diseases in immunocompromised individuals. HCMV triggers an extensive reorganization of organelle structure for energy production, intracellular trafficking, and generation of the virion assembly compartment. However, the viral proteins that target distinct organelles or the specific organelle proteins recruited for these morphological changes remain in large part unknown. Here, we report the first global proteomic study of cellular organelles during viral infection. First, using time-lapse microscopy and fluorescent confocal microscopy, we monitored changes in organelle morphology during HCMV infection of primary human fibroblasts. We observed the signature mitochondria fission, rearrangement of the Golgi apparatus around the viral assembly complex, increased lysosome size, and increased density of the endoplasmic reticulum. Next, organelle densities were examined by ultracentrifugation, while changes in their compositions were determined by quantitative mass spectrometry. Labeling with tandem mass tags was combined with a label-free approach to gather spatial and temporal information of organelle composition. Dimensional reduction algorithms and supervised machine learning allowed us to confidently assign proteins to organelles throughout the time course of infection. Importantly, we discovered viral proteins with temporally-regulated localizations at different organelles, including the previously uncharacterized HCMV protein, pUL13. We next investigated the function of pUL13, demonstrating its requirement for viral replication and spread. Altogether, our study highlights mass spectrometry as an important component of discoveries in virology.

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Bernhard KUSTER

Proteomics and Bioanalytics at the Technical University of Munich DE

"Chemical proteomics reveals the target landscape of clinical kinase inhibitors"

Kinase inhibitors have developed into important cancer drugs because de-regulated protein kinases are often driving the disease . Efforts in biotech and pharma have resulted in more than 30 such molecules being approved for use in humans and several hundred are undergoing clinical trials. As most kinase inhibitors target the ATP binding pocket, selectivity among the 500 human kinase is a recurring question. Polypharmacology can be beneficial as well as detrimental in clinical practice, hence, knowing the full target profile of a drug is important but rarely available. We have used a chemical proteomics approach termed kinobeads to profile 240 clinical kinase inhibitors in a dose dependent fashion against a total of 320 protein kinases and some 2,000 other kinobead binding proteins. In this presentation, I will outline how this information can be used to identify molecular targets of toxicity, re-purposing existing drugs or combinations for new indications or provide starting points for new drug discovery campaigns.

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Ana MARTÍNEZ

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"Understanding Ground State Pluripotency using Quantitative Proteomics"

Martinez-Val, A., Lynch, C., Serrano, M., Muñoz, J.

Typically, mESCs cultured in serum are highly heterogeneous and cells fluctuate between a "primed" and a "naïve" state of pluripotency. Whilst the primed state is prone to differentiation, the naïve state shows higher maintenance of its self-renewal capacity. Also, mESCs in the naïve state are defined by the uniform expression of pluripotency markers, the reactivation of the X chromosome and the transcriptional pausing of RNApol II. Thus, naïve mESCs are considered as the ground state of pluripotency and an in vitro surrogate of pre-implantation embryos. Inhibition of GSK3 and ERK (known as 2i) can capture this ground state indefinitely in culture. Interestingly, inhibition of CDK8 (a regulator of RNApol II) also seems to stabilize mESCs towards the ground state. Whether these two mechanisms, i.e. 2i and CDK8i, converge in a similar ground state it is not clear.

Here, we used quantitative proteomics and phosphoproteomics to characterize the molecular mechanisms regulated by 2i and CDK8i. Our results show a significant degree of similarity in the proteomes of mESCs treated with 2i and CDK8i (e.g: pluripotency maintenance and DNA methylation). Integration of our proteomic signatures with published transcriptomic data from in vitro and in vivo mESCs revealed the resemblance of these cells to other naïve mESCs and to the pre-implantation epiblast. Moreover, despite 2i and CDK8i act at different regulatory levels, the early phospho-proteome of both treatments highly overlaps. Together, these observations suggest that direct inhibition of RNA pol II activity, by means of CDK8i, also induces the ground state of pluripotency.

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Jordi RAMBLA

Centre for Genomic Regulation, Barcelona ES

"Sharing sensitive genomic human data"

Data privacy vs data sharing issues are a known topic in Human Genomics. Although it is not yet a solved problem for genomics, the field has found and dealt with the first wave of issues and it is now addressing the second wave. In this talk, we will summarize the known issues and lessons learned on the way.

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Henry RODRIGUEZ

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"Proteogenomics of Cancer: New Oportunities in Cancer Biology and Precision Medicine"

The ability to interrogate cancer at the proteogenomic level (interplay between the proteome and genome) will transform oncology care from one that relies mainly on trial-and-error treatment strategies based on the anatomy of the tumor, to one that is more precisely based on the tumor's molecular profile. Understanding this molecular interplay and publicly releasing proteogenomic data sets and targeted assays to create community resources is anticipated to accelerate our understanding of cancer and its treatment. This seminar will discuss how genomics, transcriptomics, and proteomics must all be brought together in the quest to understand the etiology of cancer, in addition to highlighting efforts by the U.S. National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC) program in this area of biomedical research. In addition, this seminar will highlight the recently announced White House Cancer Moonshot APOLLO program (Applied Proteogenomics OrganizationaL Learning and Outcomes) and the international proteogenomic partnerships. APOLLO brings together the U.S. National Cancer Institute, U.S. Department of Defense, and the U.S. Department of Veterans Affairs to create the nation's first healthcare system in which cancer patients will be routinely screened for genomic abnormalities and proteomic information with the goal of matching their tumor type to a specific targeted therapy. The international proteogenomic partnerships span 7 countries and 14 institutions.

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Anna SANTAMARIA

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"Plk1 phosphoproteomics: from mitotic control to cancer development"

Plk1 belongs to the polo-like kinase family, of which five members (PlK1–5) have been identified in human. PlK1 stands out as a promising drug target in oncology. PlK1 is essential for cell division, and its inhibition leads to mitotic arrested cells with monopolar disorganized spindles, eventually resulting in cell death. Interestingly, non-transformed cell lines survive inhibition of PlK1, providing a possible basis for tumour selectivity.

A common feature of polo-like kinases is the presence of a highly conserved polo-box domain (PBD) in the carboxy-terminal part of the protein. Through its PBD, PlK1 associates with a large number of proteins, some of which mediate its recruitment to defined cellular structures, such as the centrosomes, kinetochores and the spindle midzone and our previous work has contributed to the identification of many of the spindle-associated Plk1 substrates.

The therapeutic window for drugs that target Plk1 is not yet fully understood, but currently available Plk1 inhibitors have been indicated for treatment of several solid tumors. Among those, hormone-refractory prostate cancer (CRPC), known to have undergone a genetic reprogramming to selectively upregulate the expression of M-phase cell cycle genes and in which we and others have found Plk1 to be overexpressed. We have recently obtained a list of protein candidates whose expression is altered in a Plk1-dependent manner during the progression of PCa to an androgen independent state, suggesting some of the Plk1 downstream targets as promising therapeutic targets for molecular intervention of CRPC
patients.