PROTEOMICS CORE UNIT

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**Titulado Superior* (Advanced Degree)

OVERVIEW

Proteomics is acquiring a critical role in the comprehensive understanding of human biology. The fast development in mass spectrometry-based proteomics instrumentation and data analysis pipelines has helped the scientific community to dig (even) deeper into the proteome. In the last decade, the main output of differential proteomics studies has evolved from long lists of proteins to the generation of new hypotheses, allowing proteomics to become functional. For example, cancer proteomics has unravelled key data in mechanistic studies on tumour growth and metastasis, contributing to the identification of clinical biomarkers and novel therapeutic targets. Several cancer proteome databases have been established and are being shared worldwide. The CNIO Proteomics Core Unit develops and applies state-of-the-art proteomics, informatics, and related technologies, for direct interrogation of protein expression, modification, and function in cell-based models of human cancer. We aim to provide valuable guidance for experimental strategies, which are critical for cancer research success.

RESEARCH HIGHLIGHTS

In collaboration with the Experimental Oncology Group, the Unit has measured stoichiometric changes in the RHC complex due to 8 RAF1 and 1 CDC37 single mutations. We observed that the modification of key interface residues between both RAF1 and CDC37 proteins reduced RAF1 protein levels present in the complex. Global analysis of protein phosphorylation was also performed, and novel RAF1, CDC37 and HSP90 phosphorylation sites were elucidated when forming this complex. Together with the Cell Division and Cancer Group, the Unit performed a global proteome analysis of neural differentiation in CDC14-null cells and elucidated UTF1 *in vitro* phosphorylated sites. The Unit also teamed up with the Breast Cancer Clinical Research Unit to reveal a new physical interactor of Filamin A, CLIP170, which plays a role in microtubule stabilisation and may explain the increased sensitivity to paclitaxel in tumours with elevated CDK4. With the Microenvironment and Metastasis Group, the Unit characterised plasma circulating small extracellular vesicles derived from melanoma patients compared to proteins detected in plasma samples. In collaboration with the Medical University of Dresden (Germany), the Unit used Tandem Mass Tag (TMT) isobaric labelling proteomics and phosphoproteomics to identify a novel treatment approach for RTK/MAPK pathway altered in gastric cancer patients. With M. Serrano's group at *IRB* Barcelona, we used label-free proteomics to reveal the profound changes of the lysosomal proteome in senescent cells and studied the "surfaceome" of 2 diploid primary fibroblasts and 2 cancer cell lines in response to the senescence inducers doxorubicin and palbociclib. Aiming to investigate the effect of different variables in the performance of proteome-wide phosphoprotein analysis protocols, the Unit has formed part of a multicentre collaboration launched by *ProteoRed-ISCIII*. Finally, the Unit setup a new cross-linking mass spectrometry-based workflow to fit the needs of the Structural Biology Programme (FIGURE 1). This emerging technology interrogates protein structure and helps reveal novel protein-protein interactions. The protocol, robust and widely applicable, is based on protein cross-linking with MS-cleavable reagents, enzymatic digestion followed by high pH fractionation, and LC-MS/MS analysis. The output allows the identification of cross-links, assessing spatial and morphology constraints for recombinant purified proteins and complexes. ■

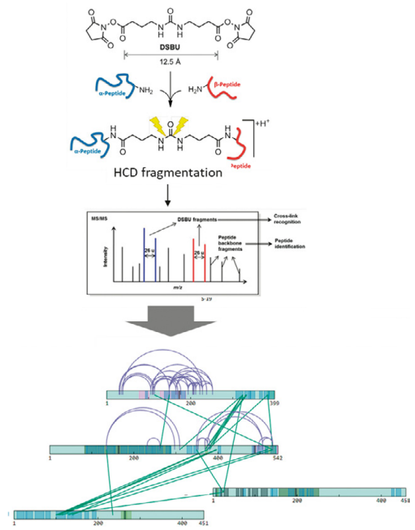

FIGURE 1 Schematic showing the workflow used for cross-linking-based mass spectrometry. Covalently bound peptides derived from cross-linked proteins are identified, providing 3D structure analysis of proteins and protein complexes.

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