PROTEOMICS CORE UNIT

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OVERVIEW

Proteins are the molecular effectors of cells and catalyse almost all biological processes. The levels of protein abundance, together with their modification states and interactions, adapt dynamically to external or internal (genetic) stimuli and thus define the cell's functional state and determine its phenotype. Mass spectrometry-based proteomics is the most powerful tool to study the proteome, providing fundamental basic biology information. In addition, recent improvements in sensitivity and throughput now enable the analysis of larger cohorts of samples including biopsies, thereby making proteomics a part of the clinical research toolbox. All these efforts are providing new insights into the molecular mechanisms underlying cancer development as well as the identification of novel biomarkers.

"The new generation of mass spectrometers enables the analysis of complex samples to unprecedented depth levels, thereby becoming a real alternative to RNAseq for sample profiling."

RESEARCH HIGHLIGHTS

Throughout 2018, the Proteomics Unit has acquired 2 novel Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) platforms. The Q Exactive Plus and the Q Exactive HF-X, the latter of which is the fastest instrument on the market, are now part of the equipment available for research projects in our Unit. Both mass spectrometers are coupled to nanoHPLC systems with plug-and-play nESI interfaces. The application of this technology in the CNIO will have a major impact on the characterisation of complex proteomes as well as on the analysis of small protein amounts (e.g. FACS sorted cells, post-translational modifications...). In addition, the high resolution of the Orbitrap detector enables the analysis of TMT11-plex, increasing the range of possibilities during the experimental design with the inclusion of biological replicates and/or different conditions. Furthermore, if coupled to modified pre-fractionation strategies based on basic pH reverse phase HPLC, full proteome coverage can be achieved across 11 samples (FIGURE). The Unit has also continued to work in close collaboration with CNIO Research Groups in several projects. To highlight some of them, together with the Brain Metastasis Group, we analysed the secreted factors of a subpopulation of pSTAT positive astrocytes that mediate brain metastasis. In collaboration with the Cell Division and Cancer Group, we performed a time course phosphoproteome analysis of activating platelets and have identified important defects in the phosphorylation of actin cytoskeleton proteins in a model of thrombocytopenia, which is caused by mutations in MASTL, a cell cycle kinase. Also, in a multi-omic project led by the Melanoma Group, we performed a proteomic analysis to define the interactors of sequestosome, aka p62, which have revealed unexpected roles for this protein in extending the mRNA half-life of several pro-metastatic factors. ■

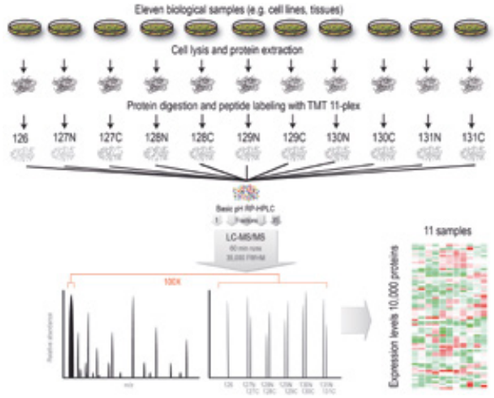

Figure Samples are digested and labelled with new isobaric tagging TMT 11-plex. The pull of peptides is then pre-fractionated by High pH and 35 non-concatenated fractions are analysed on a Q Exactive instrument at high resolution using short gradients. This enables the identification of nearly full proteome coverage.

PUBLICATIONS

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