OMICS International
conferenceseries.com

J Proteomics Bioinform 2015, 8:8
http://dx.doi.org/10.4172/0974-276X.S1.077

5th International Conference on

Proteomics & Bioinformatics

September 01-03, 2015 Valencia, Spain

A dynamic picture of the ubiquitinome upon proteasome inactivation

Jeroen A A Demmers
Erasmus University Medical Center, The Netherlands

The 26S proteasome is a 2.5 MDa protein complex, which degrades unneeded and damaged proteins in the cell. As such, it is critical in regulating proteostasis and controls key regulator abundance levels. Malfunctioning of the ubiquitin–proteasome system has been implicated in diseases such as cancer and neurodegenerative disorders. On the other hand, in cancer therapeutics the induction of apoptosis by proteasome inhibition using drugs is widely used. Current strategies are directed towards the development of more selective inhibitors that target the proteasome regulatory subcomplex and have less side-effects. We take a proteomics approach to dissect the molecular mechanisms of the proteasome regulatory subcomplex, which is essential for the development of better proteasome inhibitors. Targeted proteasome inactivation by selective RNAi knockdown or drugs is monitored at the proteome and ubiquitinome levels using a SILAC approach in *Drosophila*. Over 5,000 proteins and 10,000 diGly peptides were identified and quantified. After brief inactivation by drugs, proteins involved in stress response, cell cycle regulation, apoptosis and the UPS were upregulated (e.g., Hsp proteins) and accumulated. After prolonged inactivation, the abundances of several 100s of proteins were altered. Similar effects were observed after inactivation of the proteasome with RNAi knockdown of different subunits. Protein ubiquitination dramatically increased upon proteasome inactivation. Interestingly, many proteins showed dynamic ubiquitination changes in opposite directions on different target lysine residues within the same protein. Proteomic analysis of individual RNAi knockdown of three proteasome bound deubiquitinating enzymes indicated that each of them has a different and specific function. Finally, proteasome interactome profiling under different experimental conditions using LFQ based quantitation suggested that the proteasome itself is a dynamic complex that recruits different partners and/or (sub)complexes under specific conditions. Global analysis of the dynamic proteome and ubiquitinome after proteasome inactivation gives detailed insight into regulatory mechanisms of the proteasome.

j.demmers@erasmusmc.nl

Mass cytometry-mass spectrometry meets cytometric applications

Sabine Baumgart
German Rheumatism Research Centre, Germany

Single-cell mass cytometry is a promising technology for a multiparametric analysis of individual cells in heterogenous biological samples such as blood, bone marrow or biopsy samples. The technology is based on atomic mass spectrometry detection but the read out is similar to flow cytometry. Cells are specifically labeled with metal-isotope tags attached to antibodies instead of fluorophores used in traditional flow cytometry approach. Fixed cells are introduced into plasma region of a mass cytometer (CyTOFTM). Metal-isotopes are atomized, ionized and separated in the time-of-flight mass analyzer before detection. Currently, the overall restricted number of antibodies being used in one panel in the traditional flow cytometry approach hampers the optimal immunophenotyping characterization. This becomes more critical in well invested studies finding biomarkers for the diagnosis of certain diseases and if the number of cells in clinical samples is restricted. Mass cytometry technology overcomes this limitation and can routinely detect about 40 markers per individual sample with minimal signal overlap common to atomic mass spectroscopy. Mass cytometry enables comprehensive profiling of cellular phenotype, signaling state and cytokine expression with the ability to measure simultaneously over 100 markers. The aim of the present study was to develop a 29 marker panel for the analysis of major and minor leukocyte populations including their differentiation-dependent subsets and appropriate activation marker molecules in whole blood samples depleted for erythrocytes. It will be used for a comprehensive immunophenotyping of human blood samples to monitor immune responses in rheumatic patients treated with anti-inflammatory biological and other autoimmune diseases.

Sabine.Baumgart@drfz.de