A.41
An accurate large-scale human E3 ligase concentration map for next-generation TPD-based precision medicine created by MaxQuantAtlas
Daniela Ferretti1, Yatao Shi1, Pavel Sinitcyn2, Shivani Tiwary2, Chris Browne1, Scott Rusin1, Eric Kuhn1, Susanne Breitkopf1, Dirk Walther1, Juergen Cox2, Kirti Sharma1

1Kymera Therapeutics, 200 Arsenal Yards Blvd., Watertown, MA 02472, USA, 2Computational Systems Biochemistry, Max-Planck Institute of Biochemistry, 82152 Martinsried, Germany

Targeted protein degradation (TPD) has emerged as a promising new therapeutic modality. Heterobifunctional small molecule degraders hijack the body’s natural cellular degradation machinery by binding an E3 ubiquitin ligase and a target protein of interest, resulting in degradation of the latter. Given the human genome encodes at least 600 E3 ligases with diverse tissue expression patterns, there is potential to apply TPD selectively to targets in specific tissues, cell types and subcellular compartments. Such an approach can unlock opportunities for many drug target classes for which unwanted on-target pharmacology limits clinical application. There is a need for accurate mapping of E3 expression across both healthy and diseased tissues for data-driven investment in E3s with tissue sparing potential.

An ideal mapping approach would provide rapid access to reliable abundance estimates of all expressed E3 ligases (and target proteins) without significant upfront investment on either reagent or data generation. To this end, with the widespread use of MS-based shotgun proteomics, countless datasets of different human cell types and tissues with deep proteome coverage are constantly being added to public repositories providing valuable quantitative information on proteome-wide protein copy numbers. However, it remains largely underused because of technical challenges to compare protein levels across individual studies.

Here we introduce MaxQuantAtlas, a quantitative proteomics software platform designed for the integration of MaxQuant-processed proteomics datasets over samples acquired with label-free and label-based quantification strategies and instrument types. MaxQuantAtlas enables assembly of a first-in-class human protein concentration atlas over cell lines, primary cells, healthy and diseased tissues and we find them clustering in biologically meaningful ways, independent of quantification and acquisition technologies. This software platform yields quantitative readouts of E3 expression patterns that are in good agreement with results from the precision method. Using comparative analyses of expression patterns from our protein concentration map, we are able to identify differentiated opportunities for selective pairings of E3 ligases with therapeutic targets of interest. We will provide examples of our LED (ligandability, expression, degradation) strategy in play as we select tissue sparing E3 ligases and showcase novel E3 ligase with broad utility for solid tumors.

100321, https://doi.org/10.1016/j.mcpro.2022.100459

A.42
MS-based Detection of Pathogen Antigen Through Immunoprecipitation of Its Proteolytic Peptides
Qingbo Shu1, Jia Fan1, Christopher J. Lyon1, Tony Hu1
1Tulane University, New Orleans, LA 70112

Detecting pathogen-derived proteins in blood through immunoassays enables microbiological confirmation of infection and tracking the dynamics of pathogen burden. However, their relatively low abundances in blood and the specified sample inactivation requirement challenge the development of an immunoassay with high sensitivity, specificity and adaptability. Here we describe robust criteria to select species-selective peptides from circulating pathogen-derived proteins as antibody targets for biomarker detection in small blood volumes, and to differentiate these immunoprecipitated peptide targets from non-specific background in trypsin-digested serum or plasma samples. We report that this approach can detect tuberculosis irrespective of infection site and disease burden, and distinguish between Ebola virus species exhibiting different virulence or requiring different treatments by target peptide sequence variations. This represents a significant advance over existing diagnostics for infectious diseases that require pathogen-rich or culture samples for specific identification, and frequently cannot distinguish viable and non-viable pathogens to assess treatment responses.

Our standard serum processing approach employs a temperature-assisted denaturing buffer. Serum samples are diluted with 5 to 10 volumes of denaturing buffer and heated at 100°C for 5 minutes, to allow protein denaturation, then supplemented with trypsin at a 1:600-1:800 ratio of trypsin-to-protein (10 μg trypsin to 100 μL serum) given an estimated serum protein concentration range of 60-80 g/L, which is less than the 1:100 trypsin-to-protein ratio utilized when a urea buffer is employed to denature protein. The denaturing condition also improves enrichment efficiency by increasing the dilution of serum proteins in the denaturation/digestion reaction. It utilizes detergent conditions that are compatible with the immunofinity procedure, to avoid the need for buffer exchange with its associated material losses and to reduce non-specific interactions with the antibody-conjugated capture matrix during the immunoaffinity reaction. Moreover, rigorous criteria for result interpretation are applied to avoid false positive identification and imprecise quantification, and the method was validated in clinical samples. It indicates that a common design approach can identify pathogen-specific peptide biomarkers that can be employed to sensitively detect, discriminate and quantify intracellular and extracellular pathogens either individually or in multiplex analyses.

100321, https://doi.org/10.1016/j.mcpro.2022.100460