A Method to Identify Actively Degraded Proteins

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Overview

This technology provides a method to identify the full landscape of proteins that are actively being degraded by the proteasome, offering a way to identify novel targets in various biological conditions and contexts.

Background and Unmet Need

The cell's proteome (protein profile) is constantly changing due to active degradation by the proteasome system, which is the main proteolytic system in the cell. Degradation by the proteasome system is a highly-regulated process implicated in numerous cellular events including transcription, differentiation, proliferation, and death. Identifying the cellular "active degradome", meaning all proteins actively being degraded by the proteasome at a given time, can therefore reflect the cellular condition, provide substantial insights into cellular programs and state, and present novel therapeutic possibilities. Current methods to detect the cell's active degradome, which mainly rely on mass spectrometry (MS) analysis, have low sensitivity and fail to detect low abundance proteins and proteins with rapid turnover (e.g. transcription factors). Therefore, there is an unmet need for a method to better detect, analyze, and profile degraded proteins inside cells.

The Solution

The group of Dr. Yifat Merbl developed MAPP (Mass Spectrometry Analysis of Proteolytic Peptides), a unique method to isolate and identify the peptides captured inside or near the proteasome.

Technology Essence

The proteasome is isolated by immunoprecipitation under reversible cross-linking conditions that maintain the peptides that are being degraded within it. Subsequently, solid-phase extraction and MS analysis identifies these peptides (Figure 1). MAPP can be used to study patterns of protein cleavage and proteasomal regulation induced by defined stimuli or under different pathologies such as cancer and inflammation. For example, since proteasomes are known to be upregulated in cancer cells, MAPP can be used on biopsies to identify specific "fingerprints" of the transformed tumor, enabling personal tailoring of the most potentially effective treatment. Another example is the immunoproteasomes, a unique proteasome that is activated by cells under inflammatory conditions. Applying MAPP on cells following a viral or bacterial infection will identify an infection-induced 'active-degradome' signature. This can be used to identify pathogenic proteins that are targeted for either antigen presentation or other cellular functions, which holds significant therapeutic potential. Of note, proteasomes can also be isolated from biological fluids (i.e. blood, cerebrospinal fluid); therefore, proteasome profiling provides an accessible tool for analyzing different pathologies.

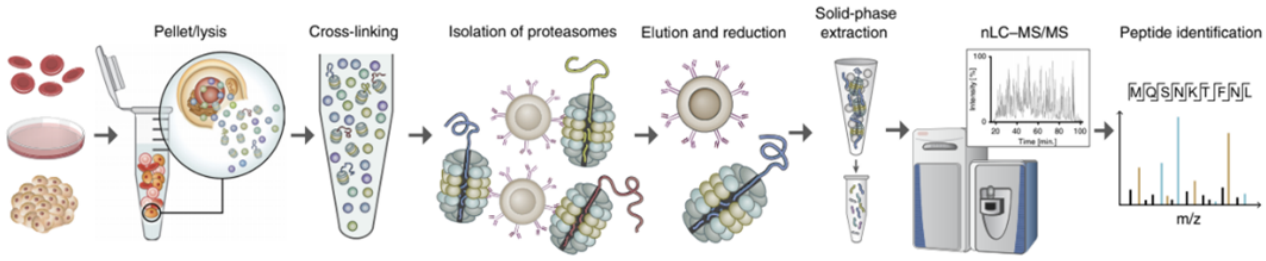

Fig 1. MAPP method workflow (Wolf-Levy H, Nat Biotech, 2018)

Applications and Advantages

- Personalized medicine applications for various pathologies, including cancer, bacterial and viral infections, and autoimmune diseases.
- Sensitive - enables the detection of less abundant proteins and proteins undergoing rapid turnover.
- Unbiased sampling.
- Versatile - can be used in profiling multiple types of specimens, including primary cells, and in a variety of contexts, from basic cell biology to clinical applications.
- Novel indications - may be used to study the regulatory steps from peptide cleavage to human-leukocyte-antigen presentation.

Development Status

Dr. Merbl and her team developed protocols for their novel method and proved it to be more sensitive compared to standard mass spectrometry assays. In vitro studies demonstrated MAPP can detect degradome changes in cells in response to stimuli that standard proteomics could not.

To demonstrate clinical importance, the team compared the degradome profiles of peripheral blood mononuclear cells (PBMCs) derived from systemic lupus erythematosus (SLE) patients vs. healthy donors. The team could detect 100 unique proteins despite minute amounts of biological samples. Principal component analysis (PCA) of MAPP-identified proteins effectively segregated healthy and SLE PBMCs, whereas that of standard proteomics-identified proteins did not. The results of this work were published in the prestigious journal, Nature Biotechnology1.

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References:

Wolf-Levy H, Javitt A, Eisenberg-Lerner A, et al. Revealing the cellular degradome by mass spectrometry analysis of proteasome-cleaved peptides. Nat Biotechnol. Published online October 22, 2018. doi:10.1038/nbt.4279

Patent Status

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