Replacing the Western: an Improved Way to Measure Cell Signaling

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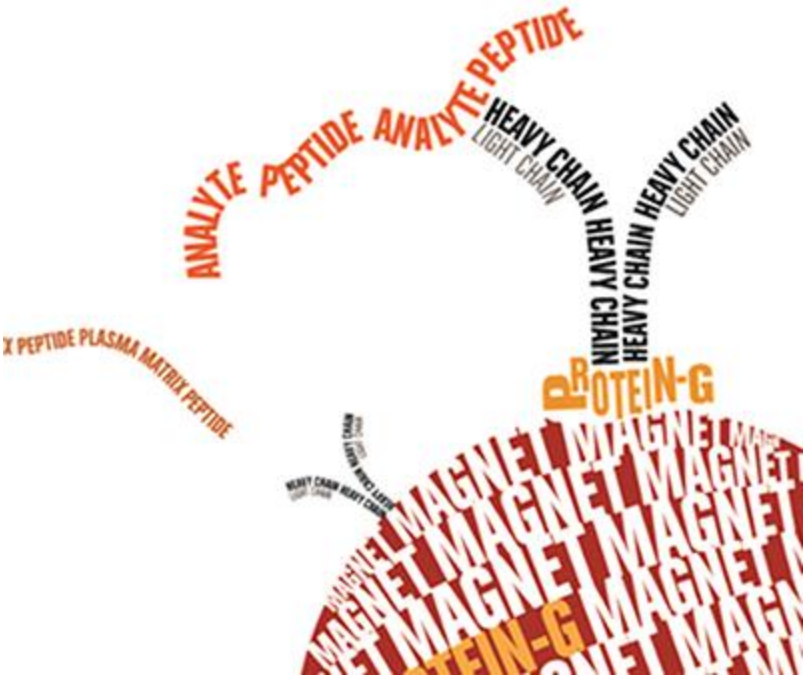

Immunoaffinity enrichment of a target peptide using the immuno-MRM assay developed in the Paulovich Lab.

Illustration by Brad Singley

Dysfunctional cell signaling is a common attribute in many disorders, and there is great interest in understanding the complex pathways that govern interactions and responses within our cells. These mechanisms can be studied through quantification of protein levels as well as covalent modification to proteins, so called post-translational modifications (PTM). A PTM with both biological and clinical relevance is phosphorylation - the addition of a phosphate group, which alters the function and activity of the target protein. Studies of phosphorylation signaling could help identify potential new drug targets, but a lack of adequate tools has stood in the way of progress - a situation that may be about to change. In a recent publication in *Molecular & Cellular Proteomics*, Drs. Jeffrey Whiteaker and Amanda Paulovich from Fred Hutch's Clinical Research Division describe a new multiplex assay that could provide the answer to many a researcher's litanies.

Countless scientific advances have been made courtesy of traditional analytical techniques like western blotting and the enzyme-linked immunosorbent assay (ELISA), but these one-analyte-at-a-time assays fail to live up to the demands placed by advanced modern-day "omics" technologies

(e.g. genomics, proteomics). Higher throughput, improved specificity, and better reproducibility are needed - preferably at a lower cost. Advances in the field of mass spectrometry (MS) has led to expansion of targeted MS, enabling precise quantification of protein-specific peptide sequences, the most commonly utilized form being multiple reaction monitoring (MRM). MRM-based assays provide direct measurement of the analyte and are recognized for yielding reliable output that can be reproduced independent of laboratory, thanks to the use of stable, synthetic internal standards. Compared with other techniques, these assays are highly specific, easily multiplexable, and comparatively quick and cheap.

The Fred Hutch investigators set out to combine MRM with antibody-based affinity-enrichment using immobilized antibodies on magnetic beads, an immuno-enrichment method previously optimized by the Paulovich Lab (Whiteaker et al. 2007). To demonstrate proof-of-concept, an immuno-MRM assay was tested for quantification of phospho-signaling in an important network, the DNA damage response (DDR) network, which has been shown to commonly display mutations in tumors. Successful monitoring of variations in DDR activity could have major effects on basic, clinical, and public health studies. The resulting multiplex assay proved applicable for a wide range of samples and conditions, efficiently replacing no less than 69 western blots with one single 40-minute run.

Dr. Whiteaker explained that the results were superior compared with those of semi-quantitative, often poorly specific, traditional methods, and that this moderate- to high-throughput MRM assay proved to be just what they hoped for: quantitative, precise, and specific. "Reliable methods for quantifying proteins and post-translational modifications are necessary tools for understanding the molecular basis of cellular signaling in diseased cells and profiling pharmacodynamic responses of new and existing therapeutics," he continued, underlining the broad impact of the innovation.

From here, there are multiple possibilities to further advance the technique. "We are very excited to continue developing these assays, expanding assay content, and getting this technology into the hands of more biologists," Dr. Whiteaker concluded. There should be quite a few researchers out there that share his gusto.

Whiteaker JR, Zhao L, Yan P, Ivey RG, Voytovich UJ, Moore HD, Lin C, Paulovich AG. 2015. Peptide immunoaffinity enrichment and targeted mass spectrometry enables multiplex, quantitative pharmacodynamic studies of phospho-signaling. *Molecular & Cellular Proteomics*. DOI: 10.1074/mcp.O115.050351. [Epub ahead of print]

See also: Whiteaker JR, Zhao L, Zhang HY, Feng LC, Piening BD, Anderson L, Paulovich AG. 2007. Antibody-based enrichment of peptides on magnetic beads for mass-spectrometry-based quantification of serum biomarkers. *Anal Biochem*. 362(1):44-54.