Reliable Methods Developed to Quantify the Human Proteome

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The ability to detect disease early or tailor personalized patient treatments depends on the identification and validation of novel biomarkers. Large systems biology studies have identified genetic biomarkers, such as DNA mutations, that are now used in the clinic to influence patient care. Protein levels are also used for diagnostics, but assays to reliably and reproducibly measure protein abundance exist for only 5% of the proteins encoded by the human genome. This deficit hinders the ability of researchers to look globally at the proteome for novel protein biomarkers of disease. A new study published in *Nature Methods*, by a team of international collaborators led by Dr. Amanda Paulovich from the Clinical Research Division, demonstrates the feasibility of large-scale efforts to standardize protein measurements. "We imagine a day when any human protein of interest can be reliably quantified (and thus effectively studied) by any researcher in the world," says Dr. Paulovich.

Researchers from the Paulovich Laboratory, the Broad Institute in Cambridge, Mass., and the Seoul National University and Korea Institute of Science and Technology, South Korea modeled what a global assay development effort might look like. They used a sensitive method called multiple reaction monitoring mass spectrometry (MRM-MS) to measure protein levels. MRM-MS is a targeted approach to measuring proteins using specialized instruments that can be "tuned" to look for small protein fragments of interest in biological specimens. Proteins are digested with a protease, such as trypsin, to generate smaller peptide fragments. One or more unique peptides are identified for each protein, and used as a surrogate to quantify that protein's abundance. This method increases sensitivity and reproducibility compared to traditional MS approaches by filtering out the noise.

In this pilot study, the researchers configured and validated across three laboratories 645 novel MRM assays representing 319 proteins (equivalent to 1.5% of the human proteome). These proteins were chosen because they are differentially expressed across 30 different human breast cancer cell lines. The MRM assays were multiplexed in four groups (ranging from 156-169 peptides) with median assay precision of 5.4%, and successful reproduction of assay results across the three laboratories ($0.96 \leq R^2 \leq 0.99$). Importantly, the researchers demonstrated that MRM-based peptide measurements in individual breast cancer cell lines were able to discriminate between molecular

subtypes of breast cancer, including HER2, estrogen receptor (ER), and basal-luminal status. Kennedy *et al*. then compared mRNA expression levels to protein levels in the different breast cancer cell lines, confirming genome-driven changes were altered in the measured proteomes for specific cells. Importantly, the researchers found novel changes in protein levels that were not found when looking at mRNA expression levels, which could identify potential disease genes specific to the different breast cancer subtypes. Taken together, these results demonstrate the potential of developing MRM assays to measure the entire proteome in search for novel biomarkers.

While the current study used cell lysates, the MRM assay platform can be applied to protein extracts from any type of biospecimen, including human tissues. Previously, Dr. Paulovich and collaborators used MRM-MS to measure human proteins in plasma (Whiteaker *et al*., 2011). However, the limited yield of protein from a biopsy or surgical specimen and the presence of multiple cell types encountered in tumor tissue samples are two hurdles that need to be overcome for clinical applicability of the approach. According to Dr. Paulovich, "Broadly available, standardized tools for quantifying human proteins would fundamentally transform biomedical research and lead to improvements in patient diagnosis and treatment by facilitating precision medicine. This will enable the translation of basic research into tangible medical benefit to patients and society."

Kennedy JJ, Abbatiello SE, Kim K, Yan P, Whiteaker JR, Lin C, Kim JS, Zhang Y, Wang X, Ivey RG, Zhao L, Min H, Lee Y, Yu M-H, Yang EG, Lee C, Wang P, Rodriguez H, Kim Y, Carr SA, Paulovich AG. 2014. Demonstrating the feasibility of large-scale development of standardized assays to quantify human proteins. *Nature Methods* 11, 149–155

See also: Whiteaker JR, Lin C, Kennedy J, Hou L, Trute M, Sokal I, Yan P, Schoenherr RM, Zhao L, Voytovich UJ, Kelly-Spratt KS, Krasnoselsky A, Gafken PR, Hogan JM,Jones LA, Wang P, Amon L, Chodosh LA, Nelson PS, McIntosh MW, Kemp CJ, Paulovich AG. 2011. A targeted proteomics-based pipeline for verification of biomarkers in plasma. *Nature Biotechnology* 29:625-34.

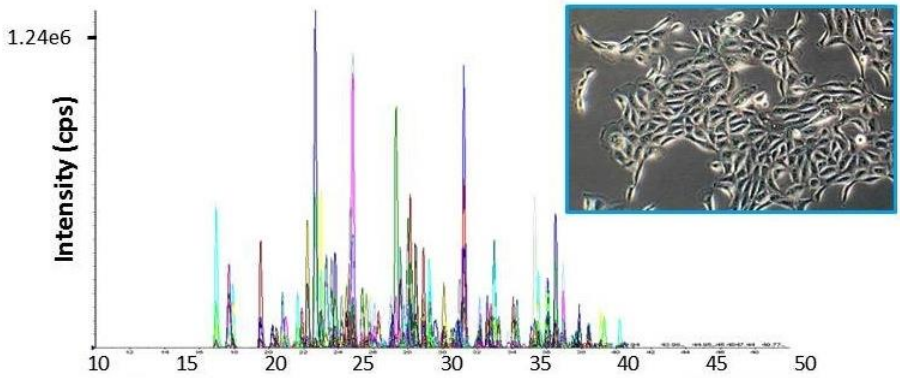

Image provided by Dr. Amanda Paulovich

A one hour targeted mass spectrometry run allows for quantification of 150 peptides from 75 proteins expressed in breast cancer cell lines (inset).