PROTEOMICS

Clinical proteomics on target

A multilaboratory study designed to assess the reproducibility of multiple reaction monitoring (MRM) mass spectrometry–based proteomics demonstrates the promise of this technology for disease biomarker verification.

Mass spectrometry–based proteomics has long been touted as a powerful technology for discovering biomarkers for the early diagnosis of diseases like cancer, and indeed, many studies have reported putative biomarker candidates. However, distinguishing true disease biomarkers above the noise of individual biological variation requires more sensitive methods, such as immunoassays, which require time and expense to develop and are limited by the availability of good antibodies and multiplexing capacity. As a result, the translation of these biomarker candidates to real-life application in a clinical setting has lagged far behind.

Quantitative, targeted proteomics approaches, however, are now emerging as promising alternatives to immunoassays. In particular, the combination of stable isotope dilution and an approach called multiple reaction monitoring (MRM) has shown great promise as a method to sensitively and reproducibly detect specific proteins in a complex background. The mass spectrometer can be set to monitor specific peptide precursor and fragment ions, called MRM transitions, which are uniquely representative of a particular protein. However, though targeted proteomics approaches are rapidly gaining traction, "it was unclear whether these methods could be made to be reproducible across laboratories," says Steven Carr of the Broad Institute. Carr was the principle investigator of a multilaboratory study, sponsored by the Clinical Proteomic Technology Assessment for Cancer (CPTAC) group of the US National Cancer Institute, to assess the reproducibility of MRM and to evaluate its potential as a robust method for biomarker verification.

The CPTAC group developed MRM assays for seven target proteins. They spiked these proteins into human plasma, which is relevant because of the high interest in finding disease biomarkers in blood, but which is also perhaps the most complex and interference-prone background. "You could say that we started with the worst-case scenario and asked the question, How well did we do?" says Carr. The CPTAC group sent the protein samples and the MRM assay configurations to eight participating labs, all from different institutions, and asked the researchers to detect and quantify the proteins in the samples.

Notably, the collective results showed that on the whole, the MRM assays were quite reproducible across labs and instrumental platforms. Though Carr's group has been using MRM assays for some time now, many of the other participating groups were novices to this technology. "The fact that you can get this kind of performance shows that these technologies actually are transportable across labs, and with some level of training you can trust the results from [novice] laboratories as much as you can from an expert lab," notes Carr.

The results also clearly demonstrate the potential of MRM–mass spectrometry as a filter for assessing putative biomarkers to refine the list of candidates that enter the clinical validation stage. Though Carr emphasizes that the point of this study was not to push the sensitivity of MRM to a clinical assay level, the results from the eight labs are promising, especially considering that multiplexed detection is possible with MRM. "If you could retain this kind of performance with a thousand-fold higher sensitivity, I think you could conceptually replace some significant number of assays routinely used in the clinic with an MRM-based methodology," he says.

Though this study focused on biomarker verification, the results also indicate the promise of MRM technology for targeted proteomics applications for basic biological research, offering the ability to track specific, low-abundance targets such as signaling peptides or modified proteins in complex backgrounds. Moreover, notes Carr, "All of the reagents, the methods and the datasets that we generated, I think are a pretty significant community resource."

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RESEARCH PAPERS

Addona, T.A. *et al.* Multi-site assessment of the precision and reproducibility of multiple reaction monitoring–based measurements of proteins in plasma. *Nat. Biotechnol.* **27**, 633–641 (2009).

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