LABEL-FREE MASS SPECTROMETRY-BASED PROTEOMICS FOR BIOMARKER DISCOVERY AND VALIDATION IN TISSUES AND BIOFLUIDS

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Label-free mass spectrometry-based proteomics applied to biomarker-rich sub-cellular compartments in (pre)-clinical samples and proximal biofluids is emerging as a powerful, versatile approach for discovery of tissue-derived biomarkers with close association to the disease.

Our label-free workflow is based on 1D gel electrophoresis in gradient mini-gels, in gel-digestion, nanoLC-MS/MS, and spectral counting-based quantitation. We have shown that this workflow is reproducible and outperforms other commonly used workflows in terms of the total number of identified proteins and the total number of reproducible identified proteins [1].

Analyzing spectral count data generated in these studies is, however, not straightforward as commonly used techniques for genomics data analysis are not suitable. We have shown that the beta-binomial test performs favourably in comparison with other methods on several datasets in terms of both true detection rate and false positive rate [2]. This test is now routinely used in all our projects for significance analysis and available for down-load (www.oncoproteomics.nl).

Over the course of the past 4 years, using analyses of technical and biological replicates in feasibility studies, we have shown for different types of (pre)clinical samples that spectral counting provides a reliable strategy for label-free protein quantitation. This applies to samples that were obtained via a relatively simple workflow (cancer cell secretome, [1]) as well as for samples obtained via more complex workflows (depleted cerebrospinal fluid, [3], nuclear fractions, [4]).

For colon cancer, promising imageable biomarker candidates have been discovered and confirmed in tissue microarrays and SRM-MS-based validation of selected nuclear proteins and secretome differential proteins as serum and stool-based markers is underway.

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