

An Integrated Pipeline for Mass Spectrometry-Based Discovery and Confirmation of Biomarkers Demonstrated in a Mouse Model of Breast Cancer

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Biomarkers have the potential to significantly improve the quality of patient care; however, the number of new biomarkers making it into the clinic is small. One problem facing biomarker development is the lack of a coherent pipeline for qualifying the large numbers of candidate biomarkers discovered for costly validation studies. We demonstrate a mass spectrometry-based biomarker pipeline encompassing unbiased discovery and targeted high-throughput confirmation. To minimize biological variation and facilitate testing of proteomic approaches, we employed a mouse model of breast cancer. Specifically, LC-MS/MS was performed on tumor and normal mammary tissue from a conditional HER2/Neu-driven mouse model of breast cancer, identifying 6,758 peptides representing >700 proteins. A novel statistical approach (SASPECT) for prioritizing proteins differentially represented in LC-MS/MS datasets was developed to identify proteins over- or under-represented in tumors. Using a combination of antibody-based approaches and multiple reaction monitoring-mass spectrometry (MRM-MS), we confirmed the overproduction of multiple proteins at the tissue level, identified fibulin-2 as a *plasma* biomarker, and extensively characterized osteopontin as a plasma biomarker capable of early disease detection in the mouse. Our results show that a staged pipeline employing shotgun-based comparative proteomics for biomarker discovery and multiple reaction monitoring for confirmation of biomarker candidates is capable of finding novel tissue and plasma biomarkers in a mouse model of breast cancer. Furthermore, the approach can be extended to find biomarkers relevant to human disease and usher them more efficiently towards clinical validation.