Discovery of candidate tumor marker and Absolute quantification using QCAT and NanoLC-MRM

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High-quality absolute quantification of proteins in the cell/tissue can be an effective route to overcome the difficulties associated with present methods for comparative proteomics, whether based on gels or mass spectrometry. Here, we used the proteotypic peptide to bio-synthesize heavy isotope-labeled, internal standard peptides using the concatenated peptide standard (QCAT) and quantified all the proteins by nanoLC-MRM.

For QCAT protein, we identified several differentially expressed proteins suggesting that these could be pursued as potential biomarkers in human breast cancer. The peptides sequence of each of selected proteins was used to design a gene that expresses artificial protein in a heterologous expression system, permitting metabolic labeling with stable isotopes. These labeled QCAT and their endogenous marker proteins were analyzed by LC-MS/MS using MRM. Potential marker protein concentrations in breast cancer tissue were determined by comparing MS/MS peak areas of the endogenous peptides to the isotopic labeled internal standard peptides.