Biomarker discovery and validation – from shotgun proteomics to targeted methods

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Introduction. Proteomics biomarker research is moving from the discovery stage using shotgun proteomics to targeted protein analysis methods using selected reaction monitoring (SRM) or parallel reaction monitoring (PRM) mass spectrometry in combination with liquid chromatography. In this lecture, I will give an overview of methodological advances in the area of clinical biomarker discovery and validation. Examples will comprise the discovery of a biomarker for cervical cancer and the validation of a biomarker for chronic obstructive pulmonary disease (COPD).

Results. Samples for discovery, shotgun proteomics were obtained from biopsies of cervical cancer patients after laser capture microdissection (LCM) of cancerous tissue, healthy epithelium and stroma. Differential analysis resulted in detection of a number of regulated proteins in cancerous tissue of which members of the mini-chromosome maintenance complex (MCM) showed strong discrimination providing leads for further validation. Initial results by Parallel Reaction Monitoring (PRM) LC-MS/MS show that MCM-3 allows discrimination of cancerous tissue from healthy tissue with high specificity and sensitivity in tissue biopsies as well as in cytological samples used for population screening. MCM-3 analysis may thus be combined with routine screening procedures (e.g. for high-risk Human Papilloma Virus (HPV)) using cytological samples to reduce the number of false positives.

The soluble receptor of advanced glycation end products (sRAGE) is being advanced for FDA qualification as a biomarker related to lung function decline due to emphysema in COPD. I will describe the development and validation of three LC-MS/MS methods that allow the accurate and precise quantitation of sRAGE in serum at the ng/mL level and relate the results to various phenotypes of COPD. Two of the methods are based on enriching sRAGE with affinity ligands while one uses strong-cation-exchange solid-phase extraction (SPE). The described methods fulfill all criteria of a validated bioanalytical method according to EMA and FDA guidelines. While we show that sRAGE levels correlate with disease severity (GOLD stage), we were confronted with a confounding factor, namely that sRAGE levels are affected by acute cigarette smoke exposure prior to sample collection.

Conclusion. Our results show that a highly discriminatory protein biomarker for cervical cancer was discovered by shotgun proteomics in extremely small samples obtained by LCM. The results show further that highly sensitivity LC-MS/MS methods can be developed and fully validated according to international regulatory guidelines and that enrichment with affinity ligands is not always required to reach pM sensitivity.

Rainer Bischoff is Professor of Pharmacy at the University of Groningen, Department for Pharmacy, Analytical Biochemistry. In this position he focuses on developing novel methods for protein, peptide and metabolite analysis. The research group has collaborations with various medical research groups in the area of disease biomarkers, with chemistry groups in the area of targeted protease inhibitors and with informatics groups concerning data processing and statistical data analysis (see: www.biomac.nl).