Cutting edge technologies for the detection of protein biomarkers

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New mass spectrometric (MS) proteomics technologies are currently under investigation which have the potential to reach the clinical laboratory in the near future. Of these, two very promising techniques have already gained much interest in the clinical research community, namely biomarker profiling by MALDI-MS and MALDI imaging technology.

Biomarker profiling

The discovery and validation of new protein biomarkers is still a challenge. The importance of biomarkers lies in their potential as indicators for the early detection of disease and the early determination of prognosis, so increasing the chances of a better ultimate disease outcome.

One of the prerequisites of a protein-based biomarker technique is the possibility to screen body fluids from patient cohorts in a quick and convenient assay with high information content. Recently, several clinical proteomics applications have demonstrated the power of mass spectrometry as a tool for the discovery of biomarker candidates in various body fluids such as serum, plasma, CSF and urine.

Defined pre-analytical standardisation is a relevant requirement for subsequent analysis [1]. The CLINPROT system is built on the speed, sensitivity, high resolution and accuracy of MALDI-TOF mass spectrometry. By means of a simple prefractionation in parallel of body fluids using functionalised magnetic beads, the direct assessment of very complex protein profiles can be easily and reproducibly carried out [2]. Hundreds to thousands of patient samples can be screened in a matter of days to weeks either manually or by automation.

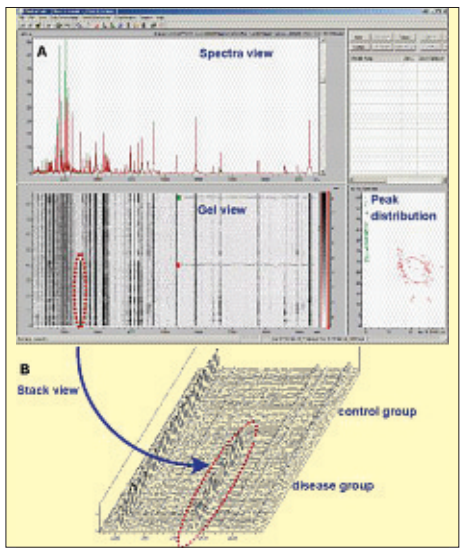

Figure 1. Upper Frame: Graphical user interface of ClinProTools 2.0 software. Lower Frame: Stack-Plot representation.

As many of the previously established single biomarkers show only low sensitivity and/or specificity, the goal of more recent approaches is to reveal panels or combinations of peptides/proteins to achieve a higher success rate in patient classification. The new ClinProTools 2.0 software package is specifically designed to fulfil exactly the workflow for a biomarker-profiling project. Disease-related changes in complex proteome patterns can be visualised with refined and easy-to-use tools allowing the analysis of cohorts of clinical samples [Figure 1]. Standard statistics and sophisticated multivariate statistics tools (Genetic Algorithm and Support Vector Machine) complete the biomarker detection workflow with an output of the best protein biomarker peak combinations resulting in classification of unknown samples.

Biomarker identification

These MS applications in clinical proteomics are not only limited to the discovery of biomarkers - the MS/MS capabilities of Bruker Daltonics' unique LIFT technology have also been shown to be a straightforward and elegant solution for direct protein identification from complex profiles [3]. The unambiguous identification of biomarkers opens up several opportunities for the clinical laboratory e.g. to develop assays or to validate themarker sets by independent immuno-based technologies.

MALDI imaging

Information about the local distribution of proteins in tissue slices is of great importance and forms part of the daily routine diagnosis in the clinical laboratory. Currently several different staining techniques and/or immuno-based methods are used for this.

MALDI imaging is based on the MS detection power and starts by simply applying a MALDI matrix (e.g. sinapinic acid) to a tissue slice followed by acquisition of spectra from many spots of the tissue surface by MALDI-MS. Capturing spectra in a scanning mode across tissue samples provides the intensity distribution of any peptide/protein. As a result, detailed information is generated in the form of a coloured image of the spatial distribution of proteins [4]. Figure 2B shows such a differential expression pattern from a human gastric cancer.

This imaging approach completes the biomarker discovery workflow by simply localising specific peptide/protein signals directly in the tissue. In clinical proteomics, MALDI imaging gives the advantage of either being able to discover biomarkers that show reproducible up- or down-regulation in the tissue, or studying the effect of drug treatment. Other possible applications involve the monitoring of disease progression in cancer and the evaluation of the efficiency of surgical removal through the direct visualisation of cancer biomarkers.

The analysis of imaging data is supported by the easy-to-handle flexImaging software solution from Bruker Daltonics. A massive reduction of the scanning time for the imaging approach is made possible by the system's smartbeam laser technology, which combines high acquisition speed with the high MALDI imaging spectral quality and sensitivity provided by a nitrogen laser.

Added value for clinical proteomics is achieved by combining MALDI imaging with the biomarker profiling approach. In this, the functionalised magnetic beads serve as a tool for upscaling and fractionation of tissue for downstream biomarker identification by peptide mass fingerprinting and MS/MS. Moreover, the ClinProTools 2.0 software can be used for the analysis of the statistical significant protein peaks from tissue samples [Figure 2C]. In this "Pseudo-Gel view", aligned mass spectra directly from MALDI imaging experiments clearly indicate putative biomarkers (arrow), not present in the control-groups.

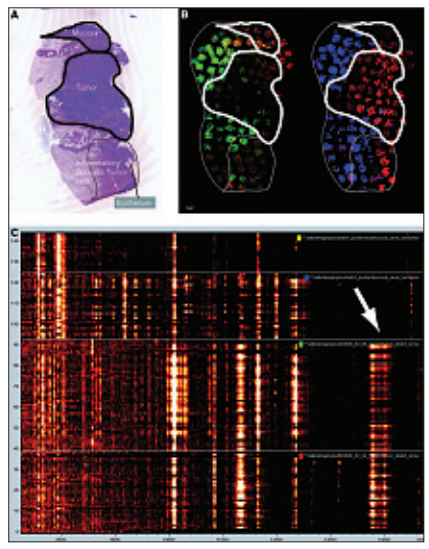

Figure 2. MALDI image from human gastric cancer tissue. (A) Histologically stained reference section. (B) MALDI images showing various specific mass signals for tumour and non-tumour tissue. (C) Pseudo-Gel view of mass spectra from different tissue domains clearly indicates putative biomarkers (arrow) in the disease groups.

References

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