Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 847 (2007) 1–2
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Editorial

Biomarker discovery by mass spectrometry symposium, May 18–19, 2006

The meeting brought 120 participants and 20 industrial exhibitors together to discuss the latest developments in biomarker discovery.

The challenge of the wide dynamic range that analytical methods need to cover (appr. 10–12 orders of magnitude) and the so far unknown complexity of biofluids makes the development of methods that "do it all" impossible. This was highlighted by Dr. Denis Hochstrasser (University Hospital, Geneva, Switzerland). To make matters worse post-translational modifications and splice variants can increase this complexity further as stressed in the presentation of Dr. Helmut Meyer (University of Bochum, Germany). Thus, we are presently seeing the much cited "tip of the iceberg". Suggestions to overcome this limitation ranged from affinity-based protein enrichment strategies to more targeted analyses in diseased tissue rather than body fluids.

While throughput remains an important issue in present-day biomarker discovery strategies, there is still a place for two-dimensional gel electrophoresis (2DGE) as shown by Dr. Fountoulakis from Hoffman-La Roche (Basel, Switzerland) and the Foundation for Biomedical Research of the Academy of Athens (Greece). He studied neurodegenerative disorders by 2DGE and MALDI-TOF-MS. When it comes to post-translational modifications, there is also a place for high-resolution, "top-down" mass spectrometry as shown by Dr. Michael T. Boyne from the University of Illinois, USA on the example of histones.

Affinity interactions may be one way out of the throughput dilemma but they rely on high-quality, specific affinity ligands, for example, antibodies. A large-scale project to generate specific antibodies against each protein represented in the human genome was presented by Dr. Sophia Hober of the Royal Institute of Technology (Stockholm, Sweden). Another approach based on recombinant, single-chain antibody fragments immobilized on protein arrays was presented by Dr. Carl Borrebaeck (University of Lund, Sweden). An interesting approach using affinity-enrichment of a class of proteins was presented by Dr. Albert Heck (Utrecht University), who used immobilized cyclic nucleotide monophosphates to pull down proteins that interact with cGMP or cAMP from the lysate of a human cell line (HEK293). In a subsequent experiment this approach was applied to rat ventricle heart tissue cells, which resulted in the selective enrichment of cAMP/cGMP binding proteins. These AKAPs are believed to localize PKA to different cell compartments.

While "top-down" proteomics remains the realm of a few specialized labs, much use is made of the so-called "shotgun or bottom-up" method as presented by Dr. Andrew Emili (University of Toronto, Canada), who analyzed enzymatic digests of mouse serum from a strain that is prone to heart failure. Quantification is an important feature of any biomarker discovery methodology, since putative biomarkers are proteins or peptides that change in concentration. Dr. Simon Gaskell (University of Manchester, UK) gave an overview over stable isotope labeling approaches to improve the reliability of quantifications in highly complex samples obtained by the "bottom-up" approach. This was followed up by Dr. Liljana Pasa-Tolic (Pacific Northwest National Laboratory, Richland, USA), who combined stable isotope labeling with FT-ICR-MS. Dr. Peter Roepstorff (University of Odense, Denmark) put the emphasis again on the sample preparation and separation aspects of proteomics highlighting that chromatographic materials that are not so commonly used, such as hydrophilic interaction media or graphitized carbon, may serve specific purposes, for example, when it comes to the analysis of glycoproteins.

Another advanced technique that can be applied for specific, sensitive detection of proteins is proximity ligation as introduced by Dr. Ulf Landegren (Uppsala University). As little as a hundred protein molecules in a sample can be detected in this manner.

Validation of the relevance of discovered biomarker candidates is a critical part of the overall process. Are the identified proteins expressed by the diseased tissue? Are they functionally linked to the disease process? Do the observed differences hold true in a larger, possibly more heterogeneous study population? Can the proteins be localized by immunohistochemistry to the diseased tissue? Many questions to which the answers are often still missing.

A novel tissue imaging technique using MALDI-MS was discussed by Dr. Markus Stoeckli (Novartis, Basel, Switzerland), where peptides, small proteins and metabolites can be detected in tissue sections. This method was successfully applied to detect

1570-0232/$ – see front matter
doi:10.1016/j.jchromb.2006.12.014

β-amyloid peptides in preparations of brain tissue, for example, in a model of Alzheimer's disease in mice. Dr. Stoeckli concluded, however, that currently this is not a technique that can be used for routine applications although the first commercial instruments are being launched.

Progress with validation was presented by Dr. Theo Luider (Erasmus Medical Centre, Rotterdam, The Netherlands) with respect to biomarkers for pre-eclampsia using laser capture microdissection of cordial tissue and mass spectrometry. Identification of the most interesting peptides was done by nano-LC FT-ICR-MS. Two peptides with a putative link to pre-eclampsia were identified as lactogen choriomammotropin. A precursor that controls the growth of tissues and blood vessels and calcycline (S100A6) that appears to stimulate the excretion of the lactogen.

Most biomarker discovery studies are presently facing the challenge of finding meaningful differences in data sets with millions or billions of data points obtained from only a hundred or even less samples. This situation is rendered even more difficult due to possibly large variations between individuals and variability introduced due to sample handling. Dr. Helmut Meyer showed that changes in protein levels between tissues of different patients can be tenfold and Dr. Rainer Bischoff (University of Groningen, The Netherlands) highlighted a number of parameters like the blood collection tube or the level of hemolysis that can affect the serum profile. Dr. Andrew Emili showed that spiking of plasma with peptides yielded good discrimination only when biological variation was excluded. However, when the peptides were spiked in plasma samples from different patients no discrimination was obtained due to patient-to-patient variability.

Some of the biggest challenges lie today in data processing and statistical analysis. Dr. Bischoff outlined a procedure based on meshing and time alignment (warping) followed by supervised classification and multivariate statistics. Dr. Age Smilde (University of Amsterdam, The Netherlands) discussed approaches for validation of the resulting statistical models in terms of robustness and generalizability. One important finding was that double-stage cross validation should be used to avoid overfitting.

In conclusion, this symposium highlighted many methodological advances in the area of biomarker discovery but it also showed that many hurdles have still to be taken. It is interesting to see that the biomarker community has developed a healthy critical attitude with respect to the obtained results but it is also evident that the first validated biomarker candidates start to emerge. We all hope that these results will ultimately benefit patients to receive more appropriate treatments and to being diagnosed at a time point that will allow a successful therapy.

R. Bischoff*
Theo M. Luider
Roel J. Vonk
Ron A. Heeren
Sander Piersma
Amsterdam, The Netherlands

* Tel.: +31 50 3633336; fax: +31 50 3637582.
E-mail address: r.p.h.bischoff@rug.nl (R. Bischoff)
Available online 3 January 2007