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The promise of biomarkers: research and applications

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Proteomics: From Technologies to Targets and Biomarkers
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NMI Natural and Medical Sciences Institute (Germany) and BioChipNet

The workshop *Proteomics: From Technologies to Targets and Biomarkers*, held on 2 February 2005 in Tübingen, Germany, was organized jointly by the NMI Natural and Medical Sciences Institute (Germany) and BioChipNet (www.biochipnet.de). It provided an overview of proteomics approaches that can be used to identify differentially expressed proteins that correlate with a disease state or drug response. Although a variety of proteomic technologies are available for the discovery and identification of potential biomarkers, a promising biomarker candidate remains elusive.

What is a biomarker?

Thomas Joos opened the workshop with an overview of the potential and pitfalls of protein microarrays, and provided answers to the question – what do we expect from an ideal biomarker? According to the FDA, an ideal biomarker must be specifically associated with a particular disease or disease state and be able to differentiate between similar physiological conditions. In addition, it would be desirable if standard biological sources, such as serum and urine, could be used for identifying biomarkers. A rapid, simple, accurate and inexpensive detection of the relevant marker should be available, together with a measurable and standard baseline as a reference point. Furthermore, an ideal biomarker should have a predictable expression level: this would demonstrate a clear association between measurable states and potential conditions [1].

Protein arrays and biomarkers

Protein microarray technology has proved to be a powerful tool in biomarker discovery and validation processes. It has already been successfully applied in the identification, quantification and functional analysis of proteins in basic and applied proteome research [2,3]. These miniaturized and parallelized assay systems have demonstrated their great potential to replace state-of-the-art singleplex analysis systems. Bead-based systems provide an interesting alternative to planar microarrays, particularly when the number of parameters to be determined in parallel is low and the number of samples is high. However, before protein microarrays find their way into routine and high-throughput applications, their robustness, sensitivity and automation must be tested thoroughly and they must be available at an affordable price. Protein microarrays have this potential and it will, in future, be possible to use them to determine simultaneously a variety of parameters from a minute sample volume, as well as in the discovery and validation of biomarkers. However, such processes require time and money, particularly if global approaches (i.e. analyzing the whole transcriptome) are anticipated. Nevertheless, for focused protein-profiling approaches searching for only a few parameters in parallel, current technologies are already mature enough to deliver reliable datasets. By contrast, array-based proteomic approaches will, most probably, take another ten years and millions of dollars of investment before this technology fulfills its potential.

Proteomic-based biomarker discovery

Hanno Langen (F. Hoffman-La Roche AG) regards proteomics as a key technology for the discovery of biomarkers for pharmaceutical and diagnostic research. Non-invasive measurement (e.g. in serum) is the key feature of a biomarker and Langen showed that biomarkers can be identified in diseased tissue. Multidimensional protein fractionation schemes are used to achieve appropriate sensitivity. Langen described a large-scale biomarker study in which the proteomic process was completely automated to generate reliable data sets. Using breast cancer tissue samples, several differentially expressed proteins were identified; next, sandwich immunoassays were developed and serum samples from patients suffering from breast cancer were screened. This approach led to the discovery of breast cancer-specific biomarker candidates. Hoffman-La Roche hopes to be able to offer several cancer-specific tumor markers in the future.

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Antibody-based biomarker discovery

An alternative approach to the analysis of differential protein expression was presented by Mathias Uhlen [Royal Institute of Technology (KTH), Sweden], who is the coordinator of the Swedish Human Proteome Resource (HPR) project; the HPR hopes to establish an affinity proteomics approach to enable systematic exploration of the human proteome. Researchers have developed a technology platform that combines the high-throughput generation of affinity-purified monospecific antibodies with protein profiling using tissue arrays. Uhlen described the basic methods used by the AlbaNova University Center at KTH for high-throughput cloning, protein expression and characterization and affinity purification of antibodies. The affinity proteomics approach involves the production of antibodies to human target proteins using a high-throughput method known as protein epitope signature tags (PrESTs). Tissue arrays (comprising healthy and diseased tissue and a large number of different cell lines) containing characterized monospecific antibodies were used to screen for the expression of corresponding proteins. Uhlen presented immunohistochemistry slides showing the differential expression of proteins for which no function has been identified. The specificity of the staining could be determined using two antibodies that bind to different epitopes of the same protein. Nevertheless, final analysis of the staining must be carried out by a pathologist. The images and datasets will be made available to the scientific community in a public database (www.hpr.se) [4]. Although there is a lot of work involved, a wealth of information can be obtained with such a global analytical research tool.

Biomarkers and the plasma proteome

In contrast to the affinity-based strategy, Keith Rose (University Medical Centre, Geneva, Switzerland) presented an industrial-scale approach to analyzing the human plasma proteome. Rose described a screening strategy that employs state-of-the-art technologies to separate, identify and characterize proteins using large volumes (from 10 ml to 2500 ml) of pooled serum, and other body fluids, from patients and controls. Over 1000 different proteins from human plasma and serum have been analyzed and identified using several fractionation and separation schemes [5,6]. Rose presented a strategy that facilitates the synthesis of chemically selected proteins using an efficient capping methodology combined with an appropriate ligation approach. The suggested strategy can be used to synthesize chemically proteins that are ≥160 residues in length that can then be used for a variety of biological assays, for example, substrates, factors or co-factors in enzymatic assays, or receptor–ligand binding assays.

Leigh Anderson (Plasma Proteome Institute) closed the workshop with a discussion on the human plasma proteome, which is the broadest and deepest subset of the human proteome and contains the most promising source of biomarkers of disease (i.e. diagnosis, prognosis and risk), toxicity and therapeutic response. Current proteomics technology faces two major challenges in finding these biomarkers. First, the enormous range of plasma biomarkers (>10 orders of magnitude difference in concentration between albumin and interleukin-6) substantially exceeds the capability of present analytical platforms (which cover 3–4 orders of magnitude). Second, the available platforms appear to expose different subsets of plasma components [7], which makes it difficult to measure routinely any specified set of biomarkers on a single platform. These limitations suggest the need for an alternative, candidate-based approach to biomarker development that is capable of the high-throughput, high-sensitivity measurement of protein panels.

The Plasma Protein Institute is exploring the use of a mass spectrometric approach to quantifying peptides and proteins in complex samples – stable isotope standards and capture by anti-peptide antibodies (SISCAPA) – that uses anti-peptide antibodies immobilized to nanoaffinity columns to concentrate the digested target peptide together with spiked stable-isotope-labeled internal standards of the same sequence. On elution from the anti-peptide antibody supports, the peptides (natural and labeled) are quantified using electrospray mass spectrometry. SISCAPA is therefore limited to sequence-defined (predetermined) analytes, but offers the possibility of greatly increased sensitivity [8]. Similar to the approach outlined by Uhlen, the method described by Anderson involves the generation of highly specific antibodies, which is no longer an obstacle because the sequencing of the entire human genome has generated the information (which is freely available) needed to undertake such multivariate approaches.

Conclusions

Focusing on a popular topic, and with high-caliber speakers, the half-day workshop provided professional answers to many questions anyone might have had about the discovery and validation of biomarkers and their clinical and basic science applications. Questions that will be tackled at the next meeting, which will take place in 2006, include cutting-edge issues relating to the growing importance of biomarkers in personalized medicine, disease detection and the monitoring of drug effects.

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