Multidimensional proteomics

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The drug discovery technology field has gone overboard in recent years with 'omics': genomics, proteomics, transcriptomics, metabolomics and glycomics, just to name a few. Although this naming trend is losing steam, the next one on the horizon appears to be derivatives of dimension, as in two-dimensional (2D) electrophoresis, three-dimensional proteomics, multidimensional protein identification and even orthogonal chromatography. A broad selection of these was discussed at IBC Life Sciences' *Proteomics and the Proteome* conference (June 30 – July 2, 2003, Basel, Switzerland).

Combining many dimensions at the same time, Steven Bodovitz (Select Biosciences; http://www.selectbiosciences.com) presented an overview of the proteomics industry. The central thesis revealed that the industry could become a victim of its own success. Proteomics technologies have dramatically accelerated the study of proteins, resulting in the discovery of large numbers of potential biomarkers and drug targets. Further advances, primarily in mass spectrometry, are expected to increase the rate of discovery, but this success is creating a bottleneck. High-throughput and/or high-output technologies are used to discover potential biomarkers and drug targets, but the validation methods are low-throughput and low-output. For the current rate of discovery to continue, the proteomics industry needs new methods and technologies to speed validation. Fortunately, multiple emerging technologies are poised to meet this need. Even the most pessimistic scenario for the market shows strong demand driving the market from $1.1 billion in 2001 to almost $2 billion in 2008. The most optimistic scenario, by contrast, shows growth to more than $3 billion in 2008.

Biomarker and drug target discovery

Evidence of success in the discovery of potential biomarkers and drug targets was abundant at the conference. Howard Schulman (SurroMed; http://www.surromed.com) presented his company's comprehensive and integrated biomarker discovery platform that identifies and quantitates thousands of proteins from serum, CSF and even cell membranes. Schulman showed compelling data on protein biomarkers for rheumatoid arthritis and ovarian cancer. Odile Carrette from the Laboratory of Clinical Chemistry at the University Hospital, Geneva (http://www.hug-ge.ch) used mass spectrometry to identify a panel of potential biomarkers for the diagnosis of dementia that correctly classified nine out of ten Alzheimer's disease patients. Pavel Gromov (Institute of Cancer Biology, Danish Cancer Society; http://www.cancer.dk) used 2D-gel electrophoresis to identify proteins that are upregulated in bladder cancers. Jean-Francois Haeuw (Pierre Fabre; http://www.pierre-fabre.com) extracted and purified epithelial cells from normal and tumoral colons and identified potential biomarkers. Daniel Chelsky (Caprion Pharmaceuticals; http://www.caprion.com) used immunomagnetic isolation of plasma membranes coupled with liquid chromatography and mass spectrometry to focus on protein variants between normal and tumoral colon that are most accessible for potential therapeutic intervention. Hanno Langen (F. Hoffman-La Roche AG; http://www.roche.com) added a third dimension to his proteomics research by coupling microdissection with 2D-gel electrophoresis and mass spectrometry and has identified more than 100 potential biomarkers.

Successes in discovery, however, yield to challenges in validation. Christian Rommel (Serono Pharmaceutical Research Institute; www.spri.serono.com) said that elucidating the basic biology of five potential targets can keep the lab busy for five years. Johannes Voshol (Novartis Pharma; http://www.novartis.com) concurred, stating that it takes two years of validation to prove the value of a single finding.

Functional proteomics

Two- and even three-dimensional proteomics typically compare protein expression between two samples, but function is an equally important dimension. Gerald Beste (Xerion Pharmaceuticals; http://www.xerion-pharma.com) discussed Chromophore-Assisted Laser Inactivation (CALI), in which a targeted antibody can inactivate a specific protein or protein subunit. The technology can be used to validate potential drug targets and to generate therapeutic antibody leads. Jean-Jacques Yarmoff (Hybrigenics; http://www.hybrigenics.com) presented a proprietary yeast two-hybrid

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technology for identifying protein pathways. The technology has been used to map transforming growth factor-beta (TGF-β) interactions and has the potential to identify key regulator molecules.

Protein microarrays

Functional studies are important for validating potential biomarkers and drug targets, but the key challenge for widening the potential bottleneck is throughput. Parallelized screening methods, such as protein microarrays and bead arrays, enable simultaneous analysis of thousands of parameters within a single experiment. Thomas Joos (University of Tuebingen; http://www.uni-tuebingen.de) presented an overview of these methods. The fundamental principles of miniaturized and parallelized microspot assays were described more than a decade ago by Roger Ekins, who demonstrated increased sensitivity as a function of decreased spot size. Many companies and researchers are now putting this principle into practice, integrating a wide range of technologies, from surface chemistry to protein capture to quantitative detection.

Several examples of recent progress in protein microarrays were presented at the conference. Barry Schweitzer (Protometrix; http://www.protometrix.com) demonstrated protein microarray technology with thousands of purified proteins immobilized in their active conformations. The applications of this technology are numerous and include pathway mapping, antibody specificity profiling, small molecule selectivity analysis and kinase substrate identification. Richard Schasfoort (MESA & Research Institute, University of Twente; http://www.el.utwente.nl/mesa) presented the first generation of a protein biochip that combines multiplexed surface plasmon resonance (SPR) detection and microfluidics. The platform integrates isoelectric focusing with gel electrophoresis through the incorporation of SPR and mass spectrometry and has the potential to provide greater resolution than 2D-gel electrophoresis. Mike Schutkowski (Jerini; http://www. jerini.com) discussed his company's peptide arrays and demonstrated powerful applications in studying kinase specificity, activation, receptor interactions and autophosphorylation.

Tissue arrays

In addition to these proteomics technologies that typically isolate proteins and then reconstruct the molecular interactions, several researchers presented tissue array technologies that show proteins in their native cellular and subcellular locations. John McCafferty (Wellcome Trust Sanger Institute; http://www.sanger.ac.uk) discussed the considerable progress made by his institution in developing an integrated, automated platform for generating antibodies, preparing tissue microarrays, immunostaining and capturing and analyzing data. Guido Sauter (University Hospital, Basel; http://www.unibas.ch) further expanded on the technical challenges of finding the best antibodies to use for tissue arrays. The primary application for Sauter's arrays are in pathology, but the main challenge will be convincing pathologists to adopt a radically different, but much more objective methodology based on automated analysis instead of manual scoring. Richard Caprioli (Mass Spectrometry Research Center at the Vanderbilt University School of Medicine; http://ms.mc.vanderbilt.edu) is also using tissue samples, but instead of immunostaining, his approach maps proteins from specific regions by desorption, ionization and mass spectrometry. Compared to the other methods, this approach loses some of the spatial resolution and sensitivity of the antibody-based staining approaches, but is able to examine differentially regulated signatures for a much larger number of proteins.

Glycomics

Even tissue arrays are isolating because they do not examine protein glycosylation. Ten Feizi (Imperial College London, http://www.ic.ac.uk) explained that there are an ever-increasing number of receptor-ligand interactions that are known to operate through binding to specific oligosaccharides. Tillman Gerngross (GlycoFi; http://www.glycofi.com) discussed therapeutic proteins that do not function without proper glycosylation. He described an array of genetically engineered yeast strains that can properly glycosylate human proteins. Ralf Riggin (Eli Lilly; http://www.lilly.com) described the detailed analyses that his research group performs to ensure that recombinant Xigris™, used for the treatment of sepsis, is properly glycosylated in order to maintain its activity and to comply with FDA standards. All of these speakers demonstrated the significance of glycoslyation, indicating that researchers should incorporate this dimension of proteomic analysis earlier in their studies, although new tools are needed to facilitate this. This challenge was addressed by Nicolle Parker (Proteome Systems; http://www.proteomesystems.com) who presented a suite of new tools for glycan mass fingerprinting.

Every dimension of proteomics provides important information, but the ultimate goal is to integrate all of the different types of data. The key challenge is linking the information together, making proteomics truly multidimensional.