Proteomics for drug target and biomarker identification

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The IBC conference on applications of proteomics for drug target and biomarker identification was held from 30 June to 2 July 2003 in Basel, Switzerland. With parallel sessions, a tremendous amount of information on many subjects, including bioinformatics, was presented in three days. Only a few selected presentations on protein microarrays, role of biomarkers, and glycoproteomics are presented in this report. Proteomic technologies are described in detail elsewhere [1].

Protein and tissue microarrays

Investigation of gene function involves the measurement of target protein and the study of protein–protein and protein–DNA interactions using protein and antibody microarrays. John McCafferty (Wellcome Trust Sanger Institute, http://www.sanger.ac.uk) presented the keynote address on the use of microarrays in gene and protein expression. Protein microarrays are required to show high sensitivity and specificity and to be reproducible. Other important features include the detection of folded proteins and their functions. Protein microarrays have been used mostly for research but have potential as diagnostic tools. Gene expression can be analysed in tissue sections. Tissue arrays are conceptually different from DNA and/or protein chips; they have fewer data points but still have high information content as they retain cellular as well as subcellular information. Protein expression can be optimised by cloning PCR products into expression vectors. Potent high-affinity antibodies can be obtained from phage display libraries and are a source of drug leads. The process is amenable to automation. Data from image analysis is analysed by informatics software. The Atlas of Gene Expression (http://www.sanger.ac.uk/Teams/Team86/) project provides information on the expression levels and localisation of protein products by using recombinant antibodies as probes to localise protein in tissues via immunocytochemistry.

Thomas Joos (University of Tübingen, http://www.uni-tuebingen.de/) presented a thorough review of protein microarray and microfluidic technology, a part of which has been previously published from his institute [2]. Solid-phase assay systems are highly parallel, miniaturised and sensitive and require low sample consumption. There are several miniaturised ligand-binding assays, including planar microarrays and bead-based systems (Luminex xMap, http://www.luminexcorp.com). Planar wave guide (Zeptosens) and surface acoustic wave (Advalytix) technologies are used for detection. Several protein engineering technologies are available but a high number of binders leads to a bottleneck, as their characterisation needs time.

Barry Schweitzer (Protometrix, http://www.protometrix.com) described ProtoArray™, a comprehensive protein microarray format that can be used to evaluate protein function and to identify agents that interact with proteins of interest. ProtoArrays™ are a cost-effective, miniaturised, high-throughput technology that can be used to screen up to several thousand proteins simultaneously for drug binding, molecular interactions or enzymatic activity. They have important applications in protein characterisation, drug target discovery and drug development. The Yeast ProtoArray™ is the world's first proteome microarray. It contains almost 5000 *Saccharomyces cerevisiae* proteins, which are double-spotted onto a surface-modified glass microscope slide alongside several hundred internal and experimental controls.

In vivo proteomics for anticancer strategies

The development of anticancer therapies with better discrimination between tumour cells and normal cells is the most important goal of modern cancer research, as most chemotherapeutic agents do not preferentially accumulate at tumour sites. Ligand-based tumour targeting based on high-affinity monoclonal antibodies enables excellent localisation to the tumour environment [3]. Dario Neri (Swiss Federal Institute of Technology, http://www.eth.ch/) and his collaborators have developed a monoclonal antibody that is specific for the EDB (extra domain B) of fibronectin, a marker of angiogenesis, and is capable becoming selectively localised to new blood vessels, although this is a slow process as the marker is located in albumin. He argued that *in vivo* biotinylation of tumour-bearing mice coupled with state-of-the-art proteomic technologies is the most efficient method available for the discovery of selective markers of tumour neovasculature. Terminal perfusion of tumour-bearing cells was shown to be feasible.

Biomarkers and pharmacoproteomics

Proteomic technologies are playing an important role in the discovery of disease biomarkers that have diagnostic value as well as the potential to be targets for drug discovery. Odille Carrette (Geneva University Hospital, http://www.hug-ge.ch/) presented the results of a study using protein array technology that demonstrated the presence in the cerebrospinal fluid (CSF) of sensitive polypeptide biomarkers for Alzheimer's disease. Cystatin C and β-2-microglobulin were upregulated and VGF, a

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neuroendocrine secreted polypeptide, was downregulated. These biomarkers could be used for diagnosis, for assessment of the severity and progression of the disease, and as a basis for new therapeutic approaches.

Howard Schulman (SurroMed, http://www.surromed.com/) described methods for the multidimensional proteomic and metabolomic analysis of serum and CSF. High-throughput LC-MS (liquid chromatography mass spectrometry) can be performed on human CSF. The company's DeepLook™ technology enables analysis of membrane proteins. Finally, bioinformatics is used for discovery of biomarkers from the integrated datasets. He presented as an example the identification of biomarkers for response versus non-response in a study of anti-TNFα (tumour necrosis factor α) in rheumatoid arthritis patients.

Jennifer Sutton (Protea Biosciences, http://www.proteabio.com/) discussed strategies for identifying disease-related protein biomarkers using solution-based LC-MS. Advantages of this technique include increased sensitivity, which means that that less material is required, the ability to analyse complex protein mixtures, improved dynamic range, and the ability to identify post-translational modifications. The company's strategy for biomarker identification is to analyse biological fluids in the initial phase and to identify the markers from the disease tissues in the secondary phase. In the final phase, an understanding of the marker is obtained at the molecular level and protein–protein interactions are investigated.

Glycoproteomics

Glycoproteins have a predominant role in cell–cell and cell–substratum recognition events in multicellular organisms. There is increasing recognition of the importance of post-translational modifications such as glycosylation as a means of diversifying proteins and as potential modulator of their function in health as well as in disease. The term 'glycome' is defined, in analogy to the genome and proteome, as the whole set of glycans produced in a single organism.

Ten Feizi (Imperial College, London, http://www.ic.ac.uk/) started the session on glycoproteomics with a review of oligosaccharide receptors, which mediate critical processes such as protein folding and trafficking. The challenge is to discover carbohydrate-recognising proteins. The characterisation of carbohydrate ligands requires sensitive high-throughput technologies to analyse protein–carbohydrate interactions in order to detect oligosaccharide sequences bound within the glycomes. A carbohydrate microarray system was described that can be used to generate the large repertoires of immobilised oligosaccharide probes required for the detection of protein–carbohydrate interactions [4]. The arrays are obtained from glycoproteins, glycolipids, proteoglycans, polysaccharides, whole organs or from chemically synthesised oligosaccharides. Carbohydrate-recognising proteins single out their ligands not only in arrays of homogeneous oligosaccharides but also in arrays of heterogeneous oligosaccharides. In addition to their roles in protein expression systems, mass spectrometry and bioinformatics, such arrays could form the basis of methods for the identification of oligosaccharide-recognising proteins in the proteome and for the mapping of the complementary recognition structures in the glycome. Such knowledge can be applied not only to inhibit or facilitate protein–carbohydrate interactions *in vivo* but also to direct glycosylated drugs to specific target cells or tissues.

Nicolle Packer (Proteome Systems, http://www.proteomesystems.com/) showed how specific protocols and bioinformatics methods using the hardware and software from the Company's proteomics platform ProteomIQ have been applied to glycomics. These technologies cover all areas of glycan analysis, from glycoprotein sample preparation to the analysis of glycans from glycoprotein isoforms separated by gel electrophoresis. Mass spectrometric data on glycan fragments are interpreted by specific bioinformatic software integrated with the company's glycan database, GlycoSuite DB, to automatically generate the corresponding oligosaccharide structures.

Ralph Riggin (Eli Lilly and Co, http://www.lilly.com/) presented a characterisation strategy for glycoprotein biopharmaceuticals during development. Oligosaccharide analysis involves the reduction of glycoprotein to oligosaccharide and protein, the precipitation of protein to leave oligosaccharide, and finally direct analysis by HPLC/electrospray mass spectrometry [5]. One example of the application of this technique was the determination of the sialic acid content of a glycoprotein by using LC/MS and obtaining mass data for the glycoprotein. Oligosaccharide formulas and the number of sialic acid residues was calculated for each oligosaccharide. The sialic acid content of the glycoprotein was then calculated from the relative percentage content of each oligosaccharide.

Concluding remarks

IBC has maintained its tradition of presenting the best proteomic conferences. The technologies presentations were of high standard. There was an opportunity to learn about cutting-edge technologies from academic researchers as well as from those in the commercial sector. Perhaps the only possible criticism of the conference was the inadequate coverage of the commercial aspects of proteomics, which are important for the growth and survival of companies developing proteomic technologies.

References

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