

Meeting report

## New perspectives on an old disease: proteomics in cancer research

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A report on the American Association for Cancer Research Conference 'Advances in Proteomics in Cancer Research', Amelia Island, USA, 27 February-2 March 2007.

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Cancer seems to have afflicted humans throughout recorded history. The earliest descriptions are in Egyptian papyri written between 3000 and 1500 BC. Cancer was named, some 2,400 years ago, after the Greek word *karkinos*, a crab, which Hippocrates thought a tumor resembled. More than two millennia later, cancer remains among the leading causes of death in industrialized countries. The name cancer covers an extremely heterogeneous set of diseases with different pathologies, prognosis and treatments. In many cases, histological or molecular standards for diagnosis and categorization are missing. An American Association for Cancer Research conference in February brought together experts covering diverse facets of proteomics and cancer research with the aim of reviewing progress in the quest for new cancer biomarkers.

Raymond DuBois (Vanderbilt-Ingram Cancer Center, Nashville, USA) opened the conference with a keynote address in which he emphasized the critical importance of early detection in cancer control and prevention. Most cancer can be effectively treated if detected early. The identification of unique molecular signatures in developing cancers is expected to pave the way toward more tailored and personalized treatments.

### Plasma as a source of cancer biomarkers

Blood plasma has attracted great attention as a potential source of protein biomarkers. It is readily accessible through minimally invasive methods and, most importantly, it circulates through virtually all tissues. Current efforts profiling human plasma proteomes are motivated by two

assumptions. First, the state of a tissue and its progression towards disease are reflected in its protein content. Second, these tissue-specific markers can be efficiently detected in the plasma. Gilbert Omenn (University of Michigan, Ann Arbor, USA) presented an update from the Human Proteome Organization (HUPO) Plasma Proteome Project. It now covers more than 3,020 non-redundant gene products, corresponding to more than 7,000 proteins or isoforms. Interestingly, besides the proteins that are primarily active in the plasma, many of the proteins found in plasma were released into the circulation by organs and cells throughout the body. The current dataset already represents an invaluable source of information, but Omenn believes that further progress will entail the integration of new approaches. The quantification of proteins in plasma remains a challenging enterprise because of the complexity of these samples and their extreme dynamic range, covering more than ten orders of magnitude of concentrations. The most interesting proteins, the ones originating from tissues or tumors, are expected to be present at extremely low concentration.

The next phase of the HUPO Plasma Proteome Project includes cross-analysis of the original sources of biomarkers: the different organs, tumors and more proximal biofluids (tears, urine and saliva). Along those lines, Julio Celis (Danish Cancer Society, Copenhagen, Denmark) presented a remarkable proteomics profiling of breast apocrine cystic lesions that led to the successful identification of two proteins differentially expressed in these lesions, 15-hydroxyprostaglandin dehydrogenase and 3-hydroxymethylglutaryl-CoA reductase.

Complementary to the current 'scanning' or shotgun approaches that aim at detecting all proteins in the plasma, new strategies are gaining momentum that rely on the optimized, quantitative detection of pre-selected analytes using targeted mass spectrometry (MS). For this type of approach, Bruno Domon (Institute of Molecular Systems Biology, ETH Zurich, Switzerland) proposed a two-step

strategy, further demonstrated by Ruedi Aebersold (also at the Institute for Molecular Systems Biology), that alleviates some of the current limitations. The first step consists of the in-depth analysis of tissue and tumor samples using high-performance instruments such as Fourier transform mass spectrometers. The complexity of the samples is reduced and their enrichment in low-abundance proteins is achieved by fractionation procedures that specifically target glycoproteins - glycosylation is characteristic of cell-surface and secreted proteins, which are the most likely to enter the bloodstream. Using a hydrazide-based chemistry to selectively enrich for *N*-linked glycopeptides by affinity purification, a 20-fold reduction in sample complexity was achieved. Many of the glycosylated proteins identified in solid tissues could also readily be detected in the plasma, confirming the general validity of the concept. In the second step, Domon and Aebersold propose to specifically quantify a pre-selected set of particularly interesting or discriminating tryptic peptides in plasma using targeted analytical MS methods such as multiple reaction monitoring (MRM). In contrast with the widely adopted scanning MS approaches that aim at identifying all proteins in a sample, MRM relies on the optimized quantitative detection of selected proteins with increased sensitivity.

Daniela Dinulescu (Brigham and Women's Hospital, Boston, USA) described genetically engineered mouse models of ovarian cancer that faithfully mimic the clinical disease. In collaboration with Samir Hanash (Fred Hutchinson Cancer Research Center, Seattle, USA), plasma derived from mice that showed microscopic ovarian cancer lesions or metastases was analyzed by a quantitative proteomics approach called the intact protein analysis system (IPAS). This combines protein labeling with Cy dyes, three-dimensional protein separation based on charge, hydrophobicity and molecular mass, and protein identification by shotgun methods. As well as proteins already described in ovarian tumors, which validated the general strategy, Dinulescu reported a shortlist of 20 new candidate biomarkers that are currently being validated in patients with ovarian cancer.

### Profiling of tissues and tumors

The molecular profiling of human biopsies is frequently complicated by their inherent biological heterogeneity. Methods based on the profiling of proteins across tissues or tumor sections give useful clues to the spatial distributions of the candidate markers, but these methods have in the past been limited by the lack of specific affinity reagents. Matthias Uhlen (Royal Institute of Technology, Stockholm, Sweden) presented an antibody-based proteomics approach that aims at the production of specific affinity agents for all human proteins. So far, more than 1,500 antibodies have been produced and used to profile proteins across 48 human tissues, 20 different cancers and 46 cell lines. The resulting Human Protein Atlas (<http://www.proteinatlas.org>) provides

more than one million high-resolution images annotated by pathologists.

Classic visualization or proteomic strategies usually fail to directly inform on the activation state of proteins. To address this point, Roger Tsien (University of California San Diego, La Jolla, USA) has synthesized a novel series of imaging agents based on activatable cell-penetrating peptides, which are specifically designed to visualize matrix metalloprotease activity *in vivo*. The peptides consist of a polyanion (nine glutamate residues), an MMP-specific cleavable linker and a polycation (nine arginine residues), linked to the fluorescent dye Cy5. The complete peptide is impermeable to cell membranes, but on cleavage of the polyanion by MMP, the resulting Arg<sub>9</sub>-Cy5 is readily taken up by neighboring cells, where fluorescence accumulates. Tsien described the successful use of the activatable cell-penetrating peptides to visualize the activity of matrix metalloproteases around tumors in several xenografted and genetically engineered mouse models of cancer. The general method holds great promise not only for the diagnosis of cancerous lesions, but also for the specific delivery of toxic chemotherapeutic agents to tumors.

Mass spectrometry is also taking center stage in direct imaging. Richard Caprioli (Vanderbilt University Medical Center, Nashville, USA) reported recent applications in imaging mass spectrometry, which is based on matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), directly on frozen sectioned tissues or tumors. Caprioli showed that the protein patterns obtained could be correlated with lung tumor classification and with patient survival trends. He also presented some interesting developments that aim at integrating histology with the mass spectrometry profiles. These included the selection of tissue-staining procedures compatible with mass spectrometry and the use of conductive glass slides for microscopy that also serve as target plates for MALDI-MS.

### Charting and quantifying changes in protein phosphorylation

Cell signaling is often mediated by post-translational modifications that modify protein conformation, localization, activity and stability. Generally, the deregulation of these processes leads to disease, including cancer. After decades of 'one by one' studies, systems-wide analyses are now being attempted.

Donald Hunt (University of Virginia, Charlottesville, USA) presented a new peptide fragmentation strategy adapted to the mass-spectrometric study of post-translational modifications. Traditionally, the peptide backbone is fragmented during the MS procedure to determine the amino-acid sequence and deduce potential sites of modification. The efficiency of the fragmentation, however, is often dependent

on the amino-acid composition and the presence of modifications. Hunt coupled the electron transfer dissociation (ETD) of peptides to a second ion/ion reaction, the proton transfer charge reduction (PTR), designed to reduce charge complexity. The ETD-PTR method not only circumvents the traditional restrictions associated with peptide fragmentation but also preserves the phosphoryl group on phosphoserine and phosphothreonine residues. Hunt demonstrated the method with the global analysis of protein phosphorylation in a model eukaryote, *Saccharomyces cerevisiae*; more than 1,200 phosphorylation sites were identified on 629 proteins.

Integrated strategies aimed at charting the temporal dynamics of protein phosphorylation on the scale of entire proteomes were presented by Matthias Mann (Max-Planck-Institute for Biochemistry, Martinsried, Germany). These combine quantitative methods, such as stable-isotope labeling by amino acids in cell culture (SILAC), with the specific enrichment of phosphopeptides by strong-cation chromatography or titanium oxide. Mann reported the most comprehensive time-resolved changes determined so far in the phosphoproteome in HeLa cells following stimulation with epidermal growth factor (EGF). He presented ongoing work aimed at charting the cross-talk between EGF and tumor necrosis factor- $\alpha$ . Last but not least, the approach holds great promise for the charting of the effects or mode of action of kinase inhibitors.

In conclusion, the conference provided a comprehensive update on the emerging approaches and methods in the field of proteomics that are channeling the current quest for new cancer biomarkers. These new developments put us well on the way towards the comprehensive profiling of proteins in tumors and healthy organs. It may not be long before the identification of unique molecular patterns or signatures in developing cancers opens the way to more tailored and personalized treatments.

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