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Cancer proteomics—an evolving battlefield

Conference on Cancer Proteomics 2009: Mechanistic Insights, Technological Advances & Molecular Medicine

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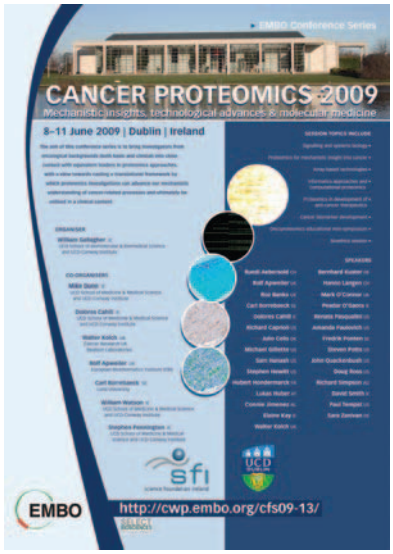

Cancer Proteomics 2009: Mechanistic Insights, Technological Advances & Molecular Medicine took place at University College Dublin, Ireland, between 8 and 11 June 2009, and was organized by W. Gallagher.

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Introduction

The first conference of the EMBO Cancer Proteomics series aimed to bring investigators from oncological backgrounds (both basic and clinical) into close contact with equivalent leaders in proteomics approaches, to establish a translational framework by which proteomics can advance our mechanistic understanding of cancer-related processes and which, ultimately, can be used in a clinical context. This conference made significant advances towards these goals and has set the stage for further meetings in this series that will continue to build towards the integration of basic proteomic sciences with the needs of clinical oncology.

Integration of basic and clinical approaches

Throughout the relatively short history of the application of proteomic methodologies to problems in cancer biology, a division has been apparent: efforts have focused on two equally important, but somewhat separate, areas. Investigators have endeavoured to use proteomic approaches to unravel the functional and mechanistic properties of cancer cells that might ultimately lead to a more complete understanding of cancer processes and, eventually, the identification of therapeutic targets. Shortly after investigations into this first area began, however, a second focus of cancer proteomics research became apparent: namely, the quest for biomarkers that could be used in clinical oncology. The idea that these historically divergent themes in cancer proteomics should be treated as one was put forward by R. Aebersold (Zurich, Switzerland) in his keynote address. Aebersold proposed that biomarkers are "footprints left by disease-perturbed networks" in the cancer cell and that, as such, research into the systems/mechanisms and biomarkers should be done together rather than in isolation. A generalized workflow schematic is shown in Fig 1.

Significant advances in technology and applications are blurring the border between clinical and systems approaches. In the past, deep proteomic investigations required substantial resources and could only be carried out with small numbers of samples in model systems. However, by using new techniques, it is now possible to perform large-scale studies with deep quantitative proteome coverage in animal models and clinical samples at both the tissue and biofluid levels (Hanash *et al*, 2008). An example of the admixture of biomarker discovery and systems-based approaches was presented in the closing keynote address by S. Hanash (Seattle, WA, USA). Researchers in Hanash's group—working on mass spectrometry-based plasma proteomics in transgenic mouse models of breast cancer (for example, ERBB2, also known as HER2/Neu)—used pathway analysis to determine the differential regulation of proteins in systems directly related to an overexpressed gene that drives tumour formation. Their ability to detect these proteins in plasma is a strong validation of

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systems approaches as well as biofluid-based biomarker discovery for cancer research.

Significant advances in proteomics measurement technologies have been made in the past few years. The majority of these have been in mass spectrometry, which remains the dominant platform in the field, although—as discussed below—other technologies are also beginning to make a substantial impact. A landmark article from the laboratory of Matthias Mann recently outlined the measurement by mass spectrometry-based methods of every expressed protein in haploid and diploid yeast (de Godoy *et al*, 2008). It cannot be long before these techniques are extended to more complex mammalian systems, allowing many of the problems faced by cancer biologists and physicians to be addressed.

Functional proteomics and cancer cell signalling

Proteomics is increasingly recognized as a method by which to decipher the molecular mechanisms underlying cancer cell growth and metastasis. The objectives are twofold: to understand the basic mechanisms of cancer initiation and progression, and to identify new therapeutic targets. Some information about the activity of oncogenic and tumour suppressor proteins has been obtained through proteomics. This was illustrated in discussions of growth factor receptor signalling by W. Kolch (Dublin, Ireland), of post-translational modifications in cancer cells, such as SUMOylation, by F. Golebiowski (Dundee, Scotland), and of phosphorylation, by S. Zanivan (Martinsried, Germany). Although it is too early to know if the proteins identified so far will be of clinical value—the time between the identification of a target and its eventual clinical use can average about ten years—proteomics-based approaches have proven their efficacy and are increasingly used as a tool to decipher the basic mechanisms of carcinogenesis. Therefore, it can reasonably be envisioned that the proteomics pipeline will soon expand the possibility of targeted therapies for cancer.

With the increasing number of tumour samples and experimental models of cancer being studied, we are entering a new phase of proteomics-driven cancer research that will be dominated by the concepts of deep proteome analysis and the definition of protein–protein interaction networks that lead to tumour cell deregulation and cancer progression. The results of this approach—which could be called 'systems proteomics'—are being integrated with information obtained from genomics and pathophysiological studies, thereby providing a framework which is likely to become the standard model for future investigations in cancer proteomics. The increasing amount of data collected about the proteomes of different cancers generates a concomitant need for better data integration. Just as information about the genome alone does not allow us to comprehend complex biological systems, the proteomic view alone is also insufficient. Although proteins are the functional products of gene transcription, they are not the final outcome but rather intermediates that carry the information necessary to drive cells towards the phenotypes and behaviours that genes ultimately encode. In the light of this, it becomes clear that a better understanding of complex biological systems—such as cancer—requires more integrated strategies. A common point mentioned during the meeting was the need for better integration at the molecular level; putting together genomic, transcriptomic and proteomic data would give access to a global view of molecular changes occurring in cells during the initiation and progression of cancer, as well as a potential way forward for rational therapeutic intervention.

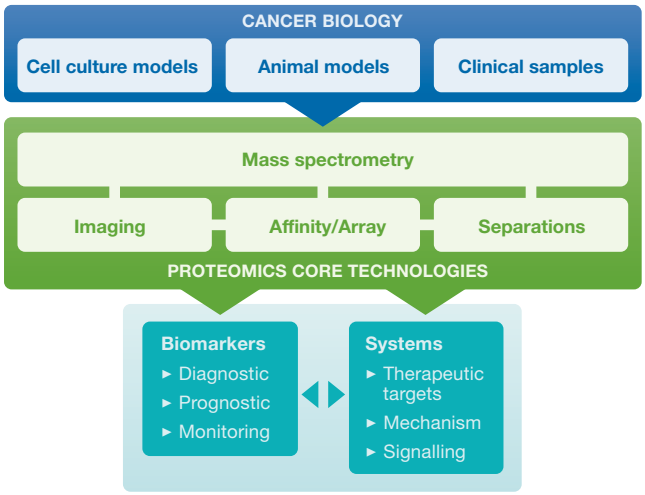

Fig 1 | Generalized workflow for cancer proteomics studies. Model systems for the study of cancer biology and clinical samples provide the material for cancer proteomics studies. Several core technologies are in use and under development in the proteomics field, with mass spectrometry as the dominant platform. The goals of these studies are often focused on either revealing 'biomarker' candidates or elucidating 'systems/mechanistic' information. There is an increasing trend towards integrating these approaches to gain a more complete understanding of tumour establishment and progression.

Emerging technologies—affinity and arrays

High-throughput proteomic profiling approaches have uncovered an array of biomarkers that could putatively be used for the improved diagnosis of cancer and prediction of disease outcome. However, the translation of these potentially informative markers to clinical use has lagged somewhat behind the pace of discovery. Various new affinity-based and array-based approaches will aid the identification and validation of cancer molecular fingerprints and significantly advance our understanding of the underlying disease. Tissue microarrays (TMAs), which were discussed by S. Hewitt (Bethesda, MD, USA), can be used to validate candidate markers and also to provide an alternative discovery platform; protein arrays allow the profiling of the autoantibody repertoire of cancer patients and were presented by D. Cahill (Dublin, Ireland); and antibody microarrays enable the simultaneous analysis of complex proteomes, as explained by C. Borrebaeck (Lund, Sweden). Indeed, Borrebaeck presented the first study to demonstrate that it is possible to predict tumour relapse in breast cancer by using a simple blood test based on a focused antibody array.

The generation and use of antibodies for protein profiling on a global scale has proven to be an intuitive approach; it has enabled a functional exploration of the human proteome using a wide range of assays, including the analysis of protein expression in multiple tissues and cells. Indeed, tissue and cell microarrays coupled with associated automated image analysis algorithms have rapidly emerged as the method of choice for translational pathology and the validation of cancer biomarkers (Hewitt, 2009). The results of one such continuing endeavour are presented in the Human Protein Atlas, which was described by F. Ponten (Uppsala, Sweden) and is available through a web-based application (www.proteinatlas.org;

Uhlen *et al*, 2005; Berglund *et al*, 2008). Through this portal, proteins with unique expression patterns can be identified as potentially useful diagnostic, prognostic or predictive clinical biomarkers (Bjorling *et al*, 2008; Ponten *et al*, 2008).

Furthermore, the use of next-generation antibody-based probes, such as surface-enhanced Raman scattering nanoparticles, offer the intriguing possibility of multiplexing protein localization in tissues, therefore further expanding the potential of TMA-based approaches (Lutz *et al*, 2008). Moreover, in the absence of specific antibodies, alternative detection strategies such as Fourier transform infrared spectroscopy—which enables protein expression to be mapped in tissues without the use of staining (Fernandez *et al*, 2005)—can be used. When tissue is limited, the repeated use of TMAs is possible through tissue immunoblotting (Chung & Hewitt, 2009). In an excellent presentation, R. Caprioli (Nashville, TN, USA) discussed the use of MALDI-TOF mass spectrometry for the direct analysis of tissue specimens (Seeley & Caprioli, 2008). By using this technology, an image of a tissue specimen corresponding to every feature in the mass spectrum can be created. An application of this technology showed the potential to redefine tumour margins when compared with standard pathological analysis. A second impressive application described by Caprioli was the construction of three-dimensional maps from analysed tissue sections, and the co-registration of these images with magnetic resonance images and/or optical images of the tissue.

Taking aim—development of cancer biomarkers

In the past decade, there has been much discussion about the potential of proteomics as a platform for biomarker discovery and validation. Despite the dedication of significant resources, proteomics has yet to yield a biomarker that is used routinely in the clinic. There are many biological and technical challenges to proteomic biomarker research, including sample complexity, dynamic range of protein concentration, reproducibility and throughput. The analysis of plasma and serum still remains the greatest challenge. Although blood-based assays would be the simplest to implement in a clinical scenario, the protein abundance range covers 10–12 orders of magnitude (Anderson & Anderson, 2002), whereas the dynamic range of typical mass spectrometers is only 3–4 orders of magnitude.

The dominance of several main proteins hinders the deep mining of the proteome. In this respect, M. Gillette (Cambridge, MA, USA) and A. Paulovich (Seattle**,** WA, USA) discussed some of the approaches towards developing robust workflows and facilitating protein quantitation at the low ng/ml range, such as protein depletion, fractionation and enrichment strategies. Their workflows attempt to focus not only on sensitivity but also on reproducibility, as well as addressing the bottleneck of validating hundreds or thousands of candidate markers generated from discovery data. Much of this research is conducted under the umbrella of the Clinical Proteomic Technology Assessment for Cancer programme, a multicentre US National Institutes of Health initiative that aims to develop and standardize robust methods in cancer proteomics. Gillette demonstrated that the simultaneous screening of hundreds of candidates that are present in low concentrations is now possible by using accurate inclusion mass screening (Jaffe *et al*, 2008) combined with strong cation exchange fractionation. This is a significant step towards bridging the gap between the discovery of many candidates and their clinical validation.

Once candidates are qualified, they can be quantified by using multiple reaction monitoring. Paulovich showed that this level of quantification can also be achieved with peptide antibody capture methods, as opposed to fractionation (Whiteaker *et al*, 2007). The sensitivity of both methods cannot yet rival that of the gold-standard ELISA tests that are routinely employed in the clinic; however, the ability to multiplex hundreds of assays will undoubtedly add substantial value, at least in validating candidate biomarkers in large patient cohorts and potentially for routine clinical use. The development of these new technologies and workflows looks to address current challenges in proteomic biomarker research and will hopefully drive real progress towards clinical feasibility.

The battle ahead—outlook for cancer proteomics

Proteomics has already delivered significant data in terms of a mechanistic understanding of cancer and of the identification of proteins of potential interest for diagnosis and treatment. However, the difficulties ahead should not be underestimated. First, the proteome is highly complex and current tools cannot yet provide a definitive solution for its exploration. Second, cancer is a multifactorial disease so diverse that a great deal of time and effort will be necessary to define its associated proteome modifications and to translate these into practical applications for the clinic. A worldwide organization such as the Human Proteome Organisation (www.hupo.org) is required to integrate different areas of expertise, and to provide the framework for a long-running programme for proteomics. Alongside this, forums that are focused on specific applications of proteomics to relevant biological platforms are required. This is the purpose of the EMBO Cancer Proteomics conference series, of which Cancer Proteomics 2009 was the opening event. Its continuity will be ensured by the next two conferences in this series, which are due to take place at intervals of two years in the UK (Cancer Proteomics 2011: Systems Biology and Data Integration) and Sweden (Cancer Proteomics 2013: Towards Clinical Implementation).

Proteomics remains a maturing field but there are substantial reasons to be optimistic about its ability to deliver significant value in the near future. The rate at which developments in proteomic technologies are made and integrated into the cancer biology community is increasing steadily. Technologists and vendors are expending great effort to make high-performance proteomics, in particular high-performance mass spectrometry (Mann & Kelleher 2008), available to non-specialist laboratories. We are soon to enter the era of global protein measurements that the term 'proteomics' implies, and the focus of efforts on cancer will surely make it one of the first disease areas to benefit from this boon.

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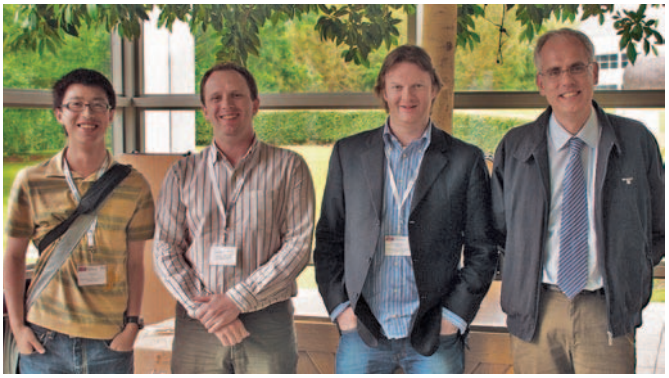

(Left to right) Thomas Y.K. Lau, Ben C. Collins, Darran O'Connor & Hubert Hondermarck