Tumor markers: discovery to practice

Wei-Wei Zhang, GenWay Biotech, 10130 Sorrento Valley Road, Suite C, San Diego, CA 92121, USA; tel: +1 858 458 0866 ext.101, fax: +1 858 458 0833, e-mail: wzhang@genwaybio.com

The *Tumor Markers: Discovery to Practice* conference was held in Santa Barbara (CA, USA) between 28 February and 4 March 2003 and specialized in new tumor markers that are currently in R&D or clinical validation studies, and new technologies for cancer diagnostic tests. The conference was organized by The University of Texas MD Anderson Center (http://www.mdanderson.org).

Basic concepts

The conference chairman, Herbert A. Fritsche (Texas MD Anderson Cancer Center), overviewed recent advances in biotechnology that fueled the discovery process and generated many new potential tumor markers. The progress was mainly from the completion of human genome sequencing and applications of gene expression profiling with DNA microarrays [1]. Two-dimensional (2D) gel electrophoresis coupled with MS, multi-dimensional HPLC, LC–MS–MS, surface enhanced laser desorption-ionization time-of-flight MS (SELDI-TOF MS) [2], helped to analyze the potential markers at the protein level. Tissue microarray applications also introduced a multiplex approach to validate gene-expression profiles at the protein level *in situ* [3]. New areas for tumor marker investigations include methylated DNA gene promoter regions and alternatively spliced mRNA. A newly developing field of tumor markers is the 'circulating epithelial cell'. There is much controversy in this area related to the detection methods used for circulating cells, the malignant nature of the circulating epithelial cell, and its metastatic potential. Highly sensitive RT-PCR analysis of these cells could provide new insights into their use as cancer diagnostics.

Sudhir Srivastava from the National Cancer Institute (NIH; http://www.nci.nih.gov) discussed the importance of discovering new tumor markers and early detection by understanding the variations and complex situations in cancer proteomics [4]. Protein expression and function are subject to modulation through transcription, as well as post-transcriptional and translational events. More than one RNA can result from one gene through a process of differential splicing. Additionally, there are >200 post-translational modifications that proteins could undergo that affect function, protein–protein interaction, stability, targeting and half-life, all contributing to potentially large numbers of protein products from one gene. At the protein level, distinct changes occur during the transformation of a normal cell into a neoplastic cell that range from altered expression, differential protein modification, changes in specific activities and aberrant localization, all of which affect cell function. Srivastava introduced the US National Cancer Institute's Early Detection Research Network consortium, in which genomics and proteomics approaches are being employed for the rapid discovery and evaluation of cancer biomarkers for early detection and risk assessment.

Genomics and proteomics

The conference provided a portfolio of presentations and discussions on the progress in genomics and proteomics that is bringing tumor marker discovery to a higher level [5,6]. DiaDexus' (http://www.diadexus.com) genomics–proteomics strategies, using bioinformatics to curate and prioritize 30,000 differentially regulated sequences and screening them via hybridizing with panels of RNA derived from human tumor and normal tissues, were shared by Jackie Papkoff. The differential expression profiling was confirmed by quantitative PCR and MS analysis on the samples of panels of tumor and matched normal adjacent tissues, as well as a set of normal tissues.

Stephen Naylor (Beyond Genomics; http://www.beyondgenomics.com) also discussed approaches of using combined genomics and proteomics methods to quantify and identify differentially expressed proteins as a function of disease or treatment of disease. The essence of what is required to perform differential proteomics was described, by discussing the formidable challenges in wide dynamic ranges of protein concentrations in sample preparation and analytical limitations in detection sensitivity.

Brigitta Brinkman from Exonhit Therapeutics (http://www.exonhit.com) reported the development of an innovative approach to qualitative genome analysis that enables the identification of the changes that take place during RNA processing. The methodology, designated DATAS (Differential Analysis of Transcripts with Alternative Splicing), provides insight into the alternative RNA processing events that occur as part of disease

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progression or following exposure to certain drugs. Susan Contrell (Epigenomic; http://www.epigenomics.com) reported on the discovery of cancer markers with analysis of methylated DNA in tissue and serum samples from cancer patients. One marker, Calcitonin, has previously been shown to be aberrantly methylated in cancer, particularly leukemia. Methylation-sensitive microarrays were used to identify new markers, which were validated by real-time PCR analysis.

Phillip Bernard from Huntsman Cancer Institute (http://www.huntsmancancer.org) discussed a large-scale analysis of clinical samples on different types of solid tumors using quantitative real-time PCR for expression profiling and for detecting gene amplifications and detection. The melting curve analysis immediately following PCR to identify small mutations, down to single base changes, was also reported.

MS and 2D gel approaches

Some results of clinical sample analysis using SELDI-TOF MS were presented by Daniel Chan (Johns Hopkins Medical Institutions; http://www.hopkinsmedicine.org), showing several cases of cancer biomarker discovery from serum or plasma of ovarian, breast and prostate cancers. The strength of the method was characterized by high-throughput and ease of sample preparation, and the limitation of the method was characterized by a large number of variables (the mass peaks).

Karrin Rodland (Pacific Northwest National Laboratory; http://www.pnl.gov) also presented novel MS approaches to characterize the proteome by using multi-pronged methodology, based upon a combination of instrumentation, separation technologies and unique affinity reagents. By combining strong cation exchange of tryptic peptides from albumin- and immunoglobulin-depleted serum with reverse-phase LC and ion-trap MS, >490 low-abundance proteins in human plasma sample were identified.

David Beer (University of Michigan Comprehensive Cancer Center; http://www.cancer.med.umich.edu) reported a case of 2D-classifier for Stage I lung adenocarcinoma. A total of 682 individual protein spots were quantified in 90 lung adenocarcinomas using quantitative 2D-gel analysis. Of 46 survival-associated proteins, 33 were identified using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS or nanoflow capillary LC coupled with electrospray tandem spectrometry. The expression of 12 candidate proteins was confirmed as tumor-derived with immunohistochemical analysis and tissue arrays using tumors from this study.

Tissue banking

Michael Misialek (Impath Predictive Oncology; http://www.impath.com) addressed the importance of creating a cancer biorepository and tissue bank, which are becoming a platform for novel biomarker discovery. A large-scale operation is in process with a planned collection of 50,000 patient cases with complete demographics, clinical data and 3-year outcome analyses. The samples will be ethically obtained after informed consent and formal Institutional Review Board review. The establishment of the tissue bank is a crucial resource for tumor marker discovery and validation in such downstream applications as immunohistochemistry, tissue and DNA microarrays, flow cytometry, image analysis, serum analysis, molecular genetics and cytogenetics, northern and Southern hybridization, PCR, fluorescence *in situ* hybridization (FISH), western blotting, drug resistance assays, cell culture and, most importantly, for future technological advances.

New tumor markers

Eleftherios Diamandis (Mount Sinai Hospital; http://www.mtsinai.on.ca) addressed a comprehensive study of kallikreins as tumor markers. Human kallikreins are a family of secreted serine proteases, which was recently identified encoded by a cluster of genes on human chromosome 19q13.4 [7]. Among these genes is prostate-specific antigen (PSA), the premier biomarker for the diagnosis and monitoring of prostatic carcinoma. It was found that many kallikrein genes were over- or underexpressed at the mRNA level, in various malignancies, including those that are endocrine-related, such as ovarian, breast and prostate cancer. More specifically, elevations of human kallikreins 5, 6, 7, 8, 10 and 11 in the serum of patients with ovarian carcinoma were observed. Human kallikreins 4, 5, 10, 11 and 15 were also found to be elevated in breast and prostate cancers.

John Robertson (Nottingham City Hospital; http://www.ncht.org.uk) presented the discovery of auto-antibodies as tumor markers. Richard Babaian and Barton Grossman (Texas MD Anderson Cancer Center) discussed prostate cancer markers by applying new assays to measure truncated and cleaved isoforms of free PSA, and gave an update on bladder cancer marker studies, respectively.

Ingegerd Hellstrom from the Pacific Northwest Research Institute (http://www.pnri.org) further reported two new assays for diagnosis of ovarian carcinoma. One of the assays applied a combination of ELISA for measuring both CA125 and soluble MPF-related molecules [8]. The other was HE4 protein-based ELISA, which was shown to have an advantage over the CA125 assay. Daniel O'Shannessy (Fujirebio Diagnostics;

http://www.fujirebiodiagnostics.com) reported on diagnostic potential of surviving – an inhibitor of apoptosis, and Raymond Houghton from Corixa Corporation (http://www.corixa.com) discussed real-time RT-PCR assays for the detection of disseminated tumor cells in breast and prostate cancer.

Circulating cells

Blood-borne distant metastasis is the leading cause of cancer-related deaths. New initiatives and approaches have been developed to look into the onset of this fundamental process in cancer patients, using ultrasensitive immunocytochemical and molecular assays that are able to detect even single metastatic cells. Stephan Braun (Leopold-Franzens-Universitaet, Innsbruk; http://www.uibk.ac.at) discussed the clinical impact of occult metastatic cells in breast cancer. A novel device, Rare Cell Detection (RCD) system, which enables the counting of tumor cells in the blood using an inexpensive disposable that prepares a relatively large sample of whole blood for automated microscopic examination in a single step, was presented by Herbert Bresler (Battelle Healthcare Institute; http://www.battelle.com).

Paul Ts'o (Cell Works; http://www.cell-works.com) also presented the BloodBiopsy™ as a universal test system for the quantitative measurement and characterization of circulating cancer cells for various types of cancers. The BloodBiopsy™ is a procedure for isolation and immunochemical-staining to detect rare cells in blood. Tim Allen-Mersh from Imperial College London (http://www.ic.ac.uk) reported on the detection of circulating tumor cells at 24 hours after primary resection to predict colorectal cancer recurrence.

Conclusions

The message from the conference is clear: the field of tumor markers from discovery to practice is quickly advancing, thanks to recent developments in new technologies and approaches. It is hoped that by applying and further developing the multiplex and high-throughput methodologies of genomics and proteomics, more tumor markers will be discovered, validated and put to use in meeting the urgent clinical needs of cancer patients.

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Textbook of Drug Design and Discovery

Edited by Povl Krogsgaard-Larsen, Tommy Liljefors and Ulf Madsen, Taylor and Francis 2002, 572 pages in paperback, £32.00, ISBN 0-4152-8288-8

The editors of this textbook include this line in the preface to the book. 'In order to attract the attention of intelligent students, the creative and fascinating nature of drug design must be the underlying theme of basic and advanced student courses in medicinal chemistry.'

This textbook is comprised of 17 chapters and 572 pages. The text is reasonably easy to read and the chapter, section and subsection headings are clear. This is the third edition of this textbook.

The *Textbook of Drug Design and Discovery* gives an excellent introduction into the required basics in many important areas in this field. With few exceptions, each chapter provides a good introductory section and more in-depth discussions of each topic. Many studies from industry are presented to exemplify or support various important points and topics. The collection of these chapters highlights the complexities and difficulties inherent in this field.

Experts in the field author each chapter, highlighting to readers that different perspectives and expertise will be encountered and that they should often be embraced, rather than ignored. With this type of organization, the editors must bring together the writing styles, ensure a consistent

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