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Advanced ProteinChip® Array Analysis using the Ciphergen ProteinChip® Interface with QSTAR™ MS/MS Technology

R. BOGUMIL

Ciphergen's ProteinChip® Arrays based on SELDI technology are widely used for the discovery, characterization and identification of proteins associated with a particular disease state. For comparative protein-profiling studies, arrays with chromatographic surfaces (ion exchange, reverse phase, IMAC etc.) are utilized to bind large classes of proteins and fractionate and retain them according to the surface type. During the discovery phase of a project, multiple ProteinChip Array surfaces and wash conditions can be empirically explored with a limited sample set to effectively reveal candidate biomarkers. Subsequently, defined conditions can be used to validate the biomarkers and to monitor disease processes by screening large banks of samples such as tissue extracts or physiological fluids (serum, urine, CSF, etc.)
For the identification and more detailed characterization of the disease-associated proteins, sophisticated protein-identification techniques are required. By using the ProteinChip Interface (PCI), the arrays can be directly analyzed with QSTAR Tandem MS technology. The PCI Interface is an exchangeable UV-laser desorption-ion source and combines the benefits of SELDI technology with the power of mass spectrometry. The mass mapping for protein identification can be achieved with high mass resolution and the MS/MS capabilities allow the sequencing of selected peptides by Collision Induced Dissociation (CID) for unambiguous identification.
In the case of low-molecular biomarkers, a direct identification from crude biological samples is possible. For larger markers above the MS/MS range of the instrument, partial enrichment is often needed. In this case the chromatographic surface type and buffer conditions used during the protein profiling suggest further purification procedures, either on-chip or by micro-chromatography. After enrichment of the protein of interest on-chip, the digest with proteolytic enzymes can also be performed on spot and the generated peptides analyzed with MS/MS.
The combination of ProteinChip Arrays with Tandem MS is a promising tool for further advanced applications. Some examples here are identification of phosphorylation sites and other posttranslational modifications or epitope-mapping experiments. By using pre-activated arrays, specific proteins can be covalently coupled and their interaction partner can be specifically enriched from complex biological samples and identified by limited on-spot proteolysis coupled with MS/MS analysis.

Ciphergen Biosystems GmbH

E-mail: rbogumil@ciphergen.com

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Cancer Proteomics Using SELDI Mass Spectrometry

A.J. RAI, Z. ZHANG, J. LI, J. KOOPMAN, Y. WANG and D.W. CHAN

The discovery of serum biomarkers is important in the diagnosis of cancer. High throughput proteomic techniques such as protein chips, in combination with suitable bioinformatics tools can allow for the identification of biomarkers useful for clinical diagnostics. One example is that of surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry. As is characteristic of high-throughput expression data, protein-array data is often characterized by a large number of variables relative to a small sample size. In analyzing such data to screen for disease-associated biomarkers, it is important to extract as much information as possible from a limited number of samples and to avoid selecting biomarkers whose performances are influenced mostly by non-disease related artifacts in the data. In such instances, the identity of the protein peaks may reveal critical information on aberrant activation of signal-transduction pathways, and can lend support to the physiological relevance of the candidate biomarkers. Several cases of cancer-biomarker discovery were presented, including ovarian, breast, and pancreatic cancers.

The Johns Hopkins University School of Medicine, Dept of Pathology, Baltimore, USA

E-mail: arai@jhmi.edu