## Editorial

# Proteomics – a new approach in biomedical studies

The field of clinical proteomics has rapidly evolved during the past few years and is continuously growing as new methodologies and technologies emerge. The focus of this article is to present a short introduction into the field of proteome analysis as well as its applications to human disease biomarker discovery.

Human body contains approximately 1 million of proteins that are products of over 20 thousands of genes in the human genome. The expression of functional proteins involves many posttranslational modifications. Phosphorylation of proteins is important in the regulation of most cellular responses to external stimuli (1). The analysis of phosphorylated proteins and the characterization of phosphorylation sites under different biological conditions are necessary to understand the complex signalling networks that regulate all major cellular processes.

Since proteins govern cellular structure and biological function, for the full understanding of physiologic and pathophysiologic processes, the whole proteome should be studied. Proteome has been defined as "proteome complement of the genome" related to all proteins of the human body, alternatively to all proteins of a given tissue, cell or body fluid. Most proteins act in complexes and form a very complex interaction network. Some proteins interact only with the few others. The proteins of subcellular localization interact mostly with others of the same localization. Failures in protein interaction network, such as gain or loss of interaction, lead to human diseases. Proteomic approach enables the study of proteomes involving the global analysis of protein expression profiles, the identification of them and their function in any organ, tissue, cell or cell organelle. Modern proteomics, by its ability to detect dynamic changes in protein expression, localization and modification, has become a powerful tool to map signal transduction pathways and deliver the functional information that will promote insights in cell biology and systems biology.

Over the past decade a great progress in technologies for study cellular proteins has been made that may have potential applications in oncology and other human diseases. Proteomic technologies are used for protein profiling, identification and quantification in tissues, microdissected cells and body fluids. This approach allows to elaborate the putative biomarkers for a variety of human diseases. Experimental approaches involve the application of two-dimensional electrophoresis (2D-PAGE), multi-dimensional liquid chromatography,

MALDI-TOF MS (matrix-assisted laser desorption ionization time–of-flight mass spectrometry), SELDI-TOF-MS (surface-enhanced laser desorption ionization mass spectrometry) and protein/antibody arrays (2), as well as the bioinformatic and statistical tools pertinent to the analysis of proteomics data.

Using 2D-PAGE is limited because of very wide concentration range of proteins present in biological samples such as serum (3). Proteins present in high concentration "mask" those potential biomarkers of diseases that are present only in trace amounts thus the new technologies are used that allow to capture selectively those proteins from the biological material that occur in the highest concentrations. MALDI –MS is based on desorption and ionization of peptide fragments attached to the matrix surface by the short impulse of laser light and then analysis of ionic beam by mass spectrometry. SELDI-TOF-MS technology enables initial concentration of biological material, purification from interfering substances such as desalting, separation of different fractions and then separate analysis of peptide patterns (4).

Protein biochip array technology uses a solid matrix with specific ligands (antibodies, antigens) attached at pre-defined sites on the surface. At present, over 20 test regions can be pre-fabricated onto the biochip surface but the capacity of tests per biochip can increase several times. This technology utilizes the basic principles of immunology : competitive, sandwich or antibody capture immunoassays. The chemiluminescent signals are simultaneously measured for the full array of tests on each chip.

Reverse phase protein microarrays are used to monitor biological response of the cells by identifying the dysregulated proteins in the individual patients (5). In this technology a cellular proteome is immobilized on a matrix with subsequent immunodetection of total and activated forms of cell signaling proteins. The intensity of signals generated by the protein spots is correlated with biological and clinical information as diagnostic and prognostic indicators.

Proteomic approach allows monitoring of disease process by simultaneous analysis of hundreds of proteins or peptides in the human body fluids that leads to discovery of clinically relevant combinations of disease biomarkers. Analysis of serum protein profiles and protein interaction networks generates enormous amount of data that are stored in large data bases, at present mostly available online.

The achievements of proteomics allow understanding of different biological processes and basis for several diseases. In neurodegenerative diseases, such as Alzheimer disease proteomic analysis of cerebrospinal fluid facilitates to understand the underlying pathology.

The abnormal expression of apolipoproteins A and E in cerebrospinal fluid as well as production and accumulation of these apolipoproteins in the neurons of the central nervous system has been found (6). Recent data have shown reduced expression of 1-38 and 1-42 amyloid β- peptides in cerebrospinal fluid analyzed by Aβ-SDS-PAGE/immunoblot in patients with Alzheimer disease (7).

The etiology of IgA nephropathy, the most common form of immune complex-mediated glomerulonephritis, has not been elucidated yet. Many patients with IgA nephropathy develop chronic renal failure. In patients with IgA nephropathy, at normal protein concentration in the urine, proteomic analysis enabled to detect specific protein profiles related to kidney dysfunction (8). Urine protein patterns may also serve as diagnostic tools in urinary bladder cancer, prostate cancer and many other diseases (9,10,11).

Very recent data on proteomic analysis have shown the wide clinical applications : in the study of inflammatory bowel diseases, in the diagnosis and prognosis of different types of cancer (breast, esophageal, ovarian, thyroid), in the diagnosis and prognosis of sepsis and ischemic and traumatic brain injury, in the diagnosis of pathophysiologic conditions of pregnancy. The proteomic approach offers a powerful tool for discovery of novel more effective drugs that may be used for individualized therapy, especially in the treatment of cancer.

In spite of very high costs, the impact of modern advanced proteomic technologies for clinical diagnosis and prognosis of human diseases in the future remains undisputed.

**Grazyna Sypniewska**
**Department of Laboratory Medicine, Collegium Medicum, Nicolaus Copernicus University , Bydgoszcz, Poland**
**Email address : odes@cm.umk.pl**

References :

1.Nita-Lazar A., Saito-Benz H., White F.M.: Quantitative phosphoproteomics by mass spectrometry : past, present and future. Proteomics 2008, 8,

2. Alex P., Gucek M., Li X.: Applications of proteomics in the study of inflammatory bowel diseases: Current status and future directions with available technologies. Inflamm Bowel Dis 2008, 8,

3. Dumnicka P.: Proteomics. Badanie i diagnoza 2007, 13,9-11

4. Drozdz R., Tisonczyk J.: From Tiselius to proteomics-New perspectives of urine protein analysis. In Vitro Explorer 2007, 6, 15-21

5. Espina V., Wulfkuhle J.D., Calvert V.S., Petricoin E.F. 3rd, Liotta L.A. : Reverse phase protein microarrays for monitoring biological responses. Methods Mol boil 2007, 383, 321-36

6. Maezawa I., Jin L.W., Woltjer R.L., Maeda N., Martin G.M. et al : Apolipoprotein E isoforms and apolipoprotein AI protect from amyloid precursor protein carboxy terminal fragment- associated cytotoxicity. J Neurochem 2004, 91, 1312-21

7.Bibl M., Lewczuk P., Esselmann H., MollenhauerB., Klafki H.W. et.al: CSF amyloid-β 1-38 and 1-42 in FTD and AD: Biomarker performance critically depends on the detergent accessible fraction. Proteomics 2008,8,4630-36

8. Park M.R., Wang E.H., Jin D.C., Cha J.H., Lee K.H. et al : Establishment of a 2-D human urinary proteomic map in IgA nephropathy. Proteomics 2006,6,1066-76

9. Lokeshwar V.B., Selzer M.G., : Urinary bladder tumor markers. Urol Oncol 2006, 24, 528-37

10. Downes M.R., Byrne J.C., Dunn M.J.: Application of proteomic strategies to the identification of urinary biomarkers for prostate cancer : a review. Biomarkers 2006, 11,406-16

11.Pisitkun T., Johnstone R., Knepper M.A. : Discovery of urinary biomarkers. Mol Cell Proteomics 2006, 5, 1760-71