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MASS SPECTROMETRY BASED PROTEOMICS

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Abstract

Despite the progress of medical sciences of the past decades, many cellular disorders and pathological alterations related to different diseases have remained unsolved. The need for early recognition of the warning signs of particular diseases, cellular dysfunction and systemic inflammation translated by early biomarkers would be the real support for prevention, diagnosis and adequate strategies for treatment that can selectively target the cellular and molecular mediators. Lately the focus is directed toward the rapid transfer of proteomics research data generated with the top mass spectrometry technology to clinical practice for the benefit of human patients.

Key words: proteomics, mass spectrometry, life sciences, personalized medicine.

THE FIRST DRAFT OF HUMAN PROTEOME MAP

The sequencing of human genome and of other numerous pathogens has opened the doors for proteomics by providing a sequence-based framework for searching and uncovering the proteome (the entire proteins complement of a genome). Why are proteins so important and why the proteomics technologies are increasingly developed and applied to study clinical samples in the research for diagnostic biomarkers and therapeutic targets?

A suggestive answer could be given by examination of the life cycle of a frog. While the adult frog develops from the fertilized egg passing through tadpole, young frog (froglet) does not change its genome but its proteome is changing as different proteins are expressed to produce very different creatures at different times. In a similar way, under various stress factors (either internal or external) the posttranslational modification of different proteins (by phosphorylation, de-phosphorylation, acetylation, ubiquit, etc…) could induce structural and/ or conformational modification of different proteins with dramatic consequences on the proper functionality, leading toward cellular and tissue dysfunction and in the end to disease.

As a result, there is high interest in applying proteomics to foster a better understanding of disease processes, develop new biomarkers for diagnosis and early detection of recognition signs of dysfunction, but also to accelerate drug development. New drug targets, improved diagnostics and the potential ability to monitor personalized therapy are becoming ensured by the human proteome mapping. The Human Proteome Project (HPP) was initiated in 2010 by The Human Proteome Organization (HUPO), (www.hupo.org), an international scientific organization representing and promoting proteomics through international cooperation and collaborations. Already in 2014, two groups, one from Germany and the other from the USA and India, published landmark studies in Nature which presented drafts of the first human proteome map using high-resolution Fourier-transform mass spectrometry (1,2).

With a lot of hard work and computing power, the first two public databases of human proteome were provided. To achieve this, the researchers extracted all of the protein from many different samples of human tissues, as well as a number of cell lines. The proteins in that purified mixture were then broken into small pieces that were first ionized and analysed by mass spectrometry revealing the sequence of amino acids forming each of those pieces. These pools of protein fragments can be compared with the human genome to make a map, showing which genes in which tissues are expressed and producing protein. Proteins encoded by 17.294 of human genes (~84% of the total annotated protein - coding genes) from 17 adult tissues, 7 fetal tissues and 6 primary hematopoietic cell lines were identified. The project was based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) by utilizing high resolution and high accuracy Fourier transform mass spectrometry. All mass spectrometry data including precursors and higher energy collisional dissociation (HCD)-derived fragments were acquired on the Orbitrap mass analysers.

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MASS SPECTROMETRY IN LIFE SCIENCES

Mass spectrometry is now widely viewed as the analytical technique of choice in the life sciences as it offers a high degree of sensitivity and the ability to search data against rapidly expanding protein and expressed sequence tag databases. The basic concept of mass spectrometry methodology, irrespective of the type of equipment produced so far, is the movement of a charged molecule in the electromagnetic field as described by the Lorentz physics law (www.wikipedia.org). Thus the mixture of macromolecules to be analysed should be transformed in gaseous ions that will be detected according to mass over charge (m/z) ratio and then separately detected. This ionization technique has become a preferred method for the high-throughput analysis of proteins by peptide mass fingerprinting, as an important aspect of early proteomics research. In terms of their capacity to analyse protein chemistry, mass spectrometers are unmatched. In the last decades the technological progress registered in mass spectrometry was supported by 4 Nobel Prize contributions (3-6) in the field of ionization methods that allowed increasing application of this methodology in biomedical requests, in the search for diagnostic biomarkers and therapeutic targets. All these were possible due to the unprecedented development of the bio-informatics and computational power that facilitated the classification, sorting and differential analysis of the huge amount of information generated by mass spectrometry analysis.

UNMATCHED PERFORMANCES OF PROTEOMICS BASED ON MASS SPECTROMETRY

The key parameters in proteomics research based on mass spectrometry are high sensitivity, mass accuracy and resolution (< 1fmol, < 1ppm and >1.000.000 respectively), speed and the possibility to analyse complex probes containing a mixture of macromolecules, in very small quantities (7). Thus, we measure the masses of these proteins and peptides that give us clues about the identity of these proteins, and what happens to them, and also we can quantitate them. This technique has turned out to be very powerful in the many areas of biomedical sciences because we can measure not only one molecule, we can measure hundreds and thousands at the same time. In this way, a much broader picture of what is actually happening in the analysed sample could be obtained. Although the blood is by far the most complex tissue of our body containing several thousand distinct proteins with high dynamic range in more than 10 orders of magnitude in concentrations (8, 9), it is possible to evidence specific changes in a distinct and defined pattern of polypeptides allowing enormous improvements in diagnosis and therapy for many wide spread diseases, for example neurological and endocrine disorders, cardiovascular or renal diseases, various type of cancer in just one step for many patients. Progresses in these directions are made every day and some discovered biomarkers were already transferred and successfully used in clinics.

Proteomics researchers frequently utilise two technologies: 2D-electrophoresis or high resolution chromatography to separate the proteins/peptides for analysis and mass spectrometry (MS) for protein identification and characterisation. Its use in peptide sequencing, identification of posttranslational modification and characterization of multi-protein complexes are proofs of the growing relevance of MS in the proteomics developments in particular and in life sciences in general. The comparative analysis can provide molecular profiles and any molecular features differences of targeted proteins. The individual particularities of diseased individuals (cancer, endocrine, neurological, inflammatory chronic illnesses,..) could be measured more precisely with high sensitivity and also quantitatively. The combination of peptides and proteins forms biomarker patterns, which are more capable to display the health status of an organ or organism with improved significance.

FINDING NEW MOLECULAR BIOMARKERS

The application of proteomics to find new biomarkers is now widely used in practically all diseases that are known because it allows finding out where the differences could lie at molecular level. In particular it has been successful in areas of infectious diseases such as HIV (10), herpes simplex virus infection (11), and also the hepatitis virus (12). Biochemical and mass spectrometry studies also proved that high-lipid stress induces profound changes in protein composition of membrane microdomains and modifies the cellular response, supporting the systemic inflammatory onset of atherosclerosis (13). However, the area that could benefit most from this biomarker research is probably cancer because it would be very useful to obtain biomarkers to discriminate people who develop cancer in a very early stage (14). Tumour development induced profound dysfunctions at molecular level of a large number of proteins distributed in numerous cellular compartments, which are dependent on the type of cancer examined. For example, the mass spectrometry comparative analyses of proteins differentially expressed in papillary thyroid

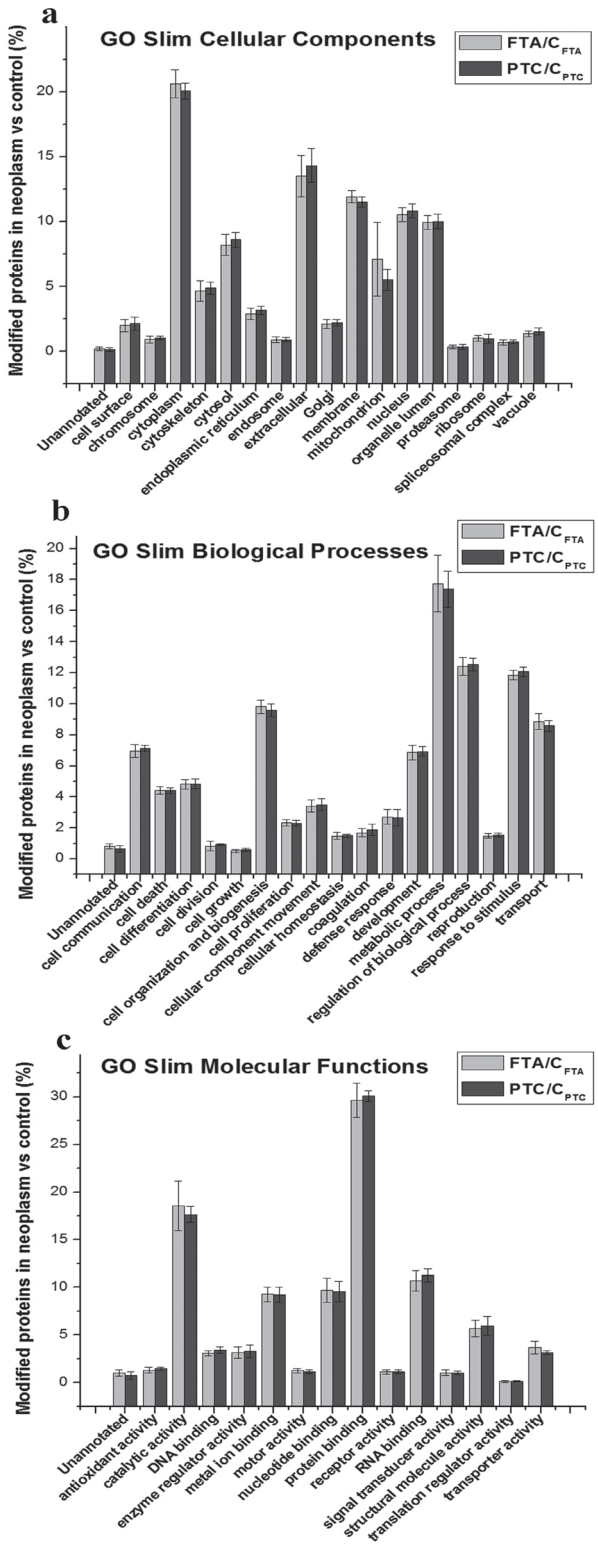

Figure 1. Mass spectrometry differential analyses of proteins expressed in folicular tyroid adenoma (FTA) and papilary thyroid carcinoma (PTC) classified by Cellular Component (a), Biological Process (b) and Molecular Function (c), according to Gene ontology (GO) data base. FTA/CFTA and PTC/CPTC represent the number of proteins identified in tumoral tissues (FTA and PTC) over corresponding adjacent non-tumoral tissue controls (CFTA and CPTC) respectively. Note, the over represented categories and the differrences that discriminated the two pathologies examied. P<0.05.

cancer (PTC) *versus* follicular thyroid adenoma (FTA) showed significant differences associated with Cellular Components, Biological Processes or Molecular Functions according to Gene Ontology data base (slim version) classification (Fig.1). Careful identification and selection of the molecular markers will allow in the near future the unbiased discrimination between the PTC *versus* FTA pathology.

Progress in proteomics proves extremely powerful and informative in areas of basic biomedical research because it uncovers molecular details of biological processes, particularly in diseases and will open up the possibility to develop a drug that can stop that process. Biomarkers could be very useful for physicians to make decisions on how to treat every patient according to his particularities. All this potential production of proteomics research are solid basis for the development of personalized medicine and the rapid transfer of basic knowledge toward clinical applications.

PROTEOMICS, THE BASIS OF PERSONALIZED MEDICINE

In healthy individuals, a distinct polypeptide pattern exists. Polypeptides in body fluids (urine, serum, cerebrospinal fluid, etc.) control the fate of cells, and consequently of organs and organisms. Hence, a thorough display of the proteins present in body fluids at any given physiological situation should give insights into the regulatory mechanisms of most diseases and support exact and unbiased diagnosis. Any changes in these polypeptides are indicative or, in some cases, even the cause for most of the common diseases. A strategy of choice is to utilise the peptide pattern found in body fluids to obtain information about the stage of disease and the general health of the patient. In case of an existing disease, proteins are missing, emerge in different formations, are modified or additional proteins appear. Consequently, recovery of the normal pattern indicates a successful therapy, which predestines our approach for control of therapy and monitoring of drugs in preclinical and clinical research. The dataset from individual analyses can be compiled to generate a typical proteome pattern based on individual analyses that will be processed by powerful software enabling automated and standardized data interpretation. The field is developing every day and new approaches in clinical proteomics become available for high throughput screening (15).

Mass spectrometry based proteomic approaches hold high promises to the endocrinology disorders and other disease associated with dysfunctions of the

secretory glandular tissues (16). For instance, the rapid evaluation of the endocrine status of a patient enables the differentiation of thyroid (17, 18), pancreatic (19, 20) or other neuroendocrine (21) dysfunction years in advance. In addition, the early classification of patients with high risk to develop certain disease, or the close monitoring of the response to the treatment applied, can be explored in a single measurement for different parameters or questioning related with potential side-effects of a pharmaceutical drug for the treatment. Examining the efficacy and side-effects of newly developed pharmaceutical substances will help the pharmaceutical industry to minimize the duration and the size of cohorts for (pre)clinical studies. Biomarkers or polypeptide patterns are suitable for precise description of the appropriate function of an organ and its alterations, and allow displaying dose related therapeutic effects of administered drugs. In the near future, identification of specific polypeptide patterns will facilitate the selection of an appropriate therapy for the personalized benefit of each patient.

Conflict of interest

The author declares no conflict of interest.

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