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Proteomics and Metabolomics for Mechanistic Insights and Biomarker Discovery in Cardiovascular Disease

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ABSTRACT

In the last decade, proteomics and metabolomics have contributed substantially to our understanding of cardiovascular diseases. The unbiased assessment of pathophysiological processes without a priori assumptions complements other molecular biology techniques that are currently used in a reductionist approach. In this review, we highlight some of the "omics" methods used to assess protein and metabolite changes in cardiovascular disease. A discrete biological function is very rarely attributed to a single molecule; more often it is the combined input of many proteins. In contrast to the reductionist approach, in which molecules are studied individually, "omics" platforms allow the study of more complex interactions in biological systems. Combining proteomics and metabolomics to quantify changes in metabolites and their corresponding enzymes will advance our understanding of pathophysiological mechanisms and aid the identification of novel biomarkers for cardiovascular disease.

La proteómica y la metabolómica: los mecanismos de la enfermedad cardiovascular y el descubrimiento de biomarcadores

Palabras clave:
Proteómica
Metabolómica
Espectrometría de masas
Espectroscopia de resonancia magnética

RESUMEN

En la última década, la proteómica y la metabolómica han realizado aportaciones sustanciales al conocimiento de las enfermedades cardiovasculares. La evaluación no sesgada de los procesos fisiopatológicos sin partir de presunciones establecidas *a priori* complementa otras técnicas de biología molecular que se emplean en la actualidad con un enfoque reduccionista. En la presente revisión, se resaltan algunos de los métodos de las ciencias «ómicas» que se emplean para evaluar los cambios de las proteínas y los metabolitos en la enfermedad cardiovascular. Es muy infrecuente que una función biológica específica discreta se atribuya a una sola molécula; es más habitual que se lleve a cabo con la aportación combinada de muchas proteínas. A diferencia del enfoque reduccionista, en el que se estudia individualmente las moléculas, las plataformas «ómicas» permiten el estudio de interacciones más complejas en sistemas biológicos. La combinación de la proteómica y la metabolómica para cuantificar los cambios de los metabolitos y sus correspondientes enzimas hará avanzar nuestro conocimiento de los mecanismos fisiopatológicos y facilitará la identificación de nuevos biomarcadores de enfermedad cardiovascular.

BACKGROUND

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in industrialized countries. Prediction of cardiovascular events relies on monitoring conventional risk factors such as age, sex, smoking habits, diabetes, and hypertension.[1] Many of these risk factors are highly prevalent in the population, and even the best algorithms for acute coronary events fail to predict the majority of cases of CVD over a 10-year period.[2] In addition to the difficulties of prediction, CVDs such as heart failure may represent a heterogeneous spectrum of etiologies, pathological stages, and genetic backgrounds. New biomarkers are urgently needed to stratify patients and personalize treatments. Currently, biomarker assessment is based on the quantification of a few proteins or metabolites.[3] High throughput platforms such as proteomics and metabolomics can offer simultaneous readouts of hundreds of proteins and metabolites. In this review, we summarize the proteomics and metabolomics platforms that are currently applied in cardiovascular research and that may lead to the identification of new biomarkers with clinical utility.

PROTEOMICS

The first complete sequence of the human genome was published in 2001. Unexpectedly, this sequence contained only

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Abbreviations

CVD: cardiovascular disease
LC: liquid chromatography
MRS: magnetic resonance spectroscopy
MS: mass spectrometry

about 20 000 to 25 000 open reading frames that encode proteins.[4] However, gene products are subject to alternative splicing and RNA editing, resulting in a variety of different protein isoforms.[5] The objective of proteomics is to interrogate the proteome.[6] The proteome encompasses the entire set of proteins expressed by a cell, tissue or organism, including their posttranslational modifications. Proteomics is the global analysis of gene expression using a variety of techniques to identify and characterize proteins. The term *proteomics* was first coined by Marc Wilkins in 1994 in analogy to genomics, the analysis of genes.

The first proteomic techniques were developed in the 1970s[7] and their use has continuously evolved. It was not until the mid-1990s that proteomics was applied to the study of CVDs, with the pioneer studies of Knecht et al.[8] and Jungblut et al.[9] At that time, a major hurdle was the identification of proteins. Initially, Edman sequencing was used but this technique has been superseded by the advent of biological mass spectrometry (MS). The first Nobel Prize for MS was awarded to F.W. Aston in 1920. His mass spectrometer allowed separation of different isotopes. More recently, two inventions made it possible to analyse biomolecules (DNA, peptides, proteins) by MS: in 1987, M. Karas and F. Hillenkamp invented MALDI (matrix-assisted laser desorption/ionization). In MALDI-MS, a matrix (eg, α-cyano-4-hydroxycinnamic acid) is mixed with an analyte (eg, peptides). The analyte is desorbed from the matrix with a laser shot and is ionized.[10] In 1989, J.B. Fenn invented electrospray ionization.[11] He was awarded the Nobel Prize in Chemistry in 2002. In electrospray ionization, the analyte is ionized from a liquid phase into the gas phase. Thus, liquid chromatography (LC) systems could be directly interfaced to mass spectrometers. LC-tandem MS (LC-MS/MS) is the current gold standard in proteomics. LC first separates peptides, which is essential because most mixtures are far too complex to be analyzed by MS without prefractionation. The tandem mass spectrometer then records the masses of the intact peptides (full MS) before one precursor ion is selected and fragmented. Fragmentation is commonly induced by collision with argon or nitrogen. More recent methods also use electrons, a softer fragmentation method that preserves posttranslational modifications.[12] The fragments are recorded in an MS/MS spectrum and the fragmentation pattern reveals a specific Δ mass for each amino acid in the peptide. Most early proteomics workflows were based on an initial step of protein separation, which involved techniques such as gel electrophoresis. As the mass spectrometers became faster and more sensitive, protein mixtures of increasing complexity were digested and analyzed directly without prior fractionation at the protein level. The latter method is termed bottom-up proteomics. In contrast, top-down proteomics analyses intact proteins by MS. However, this method is still limited to single proteins in solutions.

Tandem Mass Spectrometry

The bottom-up techniques are currently the workhorse to analyze biological samples: in "shotgun proteomics", proteins in complex mixtures are analyzed using a combination of high-performance LC and MS/MS. A potential caveat of this technique is that the mass spectrometer selects the most abundant precursor ion for fragmentation. Thus, abundant proteins are more likely to be detected than scarce proteins. Undersampling is particularly an issue in samples such as plasma or serum that represent the most complex proteome of the human body with proteins spanning 10 to 12 orders of magnitude in linear dynamic range.[13] Current mass spectrometers only resolve 4 to 5 orders of magnitude. While a single peptide can be sufficient to unambiguously identify a protein, multiple peptides of the same protein are required for reliable quantification in shotgun proteomics. To date, most proteomics studies analyzing plasma samples have failed to reveal new biomarkers because low-abundant proteins are difficult to detect in the presence of very high-abundant components such as albumin. The issue of undersampling of the plasma proteome could be partially overcome by techniques involving depletion of abundant plasma proteins. An alternative strategy is to use diseased tissue, in which the biomarkers are more enriched. More targeted strategies can be used in plasma/serum once the biomarker candidate has been identified.

Multiple Reaction Monitoring

Multiple reaction monitoring on a triple-quadrupole mass spectrometer (QqQ-MS) is applied to peptides or metabolites identified in discovery experiments.[14,15] A QqQ-MS provides an effective, accurate way of quantifying a few selected target molecules even in complex mixtures such as plasma or serum.[15] If the molecule of interest can be ionized by electrospray ionization, the QqQ-MS will be interfaced to an LC system for separation of the analyte, as mentioned above. The precursor molecule is then selected in the first quadrupole. The second quadrupole is used as a collision chamber for fragmentation. The product ions are detected in the third quadrupole and their intensity is indicative of abundance of the parent molecule.[16] Due to its high specificity, multiple reaction monitoring is used for validation of biomarker candidates identified in discovery experiments,[17] but it has also been successfully used to confirm specific protein cleavage sites.[18] In addition, QqQ-MS is the method of choice for targeted metabolomics studies using standard metabolites as internal calibrators.[19]

METABOLOMICS

Metabolites are the end products of all processes occurring in cells, and metabolite levels in disease reflect the adaptation of biological systems to pathological conditions. It is currently estimated that over 2000 different metabolites can be endogenously synthesized.[20] Additionally, exogenous metabolites, such as vitamins, for example, are incorporated as part of the diet. Another major contributor to the metabolite pool is the gut flora. Like proteomics, the aim of metabolomics is to characterize the small molecule complement of a given sample and to interrogate the metabolic networks under normal and pathological conditions in a qualitative and quantitative manner. Metabolomics technologies have been applied to different clinical research areas including biomarkers and drug discovery,[21,22] toxicology,[23] and nutrition.[24] The first metabolomics studies were published early in the 2000s and were rapidly applied to cardiovascular research. As in proteomics, MS is the method of choice for metabolite analyses, but magnetic resonance spectroscopy (MRS) is also widely used.[25]