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Teaser This review describes the methodological and technological improvements in the field of proteomics and how proteomic-based techniques represent a powerful tool to globally analyse the spectrum of pharmacological drug targets.

Technological advances and proteomic applications in drug discovery and target deconvolution: identification of the pleiotropic effects of statins

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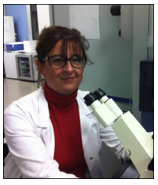
Proteomic-based techniques provide a powerful tool for identifying the full spectrum of protein targets of a drug, elucidating its mechanism(s) of action, and identifying biomarkers of its efficacy and safety. Herein, we outline the technological advancements in the field, and illustrate the contribution of proteomics to the definition of the pharmacological profile of statins, which represent the cornerstone of the prevention and treatment of cardiovascular diseases (CVDs). Statins act by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, thus reducing cholesterol biosynthesis and consequently enhancing the clearance of low-density lipoproteins from the blood; however, HMG-CoA reductase inhibition can result in a multitude of additional effects beyond lipid lowering, known as 'pleiotropic effects'. The case of statins highlights the unique contribution of proteomics to the target profiling of a drug molecule.

Introduction

Over the past few decades, the concept of designing selective ligands to act on individual drug targets, with the drug as a selective 'key' that fits into the 'lock' of a specific target, has become the dominant paradigm in drug discovery. This assumption has long influenced many aspects of drug discovery strategies, target identification, screening, drug design, and clinical trial design, including disease classification. However, a growing body of evidence from postgenomic biology has revealed a more complex picture of drug action, and it has been realised that many effective drugs, such as those used in oncology, psychiatry, infection, and cardiovascular medicine, act on multiple rather than single targets [1]. The serendipity of these multi-target effects of drug action and awareness of biological networks led to the novel concept of 'network pharmacology', a term coined by Hopkins in 2008 [1] to emphasise the paradigm shift from a one target–one drug to a network targeted–multi-target drug approach. The latter approach differs from traditional drug discovery approaches, usually based on specific targeting of single proteins, because it addresses the ability of a drug to



Cristina Banfi was awarded her PhD in biotechnology applied to pharmacology by the University of Milan; she is currently head of the Proteomic Unit at the Centro Cardiology Monzino in Milan. Her research involves basic research in pharmacological, biochemical, and molecular biology, as well as clinical research with patients at high risk for atherosclerosis and cardiovascular events. During her career, she has developed proteomic strategies using state-of-the-art techniques and instrumentations for the analysis of differential expressed proteins in human specimens (tissue, body fluids, and circulating cells), in in vitro cultured cells, and in animal models of heart ischaemia and stroke, with the aim of uncovering new therapeutic targets for the treatment and prevention of cardiovascular diseases, as well as new diagnostic biomarkers for early disease detection.

Elena Tremoli is the scientific director of the Centro Cardiologico Monzino and a professor of pharmacology. She has authored over 400 scientific articles on topics that include: the identification and development of new markers of atherothrombosis; the regulation of inflammatory and thrombotic system; the monitoring of the effectiveness of old and new therapies and identification of coagulation and platelet activation markers; and the morphology and function of the vascular wall by imaging. She received special recognition from the International Society of Atherosclerosis on the 20th anniversary of the description of carotid intima-media thickness as a diagnostic tool, and by the European Society for Non-Invasive and Preventive Cardiology for her outstanding contributions to non-invasive cardiovascular imaging and preventive cardiology.

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http://dx.doi.org/10.1016/j.drudis.2017.03.001

GLOSSARY

2D difference gel electrophoresis (2D-DIGE) 2D co-separation of protein samples labelled with different fluorescent dyes.
2D liquid chromatography (2D-LC) a preliminary separation technique generating fractions that are further fractionated on a second column using an orthogonal chromatography technique.
2D gel electrophoresis (2-DE) currently used in reference to the gel-based separation of proteins by their isoelectric point in one dimension followed by a molecular weight separation by SDS-polyacrylamide gel electrophoresis perpendicular to the first dimension.
Biomarker any molecular species found to correlate with a particular phenotype or perturbation of a biological system. Co-variant analysis of multiple biomarkers or patterns usually results in higher correlation confidence.
Data mining the ability to query very large databases to satisfy a hypothesis ('top-down' data mining); or to interrogate a database to generate new hypotheses based on rigorous statistical correlations ('bottom-up' data mining).
Data-independent acquisition (DIA) method of molecular structure determination in which all ions within a selected m/z range are fragmented and analysed in a second stage of tandem MS.
Electrospray Ionisation (ESI) a process in which ionised species in the gas phase are produced from a solution by means of spraying the solution from a narrow-bore needle tip under atmospheric pressure in the presence of a high electric field.
Fourier transform ion cyclotron resonance (FTICR) ion trap mass analyser based on the principle of ion cyclotron resonance, in which an ion in a magnetic field moves in a circular orbit at a frequency specific of its m/z value.
High-performance liquid chromatography (HPLC) a technique for separating proteins and other molecules of a solution, using a solvent as the mobile phase, and chemically coated material packed into a column as the stationary phase.
Ion-mobility spectrometry (IMS) analytical technique used to separate and identify ionised molecules in the gas phase based on their mobility in a carrier buffer gas. First, the ion mobility spectrometer separates ions according to their mobility, then the mass spectometer discriminates ions according to their m/z.
Isobaric tags for relative and absolute quantification (iTRAQ) a multiplexing isobaric labelling method used to determine the amount of proteins from up to eight different samples simultaneously in a single experiment.
Isotope-coded affinity tag (ICAT) proteomic technique based on the use of isotopic reagents for labelling two different populations of proteins.
Lipoproteins large macromolecular complexes comprising lipids and proteins that transport poorly soluble lipids (primarily triglycerides, cholesterol, and fat-soluble vitamins) through body fluids (plasma, interstitial fluid, and lymph) to and from tissues. Plasma lipoproteins are divided into five major classes that vary in density, size, and protein composition. Low-density lipoproteins (LDL) comprise a moderate proportion of protein with little triglyceride and a high proportion of cholesterol; an elevated plasma level of LDL is associated with increased probability of developing atherosclerosis. High-density lipoproteins (HDL) comprise a high proportion of protein with little triglyceride and cholesterol; an elevated plasma level of HDL is correlated with reduced risk of atherosclerosis.
Liquid chromatography-mass spectrometry (LC-MS) a technique that combines the physical separation capabilities of liquid chromatography (HPLC) with the mass analysis capabilities of MS.
Matrix-assisted laser-desorption ionisation (MALDI) common form of soft ionisation for protein analysis. It involves the formation of gas-phase ions from molecules that are present in a solid matrix that is irradiated with a pulsed laser.
Orbitrap ion trap in which the ions are orbitally trapped and oscillate along the trap axis. The oscillation frequency is inversely proportional to the square root of m/z.
Peptide-mass searching a method to identify proteins contained within a sequence database using an algorithm to match a set of peptide masses generated from the protein of interest with MS, using specific cleavage reagents (either enzymatic or chemical), with theoretical peptide masses calculated from each sequence entry in the database if the database sequences had been cleaved with the same specificity as the reagent in the experiment.
Pleiotropy/pleiotropic effects In pharmacology, pleiotropy (from Greek pleion 'greater in quantity, the more part, very many' + trope 'turn, turning') refers to the action(s) of a drug, usually unanticipated, other than those for which the agent was specifically developed. These effects can be related or unrelated to the primary mechanism of action of the drug, and can be beneficial, neutral, or undesirable (such as adverse effects or toxicity).
Proteome 'the total protein complement of a genome': that is, the complete set of proteins expressed by a cell, tissue, or organism.
Quadrupole time of flight (QTOF) it combines the ion-handling capacity of a quadrupole instrument with the resolving power and duty cycle of a TOF analyser.
Secretome the part of the proteome comprising all the proteins secreted by a cell, tissue, or organism at any given time or under certain conditions. The secretome constitutes an important class of proteins that control and regulate a multitude of biological and physiological processes.
Shotgun proteomics identifies all proteins present in complex mixtures using a combination of HPLC and with MS. Shotgun proteomics comprises four steps: (i) preparation and proteolytic digestion of the sample; (ii) separation of peptides; (iii) analysis by MS; and (iv) informatics analysis of the MS data.
Stable isotope labelling with amino acids in cell culture (SILAC) a MS-based quantitative proteomics method that involves the metabolic in vivo incorporation of a light or heavy label into the proteins.
Statins also known as HMG-CoA reductase inhibitors; the most effective agents for treating dyslipidaemia; they are reversible competitive inhibitors of HMG-CoA reductase, the enzyme that catalyses the rate-limiting step in cholesterol biosynthesis. Statins currently available include: atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin.
Tandem mass spectrometry (MS/MS) involves multiple steps of MS selection, with some form of fragmentation occurring between stages. Ions formed in the