REVIEW 10.1111/1469-0691.12029

Applying proteomic technology to clinical virology

C. Mancone[1,2], F. Ciccosanti[1], C. Montaldo[1,2], A. B. Perdomo[1], M. Piacentini[1,3], T. Alonzi[1], G. M. Fimia[1] and M. Tripodi[1,2]

1) *'Lazzaro Spallanzani' National Institute for Infectious Diseases I.R.C.C.S.,* 2) *Department of Cellular Biotechnologies and Haematology, Istituto Pasteur-Fondazione Cenci Bolognetti, Sapienza University of Rome and* 3) *Department of Biology, University of Rome 'Tor Vergata', Rome, Italy*

Abstract

Developing antiviral drugs, vaccines and diagnostic markers is still the most ambitious challenge in clinical virology. In the past few decades, data from high-throughput technologies have allowed for the rapid development of new antiviral therapeutic strategies, thus making a profound impact on translational research. Most of the current preclinical studies in virology are aimed at evaluating the dynamic composition and localization of the protein platforms involved in various host–virus interactions. Among the different possible approaches, mass spectrometry-based proteomics is increasingly being used to define the protein composition in subcellular compartments, quantify differential protein expression among samples, characterize protein complexes, and analyse protein post-translational modifications. Here, we review the current knowledge of the most useful proteomic approaches in the study of viral persistence and pathogenicity, with a particular focus on recent advances in hepatitis C research.

Keywords: Biomarker discovery, HCV, mass spectrometry, post-translational modifications, proteomics
Original Submission: 12 July 2012; **Revised Submission:** 31 August 2012; **Accepted:** 4 September 2012
Article published online: 24 September 2012
Clin Microbiol Infect 2013; **19:** 23–28

Corresponding author: M. Tripodi, 'Lazzaro Spallanzani' National Institute for Infectious Diseases I.R.C.C.S., Rome, Italy
E-mail: tripodi@bce.uniroma1.it

Introduction

Our understanding of host–virus interaction has recently been advanced by system-wide analyses. 'Omics' technologies such as genomics and transcriptomics have greatly enhanced the systemic evaluation of gene expression. Despite their usefulness, genomics and transcriptomics approaches have certain limitations. First, gene mutations and mRNA expression levels determined quantitatively often do not mirror the real situation in terms of mature proteins. Moreover, translational and post-translational regulation affect not only the level of a mature protein but also its function. In fact, the effects on protein structure and biological function of alternative splicing, post-translational modifications and virus–host interactions cannot be easily predicted by genomics and transcriptomics studies. In light of this, mass spectrometry (MS)-based proteomics is a unique and necessary tool for system-wide, structural and functional analysis of the effectors of biological functions.

MS-based Proteomics

Proteomics offers a great variety of applications. Each experimental proteomic analysis can be summarized in three major steps: (i) sample preparation; (ii) protein separation; and (iii) identification. A downstream bioinformatic analysis can also be performed for data processing (Fig. 1).

(i) The first requirement for successful proteomic analysis is appropriate and specific protein sample enrichment by fractionation techniques. Indeed, many of the crucial viral life-cycle steps are associated with defined subcellular complexes; moreover, the more attractive therapeutic targets or prognostic markers are either specific cell membrane components, or circulate in biological fluids at low concentrations [1,2].

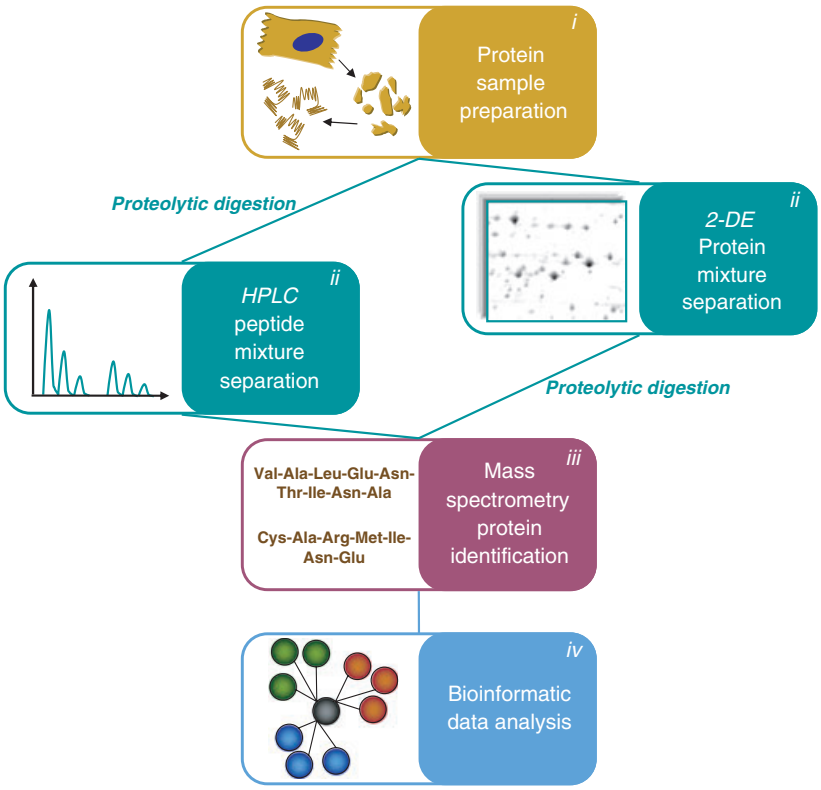

FIG. 1. MS-based proteomic workflow. Experimental proteomic analysis consists of several steps, listed in the different panels: sample preparation (i), protein or peptide mixture separation (ii), protein identification by MS (iii), and bioinformatic analysis (iv). 2-DE, two-dimensional gel electrophoresis.

(ii) The sample preparation step leads to a complex mixture of different proteins. Proper separation of these mixtures is essential for the accurate identification and quantification of proteins by MS. Traditionally, two-dimensional gel electrophoresis (2-DE) has been considered to be a suitable protein separation method. In the past few years, this technique has been complemented by a quantitative application (two-dimensional difference in-gel electrophoresis (DIGE)) that allows for analysis of the relative changes in protein abundance among different samples [3]. However, owing to recent technical advances, high-resolution nano-liquid chromatography is gradually replacing both 2-DE and DIGE. This is because the low volume and low flow rate of nano-liquid chromatography allows for analysis of proteins on an attomole level, significantly reducing solvent waste and enhancing MS-based protein identification [4].

(iii) MS is the core of proteomics. The biological applications of MS have grown exponentially since the discovery of matrix-assisted laser desorption ionization and electrospray ionization techniques [5]. Protein identification is the primary aim of MS in proteomic applications. Because of the difficulties in analysing the mass of entire proteins, identification is performed on peptides obtained from them by proteolytic cleavage.

The development of quantitative techniques has extended MS applications in proteomics. In fact, high-throughput relative and absolute protein abundance measurements are now possible in liquid chromatography/MS configurations with a variety of isotope-mediated approaches, such as isotope-coded affinity tags, isotope-coded protein labelling, the accurate mass and time tag approach, isobaric tags for relative and absolute quantitation, and stable isotope labelling of amino acids in cell culture [6].

Proteomic Applications in Preclinical and Clinical Virology

Viral replication and propagation cause host cellular proteome variations. The identification of proteins whose presence is affected by the presence of a virus may serve for either diagnostic or prognostic purposes, or for the discovery of novel therapeutic targets (Fig. 2). Biomarker investigations are normally performed on biological fluids such as serum, plasma, and saliva. An excellent example of this can be observed in an investigation by Wiederin *et al.* [7] on sera of human immunodeficiency virus-1-associated dementia patients that allowed for identification of two differentially expressed proteins (prealbumin and gelsolin) as early biomarkers in human immunodeficiency virus-induced neurodegenerative disorders.

Interestingly, biomarker identification can also be obtained with tissues or cell cultures as a protein source. For