Mass Spectrometry-Based Proteomics

Subjects: Immunology

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Omics technologies provide the tools required to investigate DNA, RNA, proteins, and other molecular determinants. These technologies include genomics, transcriptomics, proteomics, and metabolomics. However, proteomics is one of the main approaches to studying allergic disorders' pathophysiology. Proteins are used to indicate normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Proteomics studies the complete set of proteins present in a live organism at a specific time or condition, including expression, structure, functions, interactions, and modifications, which are crucial for early disease diagnosis, prognosis, and monitoring of disease development.

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1. Introduction

The World Health Organization (WHO) estimates that around 25% of the world's population suffers from respiratory allergic diseases [1]. Airborne allergens cause inflammation of the airways, and the most common allergens are house dust mites, pollen, proteins in animal hair, and animal urine. Air pollutants can aggravate allergy symptoms. The most important are particulate matter (PM_{10} and $PM_{2.5}$), ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), and sulfur dioxide (SO_2), among others [1]. Pollutants penetrate the airways, triggering airway inflammation and exacerbating respiratory symptoms. Barrier dysfunction in the lung allows allergens and environmental pollutants to activate the epithelium further and produce cytokines that promote the induction and development of immune responses [2]. Therefore, respiratory allergies are more frequent in cities with high air pollution. Additionally, climate change extends the flowering period and pollen production of many tree species, resulting in chronic healthy affectations [3]. Pollen can also cause cross-allergies with some foods because they have similar proteins. For example, in oral allergy syndrome (OAS), people with a respiratory allergy who eat fresh and raw fruits and vegetables can suffer an allergic reaction in the lips, mouth, and throat [4]. Respiratory allergy is a type I hypersensitivity reaction mediated by IgE. The IgE-mediated mechanism involves a sensitization step in which Th2 cells produce cytokines such as IL-4, IL-5, and IL-13, which produce eosinophilia and induce specific IgE production. The IgE molecules bind to FcεRI receptors on mast cells (MCs) and basophils. This bind triggers a complex cascade signaling that leads to the release of inflammatory and vasoactive mediators such as histamine, leukotrienes, and vasopressin, among others, which cause the clinical response [5].

Biomarkers are defined as characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Clinical

biomarkers offer some advantages: they are less expensive and usually measured quickly [6]. Unlike genes or transcripts, proteins are the most informative biomarker, are differentially expressed during disease states, and can undergo changes in protein folding and post-translational modifications relevant to understanding disease pathophysiology. Proteins can be measured and evaluated to compare the normal versus pathogenic biological processes or pharmacologic responses to develop therapeutic interventions. Mass spectrometry (MS) is the core technology used for current proteomics studies. It is helpful to discover new proteins as indicators of pathogenic processes or pharmacologic responses to treatment in allergenic diseases.

2. MS-Based Proteomics

Proteomics studies the complete set of proteins present in a live organism at a specific time or condition, including expression, structure, functions, interactions, and modifications, which are crucial for early disease diagnosis, prognosis, and monitoring of disease development [7][8]. Although other techniques are relevant, MS has been the leading technology for proteomic analysis. As a result, the human proteome map was constructed employing MS [9][10]. With recent advances in instrumental devices, bioinformatics pipelines, and machine learning algorithms, proteomics has expanded to identify and analyze thousands of proteins with quantification capabilities [11]. MS determines the mass-to-charge (*m*/*z*) ratio of gas-phase ions produced in an ionization source such as in electrospray ionization (ESI) [12] and the matrix-assisted laser desorption ionization (MALDI) [13]. In addition, liquid chromatography (LC) coupled to tandem MS is the most common method to large-scale characterize proteins in complex biological samples [14][15][16].

In MS-based methods for proteomics, it can be identified two approaches: bottom-up and top-down. Bottom-up is also called shotgun proteomics, and it is employed to identify proteins, post-translational modifications, and quantify biomarker discovery and diagnostic screening [17]. A mixture of proteins is enzymatically digested with a protease into mixtures of peptides before separating by LC. Then, the peptides are ionized and separated according to *m*/*z* in a first MS to be immediately split into fragmentation ions for the MS2 or MSn depending on the instrument capabilities. The mass spectra generated are compared with theoretical MS/MS patterns from databases and scores based on peptide-spectrum matches (PSMs). Additionally, de novo sequencing is possible. In this step, the typical analysis software includes MASCOT, SEQUEST, and X! Tandem [18]. Despite this approach being the most common method for proteome screening, there are limitations, including the fact that most proteins are identified based on few peptides, protein isoforms and post-translational modifications are often missed, and low-abundance proteins often will be lost or suppressed by other high-abundance proteins.

The other top-down MS approach can directly sequence proteins by LC-MS/MS. Here the intact proteins are chromatographically separated and detected directly without enzymatic digestion by ESI or MALDI. Then, ions generated in the ionization source are fragmented and analyzed in tandem mass spectrometry. This strategy provides more information on identifying and quantifying the protein isoforms, sequence variants, and post-translation modifications. However, routine identification of only the highest abundance proteins makes it difficult to characterize lower abundance proteins [19][20][21]. On the other hand, the quantification of proteins is crucial for understanding the complex biochemical mechanisms involved in a human disease condition. Protein levels in

response to the environment, differential expression analysis, and protein–protein interaction reflect the body's steady state. The proteomics techniques can be relative or absolute quantitation. The commonly used methods include label-free quantification (LFQ), the most widely used strategy for proteome quantification due to its simplicity and minimal interference. However, only relative quantification of proteins is possible with this methodology; no other biomolecule is added to the sample. It is usually employed in the clinical practice of searching biomarkers in cancer research when tumor versus normal tissues are compared [22]. Relative and absolute quantification is possible with stable isotope labeling with amino acids in cell culture (SILAC). Isotopes of Lys and Arg (13C or 15N) are added to the cell medium, labeling proteins for detection in MS1 by mass spectrometry. SILAC was used to find individual biomarkers in clinical practice [23].

Another quantitative method is isobaric tags for relative and absolute quantification (iTRAQ). It consists of comparing a reporter group of peptides with a balanced group. Qualitative and quantitative analysis can be performed simultaneously [24]. Another technique consists of the use of tandem mass tags (TMT). Labeling proteins with a reporter, normalizer, and an amine-reactive group allows analyzing different samples in a multiplex run with high precision and fewer missing values than LFQ [25]. Targeted proteomics detect low-abundance and specific proteins on multiple-, selected- or parallel-reaction monitoring (MRM, SRM, and PRM, respectively). These acquisition methods target specific peptide sequences and quantify protein isoforms and post-translational modifications, producing more reproducible and precise results [26]. An example of this quantitative technique is Absolute Quantification (AQUA), which incorporates synthetic peptides containing stable isotopes as internal standards. Then, the ratio between endogenous and the synthetic peptide is used to calculate the absolute quantitation of desired protein [27].

Besides MS, gel-based proteomics and immunological methods remain useful for allergen identification and respiratory illnesses. Two-dimensional electrophoresis (2-DE) consists of focusing proteins according to their isoelectric point (IEF) and by a molecular weight [28][29]. The presence or absence of spots provides valuable information about the dysregulation, level expression, quantity, and misshaping of proteins related to a respiratory disorder. However, 2-DE is technically laborious and challenging to replicate, and frequently more than one protein is in the same spot; thus, quantification is not precise. Additionally, western blotting is performed to detect IgE-reactive spots employing sera from allergic patients subsequently characterized by MS. Proteomics for respiratory allergies often depend on the sample type to be analyzed. These include blood cells, plasma, serum, sputum, bronchoalveolar and nasal lavage fluid (NLF), exhaled breath condensate, and biopsies of the lung and nasal polyps (NPs) [26].

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