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Review

Proteomics of bronchoalveolar lavage fluid

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Abstract

Lung diseases are essentially multi-factorial diseases that require a global analysis, and thus, cannot be understood through the sole analysis of individual or small numbers of genes. Proteome analysis has rapidly developed in the post-genome era and is now widely accepted as the obligated complementary technology for genetic profiling. It has been shown to be a powerful tool for the study of human diseases and for identifying novel prognostic, diagnostic and therapeutic markers. During last years, proteomic approaches applied to lung diseases are centred on the analysis of proteins in samples, such as cell cultures, biopsies and physiological fluids like serum and, especially, bronchoalveolar lavage fluid (BALF). BALF is presently the most common way of sampling the components of the epithelial lining fluid (ELF) and the most faithful reflect of the protein composition of the pulmonary airways. This review focuses on the state of the investigations of BALF proteome and its powerful contribution both to a better knowledge of the lung structure at the molecular level and to the study of lung disorders at the clinical level.

Keywords: Proteomics; Bronchoalveolar lavage fluid

Contents

Abbreviations: 2-DE, two-dimensional gel electrophoresis; AOP2, antioxidant protein 2; BALF, bronchoalveolar lavage fluid; CC16, clara cell protein 16; ELF, epithelial lining fluid; ESI–MS–MS, electrospray ionisation tandem mass spectrometry; ICAT, isotope-coded affinity tag; LCM, laser capture microdissection; LPS, lipopolysaccharide; MALDI-TOF, matrix absorption laser desorption ionisation-time of flight; Multi-LC, multidimensional liquid chromatography; OVA, ovalbumin; SELDI, surface-enhanced laser desorption ionisation; SPA, surfactant protein A

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1. Introduction

Respiratory diseases are important health problems. The lung is the primary target of ambient air pollutants that can produce various health effects, including decrement of lung functions, exacerbation of asthma and increased risks of respiratory infections. Whether caused by chemical, physical or biological environmental compounds, damage to the lungs and airways, often leads to morbidity or mortality [1]. Lung diseases are essentially multi-factorial diseases, and thus, require a global analysis of such disorders [2]. With the DNA sequences of more than 97 genomes completed, as well as a draft sequence of the human genome, it is now evident that the complexity of tissues and, more generally, organisms is only to a small degree the result of direct gene expression from the genome [3–5] and it is clear that the famous dogma of one gene-one protein is no more correct. One gene can give rise to a whole family of gene products by numerous processes, such as alternative mRNA splicing, RNA editing [6] and post-translational protein modification [7]. Moreover, the correlation between mRNA and protein concentration is insufficient to predict protein expression levels from quantitative mRNA data [8]. Therefore, the direct measurement of protein expression is essential to analyse biological processes in normal and disease conditions. The protein complement of a specific genome is termed “proteome”. In contrast with the genome, the proteome is dynamic and constantly changing in response to various environmental factors and other signals, thus giving rise to near-infinite dimensions of states [9]. Elucidating how the proteins complement changes in a cell type during development in response to environmental stimuli and in diseases is crucial to understand how these processes occur at a molecular level. There is also a wide-ranging interest in using the proteomic approach and its recent advances in protein analysis techniques to define markers of diseases and allow early diagnoses, a crucial step in the treatment of these disorders. In combination with genomic and transcriptomic approaches, protein profile analysis of clinical samples will lead to a better understanding of the mechanisms of lung diseases and contribute to the discovery of new therapeutics for the prevention and the treatment of diseases [10].

The core element of the classical proteome research combines the multidimensional separation of polypeptides by two-dimensional gel electrophoresis (2-DE) [12] or by multidimensional liquid chromatography (multi-LC) [13] from a physiological complex mixture and their identification by mass spectrometry, MALDI-TOF or ESI–MS/MS [14]. In 2-DE, proteins are separated according to their isoelectric point in the first dimension and to their molecular weight in the second one. Once stained, the resulting two-dimensional protein maps can be compared, the objective being, in differential-display proteomics [12], to search for and identify proteins that are up- or down-regulated in a disease-specific manner for, e.g. further use as diagnostic markers. The rapid evolution of mass spectrometry has made it a key technique for the investigation of the proteome [14,15]. At the beginning, this technology has led to the identification of a large part of the proteins of interest. The coupling of liquid chromatography with MS had a great impact on identification and quantification of small, minor and hydrophobic proteins and has proven to be an important alternative method to 2-DE gels [16]. Typically, proteins in a complex mixture are separated by ionic and reverse phase column chromatography coupled to MS or MS–MS analysis. The complexity of the proteome and the separation limits of both 2D gel electrophoresis and liquid chromatography allow only a fraction of that proteome to be analysed [17]. An alternative approach is to reduce the complexity of the protein sample prior to protein separation and characterization. In this context, the Aebersold group has developed a pair of isotope-coded affinity tag (ICAT) reagents to differentially label protein samples on their cysteine residues and allow their specific purification by affinity [18]. Other promising mass spectrometry techniques are the protein profiling methods. Protein profiling is the screening of samples by mass spectrometry with limited or not sample preparation. The resulting profiles of different samples (body fluids, cell lysates or tissue samples) can be compared, and differences in the relative abundance of proteins can be identified. These techniques provide a complementary method to 2D-PAGE or multi-LC for protein visualisation and quantification. For protein profiling, surface-enhanced laser desorption-ionisation (SELDI) [19] and imaging mass spectrometry (IMS) are currently being evaluated [20].

2. Lung proteome

The lung proteome is a dynamic group of specialized proteins related to pulmonary functions. The complexity of the lung proteome is essentially due to the presence of multiple different types of cells and the large contact with environmental compounds, such as industrial pollutants or pathogens [11]. The effects of chemical, physical and biological exposures alter the expression and the structure of the proteome, which becomes manifest in long-term adverse health effects and diseases. The complexity of the lung proteome requires an investigation at different levels (subproteomes) to define

patterns of protein expression in particular cells (i.e. tissues: biopsies), in vitro cell culture system (treated versus control), and biological fluids like serum, urine or lavage (disease versus no disease).

Last years, proteomic approaches applied to lung diseases are centred on the separation of proteins in samples such cell cultures, biopsies and physiological fluids, especially serum and bronchoalveolar lavage fluid (BALF). This review focuses on the state of the investigations of the BALF proteome.

2.1. Lung tissues proteome

During these last years, lung tissue proteome works have been essentially devoted to tumor biopsies and have shown that different histopathological tumor types exhibit significantly different protein expressions. First, Hirano et al. [21] observed from biopsies resolved by 2-DE gels that a pair of polypeptides (TAO1, TAO2) was significantly up-regulated in primary lung adenocarcinomas. In the same context, proteome analysis of lung adenocarcinoma led to the identification of a set of new prognostic biomarkers and indicated that protein expression profiles can diagnose patients with early-stage lung cancer [22,23]. More recently, proteomic patterns obtained directly from small amounts of fresh frozen lung-tumor tissue have been used to accurately classify and predict histological groups as well as nodal involvement and survival in resected non-small cell lung cancer [24]. This approach is greatly enhanced with laser capture microdissection (LCM) [25] that allows the selection of a homogenous population of cells based on morphology and histology. In this study, the authors showed that the profile of mass spectrometry spectra allowed classification of surgically resected lung-tumors into groups and showed good correlations with prognosis [24]. A comprehensive lung cancer database consisting of transcriptomic and proteomic informations for different types and stages of lung cancer has been developed by Oh et al. [75].

2.2. Lung cell proteome

Additional informations on lung proteome can be obtained by cell isolation and proteome analysis of the isolated cells. Since 10 years, major investigation have been realised on isolated lung cells like macrophages [26], lung fibroblast cells [27], isolated ciliary axonemes [28], cell culture of mesenchymal type [29], transformed human bronchial epithelial cell line [30] and cancer cells [24]. Different comprehensive 2-DE gel databases have been started: recently, the group of Malmstrom et al. [31] has undertaken an encompassing large-scale protein expression profiling and annotations of human primary lung fibroblast cells in both activated and resting cells. In the same context, Ostrowski et al. [28] have initiated an extensive proteomic analysis of isolated ciliary axonemes by 2-DE gel electrophoresis coupled to LC–MS–MS technology. Interesting informations have been obtained on the modifications of the lung cell proteome after different stimuli, such as acute exposure to ozone [26] and Jet fuel exposition [32], transforming growth factor beta addition [27], endothelin-1 stimulation [31] and arsenic transformation [33]. The set of these studies have led to significant advances in the molecular mechanisms of different lung pathologies like asthma, fibrosis and lung cancer.

2.3. Plasma/serum proteome

During last years, it has been demonstrated that the serum is a convenient protein-rich information reservoir that may show a systemic response to a specific disease. The protein profile or biomarkers from serum may faithfully reflect a diseased lung or an acute-phase response to the disease [34]. Recently, Xia and co-workers evaluated the serum proteome of patients with non-small cell lung cancer. In this study on 28 patients, two proteins are up-regulated, while three are down-regulated [35]. Subsequently, these authors showed that the SELDI technology combined with the artificial intelligence classification algorithm can facilitate the discovery of better biomarkers and provide a useful tool for early diagnosis in the future. Another study, using serum profiling technology, showed that serum amyloid A protein can not only be associated with lung cancer but also with nasopharyngeal cancer [36]. It is clear that more recent potential biomarkers found in the plasma or serum proteome are not lung-specific proteins and require a validation. The modulation of the level of non-specific proteins in the serum or plasma could be the reflection of common mechanisms (i.e. inflammatory process) or exposure leading to several different diseases. Nevertheless, the advantage of plasma proteome analysis is that blood samples are readily accessible. In theory, plasma should contain a large part of, if not all, the human proteins and should, therefore, be an ideal target for a lung proteome approach. However, the dynamic range of protein concentrations in plasma is even greater than in other body fluids like the epithelium lining fluid, and unfortunately the concentration of specific pulmonary proteins in plasma is relatively low [37]. Moreover, lung tissues and epithelium lining fluid much better reflect the local environment from which they are coming and offer insight into the molecular mechanism of disease initiation and progression. Thus, BALF and tissue samples are more ideal sources of lung biomarkers identification.

3. BALF proteome

3.1. Epithelial lining fluid (ELF) and BALF

The airways and, particularly, the alveoli are covered with a thin layer of ELF, which is a rich source of many different cells and soluble components of the lung that play important functions by protecting the lung from undue aggressions and preserving its gas-exchange capacity [38]. Therefore, the protein composition of the ELF most faithfully reflects the effects of the external factors that hit the lung and is of primary

importance in the early diagnosis, the assessment and the characterization of lung disorders as well as in the search for disease markers [39]. Numerous chemical, physical and biological exposures and, finally, lung diseases induce biochemical modifications of this ELF [40]. Diverse techniques are now used to sample the distal airways. Induction of sputum with hypertonic saline [41], nasal lavage [42] and condensation of exhaled breath [43] are more or less non-invasive techniques that open the way to only a relatively small subset of the lung proteome. However, the oldest and most common means used up to now to get samples of ELF that most accurately reflects its global protein composition is the bronchoalveolar lavage (BAL), which is a safe but invasive technique performed during fiberoptic bronchoscopy, and thus, cannot be performed in all patients [40]. BALF contains cells (alveolar macrophages, lymphocytes, neutrophils, eosinophils, sometimes plasma cells, squamous epithelial cells, bronchial epithelial cells, type II pneumocytes, basophils and mast cells) and a wide variety of soluble components (lipids, nucleic acids and proteins/peptides) originating from ELF [44]. ELF analysis has become an established technique to study the cellular and molecular components of the lung and to investigate their relative changes in different respiratory diseases [45,46].

3.2. History of BALF proteome

The development of technologies like mass spectrometry and 2-DE gel separation, and the recent progresses in the sample preparation of the BAL fluid has boosted the BALF proteome research which is now in an exponential growth phase. Proteome analysis of BALF has been performed for the first time in 1979 by Bell [47]. These authors realised the first 2D-PAGE BALF database and the first differential proteome analysis between BALF samples from smoker and non-smoker patients. The use of antiserums monospecific for normal plasma components and the comparison with the serum 2D-PAGE protein map have shown that most of the proteins found in BALF are serum proteins. Interestingly, this study reveals significant differences between smokers and non-smokers showing the potential medical interest of this approach: an increased level of IgG, C4 and C3 and a decrease of α2-thioglycoprotein, α1-acid glycoprotein and Gc-globulin were observed in smoker samples. Later, other works, essentially realised by the group of Lenz, confirmed the BALF proteome research potential [48–50]. These authors compare the BALF proteomes from patients with idiopathic pulmonary fibrosis, sarcoidosis and asbestosis with controls. If these authors observed statistically significant variations of plasma proteins in the sarcoidosis sample (increase of IgG, IgA), other interesting proteins especially characterised by a low-molecular weight and significantly increased in asbestosis patients still remain non-identified. Long-standing difficulties in the identification and quantitative analysis of proteins of lower abundance have been responsible for the limited clinical applicability of the proteome analysis of BAL fluid in the past. The emergence of the N-terminal automated Edman sequencing method, and, more recently, the mass spectrometry technology for "high-throughput" protein identification from gels have led to a tremendous increase in the number of identified protein from BAL fluid, and thus, to a much better proteome description of this fluid. The groups of Lindahl et al. [42,51–53], Wattiez and co-workers [44,54,55], Magi et al. [56], and Sabounchi-Schutt et al. [57,58] have been the principal investigators in the development of a current master gel of BALF proteins. Classically, master gels of BALF proteins comprise >1200 protein spots visualised by silver staining (Fig. 1). Moreover, the application of narrow-range immobilized pH gradient (IPG) strips has further increased the resolution of 2D-PAGE, and thus, increased the number of identified proteins characterised by a low level. Recently, in combination with the paper bridge sample application, Sabounchi-Schutt et al. [57] identified 12 "non-described" proteins not detected in the plasma in the narrow pH range 4.5–5.2, from an individual healthy BALF sample.

3.3. The power of BALF proteome in the study of lung disorders

All these studies show that the proteome of BALF contains numerous different classes of proteins that reflect the great diversity of their cellular origins and functions (Fig. 2). Soluble proteins in BALF may originate from a broad range of sources, such as diffusion from serum across the air-blood barrier (i.e. albumin, transferrin, α1-antitrypsin and immunoglobulins A and G) and the production by the different cell types (Fig. 3).

A comparison between serum and BALF proteomes reveals that a certain number of proteins are characterized by a higher level in BALF than in plasma, suggesting that they are specifically produced in the airways (Fig. 4). These proteins are, therefore, good candidates for becoming lung-specific biomarkers. Interestingly, most of these lung-specific proteins could be detected in plasma by the use of specific antibodies. For example, an increase in the level of serum lung-specific proteins SP-A and SP-B reflects increased damage to the alveolocapillary barrier [37].

3.3.1. Differential-display proteomics

One of the major interests of the proteomic approach is the comparison between proteomes (differential-display proteomics) of different state of the lung (i.e. patients with different lung diseases and control), to obtain a better understanding of pathogenetic mechanisms and events operating at alveolar level in different types of lung diseases. The aim of this investigation is to define proteins or group of proteins significantly associated with disease, or specific for clinical outcome. Differential-display proteomics thus requires high reproducibility in sample preparation and separation technologies. Major problems associated with BALF protein samples are the low protein content and its high salt concen-

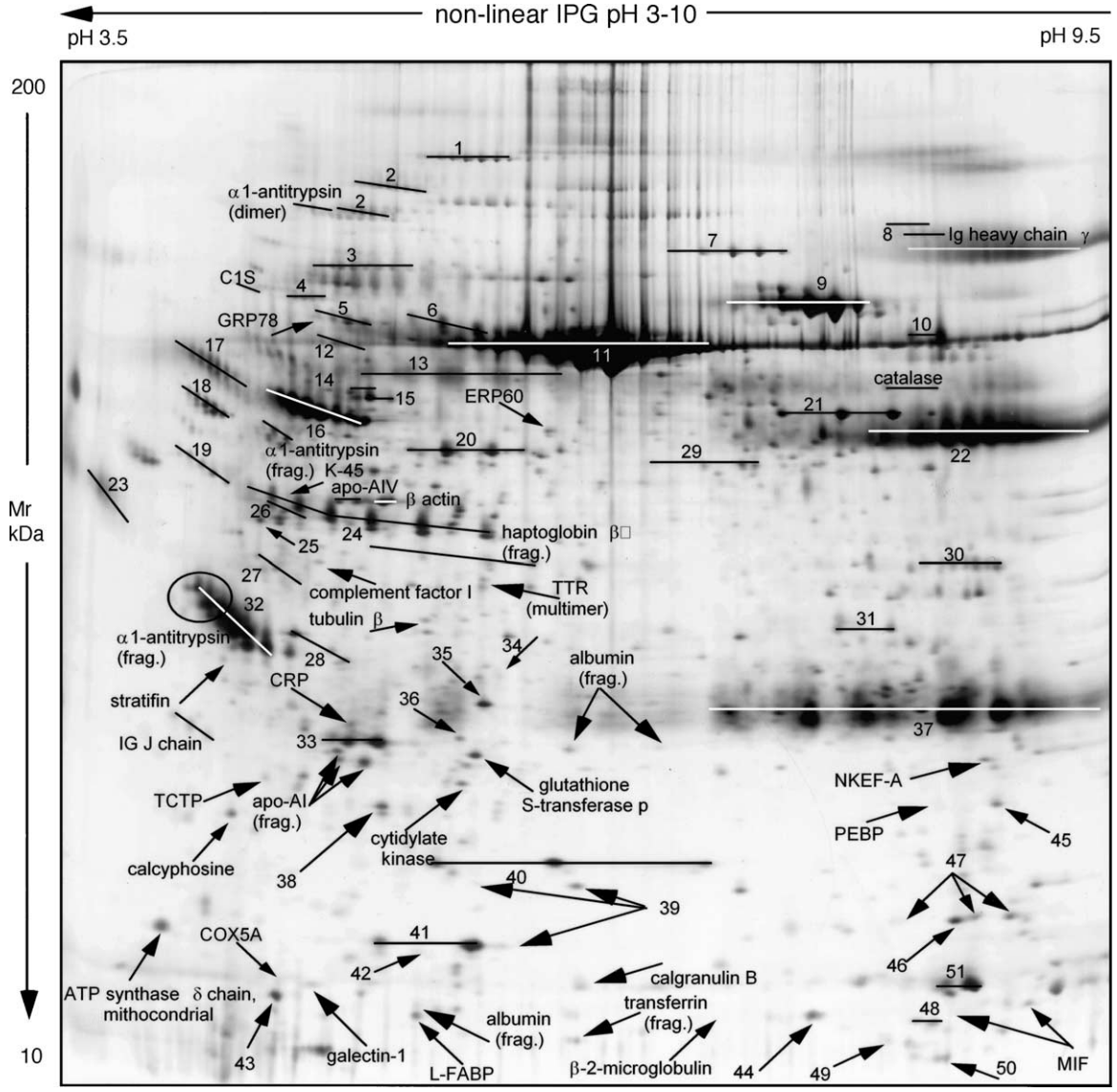

Fig. 1. 2D electrophoretic pattern of BALF from an idiopathic pulmonary fibrosis patient, performed using a non-linear immobiline gradient 3–10, followed by 9–16% SDS-PAGE. Proteins were detected by silver staining. (1) α-2-macroglobulin; (2) ceruloplasmin; (3) immunoglobulin A-S chain; (4) prothrombin; (5) α1-B-glycoprotein; (6) hemopexin; (7) complement factor B; (8) plasminogen; (9) transferring; (10) complement c3 β; (11) albumin; (12) α-2-antiplasmin; (13) immunoglobulin heavy chain α; (14) antithrombin III; (15) Vitamin D-binding protein; (16) α1-antitrypsin; (17) α1-antichymotrypin; (18) α2-HS-glycoprotein; (19) leucine-rich α2-glycoprotein; (20) fibrinogen γ, A chain; (21) fibrinogen β chain; (22) immunoglobulin heavy chain γ; (23) orosomucoid 1; (24) haptoglobulin 1 β chain; (25) complement C3; (26) Zn-α2-glycoprotein; (27) clusterin; (28) α1-microglobulin; (29) immunoglobulin heavy chain μ (intermediate segment); (30) immunoglobulin heavy chain γ (intermediate segment); (31) complement C4; (32) pulmonary surfactant-associated protein A; (33) apolipoprotein A-I; (34) cathepsin D heavy chain; (35) proapolipoprotein AI; (36) serum amyloid P-component; (37) immunoglobulin light chain κ, λ; (38) serum retinol binding protein; (39) superoxide dismutase (Cu–Zn); (40) haptoglobin α; (41) transthyretin; (42) immunoglobulin binding factor; (43) thioredoxin; (44) β2-microglobulin; (45) phosphatidylethanolamine binding protein; (46) peroxisomal antioxidant enzyme; (47) peptidyl-prolyl *cis–trans* isomerase A; (48) calgranulin A; (49) GTP cyclohydrolase I feedbackregulatory protein; (50) ubiquitin; (51) haemoglobin β chain. *Abbreviations*: C1S, complement C1S; GRP78, 78 kDa glucose-regulated protein; ERP60, protein disulfide isomerase A3; TTR, transthyretin; K-45, serum paraoxonase-arylesterase 1; CRP, C-reactive protein; TCTP, translationally controlled tumor protein; cox5A, cytochrome c oxidase polypeptide VA; L-FABP, fatty acid binding protein liver; NKEF-A, thioredoxin peroxidase 2; FEBP, phosphatidylethanolamine binding protein; MIF, macrophage migration inhibitory factor (Magi et al. [56], reprinted with permission from Wiley, VCH].

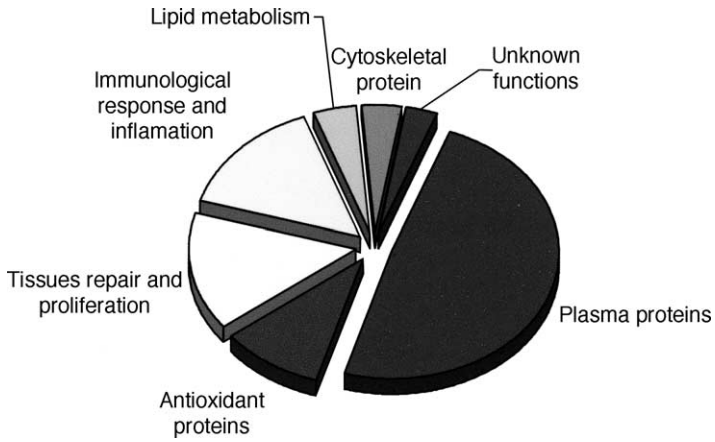

Fig. 2. Overview of the different identified proteins in BALF. Many proteins identified in BALF are plasma proteins, probably originating from it by passive diffusion due to the permeability of the air-blood barrier. However, among these proteins, some are synthesized in the lung too (i.e. α1-antitrypsin, α-e macroglobin, apolipoprotein A1, β2-microglobulin, ceruloplasmin, complement factors 3 and 4, immunoglobulins), probably to provide local extra-protection against invading micro organisms, oxidative damages and proteases released during inflammatory processes taking place in the lung. Other constituents are antioxidant proteins, cytoskeletal proteins, or proteins involved in tissue repair and proliferation, in immunological and inflammation responses and in lipid metabolism.

tration that comes from the phosphate-buffered saline used for the lavage procedure [45]. In this field, great efforts have been made, these last years, to optimize the process of sample preparation using a variety of techniques, including dialysis, ultramembrane filtration, precipitation and gel filtration. A pilot study dedicated to the BAL fluid analysis with special emphasis on sample preparation has been realised by Plymoth et al. [59]. It is clear that these advances in technology and in sample preparation allowed a drastic increase in the number of applications of the BALF proteome research particularly in the differential-display proteomic research.

3.3.2. Fibrosing interstitial lung diseases

Among the applications of BALF differential-display proteome analysis, many studies have been dedicated to different lung pathologies, such as sarcoidosis [44,49,51,54–56,58], bacterial pneumonia [54], idiopathic pulmonary fibrosis [44,49,51,54–56], lupus erythematosis [55], Wegener's granulomatosis [55], hypersensitivity pneumonitis [60], chronic eosinophilic pneumonia [55], alveolar proteinosis [61], asbestosis [55], lipoid pneumonia [55], cystic fibrosis before and after α1-antiprotease treatment [62] and, recently, acute lung injury [63]. Among these different pathologies, some fibrosing interstitial lung disease like sarcoidosis and idiopathic pulmonary fibrosis have been most studied combining the 2D electrophoresis separation and mass spectrometry technologies [44,49,51,54–56,58]. First, plasma proteins are found in higher concentration in BALF of sarcoidosis. This may be due to altered alveolar membrane integrity during alveolitis in the course of the disease [64]. Many low-Mr proteins are produced locally by different cell types. Some of them may be a product of cell damage, which is severe in idiopathic pulmonary fibrosis (IPF), others are produced by active secretion and have various functions like, for example, proteins involved in inflammatory processes and with antioxidant activity. It may be too early to decide which of these proteins are actual diagnostic and prognostic markers; however, the differences found between sarcoidosis and IPF give the hope that, in a near future, groups of proteins could be used to diagnose or evaluate the evolution of theses diseases, for example, as indicators of progression towards lung fibrosis. If small differences were observed between all these studies, they probably reflect differences in sample preparation and individual variability. Nevertheless, these independent researches realised on different cohorts reveal the great medical potential of this approach.

It is clear that the level of a single BALF protein represents the integration of a multitude of different mechanisms involved in its synthesis, release and/or clearance, therefore, measuring changes in the levels of only one particular protein species gives insights only into one particular piece involved in the puzzle of a defined lung disease (Fig. 3). Therefore, single genes or single proteins could not predict a specific outcome as a set of genes or proteins in BALF samples can do. For example, increase of BALF surfactant protein A may result from increase of the synthesis and/or release by secreting cells or by impaired clearance by alveolar macrophages, mucociliary transport, degradation, and absorption into the bloodstream. Increased synthesis and/or release are the most plausible mechanisms explaining BALF SP-A increase in patients with sarcoidosis [65]. The analysis of the BALF proteome may, thus be helpful to quantify the overall molecular changes associated with lung injuries but may also offer the possibility to directly monitor therapeutic interventions. A good example of this approach has been realised by Griese et al. [62] with cystic fibrosis patients. In this pathology, the chronic neutrophilic inflammation of the airways results in proteolytic degradation of lung tissue early in the course of the disease. This pilot study demonstrates that inhalation of α1 protease inhibitor is associated with biochemical changes consistent with reduced proteolysis of SP-A and other proteins.

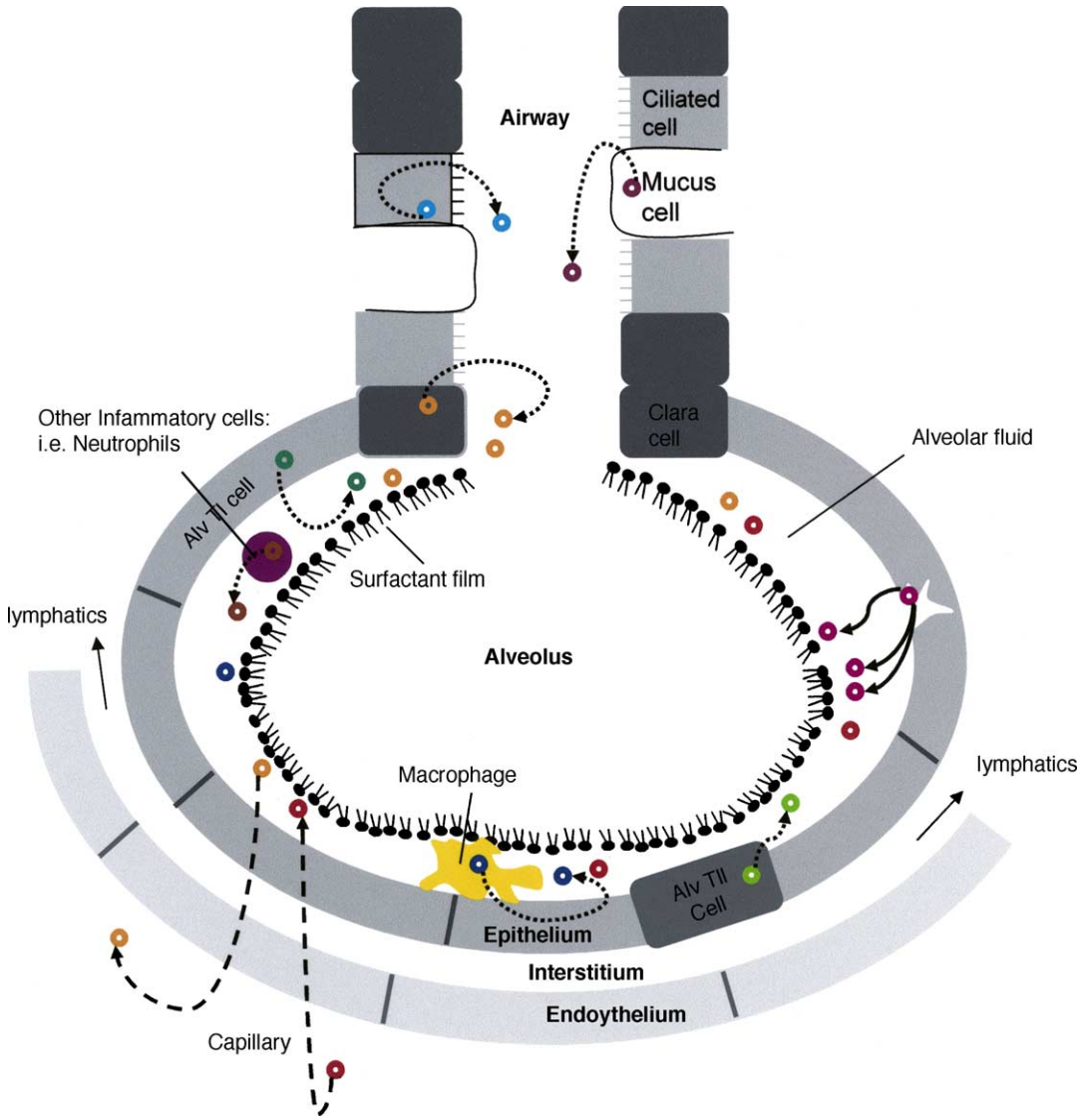

Fig. 3. Schematic diagram of the different origins of the proteins present in the bronchoalveolar lavage fluid.

3.3.3. Allergic asthma

A proteome approach of BALF has also been used to better understand disease mechanisms or patient susceptibility in the case of exposure to different environmental compounds. In this context, recent studies have investigated the changes in protein expression patterns that occur in lung tissues and airspaces following challenge using model animals (rat and mouse).

Allergic asthma is a chronic inflammatory disease involving a multitude of cell types. In asthma, reversible airway obstruction is characterised by airway inflammation and hyperresponsiveness, bronchoconstriction, increased mucus secretion and increase in lung vessels permeability [66,67]. Despite the great research efforts made, the underlying mechanisms for the development of the severe symptoms remain poorly defined. The first report on the asthmatic state of the human BAL and nasal lavage fluids proteomes has been presented by Lindhal et al. [42]. These authors show that the levels of the lipocalin-1, cystatin S, IgBF and transthyretin have changed in individuals that suffer from upper airway irritation or asthma.

A recent proteome study of samples from lung and BALF of a mouse model for allergic lung inflammation clearly shows an increase of different proteins during the allergen-induced inflammation. Many of these proteins are known to be associated with hypoxia and cell stress, indicating a link between allergen-induced inflammation and increases of hypoxia-related gene products [68]. In an other study, Signor et al. [69] evaluated the BALF proteome of rats treated with allergen (ovalbumin: OVA) or endotoxin (lipopolysaccharide: LPS). Their study shows that the BALF content of proteins derived from plasma or produced locally in the lung is significantly different in animals after allergen or endotoxin treatments compared to control (Fig. 5). Increase (T-kininogen I and II, α1-antitrypsin, calgranulin A, fetuin A and B, haptoglobulin) and decrease (clara cell

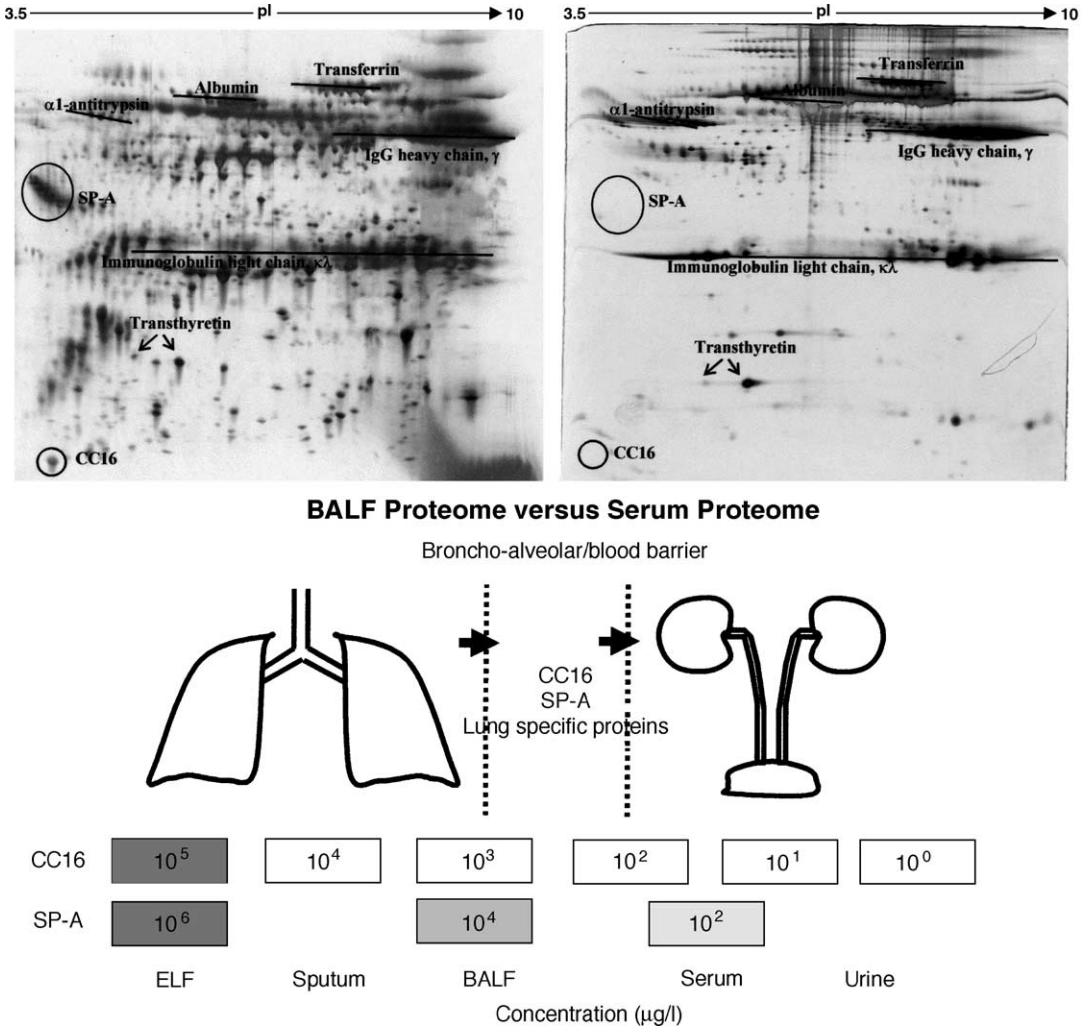

Fig. 4. Comparison between bronchoalveolar lavage fluid and plasma proteomes. Several proteins are present in BALF and not in plasma, suggesting that they are specifically produced in the airways. These proteins are, therefore, good candidates for becoming lung-specific biomarkers. Lung-specific proteins, such as surfactant protein A (SP-A) and clara cell protein 16 (CC16) are elevated in BALF proteome. Concentration of both proteins is indicated in different physiological fluids.

16 kDa secretory protein, pulmonary surfactant protein B) of proteins were observed in both treatments compared to control. In contrast, pulmonary surfactant associated protein A was decreased in the OVA-challenge and was not significantly affected in the LPS-challenge. Clearly, these results obtained on animal models are essential for the identification of potential inflammation biomarkers and for a better understanding of the mechanisms involved in the pathogenesis of lung inflammatory diseases.

3.3.4. Post-translational protein modifications

Most important, the proteomic approach allows the detection of post-translational modifications of BALF proteins [70] that could be associated with lung injuries. Clearly, the identification of these modifications of BALF proteins could contribute to understand of the mechanistic basis of lung disease pathogenesis as demonstrated by the team of Griese and co-workers [71].

Surfactant protein D (SP-D) is a host defence molecule that has been shown to interact with cystic firosis (CF)-associated pathogens. Griese and co-workers [71] showed that SP-D is a target of proteases present in BALF of CF and concluded that host defence is probably impaired due to the proteolysis of SP-D, which event contributes to the suppurative lung disease in CF. Recently, unequivocal identification of modifications and degradation products of surfactant proteins has been facilitated by the high-resolution mass spectrometric proteome analysis using Fourier transform-ion cyclotron resonance mass spectrometry (FT–ICR MS) [72]. Isoforms of specific BALF proteins, such as SP-A have also been detected by use of immunoaffinity detection [61].

3.3.5. Susceptibility to oxidants

On the other hand, BAL fluid can be used to detect a specific set of proteins related to the susceptibility to toxic aggression leading to diseases. Oxidative stress is associated

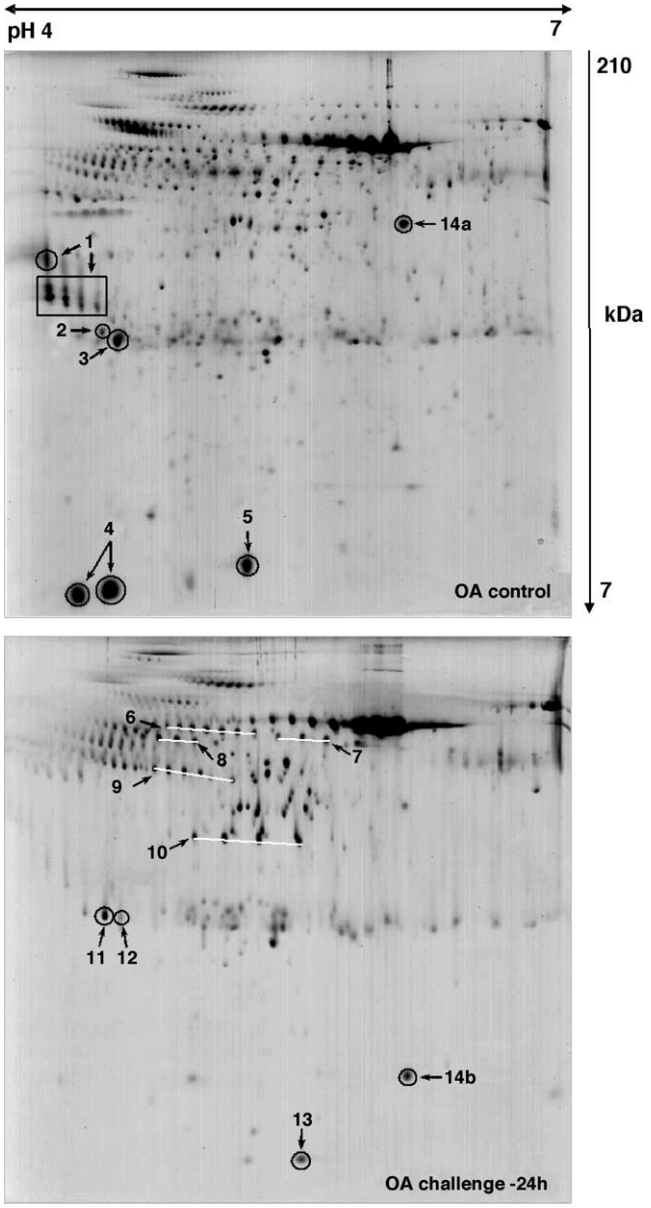

Fig. 5. Coomassie-stained 2-DE gels of rat BALF proteins from control animals and after OA challenge. The proteins that present changes are numbered. (1) SP-A; (2) c-reactive protein; (3) SP-A; (4) CC-16; (5) SP-B fragment; (6) T-kininogen I and II; (7) fetuin B; (8) fetuin A; (9) α1-antitrypsin; (10) haptoglobin; (11) c-reactive protein; (12) SP-A; (13) calgranulin A; (14a) Transthyretin, chain A (multimer); (14b) transthyretin (monomer) (Signor et al. [69], reprinted with permission from Wiley, VCH).

with a range of inflammatory lung diseases, including asthma. Previous studies have shown that the pulmonary response to an oxidant, such as ozone (O_3) greatly varies among strains of mice, but the factor(s) and the mechanism(s) responsible for this differential susceptibility have not yet been clearly identified. In this context, the susceptibility to oxidative stress of ozone-sensitive and resistant strains of mice has been studied by proteome analysis of BAL has been studied [73]. The exploration of the molecular bases for this differential O_3 susceptibility has been undertaken by the quantitative analysis of the expression of proteins associated with the ELF from two strains of mice, C57BL/6J and the C3H/HeJ, respectively, described as O_3-sensitive and O_3-resistant. Proteomic approach reveals two major differences between the BALF 2-DE protein maps obtained from C57BL/6J and C3H/HeJ strains. The lung is continuously exposed to oxidative stress and the authors suggest that antioxidant protein 2 (AOP2) and CC16 might participate in the protection of the pulmonary tract against O_3-induced lung injury [74].

4. Future directions of BALF proteome research

The studies presented in this review demonstrate that lung proteome can be approached by the analysis of clinical samples like serum, biopsies and BAL fluid. In particular, numerous recent works demonstrate the great interest of the proteome studies of BALF and are a reflect of the considerable technological progresses made in that field. Theses advances offer a better image of the proteome of the BALF and the function of these different constituents. In spite of the invasive character of the procedure needed to sample this fluid, BAL fluid represents an important source of new potential lung biomarkers. Most of the studies are already relevant mainly in the detection of new biomarkers of different lung alterations and in the better understanding of the mechanisms involved in the pathogenesis of different lung diseases.

To be most efficient, like the “plasma proteome project” recently initiated by the Human Proteome Organization (HUPO), BALF proteome research needs an increased international cooperation with the aim of promoting better rationalization of BALF proteomic studies. As for the plasma, even more efforts will be necessary to identify and mainly quantify minor proteins and their modifications. A better rationalization in the sample preparation and in the characterization of patients (age, sex, smoking or not smoking, status of lung pathologies . . .) for future differential-display proteome analysis also seems to be necessary. From the technological point of view, the recent developments of mass spectrometry mainly in protein profiling, and protein microarray coupled to the bioinformatics open new perspectives for the BALF researches and its clinical concern in the future.

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