Chapter 14
Proteomic Analysis of the Asthmatic Airway

John E. Wiktorowicz and Mohammad Jamaluddin

Abstract Proteomic investigations in general utilize varied technologies for sample preparation, separations, quantification, protein identification, and biological rationalization. Their applications range from pure discovery and mechanistic studies to biomarker discovery/verification/validation. In each specific case, the analytical strategy to be implemented is tailored to the type of sample that serves as the target of the investigations. Proteomic investigations take into consideration sample complexity, the cellular heterogeneity (particularly from tissues), the potential dynamic range of the protein and peptide abundance within the sample, the likelihood of posttranslational modifications (PTM), and other important factors that might influence the final output of the study. We describe the sample types typically used for proteomic investigations into the biology of asthma and review the most recent related publications with special attention to those that deal with the unique airway samples such as bronchoalveolar lavage fluids (BALF), epithelial lining fluid and cells (ELF), induced sputum (IS), and exhaled breath condensate (EBC). Finally, we describe the newest proteomics approaches to sample preparation of the unique airway samples, BALF and IS.

Keywords Proteomics • Sample prep • Size-exclusion chromatography • Asthma • Airway inflammation • Bronchoalveolar lavage • Epithelial lining fluid • Induced sputum • Exhaled breath condensate • Bronchoscopic microsampling

J.E. Wiktorowicz, Ph.D. (✉)
Department of Biochemistry & Molecular Biology, University of Texas Medical Branch, 2.208A Basic Science Bldg, 301 University Blvd, Galveston, TX 77555-0635, USA
e-mail: jowiktor@utmb.edu

M. Jamaluddin, Ph.D.
University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-1060, USA
e-mail: mjamalud@utmb.edu

A.R. Brasier (ed.), *Heterogeneity in Asthma*, Advances in Experimental Medicine and Biology 795, DOI 10.1007/978-1-4614-8603-9_14,

14.1 Introduction

Proteomic investigations utilize many technologies for sample preparation, separations, quantification, protein identification, and biological rationalization. Their applications range from pure discovery and mechanistic studies to biomarker discovery/verification/validation. In each case, the analytical strategy to be implemented is tailored to the type of sample that serves as the source of the investigations. In general, these may include tissues, cells (primary or continuous), or biofluids. Proteomic investigations take into consideration sample complexity, the cellular heterogeneity (particularly from tissues), the potential dynamic range of the protein and peptide abundance within the sample, the likelihood of posttranslational modifications (PTM), and other important factors that might influence the final output of the study.

Interestingly, proteomic samples types derived from the human airway encompass the entire range of sample types, from natural or derived biofluids such as induced sputum and bronchoalveolar lavage (BALF), respectively; to tissues such as bronchial biopsies; and primary cells such as airway epithelial, alveolar macrophages, and dendritic cells by sampling the epithelial lining fluid (ELF) or recovered from BALF. As a consequence, a review of proteomic technologies applied to airway investigations serves as a comprehensive survey of proteomic technologies in general.

Although the temptation is great to create a complete catalog of all the technologies historically applied to airway investigations, many recent reviews (>2009) address these issues to various degrees (Holguin and Fitzpatrick 2010; Lin et al. 2010; O'Neil et al. 2011a, b; Park and Rhim 2011; Wiktorowicz et al. 2011). We focus on the most recent examples of airway proteomic investigations and refer the reader to other reviews where appropriate. With regard to the most recent publications (>2009) of application of proteomics to asthma, studies are spread evenly between animals models (Haenen et al. 2010; Nagai et al. 2011; Quesada Calvo et al. 2011; Xu et al. 2010; Zhang et al. 2009), human patients (Cederfur et al. 2012; Fitzpatrick et al. 2009a, b; Gharib et al. 2011; O'Neil et al. 2011a, b), and tissue culture (Ali et al. 2011; Altraja et al. 2009, 2010; Cao et al. 2012; Kroeker et al. 2012), primary or continuous.

Airway sampling techniques include those that directly sample the mucosal surface, such as bronchial biopsy or bronchoscopic microsampling (BM) by brush biopsy, or those that indirectly sample the surface, such as bronchoalveolar lavage (BALF), induced sputum (IS), or exhaled breath condensate (EBC).

14.2 Sample Types, Collection, and Studies

14.2.1 Bronchoscopic Microsampling

Bronchial biopsy is an invasive procedure performed to collect a small amount of tissue from a specific region of the lung. This can include excising a tissue sample, or scraping epithelial or other cells that line the airway surfaces by brush biopsy.

In general, a trained pulmonologist performs this procedure using a bronchoscope that is threaded through right or left nostril into the trachea and bronchi of the anesthetized subjects. A small forceps is passed through the channel of the bronchoscope to excise tissues from the bronchi. Generally, multiple samples are collected and pooled for proteomic studies.

Bronchial tissue is an excellent material for unbiased (discovery) proteomic analysis for changes in protein expression in asthmatics. Most of the recent proteomic studies were performed with lung tissues from animal models or from primary tissue cultures, while one notable example utilized human bronchial biopsies. Recently, O'Neil et al. analyzed the global proteome of bronchial biopsies from normal and asthmatic subjects, treated either with an inhaled glucocorticoid steroid or placebo compared pair-wise pre- and posttreatment (O'Neil et al. 2011a, b). Using isobaric tag technology (iTRAQ) and 2D LC-MS/MS to quantify peptides, they identified from ~1,200 to ~1,700 proteins in total. For pre- and postplacebo-treated asthmatics, 115 proteins were analyzed and 3 were identified as statistically significant. In a pair-wise comparison of pre- and poststeroid-treated asthmatics, ~1,200 to ~1,400 proteins were quantified, with 141 analyzed statistically after steroid treatment, with 7 judged significantly changed. The highest scoring pathways by Ingenuity pathway analysis (IPA) for this cohort included prothrombin activation, actin motility, and actin signaling. Although demonstrating eigenvalues below that generally considered compelling (<38 % for components 1 and 2), principal component analysis (PCA) demonstrated tight clustering of healthy controls, with greater scatter of asthmatics, regardless of treatment. This is not only surprising, since asthma is generally recognized as a heterogeneous disease (Moore et al. 2010), but also spotlighting the small sample size of the study (22 patients in total). Nevertheless, this study shows that a small bronchial biopsy sample can be utilized for proteomic analysis and relevant information on the functional biological pathways involved in asthma pathogenesis can be obtained. In addition, the therapeutic effect of glucocorticoid steroids on the proteome level can be analyzed. The major challenge to proteomic analysis of bronchial biopsy samples is the lack of sufficient patient volunteers.

14.2.2 Epithelial Cells and Epithelial Lining Fluid

The airway epithelium constitutes the largest surface area in the human lung and its role in asthma pathogenesis has long been investigated. As epithelial cells and other cells that typically inhabit this environment are sentinels of the airway, it is well recognized that they play an important role in initiating and maintaining asthma pathogenesis by secreting a variety of inflammatory mediators in response to various insults such as allergens and noxious chemicals (Lim et al. 2004). Epithelial lining fluid (ELF), which forms a thin aqueous layer on the bronchial and alveolar epithelial cells, is a suitable material for proteomics as it contains many proteins that are believed to be involved in epithelial functions in normal and diseased state. ELF functions as a protective barrier between epithelium and external environment, and

accumulation of cellular products occurs in ELF during epithelial activation or injury by extracellular or intracellular factors.

ELF is obtained using a bronchoscopic microprobe containing an adsorptive tip that is lowered into the trachea until the lining is contacted (Franciosi et al. 2011). Fluid and cells thus obtained are recovered from the tip without dilution and analyzed. For proteomics studies, ELF has many advantages over BALF and induced sputum (IS). Unlike BALF, it is obtained undiluted and can be collected from the site of disease area without contamination with bacteria and other body fluids. One proof-of-concept study with ELF from rabbits identified 43 proteins, many of which have been previously reported as associated with lung disease (Kipnis et al. 2008). In addition, a recent study indicated that children with persistent bronchial asthma have greater level of nitric oxide oxidation products in ELF (Fitzpatrick et al. 2009a, b). Recently, Altraja et al. have studied the effect of the proinflammatory mediator, leukotriene E_4 (LTE_4), and remodeling factor, TGF-β_1, on the bronchial epithelial cell proteome as these mediators function in an autocrine fashion (Altraja et al. 2009). BEAS-2B culture cells were separated by 2D electrophoresis (2-DE), quantified, and proteins of interest excised and digested, and identified by MALDI-TOF MS. LTE_4 treatment downregulated β_1-tubulin (a marker of ciliated cells), HSP90α, and stress-70 protein, while TGF-β_1 upregulated antioxidant enzyme superoxide dismutase 1, profibrotic enzyme, protein disulfide-isomerase, and heat shock 70-kDa protein 9B, and downregulated lamin A/C, GAPDH, oncogene DJ-1, hnRNP A2/B1, and stress-70 protein.

Of particular interest is HSP90α, which is linked to the glucocorticoid receptor (GR) in a functional complex and is also downregulated by LTE_4 in this study. The authors make the case that HSP90α (α chaperonin) is known to play a special role in regulating the activity of GR, as well as the synthesis of prostaglandin E_2 (PGE_2), and that its downregulation in response to LTE_4 may cause inactivation of GR in bronchial epithelial cells as well as diminishing the bronchoprotective effect of PGE_2. This study clearly indicated that a proteomics approach can identify those proteins that play important roles in epithelial remodeling and illuminate airway pathology.

In a targeted approach to assess the response to increased formation of reactive oxygen species of patients with severe asthma, Fitzpatrick et al. (2009a, b) examined ELF from control and severely asthmatic children measuring reduced and oxidized glutathione (GSH and GSSG, respectively), as well as GSH reductase, GSH-S-transferase, and other indicators of oxidative stress. In comparison to carefully selected controls, children with severe asthma demonstrated lower GSH, increased GSSG, but no change in the glutathione cycle enzymes. In addition, increased malondialdehyde, 8-isoprostane, and hydrogen peroxide were observed in severe asthmatics.

These results were further analyzed using univariate logistic and linear regression analyses to determine the association between altered GSH:GSSG and clinical markers of asthma severity. Hospitalization (<1 year), prednisone use and dose, fraction of exhaled NO (F_{eno}), baseline FEV_1 and FEV_1 bronchodilator reversibility were tested as dependent variables against GSSG as a predictor. Of these dependent variables,

hospitalization ($p=0.001$) and baseline F_{eno} ($p=0.32$) were found to be significantly predicted by GSSG percentage. With these results, the authors conclude that severe asthmatic children exhibit increased biomarkers of oxidative stress (increased GSSG, malondialdehyde, isoprostane, and hydrogen peroxide) in their ELF and that interventions that increase GSH in the ELF might serve as effective treatments.

14.2.3 Bronchoalveolar Lavage Fluids

Historically instillation and recovery of bronchoalveolar lavage fluid (BALF) is the oldest technique commonly used to sample ELF and cells from the airway surfaces, and its analysis in human airway disease has been reviewed extensively since 2009 (Lin et al. 2010; Rottoli et al. 2009; Simpson et al. 2009; Vento et al. 2010). BALF is ideal in many respects for proteomic study, in that it contains different immune and nonimmune cells (alveolar macrophages, lymphocytes, neutrophils, eosinophils, squamous epithelial cells, bronchial epithelial cells, type II pneumocytes, basophils, and mast cells) and different soluble components (proteins, lipids, and nucleic acids).

Some of the soluble components are in common with plasma due in part to leakage from the plasma, and therefore the much discussed use of plasma as a sample source for proteomics studies enumerates several challenges likewise for BALF. Among these are the 10–12 orders of dynamic range in protein and peptide abundance; the interference of high-abundance proteins in the detection and quantification of the often more informative mid- and low-abundance proteins, and the tendency of these high-abundance proteins to absorb the mid- and low-abundance proteins (Gundry et al. 2007, 2009; Seferovic et al. 2008). Finally, since 12 % of serum peptides are naturally occurring (Richter et al. 1999) (i.e., not a result of artifactual proteolysis), the need to discriminate them from artifact requires preseparation fractionation (Brasier et al. 2012a; Wiktorowicz et al. 2011).

Using conventional techniques, different components of BALF can be separated and utilized for a comprehensive asthma proteomic study. In the last two decades, many proteomic analyses of BALF from healthy control and asthma patients have been conducted and databases of BALF proteins constructed (Li et al. 2009; Noel-Georis et al. 2002). However, most of the proteins identified have not attained the status of asthma biomarkers due to lack of verification by alternate methods. As BALF is a mixture of cells, and plasma proteins, lipids, nucleic acids, and salts, its use for proteomic analysis requires careful consideration of sample prep treatments. We have developed an approach that minimizes the confounding tendency of high-abundance proteins to adsorb lower-abundance proteins, allowing plasma protein immunodepletion strategies without loss of informative proteins, and provides reproducibly generated protein and peptide pools from the same biofluid sample, permitting the discrimination between natural and artifactual peptides. This approach has been in extensive use in our NIAID funded Clinical Proteomics Center for Infectious Disease and Biodefense and is described below in detail (Brasier et al. 2012a).

In a more targeted approach, Cederfur et al. have studied the glycoproteomic profile of BALF from asthmatics using galectins (small carbohydrate-binding proteins) and identified about 160 galectin-enriched proteins including several surfactant proteins (SP-A2, PIGR, and SP-B) (Cederfur et al. 2012). This study indicated that galectins, which are implicated in inflammatory lung diseases, can be used to dissect the lung glycoprotein dynamics in asthmatics.

In another targeted approach, a global feasibility study of the BALF phosphoproteome was performed to begin to assay the role of PTMs in biofluids (Giorgianni et al. 2012). While not the first to establish the importance of PTMs in biomarker discovery of biofluids (Brasier et al. 2012a), this study is the first to examine the presence of phosphorylated proteins in BALF. The authors enriched their sample by immobilized metal affinity chromatography (IMAC), followed by LC-MS/MS. From their analysis of normal subjects, they were able to identify 36 phosphopeptides containing 26 different phosphorylation sites, which were mapped to 21 phosphoproteins. Among these are included vimentin, plastin-2, ferritin heavy chain, kininogen-1, filamin, and, notably with regard to LTE_4 downregulation of GR discussed above, HSP90α and β. Many of the proteins identified have precedence in the literature of lung perturbations due to disease, smoking, and other environmental stresses.

Clearly, although the perfect approach to analyzing the proteome of BALF remains to be established, the value of such analyses in illuminating functional pathways and in generating candidate biomarkers is well worth the effort, with an intriguing future.

14.2.4 Induced Sputum

While BALF represents a diluted sample of the ELF, induced sputum samples the secretions of lung airways. Sputum induction is used for the noninvasive assessment of chronic inflammatory lung diseases such as asthma, COPD, and cystic fibrosis. Sputum is induced by delivering nebulized saline (3 % saline) to the lung, which irritates central airways and results in coughing. The major components of induced sputum are cells and fluids rich in many proteins. Apart from its diagnostic application, in recent years induced sputum has received a considerable attention for its use in biomarker discovery through global proteomic investigations in lung disorders (for recent review, see ref. Nicholas and Djukanović 2009). Since 2009, there have been a few proteomic studies using IS to identify new biomarkers of asthma and COPD (Gharib et al. 2011; Terracciano et al. 2011).

Recently, Gharib et al. (2011) reported identification and quantification of protein abundance using a shotgun label-free proteomic approach of asthmatic and healthy IS, followed by functional annotation by gene ontology and pathway analysis by IPA. In all, 254 proteins were identified in healthy control and asthmatic patients. Of these 17 proteins S100A8, S100A9, SCGB1A1, and SMR3B were

reduced, while serpin A1 was elevated in asthmatics versus controls and permitted "robust" classification of healthy controls from asthmatic patients. Western blots confirmed the increase of serpin A1, and decreases in SCGB1A1 and SMR3B in asthmatics relative to controls. The top functional categories mapped to these proteins include defense response, protease inhibitor activity, immunity, inflammation and wound response, and complement activation. However, in attempting to extract principle components representing of protein abundance variation of asthma and control samples by PCA, the data did not exhibit any significant clustering that corresponded to the clinical phenotypes. As with all proteomic strategies with limited fractionation prior to MS, the authors observe that their shotgun approach is biased toward detection of larger, more abundant proteins.

In a more recent study, the peptidome of asthmatics was compared to patients suffering from COPD by fractionating and extracting peptides from mesoporous silica beads with variable selectivity coupled with MALDI-TOF MS (Terracciano et al. 2011). Because these beads are rationally designed nanoporous network, the smallest of which has a pore diameter of 2.7 nm, only peptides of <4,500 were detected. With this fractionation/MALDI-TOF approach—sharing striking similarities to surface-enhanced laser desorption ionization (SELDI)—the authors report detection of >400 peptides. Comparison of healthy, asthmatic, and COPD ISs yielded 6 m/z peaks corresponding to three α-defensins, and three peaks of unknown identity that allowed discrimination between the three states by virtue of t-test statistics ($p \leq 0.05$).

14.2.5 Exhaled Breath Condensate

Exhaled breath condensate (EBC) is relatively a new and simple airway sampling approach that has been applied in the study of asthma and COPD, with the attraction of its noninvasiveness and risk-free collection. Because of these features, samples can be collected from a wide range of subjects, from youth to the elderly. EBC is composed of water-saturated aerosols from the airway lining fluid (ELF) containing water soluble gases, leukotrienes, isoprostanes, hydrogen peroxide, and proteins (Montuschi 2007). It is typically collected when subjects breathe into a mouthpiece through a one-way valve connected to a cooled condensing collection device. This condensed liquid may then be recovered for analysis. Although simple in concept and in operation, however, unless saliva is simultaneously collected for comparison and detected proteins that are in common ignored, the final analysis may be confounded by this contamination.

In a relatively large-scale study of healthy ($n = 30$) and asthmatic ($n = 40$) children, EBCs samples were concentrated, trypsin digested, separated by reversed-phase LC, and peptides quantified by MALDI-TOF/TOF. Saliva contamination was judged by the presence of salivary α-amylase, and those samples were removed from the analytical pool. Support vector machine analysis was performed to identify

the minimal model necessary to correctly classify 100 % of the subjects, regardless of their status. Ten peptides were identified—five more abundant in the control and five in the patient group. Upon validation with new samples, only eight peptides were detected with a classification accuracy of 80 %. Despite the promise of the first relatively successful proteomics study of EBCs, a major fault in this study was the inability to identify any of these peptides due to their low abundance. Nevertheless, this preliminary study presages successful use of EBCs for proteomic analysis, one without patient discomfort or risk.

14.3 Analytical Procedures

Proteomic analysis entails several steps and the technologies employed vary according to sample types. As indicated in Sects. 14.2 above and 14.3.1 below, biofluids require special processing compared to tissue or cellular material. In general, separations may involve HPLC using reversed phase (RP) chromatography; two dimensional chromatography for greater depth of inquiry with tandem RP and strong cation exchange (SCIEX) supports; one dimensional, size-based electrophoresis with SDS; two-dimensional electrophoresis with isoelectric focusing (IEF), followed by size-based electrophoresis; and preparative electrophoresis, based on solution IEF, or size fractionation (for recent review, see Wiktorowicz et al. 2011). An extensive survey of the general analytical procedures used in proteomics is beyond the scope of this chapter, and the reader is referred to recently published books on proteomic technologies (Lovric 2011; Mishra 2010).

This section, however, focuses on sample preparation techniques that are uniquely applicable to the airway samples, namely BALF and induced sputum.

14.3.1 BALF

In many respects, BALF is similar to plasma since plasma leakage occurs through airway tissues during injury, inflammation, and disease. For proteomic investigations using BALF, this means that similar challenges exist as those using plasma (or serum).

Since targets for proteomic analyses may be proteins, peptides, or both—and they may be complexed with other more abundant, but less informative molecules—one strategy is to create individual protein and peptide pools from the same sample for subsequent separation and analysis by separation of urea-denatured BALF by size-exclusion chromatography (SEC) (Brasier et al. 2012a). Furthermore, since native peptides constitute about 12 % of the plasma "peptidome" (Richter et al. 1999), this approach permits discrimination between derived peptides (peptides

derived from proteolysis of circulating proteins) and native peptides—since native peptides will be uniquely present in the peptide pool, whereas derived peptides will also appear as part of a protein in the protein pool. Since samples to be compared cannot be multiplexed, reproducible fractionation and creation of protein and peptide pools can be accomplished by incorporating a fluorescently labeled internal standard (e.g., Alexa-488 thaumatin—MW = 23 kDa) into the sample, with SEC systems equipped with UV monitors that electronically trigger fraction collection upon detection of the internal standard. This fractionation-pooling approach uniquely sets the stage for the discovery of higher quality candidate biomarkers, where it has been used extensively in the NIAID Clinical Proteomics Center for Infectious Disease and Biodefense at UTMB (Brasier et al. 2012a, b).

After antibody depletion of the 14 most highly abundant proteins, the remaining proteins are then saturation fluorescence labeled (Pretzer and Wiktorowicz 2008; Tyagarajan et al. 2003) to yield highly quantitative and accurate protein abundance estimates (CV < 0.08), separated and quantified by 2-DE, and the picked proteins (abundance ratio $\geq|1.25|$, $p < 0.05$) identified *via* LC-MS/MS as described above.

The peptide pools to be compared are individually labeled with $^{16/18}O$ by trypsin-mediated exchange in the presence of heavy (control) or light (case) water for 24 h. Peptides are quantified and identified by a nanoLC tandem electrospray MS/MS. As stated previously, the identities of the proteins and peptides in the pools are then compared to eliminate peptides from the candidate status by their coincident appearance of their precursor proteins in the protein pool.

Procedure: Alexa-488 thaumatin is produced by mixing 200 μL 8 M urea–Tris buffer (pH 7.5) with 50 μg thaumatin (Sigma-Aldrich, St. Louis, MO). To this is added 0.8 μL TCEP (Sigma–Aldrich) and the mixture incubated for 30 min at room temperature. The whole solution is then added to a tube with ~0.34 μmol dried Alexa-488 (Invitrogen, Carlsbad, CA) and incubated in the dark at room temperature for 120 min. The reaction is quenched by adding 1.2 μL of 2-mercaptoethanol and incubated for 30 min at room temperature. After aliquoting the solution into microcentrifuge tubes, the labeled protein is stored at −80 °C until needed.

To 100 μL of concentrated BALF containing at least 200 μg of protein is added enough urea to make an 8 M solution. Also added is 50 μg of Alexa-thaumatin. This solution is pumped at a flow rate of ~20 mL/h into a 1.5 × 30 cm column packed with Superdex75™ (GE Healthcare, Piscataway, NJ) for which the void and exclusion volumes have been determined (GE Healthcare mfg recommendations). After the free dye has eluted, the fractions after the void volume to the end of the first thaumatin peak (~17 kDa) are pooled and termed the "protein pool." Fractions from the end of the thaumatin peak to the beginning of the free dye peak are pooled and termed the "peptide pool."

The protein pools may be analyzed by conventional 2D electrophoresis, while the peptide pools by either label-free LC-MS/MS or stable isotope labeled and analyzed by LC-MS/MS.

14.3.2 IS

Extraction of proteins or peptides from induced sputum is complicated by the glycoproteins MUC5B and MUC5AC, which impart viscosity to mucin. Most protocols decrease the viscosity by treatment with disulfide reducing agents and centrifugation to separate associated cells. Supernatants are then analyzed by conventional separations and MS.

Procedure: As a recently published example of a typical approach (Terracciano et al. 2011), IS samples were treated with PBS containing 5 mM DTT. After addition of a protease inhibitor cocktail at 22.5 mL/g of sputum, the samples were incubated for 30 min at room temperature. The samples were then filtered through a 70-μm mesh filter and centrifuged at 400×*g* for 10 min at 4 °C to pellet the cells. The supernatants were removed and recentrifuged at 12,000×*g* for 10 min at 4 °C, before being aliquoted and stored at −80 °C until needed for analysis.

14.4 Future Directions

Because of its widespread use and ease of administration and recovery, BALF provides the lion's share of samples for the study of asthma classifiers. However, since BALF samples the ELF, whose volume may be variable due to inflammation or other factors, dilution of ELF constituents may confound quantitative comparisons of BALF. A means for normalization is needed, and one might envision combining ELF microprobe estimation of ELF volume (Franciosi et al. 2011) accompanied by measurement of total protein. This value could then be used to normalize the protein constituents recovered in BALF from the same patient.

More generally, discovery proteomics remains a technology-driven discipline, whose promise is based on the ability of academic and industry researchers to address its very real and daunting challenges. In comparison to the genome, where the complexity largely understood, the complexity of the proteome—due to alternative splicing, posttranscriptional, and translational modifications—present in even a single cell type is still subject to speculation. Moreover, the challenge is further exacerbated by the enormous dynamic range of protein concentrations in biological samples, and not the least of which is requirement for high sensitivity to permit detection of hundreds, if not tens of copies of a given protein per cell from small numbers of cells (<10,000), to name a few of the pressing challenges. So, while great progress has been made, there is still a long way to go.

Nevertheless, promising technologies that exploit new developments in the fields of microfluidics, nanotechnology, and chemistry fuel the optimism that proteomics research will yield breakthroughs in therapeutic discovery and translational medicine of this heterogeneous disorder.

References

Ali M, Lillehoj EP, Park Y et al (2011) Analysis of the proteome of human airway epithelial secretions. Proteome Sci 9:4

Altraja S, Jaama J, Valk E et al (2009) Changes in the proteome of human bronchial epithelial cells following stimulation with leucotriene E4 and transforming growth factor-beta1. Respirology 14:39–45

Altraja S, Jaama J, Altraja A (2010) Proteome changes of human bronchial epithelial cells in response to pro-inflammatory mediator leukotriene E4 and pro-remodelling factor TGF-beta1. J Proteomics 73:1230–1240

Brasier AR, Garcia J, Wiktorowicz JE et al (2012a) Discovery proteomics and nonparametric modeling pipeline in the development of a candidate biomarker panel for dengue hemorrhagic fever. Clin Transl Sci 5:8–20

Brasier AR, Ju H, Garcia J et al (2012b) A three-component biomarker panel for prediction of dengue hemorrhagic fever. Am J Trop Med Hyg 86:341–348

Cao R, Wang TT, DeMaria G et al (2012) Mapping the protein domain structures of the respiratory mucins: a mucin proteome coverage study. J Proteome Res 11:4013–4023

Cederfur C, Malmstrom J, Nihlberg K et al (2012) Glycoproteomic identification of galectin-3 and -8 ligands in bronchoalveolar lavage of mild asthmatics and healthy subjects. Biochim Biophys Acta 1820:1429–1436

Fitzpatrick AM, Brown LA, Holguin F et al (2009a) Levels of nitric oxide oxidation products are increased in the epithelial lining fluid of children with persistent asthma. J Allergy Clin Immunol 124:990–996, e1–9

Fitzpatrick AM, Teague WG, Holguin F et al (2009b) Airway glutathione homeostasis is altered in children with severe asthma: evidence for oxidant stress. J Allergy Clin Immunol 123:146–152, e8

Franciosi L, Govorukhina N, Ten Hacken N et al (2011) Proteomics of epithelial lining fluid obtained by bronchoscopic microprobe sampling. Methods Mol Biol 790:17–28

Gharib SA, Nguyen EV, Lai Y et al (2011) Induced sputum proteome in healthy subjects and asthmatic patients. J Allergy Clin Immunol 128:1176–1184, e6

Giorgianni F, Mileo V, Desiderio DM et al (2012) Characterization of the phosphoproteome in human bronchoalveolar lavage fluid. Int J Proteomics 2012:460261

Gundry RL, Fu Q, Jelinek CA et al (2007) Investigation of an albumin-enriched fraction of human serum and its albuminome. Proteomics Clin Appl 1:73–88

Gundry RL, White MY, Nogee J et al (2009) Assessment of albumin removal from an immunoaffinity spin column: critical implications for proteomic examination of the albuminome and albumin-depleted samples. Proteomics 9:2021–2028

Haenen S, Vanoirbeek JA, De Vooght V et al (2010) Proteome analysis of multiple compartments in a mouse model of chemical-induced asthma. J Proteome Res 9:5868–5876

Holguin F, Fitzpatrick A (2010) Obesity, asthma, and oxidative stress. J Appl Physiol 108:754–759

Kipnis E, Hansen K, Sawa T et al (2008) Proteomic analysis of undiluted lung epithelial lining fluid. Chest 134:338–345

Kroeker AL, Ezzati P, Halayko AJ et al (2012) Response of primary human airway epithelial cells to influenza infection: a quantitative proteomic study. J Proteome Res 11:4132–4146

Li SJ, Peng M, Li H et al (2009) Sys-BodyFluid: a systematical database for human body fluid proteome research. Nucleic Acids Res 37:D907–D912

Lim JR, Gupta SK, Croffie JM et al (2004) White specks in the esophageal mucosa: an endoscopic manifestation of non-reflux eosinophilic esophagitis in children. Gastrointest Endosc 59:835–838

Lin JL, Bonnichsen MH, Nogeh EU et al (2010) Proteomics in detection and monitoring of asthma and smoking-related lung diseases. Expert Rev Proteomics 7:361–372

Lovric J (2011) Introducing proteomics: from concepts to sample separation, mass spectrometry, and data analysis. Wiley, West Sussex
Mishra NC (2010) Introduction to proteomics: principles and applications. Wiley, Hoboken, NJ
Montuschi P (2007) Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications. Ther Adv Respir Dis 1:5–23
Moore WC, Meyers DA, Wenzel SE et al (2010) Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 181:315–323
Nagai T, Nakao M, Shimizu Y et al (2011) Proteomic analysis of anti-inflammatory effects of a kampo (Japanese Herbal) medicine "Shoseiryuto (Xiao-Qing-Long-Tang)" on airway inflammation in a mouse model. Evidence-based complementary and alternative medicine. eCAM 2011:604196
Nicholas B, Djukanović R (2009) Induced sputum: a window to lung pathology. Biochem Soc Trans 037:868–872
Noel-Georis I, Bernard A, Falmagne P et al (2002) Database of bronchoalveolar lavage fluid proteins. J Chromatogr B 771:221–236
O'Neil SE, Lundback B, Lotvall J (2011a) Proteomics in asthma and COPD phenotypes and endotypes for biomarker discovery and improved understanding of disease entities. J Proteomics 75:192–201
O'Neil SE, Sitkauskiene B, Babusyte A et al (2011b) Network analysis of quantitative proteomics on asthmatic bronchi: effects of inhaled glucocorticoid treatment. Respir Res 12:124
Park CS, Rhim T (2011) Application of proteomics in asthma research. Expert Rev Proteomics 8:221–230
Pretzer E, Wiktorowicz JE (2008) Saturation fluorescence labeling of proteins for proteomic analyses. Anal Biochem 374:250–262
Quesada Calvo F, Fillet M, Renaut J et al (2011) Potential therapeutic target discovery by 2D-DIGE proteomic analysis in mouse models of asthma. J Proteome Res 10:4291–4301
Richter R, Schulz-Knappe P, Schrader M et al (1999) Composition of the peptide fraction in human blood plasma: database of circulating human peptides. J Chromatogr B Biomed Sci Appl 726:25–35
Rottoli P, Bargagli E, Landi C et al (2009) Proteomic analysis in interstitial lung diseases: a review. Curr Opin Pulm Med 15:470–478
Seferovic MD, Krughkov V, Pinto D et al (2008) Quantitative 2-D gel electrophoresis-based expression proteomics of albumin and IgG immunodepleted plasma. J Chromatogr B Analyt Technol Biomed Life Sci 865:147–152
Simpson RJ, Lim JW, Moritz RL et al (2009) Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics 6:267–283
Terracciano R, Preiano M, Palladino GP et al (2011) Peptidome profiling of induced sputum by mesoporous silica beads and MALDI-TOF MS for non-invasive biomarker discovery of chronic inflammatory lung diseases. Proteomics 11:3402–3414
Tyagarajan K, Pretzer EL, Wiktorowicz JE (2003) Thiol-reactive dyes for fluorescence labeling of proteomic samples. Electrophoresis 24:2348–2358
Vento G, Tirone C, Aurilia C et al (2010) Proteomics and neonatal infection. Minerva Pediatr 62:47–49
Wiktorowicz JE, Soman K, Haag A (2011) Discovery strategies for proteomic profiling of airway diseases. Curr Proteomics 8:97–110
Xu YD, Cui JM, Wang Y et al (2010) The early asthmatic response is associated with glycolysis, calcium binding and mitochondria activity as revealed by proteomic analysis in rats. Respir Res 11:107
Zhang L, Wang M, Kang X et al (2009) Oxidative stress and asthma: proteome analysis of chitinase-like proteins and FIZZ1 in lung tissue and bronchoalveolar lavage fluid. J Proteome Res 8:1631–1638