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Review

Integrating *Omics* Technologies to Study Pulmonary Physiology and Pathology at the Systems Level

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Key Words
Genomics • Proteomics • RNA-Seq • ChIP-Seq • Epigenomics • Systems Biology • Network Biology • Interactomes • Transcriptomes • Diseasosomes

Abstract
Assimilation and integration of "omics" technologies, including genomics, epigenomics, proteomics, and metabolomics has readily altered the landscape of medical research in the last decade. The vast and complex nature of omics data can only be interpreted by linking molecular information at the organismic level, forming the foundation of systems biology. Research in pulmonary biology/medicine has necessitated integration of omics, network, systems and computational biology data to differentially diagnose, interpret, and prognosticate pulmonary diseases, facilitating improvement in therapy and treatment modalities. This review describes how to leverage this emerging technology in understanding pulmonary diseases at the systems level –called a "systomic" approach. Considering the operational wholeness of cellular and organ systems, diseased genome, proteome, and the metabolome needs to be conceptualized at the systems level to understand disease pathogenesis and progression. Currently available omics technology and resources require a certain degree of training and proficiency in addition to dedicated hardware and applications, making them relatively less user friendly for the pulmonary biologist and clinicians. Herein, we discuss the various strategies, computational tools and approaches required to study pulmonary diseases at the systems level for biomedical scientists and clinical researchers.

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Introduction

Biological systems function as a network of interconnected and mutually dependent components, constituting the unified whole. Completion of the Human Genome Project reinforced this idea and signaled a paradigm shift in analyzing biological phenomena, introducing a holistic approach of systems biology a decade ago [1-6]. Increasingly, technology provides omic scale data, while systems-level models and measurements predict and validate biological phenomena [3, 7]. Thus, the pervasive nature of systems biology in molecular diagnostics, genetic and proteomic mapping of diseases is well recognized [7].

Systems biology integrates data from multiple sources into predictive molecular models analogous to the study of organ pathophysiology, including the lung [8]. Lung cancer, chronic obstructive pulmonary disease (COPD), asthma and idiopathic pulmonary fibrosis (IPF) are highly complex diseases with overlapping pathologies associated with lung remodeling and altered molecular and physiological functions. Genomics, epigenomics, proteomics and metabolomics are thus used to ascertain the molecular mechanisms underlying these functional alterations (Fig. 1). Attempts have been made to integrate data from these technologies using a systems biology approach in pulmonary medicine [8-10]. In the present review, we summarize and provide guidelines to integrate the different omics based technologies currently applied to pulmonary diseases. Detailed work flows and resources for analyzing data obtained from omics approaches provide an easy access and understanding for biologists and clinicians alike who are just entering in the field of systems biology.

Genomics & Epigenomics in Pulmonary Biology

Genomics

Large-scale genome sequencing and microarray projects have resulted in paradigm shifting approaches to understanding human diseases, including pulmonary disorders, as evidenced by a steady increase in publications (Fig. 2). Transcriptional profiles of disease phenotypes have contributed towards a better understanding of the molecular mechanisms driving pulmonary pathologies such as idiopathic pulmonary fibrosis (IPF), asthma, COPD, and pulmonary hypertension [11-18]. Currently established work flows for genomics experiments involve data generation, analysis and more recently systems level analysis (Fig. 3). Herein, we provide details of current and future technologies used in the area of genomics.

Microarray technology in pulmonary biology. DNA microarrays have defined genome-wide signatures of smoking and smoking cessation on bronchial and nasal epithelium [19-21], identifying novel molecules that serve as diagnostic markers in smokers with clinical suspicion of lung cancer [22, 23]. Genes regulating collagen synthesis/deposition were similarly defined for early detection of fibrotic changes in the lung [24, 25]. Likewise, prognostic gene signature during progression and resolution of acute lung injury (ALI) were identified from the microarray profiles of patient lungs [26, 27]. Metastasis signatures are similarly obtained from transcriptomic data on critical lung cancer genes [28-31]. A detailed account of genomics driven understanding of lung disorders has been published [32]. Genome wide association studies (GWAS) based on data from DNA microarray analysis have aided search for single-nucleotide polymorphisms (SNPs) within a population, which has successfully predicted pharmacogenetic factors associated with diseases like asthma and response to bronchodilators [33-37].

Microarray data analysis can be performed using a number of free and commercial tools (Table 1). Tools like Database for Annotation, Visualization and Integrated Discovery (DAVID) v.6.7 [38, 39], GenePattern 2.0 [40], Galaxy [41], Cytoscape [42], GenomeSpace (http://www.genomespace.org) and Bioconductor (http://www.bioconductor.org/) are free to use and have been routinely cited in published literature. Most cited commercial software tools include Ingenuity Pathway Analysis (IPA) (www.Ingenuity.com), GeneGO, MetaCore (http://

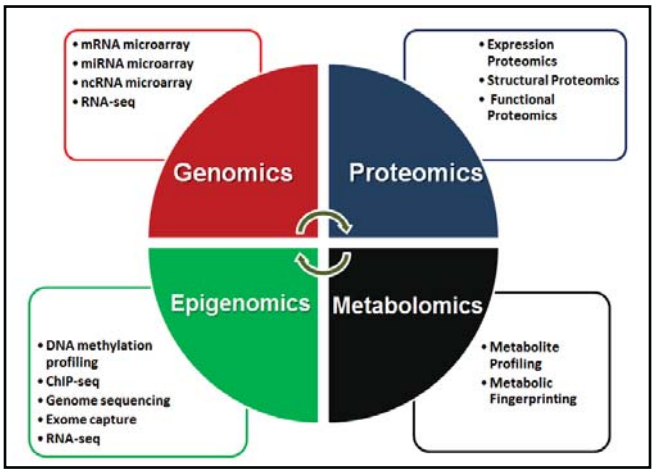

Fig. 1. Global screening strategies and techniques driving "omics" era. Genomics applications analyze mRNA, miRNA and other small RNA moieties, including lncRNA by microarray and RNA-seq technologies. These allow genome-wide interrogation of biological phenomena, as well as detect expression changes in the transcriptome. Proteomics approaches deal exclusively with changes at the proteome level. Existing proteomic techniques study structural and functional characteristics of proteins in addition to detecting changes in the global protein expression levels of an organism. In combination with genomics, proteomic analysis can offer detailed insights into the transcriptional and translational events occurring in a biological system. Detecting, identifying and quantifying small molecule metabolites that are central to living systems using metabolomic approaches is vital for a systems level understanding and can be integrated with other work flows. In order to truly understand gene regulation, analysis of epigenetic events that at the chromosomal level is indispensable. The integration of protocols epigenetic like DNA methylation and ChIP with genomic approaches like microarray and sequencing allow rapid and global analysis of the epigenome. Although, "omic" technologies are specific in terms of the processes they analyze, integration of one or more of these technologies is the cornerstone of systems approach to biological systems.

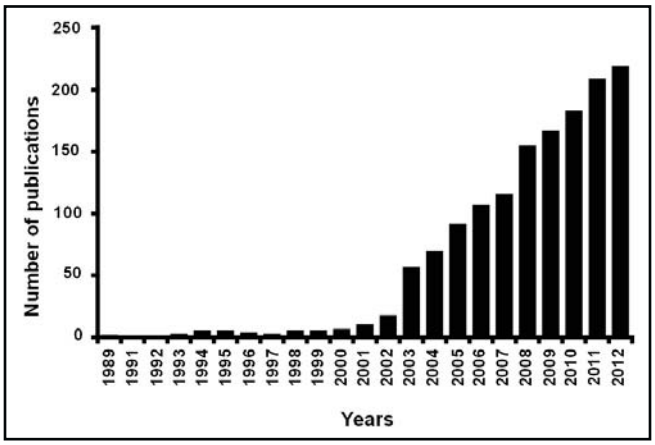

Fig. 2. Emergence of Genomics in pulmonary biology. PubMed search for the co-occurrence of the terms "Genomics and pulmonary biology" identified 800 publications featuring these key words in the last decade. Interestingly, this number reflects a 20-fold increase in the number of publications with similar key words from the previous decade. The dramatic increase can be attributed to the availability of cheaper technologies, highly developed databases and analysis platforms with user friendly interface.

portal.genego.com/) Partek 6.3 (www.partek.com). These enables biologists to discover visualize and explore therapeutically relevant transcriptional networks. Researchers must download, analyze and compare existing microarray datasets from public repositories like Gene Expression Omnibus [43, 44], ArrayExpress [45], and Stanford Microarray Database [46] to strengthen hypothesis and avoid redundant experimentation as shown by Sirota et al. [47], who created a set of 100 disease signatures from the Gene Expression Omnibus and identified many novel drug–disease gene pairs.

miRNA signature profiling in pulmonary diseases. A host of miRNAs have been identified as critical players in the development, progression and status of multiple lung pathologies [48-55] including in IPF [56, 57] and smokers lung [58]. These profiles are distinct from the miRNA profiles in chronic obstructive pulmonary disease (COPD), cancer, asthma and cardiovascular disease [59-65]. For example, targeting an miRNA let-7c has been proposed as it is a pathogenic link between COPD and lung cancer [66]. About 200 miRNAs are

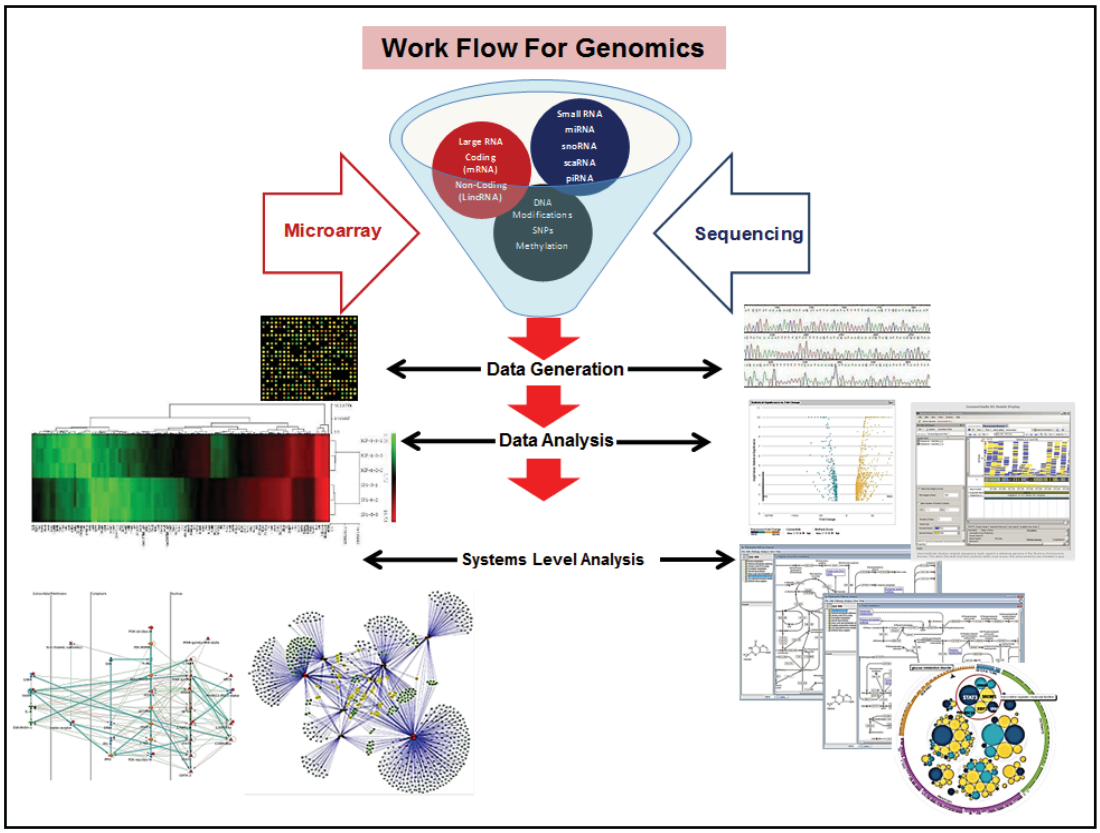

Fig. 3. Workflow for genomics data obtained from microarray and RNA-seq. RNA or DNA extracted from different sources (cells/tissues) is the starting point for conventional genomics work flow. RNA/DNA is used for microarray or RNA-seq analysis as per recommended protocols. These methods allow users to study large and small RNA populations as well as DNA modifications. The second step of genomic workflow comprises of retrieving data using different sample processing software that are unique to a given application. Standard outputs from the software comprise of signal intensity values (microarray), sequence reads (RNA-seq), annotations and accession identifiers for the different genes that are being studied. This information serves as the input for the third step of the workflow, which is data analysis. The raw data obtained has to converted into biologically relevant information like differential gene expression data (fold change values), sequence alignment files (RNA-seq) and clusters of similarly affected targets (hierarchical clustering and volcano plots). The last and most important step of genomic work flows is systems level analysis. Using the information generated from the data analysis step, users can derive additional details about the alterations in biological pathways, networks and diseases that can be correlated to the biological phenomena being studied.

reported to be altered in steroid naïve asthmatic subjects, thereby establishing a potential link between inflammation and aberrant miRNA expression in patients with asthma [67-69]. Altered miRNAs in IPF have been extensively studied in pathogenesis of IPF and pulmonary hypertension [70-72]. While such molecular information is routinely generated, the precise mechanisms and roles of these miRNAs can only be ascertained by a combination of systems biology driving traditional experimental approaches [73].

Indeed, systems level analyses using miRNA expression profiles of lung cancer and interstitial lung diseases (ILDs) are being used to understand pathogenesis and progression of pulmonary disorders [9, 10, 74-79]. A number of computationally and experimentally derived databases of miRNA have been published [80-92] and are listed in Table 2. Approaches combining high-throughput experimental data together with sequence-based putative miRNA predictions have also been successfully applied [92-96]. Users can retrieve

Table 1. Resources for processing and analyzing genomics data

	Software tools	Microarray Analysis
1.	(DAVID) v.6.7	http://david.abcc.ncifcrf.gov/
2.	GenePattern 2.0	http://www.broadinstitute.org/cancer/software/genepattern/
3.	Galaxy Project	http://galaxyproject.org/
4.	GenomeSpace	http://www.genomespace.org
5.	Bioconductor	http://www.bioconductor.org/
6.	Cytoscape	http://www.cytoscape.org/
7.	Ingenuity Pathways Analysis	www.Ingenuity.com
8.	GeneGO, MetaCore	http://portal.genego.com/
9.	Partek 6.3	www.partek.com
	Software tools	RNA-seq Analysis
1.	Galaxy Project	http://galaxyproject.org/
2.	GenePattern 2.0	http://www.broadinstitute.org/cancer/software/genepattern/
3.	R-workbench	http://www.ebi.ac.uk/Tools/rcloud/
4.	Palmapper	http://raetschlab.org/suppl/palmapper
5.	mTim	http://raetschlab.org/suppl/mtim
6.	rQuant	http://raetschlab.org/suppl/rquant
7.	rDiff	http://cbio.mskcc.org/public/raetschlab/user/drewe/rdiff/

Table 2. List of databases and tools for analyzing miRNA

Databases	Web Links
1. miRBase	http://www.mirbase.org/
2. miRNAmap	http://mirnamap.mbc.nctu.edu.tw/
3. miRWalk	http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/index.html
4. miRDB	http://mirdb.org/miRDB/
5. TarBase V.5c	http://diana.cslab.ece.ntua.gr/tarbase/
6. miRGen	http://www.diana.pcbi.upenn.edu/miRGen.html
7. miROrtho	http://cegg.unige.ch/mirortho
8. mir2disease	http://www.mir2disease.org/
9. miRanda	http://www.microrna.org/microrna/home.do
10.TargetScan 6.2	http://www.targetscan.org/
11.miRanda	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/
12.DIANAmicroT	www.microrna.gr/microT
13.PicTar	http://pictar.mdc-berlin.de/
14.miRecords	http://mirecords.umn.edu/miRecords/
15.mirTarBase	http://mirtarbase.mbc.nctu.edu.tw/
16.miRWalk	http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/
17.miRNAMap	http://mirnamap.mbc.nctu.edu.tw/

miRNA and mRNA gene expression profiles from NCI-60 (a set of 60 human cancer cell lines derived from diverse tissues) and use a tool, called CellMiner [97], to query these microarray datasets. Recently, transcriptome scale study on miRNA-lncRNA interactions have also added a new dimension to genomics analysis [98]. Such an approach has not been applied to study pulmonary disorders and will definitely contribute to a better understanding of disease regulation and progression. LncRNAs (long non coding RNAs) are a class of non-protein coding RNAs that interact with other classes of RNAs including mRNAs and miRNAs and modulate their regulatory role via physical interactions [99]. Some of the more recent findings on the role of LncRNA in lung cancer have been reviewed recently [100]. The availability of high density LncRNA microarrays and network driven systems approach [101, 102] for studying the role of LncRNAs in human diseases makes it a promising approach for pulmonary biologists.

RNA-seq: Next generation technology for studying pulmonary disorders. RNA-seq is superior to previously developed transcriptomic methods. Unlike microarrays, RNA-seq detects known and unknown transcripts with low signal/noise ratio and high degree of

Table 3. Protein-Protein Interactions Databases

Databases	Web Links
1.STRING 9.05	http://string-db.org
2.Molecular Interaction Database (MINT)	http://mint.bio.uniroma2.it/mint/Welcome.do
3.Database of Interacting Proteins (DIP)	http://dip.doe-mbi.ucla.edu/dip/Main.cgi
4.IntAct	http://www.ebi.ac.uk/intact/
5.HPRD	http://www.hprd.org/
6.BioGRID 3.2	http://thebiogrid.org/
7.PrePPT	http://bhapp.c2b2.columbia.edu/PrePPI/

sensitivity [103]. Recently, RNA-seq identified novel transcripts associated with smoking and lung cancer in small airway epithelium, and changes in RNA in peripheral lung in emphysema and IPF [104-107]. RNA-seq also identified novel and confirmative genes linked to asthma, indicating differences in biological processes in the airways of asthma patients [108]. In RNA-seq, differential gene expression data sets are analyzed by Software suites like GenePattern 2.0 [40], Galaxy [41] and Bioconductor [109]. The Bioconductor package ArrayExpressHTS [110] and R-workbench generates binary alignment (BAM) format [111], allowing users to process and analyze raw RNA-seq data. The Ensembl genome browser [112] can be used to display the BAM files containing the sequence alignment data. Currently ArrayExpress provides approximately 1200 BAM files for 125 RNA-seq experiments, from 14 different species [45]. Commercial software suites like NGS (Next Generation Sequencing; Genomatix) also provide a comprehensive promoter and transcript annotation database along with high end GUI (Graphic User Interface) for visualization and analysis of RNA-seq data (Table 1).

Epigenomics

Epigenetic alterations in idiopathic pulmonary fibrosis (IPF) and COPD have recently received considerable attention [113-120]. DNA-methylation profiles identified 870 genes and 625 CpG islands differentially methylated in IPF tissues [121, 122]. Surprisingly, a significant overlap was found between lung cancer and IPF, providing valuable insight into common pathways driving these two distinct diseases. Epigenetics approach also indentified changes in miRNAs and fibroblast signature for genes regulating extracellular matrix in IPF and lung cancer [123-125]. An epigenomic approach focusing on DNA methylation to identify potential biomarkers for lung cancer was recently published [125]. While methylation of DNA is associated with decreased gene expression, acetylation of histones results in relaxation of chromatin facilitating gene transcription. Therefore, studying histone modifications becomes paramount to understand regulation of pulmonary diseases, as elegantly demonstrated in a recent finding that implicates histone modifications in decreased Fas expression and apoptosis resistance in fibrotic lung fibroblasts [126]. Further, the identification of distinct posttranslational histone modification patterns in histone H3 and histone H4 in lung cells [127], which may be considered as usable biomarkers for Cigarette Smoke-induced chronic lung diseases, supports the importance of epigenomics in pulmonary disorders. A more comprehensive summary of the latest experimental and translational epigenetic studies in pulmonary disorders can be found elsewhere [128].

Epigenomic work flows for data generation and analysis are similar to genomic studies (Fig. 4). Repitools, a part of Bio-conductor ver. 2.12, is used for enrichment-based epigenomic data analyses. Standard features include summarization and visualization of epigenomic data across promoters in relation to gene expression context, detection of regions of differential methylation/binding and, options for quantifying methylation. Epigenomix, a tool within Bio-conductor package performs integrative analysis for gene expression and histone modification data sets obtained by chromatin immunoprecipitation (ChIP) sequencing (ChIP-seq). Methylated DNA immunoprecipitation (MeDIP) is a global purification technique used to enrich methylated DNA sequences [129]. MEDIPS is a Bioconductor package that is developed for analyzing data derived from methylated DNA immunoprecipitation (MeDIP) experiments followed by sequencing (MeDIP-seq). MEDIPS also provides several

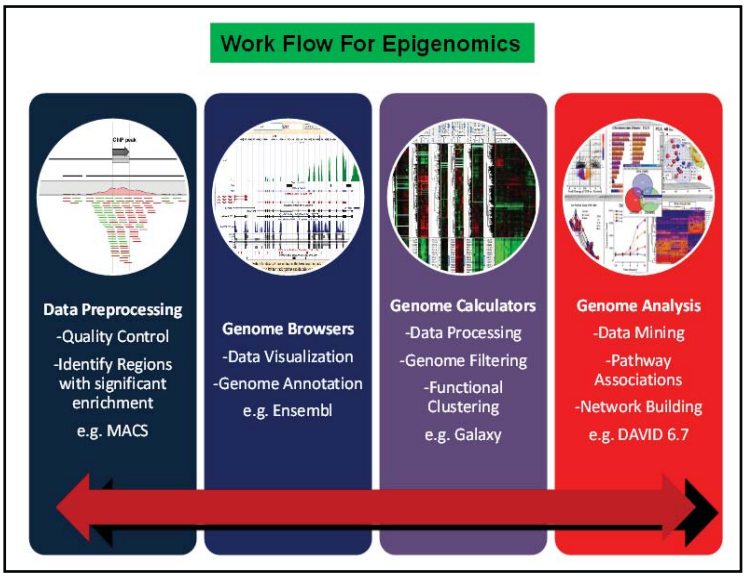

Fig. 4. Work flow for processing and analyzing epigenomics data. Epigenomics shares a number of technologies and applications with genomics and proteomics, which allows users to overlap work flows for data generation and analysis. While initial steps of data retrieval and processing are highly similar to microarrays, there are additional steps like using genome browsers for alignment and annotations (similar to RNA-seq). Downstream analysis after data processing comprise of data clustering, pathway mining and network analysis to predict the most significantly altered candidates. For certain applications, software tools available on the Bioconductor package can integrate conventional genomic and proteomic workflow with output obtained from quantitative sequencing data (e.g. ChIP-seq, MBD-seq and CMS-seq). Given that the technology is still in early stages of development, users need to select different tools from different sources to put together user-defined work flows.

functionalities for the analysis including calculation of differential coverage between groups of samples as well as saturation and correlation analyses.

To predict absolute and relative methylation levels in MeDIP-microarray experiments a software package called MEDME is used. Bisulfite sequencing is another widely used technique that determines pattern of methylation following bisulphite treatment of DNA [130]. Data analysis for this follows a two-step analysis process, methylation calling and visualization. Bismark [131] is a program that can map bisulfite treated sequencing reads to a genome of interest and perform methylation calls in a single step, with output that can be viewed on genome viewer. SeqMonk, IGV and TABLET [132] are tools for visualization and analysis of high throughput mapped sequence data. For downstream analysis of epigenomics data, software like Galaxy, Database for Annotation, Visualization and Integrated Discovery (DAVID) v.6.7 [38, 39], Ingenuity Pathways Analysis (IPA) (www.Ingenuity.com), GeneGO, MetaCore (http://portal.genego.com/), Partek 6.3 (www.partek.com) allow users to correlate epigenomics data with pathway and biological networks. NCBI epigenomics is a curated resource for whole-genome epigenetic data sets that processes raw data maps to genomic coordinates that can be visualized using genome browsers or downloaded for local analysis [133, 134].

Proteomics & Metabolomics in Pulmonary Biology

Proteomics

Since most biological activities are driven by alterations in protein function proteomic approaches are increasingly used to understand pulmonary disorders [135-139]. A recent study using 2-dimensional difference in-gel electrophoresis (2D-DIGE) coupled with MALDI-ToF/ToF MS identified hyperoxia induced temporal alterations proteome, highlighting the role of proteins in translational regulation [140]. The human Bronchoalveolar Lavage Fluid (BALF) phosphoproteome recently indicated a diverse group of proteins, hitherto unknown for their biological functions [141]. Protein profiles specific to Ventilator-associated pneumonia (VAP) were readily identified in BALF, offering promising candidates for

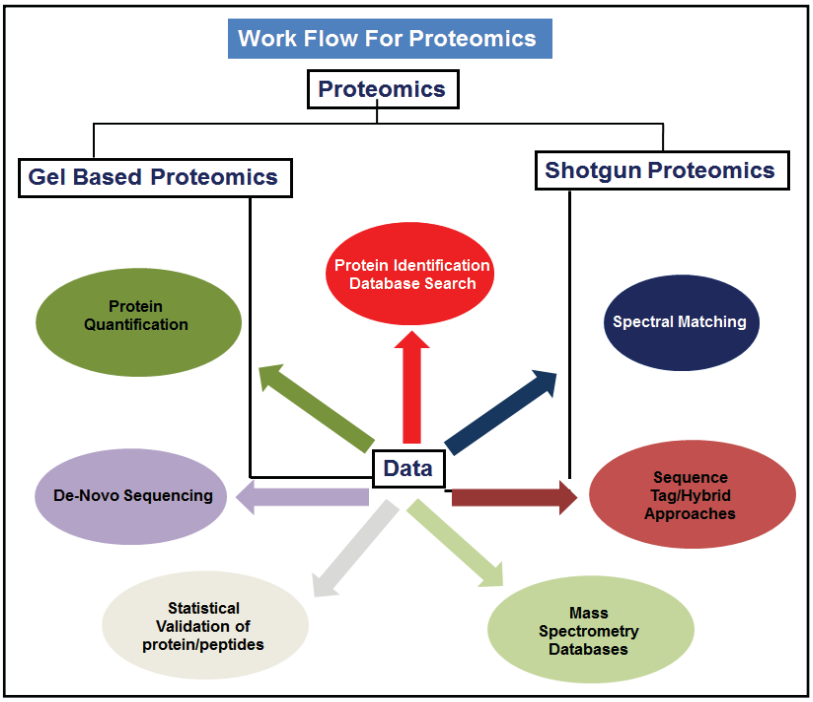

Fig. 5. Proteomic approaches and data analysis work flows. Proteomic analysis can be broadly divided into gel-based and shotgun-based approaches. The data obtained from either of these two methods can be analyzed in different ways depending on the biological properties being probed. Most proteomic analysis is highly sequential in nature and work flows have to be integrated by the user to customize data processing and analysis. Identification of proteins is the preliminary step for analyzing proteomic data. This can be done by querying known databases, analyzing and calculating the masses of peptides and analyzing known posttranslational modifications. Data obtained from high end technologies like mass spectrometry require synergistic tools which can generate databases, perform spectral matches, calculate false discovery rates (FDR) and quantify proteins.

Table 4. Resources for protein sequence, structure and function

	Databases	Web Links
1.	NCBI	http://www.ncbi.nlm.nih.gov/protein/
2.	Protein Information Resource (PIR)	http://pir.georgetown.edu/
3.	UniProtKB -UniProtKB/Swiss-Prot (Manual, Reviewed) -UniProtKB/TrEMBL (Automated, Unreviewed)	http://www.uniprot.org/
4.	HPRD	http://www.hprd.org/query
5.	Human ProteinPedia	http://www.humanproteinpedia.org/index_html
6.	nExtProt 3.0	http://www.nextprot.org/db
7.	PRIDE	http://www.ebi.ac.uk/pride/

understanding the mechanism driving VAP [142]. Likewise, protein signatures of sarcoidosis, IPF, pulmonary langerhans cell histiocytosis, PF due to systemic sclerosis were highlighted, and using systems biology platforms, common and distinct pathways were identified [143]. Intriguingly, phosphoproteomics on lung tissues of lung cancer and COPD patients revealed that activation of the NF-κB pathway was the most relevant signaling link between the two diseases, providing clues to developing therapies [144-148].

Complex proteomic methodologies generate immense data, which requires multiple downstream analysis workflows (Fig. 5). Unlike genomics, the under-developed tools for proteomic analysis have many pitfalls [149-151]. However, use of databases such as NCBI, UniProtKB, Swiss-Prot, Protein Information resource (PIR) and OWL, has streamlined protein identification pipeline (Table 4). PROSITE [152] and SWISS-MODEL Repository [153] are used to define domains, families, and structures. Proteins are identified by matching empirically acquired information against a protein database using tools like Compute pI/Mw, ProtParam, PeptideCutter and ProtScale, which includes PTMs in mass calculations [154]. TagIdent [154-156], AACompIdent [154, 157, 158] and AACompSim [154, 157] are online

tools that accept multiple data types for protein identification, calculating protein isoelectric point (pI), molecular weight and isoforms of interest, AA composition and sequence tag. Tools such as MOWSE can query user provided peptide or sequence data against the protein databases and rank the results according to a scoring algorithm [159].

While, Mascot, ProFound and SEQUEST are commercial suites [160-162], open source tools like Protein Prospector, X!Tandem 4 and the Open Mass Spectrometry Search Algorithm (OMSSA) [163, 164] interpret MS-MS, and ICAT data (http://prospector.ucsf.edu/prospector/mshome.htm) in equally robust and reliable manner. For versatile tasks, including protein database generation, spectral reduction, peptide false discovery rate (FDR) analysis, peptide quantitation, protein parsimony, protein FDR analysis and protein quantitation proteomic work flows are not well developed. For instance, FDR analysis is critical for maximizing sensitivity while simultaneously controlling specificity. We suggest use of Coon OMSSA Proteomic Analysis Software Suite (COMPASS): a free and open-source software pipeline for high-throughput analysis of proteomics data, designed around the Open Mass Spectrometry Search Algorithm, which provide a viable alternative [165].

Researchers can also access data from exiting proteomics experiments through a number of online resources. Databases like PeptideAtlas [166], Global Proteome Machine Database (GPMDB) [167], MaxQB [168], provide a repository of proteomics data with varying options [169]. Most databases preprocess the data; therefore, to obtain raw data, the PRoteomics IDEntifications (PRIDE) database [170-173] is widely used. PRIDE stores protein and peptide identifications (IDs) and quantitative values (including PTMs), analyzed as mass spectra and the related technical and biological metadata. PRIDE also features several popular tools and allows easy integration of user data into different workflows. Repositories like Model Organism Protein Expression Database (MOPED), PaxDB [174, 175], is focused on protein expression information while MassBank [176] and neXtProt [177, 178] represent an extra layers of information on top of the experimental data from MS.

The rapid emergence of next generation technologies including new MS methods detect proteins with high sensitivity [179]. With triple quadruple approach, researchers can process samples by selected reaction monitoring (SRM), also known as multiple reaction monitoring (MRM) for targeted proteomic analysis [180, 181]. A major resource is SRMAtlas, which is a compendium of targeted proteomics assays run by the Institute for Systems Biology in Seattle, WA. A superior technique to SRM is called SWATH, which allows users to analyze more proteins and peptides as compared to the SRM approach and employs complex mass spectra generated by data-independent acquisition to query for the presence of specific peptides [182]. Recent developments and clinical application of proteomics can be found elsewhere [183-185]. In summation, the emergence of these high end proteomic platforms and development of sophisticated data analysis tools will greatly accelerate research in pulmonary biology.

Metabolomics

Metabolites from tissues and bio-fluids, including BALF are identified and quantified using nuclear magnetic resonance (NMR) spectroscopy, Fourier transformed infrared spectroscopy (FT-IR), Fourier transformed ion cyclotron resonance mass spectroscopy (FTICR-MS) and mass spectrometry (MS) [186-191], gas chromatography mass spectrometry (GC–MS) and liquid chromatography based mass spectrometry (LC–MS) [192]. The information derived from these consists of metabolic intermediates, secondary metabolites, hormones, and other signaling molecules, forming the "*metabolome*" [193]. Analysis of metabolic responses to pharmacological interventions to diseases is defined as "*metabonomics*" [194], which aims to refine therapy and diagnosis as shown in lung cancer diagnosis [195]. However, these analysis are not trivial and necessitates the use of multiple techniques [196]. Metabolome is thus analyzed by targeted and global analysis (metabolite profiling and fingerprinting) (Fig. 6). The targeted approach being quantitative with *a priori* knowledge, while global approach generates qualitative profiles of all metabolites, identifying a unique pattern characterizing a snapshot or a fingerprint of the metabolism [190].

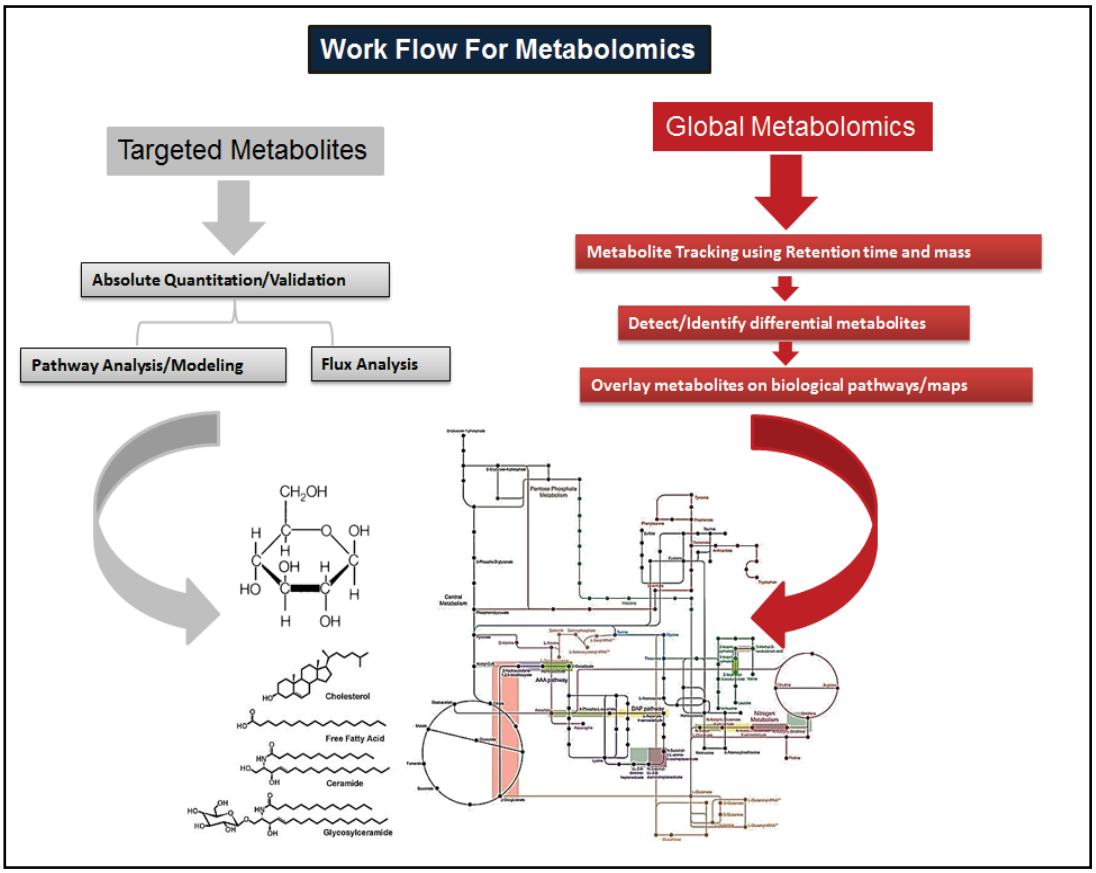

Fig. 6. Targeted and global metabolomics approach for metabolite identification and quantification. Data analysis workflows for global metabolomics are comparable to genomics and proteomics since many of the techniques generate raw data that are of similar format (e.g. KEGG for pathway analysis). Software tools like MetaboAnalyst 2.0 can be used to integrate different "omics data" with metabolomics outputs for a systems level analysis.

Pattern recognition tools and discriminate analysis techniques together classify these fingerprints and associate it with a biological response [197]. This approaches have indeed characterized biochemical fingerprints of airway lining in childhood asthma patients and provided fingerprints of exhaled breath condensate in different clinical settings [198, 199]. Capillary Electrophoresis coupled to Time-of-Flight Mass Spectrometry (CE–TOF-MS) identified and validated metabolic biomarkers in ventilator-induced lung injury (VILI) [200]. Despite early success, sample collection, preparation, and analysis of NMR data remain challenging [201]. Metabolite profiling, an extension of functional genomics, assesses metabolic state of cells and aid in identifying disease genes where no apparent phenotype exists. For example, fingerprint of exhaled breath condensate (EBC) to discern severe asthma phenotype [193, 197, 202, 203].

Integration of metabolome to global *omics* identified novel dysregulated phospholipids as biomarkers that associate with asthma risk alleles [204]. Potential cancer drug targets have also been identified by integrating cancer gene co-expression network and metabolic networks [205]. Thus, users can integrate "omic" profiles to better understand pulmonary disorders by combining genomic, proteomic and metabolic signatures.

The sheer volume of data and complexity of metabolome requires a combination of mathematical, statistical and bioinformatical tools for analysis (Table 5) as reviewed previously [206, 207]. The Automated Mass Spectral Deconvolution, Identification System 2.63 (AMDIS) [208] and Mzmine [209] are the tools used to process raw data obtained by

Table 5. Software tools for epigenomics data processing and analyses

Software Tools	Methylation Analysis
1. Batman	http://td-blade.gurdon.cam.ac.uk/software/batman
2. BDPC	http://biochem.jacobs-university.de/BDPC
3. Epinexus	http://epinexus.net/home.html
4. MethDB	http://www.methdb.de
5. methPrimerDB	http://medgen.ugent.be/methprimerdb
6. MethyCancer Database	http://methycancer.psych.ac.cn
7. Methylumi	http://www.bioconductor.org/packages/bioc/html/methylumi.html
8. mPod	http://www.compbio.group.cam.ac.uk/Projects/p4meth.html
9. PubMeth	http://www.pubmeth.org
10.QUMA	http://quma.cdb.riken.jp
11.TCGA Data Portal	http://cancergenome.nih.gov/dataportal
Software Tools	Bisulphite Sequencing Analysis
1. BSMAP	http://code.google.com/p/bsmap
2. MethBLAST	http://medgen.ugent.be/methBLAST
3. methPrimer	http://www.urogene.org/methprimer
4. Methyl Primer Express	http://www.appliedbiosystems.com/methylprimerexpress
5. CpGviewer	http://dna.leeds.ac.uk/cpgviewer
Software Tools	CpG Island Analysis
1. CpGcluster	http://bioinfo2.ugr.es/CpGcluster
2. CpGFinder	http://linux1.softberry.com
3. CpG Island Explorer	http://bioinfo.hku.hk/cpgieintro.html
4. CpG Island Explorer	http://cpgislands.usc.edu
5. CpG Promoter	http://www.cshl.edu/OTT/html/cpg_promoter.html
6. CpG ratio/GC content Plotter	http://mwsross.bms.ed.ac.uk/public/cgi-bin/cpg.pl
7. EMBOSS CpGPlot/CpGReport/Isochore	http://www.ebi.ac.uk/Tools/emboss/cpgplot/index.html

Table 6. Software tools for analyzing and interpreting metabolomics data

Software Tools	Web Links
1. BioSpider	http://www.biospider.ca/
2. COLMAR	http://spinportal.magnet.fsu.edu/
3. FiD	http://www.cs.helsinki.fi/group/sysfys/software/fragid/
4. HORA suite	http://www.paternostrolab.org/
5. MeltDB 2.0	https://meltdb.cebitec.uni-bielefeld.de/cgi-bin/login.cgi
6. MetaboAnalyst 20	http://www.metaboanalyst.ca/MetaboAnalyst/faces/Home.jsp
7. MetaboMiner	http://wishart.biology.ualberta.ca/metabominer/
8. MolFind	http://metabolomics.pharm.uconn.edu/Software.html
9. OpenMS 1.11.1	http://open-ms.sourceforge.net/openms-1-11-1-released/
10. SetupX	http://fiehnlab.ucdavis.edu/projects/binbase_setupx/
11. Seven Golden Rules	http://fiehnlab.ucdavis.edu/projects/Seven_Golden_Rules/Software/
12. XCMS	http://metlin.scripps.edu/xcms/
13. MSEA	http://www.msea.ca
14. MBRole	http://csbg.cnb.csic.es/mbrole
15. MPEA	http://ekhidna.biocenter.helsinki.fi/poxo/mpea/
16. IMPaLA	http://impala.molgen.mpg.de
17. BioCyc – Omics Viewer	http://biocyc.org
18. MetPA	http://metpa.metabolomics.ca
19. Reactome	http://www.reactome.org
20. Cytoscape	http://www.cytoscape.org/

GC-MS and LC-MS. Recent additions include, MeltDB, a web-based software platform for the analysis and annotation of datasets from metabolomics experiments [210], while software suites like MetaboAnalyst 2.0 offer easy to use and visualize interface for researchers. MetaboAnalyst accepts a variety of metabolomics data types and consists of different functional modules - data processing, functional enrichment, statistical and metabolic pathway analysis [211]. XCMS software tool [212] is a unique tools that integrates peak detection, statistical analysis, and subsequent visualization of raw data for verification purposes and can be used for analyzing metabolite data from LC/MS, peptide digests, and GC/MS. Pathway analysis and data visualization tools form critical components of metabolomic data analysis. Prominent options include KEGG (http://www.genome.ad.jp/kegg/) and MetaCyc (http://metacyc.org/).

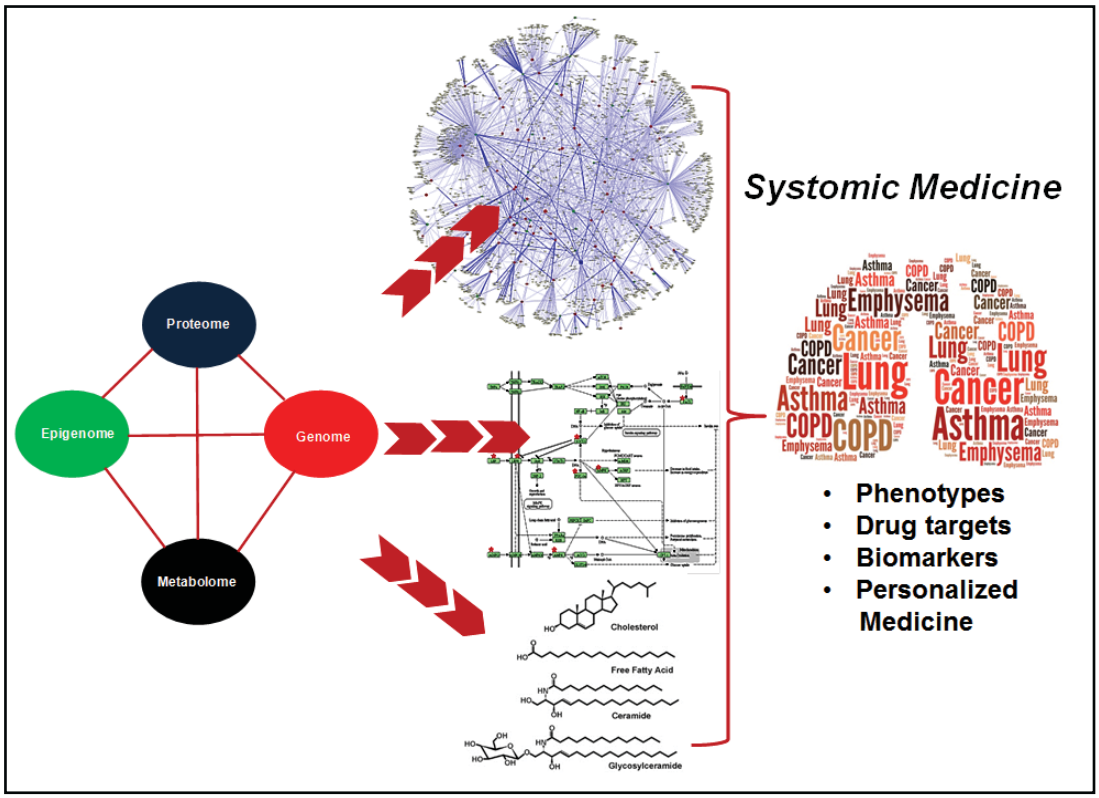

Fig. 7. Systomics: Integrative approach for pulmonary medicine. The confluence of genomics, proteomics, metabolomics and epigenomics drives systems driven approach for studying pulmonary disorders. Integration of work flows and data obtained from these will contribute to a more holistic understanding of the onset and progression of pulmonary diseases. This in turn will allow identification of novel disease phenotypes and assist drug designing strategies based on unique biomarkers. In summation, the integration of omics approaches will pave the way for personalized medicine in pulmonary medicine.

Databases that catalogue information about the human metabolome are vital in deducing biological interpretations from a metabolomics experiment. The human metabolome database (HMDB) [213-215] version 3.5 (http://www.hmdb.ca) contains) contains 41,469 metabolite entries including both water-soluble and lipid soluble metabolites as well as metabolites that would be regarded as either abundant (> 1 uM) or relatively rare (< 1 nM). Additionally, 5,688 protein (and DNA) sequences are linked to these metabolite entries. The database supports extensive text, sequence, chemical structure and relational query searches all displayed in interactive easy to comprehend viewing applets.

Conclusion

Different omics approaches attempt to unravel the high degree of complexity in biological systems and explain the onset and progression of diseases, revealing mechanisms that can be used for targeted interventional therapies. The sheer breadth of information generated by these technologies from different vantage points (RNA, DNA, proteins, chromatin, and metabolites) necessitates integration to arrive at a holistic picture of a given disease. Thus, the use of systems biology approach that integrates results obtained from high throughput technologies is fast becoming a central theme in medical research, especially in context of personalized medicine [216]. A *"systomics"* approach would combine data obtained from genomic, proteomic, epigenomic and metabolomic data to study and treat human diseases. For instance, systomic medicine can allow researchers and clinicians to study closely

related pulmonary remodeling disorders like COPD, asthma, lung cancer and emphysema that have several overlapping phenotypes and symptoms (Fig. 7). Integration of data from the different omics platforms would allow the users to better understand the cause and effect relationships between molecular disruptions (e.g. gene mutations, alterations in protein expression and structure and fluctuating levels of metabolites) and help understand progression of irreversible lung remodeling diseases at the molecular level at a holistic level. This will allow us to identify common causative molecular events in these lung diseases, identifying novel drug targets and biomarkers that could pave the way for personalized medical treatment of pulmonary disorders based on individual genetics, age, environment and physiology.

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