Multi-omics to study chronic respiratory diseases and viral infections

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Integrating omics layers reveals complex biological systems, unveiling vital insights into chronic respiratory diseases and COVID-19. This holistic approach is crucial for developing more effective treatments and understanding intricate relationships. https://bit.ly/3VKFzJH

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Abstract

Despite recent advances, the underlying mechanisms of the development and progression of many chronic respiratory diseases remain to be elucidated. Factors such as heterogeneity and complexity of human diseases and difficulty interpreting large datasets hinder research into chronic respiratory diseases. Omics assesses the changes in specific biological entities, such as mRNA expression, epigenetics/epigenomics, genomics, proteomics, metagenomics and metabolomics, and provides valuable insights into the roles of these processes in chronic respiratory diseases. High-throughput omics at bulk, single-cell and spatial levels empower the exploration of disease-related changes through untargeted data-driven statistical methods. Multi-omics is the exploration and integration of multiple biological processes, which compared to a single-omics, can provide a substantially greater and more holistic overview of the pathogenic mechanisms that underpin complex diseases. Multi-omics analysis can comprehensively characterise the mechanisms that drive chronic respiratory diseases, capturing unique biological signatures and cellular interactions at different omics levels. Use of these methods has begun to identify key factors and biomarkers in chronic respiratory diseases. Here, we review current omics approaches and highlight recent advances in respiratory research achieved using multi-omics and integrative methods. Our review provides a valuable resource for researchers and clinicians in this area.

Introduction

Recent advances in high-throughput omics methods, including transcriptomics, epigenomics, proteomics, microbiomes, and metabolomics have produced novel insights into the molecular and cellular mechanisms underlying chronic respiratory diseases, including bronchial asthma, COPD, idiopathic pulmonary fibrosis (IPF), and respiratory viral infections like coronavirus disease 2019 (COVID-19), rhinovirus and respiratory syncytial virus (RSV) and influenza viruses. The application of different omics to well-characterised clinical cohorts [1] has led to the development of methods to integrate each of these levels, termed multi-omics, which involves generating, analysing and integrating different modalities to comprehensively understand biological processes driving disease (figure 1) [1]. Multi-omics has advanced respiratory research by analysing big data collected from microarrays, bulk, single-cell and now spatial RNA sequencing (RNA-seq), proteomics, epigenetics/epigenomics, metagenomics, meta-transcriptomics and metabolomics, among others [2]. Multi-omics studies of chronic respiratory diseases have revealed key alterations in immune signalling, epithelial remodelling and metabolic pathways. These findings are crucial for understanding the biological changes underlying disease onset and progression and the response to therapeutic interventions [3]. Many datasets of the same omics level are publicly available and can be combined, resulting in greater statistical power, more reproducible findings and enhanced interpretation. Thus, based on single-omics profiles of patients and optimal animal models of disease [4, 5], more effective treatments can be developed [6–8].

Multi-omics is proposed to overcome the inherent limitations of each single omics. This is greatly improved when samples are matched from the same patient or use assays that generate multiple layers of

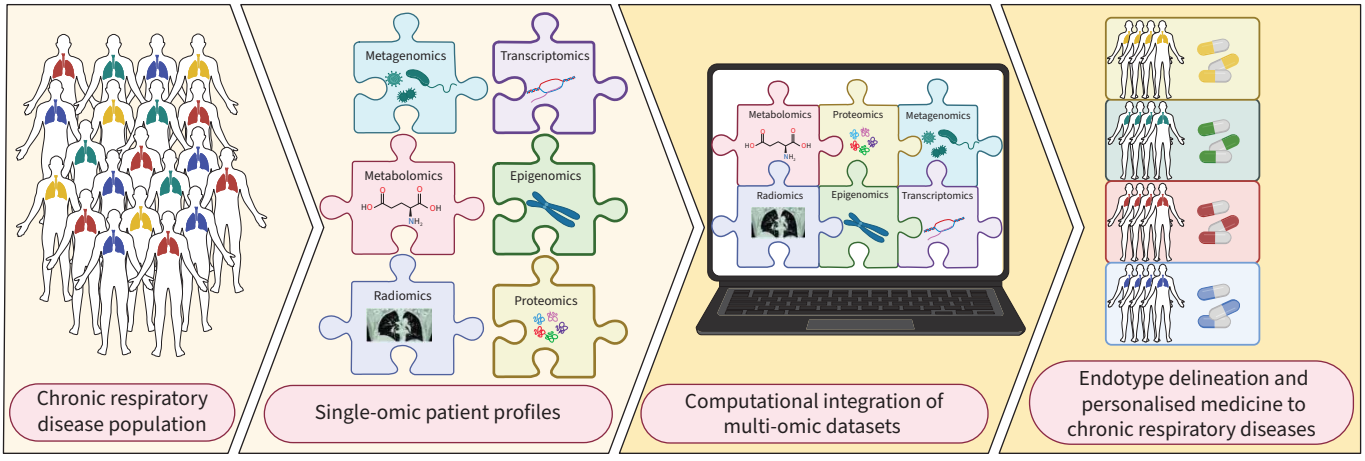

FIGURE 1 General overview of multi-omics layers including transcriptomics, proteomics, metagenomics, metabolomics, radiomics and epigenomics. Multi-omics level integration provides a comprehensive understanding of chronic respiratory diseases, can interconnect data sources, and guide tailored treatment strategies, improving precision and personalised medicine.

omics data from the same sample. This is further enhanced when sophisticated computational biology is applied [3, 9]. Such integration of technologies produces a more comprehensive biological picture, providing answers that single omics may not. However, while multi-omics has shown promise in identifying disease endotypes and uncovering biological diversity, translating these molecular insights into effective treatments remains challenging. The high cost, technical complexity, and need for extensive validation in clinical settings hinder the widespread application of these technologies. Nevertheless, continued advances in computational methods, standardised protocols and large-scale clinical studies could help overcome these obstacles, paving the way for multi-omics to become a powerful tool in precision medicine [10].

Current single-omic techniques

Epigenetics/epigenomics

Gene expression is regulated by complex and reversible modifications to DNA and histones, which influence transcriptional activity without altering the underlying DNA sequence. These modifications, studied through epigenomics, have critical roles in cellular identity and disease progression. Among them, methylation quantitative trait loci are single-nucleotide polymorphisms associated with variations in DNA methylation levels [11]. To analyse DNA methylation, several bulk techniques have been widely adopted. These include methylation arrays and sequencing-based approaches such as whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing [12–14]. Chromatin accessibility, another key epigenetic feature, is commonly assessed using DNase-seq (to detect DNase I hypersensitive sites), ATAC-seq, and FAIRE-seq (formaldehyde-assisted isolation of regulatory elements) [15]. Histone modifications are typically profiled using chromatin immunoprecipitation followed by sequencing (ChIP-seq) [16].

Recent advances in single-cell (sc) epigenetic profiling have enabled unprecedented resolution in understanding cellular heterogeneity. Techniques like scATAC-seq now employ combinatorial indexing and automated workflows to enhance throughput and sensitivity. Integrative platforms combining scRNA-seq with scATAC-seq provide comprehensive insights into gene regulation and cellular states, supporting scalable multi-omics analysis. Moreover, emerging methods such as scM&T-seq (single-cell methylome and transcriptome sequencing), snmCT-seq (profiles transcriptome, DNA methylome and chromatin accessibility of single nuclei using molecular partitioning), and scTrio-seq (captures genome, methylome and transcriptome data simultaneously) enable parallel profiling of multiple epigenetic layers. Additional assays like scNOMe-seq (nucleosome occupancy and methylome sequencing) and scCOOL-seq (integrating chromatin accessibility, nucleosome positioning and DNA methylation) offer high-resolution chromatin mapping. Other single-cell techniques such as bisulfite-sequencing and scWGBS provide enhanced coverage and accuracy for DNA methylation analysis [17, 18].

Thus, epigenetic changes may offer potential biomarkers for disease susceptibility and progression. However, the reversibility of epigenetic modifications suggests that therapies targeting epigenetic regulation could have profound implications, though the full therapeutic potential for chronic respiratory diseases remains underexplored.

Transcriptomics

Transcriptomics measures mRNA expression levels, which is the most used omics approach and, along with other omics techniques, has helped better understand chronic respiratory diseases (table 1). The early version of this technique was microarray-based [19], where >10 000 distinct DNA probe libraries are printed onto a chip that binds corresponding sample fragments that are detected using labelled molecules that bind to specific RNA sequences [20]. It assesses mRNA expression on a large scale as well as long noncoding (lnc) and micro-(mi)RNAs [21–24]. However, arrays capture only a portion of the transcriptome that is known and have a small dynamic range compared to more recent RNA-seq [25]. RNA-seq is the predominant method for quantifying large mRNA, mi-RNA and lncRNA (appropriate extraction and library preparation are needed) [26, 27]. It enables studies of entire transcriptomes and their dynamics and depending on the method of library preparation can provide detailed information on alternative splicing, and RNA editing. This improves understanding of gene regulation, protein diversity and the functionality of RNA processing molecules. This technology has been used to show how diverse RNA molecules actively contribute to cellular function and regulation, significantly advancing insights into their roles in health and disease. More recently, scRNA-seq has enabled assessment of cellular heterogeneity and dynamics in complex tissues and diseases [28, 29]. It achieves remarkably detailed characterisation of structural and infiltrating immune cells in healthy and diseased respiratory tracts [30]. This identifies previously unrecognised cells and their roles in chronic respiratory diseases [28, 31–36]. *In vitro* air–liquid interface cultures have provided further evidence to identify rare cell types, including ionocytes in primary

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TABLE 1 Key features of different omics techniques

	Description	Pros	Cons	Applications
Epigenetics				
DNA methylation	Whole-genome bisulfite sequencing to detect DNA methylation patterns	Nongenetic inheritance Reversibility (epigenetic changes are reversible)	Epigenetic changes are reversible Targeting specific changes is difficult Epigenetic mechanisms are intricate and complex to understand Nongenetic inheritance needs further exploration	Study gene regulatory networks Cancer research Personalised medicine
Epigenomics				
ATAC-seq	Technique to detect open chromatin regions, offering more efficient processing compared to ChIP-seq, and can be applied at the single-cell level	Detection of open chromatin More efficient processing compared to ChIP-seq No antibody required Can do at single-cell level	Lower resolution compared to ChIP-seq Data quality can be affected by sample preparation Not as effective for studying protein–DNA interactions	Chromatin accessibility study Transcriptional regulation
ChIP-seq	Measures protein–DNA interactions by identifying histone modifications and transcription factors	Identify protein–DNA interactions Higher resolution compared to ATAC-seq	Labour-intensive Crosslinking artefacts can affect data accuracy Data quality dependent on antibody quality Higher resolution compared to ATAC-seq	Protein–DNA interaction study Gene and epigenetic regulation
Transcriptomics				
Microarray	A high-throughput technique used to measure the expression of thousands of genes simultaneously	High-throughput analysis of gene expression Established technology with widespread use	Limited to known probes; does not capture novel or rare transcripts Limited dynamic range Prone to cross-hybridisation	Biomarker discovery Pathway analysis
RNA-seq	Sequencing-based method to quantify gene expression by sequencing RNA transcripts	High sensitivity and broad dynamic range Detects alternative splicing and novel transcripts	Requires high computational resources for data analysis Library preparation biases can affect results	Biomarker discovery Pathway analysis
Single-cell RNA-seq	Allows RNA sequencing at single-cell resolution to measure gene expression heterogeneity	Provides insights into cellular heterogeneity Enables identification of rare cell populations High reproducibility and low input requirements	Limited sensitivity for low-input samples Higher cost per cell compared to bulk RNA-seq	Single-cell genomics Cell lineage tracing Differentiation studies
Spatial transcriptomics	Combines transcriptomic profiling with spatial information to study gene expression in tissue sections	Captures spatial gene expression within tissue context Allows mapping of gene expression patterns *in situ*	Requires high-quality tissue preservation; data analysis can be challenging due to high dimensionality Sensitivity may vary depending on the platform	Spatial transcriptomics studies Understanding tissue heterogeneity
Proteomics				
MS-based proteomics	Expensive, requires high technical expertise; challenges with data interpretation	High sensitivity and specificity High coverage	Expensive, requires high technical expertise; challenges with data interpretation	Biomarker discovery Drug discovery Disease mechanisms

Continued

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TABLE 1 Continued

	Description	Pros	Cons	Applications
Spatial proteomics	Combines MS with imaging to map protein distribution across tissue sections	Provides a comprehensive view of protein distribution and localisation High resolution and high sensitivity	Requires high-resolution tissue sections; complex data analysis	Provides insights into cellular diagnosis with applications in disease diagnosis and treatment
Metabolomics				
MS-based metabolomics	MS used to analyse small molecules (metabolites) in biological samples	Can be compatible with imaging to make a spatial map of metabolites	Ionisation bias Complex sample preparation	Typically used for broad metabolite coverage
HPLC	Separation technique used for metabolite profiling, often combined with UV or MS detection	Compatible with MS Separation power	More expensive Sample matrix effect and interference from co-eluting compounds can affect data quality	Typically used for sample clean-up and fraction before MS analysis
NMR spectroscopy	Noninvasive technique used to identify and quantify metabolites based on their nuclear magnetic properties	Minimal sample preparation required Nondestructive process Provides quantitative value of metabolites without standards	Lower sensitivity Not high-throughput	Used when samples are needed for other processes after metabolomic profiling
Capillary electrophoresis	A separation technique that uses an electric field to separate metabolites based on their size and charge	Requires only small sample volumes Compatible with MS	Use limited to certain settings	Typically used for separation of small and charged molecules
Microbiome				
16S rRNA gene sequencing	Sequencing of the 16S ribosomal RNA gene to profile microbial communities in various samples	Cost-effective Targeted approach Broad taxonomic coverage	Provides limited resolution at species and strain levels due to short length of 16S rRNA Subject to PCR amplification biases and primer selection Cannot detect nonbacterial microbes	Typically used for taxonomic profiling of microbial communities
Metagenomics, metatranscriptomics, metaproteomics	Techniques for profiling the entire genomic, transcriptomic and proteomic content of microbial communities	Unbiased approach Strain-level detection Potential to identify novel molecules	Expensive Reconstruction of microbial genomes/transcriptomes/proteomes can be challenging, especially for low-abundance microbes and complex communities	Typically used for host–microbiome interactions

ATAC: assay for transposase-accessible chromatin; seq: sequencing; ChIP: chromatin immunoprecipitation; MS: mass spectrometry; HPLC: high-performance liquid chromatography; NMR: nuclear magnetic resonance; UV: ultraviolet.

bronchoepithelial cells [37]. In parallel, scRNA-seq has revealed additional cellular diversity across other lung compartments. One study subclassified pulmonary capillary endothelial cells into distinct populations, including aerocytes and general capillary endothelial cells [38]. Atypical basaloid cells were identified that co-express markers of basal epithelial, mesenchymal, senescence and development and are situated at the periphery of myofibroblast foci in IPF lungs [35]. Following the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), scRNA-seq has been used on COVID-19 patient samples to reveal distinct alveolar macrophage and monocyte populations associated with disease severity [39, 40] and mechanisms of increased susceptibility to infection in COPD [28]. Nevertheless, standard scRNA-seq methods often introduce dissociation-induced biases. These arise during tissue processing, where enzymatic digestion can disproportionately capture more robust, dissociation-tolerant cells (typically immune cells) while under-representing fragile or tightly adherent cell types such as epithelial or stromal cells. Additionally, transcriptomic stress responses may be artificially induced during cell isolation. Single-nucleus (sn)RNA-seq addresses many of these limitations. By isolating nuclei instead of whole cells, snRNA-seq reduces dissociation bias, minimises the over-representation of immune cells, and enables the capture of more delicate or structurally embedded cell types, improving representation of the full cellular landscape of tissues [41].

Overlay of computational methods maximises the interpretation of single-cell data and is being extended to multi-omic analysis. Cell–cell communication (CCC) can be inferred using reference databases of known ligand/receptor pairs and then overlaying cell type (*e.g.* CellPhoneDB [42]) or condition (*e.g.* NicheNet [43]). NicheNet infers CCC by predicting how ligands secreted by one cell type influence gene expression in a target cell. It uses ligand–receptor interaction databases and gene expression data to identify and rank potential signalling pathways and key regulatory genes involved in cellular communication [43].

Trajectory inference methods analyse scRNA-seq data to reconstruct the dynamic developmental paths that cells follow over time. By modelling cellular progression through various states, these methods capture how cells transition from one state to another, such as from progenitor to differentiated cell types. With ∼70 methods currently available, approaches range from graph-based techniques to tree-based models, which include multifurcation and bifurcation structures as well as linear and cyclic trajectories, each suited to capturing different developmental or differentiation patterns. These methods provide insights into complex cellular processes, enabling a deeper understanding of their development and disease processes [44].

Where scRNA-seq data from identical samples is unavailable, leveraging publicly accessible reference atlases like the Human Lung Cell Atlas is a viable alternative [30]. Reference datasets for mice (and accurate mouse models [5, 45]) and humans are crucial foundations for annotating, inferring and interpreting multi-omics data in relevant contexts. As more scRNA-seq data are generated, tools are emerging to integrate datasets across laboratories, increasing statistical power to detect subtle changes in RNA and enabling more in-depth analysis [46, 47].

Proteomics

Proteomics explores protein interactions, composition, structures and cellular activity [2, 48, 49]. It uses tissue array and mass spectrometry (MS) (matrix-assisted laser desorption/ionisation (MALDI)-time-of-flight (TOF)/MS, liquid chromatography (LC)-MS/MS, surface-enhanced laser desorption/ionisation (SELDI)-TOF/MS, and two-dimensional electrophoresis (2-DE)/MS) to identify and verify proteins [50, 51]. Tissue array is rapid, but has target selection challenges, while MALDI-TOF/MS is favoured for accuracy and is untargeted. Untargeted LC-MS/MS is high-throughput with limitations in sensitivity, sample preparation requirements, variability and cost. ELISA and immunoblot are used for protein verification and quantification, with ELISA preferable for rapidity, sensitivity and accuracy [50, 52]. MS-based techniques are utilised to generate comprehensive data in chronic respiratory diseases [2, 53]. Their use has revealed disease-associated biomarkers (*e.g.* TPP1, CTSD, DPYSL2, TGM2) and therapeutic targets (RNA biosynthesis and binding proteins, S100A1, mitochondrial dysfunction, oxidative stress) [53–55] in experimental and human COPD, asthma, IPF and bronchiectasis [56]. Among the emerging high-throughput proteomics platforms, Olink has gained prominence for its ability to achieve multiplex protein biomarker analysis with high sensitivity and specificity. It uses a proprietary proximity extension assay, where pairs of oligonucleotide-labelled antibodies bind to specific target proteins. Upon binding, the proximity of the antibody pairs allows their attached DNA strands to hybridise and be extended by DNA polymerase, generating unique DNA barcodes. These barcodes are then quantified using quantitative PCR or next-generation sequencing, enabling precise protein detection across hundreds of targets from minimal sample volumes [57]. Complementing this, the SomaScan assay, an aptamer-based proteomics platform, has been employed recently to identify plasma biomarkers associated with COPD and to predict emphysema

risk [58]. Unlike antibody-dependent methods, SomaScan uses chemically modified DNA aptamers (SOMAmers) that bind selectively to thousands of proteins, providing extensive proteomic coverage with high specificity in complex biological samples [58].

Integrating RNA-seq with proteomics provides a more comprehensive understanding of chronic respiratory diseases. RNA-seq delivers high-resolution insights into gene expression at the mRNA level, and proteomics complements this by confirming protein-level changes and uncovering post-transcriptional regulatory mechanisms that are not captured by transcriptomic analysis alone [59].

Metabolomics

Metabolomics studies small-molecule metabolites involved in cellular metabolism [60, 61]. The most common methods used are nuclear magnetic resonance, MS, Raman spectroscopy and Fourier-transformed ion cyclotron resonance MS. MS-based techniques employ differential separation of molecules using gas or LC, or capillary electrophoresis, depending on the physicochemical properties of the molecules being assessed. A major challenge is the accurate annotation of metabolites, and the method used to confirm their identity must be transparent. Targeted approaches measure a predefined set of metabolites employing standard chemical libraries for higher certainty of annotation [62]. Untargeted approaches employ high-resolution MS for discovery, where metabolites are annotated using spectral databases [63] and/or molecular networking [64]. Cell-based metabolomics uses fluorescence-activated cell-sorting and immunomagnetic separation to isolate specific cell subsets, enabling targeted metabolite analysis [65]. The metabolic coverage of a method depends on the chemistry selected for the analysis, and issues of coverage and incorrect annotation can bias pathway mapping [66]. Several studies applied these techniques to chronic respiratory diseases and identified biomarkers in COPD [67–72]. One study showed that a childhood asthma subtype marked by early onset and heightened airway resistance is distinguished by diminished sphingolipid levels linked to 17q21 genetic variations and activity of serine palmitoyltransferase [73]. Another identified sphingomyelins and glycosphingolipids (including ceramide d18.1.N16.0, ganglioside GM3 d18.1.N16.0 and sphingomyelin d18.1.N16.0) were associated with COPD features [74]. Various traditional metabolome profiling approaches, particularly mass spectrometry techniques, have been applied to COPD. In the large SPIROMICS cohort, analysis of sputum supernatant metabolites revealed that sialic acid, hypoxanthine, xanthine, methylthioadenosine, adenine and glutathione were strongly associated with COPD patients experiencing exacerbations compared to those without. These findings suggest that metabolome-based biomarkers can differentiate between disease states and predict exacerbation risk. However, the predictive power of these biomarkers in terms of sensitivity, specificity and their integration into clinical practice remains an area of active investigation. Further studies are needed to validate these findings in independent cohorts and to assess their utility in guiding personalised treatment strategies or predicting long-term outcomes in COPD [75].

Challenges persist in conducting metabolomics studies in chronic respiratory diseases, primarily due to the lack of standardised sampling methods. For example, collecting airway epithelial lining fluids *via* bronchoscopy using normal saline lavage often results in a dilution of these fluids up to 100-fold. This varies between samples and is frequently overlooked in metabolomics studies, leading to inconsistencies.

With increasing recognition of the pivotal roles of metabolites in health and disease, spatial metabolomics is now increasingly being used to profile metabolites, lipids, drugs and other small molecules in cells, tissues and organisms [76].

Radiomics

Radiomics is a quantitative medical imaging approach that uses advanced mathematical analysis to extract textural information, offering additional information over visual interpretation. It enhances clinical decision-making and is being applied to early tumour detection, survival prediction, and assessing therapeutic response. There are technical challenges, a lack of standard criteria, and limited real-world experience that impact clinical outcomes [77]. Radiomics has been applied to chronic respiratory diseases, including nodules, lung cancer, obstructive and restrictive conditions, and interstitial lung diseases/IPF [78–81]. Radio-transcriptomics, integrating radiomics with transcriptomics, is a novel strategy offering insights into the dynamic interplay between tumour cell populations and the microenvironment. Demonstrating efficacy in predicting thrombosis and death in COVID-19 patients and responses to dexamethasone, radio-transcriptomics shows promise in developing noninvasive imaging biomarkers. The incorporation of single-cell or single-nuclei RNA-seq data may enhance the representation of disease heterogeneity. Ongoing advances in imaging and RNA-seq technologies, coupled with *in silico* methods, hold potential for constructing advanced models with clinical and translational value [82].

Metagenomics

Understanding the composition and function of respiratory microbial communities is achieved using 16S rRNA and metagenomic approaches. They define the phylogenetic, taxonomic and genetic characteristics of taxa and decipher the roles of the microbiome in health and disease [83]. Sequencing of bacterial 16S rRNA amplicons enables cost-effective and rapid taxonomic classification using PCR amplification with primers targeting variable regions of the bacterial 16S ribosomal gene. This requires only modest sequencing depth as it assesses a specific bacterial genome region [84]. Shotgun metagenomics involves whole DNA sequencing of the genetic content within samples, enabling the assessment of bacteria, DNA viruses and eukaryotic DNA (including fungi). It produces an unbiased and more in-depth taxonomic analysis by sequencing diverse regions of microbial genomes [85].

Now, microbiome data is being integrated with other omics to generate a holistic view of chronic respiratory diseases [86] in discovery and clinical investigations [87]. This suggests the potential of integration to identify distinct molecular subphenotypes, enabling more targeted and personalised treatment. It emphasises the importance of integrating data from various molecular levels and anatomical locations for a comprehensive understanding of chronic respiratory diseases.

Spatial omics

scRNA-seq, proteomics and other omics have unveiled many valuable biomolecular changes in chronic respiratory diseases, but lack spatial context due to the need for tissue dissociation. Spatial transcriptomics has emerged that preserves cell location and reveals spatially resolved cell interactions in chronic respiratory disease pathogenesis [87]. It involves sequencing- and imaging-based methods that are targeted or untargeted (*e.g.* whole transcriptome *versus* specific panels) [87, 88]. Platforms like Visium and GeoMx employ whole-transcriptome RNA-seq with trade-offs in RNA capture efficiency and single-cell resolution. SCRINSHOT, Xenium and CosMx use single-molecule fluorescence *in situ* hybridisation and imaging with machine learning for targeted single-cell resolution [89, 90]. Platform selection involves balancing whole transcriptome coverage and single-cell resolution. One study used Visium to spatially resolve transcriptomes and cell types in healthy human lungs, providing a reference study for chronic respiratory disease research [91]. Another used GeoMx to reveal interactions between cytotoxic lymphocytes and inflammatory macrophages in COVID-19 [92]. GeoMx was also used to define transcriptional distinctions between fibroblastic foci, fibrous regions and normal areas in IPF [93] and identify new fibrogenic biomarkers (*i.e.* MMPs, CDK4/6 inhibitors). SCRINSHOT was used to generate a reference spatially resolved map of developing human lung tissue [94]. scRNA-seq data can be integrated with spatial information computationally, quantifying the proportions of various cell types in given spatial regions [95]. Applying scRNA-seq and spatial transcriptomics to the same biological samples from a single experiment gives utmost precision in cell type identification. Where this is not possible, reference atlases are used [30]. These combinations can be applied to any omics.

Approximately 50% of proteins have multiple subcellular locations, contributing to intricate spatial proteomic landscapes [96]. High-throughput imaging and quantitative MS decipher these subcellular protein networks. Untargeted MS proteomics and imaging (MSI) provides complementary spatial proteomics data applicable to serial tissue sections [97, 98]. Understanding the dynamic spatial proteome is challenging, but yields valuable insights into cellular diagnosis, with applications in disease diagnosis and treatment [99].

Serial-section analysis is often necessary, particularly for untargeted studies, because single-omics methods are typically destructive and cannot preserve the sample for further analysis. However, some spatial platforms overcome this limitation by enabling simultaneous detection of targeted proteins, including 10x Genomics, NanoString, SM-Omics, spatial-CITE-seq and SPOTS [100–102]. Computational integration of spatial transcriptome and proteome data is challenging. It is typical to define the concomitant presence of transcripts and proteomes and perform pairwise correlations. One investigation involving bulk RNA-seq and untargeted proteomics in human lung cells revealed that only ∼40% of mRNA–protein pairs had matched expression, and correlations were relatively low (Spearman rank coefficient ∼0.4) [103]. This indicates nonlinearity between different biomolecular layers in the lungs. Thus, when working with multiple types of data, finding common features that exist across them and using them as integration anchors helps ensure that integration is meaningful. It may be possible to integrate RNA-seq and proteomics with metabolites, lipids and other omics to further understand chronic respiratory diseases.

Recently, spatial metabolomics was used to identify glycogen as a target in IPF [104]. Accompanying MSI generates extensive hyperspectral imaging data, prompting the development of specialised computational methods to evaluate metabolomics in image analysis [76]. Another study used this to find blunted N-linked

glycan in fibrotic compared to wild-type mice showing that lysosomal utilisation of glycogen is required for the progression of pulmonary fibrosis [104]. Finally, desorption electrospray ionisation-multiple-reaction-monitoring mass spectrometry was recently developed to perform spatially resolved quantification of oxylipins in lung tissue [105].

Characterisation of biological processes

Multi-omics and computational methods have been developed recently to infer biological processes from data, identifying signalling pathways and potential protein–protein interactions (PPIs) [106–110], *e.g.* BioGRID [111], IntAct [112] and STRING [113]. PPIs are essential in all cellular processes [114], and aberrant ones are involved in various chronic respiratory diseases. However, the text-mining approaches used have limitations in accuracy and context-dependent information. Additionally, reliance on pre-existing knowledge may bias the source, and lead to unreliable predictions [106]. Targeting PPIs is an important strategy for developing new drugs. Computational methods can virtually screen libraries of compounds against protein active sites to predict their likelihood of binding and identify PPI modulators that may be drugs [115]. Such techniques were used to develop PPI modulators of MDM2/p53 [116] and TCF/β-catenin [117].

Disruption in cell signalling pathways can lead to improper decoding of cellular messages and cause disease. Thus, identifying and characterising intercellular signalling pathways is another area of intense research [118]. Construction of networks wherein genes exhibiting co-expression patterns are interconnected can identify essential disease pathways and signatures and factors that control them. Computational methods like weighted gene co-expression network analysis [119], co-expression centrality analysis [120], and gene whole co-expression network analysis [121] are employed to construct these networks (figure 2). One study developed a multiplex network approach to study 20 million gene relationships and identified distinct phenotypic modules in 3771 rare diseases [122]. Thus, these methods provide a holistic understanding of the collective involvement of groups of genes in specific biological processes or pathways associated with disease.

This is achieved using computational methods, such as transcription factor enrichment analysis, which detects positional motif enrichments related to transcription [123, 124]. Other methods include ChEA3 [125] and VIPER [126]. Other studies used network and enrichment tools to infer new transcription factors

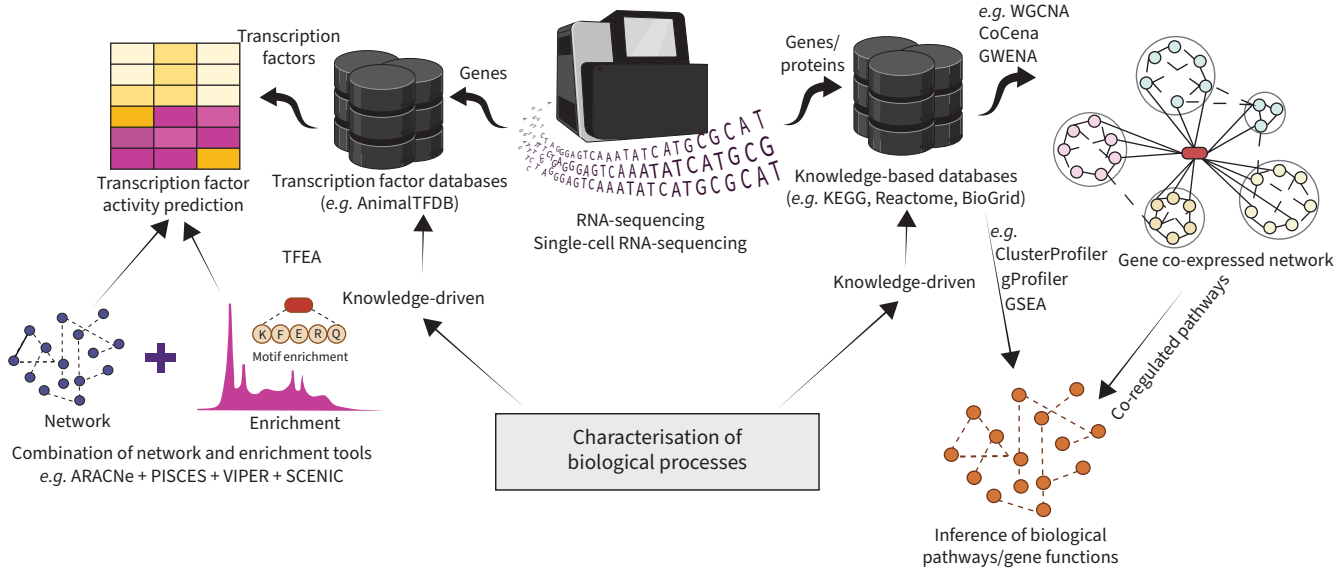

FIGURE 2 Methods used to characterise biological processes. Knowledge-based databases (*e.g.* Kyoto Encyclopedia of Genes and Genomes (KEGG)/Reactome) are used to predict biological pathways using gene and protein information and/or to construct co-expressed networks and infer co-regulated pathways. Moreover, transcription factor databases (*e.g.* Animal transcription factor database (AnimalTFDB)) are used to infer transcription factor activity using various methods such as transcription factor enrichment analysis (TFEA) or a combination of network and enrichment methods (*e.g.* ARACNe, VIPER, PISCES, SCENIC). WGCNA: weighted gene co-expression network analysis; CoCena: co-expression centrality analysis; GWENA: gene whole co-expression network analysis; GSEA: gene set enrichment analysis. Figure created using BioRender.com.

from scRNA-seq data [127] and ARACNe [128], PISCES, VIPER [129] and SCENIC [130] to infer transcription factor activity and enrichment in different cells and diseases [129] (figure 2). Regulating gene expression involves complex transcriptional programmes driven by transcription factors and epigenetic events, but defining this coordinated control of cell-type-specific gene expression remains challenging. New computational methods (*e.g.* AnnoMiner) are being developed to annotate and integrate transcription factor and epigenetic data that can help assess gene regulation and identify functional regulatory elements, which in turn can enhance the understanding of how transcription factors and epigenetic modifications control gene expression [131]. In short, these methods have substantially improved our understanding of biological processes by identifying key molecules driving diseases. However, findings are predictions and require validation *in vivo* (animal, human) and *in situ* to ensure accuracy and reliability.

Multi-omics integration

Multi-omics data can be measured at different resolutions: at the sample level (bulk) or the cellular level (single-cell). The choice of multi-omics data integration methods must be tailored to the resolution and the type of data that is measured. Thus, different methods are required for different data types (figure 3) [132].

Integrating multi-omics data at the sample level

Bulk multi-omics data provides an average profile across cells within a sample. Thus, bulk multi-omics data integration is used to understand variation at the tissue or sample level. In disease research bulk multi-omics integration can be used to discover biomarkers (such as transcripts, DNA, genes, proteins and metabolites that indicate whether a biological activity is normal or abnormal [133]) or stratify patients. Bulk data integration methods can be classified into five categories: regression/association-based methods, multiple kernel learning, Bayesian-based methods, network-based methods and matrix factorisation-based methods.

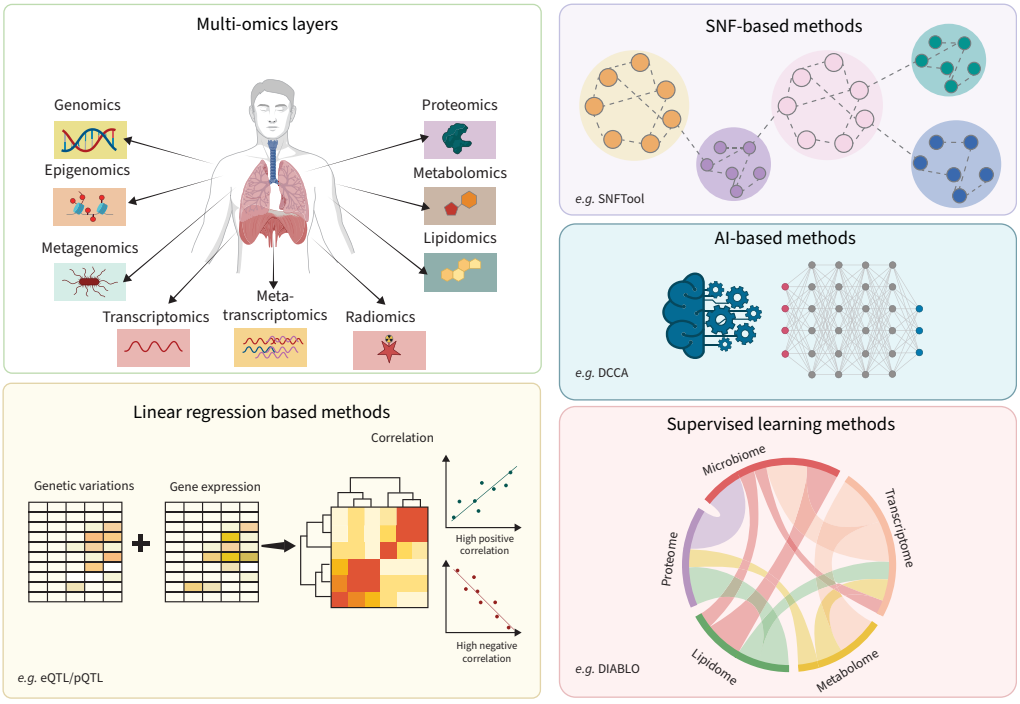

FIGURE 3 Various multi-omic layers including genomics, epigenomics, metagenomics, transcriptomics, meta-transcriptomics, radiomics, proteomics, metabolomics and lipidomics are currently being used to study respiratory diseases. Current multi-omics methods include correlation approaches for two-layered integration, similarity network fusion (SNF) that involves constructing similarity networks independently for each data type and then fusing these networks to create an integrated similarity network. Artificial intelligence (AI) (*e.g.* machine learning/neural network)-based multi-omics methods that can generate missing data based on the learned latent representation of other omics layers (*i.e.* transcriptomics, epigenetics), and supervised learning methods are being adapted such as mixOmics, Anvi'o, and integrOmics. eQTL: expression quantitative trait loci; pQTL: protein quantitative trait loci. Figure created using BioRender.com.

Regression/association-based methods [134, 135] contribute to biomarker discovery by identifying associations/correlations between different omics layers [136]. Association-based approaches [135, 137] have been used to link single-nucleotide polymorphisms to quantitative trait loci (QTLs) for gene expression (eQTLs). These associations help to identify genetic factors responsible for certain diseases and uncover the pathways impacted by these factors [137, 138]. Multiple-kernel learning methods [139] are used to integrate different types of omics data (*e.g.* genomics, proteomics, transcriptomics) by creating separate "kernels" for each data type and then combining them into a single, unified kernel matrix for analysis. In the weight-boosted kernel [140], features significantly upregulated or downregulated in samples are given higher weights, because they reflect key disease-related molecular changes. This ensures these biologically relevant features have greater influence in constructing kernels for each omics data type. These features are assigned higher weights, meaning they have more influence when constructing the kernels for each omics data type [141]. Separate kernels are integrated into a unified matrix, which is used for clustering to identify disease subtypes. This approach helps to prioritise the most relevant and informative data sources, improving the accuracy and robustness of biomarker identification and improving disease classification [140]. Thus, this method enhances the potential for more personalised and effective therapeutic strategies [139].

Integrating between five and seven omics data-blocks has proven effective in diagnosing COPD by providing comprehensive maps of the molecular alterations associated with the disease. This approach enables the identification of complex biomarkers and molecular signatures that are more specific and sensitive than traditional diagnostic methods, facilitating a deeper understanding of disease heterogeneity and enabling the differentiation of COPD subtypes. While spirometry remains a standard diagnostic tool, multi-omics provides insights into disease mechanisms, progression and potential therapeutic targets that cannot be achieved by spirometry alone. These insights can inform personalised treatment strategies, ultimately improving patient outcomes. However, the clinical utility of multi-omics is currently limited due to high costs, technical complexity, and the need for further validation in larger cohorts. Despite these challenges, the potential to enhance disease understanding and guide more targeted therapies justifies investment in these technologies, especially as advances in computational methods and reduced costs increase accessibility and clinical applicability in the future [86]. For patient stratification, Bayesian-based methods [142] formulate probabilistic models that capture the distribution and correlation of various omics datasets. Probabilistic stratification uses Dirichlet process (infinite mixture) models to integrate copy number variation and gene expression data, enabling joint classification of patients into disease subgroups [142]. While initially applied to cancer, similar Bayesian approaches have been extended to complex chronic respiratory diseases. Shaddox *et al.* [143] applied a Bayesian hierarchical graphical modelling framework to gene expression and metabolite data from subjects with varying COPD severity, capturing complex inter-omics relationships pertinent to disease progression. These applications underscore the versatility of such models in integrating heterogeneous omics data to elucidate disease mechanisms and support precision medicine in chronic respiratory diseases. Network-based methods [144] construct networks where nodes represent biological entities (*e.g.* genes, proteins, cells) and edges represent interactions or associations between them. These methods capture complex, nonlinear dependencies in biological systems. In COPD, one study used a network-based approach to integrate transcriptomics, proteomics, and PPI data, improving the accuracy of disease classification. Key genes like CXCL11 and TLR2 were identified, and pathways such as glycosaminoglycan signalling were enriched, providing insights into molecular mechanisms [145]. PARADIGM [144], a pathway-based multi-omics integration method, was used to successfully identify consistent pathway-level activities in subsets of patients with glioblastoma multiforme, that are often overlooked when analysing genes individually. While initially developed for cancer, this approach can also be applied to chronic respiratory diseases where understanding pathway perturbations may help uncover molecular endotypes. Matrix factorisation methods [146] project the variations among different omics layers onto a dimension-reduced space. iCluster+ [146] has been used to integrate DNA methylation with other omics data, revealing two distinct subsets in lung adenocarcinoma. This highlighted the role of chromatin maintenance in the disease and provided insights into its molecular subgroups [147].

Integrating RNA-seq and proteomics provides a comprehensive view of gene expression by linking transcriptomic data to protein abundance, but it faces several methodological challenges. RNA-seq measures mRNA levels using high-throughput sequencing, while proteomics employs MS to quantify proteins. Correlation analysis, pathway enrichment and machine learning are commonly used to connect transcript and protein data, enabling insights into gene regulation and post-translational modifications [148]. However, this integration is complicated by differences in sensitivity and dynamic range between RNA-seq and MS, as well as the fact that mRNA levels do not always correlate directly with protein abundance due to translational control and protein degradation. Moreover, proteomics may fail to detect low-abundance proteins or those with low ionisation efficiency, while RNA-seq might miss short-lived or

rare transcripts [149]. While bulk data does not achieve the resolution of single-cell data, bulk datasets typically exist at a far larger scale of sample sizes. This can be highly beneficial, as integration across many samples often results in more generalisable predictions, which is crucial to discovering robust biomarkers and patient subgroups.

Integrating multi-omics data at the cell level

Single-cell approaches benefit from analysing cellular heterogeneity, whereas traditional bulk methods assess data at the sample level and may require deconvolution for cellular insights [145]. Multi-omics integration methods model variations at both the cellular and feature levels, which is particularly important for chronic respiratory diseases like asthma and COPD [150]. In asthma, single-cell multi-omics identified distinct subtypes by analysing cellular interactions and inflammatory pathways, improving the understanding of disease heterogeneity and potential therapeutic targets [151]. In another study, COPD was primarily associated with molecular alterations in monocytes, which induced pro-inflammatory effects on alveolar epithelial cells. Additionally, aged monocytes and club cells contributed to COPD development, while smoking-related macrophage dysfunction disrupted the sphingolipid balance [152]. These analyses rely on joint embeddings produced by multi-omics data integration methods. Such methods, known as global integration strategies [153], are designed to detect larger-scale patterns of covariation across different modalities, enabling the unsupervised identification of global shifts in cellular states. Global data integration methods can be categorised into three primary approaches: neural network-based, matrix factorisation-based, and network-based. Neural network-based methods [154] use artificial neural networks to model complex relationships and patterns in data. These methods, which include architectures like variational autoencoders, deep canonical correlation analysis, and other deep learning models, are designed to learn low-dimensional representations of data through multiple layers of nonlinear transformations. DeepSpiro, a deep learning based model, was developed to predict future COPD risk using volume–flow spirometry data. By incorporating modules for smoothing, encoding key variability patterns, integrating heterogeneous data, and explaining predictions, DeepSpiro successfully predicted undiagnosed high-risk cases with long-term accuracy, demonstrating its effectiveness for early COPD detection and risk stratification [155]. Matrix factorisation based methods [156–158] decompose original matrices into two low-dimensional submatrices using linear approaches. One submatrix captures signature information (*e.g.* genes), while the other records cell information [159]. These methods help reduce the complexity of large datasets while retaining the essential features that describe the biological processes. Network-based methods [95] construct networks where nodes represent biological entities (*e.g.* genes, proteins, cells) and edges represent interactions or associations between these entities. These methods capture complex, nonlinear dependencies in biological systems. In COPD research, a study used a network-based approach to integrate transcriptomics, proteomics and PPI data, improving the accuracy of disease classification. Key genes like CXCL11 and TLR2 were identified, and pathways such as glycosaminoglycan signalling were enriched, providing insights into molecular mechanisms [160]. Feature-level variation can be understood by inferring gene-regulatory relationships, a process known as local integration [153]. This approach identifies potential interactions between specific features across diverse molecular layers. While many local integration methods rely on regression models [161], distinguishing true feature interactions from false associations is a key challenge that can occur due to confounding factors or upstream influences [153]. To address this, linear mixed models are widely used, incorporating fixed and random effects into the analysis [162, 163]. Furthermore, mapping eQTLs using single-cell expression profiles enables the identification of cell-type-specific eQTLs in rare cell populations [164]. Tools such as CellOracle [165] and SCENIC+ [166] are also available for inferring gene regulatory networks. The aforementioned single-cell multi-omics integration methods rely on jointly measured multi-omics profiles per cell. However, most available single-cell data across omics modalities have been measured unimodally. To leverage less expensive unimodal technologies for multimodal data integration, the different modalities must first be aligned. This can be achieved using manifold alignment methods [167–170] to infer a lower-dimensional structure within multiple complex datasets. However, this integration task is complicated by the incomplete understanding of the properties of latent biological manifolds [153]. In this case, matched multimodal assays can serve as gold-standard datasets to address these challenges and benchmark integration strategies [171].

Multi-omics data integration across resolutions

As single-cell datasets expand to profile hundreds of patients, studies can correlate single-cell gene expression with data obtained from bulk profiling [172]. Furthermore, integrating spatial and single-cell transcriptomics is crucial for understanding cell-to-cell communication [173]. In this context, we treat bulk RNA and single-cell RNA as separate modalities.

Integrating single-cell gene expression with bulk data modalities can help to identify the cell-type composition of disease-relevant tissues [174]. This can be achieved by deconvolution methods. These

methods often use regression-based techniques, such as non-negative least squares [175] and support vector regression [176], to map bulk gene expression profiles to specific cell types. This allows the identification of cellular targets for treatment and offers a better understanding of disease mechanisms [174]. Furthermore, to better understand interindividual variation, in the Human Lung Cell Atlas, demographic covariates (such as age, sex and body mass index) are modelled on single-cell gene expression with generalised linear mixed models [30]. While scRNA-seq identifies cell subpopulations within the tissue, it does not capture spatial distribution or local networks of intercellular communication [177]. Spatial techniques that localise RNA within tissue, including next-generation sequencing based methods, *in situ* sequencing (high-plex RNA imaging) and *in situ* hybridisation-based methods, do not yet offer single-cell resolution. Thus, integrating single-cell and spatial data is necessary to achieve maximal resolution in tissue. Two primary approaches for integrating scRNA-seq and spatial data are deconvolution and mapping. Like bulk deconvolution described earlier, spatial deconvolution identifies cell subpopulations by separating mRNA transcript mixtures at each capture spot. Methods [178–182] typically require an annotated scRNA-seq dataset with known cell-type markers for deconvolution [172]. Regression-based deconvolution methods merge scRNA-seq data with capture spot data to generate a matrix that profiles capture spots with overlaid scRNA-seq cell subtypes. This matrix is used to identify the scRNA-seq subtype profiles that most accurately explain the composition of each capture spot mixture [177]. Probabilistic distribution models are used to characterise each capture spot by assessing how well each cell type's model fits the transcript distribution of the spot. Recent methods, such as BayesTME [88] and SpatialGlue [183] perform deconvolution without reference to scRNA-seq data. For high-plex RNA imaging data, cluster-based mapping methods [47, 184, 185] integrate scRNA-seq and spatial data into a shared low-dimensional space. Meanwhile, other methods [186–188] create joint embeddings that can be used for downstream analysis, such as identifying spatially variable genes, defining spatial domains and analysing spatial heterogeneity. These methods further enhance the understanding of communication between adjacent cells and position-specific states [189].

Multi-omics to understand chronic respiratory diseases and viral infections

Multi-omics is being used to make significant advances in understanding chronic respiratory diseases by uncovering detailed molecular mechanisms through integrating data from single omics [1]. While multi-omics integration has identified potential biomarkers for chronic respiratory diseases, translating these findings into clinical interventions remains challenging. Variability in patient populations, the complexity of chronic respiratory diseases, and the need for rigorous clinical validation hinder the widespread application of multi-omic-based therapies. However, opportunities exist to refine patient stratification and develop more targeted treatments. Here, we review progress in COPD, asthma, IPF and viral infection including COVID-19, rhinovirus, RSV and influenza viruses (table 2).

COPD

Multi-omics approaches, including transcriptomics, metagenomics, metabolomics, lipidomics and proteomics, have been extensively applied to both pre-clinical COPD animal models and human COPD samples, providing important insights into disease mechanisms and defining therapeutic targets [28, 53, 195, 215–221]. Lipidomic and metabolomics have identified early-stage COPD biomarkers (*e.g.* sialic acid, hypoxanthine, xanthine, methylthioadenosine, adenine, glutathione) enabling personalised medicine strategies such as chronotherapy and improving treatment efficacy [222].

Epigenetic profiling of blood and lung tissues unveiled the effects of smoking in patients with mild-to-moderate COPD. In severe COPD, marked demethylation was identified, showing associations with increased expression of specific transcripts [223]. A combination of ATAC-seq and ChIP was used to elucidate the epigenetic regulation of monocyte extravasation to the lung in COPD by initially identifying the target of interest, PRMT7, applying ATAC-seq and then further validating using the PRMT7 promoter using ChIP-seq data [224].

Proteomics and metabolomics studies associated proteins (*e.g.* fibulin-1, tripeptidyl-peptidase 2) and metabolites (*e.g.* theophylline, hypoxanthine, cadherin-5) with advanced COPD diagnosis, highlighting diagnostic targets and therapeutic potential [195, 225].

Metagenomics and metabolomics have revolutionised our understanding of the lung and gut microbiome in COPD [226–230]. We discovered a disease-associated network in patients with COPD connecting *Streptococcus parasanguinis_B* with N-acetylglutamate and its analogue N-carbamoylglutamate [195]. Others found that hypoxanthine, theophylline, palmitoylethanolamide and CDH5 could identify patients with advanced COPD [231].

TABLE 2 Key examples of multi-omics applications in chronic respiratory diseases and viral infections

First author (year) [reference]	Key findings	Key omics approaches	Relevance
COPD			
Skerrett-Byrne (2021) [53]	Significant shifts in RNA biosynthesis involving HNRNPC and MSI2 identified, with inflammatory modulators like S100A1 validated in human COPD.	Proteomics, transcriptomics	Provides insights into conserved molecular changes in experimental models and human COPD
Schwartz (2023) [190]	Integrated DNA methylation and RNA-seq analysis uncovered novel transcriptomic and epigenetic signatures associated with COPD onset, including enriched binding sites for transcription factors TCF21 and FOSL2/FRA2. Analysed samples from 3 non-COPD individuals, 3 with mild COPD (GOLD stage I), and 5 with moderate to severe COPD (GOLD stage II–IV). Spearman correlation was performed to define associations between gene expression and differentially methylated regions.	Epigenomics, transcriptomics	Highlights the role of epigenetic regulation in COPD onset and progression
Chotirmall (2022) [191]	Azithromycin enhances anti-inflammatory bacterial metabolite production, linking respiratory microbiota to COPD.	Metagenomics, proteomics, metabolomics	Links microbiome changes to inflammation and COPD progression, suggesting therapeutic targets
Lynch (2015) [192]	Multi-omics identified emphysema- and airway-predominant COPD subtypes.	CT imaging, transcriptomics, proteomics	Helps refine COPD classification and potential subtypes for targeted therapies
Li (2025) [193]	Identified 248 early COPD-associated proteins, many involved in inflammation. Found 137 early COPD-associated metabolites indicating early metabolic disturbances.	Proteomics, metabolomics	Highlights early molecular changes in COPD Supports use of proteomic and metabolomic biomarkers for early diagnosis and intervention in chronic respiratory diseases
Yan (2022) [194]	Microbial dysbiosis in COPD with decreased *Prevotella* and tryptophan-derived metabolite IAA. Reduced IAA impairs IL-22 signalling. IAA restoration *via Lactobacillus* reduces inflammation in mice.	Metagenomics, metabolomics, transcriptomics, proteomics	Demonstrates how host–microbe interactions drive COPD pathology Identified IAA and microbial modulation as potential therapeutic avenues
Bowerman (2020) [195]	Identified bacterial species, including *Streptococcus* spp. and members of Lachnospiraceae, correlated with reduced lung function. Untargeted metabolomics revealed a COPD-associated signature comprising lipids, xenobiotics and amino acid related metabolites.	Metagenomics, metabolomics	Highlights the association between gut microbiome/metabolome alterations and COPD, suggesting potential biomarkers and therapeutic targets *via* the gut–lung axis
Budden (2024) [196]	Used proteomics, metabolomics and microbiome analysis to show that faecal transfers and complex carbohydrate supplementation improved COPD by modulating glucose and starch metabolism and altering bacterial abundance (Muribaculaceae, Desulfovibrionaceae, Lachnospiraceae).	Metagenomics, proteomics, metabolomics	Demonstrates the contribution of the gut microbiome to COPD pathogenesis and its potential as a therapeutic target through dietary interventions
Asthma			
Guang (2023) [127]	Integration of clinical, plasma and metagenomic/metabolomic data reveals clinical heterogeneity in allergic asthmatic children.	Metagenomics, metabolomics	Identifies biomarkers for stratified treatment approaches
Abdel-Aziz (2022) [197]	Elevated eicosanoids (11-dehydrothromboxane-B2, prostaglandin-E2) linked to microbiome–inflammation axis, correlating with PTGS2 gene expression.	Metabolomics, transcriptomics, proteomics	Suggests microbiome–inflammation interactions as therapeutic targets
Sunata (2024) [198]	IL-4 stimulation induces IL1RL1-high eosinophils with upregulated cysteinyl leukotriene metabolism (*e.g.* increased LTC4S, GGT5 expression), leading to elevated leukotriene D4 production *via* the STAT6 pathway.	Transcriptomics, proteomics, lipidomics	Identifies a unique eosinophil phenotype linked to type 2 inflammation in asthma and chronic rhinosinusitis, providing novel insights into IL-4-mediated inflammatory pathways and potential therapeutic targets
IPF			
Allen (2017) [199]	Epigenetic drivers in lung fibroblasts identified, including twist transcription factor-1 and forkhead box protein-A1 expression.	Transcriptomics, epigenomics	Sheds light on pathogenic roles of epigenetic modifications in IPF progression

Continued

https://doi.org/10.1183/16000617.0286-2024

TABLE 2 Continued

First author (year) [reference]	Key findings	Key omics approaches	Relevance
Wen (2025) [200]	A multi-omics approach identified 6 genes (ANO9, BRCA1, CCDC200, EZH1, FAM13A, SFR1) as potential therapeutic targets in IPF.	Genomics, transcriptomics	Key therapeutic targets identified for further validation and potential therapy development
Zhang (2024) [201]	Role of Mo-AMs in IPF highlighted through transcriptional and epigenetic profiling.	scRNA-seq, ATAC-seq	Identifies macrophages as targets for fibrotic treatments
COVID-19			
Ng (2021) [202]	Plasma proteomics and metabolomics identified dysregulated complement pathways, suggesting therapeutic potential for complement-targeting interventions.	Proteomics, metabolomics	Reveals complement system dysregulation as a therapeutic target
Ma (2022) [203]	Integration of multi-omics with next-generation sequencing identified Sigma1/Sigma2 modulators and an Nsp16 mutation as potential therapeutic candidates.	Multi-omics, next-generation sequencing	Unveils new antiviral drug targets and potential vaccine candidates
Su (2020) [204]	Blood-based multi-omics revealed immune dynamics, with plasma lipids and cytokines linked to disease severity.	Proteomics, metabolomics	Identifies biomarkers for COVID-19 severity and early intervention
Barh (2020) [205]	Integrated omics identified potential drug repurposing candidates (betamethasone, statins, vitamin D, zinc) for COVID-19.	Transcriptomics, proteomics, host genetics	Provides a framework for repurposing existing drugs and improving treatment strategies
Rhinoviruses			
Djeddi (2024) [206]	Rhinovirus infections exacerbate asthma by triggering immune responses that lead to airway inflammation and hyperresponsiveness.	Transcriptomics, GWAS	Highlights the role of nonciliated epithelial cells in rhinovirus-induced asthma exacerbations
Raita (2021) [207]	Identified endotypes in infants with rhinovirus bronchiolitis, characterised by specific viral profiles, microbiome compositions, and immune responses. Certain endotypes were associated with an increased risk of developing childhood asthma.	Metagenomics, transcriptomics, metabolomics	Highlights the heterogeneity in bronchiolitis and the potential for early identification of infants at high risk for asthma development through integrated omics approaches
RSV			
Huang (2023) [208]	Elevated serum collagen levels suggest airway remodelling, disruptions in lipid metabolism and complement system activation.	Proteomics, metabolomics	Highlights potential therapeutic strategies for managing RSV-induced airway remodelling
Raita (2021) [209]	Identified 4 endotypes of RSV bronchiolitis based on immune responses, microbial and metabolic profiles.	Genomics, transcriptomics, microbiome, metabolomics	Identifies high-risk infants for asthma development, enabling early identification for targeted interventions
Influenza viruses			
Zhu (2023) [210]	Built *H2Flu*, a curated database of human host genes/proteins associated with 14 IAV subtypes plus influenza B/C, a resource for influenza research.	Genomics, transcriptomics, proteomics data	Enables efficient identification and prioritisation of influenza host factors
Riese (2022) [211]	Identified molecular and immune pathway signatures that differentiate responders *versus* nonresponders to seasonal flu vaccines.	Transcriptomics, proteomics, immune profiling	Guides personalised vaccine strategies and better formulation design
Martin-Sancho (2021) [212]	Discovered that IAV M2 protein interacts with TBC1D5 to avoid lysosomal degradation revealing viral autophagy evasion.	Genetic CRISPR screens, transcriptomics, proteomic	Elucidates mechanisms of viral persistence and potential antiviral targets
Platt (2022) [213]	Showed IAV binding to *Streptococcus pneumoniae* alters bacterial physiology and virulence, offering insight into viral–bacterial synergy in pneumonia.	Transcriptomics, proteomics, phenotypic assays	Co-infection with IAV and bacterial pathogens is a major driver of exacerbations in chronic respiratory diseases such as COPD and asthma; this study enhances understanding of mechanisms behind infection-triggered disease worsening
Hu (2022) [214]	Found specific shifts in oropharyngeal microbiota and metabolites linked to disease severity in children with IAV pneumonia.	Microbiome profiling (16S/ shotgun), metabolomics	Potential for noninvasive biomarkers and therapeutic targeting

IPF: idiopathic pulmonary fibrosis; COVID-19: coronavirus disease 2019; RSV: respiratory syncytial virus; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CT: computed tomography; IAA: indole-3-acetic acid; IL: interleukin; IL1RL: IL-1 receptor-like; Mo-AM: monocyte-derived alveolar macrophage; scRNA-seq: single-cell RNA sequencing; ATAC-seq: assay for transposase-accessible chromatin sequencing; GWAS: genome-wide association study; IAV: influenza A virus.

Airway colonisation by *Staphylococcus aureus* was linked to worsened lung function by activating homocysteine through the AKT1-S100A8/A9 axis. In a mouse model, bacteriophage elimination of *S. aureus* restored lung function, suggesting microbiome-modifying therapies as novel strategies to suppress COPD progression [232]. Dysbiosis of the lung microbiome has also been associated with disease progression and exacerbations, with specific microbial signatures associated with neutrophilic or eosinophilic endotypes [75, 187]. Cigarette smoke and COPD were shown to cause pathological changes in the gut that also impacted the lung. The authors then defined the gut microbiome and metabolome differences in human and experimental COPD [195, 196]. Dysbiosis of the lung microbiome has also been linked to disease progression and exacerbations, with specific microbial signatures associated with neutrophilic or eosinophilic endotypes [195, 233]. Faecal microbiome transfers alleviated experimental COPD and associated gut pathology. Cigarette smoke-associated microbiota induced lung inflammation and were associated with disease, and proteomics and metabolomics showed that they had reduced glucose and starch metabolism. Dietary complex carbohydrates improved COPD in mice and patients. Lactobacilli depletion and reduced indole-3-acetic acid levels disrupted interleukin-22 pathways, leading to epithelial cell apoptosis [194]. Furthermore, *S. parasanguinis* B was linked to distinct metabolomic profiles in COPD patients, indicating the potential of targeting both lung and gut microbiomes for therapy [53, 195].

Multi-omics studies have also shed light on adaptive immune dysregulation in COPD. Integrated analyses of large datasets identified immune cell deficiencies, particularly $CD4^+$ T-cells, as predictors of acute exacerbations, suggesting that immune monitoring could improve management [234]. Spatial transcriptomics and computed tomography imaging revealed increased B-cell-related gene expression in severe emphysema, with abnormal B-cell activity associated with emphysema severity [235]. Patients with Global Initiative for Chronic Obstructive Lung Disease 1–2 COPD showed distinct B-cell activation signatures, linking B-cell maturation and antibody production to emphysema development. In addition, blood-based multi-omics identified biomarkers and predictive models combining clinical and protein data, enabling early diagnosis and potential therapeutic targeting of emphysema in COPD [235, 236].

These findings show the value of multi-omics in understanding molecular mechanisms, identifying biomarkers and guiding therapeutic development in COPD. By integrating diverse omics layers, these approaches offer a holistic understanding of COPD pathogenesis, progression, and potential interventions.

Asthma

Asthma has been extensively explored using multi-omics approaches, including transcriptomics, proteomics, genomics and metabolomics, providing insights into its complexity and clinical heterogeneity [215, 282]. Studies like U-BIOPRED and the Severe Asthma Research Program have advanced our understanding of genetic predisposition, disease heterogeneity and treatment responses [120]. A large-scale meta-analysis of global asthma genome-wide association study (GWAS) identified five novel genetic loci and two new associations at known loci, enhancing our understanding of asthma susceptibility and paving the way for personalised therapies [121]. Epigenomic investigations revealed cell lineage specific differences and asthma-associated DNA methylation changes, such as in lymphotoxin-α and tumour necrosis factor genes, suggesting that epigenetic mechanisms may be potential therapeutic targets [122]. A recent study combined eicosanoids, transcriptomics and proteomics to show higher levels of 11-dehydrothromboxane-B2 and prostaglandin-E2 in microbiome-driven asthma, correlating with upregulated PTGS2 gene expression in sputum. This suggests that a microbiome–inflammation axis could guide targeted anti-inflammatory interventions [197]. These findings collectively provide actionable insights into asthma mechanisms, inform precision medicine strategies and identify novel therapeutic opportunities.

IPF

Multi-omics approaches have transformed our understanding of the pathogenesis and therapeutic opportunities in IPF by uncovering novel mechanisms and cellular processes. Genomic studies identified rare and common genetic variants, shedding light on disrupted biological pathways in inherited forms of pulmonary fibrosis, such as telomere biology, surfactant metabolism and immune function, which suggest genetic predispositions that could guide personalised treatments [237, 238].

scRNA-seq has revealed the cellular heterogeneity in IPF lungs, uncovering previously unrecognised fibrotic and immune cell populations, including a $KRT5^-/KRT17^+$ pathogenic epithelial population that contributes to extracellular matrix production, highlighting its potential as a therapeutic target [35, 239]. Novel fibrosis-specific immune, stromal, endothelial and epithelial cell states have been identified, which persist in human fibrosis and transiently appear during lung regeneration in mice, suggesting their crucial and potentially targetable role in disease progression [34, 240–242].

An integrative IPF cell atlas compiling published scRNA-seq datasets has improved our understanding of molecular and cellular relationships in IPF [243]. Combining transcriptomics, epigenomics and proteomics characterised differentially methylated regions, lncRNAs, and their regulation of key mediators such as matrix metalloproteinase-7, revealing epigenetic drivers of disease progression [244].

Integrative studies have identified potential tissue biomarkers, such as butyrophilin-like-9 and plasmolipin, that distinguish clinically relevant molecular subtypes [245]. Blood-based multi-omics from the IPF-PRO Registry revealed two molecular subtypes with distinct prognostic implications; subtype-1 was associated with more severe disease and shorter progression time, providing a framework for risk stratification and personalised care [246]. Multi-modal integration of scRNA-seq and proteomics from bronchoalveolar lavage fluid and plasma identified peripheral protein biomarker signatures reflecting lung cell state changes, which predicted disease status and functional decline [247].

Lastly, combining whole-exome sequencing, bulk RNA-seq and scRNA-seq provides unique insights into genomic and transcriptomic interactions, offering a holistic understanding of IPF [248]. Multi-omics approaches have also facilitated the discovery of numerous therapeutic targets, which have been validated in cells and tissues from IPF patients, supporting the development of targeted therapies. A recent study employed a comprehensive multi-omics approach, integrating genomic and transcriptomic data, to uncover six genes ANO9, BRCA1, CCDC200, EZH1, FAM13A and SFR1 as potential therapeutic targets for IPF [200, 201]. Moreover, a recent study identified TRAF2- and NCK-interacting kinase as a target for fibrosis treatment and used artificial intelligence (AI)-driven drug discovery to identify INS018_055, a small-molecule inhibitor, as a potential therapeutic. INS018_055 was then used to demonstrate significant antifibrotic and anti-inflammatory effects in pre-clinical models and showed promising safety and pharmacokinetic profiles in phase I clinical trials, positioning it as a potential therapy for fibrotic diseases [249].

These findings collectively advance the field by linking molecular discoveries to clinical outcomes, enabling biomarker development, molecular subtyping and the identification of actionable therapeutic targets.

COVID-19

With the COVID-19 endemic and the emergence of long COVID, the development of effective therapies is urgently needed. Omics studies have provided critical insights into how SARS-CoV-2 hijacks host cellular machinery to induce pathogenesis, uncovering pathways and potential therapeutic targets [28, 250]. scRNA-seq revealed that differentiated COPD bronchoepithelial cells exhibit higher susceptibility to infection due to increased viral co-receptor expression and impaired antiviral responses, and exacerbating COPD by disrupting protease balance. This identified co-receptor inhibitors as potential therapies for patients with both COPD and COVID [28].

While single-omics studies have provided valuable insights, they often fail to capture the complexity of COVID-19. The evolving dynamics of the disease, shaped by widespread immunity and the virus's endemicity, demand a multi-omics approach for deeper understanding [251]. Multi-omics analyses of lower airway microbiomes from critically ill COVID-19 patients revealed that high viral loads and impaired adaptive immunity were linked to poor outcomes [203]. They also identified microbial and transcriptomic signatures that could serve as prognostic markers or therapeutic targets. Metagenomics and metatranscriptomics of bronchoscopy samples showed that elevated levels of *Mycoplasma salivarium* and distinct airway transcriptomic profiles were associated with worse clinical outcomes, emphasising the critical role of microbial–immune interactions in severe disease [252].

Integration of multi-omics with next-generation sequencing and reverse genetics has advanced the identification of novel therapeutic targets. Studies revealed Sigma1/Sigma2 modulators as potential antiviral drug targets and identified an Nsp16 point mutation that attenuates SARS-CoV-2 while eliciting protective immunity, suggesting its potential as a vaccine candidate [203, 253].

Blood-based multi-omics investigations have elucidated immune dynamics during disease progression, linking decreases in plasma lipids and amino acids, elevated pro-inflammatory cytokines, and exhausted $CD4^+$ T-cells to severe disease [204]. Circulating cytokines, lipids and metabolites have been identified as biomarkers for COVID-19 severity and hospitalisation risk, enabling better risk stratification and early intervention [254].

By linking these findings to actionable outcomes, such as identifying biomarkers, guiding therapeutic interventions and advancing vaccine development, multi-omics approaches provide a holistic framework to improve the management and treatment of COVID-19.

Rhinoviruses

Rhinoviruses, members of the Picornaviridae family, are small, non-enveloped viruses with single-stranded RNA genomes and are the leading cause of the common cold. These highly contagious viruses primarily infect the upper respiratory tract and are responsible for ∼90% of acute respiratory tract infections, ranging from mild colds to severe lower respiratory illnesses such as pneumonia [255]. Rhinoviruses are common causes of exacerbating conditions like COPD [256] and asthma [257]. They do so by triggering immune responses that lead to airway inflammation and hyperresponsiveness. Airway epithelial cells are central to initiating these responses, and recent studies suggest that modulating epithelial pathways may offer therapeutic potential in managing rhinovirus-induced asthma exacerbations [257]. The application of multi-omics approaches, integrating genomics, transcriptomics, proteomics and metabolomics has significantly advanced our understanding of rhinovirus biology and its interactions with host systems. Notably, a recent study integrating GWAS with transcriptomic profiling identified that rhinovirus infections interact with asthma-associated genetic risk factors predominantly through nonciliated airway epithelial cells. These findings highlight a critical mechanism in childhood-onset asthma and point to nonciliated epithelial cells as potential targets for mitigating virus-induced exacerbations [206].

RSV

RSV is highly contagious and primarily affects the respiratory tract. It is a leading cause of lower respiratory infections, including bronchiolitis and pneumonia, especially in infants, the elderly and immunocompromised individuals. RSV is also linked to an increased risk of wheezing and the development of asthma later in life. Due to its significant global disease burden, particularly in children aged <5 years, RSV is a critical target for vaccine and therapeutic development [258]. This highlights the importance of multi-omics (genomics, transcriptomics, proteomics, metabolomics) in advancing predictive, preventive and personalised medicine for RSV. By providing a comprehensive view of host–pathogen interactions and individual variability, multi-omics can enhance early risk assessment, enable targeted interventions, and inform patient-specific therapies, ultimately improving outcomes and reducing healthcare burdens [259]. A study employing integrated proteomic and metabolomic analyses explored the host responses in children with RSV pneumonia. By analysing serum samples from affected children, healthy controls and those with other infections, the researchers identified several differentially expressed metabolites, with six showing promise as diagnostic biomarkers. Notably, neuromedin N and histidylproline diketopiperazine were validated in an independent cohort. The study also revealed elevated serum collagen levels, suggesting potential airway remodelling, and highlighted disruptions in lipid metabolism and complement system activation. These findings provide valuable insights into RSV pathogenesis and could inform future diagnostic and therapeutic strategies [208]. In another study, a comprehensive multi-omics approach including clinical data, viral profiling, airway microbiome analysis, transcriptomics, and metabolomics was used to identify biologically distinct endotypes in infants hospitalised with RSV bronchiolitis. The authors identified four endotypes, each defined by unique microbial compositions, immune responses and metabolic profiles. One endotype, marked by high interferon responses, IgE sensitisation and co-infection with rhinovirus, exhibited a significantly increased risk of developing asthma by the age of 5 years. These findings emphasise the role of multi-omics (genomics, transcriptomics, proteomics, metabolomics) in supporting predictive, preventive and personalised (3P) medicine in RSV infection management. By capturing a comprehensive picture of host–pathogen interactions and individual variability, multi-omics tools can guide early risk assessment, targeted interventions and patient-tailored therapies, ultimately improving outcomes and reducing healthcare burdens. In addition, they highlight the potential of integrated omics in early identification of infants at high risk for asthma and suggest that targeted preventive strategies could be developed based on these insights [209].

Influenza viruses

Influenza viruses, particularly influenza A and influenza B, are the primary causative agents of seasonal flu in humans. While influenza A viruses (IAV) are responsible for both seasonal epidemics and global pandemics due to their high mutation rate and ability to infect multiple species, influenza B viruses are largely restricted to humans and tend to cause milder, seasonal outbreaks [260, 261]. IAVs are highly contagious enveloped RNA viruses that pose a significant public health challenge. A recent study used a comprehensive multi-omics approach integrating genome-wide CRISPR screens, transcriptomics and proteomics to uncover host factors that restrict IAV replication. This identified TBC1D5, a key regulator of autophagy, as a potent antiviral factor that promotes lysosomal degradation of viral components. Notably, the viral M2 protein was found to bind TBC1D5, disrupting its interaction with Rab7 and thereby evading autophagic clearance, facilitating viral survival and replication. These findings reveal a novel immune evasion mechanism and suggest TBC1D5 as a target for host-directed antiviral therapy [212]. Another multi-omics study examined the basis of reduced vaccine responsiveness in the elderly population by integrating transcriptomics, proteomics, cytokine profiling and immune phenotyping. It identified distinct baseline immune and molecular signatures that predicted vaccine responsiveness. Individuals who

responded well exhibited upregulation of genes involved in B-cell activation, interferon signalling and cellular metabolism, whereas nonresponders had elevated expression of inflammatory and stress pathways. These insights highlight the heterogeneity of immune responses in ageing populations and offer potential biomarkers and therapeutic targets for enhancing influenza vaccine efficacy [211].

Future directions for the application of multi-omics to chronic respiratory diseases and viral diseases

There are several promising areas that should be explored to improve patient outcomes. First, integrating multi-omics data from chronic respiratory disease patients could help capture dynamic disease progression over time, allowing for more precise identification of biomarkers and therapeutic targets. This approach could reveal subtle molecular changes that single-omics studies may miss, aiding earlier diagnosis and more personalised treatment. For viral diseases, multi-omics can offer a deeper understanding of viral–host interactions, immune responses and long-term consequences [262]. Moreover, advancing longitudinal multi-omics studies can provide a more comprehensive picture of disease progression over time, especially for conditions like long COVID, and other post-viral syndromes, by integrating genomic, transcriptomic and microbiome data. These efforts are crucial to identify biomarkers that predict disease outcomes, enabling the development of targeted therapeutic strategies [263]. By combining genomic, transcriptomic, proteomic and microbiome data, researchers can uncover mechanisms of immune evasion, viral replication and host susceptibility. This would help to develop more effective treatments, but also identify patients at higher risk of severe outcomes. Combining multi-omics with clinical and environmental data can further refine precision medicine approaches, facilitating better diagnosis and more effective treatments. As computational tools and validation methods improve, these multi-omics approaches will play an increasingly vital role in developing personalised therapies for both chronic respiratory diseases and viral diseases [30, 264, 265].

Summary and challenges

Human studies face key limitations: they often rely on samples taken at a single time point, use lung tissue from end-stage disease (making it difficult to study how the disease develops and progresses), and analyse peripheral fluids (like blood or sputum) that do not fully capture what is occurring in the lungs [266]. These limitations are alleviated by the use of animal models that replicate key features of human disease, enabling longitudinal assessment of the development, progression, exacerbations and treatment responses using comparable exposure methods and systemic outcomes [5, 45, 53, 267]. Findings from animal models should be validated and translated in complementary human *ex vivo* lung models, including organoids and precision-cut lung slices [268–273]. They should be combined with spatial omics technologies to enable longitudinal assessment of disease-related changes. In our recent work, we used scRNA-seq and spatial proteomics to identify five neutrophil subtypes in COPD, of which only one was disease-associated [218]. Using a validated mouse model, we traced the emergence of these pathogenic neutrophils from the bone marrow to the bloodstream and into the lung, revealing a systemically driven disease mechanism with therapeutic potential. These insights cannot be obtained through *ex vivo* or *in vitro* models [274].

Challenges remain in handling heterogeneous clinical metadata, variable sample and file sizes, missing data, batch effects and insufficient sequencing depth. Effective integration of different omics layers requires detailed characterisation of patient populations to address variability in disease phenotypes. Variability across independent cohorts due to differences in patient demographics, disease severity and data processing protocols further complicates data harmonisation. In addition to these technical and biological sources of variability, the extent and quality of sample and metadata collection play a crucial role in the utility of multi-omics data. Many studies lack standardised clinical annotations, longitudinal sampling, or representation across diverse demographic groups. Enhanced collection of detailed metadata including comorbidities, treatment history, environmental exposures and disease progression along with the inclusion of a broader range of biological samples (*e.g.* bronchoalveolar lavage fluid, biopsies, nasal swabs and time-series samples) will greatly enhance translational potential. Establishing standardised protocols and harmonised metadata frameworks across studies is essential to enable meaningful integration and interpretation of datasets in clinical research. However, this variability can also contribute to improved generalisability by reflecting a wider range of disease manifestations, thus enhancing the relevance of findings across diverse patient populations. Disparities in omics layers and sample sizes can limit statistical power and generalisability, while high resource demands for data integration and potential cohort selection bias may restrict the applicability of findings to the broader chronic respiratory disease population [86, 275]. Much of this will be resolved by continued enhanced high-quality data collection.

Data cleaning, filtration, quality control and benchmarking before integrating is also essential. Improved analysis will be facilitated by acquiring more comprehensive and accurately curated patient metadata. Omics data also originates from various sources; therefore, benchmarking and establishment of gold standard methods of data collection, terminology and formatting are critical. To address these issues,

universal platforms are being developed to enable access and integration of data from different groups (*e.g.* Human Lung Cell Atlas) [30, 264, 265], which reduces the need to run new experiments. Moreover, long-read sequencing may be a game-changer in genetic analysis, reducing costs while capturing a more complete genomic picture enhancing our understanding of genetic heterogeneity [276].

Various AI and statistical methods have been developed to address the challenges of integrating multi-omics datasets. These approaches require high-performance computing, advanced software, and rapidly evolving machine learning and AI technologies [277]. Different programming languages, such as Python, R and Perl, are used to create new integration tools [277, 278]. However, no gold-standard workflows have yet emerged, making it difficult to achieve consistent and reproducible analyses [279]. Data protection and legal restrictions further complicate cross-jurisdiction studies [280]. Swarm learning has emerged as a solution, integrating data while maintaining confidentiality through cutting-edge computing and blockchain-based peer-to-peer networking for onsite analysis [281].

In summary, multi-omics provide new insights into the molecular mechanisms of chronic respiratory diseases, improving our understanding of disease heterogeneity and revealing potential therapeutic targets. Techniques like transcriptomics, proteomics, epigenomics, metagenomics and metabolomics produce detailed maps of transcriptional, protein, microbiome and metabolic changes. Longitudinal studies, combined with multi-omics integration are essential to identify biomarkers and therapeutic targets. Despite these advances, translating molecular insights into effective treatments remains challenging due to the need for further validation, the high costs of multi-omic analyses, and the lack of robust computational tools. These obstacles, along with the complexity of chronic respiratory diseases, hinder the optimal use of multi-omics data in clinical settings. However, with improvements in computational tools and continued validation, multi-omics holds the potential for more personalised and targeted treatments in the future [263].

Advancing multi-omics: what is next?

Opportunities in multi-omics research

- There is a substantial opportunity to improve the reliability and effectiveness of integrative multi-omics methods. By refining computational tools and analytical frameworks, researchers can enhance the robustness and clinical relevance of multi-omics analyses.
- Addressing batch effects and data heterogeneity remains a key challenge, but also an opportunity. Emerging computational solutions have promise, and continued development of advanced integration techniques will significantly improve data harmonisation and interpretation across studies.
- Reducing the costs and logistical barriers of multi-omics research will enable larger, more inclusive studies. This will increase statistical power, broaden the diversity of study populations, and enhance the generalisability and clinical impact of findings.
- Improving the collection of comprehensive metadata including comorbidities, treatment history, environmental exposures and disease progression alongside more diverse biological samples, represents a major opportunity to boost the translational potential of multi-omics data and bridge the gap between bench and bedside.

Strategic recommendations for advancing multi-omics research

- Promote open data sharing: depositing multi-omics data into globally accessible platforms is essential for enabling large-scale, cross-disciplinary analyses. This practice fosters international collaboration and accelerates the discovery of deeper, more comprehensive insights into complex diseases including chronic respiratory diseases.
- Establish standardised protocols: standardising data collection protocols and harmonising metadata frameworks across studies is crucial to improve dataset integration. It will reduce variability, enhance reproducibility and ensure consistent interpretation of results in clinical and translational research.
- Foster multidisciplinary collaboration for endotype discovery: understanding and classifying disease endotypes is vital for developing accurate diagnostics and targeted therapies. Achieving this requires close collaboration between scientific, clinical, and industry experts. The Horizon Europe MSCA Doctoral Network “RESPIRE-EXCEL” aims to train future leaders in translational medicine. It focuses on identifying disease mechanisms, developing predictive biomarkers, detecting individual biomarker activity, designing endotype-specific treatments, and advancing precision medicine strategies for sustained disease remission.

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