

Advancements in omics technologies: Molecular mechanisms of acute lung injury and acute respiratory distress syndrome (Review)

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Abstract. Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is an inflammatory response arising from lung and systemic injury with diverse causes and associated with high rates of morbidity and mortality. To date, no fully effective pharmacological therapies have been established and the relevant underlying mechanisms warrant elucidation, which may be facilitated by multi-omics technology. The present review summarizes the application of multi-omics technology in identifying novel diagnostic markers and therapeutic strategies of ALI/ARDS as well as its pathogenesis.

Contents

1. Introduction
2. Multi-omics for the discovery of biomarkers for ALI/ARDS
3. Integration of multi-omics
4. Single-cell sequencing identification of novel cellular subpopulations in ALI/ARDS
5. Conclusion

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1. Introduction

Acute lung injury (ALI) is characterized by a marked decrease in lung function due to non-cardiac inflammatory injuries (1). Its more severe form, acute respiratory distress syndrome (ARDS), is pathologically characterized by widespread alveolar damage, increased lung vascular permeability and diffuse alveolar edema (2). These changes lead to substantial alveolar flooding and subsequent hypoxemia, compromising oxygen exchange (3,4). In clinical practice, accurately diagnosing ALI/ARDS and determining its severity can be challenging, especially due to individual variability between patients. To improve treatment outcomes, standardizing assessments for pulmonary and extrapulmonary involvement in ARDS is key, ensuring that each patient receives the most suitable and effective treatment (5). The Berlin Definition provides key diagnostic criteria for ARDS, including timing (onset within 1 week of a known clinical insult or new or worsening respiratory symptoms), chest imaging (bilateral opacities on imaging that are not fully explained by effusion, lobar/lung collapse or nodules), origin of edema (respiratory failure not fully explained by cardiac issues or fluid overload; objective assessments such as echocardiography may be required to rule out hydrostatic edema if no ARDS risk factor is present) and oxygenation (6). In addition to these tests, imaging is widely applied for diagnosis. In the middle and late stages of ARDS, chest radiographs detect blurred edges, bilateral patchy appearance and fusion into large patchy ground glass or solid infiltrating shadows. Pulmonary interstitial fibrosis is typically visualized at a later stage. Computed tomography (CT) technology can further improve the accuracy of examination, however the application of CT is limited by the high doses of radiation and risk of transport of critically ill patients. Meanwhile, chest radiography and CT have limited ability to identify early lung lesions. Lung ultrasonography can be employed for the auxiliary examination of ARDS. This technique is widely used owing to its convenience, safety and ability to dynamically evaluate lesions. However, the relatively low specificity and technical

requirements increases the probability of a false positive. In addition, Swan-Ganz catheterization, as an invasive adjunct, is used for the diagnosis of ARDS (7,8). However, due to damage incurred to patients and further complications caused by this technology, it is presently not used as a routine examination method (9). Diagnostic criteria for ARDS, such as increased pulmonary vascular permeability and diffuse alveolar injury, are difficult to determine in clinical practice. Meanwhile, it is often a challenge for clinicians to diagnose ALI/ARDS and assess the severity of the condition due to issues such as individual heterogeneity of patients (10). Therefore, there is need to standardize the assessment of pulmonary and extrapulmonary involvement in ARDS to ensure patients can receive appropriate and effective individualized treatment (5). At the pathophysiological level, alveolar-capillary barrier destruction, abnormal pulmonary inflammation, oxidative stress and micro-circulation disturbance occur during ALI/ARDS (11-13). These hallmarks of pathogenesis reveal the changes of the molecular structure. In addition, the biomarkers found in the pathological process are summarized in Table I. Combining insights from biomarkers and hallmarks provides a more individualized and adaptive approach to disease treatment, yielding better outcomes and minimal side effects.

In the United States, the mortality rate of ALI/ARDS 38.5-41.1%, and nearly 80% in individuals aged ≥ 80 years (24-26). Currently, the standard treatment approach focuses on mechanical ventilation with lung-protective strategies and addressing underlying causes (27). In the absence of targeted therapy, ventilation strategies emphasize low tidal volumes (6 ml/kg) and the lung-protective ventilation strategy, which involves maintaining low airway pressure (<30 cm H₂O), applying moderate positive end-expiratory pressure and allowing a controlled increase in Partial Pressure of CO₂ to enhance alveolar ventilation and oxygenation (28). However, high airway pressures can lead to complications such as pneumothorax, interstitial emphysema and ventilator-induced lung injury due to barotrauma from excessive pressure to the lungs during mechanical ventilation (29,30). Moreover, in the advanced stages of ALI, where lung tissue becomes more consolidated, mechanical ventilation may lose its effectiveness. Beyond the immediate clinical challenges of ALI/ARDS, long-term impacts include psychological trauma and financial strain on patients and families (31). Therefore, an early intervention is required to prevent and slow the progression of ALI/ARDS and new techniques are needed to improve diagnostic biomarker sensitivity and identify novel therapeutic targets.

Recent advances (32-35) in single-cell and spatial omics technologies have transformed understanding of cellular subtypes and functional states. However, because cells are complex entities containing DNA, RNA, and proteins, no single omics technology can fully capture the dynamic pathological changes occurring throughout a cell life cycle. Therefore, to determine the transcriptional regulatory mechanisms of heterogeneous genes involved in ALI/ARDS treatment, multi-omics approaches must be integrated at the single-cell level. Single-cell multi-omics technology enables simultaneous measurement and analysis of diverse molecular types within the cell, yielding greater biological insights than analyzing each molecular layer separately in different cells. Multi-omics technologies are becoming increasingly

prevalent in ALI/ARDS research, offering insight into the underlying pathological processes (17,36-38). Recent advances in single-cell multi-omics allow assessment of cellular heterogeneity, advancing understanding of molecular and cellular dynamics in ALI/ARDS (39,40). This facilitates the exploration of interactions among histological layers, offering a systematic view of cell states and fates and augmenting understanding of ALI/ARDS onset and progression (41). Moreover, single-cell multi-omics technology allows quantitative assessment of dynamic interactions among biological components. Investigating these processes at the single-cell level facilitates recording, modeling and designing of large genomic datasets (42). Concurrently, progress in genomics, proteomics, peptidomics, metabolomics and related fields, including nanotechnology, bioinformatics and antibody chip technology, offers methods for rapidly identifying and screening biomarkers, thereby boosting clinical diagnosis and treatment options. The integrated application of multi-omics enables investigation of ALI/ARDS pathogenesis and drug targets (Fig. 1) (43).

Continuous research yields large amounts of data arising from diverse sources. To meet the increasing demand for personalized data processing, artificial intelligence (AI) is being developed and applied as an effective and reliable solution. Optimized data visualization functionality facilitates analysis and is time-saving (44). In addition, by integrating data from various sources, a more comprehensive understanding of disease complexity and novel therapeutic targets and strategies may be achieved. However, medical data mining continues to face challenges, such as disease diversity, heterogeneity of treatments and outcomes and complexity in collecting, processing, and interpreting data (45).

Studies employing omics methods have identified racial- and sex-based genetic differences in various diseases (46,47), highlighting disparities in disease susceptibility and prevalence. These genetic variations contribute to differences in conditions such as essential hypertension (48), ovarian cancer (49) and pulmonary nodules (50). A study analyzing data from nearly 40 million ARDS-associated deaths demonstrated the influence of genetic variations on ARDS mortality rates, with African American males experiencing the highest mortality (51). Moreover, through a comprehensive literature review, Flores *et al* (52) observed a positive association between genetic variations and susceptibility to ALI/ARDS, as well as its outcomes, primarily through association studies. These findings emphasize that genetic differences play a crucial role in the clinical management and diagnosis of ALI/ARDS, offering new insights into the underlying pathophysiology. Thus, ALI/ARDS results from complex interactions between genetic and non-genetic factors. This condition exhibits high heterogeneity, influenced by individual genetic differences, cell-cell interaction and fluctuations in internal and external environments. These factors regulate lung inflammation and affect the incidence and progression of ALI/ARDS (53). Advancements in multi-omics, especially single-cell multi-omics, provide more detailed understanding of the regulatory and causal associations between genetic factors and cellular functions in the development and progression of ALI/ARDS.

From the perspective of multi-omics technology, the present review systematically summarizes and reviews

Table I. Biomarkers of ALI/ARDS therapy.

First author, year	Biomarker	Samples	Results	(Refs.)
Aisiku <i>et al</i> , 2016	IL-6, IL-8	Plasma	IL-6 and -8 are associated with inflammation	(14)
Tang <i>et al</i> , 2024	CD274	M1 macrophages	CD274 is a negative regulator of macrophage M1 polarization and pro-inflammatory cytokine secretion	(15)
Zhao <i>et al</i> , 2023	NETs	Airways	NET-mediated inflammatory lung injury may serve as a new therapeutic target for ARDS/ALI	(16)
Cui <i>et al</i> , 2022	miR-29a-3p	Plasma and lung tissue	miR-29a-3p is a potential target for improving ALI/ARDS	(17)
Wang <i>et al</i> , 2023	GPR84	Neutrophils from BALF	Activation of GPR84 induces production of reactive oxygen species by neutrophils and suggests GPR84 as a potential drug target for ALI	(18)
Gong <i>et al</i> , 2023	HP, LTA4H, S100A9, SAA1, SAA2 and SERPINA3	Lung tissue and blood	HP, LTA4H, S100A9, SAA1, SAA2 and SERPINA3 proteins in the blood are sensitive and non-invasive biomarkers associated with pathological changes in the lung and pathological changes in blood and may serve as targets for early detection and treatment of ARDS, particularly in the hyperinflammatory subphenotype	(19)
Korde <i>et al</i> , 2023	miR-1	Endothelial cells	miR-1 is a cytoprotective coordinator of the endothelial response to acute injury and has prognostic and therapeutic potential	(20)
Wang <i>et al</i> , 2024	VCAM1	Endothelial cells	Endothelial extracellular vesicles expressing VCAM1 promote sepsis-associated ALI by targeting and reprogramming monocytes, providing potential novel targets and strategies for precision treatment	(21)
Helou <i>et al</i> , 2023	LAIR-1	Macrophages	LAIR-1 may serve a role in the pathogenesis of ALI/ARDS by regulating macrophages, thereby providing a basis for novel therapeutic targets	(22)
Park <i>et al</i> , 2023	miR-6238	Lung epithelial cells and macrophages	miR-6238 is a novel regulator of lung inflammation and putative biomarker to distinguish PM-induced from LPS/PTG-mediated ALI	(23)

ALI, acute lung injury; ARDS, Acute respiratory distress syndrome; PD-L1, Programmed cell death ligand 1; NET, Neutrophil extracellular trap; miR, MicroRNA; GPR84, G protein-coupled 84; BALF, Bronchoalveolar lavage fluid; HP, Hemoperfusion; LTA4H, Leukotriene A4 hydrolase; S100A9, S100 calcium binding protein A9; SAA1, Serum amyloid A1; SERPINA3, Serine protease inhibitor clade A member 3; VCAM, Vascular cellular adhesion molecule; LAIR, Immunoglobulin-like receptor; PM, Particulate matter; LPS, Lipopolysaccharide; PTG, Post Traumatic Growth.

the pathological mechanism and targets of ALI/ARDS. To the best of our knowledge, the present study is the first to review single-cell-based ALI/ARDS (such as the detection of heterogeneous genes) and multi-omics disease research and comprehensively summarize integration technology of multi-omics, as well as the association between these genetic susceptibility targets and ALI/ARDS pathogenesis (Table II), providing a foundation for further investigation into the mechanisms underlying this condition.

2. Multi-omics for the discovery of biomarkers for ALI/ARDS

Numerous biomarkers and therapeutic targets have been used in diagnosis and treatment of ARDS (Table I). Current studies typically use samples of serum and lung tissues from biopsy

and bronchoalveolar lavage fluid (BALF) to identify molecular biomarkers for ALI/ARDS (82,83). Lung tissue biopsy induces potential complications and increases the infection rate, hence liquid samples are used for serial sampling over time. Certain biomarkers tailored to specific clinical needs have shown potential for future clinical application, as shown in Table I, while high-throughput technologies minimize selection bias and offer more comprehensive assessment of biomarkers.

Genomics for ALI/ARDS. Genomics, as the earliest and most established omics technology, serves a foundational role in the omics field. Genetic susceptibility of diseases and large-scale data analysis facilitate disease detection. With continuous advancements in genomics technology and understanding of the pathogenesis of ALI/ARDS, numerous genes associated with risk and severity of ALI/ARDS have been identified (84),

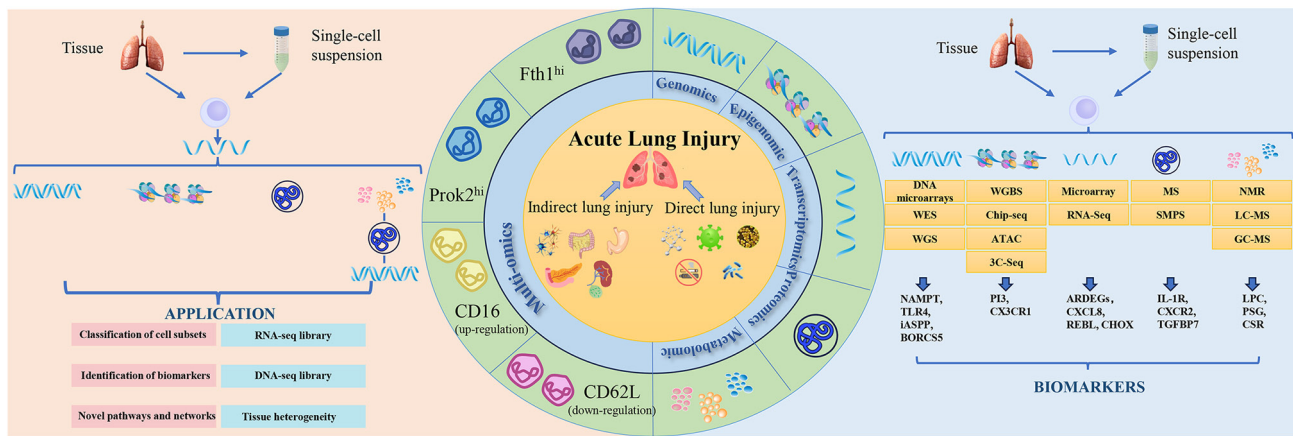


Figure 1. Application of single cell multi-omics technology in the pathogenesis of acute lung injury/acute respiratory distress syndrome. Seq, Sequencing; Fth1^{hi}, ferritin heavy chain 1 Gene; Prok2^{hi}, Prokineticin 2; WES, Whole exome sequencing; WGS, Whole Genome Sequencing; WGBS, Whole Genome Bisulfite Sequencing; ATAC, Assay for Targeting Accessible; 3C, Chromosome Conformation Capture; MS, Mass Spectrometer; SMPS, Switched-mode Power Supply; NMR, Nuclear Magnetic Resonance Spectroscopy; LC-MS, Liquid Chromatograph Mass Spectrometer; GC, Gas chromatography; NAMPT, Nicotinamide phosphoribosyl transferase; iASPP, Protein phosphatase 1 regulatory subunit 13 like Gene; TLR, Toll-like receptor; BORCS, BLOC-1 Related Complex Subunit; PI3, Peptidase inhibitor 3; CXCR1, C-X-C Motif Chemokine Receptor 1; ARDEG, Autophagy-related differentially expressed genes; CXCL8, Interleukin-8; (REBL), RELB Proto-Oncogene, NF-κB subunit; CHO, Cholesterol oxidase, Microorganism; IL-1R, Interleukin-1 Receptor; TGFβ, Transforming growth factor-β; LPC, Lysophosphatidyl choline; PSG, Polysaccharide; CSR, Clinical Study Report.

such as angiopoietin-2 (85) and pre-elafin (PI3) (86). These genes, which are associated with susceptibility to ARDS, provide understanding of its pathogenesis and contribute to the identification of new biomarkers and therapeutic targets. Gene-based therapy is becoming more prominent in clinical practice (87-89). In recent years, studies have implicated ferroptosis in the pathogenesis of ALI/ARDS by promoting the accumulation of reactive oxygen species (90,91). Studies (54,59) have reported genetic targets using both candidate gene and genome-wide association study (GWAS) approaches, including nicotinamide phosphoribosyl transferase, toll-like receptor 4 (TLR4), myosin light-chain kinase (MYLK) and macrophage migration inhibitory factor (59). Genomic methods such as DNA microarray and reverse transcription (RT)-PCR confirmed that the inhibitor of apoptosis-stimulating protein of p53 protects against ALI by inhibiting ferroptosis, thereby mitigating oxidative stress via the nuclear factor erythroid 2-related factor 2 (Nrf2)/hypoxia-inducible factor α /transferrin signaling pathway (54). These studies (67,92) offer insights and potential therapeutic avenues for clinical treatment (). Furthermore, through meta-analysis and GWAS of ARDS, Guo *et al* (67) identified a novel susceptibility locus with two annotated genes BLOC-1 Related Complex Subunit 5 and Dual specificity phosphatase 16. These genes are involved in immune-inflammatory processes and serve as functional genetic predictors of susceptibility to ARDS.

In addition, the mechanism by which microRNAs (miRNAs or miRs) contained in exosomes participate in intercellular communication and serve an immunomodulatory role in ALI/ARDS has investigated: Shen *et al* (93) found that miR-125b-5p is enriched in exosomes of adipose-derived stem cells (ADSCs) through miRNA microarrays and analysis of databases such as miRDB, miRtarbase, starBase and TargetScan (93). In Cecum Ligation And Puncture (CLP)-induced sepsis models (Six-to eight-week-old male healthy BALB/C mice), ADSC exosomes can alleviate lung tissue damage and reduce mortality rates by significantly

increasing expression of Nrf2 and glutathione Peroxidase 4 (GPX4) (94,95). miR-125b-5p in ADSC exosomes can alleviate inflammation-induced pulmonary microvascular endothelial cell(PMVEC) ferroptosis by regulating the Keap1/Nrf2/GPX4 pathway, thereby improving ALI in sepsis (93). Consistently, Ma *et al* (96) synthesized a novel engineered exosome (N-exo) and found that N-exo containing miRNA 182-5p significantly improved ALI *in vivo* and *in vitro* by targeting the NADPH oxidase 4/Dynamin-related protein 1/NLRP3 signaling pathway (96). Additionally, Fan *et al* (97) used Illumina HiSeq 2500 to sequence benzo(a)pyrene (BaP)-associated miRNAs in plasma samples and screened differentially expressed miRNAs; hsa-miR-122-5p/Tumor protein 53 (TP53) axis regulates WNT5A through the non-canonical Wnt signaling pathway, thereby participating in BaP-induced lung epithelial injury (97). Wang *et al* identified and validated gene expression changes associated with pathogenesis of hemorrhagic shock-induced ALI/ARDS, including mRNA, miRNA, long non-coding (lnc)RNA and circular (circ)RNA, through high-throughput sequencing analysis and RT-quantitative (q)PCR; regulatory networks of lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA may serve a key role in pathological processes, such as NOD-like receptor signaling pathway, JAK/STAT signaling pathway, cytokine/cytokine receptor interaction and so on (98). Whole exon sequencing refers to sequencing of all exons of a gene, although the exon region only accounts for ~1% of the whole genome and contains 85% of the disease-causing mutations. Therefore, whole exon sequencing has contributed toward understanding individual genomes to facilitate development of personalized treatment and prevention strategies. Compared with whole genome sequencing, its cost and technical requirements are lower (48). There have been recent reports on clinical cases using this technique to verify the metastatic origin of lymph node cancer cells in multiple types of primary lung cancer (99,100). In addition, Zhang *et al* (101) used whole exon sequencing to reveal diverse enriched pathways (such as p53/SLC7A11-cystine

Table II. Omics applications in ALI pathogenesis.

First author, year	Technology	Data type	Results	(Refs.)
Du <i>et al</i> , 2021	GWAS, RNA-seq, Mendelian randomization analysis	DNA, mRNA	Identification of a novel susceptibility locus in ARDS, encompassing BORCS5 and DUSP16. These genes, implicated in immune-inflammatory processes, serve as functional genetic predictors of susceptibility to ARDS	(38)
Jiang <i>et al</i> , 2015	DNA microarray	DNA	Genomic approaches, including DNA microarray analysis, were used to validate the inhibitory role of iASPP in ferritin deletion, which mitigates oxidative stress via the Nrf2/HIF-1/TF signaling pathway, underscoring the protective effect of iASPP against ALI	(54)
Klein <i>et al</i> , 2015	Droplet-based RNA-seq	mRNA, DNA	Mouse embryonic stem cells were used to observe structure and the heterogeneous onset of differentiation following leukemia inhibitory factor withdrawal.	(55)
Xu <i>et al</i> , 2017	Isotope tagging for relative and absolute quantification	Protein	Hyaluronic acid mitigates ARDS by suppressing cytokine storm, enhancing alveolar barrier permeability and restoring the balance of the proteomic environment of the alveolar exudation phase disorderhomeostasis to the disrupted proteomic environment during the exudative phase	(56)
Huang <i>et al</i> , 2017	GWAS	DNA	Pulmonary expression of KLF2 is reduced in acute lung injury, while KLF2 can indirectly activate Rac1 in HMVECs through the RAPGEF3/EPAC1 pathway, thereby promoting endothelial barrier function.promotes endothelial barrier function, indirectly activating Rac1 in HMVECs via the RAPGEF3/EPAC1 pathway	(57)
Montoro <i>et al</i> , 2018	Plate-based scRNA- seq, droplet-based scRNA-seq,	DNA, mRNA	Identification of novel cell types within the mouse tracheal epithelium, offering insight into potential treatment for cystic fibrosis and understanding of lung biology	(58)
Lynn <i>et al</i> , 2019	GWAS, mRNA microarray	DNA, mRNA	Identification of genetic targets, including NAMPT, TLR4, MYLK and MIF	(59)
Zhang <i>et al</i> , 2019	DNA microarrays	DNA, mRNA	Multiple hypermethylation sites in PI3 and CX3CR1 are associated with the downregulation of gene expression	(60)
Adrover <i>et al</i> , 2020	LC-MS	Protein	Cell-intrinsic program driven by the receptor CXCR2 and circadian regulators decreases inflammation and protein stores by altering the neutrophil proteome	(61)
Li <i>et al</i> , 2020	UPLC-triple- TOF/MS	Metabolites in serum	PSG exhibits a protective effect against ALI by preserving lung histology and decreasing levels of pro-inflammatory cytokines and nitric oxide in both serum and lung tissue	(62)
Hu <i>et al</i> , 2020	UPLCQTOF-MS	Metabolites in plasma	CSR regulates abnormal sphingolipid metabolic pathways. WSR-mediated treatment of ALI primarily involves reversal of abnormalities in retinol and tryptophan metabolic pathways	(63)
Boyd <i>et al</i> , 2020	Meta-analysis, scRNA-seq, spatial transcripto mics	DNA, mRNA	Distinct fibroblast activation states are triggered by respiratory viral infection and serve a role in immunopathology. Targeting the extracellular matrix protease activity of these fibroblasts may present a promising therapeutic avenue for preserving lung function and enhancing outcomes in severe respiratory infection	(64)
Wang <i>et al</i> , 2021	RNA-seq, LC-MS/MS, snRNA-seq	RNA, Protein	Comprehensive cellular and molecular profiles of the lung of patients with COVID-19 provides insights into SARS-CoV-2-related lung injury and facilitates the identification of biomarkers and development of symptomatic therapy	(66)
Guo <i>et al</i> , 2021	Proteomics LC-MS/MS, RNA-seq	Protein RNA	Quantitative redox proteomics methods demonstrate FABP5 is significantly more highly expressed in macrophages than the top five glutathione-based proteins in the metabolic process, suggesting S-glutathionylation of FABP5 is involved in the pathogenesis of ALI by modulating macrophages	(67)

Table II. Continued.

First author, year	Technology	Data type	Results	(Refs.)
Jiang <i>et al</i> , 2020	scRNA-seq	DNA, mRNA	In monocyte clusters of patients with ARDS, expression of SOCS3 is downregulated, which coincides with pro-inflammatory traits and the upregulation of IFN-induced genes, including RAB11A. RAB11A inhibits exocytosis of neutrophils, particularly in CD16 ⁺ cells	(65)
Dong <i>et al</i> , 2021	GSMR, Proteomics	Protein	Plasma IGFBP7 is a novel protein target implicated in the pathogenesis of platelet dysfunction in ARDS	(68)
Wendisch <i>et al</i> , 2021	scRNA-seq, Pproteomics	Proteomics, mRNA, DNA	SARS-CoV-2 induces pro-fibrotic reactions in macrophages, resulting in notable fibroproliferative ARDS	(69)
Bradic <i>et al</i> , 2022	Genomics	DNA	In a methylation profile of patients with COVID-19 and ARDS, probes targeting COVID-19 predictors, including genes associated with interferon regulation and viral response, are hypomethylated, suggesting increased gene expression during severe infection	(70)
Nan <i>et al</i> , 2022	UHPL-CMS/MS	Metabolites in plasma	Non-targeted metabolomics identified myristoyl lysophosphatidylcholine in the plasma of patients with pneumonia; its concentration is inversely associated with the disease severity	(71)
Whitney <i>et al</i> , 2022	scRNA-seq	DNA, publicly available genomic databases	In pediatric ARDS, scRNA-seq offers great potential for identifying cell types and markers in genes from multiple affected tissues and circulating immune cells. which lacks targeted therapy due to unclear pathobiology, scRNA-seq identifies comprehensive, unbiased pathophysiological mechanisms, paving the way for targeted therapy	(72)
Wang <i>et al</i> , 2022	scRNA-seq, SMART-seq	DNA, mRNA	Distinct neutrophil populations (Fth1 ^{hi} and Prok2 ^{hi} Neu) were identified in LPS-induced ALI mouse lung, suggesting the potential role of Fth1 ^{hi} Neu in pathology and prognosis	(73)
Tang <i>et al</i> , 2023	Genomics, KEGG analysis	DNA	Targeting RUNX1-dependent mitophagy alleviates AT2 damage and pulmonary inflammation in ARDS. SARS-CoV-2 exploits mitophagy for survival, therefore the role of RUNX1 in mitophagy may offer insight into ARDS associated with SARS-CoV-2 infection	(74)
Lai <i>et al</i> , 2023	scRNA-seq	mRNA	Ectopic expression of PRMT4 protein can trigger significant lymphocyte death via cell death signal transduction mediated by caspase 3. PRMT4 can be suppressed by short hairpin RNA, blocking the activation of caspase 3 induced by LPS. This highlights PRMT4 as a potential therapeutic target for sepsis.	(75)
Li <i>et al</i> , 2024	snRNA-Seq	RNA	ARDS-associated DEGs participate in cellular responses to DNA damage stimuli, inflammatory reactions and cellular senescence pathways	(76)
Gong <i>et al</i> , 2024	Proteomics RNA-seq	DNA, mRNA	Exosomal TNC binds TLR4 on macrophages, leading to increased production of reactive oxygen species, subsequent mitochondrial damage, activation of the NF-κB signaling pathway and the induction of DNA damage response	(77)
Xia <i>et al</i> , 2024	Transcriptomics	mRNA, sgRNA	GWIT-iCPPs promote heart and lung development. Administration of iCPP-derived exosomes directly into the lung induces dose-dependent alleviation of LPS-induced ALI in mice. This effect is achieved through decreased lung inflammation, enhancement of endothelial function and restoration of both capillary endothelial cells and the epithelial barrier	(78)
Liu <i>et al</i> , 2024	miRNA-seq, Proteomics	miRNA	Mesenchymal stem cell-derived exosomes treat ALI by inhibiting alveolar macrophage apoptosis and decreasing inflammatory response via pyroptosis-targeted miRNA and delivery of immunomodulatory proteins via exosomes	(79)

Table II. Continued.

First author, year	Technology	Data type	Results	(Refs.)
Pollenus <i>et al</i> , 2024	scRNA-seq, DEG analysis	DNA, mRNA	Identification of endothelial cell killing and resolution pathways in an experimental mouse model of malaria-associated ARDS. MultiNicheNet interaction analysis identified significant alterations in ligand-receptor interactions during disease remission	(80)
Mitsuyama <i>et al</i> , 2024	scRNA-seq, miRNA-seq	mRNA, miRNA	miRNAs regulated the PD-1 and PD-L1 cancer immunotherapy pathway and Th2 pathway through miRNA interference action of mRNA. Integrated analysis of mRNAs and miRNAs showed that T cells are dysfunctional in ARDS patients.	(81)

ALI, Acute lung injury; iASPP, Protein phosphatase 1 regulatory subunit 13 like Gene; HIF, Hypoxia-inducible factor; TF, Transferrin; seq, Sequencing; ARDS, Acute respiratory distress syndrome; GWAS, Genome-wide association study; KLF2, KLF transcription factor 2; Rac1, Ras-related C3 botulinum toxin substrate 1; HMVEC, Human Umbilical Vein Endothelial Cells; RAPGEF3/EPAC1, Rap guanine nucleotide exchange factor 3; sc, Single cell; GWAS, Genome-wide association study; SNP, Single nucleotide polymorphism; NAMPT, Nicotinamide phosphoribosyltransferase; TLR4, Toll-like receptor 4; MYLK, Myosin light-chain kinase; MIF, Macrophage migration inhibitory factor; PI3, Peptidase inhibitor 3 PI3; CX3CR1, CX3C chemokine receptor1; LC-MS, Liquid Chromatograph Mass Spectrometer; UPLC, Ultra Performance Liquid Chromatography; TOF, Time of Flight Mass Spectrometer; PSG, Polysaccharide; CSR, crude *Scutellariae radix*; WSR, wine-processed *S. radix*; SOC3, Suppressor Of Cytokine Signaling 3; RAB11A, Member RAS Oncogene Family; BORCS5, BLOC-1 Related Complex Subunit 5; DUSP16, Dual specificity phosphatase 16; sn, single-nucleus; COVID, Coronavirus disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; FABP5, Fatty acid binding protein 5; GSMR, Generalised Summary-data-based Mendelian Randomisation; IGFBP7, Insulin Like Growth Factor Binding Protein 7; UHPL-CMS, Ultra-high performance liquid chromatography-MS; SMART, Simple Modular Architecture Research Tool; Fth1^{hi}, Highly Ferritin heavy chain; Prok2^{hi} Neu, (Highly Prokineticin 2 neutrophils; LPS, Lipopolysaccharides; KEGG, Kyoto Encyclopedia of Genes and Genomes; RUNX1, RUNX Family Transcription Factor 1; AT2, Angiotensin II; PRMT4, coactivator Associated Arginine Methyltransferase 1; DEG, Differentially Expressed Genes; TNC, Tenascin-C; GWIT-iCPP, Cardiopulmonary mesoderm progenitors expressing GLI1, WNT2, ISL1, and TBX5; sg, Single guide; miRNA, MicroRNA.

uptake-ferroptosis pathway and embryonic development pathway related to p53 and Mdm2 in normal lung tissue and pre-invasive and invasive adenocarcinoma (101). Whole exon sequencing is primarily employed in genetic diseases and rarely used in ALI/ARDS (50). However, with the maturity and popularity of this technology as well as combination of emerging technologies such as data mining, whole exon sequencing to provide personalized treatment for ALI/ARDS patients may be a possible application to facilitate future development of gene-targeted treatment.

Genomics facilitates understanding of the genetic heterogeneity and diversity of cells in patients with ALI/ARDS. It has been hypothesized that the ALI/ARDS susceptibility gene or promoter loci obtained by genomics can be inhibited or activated to prevent the occurrence and development of disease (51), suggesting a potential method to prevent or cure ALI/ARDS. However, the clinical and diagnostic applications of these identified genes (such as angiopoietin-2 gene (85) and PI3 (86)) have insufficient specificities and sensitivities. Single nucleotide polymorphisms (SNPs) or single nucleotide structural variations have not been fully studied.

Epigenomics for ALI/ARDS. When compared with genomics, epigenomics provides a deeper understanding of the genetic mechanisms and processes of gene expression in ALI/ARDS. Epigenomics refers to investigation of gene activity regulation and expression changes that are not reliant on the DNA sequence (102). In ARDS, expression of cell-specific genes is distinguished based on their epigenomic features, such as

DNA methylation, histone modification and local chromatin configuration (103). Epigenomics can reveal reversible and dynamic genetic changes and analyze epigenetic marks in different cell types to provide targets for the treatment of ALI/ARDS.

A growing number of studies are exploring epigenetic changes in ARDS, moving beyond traditional examination of the genome or transcriptome (69,104,105). DNA methylation, a key epigenetic modification, serves a pivotal role in ARDS pathogenesis by directly inhibiting transcription and expression by preventing specific transcription factors from binding their target sequences on candidate genes, thereby altering gene activity (106,107). DNA methylation variations may serve as specific diagnostic biomarkers for ARDS. Zhang *et al* (60) identified hypermethylation sites in peptidase inhibitor 3 (PI3) and microglial fractalkine receptor (CX3CR1) genes, which were associated with downregulated gene expression in ARDS, suggesting their involvement in pathophysiology. Additionally, a methylation profile study of patients with coronavirus disease 2019 (COVID)-19 and ARDS found hypomethylated probes linked to COVID-19 predictors, including genes participating in interferon regulation and viral response (70). This indicated increased gene expression during severe infection, highlighting potential biomarkers for ARDS in the context of COVID-19 (70). Further supporting the role of DNA methylation in ARDS, an integrative omics study identified the rs7967111 genetic variant at 12p13.2, which was significantly associated with increased risk of ARDS (38). Sensitivity analysis and

pleiotropy evaluation suggest that this locus might act as a pathogenic ARDS mutation, interfering with the cascade of DNA methylation and histone modification (38). Moreover, in a lipopolysaccharide (LPS)-induced ALI mouse model, increased DNA methyltransferase levels were observed, along with increased 5-methylcytosine levels, indicating higher DNA methylation levels (17). Genome-wide epigenomic analysis of lung tissue has revealed that several inflammation-associated genes (such as IL-10, IL-1 β and CXCR2/5/6) (108) are downregulated through methylation, further supporting the role of epigenetic alteration in the disease process.

DNA methylation and histone modifications shape gene expression patterns (109). Histone modifications influence DNA accessibility by altering local chromosomal structure or regulating the binding of effector proteins that control transcription (110). Wu *et al* (111) investigated the mechanism of lactate-regulated sepsis-induced ALI through omics technology. METTL3, a key modifying enzyme responsible for m6A, is upregulated in sepsis-associated multiple organ dysfunction (111). Lactate promotes METTL3 transcription by upregulating p303-mediated H3K18la in the METTL3 promoter region, which increases m6A modification to stabilize Acyl-CoA synthetase long-chain 4 (ACSL4) mRNA, inducing mitochondrial damage and subsequent induction of ferroptosis in alveolar epithelial cells (111). The regulatory pathway of histone modifications suggests that targeting modification sites may be an effective therapeutic approach to alleviate ALI/ARDS.

Given the relative ease of DNA isolation and stability of DNA methylation marks, epigenetic signatures hold promise as biomarkers for ARDS. As it has been confirmed that DNA methylation or histone modification at certain sites is related to the degree of ALI/ARDS, artificial DNA methylation regulation in the lung may have an inhibitory effect on progression of ALI/ARDS (104). Currently, epigenomics often only focuses on the effects of a single gene or pathway, while the disease mechanism of ALI/ARDS involves multi-factor interaction, which requires validation studies in this field (112-114). Occurrence and development of ALI/ARDS is a dynamic and rapid process. Although epigenetic modifications have different effects at different stages, dynamic and long-term follow-up studies lack explanation of the dynamic characteristics. In addition, the limited sample size may prevent reflection of the pathological process. Therefore, large-scale studies and long-term follow-up are required.

Transcriptomics for ALI/ARDS. Transcriptomics aims to capture both coding and ncRNA and quantify gene expression heterogeneity among cells, lung tissue and organs. RNAs are messengers that function between DNA and proteins; RNAs in single cells convert genetic information stored in DNA into proteins (115). The transcriptome can serve as an indirect indicator of protein expression, suggesting genomic activity in real-time (116). Transcriptomics analysis involves comprehensive examination of modifications in gene expression, offering insight into the regulatory mechanisms governing key genes and pathways in disease progression.. Transcriptional profile of various types of cell (such as endothelial cells and epithelial cells) in ALI/ARDS can determine the prognosis and outcome of ALI/ARDS (117,118).

Through comparative gene expression analysis between patients with ALI/ARDS and healthy controls, several differentially expressed genes (DEGs) that are closely associated with the disease have been identified (76). Among these markers, certain genes and transcription factors (such as cytoskeleton associated protein 2 Gene (CKAP2), purinergic receptor P2Y, G-protein coupled, 14 (P2RY14), Retinol Binding Protein 2 (RBP2) and Thymidylate Synthetase (TYMS)) serve key roles in disease onset and progression; others serve as molecular markers associated with disease prognosis (76). These markers enhance understanding of ALI/ARDS pathogenesis and offer new avenues for early diagnosis, disease monitoring and the development of personalized treatments.

Transcriptomic technology has highlighted major alterations in immune dysfunction-associated gene expression in lung parenchyma. C-reactive protein and serum and amyloid A1 are among the most upregulated genes at both transcriptional and translational levels, making them potential predictive markers for diseases such as COVID-19 (66). Similarly, in patients with ALI/ARDS, the regulation of immune responses plays a key role in disease severity. Decreased number and proportion of regulatory T cells are associated with worsened disease outcome, while elevated levels of pro-inflammatory cytokines contribute to disease exacerbation. If the regulation process of transcription and expression of pro-inflammatory cytokines is inhibited, the severity of ALI/ARDS may be decreased, and imbalance of inflammatory response may be regulated to a balanced state so as to slow progress of ALI/ARDS (119,120). Furthermore, transcriptomics holds considerable promise for developing novel therapeutic strategies for ALI/ARDS. For example, Ma *et al* (90) used the Gene Expression Omnibus database to analyze ALI/ARDS models and identified immune-mediated ferroptosis genes involved in oxidative stress and metabolic processes, suggesting potential targets for immunotherapy aimed at modulating underlying pathophysiology. Transcriptome studies in lung tissue have also revealed that furosemide mitigates inflammatory responses and oxidative stress by modulating the thioredoxin-interacting protein/NOD-like receptor thermal protein domain-associated protein 3 (NLRP3)/gasdermin D pathway, influencing inflammasome activation and pyroptosis (121-123). Wang *et al* performed high-throughput analysis of hemorrhagic shock-induced ALI/ARDS, identifying a network of lncRNA-miRNA-mRNA (including 12 lncRNAs, 5 miRNAs and 15 mRNAs) and circRNA-miRNA-mRNA interactions (including 16 miRNAs and 39 mRNAs and 10 circRNAs) associated with ALI/ARDS (98). The discovery of these regulatory changes facilitates development of novel treatments targeting the metabolic processes in ALI/ARDS. miRNA has become one of the therapeutic strategies of choice. Downregulated the aforementioned miRNAs (such as MAP2K3, Copine 1 and Cortactin(Map2k3, Cpn1, and Ctnn)) may serve as a treatment of ALI/ARDS.

The large amount of data involved in transcriptomic techniques presents difficulties in processing and analysis. The standardization of data processing and analysis is Standardization of data processing and analysis is challenging due to the need to apply different experimental methods. Therefore, development of efficient data processing tools and algorithms and the exploration of novel data integration and

standardization methods are required. Advancing the integration of transcriptomic techniques and other omics techniques to understand the function and regulatory networks of biological systems can facilitate further research. Moreover, the clinical implementation of transcriptomic markers necessitates further validation and optimization to ensure accuracy and reliability.

Compared with traditional cell population analysis, transcriptomic data offer a novel perspective on understanding gene expression, revealing regulatory mechanisms of key genes and pathways involved in disease processes (124). Advances in cell capture, phenotyping, molecular biology and bioinformatics continue to expand the potential for transcriptomics in biological and medical applications (125). Further technological progress and research may identify clinically relevant transcriptomic markers for ALI/ARDS to redefine treatment approaches, improving patient outcomes and quality of life.

Proteomics for ALI/ARDS. Proteins are the primary building blocks of life and maintain most cellular functions, including replicating DNA, facilitating transduction of genetic information, catalyzing metabolic reactions and driving cellular motility (126). Because protein profiles reflect organ-specific information more accurately than DNA or RNA alone, proteomics may reveal mechanisms that cannot be identified at the genomic level and are directly associated with clinical phenomena. Deep proteome profiling has identified biomarkers linked to ALI/ARDS progression, such as caveolin-1 (127), MMPs (128), vascular endothelial growth factor (VEGF) (129), receptor of advanced glycation end products (130), tumor necrosis factor (TNF- α) and IL-8 (131), providing insights into diagnosis, prognosis and mechanistic understanding of the disease at all stages (132). In 2003, proteomics was proposed to analyze altered protein expression comprehensively, allowing early biomarker discovery, refined therapeutic strategies and accelerated drug development (133). Increasingly, proteomics studies use diverse sample types, such as BALF (134), lung tissue (135), blood and exhaled breath condensate (136), offering flexibility in identification of key proteins and pathways associated with ALI/ARDS. This approach enhances understanding of inflammatory and repair signaling pathways and specific proteins relevant to ALI/ARDS pathology with potential clinical applications as biomarkers.

ALI/ARDS triggers complex changes in lung protein expression; >100 protein biomarkers have emerged as potential therapeutic targets (137). In 2004, Bowler *et al* (138) profiled proteins in plasma and pulmonary edema fluid of patients with ALI, identifying >300 distinct proteins (138). These biomarkers, analyzed through methods such as protein-protein interaction networks, data-independent acquisition proteomics and parallel-reaction monitoring, demonstrate promising diagnostic and prognostic potential (19). Studies have highlighted the involvement of proteins such as caveolin-1 (127), MMPs (128), vascular endothelial growth factor (VEGF) (129), receptor of advanced glycation end products (130), tumor necrosis factor (TNF- α) and IL-8 (131). These proteins are primarily expressed by alveolar epithelial or endothelial cells. Therefore, regulation of protein expression in epithelial or endothelial cells during ALI/ARDS may be a strategy for

future treatment. However, a holistic approach is needed to capture the dynamic, multifaceted changes that characterize ALI.

Recent advances in mass spectrometry (MS) technology have enabled high-throughput protein sequencing, leading to broader application of proteomics in ALI/ARDS research (139,140). MS-based proteomics techniques include isotope-encoded affinity tagging, stable isotope labeling of amino acids in cell culture, isobaric tagging for relative and absolute quantification (iTRAQ), gas chromatography and liquid chromatography (LC). Xu *et al* (56) used iTRAQ to identify proteins differentially expressed in endotoxin-induced porcine ARDS models, noting that IL-1 receptor antagonist protein, α -trypsin-interacting inhibitor heavy chain H4, MMP-1 and MMP-10 are highly expressed after LPS-induced ARDS and decreased following hemadsorption (HA) treatment. This indicates HA treatment could mitigate ARDS by curbing cytokine storms, improving alveolar barrier integrity and restoring proteomic balance in the exudative phase. Clinical verification is required to determine whether HA can improve disease progression of patients with ALI/ARDS (56). Adrover *et al* (61) used LC-MS to reveal that a CXCR2- and circadian-regulated program in neutrophils decreases inflammation by modulating proteome profiles. This depletes granule content and extracellular trap formation, leading to a reduction in inflammatory proteins (61). Dong *et al* (68) conducted proteomic assay and Mendelian randomization to identify plasma insulin-like growth factor binding protein 7 as a novel biomarker involved in platelet function in ARDS pathology, providing a promising target for further experimental and clinical research.

Proteomics methods are relatively complex and certain proteins may exhibit instability, which limits their application. Thus, optimizing proteomic analysis methods may be a future research focus. Additionally, proteomics can be integrated with other omics techniques to mitigate its limitations, thereby advancing understanding of complex biological systems such as ALI/ARDS.

Protein translational modification (PTM). The application of proteomics has identified protein biomarkers for ALI/ARDS diagnosis and prognosis (such as fatty acid-binding protein 5 (67), Chromosome Segregation 1 Like) (141). However, the human proteome is dynamic and certain protein biomarkers exhibit structural diversity after modification. PTMs are chemical modifications that are primarily catalyzed by enzymes that play key roles in the functional proteome and are the key mechanism underlying increased proteomic diversity (142). PTMs are commonly involved in complex and dynamic cellular processes by regulating cellular activity, localization and interactions with other cell molecules such as proteins, nucleic acids, lipids and cofactors (143). PTMs include phosphorylation, glycosylation, ubiquitination, methylation, acetylation, lipidation and protein hydrolysis, and are involved in almost all aspects of normal cell biology and pathogenesis (144). Moreover, there is a growing body of evidence implicating PTMs in a number of human disease mechanisms, which makes the utilization and understanding of PTMs key in the discovery of molecular mechanisms and therapeutic targets for ALI/ARDS (144,145).

Phosphorylation. Protein phosphorylation is one of the most studied and common PTMs, occurring primarily on threonine, serine, and tyrosine residues (146-148). Protein phosphorylation serves a major role in regulating the cell cycle, division, apoptosis and signal transduction as a key mechanism of cellular signal transduction (149). Zhao *et al* (150) demonstrated the importance of MAPK and the JAK/STAT pathway by enriching the transcriptome of lung tissue, followed by protein immunoblotting to demonstrate that Xie-Bai-San (XBS) inhibits phosphorylation of extracellular signal-regulated kinases (ERKs) and STAT3 to combat ALI (150). PTMs occur on different amino acid side chains or peptide bonds and are usually mediated by enzymatic activity (146). Li *et al* (151) found that histone deacetylase, an important epigenetic modifying enzyme, can promote ALI by upregulating the phosphorylation of Rho-associated protein kinase 1 (151).

In conclusion, protein phosphorylation and dephosphorylation are the most prevalent key regulatory mechanisms that regulate and control protein viability and function. Protein phosphorylation plays an important role in the pathological mechanisms of ALI/ARDS and may serve as a biomarker and potential therapeutic target for ALI/ARDS. However, most of the biomarkers in phosphoproteomics are still in the preclinical stage and there are only few methods available to detect protein phosphorylation with low specificity, such as mass spectrometry and quantitative phosphoproteomics (152). Therefore, there is need for larger prospective cohort studies on phosphoproteomics, as well as development of assays and reduction of assay cost to facilitate their application in clinical practices.

Lactate proteomics. With the development of PTMs in recent years, lactate proteolysis has become a focus of research (146). Lactate is a key metabolite of glycolysis, and lactate proteolysis is crucial for lactate functioning, which is involved in glycolysis-related cellular function and macrophage polarization (153). Lactate proteolysis is a protein modification induced by lactate accumulation, which can affect gene transcription by altering spatial conformation of histones and thereby regulating the expression of associated genes (154). Increased production of lactate under hypoxia and mitochondrial dysfunction and decreased clearance due to renal and hepatic injury facilitate the accumulation of lactate in septic patients; therefore, high levels of serum lactate are a key biomarker of sepsis prognosis (155).

Lactation is involved in inducing hyperpermeability of endothelial cells. Fan *et al* (156) found that lactate causes vascular permeability and exacerbates organ dysfunction in CLP-induced sepsis. Lactic acid-induced ERK-dependent activation of calpain 1/2 for hydrolytic cleavage of vascular endothelial (VE)-calmodulin leads to enhanced endocytosis of VE-calmodulin in endothelial cells. Lactate-induced phosphorylation of ERK2 promotes the dissociation of ERK2 from VE-calmodulin. *In vivo* inhibition of lactate production or gene depletion of the lactate receptor G protein-coupled receptor 81 (GPR81) attenuates vascular permeability and multiorgan injury and improves survival outcomes in polymicrobial sepsis. Overexpression of heat shock protein A12B (HSPA12B) prevents lactate-induced disassembly of

VE-calmodulin (156). Lactate promotes vascular permeability by decreasing expression of VE-calmodulin and tight junction proteins, and the deleterious effects of lactate on vascular hyperpermeability are mediated by HSPA12B- and GPR81 (a specific receptor for lactate)-dependent signaling (156). Lactation is involved in the regulation of macrophage polarization: Yao and Yang (157) revealed the involvement of lactation in the regulation of gene expression during M1 macrophage polarization (157). Wang *et al* (158) revealed that lactic acid enhances the lactation of pyruvate kinase isozyme type M2 and promotes the transition of pro-inflammatory macrophages to a reparative phenotype. Further, Dichtl *et al* (159) suggested that lactylation could be a consequence of macrophage activation rather than its cause. However, Wang *et al* (160) revealed that proline lactylation promoted M1 macrophage polarization which in turn reduces lactic acid. Thus, macrophage polarization is important in numerous diseases, and the association between lactation and macrophage activation warrants further in-depth study (154).

Ubiquitinated proteomics. Ubiquitination is a key PTM mediated by three enzymes (E1, E2 and E3) that are involved in the degradation of proteins as well as in the regulation of cellular functions, such as cell cycle, proliferation, apoptosis, differentiation, metastasis, gene expression, transcriptional regulation, signaling, repair of damage and inflammatory immunity (161). Ubiquitination serves an important role in the pathogenesis of ALI and other types of lung diseases, and the expressions of various inflammatory and anti-inflammatory factors are regulated by ubiquitination. For example, the deubiquitinating enzyme Ubiquitin Specific Peptidase 13 (USP13) stabilizes the anti-inflammatory receptor immunoglobulin interleukin-1-related receptor (IL-1R8/SigIRR) to inhibit lung inflammation (162). Qian *et al* (163) found E3 ubiquitin ligase, tripartite motif-containing 47 (TRIM47), is highly expressed in vascular endothelial cells and the knockdown of TRIM47 inhibits the transcription of pro-inflammatory cytokines, thereby suppressing LPS-induced lung inflammation and ALI (163). Li *et al* (164) found that inflammatory cytokine secretion is decreased by inhibiting Nrf2 ubiquitinated proteasomal degradation, which attenuates LPS-induced ALI (164). There is growing evidence of the involvement of ubiquitination in development of ALI/ARDS, but studies of ALI/ARDS based on ubiquitination proteomics are not comprehensive (165,166). Optimization of ubiquitination proteomics methodology and may identify ubiquitination biomarkers.

Protein phosphorylation, endocytosis and ubiquitination are the most prevalent key mechanisms that regulate and control protein viability and functions. Protein phosphorylation, endocytosis and ubiquitination serve key roles in the pathological mechanisms of ALI/ARDS and may serve as biomarkers and potential therapeutic targets. However, most biomarkers for modified proteomics are still in the preclinical stage, with few assays and low specificity. Therefore, there is need for larger prospective cohort studies of modified proteomics, as well as development of additional assays and decreased costs to facilitate application in clinical practice.

Metabolomics for ALI/ARDS. Metabolomics involves qualitative and quantitative analyses of low-molecular weight

metabolites or small molecule chemicals involved in metabolism and present in an organism or cell to reveal the association between dynamic metabolic changes and pathophysiological processes influenced by internal and external factors (167). Metabolomics uses information modeling and system integration to evaluate indicators, thereby offering insights into dynamic metabolic changes in organisms. Metabolomics applications involve techniques to elucidate large differences in polarity, charge and size of multiple metabolites, and the most common techniques are based on nuclear magnetic resonance spectroscopy and MS (168). Metabolomics can analyze changes in cell metabolic state in real-time and reveal the metabolic pathways in development of ALI/ARDS to facilitate the discovery of novel biomarkers for early diagnosis and prognosis evaluation.

Moreover, advancements in MS technology have enhanced the speed of small molecule analysis in cell metabolomics, enabling high-throughput metabolomics and novel routes for systems biology, functional genomics, drug discovery and personalized medicine (169,170). For example, using an LC-MS platform, Evans *et al* (171) analyzed BALF samples from patients with ARDS and healthy controls, identifying 37 biomarkers primarily affecting amino acid metabolism, glycolysis, gluconeogenesis, fatty acid biosynthesis and phospholipid and purine metabolism. Similarly, Nan *et al* (71) used ultra-high-performance LC-MS/MS to examine the metabolomic profiles of patients with community-acquired pneumonia (CAP). The aforementioned study distinguished acute and remission phases of CAP and identified myristoyl lysophosphatidylcholine (LPC) in patient plasma, a metabolite inversely associated with disease severity, suggesting its potential as a biomarker and therapeutic target for pneumonia. Additionally, Fan *et al* (172) used metabolomics to differentiate and diagnose ALI in patients with acute aortic dissection, identifying β -hydroxybutyrate and TNF- α as independent risk factors for ALI. The aforementioned study also highlighted elevated levels of pyruvate, alanine, malondialdehyde and lactic acid, along with changes in amino acid profiles, providing valuable insight into ALI mechanisms and potential biomarkers for diagnosis and treatment (172).

Large-scale metabolomics research is increasingly used to explore individual responses to environmental stimuli based on genetic background and time-dependent variations (173,174). Beyond observing metabolic shifts following drug administration, metabolomics offers a detailed and accurate view of drug mechanisms and therapeutic efficacy. Li *et al* (62) employed Ultra Performance Liquid Chromatography (UPLC-triple-time of flight (TOF)/MS to analyze the metabolic effects of *Ganoderma atrum* polysaccharide (PSG) on rats; significant alterations in histidine, nitrogen, tryptophan and glycerol phospholipid metabolism were observed, which supported the protective effect of PSG against ALI, preserving lung histology and decreasing pro-inflammatory cytokines and NO in serum and lung tissue. Similarly, using Ultra Performance Liquid Chromatography Quadrupole TOF-MS (UPLC-QTOF-MS), Hu *et al* (63) examined plasma samples from LPS-induced ALI rats treated with crude *Scutellariae radix* (CSR) and wine-processed *S. radix* (WSR) and found 16 biomarkers in LPS-induced rat plasma; CSR influenced ALI by regulating abnormal sphingolipid metabolic pathways,

whereas WSR-treated samples primarily showed adjustments in retinol and tryptophan metabolism, indicating mechanisms of these treatments.

However, metabolomics research has problems. For example, small sample sizes may prevent detection of significant differences. Differences in the metabolome of patients with ALI/ARDS caused by different diseases may affect the further report of metabolomics. For metabolomics to become a reliable tool in intensive care, issues associated with sample collection and processing, multivariate data analysis and patient selection must be addressed (175). For example, patients tend to choose traditional clinical application techniques and there is instability of metabolic markers (176). Future research should improve speed and precision of metabolomics in detecting metabolites across large samples. Developing methods for accurately analyzing small samples is key for expanding metabolomic clinical utility.

3. Integration of multi-omics

The development of single-cell multi-omics technology facilitates analysis of genomics, proteomics, transcriptomics and chromatin accessibility at an individual cell level (Fig. 2) (177). This integrative approach is key in studying the immune response dynamics in patients with ARDS and animal models. Single-cell multi-omics not only helps identify biomarkers associated with causes and treatment of ARDS but also provides key insights into patient-specific disease progression and outcomes.

Integration of transcriptome and genome. Gene expression profiling through genomic or transcriptomic sequencing serves a central role in understanding disease genetics (178,179). However, relying solely on genomics or transcriptomics limits insight due to individual variations in DEGs across populations and lack of clear gene-disease associations. Integrating genome and transcriptome data provides a more comprehensive approach to investigating complex diseases, bridging genetic variations and disease phenotypes.

Combining genomics and transcriptomics has advanced understanding of the molecular mechanisms driving ALI/ARDS, especially pathways involved in inflammation (180). Furthermore, by examining gene expression dynamics, integrated genomics and transcriptomics offer valuable insight into potential targets for disease treatment. Compared to using one of these technologies alone, transcriptomics and genomics are more likely to reveal responses and expression pathways of specific cell populations at the cell level. The increased understanding of inflammatory pathways at the cellular level is more conducive to implementation of therapeutic strategies for specific cell types, such as lymphocytes, monocytes and endothelial cells (181). Jiang *et al* (65) used single-cell RNA sequencing (scRNA-seq) and differential gene analysis to compare peripheral blood mononuclear cells from patients with pneumonia and sepsis with early ARDS with those without ARDS; downregulated cytokine signaling 3 (SOCS3) expression in monocytes from patients with ARDS was observed, alongside increased expression of IFN-induced pro-inflammatory genes (such as Ras-related protein in

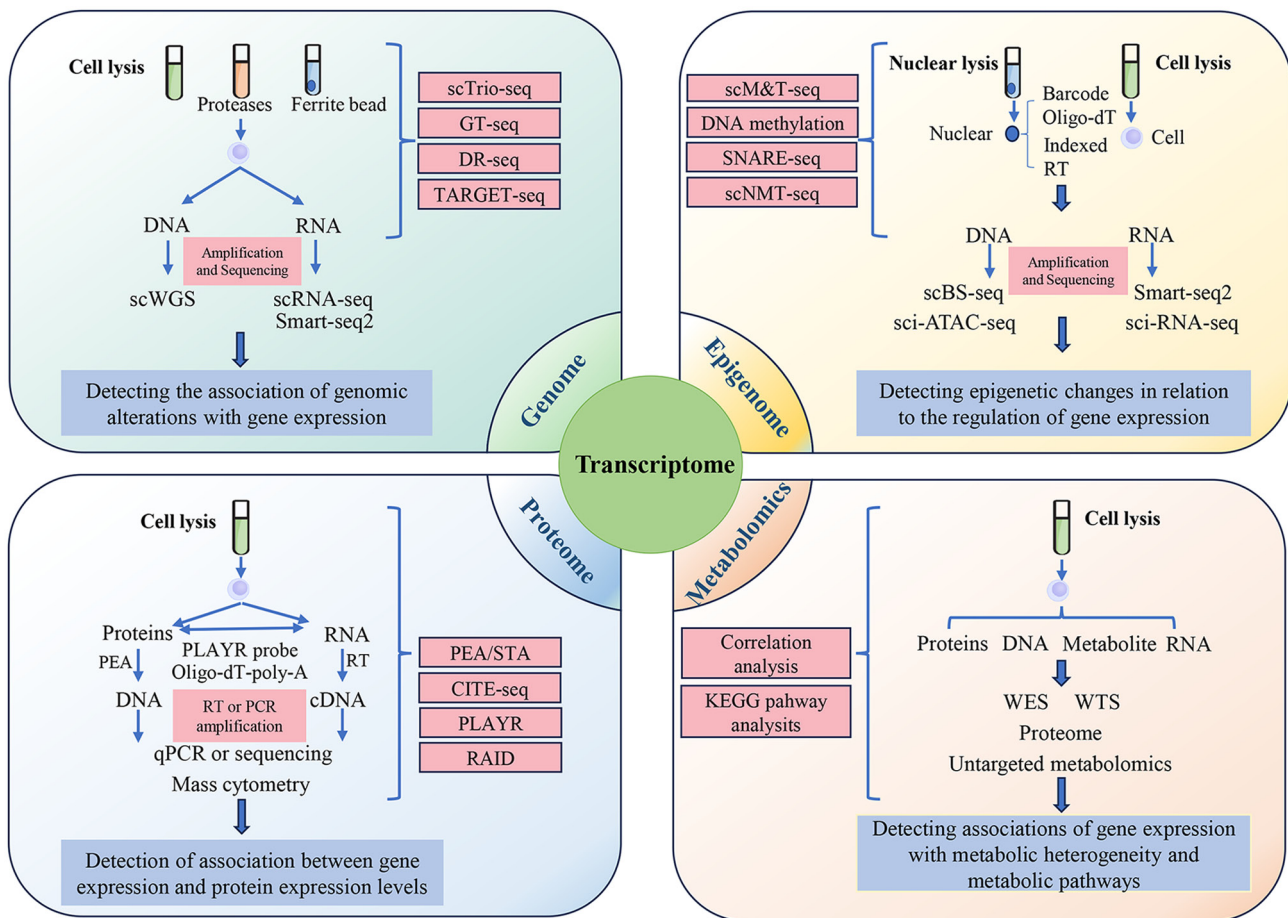


Figure 2. Single-cell multi-omics sequencing technology. By isolating individual cells and using various sequencing technologies, single-cell multi-omics analysis offers an in-depth understanding of cell type-specific gene regulation. Detection and synthesis of multiple molecules within the same cell, as well as integration of data from different molecular types, provide a comprehensive view of cellular processes. sc, Single cell; Trio, Triple omics; seq, Sequencing; GT, Genotyping-in-Thousands by sequencing; DR, gDNA-mRNA; WGS, Whole Genome Sequencing; Smart, Simple Modular Architecture Research Tool; M&T, methylome and transcriptome; SNARE, Soluble N-ethylmaleimide-sensitive factor attachment protein receptor; NMT, N-methyltransferase; RT, real-time; BS, Bisulfite; ATAC, Assay for Transposase-Accessible Chromatin; SCI, Small circular interfering; PEA, Proximity Extension Assay; PLAYR, The proximity ligation assay for RNA; q, Quantitative; STA, Static Timing Analysis; CITE, Cellular Indexing of Transcriptomes and Epitopes; RAID, Redundant Array of Independent Disks; KEGG, Kyoto Encyclopedia of Genes and Genomes; WES, Whole exome sequencing; WTS, Whole transcriptome.

brain 11A (RAB11A)), which may inhibit exocytosis of neutrophils, particularly in CD16⁺ cells, suggesting that monocytes in patients with ARDS exhibit dysregulated gene expression patterns beyond inflammation-associated genes. This finding may suggest that improving the monocyte intrinsic regulatory system can alleviate ALI/ARDS (65). Other studies have demonstrated the use of integrated genomics and transcriptomics in understanding cell-specific responses (57,75,80,89,182). Lai *et al* (75) performed scRNA-Seq to study expression of the protein arginine methyltransferase 4 (PRMT4) gene, known to mediate lymphocyte apoptosis, in activated T lymphocytes, finding its upregulation induces caspase 3-mediated cell death signaling. Short hairpin (sh)RNA-mediated PRMT4 knockdown inhibits LPS-induced caspase 3 activation, suggesting it as a promising therapeutic target. Huang *et al* (57), through multi-network analysis, explored how Kruppel-like factor 2 (KLF2) regulates endothelial integrity in ALI. Overexpressed KLF1, a regulator of ARDS-associated genes associated with pulmonary microvascular endothelial cytokine storm, oxidation and coagulation, was found to improve LPS-induced ALI. Conversely, expression of pulmonary KLF2, a key

ARDS-associated gene regulator, decreased. KLF2 improves endothelial barrier function and activates the Ras-related C3 botulinum toxin substrate 1 (Rac1) pathway in human microvascular endothelial cells via the Rap guanine nucleotide exchange factor 3/exchange protein directly activated by cAMP 1 pathway. Furthermore, DEG analysis and scRNA-Seq in a mouse malaria-associated ARDS model revealed an increase in endothelial cell number during disease regression. Therefore, promoting the proliferation of endothelial cells may be an interesting novel treatment for malaria-associated ARDS in combination with antimalarial drugs (80). MultiNicheNet interaction analysis has unveiled substantial modifications in critical ligand-receptor interactions during ARDS remission, offering potential targets for novel therapeutic strategies (80). Clinical studies using mRNA and miRNA genomics and transcriptomics show T cell dysfunction in patients with ARDS, highlighting the therapeutic importance of regulating immune pathways (81,182).

Combined analysis of transcriptomics and genomics enhances ability to diagnose and treat ALI/ARDS. Nonetheless, further exploration of specific molecular mechanisms is needed to realize this potential.

Integration of transcriptome and epigenome. In the complex pathology of ALI/ARDS, finding novel diagnostic markers is key (183). Compared to using one of these technologies alone, integrating transcriptomic and epigenomic analyses provides deeper insight into disease pathogenesis and potential therapeutic targets (184,185). In ALI/ARDS, transcriptomics helps identify gene expression changes and functional pathways, define gene function and map regulatory networks (186). However, transcriptome data alone are not sufficient to reveal the disease complexity. Unlike the genome, which solely contains genetic information, the epigenome expresses genetic information through modifications. Because cis-acting elements cannot encode proteins, trans-acting elements such as external transcription factors (TFs) and epigenetic modifications serve key regulatory roles in gene expression. Epigenomics provides additional context by capturing gene regulation via the aforementioned elements. Thus, compared to using one of these technologies alone, combining these data allows a more comprehensive view of transcriptional regulation, including pathway activity and transcription factor interactions (187).

Integrating transcriptome and epigenome data is valuable for understanding the association between ALI/ARDS and genetic polymorphisms. The identification of genetic polymorphisms can identify increased risk of disease within a certain group. Tejera *et al* (188) investigated the association between genetic variants and ARDS susceptibility in a cohort of 449 patients with ARDS and 1,031 at-risk patients who did not meet the criteria for ARDS during hospitalization and genotyped NPs, particularly the variant rs2664581; ELISA of plasma PI3 levels revealed that rs2664581 (or associated SNPs in linkage disequilibrium) may impact PI3 capacity to inhibit heightened human neutrophil Elastase activity, a genetic variant linked to ARDS risk. Notably, different rs2664581 genotypes resulted in varying plasma PI3 levels, with the Hap2 (TTC) haplotype, containing the variant allele rs2664581, identified as a risk factor for ARDS. Moreover, plasma PI3 level was significantly lower in patients with ARDS with a allele homozygosity than in those with the C allele variant. These findings underscore the association between PI3 gene polymorphisms, ARDS susceptibility and circulating plasma PI3 levels, thereby emphasizing the role of genetic and epigenetic factors in disease risk and highlighting PI3 as a potential biomarker for targeted therapeutic intervention.

Integration of transcriptome and epigenome analysis offers deeper understanding of ARDS pathogenesis to unveil new potential inflammatory targets for its prevention and treatment. Sun *et al* (189) expanded on previous findings (190) linking variants of MYLK gene to severe asthma susceptibility by investigating the role of the non-muscle (nm) MYLK promoter region in ARDS. The 2512-bp DNA of this region was synthesized based on the NM_053025 sequence. Site-directed mutagenesis was used to modify the nmMYLK DNA sequence, which was sequenced to examine its function in ARDS. The aforementioned study also conducted electrophoretic mobility shift assays to detect TF binding to nmMYLK and tested promoter demethylation using 5-aza-2'-deoxycytidine in an LPS-induced ALI mouse model to verify the role nmMYLK. The aforementioned study revealed that exogenous and endogenous inflammatory factors, such as cytokines, mechanical stress, hypoxia and DNA

demethylation, can significantly increase nmMYLK promoter activity, influencing ARDS-associated SNPs by altering TF binding and increasing nmMYLK expression, potentially affecting inflammatory severity in ARDS (189). Increasing activity of this site may serve a crucial role in suppression of inflammation in clinical ALI/ARDS cases.

Integrating transcriptome and epigenome data helps clarify the role of plasma cytokine levels. Kovacs-Kasa *et al* (191) observed elevated plasma cytokine levels in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-infected patients but these cytokines alone did not increase pulmonary microvascular endothelial permeability directly. The plasma factors causing pulmonary microvascular injury and increased endothelial permeability remain unknown. Electric cell-substrate impedance sensing assessment of plasma factors demonstrates that thrombin, angiopoietin 2, VEGF, complement factors C3a and C5a and spike protein contributed to increased endothelial permeability, although SARS-CoV-2 plasma induces milder, shorter-lived endothelial injury compared with plasma from patients with unconfirmed SARS-CoV2 infection. Among 15 cytokines analyzed, plasma levels of the pro-inflammatory cytokines IL-1b, IL-2, IL-6, IL-8 and IL-17 are elevated in patients with novel CoV, with IL-10 exhibiting the greatest increase compared with the normal control group (191). Pretreatment with thrombin inhibitors, neutralizing antibodies against cytokines, C3a and C5a receptor antagonists or angiotensin-converting enzyme 2 (ACE2) antibodies alone or in combination does not significantly decrease SARS-CoV-2-induced endothelial permeability, underscoring the complexity of SARS-CoV-2-associated endothelial injury (192).

Integrating transcriptome and epigenome analyses is instrumental in identifying drug targets and underlying mechanisms. Xian *et al* (193) combined shRNA lentivirus silencing, RNA isolation, qPCR, immunofluorescence, confocal microscopy and chromatin immunoprecipitation to investigate the effect of metformin on ARDS; the primary molecular target, Electron Transport Chain Complex I (ETCCI), exerts an inhibitory effect by decreasing ATP production. Metformin prevents NLRP3 inflammasome activation, macrophage infiltration and collagen deposition by inhibiting TLR-induced mitochondrial DNA (mtDNA) synthesis (193). These results indicate that metformin, an affordable and well-tolerated drug, may be repurposed to prevent and alleviate LPS-induced ARDS, highlighting it as a potential therapeutic agent.

In summary, integrating transcriptomics and epigenomics facilitates understanding of the association between gene polymorphisms, changes in plasma cytokine levels and gene modifications in ARDS. These insights are key for the future of disease diagnosis, treatment and prognosis assessment. However, challenges remain, including the need for advanced technology and equipment for data acquisition and processing, ongoing optimization and updating of bioinformatics methods and the translation of research findings into applications for clinical practice.

Integration of transcriptomics and proteomics. Transcriptomics involves comprehensive analysis of the entire transcriptional activity within cells at specific developmental or physiological stages, allowing for the detection

and quantification of specific nucleic acid sequences (194). By contrast, proteomics is the systematic study of all proteins expressed within a genome to unravel the molecular networks governing complex biological processes at the protein level (139). Integrating transcriptomic and proteomic data offers holistic understanding of biological samples, providing critical insight into physiological processes and disease mechanisms. In ALI/ARDS research, this combined approach has aided in understanding disease pathogenesis and identifying potential therapeutic targets.

As a key reaction in ALI/ARDS, inflammatory response plays an important role in progression and prognosis: Activation of inflammatory responses aggravates ALI/ARDS severity (9,77,195). Understanding the molecular mechanisms underlying the inflammatory response holds promise for improving diagnosis and treatment of ALI/ARDS. Considering the heterogeneity and limited research findings (196), DNA microarray analysis and genotyping have been integrated with proteomics to study ALI pathogenesis. Tang *et al* (197) recently analyzed the inflammatory response characteristics of ALI/ARDS in obesity by targeting the transcriptional profile of lung tissue in high-fat-induced obese ALI mice. The biological functions of DEGs and expression characteristics of protein were explored through transcription profiling, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis. The comprehensive transcriptome and proteomics verified that lncFirre is an effective therapeutic target for obesity-associated ALI and revealed its inflammatory signaling mechanism in ALI. Moreover, inflammation-associated lncRNAs played a crucial role in the inflammatory pathway of ALI, which provides precise targets for drug development (198). Furthermore, by merging proteomic with transcriptomic data, studies have identified key tissue and circulating biomarkers that predict disease progression or deterioration in patients with idiopathic pulmonary fibrosis and ALI (199–201). Using a combination of RNA-seq and SomaLogic proteome assay, significant increases in pathways such as mast cell chemokines, T helper-2 (Th-2) axis and Wnt signaling were identified, providing valuable diagnostic insights for ALI/ARDS (199). Sinha *et al* (200) employed scRNA-Seq and plasma proteomics to investigate pathological mechanisms underlying ARDS. The aforementioned study characterized a COVID-19-enriched neutrophil state, its mechanism of action and dexamethasone-mediated modulation of neutrophil function to support the development of targeted immunotherapies for severe COVID-19. It remains unclear whether dexamethasone improves ARDS caused by COVID-19 in a manner similar to that in patients with ARDS from other causes. Moreover, by applying scRNA-Seq and unbiased serum proteomics, Ramaswamy *et al* (201) investigated multisystem inflammatory syndrome in children and COVID-19 in adults and healthy individuals, revealing specific alterations, such as heightened cytotoxic gene expression in natural killer and CD8⁺ T cells, which could aid in early disease diagnosis and prognosis assessment. Thus, transcriptomics and proteomics are pivotal in examining immune response-associated characteristics and mechanisms in ARDS progression.

Oxidative stress, a key factor in ALI/ARDS pathogenesis, has been studied using transcriptomic and proteomic tools. Application of quantitative redox proteomics has revealed that

S-glutathione promotes interaction between fatty acid binding protein 5 and peroxisome proliferator-activated receptor (PPAR) β/δ , activating PPAR β/δ target genes and suppressing LPS-induced macrophage inflammation (67). Additionally, a comparison of ALI mouse models underscores the key role of S-glutathione in cellular antioxidant defense during ALI (67). MMPs serve as key mediators and effectors of alveolar-capillary membrane damage and repair due to oxidative stress in ALI/ARDS. They contribute to degradation of non-structural extracellular matrix (ECM) components in experimental lung injury, including syndecan-1/keratinocyte-derived chemokine and macrophage inflammatory protein-1 α (128). Further studies have highlighted Cav-1 as a regulatory factor in apoptotic pathways in different stages of lung injury (202,203). Cav-1 interacts with or is upregulated by death receptor agonists such as Fas ligands, TNF and TNF-related Apoptosis Inducing Ligand. Under normal conditions, it interacts with the key autophagy marker protein light chain 3B, a relationship that is disrupted by reactive oxygen species (ROS). This suggests Cav-1 is involved in regulating autophagy-mediated cell death during ALI/ARDS (204–206). In summary, a combination of transcriptomics and proteomics can assess the extent of oxidative stress. Targets of oxidative stress are expected to provide a new way for the treatment of ALI/ARDS.

Integration of genomics, transcriptomics, metabolomics, and proteomics. The potential of single-cell multi-omics technology in diagnosing, assessing treatment strategies, predicting prognosis and outcomes of ALI/ARDS continues to expand (207). Integrating metabolomics with other omics data has become essential in understanding diseases and developing effective treatments (172,175,208). Analyzing the association between DEGs obtained from transcriptome data with differential metabolites can examine organismal variations at causal and effectual levels. This combined approach allows identification of pivotal genes, metabolites, metabolic pathways and regulatory networks that underlie complex disease mechanisms. Additionally, genomics aids in identifying ALI/ARDS susceptibility-associated genetic targets (209,210). Single-cell multi-omics techniques may overcome the inherent limitations of individual omics approaches, providing more precise, reliable data for comprehensive understanding of disease mechanisms.

Single-cell multi-omics technology has recently made notable contributions to the study of ALI/ARDS and other diseases, especially in elucidating cellular and organelle-level molecular mechanisms in ALI/ARDS (207,211). Zhang *et al* (212) used such as glucose uptake and mitochondrial calcium measurement, fluorescence staining, protein blot analysis, apoptosis assay, ROS flow cytometry analysis, small interfering RNA duplex transfection, total RNA isolation and real-time RT-PCR to explore the mitochondrial pathway involved in hyperoxia-induced lung injury, which revealed a novel TLR4/stanniocalcin 1 (STC1)-mediated mitochondrial pathway that serves both homeostatic and oxidant-induced cytoprotective roles in lung endothelial cells. Similarly, Wang *et al* (213) explored the potential molecular mechanism of AU-rich binding factor 1 (AUF1, in regulating iron homeostasis in sepsis-induced ALI: Co- and RNA immunoprecipitation and measurements of cell viability, lipid

peroxidation, iron accumulation and total glutathione levels demonstrated that activating the AUF1 pathway may alleviate sepsis-induced ALI. Single-cell multi-omics has demonstrated elevated iron and Malondialdehyde levels in lung tissue, along with marked NRF2 downregulation and activating transcription factor 3 (ATF3) upregulation, further validating AUF1 therapeutic potential in alleviating CLP-induced ALI.

Exosomes have promise in mitigating ALI/ARDS (78,79,214). Derived from human mesenchymal stem cells and other sources, exosomes have been studied for therapeutic effects on macrophage function and inflammatory response in LPS-induced ALI (78,79,214). RNA extraction, RT-qPCR, western blotting and ELISA have demonstrated that human mesenchymal stem cell- derived exosomes (hMSC-exos) derived exosomes effectively attenuate glycolysis and sepsis-triggered inflammatory responses in macrophages, thereby decreasing lung damage (215). Adipose-derived exosomes, a promising therapy for ALI, are potent protectors against LPS-induced ALI in mice. Alongside exosomes, inflammasomes are key in ALI/ARDS progression (216). A 4-benene-indol derivative significantly inhibits NLRP3 inflammasome activation in LPS-induced ALI, suggesting a therapeutic target for sepsis-related ALI (217). By performing RNA isolation, real-time qPCR, flow cytometry, immunoprecipitation and protein blot analysis, Xie *et al* (218) identified High mobility group box 1 (HMGB1) as a factor that impedes macrophage-mediated exocytosis and prolongs inflammation by inhibiting member RAS oncogene family (Rab43)-regulated anterograde transport of CD91, indicating that restoring Rab43 may be beneficial in reducing inflammation and mitigating ALI/ARDS in humans (218). In a study involving patients with severe COVID-19, CD163-expressing monocyte-derived macrophages acquired a pro-fibrotic transcriptional phenotype during COVID-19 ARDS (69). Gene set enrichment and computational data integration revealed significant similarities between COVID-19-associated macrophages and profibrotic macrophage populations in idiopathic pulmonary fibrosis. This provides a deeper understanding of the ALI/ARDS mechanism and potential therapies (69). The aforementioned comprehensive investigations used quantitative proteomics, immunohistochemistry, scRNA-seq, clinical CT imaging and clinical evaluation to analyze transcriptional profiles of lung tissue cells, highlighting macrophage accumulation, monocyte-derived macrophage fibrosis and robust intercellular signaling in the ALI/ARDS pathophysiology. In addition, analysis of transcriptional and proteomic profiles in COVID-19-associated macrophages revealed that lung macrophages in COVID-19 express a range of scavenger receptors and proteins involved in exocytosis, including macrophage mannose receptor (MRC1), CD163, among others, as well as high levels of transforming growth factor- β 1 (TGFB1) and Transforming Growth Factor Beta Induced (TGFB1). These genes may directly or indirectly contribute to the profibrotic function of macrophages. Moreover, integrative study not only detailed cell-specific responses but also suggested that the presence of CD163/Legumain LGMN-M ϕ during convalescence may be the key to unraveling the mechanisms of fibrosis regression and reversing lung injury (69). Further exploration is warranted to elucidate the molecular mechanisms underlying virus- and macrophage-induced fibrosis *in vivo*.

Despite their advantages, omics techniques currently require cell isolation from tissue, which can disrupt inter-cellular interactions. However, advancements in spatial multi-omics hold promise for overcoming these limitations by allowing spatially resolved understanding of molecular interactions within tissues (207).

AI-based multi-omics analysis. With continuous advancements in biotechnology, multi-omics technology may provide more precise characterization of the heterogeneity and prognosis of disease. Multi-omics technology and AI algorithms promote the development of precision medicine (116). Large-scale data from multi-omics technology is difficult to integrate (219). Poirion *et al* (220) proposed a unique computational modeling framework, DeepProg, which uses patient survival as the target model to predict novel patient survival risk. DeepProg is run by processing multiple types of omics dataset through a combination of deep learning and machine learning algorithms. This approach has advantages of high sensitivity and high specificity in cancer classification (221), survival characteristics (222) and treatment planning (223). The high predictability based on deep learning is primarily attributed to its ability to automatically capture non-linearities and perform dimensionality reduction and hierarchical representation. In addition, Cui *et al* (224) constructed a basic model, scGPT, that can reveal complex biological interactions in the single-cell domain by harnessing the power of pretrained transformers on a large amount of single-cell data. The data entered in the model in advance by the developer can then be transferred to downstream tasks through fine-tuning, such as cell type annotation, perturbation prediction and multi-batch and multi-group integration. The pretrained model demonstrates strong extrapolation capability to present meaningful clustering patterns on unseen datasets (138).

In summary, AI-based data integration studies of multi-omics data are primarily classified into the following four categories: i) Feature selection and dimensionality reduction, ii) prediction of clinical outcomes; iii) clustering for subtype discovery (225). High-dimensional multi-omics techniques analyze data integration through algorithms to observe molecular-molecular interactions and biological phenomena in living organisms. To the best of our knowledge, however, there are relatively few studies on AI algorithms in ALI/ARDS disease prediction and cell subtype classification (204,205). The use of high-accuracy prediction and drug utilization models may improve disease prognosis and drug selection for ALI/ARDS as well as the development of precision medicine.

Prospects for clinical translation and application. There is extensive research on the application of multi-omics technology in ALI/ARDS (37,226,227). Application of multi-omics techniques facilitate diagnosis of diseases, such as through the upregulation or downregulation of serum markers mentioned above. In addition, multi-omics technology can clarify the disease mechanism of ALI/ARDS, such as the upstream and downstream communication between inflammatory response and reaction processes of oxidative stress (228). To translate current research into the clinical setting, biomarkers must be validated. The analysis of potential biomarkers by high-throughput omics technology requires collection of

biological samples from patients at different stages, followed by verification of feasibility and effectiveness in clinical trials. The genomic data of patients should be integrated and analyzed to construct a patient information database platform for ALI/ARDS, identify multi-omics data associated with therapeutic drugs, and adjust personalized treatment plans for patients at different stages. A good feedback mechanism should be established. When feasible treatment schemes or diagnostic methods in research are applied to clinical practice, timely feedback to researchers and continuous exploration and development of basic experimental mechanisms is key for development of long-term and effective clinical solutions. Unbiased analysis of preclinical and clinical samples should be performed. Unbiased analysis should involve careful selection of data, ensuring that the sample is representative of the population, and that the analysis does not overemphasize certain subsets of data that may lead to skewed or inaccurate results. While researchers conduct animal experiments to complete preclinical research, the use of clinical ALI/ARDS samples to correct the problems which found in animal experiments can promote clinical research progress. Combination of AI and multi-omics can determine new solutions to clinical problems through secondary analysis of datasets. Classification and regression tree and artificial neural networks have been applied in individualized treatment of ARDS and the integration of algorithms can be more helpful than existing single algorithmic approach in clinical treatment (229).

4. Single-cell sequencing identification of novel cellular subpopulations in ALI/ARDS

Advancements in multi-omics techniques such as genomics, transcriptomics and proteomics are increasingly applied at the single-cell level, providing a detailed view of cell states and disease pathobiology (39,40,230). scRNA-Seq technology enables identification of rare and complex cell populations, as well as revealing gene regulatory networks and developmental trajectories in cell lineages (231). For example, Montoro *et al* (58) discovered several novel cell types, including a pulmonary ionocyte, by characterizing transcriptional profiles in mouse lung epithelial cells. The function of the ionocyte is yet to be fully understood (58). The complex lung tissue architecture, with its diverse specialized cell types, presents challenges for traditional analytical methods in accurately characterizing specific cell subpopulations and their roles, especially in common respiratory diseases such as ALI/ARDS. scRNA-Seq offers single-cell resolution in genomics, providing a tool to accurately capture cellular states (232,233), pinpoint targets for targeted therapies and analyze pathophysiological mechanisms in various types of tissues and cell (72).

Recent studies using scRNA-seq have expanded understanding of cellular subpopulations in ALI (137,234,235). Wang *et al* (73) identified distinct neutrophil subpopulations in ALI mouse lung through scRNA-seq: Highly Ferritin heavy chain (Fth1)- and Prokineticin 2 (Prok2)-expressing neutrophils. These subpopulations serve specific roles in ALI pathology and exhibit that the Fth1^{hi} Neu population may promote the pathological development in the lung of patients with ARDS, highlighting their potential as biomarkers for disease prognosis. Similarly, by conducting single-cell

sequencing and spatial transcriptomics, Boyd *et al* (64) classified lung fibroblasts into three subpopulations: Resting, ECM-synthesizing and inflammatory (64). They found that a subset of fibroblasts remodels the lung microenvironment and promotes immune cell infiltration by expressing ECM proteases such as a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4), ultimately impairing lung function (64). Additionally, Tang *et al* (74) demonstrated that RUNX1 protein enhances mitochondrial autophagy and decreases lung inflammation during ALI through RNA-Seq of RUNX1-silenced alveolar epithelial cells, identifying a key regulatory pathway in ALI response.

Single-cell sequencing allows precise definition of lung cell types and states and presents new avenues for developing biomarkers, diagnostic tools and targeted therapies for ALI and ARDS (55). However, there are inherent challenges. Lung specimens used in single-cell studies are often small and prone to degradation, making it challenging to draw robust conclusions on cellular activity. Furthermore, scRNA-Seq tends to capture high-expression genes, leading to underrepresentation of low-expression genes in its output (236).

The rapid advancement of scRNA-seq, particularly with spatial-based single-cell technology, holds potential to deepen understanding of cellular interactions and spatial organization within lung tissue, thereby improving knowledge of lung health and disease mechanisms (237). While single-cell sequencing has already provided insights into complex biological systems (238), continued research leveraging emerging single-cell technologies is key to determine molecular mechanisms and pathophysiology underlying ALI/ARDS and advance therapeutic strategies for these diseases.

5. Conclusion

While single-cell multi-omics technology has facilitated research on ALI and ARDS, several challenges remain. First, sensitivity and resolution need improvement to accurately detect low-abundance biomolecules and rare cell subpopulations. Second, enhanced data processing methods are essential to handle the high dimensionality and complexity of single-cell data effectively. Furthermore, interpreting single-cell data within complex biological systems and translating them into clinically relevant information is challenging.

Current application of multi-omics technology in ALI/ARDS research is limited due to large amounts of data and the difficulty of cross-omics integration. Interpretation algorithms are often limited by differences in methodology and platform technology. This may be resolved by adopting more advanced algorithms to integrate multi-omics data and promoting standardization of omics technology. Other technical limitations of this technology include low single-cell resolution, challenges in sample separation, cell damage and data generation. Using single-cell RNA sequencing, separating individual cells from a heterogeneous sample is a major problem without cross-contamination, which may introduce variability, impacting the accuracy of results. No matter which kind of techniques such as microfluidics, laser capture, or even standard mechanical isolation methods, all these methods could introduce mechanical stress or damage to cells, which can alter cellular behavior, leading to biased

or inaccurate data, especially when studying sensitive or rare cell types. The molecular mechanism of ALI/ARDS involves dynamic cell responses and time-dependent processes, while omics techniques often only capture biological information at a single time point. Thus, complete understanding of dynamic processes may require multiple sampling and analytical tools with higher temporal resolution. Moreover, high cost and large databases limit the development of this technology. Extracting clinically relevant results from large datasets poses a challenge. Patient and cell-to-cell differences are often influenced by multiple factors and increasing heterogeneity increases the sample size requirement. However, difficulty of sample extraction limits the number of clinical samples available.. Therefore, the development of non-invasive sampling methods for longitudinal studies in critically ill patients may better capture the dynamics of the disease.

Despite the aforementioned challenges, advancement of single-cell multi-omics holds promise for biomedicine, particularly in understanding the pathogenesis of complex diseases such as ALI and ARDS. This technology enables precise, detailed analysis of cell heterogeneity throughout disease progression. Capturing multiple molecular layers, such as the genome, transcriptome, proteome and metabolome, within individual cells can unravel the intricate networks driving disease onset and development. In ALI and ARDS, single-cell multi-omics deepens knowledge of essential processes such as pulmonary inflammation, immune response and apoptosis and offers specific disease biomarkers for early diagnosis, prognosis and treatment.

Machine learning is a technique that enables computer systems to use data to continuously improve performance. It belongs to a branch of AI whose main purpose is to allow computers to learn from data to recognize patterns and make predictions. With the increase in available biological data, machine learning can automate analytical processes and improve efficiency and accuracy of data processing. Because biological data often contain a large number of features and complex patterns, it presents a challenge to human analysts. Machine learning can identify patterns in these complex datasets. Machine learning can also be used to build predictive models for predicting changes in gene expression and disease development. These models can facilitate predictions and guide future research, especially when experimental data is lacking (239). As aforementioned, ARDS is a syndrome rather than a specific pathological entity and is currently identified based on clinical criteria (9). Machine learning models can predict the presence or absence of disease based on patient clinical characteristics and laboratory test results, which can improve the accuracy of diagnosis. Machine learning can predict development of diseases and the prognosis of patients, aiding personalized and precision medicine practices (240). The predictive accuracy of machine learning models is affected by factors such as missing values and uncertainties involved with medical data. In addition, protecting patient privacy is also challenging for development of machine learning (241).

In future, single-cell multi-omics technologies may elucidate pathogenesis of ALI and ARDS by precisely detecting and analyzing biomolecular changes in individual cells, integrating data from different sources to gain a more comprehensive understanding of disease complexity as well as

new therapeutic targets and strategies. Additionally, interdisciplinary collaboration and integration with other technologies, such as AI and data analysis, are expected to progress disease research. Further refinement of single-cell multi-omics technology are anticipated, improving sensitivity, resolution and data analysis methods to allow more accurate detection of biomolecular changes within cells. Moreover, establishing a robust single-cell multi-omics database and resource-sharing platform may facilitate data sharing and support advancements in disease research and development. Future studies should identify novel therapeutic targets and drug mechanisms by applying single-cell multi-omics.

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Authors' contributions

ZZ, XQ, JY, JK and WH wrote the manuscript. ZZ, XQ and JY constructed the figures. JQ, GT, XF and FM revised the manuscript. XF conceived and supervised the study. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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