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Review

METTL3-Mediated N6-Methyladenosine Ferroptosis in Sepsis-Associated Acute Lung Injury — A Narrative Review

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Abstract

Sepsis-induced acute lung injury (ALI) represents a complex and life-threatening condition with limited therapeutic options. Recent research has unveiled the role of methyltransferase-like 3 (METTL3)-mediated N6-methyladenosine (m6A) modifications in exacerbating ferroptosis via m6A-insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2)-dependent mitochondrial metabolic reprogramming, shedding light on potential therapeutic targets. This study delves into the implications, challenges, and prospects of this intricate molecular pathway in sepsis-associated ALI. METTL3-mediated M6A modifications assume a pivotal role in the pathogenesis of sepsis-induced ALI. These modifications exacerbate ferroptosis, a regulated cell death process characterized by iron-dependent oxidative damage to lipids. The involvement of m6A-IGF2BP2-dependent mitochondrial metabolic reprogramming adds another layer of complexity to this mechanism, offering potential therapeutic avenues. Understanding the intricate network of METTL3-mediated m6A modifications, IGF2BP2, and mitochondrial metabolic reprogramming poses a formidable challenge. Developing interventions that modulate this pathway while minimizing off-target effects remains a significant hurdle. Patient-specific responses and identifying reliable biomarkers further complicate the clinical translation of these findings. The unraveling of this molecular pathway holds promise for personalized medicine approaches in ALI management. Early diagnosis and tailored interventions based on individual patient profiles may significantly enhance clinical outcomes. Collaboration among multidisciplinary teams, including researchers, clinicians, and drug developers, is essential to bridge the gap between laboratory discoveries and clinical applications.

Keywords: ferroptosis; METTL3; m6A modification; IGF2BP2; lung injury; biomarkers; therapeutic development

1. Introduction

One common chronic lung disease is chronic obstructive pulmonary disease (COPD), which restricts airflow and causes persistent respiratory symptoms due to anomalies in the airways and/or alveoli [1]. When it comes to global incidence and mortality rates, COPD is among the top four [1]. On the other hand, chronic obstructive pulmonary disease affects almost 250 million people worldwide making up about 6% of all fatalities globally [1]. COPD is thought to have its origins in the aberrant inflammatory response brought on by long-term exposure to cigarette smoke [2]. Airway epithelial cells may be directly injured by cigarette smoke's toxic chemicals, which may cause cell death and aberrant growth. When airway epithelial cells sustain this sort of damage, they are unable to perform their usual barrier function, leaving the airway open to infection from outside sources. Meanwhile, the inflammatory response within the airways is intensified due to the production of different inflammatory mediators by injured epithelial cells [2]. Additionally, airway remodelling is a hallmark of COPD. Cigarette smoke has the potential to cause a variety of airway remodelling phenomena, including thickening of the airway wall, proliferation of smooth muscles, and fibrosis. These changes cause the airway lumen to constrict, which in turn reduces airflow and causes symptoms like breathing problems. Its compliance can be affected by airway remodelling, making it more susceptible to outside stimuli. A chronic inflammatory response is caused by the toxic chemicals produced by smoking, which stimulate immune cells in the airways. The inflammatory response worsens airway damage and remodelling, hinders lung gas exchange performance (hypoxia and associated symptoms), and eventually causes cell death and other negative outcomes. Thickness and distortion of the airway wall are primarily caused by an imbalance in the equilibrium of proliferation and death of airway epithelial cells [3]. Consequently, investigating the causes of COPD requires a focus on cell death patterns.

Sepsis-induced acute lung injury (SI-ALI) represents a complex and life-threatening condition with limited therapeutic options. Recent research has unveiled the role of methyltransferase-like 3 (METTL3)-mediated N6-

methyladenosine (m6A) modifications in exacerbating ferroptosis via m6A-insulin growth factor 2 binding protein 2 (IGF2BP2)-dependent mitochondrial metabolic reprogramming, shedding light on potential therapeutic targets. This paper delves into the implications, challenges, and prospects of this intricate molecular pathway in sepsis-associated ALI. METTL3-mediated m6A modifications assume a pivotal role in the pathogenesis of sepsis-induced ALI.

2. Ferroptosis

Cell respiration, DNA replication, and protein synthesis are all crucial for cell development and proliferation; iron, a functional component of the majority of animals, satisfies a number of these activities. On the other hand, iron can produce reactive oxygen species (ROS) in redox cycling through the Fenton reaction, which could harm DNA and lipids in the membrane. Thus, iron may cause cell death (specifically a novel form of cell death called ferroptosis) and stimulate cell growth simultaneously [3,4]. A new type of intentional cell death called ferroptosis is emerging; it differs from necrosis, autophagy, and apoptosis in that it is characterized by lipid peroxidation and intracellular iron buildup. Oxidative stress and cell death are the end results of these processes [4]. There are a number of signaling networks and three main metabolic pathways involved in ferroptosis, including those for amino acids, iron, and lipids [5]. Ischemic stroke [6], intracerebral hemorrhage [7], cancer [8], sepsis [9], and myocardial infarction [10] are just a few of the pathogenic processes and disorders that have been linked to ferroptosis. As a result of oxidative stress, COPD develops, according to the theory that smoking makes the body's antioxidant defenses less effective. Oxidants including cigarette smoke can harm lung cells, decrease antioxidant defense capacities, accelerate cell lysis and epithelial permeability, and raise the burden of oxidants on the respiratory tract. When the antioxidant system, which includes NAD(P)H:Quinone Oxidoreductase 1 (NQO-1), glutathione (GSH), and Solute Carrier Family 7 Member 11 (SLC7A11), is unable to eliminate an excess of lipid ROS, ferroptosis ensues [7]. NQO-1, GSH, and SLC7A11 are all interconnected in cellular defense and redox homeostasis, with NQO-1 being an antioxidant enzyme, GSH a crucial antioxidant, and SLC7A11 a transporter involved in cysteine uptake and GSH synthesis. Ferroptosis-related gene expression changes are present in COPD patients and mice models, suggesting that ferroptosis may become a feasible therapeutic target for COPD [11], however, the exact mechanism is not yet known. Therefore, it is extremely important to investigate how ferroptosis works in airway epithelial cells of COPD, to understand the pivotal role ferroptosis plays in the development and progression of COPD, and to develop effective treatment methods for COPD. The aberrant expression and activity of many redox-active enzymes that either create or detoxify free radicals and lipid oxidation products leads to ferroptosis, a redox imbalance between oxidant and antioxidant production. After messenger RNA (mRNA) transcription, a frequent alteration called N6-methyladenosine (m6A) takes place. It plays a role in controlling mRNA stability, shear, localization, and transcription [12,13]. The expression and activity of m6A-modifying enzymes may be impacted by the oxidative stress and inflammatory processes caused by smoking. Smoking changes m6A modification levels, which impacts gene expression regulation, according to research [14]. This effect is more pronounced in lung tissue. To add insult to injury, smoking may alter m6A-related gene expression, which in turn impacts cell proliferation, apoptosis, and growth [15]. Using a mouse model of COPD, Hu *et al.* [16] performed Me-RIP sequencing and discovered that a change in m6A methylation can increase the risk of developing COPD. Consequently, m6A and ferroptosis are critical in COPD. Several processes in ferroptosis are regulated by abnormal m6A levels. These include the antioxidant system, iron metabolism, and lipid metabolism [17,18]. Critical regulators of ferroptosis are frequently found to have abnormal m6A levels. The ferroptosis pathways that are regulated by "writers", "readers", and "erasers" that influence the function of m6A in a dynamical manner include the antioxidant system, lipid metabolism, and iron metabolism [19,20]. Among the many forms of active extinction that make up programmed cell death are autophagy, ferroptosis, pyroptosis, apoptosis, and necroptosis. Methylases (writers) methylate m6A, and demethylases (erasers) demethylate it, making it a reversible RNA modification. The role of SLC7A11, NQO-1, and Fat Mass and Obesity-associated (FTO) protein in the development of COPD is debatable [21,22]. *FTO* is a gene that plays a role in obesity and related metabolic disorders. FTO is linked to increased food intake, reduced satiety, and higher BMI. It also influences glucose and lipid metabolism, particularly in the liver. Acute lung damage caused by sepsis is regulated by lactate in alveolar epithelial cells through m6A modification [18,19].

3. Sepsis

Sepsis has emerged as a critical global public health concern, representing a severe clinical condition that arises from the body's maladaptive response to infection, posing a substantial and immediate threat to life [23]. It was formally defined as 'life-threatening organ failure triggered by an imbalanced host immune response against various infections' [23]. A comprehensive international study centered on admitted patients to hospitals with sepsis uncovered striking statistics. Globally, the mortality rate for individuals suffering from sepsis has risen tremendously at a rate of 26.7%, despite an initial decrease in the 20th century [24,25]. The mortality rate in the subgroup of sepsis patients requiring Intensive Care Unit (ICU) admission has even further increased to about 41.9%. This condi-

tion, which is still one of the most common complications encountered in ICU, has a high fatality rate, primarily due to its intricate molecular underpinnings [26]. Additionally, research findings underscore the considerable economic burden imposed by sepsis. A separate study illuminated that the annual healthcare costs of treating approximately 230,000 septic patients in ICUs amounted to a staggering $4.6 billion [27–29]. Frequently, sepsis is also defined by an abnormal host immune response to an invading variety of pathogens. A systemic inflammatory reaction can occur that may lead to a precipitating disseminated intravascular coagulation (DIC) and multiple organ dysfunction syndromes (MODS) with, eventually, death of the patient [30]. The incremental advancements in sepsis diagnosis, treatment, and management, accompanied by ongoing guideline updates, have led to a notable reduction in mortality rates [31]. Despite these developments, the early recognition of sepsis, prevention of MODS, and improvement of patient prognosis remain pressing and unresolved challenges for physicians and healthcare institutions [30]. In multiple instances, sepsis is associated with other organ dysfunctions and injuries/damage like lung and kidney parenchyma (AKI, Acute Kidney Injury, and ALI, Acute Lung Injury). AKI and ALI both have essential clinical importance because they are life-threatening and are characterized by an overwhelming inflammatory response (IR) and oxidative stress (OS), which frequently leads to respiratory failure, even in children [30,32,33]. Specifically, the clinical understanding of ALI has continually evolved over the last century. ALI, often secondary to sepsis, is a significant source of morbidity and mortality in adult and pediatric populations [32,33]. Conspicuously, research indicates that sepsis contributes to a considerable portion of ALI cases, accounting for a range of 6% to 42%. In contrast, ALI finds frequent usage within experimental lung injury models, where it is categorized as a less severe variant of the human condition known as “Acute Respiratory Distress Syndrome (ARDS)”, as defined by the more recent ‘Berlin definition’ [34]. The diagnosis and treatment of ALI have consistently adhered to the guidelines established in the 2012 Berlin definition in the clinical practice [35].

Given ALI’s multifaceted and clinically diverse nature, current consensus on effective treatment strategies is somewhat limited. Protective ventilation therapy stands as one of the few widely accepted approaches. Unfortunately, other interventions involving anti-inflammatory agents (such as glucocorticoids, macrophage colony-stimulating factor (GM-CSF), statins, and aspirin) and medications aimed at enhancing lung function (including β receptor agonists and nitric oxide inhalation) have demonstrated only a marginal impact on reducing ALI mortality rates [36]. Sepsis-related ALI encompasses a multitude of molecular mechanisms. Therefore, it is paramount to undertake a quantitative examination of the current situation, areas of emphasis, and forthcoming opportunities concerning ALI in sepsis [34]. It is important to note that ALI can be exacerbated by ARDS, a condition characterized by hypoxemic respiratory failure, often considered a fatal consequence of severe sepsis. The incidence of ARDS has been on the rise, now reported at a rate of up to 86.2 cases per 100,000 person-years [34]. In the United States, there are approximately 200,000 reported cases of ARDS each year, and these cases are associated with a significantly high hospital mortality rate of 38.5%. Unfortunately, this mortality rate has shown limited improvement for several decades. The primary risk factor for the development of ARDS is severe sepsis, irrespective of whether it originates from a pulmonary or non-pulmonary source, accounting for a substantial 79% of cases.

Additionally, there are other contributing factors to this risk, including aspiration, exposure to toxic inhalants, lung contusion, acute pancreatitis, trauma, blood transfusion, burn injuries, and cardiopulmonary bypass surgery [34]. Moreover, a prominent histological feature of ARDS is severe diffuse alveolar damage commonly attributed to endothelial dysfunction and localized inflammation. Recently, neutrophil extracellular traps (NETs), released by polymorphonuclear neutrophils (PMNs), seem to have demonstrated to exert a robust antimicrobial function in infectious diseases like sepsis [37,38]. These authors demonstrate that NETs induce sepsis-induced (SI)-ALI through enhanced ferroptosis in alveolar epithelial cells. These authors confirm that the excessive release of NETs in patients and animal models with SI-ALI is accompanied by an up-regulation of Ferroptosis, which depends on METTL3-induced m6A modification of the hypoxia-inducible factor-1α (HIF-1α) and subsequent mitochondrial metabolic reprogramming.

Their results indicate the crucial role of NETs in the progression of SI-ALI via NETs-activated METTL3 m6A-IGF2BP2-dependent m6A modification of HIF-1α, which further contributes to metabolic reprogramming and Ferroptosis in alveolar epithelial cells. This commentary provides insights and recommendations for effectively integrating these outstanding results for personalized medicine and future research.

ARDS is a multifaceted condition frequently characterized by sudden worsening of non-cardiogenic pulmonary edema, profound hypoxemia, and the necessity for mechanical ventilation. Conversely, ALI resulting from sepsis refers to the onset of acute hypoxic respiratory failure triggered by sepsis-induced alveolar injury resulting from the dysregulated inflammatory response. Acute lung injury can stem from various (pathogens, toxins, physical trauma, OS, chemical stressors, radiation, genetic mutation, autoimmunity, environmental changes) directly influencing pulmonary function. The pathophysiology of ALI primarily involves severe inflammatory damage to the alveolar-capillary barrier, depletion of pulmonary surfactant, re-

duced adequate ventilation of lung tissue, and decreased lung compliance [35,36,39,40]. However, it is essential to note that ALI and ARDS are differentiated based on well-established criteria (please see below) [41]. Among the various causes of these conditions, sepsis and pneumonia are notably prominent etiological factors [32,33].

There has been a lot of study on SI-ALI, but the complex molecular pathways that cause the disease are still not fully understood [42]. Dysregulated RNA modifications, especially m6A, play a significant role in the development and progression of several disorders, such as cancer and conditions resembling neurodegenerative diseases [43]. But so far, nothing is known about how m6A alteration contributes to sepsis-associated ALI or what effects it may have in the future [44].

This study aims to fill a gap in our understanding by determining how the enzyme METTL3, which is responsible for m6A methylation, contributes to the progression of ferroptosis. In the setting of SI-ALI, ferroptosis is defined as a controlled cell death process that involves lipid peroxidation (LP).

This literature review delves into the complex molecular pathways that underlie the intensification of Ferroptosis in ALI caused by METTL3-mediated m6A alteration. Also, we stress that studying its interaction with IGF2BP2 could have a major effect on metabolic reprogramming in mitochondria. Little is known about its function in SI-ALI. Investigating the molecular pathways driving the development of ALI is vital for personalized therapy and future research because to its clinical significance and effect on healthcare expenses. In this research project, our goal is to help patients dealing with SI-ALI by providing important information that could improve our understanding of the disease at the molecular level and lead to new treatment options.

4. History of Sepsis-Induced-ALI

According to Geroulanos and Douka, the term Σήψη (sepsis) finds its origins in ancient Greece [45,46]. The Greek word *σήψη*, meaning 'putrefaction', is the origin of the term 'sepsis', which was initially used in a medical context over 2500 years ago in Homer's writings [46]. It was initially introduced by the renowned Greek physician *Hippocrates* to describe a phenomenon characterized by the breakdown of organic matter. Subsequently, the understanding of sepsis evolved as other medical experts, including *Aulus Cornelius Celsus* and *Galen*, elucidated that when the human body becomes infected, it exhibits distinct signs such as redness, swelling, fever, pain, and impaired functionality—collectively recognized as markers of inflammation [45]. In recent times, sepsis denotes a complex medical condition that arises when the body encounters significant challenges due to an infection. It encompasses a multifaceted array of issues within the body, encompassing its physiological processes, the occurrence of aberrations, and alterations in biochemical and molecular constituents, akin to miniature building blocks [47]. In 1914, Schottmueller made a pivotal discovery [48]. He observed that when harmful microorganisms invade the bloodstream, the body instills widespread inflammation. This systemic inflammatory response can lead to significant complications. Schottmueller wrote that "sepsis is present if a focus has developed from which pathogenic bacteria, constantly or periodically, invade the blood stream in such a way that this causes subjective and objective symptoms" [49].

Consequently, numerous research endeavors have been undertaken in subsequent decades to define the concept of sepsis precisely. These efforts have led to the use of various terms, such as septicemia, sepsis, toxemia, bacteremia, and endotoxemia, to characterize and comprehend this condition [47].

The global academic community has revised the definition and diagnosis criteria of Sepsis 3-times revisions to enhance the understanding of sepsis among clinicians, facilitate its early identification and diagnosis, and improve treatment strategies [23]. The latest definition, 'Sepsis-3' was consistently approved during the 45th Society of Critical Care Medicine (SCCM) meeting. It defines sepsis as a life-threatening condition that arises from dysfunctional host responses to infection, ultimately leading to organ dysfunction [45] (Tables 1,2).

The original documented syndrome characterized by the abrupt onset of severe respiratory distress following a discernible injury in 1967 was accompanied clinically by difficulty breathing, decreased lung compliance, widespread chest X-ray infiltrates, and unresponsive hypoxemia, even with supplemental oxygen [45]. This clinical sequence is now recognized as ARDS. The progression of ARDS is rapid and associated with high mortality. More than half of affected patients succumbed to respiratory failure within days to weeks, while a meta-regression analysis demonstrated a gradual decrease of 1.1% per year in mortality rates from 1994 to 2006, resulting in an overall pooled mortality rate of 43% across all the studies examined [25,45]. Although the American-European Consensus Conference (AECC) formally established diagnostic criteria for ARDS in 1994, these criteria encompass key elements. These elements include the acute onset of symptoms, bilateral chest radiographic infiltrates, persistent hypoxemia despite adjustments in positive end-expiratory pressure oxygen concentration, arterial partial pressure of oxygen to inspired oxygen fraction ratio (PaO_2/FiO_2) below 200, and the absence of indications suggesting left atrial hypertension.

Moreover, the AECC recognized that ARDS represents the most severe end of the spectrum of conditions falling under the broader category of acute lung injury [23,31,45]. In 2012, a redefinition of ARDS was introduced following the Berlin definition [34,39] (Table 1). This redefinition eliminated the previous classification of acute

Table 1. ALI criteria and timeline-related criteria for ARDS.

	ALI	ARDS
Onset	Acute	Acute
PaO_2/FiO_2	More than 300 mmHg	More than 200 mmHg
Chest radiograph	BI	BI
Left atrial hypertension	No	No

Notes: ALI, acute lung injury; ARDS, Acute Respiratory Distress Syndrome; BI, Bilateral infiltrates; PaO_2/FiO_2, partial pressure of oxygen to inspired oxygen fraction ratio.

lung injury, which had previously included cases not meeting the criteria for ARDS as per the AECC definition. Instead, ARDS has been reclassified into three distinct groups based on the severity of hypoxemia: Mild, Moderate, and Severe. Each category is associated with varying mortality levels [39,40]. It is important to note that this new classification primarily reflects histologic modification observed in ALI lung parenchyma and is not intended to encompass the entirety of the clinical entity.

Additionally, the histopathological counterpart of ARDS is called Diffuse Alveolar Damage (DAD). DAD represents the most severe form of lung injury and can result from various direct lung injuries, such as infections [50]. It is noteworthy that, at times, fibroblastic tissue, known as organizing pneumonia, can be observed within the airspaces during the progression of ALI [39,40]. Nevertheless, there are instances where organizing pneumonia occurs without a discernible cause. The clinical presentation of cryptogenic organizing pneumonia develops gradually over several weeks to months [51] and is discussed in the context of chronic lung diseases. It is essential to mention that DAD and other histological characteristics of acute lung injury do not precisely indicate the underlying cause [52]. When pathologists identify these features, they must conduct further investigations in the biopsy to uncover additional clues that might aid in determining the specific cause.

5. Pathogenesis of SI-ALI and "Morphology" of Ferroptosis

Sepsis extends beyond a simple systemic inflammatory reaction or immune dysfunction; it entails significant disruptions in the functioning of numerous organs throughout the body. The pathogenesis of sepsis unfolds as an exceptionally intricate phenomenon at the cellular and molecular levels. Within this complexity, a multitude of factors come into play, including disturbances in the balance of inflammatory response, abnormalities in the immune system, mitochondrial dysfunction, disruptions in coagulation pathways, alterations in the neuroendocrine-immune interaction, endoplasmic reticulum stress, autophagic processes, and an array of additional pathophysiological intricacies. These multifaceted elements ultimately lead to the development of organ dysfunction, which we will carefully examine in the following sections. Gas exchange performance is impaired in patients with SI-ALI because of inflammation and tissue damage in the lungs. Pulmonary interstitial fibrosis, lower alveolar surface tension, inflammatory factor release, and pulmonary vascular endothelial damage are pathological processes [53–55]. In SI-ALI, ferroptosis plays a critical role [21,22,56–58] due to its unique characteristics and mechanisms (Fig. 1).

It was first described in the early 2010s. It has since garnered significant attention from researchers in fields like cell biology, oncology, and neurology, which play a critical role in various physiological and pathological processes. However, Ferroptosis is characterized by the accumulation of iron-dependent lipid peroxides within cells, leading to oxidative damage to plasma membranes and cell death [56] (Fig. 2). Unlike apoptosis, which involves the controlled dismantling of cellular components, or necroptosis, characterized by inflammatory cell death, Ferroptosis exhibits distinct features. However, it has been implicated in various biological contexts, including cancer, neurodegenerative diseases, ischemia-reperfusion injury, and tissue homeostasis. Its role in cancer therapy is particularly intriguing, as targeting Ferroptosis can provide a novel strategy to inhibit the proliferation of cancer cells resistant to traditional treatments [59].

Ferroptosis contributes to the pathogenesis of ALI by exacerbating oxidative stress, lipid peroxidation (LPO), iron dysregulation, and glutathione (GSH) depletion in lung cells. This cascade of events ultimately leads to cellular damage, disruption of the alveolar-capillary barrier, and the development of ALI. Understanding the role of Ferroptosis in ALI may provide insights into potential therapeutic strategies for mitigating lung injury in conditions like ALI. However, it is essential to note that the field of Ferroptosis research in ALI is continually evolving, and more studies are needed to unravel the molecular mechanisms and develop effective interventions fully. Therefore, it plays a significant and crucial role in molecular pathogenesis in the development of ALI through the steps mentioned below.

5.1 Oxidative Stress (OS) and Lipid Peroxidation (LPO)

An imbalance between the body's generation of free radicals and its ability to counteract them is a major contributor to the development of acute lung injury (ALI). One of the key pathological mechanisms in ALI is oxidative stress,

Table 2. ARDS criteria.

	AECC (1994)	Berlin (2012)	2022 – Criteria
Timing	Acute, NOS	New/worsening $<$7 d	
Chest-X-ray/CT	Bilateral infiltrates	Bilateral infiltrates	Ultrasound (+)
Oxygenation	PaO_2-FiO_2, ALI, 300 mmHg, ARDS $<$200 mmHg	PaO_2-FiO_2, (*) 200–300 mmHg (**) 100–200 mmHg (***) $\leq$100 mmHg	SpO_2-FiO_2 ratio

Notes: The first consensus ARDS criteria were the AECC in 1994. This consensus was followed by the Berlin Consensus Criteria 18 years later in 2012. ARDS criteria are being revised in 2022. The era of precision medicine and liquid biopsy is approaching and the future of ARDS in this era will probably be quite different during this decade with the advent of biomarkers, which are under investigation. AEEC, American European Consensus Criteria; PaO_2, partial pressure of arterial oxygen; FiO_2, fraction of inspired oxygen; SpO_2, oxygen saturation; ARDS, acute respiratory distress syndrome; PEEP, positive end expiratory pressure; CT, computed tomography. The 2012 Berlin definition for Acute Respiratory Distress Syndrome (ARDS) defines it as acute onset respiratory failure within one week, bilateral opacities on chest imaging not fully explained by other conditions, and a PaO_2/FiO_2 ratio of 300 mmHg or less. The definition also requires a positive end-expiratory pressure (PEEP) of at least 5 cm H_2O and specifies three levels of severity: Mild (*) (PaO_2/FiO_2 201–300), Moderate (**) (PaO_2/FiO_2 101–200), and Severe (***) (PaO_2/FiO_2 $\leq$100).

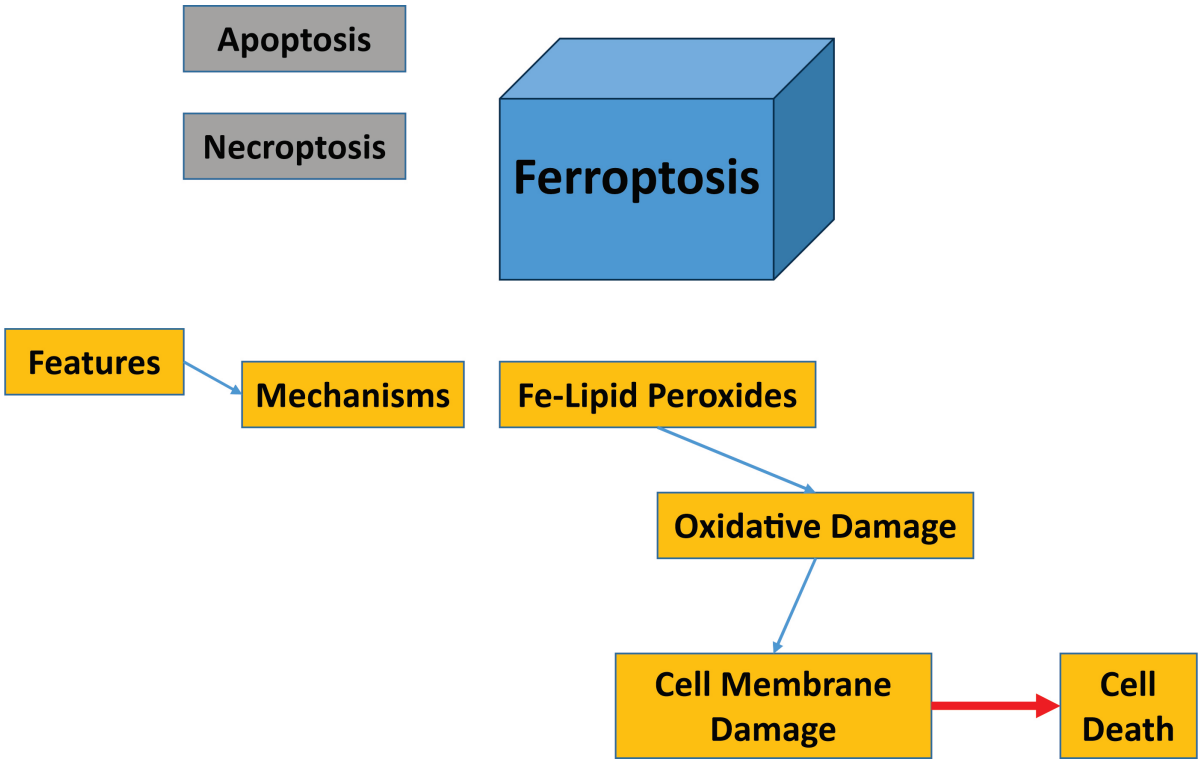

Fig. 1. Complexity of ferroptosis as a cell death pathway. The complexity of Ferroptosis as a cell death pathway, with its unique characteristics and mechanisms, includes the accumulation of iron-dependent lipid peroxides leading to oxidative damage, cell membrane damage, and eventual cell demise. It distinguishes Ferroptosis from other cell death pathways like Apoptosis and Necroptosis.

which occurs when lipids within cell membranes are broken down due to an excess of reactive oxygen species (ROS). This damages lung tissue, which in turn causes inflammation, cell death, and impaired lung function. Both OS and LPO are inserted in the context to trigger ALI. These factors associated with ALI susceptibility include pathogens, toxins, physical trauma, chemical stressors, radiation, genetic mutations, autoimmune reactions, and environmental

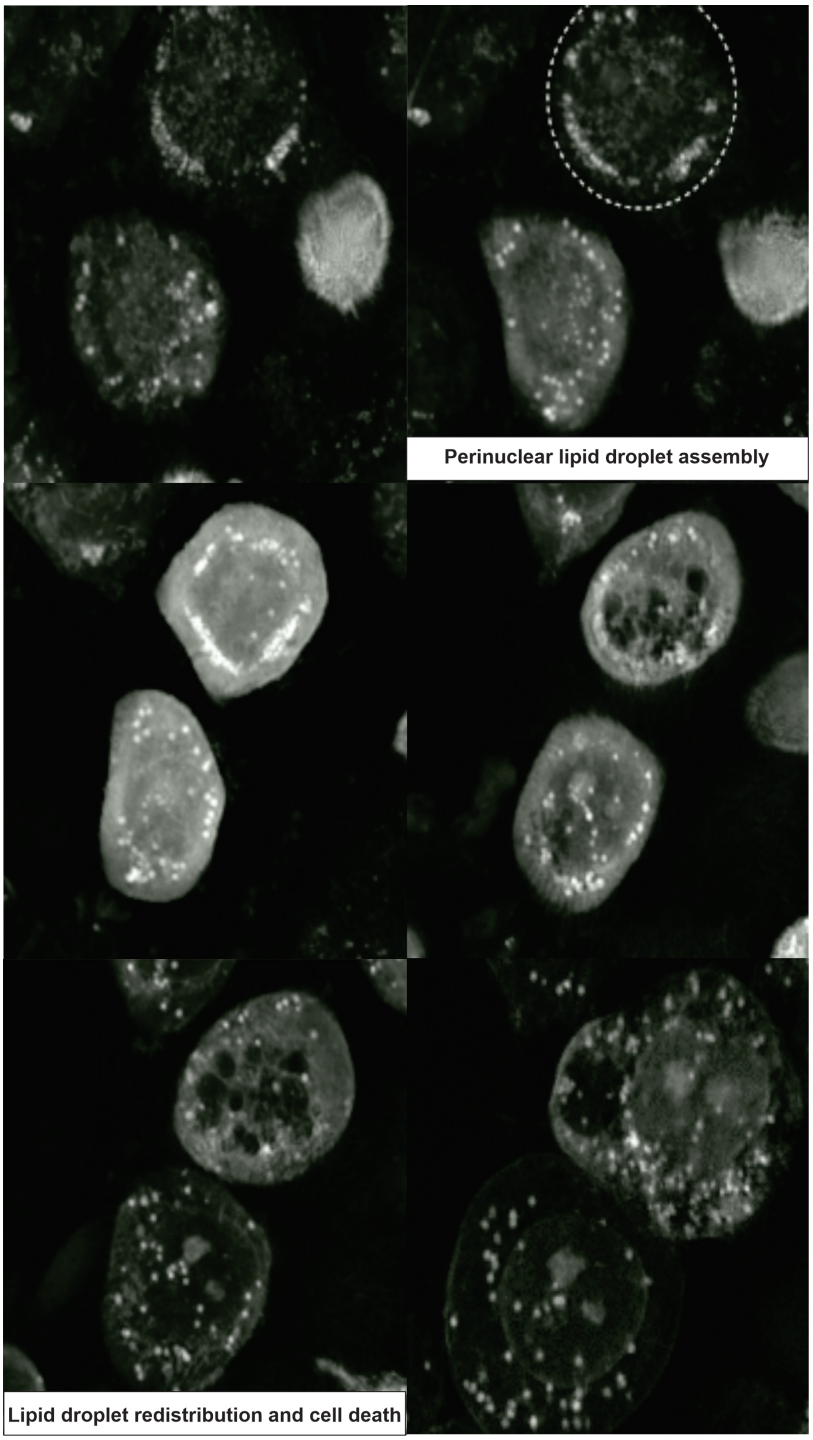

Fig. 2. Ferroptosis and subsequent cell death. The extracted refractive index images are from a YouTube footage using Nanolive's label-free imaging technology (Nanolive technology reveals Ferroptosis execution in an aggressive human prostate cancer model – – "YouTube" channel, https://www.youtube.com/watch?app=desktop&v=ATl331x9kAw). Brighter regions correspond to structures with a higher refractive index. It depicts the morphological alterations that cells experience during Ferroptosis. The cell undergoes contraction followed by swelling. Perinuclear lipid assembly is detected just prior to the onset of Ferroptosis. Upon completion of the process, lipid droplets (bright white dots) are redistributed. Technology: 3D Cell Explorer microscope by Nanolive, Switzerland. The 3D Cell Explorer is a rapid, high-resolution, non-invasive microscope for live cell imaging that can penetrate deeply into biological systems. This enables the rapid recording of exceptional 3D images of whole cells within seconds. Source of the Copyright Holder, whose permission to re-use the photographs has been granted: Nanolive.

changes [18]. These insults can induce an inflammatory response in lung tissue [22,60]. This inflammatory response often generates ROS and subsequent oxidative stress, as noted by Yi *et al*. in 2020 [61]. Within the lung microenvironment, oxidative stress can act as a catalyst for initiating Ferroptosis, a cell death pathway, by instigating lipid peroxidation. As highlighted by Conrad and Pratt in 2019 [62], this process leads to the deterioration of lipid bilayers within cell membranes.

5.2 Iron-Dysregulation

Iron is a crucial element involved in various cellular processes, but its excess or mismanagement can lead to cellular damage. In Ferroptosis, there is a significant accumulation of labile iron, particularly ferrous ions (Fe^{2+}), which are highly reactive and can catalyze the formation of harmful ROS through Fenton reactions [63]. In ALI, iron dysregulation can occur due to inflammation and tissue damage, leading to the release of iron from damaged cells and the circulation of free iron in the lung tissue [58].

5.3 Glutathione Depletion

Glutathione is a tripeptide that is the most abundant non-protein thiol in eukaryotic cells. It is found in all mammalian tissues but is especially concentrated in the liver. Glutathione is important for many cellular functions, including

(a) Redox signaling: GSH is a key determinant of redox signaling and a major defender against oxidative stress.

(b) Detoxification: GSH is vital for detoxifying xenobiotics.

(c) Protein and DNA synthesis: GSH plays a role in the synthesis of proteins and DNA.

(d) Cell proliferation, apoptosis, and immune function: GSH regulates cell proliferation, apoptosis, and immune function.

GSH is synthesized from amino acids in two enzymatic steps. The availability of cysteine, a sulfur amino acid precursor, and the activity of glutamate cysteine ligase (GCL) are key determinants of GSH synthesis. GSH is a vital antioxidant molecule that helps neutralize ROS and protect cells from oxidative damage [64]. In Ferroptosis, intracellular GSH levels are depleted, making cells more susceptible to oxidative stress and lipid peroxidation [65]. ALI is often associated with GSH depletion, as the antioxidant capacity of the lungs can be overwhelmed by the oxidative stress generated during inflammation and injury [66].

5.4 Cellular Damage and ALI Progression

As lipid peroxidation and oxidative damage to cell membranes accumulate due to Ferroptosis, lung cells become more vulnerable to injury and death [67]. In the context of ALI, such damage can potentially compromise the structural integrity of the alveolar-capillary barrier. Consequently, this disruption can lead to heightened permeability of blood vessels, allowing the escape of fluids and proteins into the alveolar spaces. The culmination of these events often results in the development of pulmonary edema.

6. METTL3-Mediated N6-methyladenosine (m6A) and ALI

METTL3, also known as methyltransferase-like 3, is an enzyme involved in RNA modification. Specifically, it catalyzes the addition of a methyl group (-CH_3) to adenosine (A) residues within RNA molecules, creating a modified form of adenosine called N6-methyladenosine (m6A). This modification occurs in various types of RNA, including messenger RNA (mRNA), with the specific feature of carrying the genetic information from DNA to the ribosome for specific protein synthesis; however, in ALI and various other cellular processes, METTL3 plays a critical role in regulating gene expression through m6A methylation. When METTL3 adds m6A marks to specific mRNA transcripts, it can impact their stability and translation, which are essential steps in protein production. Adding m6A marks can enhance the stability of specific mRNAs, preventing them from rapid degradation and increasing their lifespan within the cell [68]. Additionally, m6A modification can affect the process of mRNA translation, influencing how efficiently the mRNA is translated into a functional protein [69]. In the context of ALI, increased METTL3 activity leads to elevated m6A methylation levels on specific mRNA transcripts. These mRNA transcripts could encompass genes associated with the inflammatory response, OS, cell death pathways, and other processes pertinent to ALI. The pivotal mechanism underlying sepsis-ALI pathogenesis revolves around an inflammatory imbalance that spans the entire sepsis process [69,70]. Various pathogens can trigger this response, including bacteria, fungi, parasites, and viruses. Initially, the host's acute reaction to invasive pathogens prompts macrophages to engulf these pathogens and release an array of pro-inflammatory cytokines. This situation sets the stage for activating cytokine storms and the innate immune system [69]. However, the m6A methylation of *IGF2BP2* mRNA is a crucial contributor to the development of ALI. This pathway involves specific molecular events that regulate the expression and function of IGF2BP2, a protein implicated in various cellular processes [71]. m6A methylation, a chemical modification involving adding a methyl group (-CH_3) to adenosine at its N6 position, is a widely observed post-transcriptional alteration in mRNA molecules [72]. This modification is meticulously orchestrated by METTL3, a pivotal enzyme responsible for introducing m6A marks at specific locations within mRNA transcripts. The focus of this intricate mechanism lies in the context of ALI development. METTL3 plays a central role in regulating the mRNA of IGF2BP2, thereby influencing post-transcriptional gene regulation mechanisms that could potentially affect ALI's pathogenesis [44].

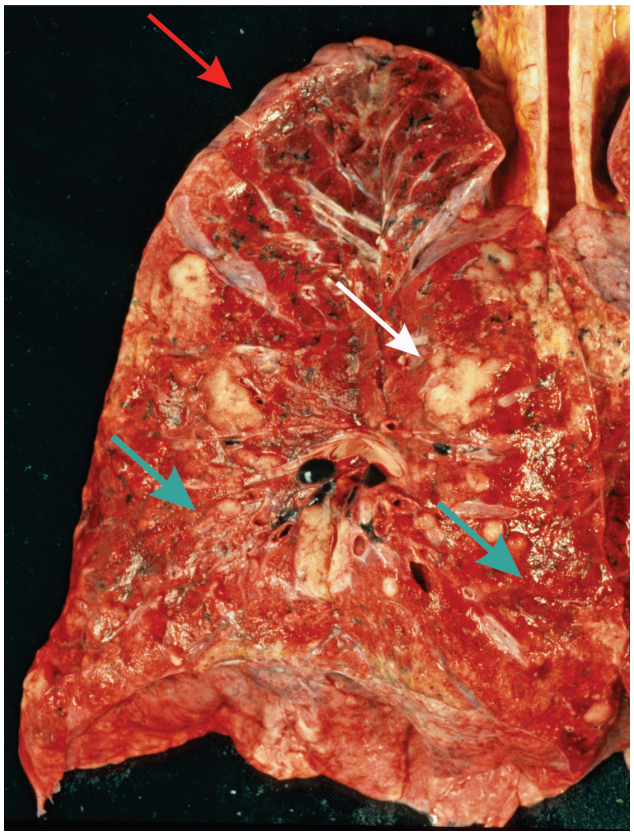

Fig. 3. Gross Photograph of a lung harboring sepsis-induced acute lung injury (SI-ALI) and metastatic foci of a pelvic chondrosarcoma. The red arrow points to a chronic obstructive pulmonary disease (COPD) area, while the green arrows point to areas of SI-ALI and the white arrow to an area of metastatic chondrosarcoma (original photograph of Dr. Sergi's archive).

In Cao *et al.*'s work [73], methyltransferase-like 14 (METTL14) stabilizes NLRP3 expression in an IGF2BP2-dependent manner, which contributes to acute lung damage. An uncontrolled inflammatory response is a common complication of ALI and its more severe variant, ARDS. The development of many inflammatory illnesses was linked to the N6-methyladenosine (m6A) alteration. There is still some mystery around the function of METTL14-mediated m6A methylation in ALI/ARDS. In a nutshell, the m6A reader IGF2BP2 stabilized NLRP3 mRNA after it detected METTL14-methylated NLRP3 transcripts. METTL14 activates the NLRP3 inflammasome through an IGF2BP2-dependent pathway, which is important for the pathogenesis of ALI/ARDS. This suggests that METTL14 and IGF2BP2 could be useful biomarkers and therapeutic targets for the treatment of ALI/ARDS. The gross photograph of the lung depicted in Fig. 3 arises from a patient with COPD (chronic obstructive pulmonary disease) and SI-ALI-accompanying metastasis of a chondrosarcoma (original photograph).

Recent research has elucidated that METTL3-mediated m6A modification of *IGF2BP2* mRNA bolsters its stability and functionality, thereby exacerbating the severity of ALI. Within this intricate pathway, one of the primary mRNA targets for METTL3-mediated m6A methylation is *IGF2BP2* mRNA, a critical player in post-transcriptional gene expression regulation. METTL3 deposits m6A marks at specific adenine residues along the *IGF2BP2* mRNA molecule, resulting in several crucial consequences. Firstly, m6A methylation augments the stability of *IGF2BP2* mRNA, rendering it more resistant to degradation and allowing it to persist within the cell for extended durations. Secondly, these m6A marks on *IGF2BP2* mRNA actively promote translation into the IGF2BP2 protein, leading to increased production of this regulatory protein within the cell. Notably, *IGF2BP2* has a well-documented role in mRNA stability and translation. It selectively binds to specific mRNA molecules, enhancing their stability and translation. In the context of ALI, it is plausible that IGF2BP2 regulates the expression of specific genes and their pathways that hold relevance for the development of this condition. The precise genes and pathways modulated by IGF2BP2 in the context of ALI; however, the specific genes and pathways influenced by IGF2BP2 in the context of ALI have yet to be clearly identified and fully understood. Additional studies are necessary to unravel the intricate molecular mechanisms and biological pathways through which IGF2BP2 operates in the development or progression of ALI. This lack of precise knowledge emphasizes the complexity of understanding the role of IGF2BP2 in ALI pathology, underscoring the need for further investigation to uncover the intricacies of this relationship. The m6A methylation of *IGF2BP2* mRNA, orchestrated by METTL3, culminates in heightened stability and translation of the IGF2BP2 protein. Elevated levels of IGF2BP2 protein can influence downstream gene expression and cellular processes, potentially contributing to the initiation or progression of acute lung injury. It is essential to acknowledge that while the role of m6A methylation in gene expression regulation is well-established, the specific mechanisms and downstream effects within the context of ALI may still be a subject of ongoing research.

Research has demonstrated that METTL3 expression is elevated in the lungs of SI-ALI patients. One investigation on acute lung injury caused by smoking found that this upregulated expression was linked to elevated m6A methylation of *IGF2BP2* mRNA [38]. By stabilizing the *IGF2BP2* mRNA and improving translation, this m6A alteration raises the amounts of IGF2BP2 protein. An essential modulator of messenger RNA (mRNA) stability and translation is IGF2BP2 [38]. It prevents the degradation of some mRNAs by binding to them. The translation of some messenger RNAs is also enhanced by IGF2BP2. Genes involved in inflammatory response, programmed cell death or apoptosis, and cell proliferation are regulated by IGF2BP2 in the context of SI-ALI [38].

Overexpression of IGF2BP2 in the lungs of mice leads to the development of SI-ALI-like symptoms, including inflammatory cell infiltration, alveolar damage, and pulmonary edema. Conversely, knocking down IGF2BP2 expression protects mice from SI-ALI [74–76].

METTL3-m6A-IGF2BP2 axis acts as a crucial contributor in the pathogenesis of SI-ALI; however, targeting this axis could be a promising therapeutic approach for SI-ALI [77]. Moreover, it is noteworthy that IGF2BP2 is recognized for its interaction with specific mRNAs involved in ferroptosis regulation, particularly those encoding proteins related to iron metabolism and lipid metabolism [78]. This interaction enhances the stability of these mRNAs, ultimately leading to increased expression of proteins that promote ferroptosis [78]. Ferroptosis is controlled cellular demise or regulated cell death, which is characterized by iron-dependent oxidative damage to lipids. It has been implicated in the pathogenesis of ALI, and the upregulation of Ferroptosis-promoting genes has been observed in ALI patients. The current evidence suggests that m6A methylation of *IGF2BP2* mRNA by METTL3 may play a pivotal role in the development and progression of ALI. By increasing the stability and translation of *IGF2BP2* mRNA, METTL3 may promote ferroptosis and other cellular processes that contribute to ALI pathology [76,79]. Investigating the specific mechanisms and downstream effects of m6A methylation in ALI could lead to the development of new therapeutic strategies for this devastating condition [75].

7. Mitochondrial Metabolic Reprogramming and Molecular Mechanisms in ALI

Mitochondrial metabolic reprogramming is a central feature of ALI that contributes to cellular dysfunction and inflammation [80]. Molecular mechanisms underlying mitochondrial dysfunction in ALI include inflammatory signaling, oxidative stress, endoplasmic reticulum (ER) stress, dysregulated autophagy and mitophagy, and the Sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK) (SIRT1-AMPK) pathway [80]. SIRT1-AMPK refers to the interaction between SIRT1 and AMPK. These are both key players in cellular metabolism and energy regulation. AMPK acts as a sensor of cellular energy status, while SIRT1 is a deacetylase that regulates various cellular processes. Understanding these mechanisms provides insights into potential therapeutic strategies, but challenges remain in translating preclinical findings into effective clinical interventions. Further research is needed to elucidate the intricate interactions between mitochondrial dysfunction and the immune response in ALI, ultimately leading to improved outcomes for patients with this life-threatening condition.

In healthy lungs, mitochondria are known as "powerhouses" of the cell due to their essential role in producing adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS). In normal lung tissue, mitochondria are abundant within type-II alveolar and endothelial cells [81]. These mitochondria play a pivotal role in preserving lung homeostasis [82,83]. These organelles produce ATP and regulate various cellular processes, including apoptosis, redox balance, calcium homeostasis, and inflammation [83,84].

A profound mitochondrial functionality and metabolism transformation accompanies ALI. This reprogramming encompasses shifts in mitochondrial respiration, heightened generation of ROS, and modifications in mitochondrial dynamics. These modifications significantly contribute to lung injury and the associated inflammatory response [80].

7.1 Impaired Mitochondrial Respiration

One of the primary changes in mitochondrial function during ALI is a decrease in mitochondrial respiration. This impairment reduces ATP production and contributes to the energy deficit observed in ALI patients [80,85]. Several factors can impair mitochondrial respiration, including oxidative damage or oxidative stress, which can damage mitochondrial proteins and membranes, impairing mitochondrial function (leading to a notable decline in the crucial functions carried out by mitochondria, such as ATP production and cellular energy regulation). Mitochondrial DNA (mtDNA)-mutations can disrupt the electron transport chain (ETC) complexes, impairing electron transport and ATP production [80,82]. Altered expression or function of ETC complex proteins can also impair mitochondrial respiration), and alterations in the ETC complexes. These defects lead to decreased ATP availability, compromising cellular repair processes and increasing susceptibility to injury.

7.2 Increased ROS Production

Mitochondrial dysfunction in ALI is closely associated with increased ROS production [86]. ROS are notably reactive entities capable of inflicting harm upon various cellular constituents, such as lipids, proteins, and DNA [87]. Within ALI, the overproduction of ROS can trigger inflammatory pathways, disrupt cellular membranes, and intensify lung injury [88]. The sources of ROS in ALI include damaged ETC complexes, peroxisomes, and dysfunctional mitochondria. ROS also plays a role in mitochondrial fission and fusion dynamics, perturbed in ALI [17,89].

7.3 Altered Mitochondrial Dynamics

Mitochondria are dynamic cellular organelles engaged in a perpetual cycle of fission and fusion processes, meticulously overseeing their morphology, distribution, and overall functionality [90]. In ALI, there is dysregulation of mitochondrial dynamics [91]. Excessive fission can lead to mitochondrial fragmentation, impairing ATP production and promoting apoptosis. Conversely, impaired fusion can result in elongated mitochondria with disrupted cristae

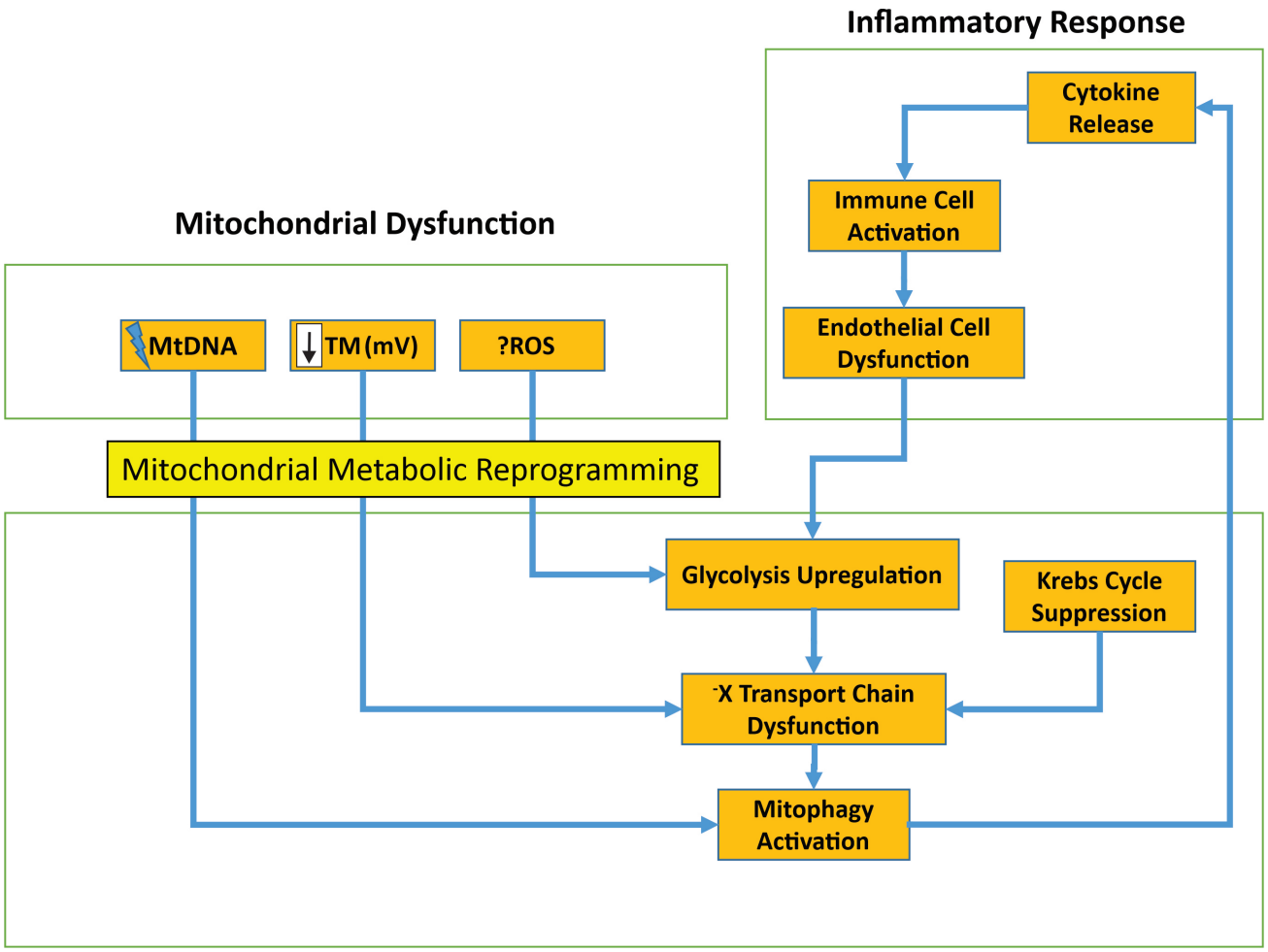

Fig. 4. Molecular mechanisms underlying mitochondrial metabolic reprogramming in ALI. This schema presents the molecular mechanisms underlying mitochondrial metabolic reprogramming in cases of Acute Lung Injury (ALI). Mitochondrial dysfunction in ALI involves a complex interplay of various cellular processes, ultimately leading to cellular dysfunction and inflammation. TM, transmembrane potential (millivolt, mV); ROS, reactive oxygen species. The lightning close to MtDNA indicates the nuclei acid changes of the mitochondrial dysfunction, as a core for the mitochondrial metabolic reprogramming. The arrow pointed down indicates that the transmembrane potential is involved showing a decrease of voltage, while the question mark before ROS indicates the variability of scientific evidence so far that involves ROS for the mitochondrial metabolic reprogramming. -X, anion.

structure, affecting respiratory capacity and ROS generation. Imbalances in mitochondrial dynamics contribute to the overall dysfunction observed in ALI [91].

7.4 Molecular Mitochondrial Dysfunction

To gain insight into the molecular mechanisms that govern mitochondrial metabolic reprogramming in the context of ALI, it becomes imperative to delve into the pivotal factors and signaling pathways associated with this phenomenon (Fig. 4).

7.4.1 Inflammatory Signaling

Inflammation is a hallmark of ALI and is crucial in mitochondrial dysfunction. Pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), can directly influence mitochondria [92]. These cytokines trigger mitochondrial ROS production and impair mitochondrial respiration by disrupting ETC complexes. Additionally, nuclear factor-kappa B (NF-κB), a central regulator of inflammation, can translocate to the mitochondria and influence mitochondrial function [93,94]. The interplay between inflammation and mitochondrial dysfunction is a bidirectional process, each exacerbating the other in ALI [80,82].

7.4.2 Oxidative Stress

Oxidative stress significantly drives mitochondrial dysfunction in ALI. As mentioned, excessive ROS production damages mitochondrial components, including mtDNA [17,22,80,82,95]. This is particularly vulnerable to oxidative damage due to its proximity to the ROS-producing ETC. Damage in mitochondrial DNA (Mt-DNA)

can initiate the release of damage-associated molecular patterns (DAMPs), including mitochondrial DNA fragments. These DAMPs have the potential to activate innate immune responses and intensify inflammation in cases of ALI [17,82].

7.4.3 ER Stress

ER stress is emerging as a critical player in mitochondrial dysfunction in ALI. The ER-mitochondrial axis regulates calcium homeostasis and lipid metabolism, which are disrupted in ALI [89]. ER stress can lead to calcium release from the ER, promoting mitochondrial calcium overload and ROS production. Additionally, perturbed lipid metabolism can affect mitochondrial function and membrane integrity. The crosstalk between ER stress and mitochondrial dysfunction contributes to the pathogenesis of ALI [89,96].

7.4.4 Autophagy and Mitophagy

Autophagy is a vital cellular process tasked with dismantling damaged organelles, including dysfunctional mitochondria, employing a specific mechanism known as mitophagy [97–99]. However, in the context of ALI, both autophagy and mitophagy undergo dysregulation. Ineffective autophagy can lead to impaired mitochondria buildup, intensifying ROS production and inflammation. Conversely, an excessive level of mitophagy can culminate in mitochondria depletion, depriving cells of the essential functional mitochondria required for energy production and cellular repair [89,97].

7.4.5 SIRT1-AMPK Pathway

The SIRT1-AMP-activated protein kinase (AMPK) pathway regulates mitochondrial function and metabolism [100,101]. SIRT1, a deacetylase reliant on Nicotinamide Adenine Dinucleotide^{+} (NAD^{+}), protects against ALI by deacetylating and activating pivotal enzymes responsible for mitochondrial biogenesis and performance [102]. Simultaneously, AMPK, a cellular energy status monitor, stimulates mitochondrial biogenesis and enhances the oversight of mitochondrial quality by facilitating autophagy and mitophagy [93,103–105]. Notably, the disruption of the SIRT1-AMPK pathway is linked with mitochondrial dysfunction in cases of ALI [95]. Although resveratrol, a common antioxidant present in red grapes, has been shown to protect against metabolic decline and stimulate mitochondrial biogenesis, the exact mechanism by which SIRT1 mediates these effects is still up for debate [100].

8. MEETL3-Ferroptosis and Mitochondrial Reprogramming in Sepsis-Induced Acute Lung Injury (SI-ALI)

In infectious diseases like sepsis, neutrophil extracellular traps (NETs) made by polymorphonuclear neutrophils (PMNs), also known as leukocytes, operate as powerful antibiotics. Fig. 5A–C (Ref. [106]) shows that NETs contribute to the development and progression of sepsis. In sepsis patients, the NET level positively correlates with illness severity. Surprisingly, a recent study found that sepsis patients had much higher levels of dsDNA and myeloperoxidase (MPO)-DNA complexes compared to healthy controls [106]. Those elevated concentrations of MPO and CitH3, biomarkers that point to the development of NETs, were confirmed by immunofluorescence (IF) imaging. These numbers were discovered in the peripheral blood of patients who did not survive, which is part of the postmortem examination or blood obtained before or during an agonal state [107–109].

This information was associated with the degree of lung damage shown by computed tomography (CT) scans. It appears that the percentage of cells secreting NETs was almost double in patients who did not survive and triple in healthy controls. These findings have also been validated in an animal model. When compared to the control group, SI-ALI animals in the CLP model had worse lung injury scores and shorter survival times. Rodents showed increased pulmonary damage in the bioassay, with alveolar wall disintegration and significant neutrophil infiltration. Compared to the control group, the SI-ALI group showed much higher levels of MPO-DNA complex and dsDNA. Indications from the data point to NETs being a major factor in ALI development. Evidence from both humans and mice points to a causal relationship between SI-NET overexpression and illness severity.

In SI-ALI animals, NET suppression decreases lung inflammation. Tissue damage and a prolonged inflammatory response might be worsened by an overabundance of NET release. For the purpose of determining if NETs, NET-inhibitors, or NET-degraders could worsen lung damage and inflammation. PAD4 is an essential enzyme in the NET synthesis process, and Cl-amidine was used to block it. The DNA structures were broken down using DNase I, and the neutrophils were directly depleted using the anti-Ly6G antibody. Both NET formation and dsDNA readings were decreased by these treatments. After NET inhibition, there was a decrease in the levels of inflammatory cytokines (TNF-α, IL-1β, and IL-6) in both plasma and bronchoalveolar lavage fluid (BAL). These results support the role of NETs in the development of ALI since they show that blocking NET formation reduced lung damage and inflammation in SI-ALI rats [106].

NETs strongly intensify the SI-ALI event through the deployment and development of Ferroptosis in lung cells at level of alveoli (alveolar epithelial cells). Ferroptosis is not a trivial event, but significant contributor to lung (alveolar) injury resulting from septicemia [20]. In experimental animals (mice) with SI-ALI, levels of ferritin, ferrous iron, and ROS were elevated. Applying Cl-amidine, an anti-Ly6G antibody, or DNase I can significantly inhibit the enzyme. Malondialdehyde (MDA), which is a result of lipid perox-

idation in membranes, showed an interesting increase after sepsis. Unfortunately, Cl-amidine, anti-Ly6G antibody, and DNase I treatment only partially reduced its levels. In a similar vein, inhibiting NETs partially restored reduced glutathione (GSH) levels and GPX4 expression in SI-ALI rats. Additionally, ferroptosis-indicating features were present in the alveolar epithelial cells of SI-ALI mice in comparison to the sham group. Mitochondrial enlargement, mitochondrial size reduction, and diminished cristae were all revealed by ultrastructural analysis using transmission electron microscopy. Reduced mitochondrial damage was seen after NET suppression [58,110].

As a result of alveolar epithelial cells, the lungs are able to function. In the unfortunate case of ALI, these cells are vulnerable to damage. Morphological changes and decreased viability were seen in HPAEpiC cells after 24 hours of exposure to NETs. The ironoptosis inhibitor ferrostatin-1 (Fer-1) attenuated these changes, suggesting that ferroptosis plays a role in NET-induced damage. It is true that ferrous iron and ROS concentrations increase with time in NET-treated cells, but GSH and GPX4 mRNA expression levels decrease; however, this trend is reversed by the addition of Fer-1. The changes were characterized by a disturbance in the cellular cytoskeleton, which Fer-1 treatment effectively reduced. In addition, NETs affected GPX4 expression in a laboratory setting. The expression of GPX4 decreases with time after HPAEpiC cells are treated with NETs. All of the above prove that ferroptosis, which is produced by NETs in alveolar epithelial cells, plays a part in SI-ALI in laboratory settings.

NETs cause Ferroptosis in pneumocytes (alveolar epithelial cells) through the tight activation of METTL3-mediated m6A modification [19,20,106]. After NET stimulation, DNase I reduced the increased m6A methylation levels. Afterwards, the results' key enzymes could be identified with the help of RNA sequencing. Cells exposed to NETs showed a small increase in IGF2BP2 and a considerable elevation in METTL3, as shown by RNA-seq. Consistent with the RNA-seq findings, the RT-qPCR study revealed a pattern. Additionally, it was shown that H3K27ac is strongly associated with the METTL3 promoter, according to data analysis done at UCSC Genome Bioinformatics (UCSC Genome Browser Home). This suggests that H3K27ac may have an effect on the transcription of METTL3. H3K27ac is synthesized by the crucial acetyltransferase p300. In addition, another research on how NETs affect p300 and H3K27ac levels found that NET therapy increased p300, METTL3, and H3K27ac expression in a time-dependent manner, which was inhibited by DNase I [19,20,106]. An inhibitor known as C646 targets p300 in particular. C646 may significantly downregulate H3K27ac and METTL3 expression, according to Western blot analysis. After 24 hours of treatment with C646, METTL3 mRNA levels were found to have reduced. Similarly, p300 and METTL3 mRNA and protein expression levels were both downregulated when p300 was silenced. Consequently, evaluating the enrichment of H3K27ac in the METTL3 promoter region relied heavily on a quantitative ChIP experiment. Reduced H3K27ac enrichment in the METTL3 promoter is a result of p300 knockdown. The results supported the idea that NET-mediated SI-ALI and METTL3 overexpression could be partially explained by p300-mediated H3K27ac activation in the METTL3 promoter.

To investigate thoroughly the delitescent role of METTL3-mediated m6A alteration in NET-induced ferroptosis, some researchers [20,106] did not arrogate it, but efficiently established models for siRNA-mediated knockdown and lentivirus-induced overexpression of METTL3 [111,112]. In contrast to the control group, mRNA levels were significantly lower and higher. Because GPX4 is a key enzyme in ferroptosis control, we found that METTL3 inhibition increased GPX4 mRNA and protein levels, whereas METTL3 overexpression reversed this effect (Fig. 5B,C).

CRISPR-Cas9 technique has been used by our group in different settings, and we found that this technique is highly reliable for molecular biology investigations [113]. In order to detect changes related to ferroptosis, CRISPR/Cas9 was utilized to generate METTL3 knockout (METTL3$^{-/-}$) HPAEpiC cells. To induce ferroptosis, NETs were exposed to METTL3$^{-/-}$ and METTL3$^{+/+}$ cells for 24 hours. Alveolar epithelial cells undergo ferroptosis after NETs stimulated p300, which improved the H3K27ac-mediated transcription of METTL3. Furthermore, mice that lack METTL3 had less severe acute lung injury when exposed to NETs. When compared to wild-type (METTL3flox/flox) mice after CLP, METTL3 CKO animals showed less severe lung injury. Minimal lung fibrosis, decreased wet/dry weight ratio, and decreased inflammatory cell infiltration were all indicators of this. Furthermore, METTL3 CKO mice showed reduced levels of inflammatory cytokines and cells in BAL samples (TNF-α, TNF-β, IL-1α), suggesting a reduction in inflammation in the lungs. Mice knocked out of the *METTL3* gene showed reduced neutrophil NET release when analyzed ultrastructurally using SEM and immunofluorescence. After CLP, the METTL3 CKO mice had a higher survival rate than the WT mice. Acute lung injury in mice caused by NETs was reduced when *METTL3* was knocked out.

In humans, the *CITED2* gene on chromosome 6 encodes the Cbp/p300-interacting transactivator 2 protein. The *CITED2* gene is an essential component of the embryonic cardiovascular system and a cardiac transcription factor [114]. Multiple heart abnormalities may emerge from CITED2 knockout studies in mice. In a research that included Tibetans who had coronary heart diseaseMETTL3 promotes the induction of HIF-1α through an m6A-IGF2BP2-dependent pathway, hence intensifying Ferroptosis. To elucidate the molecular pathways associated with METTL3-regulated ferroptosis, the researchers

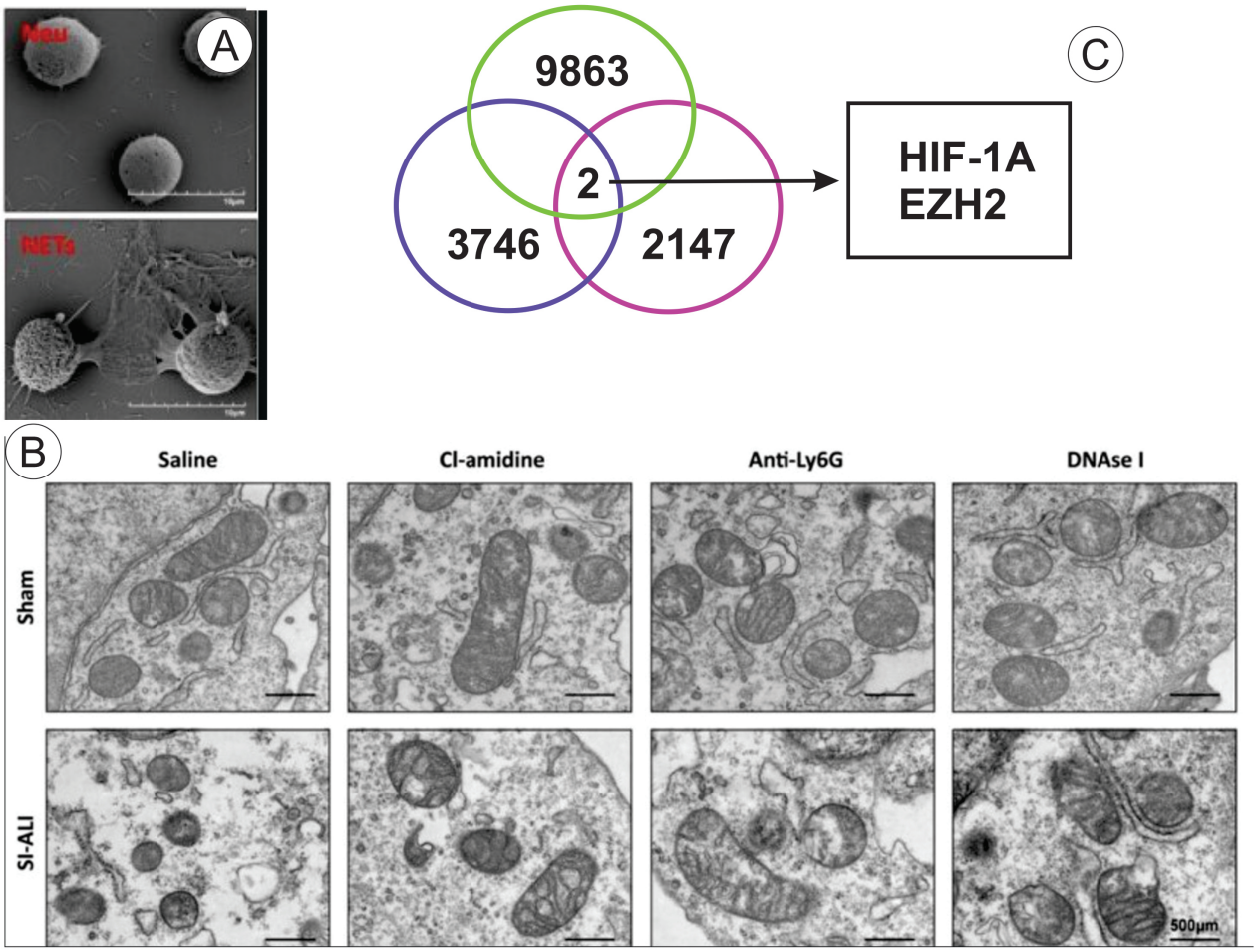

Fig. 5. MEETL3-ferroptosis and mitochondrial reprogramming in sepsis-induced acute lung injury (SI-ALI). (A) Images of scanning electron microscopy (SEM) of inactivated and activated neutrophils. They are photographed while there is evidence of NETs release (scale bar: 10 micrometers). (B) Images of transmission electron microscopy (TEM) of morphological changes revealed in mitochondria (scale bar, 500 nanometers). The original photograph reported a scale bar of 500 micrometers, which is not plausible. Note that the alveolar epithelial cells of DI-ALI mice show characteristic typical of Ferroptosis, i.e., mitochondrial shrinkage, decrease of the mitochondrial size, and small cristae. Such mitochondrial damage was found to be absent following the inhibition of neutrophil extracellular traps (NETs). (C) RNA sequencing and MeRIP-sequencing were used to identify differentially expressed genes in METTL3 overexpression and knockout cells comparing them to controls. The analysis revealed that m6A peaks of 9865 transcripts demonstrated a remarkably increased abundance. The RNA-sequencing and MeRIP sequencing revealed two transcripts, which overlapped. They were originating from two distinct genes, remarkably, one of which was HIF-1 alpha. The images are adapted and modified from Zhang *et al.* 2023 [106]. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

performed RNA sequencing in HPAEpiC cells exhibiting METTL3 overexpression and knockout levels [106]. When comparing the METTL3 overexpression and knockdown groups to their respective controls, RNA-seq analysis showed that 3748 transcripts were considerably upregulated and around 2000 transcripts were significantly downregulated. METTL3 overexpression in HPAEpiC cells and control cells using MeRIP-seq. The study found that over 9000 transcripts had an increased number of m6A peaks. Two transcripts from separate genes, including HIF-1α, overlapped with each other, according to the RNA-seq and MeRIP-seq results. The expression of *HIF-1α* was significantly enhanced by the overexpression of METTL3. The downregulation of *METTL3* significantly reduced HIF-1α mRNA and protein levels in lentivirus-transfected METTL3 knockdown cells. A higher proportion of HIF-1α mRNA displaying m6A methylation and a comparable decrease were seen after overexpressing or knocking down METTL3. But cells with the catalytic mutation METTL3 showed no improvement. The par-

ticular alteration of HIF-1α was determined by engineering mutant plasmids to recognize the expected m6A position. The stability of HIF-1α mRNA was tested by the researchers using actinomycin D to determine the effect of METTL3-mediated m6A change. According to the results, the degradation of HIF-1α mRNA was significantly decreased when METTL3 was overexpressed [19,20,106]. Since NETs could elevate the concentration of IGF2BP2, a m6A-binding protein that clearly enhances mRNA stability and translation. In fact, NETs may activate HIF-1α through a m6A-IGF2BP2-dependent pathway. As anticipated, activation of NETs increased the expression of IGF2BP2 *in vitro* [106]. Additionally, HIF-1α protein and mRNA levels were significantly reduced when IGF2BP2 was silenced. Using RNA immunoprecipitation (RIP), an additional evaluation of the relationship between HIF-1α and IGF2BP2 was conducted. The interaction between IGF2BP2 and HIF-1α mRNA was altered by the overexpression and silencing of IGF2BP2. The actinomycin D experiment showed that increasing the expression of METTL3 might partially restore the reduced stability of HIF-1α caused by the knockdown of IGF2BP2. The link between IGF2BP2 and HIF-1α mRNA was diminished as METTL3 was reduced, according to the RIP results. In addition, the binding interaction between IGF2BP2 and HIF-1α mRNA was reduced when METTL3 was knocked down, suggesting that the association between the two proteins may be METTL3/m6A dependent. In addition, decreasing IGF2BP2 and HIF-1α levels allowed GPX4 expression to be upregulated, suggesting that METTL3 increased HIF-1α levels through a m6A-IGF2BP2-dependent pathway, which in turn promoted ferroptosis by reducing GPX4 levels.

HIF-1α is a principal regulator of cellular glycolysis, and heightened glycolysis may significantly contribute to the advancement of ALI [106,115]. In cells lining the airways, METTL3 encourages glycolysis to rise while oxidative phosphorylation decreases. As METTL3 has the potential to affect ferroptosis, in order to study ferroptosis in alveolar epithelial cells by controlling metabolic pathways, researchers conducted RNA-seq analyses on cells that had been pretreated with si-METTL3 and si-NC. Dot blotting and Western blotting validated the METTL3 knockdown levels and the correlating downregulation of m6A levels. Compared to the control groups, the si-METTL3 groups showed a significant downregulation of ENO1, a glycolytic enzyme that converts 2-poly-γ-glutamic acid to phosphoenolpyruvate. This observation was confirmed by Western blotting and immunofluorescence microscopy. Several glycolytic enzymes, including LDHA, PFKP, PKM2, and HK2, had their expression levels decreased when METTL3 was inhibited. After that, we wanted to see if there was any change in aerobic oxidation, a measure of glucose metabolism, following METTL3 silencing. The level of mitochondrial complexes was found to be significantly higher in si-METTL3 cells compared to controls, which is in line with the results of RNA-seq, which showed that mRNA levels associated with complexes I to V in the mitochondria were significantly elevated. This suggests that METTL3 may inhibit oxidative phosphorylation. In NET-treated METTL3$^{+/+}$ HPAEpiC cells, there was a significant increase in the levels of glycolysis-related markers, including as ECAR, lactate production, and glycoPER. On the other side, METTL3$^{+/+}$ HPAEpiC cells showed a suppression of oxidative phosphorylation indicators such as OCR, baseline respiration, maximum respiration, and spare respiratory capacity. In conclusion, it proves that METTL3 boosts glycolysis and decreases oxidative phosphorylation in alveolar epithelial cells [19,20,106].

Finally, the inhibition of NET formation with net PAD4 knockout was also targeted [19,20,106]. Inhibiting NET formation with PAD4 deletion alleviates ferroptosis and the lung damage caused by sepsis in rats, as was clearly demonstrated. Less inflammation and damage to the lungs was observed in PAD4 knockout mice. Less inflammatory cell infiltration in lung tissues, a lower plasma cfDNA (cell-free DNA) level, a lower total cell count in BAL (broncho-alveolar lavage), and a lower wet/dry weight ratio were all indicators of this. Moreover, inhibiting NETs lowered systemic inflammation, as PAD4 KO animals had lower levels of IL-1α, TNF-α, and TNF-β compared to WT mice. When compared to wild-type mice, PAD4 knockouts showed moderate fibrosis and neutrophil extracellular trap development in the lung tissues. After PAD4 deletion, there was a rise in GSH and GPX4 levels, a decrease in ferritin, MDA, and ROS levels, and a decrease in METTL3 and HIF-1α expression levels in the lung tissues of mice. These indicators are related to ferroptosis and oxidation. Depleting PAD4 may decrease NET formation, which in turn may decrease ferroptosis and SI-ALI.

Up to this point, there is strong evidence that NET-induced ferroptosis is mediated by METTL3-m6A modification. As a result of mitochondrial metabolic reprogramming dependent on m6A-IGF2BP2, ferroptosis was exacerbated when NET-induced overexpression of METTL3 in alveolar epithelial cells. SI-ALI attacks people all around the world and kills them [116–118]. Throughout their development, leukocytes can discharge NETs, filamentous formations consisting predominantly of DNA, histones, cathepsin G, myeloperoxidase (MPO), and several antibacterial agents, to neutralize and eliminate infections. Nonetheless, NET components can purpose as DAMPs, potentially eliciting a chronic inflammatory tissue response and, eventually, tissue injury. In previous literature, NETs exacerbated systemic inflammation through the release of some cytokines, including TNF-α, IL-1β, and IL-6. These inflammatory cytokines could subsequently promote further NET development, creating a vicious cycle. NETs may also engage with platelets, initiating significant and potentially life-threatening immunothrombosis.

9. m6A and Sepsis-Induced Acute Lung Injury (SI-ALI)

When it comes to internal mRNA modifications, m6A is by far the most prevalent and conserved. Its involvement in various biological processes has been demonstrated in recent studies. As seen early, m6A modulates tumor-associated gene expression, which plays a role in cancer formation and progression. More and more research shows that m6A plays a crucial role in sepsis.

Crucially, researchers' interest in metabolic reprogramming triggered by NETs has grown. In human pulmonary artery endothelial cells, NETs induced a pro-inflammatory and pro-angiogenic response marked by increased glycolysis. Similarly, in endothelium and alveolar epithelial cells, sepsis induced a pro-inflammatory phenotype due to increased glycolysis. Metabolic reprogramming may play a role in ferroptosis, a newly recognized form of necrosis that results from ROS buildup and iron-dependent lipid peroxidation.

An imbalance between the production and clearance of ROS is a key component of ferroptosis in sepsis. There is mounting evidence that metabolic reprogramming plays a crucial role in regulating ferroptosis and the innate inflammatory response; however, the exact mechanisms by which this occurs are still unclear. It was suggested that m6A modification of *HIF-1*α led to enhanced glycolysis and impaired oxidative phosphorylation based on the reduced expression of glycolytic enzyme genes and high expression of complex I-V-associated genes in the mitochondria of *METTL3* knockdown cells. These changes in metabolism may also make it easier for alveolar epithelial cells to undergo ferroptosis and ROS accumulation. In the end, it was proven that *PAD4* deletion reduced ferroptosis and reduced lung damage. Ferroptosis, NET targeting, and METTL3 are potentially target for future treatment.

10. SI-ALI Discoveries and Therapeutic Implications

Comprehending the molecular intricacies governing mitochondrial metabolic reprogramming/controlling in ALI paves the way for potential therapeutic interventions. In preclinical and clinical studies, numerous strategies have been investigated to address this aspect [17,119].

10.1 Antioxidant Therapy

Considering the pivotal role of ROS in developing mitochondrial dysfunction in ALI, there has been a focus on exploring antioxidant treatments [17]. Compounds like N-acetylcysteine (NAC), superoxide dismutase mimetics, and antioxidants specifically targeted to mitochondria have displayed potential in preclinical experiments [120,121]. Nonetheless, translating these discoveries into effective clinical therapies continues to present a significant challenge.

10.2 Mitochondrial Biogenesis Promotion

Promoting mitochondrial biogenesis via activating the SIRT1-AMPK pathway has surfaced as a prospective therapeutic approach [100]. Compounds such as resveratrol, known for its ability to activate SIRT1, and metformin, recognized as an AMPK activator, have demonstrated favorable outcomes in preclinical studies involving ALI. However, determining their effectiveness in ALI patients warrants clinical trials [100,122].

10.3 Modulation of Autophagy and Mitophagy

Drugs that regulate autophagy and mitophagy have been explored as potential therapies for ALI. Rapamycin, an mTOR inhibitor that promotes autophagy, and mitophagy enhancers, such as urolithin A, have shown promise in preclinical studies. For instance, promising outcomes have emerged in research involving Rapamycin, an mTOR inhibitor that promotes autophagy, and mitophagy enhancers like urolithin A [123]. Rapamycin's inhibition of mTOR induces autophagy, aiding in the removal of damaged cellular components, including mitochondria. Urolithin A enhances mitophagy, explicitly targeting the elimination of dysfunctional mitochondria. These interventions can potentially maintain cellular health and address various health conditions [123]. However, achieving the precise balance between autophagy and mitophagy requires careful consideration to avoid excessive mitochondrial depletion [91].

10.4 Immunomodulation

Therapies focused on modulating the inflammatory response in ALI may indirectly influence mitochondrial dysfunction. Investigations are underway regarding the potential of immune checkpoint inhibitors, anti-inflammatory cytokines, and strategies to reduce DAMP release from damaged mitochondria [124].

11. SI-ALI Future Directions

The future of scientific exploration, many unanswered questions, and promising research avenues are in the realm of sepsis-induced ALI and the intriguing phenomenon of Ferroptosis. In this decade, we need to address unanswered questions and research opportunities in sepsis-induced ALI and Ferroptosis.

- **Enigmatic Molecular Players**: While the stage is set, there are still undiscovered characters in the intricate narrative of ALI and Ferroptosis. Identifying these hidden actors could illuminate fresh paths toward novel therapeutic targets and diagnostic markers.
- **Chronological Sequencing**: The exact sequence of events in the intricate interplay between ALI and Ferroptosis remains elusive. Does Ferroptosis precede ALI, or does it emerge consequently? Unraveling this temporal puzzle is pivotal for crafting effective interventions.

- **The Immune Enigma**: The interaction between the immune system and Ferroptosis presents a captivating enigma. How do immune cells influence the trajectory of Ferroptosis in ALI, and can this interplay be harnessed for therapeutic purposes?
- **Precision Therapies**: Despite the blueprint at hand, the development of precision therapies remains a challenge. Can we engineer interventions that modulate ALI Ferroptosis without unintended side effects?
- **Patient Stratification**: Is it plausible that distinct ALI subtypes exhibit varying susceptibility to Ferroptosis? Tailoring treatments based on individual patient profiles could be used in a new era of personalized medicine.
- **Biomarkers and Imaging**: The quest for reliable biomarkers and advanced imaging techniques to detect Ferroptosis in ALI patients is wide open. Early and accurate diagnosis could significantly enhance clinical outcomes.
- **Combination Approaches**: Given the multifaceted nature of ALI and Ferroptosis, combination therapies may hold immense potential. How can we effectively integrate established treatments with emerging agents targeting Ferroptosis?
- **Long-Term Effects**: What are the enduring consequences of Ferroptosis in survivors of ALI? Delving into the aftermath might yield strategies to ameliorate chronic lung impairment.

The scientific community is presented with a canvas of questions and opportunities in this uncharted territory of ALI and Ferroptosis. The journey promises to be both challenging and rewarding as we navigate the intricacies of these intertwined pathways.

Substantially, Ferroptosis, a relatively recent addition to the landscape of cell death mechanisms, has garnered significant attention for its potential role in various pathological conditions, including lung injury. Specifically, the interplay between METTL3-mediated m6A modifications and IGF2BP2 in regulating ferroptosis pathways has emerged as an intriguing research focus. While this area holds promise for advancing our understanding of lung injury and potentially offering new therapeutic avenues, its clinical translatability comes with challenges and exciting prospects.

- **Complex Molecular Network**: The intricate network of METTL3-m6A-IGF2BP2-mediated Ferroptosis involves multiple players and pathways. Deciphering this complexity to develop targeted interventions is a formidable challenge.
- **Biomarker Identification**: Identifying reliable biomarkers associated with this pathway's activation in lung injury patients remains a critical obstacle. These biomarkers would be essential for early diagnosis and monitoring.
- **Patient Heterogeneity**: Lung injuries are multifaceted, and patient responses can vary significantly. Understanding how METTL3-m6A-IGF2BP2-mediated Ferroptosis operates in different patient populations is essential for personalized medicine approaches.
- **Therapeutic Development**: While the concept is promising, translating this knowledge into effective treatments faces substantial hurdles. Developing drugs or interventions targeting this pathway without off-target effects is a significant challenge.

In addition to the above indicated thoughts, a few perspectives can be delineated below.

- **Precision Medicine**: The elucidation of METTL3-m6A-IGF2BP2-mediated Ferroptosis could pave the way for personalized treatment strategies. Tailoring interventions based on individual patient profiles may lead to more effective outcomes and thorough investigation is critical.
- **Early Diagnosis**: If robust biomarkers can be identified, diagnosing lung injury at earlier stages may be possible when interventions can be more effective.
- **Combination Therapies**: Combining therapies targeting this pathway with existing treatments for lung injury might enhance patient outcomes. This approach could be particularly beneficial in severe cases.
- **Research Collaboration**: Collaboration between clinicians, molecular biologists, and drug developers is crucial. Bridging the gap between laboratory discoveries/technology and clinical applications requires a multidisciplinary effort.
- **Improved Patient Care**: Ultimately, understanding the clinical translatability of METTL3-m6A-IGF2BP2-mediated Ferroptosis in lung injury can improve patient care by offering new therapeutic options and insights into disease progression.

12. Conclusive Remarks

In summary, while we lack a definitive troponin-like diagnostic marker for sepsis, I believe we are progressing towards formulating a strategy. Sepsis is a critical condition and accounts for the highest mortality rate among hospitalized patients. According to the most updated sepsis definition (Sepsis-3), a substantial population-based study examined mortality rates among patients exhibiting signs and symptoms of sepsis. This led to the description of sepsis as a life-threatening organ malfunction resulting from a dysregulated host response to infection, along with clinical characteristics, including the sequential organ failure assessment risk score. In sepsis, numerous therapy criteria exist, yet there is no guidance on diagnosis and basic scientists and/or pathologists may help in delineating it. In the initial phase of sepsis, there is a dynamic interaction between the elevation of anti-inflammatory and pro-inflammatory cytokines, which can become dysregulated. This imbalance may result in leukocyte activation, endothelial cell dysfunction, microvascular flow restriction, and co-

agulopathy. If neglected, this results in tissue damage, organ failure, and ultimately mortality. From a laboratory standpoint, the optimal sepsis biomarker must be specific to sepsis and differentiate it from noninfectious etiologies of SIRS. We need to identify a sensitive method to detect sepsis at the earliest stages of the pathobiological process, enabling us to commence goal-directed therapy, treat our patient, and enhance outcomes. The biomarker must be easily interpretable by emergency physicians, effective for risk classification and prognosis, suitable for monitoring resuscitation and antimicrobial therapy, and possess a short turnaround time. We are optimistic and anticipate that in this decade the biomarker(s) of sepsis will be available for clinicians and laboratory scientists. The clinical translatability of METTL3-m6A-IGF2BP2-mediated Ferroptosis in lung injury presents both challenges and exciting prospects. While there are obstacles to overcome, the potential for personalized medicine, early diagnosis, and improved patient care underscores continued research's importance.

Availability of Data and Materials

All PDFs are publicly available. The original slides of the gross pathology (Fig. 3) is from patients affected with SI-ALI from the Personal Archive of Consolato M. Sergi, Department of Laboratory Medicine and Pathology, University of Alberta, Canada and Division of Anatomic Pathology, Children's Hospital of Eastern Ontario, University of Ottawa, Canada.

Author Contributions

CS conceptualized the study; RK collected data, drafted the initial manuscript, and revised the manuscript. CS revised the data and performed the analysis, was responsible for the intramural funding, and revised the final draft of the manuscript. RL literature search, revised the manuscript, and BC collected the histopathological data, prepared the figure of histopathology and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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