



REVIEW ARTICLE

# Long non-coding RNA (lncRNA): A potential therapeutic target in acute lung injury

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**Abstract** Acute Lung Injury (ALI) and its severe form Acute Respiratory Distress Syndrome (ARDS) are the major cause of ICU death worldwide. ALI/ARDS is characterized by severe hypoxemia and inflammation that leads to poor lung compliance. Despite many advances in understanding and management, ALI/ARDS is still causing significant morbidity and mortality. Long non-coding RNA (lncRNA) is a fast-growing topic in lung inflammation and injury. lncRNA is a class of non-coding RNA having a length of more than 200 nucleotides. It has been a center of research for understanding the pathophysiology of various diseases in the past few years. Multiple studies have shown that lncRNAs are abundant in acute lung injury/injuries in mouse models and cell lines. By targeting these long non-coding RNAs, many investigators have demonstrated the alleviation of ALI in various mouse models. Therefore, lncRNAs show great promise as a therapeutic target in ALI. This review provides the current state of knowledge about the relationship between lncRNAs in various biological processes in acute lung injury and its use as a potential therapeutic target.

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## Introduction

Acute lung injury (ALI) is a syndrome that comprises severe hypoxemic respiratory failure along with bilateral lung infiltrates and is also linked with pulmonary as well as non-pulmonary risk factors.<sup>1</sup> It is characterized by severe inflammation that causes serious damage to the alveoli and results in poor lung compliance.<sup>2</sup> ALI is associated with significant morbidity and mortality, and its severe form, i.e., acute respiratory distress syndrome (ARDS), has even higher mortality rates. Globally, it affects around 3 million people annually and is responsible for 10 percent of ICU admissions.<sup>3</sup> Despite recent advancements in understanding and management of the disease, patients suffering from ALI/ARDS end up with a poor prognosis. Therefore, a new approach for better understanding and effective treatment strategies is urgently needed to mitigate the disease and overcome the economic burden it causes.

Long non-coding RNA (lncRNA) is a fast-growing topic in lung inflammation and injury.<sup>4</sup> In human, less than 3% of genome codes for proteins, while the rest of the genomic part consists of either introns or intergenic DNA.<sup>5</sup> Among all the transcribed parts of the genome, most are transcribed into non-coding RNA (ncRNA)<sup>6</sup> and are likely to be non-functional.<sup>7</sup> However, a certain part of it plays a vital role in gene regulation. ncRNA have been further divided into multiple categories such as miRNA, snoRNA, piRNA, and lncRNA based on their size/nucleotide length.<sup>8</sup> Long non-coding RNA or lncRNA is the "non-coding RNA that exceeds 200 nucleotides in length"<sup>9</sup> and exhibits diverse roles and functions in many important biological processes. Earlier, lncRNAs were considered as a by-product of the transcription process. However, with more research/study focusing on the lncRNA, it became evident that these RNA molecules play a crucial role in regulating many

physiological processes, including immunity,<sup>10</sup> inflammation, proliferation, cell differentiation, and cell survival.<sup>11,12</sup> In recent years, advances in next-generation sequencing (NGS) technologies have prompted an eruption of newly discovered lncRNAs, particularly in humans.<sup>13</sup>

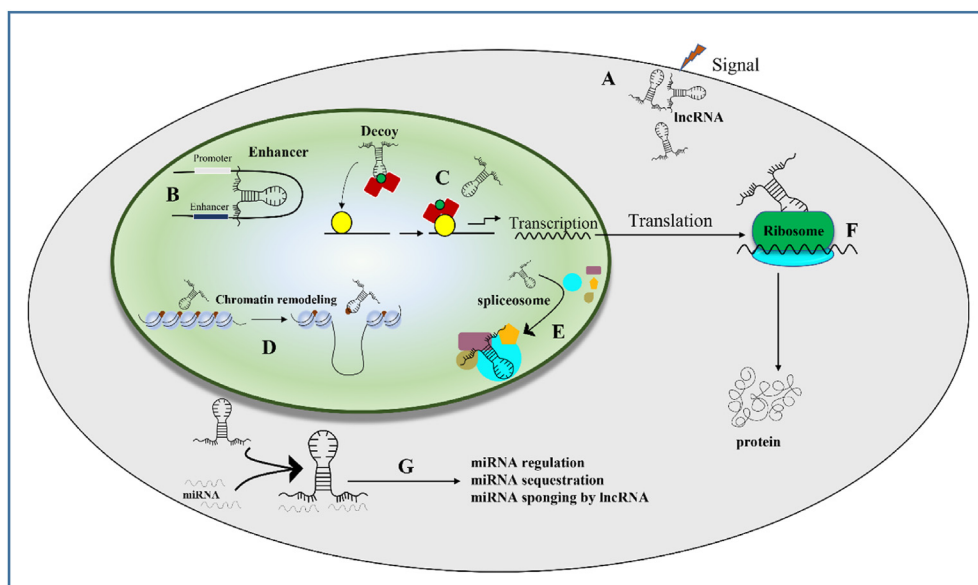
Long non-coding RNA regulates gene expression at transcriptional, post-transcriptional, epigenetic, and chromatin levels and activates or constrains the expression of target genes by directly binding to them or by recruiting transcription factors (Fig. 1 showing lncRNA activity in cellular environment).<sup>14</sup> Multiple studies suggest that dysregulation of lncRNA is associated with many human diseases such as focal ischemia, cancer, neurodegenerative diseases, and respiratory diseases.<sup>15–18</sup> Among respiratory diseases, lncRNAs have been implicated in usual interstitial pneumonia, chronic obstructive pulmonary diseases, lung cancer, pulmonary arterial hypertension, and ALI.<sup>19</sup> The scope of the present review will be limited to acute lung injury/acute respiratory distress syndrome.

## lncRNA in acute lung injury

lncRNA has provided new insights on the pathogenesis of ALI. It will aid the investigators in developing a novel therapeutic target for the treatment of acute lung injury. So far, many studies have discovered the role of various lncRNAs as a potential therapeutic target in acute lung injury, such as MALAT1, NEAT1, TUG1, THRILL, etc. (see Table 1). Some of the important lncRNAs are discussed below.

### MALAT1

MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) is 6.5 kb, abundant, evolutionary conserved, and stable lncRNA that was found overexpressed in solid



**Figure 1** General description of lncRNA activity in the regulation of cellular activities. (A) Expression of lncRNA in response to an external stimulus. (B) It regulates the promoter and enhancer region, (C) lncRNA providing a platform for transcriptional activity. (D) It also controls the chromatin remodeling; (E) splicing alternation via spliceosome, (F) translation and protein modification, and (G) microRNA regulation in many cellular activities by lncRNA.

**Table 1** lncRNAs involved in ALI/ARDS.

lncRNAs	Model and/or cells	Expression	Function	Molecular targets	References
TUG1	LPS-induced ALI Murine, PMVECs	Decreased	Alleviates inflammation and apoptosis	miR-34 b-5p/GAB1	41
MALAT1	LPS-induced ALI in HPMEC, Rat ALI/ARDS model, sepsis patients	Increased	Decreased apoptosis and inflammation	miR-181a-5p, ICAM-1, miR-425	42,43
CASC2	A549 cell line	Decreased	Increased viability, decreased apoptosis, and inflammation	miR-27b/TAB2 axis	38
NEAT1	LPS-induced ALI mice models and A549 cell line	Increased	Enhanced cell viability and reduced lactate dehydrogenase release, apoptosis, and caspase-3/9 activity. Decreased inflammation	HMGB1/RAGE signaling, miR-944/TRIM37	32,36
THRIL	Septic-induced ALI in C57BL/6 mice model and MPVECs.	Increased	Decreases the inflammation and apoptosis	miR-424/ROCK2	25
SNHG5	LPS-induced mouse model, A549 cell line	Decreased	It inhibits inflammation and promotes the cell viability	miR-205/COMMD1	44
PRNCR1	LPS-treated PVEC and mouse model	Increased	Attenuates expressions of TLR4, NF- $\kappa$ B, and inflammatory cytokines and increases cell viability	miR-330-5p/TLR4 axis	45
Hsp4	LPS-induced MLE-12 cells	Decreased	Reduced apoptosis	mTOR signaling, miR-466 m-3p/DNAjb6	46
lncRNA-5657	CLP-induced ALI mouse model, patients with sepsis-induced lung injury, NR8383 cell line	Increased	Alleviated inflammatory response	spsn2	47
CLMAT3	Monocytes isolated from blood samples of healthy controls and ALI patients, U937 macrophage cell line, C57BL/6 mice Model	Increased	Alleviates pro-inflammatory cytokines	CtBP2-p300-NF- $\kappa$ B complex	48
MEG3	LPS-induced ALI mouse alveolar and macrophage NR8383 cells	Increased	Improves inflammatory response	microRNA-7b (miR-7b)/NLR pyrin domain containing 3 (NLRP3)	49
CASC9	LPS-induced HSAECs as well sepsis-induced lung injury mouse model	Decreased	Increased the viability of HSAECs	miR-195-5p/PDK4 axis	50
XIST	Lung transplant patients, PCI-induced rat model	Increased	Alleviation of inflammation and apoptosis, cessation of NET formation	miR-21/IL-12 A	51
GAS5	LPS-induced murine alveolar epithelial cell line MLE-12	Decreased	Decreased cell inflammatory responses and apoptosis	miR-429/DUSP1 axis	52
SNGH14	LPS-induced ALI mouse model	Increased	Reduces the levels of pro-inflammatory cytokines IL-18, IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 and inhibits MH-S cell viability.	miR-34c-3p/WISP1	53
H19	LPS-induced ARDS in rats and MH-S cells	Increased	Pulmonary injury, inflammation, and fibrosis	miR-423-5p/FOXA1	54
Mirt2	LPS induced primary cultured peritoneal macrophages, RAW264.7 cells	Increased	Regulate macrophage polarization and inflammation	TRAF6, NF- $\kappa$ B, stat6 and MAPK	55

tumors and associated with proliferation, progression, metastasis, migration, invasion, survival, and acts as a prognostic marker of cancers.<sup>20</sup> Investigation in LPS treated macrophages showed upregulation of MALAT1, while it was downregulated in IL4 treated cells and showed contrasting expression in differentially treated macrophages. MALAT1 knockdown ameliorated LPS-induced M1 macrophage activation involved in lung injury and inflammation, while IL-4 induced M2 macrophages activation was enhanced in MALAT1 knockdown cells known for inflammation resolution.<sup>21,22</sup>

### THRIL

TNF and HNRNPL related immunoregulatory long non-coding RNA or THRIL have been previously reported as a critical regulator in inflammatory diseases. The expression level of THRIL increases in coronary heart disease patients with systemic inflammation.<sup>23</sup> In a recent study, overexpression of THRIL has been observed in sepsis patients. More strikingly, it positively correlates with the increased risk of ARDS, disease severity, inflammation, and high mortality.<sup>24</sup> Chen et al reported overexpression of THRIL in the tissues of sepsis-induced ALI mice, which was further validated in the sepsis cell model. They also reported attenuation in lung injury and increased survival when THRIL was downregulated. It indicates that THRIL prompts acute lung injury in sepsis.<sup>25</sup>

THRIL binds to the promoter region of the TNF- $\alpha$  forming RNA-protein complex with hnRPL, inducing the expression of TNF- $\alpha$ , thus imposes an inflammatory response.<sup>26</sup> Chen et al<sup>25</sup> reported a dramatic decrease in the protein level of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and the number of macrophages and neutrophils counts by silencing THRIL, indicating that inhibition of THRIL relieved sepsis-induced inflammation and ALI. Apoptosis is a crucial feature for inducing organ damage.<sup>27</sup> Significant apoptosis occurs in sepsis-induced ALI, and inhibition of THRIL can suppress the apoptosis and further alleviate the ALI induced by sepsis.<sup>25</sup> Evidence suggested that suppressed immune response may be linked with sepsis-induced ALI. Downregulating the THRIL may enhance immune response that can alleviate sepsis-induced ALI as suggested by inhibition of alveolar macrophage apoptosis.<sup>28</sup> Therefore, lncRNA THRIL can serve as a potential therapeutic target for acute lung injury induced by sepsis.

### NEAT1

In recent years, the role of Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) has been vastly explored in multiple diseases such as cancer and inflammatory diseases.<sup>29</sup> NEAT1 has also been implicated in inflammatory diseases. For instance, it was found upregulated in rats having chronic constriction injury, while knockdown of NEAT1 resulted in suppression of neuroinflammation as well as neuropathic pain behaviors.<sup>30</sup> In brain injury, NEAT1 has anti-inflammatory and anti-apoptotic functions.<sup>31</sup>

Zhou et al<sup>32</sup> observed a very high level of NEAT1 expression in LPS induced ALI in mouse model. Huang et al<sup>33</sup> also reported remarkably high levels of NEAT1 in sepsis patients, which was associated with augmented disease severity and poor prognosis. Alveolar epithelial cells-II (AECs) are the main injury sites in ALI/ARDS. It maintains

the alveolar-capillary barrier integrity and produces surfactant substances that decrease the pulmonary surface tension. In ALI, this mechanism is impaired and results in lower lung compliance. Therefore, protecting AECs from injury represents a vital therapeutic strategy against ALI induced by sepsis.<sup>34,35</sup>

Zhou et al investigated the role of NEAT1 on the LPS-exposed adenocarcinoma cell line. They discovered that cessation of NEAT1 reduces the adverse function of LPS on the availability of AECs. Additionally, NEAT1 inhibition restrained apoptosis, caspase-3/9 activity, and release of LDH in LPS-induced ALI, suggesting its protective roles in ameliorating LPS induced AECs damage and apoptosis.<sup>32</sup> Inflammation and cytokine storm is the leading cause of mortality and morbidity in ALI/ARDS. Controlling the inflammatory response could provide a vital therapeutic target in sepsis-induced ALI. Suppression of NEAT1 significantly reduces the pro-inflammatory cytokines IL-6, IL-1 $\beta$ , and TNF- $\alpha$ .<sup>32</sup>

Zhou et al<sup>32</sup> also reported that restoration of the HMGB1-RAGE pathway, which is excessively activated in sepsis models and patients, reverses the anti-inflammatory and anti-injury efficacy of NEAT1 knockdown in AECs exposed LPS. Evidence also suggested that NEAT1, like other lncRNA, regulates the activities of specific downstream proteins such as TRIM37.<sup>36,37</sup>

### CASC2

Cancer susceptibility candidate 2 (CASC2) is located on chromosome 10 and has been implicated as a crucial gene involved in the pathogenesis of many types of malignancies, including glioma and endometrial cancers.<sup>38</sup> Liu et al<sup>39</sup> discovered that CASC2 was downregulated in A549 cell lines treated with LPS, while accumulation of CASC2 attenuated LPS-induced ALI in *in vitro* and *in vivo* conditions. Ji L et al<sup>40</sup> established bronchopulmonary dysplasia (BPD) model by giving 14 days of hyperoxia treatment to neonates mice. Furthermore, lncRNA CASC2 has been found reduced in murine lung tissue while CAV1 protein was found elevated.

## The mechanistic function of lncRNA in acute lung injury

### lncRNA-mediated cell signaling regulation and acute lung injury

Cell signaling is vital in various cellular and physiological processes. Many cell signaling pathways have been discovered that play a pivotal role in various biological processes and gene expression. A single signaling pathway often consists of multiple signaling molecules that ultimately affect gene expression by regulating the activity of transcription factors directly or indirectly. Shreds of evidence from recent studies suggest that lncRNAs are involved in various cell signaling pathways in many diseases.<sup>12,56</sup> However, few studies are available that focus on the role of these lncRNAs in acute lung injury. Here we have discussed some pathways that have been studied so far.

Study in the past demonstrated that MALAT1 could worsen sepsis-induced inflammation and cardiac insufficiency by regulating p38 MAPK/p65 NF- $\kappa$ B signaling

pathway.<sup>57</sup> Lin et al used three study groups in mice: the control group, LPS group, and LPS+MALAT1 group, to investigate the role of lncRNA MALAT1. They checked various factors, including the expression level of p38MAPK/P65 NF- $\kappa$ B signaling pathway-related protein in the lung tissue and the BAL fluid of the mouse and reported that knockdown of MALAT1 leads to reversal of the abnormal increase in the phosphorylation of p38 MAPK and p65 NF- $\kappa$ B.<sup>58</sup> Likewise, Zhu et al<sup>59</sup> also demonstrated that MALAT1 via interaction with p65 blocks LPS-induced activation of NF- $\kappa$ B and repressed LPS-induced inflammatory injury in WI-38 cell line.

In a separate study on the mechanism of NEAT1 in ALI, investigators corroborated the activation of HMGB1/RAGE and NF- $\kappa$ B signaling in A549 cell lines treated with LPS. Suppression of NEAT1 represses this pathway while reactivates the HMGB1/RAGE signaling through HMGB1 overexpression and dampened the anti-inflammatory/injury effects of NEAT1 knockdown, implying it as a potential therapeutic target for this condition.<sup>32</sup>

### lncRNA sponging miRNA in acute lung injury

miRNA is 18–22 nucleotides small non-coding RNA that regulate gene expression post-transcriptionally, typically binding at 3' UTR of target messenger RNA (mRNA). On account of acute lung injury, miRNA act as a potential biomarker in the diagnosis and severity of the acute lung injury disease.<sup>60</sup> In brief, biogenesis of miRNA begins in the nucleus where RNA polymerase II transcribes pri-miRNA (2000 bp long, double-stranded, capped, and polyadenylated) as a precursor of miRNA, which is further cleaved and processed by RNase III-like endonuclease, drosha, and transported as pre-miRNA to the cytoplasm through exportin-5. In the cytoplasm, pre-miRNA is further processed, producing 25 nucleotides miRNA duplex (miRNA–miRNA\*) by the action of cytoplasmic RNase III Dicer. One strand of miRNA duplex is degraded, and the remaining one gets accommodated into an RNA-induced silencing complex (RISC) incorporated with Argonaut protein. miRNA loaded with RISC-Ago complex has seed region which complementarily binds to 3' or 5' UTR regions of target mRNA for its degradation resulting negative regulation.<sup>61,62</sup> It is estimated that approximately 60% of human genes are targeted and regulated by miRNA, and they can serve as a therapeutic target in various diseases.<sup>63</sup> The developing horizon of research identified and reported that lncRNA is an endogenous sponge to miRNAs that can control the expression of genes by targeting miRNAs in acute lung injury. In a study, downregulation of miR-34 b-5p by overexpressed TUG1 was protective in sepsis-induced acute lung injury in mice model.<sup>41</sup> Elevated MALAT1 was found to be associated with acute lung injury that downregulated miR-181a-5p expression. Similarly, increased level of THRIL downregulated miR-424 in sepsis-induced lung injury where knockdown of THRIL potentially enhanced miR-424 and markedly increased the survival rate of septic mice.<sup>25</sup> LPS treated A549 cell lines showed a decreased amount of lnc-CASC2 with a raised level of miR-27 b. Overexpression of

lnc-CASC2 further downregulated miR-27 b, preventing LPS induced acute lung injury.<sup>38</sup> Jinyuan et al<sup>53</sup> performed *in vivo* and *in vitro* studies and found aggravated expression of lncRNA SNHG14 adversely influenced microRNA-34c-3p expression in LPS induced acute lung injury models. It is well established that lncRNA regulates miRNA by acting as an agonist or antagomir. Furthermore, miRNA processing, their transport to mRNA, and even outside the cell could be controlled by lncRNA.<sup>64–66</sup>

## Role of lncRNA in different biological processes in acute lung injury

### Pyroptosis

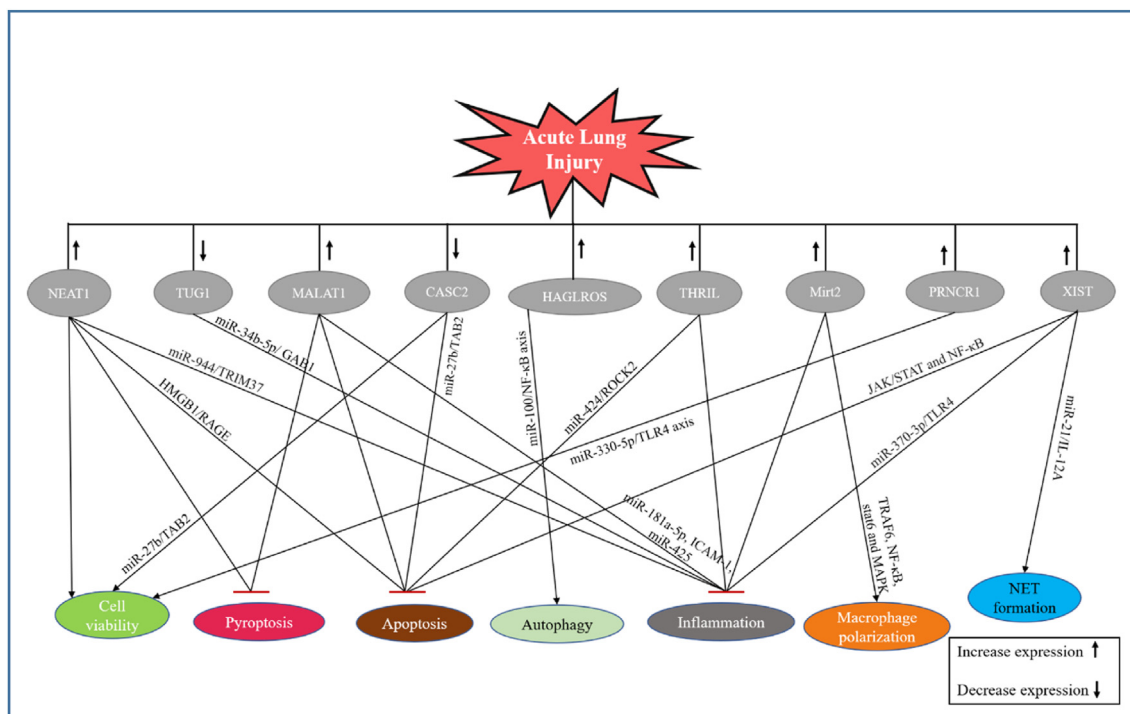
Pyroptosis is a newly discovered cell death process stimulated by both infectious and non-infectious sources, including factors produced in the host during myocardial infarction.<sup>67</sup> It is distinct from other forms of cell death both morphologically and mechanistically. The defining feature of pyroptosis is its dependency on Caspase 1, which mediates this type of cell death. It is not involved in apoptosis, and neither its degradation ceases apoptosis.<sup>68</sup>

In recent decades lncRNAs were found to be associated with pyroptosis. Pyroptosis plays a key role in the inflammatory resolution of acute lung inflammation. Guo et al demonstrated that inflammasomes activation and inflammasome-dependent pyroptosis is associated with acute lung inflammation.<sup>69</sup> Several lncRNAs have been recognized as a key regulator in acute lung inflammation, either by modulating miRNAs/downstream molecules or by directly regulating pyroptosis.<sup>70,71</sup> Luo et al investigated the molecular mechanism and also looked for the candidate lncRNA and mRNA involved in pyroptosis in LPS induced acute lung injury. They found 1503 differentially expressed lncRNA and observed that lncRNA4344 sponged miR-138-5p and promoted pyroptosis in inflammatory responses by targeting NLRP3 in LPS-induced ALI.<sup>72</sup>

MALAT1 and NEAT1 have been the most widely reported lncRNAs in acute lung injury, and it is also associated with pyroptosis (See Fig. 2). Apart from these lncRNAs, many other lncRNAs are associated with pyroptosis regulation, and still, many more are being identified. Targeting these lncRNAs associated with pyroptosis could provide a potential therapeutic target.

### Apoptosis

Apoptosis is a highly conserved “programmed cell death” found across all metazoans and is crucial for the development, maintenance of tissue homeostasis, and prevention of cancer.<sup>73</sup> Inadequate apoptosis may result in autoimmunity or cancer. On the other hand, augmented or heightened apoptosis may lead to degenerative diseases. Blebbing, cell shrinking, nuclear fragmentation, and apoptotic body formation are some of the characteristic morphological changes during apoptosis.<sup>74</sup> Our lung is a complex structure made up of various types of cells, such as



**Figure 2** The roles of lncRNAs involved in the various biological process during acute lung injury. lncRNAs act via targeting their downstream molecules, such as miRNA/proteins. TUG1 act via regulating their downstream target miR-34 b-5p/GAB1. MALAT1 has more than one downstream target molecules including miR-181a-5p, ICAM-1, miR-425. CASC2 acts via regulating miR-27b/TAB2 axis. NEAT1 has HMGB1/RAGE signaling as its downstream target. THRIL regulates the activity of miR-424/ROCK2 for functional effects. Mirt2 controls TRAF6, NF- $\kappa$ B, stat6 and MAPK signaling pathways. The target of PRNCR1 is miR-330-5p/TLR4 axis. In contrast, XIST acts via regulating the activity of miR-21/IL-12 A.

endothelial cells, epithelial cells, fibroblasts, and different leukocytes. Apoptosis of these cells can either exacerbate or ameliorate lung injury subject to cell types involved.<sup>75</sup> In the lungs of ALI patients, apoptosis of neutrophils is delayed, and various soluble factors, including GM-CSF, mediate this effect. In contrast, epithelial apoptosis is likely to be mediated by sFasL.<sup>75</sup>

The role of lncRNAs in acute lung injury and apoptosis has been studied by many researchers in the last few years. The results obtained by Liu et al demonstrated that downregulation of lncRNA MALAT1 significantly decreased the Fas level and apoptosis in the HPMEC cell line and ALI mouse model. Li et al<sup>38</sup> showed that accumulation of lncRNA CASC2 attenuated the increased apoptosis in LPS induced A549 cells. Ji et al<sup>46</sup> in a similar study found that overexpression of Hsp4 lncRNA significantly reverted LPS induced apoptosis in MLE12 cell lines. Together, these outcomes clearly show that regulation of lncRNAs could reduce ALI-induced apoptosis in cell lines and mouse models.

Until now, studies have primarily focused on the role of lncRNAs in apoptosis during ALI in general. There are two different apoptosis pathways: intrinsic (mitochondrial-dependent pathway) and extrinsic (death receptor-dependent pathway).<sup>76</sup> The Bcl-2 family proteins regulate the intrinsic pathway in addition to pro-apoptotic proteins and anti-apoptotic proteins. This pathway is ignited by the mitochondrial damage that induces the secretion of cytochrome-c that activates the Caspase-3, which is the

ultimate factor responsible for apoptosis. On the other hand, the extrinsic pathway is initiated by the interaction of Fas ligand with Fas receptor and TNF-ligand with TNF-receptor that activates the Caspase-8 culminating with the activation of Caspase-3 and initiation of apoptosis.<sup>77</sup> Therefore, the apoptotic pathway associated with the effects of lncRNAs on ALI remains enigmatic, and further studies are needed to find out more about its role.

### Autophagy

In different forms of stress, including nutrient scarcity, growth factor deprivation, infectious condition, and hypoxia, cells degrade themselves to recycle nutrients essential for cellular functions by an intracellular catabolic process known as autophagy. Autophagy initiates with the formation of double-membrane vesicles, the autophagosome, that encapsulates or engulfs damaged organelles, distorted macromolecules, and different cytoplasmic debris and ends up with the fusion with lysosomes, termed as autolysosome, for degradation.<sup>78,79</sup> The autophagic flux is the measure of cargo delivery by autophagosomes to autolysosomes.<sup>80</sup> The main purpose of autophagy is to provide cellular essentials for cell thriving. Poorly regulated autophagy processes are linked with various diseases, including acute lung injury. Although the role of autophagy in the context of acute lung injury is still indefinite and remains uncertain. However, increased pro-inflammatory cytokines in LPS treated mice manifested acute lung

injury. Alveolar epithelial cells of LPS treated mice showed a significant increase in the expression of autophagy marker LC3B. In addition, administration of autophagy inhibitors 3-methyladenine (3-MA) and chloroquine (CLQ) potentially decreased total protein in BALF and lung injury score, thus alleviating lung injury.<sup>81</sup> Stimulation of WI-38 human lung fibroblasts with LPS triggers the expression of the lncRNA HAGLROS and thereby causing cell injury.

Furthermore, downregulation of HAGLROS assuaged LPS induced cellular injury by increasing cell viability and curbing autophagy.<sup>39</sup> lncRNAs regulating autophagy in acute lung injury are not fully characterized. Therefore, elaborated research is needed to elucidate the role of lncRNA in autophagy linked with ALI.

## Inflammation

Inflammation is the sequential response by the body's immune system against harmful stimuli, including invading infectious agents, toxic substances, damaged cells, or irradiation, consequently eliminating noxious substances and initiation of the healing process by the body. Therefore, inflammation is categorized as a protective mechanism essential for health.<sup>82,83</sup> Inflammation is known to play an essential role in the pathophysiology of ALI. Various reports have exhibited that lncRNA exerts an essential function in inflammatory processes. Excessive inflammation could initiate a cascade to exacerbate further lung damage leading to macrophage activation and polarization, neutrophils recruitment, and release of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and ICAM1.<sup>84</sup> lncRNA-5657 gets upregulated in LPS treated alveolar macrophage cell lines and in lung tissue of CLP rats.

Furthermore, substantially overexpressed lncRNA-5657 results in the production of pro-inflammatory cytokines including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, whereas the reverse was noticed after silencing of lncRNA-5657.<sup>47</sup> In a study, knockdown of lncRNA MALAT1 improved LPS induced acute lung injury in mice by decreasing the level of inflammatory cytokines. In addition, the number of neutrophils and macrophages in BALF was found to be reduced.<sup>58</sup> Mice with acute lung injury showed decreased expression of lncRNA CASC2, AQP1, and increased miR-144-3p proportional to inflammation in lung tissue. Alternatively, overexpression of CASC2 by transfection in LPS treated A549 cell lines restored AQP1 protein and ameliorated acute lung injury.<sup>85</sup> Zhiming et al reported upregulation of lncRNA SNHG16 and pro-inflammatory cytokines such as (IL-6, IL-1 $\beta$ , and TNF- $\alpha$ ) in WI-38 cells after treatment with LPS. Downregulation of SNHG16 significantly increased cell viability and proportionally reduced the expression of IL-6, IL-1 $\beta$ , and TNF- $\alpha$ .<sup>86</sup>

## lncRNA effect on macrophage polarization in acute lung injury

Macrophages are versatile and multifaceted innate immune cells involved in the first line of defense. Their ability to detect, engulf and eliminate intruding pathogens make them prominent in an inflammatory response.<sup>87</sup> However, they can be subdivided into two functional categories (M1 and M2 macrophages) depending on specific external

stimuli and signals. M1 macrophages are the classically activated subtype of macrophages that are involved in pro-inflammatory response, on the other hand, alternatively activated M2 macrophages are responsible for the anti-inflammatory and angiogenic properties<sup>88</sup>. M1 macrophages are characterized by their ability to express pro-inflammatory cytokines, including TNF- $\alpha$  and IL-1 $\beta$  and iNOS (inducible NO synthase).<sup>89</sup> Besides, M2 macrophages release anti-inflammatory cytokines such as IL-10 and TGF- $\beta$ , including characteristic markers (Arg-1, Ym1, Fizz1) expression.<sup>90</sup>

Macrophage polarization plays a crucial role in the etiology of many multifactorial diseases such as atherosclerosis, sepsis, autoimmune diseases, cancer, tissue injury and repair.<sup>91</sup> Differentially expressed lncRNA in polarized macrophages (M1 and M2) provide a conspicuous result in their involvement in inflammation and regulation.<sup>92</sup> In a study, overexpression of MALAT1 in LPS treated mouse BMDM was reported. Further studies also reported similar observations in human monocytic THP-1 and human peripheral blood mononuclear cell-derived macrophages. MALAT1 demonstrated its role in regulating clec16a (C-type lectin domain family 16 member A) protein known for its role in activating pro-inflammatory macrophages. MALAT1 knockdown cells were observed with substantially decreased *clec16a* transcript. Extending work showed that *clec16a* knockdown proportionally decreased in MALAT1 knockout mice that distinctly showed the role of MALAT1 in macrophage activation and inflammation.<sup>21</sup> LPS stimulated primary cultured peritoneal macrophages (PCPM) showed Mirt2 lncRNA upregulation, whereas the opposite was observed in IL-4 treated cells. Knockdown of inflammatory pathways downstream signaling proteins such as MyD88 and TRIF partially inhibited Mirt2 expression. Immunoprecipitation assays revealed Mirt2 interaction with TRAF6 that regulates inflammatory response. Mirt2 also regulates M2 polarization via PPAR $\gamma$  and STAT-6 independent pathway<sup>55</sup> The horizon of lncRNAs can be exploited to evaluate their intricate role in macrophage polarization and their therapeutic potential in acute lung injury.

## Ferroptosis

Ferroptosis is a type of cell death induced by a small molecule called erastin; it inhibits the import of cystine, leading to depletion of glutathione and inactivation of phospholipid peroxidase glutathione peroxidase 4 (GPX4).<sup>93</sup> Cell death induced by erastin in ferroptosis is distinct from other forms of cell death such as apoptosis, necrosis, and autophagy based on morphological, biochemical, and genetic characteristics.<sup>93</sup> Many diseases have been reported to be associated with ferroptosis. Recently, the role of ferroptosis in lung diseases have also been reported.<sup>94,95</sup> Liu et al<sup>96</sup> demonstrated that Fer-1 (ferroptosis inhibitor) alleviates the LPS-induced ALI as well as the inflammatory responses *in vivo* efficiently by regulating ferroptosis.

lncRNA seems to play an essential role in the regulation of ferroptosis. Interaction between many lncRNAs and various cancer cells has been demonstrated, including lung cancer cells.<sup>97</sup> However, the role of lncRNA in the regulation of ferroptosis during acute lung injury has not been

explored. Exploring the role of lncRNA in this area could provide better therapeutic targets to manage ALI patients.

### Efferocytosis

Efferocytosis is a process in which apoptotic cells are cleared by the phagocytic cells. It is vital for normal development, tissue repair and homeostatic cell turnover. Apoptotic cells comprise many potential autoantigens and alarmins like adenosine, HSP proteins, HMG box-1 proteins. Secretion of these molecules during necrosis leads to mortality in the appropriate sepsis model, and their long-term persistence can also induce autoimmunity.<sup>98</sup> Dysfunctional efferocytosis has been reported in many lung diseases such as asthma, COPD, and cystic fibrosis; This led to the proposal that promoting efferocytosis might arrest the progression of the disease.<sup>99</sup>

Dysfunctional efferocytosis affects the prognosis in ALI/ARDS murine model. During gut ischemia-reperfusion in mice, the concentration of MFG-E8, an efferocytotic opsonin, decreases significantly while intraperitoneal administration of the same decrease inflammatory cytokines in the lung and also alleviates lung injury leading to increased survival.<sup>100</sup> In the LPS-induced ALI model, mice lacking MFG-E8 showed enhanced neutrophil infiltration, inflammatory cytokines, and reduced survival.<sup>101</sup>

The role of lncRNAs in efferocytosis has not been well studied. However, a recent study by Simion et al showed the role of macrophage-specific lncRNA MAARS in atherosclerosis and demonstrated that knockdown of MAARS is associated with increased expression of MerTK, a crucial receptor for efferocytosis, both *in vivo* and in bone marrow-derived macrophages (BMDMs). Additionally, silencing of MAARS increased the expression of mRNA SIRT1, an anti-apoptotic HuR target gene known to increase the macrophage-dependent efferocytosis.<sup>102</sup>

### Conclusion and future perspective

Acute lung injury is one of the major causes of ICU death around the world. The current treatment strategy is not effective enough to mitigate disease morbidity and mortality. New therapeutic targets are needed urgently that can effectively reduce the disease burden. In the last few decades, lncRNAs provided a new dimension to researchers. Studies have successfully demonstrated these molecules as novel potential targets in acute lung injury/ARDS/sepsis. These RNAs are involved in various pathophysiological processes such as apoptosis, inflammation, cell signaling pathways etc. However, the mechanisms underlying these processes are still needed to be explored extensively. Moreover, most of these studies are based on mouse models and cell lines, and only a few are based on patient's samples. Therefore, the role of these lncRNAs is needed to be established in a larger sample size and to find its role as a therapeutic target in human subjects.

### Conflict of interests

The authors declare that there is no conflict of interests.

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