EDITORIALS

Dampening the Fire: A Negative Feedback Loop in Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS), a severe form of acute lung injury (ALI), is a major clinical problem worldwide that is associated with high morbidity and mortality (1). Importantly, ARDS is one of the main fatal comorbidities caused by infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the coronavirus disease (COVID-19) pandemic of 2019 (2). Despite improvements in processes of care, including mechanical ventilation, fluid management, and other supportive measures, the mortality from ARDS remains high, and specific and effective therapies are not available (1). ARDS is characterized by diffuse lung inflammation, which results in endothelial and epithelial damage and subsequent increased vascular permeability, leading to alveolar damage. HMGB1 (high-mobility group box 1) protein is a ubiquitous nuclear protein; however, it can be released into extracellular space, where it elicits a potent inflammatory response (3). HMGB1 has been identified as a biomarker and mediator of the pathogenesis of a variety of lung disorders, including ARDS (4). The concentrations of HMGB1 are increased in plasma and BAL fluid of patients with ALI and a mouse model of endotoxin-induced ALI (5). Anti-HMGB1 antibody has been known to alleviate endotoxin-induced ALI, and intratracheal instillation of HMGB1 directly induces ARDS (6, 7). Therefore, HMGB1 has been considered as a promising therapeutic target for ARDS.

MicroRNAs (miRNAs), a class of small noncoding RNAs that regulate gene expression, have emerged as potential therapeutic targets in various diseases, including ARDS. The unique biochemical characteristics of miRNAs make them good candidates as disease biomarkers; they are present in a wide range of different biological fluids, and they are extremely stable and resistant to degradation. miRNAs are suitable therapeutic targets because their expression can be modulated by different available strategies. Several miRNA-targeted therapeutics have reached clinical trials (8). Differential miRNA expression profiles have been identified in patients with ARDS and animal models of ALI (9). miR-146, miR-21, and miR-17 play an important role in ARDS-associated inflammation through their regulation of genes related to the pathogenesis of ARDS (10, 11). Overexpression of miR-181b reduced lung injury and mortality in an ARDS mouse model, and miR-155$^{-/-}$ mice exhibited diminished inflammatory cytokine production and inflammatory cell accumulation in an ARDS model (12, 13).

Using samples from both human patients with ARDS and a mouse ALI model, He and colleagues report in this issue of the *Journal* (pp. 196–207) that miR-574-5p is important for alleviating ARDS through its targeting of HMGB1 (14). The authors first performed miRNA expression profiling on plasma samples from three pneumonia-related ARDS cases and three healthy control subjects and identified 45 miRNAs that were differentially expressed in plasma from patients with ARDS compared with their healthy counterparts. They focused on miR-574-5p, which was upregulated in ARDS samples and was predicted by bioinformatics analyses to have four potential binding sites to the HMGB1 mRNA 3′untranslated region (UTR). They further validated the upregulation of miR-574-5p in an additional 20 patients with ARDS as compared with 20 healthy control subjects by qRT-PCR. The authors then demonstrate that HMGB1 is a direct target of miR-574-5p using a 3′-UTR reporter assay. In addition, they show that miR-574-5p mimics decreased HMGB1 mRNA stability and abundance and protein release in LPS (also termed "endotoxin")-treated human pulmonary microvascular endothelial cells (HPMECs) or mouse monocyte/macrophage-like RAW 264.7 cells. Furthermore, miR-574-5p agomir, a specially labeled and chemically-modified double-stranded miRNA that mimics endogenous miRNA, also decreased HMGB1 protein concentrations in the lung tissue, serum, and BAL fluid of LPS-treated mice. Therefore, miR-574-5p appears to directly target HMGB1 and negatively regulates its expression *in vitro* and *in vivo*.
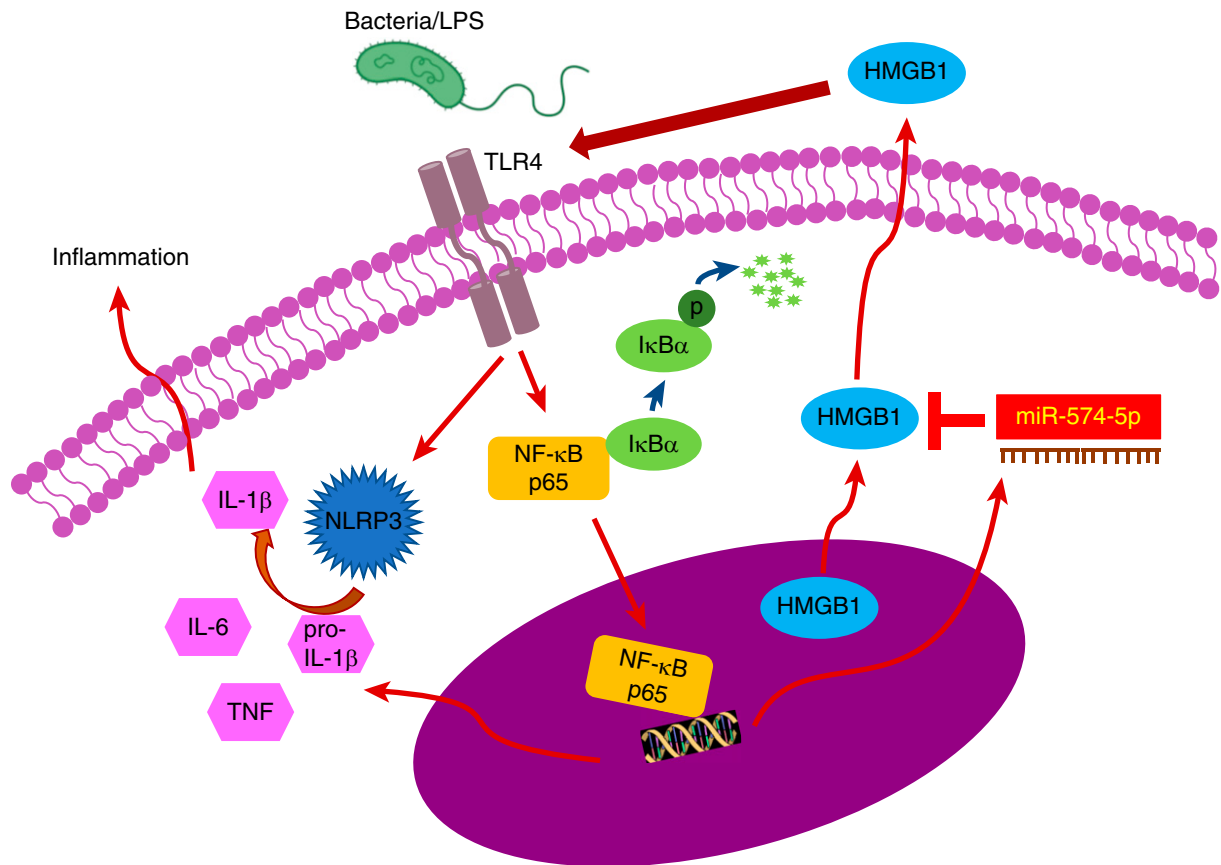
The authors then investigated whether and how miR-574-5p itself is upregulated in experimental ARDS. They show that miR-574-5p was upregulated in LPS-stimulated HPMECs and ARDS mice compared with their control counterparts. As the innate immune system senses bacterial LPS through TLR4 (Toll-like receptor 4), the authors sought to determine whether LPS induces miR-574-5p upregulation in a TLR4-dependent manner. Indeed, LPS-induced upregulation of miR-574-5p was attenuated by TAK-242, a specific inhibitor of TLR4 signaling, in HPMECs *in vitro* and in *Tlr4*-deficient mice. They then demonstrate that NF-κB is involved in LPS-induced upregulation of miR-574-5p through the use of NF-κB inhibitors (SC-51 or pyrrolidine dithiocarbamate [PDTC]) in LPS-stimulated HPMECs or ARDS mice. Importantly, the gene encoding miR-574 is located in the first intron region of *Fam114a1*-encoding Noxp20 (15), which the authors identified as an NF-κB direct target gene by chromatin IP analysis. The authors conclude that TLR4 and NF-κB are involved in the upregulation of miR-574-5p by LPS.

HMGB1 is known to bind to and activate TLR4, serving as an alarmin to mediate inflammation (3). Because the authors identified that miR-574-5p targets HMGB1, they sought to determine the functional significance of the miRNA–HMGB1 axis in ARDS. They first show that miR-574-5p mimics inhibited, whereas miR-574-5p inhibitor increased, LPS-induced inflammatory cytokine expression in HPMECs, including that of IL-6, IL-1β, and TNF-α.

Figure 1. miR-574-5p–mediated negative feedback loop for LPS–TLR4 (Toll-like receptor 4) signaling during acute respiratory distress syndrome (ARDS). In the context of LPS-induced acute lung injury/ARDS in an experimental animal model, binding of LPS to TLR4 triggers the inflammatory response through NF-κB–dependent inflammatory gene expression and NLRP3 inflammasome activation. In addition, HMGB1 (high-mobility group box 1) is released into extracellular space, where it activates TLR4 and amplifies the inflammatory response. On the other hand, NF-κB induces miR-574-5p, which targets HMGB1 mRNA for degradation, thereby restraining the inflammatory response. NLRP3 = NLR family pyrin domain containing 3.

Importantly, miR-574-5p agomir ameliorated lung inflammation and injury in LPS-induced ARDS in mice. The authors also explored the mechanism by which miR-574-5p inhibits LPS-induced inflammation. They found that miR-574-5p mimics suppressed LPS-induced NF-κB activation in both HPMECs and mice, as shown by reduced phosphorylation of IκBα and nuclear translocation of the NF-κB p65 subunit. They also observed that miR-574-5p mimics impaired the activation of the NLRP3 inflammasome in ARDS mice. Finally, the authors demonstrate that the inhibitory effects of miR-574-5p on the LPS-induced inflammatory response is mediated through HMGB1, as silencing of HMGB1 abolished the effect of the miR-574-5p inhibitor, whereas overexpression of HMGB1 abolished the effect of miR-574-5p mimics on LPS-induced NF-κB activation, inflammasome activation, and inflammatory gene expression. Based on these observations, the authors suggest that miR-574-5p serves in a negative feedback loop to limit LPS-induced inflammation by targeting HMGB1 (Figure 1).

The study by He and colleagues started from differentially expressed miRNAs in patients with ARDS and identified a negative regulatory network for LPS-induced inflammatory signaling via miR-574-5p–mediated HMGB1 targeting, which

is clinically relevant. This study not only adds to our understanding of the pathogenesis of ARDS but also holds significant therapeutic potential, especially for COVID-19 treatment given the growing evidence that the respiratory system mechanics of COVID-19–associated ARDS are similar to those in historical ARDS. Additional studies are required to determine whether miR-574-5p targets HMGB1 and additional proteins coordinately to restrain the inflammatory response in ARDS. The roles of the other differentially expressed miRNAs in ARDS identified in this study also warrant further investigation. ∎

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