

Loss of Endothelial WWOX: A Risk Factor for ARDS in Smokers?

Cigarette smoking contributes to premature mortality primarily due to cardiovascular and pulmonary diseases. Emerging evidence suggests that it also increases the risk of developing acute respiratory distress syndrome (ARDS) (1, 2).

ARDS is a clinical entity characterized by acutely worsening respiratory failure with bilateral opacities, not fully explained by cardiac failure or fluid overload. Both direct (e.g., pneumonia, aspiration) and indirect injuries (e.g., sepsis, trauma) can precipitate ARDS (3). Its initial lung injury response involves innate immune-mediated damage of the alveolar–capillary barrier and accumulation of protein-rich fluid within lung interstitium and alveoli.

Cigarette smoke (CS) exposure alters expression of thousands of genes ($\sim 3,000$, $\sim 1,500$, ~ 700 , and ~ 500 over 5 h, 8 d, and 1.5 and 6 mo, respectively) in lungs of mice. These genes are components of diverse functional pathways regulating antioxidant, apoptosis, cell survival pathways, extracellular matrix, and ubiquitin proteasomes. For example, the antioxidant and phase II detoxification genes heme oxygenase 1 (HO-1), glutamate-cysteine ligase, catalytic subunit (GCLC), and glutathione reductase 1 (GSR) were highly upregulated after 1 day of CS exposure, but their upregulation progressively subsided during the ensuing 6 months (4). Epigenetic regulation mechanisms such as DNA methylation may play an important role in such CS-altered gene expression and, with long-term CS exposure, the development of lung pathology (5).

CS exposure exacerbates lung inflammation and predisposes to experimental lung injury in mice (6) and ARDS in patients (1, 2). The contributing mechanisms are incompletely understood, but candidates strongly implicated include CS-induced alveolar permeability caused by depletion of endogenous antioxidants (7), and accumulation of alveolar macrophage populations including those in which production of antiinflammatory cytokines are reduced (6).

Integrity of alveolar epithelial cells serves as the frontline barrier against noxious exogenous stimuli such as CS, and the alveolar endothelial barrier is key to determining alveolar capillary permeability and thereby ARDS pathophysiology. In this context, an emerging potential modulator is the WW domain-containing oxidoreductase (WWOX), a chromosomal fragile-site gene related to tumor suppression. WWOX participates in signaling pathways such as apoptosis and DNA damage repair (8), and WWOX polymorphisms are associated with chronic obstructive pulmonary disease susceptibility (9). Exposure to CS extract induces hypermethylation and inactivation of WWOX in human bladder cancer cells *in vitro* (10). Knockdown of WWOX in mice by intratracheal siRNA injections induced alveolar protein leakage, neutrophil influx, and inflammatory cytokine production

and exacerbated LPS-induced lung injury. Also, in WWOX knockdown mice, the extent of protein leakage into the alveolar space was more severe than that evoked by LPS-induced inflammation and lung injury, implying a contribution of endothelial barrier disruption to WWOX depletion–associated exacerbation of lung injury (11). Also, in cultured human alveolar epithelial cells, WWOX knockdown increased neutrophil chemotaxis.

In this issue of the *Journal*, Zeng and colleagues (pp. 89–99) reveal that downregulation of WWOX contributes to exacerbation of lung injury, by actions mainly centering on the endothelial cell barrier (12). By analyzing lung tissue of current smokers versus that of former/never-smokers, they found the former showed significantly decreased WWOX gene and protein expression. They also confirmed WWOX downregulation in lungs of e-cigarette vapor (containing nicotine)–exposed mice. Furthermore, WWOX knockdown in human lung microvascular endothelial cells induced endothelial barrier dysfunction, assessed by transendothelial electrical resistance and FITC-dextran permeability assays, and it was not rescued by inhibiting JNK. The authors further show that endothelial cell (EC)-specific WWOX knockout (KO) exacerbated lung injury, using EC-specific WWOX KO mice they generated. EC-specific WWOX KO mice exhibited significant exacerbations of alveolar leukocyte infiltration and protein leakage elicited by intratracheal administration of LPS or heat-killed methicillin-resistant *Staphylococcus aureus*. Surprisingly, these exacerbations were unaccompanied by any significant changes in inflammatory cytokines such as TNF- α and KC (ketatinocyte-derived chemokine). Given this observation, EC-specific loss of WWOX appeared to contribute directly to inflammation-independent disruption of the endothelial barrier. Taken together, these results led the authors to conclude that downregulation of lung WWOX may be linked, at least in part, to increased risk of ARDS in chronic smokers (Figure 1).

These novel findings raise further intriguing questions that would possibly drive future investigations. First, how does WWOX loss contribute to endothelial barrier dysfunction during lung injury? During acute lung injury, RhoA and myosin light chain kinase induce EC contraction via actin polymerization and phosphorylation of myosin light chain, which leads to gap formation between ECs. Furthermore, TNF- α can induce junctional instability by Src-family tyrosine kinase-mediated phosphorylation of vascular endothelial cadherin (13). Also, CS-induced mitochondrial fission and oxidant stress led to endothelial barrier dysfunction (14). Given the link of EC-specific knockdown of WWOX to inflammation-independent endothelial barrier dysfunction, there may be unknown interactions between WWOX and

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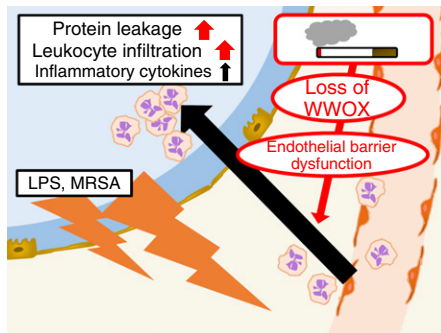


Figure 1. Proposed mechanisms of WWOX loss–mediated exacerbation of acute respiratory distress syndrome among smokers. Enhanced lung inflammation by LPS or MRSA in the lungs of smokers. Cigarette smoke (CS)-induced loss of WWOX leads to endothelial barrier dysfunction, which exacerbates the protein leakage and inflammatory cell infiltration into the alveolar space compared with the lungs without CS. MRSA = methicillin-resistant *Staphylococcus aureus*; WWOX = WW domain-containing oxidoreductase.

these effector molecules. Second, exposure to CS and e-cigarette induced downregulation of WWOX in the lung of humans and mice, respectively. Are there any cell type–specific effects of WWOX loss in the development of lung injury? In this study, the authors focused on the effects of EC-specific WWOX downregulation that is sufficient to exacerbate lung injury. However, according to the mouse single cell transcriptome data, other cell types including lung epithelial and myeloid cells express the WWOX gene more highly than endothelial cells (15). Although the depletion and/or augmentation of WWOX in ECs may restore endothelial barrier function in experimental models, the therapeutic application of targeting WWOX for ARDS would be challenging without knowing the role of other cell types expressing WWOX (e.g., lung epithelial cells, monocytes) in the development of lung injury.

In conclusion, the present work uncovers WWOX loss in the lung ECs as one of the mechanisms explaining why cigarette smoking may predispose an individual to the development of ARDS following lung infection. This novel finding raises a serious concern about e-cigarette use, which may also increase the risk of ARDS as reported by recent studies (16). Future studies should investigate the potential of WWOX as a therapeutic target in mitigating CS-induced ARDS. ■

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