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EDITORIAL

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NEDD9 provides mechanistic insight into the coagulopathy of COVID-19

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus-2 (SARS CoV-2), was the third leading cause of death in the United States in 2020 and has caused more than 6 million deaths worldwide in the last 2 years. Early in the COVID-19 pandemic, clinicians quickly noticed an increased rate of thrombotic complications in COVID-19 patients relative to those with other viral infections, an observation that was subsequently validated by numerous epidemiologic studies.[1,2] Given that these thrombotic complications contribute significantly to the morbidity and mortality of COVID-19 patients, the medical community rapidly responded with the initiation of several randomized controlled trials of different anticoagulant strategies in COVID-19 including intensified prophylactic heparin, full-strength anticoagulation, and even fibrinolytic therapy with tissue plasminogen activator.[1,3] The results of these studies, which collectively demonstrated that the risk-benefit ratio of various anticoagulation strategies varies based on the stage and severity of SARS CoV-2 infection, highlight the complexity of COVID-19 coagulopathy, particularly as these patients are also at increased risk for complications of anticoagulant therapy due to critical illness associated thrombocytopenia, platelet dysfunction, and coagulation factor deficiencies.[4] This precarious situation highlights an urgent need to understand the precise molecular mechanisms underlying COVID-19 coagulopathy so that targeted therapeutics can be developed to prevent these devastating complications.

In this issue of *Pulmonary Circulation,* Alba et al.[5] report a novel, and potentially targetable, signaling pathway that contributes to intravascular thrombosis in patients with acute respiratory distress syndrome (ARDS) due to COVID-19.[5] They identified that neural precursor cell expressed developmentally downregulated protein 9 (NEDD9), a cytoskeletal scaffolding protein that was previously appreciated for its role in the progression of malignancy, is a critical mediator of the interaction between pulmonary artery endothelial cells (ECs) and platelets under hypoxic conditions, particularly in the context of COVID-19. By performing immunofluorescence imaging analysis of lung tissue from patients with COVID-19 ARDS, non-COVID-19 ARDS, and without ARDS, the authors both confirmed increased levels of pulmonary microthrombi in COVID-19 ARDS and reported, for the first time, that an extracellular NEDD9 peptide ($N9_P$) is present at sites of pulmonary microvascular thrombosis in COVID-19 patients.[5] The physiologic significance of these findings is highlighted by a significant relationship between $N9_P$ expression and ventilatory ratio, a surrogate for dead space that can be easily calculated at the bedside, in patients with COIVD-19 ARDS, but not those with non-COVID-19 ARDS.

Subsequently, the authors performed complementary in silico and in vitro experiments to determine the mechanisms underlying these clinical findings. Given the relationship between $N9_P$ and ventilatory ratio in COVID-19 ARDS, but not non-COVID-19 ARDS, the authors first aimed to determine how SARS-CoV-2 could impact hypoxia-induced NEDD9 overexpression. Using in silico analysis, they identified that transcription factor 12, which is known to be upregulated by hypoxia and increase transcription of NEDD9, is capable of binding to the SARS-CoV-2 RNA-dependent RNA polymerase NSP12, suggesting a possible mechanism for increased N9P containing microthrombi in COVID-19 ARDS. Additionally, using a strategy of tandem immunoprecipitation–liquid chromatography–mass spectrometry, the authors identified integrin αvβ3 as a likely mediator of the interaction between $N9_P$ on the endothelial surface and activated platelets.[5] The proposed mechanism underlying intravascular thrombosis in COVID-19 ARDS is summarized in schematic form (Figure 1).

Although the study by Alba et al.[6] has identified critical mechanistic insight into the pathogenesis of the coagulopathy of COVID-19, significant work remains before the implementation of NEDD9 targeted therapeutics for COVID-19 patients. Given prior work demonstrating vascular bed-specific effects of hypoxia on NEDD9 expression,[6] additional studies are necessary to clarify whether pharmacologic agents aimed at

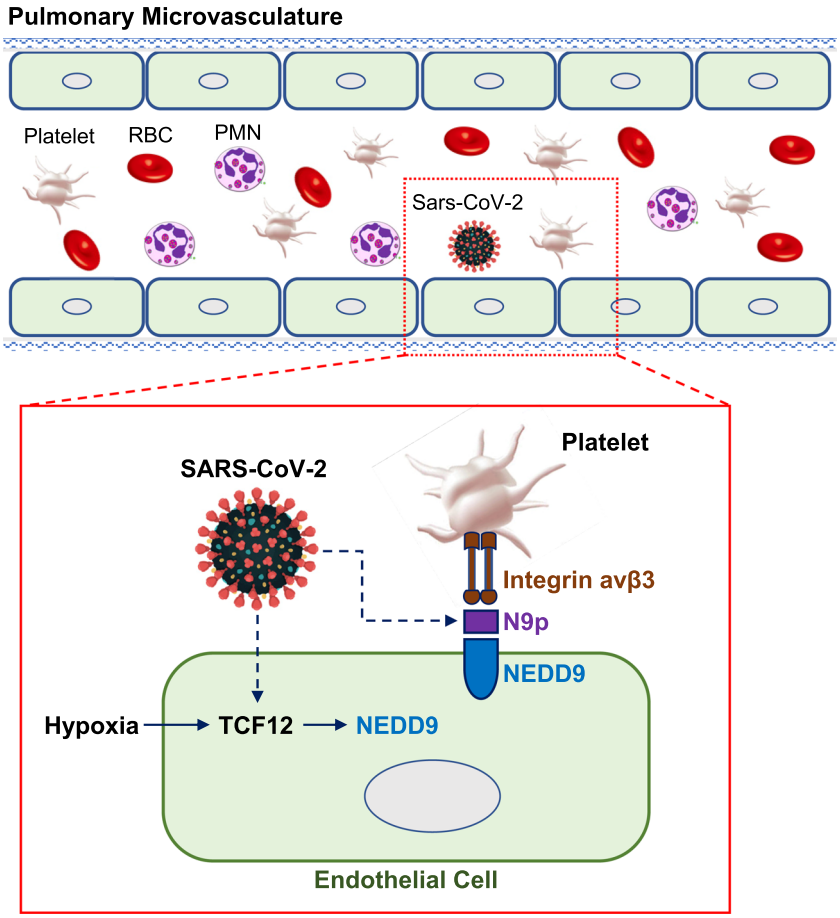

FIGURE 1 Proposed mechanism underlying the role of NEDD9 in COVID-19 coagulopathy. Hypoxia upregulates neural precursor cell expressed developmentally downregulated protein 9 (NEDD9) in lung vascular endothelial cells (ECs) via transcription factor 12 (TCF12). The extracellular NEDD9 peptide $N9_P$ binds to integrin αvβ3 on the cell surface of platelets, which results in EC-platelet adhesion and contributes to the initiation of microvascular thrombosis. Increased co-localization of $N9_P$ with fibrin in patients with coronavirus disease-19 (COVID-19), compared to those without COVID-19, suggests differential regulation of N9p in the setting of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. The SARS-CoV-2 RNA-dependent RNA polymerase, Nsp12, may also directly bind to TCF12, and thus alter NEDD expression. PMN, neutrophil; RBC, red blood cell. [Color figure can be viewed at wileyonlinelibrary.com]

blocking the interaction between ECs and platelets will have off-target effects in the vasculature of other organs. This may be particularly important in the brain microvasculature because COVID-19 patients are at increased risk of both ischemic stroke and intracranial hemorrhage.[2] Additionally, although the correlation between $N9_P$ expression in autopsy specimens and the ventilatory ratio, which was calculated within 24 h of intubation, suggests that NEDD9-mediated microvascular thrombosis begins early in the pathogenesis of ARDS, further investigation is necessary to quantify NEDD9 expression in living COVID-19 patients, perhaps using plasma NEDD9 levels. Furthermore, the extent to which NEDD9-mediated microvascular thrombosis contributes to pulmonary vascular remodeling in patients who have recovered from COVID-19 is still unknown.

In addition to identifying mechanistic insights into COVID-19 coagulopathy, this study highlights the emerging concept of ARDS heterogeneity by providing evidence of pathophysiologic differences between COVID-19 ARDS and non-COVID-19 ARDS. Given that prior attempts to treat ARDS as a single disease state have failed to identify effective pharmacologic strategies, recent translational research has shifted to focus on identifying subpopulations of ARDS, that may have differential responses to pharmacology treatments, and causal biomarkers, which can identify these populations at the bedside.[7,8] Given the central role of NEDD9 in pulmonary microvascular thrombosis, it is likely that patients with elevated NEDD9 levels, or potentially those with elevated or increasing ventilatory ratios, would be more likely to benefit from NEDD9 targeted therapeutics, such as $N9_P$ blocking antibodies. Although additional

work is necessary to test these hypotheses, this study highlights the importance of integrating mechanistic basic science and translational human subject research to further our understanding of the pathogenesis of ARDS and develop precision medicine approaches to treat this devastating condition.

In summary, this study provides strong evidence that upregulation of NEDD9, due to hypoxia, and potentially direct effects of SARS-CoV2, mediates the adhesion of platelets to the pulmonary endothelium, which is an early step in the development of thrombosis in COVID-19. This study also highlights the potential for novel mechanism-targeted therapeutic strategies, such as $N9_P$ blocking antibodies, in the prevention of in situ pulmonary thromboembolic events in COVID-19 ARDS. Furthermore, this study adds to a growing body of literature supporting that NEDD9 is a key mediator of hypoxia-induced dysregulation of vascular homeostasis, a finding which has implications in multiple cardiopulmonary disease processes including pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension.[6,9,10] Future investigation is needed to further define the complex role of NEDD9 in the pulmonary vasculature and evaluate the therapeutic potential of NEDD9 inhibitors in COVID-19 and other cardiopulmonary diseases.

KEYWORDS

ards acute respiratory distress syndromes and acute lung injury, coagulation, pulmonary vasc, viral infections and pathogenesis

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

The ethics statement is not available.

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