ELX/TEZ/IVA to the 6- to 11-year-old age group. The CF community has been waiting for the results of this study and to have ELX/TEZ/IVA for children. The goal and expectation will be much better long-term outcomes for people living with CF.

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NEDD9, a Hypoxia-upregulated Mediator for Pathogenic Platelet–Endothelial Cell Interaction in Pulmonary Hypertension

Pulmonary hypertension (PH) is a progressive cardiopulmonary syndrome with high mortality and poor prognosis. Excessive pulmonary vascular remodeling and fibrosis, due in part to the endothelium injury and platelet–endothelium interaction, is one of the major causes for elevated pulmonary vascular resistance and pulmonary arterial pressure in patients with PH and experimental animal models (1–3). Hypoxia and hemodynamic shear stress in the pulmonary vasculature are believed to activate thrombotic pathways, leading to the formation of *in situ* thrombosis in pulmonary arterial hypertension (PAH) and chronic thromboembolic PH (CTEPH) (4, 5). Emerging studies have reported the contribution of platelet activation in the formation of pulmonary vascular thrombosis and the development of pulmonary vascular remodeling. Upon activation, the platelets aggregate to the damaged pulmonary vasculature and release vasoactive mediators, angiogenic agents, growth factors, chemokines, and cytokines, many of which are implicated in the

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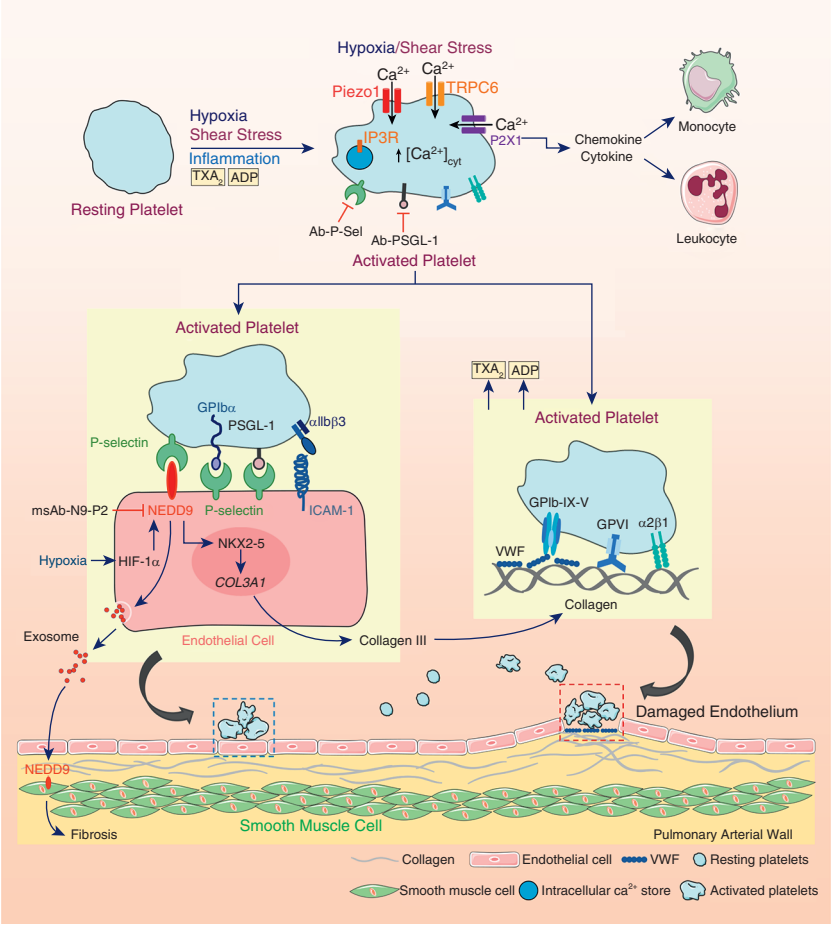

Figure 1. Proposed mechanisms showing the pathogenic role of NEDD9 in hypoxia-induced platelet activation and platelet–endothelial cell (EC) interaction and their relationship to the development of pulmonary vascular remodeling. Hypoxia, together with hemodynamic shear stress and inflammation, activates platelets by inducing Ca^{2+} influx through Piezo1 (a mechanosensitive Ca^{2+} channel) and TRPC6 (a receptor-operated Ca^{2+} channel) and increasing cytosolic $[Ca^{2+}]_{cyt}$ in platelets. Hypoxia upregulates NEDD9 in lung vascular ECs via HIF-1α, and NEDD9 binds to P-selectin on the surface membrane of activated platelets, resulting in platelet–EC adhesion. Synthesis and release thromboxane A2 (TXA_2) and ADP from activated platelets further enhance the hypoxia- and inflammation-mediated activation of platelets. NEDD9 can also enhance platelet–smooth muscle cell interaction via exosomes released from EC and cause pulmonary vascular fibrosis. NEDD9 can also upregulate collagen III via NKX2-5 and COL3A1 and cause obliterative intimal lesions in the small arteries and arterioles. NEDD9 can also up-regulate the collagen III gene (*COL3A1*) expression via NKX2-5 (homeobox protein NKX2-5), increase collagen deposition in extracellular matrix (and plasma), and cause obliterative intimal lesions in the small arteries and arterioles. Blocking NEDD9 with specific antibodies such as msAb-N9-P2 disrupts NEDD9/P-selectin interaction, inhibits platelet–EC adhesion, and attenuates pulmonary vascular remodeling and fibrosis. $\alpha_2\beta_1 = \alpha_2\beta_1$ integrin; αIIbβ3 = platelet αIIbβ3 integrin; Ab-P-Sel = antibody against P-selectin; Ab-PSGL-1 = antibody against P-selectin glycoprotein ligand-1; GPIbα = platelet glycoprotein Ib α chain; GPIb–IX–V = glycoprotein Ib–IX–V receptor complex; GPVI = platelet glycoprotein VI (collagen receptor); ICAM-1 = intercellular adhesion molecule 1; IP3R = inositol trisphosphate receptor; P2X1 = P2X purinergic receptor 1; PSGL-1 = P-selectin glycoprotein ligand-1; TRPC6 = transient receptor potential canonical 6 channel; VWF = von Willebrand factor.

pathological pulmonary vascular remodeling and microvascular thrombosis in PH and PAH (6). However, the detailed molecular mechanisms underlying the platelet and pulmonary arterial endothelial cell (PAEC) adhesion under conditions of hypoxia and PH remain largely unknown.

In this issue of the *Journal*, Alba and colleagues (pp. 1533–1545) reported a novel mechanism in which hypoxia induces an HIF-1α–dependent upregulation of the NEDD9 (neural precursor cell expressed developmentally down-regulated protein 9) in PAEC, which can bind to P-selectin on the surface membrane of the activated platelets, trigger the platelet–PAEC adhesion, and promote thromboembolic vascular remodeling (7). Blocking such NEDD9/P-selectin interaction using a specific antibody (msAb-N9-P2) that targets the extracellular NEDD9 peptide not only inhibits the platelet–PAEC adhesion but also exerts promising therapeutic effects on pulmonary embolism in mice (induced by ADP) and PH in rats with chronic pulmonary thromboembolic disease (induced by the administration of thrombogenic microspheres). Using *in vitro* and *in vivo* models and cells isolated from patients, this study provides extensive data indicating that NEDD9 is a critical mediator for the platelet–EC interaction or adhesion in the lungs. The concept or finding that HIF-1α–sensitive upregulation of NEDD9 is a critical

step for platelet adhesion to the lung vascular EC is novel and important. The identification and characterization of the upstream and downstream signaling cascades of NEDD9 in platelet–EC adhesion and their potential pathogenic role in the development of thromboembolic PH provide important information for the future development of novel therapeutic approach for occlusive intimal and neointimal lesions in CTEPH and PAH. As indicated in the study, blockade of the Nedd9–P-selectin binding using specific antibodies against NEDD9 (e.g., msAb-N9-P2) is potentially a novel therapeutic strategy for patients with CTEPH and PH/PAH associated with microvascular thrombosis and intimal lesions (7). The study demonstrates a well-organized molecular basis of the hypoxic upregulation of endothelial NEDD9 in mediating the activated platelet–PAEC adhesion through interacting with P-selectin (7) (Figure 1). Additional attention should also be paid to other potential mechanisms involved in the activation of platelets under hypoxic condition in patients with PH and PAH/CTEPH.

Platelets can be activated by many key factors associated with the development of PH, such as hypoxia, inflammation, and the circulating sheer flow. In a recent study, Delaney and colleagues (8) demonstrated an increased number and activation of platelets in the lungs of both hypoxia-induced PH mice and patients with idiopathic PAH, in line with the proinflammatory states. However, the lung inflammation is prevented in thrombocytopenic mice generated with an anti-GP1bα (rat) IgG antibody, suggesting platelets as important mediators in hypoxia-driven lung inflammation (8). As shown in Figure 1, the activated platelets can induce vascular inflammation by directly interacting with the extracellular matrix and resident vascular cells (9) or indirectly recruiting the circulating monocytes and leukocytes by releasing chemokines and cytokines (10). An increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is another key factor to drive platelet activation. Resting platelets maintain a steady $[Ca^{2+}]_{cyt}$, whereas hypoxia- and shear stress–mediated activation of TRPC6 (11), a receptor-operated Ca^{2+} channel, and Piezo1, a mechanosensitive Ca^{2+} channel, leads to increased $[Ca^{2+}]_{cyt}$ and triggers the activation of platelets. Previous studies have reported that enhanced Ca^{2+} influx through the ATP-gated P2X1 channel (12), store-operated Ca^{2+} channels (13, 14), and Ca^{2+} release from intracellular Ca^{2+} stores via IP_3 receptors (IP_3R) (15) all contribute to the $[Ca^{2+}]_{cyt}$ increase and platelet activation (16). Recently, Ilkan and colleagues demonstrated that the mechanosensitive Piezo1 channel also participates in and facilitates the shear stress–induced elevation of $[Ca^{2+}]_{cyt}$ in platelets, contributing to the platelet activation and thrombus formation under arterial shear (17). Under hypercoagulable state and high hemodynamic sheer stress (under hypoxic condition with inflammation), it is likely that the flow shear force–induced activation of Piezo1 and TRPC6 (also a mechanosensitive channel) may also contribute to the platelet activation, platelet aggregation, and, ultimately, pulmonary vascular thrombus formation. In pulmonary vascular cells, it is shown that hypoxia upregulates multiple Ca^{2+} channels (18–20). It would be interesting to study whether NEDD9, in addition to interacting directly with P-selectin in platelets, also acts together with hypoxia-sensitive and mechanosensitive Ca^{2+} channels to activate platelets, enhance platelet–EC adhesion, and mediate thrombosis-associated pulmonary vascular remodeling in CTEPH and PH/PAH with microvascular embolism.

NEDD9 is also involved in the regulation of pulmonary endothelial fibrosis by transcriptionally inducing *COL3A1* via NKX2-5, which enhances collagen III synthesis and contributes to the development of PH (21) by possibly increasing pulmonary vascular wall stiffness. Genetic depletion or siRNA knockdown of NEDD9 inhibits pulmonary vascular fibrosis and ameliorates experimental PH (21), whereas the NEDD9 antibody, msAb-N9-P2, shows promising therapeutic efficiency in animal models with ADP-induced pulmonary embolism and chronic pulmonary thromboembolic disease (21). These data imply that blocking of platelet–PAEC adhesion is a novel and ideal therapeutic approach for PH with prothrombotic diseases such as CTEPH and PAH with *in situ* microvascular thrombosis. Furthermore, the authors observed that NEDD9 was significantly increased in the plasma from patients with PAH, and the plasma NEDD9 level well predicted the disease diagnosis, suggesting that NEDD9 is potentially an excellent diagnostic and prognostic biomarker for PAH (22).

In summary, this study provides compelling evidence that hypoxic upregulation of endothelial NEDD9 mediates the platelet–PAEC adhesion, formation of pulmonary vascular thromboemboli, and development of pulmonary vascular remodeling. Given the fact that NEDD9 is a noncatalytic scaffolding protein, NEDD9 may, directly or indirectly, interact with other membrane proteins and signaling proteins (other than P-selectin) to cause platelet activation and thromboembolus-associated pulmonary vascular remodeling in patients with CTEPH and PAH. Blocking of NEDD9 with specific antibodies such as msAb-N9-P2 disrupts the platelet–PAEC adhesion and inhibits thrombosis-mediated vascular remodeling. Further research is needed to study whether pharmacological blockade of NEDD9 with extracellular antibodies or genetic modification of the NEDD9 gene is sufficient to inhibit microvascular thrombosis and emboli-associated intima lesions in patients with PAH with *in situ* thrombosis and CTEPH with microvasculopathy. ■

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Physiology May Be the Key: Cardiovascular Risk Stratification in Obstructive Sleep Apnea

It has been 40 years since Sullivan and colleagues first described the effectiveness of continuous positive airway pressure (CPAP) therapy in obstructive sleep apnea (OSA) (1). Since then, CPAP therapy has become the most popular therapy for OSA because it ameliorates many adverse consequences of OSA. It is widely accepted that CPAP therapy reduces daytime sleepiness and risk of crashes and improves quality of life, erectile function, and systemic blood pressure (2, 3).

There has been considerable interest in the impact of OSA on cardiovascular health. Abnormal breathing in OSA is associated with several physiologic insults that are implicated in the development of cardiovascular disease (CVD), such as intermittent hypoxemia and hypercapnia, sleep fragmentation, autonomic activation (4), and large intrathoracic pressure swings that have been shown to promote inflammation, endothelial dysfunction, and metabolic derangements. Observational studies have consistently shown associations between OSA and hypertension (5), coronary disease and heart failure (6), atrial fibrillation (7), stroke (8), and CVD deaths (9). Many studies have shown that CPAP therapy improves endothelial function (10) and reduces inflammatory markers (11), blood pressure (12), and early signs of atherosclerosis (13). Together, these observations suggest that CPAP therapy should reduce the risk of CVD in patients with OSA. However, several randomized controlled trials (RCT) and meta-analyses have shown no risk reduction in CVD events from the use of CPAP therapy in OSA (14). While the validity of the RCT findings and their generalizability to clinical sleep apnea populations continues to generate considerable debate, it is fair to say that the role of CPAP therapy in CVD prevention remains uncertain.

A potential explanation for the ineffectiveness of CPAP therapy in reducing CVD events in OSA observed in RCTs is that the effect of OSA on CVD could vary between individuals. Although OSA severity is generally quantified using frequency-orientated metrics such as the apnea–hypopnea index (AHI) and oxygen desaturation index, these

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