Blood Flow Disturbances in Congenital Heart Disease

Is Neuroblastoma Suppressor of Tumorigenicity 1 a Target for Preventing Pulmonary Vascular Remodeling?

Endothelial-to-mesenchymal transition (EnMT) plays a key role in the development of pulmonary hypertension (PH) involving profound cellular remodeling that affects all three layers of the pulmonary arterial wall (1). The abnormal accumulation of dedifferentiated and proliferative mesenchymal-like cells in usually nonmuscular small-diameter vessels leads to increased intimal and/or medial stiffening and pulmonary artery wall thickening. These cause the arterial lumen to narrow, resulting in an increase in pulmonary vascular resistance, which can lead to right ventricular failure and death (1, 2). Throughout this process, endothelial cells undergo important phenotypic and functional alterations, losing their cobblestone morphology and their intrinsic expression of endothelial cell–specific proteins, such as CD31/platelet endothelial cell adhesion molecule-1 and CDH5 (cadherin 5; vascular endothelial cadherin). At the same time, they acquire mesenchymal markers such as vimentin and ACTA2. In addition, they enhance the production of collagen and increase their migration and invasion into the surrounding tissue (3, 4).

EnMT is an essential and physiological process in embryonic cardiovascular development (2). Interestingly, endocardial cells with a clear endothelial cell phenotype were able to give rise to mesenchymal heart cushion cells through the process of EnMT (5). In addition, it has been shown that EnMT is an important event in aortic and pulmonary artery development (6) and in the maturation of both arteries and veins (7). It is important to note that EnMT does not normally occur in the adult under physiological circumstances (8).

Congenital heart disease (CHD) is characterized by defects at birth in the structure of the heart wall, its valves, or the great vessels. Such defects induce important blood flow disturbances in the heart and in lungs that result in abnormally high pressures in the pulmonary circulation.

Appropriate blood flow and hemodynamic signals are essential for directing the proper vascular development in humans, because mechanical forces influence the development of a balanced endothelial cell phenotype and function (9). In CHD, flow patterns and hemodynamic forces change, resulting in nonuniform and irregular shear stresses that alter the expression of endothelial genes and proteins, resulting in endothelial cell damage and leading to abnormal vascular development that contributes to the development of PH. If CHD is left unrepaired, long-term life-threatening complications such as irreversible pulmonary arterial hypertension (PAH), also known as Eisenmenger syndrome, can develop. Increased pulmonary vascular blood flow in CHD enhances pulmonary vascular remodeling. Hence, patients with CHD with Eisenmenger syndrome have a poor prognosis and no therapeutic options other than lung transplant (4).

In this issue of the *Journal*, Wen and colleagues (pp. 666–679) clearly demonstrate the importance of flow-induced vascular remodeling using an innovative flow-associated CHD-PAH animal model (4). This study also uncovers the underlying molecular mechanisms involving the glycoprotein NBL1 (neuroblastoma suppressor of tumorigenicity 1) in PAH, previously identified as playing a role in cancer through its effects on phenotype transformation, cell proliferation, and tumor progression (10, 11).

In a systematic and well-conceived study, the authors show that NBL1 promotes EnMT in human pulmonary artery endothelial cells, that it is highly expressed in remodeled pulmonary arteries in patients with PAH, and that its level is strongly associated with the severity of CHD-PAH (4). Importantly, the authors also show that NBL1-knockout rats exhibit lower pulmonary artery pressures and less vascular remodeling; a reduced expression of EnMT genes such as Snail, Slug, and Twist; and a higher expression of bone morphogenetic proteins (BMP) compared with shunt-treated wild-type rats (4).

It is known that the TGF-β (transforming growth factor-β) and BMP families play major roles in the initiation and progression of PAH (12) and that a shift in the TGF-β–BMP axis, which increases the level of TGF-β relative to BMP, can increase inflammation and contribute to the induction of EnMT (13). Hence, it is not surprising that Wen and colleagues demonstrate that NBL1 is involved in the disruption of the TGF-β–BMP axis that promotes EnMT via the canonical TGF-β pathway.

This study advances our understanding of the origins and progression of EnMT and the molecular mechanisms by which endothelial cells transdifferentiate into mesenchymal-like cells. Studies such as this one are key for the development of novel and effective therapies aimed at inhibiting EnMT early in the development of PAH.

In addition, Wen and colleagues emphasize that the development of animal models capable of reproducing the pathophysiological mechanisms or clinical causes of human PAH is necessarily complex, perhaps even more difficult in the case of CHD-PAH (4). Wen and colleagues have employed an innovative flow-associated PAH animal model involving a left cervical shunt followed by right pulmonary artery ligation (14). Remarkably, NBL1-knockout rats had reduced right ventricular and pulmonary artery systolic pressures and lessened vessel remodeling and muscularization. However, this animal model could be extended

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even further by the use of cell lineage tracing approaches. Such tracing analysis uses transgenic mice engineered to express a permanent fluorescent marker after Cre activation by tamoxifen in vascular endothelial cells (15). If applied in this model, it could provide important additional information related to the structural and functional transformations that pulmonary artery endothelial cells undergo in response to disturbed blood flow patterns that lead to the development of PH-CHD. Hence, future research can extend these results through the use of available powerful techniques to track the migration, proliferation, and differentiation of vascular cells *in vivo*. Future interesting studies could then assess the potential clinical use of NBL1 as a therapeutic target in CHD-PAH. ■

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