

Stem Cell–derived Nanovesicles for the Treatment of Pulmonary Hypertension: Are We There Yet?

Pulmonary arterial hypertension (PAH) is a progressive disease with high mortality rates and poor survival. The key pathophysiological feature of PAH is a hyperproliferation of resident pulmonary vascular cells resulting in inward remodeling of small pulmonary arteries (PAs), unresolved increase in PA pressure, right heart failure, and premature death. Hyperproliferation of pulmonary vascular cells in PAH is a complex multifactorial process that could be triggered by numerous pathological stimuli, including genetic susceptibility, epigenetic events, environmental factors, and inflammation. Chronic exposure to elevated concentrations of growth factors and proinflammatory mediators, shear stress, changes in extracellular matrix composition and stiffness, and dysregulated amounts of microRNAs may lead to increased proliferation and apoptosis resistance of pulmonary artery smooth muscle cells (PASMCs), pulmonary artery endothelial cells (PAECs), and pulmonary artery adventitial fibroblasts (1, 2). Although great progress has been made in translational and clinical research, the majority of approved therapies are vasodilators with limited antiproliferative properties, and there is a need for antiproliferative antiremodeling strategies to reverse pulmonary vascular remodeling and halt PAH progression (1).

Mesenchymal stem cells (MSCs) are nonhematopoietic multipotential stem/progenitor cells with antiinflammatory, immunomodulatory, and regenerative properties. MSCs are under active investigation as an attractive therapeutic option in the treatment of tissue injuries, aging frailty, excessive inflammation, and for alleviating organ damage. The MSC-released extracellular vesicles (MSC-EVs), loaded with microRNAs, mRNAs, and proteins, act as modulators of cell fate, cell–cell communications, and inflammatory responses. MSCs show anti-proliferative, antiinflammatory, and antiremodeling benefits in animal models of pulmonary hypertension (PH). However, transition of MSCs to clinical trials has been delayed because of serious concerns about potential adverse events accompanying intravenous MSC delivery, including formation of emboli, the potential for uncontrolled angiogenesis, and altered immune responses (3). Use of cell-free MSC-EVs demonstrated preserved antiproliferative and antiremodeling properties without associated serious side effects, and MSC-EVs were recently shown to improve vascular remodeling and reverse established PH in a severe rat SU5416/hypoxia model (3, 4). There are still some limitations related to difficulties with industrial scale production, however, which continue to be challenging because of low yield and the heterogeneity of EVs released by MSCs (5).

In this issue of the *Journal*, Hu and colleagues (pp. 61–75) aimed to overcome this limitation by fabricating exosome-mimetic

nanovesicles (MSC-NVs), which have an improved yield while preserving the therapeutic benefits of MSC-EVs (Figure 1) (6). Indeed, the authors demonstrate that MSC-NVs inhibited platelet-derived growth factor–induced proliferation and migration of PASMCs and attenuated monocrotaline-induced pulmonary vascular remodeling and PH in rats to the same extent as did MSC-EVs. Importantly, analysis of NV cargo, performed by the authors, identified two microRNAs—miR-125b-5p and miR-100-5p—as the top two enriched miRNAs in MSC-NVs. The authors then experimentally confirmed that miR-125b-5p and miR-100-5p are, at least in part, responsible for downregulating Myo1e and mTOR and inhibiting platelet-derived growth factor–induced proliferation and migration of PASMCs (6). Further reinforcing the translational significance of the study, the authors demonstrate that miR-125b-5p and miR-100-5p were downregulated in the lung tissues of patients with PAH and rats with monocrotaline-induced PH, providing a molecular basis for antiremodeling actions of MSC-NVs.

The study by Hu and colleagues provides strong evidence of translational attractiveness of MSC-NV administration as a strategy to target PASM hyperproliferation, remodeling, and overall PH. There are, however, some critical questions that remain to be answered. First, the authors show that the MSC-derived NVs localize to PASMCs *in vivo* and focus their attention on a PASM-centric mechanism for the NV's action. Yet, it remains unclear if the NVs reduce PH through their direct antiproliferative action on PASMCs. It should be noted that EVs, which have a size similar to that of the NVs described by Hu and colleagues, affect endothelial cells and can improve pulmonary vascular endothelial function (7), which, in turn, could indirectly regulate PASM proliferation (8). The authors observed an MSC-EV–dependent attenuation of endothelial-to-mesenchymal transition of PAECs induced by chronic hypoxia. Hence, it is possible that MSC-NVs reduce pulmonary vascular remodeling by restoring physiologic endothelial function, so the potential role of PAEC–PASM communication in MSC-NV antiremodeling action needs to be determined. Second, other potential target cells of nanoparticles require further evaluation. Such target cells may include inflammatory or myeloid cells, which are known targets of EVs (9, 10), as well as pulmonary artery adventitial fibroblasts and pericytes, which are important drivers of pulmonary vascular remodeling in PAH. Third, besides the target cell types, Hu and colleagues discovered that the antihypertensive effects of MSC-NVs require miR-125b-5p and miR-100-5p; yet, multiple mechanisms underlying the protective effects of EVs derived from MSCs have been reported previously, including more fundamental

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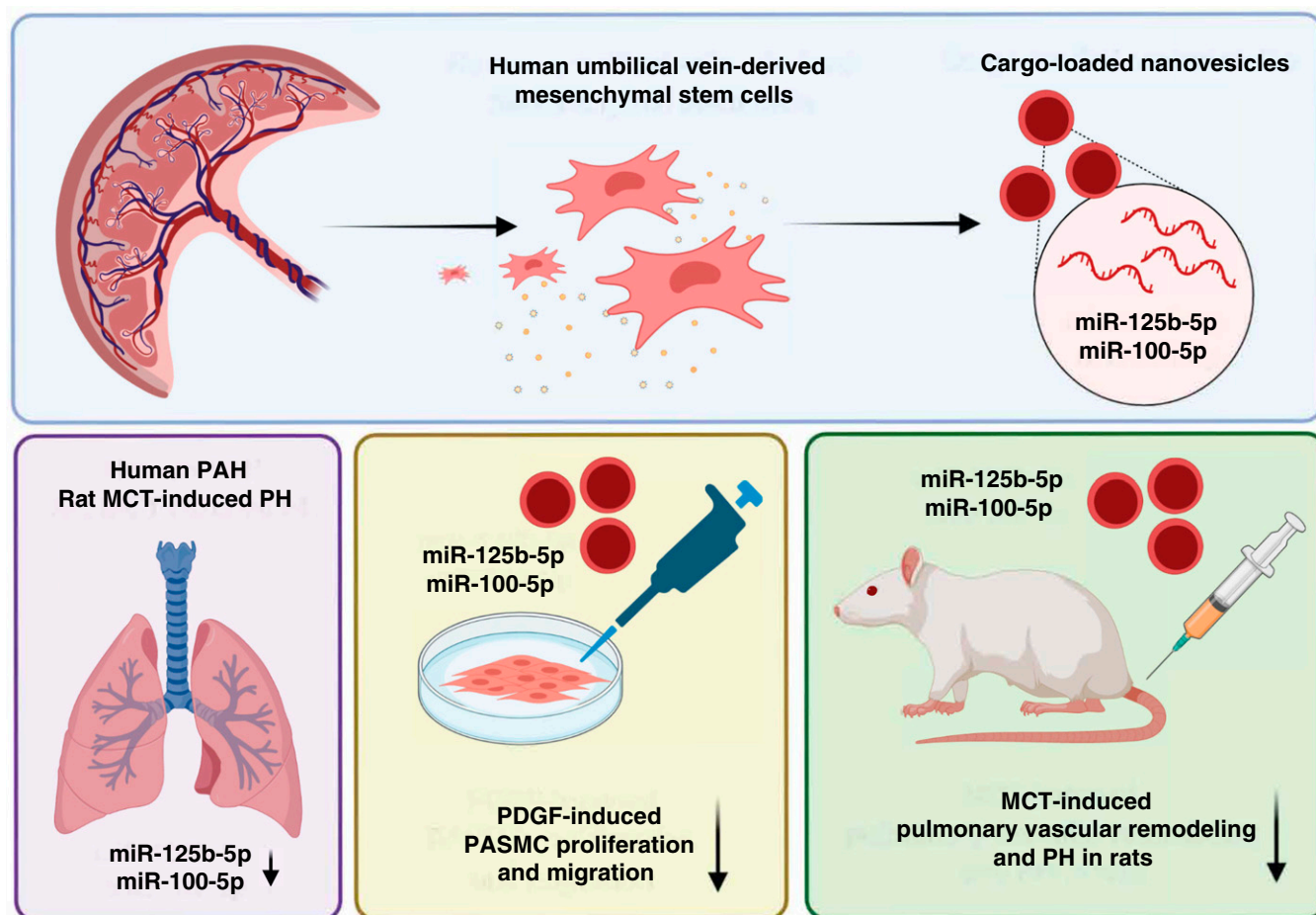


Figure 1. Antiproliferative and antiremodeling role of MSC-NVs in pulmonary hypertension as shown by the study of Hu and colleagues (6). Upper panel: Production of MSC-NVs from human umbilical cord mesenchymal stem cells. Left: Dysregulation of miR-125b-5p and miR-100-5p in lung tissue from patients with PAH and rats with monocrotaline-induced PH. Middle and right: Treatment with MSC-NVs carrying miR-125b-5p and miR-100-5p cargo decrease PDGF-induced proliferation and migration of PASM Cs and reduce pulmonary vascular remodeling and PH in rats. MCT = monocrotaline; MSC-NVs = mesenchymal stem cells-derived nanovesicles; PAH = pulmonary arterial hypertension; PASM Cs = pulmonary arterial smooth muscle cells; PDGF = platelet-derived growth factor; PH = pulmonary hypertension. Illustration created with BioRender.com.

epigenetic and transcriptomic reprogramming (9). Thus, the protective effects of MSC-NVs may similarly exert fundamental effects on PASM Cs, which calls for further mechanistic studies. Fourth, besides such questions concerning the molecular and cellular mechanisms of NVs, it would also be important to directly test if the use of NVs could overcome current drawbacks of EVs (low yield and heterogeneity), which hinder translation to a clinical scale (5). Finally, more preclinical studies are needed before translating MSC-NVs to clinical trials. PAH is a predominantly female disease; however, only male rats were tested in the study by Hu and colleagues. It should also be noted that PASM Cs in PAH undergo complex molecular reprogramming and acquire a self-supported proproliferative/prosurvival phenotype that drives pulmonary vascular remodeling without exogenous stimuli (1). Whether MSC-NVs can selectively target the “diseased” PAH PASM C phenotype remains to be determined.

The study by Hu and colleagues not only provides important information but also calls for further investigation to help answer the

next big question related to translation of MSC-derived NVs to clinical trials. Preclinical testing in multiple cellular and animal models of PH would reveal whether there are differences between entities of PH regarding a benefit from NV therapy. Such testing will need to extend to a model of occlusive pulmonary arteriopathy and large-animal models. These studies should precede any clinical evaluation of safety and efficacy in humans. In conclusion, we congratulate Hu and colleagues for their elegant and important study providing novel and exciting preclinical advances in MSC-derived NV therapy in PH. ■

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