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Nanoparticle Facilitated Gene Delivery in Congenital Pulmonary Vascular Disease: Roadmap for Other Forms of Pulmonary Hypertension

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With the advent of high throughput platforms for genomic and molecular phenotyping of pulmonary vascular diseases, there has been renewed interest in utilizing the results to advance gene therapies as precision therapeutics. When considering gene therapy in the pulmonary vasculature, there are several important considerations: determining the optimal route for delivery (i.e., intravenous vs. inhaled); selecting the delivery vector or vehicle, which ideally has high *in vivo* target tissue tropism; obtaining an acceptable transfection efficiency; achieving durable expression; and, limiting off target effects. Among the approaches gaining traction for pulmonary vascular gene delivery, nanoparticles have been shown to have several unique properties that are preferential for transducing the pulmonary vasculature.[1] Nanoparticles, defined as particles <100 nm in one direction, have been utilized to package RNAs, DNAs, viral vectors, or drugs for protected delivery to a tissue site of interest. Specifically, nanoparticles comprised of polyethelenimine, lipids, and polyethylene glycol with a positive charge been shown to target the pulmonary capillary endothelium with a high degree of efficiency when injected intravenously.[2] Given that the pulmonary capillary bed has the largest surface area of any organ (cross-sectional area=400 cm^2) and contains ~50% of the body's capillary surface area,[3, 4] intravenous administration of these engineered nanoparticles offers excellent targeted delivery of their cargo in diseases where pulmonary capillary dysfunction or loss is a predominant feature.

One such pulmonary vascular disease characterized by decreased pulmonary capillary density is alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV). This devastating congenital disorder with a reduction in pulmonary capillary surface area, pulmonary arterial hypertrophy and aberrant pulmonary veins leading to pulmonary hypertension after birth has no directed pharmacologic therapies and results in early death without transplantation.[5, 6] Investigations into the genomic etiologies of ACDMPV identified loss-of-function point mutations, copy number variations, and other heterozygous genomic variants in the gene or locus for the transcription factor Forkhead Box F1

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(FOXF1).[5] This transcription factor plays a critical role in lung maturation by regulating lung alveolar capillary development.

In the current issue of *Circulation*, Sun et al provide groundbreaking new evidence to demonstrate the promise of gene therapy as a novel therapeutic for ACDMPV.[7] They report that intravenous administration of nanoparticles containing STAT3 cDNA, a downstream target of FOXF1 that is disrupted in ACDMPV, is sufficient to ameliorate pulmonary hypertension and right ventricular hypertrophy in the preclinical mouse *Foxf1*$^{WT/S52F}$ model of ACDMPV. When administered intravenously during the neonatal period, the nanoparticles stimulated angiogenesis to increase pulmonary capillary density. This prevented the development of pulmonary hypertension and attendant right ventricular hypertrophy and dysfunction, improved lung remodeling and oxygenation, and increased survival. While the STAT3 nanoparticles did target the endothelium of the right ventricle, there was no change in right ventricular capillary density suggesting that all the benefits observed derived from improved pulmonary capillary density. These exciting findings are likely to advance gene therapy with STAT3 nanoparticles one step closer to the clinic for ACDMPV as a first-in-class therapeutic. One interesting aspect of this work is that it may have application to other pulmonary vascular or lung diseases associated with reduced capillary density.

In contrast to ACDMPV where there is a congenital failure of the pulmonary capillary bed to develop fully, it is now increasingly recognized that there is a postnatal acquired decrease in pulmonary capillary density in some patients with underlying lung or pulmonary vascular disease. For example, in patients with chronic obstructive pulmonary disease, a subset of individuals with only mild to moderate lung parenchymal disease were found to have disproportionate pulmonary arterial pruning and capillary loss. This phenomenon was associated with right ventricular dilatation and poor clinical outcomes.[8] Similarly, in patients with chronic thromboembolic pulmonary hypertension, distal vascular pruning and capillary loss has been observed and this finding correlated with cardiopulmonary hemodynamics at right heart catheterization.[9] In pulmonary arterial hypertension, a similar decrease in pulmonary capillary surface area have been suggested by analyses of diffusion capacity and lung capillary volume.[10] Thus, these while the mechanisms underlying reduced capillary density in ACDMPV these other forms of pulmonary vascular disease and pulmonary hypertension differ (failure to develop vs. regression or dropout) the pulmonary arterial and capillary bed pathologic findings and the pulmonary hypertension and right ventricular end-pathophenotypes share similarities. This suggests that nanoparticle facilitated gene therapy with STAT3 to improve angiogenesis and overall lung capillary density in ACDMPV may have some utility in other pulmonary vascular diseases. Theoretically, pro-angiogenic gene therapy on the background of combustible tobacco-related lung disease could support (sub)clinical tumor formation by providing a vascular supply via neoangiogenesis; however, the short bioactivity of the nanoparticle STAT3 payload tested in the current study is likely to mitigate this risk. The bigger question is whether the ability to restore pulmonary capillary density in these pulmonary vascular diseases improves pulmonary hypertension, right ventricular function, and clinical outcomes in adults with longer term disease. Owing to the incidence of pulmonary hypertension, this hypothesis merits consideration for testing in preclinical models.

Although there were in-depth analyses of the pulmonary arteries in ACDMPV, the pulmonary veins were not explored in the current study and the consequences of STAT3 nanoparticle therapy for the pulmonary veins remains unknown. In ACDMPV, it has been suggested that the misaligned pulmonary veins are anomalous vessels that originate from the bronchial veins and serve as shunts between the pulmonary and bronchial venous systems.[5] Analyses of autopsy specimens further reveals that the pulmonary veins may also be muscularized.[6] Although perinatal gene therapy with STAT3 with short-term bioactivity (7 days) won't rectify misalignment of the pulmonary veins, it remains plausible that it may have some effect on pulmonary venous remodeling. STAT3 nanoparticles will reach the pulmonary veins via transit through the limited number of preexisting capillary beds traversing the pulmonary arterial to the pulmonary venous circulation. As the STAT3 nanoparticles are administered via intravenous injection in the neonatal mouse model, it is also possible that STAT3 could target the pulmonary veins via the systemic circulation through the anastomoses between the bronchial veins (systemic circulation) and the pulmonary veins (pulmonary circulation). The net effect of increased pulmonary venous endothelial expression of STAT3 is yet to be determined but is likely to stimulate proangiogenic signaling pathways. In the case of the misaligned pulmonary veins, it remains to be determined if this would have advantageous or deleterious effects. Though more work is required to understand the effect of nanoparticle STAT3 gene therapy on pulmonary venous remodeling in ACDMPV, the findings may have implications for other forms of pulmonary hypertension. Pulmonary venous remodeling has been observed in pulmonary arterial hypertension and chronic thromboembolic disease and is particularly relevant in patients with pulmonary hypertension attributable to left heart disease where pulmonary venous hypertrophic remodeling is a prominent feature.[11–13]

Utilizing nanoparticle facilitated gene therapy has the potential to modulate several of the molecular, structural, and functional complexities of pulmonary arterial and capillary abnormalities associated with ACDMPV that lead to pulmonary vascular dysfunction and pulmonary hypertension. The work of Sun et al demonstrates the efficacy of tailored gene therapy with nanoparticle delivery of STAT3 to the pulmonary vasculature in ACDMPV, a rare disease without pharmacotherapies.[7] This first-in-class agent for ACDMPV further highlights the promise of nanoparticle facilitated gene therapy for congenital diseases with pulmonary vascular involvement. It also emphasizes the potential of nanoparticle facilitated gene therapy to advance as a therapeutic in other heritable or acquired pulmonary vascular diseases and pulmonary hypertension associated with reduced capillary density. Once the genomic and molecular signals underlying pulmonary vascular remodeling have been discovered, precision nanoparticle facilitated gene therapy may join the armamentarium of therapeutics available to treat other forms of pulmonary vascular disease.

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