Endothelium-protective effects of exosomal KLF2-induced micro RNAs: implications for treatment of pulmonary hypertension

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Background: Pulmonary arterial hypertension (PAH) is a severe disorder characterised by progressive pulmonary vascular remodeling, leading to right heart failure. Endothelial apoptosis and inflammation contribute to the pathogenesis of the disease. Shear stress-activated transcription factor Krueppel-like factor 2 (KLF2) maintains vascular homeostasis and exosomes from KLF2-overexpressing cells have atheroprotective effects. We hypothesised that KLF2-induced exosomal microRNAs may attenuate endothelial dysfunction in pulmonary hypertension.

Methods and Results: KLF2 overexpression in human pulmonary artery endothelial cells (HPAECs) was induced by adenoviral gene transfer. Incubation of HPAECs with exosomes purified from KLF2-overexpressing cells attenuated starvation-induced apoptosis and reduced TNF-α- and hypoxia-induced activation of NFkB. The analysis of microRNA profile in exosomes isolated from control and KLF2-overexpressing HPAECs identified 86 differentially expressed miRs. Five miRs known to be reduced in plasma of PAH patients but elevated in KLF2-overexpressing cells, were transfected into HPAECs and their effects on endothelial apoptosis and NFkB activity were studied. Only miR-181a and miR-324a mimics had endothelium-protective effects in all experimental conditions and their combined actions were stronger compared with single treatments. Inhibitors of miR-181a-5p and miR324-5p reversed protective effects of KLF2 exosomes, suggesting that these two miRs mediate homeostatic effects of KLF2. The levels of miR-181a-5p and miR-324-5p were significantly reduced in blood-derived endothelial progenitor cells (BOECs) from PAH patients (n=7).

RNA sequencing followed by pathway analysis in HPAECs transfected with miR-181a-5p and miR-324-5p (single and combined treatments), revealed a number of gene targets involved in vascular remodelling, including ETS-1, Notch4, TNF-α, IL-1, MMP10 (miR-181a) and ETS1, MAPK, SOCS1, NFATC2 (miR-324). Vascular delivery of miR181a and miR324 mimics in Sugen/Hypoxia mouse model of PAH attenuated pulmonary vascular remodelling and reduced expression of miR targets, Notch4 and ETS-1 in the lung.

Conclusion: Therapeutic supplementation of KLF-2-induced miRs may help design new treatment strategies in pulmonary hypertension.