Growth differentiation factor 6 is upregulated in pulmonary hypertension due to left heart disease and promotes pulmonary arterial remodeling

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Introduction: Pulmonary hypertension due to left heart disease (PH-LHD) is the most common form of pulmonary hypertension (PH). PH-LHD increases right ventricular (RV) afterload thus causing RV remodeling and ultimately, RV failure. While PH-LHD is initially a result of passive lung congestion, it later transforms into a reactive process with extensive pulmonary arterial (PA) remodeling. PA remodeling in other forms of PH has previously been attributed to an imbalance between bone morphogenetic protein (BMP) and transforming growth factor-β (TGFβ) signaling, resulting in increased proliferation of PA residual cells and fibrosis. Growth differentiation factor (GDF) ligands act through shared BMP and TGFβ receptors; however, the specific roles of GDFs in regulating PA remodeling in PH-LHD has so far not been addressed. **Methods and results:** To understand the regulation of PA remodeling in PH-LHD, we performed bulk RNA sequencing of PA samples isolated during heart transplantation from PH-LHD patients (n=15) and corresponding healthy heart-heart donors (n=8), as well as in an experimental PH-LHD rat model (induced by surgical aortic-banding, AoB) and sham operated controls (n=4 and 5, respectively). Analysis of differentially expressed BMP-signaling pathway genes, identified by gene ontology (GO:0030509), revealed upregulated GDF6 in both PH-LHD patient and AoB rat PA samples as compared to corresponding healthy donors or sham controls. Western blotting confirmed elevated levels of GDF6 protein in PAs of PH-LHD patients and lungs of AoB rats. Histological analysis revealed GDF6 colocalization with smooth muscle actin (α-SMA) and CD31, markers of smooth muscle cells (SMC) and endothelial cells (ECs), respectively. By Western blotting, primary PA SMCs and ECs isolated from PH-LHD PAs had a 2-fold and 1.5-fold increase in GDF6 protein levels relative to corresponding control cell types. To address functional effects of GDF6 on PA cells, we treated healthy SMCs and ECs with 300 ng/mL of recombinant GDF6 protein and analyzed cell migration and proliferation by wound-healing assay and immunofluorescent detection of the cell cycle marker Ki67, respectively. Results revealed that in both cell types GDF6 promotes cell migration and proliferation. **Conclusions:** We identified increased GDF6 expression in PA SMCs and ECs of PH-LHD patients and AoB rats. GDF6 may contribute to PA remodeling by increasing proliferation and migration of both SMCs and ECs. The molecular mechanism of GDF6 action and its functional role *in vivo* deserve further indepth study.

Acknowledgements: This research was supported by the DZHK (German Centre for Cardiovascular Research), the DFG (German Research Foundation), the BMBF (German Ministry of Education and Research), and the CSC (China Scholarship Council).