2021 Pulmonary Hypertension: Beyond Vasodilators

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1. *Aerosolized SIN3a gene therapy inhibits Sugen/Hypoxia-induced PAH by restoring BMPR2 expression through FOXK2 in pulmonary artery endothelial cells*

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BACKGROUND: Pulmonary arterial hypertension (PAH) is a fatal lung disease of multifactorial etiology, with no curative treatment. Several studies have previously suggested that the loss of BMPR2 expression is associated with poor outcomes in PAH patients. Recently, our group showed that SIN3a (Switch-Independent 3) plays a critical role in the epigenetic regulation of BMPR2 expression in human pulmonary artery smooth muscle cells (hPASMC). However, the role of SIN3a in pulmonary artery endothelial cells (hPAEC) remains to be investigated. METHODS/RESULTS: We found a significant downregulation of SIN3a expression in PAEC isolated from PAH patients. In vitro, our results showed that SIN3a inhibits cell proliferation, migration and upregulates BMPR2 independently of the methylation status by upregulating the FOXK2 transcription factor. Using a loss-of-function approach using a specific shRNA against FOXK2, we found that the loss of FOXK2 expression decreased the expression of BMPR2 in hPAECs. Interestingly, we also found enrichment in FOXK2 abundance within the BMPR2 promoter by ChIP-qPCR in PAEC. In vivo, our results showed that restoring SIN3a expression using a gene transfer approach significantly decreased SuHx-induced PAH in mice. CONCLUSIONS: Altogether, our study revealed that SIN3a plays a critical role in the regulation of BMPR2 expression through FOXK2 and identifies lung-targeted SIN3a gene therapy as a promising therapeutic strategy for treating PAH.

2. *Microbiome-Derived Molecules in Attenuation of Pulmonary Hypertension-Associated Endothelial Phenotypes*

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Pulmonary hypertension (PH) is a progressive, severe disease characterized by high blood pressure in the pulmonary circulation and excessive pulmonary vascular remodeling; endothelial cells (EC) dysfunction is recognized as a precipitating event for this remodeling. Short chain fatty acids (SCFAs) present in circulating blood and produced by human microbiome (MB) have been associated with cardiovascular diseases and hypertension. However, the role of MB-derived molecules in EC and PH is still unknown. We hypothesized that sodium butyrate NaB (a bacterial SCFA) plays a protective role of ECs under PH conditions. Our results point that NaB attenuates IL1beta and hypoxia-induced HPAEC migration at 24 and 48 hrs. NaB also reversed of PH-related adhesion molecules such as VCAM-1 and PECAM-1 in pulmonary endothelial cells upon PH conditions. NaB enhanced other cell surface proteins, such as integrins, which are known to regulate adhesion molecules in the vascular endothelium. Finally, NaB reversed IL1beta-induced reduction of mRNA and protein of the scaffolding protein EBP50, a PDZ protein that we recently implicated in endohelial-to-mesenchymal transition (EndoMT) in PH. Collectively our results demonstrate that NaB may protect against EC pathophysiological phenotypes including migration and inflammatory activation under PH-related stresses. NaB may also influence EndoMT through its effects on EBP50. These findings support the potential for therapeutic benefits of NaB for PH.

3. Glycolysis-Driven Lipogenesis Promotes Proliferation of Human Pulmonary Arterial Vascular Smooth Muscle Cells in Pulmonary Arterial Hypertension

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Pulmonary arterial vascular smooth muscle cells (PAVSMC) proliferation is a key pathological compo-nent of pulmonary arterial hypertension (PAH). Lipogenesis is linked with proliferative diseases, including cancer, but its role in PAVSMC proliferation in PAH remains to be elucidated. Here we report that ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FASN), key enzymes driv-ing biosynthesis of fatty acids, are significantly upregulated in PAVSMC from PAH lungs compared to controls, which is associated with significantly higher unstimulated growth in lipid-free conditions. 5-tetradecyloxy-2-furoic acid, an allosteric ACC inhibitor, significantly decreased PAH PAVSMC prolifera-tion, suggesting that the lipogenesis is increased in human PAH PAVSMC, which is required for unstimu-lated hyper-proliferation. Immunocytochemical analysis showed that accumulation of intracellular lipids and proliferation of PAH PAVSMC in lipid-free conditions were suppressed by the treatment with 2-Deoxy-D-glucose (2DG), a non-metabolizable analog of glucose, while co-treatment with glucose metab-olite pyruvate significantly attenuated 2DG-dependent inhibition of lipid synthesis and PAH PAVSMC proliferation. In aggregate, these data indicate a novel metabolic link between glycolysis, lipogenesis and proliferation of human PAH PAVSMC, providing new potential target pathway for therapeutic inter-vention.

4. *MST1/2-BUB3/FOXO Signaling Is Responsible for Pro-survival Phenotype of Pulmonary Vascular Cells in Pulmonary Arterial Hypertension*

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Increased proliferation and survival of pulmonary vascular cells in small pulmonary arteries (PA) are key pathological features of pulmonary vascular (PV) remodeling and pulmonary arterial hypertension (PAH). The role of HIPPO growth-suppressor components mammalian Ste20-like kinases (MST)1/2 in pro-proliferative/pro-survival phenotype of PA vascular cells in PAH is unknown. We here report that MST1/2 play pro-proliferative/pro-survival role in PA vascular smooth muscle cells (PAVSMC) and PA adventitial fibroblasts (PAAF) from human PAH lungs, and are required for PAH-specific hyper-proliferation and survival. We further demonstrate that MST1/2 are overexpressed in human PAH PAAF in the IL-6/STAT3-dependent manner, leading to PSMC6-dependent decrease of FOXO3 and PAAF hyper-proliferation. In PAH PAVSMC, MST1/2 formed disease-specific interaction with BUB3 and up-regulated BUB3 in the extracellular matrix-/USP10-dependent manner, supporting Akt-mTORC1 activation, sustained cell growth, and impaired apoptosis. In mice with established SU5416/hypoxia-induced PH, smooth muscle-specific depletion of Mst1/2 reversed PV remodeling, reduced systolic RV pressure, PA pressure, and contractility. In aggregate, our data present novel non-canonical pro-proliferative/pro-survival role for MST1/2 in PAH, identify novel mechanism of MST1/2 action, and suggest MST1/2 as a therapeutic target for PAH.

5. Biomimetic Human Small Muscular Pulmonary Arteries

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In vitro arteriolar mimetic approaches are needed to improve the study of cardiovascular disease, because animal models do not accurately recapitulate the anatomy or pathophysiology of human microvessels. Here, we describe novel, highly parallel, mass biofabrication of biomimetic tubular vessel constructs composed of biocompatible silicon oxide bilayers fabricated by photolithography and thin film deposition. The scaffold was constructed from essential components of human arterioles, including pulmonary microvascular endothelial cells (HPMEC), extracellular matrix components including fibronectin and laminin, and human pulmonary artery smooth muscle cells (HPASMC). These constructs had an average diameter of 300 microns, and lengths of 1 mm to 3 mm. Cells were incorporated into these constructs in patterns that mimicked the layering and relative alignment observed in human small muscular pulmonary arteries. HPMEC in these biomimetic vessel constructs produced more nitric oxide, and demonstrated higher phosphorylation levels of eNOS as compared with equal numbers of cells grown on flat surfaces. HPASMC were aligned at tunable angles that mimicked in vivo organization using biomolecular micropatterning. Biomimetic small muscular pulmonary arteries produced in this study exhibited improved endothelial functionality together with multi-cellular layering and anatomically accurate cellular alignment, and provide a drug screening platform for pulmonary hypertension.

6. *A Potential Role for PDZ protein EBP50 in Right Ventricular Defenses to Pulmonary Hypertension-Associated Pressure Overload*

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Introduction: Pulmonary hypertension (PH) is a fatal disorder characterized by prolonged pressure overload on the right ventricle (RV) and hence its remodeling and eventual failure. Importantly, RV failure remains the leading cause of death in PH for which no therapies exist. Our preliminary studies show that the scaffolding protein EBP50 (ERM-binding phosphoprotein 50) is implicated in RV cardiomyocyte and fibroblast subcellular processes in vitro. However, whether EBP50 plays a role in RV responses to pressure overload in vivo remains unknown. Method: Wild type (WT) and EBP50 knockout (KO) mice were subjected to pulmonary artery banding (PAB), inducing pressure overload. Hemodynamics were used for RV pressure-volume functional assessment. We obtained gene expression profile (128 genes), utilizing a PCR array custom designed using in silico network analyses of the intersection of PH-related and cardiomyopathy-related gene networks. Results: PAB induced a significant increase in RV pressure (RVP) and hypertrophy in WT mice, which were further exacerbated in KO mice under PAB. RV gene expression arrays showed 7 upregulated and 36 downregulated genes in WT PAB, some of which have not been previously connected to RV. Importantly, 5 of the 7 upregulated genes were further enhanced in KO PAB, and 3 of the 36 downregulated genes were further attenuated in KO PAB. Conclusion: Our data demonstrate a novel role for EBP50 and offer mechanistic insight into RV responses to PH.

7. Longitudinal Evaluation of Pulmonary Hypertension in Patients with Ventricular Assist Devices

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Pulmonary hypertension (PH) is common in end-stage heart failure and may exhibit short-term improvement after left ventricular assist device (VAD) implantation. This study evaluated persistence of PH after VAD as destination therapy (DT) or bridge to transplant (BTT) in 111 & 163 patients, respectively. Right heart catheterization (RHC) data was collected pre-, post-intervention, and latest RHC (laRHC). Group 2 PH (PH2) was defined as mean pulmonary artery (PA) pressure (mPAP) >= 20mmHg, PA wedge pressure (PAWP)>=15mmHg, and pulmonary vascular resistance (PVR)<3 WU. In BTT and DT, significant reductions were observed post-VAD vs pre-VAD in mPAP (mmHg: 41+/-8 to 26+/-9 in BTT and 38+/-10 to 29+/-9 in DT), PAWP (mmHg: 28+/-8 to 13+/-8 in BTT and 26+/-8 to 15+/-7 in DT) and PVR (WU: 3.2+/-1.8 to 2.4+/-1.2 in BTT and 3.0+/-1.6 to 2.7+/-1.4 in DT). BTT improved post-transplant (OHT) vs post-VAD in PVR (1.7+/-0.6 WU), but no changes in mPAP (24+/-6 mmHg) or PAWP (14+/-5 mmHg). BTT improved in mPAP (20+/-5 mmHg) and PAWP (11+/-5mmHg) at laRHC. DT showed no change in mPAP from post-VAD to laRHC (29+/-9 to 27+/-9mmHg) or PAWP (15+/-7mmHg) suggestive of PH2, despite no change in PVR (2.2+/-1.4 WU). Pre-VAD RHC exhibited PH2 with mild PVR elevation (combined PH), which mostly attenuated post-VAD in both cohorts. In BTT, RHC indices normalized after OHT, but DT showed worsening mPAP and elevated PAWP long-term consistent with PH2 requiring prolonged monitoring of DT patients for PH.

8. Inhibition of the Prolyl Isomerase Pin1 Improves Endothelial Function and Attenuates Vascular Remodelling in Pulmonary Hypertension by Inhibiting TGF-β Signalling

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Pulmonary arterial hypertension (PAH) is a devastating disease, characterized by obstructive pulmonary vascular remodelling ultimately leading to right ventricular (RV) failure and death. Disturbed TGF-β/BMP signalling, endothelial cell dysfunction, increased proliferation of smooth muscle cells and fibroblasts, and inflammation contribute to this abnormal remodelling. Peptidyl prolyl isomerase Pin1 is a critical driver of proliferation and inflammation in vascular cells, but its role in the disturbed TGF-β/BMP signalling, endothelial cell dysfunction and vascular remodelling in PAH is unknown. Methods and Results: Pin1 expression is increased in cultured pulmonary microvascular endothelial cells (MVECs) and lung tissue of PAH patients. The Pin1 inhibitor, juglone significantly decreased TGF-β signalling, increased BMP signalling, normalized their hyper-proliferative, and inflammatory phenotype. Juglone treatment reversed vascular remodelling through reducing TGF-β signalling in monocrotaline + shunt-PAH rat model. Juglone treatment decreased the Fulton index, but did not affect or harm cardiac function and remodelling in rats with RV pressure load induced by pulmonary artery banding. Conclusion: Our study demonstrates that inhibition of Pin1 reversed the PAH phenotype in PAH-MVECs in vitro and in PAH rats in vivo, potentially through modulation of TGF-β/BMP signalling pathways. Selective inhibition of Pin1 could be a novel therapeutic option for the treatment of PAH.