ID: 113 THE ENDOTHELIAL PHD2/HIF-2 AXIS REGULATES PULMONARY ARTERY PRESSURE IN MICE

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Background Pulmonary hypertension (PH), a common clinical problem characterized by increased pulmonary artery (PA) pressure, is frequently triggered by hypoxia. Key mediators of cellular hypoxia responses are hypoxia-inducible factors (HIF)-1 and -2, the activity of which is regulated by prolyl-4-hydroxylase domain (PHD)

proteins, with PHD2 being the main oxygen sensor that controls HIF activity under normoxia. Although both transcription factors are expressed in the lung, little is known about their cell type-specific roles in the pathogenesis of PH.

Methods and Results Here we used a genetic approach to investigate the role of endothelial PHD2/HIF axis in the regulation of PA pressure. Endothelial cell specific HIF activation was achieved by crossing Vecadherin (Cdh5)-Cre transgenics to *Phd2* floxed mice (*ePhd2*), while the contribution of each HIF isoform was assessed by generating double mutants lacking *Phd2 and Hif-2* (*ePhd2Hif2*) or *Phd2* and *Hif-1* (*Phd2Hif1*). Right ventricular systolic pressure (RVSP) was measured via insertion of a 1.4F Mikro-tip catheter transducer into a surgically exposed right internal jugular vein. *ePhd2* mice showed activation of HIF-signaling as shown by immunoblot analysis of lung tissue for HIF-1 and HIF-2. These mice developed spontaneous PH (RVSP, *ePhd2*: 54.3±6.9 vs *Cre-*: 24.8 ±2.2 mm Hg, P=0.005), which was associated with right ventricular hypertrophy (RVH) (Fulton Index, *ePhd2*: 0.52 vs *Cre-*: 0.28, P=0.0004) and early mortality. While morphologic analysis of *ePhd2* lungs did not demonstrate plexiform or lumen-obliterating lesions, enhanced muscularization of peripheral PAs was detected in mutants compared to controls, as indicated by an increase in the number of arteries with diameters <100 μm that stained positive for αSMA (22.1±1.6 vs. 7.6±1.5 muscularized vessels/10 hpf, P<0.0001). The PH phenotype was maintained in *ePhd2Hif1* mutants but was reversed in *ePhd2Hif2* mutants. To assess the contribution of endothelial HIF-2 in hypoxia induced PH, endothelial *Hif2* single mutants or Cre-littermates were exposed to normobaric hypoxia (10% O_2) for 4 weeks. In contrast to controls, *eHif2* mutants were protected from development of PH and RVH. Bone marrow transplantation studies showed no contribution from hematopoietic HIF-2 in hypoxia induced PH. Because hypoxia regulates endothelin 1 (EDN1), a potent vasoconstrictor but also apelin (APLN), a vasodilatory peptide acting through binding to the apelin G-protein-coupled receptor (APLNR), we assessed the role of endothelial HIF-2 axis in the regulation of these molecules. Endothelial deletion of *Phd2* resulted in 6.4-fold induction of pulmonary *Edn1* mRNA (P=0.029), but not *Apln* mRNA. In contrast, *Aplnr* was downregulated by 2.5-fold in *ePhd2* mutants (P=0.037). A similar pattern of expression was detected in *ePhd2Hif1* mice, whereas simultaneous deletion of *Hif2a* and *Phd2* reversed these changes. To investigate the differences between acute and chronic hypoxia, we examined the effects of acute HIF activation on *Edn1* and *Apln*/*Aplnr* gene expression in vivo. To model acute hypoxia, we subjected WT mice to 8% O_2 for 48 hrs and maintained controls in room air. Acute hypoxia resulted in a 4.3-fold and 1.6-fold up-regulation of *Edn1* and *Apln* transcripts respectively (P=0.0011 for *Edn1*, P=0.08 for *Apln*) while *Aplnr* was reduced by 4.3-fold (P=0.0005). We observed similar gene expression changes in mice treated with a prolyl-4-hydroxylase inhibitor (PHI) that results in global HIF activation.

Conclusions Our studies identify endothelial HIF-2 as a key transcription factor in the pathogenesis of PH and suggest that HIF-2 regulates PA pressure by modulating the expression of vasoactive molecules. Our findings identify the PHD2/HIF2 axis as a potential target for PH therapies.