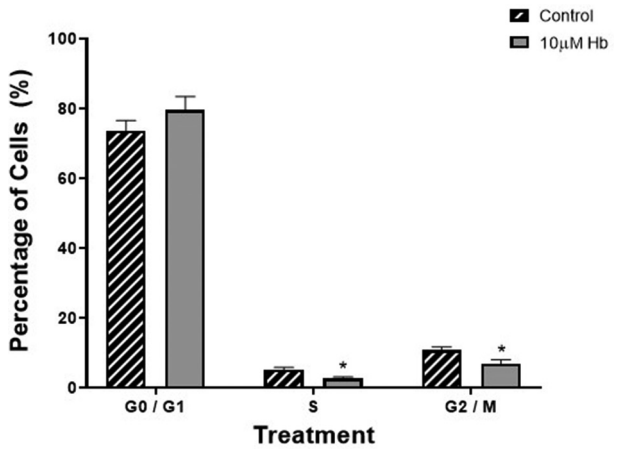

Abstract S97 Figure 1 Change in cell proliferation

Results Total intracellular and mitochondrial ROS production in HPAECs increased (~3-fold) following Hb challenge compared to control. Additionally, Nox2 mRNA expression was greater in hPAECs treated with Hb for durations of 1 or 2 hours compared to control. Hb-treated hPAECs displayed a significant decrease ($*p<0.05$) in the percentage of cells in S and G2/M phases compared to control (see figure). In contrast, the BrdU results indicated a significant increase (~1.8 fold) in proliferation in response to Hb treatment ($**p<0.005$).

Conclusions These findings suggest that hPAECs exposed to Hb undergo an increase in intracellular and mitochondrial ROS production, which is also associated with an upregulation in Nox2 gene expression. Results from the cell cycle and BrdU assays suggest contrasting proliferative responses to Hb exposure, but warrant further investigation into possible changes in apoptotic or cell repair processes. Further studies are warranted to investigate the role of these processes in the PAH disease setting.

S98 THE EFFECTS OF BMPRII LOSS ON ENDOTHELIAL SHEAR ADAPTATION IN THE PULMONARY VASCULAR ENDOTHELIUM

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Introduction Abnormal endothelial morphological adaptation to shear stress is a feature of pulmonary arterial hypertension (PAH) but the mechanisms responsible are poorly understood. In this study, we explored whether BMPRII loss mediates abnormal human pulmonary artery endothelial cell (HPAEC) adaptation to laminar shear stress and investigate gene expression of shear-sensitive Rho GTPases (RhoA, Rac1 and CDC42), known for their involvement in cytoskeletal reorganisation.

Methods HPAECs were transfected with BMPRII siRNA (siBMPRII) or control siRNA (siControl). Laminar shear stress acting on HPAECs was modelled using a parallel-plate fluid flow chamber (ibdi). siControl and siBMPRII-transfected HPAECs were exposed to unidirectional shear stress (15 dyn/cm^2) for 72 hours. Phase-contrast and confocal microscopy were used to assess cell morphology and orientation. Gene expression of RhoA, Rac1 and CDC42 were quantified using qPCR.

Results siControl-transfected HPAECs subjected to shear stress significantly elongated (length-to-width ratio 1.90 ±0.227 versus 4.12±0.133, $p<0.001$) and aligned within the direction of flow (31.7±4.82% versus 62.9±5.83%, $p<0.05$) compared with static siControl cultures, whereas that of BMPRII-silenced HPAECs exposed to flow failed to significantly elongate (1.79±0.173 versus 2.45±0.136) and align (29.6±1.97% versus 42.1±5.49%), relative to static siBMPRII HPAECs. Shear stress significantly induced the upregulation of RhoA and not Rac1 and CDC42 in siControl-transfected HPAECs, while siBMPRII-treated HPAECs subjected to flow did not exhibit significant increases in RhoA, Rac1 and CDC42 mRNA, in comparison with static counterparts, respectively.

Conclusion Inactivating mutations in the BMPRII gene may contribute to PAH by engendering abnormal pulmonary artery endothelial shear adaptation.

S99 HEPCIDIN DOWN REGULATES BMPRII IN PULMONARY ARTERY ENDOTHELIAL CELLS MIMICKING PULMONARY ARTERY HYPERTENSION PHENOTYPES

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Introduction Pulmonary arterial hypertension (PAH) is characterised by vascular remodelling of pulmonary arterioles. Disrupted iron homeostasis linked to elevated hepcidin levels has been observed in PAH patients, and disruption of the hepcidin/ferroportin axis at the level of the pulmonary vasculature cells has been shown to contribute to proliferation of pulmonary artery smooth muscle cells. A role for Pulmonary artery endothelial cells (PAECs) linked to hepcidin has not been investigated.

Objectives In this study we explored the influences of hepcidin-25 on PAEC gene expression targeting BMPRII, known to be dysfunctional in PAH.

Methods Cells were challenged with Hepcidin-25 (1 μg/mL) or for comparison IL-6 (10 ng/mL). Transcriptional regulation was analysed by RT-PCR, protein expression by immunocytochemistry.

Results Novel findings demonstrate that BMPRII mRNA expression is significantly down regulated in PAECs challenged with hepcidin-25 over a time course from 1 hour to 5 hours; figure 1 illustrates findings at 3 hours. IL-6 challenge was not able to replicate this response over the same time frame. In addition, Western blot analysis of cell lysates (n=2) showed an obvious loss of BMPRII protein expression in Hepcidin-25 challenged cells when compared to control and IL-6 challenged cell lysates.

Conclusion This is the first report linking hepcidin-25 activity to potentially dysfunctional BMPRII responses in PAECs. Given the established role of hepcidin as regulator of cellular iron levels, a role for downstream signaling linked to iron accumulation in PAECs may offer a plausible mechanism for these observations and warrants further investigation. These