Pulmonary arterial hypertension (PAH) is a disease characterized by progressive loss and remodeling of the pulmonary arteries resulting in right heart failure and death. The majority of research has focused on endothelial dysfunction in the pulmonary circulation without much attention to whether similar pathology may be found in the rest of the circulatory system. However, there is growing evidence that PAH patients also exhibit systemic endothelial dysfunction as evidenced by impaired brachial artery flow-mediated dilation, abnormal cerebral blood flow and intrinsic kidney disease. Besides abnormalities in vascular function and metabolic processes, patients with systemic sclerosis and PAH have been reported to exhibit distinctive morphological changes in kidney, skin, nailfold capillaries and sublingual vessels. The eye is a highly vascularized organ that is sensitive to systemic changes in oxygen and blood flow. Interestingly, abnormally dilated episcleral vessels were found not only in PAH patients but also in unaffected carriers before the development of the disease. A more recent study by Chyou and colleagues using the Multi-Ethnic Study of Atherosclerosis database demonstrated that a higher right ventricular mass and volume by MRI correlated with a wider diameter in retinal vessels in women compared to men independent of body size, diabetes mellitus, cholesterol, age and alcohol use. While this study did not involve PAH patients, it does allow speculation of a physiological communication between the retina and cardiopulmonary system that should be further explored. We sought to characterize the genetic and functional properties of human retinal endothelial cells (REC) along with anatomic characterization of healthy and PAH retinal vessels in human and a hypoxia mouse model of pulmonary hypertension (PH).

Loss of function mutations in BMPR2 are the most common genetic cause of PAH and are associated with loss of endothelial viability and reduced angiogenesis. Interestingly, the presence of abnormal episcleral vessels has been documented in a family of BMPR2 mutations carriers, raising the possibility that BMPR2 loss could also affect the endothelial function of ocular vessels. To test this, we used siRNA to knockdown BMPR2 in healthy RECs and compared their capacity to form tube like structures in a Matrigel assay against pulmonary microvascular (PMV) ECs transfected in a similar fashion. We found that both siBMPR2 RECs and PMVECs displayed significantly reduced tube formation compared to cells transfected with

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a nontargeting siRNA Control (siCtrl, Fig. 1a). As a next step, we sought to identify the gene expression profile of siBMPR2 RECs and compare against that of healthy RECs and siBMPR2 PMVECs using the Angiogenesis RT² Profile™ platform (cat #PAHS-024Z, Qiagen). Interestingly, angiogenesis cytokine profiles were more similar between RECs siBMPR2 and PMVECs siBMPR2 as evidenced by a close clustering in the ClusterGram heatmap.

Fig. 1. BMPR2 knockdown is associated with reduced tube formation in both RECs and PMVECs. (a) Matrigel tube formation assay of PMVECs and RECs transfected with either control (siCtrl) or BMPR2 specific siRNA (siBMPR2). **P < 0.001, unpaired t-test. (b) Retina vasculature of normoxia (left) and hypoxic (right) male C57BL6J mice. Retinal vessel area and branch points were measured with AngioTools. *P < 0.01, unpaired t-test. N = 4 mice per group. (c) Conventional funduscopic examination and AOSLO of healthy individual and two PAH patients. Notice tortuosity of vessels in PAH patient, highlighted in greater detail using AOSLO.
may alter immune regulation and maturation of vessels in the retina, which could affect the pro-angiogenic repair response to injury. At this time, we must stress that these results require further functional validation before we can conclude that they indeed represent similarities in gene expression and disease mechanisms between the retina and lung circulation.

Next, we shifted our attention to the retina of the mouse, a well-established model for the study of angiogenesis. While there has been much written on the effect of hypoxia on the adult mouse retina, comparatively little information is available concerning the impact of hypoxia on the retina. We exposed mice to 21 days of hypoxia followed by hemodynamic assessment and harvesting of the eyeballs for staining. Quantification of the retina vascular network demonstrated a significant increase in vessel area and branching with hypoxia which directly correlated with RVSP (Fig. 1b). Taken together, these studies suggest that hypoxia provides a strong angiogenic stimulus to the retina circulation which appears to be opposed to the vessel reduction seen in the lungs with chronic hypoxia.

As a final step, we sought to look at the retina of patients with PAH for evidence of structural changes in the retinal vasculature. Given the limitations of conventional ophthalmoscopy, we decided to apply adaptive optics scanning light ophthalmoscopy (AOSLO, both in reflectance confocal and multiple scattering modalities), a non-invasive imaging technique that helps with the identification of early structural and functional anomalies in retinal microvessels. As a proof of concept, we performed AOSLO on two female patients with Group 1 PH: the first was a 32 y/o NYHA functional class 2 IPAH patient being treated with sildenafil, macitentan and inhaled treprostinil; the second was a 41 y/o patient with NYHA functional class 3 Eisenmenger syndrome secondary to uncorrected ASD being treated with sildenafil, bosentan and subcutaneous treprostinil. In both patients, we found evidence of tortuosity in a large number of imaged vessels (Fig. 1c). Interestingly, these findings are similar to those described in patients with systemic hypertension and diabetes although none of the patients suffered from these disorders or had any other cardiac risk factors. Finally, it is important to note that we did not screen the patients for PAH associated mutations, as this is not routinely done in our clinic due to lack of insurance coverage.

Taken together, our findings provide exciting evidence that the retina circulation could be affected in PAH and suggest the existence of a lung-eye axis. These findings have potential far-reaching implications to the pathobiology and clinical evolution of PAH since there are no easily accessible imaging tools available to functionally or anatomically assess the pulmonary microcirculation or stage the degree of vascular remodeling in our patients. Thus, it is tantalizing to speculate that assessment of the retina circulation would allow serve as a surrogate for assessment of vascular changes in the lungs that is not captured by the

| Table 1. Gene expression profile of siCtr and siBMP2 RECs and PMVECs using RT² Profiler™ Array. |
|-----------------------------------------------|
| Gene symbol | siBMP2 vs. siCtr RECs fold regulation | siBMP2 RECs vs. siBMP2 PMVECs regulation |
|-----------------------------------------------|
| CXCL10 | 8.18 | |
| ADGRB1 | 4.14 | |
| HGF | 3.64 | |
| CCL11 | 3.33 | |
| IFNA1 | 3.28 | |
| PLG | 7.34 | |
| PROK2 | 6.18 | |
| PF4 | 3.12 | |
| ANGPT1 | 41.19 | |
| IFNA | 18.56 | |
| PTGS1 | 12.98 | |
| CCL2 | 10.04 | |
| CCL11 | 6.7 | |
| PROK2 | 5.81 | |
| COL4A3 | 5.78 | |
| ADGRB1 | 5.41 | |
| MMP2 | 5.17 | |
| TIMP3 | 353.12 | |
| TGFA | 14.75 | |
| TNF | 13.25 | |
| CXCL10 | 6.2 | |
| IL1B | 5.96 | |
| TGFβ2 | 4.88 | |
| TYMP | 4.76 | |
| CXCL6 | 3.17 | |

(Supplement Fig. 1). This raises the possibility that RECs in patients with BMP2 mutation may share similar angiogenesis gene profiles with PMVECs.

Table 1 demonstrates genes that were >3-fold differentially regulated in siBMP2 versus siCtrl RECs. Interestingly, the gene with the highest upregulation (8.18-fold) in siBMP2 RECs was CXCL10, which codes for an angiostatic and pro-inflammatory cytokine. On the other hand, BMP2 knockdown led to >6-fold downregulation in two proangiogenic genes: PLG (plasminogen, −7.34) and PROK2 (prokineticin, −6.18-fold). Next, we compared the expression profile of siBMP2 RECs against siBMP2 PMVECs. Compared to siBMP2 PMVECs, there were marked increases in angiogenic and immunelated genes such as ANGPT1 (angiopoietin 1, 41.19-fold) and INFA (Interferon A, 18.56-fold), whereas TIMP3 (−353.12-fold), TGFA (−14.75-fold) and TNF (−13.25-fold) exhibit the largest downregulation. These results suggest a model in which loss of BMP2 in RECs
current standard diagnostic workup. Future studies are required to determine whether assessing the retinal micro-circulation could serve as a biomarker and help monitor treatment response in patients with PAH.

Authors’ contributions
All authors made substantial contributions to conception, design and drafting of the manuscript. All authors approved the final version of the manuscript.

Conflict of interest
The author(s) declare that there is no conflict of interest.

Ethical approval
This work is approved by Stanford University, IRB #38931.

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References
1. Riccieli V, Vasile M, Iannace N, et al. Systemic sclerosis patients with and without pulmonary arterial hypertension: a nailfold capillaroscopy study. *Rheumatology (Oxford)* 2013; 52: 1525–1528.
2. Chyou AC, Klein BEK, Klein R, et al. Retinal vascular changes and right ventricular structure and function: the MESA-right ventricle and MESA-eye studies. *Pulm Circ* 2019; 9: 2045894018819781.
3. Southgate L, Machado RD, Graf S, et al. Molecular genetic framework underlying pulmonary arterial hypertension. *Nat Rev Cardiol* 2020; 17: 85–95.
4. Watanabe M, Makino S and Obata H. Bilaterally dilated episcleral vessels in patients with heritable pulmonary arterial hypertension. *J Gen Fam Med* 2017; 18: 464–465.
5. Angiolillo AL, Sgadari C, Taub DD, et al. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. *J Exp Med* 1995; 182: 155–162.
6. Comar M, Zanotta N, Zanconati F, et al. Chemokines involved in the early inflammatory response and in tumoral activity in asbestos-exposed workers from an Italian coastal area with territorial clusters of pleural malignant mesothelioma. *Lung Cancer* 2016; 94: 61–67.
7. Kurebayashi H, Goh T, Shimada M, et al. Prokineticin 2 (PROK2) is an important factor for angiogenesis in colorectal cancer. *Oncotarget* 2015; 6: 26242–26251.
8. Oh CW, Hoover-Plow J and Plow EF. The role of plasminogen in angiogenesis in vivo. *J Thromb Haemost* 2003; 1: 1683–1687.
9. Cantaert T, Baeten D, Tak PP, et al. Type I IFN and TNFalpha cross-regulation in immune-mediated inflammatory disease: basic concepts and clinical relevance. *Arthritis Res Ther* 2010; 12: 219.
10. Chen YY, Brown NJ, Jones R, et al. A peptide derived from TIMP-3 inhibits multiple angiogenic growth factor receptors and tumour growth and inflammatory arthritis in mice. *Angiogenesis* 2014; 17: 207–219.
11. Stahl A, Connor KM, Sapieha P, et al. The mouse retina as an angiogenesis model. *Invest Ophthalmol Vis Sci* 2010; 51: 2813–2826.
12. Burns SA, Elsner AE, Chui TY, et al. In vivo adaptive optics microvascular imaging in diabetic patients without clinically severe diabetic retinopathy. *Biomed Opt Express* 2014; 5: 961–974.
13. Chui TY, Pinhas A, Gan A, et al. Longitudinal imaging of microvascular remodelling in proliferative diabetic retinopathy using adaptive optics scanning light ophthalmoscopy. *Ophthalmic Physiol Opt* 2016; 36: 290–302.