Differential Cell-Matrix Responses in Hypoxia-Stimulated Aortic Vs. Mitral Valves

Matthew C. Sapp[a], Varun K. Krishnamurthy[a], Daniel S. Puperi[a], Saheba Bhatnagar[a], Gabrielle Fatora[a], Neelesh Mutyala[a], K. Jane Grande-Allen[a,1]

[1]Address for correspondence:
K. Jane Grande-Allen, Ph.D.
Rice University
Dept of Bioengineering, MS 142
6100 Main St.
Houston, TX 77005
Phone: 713-348-3704
Fax: 713-348-5877
Email: grande@rice.edu

Journal of the Royal Society Interface

Appendix A: Preservation of endothelium in hypoxic stimulated valves

The valve endothelium functions to protect the leaflet from platelet adhesion and helps to regulate quiescence in valvular interstitial cells (VICs). The endothelium consists of valvular endothelial cells (VECs) and covers the entire surface of the valve leaflet. Antibodies directed against CD31 (Abcam, 1:25), a protein commonly expressed on endothelial cells, were used to identify VECs in radial sections of aortic and mitral valve leaflets. Fresh tissue leaflets were fixed after harvest and acted as a no-culture control. Hypoxic simulated leaflets (20% oxygen or 13% oxygen) were cultured for 2 weeks and then fixed and stained. CD31 staining identified the endothelium of fresh and cultured valve leaflets as shown in figure S1.

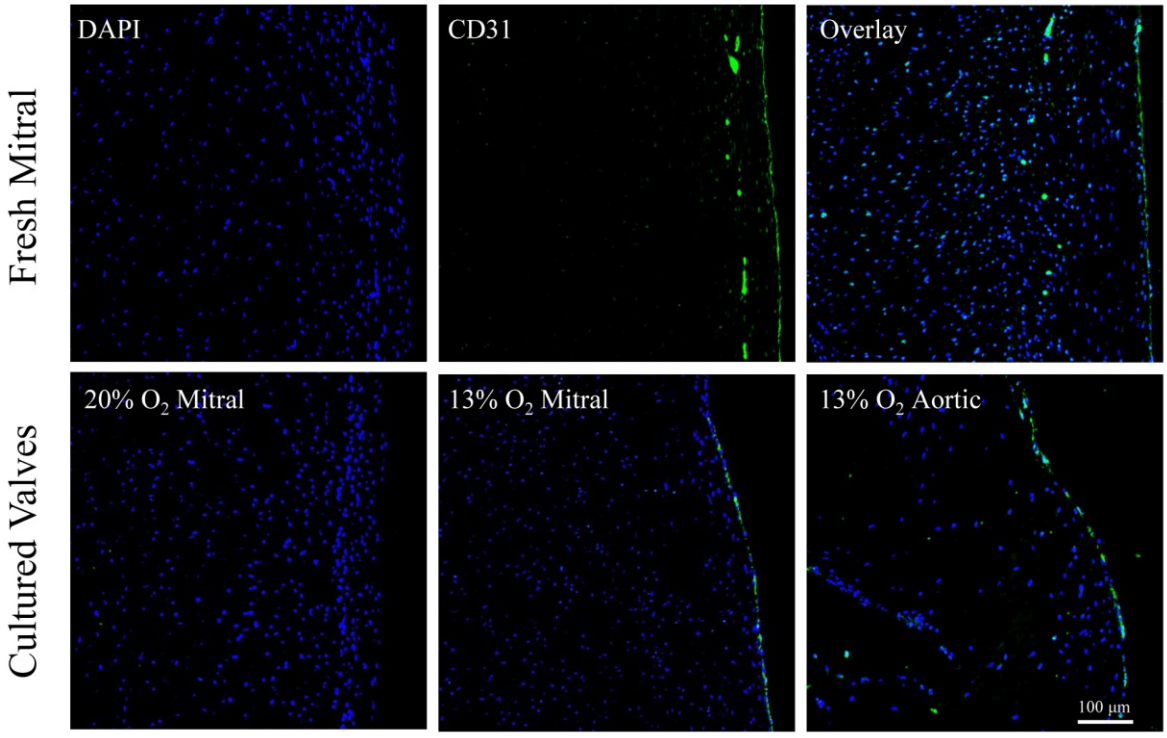

Figure S1: CD31 staining of the valve leaflet endothelium. DAPI stained all cell nuclei blue. The endothelium was intact in mitral fresh tissue controls. In all hypoxic stimulated aortic and mitral leaflets, the endothelium was partially missing or completely absent.

Appendix B: Colocalization of HIF-1α and angiogenesis-associated factors

Expression of hypoxia inducible factor-1 alpha (HIF-1α) often indicates the presence of hypoxia and is a known promotor of angiogenesis. Hypoxic stimulated (13% oxygen) mitral valve anterior leaflets were stained with antibodies directed against HIF-1α (Abcam, 1:50), chondromodulin-1 (Chm-1) (Abcam, 1:50), and vascular endothelial growth factor receptor 2 (VEGFR2) (Abcam, 1:50). Sections co-stained with HIF-1α and Chm-1 are given in figure S2*a*, and colocalization of HIF-1α and VEGFR2 is shown in figure S2*b*.

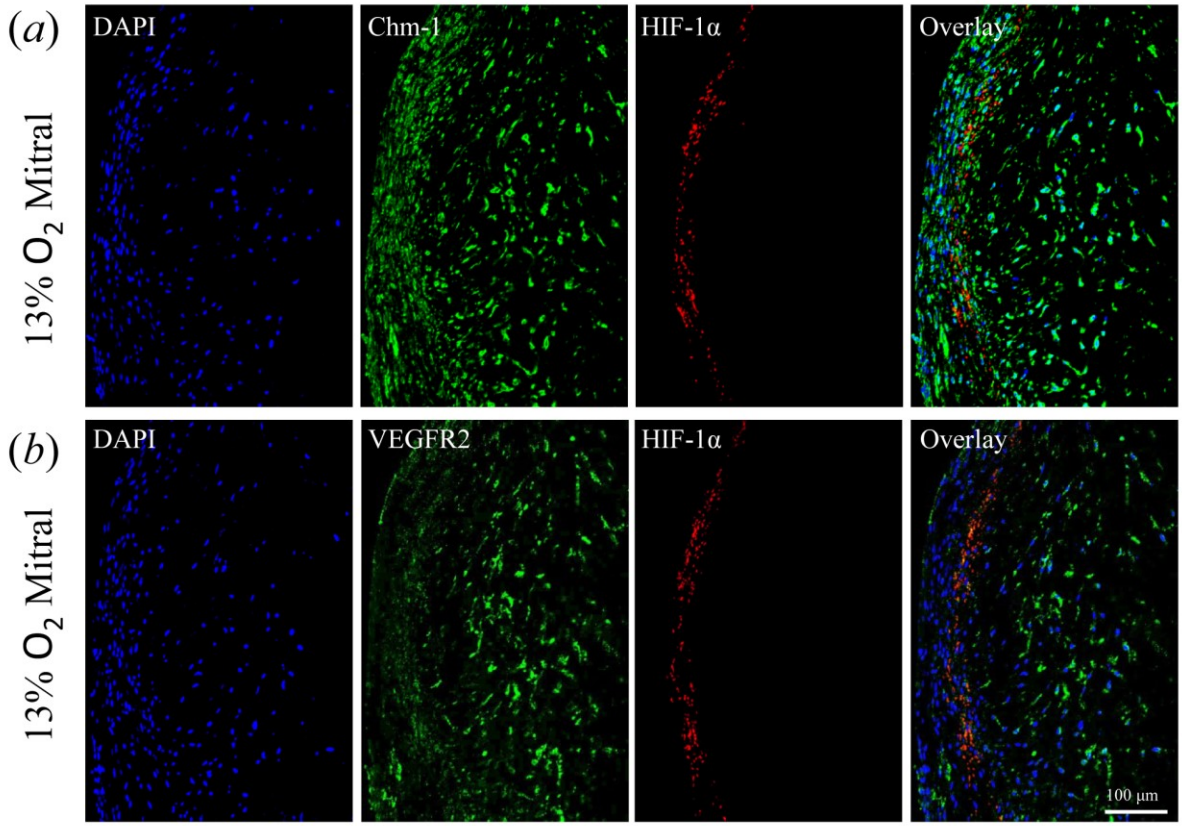

Figure S2: Association of pro- and anti-angiogenic factors with HIF-1α expression. (*a*) Chm-1 expression rarely co-localized with HIF-1α yet Chm-1 was strongly expressed in VICs near HIF-1α positive VICs. (*b*) VEGFR2 showed moderate co-localization with HIF-1α but was more strongly expressed near the center of the leaflets and in intact valve endothelium.