PROGRESS REPORT

PI: Volker H. Haase

Title of Project: Identification of novel oxygen-dependent interstitial molecular markers of renal disease progression

SIGNIFICANCE

The development and progression of chronic kidney disease (CKD) and diabetic nephropathy (DN) is frequently associated with altered oxygen metabolism secondary to mitochondrial dysfunction in epithelial cells. This pilot grant application investigated the hypothesis whether such changes in oxygen metabolism also activate oxygen-regulated signaling pathways in neighboring microvascular cells. The overall aim of this pilot and feasibility grant application was to address this hypothesis and to provide novel insights into the regulation of microvascular plasticity.

A key mediator of cellular hypoxia responses is the prolyl-4-hydroxylase domain (PHD)/hypoxia-inducible factor (HIF) oxygen-sensing pathway, which enables cells to survive hypoxic environments. PHD proteins are Fe(II)- and 2-oxoglutarate (2OG)-dependent dioxygenases that function as oxygen sensors in this pathway and control the activity of HIF-1 and HIF-2, pleiotropic heterodimeric transcription factors that regulate a multitude of biological processes including erythropoiesis and iron metabolism, anaerobic glucose metabolism, angiogenesis, cell growth and proliferation.

Data from our and other laboratories have established that endothelial and perivascular HIF-2 mediates key aspects of microvascular HIF responses in the kidney, including erythropoietin synthesis (*Kobayashi et al.,* JCI, 2016; **126:** 1926-1938; *Kapitsinou et al.*, JCI, 2014; **124:** 2396-2409.). However, little is known about the role of endothelial or perivascular HIF homologs in the pathogenesis and progression of CKD, and to what degree these transcription factors are involved in the regulation of microvascular plasticity.

ACCOMPLISHMENTS

The goal of this pilot and feasibility grant was to generate preliminary data on a) the role of vascular HIF-2 signaling in the development of CKD and DN and b) to characterize HIF-2-dependent molecular, i.e. transcriptomic responses, in the renal microvasculature.

Under aim 1 we initially proposed to study the effects of streptozotocin (STZ) induced diabetes mellitus in mice with endothelial cell-specific inactivation of HIF-2 or PHD2 taking advantage of a constitutively expressed *VeCadherin* (*Cdh5*)-*Cre* transgenic line. PHD2 is the main regulator of HIF signaling in most cells, including endothelial and pericytes and perivascular fibroblast-like cells. However, we found that *Cdh5-Phd2*$^{-/-}$ mice developed severe pulmonary hypertension (*Kapitsinou et al.,* MCB, 2016; Figure 1). We therefore decided to utilize a tamoxifen-inducible *Cdh5-Cre* transgene to target the endothelial PHD/HIF axis in adults. These studies are currently still ongoing.

Aim 2 aimed at characterizing HIF-dependent responses in the renal microvasculature under renal injury conditions. The ultimate goal of work carried out under this aim was to a) identify and characterize subpopulations or renal microvascular cells based on molecular mRNA signatures and b) to examine the molecular changes in these subpopulations that associate with renal injury taking advantage of RNA sequencing technology. We have now successfully established single cell sequencing protocols in our laboratory and have begun to characterize the hypoxic signatures in single microvascular cells (Figure 2). This work is still ongoing. Preliminary data will be used to apply for federal grant support.

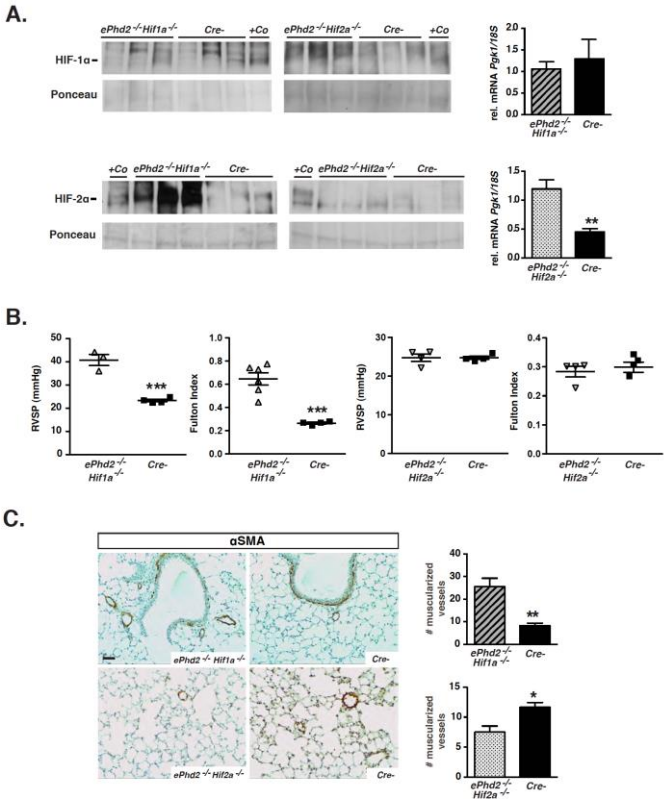

Figure 1. Pulmonary hypertension in endothelial cell-specific *ePhd2*$^{-/-}$ mice is dependent on HIF-2. **(A)** Shown are HIF-1α (top) and HIF-2α protein levels (bottom) as detected by immunoblot in nuclear pulmonary extracts from *ePhd2*$^{-/-}$*Hif1a*$^{-/-}$ mice, *ePhd2*$^{-/-}$ *Hif2a*$^{-/-}$ and their *Cre-* littermate controls. Nuclear protein extracts from the kidney or liver of a PHI-treated mouse were used as positive controls (+Co). Ponceau staining was used to assess for equal protein loading. Right panels depict *Pgk1* transcript levels in lungs from *ePhd2*$^{-/-}$ *Hif1a*$^{-/-}$, (n=6) and *ePhd2*$^{-/-}$ *Hif2a*$^{-/-}$ mice (n=5). **(B)** Right ventricular systolic pressure (RVSP), Fulton index in *ePhd2*$^{-/-}$ *Hif1a*$^{-/-}$ and *ePhd2*$^{-/-}$ *Hif2a*$^{-/-}$ mice. **(C)** Representative images of lungs stained for αSMA and quantification of muscularized vessels expressed as number/10 hpf in *ePhd2*$^{-/-}$ *Hif1a*$^{-/-}$, *ePhd2*$^{-/-}$ *Hif2a*$^{-/-}$ and control mice. Bars represent mean values ± SEM; *, P<0.05; **, P<0.01; ***, P<0.001. Scale bars indicate 50 μm.

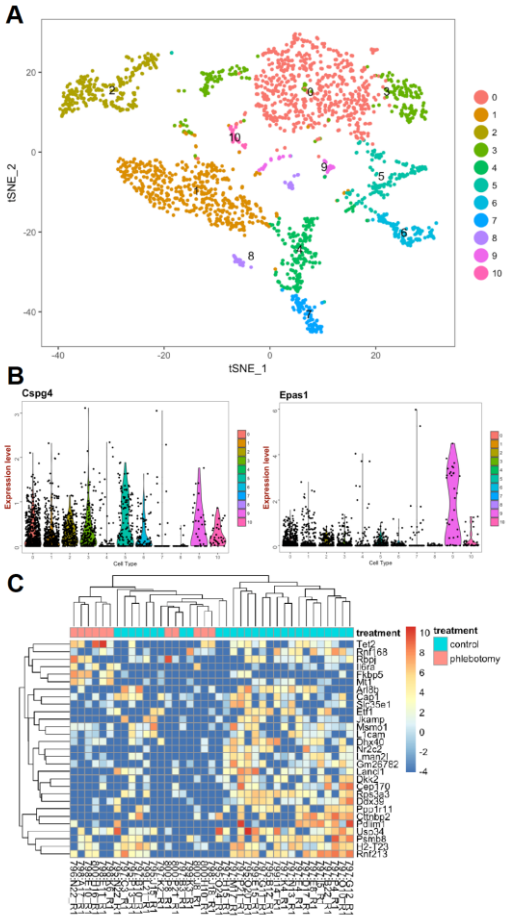

Figure 2. **Differential gene expression in hypoxic NG2^{+} perivascular cells.** Shown are the results from a preliminary single cell RNA sequencing study of FASC-sorted perivascular cells isolated from *NG2-dsRed* transgenic mice (*Zhu et al*., Development, 2008; **135:** 145-157). Using this approach we identified 10 sub-populations of cells (A), 7 of which expressed *Cspg4* (B). ~70% of all cells analyzed expressed *Cspg4*$^{+}$. Group 9 represents a small group of *Cspg4*$^{+}$ cells that expressed high levels of *Hif2a* (*Epas1*), *vimentin*, *S100a4* and *Acta2*. Panel C shows the top 30 genes with at least 1.5-fold difference in expression levels when control was compared to cells isolated from hypoxic animals. Glycolytic genes *Pfkfb3* (PFKFB3 catalyzes the production of Fru-2,6-BP) and *Aldolase b* were increased in cells from anemic animals (1.4 fold, not shown in heat map).