Prolyl hydroxylase 3 knockdown accelerates *VHL*-mutant kidney cancer growth *in vivo*

Von Hippel Lindau (VHL) inactivation, which is common in clear cell renal cell carcinoma (ccRCC), leads directly to the disruption of oxygen homoeostasis. VHL works through hypoxia-inducible factors (HIFs). Within this VHL-HIF system, prolyl hydroxylases (PHDs) are the intermediary proteins that initiate degradation of HIFs. PHD isoform 3's (PHD3) role in ccRCC growth *in vivo* is poorly understood. Using viral transduction, we knocked down expression of PHD3 in the human ccRCC cell line UMRC3. Compared with control cells transduced with scrambled vector (UMRC3-SC cells), PHD3-knockdown cells (UMRC3-PHD3KD cells) had increased cell invasion and tumor growth and were responsive to sunitinib (Tumor Growth Figure). PHD3 knockdown reduced HIF2α expression and increased phosphorylated epidermal growth factor (EGFR) expression in untreated tumor models. However, after sunitinib treatment expression of HIF2α and phosphorylated EGFR in tumor tissue did not significantly differ (Densitometry Table).   In addition, PHD3 knockdown changed the overall redox state of the cell.   Concentrations of free glutathione are significantly higher in UMRC3-PHD3KD tumors compared to UMRC3-SC tumors. UMRC3-PHD3KD cells proliferate faster when grown in the presence of 1.5 mM hydrogen peroxide compared to control cells.   Our findings illustrate 1) that PHD3's ability to affect HIF2α expression is variable, 2) reduction of PHD3 expression leads to faster tumor growth in a ccRCC animal model and 3) PHD3 plays an important role in the redox state of the cell and enhances UMRC3 cells ability to grow in a toxic microenvironment. Our results provide an improved understanding of PHD3's potential role in ccRCC progression and the possible role determining PHD3 expression in ccRCC could guide clinical therapeutic decisions.

Tumor Growth Figure: no treatment (NT), sunitinib-treated (TR), * P < 0.02, ** P < 0.001
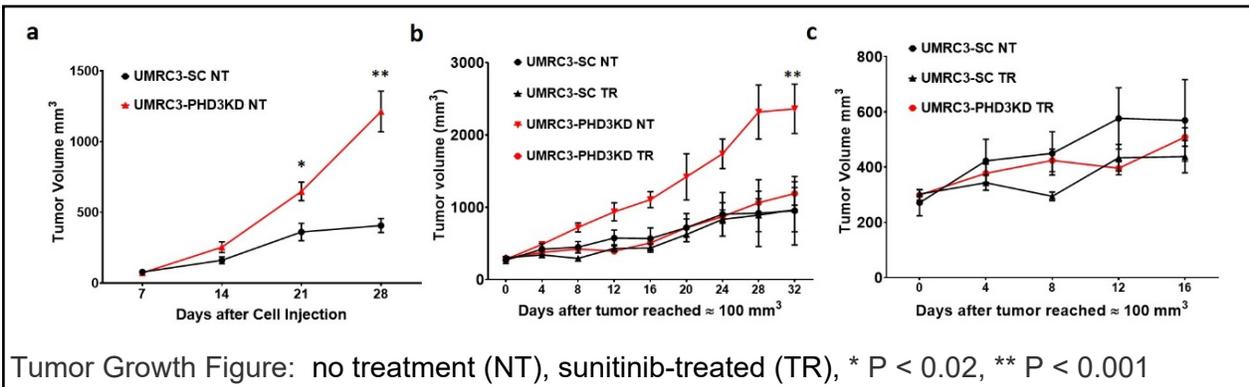
Tumor Type	PHD3/ β-actin	HIF1α/ β-actin	HIF2α/ β-actin	pEGFR/ β-actin	EGFR/ β-actin
UMRC3-PHD3 NT	0.38 $\pm$ 0.15	0.40 $\pm$ 0.11	* 0.50 $\pm$ 0.15	* 0.13 $\pm$ 0.02	5.59 $\pm$ 0.03
UMRC3-SC NT	0.59 $\pm$ 0.03	0.44 $\pm$ 0.11	* 0.95 $\pm$ 0.27	* 0.05 $\pm$ 0.01	5.32 $\pm$ 0.43
UMRC3-PHD3 TR	* 0.21 $\pm$ 0.09	0.23 $\pm$ 0.19	0.92 $\pm$ 0.18	0.21 $\pm$ 0.07	0.50 $\pm$ 0.08
UMRC3-SC TR	* 0.54 $\pm$ 0.06	0.25 $\pm$ 0.10	0.77 $\pm$ 0.42	0.23 $\pm$ 0.03	0.52 $\pm$0.07

Densitometry Table, * indicates statistically significant differences P < 0.05