Efficient hemangioma formation in virally-transduced *VHL* floxed mice is serotype-dependent

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Background: Retinal hemangiomas are often the first manifestations of VHL Syndrome, frequently present as large, highly vascular tumors characterized by giant foamy cells of unidentified origin that threaten vision due to hemorrhage of vascular protrusions and exudation. Mouse models of retinal hemangiomas have provided few insights into tumor formation. Homozygous loss of the *VHL* gene in erythroid lineages caused leaky vasculature and tumorlet formation, whereas AAV2-mediated retinal loss of VHL caused leaking vasculature with hemangioma in some retinas. To establish a more consistent model of retinal hemangiomas, we evaluated AAV2, AAV6 and AAV9-mediated deletion of VHL and the functional consequences on retinal vasculature.

Methods: Intravitreal injections of AAV2, AAV6 or AVV9 virus expressing Cre recombinase and/or a reporter gene under a general promoter were performed in wild type or mice homozygous for the floxed *VHL* allele. Retinas were monitored by indirect ophthalmic endoscopy and fluorescence angiography (FA) over the course of 8 weeks. Confocal microscopy of retinal whole mounts was used to assess expression of the reporter gene and vascular changes.

Results: Virus-mediated gene expression was observed in a variety of retinal cell types at 6- and 8-weeks post-injection for all serotypes. Abnormal vasculature assessed by FA was diagnostic for retinas transduced by AAV6-Cre compared to controls but not for retinas transduced with AAV2- or AAV9-Cre. Hemangiomas were observed in 0% of the wholemount retinas transduced with AAV9-Cre (n=4), AAV6-eGFP (n=6), or no virus (n=2) in floxed *VHL* mice or in AAV2-Cre (n=22) in wild type mice. By contrast, hemangiomas were observed in 40% of wholemount retinas transduced with AAV2-Cre (n=20) and 75% of retinas transduced with AAV6-Cre (n=20). AAV6-Cre induced hemangiomas were more numerous and predominantly located along large retinal vessels, consistent with patient presentation.

Conclusions: We previously developed a novel ocular mouse model of VHL Syndrome using an unbiased targeting of retinal cells with virally-expressed Cre recombinase to genetically excise *VHL*. The frequency of hemangioma formation was relatively low and lacked a clear *in vivo* diagnostic. Using a different AAV serotype, the efficiency of hemangioma formation was

increased from 40-75%, and a clear diagnostic phenotype by FA was identified, thereby generating a useful model for future studies. A major use of this model system will be to enable identification of key cell types in the initiation and progression of disease.