Investigating vascular-microglia interactions in a mouse model of retinal hemangioblastomas

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Background: Retinal hemangioblastomas are often the first manifestations of VHL Syndrome and have significant consequences for vision. At presentation, retinal hemangioblastomas are often large, highly vascular tumors characterized by giant foamy cells of unidentified origin, located in the superficial layers of the retina often projecting into the vitreous. Retinal hemorrhage and exudation can lead to retinal damage and detachment resulting in vision loss. We have generated a mouse model of retinal hemangioblastomas in which the effects of loss of expression of the VHL gene is limited to the eyes so as to not compromise their overall health and survival.

Methods: Intravitreal injections of AAV2 virus expressing Cre recombinase and eGFP under a general promoter were performed in mice homozygous for the floxed *VHL* allele. Nested PCR was performed to confirm Cre-mediated excision in treated eyes. Confocal microscopy of retinal whole mounts or sections was used to assess expression of eGFP, and vascular microglia changes. Retinas were monitored by indirect ophthalmic endoscopy and fluorescence angiography (FA) over the course of 8 weeks.

Results: Virus-mediated GFP expression occurred within three days. Numerous retinal phenotypes were characterized in both eyes that received Cre recombinase or control virus. Similarly, intermittent or chronic vascular leakage were observed by FA in both experimental and control groups. However, vascular changes were specific to retinas receiving Cre recombinase, including vascular tumors observed by indirect ophthalmoscopy and hemangioblastomas ranging from small incipient tumors to established large vascularized hemangioblastomas predominantly in or above the superficial vascular layer, with activated microglia in presumptive growth areas.

Conclusions: Vascular alterations, consistent with exophytic retinal hemangiomas described in VHL patients prior to formation of overt hemangiomas, are observed as an early phenotype in this novel ocular mouse model of VHL Syndrome. We hypothesize loss of *VHL* in vascular endothelial cells and/or microglia allow tumor formation characterized by hyperproliferation of vasculature and activation of microglia. This unbiased targeting of retinal cells with virally-expressed Cre recombinase to genetic excise *VHL* enables us to identify key cell types in the initiation and progression of disease. A major use of this model system will be determining and testing new strategies for hemangioma prevention and treatment.