A New in vivo Model for VHL Retinal Hemangiomas

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Retinal Hemangioblastomas in Patients

- Retinal hemangioblastomas are typically large, highly vascular and disrupt vision at presentation
- Characterized by giant foamy cells of unidentified origin, typically located in the superficial retinal layers and projecting into the vitreous
- Retinal hemorrhage and exudation can lead to vision loss
- Tumor cells but not vascular cells in the lesion show LOH*

Mouse models of Retinal Hemangiomas

Mice with the VHL gene flanked by lox P (flox) have no overt phenotype

VHL promoter and exon 1 deletion mediated by Cre recombinase

Loss of VHL using general or specific promoters has identified numerous roles in the retina or choroid

Mouse Models of Retinal Hemangiomas

Loss of VHL using specific promoters has shown:
- hemangioma formation (hematopoietic and neural promoter)
- lethality during development aniridia, microphthalmia and RPE apoptosis (melanocyte promoter)
- slow degeneration in aged retinas and delayed retinal degeneration from light damage (rod-specific promoter)
- abnormal vascular development and retinal degeneration (retinal neuronal progenitor promoter)
- increased vascular proliferation and VEGF expression (astrocyte specific promoter)
The Potential of Mouse Models

Our goal is to identify the initiating cell types:
- giant foamy cells of unidentified origin, typically located in the superficial retinal layers and projecting into the vitreous
- Tumor cells but not vascular cells in the lesion show LOH*
- hemangioma formation (hematopoietic and neural promoter)

The Players:
- Retinal neurons
- RPE
- vascular endothelial cells
- astrocytes
- microglia

Retinal Expression of eGFP

Three viral doses were tested for eGFP expression

10^10 vg

10^8 vg

Expression was observed at the 10^10 vg

Methodology

- Intravitreal injections deliver Cre recombinase to the retina
- AAV2 serotype has general retinal tropism
- General promoter directs expression “agnostically”
- eGFP to visualize and verify viral expression

Vascular Leaking - Fluorescence Angiography

Fluorescence angiography reveals leaky retinal vasculature, typically indicative of angiogenesis

Normal  Localized leak  possible anastomosis  Large leak
Fluid-filled cavities, vascular-fibrillary lesions appear in the near retinal pigment epithelium and photoreceptors

Abnormal vascularization and macrophage/microglia accumulation between the retinal pigment epithelium and photoreceptor

Abnormal vasculature is observed in the superficial layer
Multi-layered IB4-positive (vascular endothelial, red) cells
Microglial (Iba1-positive, green) surrounding blood vessels

Abnormal vasculature is observed in the superficial layer
lumen contains red blood cells
Summary of Phenotypes

<table>
<thead>
<tr>
<th>Method</th>
<th>Description of abnormality</th>
<th># of eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect Ophthalmoscope</td>
<td>Vascular/Avascular, Flat/Elevated</td>
<td>15 / 34</td>
</tr>
<tr>
<td>Fluorescence Angiography</td>
<td>Intermittent/chronic, lesion</td>
<td>5 / 17</td>
</tr>
<tr>
<td>Sd-OCT</td>
<td>Infiltration, inclusion, altered thickness, fluid-filled cavity, disorganization of layers</td>
<td>17 / 17</td>
</tr>
<tr>
<td>Histology</td>
<td>Vascularization, macrophage/microglia growths</td>
<td>4 / 11</td>
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<td>Immunofluorescence microscopy</td>
<td>Increased # of superficial blood vessels</td>
<td>3 / 6</td>
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<tr>
<td></td>
<td>Large superficial blood vessels</td>
<td>4 / 6</td>
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</tbody>
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Conclusions – A Novel in vivo Mouse Model of Retinal Hemangiomas

An “agnostic” approach to effect homozygous loss of the VHL gene

Functionally and structurally abnormal vasculature are observed

Abnormal vasculature with microglial activation occurs within 6 weeks

This mouse model of von Hippel Lindau disease can be used to identify if vascular endothelial cells and/or microglia are integral to hemangioma formation

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