The University of Utah
Department of Ophthalmology

Laser-induced CNV in Rap1b-deficient Mice

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Introduction

Choroidal neovascularization causes central vision loss in neovascular age-related macular degeneration (AMD), central serous retinopathy, degenerative myopia, and inflammatory choroiditis. An important step is the migration of choroidal endothelial cells (ECs) across the retinal pigment epithelium (RPE) into the sensory retina. Understanding the mechanisms of this step is important.

We were interested in Rap1, a member of the Ras family of small GTPases, because it is involved in the barrier integrity of endothelial and epithelial cells[1-6]. There are two isoforms. Rap1a has also been important in pathologic angiogenesis, whereas Rap1b is involved in physiologic developmental angiogenesis[7-9]. Both are activated by guanine nucleotide exchange factors (GEFs) and inactivated by GTPase activating proteins (GAPs) and appear important in barrier integrity.

We proposed that active Rap1 in RPE would increase barrier integrity and restrict CNV from the sensory retina. This may lead to a potential therapy to reduce vision loss from sensory retinal CNV.

Purpose

To determine the extent and volume of CNV induced by laser injury in Rap1b knockout mice compared to litter-matched control.

Methods

Laser-induced CNV Model

A 532nm OcuLight GL laser (0.1sec, 100um, 150mW; Iridex, CA) was used to cause injury to Bruch's membrane in adult 3 month old Rap1b knockout (provided by M. Chrsanowska-Wodnicka, Blood Research Institute, WI) or wild type (WT) littermates (C57B16). Four to six laser spots were delivered to each eye, avoiding major vessels. Rupture of Bruch's membrane was confirmed by a cavitation bubble. Volumes of CNV were measured one week after laser[10].

Retinal images: sd-OCT

Retinas were imaged using a spectral-domain optical coherence tomography unit (sd-OCT; Bioptogen, NC) prior to and 1, 2, 3 and 4 weeks following laser.

Fluorescein Angiography

Fluorescein angiograms were taken using the Micron Ⅲ (Phoenix Research Laboratories, Inc. CA) at one week following laser.

Assessment of CNV

Choroidal flat mounts were dissected and stained using isolectin B4 (GS-1B4, Alexa Flur 568, Invitrogen, CA). Confocal microscopy (Olympus, Japan) and image-analysis software (Volocity; Improvision Inc, UK) were used to obtain CNV volumes for each eye. Images were measured by two masked reviewers. Lesions with obvious hemorrhage or bridging CNV were excluded. The averaged lesions/eye for Rap1bKO and WT were analyzed using the Mann-Whitney's U- test.

Results

sd-OCT after Laser Injury

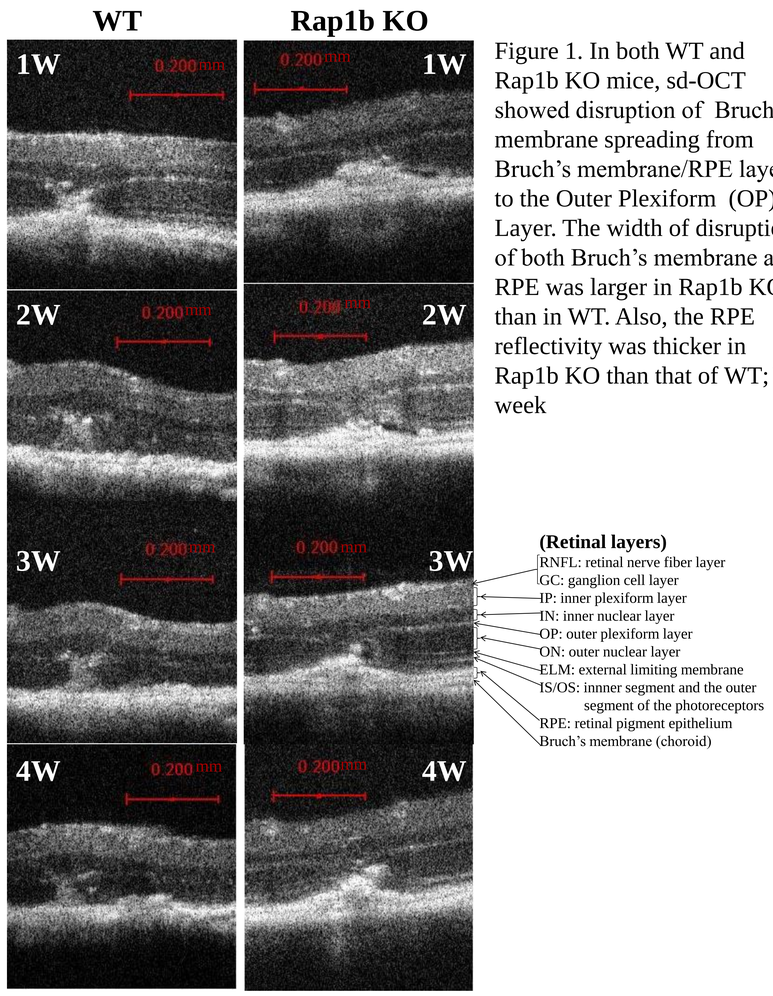

Figure 1. In both WT and Rap1b KO mice, sd-OCT showed disruption of Bruch's membrane spreading from Bruch's membrane/RPE layers to the Outer Plexiform (OP) Layer. The width of disruption of both Bruch's membrane and RPE was larger in Rap1b KO than in WT. Also, the RPE reflectivity was thicker in Rap1b KO than that of WT; W-week

Lectin-stained CNV in Choroidal Flatmounts Following Laser Injury at One Week

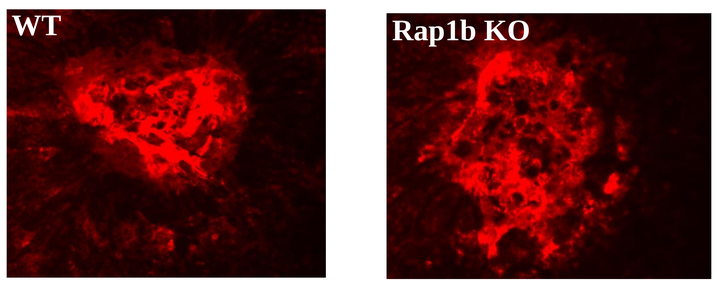

Figure 2. The maximum projection of stained choroidal flat mounts was larger in Rap1b knockout than WT mice.

Fluorescein Angiography

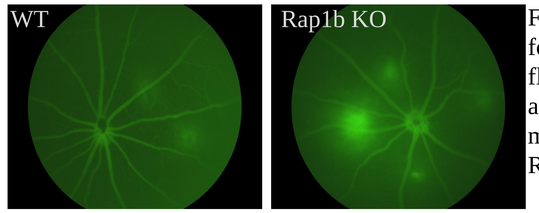

Figure 3. One week following laser, fluorescein angiography showed more leakage in Rap1b KO than WT.

Laser-induced CNV

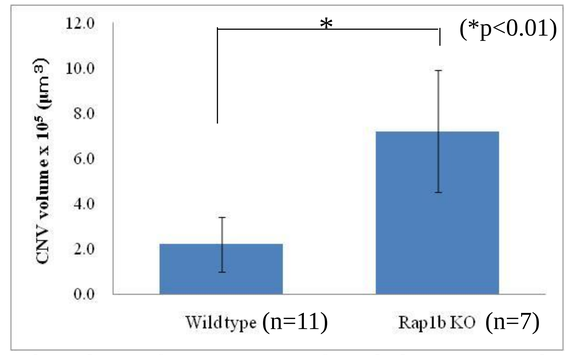

Figure 4. The volume of CNV was significantly larger in Rap1b KO mice ($7.2\pm2.7 \times 10^5$ um^3) compared to WT ($2.2\pm1.2 \times 10^5$ um^3) 1 week after laser.

Conclusion

- Laser induced CNV in Rap1b KO mice was larger than in WT mice, implying that Rap1b may be important in containing the size of CNV induced by laser injury.
- Studies are in progress to define the role of the Rap1a isoform in CNV formation and to understand the molecular mechanisms involved.

References

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