Angiographic Changes of Blood Vessels in the Photoreceptor Degenerative Mouse Retina

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Abstract

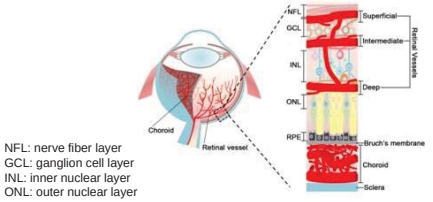
Blood vessel degeneration is critically involved in nearly all types of retinal degenerative diseases. It is commonly observed in humans and animals diagnosed with diseases caused by the degeneration of photoreceptor cells, such as retinitis pigmentosa (RP). However, the mechanisms of their formation and development are poorly understood. We used immunocytochemical, confocal imaging and computer remodeling techniques to map the angiographic changes in the photoreceptor degenerative mouse model, *pde6b* (rd1) mice. We found that mechanical instability and rewiring in the distal retina of rd1 mice could be mechanisms for the initiation and development of these tortuous vessels. We also found that in the late stages of retinal degeneration, severe tortuosity leads to ischemic attack in the distal retina. This study provides valuable insights into understanding the pathologic changes of retinal blood vessels in the development of diseases in humans and animals.

Method

FVB/N (*rd1*^{PDE6b}) and wild-type mice were obtained from the Jackson Laboratory. The mice were kept in the animal facility under a 12-h dark/light cycle. The mice used in the experiments were euthanized by intraperitoneal injection of a mixture of katamin (200mg/kg) and xylazine (10mg/kg). All procedures were performed in accordance with the National Institute of Health guidelines for the care and use of laboratory animals, approved by the Committee on Animal Research (IACUC) of Florida Atlantic University.

Freshly enucleated eyes were fixed for 20 min in a phosphate buffered saline (PBS) solution containing 4% paraformaldehyde. After removing the cornea and lens, retinal tissues were removed from the eyecups and placed in the fixative for another 15 min. The fixed retinas were washed in PBS solution, then incubated in the solution with a primary antibody, anti-CD-144, in 1:1000 dilation, for 3 days at 4°C. After three 15min washes with PBS solution, the retinas were incubated in a fluorescent secondary antibody, either Alexa 488 or Cy-3 at a concentration of 1:1000, over night at 4°C. The retinas were subsequently rinsed with PBS solution, mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and viewed with a confocal laser-scanning microscope (LMS 700, Zeiss, Munich, Germany). Images were acquired with 20x objectives, and processed with the Zeiss Microscope Software Zen.

An Illustration of the Ocular Vasculature



NFL: nerve fiber layer
GCL: ganglion cell layer
INL: inner nuclear layer
ONL: outer nuclear layer
RPE: retinal pigment epithelium

Figure 1. Left: A schematic cross-section through an eye showing the retinal vasculature lining the inner surface of the retina and the choroid vessels. Right: An enlarged cross sectional illustration of the eye showing detailed structure of the retinal and choroidal vasculature. Three layers of retinal vessels are embedded among retinal neurons: the superficial retinal vasculature lies in the NFL; the intermediate and deep retinal vascular networks align along each sides of the INL. The choroidal vessels between RPE and sclera serve to supply blood to the outer portion of the retina.

Neurovascular Changes in the *rd1* Mouse

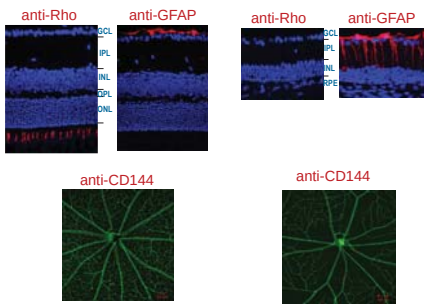


Figure 2. Loss of photoreceptors and significantly increasing of GFAP expression in the retinas of adult *rd1* mice, immune-labeled by anti-rhodopsin and anti-GFAP. Immuno-labeling of anti-CD144, a marker for vascular endothelial-cadherin (VE-cadherin), shows angiographic changes in the *rd1* retina.

Loss of Blood Vessels in the Photoreceptor Degenerated Retina

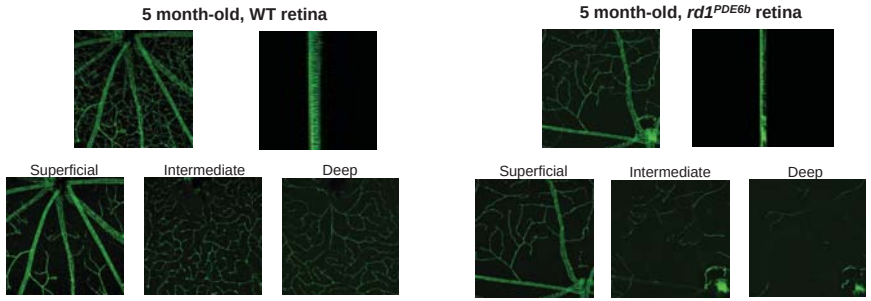


Figure 3. Immuno-labeling of vascular endothelia-cadherin by anti-CD144 in flat-mounted retinas depicted structures of blood vessels in the adult mouse retinas from 5 month-old healthy controlled and photoreceptor degenerated (*rd1*) mice. Loss of blood vessels at the intermediate and deep layers of the *rd1* retina detected by the antibody.

Results

1.Loss of photoreceptors in adult rd1 mouse retina, labeled by antibodies against rhodopsin molecules in photoreceptor outer-segments.

2.Photoreceptor degeneration accompanied by significant increasing of GFAP (Glial fibrillary acidic protein) expression in the rd1 retina.

3.Angiographic changes are observed to have occurred in the adult rd1 retinas.

4.Significant loss of blood vessels in the distal retina layers, but less affected in the inner retina layers.

Conclusion

1.The rd1^{PDE6b} mouse is a retinal degenerative disorder model in which blood vessel degeneration contributes significantly to retinal atrophy, ultimately leading to loss of vision.

2.In this study we used the antibody against endothelia-cadherin to demonstrate the angiographic changes and loss of blood vessels in the rd1 mouse retina. Our results provide further evidence supporting that the rd1 mouse retina is an accessible model that can be used for studying neurovascularization and protection in retinal degenerative diseases.

3.Given the increasing incidence of blindness in retinal degenerative diseases, there is an urgent need for strategies promoting blood vessel survival and protection in retinal degenerative diseases.

Acknowledgement

This work was supported by:

The National Science Foundation (IOS1021646, Wen Shen)
The National Eye Institute, NIH (EY14161, Wen Shen)