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Analysis of Damage and Wound Healing in the Retinal Pigmented Epithelium

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Abstract

Previous studies of retinal pigment epithelium (RPE) morphology found cell-level and spatial patterning differences in many quantitative metrics in comparing normal and disease conditions. However, most of these studies examined eyes from deceased animals. Here we sought to compare noninvasively imaged RPE cells from live mice to histopathology. We describe changes to improve noninvasive imaging of RPE in the live mouse. In retinal diseases, there can be invasion by Iba1-positive cells, which can be detected by noninvasive imaging techniques. Here we can detect potential Iba1-positive cells at the level of the RPE noninvasively.

Keywords

Best1; Retinal pigmented epithelium (RPE); Histopathology; Flat-mounting; Confetti mouse line; Transgenic mice; Cre; Noninvasive imaging; Inherited retinal diseases

70.1 Introduction

Under normal circumstances the retinal pigment epithelium (RPE), as in many other epithelial sheets throughout nature, takes on a regular pattern of hexagonally shaped cells. The RPE sheet is attached to Bruch's membrane and consists of a single-cell-thick layer between outer segments of photoreceptors and the choroid. Near the apical face of the RPE, tight junctions between adjacent cell borders constitute the major outer blood-retinal barrier.

When the RPE sheet is damaged acutely or chronically, the sheet may take on different appearances depending on the kind of damage, its extent, and the length of time since damage. In many cases, the RPE may look quite normal as the RPE sheet, like other epithelial sheets, are highly adapted to promote rapid repair of barrier functions. This robust nature of the sheet appears to be highly conserved throughout nature.

Long-term chronic diseases may result in the slow, gradual accumulation of damage to the RPE sheet. In age-related macular degeneration, RPE is thought to be the initial site of pathology. Changes to the RPE may lead to loss of barrier function compromising both overlying neural retina and underlying choroid. Thus, it would be useful to examine noninvasively early and acute damage to the RPE sheet. We have been able to detect early events by using analyses from dead tissue. These changes suggest potential mechanisms, but we need to establish correlates in the living eye compared to histopathological images. As prerequisites to studying wounding and healing of the RPE sheet, here we consider whether and how we might be able to:

1. Image the RPE cell in mouse noninvasively
2. Register noninvasive RPE images with histology and confocal flat mounts
3. Mark RPE cells to track changes in cell shape and movement over time noninvasively

Here we describe studies in mouse models to address these needs.

70.2 Methods

Transgenic mice were obtained from Jackson Labs and were on a C57BL/6J background, and PCR-based genotyping was conducted periodically. Equal numbers of male and female mice were used. All procedures were approved by the Emory IACUC and in compliance with ARVO guidelines on the use of animals. Methods and materials are fully described (Jiang et al. 2013; Boatright et al. 2015; Bhatia et al. 2016).

70.3 Results

As an example of histopathological processes in the retina (Fig. 70.1a), we stained flat-mounted neural retina with Iba1 in an early stage of retinal degeneration, the IRBP knockout mouse (Markand et al. 2016). The IRBP KO mouse is a model of RP66 and vision-independent severe early onset myopia. The image showed order in putative microglia during the early stages. The pattern of Iba1 indicated a regularity in spacing that minimized cell-cell contact (contact inhibition) but with tight packing and a countable number of microglia.

In a second example, in the RPE (Fig. 70.1b), we stained for the nuclear marker Lsd1 and the tight junction marker, ZO-1. This analysis demonstrated that about 50% of mouse RPE cells are mononucleate and 50% binucleate. The regular tiling of the RPE cells in a normal circumstance was evident.

Noninvasive imaging can be used in conjunction with transgenic mice and fluorescent markers as aids to measuring experimental outcomes. We detected the expression of a green fluorescent protein (ZsGreen1) expressed in RPE cells without the aid of excitation or emission filters and imaged with plain white light on a MicronIV (Phoenix Labs, Inc) fundus camera (Fig. 70.2).

Following procedures established by Krebs and coworkers (Krebs et al. 2016), we made use of a simple “shift and add” astronomy-based approach (Fried 1966) to increase signal to noise. This technique recovered noticeably sharper images of the fundus with white light (Fig. 70.3a prior to image stacking and Fig. 70.3b after combining 100 images). The increase in resolution allowed detection of features that were previously smudged, fuzzy, and likely to have been dismissed as imaging artifacts. Collection of 100 images took 2 seconds, indicating that this kind of image acquisition is convenient to do routinely. It was enticing that the granular size of the smallest features in the color fundus image (Fig. 70.3b) approached “cellular” dimensions.

To mark individual RPE cells, we employed “confetti” mice. In these lines of mice, a single fluorescent color per cell is expressed in a stochastic manner from a selection of four possible colors. The colors are driven with the use of Best1-Cre drivers (Le et al. 2008; Iacovelli et al. 2011), which express Cre recombinase only in the RPE. The central points (Fig. 70.4) here are (a) cell-level resolution and (b) easy detection through an intact normal mouse retina, which (c) allows us to follow the movement of single RPE cells over time. More colors can be detected by confocal microscopy (Fig. 70.5), and adjacent cells are obvious.

Light-induced retinal damage is a classical model in vision research, and we have detected primary or bystander damage to RPE as illustrated in Fig. 70.6, which showed a correspondence of Iba1-positive cells at the RPE with white dots by cSLO.

70.4 Discussion

Here we employed novel in vivo imaging of color-coded RPE cells together with image analyses and histopathological techniques to allow us to consider mechanisms during damage and repair of the RPE. These preliminary observations suggest that the RPE responds to damage or disease, restoring the BRB via definable protective mechanisms. Clever “shift and add” software (Krebs et al. 2016) markedly improved resolution of off-the-shelf imaging equipment. Additional powerful image processing techniques, including deconvolution (Hope et al. 2016; South et al. 2018), await testing.

Single RPE cells can be imaged noninvasively in readily available mouse lines expressing various fluorescent proteins selectively in RPE cells. Confetti mice worked well given sufficient time to develop fluorescent colors in RPE cells (Le et al. 2008; Iacovelli et al. 2011) which can be traced and followed over time, ranging from hours to years.

C57BL/6J mouse RPE can be damaged by sufficient levels of “white” light. Our preliminary findings suggest that young mice are more prone to RPE damage and invasion of microglia or macrophages than older mice. Damage to RPE was coincident with Iba1-positive “activated microglia” in the RPE sheet. Following light damage, we detected many white dots by BAF cSLO noninvasively, and we found many Iba1-positive immunostained cells by confocal microscopy of RPE flat mounts. Each Iba1-positive cell in the RPE corresponded one-to-one with a white dot in the cSLO image.

The types of damaged shapes of the RPE cell varied widely (data not shown) among different methods of damage induction, whether experimental, genetic, or temporal. Damage in RPE cells can persist for a long time (>60 days), but there is clear evidence for at least some recovery of the RPE sheet.

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References

- Bhatia SK, Rashid A, Chrenek MA et al. (2016) Analysis of RPE morphometry in human eyes. *Mol Vis* 22:898–916 [PubMed: 27555739]
- Boatright JH, Dalal N, Chrenek MA et al. (2015) Methodologies for analysis of patterning in the mouse RPE sheet. *Mol Vis* 21:40–60 [PubMed: 25593512]
- Fried DL (1966) Optical resolution through a randomly inhomogeneous medium for very long and very short exposures. *J Opt Soc Am* 56:1372
- Hope DA, Jefferies SM, Hart M et al. (2016) High-resolution speckle imaging through strong atmospheric turbulence. *Opt Express* 24:12116–12129 [PubMed: 27410132]
- Iacovelli J, Zhao C, Wolkow N et al. (2011) Generation of cre transgenic mice with postnatal RPE-specific ocular expression. *Invest Ophthalmol Vis Sci* 52:1378 [PubMed: 21212186]
- Jiang Y, Qi X, Chrenek MA et al. (2013) Functional principal component analysis reveals discriminating categories of retinal pigment epithelial morphology in mice. *Invest Ophthalmol Vis Sci* 54:7274–7283 [PubMed: 24114543]
- Krebs MP, Xiao M, Sheppard K et al. (2016) Bright-field imaging and optical coherence tomography of the mouse posterior eye. *Methods Mol Biol* 1438:395–415 [PubMed: 27150100]
- Le Y-Z, Zheng W, Rao P-C et al. (2008) Inducible expression of cre recombinase in the retinal pigmented epithelium. *Invest Ophthalmol Vis Sci* 49:1248–1253 [PubMed: 18326755]
- Markand S, Baskin NL, Chakraborty R et al. (2016) IRBP deficiency permits precocious ocular development and myopia. *Mol Vis* 22:1291–1308 [PubMed: 27829784]
- South FA, Liu Y-Z, Bower AJ et al. (2018) Wavefront measurement using computational adaptive optics. *J Opt Soc Am A Opt Image Sci Vis* 35:466–473 [PubMed: 29522050]

Fig. 70.1.

Destructive analyses showing patterning. **(a)** Regular patterning of Iba1-positive microglia (stained green) illustrating principles of exclusion zones and contact inhibition. Countable numbers of microglial cell bodies are easily recognized. The IRBP knockout mouse exhibits a clear initiation of gliosis at P30. **(b)** Normal mouse RPE cells are often binucleate but never more than two. *Lsd1*, a key epigenetic enzyme of eye development, was detected in all RPE nuclei in a pattern consistent with euchromatin and clearly delineated one nucleus from the next. ZO-1 tight junctions in normal RPE were stretched but not taut, as expected in the normal RPE sheet

Fig. 70.2.

Color fundus photography of a mouse expressing ZsGreen1 in RPE cells. This indicated a high level of expression and the show-through of the signal from the RPE through the neural retina, even in conventional white light

Fig. 70.3.

Color fundus photography of a normal C57BL/6J mouse. **(a)** A single image. Blood vessels can be detected. Some granularity is detected. The optic nerve is evident. Appropriate red color throughout. **(b)** Following registry-alignment of 100 images, they were added together and averaged. Much more detail is evident. Blood vessels are sharp. Granularity is recognized

Fig. 70.4.

MicronIV noninvasive imaging. Two color channels showing sharply distinct green and red structures at about the size expected for RPE cells. **(a)** Green channel (eGFP), **(b)** red channel, RFP, **(c)** green and red channels merged, showing no overlap of green and red structures, later shown to be individual RPE cells. The absence of overlap was predicted based on a single rearrangement event per RPE cell giving a checkboard appearance

Fig. 70.5.

Confocal imaging of confetti mice after selective RPE cell expression of Cre. The single allowed Cre recombinase rearrangement revealed immediately adjacent RPE cells with different colors. In (a), YFP is expressed in cytosol. In (b), RFP is expressed in plasma membrane. In (c), CFP is expressed in cytosol. In (d), the three channels are merged showing that no cell expressed more than one color, and adjacent cells expressed different colors generally

Fig. 70.6.

Following light-induced retinal damage, Iba1-positive cells (red) were detected on the apical face of the RPE sheet (**a**). White dots from blue autofluorescence were detected throughout the retina and RPE (**b**); and Iba1-positive cells aligned with some of the white dots (labeled 1–6 in each panel)