60 Pathoconnectome Analysis of Müller Cells in Early Retinal Remodeling

Rebecca L. Pfeiffer, James R. Anderson, Daniel P. Emrich, Jeebika Dahal, Crystal L. Sigulinsky, Hope A. B. Morrison, Jia-Hui Yang, Carl B. Watt, Kevin D. Rapp, Mineo Kondo, Hiroko Terasaki, Jessica C. Garcia, Robert E. Marc, and Bryan W. Jones

Abstract

Glia play important roles in neural function, including but not limited to amino acid recycling, ion homeostasis, glucose metabolism, and waste removal. During retinal degeneration and subsequent retinal remodeling, Müller cells (MCs) are the first cells to show metabolic and morphological alterations in response to stress. Metabolic alterations in MCs chaotically progress in retina undergoing photoreceptor degeneration; however, what relationship these alterations have with neuronal stress, synapse maintenance, or glia-glia interactions is currently unknown. The work described here reconstructs a MC from a pathoconnectome of early retinal remodeling retinal pathoconnectome 1 (RPC1) and explores relationships between MC structural and metabolic phenotypes in the context of neighboring neurons and glia. Here we find variations in intensity of osmication inter- and intracellularly, variation in small molecule metabolic content of MCs, as well as morphological alterations of glial endfeet. RPC1 provides a framework to analyze these relationships in early retinal remodeling through ultrastructural reconstructions of both neurons and glia. These reconstructions, informed by quantitative metabolite labeling via computational molecular phenotyping (CMP), allow us to evaluate neural-glial interactions in early retinal degeneration with unprecedented resolution and sensitivity.

Keywords

Retinal remodeling · Müller cells · Connectomics · Pathoconnectome · Ultrastructure · Early retinal degeneration

R. L. Pfeiffer (✉) · J. R. Anderson · D. P. Emrich · J. Dahal · C. L. Sigulinsky · H. A. B. Morrison · J.-H. Yang · C. B. Watt · K. D. Rapp · J. C. Garcia · B. W. Jones
Departments of Ophthalmology, Moran Eye Center, University of Utah, Salt Lake City, UT, USA
e-mail: r.pfeiffer@utah.edu

M. Kondo
Departments of Ophthalmology, Mie University Graduate School of Medicine, Tsu, Japan

H. Terasaki
Departments of Ophthalmology, Nagoya University, Graduate School of Medicine, Nagoya, Japan

R. E. Marc
Departments of Ophthalmology, Moran Eye Center, University of Utah, Salt Lake City, UT, USA

Departments of Ophthalmology, Signature Immunologics, Torrey, UT, USA

C. Bowes Rickman et al. (eds.), *Retinal Degenerative Diseases*, Advances in Experimental Medicine and Biology 1185, https://doi.org/10.1007/978-3-030-27378-1_60

60.1 Introduction

MCs provide trophic and structural support, regulate water and ion homeostasis, remove metabolic waste, play central roles in glucose metabolism, and recycle amino acid neurotransmitters in retina (Reichenbach and Bringmann 2010). Combined, these functions make MCs indispensable to retinal health as selective ablation induces rapid, progressive loss of photoreceptors, neovascularization, and structural abnormalities in retina (Shen et al. 2012).

The retinal degenerative diseases (RDD), age-related macular degeneration (AMD), and retinitis pigmentosa (RP) phenotypically present with progressive photoreceptor loss, leading to retinal remodeling and neurodegeneration. Retinal remodeling is a phased revision of retinal topology and circuitry that compromises existing retinal networks via loss of existing synaptic connectivities as well as generation of novel synapses. Morphological changes in neurons occur through neurite sprouting, with changes in receptor expression by subsets of neurons (comprehensive review of remodeling in (Jones et al. 2012)). Ultimately, remodeling progresses to neurodegeneration as a final common pathway in RDD. MCs play central roles in remodeling with structural, protein, and metabolic changes (Fariss et al. 2000; Jones et al. 2003, 2016a, b; Pfeiffer et al. 2016). Structural changes are among the earliest observed in the retina, with hypertrophy becoming evident throughout MC columns. Protein changes include increases in glial fibrillary acidic protein (GFAP) (Bignami and Dahl 1979; Erickson et al. 1987) and loss of glutamine synthetase (GS) (Reichenbach and Bringmann 2013). Metabolic changes include increased heterogeneity between MCs, particularly in taurine (τ), glutamine (Q), and glutamate (E) levels within MCs (Jones et al. 2011; Pfeiffer et al. 2016).

Our goal of this project is to describe interactions between MCs and their surrounding neighbors in early RDD, using combined serial section transmission electron microscopy (ssTEM) and CMP. At this stage, we have reconstructed a single MC from a transgenic (Tg) rabbit model of retinitis pigmentosa (P347L) and initiated reconstruction of MCs from a previously published wt rabbit connectome: RC1 (Anderson et al. 2011).

60.2 Materials and Methods

60.2.1 Tissues Used for RPC1 and RC1 Volumes

The heterozygous Tg P347L rabbit used for creating the retinal pathoconnectome 1 (RPC1) volume was generated as a model of human autosomal dominant retinitis pigmentosa (adRP) (Kondo et al. 2009). These rabbits express a mutated rhodopsin gene, caused by a C-to-T transition at proline 347. This mutation leads to trafficking defects in rhodopsin, causing rod photoreceptor degeneration. As with human adRP, cones are initially preserved but eventually degenerate following rod degeneration (Jones et al. 2011). The tissue for RPC1 was obtained from a 10 month Tg P347L rabbit, in a region where rod photoreceptors are still present, for analysis of early retinal remodeling.

Retinal connectome 1 (RC1) (Anderson et al. 2011) was taken from a light-adapted Dutch-belted rabbit, with no indications of retinal disease.

All animal experiments were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, with approval of the Institutional Animal Care and Use Committee at the University of Utah. To acquire tissues for connectome volumes, animals were tranquilized with intramuscular ketamine/xylazine, subsequently 25% (RC1) or 15% (RPC1), and intraperitoneal urethane was administered, followed by bilateral thoracotomy.

60.2.2 Preparation of RPC1 Volume

Following euthanasia, retinal tissues were harvested and fixed in mixed aldehyde solutions (1% formaldehyde, 2.5% glutaraldehyde, 3% sucrose, 1 mM $MgSO_4$ in cacodylate buffer (pH 7.4)),

then osmicated, dehydrated, resin embedded, and sectioned at 70 nm. Sections were placed on formvar grids, stained, and imaged at 2.18 nm/px on a JEOL JEM-1400 TEM using SerialEM software. One section was reserved every 30 sections for CMP, where it was placed on a slide and probed for small molecules, glutamate (E), glutamine (Q), glycine (G), GABA (γ), taurine (τ), and glutathione (J), or proteins, glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS). All sections were sequentially aligned and interleaved into the final volume of 946 serial sections from the outer plexiform layer to vitreous. Details of RC1 preparation can be found in (Anderson et al. 2011). RPC1 and RC1 were evaluated and annotated using the Viking software suite.

60.3 Results

60.3.1 RPC1 Features

RPC1 is selected near the visual streak. Although rods are still present, they demonstrate disorganization and shortening of outer segments and swollen mitochondria indicative of cell stress. Further examination of inner retina demonstrates MC hypertrophy and regional hyperosmication, both consistent with previous observations of early degenerate retina (Jones et al. 2011).

60.3.2 Three-Dimensional Reconstruction of RPC1 MC 2628

Alterations in MC morphology have been observed following stress to photoreceptors (Erickson et al. 1983; Fisher and Lewis 2003; Jones et al. 2011) revealing that MC responses to retinal stress appear universal. Mechanisms of injury, trauma, or disease are varied, but all retinal stress shows MC responses of hypertrophy (Erickson et al. 1983; Marc et al. 1998; Jones et al. 2016a, b; Pfeiffer et al. 2016). Here we describe a reconstructed MC within RPC1 characterizing its morphology and illustrate its relationship with other cells, metabolism, and structure (Fig. 60.1). MC 2628 shows stereotypical hypertrophy of MC columns in addition to extensive branching and interdigitating of its endfeet. Although branching of endfeet is common in many species' MCs, Golgi studies of MCs in rabbit show limited branching near the visual streak (Reichenbach et al. 1989).

60.3.3 RPC1 MC Metabolic Heterogeneity

RPC1 MCs demonstrate metabolic heterogeneity consistent with previously described work (Jones et al. 2011, 2016a; Pfeiffer et al. 2016). CMP reveals wide variation in τ and Q metabolites, both within the inner nuclear layer and ganglion cell layers (Fig. 60.2). While there is subtle variation in these metabolites in the MCs endfeet of normal retina, in pathological retina, these metabolite concentrations are far more variable. In addition, 3D reconstruction of a MC within RPC1 demonstrates levels of Q vary not only between cells but also in the branched endfeet.

60.3.4 RPC1 MC Osmication Variability

RDD demonstrates high variability in osmication of MC cytosol. During tissue preparation for TEM, osmium tetroxide (OsO_4) becomes bound to lipids, notably phospholipids within cell membranes, providing increased contrast. Due to OsO_4's affinity for phospholipids, structures other than cell/organelle membranes may also become osmicated. MCs in RPC1 demonstrate high levels of osmication variability, between cells, but also within individual MCs (Fig. 60.3). The lipid/phospholipid structure responsible for osmium variability in MC cytosol is currently unknown but suggests possible investigative pathways relevant to RDD. Coincidence between low osmium staining and high levels of small molecule metabolites is also unknown.

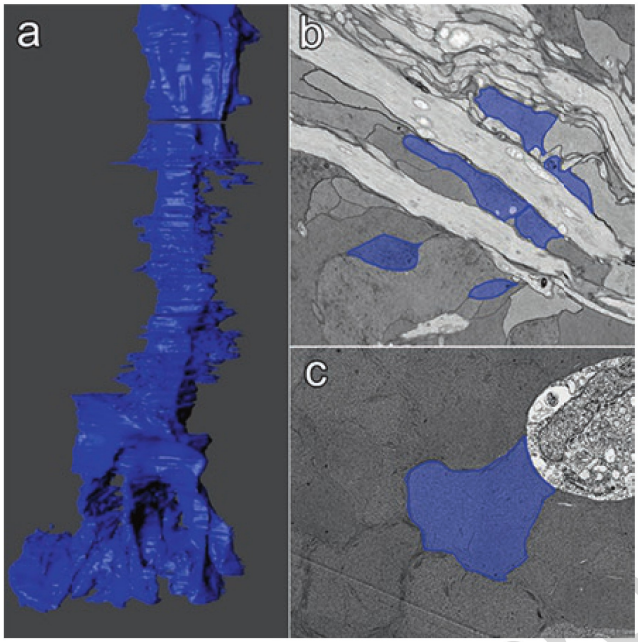

Fig. 60.1 MC structure changes in early retinal degeneration with rod photoreceptors still present. (**a**) 3D reconstruction of cell #2628 from the RPC1 volume. The top of the image is in the inner nuclear layer, while the bottom is the MC endfeet where they terminate at the vitreous. (**b**) Horizontal section through the GCL of RPC1. Areas shaded in blue are cross sections through the endfeet of 2628. (**c**) Horizontal section through GCL of RC1. Blue-shaded area demonstrates the endfoot of a single MC

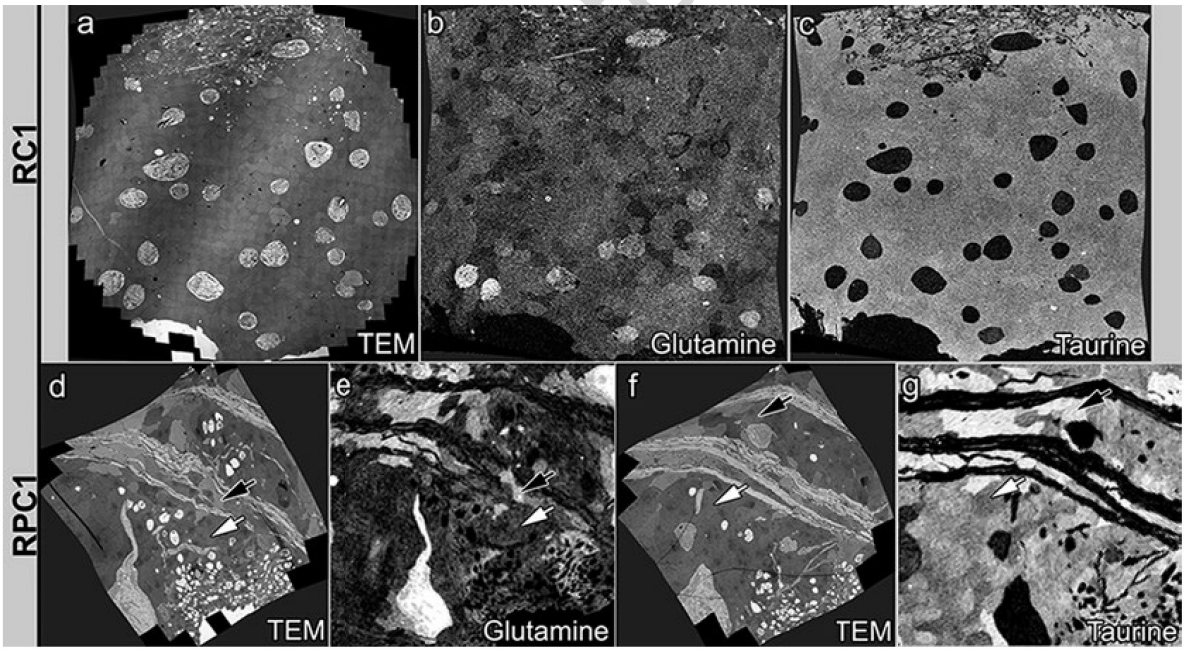

Fig. 60.2 Metabolic labeling in RC1 (top row) and RPC1 (bottom row). (**a**) TEM section through the GCL of RC1. (**b**) Q CMP overlay of 2(a). (**c**) τ CMP overlay of 2(a). (**d**) TEM section from GCL of RPC1. (**e**) Q CMP overlay of TEM section shown in 2(d). (**f**) TEM section from GCL of RPC1. (**g**) τ CMP overlay of TEM section shown in 2(f). (**e**–**g**) Black arrows indicate MCs with high levels of amino acid content. White arrows indicate MCs with lower levels of amino acid content

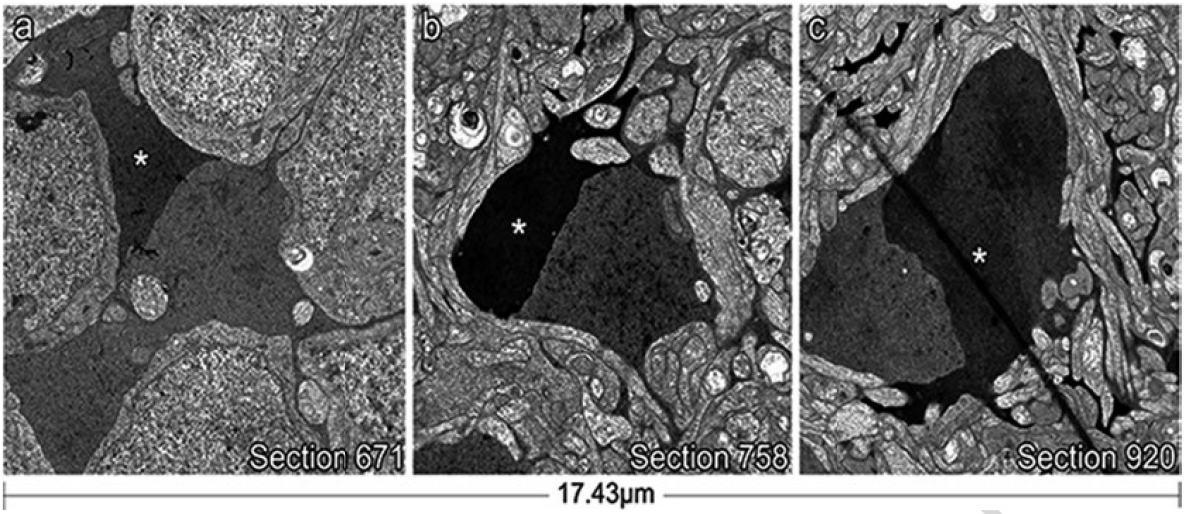

Fig. 60.3 Osmium content variability within MCs in RPC1. (**a**–**c**) Single MC indicated by asterisk. (**a**) Section 671 from RPC1 volume, found within INL. (**b**) Section 758 from RPC1 volume, found within apical region of the IPL. (**c**) Section 920 from RPC1 volume, basal to section 758 in IPL

60.4 Discussion

Here we describe MC changes in a pathoconnectome of early retinal remodeling. We find increased metabolic variability, structural abnormalities, and variations in OsO_4 staining between and within MCs. The impetus for this investigation was to test the hypothesis that variability in MC metabolites may be caused by interactions with neighboring cells, especially those regions of MCs adjacent to synaptic contacts between neurons. RPC1 MC *2628* has 2164 polygon annotations composed of 827,933 vertices, making this one of the most complex cells ever to be reconstructed at ultrastructural resolution. Annotation and analysis of neurons and glia contacts in RC1 and RPC1 is ongoing.

Q and τ immunoreactivity varies across MCs early in RDD as opposed to possessing homogeneous signals in normal retina. Effectively, variations in Q and τ reveal multiple metabolic states across MCs in RDD. Whether or not these are stable or metastable states is unknown. Interestingly, GS, the central enzyme for E to Q metabolism, also exhibits variable expression across MCs in RDD (data not shown). GS variation may explain altered levels of Q and suggests multiple metabolic pathways may be impacted in early retinal degeneration.

Osmication in MCs is highly variable both intra- and intercellularly. The mechanisms leading to this variability are unknown. We surmise, based on mechanisms of OsO_4 binding of phospholipid head groups in tissues, that lipids are involved. That said, the lipid species increased in response to RDD, and its function remains unknown.

In addition to lipid and small molecule variability, MC morphology in early RDD is also altered. Accompanying characteristic MC hypertrophy, we find increased branching and intertwinement of MC endfeet, a morphology not normally found in rabbit retinas. We believe this branching is the beginning of chaotic glial entanglement initially characterized by Fisher and Lewis (Fisher and Lewis 2003). The impact of glial entanglement is currently unclear. How this process may affect metabolic interactions between glia and neurons is an unanswered question, which this project eventually hopes to address.

In conclusion, this chapter describes our early findings in RPC1, a pathoconnectome of early retinal remodeling. Future directions include continued analysis of MC structure and interactions with neuronal and glial components of degenerate and healthy retinas, evaluation of cell-cell interactions, and their relationship to variable

metabolic phenotypes found in degenerate retina. Our hope is that this work will serve as a scaffolding to better understand mechanisms of early RDD from metabolic and morphological perspectives and what role MCs and their interactions with their neighbors play within the local retinal environment as retinal degeneration and remodeling ensue.

References

Anderson JR, Jones BW, Watt CB et al (2011) Exploring the retinal connectome. Mol Vis 17:355–379

Bignami A, Dahl D (1979) The radial glia of Müller in the rat retina and their response to injury. An immunofluorescence study with antibodies to the glial fibriallary acidic (GFA) protein. Exp Eye Res 28:63–69

Erickson PA, Fisher SK, Anderson DH et al (1983) Retinal detachment in the cat: the outer nuclear and outer plexiform layers. Invest Ophthalmol Vis Sci 24:927–942

Erickson PA, Fisher SK, Guerin CJ et al (1987) Glial fibrillary acidic protein increases in Muller cells after retinal detachment. Exp Eye Res 44:37–48

Fariss RN, Li ZY, Milam AH (2000) Abnormalities in rod photoreceptors, amacrine cells, and horizontal cells in human retinas with retinitis pigmentosa. Am J Ophthalmol 129:215–223

Fisher SK, Lewis GP (2003) Muller cell and neuronal remodeling in retinal detachment and reattachment and their potential consequences for visual recovery: a review and reconsideration of recent data. Vis Res 43:887–897

Jones BW, Kondo M, Terasaki H et al (2012) Retinal remodeling. Jpn J Ophthalmol 56:289–306

Jones BW, Pfeiffer RL, Ferrell WD et al (2016a) Retinal remodeling in human retinitis pigmentosa. Exp Eye Res 150:149–165

Jones BW, Pfeiffer RL, Ferrell WD et al (2016b) Retinal remodeling and metabolic alterations in human AMD. Front Cell Neurosci 10:103

Jones BW, Watt CB, Frederick JM et al (2003) Retinal remodeling triggered by photoreceptor degenerations. J Comp Neurol 464:1–16

Jones BW, Kondo M, Terasaki H et al (2011) Retinal remodeling in the Tg P347L rabbit, a large-eye model of retinal degeneration. J Comp Neurol 519:2713–2733

Kondo M, Sakai T, Komeima K et al (2009) Generation of a transgenic rabbit model of retinal degeneration. Invest Ophthalmol Vis Sci 50:1371–1377

Marc RE, Murry RF, Fisher SK et al (1998) Amino acid signatures in the detached cat retina. Invest Ophthalmol Vis Sci 39:1694–1702

Pfeiffer RL, Marc RE, Kondo M et al (2016) Muller cell metabolic chaos during retinal degeneration. Exp Eye Res 150:62–70

Reichenbach A, Bringmann A (2010) Muller cells in the healthy retina. In: Muller cells in the healthy and diseased retina. Springer Science+Business Meddia, LLC

Reichenbach A, Bringmann A (2013) New functions of Muller cells. Glia 61:651–678

Reichenbach A, Schneider H, Leibnitz L et al (1989) The structure of rabbit retinal Muller (glial) cells is adapted to the surrounding retinal layers. Anat Embryol (Berl) 180:71–79

Shen W, Fruttiger M, Zhu L et al (2012) Conditional Mullercell ablation causes independent neuronal and vascular pathologies in a novel transgenic model. J Neurosci 32:15715–15727