Published in final edited form as:

Hum Gene Ther. 2019 August 01; 30(8): 921–922. doi:10.1089/hum.2019.116.

Visualization of Retinal Morphology in rd12 Mice by Scanning Electron Microscopy

Bertin Mary, Giridhara R. Jayandharan*

Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, Uttar Pradesh, India

Abstract

We have evaluated the retinal morphological changes in normal C57BL/6/J mice and a retinal degenerative disease (rd12 mice) model using scanning electron microscopy. A method of sample preparation for electron microscopy was developed and the cryosectioned retina was used to study the retinal thickness. Our data demonstrate variation in the neural retina texture between rd12 mice and C57BL/6/J mice.

Keywords

scanning electron microscopy; rd12 mice; retina

Introduction

PRECLINICAL EVALUATION OF gene therapy for Leber congenital amaurosis type 2 has largely relied on the use of *rd12* mice, which recapitulates several anatomical and phenotypic characteristics of the human disease.^{1,2} Although the success of the therapeutic intervention in this model is measured by several endpoint assays (*e.g.*, electroretinogram analysis), there is lack of adequate information on the neural layer architecture after gene therapy. Spectral domain ocular coherence tomography has been commonly used to study the retinal thickness of the posterior segment of murine retina such as that of C57BL/6J mice ($\sim 210 \pm 1.09 \mu\text{m}$).³ Although this is an excellent method for noninvasive data acquisition, the requirement of a dedicated equipment and its low resolution in capturing the ultra-structure and the texture of the retina necessitate the availability of a simple technique to measure retinal thickness in murine models. Thus, we have utilized high-resolution scanning electron microscopy (SEM) to investigate the retinal architecture in rd12 mice in comparison with that of normal mice.

*Correspondence: Dr. Giridhara R. Jayandharan, Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208 016, Uttar Pradesh, India. jayrao@iitk.ac.in.

Author Disclosure

No competing financial interests exist.

Methods

Eyeballs from 3- to 5-month-old C57BL6/J and rd12 (B6(A)-*Rpe65*^{rd12}/J; The Jackson Laboratory, Bar Harbor, Maine) mice ($n = 4$ eyes/group) were collected by enucleation and fixed in 4% paraformaldehyde for 30 min.⁴ Fixed eyeballs were dehydrated using a sucrose gradient (10%, 20%) for 30 min each at 4°C and a final dehydration in 30% sucrose solution overnight at 4°C. The dehydrated eyeballs were later embedded in polyfreeze medium (Sigma Aldrich, St. Louis, MO) and serial cryosections of 10 μm thickness were collected in glass coverslips. The retinal sections were further washed with phosphate-buffered saline to remove the optimal cutting temperature compound (Polyfreeze) and dried in room temperature. The dried sections were gold coated for 90 s in a gold sputter coating unit (JEC 3000; JEOL, Peabody, MA) and imaged in a field-emission scanning electron microscope (Nova nanoSEM 450, FEI; Thermo Fisher Scientific, Waltham, MA). Statistical comparison between the images acquired from C57BL6/J and rd12 mice groups was performed with GraphPad Prism software (GraphPad, La Jolla, CA) by student's t -test. A p -value < 0.05 was considered to be statistically significant.

Results and Discussion

SEM imaging of murine retina captured the retinal layer cross-section morphology and further differentiation between retinal architecture was possible by this method. Figure 1 shows the retinal architecture of a 3-month-old rd12 mouse. To further quantify the variations in retinal thickness between C57BL6/J and rd12 mice (Supplementary Figs. S1 and S2), we measured the total retinal thickness (TRT) of the sections from inferior, nasal, temporal and superior retina. The mean TRT was computed from 36 data points for each murine eye from three sections (four images per section were taken and three TRT measurements per image were made) for a total of four eyes per group. This analysis demonstrated that the mean TRT measurements between the C57BL6/J and rd12 mice were significantly different by SEM analysis. The mean TRT values were $200 \pm 14 \mu\text{m}$ in C57BL6/J mice and $151 \pm 6 \mu\text{m}$ ($p < 0.05$) in rd12 mice at 3 months of age. Similarly, in 5-month-old C57BL6/J and rd12 mice, the mean TRT values were 191 ± 14 and $155 \pm 23 \mu\text{m}$ ($p < 0.05$), respectively. Our data confirm previous findings⁵ that a time point between 3 and 5 months of age is ideal to test retinal gene delivery into the rd12 mice. Further SEM analysis of murine retina is a simple yet sensitive approach to visualize not only retinal degeneration but also to capture the morphology of individual cells within the neural retina. Thus, this method should be a valuable tool to follow up the efficacy of novel gene or cell therapy strategies tested in rd12 mice, to document their impact at a cellular level (*e.g.*, retinal pigmented epithelial cells), and to enable accurate depiction of morphological and phenotypic reconstitution, if any, of the neural retina after an intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

G.R.J. is supported by a research grant from Wellcome Trust DBT India Alliance fellowship and an initiation grant (2014-256) from IIT Kanpur. B.M. is supported by a Ministry of Human Resource and Development PhD fellowship. We thank Dr. Sankalp Verma and Dr. Vivek Verma, Material Science and Engineering Department, IIT Kanpur, for help in SEM analyses.

References

1. Pang JJ, Chang B, Hawes NL, et al. Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human leber congenital amaurosis (LCA). *Mol Vis*. 2005; 11:152–162. [PubMed: 15765048]
2. Jacobson SG, Cideciyan AV, Ratnakaram R, et al. Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. *Arch Ophthalmol*. 2012; 130:9–24. [PubMed: 21911650]
3. Ferguson LR, Dominguez JM 2nd, Balaiya S, et al. Retinal thickness normative data in wild-type mice using customized miniature SD-OCT. *PLoS One*. 2013; 8:e67265. [PubMed: 23826252]
4. Petrs-Silva H, Dinculescu A, Li Q, et al. Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. *Mol Ther*. 2011; 19:293–301. [PubMed: 21045809]
5. Jacobson SG, Aleman TS, Cideciyan AV, et al. Identifying photoreceptors in blind eyes caused by RPE65 mutations: prerequisite for human gene therapy success. *Proc Natl Acad Sci U S A*. 2005; 102:6177–6182. [PubMed: 15837919]

Figure 1. Retinal architecture of rd12 mice. Pseudocolored SEM image of rd12 mice retina (3 months old) at $2,000\times$ magnification showing degeneration in the outer segments. Colorization of the SEM image was done by MountainsMap® SEM software. The original image is presented in Supplementary Fig. S1B and the scale bars were derived in comparison with the original image. SEM, scanning electron microscopy. Color images are available online.