The ROYAL COLLEGE of OPHTHALMOLOGISTS

www.nature.com/eye

BRIEF COMMUNICATION

Basic-science observations explain how outer retinal hyperreflective foci predict drusen regression and geographic atrophy in age-related macular degeneration

Qitao Zhang[1] ✉ and Jason M. L. Miller[1] ✉

Eye (2022) 36:1115–1118; https://doi.org/10.1038/s41433-021-01748-y

The appearance of hyperreflective foci (HRF) above drusenoid pigment epithelial detachments (d-PEDs) on optical coherence tomography (OCT) imaging strongly predicts drusen collapse and subsequent progression to geographic atrophy (GA) in age-related macular degeneration (AMD) [1–6] (Fig. 1A, B). Here, we provide literature and laboratory evidence supporting the hypothesis that HRF represent RPE undergoing a pathologic epithelial-to-mesenchymal transition (EMT) and that this phenotypic change results in a decrease in the RPE's secretion of drusen-sustaining components, leading to drusen collapse just before GA onset.

Most evidence points to HRF above d-PEDs as RPE migrating above the RPE monolayer into the neural retina [7–9]. RPE cells that lose contact with the RPE monolayer undergo a phenotypic change, termed an EMT [10]. EMT results in RPE proliferation and increased fibrotic capacity [10, 11], accompanied by a decline in photoreceptor-supporting functions [12]. The extreme clinical example of RPE EMT is proliferative vitreoretinopathy, where RPE released from the monolayer undergoes massive proliferation and fibrosis [11], but more subtle RPE EMT has been demonstrated in AMD as well [10, 13]. Mild RPE EMT may lead to photoreceptor loss and an inability to fill-in gaps in the RPE monolayer after EMT-RPE migrates into the neural retina, thereby triggering GA [10].

d-PED collapse often heralds GA onset [8, 14, 15]. To understand this phenomenon, we first note that d-PEDs are dynamic structures representing an equilibrium between ongoing deposition and egress of drusen material [15]. As RPE sickens, its secretion of the most abundant apolipoprotein component of drusen, apolipoprotein E (apoE) [14], decreases [15], causing a shift in d-PED equilibrium toward egress prior to GA onset.

Here, we hypothesize that RPE undergoing EMT, clinically indicated by HRF above d-PEDs on OCT, also leads to a decrease in secretion of drusen components, further promoting drusen regression prior to GA. To test this hypothesis, we utilized our primary human RPE culture system, previously shown to mimic in vivo RPE [16]. Under normal conditions, our primary human RPE culture maintains a cobblestone morphology (Fig. 1Ci, left). When plated at low density, however, RPE cells fail to make strong cell–cell contacts, triggering EMT and a fibroblastic morphology (Fig. 1Ci, right). EMT-RPE is marked by expression of vimentin (Fig. 1Cii), which is also expressed in degenerating RPE from AMD eyes, further bolstering the concept that AMD involves an RPE EMT shift [17]. Our EMT-RPE also lose their tight-junction integrity, which is critical for establishing the outer retinal–blood barrier (Fig. 1D). Importantly, unlike epithelial RPE cultures, EMT-RPE cultures secrete significantly lower levels of drusen components, including apoE and TIMP-3 (Fig. 1E).

Together, these data support the following model (Fig. 2): as RPE sickens in dry AMD, it undergoes an EMT. Some EMT-RPE may remain in the RPE monolayer but other EMT-RPE migrates into the neural retina, manifesting as HRF on OCT imaging that predict AMD progression. The subsequent decreased secretion of drusen components by sick and EMT-RPE, along with continued efflux of drusen components into the choroid, results in d-PED collapse, followed by GA.

[1]Kellogg Eye Center, University of Michigan, Ann Arbor, MI, USA. ✉email: qitaoz@med.umich.edu; miljason@med.umich.edu

Received: 6 July 2021 Revised: 3 August 2021 Accepted: 5 August 2021
Published online: 20 August 2021

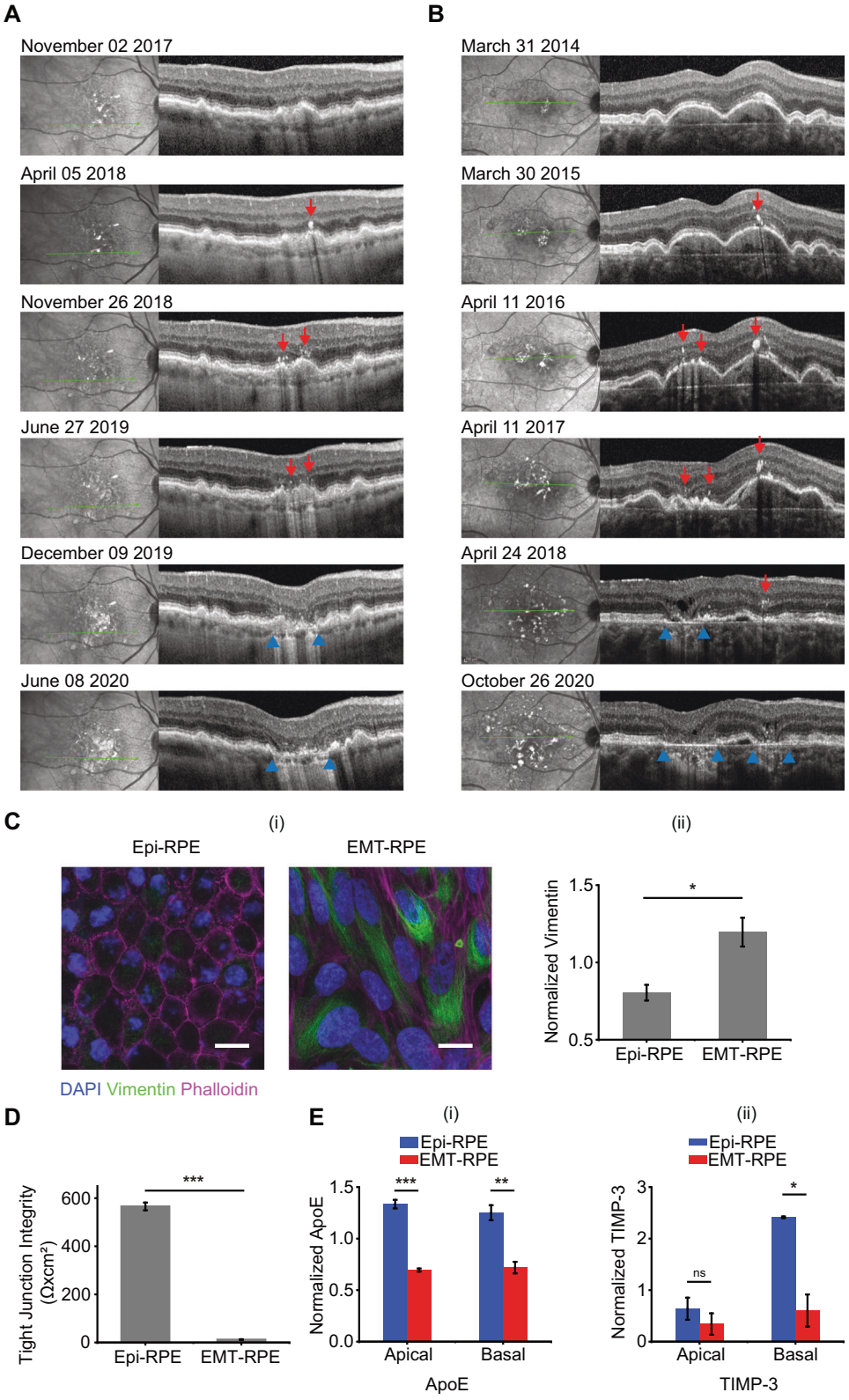
A
November 02 2017
April 05 2018
November 26 2018
June 27 2019
December 09 2019
June 08 2020
B
March 31 2014
March 30 2015
April 11 2016
April 11 2017
April 24 2018
October 26 2020
C
(i)
Epi-RPE
EMT-RPE
DAPI Vimentin Phalloidin
(ii)
Normalized Vimentin
1.5
1.0
0.5
*
Epi-RPE
EMT-RPE
D
Tight Junction Integrity (Ωxcm²)
600
400
200
0
***
Epi-RPE
EMT-RPE
E
(i)
Epi-RPE
EMT-RPE
Normalized ApoE
1.5
1.0
0.5
0.0
***
**
Apical
Basal
ApoE
(ii)
Epi-RPE
EMT-RPE
Normalized TIMP-3
3
2
1
0
ns
*
Apical
Basal
TIMP-3

Fig. 1 Hyperreflective foci (HRF) on OCT imaging in AMD represent RPE undergoing an epithelial–mesenchymal transition (EMT), resulting in decreased RPE secretion of drusen components, followed by collapse of the drusenoid PED (d-PED) underlying the HRF and then GA. A An 84-year-old female with intermediate AMD in the right eye and disciform scar in the left eye was followed for nearly 3 years. Near-infrared reflectance (NIR) on left and OCT B-scan on right demonstrating appearance of HRF over d-PEDs (red arrows) followed by collapse of d-PEDs and onset of GA (bounded by blue arrowheads). **B** A 60-year-old male with intermediate AMD in both eyes was followed for more than 6 years, again demonstrating HRF formation (red arrows) followed by d-PED collapse and subsequent GA (blue arrowheads) in the right eye. **C** (i) Primary human RPE was cultured as previously described [16]. Typical culture (epithelial RPE—Epi-RPE) demonstrates a cobblestone morphology and absence of the EMT marker vimentin (left). In contrast, culturing freshly split RPE onto microporous supports at a 1:20 dilution for 6–10 days results in confluent cultures but an EMT transition (EMT-RPE) marked by an elongated, spindle morphology and expression of vimentin (right). Blue = DAPI (nuclei marker), green = vimentin (EMT marker; #OMA1-06001, Thermo, 1:100), pink = phalloidin (actin cytoskeleton marker). Scale bar = 15 μm. (ii) Normalized quantification of vimentin protein expression by Western blot in epithelial RPE (Epi-RPE) and EMT-RPE ($n = 3$; #5741, Cell Signalling Technology, 1:1000). **D** EMT-RPE lose tight-junction integrity, a key RPE phenotype critical for maintaining the outer retinal–blood barrier. Tight-junction integrity measured by trans-electrical resistance ($\Omega \times cm^2$), as previously described [16] ($n = 4$). **E** Secretion of drusen components is decreased in EMT-RPE. Apolipoprotein E (apoE), the most abundant apolipoprotein in drusen, is secreted above (apical) and below (basal) the RPE, and EMT-RPE secretes lower amounts on both sides. TIMP-3, a major Bruch's membrane remodeling protein and component of drusen, is normally secreted basally. In EMT-RPE, its secretion is negligible. ApoE and TIMP-3 secretion were measured in the media above and below the RPE by Western blot, subtracted from background signal, and subsequently normalized ($n = 3$; apoE = #AB947, Millipore, 1:2000; TIMP-3 = #AB6000, Millipore, 1:1000). Student *T* test for all statistical analysis, ***<0.001, **<0.01, *<0.05 (color figure online).

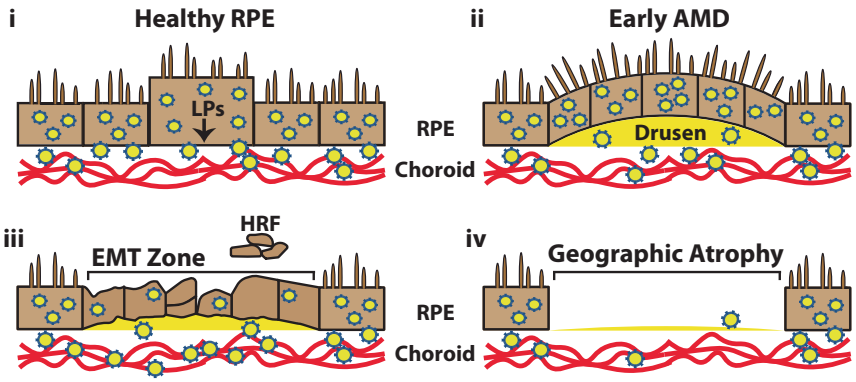

Fig. 2 Model linking RPE epithelial–mesenchymal transition (EMT) to OCT hyperreflective foci (HRF), drusenoid PED (d-PED) collapse, and subsequent GA. (i) Healthy RPE normally secretes abundant amounts of drusen-sustaining material, such as lipoproteins (LPs) containing apolipoprotein E (apoE). (ii) In early dry AMD, drusen forms under the RPE, composed of lipid- and protein-rich material secreted normally by the RPE. Drusen accumulates because secretion occurs faster than efflux of the drusen material into the choroidal circulation. (iii) As the RPE sickens, it undergoes morphologic and migratory changes suggestive of an epithelial-to-mesenchymal transition (EMT). Instead of a smooth monolayer, the RPE becomes dysmorphic and certain RPE cells migrate into the neural retina, apparent on OCT as hyperreflective foci (HRF). HRF are a presumed marker of RPE EMT and have been well-established as one of the strongest predictors of progression to geographic atrophy. Our basic science data show that RPE undergoing EMT secrete less drusen material, allowing efflux of the remaining drusen into the choroidal circulation. This leads to collapse of drusenoid pigment epithelial detachments (d-PEDs) as the RPE becomes progressively sicker. (iv) As the RPE fully dies in the area of prior EMT, the d-PED also disappears via choroidal efflux, leaving a zone of geographic atrophy.

REFERENCES

1. Christenbury JG, Folgar FA, O'Connell RV, Chiu SJ, Farsiu S, Toth CA. Progression of intermediate age-related macular degeneration with proliferation and inner retinal migration of hyperreflective foci. Ophthalmology. 2013;120:1038–45. https://doi.org/10.1016/j.ophtha.2012.10.018.
2. Fleckenstein M, Charbel Issa P, Helb H-M, Schmitz-Valckenberg S, Finger RP, Scholl HP, et al. High-resolution spectral domain-OCT imaging in geographic atrophy associated with age-related macular degeneration. Invest Ophthalmol Vis Sci. 2008;49:4137–44. https://doi.org/10.1167/iovs.08-1967.
3. Ouyang Y, Heussen FM, Hariri A, Keane PA, Sadda SR. Optical coherence tomography-based observation of the natural history of drusenoid lesion in eyes with dry age-related macular degeneration. Ophthalmology. 2013;120:2656–65. https://doi.org/10.1016/j.ophtha.2013.05.029.
4. Sleiman K, Veerappan M, Winter KP, McCall MN, Yiu G, Farsiu S, et al. Optical coherence tomography predictors of risk for progression to non-neovascular atrophic age-related macular degeneration. Ophthalmology. 2017;124:1764–77. https://doi.org/10.1016/j.ophtha.2017.06.032.
5. Nassisi M, Fan W, Shi Y, Lei J, Borrelli E, Ip M, et al. Quantity of intraretinal hyperreflective foci in patients with intermediate age-related macular degeneration correlates with 1-year progression. Invest Ophthalmol Vis Sci. 2018;59:3431–9. https://doi.org/10.1167/iovs.18-24143.
6. Sitnilska V, Kersten E, Altay L, Schick T, Enders P, de Jong EK, et al. Major predictive factors for progression of early to late age-related macular degeneration. Opthalmologica. 2020;243:444–52. https://doi.org/10.1159/000507196.
7. Ho J, Witkin AJ, Liu J, Chen Y, Fujimoto JG, Schuman JS, et al. Documentation of intraretinal retinal pigment epithelium migration via high-speed ultrahigh-resolution optical coherence tomography. Ophthalmology. 2011;118:687–93. https://doi.org/10.1016/j.ophtha.2010.08.010.
8. Balaratnasingam C, Messinger JD, Sloan KR, Yannuzzi LA, Freund KB, Curcio CA. Histologic and optical coherence tomographic correlates in drusenoid pigment epithelium detachment in age-related macular degeneration. Ophthalmology. 2017;124:644–56. https://doi.org/10.1016/j.ophtha.2016.12.034.
9. Curcio CA, Zanzottera EC, Ach T, Balaratnasingam C, Freund KB. Activated retinal pigment epithelium, an optical coherence tomography biomarker for progression in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2017;58: BIO211–26. https://doi.org/10.1167/iovs.17-21872.
10. Zhou M, Geathers JS, Grillo SL, Weber SR, Wang W, Zhao Y, et al. Role of epithelial–mesenchymal transition in retinal pigment epithelium dysfunction. Front Cell Dev Biol. 2020;8:501. https://doi.org/10.3389/fcell.2020.00501.
11. Tamiya S, Kaplan HJ. Role of epithelial–mesenchymal transition in proliferative vitreoretinopathy. Exp Eye Res. 2016;142:26–31. https://doi.org/10.1016/j.exer.2015.02.008.
12. Zhao C, Yasumura D, Li X, Matthes M, Lloyd M, Nielsen G, et al. mTOR-mediated dedifferentiation of the retinal pigment epithelium initiates photoreceptor degeneration in mice. J Clin Invest. 2011;121:369–83. https://doi.org/10.1172/JCI44303.
13. Ishikawa K, Kannan R, Hinton DR. Molecular mechanisms of subretinal fibrosis in age-related macular degeneration. Exp Eye Res. 2016;142:19–25. https://doi.org/10.1016/j.exer.2015.03.009.
14. Spaide RF, Ooto S, Curcio CA. Subretinal drusenoid deposits AKA pseudodrusen. Surv Ophthalmol. 2018;63:782–815. https://doi.org/10.1016/j.survophthal.2018.05.005.

15. Miller JML, Zhang Q, Johnson MW. Regression of drusen or vitelliform material heralding geographic atrophy: correlation between clinical observations and basic science. Graefes Arch Clin Exp Ophthalmol. 2021. https://doi.org/10.1007/s00417-021-05101-7.
16. Zhang Q, Presswalla F, Feathers K, Cao X, Hughes BA, Zacks DN, et al. A platform for assessing outer segment fate in primary human fetal RPE cultures. Exp Eye Res. 2018;178:212–22. https://doi.org/10.1016/j.exer.2018.10.008.
17. Guidry C, Medeiros NE, Curcio CA. Phenotypic variation of retinal pigment epithelium in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2002;43:267–73.

ACKNOWLEDGEMENTS

The authors acknowledge the Kellogg Eye Center's Pre-Residency Fellowship program for funding the basic science aspects of this study. The authors also gratefully acknowledge philanthropic donations from Barbara Dunn as well as Dee and Dickson Brown.

AUTHOR CONTRIBUTIONS

QZ designed and executed all experiments. JMLM conceived of the study and wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Q.Z. or J.M.L.M.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.