



Original Article

Assessment of retinal pigment epithelial cells in epiretinal membrane formation

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Abstract

Background: The definite etiology of epiretinal membrane (ERM) is unknown. Clinically, ERM may cause metamorphopsia and decreased vision during the disease exacerbation. Several theories of pathogenesis emphasize a glial tissue origin. However, in some studies, surgically removed ERM specimens were found to contain retinal pigment epithelial (RPE) cells. The actual mechanism by which RPE cells gain access into the inner retina and what roles they play in the formation of ERM remain controversial. The purpose of this study was to evaluate the incidence of RPE cells in ERM and discuss the possible mechanisms.

Methods: A retrospective review of the histological findings in 23 surgically removed specimens of ERM was done. The samples were studied using light microscopy and immunohistochemistry.

Results: Glial cells were the main components in all 23 cases, and RPE cells were found in five of the specimens. Two of these five cases were clinically diagnosed as idiopathic macular pucker, whereas the other three cases were identified as macular pucker associated with previous retinal detachment. A much higher density of myofibroblasts was noted in these five specimens than in the other 18 cases.

Conclusion: The incidence of RPE cells found in ERM is 21.7% (5 out of 23 specimens). A strong association between RPE cells and myofibroblasts in cases of ERM with or without retinal detachment indicates that RPE cells may contribute to the formation of ERM via a wound healing process.

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Keywords: epiretinal membrane; myofibroblast; retinal detachment; retinal pigment epithelial cells; wound healing

1. Introduction

Epiretinal membrane (ERM) is a general classification of several vitreoretinal interface diseases, such as vitreomacular

traction syndrome, cellophane, and macular pucker, which can be identified by fundoscope, optical coherence tomography, and photography. Patients with ERM may have trivial symptoms or suffer from distorted and decreased vision, depending on the severity of the disease. Histopathologically, ERM has been characterized as a preretinal fibrotic lesion containing a layer of glial cells on the retinal surface from light and electron microscopic findings of enucleated or autopsy eyes.¹ It is also sometimes said to have an idiopathic origin when there is no association with other ocular diseases, such as retinal

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detachment, intraocular inflammation, trauma, or retinal vascular diseases.² The origin of the cells in ERM was considered by Foos³ as accessory glial cells migrating from the nerve fiber layer to the surface of the internal limiting membrane (ILM). Other types of ocular cells, including fibroblasts, hyalocytes, RPE cells, and macrophages, identified by electron microscopy and immunohistochemistry, appear to play a crucial role in the formation of ERM.^{2,4–6} ERM in cases of proliferative vitreoretinopathy (PVR) resulting from retinal tears, trauma, infection, or blunt injury is usually complicated by the presence of RPE cells. These cells are present in variable numbers and can be easily recognized morphologically as multilayered cohesive cells, with opaque eosinophilic cytoplasm and, often, the presence of pigment. Along with the RPE cells, variable numbers of macrophages and lymphocytes can be present, and the membranes are often thick and contain collagen, indicating a putative tissue repair process.^{7–9} The presence of RPE cells in idiopathic ERMs was reported in some early histopathologic studies.^{10–12} However, the actual mechanism through which RPE cells gain their access into the inner retina remains unresolved. In this study, we evaluated the incidence of RPE cells in ERM and discuss the possible mechanism.

2. Methods

We conducted a retrospective review of specimens from 23 patients with a clinical diagnoses of macular pucker who underwent the removal of ERM via vitrectomy surgery by the same surgeon (S.-J.C.). The specimens were submitted as flat-mount slides, for which the removed tissue had been immediately placed into fixative solution of 2% paraformaldehyde for at least 24 hours. Afterwards, the specimens were extended onto glass slides as whole mounted membranes to show their maximum area and then stained with hematoxylin and eosin (H&E) and studied using both light microscopy and immunohistochemistry. Patients short of adequate tissue for pathologic diagnosis and those without clear operative clinical diagnoses were excluded from this study. Furthermore, cases with the clinical diagnosis of macular hole, vitreomacular traction syndrome, or proliferative diabetic retinopathy were also excluded.

3. Results

A total of 23 specimens of vitrectomized eyes with ERM peeling flat-mount slides were reviewed. There were glial cells in all 23 cases (100%), which were confirmed by positive immunohistochemistry staining of glial fibrillary acidic protein (GFAP). Second to glial cells was myofibroblasts, which were found in 17 specimens (73.9%), and macrophages, which were noted in nine cases (39.1%). Finally, RPE cells were identified in five cases (21.7%). Of these five cases, three were associated with previous retinal detachment and retinal tear, whereas the other two cases were clinically diagnosed as idiopathic macular pucker (Table 1). The cell number in our study was defined as high when a density of > 20 cells were

counted under high power field (400×), as moderate for 10–20 cells, and as low for < 10 cells. The RPE cells present in our specimens did not show multilayered cohesive cells as previous studies reported but were easily identified by abundant cytoplasm packed with melanin pigments. Some inflammatory cells and macrophages were seen accompanying the RPE cells, and the ERMs were often thick and contained collagen, indicating a putative tissue repair process. Furthermore, we found that RPE cells presenting in five cases were all associated with high density of myofibroblasts (Fig. 1). By contrast, the other 18 specimens in which RPE cells were not present only showed low to moderate density of myofibroblasts (Fig. 2).

4. Discussion

The formation of ERM characterizes a number of pathological changes occurring in the vitreoretinal interface. Snead and colleagues⁴ have classified ERM into three distinct types: simple ERM, which is thought to be idiopathic, PVR/tissue repair ERM associated with previous retinal tear, trauma, infection, or blunt injury, and neovascular ERM caused by proliferative diabetic retinopathy. The second type of ERM was found to contain RPE cells with variable amounts of extracellular stroma. This is because RPE cells can migrate through retinal tears and become attached to the inner retina after retinal detachment.^{13–16} Other than PVR/tissue repair ERM, the etiology of idiopathic ERM is still debated. There are no detectable retinal tears through which RPE cells can gain their access into the inner retina. However, RPE cells in idiopathic ERMs have been demonstrated in previous histopathologic reports.^{10–12}

Several theories of mechanism regarding ERM formation have been proposed, but none can fully explain all features involving the formation process. First, it is possible that the RPE cells may migrate through occult breaks into the inner retina.¹⁰ However, there is no direct evidence supporting this theory. Moreover, the cases in our study were carefully examined by indirect ophthalmoscopy pre- and post-operatively. Thus, it is unlikely that an occult break was overlooked. Similarly, no findings of subclinical or healed retinal breaks were demonstrated in a histopathological study of large-scale autopsy reports.¹⁷

The second possible theory is that other cell types such as glial cells may undergo transformation into RPE cells. These GFAP-positive cells could be of hyalocyte or Müller-cell origin.^{18,19} Clinically, hyalocytes are associated with the pathogenesis of various vitreoretinal diseases, including PVR,

Table 1
The cell types found in the 23 epiretinal membrane (ERM) specimens.

Cell type	Number (ratio) of specimens
Glial cell	23/23 (100%)
Myofibroblast	17/23 (73.9%)
Macrophage	9/23 (39.1%)
Retinal pigment epithelial (RPE) cell	5/23 (21.7%)

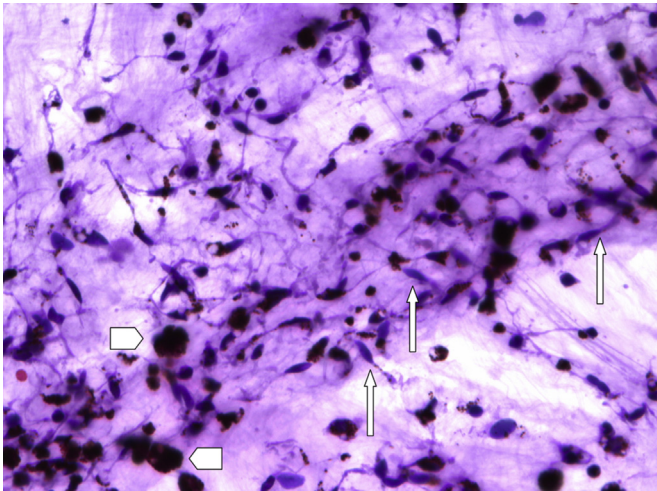


Fig. 1. Along with the retinal pigment epithelial (RPE) cells (arrowheads), numerous myofibroblasts are identified (arrows). Hematoxylin and eosin (H&E), 400 \times .

with its myofibroblastic transdifferentiation.^{20–23} Therefore, hyalocytes might be a primary component of ERM, because they abound in the posterior hyaloids.⁵ Furthermore, hyalocytes involved in the residual outer layer of the posterior vitreous cortex after anomalous posterior vitreous detachment with vitreoschisis is thought to be one of the cellular origins of idiopathic ERM, as previously suggested by Sebag et al.^{24,25}

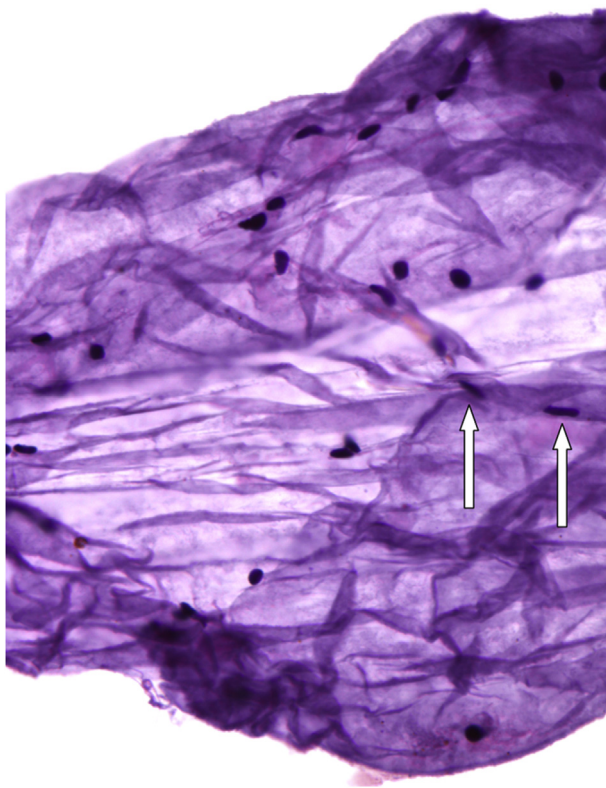


Fig. 2. In specimens in which retinal pigment epithelial (RPE) cells were not present, only a few myofibroblasts are discovered (arrows). Hematoxylin and eosin (H&E), 400 \times .

Bu and his coworkers⁶ recently reported active and dynamic involvement of Müller cells in the pathogenesis of macular hole and subsequent ERM formation. Müller cells can be activated by various pathogenic factors, such as mechanical traction, retinal trauma, hyperglycemia, and the release of cytokines and growth factors due to blood–retinal barrier breakdown.^{18,26,27} Therefore, we can postulate that both hyalocytes and Müller cells can transdifferentiate into a myofibroblast phenotype that plays an important role in the formation of ERM.^{5,28}

Finally, RPE cells are thought to gain access into the inner retinal surface via a defect in the ILM.¹⁰ This theory is suggested by the fact that the formation of ERM is a gliotic and fibrotic process where glial cells play an active role by migration, proliferation, and transdifferentiation into a myofibroblast phenotype.⁶ Myofibroblasts are found at sites of wound healing and chronic inflammation, and are believed to play a pivotal role in the healing process and in the pathogenesis of fibrosis.²⁹ Several studies demonstrated that circulating fibrocyte traffic to sites of tissue injury, and differentiate into myofibroblasts and contribute to wound healing and fibrosis.^{30–32} These cells emerge at the tissue site during wound repair, resulting in hypertrophic scars and keloids, airway remodeling in asthma, interstitial pulmonary fibrosis, systemic fibroses, intimal hyperplasia, atherosclerosis, reactive fibrosis in chronic pancreatitis and cystitis, and tumor-induced stromal reaction.^{33,34} Furthermore, fibrocytes are shown to function as precursors of myofibroblasts in PVR membranes.²⁹

This explains the findings in our study that RPE cells are strongly associated with myofibroblasts. In ERMs secondary to previous retinal detachment, RPE cells migrate into the inner retina via retinal tears. The high density of myofibroblasts in these cases indicates both a wound repairing process during re-attachment of the retina, and also a PVR membrane formation process. By contrast, in idiopathic ERMs, RPE cells may gain their access to the inner retinal surface through a discontinuous ILM. Along with these RPE cells, a large number of myofibroblasts can also be discovered. This phenomenon implies the healing process of the ILM.

In conclusion, the incidence of RPE cells in ERM in our study is 21.7% with or without a previous history of retinal tear. Strong association between the RPE cells and myofibroblasts indicates that the formation of ERM is a gliotic and fibrotic healing process. However, because our study was limited by a small sample size, the exact reason why RPE cells occur in ERM needs further investigation.

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