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ORIGINAL PAPER

Comparison of histopathological findings between idiopathic and secondary epiretinal membranes

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Abstract To evaluate the histopathological findings of idiopathic and secondary epithelial membranes (ERMs). This study involved 19 ERM cases that underwent pars plana vitrectomy (PPV). ERM specimens were obtained from each patient during PPV and immediately fixed in 10 % formalin. Paraffin sections were stained with hematoxylin eosin (HE) and immunohistochemical analysis was performed with glial fibrillary acidic protein (GFAP), Ki-67, CD34, and nestin antibodies. The 19 ERM cases included 11 idiopathic ERM cases and 8 secondary ERM cases i.e., 2 eyes that underwent PPV for retinal detachment and 6 eyes that underwent PPV for proliferative diabetic retinopathy. HE staining showed that some of the idiopathic ERM specimens consisted of internal limiting membrane. In contrast, numerous invasive cells were observed in the secondary ERM specimens compared to the idiopathic ERM specimens. Immunohistochemical analysis revealed GFAP-positive cells in 4 of the 11 idiopathic ERMs cases, yet no nestin-, Ki-67-, or CD34-positive cells in those cases. In contrast, there were 4 GFAP-positive cases, 2 Ki67-positive cases, 3 CD34-positive cases, and 7 cases including nestin-positive cells. The findings of this study indicate that there are different histological characteristics between idiopathic and secondary ERM and that mature nestin-positive cells in the retina might be related to secondary ERM formation.

Keywords Epiretinal membrane · Vitrectomy · Ki-67 · CD34 · GFAP · Nestin

Introduction

Epiretinal membranes (ERMs) can be idiopathic or secondary. The onset of secondary ERM is often a complication of other eye-related diseases, such as uveitis, rhegmatogenous retinal detachment, diabetic retinopathy, and retinal vein occlusion. Reportedly, there are two proposed etiologies for idiopathic ERM, i.e., (1) a theory involving the migration and proliferation of glial cells in the sensory retina due to rupture of the internal limiting membrane (ILM) [1–4], and (2) a theory that a foundation of residual vitreous cortex on the posterior precortical vitreous pocket leads to cell proliferation [5]. In secondary ERM, in addition to the proposed etiologies shown above, it is believed that retinal pigment epithelium (RPE) cells begin to migrate and proliferate from a retinal tear and that cytokines released into the vitreous cavity due to

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intraocular inflammation and/or retinal ischemia contribute to membrane formation [6]. The membrane is reportedly compromised of glial cells, hyalocytes, macrophages, fibroblasts, and collagen in varying proportions [7]. However, the pathophysiology in these two types of ERM remains unclear.

In this present study, we investigated histological differences between idiopathic and secondary ERMs by immunohistochemical analysis with glial fibrillary acidic protein (GFAP), Ki-67, CD34, and nestin antibodies.

Materials and methods

In this study, 19 consecutive ERM cases underwent pars plana vitrectomy (PPV) at Osaka Medical College, Takatsuki-City, Japan between 2008 and 2011, and informed consent was obtained from all patients prior to their involvement in the study. Of those 19 cases, there were 11 patients with idiopathic ERM and 8 patients with secondary ERM [i.e., 2 eyes with ERM post-PPV due to retinal detachment (RD) and 6 eyes with proliferative diabetic retinopathy (PDR)].

Samples were obtained from each patient during PPV, and the tissues were fixed in 1 % buffered paraformaldehyde. Sections were then counterstained with hematoxylin eosin (HE) before dehydration, cleared in xylene, and mounted. Formalin-fixed and paraffin-embedded tissues were then examined by immunohistochemistry with GFAP, Ki-67, CD34, and nestin antibodies (Table 1). Immunopositivity was then evaluated according to the number of positive cells; i.e., no positive cells (−) and few positive cells (+).

Results

HE findings

Most of the component cells were spindle-shaped fibroblast-like cells, macrophages, and undifferentiated cells. Some of the idiopathic ERM specimens consisted of ILMs. The RD ERM (Case S-1) consisted of both from glial and RPE cells. The PDR specimens likely consisted of endothelial cells forming a microvascular cavity. The secondary ERM specimens included a higher number of cells than in the idiopathic ERM specimens (Fig. 1).

Immunohistological staining

None of the idiopathic ERM specimens showed Ki-67-, CD34-, or nestin-positive cells (Table 2). Four of the 11 idiopathic ERM specimens and 5 of the 8 secondary ERM specimens showed GFAP-positive cells (Table 3). Of the 8 secondary ERM specimens, 2 showed positive with Ki-67 (Fig. 2), 3 showed positive with CD34 (Fig. 3), and 7 showed some nestin-positive cells (Fig. 4). Nestin expression in the PDR ERM specimens was stronger than in the RD ERM specimens.

Discussion

ERM can be classified as idiopathic or secondary depending on the initiating event of the ERM formation. Idiopathic ERM results from the glial proliferation secondary to a break in the ILM occurring during the process of posterior vitreous detachment [1–4]. Another pathogenesis is due to the residual vitreous cortex on the posterior precortical vitreous pocket leading to cell proliferation [5]. On the other hand, secondary ERM results from an already existing ocular pathology like uveitis, diabetic retinopathy, rhegmatogenous retinal detachment, and retinal vein occlusion. Since the primary mechanisms for the ERM formation differ in idiopathic and secondary ERMs, the features of the membranes may also differ.

In this study, 11 idiopathic ERM specimens and 5 of the 8 secondary ERM specimens showed GFAP-positive cells. As with the findings of previous reports [1, 2], our results showed that glial cells are involved

Table 1 Antibody type, species, dilution, and manufacturer of the antibodies used in the study

Antibody	Species	Dilution	Manufacturer
Nestin (RPA48681)	Polyclonal rabbit	1:400	Reprokine, Research immunity, NY
Ki-67 (M7240)	Monoclonal mouse	1:100	DAKO, Carpenteria, CA
CD34 (M7165)	Monoclonal mouse	1:100	DAKO, Carpenteria, CA
GFAP (Z0334)	Polyclonal rabbit	1:1000	DAKO, Carpenteria, CA

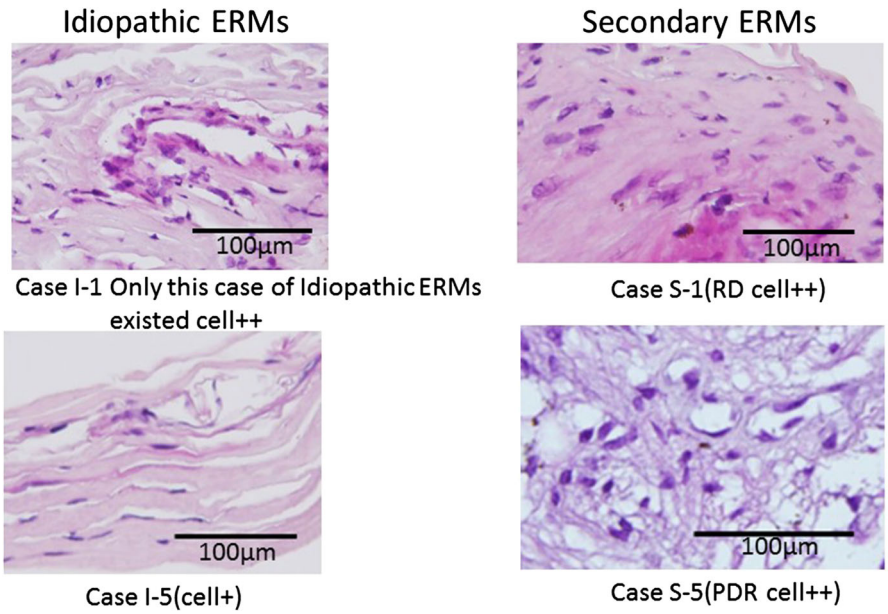

Fig. 1 Epiretinal membranes (ERMs) stained with HE Idiopathic ERMs: (*Top left*) Case I-1. Only this idiopathic ERM case showed the existence of cell++. (*Bottom left*) Case I-5 (cell+). Secondary ERMs: (*Top right*) Case S-1 [retinal detachment (RD) cell++]. (*Bottom right*) Case S-5 [proliferative diabetic retinopathy (PDR) cell++)

Table 2 Results of hematoxylin eosin (HE) and immunohistochemical analysis with nestin, Ki-67, CD34, and glial fibrillary acidic protein (GFAP) antibodies in idiopathic epiretinal membranes (ERMs)

Case	HE	GFAP	Ki67	CD34	Nestin
I-1	++	+	−	−	−
I-2	+	+	−	−	−
I-3	+	+	−	−	−
I-4	+	+	−	−	−
I-5	+	−	−	−	−
I-6	+	−	−	−	−
I-7	+	−	−	−	−
I-8	+	−	−	−	−
I-9	+	−	−	−	−
I-10	+	−	−	−	−
I-11	+	−	−	−	−

HE: ++ = more than 100 cells/field (×400), + = less-than 100 cells

GFAP: ++ = numerous positive cells, + = a few positive cells

CD34: + = the existence of positive cells without capillary formation cells

Nestin: ++ = most of the cells were positive, + = only a few positive cells

Table 3 Results of HE and immunohistochemical analysis with nestin, Ki-67, CD34, and GFAP antibodies in the secondary ERMs

Case		HE	GFAP	Ki67	CD34	Nestin
S-1	RD	++	+	+	−	+
S-2	RD	++	−	−	−	+
S-3	PDR	++	++	−	−	++
S-4	PDR	+	++	−	−	++
S-5	PDR	+	+	+	+	++
S-6	PDR	+	+	−	−	++
S-7	PDR	+	−	−	−	++
S-8	PDR	+	−	−	−	−

HE: ++ = more than 100 cells/field (×400), + = less-than 100 cells

GFAP: ++ = numerous positive cells, + = only a few positive cells

CD34: + = the existence of positive cells without capillary formation cells

Nestin: ++ = most of cells were positive, + = only a few positive cells

in the development of both ERM types; however, distinct differences of histological findings were found between idiopathic and secondary ERMs. RD ERM consisted of two types of cells derived from glia and RPE. Specimens with PDR likely consisted of endothelial cells forming a microvascular cavity.

The finds of this study also revealed that the secondary ERM specimens included a higher number of cells than in the idiopathic ERM specimens. Moreover, none of the idiopathic ERM specimens showed Ki-

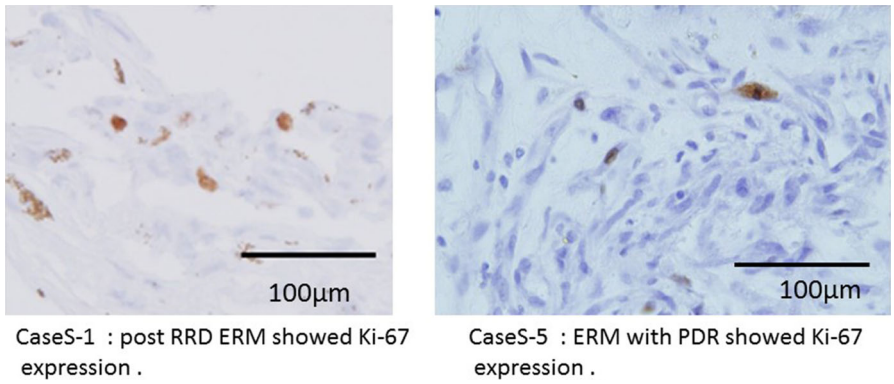

Fig. 2 Secondary ERMs stained with Ki-67. Case S-1: A post-RD ERM case showing Ki-67 expression (*left-side image*). Case S-5: A PDR ERM case showing Ki-67 expression (*right-side image*)

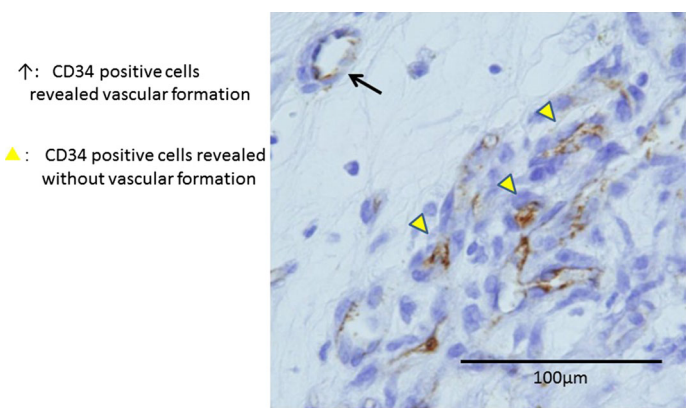

Fig. 3 Secondary PDR ERM stained with CD34 (Case S-5: PDR ERM). CD34 positive cells revealed vascular formation (*black arrows*). CD34-positive cells revealed without vascular formation (*yellow triangles*)

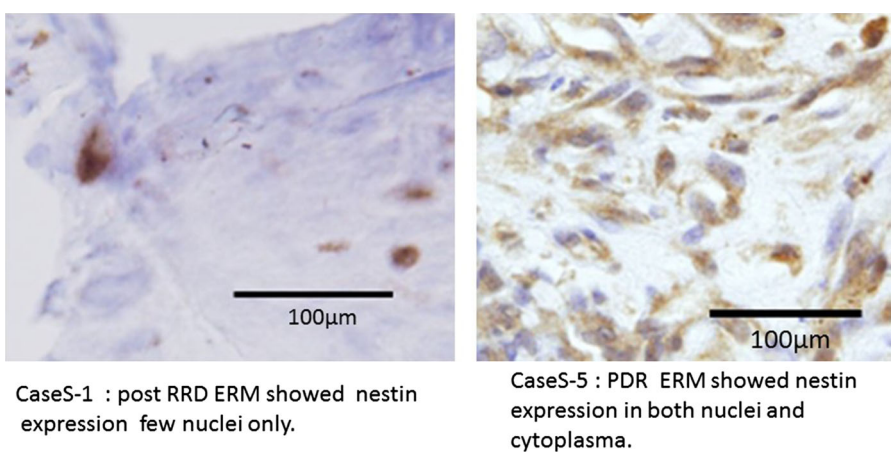

Fig. 4 Secondary ERMs stained with nestin. Case S-1: Post-RRD ERM showing nestin expression: only a few nuclei (*left-side image*). Case S-5: PDR ERM showing nestin expression in both nuclei and cytoplasm (*right-side image*)

67- or CD34-positive cells, yet 2 of the secondary ERM specimens were positive with Ki-67 and 3 showed CD34-positive cells. Interestingly, 7 of the 8 secondary ERM specimens included nestin-positive cells, while none of the idiopathic ERM specimens did. In addition, nestin expression in the PDR ERM specimens was stronger than in the RD ERM specimens.

The Ki-67 protein is a cellular marker that is strictly associated with cell proliferation [8]. During interphase, the Ki-67 antigen can be exclusively detected

within the cell nucleus, whereas in mitosis, most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (i.e., G1, S, G2, and mitosis), yet is absent from resting cells (G0). In the clinical setting, we often experienced that secondary ERMs were more aggressive (faster-growing) than idiopathic ERMs and the Ki67 expression data in this study mirrors those findings.

CD34 is a cluster of differentiation in a cell surface glycoprotein, and functions as a cell-to-cell adhesion factor. The CD34 protein is a member of a family of single-pass transmembrane sialomucin proteins that show expression on early hematopoietic and vascular-associated tissue [9]. Recent findings suggest that CD34 may also play a more selective role in chemokine-dependent migration of eosinophils and dendritic-cell precursors [10]. In the clinical setting, we experienced that compared with idiopathic ERMs, secondary ERMs are sometimes firmly stuck to the retinal surface during vitrectomy, and the high expression of CD34 in secondary ERMs might be related with those perioperative findings.

Nestin, a neural stem-cell marker, is reportedly mainly expressed in Müller glial stem cells during the embryonic stage [11–14]. In the adult human retina, nestin staining has shown that the ora serrata may be a growth or germinal zone equivalent to the anatomically similar ciliary margin zone (CMZ). The CMZ is a cell proliferation area that has been identified in numerous species, including fish, reptiles, birds, marsupials, and mammals, and gives rise to new retinal cells [15, 16]. The expression of nestin is also known to be upregulated under ischemic and traumatic pathological conditions in the adult rat retina [17–20]. It remains unclear why such changes occur in humans. In 2003, Mayer et al. reported the presence of nestin in not only the CMZ of the adult human retina, but also in the optic nerve head, the posterior retina, and ERMs [21]. However, it is not clear whether the ERMs in that report were idiopathic or secondary in nature.

In this present study, no nestin-positive cells were observed in all of the idiopathic ERMs, yet they were observed in 7 of the 8 secondary ERMs. If indeed there are a large number of nestin-positive undifferentiated cells in secondary ERMs, then this may be related to the onset and pathogenesis of secondary ERM, including RD ERM and PDR ERM.

In conclusion, the findings of this study indicate that there are different histological characteristics between idiopathic and secondary ERM and that the pathogenesis of secondary ERM might be involved to intraocular inflammation and/or retinal ischemic change.

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Compliance with ethical standards

Conflict of interest statement The authors have no conflicts of interest to declare.

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