Chapter 4

Glial Cells and Collagens in Epiretinal Membranes Associated with Idiopathic Macular Holes

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Abstract

Purpose: To investigate the identity of collagens and cellular components in the epiretinal membrane (ERM) associated with full thickness idiopathic macular hole (FTMH) and their clinical relevance.

Methods: Pars plana vitrectomy with peeling of the internal limiting membrane (ILM) and ERM was performed by two surgeons in 40 eyes with idiopathic macular holes. The clinical data were reviewed and the surgical specimens were processed for flat-mount and immuno-histochemical analysis.

Results: ERM is a GFAP-positive gliotic and fibrotic scar which contains newly formed collagen type I, III and V. Type VI collagen was not observed. Co-localization studies found cells co-expressing GFAP/CRALBP, GFAP/α-SMA, and α-SMA/CRALBP, which are consistent with transdifferentiation of Müller cells into fibroblasts and myofibroblasts. The clinically significant ERMs can be divided into two groups according to the amount of cells in the ERM: sparse cellular proliferation and dense cellular proliferation. The latter group is associated with a higher chance of surgical difficulty during ILM peeling ($p=0.006$). Pre and post-operative visual function were not affected by the density of the cellular proliferation.

Conclusion: Retinal glial cells, probably transdifferentiated Müller cells, are involved in the formation of FTMH-associated ERMs by a gliotic and fibrotic process. Such ERMs contain newly formed type I, III and V collagens depositions. The cell density of the ERM affects its biomechanical properties and determines the difficulty of ERM peeling.
Glial Cells and Collagens in Idiopathic Macular Hole

Introduction

During the development of an idiopathic full thickness macular hole (FTMH), a fibrocellular epiretinal membrane (ERM) on the inner surface of the inner limiting membrane (ILM) may occur as a secondary event. The contraction exerted by the ERM contributes to the enlargement of the hole, increasing the rigidity of the retina and preventing tissue approximation. The surgical technique for FTMH involves creating a complete posterior vitreous detachment (PVD) to release the anteroposterior traction and peeling of the ILM. In case of ERM formation, the latter maneuver will ensure the removal of the ILM and a complete removal of the associated ERM. This is beneficial in further improving the anatomical success rate by releasing tangential traction forces and restoring the flexibility of the retina for reattachment.

Moreover, the clinical data shows that ILM peeling carries a better primary anatomical outcome in later stages of FTMH, where the presence of ERM is more common. ERM associated with FTMH is an avascular fibrocellular proliferation which contains various types of cells and extracellular matrix (ECM) proteins. The cellular components include glial cells, fibroastrocytes, fibroblasts, myofibroblasts, hyalocytes, RPE cells and macrophages, which have been identified by electron microscopy and immunohistochemistry. While much attention has been paid to the cellular components, the origin and relationship of the epiretinal cells has not yet been clarified and the ECM components of the ERM such as collagens have not been fully identified. To date, only collagen types II and IV have been reported, and they are mainly native collagens of the vitreoretinal interface. Other collagens described in vitreous include type V/XI, IX and VI collagens. Recent advancements in matrix biology revealed that collagens not only provide a scaffold for cellular proliferation and migration, but they also bind to the cellular membrane receptors and play essential roles in fibrosis development, matrix
remodeling and cell signaling.\textsuperscript{17} Type I, III, and V collagens belong to the fibrillar collagen subfamily that mainly provides mechanical functions. These types of collagen are the major constituents of skin, blood vessels, muscles and fibrotic scar tissues, which depend on a collagen-rich matrix around the contractile cells for proper functioning, but they are not commonly present in the normal vitreoretinal interface.\textsuperscript{16,18} Type I, III and V collagens can regulate cell migration and proliferation through their interaction with integrin expressed on nerve and glial cell membranes.\textsuperscript{17,19} Type VI collagen belongs to the anchoring collagen subfamily and is present in vitreous and ILM.\textsuperscript{16,20} Besides its property to maintain the ECM structure in other tissues, type VI collagen is also involved in the transdifferentiation of fibroblasts to myofibroblasts, which is important in fibrosis, scar contraction and matrix remodeling.\textsuperscript{21,22}

We hypothesize that the collagens present at the vitreo-retinal interface and ERM affect the mechanical properties of these membranes and alter the adhesion of both tissues to each other and to their surrounding tissues, which may have an impact on visual function and on technical aspects of vitreo-macular surgery. To gain more insight in the pathogenesis of FTMH and ERM-formation in this disease process, we studied the identity of the collagens in FTMH-associated ERM and their relation to activated glial cells. We used immunohistochemistry on flat mounted specimens. We performed combined glial cell and myofibroblast marker labeling studies to pinpoint a link between part of the identified cells. This knowledge may be beneficial in developing pharmacological approaches to vitreo-macular interface diseases and in fine-tuning existing surgical therapies for FTMH.

**Material and Methods**

Tissue samples were collected from (1) human donor eyes and (2) patients during FTMH surgery. The human donor tissue was used to compare the intensity of the GFAP staining.
1. Donor eyes

Three human donor eyes from 2 donors (male, 38 and 65 years old) without a known history of ocular disease were obtained from the Cornea Bank in Beverwijk, the Netherlands. The eyes (without cornea) were dissected frontally through the equator into two halves. The vitreous was removed by scissors to gain access to the posterior pole. With the aid of Brilliant Blue G 250 (DORC, Zuidland, The Netherlands) and end-gripping forceps, the ILMs were peeled from the posterior pole from an area of about 2 disc diameters in size and processed for flat mount analysis and immuno-histochemistry.

2. Patients

Forty patients (26 females, 14 males) who were referred to the Ophthalmology Department of the University Medical Center Groningen for FTMH operation were involved in the study between October 2007 and October 2010. All included patients had been properly informed and had signed an informed consent form agreeing to the use of the ILM for research purposes. This procedure was approved by the Medical Ethical Committee of the University of Groningen and is in accordance with the tenets of the declaration of Helsinki. The mean age of the patients was 69.5 years (range 55 to 84). Macular holes were classified according to Gass as stage II (n=8), stage III (n=29), and stage IV (n=2). One patient was classified as stage II/III, since no preoperative optical coherence tomography (OCT) record was available and this patient had no complete PVD. At each patient’s first visit, a full ophthalmic examination was carried out. In addition, in most patients (39/40) an OCT was performed. We checked for the presence of a total PVD and/or an ERM by evaluating the preoperative OCT and the intra-operative findings as documented in the surgical report. Clinical data of the patients including the status of the lens, history of previous ocular disease, trauma, operations, pre- and post-operative best corrected visual acuity (BCVA) and the difficulty of ILM/ERM peeling
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were documented. Criteria of difficult ILM/ERM peeling were subjectively documented by the surgeons as: ① The presence of a strong adhesion between the ILM/ERM complex and the underlying retina or ② Fragility of the ILM/ERM complex.\(^{24}\)

3. General Procedures

The surgical technique involved a standard three ports trans pars plana vitrectomy, creation of a PVD if necessary and ILM/ERM peeling with the aid of indocyanine green. Peeling was performed with an end-gripping forceps, intending to remove the ILM or ERM plus ILM over an area of more than 1.5 disc diameters surrounding the macula. After fluid–air exchange, the vitreous cavity was perfused with a sulfur hexafluoride (SF\(_6\) / oxygen mixture to achieve 20% SF\(_6\) gas tamponade. Postoperatively, patients were recommended to maintain a face-down position for three days. All vitrectomy procedures were performed by two experienced vitreoretinal surgeons (V. W. Renardel de Lavalette and E.A. Huiskamp). The ILM samples were either immediately processed for flat mount analysis or stored in balanced saline solution at -80˚C until further use. The laboratory investigator was masked from the clinical records during the period of tissue processing and image analysis.

4. ILM/ERM flat mount and immunofluorescence

The ILM specimens were placed on a silicone elastomer (SYLGARD\(^{®}\) 184, Dow Corning, USA) coated petri dish in 200µl of 1% phosphate buffered saline containing 2% Tween 20 (Bio-Rad, Veenendaal, the Netherlands) (PBST). Under a stereomicroscope, the ILMs were flattened using fine glass sticks and pinned to the dish with stainless steel pins (Austerlitz Insect Pins\(^{®}\), Fine Science Tools Inc, California, USA). Subsequently, the flattened ILMs were fixed in 2% paraformaldehyde for 30 minutes, washed three times with PBST for 15 minutes, blocked with PBST containing 5% bovine serum albumin (BSA, Sigma, St. Louis, USA)
for 60 minutes and incubated overnight at 4°C with a mixture of two primary antibodies (both diluted to 1:200). Primary antibodies used include rabbit (polyclonal, Abcam, Cambridge, UK) or mouse (monoclonal, Sigma, St. Louis, USA) anti-glial fibrillary acidic protein antibody (GFAP, glial cell marker); rabbit anti-cellular retinaldehyde-binding protein antibody (CRALBP, Müller cell marker, UW55, a kind gift from J.C. Saari, University of Washington, Seattle, Wash. USA), mouse anti-α-smooth muscle actin (α-SMA, myofibroblast marker, monoclonal, Sigma, St. Louis, USA) and rabbit anti type I, III, V and VI collagen antibody (polyclonal, Abcam). Different combinations of antibodies were made, and only primary antibodies obtained from different hosts were combined in the same procedure. Then, the samples were rinsed three times with PBST for 15 minutes and incubated for four hours at 4°C with two fluorescent labelled secondary antibodies (diluted 1:200) combined with 4’,6-diamidino-2-phenylindole (DAPI, Sigma) (diluted 1:200). Secondary antibodies used include donkey anti-rabbit antibody conjugated with RedX, donkey anti-goat antibody with FITC and donkey anti-mouse with FITC conjugation (Jackson ImmunoResearch Laboratories, Inc. Pennsylvania, USA). After incubation, the ILMs were washed three times with PBST for 15 minutes and removed from the petri dish. Using a stereomicroscope, the ILMs were flattened on a glass slide in a drop of antifadent (AF1, Citifluor Ltd, London, UK) and sealed with a cover-slip. Negative control samples underwent the entire procedure, except for the application of the primary antibodies.

5. Photodocumentation and Statistical Analysis

A fluorescent microscope (Leica DMR, Wetzlar, Germany) and confocal laser scanning microscope (CLSM, Leica TCS-SP2, Wetzlar,) were used to document the samples. Image-J software was used to measure the cell counts and the area of the specimens. The data was analysed by PASW Statistics 18 (SPSS Inc., Chicago, IL). P<0.05 was considered statistically significant.
Table 2: Summary of the surgical samples and antibodies used in the study.

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Number of Samples</th>
<th>Positive expression / number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With ERM</td>
</tr>
<tr>
<td>GFAP</td>
<td>40</td>
<td>30/30</td>
</tr>
<tr>
<td>CRALBP</td>
<td>6</td>
<td>3/3</td>
</tr>
<tr>
<td>α-SMA</td>
<td>4</td>
<td>4/4</td>
</tr>
<tr>
<td>Type I Collagen</td>
<td>4</td>
<td>2/2</td>
</tr>
<tr>
<td>Type III Collagen</td>
<td>8</td>
<td>6/6</td>
</tr>
<tr>
<td>Type V Collagen</td>
<td>5</td>
<td>2/2</td>
</tr>
<tr>
<td>Type VI Collagen</td>
<td>10</td>
<td>0/9</td>
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</tbody>
</table>

**Results**

**1. General findings**

Thirty-six patients had a clear visualization of the fundus, which allowed the vitrectomy to be performed without lens extraction. Four patients had combined procedures consisting of vitrectomy and cataract extraction. The latter were excluded from the visual outcome analysis because their preoperative visual loss was caused by macular hole and cataract. The mean preoperative BCVA was 0.78 ± 0.33 logMAR units (Snellen VA=20/120). The mean postoperative BCVA at 3 months was 0.48 ± 0.30 logMAR units (Snellen VA=20/60) and 0.30 ± 0.28 logMAR units (Snellen VA=20/40) at final follow-up (range: 3 to 52 months). The primary closure rate was 97.5% (38/40). Two cases (1 stage II, 1 stage III) had a second surgery with intravitreal gas tamponade for persistent macular hole. There were no severe intra- or post-operative complications such as subchoroidal hemorrhage, infectious endophthalmitis or retinal detachment. Thirteen of the 36 sole vitrectomy patients had uneventful phacoemulsification and intraocular lens implantation because of cataract progression during the follow-up period.
According to the baseline clinical findings and the cellular density of the membrane, the cases could be divided into three groups. Group 1: no clinically significant ERM (10 cases), which had no evidence of ERM by preoperative binocular indirect ophthalmoscopy, OCT and intraoperative findings. In these cases, microscopic evaluation showed that the surgical samples only contained ILM with a mean cell density of 5 cells/mm$^2$ (range 3~7, SD 2 cells/mm$^2$). Groups 2 and 3 both had clinically detectable ERMs based on OCT and/or intraoperative findings. Of these 30 cases, 15 showed few GFAP positive cells, which is referred to as sparse cellular proliferation (Fig 1 A & C) and the other 15 contained a higher cellular density and a significant GFAP positive membrane, which is referred to as dense cellular proliferation (Fig 1 B & D). In twelve cases in groups two and three each, photographic quality was such that a cell density calculation could be performed. Mean cell density in sparse cellular proliferation was 37 cells/mm$^2$ (range 9~83, SD 32 cells/mm$^2$) compared with 389 cells/mm$^2$ (range 114~1244, SD 340 cells/mm$^2$) in dense cellular proliferation.

Between the three groups, no statistically significant difference was observed regarding preoperative and postoperative BCVA, rate of cataract surgery during the follow-up period and rate of macular hole reopening. Difficulties during ILM/ERM peeling were documented by the surgeons in 9 cases which all belonged to the dense cellular proliferation group. No peeling difficulties were reported in the rest of the surgeries (Table 1). With Fisher’s exact test, the presence of dense cellular proliferation was statistically significantly related to the occurrence of surgical difficulties ($p=0.006$), whereas a clinically detectable ERM as such was not significantly related to surgical difficulties ($p=0.081$).
Figure 1: ERM specimens of sparse and dense proliferation. Fluorescent microscopic images of ERMs with sparse (A & C) versus dense (B & D) cellular proliferation. DAPI stains nuclei (blue) and anti-GFAP stains glial cells (green).

Table 1: The classification of ERMs and clinical data

<table>
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<tr>
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<th>No ERM</th>
<th>Clinically significant ERM</th>
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<tr>
<td></td>
<td></td>
<td>Sparse proliferation</td>
</tr>
<tr>
<td>Number of cases</td>
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<td>15</td>
</tr>
<tr>
<td>Stage of MH</td>
<td>Stage II</td>
<td>Stage III</td>
</tr>
<tr>
<td>Number of cases</td>
<td>3</td>
<td>7</td>
</tr>
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<td>Difficult ILM peeling</td>
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<td>0</td>
</tr>
<tr>
<td>BCVA (logMAR)</td>
<td>0.84±0.29</td>
<td>0.76±0.27</td>
</tr>
<tr>
<td>Final VA (logMAR)</td>
<td>0.28±0.38</td>
<td>0.35±0.26</td>
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</table>

Thirty surgical FTMH cases were associated with a clinically detectable ERM, 15 of these showed massive cellular proliferation on IHC. Nine of these 15 cases caused difficulties in ILM peeling. There were no reports of surgical difficulties in the non-ERM or sparse cellular proliferation cases. a: Dense cellular proliferation is highly related to difficulties during ILM peeling (\(p=0.006\)), whereas the sparse cellular proliferation is not (\(p=0.081\)).

2. Immunohistochemical analysis of the surgical samples

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Donor ILMs

The ILMs appeared as transparent sheets under the microscope and showed only a few nuclei by DAPI-staining in the perimacular area (Fig. 2A). Two samples (donor 1 and 2) showed positive GFAP staining outside the central macular area, which represents the Müller cell end-feet (Fig. 2B).

![Figure 2: Overview of a donor ILM sample by merging individual images of donor 2 (female, 65 years). A: DAPI staining shows a few nuclei in the perimacular ILM (arrowhead) but not at the centre of the macula. The central dark area corresponds to the macular ILM (arrow). B: GFAP positive filaments (green) representing Müller cell end-feet are arranged in the perimacular area (arrows). Bar=1000µm](image)

All tested ERMs were positive for GFAP with varying intensity compared with the donor ILM (Fig. 2 and 3). In 12 cases, the mean ratio of GFAP-positive glial cells to total cell count could be determined and was estimated as 81% (range 48% to 100%).

Glial cells in the fibrocellular membrane

The cell type specific antibody combinations used for double labelling were: GFAP/CRALBP for activated Müller cells (n=4), GFAP/α-SMA for glia originated myofibroblasts (n=4) and CRALBP/α-SMA for Müller cell originated myofibroblasts. In ERM specimens, CRALBP positive cells, GFAP positive cells and cells co-expressing
GFAP and CRALBP were revealed. This indicates the presence of Müller cells, glial cells and activated Müller cells, respectively (Fig. 4A). Co-expression of CRALBP and GFAP was found in the direct vicinity of CRALBP positive cells. This finding suggests a dynamic process of Müller cell activation in the formation of ERM. Furthermore, some GFAP and CRALBP positive cells were found to be co-localized with α-SMA (Fig. 4 B & C), which suggests a transdifferentiation of glial cells and Müller cells to a myofibroblast phenotype. To confirm true co-localization (as opposed to false co-localization due to overlapping cells in different cell layers) some specimens (n= 2 for GFAP/CRALBP, n= 2 for GFAP/α-SMA and n= 2 for CRALBP/α-SMA colocalization, respectively) were checked by using confocal laser scanning microscopy and true co-localisation was confirmed (Fig. 4).

Figure 3: Glial proliferation on the ERM associated with the ILM. DAPI for staining of nuclei (blue), and anti-GFAP staining for glial cells (green). A & C: GFAP positive cells at the rim of the macular hole (stage 3 macular hole, 100x, bar=100µm). B:
Overview of a sample (stage 3) with extensive GFAP positive staining of the membrane on the vitreal side of the ILM, bar=50 µm). D: confocal laser scanning microscopy image shows GFAP positive filaments (bar=50µm).

Figure 4: Double labeling of flat-mounted ILM/ERM samples with GFAP, CRALBP and α-SMA. A: confocal laser scanning microscopy (CLSM) image of GFAP/CRALBP double labeling. Some cells only express CRALBP (red), some only GFAP (green) and some express both GFAP and CRALBP (yellow), indicating that a portion of the retinal Müller cells in the gliotic scar are activated. Bar=50 µm. B: Colocalization of α-SMA (green) and GFAP (red). Bar= 25 µm. C: CLSM study confirmed the co-localization (yellow) of α-SMA (green) and GFAP (red). Bar= 50 µm.

Collagens in the fibrocellular membrane

The ERMs were positive for type I, III and V collagens (Table 2). With the advantage of the flat-mount technique, the organization of the collagen fibres could be visualized. Type I collagen showed a typical fibrillar appearance with few branches, forming a network structure (Fig. 5A). Type III collagen was present as fine and solitary filaments which were only visible under 1000x magnification (Fig. 5B). Type V collagen had the appearance of a delicate meshwork (Fig. 5C). We did not find positive staining for type VI collagen in the ERMs.

Association of collagens and activated glial cells
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With the double labelling technique, we found that the newly formed type I, III and V collagens are frequently present in the vicinity of GFAP positive glial cells (Fig 5A & 6), suggesting that these cells are producing the collagens, digesting them or doing both.

**Figure 5:** Type I, III and V collagen in ERM.
A: confocal laser scanning microscopy image of ILM with fibrocellular membrane labeled with antibodies to type I collagen (red) and GFAP (green, glial cells), DAPI for nuclear staining (blue). Type I collagen positive fibers form a network (arrow) which is associated with the glial cells (arrow head). Bar=50µm. B & C: Fluorescent microscopy images of ERM stained for type III and V collagens (red). Left: Type III collagen is present as fine filaments (arrows). Bar=10µm Right: Type V collagen forms a network. Bar=25µm.

**Figure 6:** Colocalization of glial cells and collagens. Association of the newly formed collagens with GFAP positive cells (green). A: Type III collagen (red) bar = 25µm. B: Type V collagen (red) bar = 50µm. DAPI for staining of nuclei (blue).
Discussion

In this clinico-pathological study, our main findings are: 1. Dense cellular proliferation of ERMs associated with FTMH is associated with a higher chance of ILM peeling difficulty. 2. ERM-associated cell proliferation consists mainly of GFAP-positive cells, which probably represent activated Müller cells. 3. Müller cells can transdifferentiate into myofibroblasts, thus adding to membrane contractility. 4. Activated Müller cells are closely associated with the newly formed types I, III and V collagens in the ERMs, suggesting they are producing and/or digesting them.

In the present study, we found no evidence of a difference in baseline visual function, visual prognosis and rate of cataract surgeries among the groups with different severity of cellular proliferation in their ERMs. Seventy percent of the patients achieved a functional visual acuity of more than 20/40 with appropriate follow-up management. A growing body of evidence suggests that a vitrectomy combined with ILM peeling has an important clinical benefit regarding the anatomical closure rates in FTMH.\textsuperscript{25} Our results also indicate that the severity of the ERM formation does not affect the overall visual outcome of patients who underwent ILM/ERM peeling for FTMH.

In case of a clinically detectable ERM with a histologically dense cellular proliferation, significantly more cases were reported to be difficult during ILM peeling. The relationship between the denser cellular membranes and the observed increased surgical difficulty may be explained in two different ways. One hypothesis would be that cells from the ERM are responsible for a stronger adhesion between the ERM/ILM complex and the underlying inner retinal tissue. Alternatively, increased surgical difficulty itself could result in a deeper cleavage plane with significantly more damage to the superficial retina. The latter would be reflected in
a higher cellular density of the removed ERM/ILM/retina complex. Because of the large amount of cells and cell nuclei observed in dense cellular proliferation, one would expect the latter situation to result in a lower postoperative visual acuity. Since we did not observe a significant difference in postoperative visual acuity between the dense and the sparse cellular proliferation groups, this would argue against the second hypothesis.

We identified the presence of type I, III and V collagens in FTMH associated ERMs. These fibrotic collagens are able to form adhesions with the underlying retinal Müller cells via integrins on the cell surface. Adhesions will be facilitated in case of local thinning and/or local absence of the ILM because Müller cell extensions can then more easily penetrate the ILM.

Kenawy et al suggested that the epi- and intra-retinal gliosis and the upregulation of GFAP might cause the increased adhesion by strengthening the cell-collagen matrix interactions.

Besides an increased adhesion to surrounding tissues, newly formed collagens can alter the rigidity and digestibility of the ERM as well. Stalmans et al reported that the overall resolution rate of vitreo-macular adhesion following an intravitreal microplasmin injection was 8.7% in case of ERM formation and 37.4% in patients without an ERM. This report suggests that the efficacy of microplasmin could be decreased in the presence of an ERM. Fibrotic collagens have been found to contain higher amounts of hydroxyallysine derived cross-links compared to those of normal tissue. The elevation of hydroxyallysine cross-links in collagen fibers leads to an increase in mechanical strength and a reduction of collagen digestibility. Moreover, the upregulation of the intermediate filament, GFAP, has been found to increase the stiffness of reactive glial cells, which can further influence the mechanical properties of the ERM. Therefore, the presence of the resilient fibrotic collagens and rigid intermediate filaments at the vitreo-macular interface could form rigid and degradation-resistant fibrotic tissue and thereby compromise the
effect of proteolytic enzymes released during the matrix remodeling process or introduced into the vitreous cavity as an adjunctive during pharmacological vitreolysis. A more complete understanding of the molecular mechanisms of vitreoretinal adhesion in healthy and diseased vitreo-retinal interfaces will be beneficial for the further advancement of surgical and non-surgical approaches to vitreo-macular interface diseases.

Cells found in these ERM specimens may have originated from the posterior vitreous cortex, the ERM and the inner retinal layer.\textsuperscript{37, 38} The GFAP positive cells could be of Müller cell and hyalocyte origin.\textsuperscript{39, 40} Our study found supportive evidence for the active and dynamic involvement of Müller cells in the pathogenesis of FTMH and subsequent ERM formation. As the predominant cell type at the fovea centralis,\textsuperscript{41, 42} 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.\textsuperscript{39, 43, 44} We found that GFAP positive cells are consistently expressed in all FTMH-associated ERMs and we observed their co-localization with CRALBP and α-SMA. CRALBP is a well-accepted marker for normal adult human Müller cells, while GFAP is a hallmark of Müller cell activation.\textsuperscript{45} The presence of GFAP positive, CRALBP positive and GFAP-CRALBP positive cell clusters indicates that the formation of an ERM is a dynamic process involving the activation and transdifferentiation of Müller cells. Previously, Schumann et al\textsuperscript{38} reported that GFAP positive cells were the predominant cell type in the ERM of FTMH. They found that the GFAP positive cells were positive for CRALBP and hyalocyte markers and therefore suggested a possible Müller cell and hyalocyte origin of these cells. Both Müller cells and hyalocytes can transdifferentiate into a myofibroblast phenotype, thus playing an important role in matrix production and contraction.\textsuperscript{46, 47} However, these authors did not find a co-localization of GFAP and α-SMA in their ERM specimens. Reasons for these slightly
different observations can be: (1) although the co-localization of GFAP and α-SMA was present in all four tested samples in our series, the number of cells that simultaneously express both antigens was small and (2) transdifferentiation is a dynamic process, therefore cells co-expressing both markers may be absent at the time the ERM is peeled.

A possible limitation of our study might be that markers such as GFAP and CRALBP stain the entire cell body. Therefore, cells from different cell layers overlying each other could mimic co-localization. To avoid a misinterpretation, we checked a number of samples by using confocal scanning laser microscopy and confirmed true co-localization for α-SMA/GFAP, α-SMA/CRALBP and GFAP/CRALBP.

Conclusions

We found that FTMH-associated ERMs are mainly formed by gliotic tissue and contain newly formed type I, III and V collagens depositions. The formation of such ERMs is due to a gliotic and fibrotic process, where retinal glial cells, probably the Müller cells, play an active role by migration, proliferation and transdifferentiation. The biomechanical properties of the newly formed collagens in the ERM and the density of the associated cells can induce an enhancement of the vitreo-retinal adhesion. This may affect the ease of ERM peeling and it will probably also affect the potential success of pharmacological vitreolysis. A better understanding of the underlying mechanisms of vitreo-retinal adhesion in pathological conditions can help to optimize therapeutic strategies.
References


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