*LETTER TO THE EDITOR*

# UPDATE ON THE CELLULAR, GENETIC AND CYTOKINE BASIS OF EPIRETINAL MEMBRANE PATHOGENESIS

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To the Editor,

Epiretinal membrane (ERM) is a layer of pathologic tissue which grows on the internal surface of the retina at the vitreoretinal interface (1). It was first described in 1865, and it can be either idiopathic, or secondary to some pathologic conditions (2). The progression of a secondary ERM is generally less aggressive and seldom causes traction retinal detachment as do primary ERMs (3). ERM consists of an inner cellular layer with one or multiple cell layers and an outer extracellular matrix (ECM) layer that contains bundles of extracellular fibrils, usually randomly oriented (1).

ERMs are characterized by a number of pathological changes that occur in the vitreoretinal junction and have varying degrees of clinical significance (4). On ophthalmoscopic examination, ERM presents as an obvious transparent, translucent, or even pigmented membrane, or as a glistening light reflection if it is thin. Although peripheral ERMs do exist, it is the macular ERMs that are, visually, more disturbing (1, 2). ERMs can involve the macular or perimacular regions and cause a reduction in vision, blurred vision, micropsia, metamorphopsia and occasionally monocular diplopia (2). They may also cause vitreoretinal traction or even tractional retinal detachment. The symptoms felt by patients can differ, depending on the duration and severity of ERMs (1). The incidence and the prevalence of ERMs increases with age. The ERM incidence is estimated close to 20% for the total population by the age of 70. The prevalence of macular ERMs in individuals under the age of 60 years is estimated at 2% and in individuals over 70 years at 12%. Other population studies indicate an overall prevalence of 7% to 11.8%, and a 5-year incidence of 5.3% (2). The purpose of the present mini review is to concentrate on the genes, cells and cytokines that are involved in the ERM pathogenesis, idiopathic and secondary.

## *Factors affecting prevalence*

Of interest is the great discrepancy in the prevalence of idiopathic ERM among different ethnic groups which ranges from 1.02% to 28.9%. The prevalence of idiopathic ERM in the United States and Australia is higher than that in Asia, which indicates that genetic and lifestyle factors may play a role in ERM occurrence. Finally, the observed

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higher prevalence of idiopathic ERM in diabetes, hypercholesterolemia, and vascular narrowing or occlusion indicates the possible involvement of metabolic factors (1). However, despite its high prevalence, the pathophysiology of ERM has not been fully elucidated (2).

*Types of ERMs*

Depending on the initiating event of the ERM formation, ERMs can be classified as idiopathic or secondary. Fibro glial proliferation secondary to a break in internal limiting membrane (ILM) occurring during the process of posterior vitreous detachment is mainly associated with idiopathic ERM formation (5). On the other hand, secondary ERM results from an already existing ocular pathology such as proliferative diabetic retinopathy (PDR), retinal breaks, retinal detachment, central or branch retinal vein occlusion, proliferative vitreoretinopathy (PVR), ocular trauma, ocular inflammation (e.g., uveitis) and high myopia (5, 6). In addition, several ocular surgeries, such as lensectomy and retinal detachment surgery or argon laser photocoagulation, may also be associated with ERM formation. Nonetheless, as idiopathic and secondary ERM are characterized by different primary formation mechanisms, the features of these membranes may also differ (3, 5). Moreover, the molecular mechanism formation of both primary and secondary ERMs is still poorly understood (1).

*Theories of ERM pathogenesis*

Based on the evidence presented, the pathogenesis of ERM is associated with the migration of glial cells that are derived from retinal tissue to the surface of the retina and arise from microdefects in the ILM that occur during posterior vitreous detachment (PVD) (7). Another theory proposes that the pathogenesis of ERM is associated with the growth and fibrous metaplasia of the vitreous cells that remain on the retina surface after PVD. A third theory proposes that the retinal pigment epithelial (RPE) cells migrate to the vitreous cavity through the retinal break and settle on the retinal surface, forming the ERM that occurs after rhegmatogenous retinal detachment (2,7).

*Cells and cytokines involved in ERM pathogenesis*

ERMs are characterized by the cellular migration and proliferation on the inner retinal surface (1), ECM formation and tissue contraction (4). Histopathological studies demonstrated that various cell types such as RPE cells, glial cells, endothelial cells, fibrocytes, fibrous astrocytes, macrophages and myofibroblast-like cells, as well as trophic and transcription factors may be associated with the formation of ERMs, although the pathogenic mechanisms are still unknown. RPE cells are hexagonal quiescent cells that do not proliferate or migrate under physiological conditions and represent various cell types including glial cells that are involved in the fibrotic reaction of the detached retina (4, 6). These cells form a scar-like thin layer as an immune response to protect the retina (6).

Another type of cells, especially in secondary (PDR) ERM, are fibroblasts, whose origin is still not fully elucidated, but they synthesize the ECM. Moreover, they construct the structural framework of the membrane and cause tractional retinal detachment (8).

ERM progression is considered to be a fibrotic process due to the increased collagen deposition and membrane contraction. Collagen types I, II, III, IV, V, and VI are important components of ERM indicating the vital role of collagen in ERM construction. Type VI collagen in particular, which forms a fine fibrillar network in idiopathic ERM, has been identified as a crucial component of ERM (9).

Müller cells are also considered to have a central role in ERM contraction by producing various collagens and expressing a-SMA which is also involved in the membrane contraction (9). Müller cells are actively involved in idiopathic ERM formation by migration, proliferation, and transdifferentiation. Müller glial cells (MGCs) acquire migratory ability and exhibit a fibroblast-like phenotype, contributing to the formation of ERM, stretching the retina and causing retinal detachment and vitreous hemorrhage. MGCs also contribute to the diabetic ERM formation, a progress associated with reactive gliosis, fibrosis, and migration (8). Laminocytes are also another fundamental cell type involved in the pathology of idiopathic ERMs and

are dispersed in reduced numbers in eyes containing a PVD (4).

Both retinal Müller cells and hyalocytes can produce and activate transforming growth factor beta (TGFb) during the idiopathic ERM formation. TGFb upregulates the expression of a-SMA in the epiretinal cells, contributing to the ERM contraction. The retinal Müller cells that express a-SMA are responsible for the membrane contraction while the retinal Müller cells that do not express a-SMA could continue their production of collagens to form the ECM network of the idiopathic ERM (9). There is also evidence that during the formation of ERM cytokines, ECM proteins and growth factors may be involved in the intracellular signaling and histologic changes (6).

Therefore, cytokines, like insulin-like growth factor (IGF), are involved in the progression of ERMs. The IGF system which is associated with inflammation process, is also involved in the stimulation of ERM contraction and it is suggested that growth factors are involved in the progression of ERMs, especially during PDR (1, 4). Older reports indicated that specific cytokines play a role in ERM formation given that they are expressed in idiopathic ERMs. Among them are the vascular endothelial growth factor (VEGF), interleukin-6 (IL6), TGFb, the connective tissue growth factor, chemokine CCL2 and tumor necrosis factor alpha (TNFa) (4). On the other hand, other T-cell cytokines or T-cell-associated factors, such as IFNG, RORC, IL17A and TBX21, have no correlation with ERM formation (6).

*Genes involved in ERM pathogenesis*

Biological events are due to changes in the expression of crucial genes and extensive changes take place in gene expression, during the onset and progression of diseases. By comparing gene expression profiles under different conditions, genes that play crucial role in a particular disease process can be identified (3). Thus, analyzing gene expression can enhance the understanding of the formation of ERM. Tables I and II summarize the genes, cells and cytokines involved in Idiopathic and secondary ERM, respectively.

There are many studies about the expression of a variety of genes in ERM, idiopathic or secondary (6). There are reports which mention that at least 52 genes are highly represented in either PVR-ERMs or other secondary ERMs. 23 genes are expressed at higher levels in the PVR-ERMs and 29 genes at higher levels in other secondary ERMs, while, especially, 4 genes, ZNF713, FN1, MALAT1 and PARP8 are abundantly represented in secondary ERMs. Genes related to proliferation and cell adhesion are highly expressed in secondary (PVR) ERMs, while in other secondary ERMs genes related to ribosomes, metabolism, and signaling are preferentially upregulated. Ten of the cell adhesion genes that are expressed in secondary (PVR) ERMs are COL1A1, COL1A2, COL3A1, LGALS1, POSTN, SPARC, THBS1, TIMP3, FN1 and DCN. These observations are in good agreement with morphological studies indicating that the amount of ECM in ERMs is positively correlated with the disease process, and it is greater in secondary (PVR) ERM than in slowly progressing ERM. There are also several genes related to proliferation, (e.g., MALAT1, CD320, SERPINE1 and STAT3) that are upregulated in secondary (PVR) ERMs, while there are possible relationships to other genes/ proteins including an additional 60 genes that have not been detected in secondary ERMs (3).

The MALAT1 gene is also associated with ERM formation. It is a long, non-coding RNA that regulates the processing pre-mRNAs in mammalian cells and plays an important role in the progression of secondary (PVR) ERM. Apparently, it is the most highly expressed gene in secondary ERM. It regulates cell motility through the concomitant regulation of the expression of motility-related genes by transcriptional and/or post-transcriptional regulation. It is also associated with metastasis. MALAT1 has not been shown to be synthesized by secondary (PVR) ERM. Nonetheless, the need for extensive migration of secondary ERMs on the retina explains the strong expression of MALAT1 in secondary ERMs (3).

The expression levels of RELA (v-rel avian reticulo endotheliosis viral oncogene homolog A), GFAP (glial fibrillary acidic protein), TNC (tenascin C) and other cytokine-encoding genes such as IL6, TGFb2, VEGFA, and CXCL1 (chemokine C-X-C

motif ligand 1) are increased, especially in idiopathic ERM eyes irrespective of sex. RELA is a subunit of the nuclear factor kappa B (NF-κB) heterodimer, a transcription factor that can be activated by various stimuli, like bacterial and viral infections, hypoxia and proinflammatory cytokines, including IL1β and TNFα, which play a vital role in inflammation, immune response, cellular proliferation and apoptosis. NF-κB and IL8 are expressed in the vascular endothelial and glial cells and are also associated with ERM formation (6). Finally, it is possible that advanced age is associated with upregulation of RELA in endothelial and glial cells and in ERM formation.

Numerous NF-κB target genes, including proinflammatory cytokines, such as TNFα, IL1β

**Table I.** *Idiopathic ERM. Genes, cells and cytokines involved in pathogenesis*

| **GENES** | **Cells** |
|---|---|
| CXCL1 (6) | Müller glial cells (8) |
| | Laminocytes (4) |
| | Hyalocytes (9) |
| GFAP (6) | Collagen type II (9) |
| IL6 (6) | Collagen type IV (9) |
| RELA (6) | Collagen type I (9) |
| TGFb2 (6) | Collagen type V (9) |
| TNC (6) | Collagen type III (9) |
| VEGFA (6) | Collagen type VI (9) |

**Table II.** *Secondary ERM. Genes, cells and cytokines involved in pathogenesis*

| **GENES** | **Cells** | **Cytokines** |
|---|---|---|
| CD320 (3) | | |
| COL1A1 (3) | Fibroblasts (8) | Growth Factors (9) |
| COL1A2 (3) | Müller glial cells (8) | IGF (1,4) |
| COL3A1 (3) | | TGFβ2 (9,12) |
| DCN (3) | | Other Cytokines (9) |
| FN1 (3) | | TGFb (11) |
| LGALS1 (3) | | VEGF (11) |
| MALAT1 (3) | | Fibrogenic (11) |
| NF-κB mRNA (10) | | |
| POSTN (3) | | |
| PPM1D (10) | | |
| SERPINE1 (3) | | |
| SP1 mRNA (11) | | |
| SPARC (3) | | |
| STAT3 (3) | | |
| TGFb2 (6) | | |
| THBS1 (3) | | |
| TIMP3 (3) | | |

and IL6 (which are important contributors to the pathological process of DR and to the formation of ERMs) can be upregulated by activated glial cells. NF-κB can also contribute to the proliferation of glial cells. It is noteworthy that patients with secondary (PDR) ERMs have significantly higher NF-κB mRNA expression levels than patients with idiopathic ERMs.

Secondary (PDR) ERMs are related to the expression of Wild-type p53-induced phosphatase 1 (Wip1), which is encoded by the PPM1D gene and plays a key role in stress signaling, cell cycle progression, cell survival, inflammation and proliferation. However, although Wip1 may be associated with ERM formation, little is known about this relationship (10). SP1 mRNA is also highly expressed in secondary (PDR) ERM, while the SP1 protein is mainly co-localized with VEGF. SP1 regulates the promoter activity and expression of genes encoding angiogenesis-related factors and thus plays an important role in the angiogenesis of PDR, highlighting the role of this gene in ERM. SP1 can regulate the expression of a wide variety of genes, including TGFb, VEGF, fibrogenic cytokine, and many matrix genes. These cytokines can be detected in the vitreous fluid and secondary ERMs, especially these obtained from patients with PDR (11).

The expression levels of pro-inflammatory genes and the genes expressed during angiogenesis and wound healing process, like IL6, TGFb2, VEGFA, CXCL1, RELA, GFAP, and TNC are significantly higher in eyes with idiopathic ERM. Moreover, there is a positive correlation between TGFb2 and IL6, CXCL1 or RELA. TGFb2, which is associated with intraocular fibrosis, is upregulated in eyes with idiopathic and secondary ERM. It induces the transformation of hyalocytes, myofibroblastic cells and RPE cells.

The expression of TGFb1 is also elevated in ERM vitreous, but only the expression of TGFb2 is increased in idiopathic ERM. In addition, the correlation of TGFb2 is more significant in the case of idiopathic ERM than that of TGFb1. In the presence of TGFb2 there is strong contractile activity of collagen gels and αSMA (ACTA1) overexpression in hyalocytes. ERM foci are composed of αSMA-positive cells with a periphery of GFAP-positive cells (6). Thus, the expression level of ACTA1 (the gene encoding αSMA) may be very low. In addition, the gene expression levels of VEGFα seem to be significantly higher in ERMs (3).

Other reports suggest that GFAP-expressing glial cells (Müller cells or astrocytes) are associated with idiopathic ERM. Additionally, retinal glial cells were reported to produce VEGF. Furthermore, there is an apparent association between VEGFA and GFAP gene expression levels in idiopathic ERM eyes. TNC, a gene that encodes the extracellular protein TNC, is involved in inflammation, wound healing, and the sprouting of endothelial cells during angiogenesis. So it is possible that TGFb2 and three factors (IL6, CXCL1, and NF-κB) stimulate GFAP positive cells related to fibrosis, while angiogenic factors (VEGFA and TNC) may augment idiopathic ERM formation.

The levels of genes encoding cytokines also seem to change in ERM eyes. As mentioned above, genes like TGFb2, CXCL1, GFAP, VEGFA, IL6, RELA and TNC seem to be significantly upregulated in the idiopathic ERM eyes, while ACTA1 and IL17A are not detected in the irrigation solution obtained from idiopathic ERM. Finally, the levels of IFNG, CCL2, CCL5, CXCL10, TGFb1, TNF, TBX21, STAT3, RORC and POSTN don't differ in idiopathic ERM (6).

*Management of ERM*

In recent years, there has been much debate about the management of idiopathic and secondary ERMs, whereas it seems that several cytokines and genes may be therapeutic targets in the future. At present the options are limited and consist of prevention, watchful waiting or vitrectomy surgery, especially in idiopathic ERMs (1, 12). However, it is not known whether the surgery for idiopathic ERMs should be aimed at an early stage with minimal symptoms or it can be safely delayed. Vitrectomy and ERM peeling have been used in patients with symptomatic visual disturbances. Nonetheless, there are cases that ERMs recurred and a second surgical intervention is needed, mainly because of incomplete removal. Thus, the removal of ILM in concert with ERM peeling may be beneficial but remains controversial (12). Moreover, there are several pharmaceutical

treatments including the use of anti-inflammatory and vitreolytic agents that improve the visual function and prevent complications of idiopathic ERMs (1, 12). In addition, as the formation of ERMs is associated with the procedure of fibrosis the verification of the role of fibrosis may contribute to find new treatment options (1).

The emergence of certain genes and cytokines as important factors for the development and progression of ERMs has led to the development of new, modern therapeutic methods. Thus, therapeutic agents have been created against targets such as TGFβ2, which has a crucial role in the formation of both idiopathic and secondary ERM, to prevent the formation and progression of ERMs. Nonetheless, further study is needed to develop more effective treatment methods targeting several genes and cytokines (9, 12).

## CONCLUSIONS

As mentioned above, of the three main theories proposed for the pathogenesis of ERM, two propose cellular migration and one cellular proliferation as the relevant mechanism. This is the reason the present mini review concentrated on the genes, cells and cytokines that are involved in the ERM pathogenesis.

Although the issue of ERM pathogenesis is still not resolved, certain features of ERM are already apparent from the findings to date: i) With the exception of the TGFb2 (5) gene there is no overlap between the genes identified so far as being involved in idiopathic and secondary ERM (Tables I and II). This may imply that the pathogenesis of each of these forms of ERM may be of different origin although they may share similar pathways of expression; ii)Although Müller glial cells and fibroblasts or collagen cells are involved both in idiopathic ERM and secondary ERM, based on our present knowledge, in each case, they use different sets of cytokines; iii) Very little is known about the pathogenesis of secondary ERM (Table II) or what triggers its pathogenesis.

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