Published in final edited form as:

Curr Eye Res. 2011 June ; 36(6): 546–552. doi:10.3109/02713683.2011.561468.

Epithelial Membrane Protein-2 (EMP2) and Experimental Proliferative Vitreoretinopathy (PVR)

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Abstract

Purpose—Proliferative vitreoretinopathy (PVR) is believed to result in part from de-differentiation of retinal pigment epithelium (RPE) with cellular migration in the vitreous cavity, membrane formation, and contraction in an aberrant wound-healing strategy. In an *in vitro* collagen-gel contraction assay, epithelial membrane protein 2 (EMP2) controls contraction through activation of focal adhesion kinase (FAK) in a RPE cell line (ARPE-19). The purpose of this study was to investigate how blocking or altering the level of EMP2 expression changed clinical PVR in an *in vivo* model.

Methods—Using the ARPE-19 cell line, the levels of EMP2 modulated through stable transfections of an EMP2 overexpressing construct, EMP2 ribozyme, or vector alone. These transfected cell lines were used in a rabbit model of PVR. The severity of PVR was classified by two masked observers. An EMP2 blocking antibody was also used to decrease functional EMP2 in the PVR model. Immunohistochemistry was used to evaluate EMP2 expression *in vivo*.

Results—The transfectants with lower levels of EMP2 had significantly less PVR severity than the degree of PVR induced by wild-type cells ($p = 0.05$). Also, the transfectants with a low-level of EMP2 expression showed a strong trend of less PVR severity than the high-levels EMP2 transfectants ($p = 0.06$). Blocking EMP2 with a specific polyclonal antibody significantly decreased the level of PVR severity ($p = 0.02$). PVR membranes were found to be positive for EMP2 expression.

Conclusions—These *in vivo* studies support a direct correlation between EMP2 expression and severity of PVR. These results validate the potential for controlling RPE biology through a change in EMP2 expression, and provide a potential therapeutic target for this disease.

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Declaration of interest: US Patent Application No. 61/263,228: Epithelial Membrane Protein-2 (EMP2) and Proliferative Vitreoretinopathy (PVR); Filed 11/20/09.

Keywords

Epithelial membrane protein 2; Fibrosis; Integrin regulation; Proliferative vitreoretinopathy; Retina; Rhegmatogenous retinal detachment; Retinal pigment epithelium; Therapy; Vitreous membranes

INTRODUCTION

The most common reason for failure in the therapy of retinal detachment or open globe injuries is the proliferation and contraction of cellular membranes that form in the vitreous cavity, termed proliferative vitreoretinopathy (PVR).¹ It is estimated that PVR occurs as a complication of up to 5–20% of cases of rhegmatogenous retinal detachment.^{1,2} In ocular trauma, the risk of PVR is very high. A review of veterans who had suffered ocular trauma with intraocular foreign bodies in Iraq during the years 2003–2005 revealed PVR as the cause for poor vision in 21% of the patients.³

Clinical studies have identified multiple risk factors for the development of PVR, including vitreous hemorrhage, intraocular gas, large or long-standing retinal detachments, and surgical failures, especially if they are associated with extensive use of cryotherapy or photocoagulation.^{1,2} It is possible that the extent of ocular/retinal damage leads to increased cytokine production or other signaling to cause increased PVR. Cytokines, such as IL-8, MCP-1, IL-1, IL-6, TNF- α , and IFN- γ , have been found to be increased in PVR; however, cytokine levels do not directly correlate with PVR severity.⁴

The retinal pigment epithelium (RPE) is believed to be one of the critical cell types implicated in PVR. Pathogenesis underlying PVR is complex; however, it is likely that, following trauma or retinal detachment, RPE cells are released into the vitreous or are stimulated to migrate from their subretinal location. These cells then migrate, proliferate, de-differentiate, and undergo an epithelial-to-mesenchymal transformation (EMT), to help create the preretinal membranes of PVR.⁴ It is likely that the RPE cells produce membrane contraction that generates tractional forces that often lead to recurrent retinal detachments and additional vision loss.^{2,5}

Control of the RPE and prevention of membrane contraction is a primary goal in the prevention of the devastating PVR response. In an *in vitro* experimental model, our laboratory identified activation of focal adhesion kinase (FAK) through ligation of integrins ($\alpha 1$, $\alpha 2$, and $\alpha 3$) as a critical control point for collagen gel contraction.⁶ The tetraspan (4-TM) superfamily is a key class of proteins that determine the types of intracellular trafficking and signaling molecules assembled with integrins and other receptor complexes.⁷ The particular tetraspan family members are highly controlled in individual cell types and states of differentiation or activation. Accordingly, combinatorial expression of tetraspans is an important contributor to integrin deployment and signaling quality. Epithelial membrane protein-2 (EMP2) is a tetraspan family member, which acts to control specific integrin deployment and signaling through FAK.⁸ EMP2, highly expressed in RPE,⁹ is a member of the growth arrest specific gene 3/peripheral myelin protein 22 (GAS3/PMP22) 4-TM protein family with distinctive biochemical and physiological roles.^{9–15}

In this study, we used the rabbit model of PVR to study the role EMP2 expression plays in the development and progression of PVR and retinal detachment. We found that decreased expression of EMP2 or blocking EMP2 by antibodies resulted in decreased PVR formation.

METHODS AND MATERIALS

Antibody Reagents

Rabbit polyclonal anti-human EMP2 antibodies were produced following immunization of animals with a multiple antigen peptide conjugated to the second extracellular loop of human EMP2 (EDIHDKNAKFYPVTREGSYG) (Research Genetics, Huntsville, Alabama, USA).⁹ The polyclonal rabbit anti-human EMP2 antibody has been found to be cross-reactive with rabbit EMP2 (unpublished data).

Cell Lines

Stable ARPE-19 Cell Line—ARPE-19 is a spontaneously arising human retinal pigment epithelia (RPE) obtained from ATCC (CRL-2302). ARPE-19 cells were transfected with one of the following constructs: pEGFP-N3 = vector control, pEGFP-N3-EMP2 = EMP2 over-expressing construct, pEGFP-N3-Ribo1 = EMP2 reduced expression construct. ARPE-19 cells were plated 24 hr prior to transfection. The cells were transfected with one of the above constructs using FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, Indiana, USA). Stable clones were selected using Geneticin (700 mg/ml, Invitrogen Life Technologies, Carlsbad, California, USA); these stable cell lines are referred to as ARPE-19/V, ARPE-19/EMP2, or ARPE-19/Ribo. Expression of EMP2 by these cell lines was previously reported.⁸

Growth Media—ATCC Medium #30–2006: A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium containing 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES, and 0.5 mM sodium pyruvate, 90%; fetal bovine serum #30–2020, 10%.

Collagen Gel Contraction—Collagen gel contraction assays were performed as previously reported.⁶ Briefly, collagen gels were prepared by combining collagen type I (BD Biosciences, San Diego, California, USA), 10× DMEM, and DMEM/F12. The final concentration of the collagen type I mixture was 2.5 mg/ml. Five hundred μ l of the collagen solution was added to each well of a 24-well plate and incubated at 37°C in 5% CO₂ for 1 hr. Cultured ARPE-19 cells were harvested and resuspended in serum-free DMEM/F12 at a final concentration of 5×10^5 /ml. ARPE-19 cells were pretreated with anti-EMP2 or control antibody for 2 hr at a 1:10 dilution. These experiments used serum, and a specific antibody concentration for anti-EMP2 was not determined. Serum concentration was used that provided an optimum response *in vitro*. However, given that the standard IgG concentration for rabbit serum is 5–10 mg/ml, we used a 1:10 dilution (0.5–1.0 mg/ml) of anti-EMP2 polyclonal antibody and preimmune control antibody to pretreat the ARPE-19 cells.¹⁶ The cells are then seeded onto the collagen gel at a concentration of 2.5×10^5 cells per well and the percent contraction was measured at 24 hr. The area of each gel was obtained by taking a picture of the gel using image capture (Gel Doc, Bio-Rad, Hercules, California, USA) and quantified using NIH Image J (Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA) software. To measure the area of the gel, the oval measuring tool was used to outline each gel. The area of the gel at time zero was compared to the area of the gel after 24 hr, generating a percent contraction for each sample. Each experiment included at least six replicates, and at least three independent experiments were performed with comparable results. A Student's *t*-test (unpaired, two-tail) was used and a *P* < 0.05 was judged to be statistically significant.

Animal Model of PVR—Rabbits used in this study were treated in accordance to institutional guidelines approved by the UCLA Institutional Review Board. PVR was induced only in the right eye of albino New Zealand rabbits as previously described.¹⁷

Briefly, using a 30-gauge needle 0.4 ml of C3F8 gas (100%) was injected into the vitreous cavity 4 mm posterior to the corneal limbus after anesthesia was induced. Anterior paracentesis was performed with a microblade to prevent elevated intraocular pressures. Two days later ARPE-19 cell preparations re-suspended in growth media were injected in a volume of 0.1 cc. The cells were either wild-type, modified ARPE-19 cells over-expressing EMP2 or under-expressing EMP2 by ribozyme transfection.⁸ EMP2 expression in transfected cells was determined by Western blot immediately prior to use. In blocking experiments, ARPE-19 cells were pre-incubated with polyclonal anti-EMP2 at 1:10 for 1 hr prior to intraocular injection. The cells were injected with the antibody solution into the vitreous of the right eye. The left eye served as a control. The rabbits were examined weekly in a masked fashion by indirect ophthalmoscopy for PVR development and inflammation. At 5 weeks the animals were sacrificed using Pentobarbital (100 mg/kg). Globes were placed in 10% formalin and a superior pars plana incision was made with a #11 blade to allow access of the fixative in the intraocular space. The globes were fixed for 3 days, bisected, and scored in a masked fashion for gross pathology using the PVR scale established by Fastenberg (Table 1).^{18,19} The globes were embedded in paraffin, sectioned, and then stained with hematoxylin and eosin.

Statistical Analysis

A Student's *t*-test (unpaired, two-tail) was used and a $P < 0.05$ was judged to be statistically significant.

Immunohistochemistry was done to detect EMP2 expression. Paraffin sections were rehydrated and blocked with 5% NGS followed by Rabbit to Rabbit block (ScyTek Laboratories, Inc., Logan, Utah). Sections were incubated with primary antibody (polyclonal rabbit anti-human EMP2 at a dilution of 1:2000). After TBS-T washes, sections were incubated with a Vectastain Rabbit IgG Elite ABC Kit (Vector Labs Burlingame, CA), followed by additional washes and visualized with Vector VIP (Vector Labs). Methyl Green (Vector Labs) was used for nuclear staining.

RESULTS

Anti-EMP2 Polyclonal Antibody Blocks ARPE-19 Gel Contraction

An *in vitro* correlate of PVR, collagen gel contraction assay, was used to determine the inhibitory concentration of the anti-EMP2 antibody. In this assay, polyclonal anti-EMP2 at 1:10 significantly blocked gel contraction by the ARPE-19 cells compared to control antisera (Figure 1).

Increased PVR Formation with Increased EMP2 Expression

Using the well-characterized rabbit model of PVR, we induced PVR with intravitreal injections of ARPE-19 cells as described.¹⁷ The cells were either wild-type, modified ARPE-19 cells over-expressing EMP2 or under-expressing EMP2 by ribozyme transfection.⁸ Eyes were graded for the degree of PVR formation by clinical and gross examination. Clinical examination by indirect ophthalmoscopy by a masked observer occurred weekly after injection. At the termination of the experiment, 5 weeks after injection, two masked observers conducted a gross examination. Clinical evaluation revealed increased EMP2 expression, which led to earlier onset of PVR severity as compared to wild type cells (Figure 2A). Of note, the ribozyme group had only trace PVR at all early time points tested (day 11 and day 19). Rabbits injected with EMP2 over-expressing cells had significantly more PVR at week 3 than wild type cells ($p = .02$) (Figure 2A). By week 4, the control cells established similar levels of PVR as compared to the over-expressing cells. At 5 weeks, however, gross examination of the eyes revealed that rabbits

that received cells that under-expressed EMP2 (ribozyme group) produced significantly less severe levels of PVR than those given the control (1.3 vs. 2.5, respectively, $p = 0.05$) (Figure 2B). In addition, there was a strong trend found of less PVR in the ribozyme group than the group receiving the EMP2 over-expressing cells (1.3 vs. 2.9, respectively, $p = 0.06$). Rabbits receiving EMP2 over-expressing cells had slightly more PVR formation than those receiving wild-type cells (2.9 vs. 2.5); however, this was not statistically significant ($p = 0.24$). Increased EMP2 expression was associated with an increased PVR severity and, concordantly, decreased EMP2 levels reduced PVR severity. Figure 3 shows typical gross histologic specimens from the three different groups of rabbits; all nine eyes were examined with similar findings.

All PVR Membranes Express EMP2

Histologic examination of the rabbit eyes that received EMP2 over-expressing cells confirmed PVR membrane formation. The *in vivo* PVR membranes were found to be positive for EMP2 expression at 5 weeks by immunohistochemistry (Figure 4). The *in vivo* level of EMP2 expression was not quantified in these experiments.

PVR Membrane Formation Blocked with EMP2 Blocking Antibody

ARPE-19 cells were pre-incubated with either polyclonal anti-human EMP2 antibody, control antibody (pre-immune sera), or media alone. Eyes were again examined 5 weeks after cell injections. The degree of PVR formation was graded by two masked observers. Blockade of EMP2 in the ARPE-19 cells using the anti-human polyclonal EMP2 antibody decreased PVR severity as compared to untreated cells or cells exposed to the preimmune control antibody (2.6 vs. 4.3, respectively, $p = 0.02$) (Figure 5). Of note, the anti-EMP2 treated group was found to clinically have less PVR at all times point evaluated, while the pre-immune treated group (control) and the wildtype group were essentially the same (data not shown). Additionally, histologic evaluation confirmed decreased PVR formation (Figure 6). EMP2 was expressed in all the pre-retinal membranes including the membranes of the anti-EMP2-treated animals (Figure 6).

DISCUSSION

Changes in the RPE mediate, in part, the formation of PVR. Initially, during the development of PVR, RPE cells are thought to bind to the retinal surface, migrate, proliferate, and form membranes.²⁰ This process is then followed by membrane contraction and tractional retinal detachment.

In this study, we extend the *in vitro* evidence that EMP2 plays an important regulatory role in membrane contraction and present evidence for the relevance of EMP2 expression *in vivo* in the rabbit PVR model. Gel contraction assays showed increased contraction of collagen gel with increased EMP2 expression.⁸ Here, we found that the expression levels of EMP2 in the RPE cells caused the PVR correlates with the severity of PVR in the rabbits. Independent evidence for the relevance of EMP2 in PVR was obtained using a blocking antibody against EMP2, which, in contrast to the control antibody, decreased PVR severity. Of note, while Figure 4 confirms continued EMP2 expression in the eyes receiving wild type and EMP2 over-expressing cells, quantification of the level of EMP2 *in vivo* was not done. Therefore, it is possible that the levels of EMP2 expression by the cells did not remain constant throughout the experiment.

There are multiple models of PVR, and in this report, we have studied the well-established PVR model in rabbits using an intravitreal injection of RPE cells. Of note, of all the animal models of PVR, injecting cells into vitreous is chosen most commonly.²¹ However, future

studies would be helpful to determine if EMP2-blockade is also effective in other models of PVR, including those that experimentally induced through retinal detachment, as the pathogenesis for these models involve the recruitment of additional cells (i.e., Mueller cells) and soluble factors.^{22–27} The RPE cells that contribute to PVR formation have been shown to undergo an EMT, by which epithelial cells transdifferentiate into a mesenchymal phenotype with more fibroblast-like properties including reduced intercellular adhesion and increased motility.^{28–30} The cell line used in this model of PVR has been shown to undergo EMT *in vitro*.³¹ Moreover, we previously reported increased expression, a marker of this transition, α -smooth muscle actin, in cells that have increased EMP2 expression.⁸ This relationship requires additional study.

EMP2 is only expressed in discrete location in the eye including high expression in RPE cells; however, its function is only beginning to be understood.⁹ As a member of the tetraspanin family (CD9, CD53, CD81, CD82), modulation of integrin and other cell surface receptors is common. EMP2 has been previously shown to be important in regulating cellular contractile capacity through facilitating the activation of the FAK/Src signaling complex.^{6,8} In addition, activation of the FAK/Src complex has been proven to be through the physical association between EMP2 and the FAK/Src complex.⁸ These *in vitro* studies found that EMP2 and the FAK/Src signaling complex were critical components in RPE-mediated gel contraction. EMP2 expression is high in wild type APRE-19 cells compared with cells from other tissues.⁸ This high expression may potentially explain why the over-expression of EMP2 in APRE-19 cells and its effect on PVR was only seen at early time points (Figure 2A) and was less significant at later time points (Figure 2B), as the biologic effect of high expression may be maximized for the wild type cells.

Although we predicted that the RPE cells would be concordant in both *in vivo* and *in vitro* studies, there are many potential differences between the previously reported observations and the present investigation. First, the *in vitro* studies use collagen as scaffold for the RPE cells, whereas the RPE cells in the *in vivo* vitreous is exposed to hyaluronic acid and many collagen types, including II, V/XI, and IX. In addition, there are many cell types that contribute to the PVR membrane *in vivo* including inflammatory cells, Mueller cells, and fibroblasts.^{1,2,4,32} *In vivo* during PVR membrane formation, the cells are known to be exposed to many cytokines, and pre-retinal membranes have been found to contain cytokines, including vascular endothelial growth factor (VEGF), (PDGF), IL-6, IL-8, TNF- α , and TGF- β .³³ These cells and the milieu of cytokines made by these cells will certainly influence the cell and membrane behavior. As previously reported *in vitro*, EMP2 over-expression is associated with increased gel-contraction and only a mild decrease in proliferation (less than 10%) and migration.⁸ In this *in vivo* model where RPE cells are placed into the vitreous, EMP2-mediated contraction appears to play a more critical role in the development of PVR.⁸ It is also important to point out that how the host cells and cytokines are contributing to the PVR development in our studies has yet to be determined.

Today, despite surgical advances, patients too frequently lose vision secondary to PVR and tractional pre-retinal membranes. These studies support further investigation into EMP2 as a potential regulator of PVR membrane formation, and targeting EMP2 may prove to be a novel therapeutic strategy in the context of PVR. Additional studies are needed to define whether there is a critical timing for EMP2 blockade to prevent or reduce PVR formation. For patients, it would be helpful to have an agent that could prevent PVR formation, which could be administered either at the time of trauma, or in patients with high-risk retinal detachments (e.g., large inferior tear), or at the time of repeat surgery for PVR. An agent that reduces or halts active PVR would also be helpful in patients with active PVR as a potential adjunct to surgery. We have detected EMP2 expression in PVR membranes from patients.³⁴ Therefore, additional studies are needed to determine when EMP2 blockade is effective

during active PVR formation. Moreover, as EMP2 is expressed in the cornea, iris, RPE, and retina, it will be important to study the safety of potential therapeutic interventions. While no histologic toxicity was detected in these studies, only the RPE cells were directly exposed to the blocking antibody. These future investigations would help define the potential utility for EMP2 blockade in the treatment of PVR.

Acknowledgments

The authors acknowledge support via unrestricted departmental funds from Research to Prevent Blindness, Foundation Fighting Blindness (DGT), and a Veterans Administration Merit Grant (LKG). NIH NEI R01 Grant Number: 1R01EY019909-01A1.

REFERENCES

1. Pastor JC. Proliferative vitreoretinopathy: An overview. *Surv Ophthalmol*. 1998; 43:3–18. [PubMed: 9716190]
2. Pastor JC, de la Rua ER, Martin F. Proliferative vitreoretinopathy: Risk factors and pathobiology. *Prog Retin Eye Res*. 2002; 21:127–144. [PubMed: 11906814]
3. Colyer MH, Weber ED, Weichel ED, et al. Delayed intraocular foreign body removal without endophthalmitis during Operations Iraqi Freedom and Enduring Freedom. *Ophthalmology*. 2007; 114:1439–1447. [PubMed: 17331579]
4. Asaria RH, Charteris DG. Proliferative vitreoretinopathy: Developments in pathogenesis and treatment. *Compr Ophthalmol Update*. 2006; 7:179–185. [PubMed: 17007731]
5. Kroll P, Rodrigues EB, Hoerle S. Pathogenesis and classification of proliferative diabetic vitreoretinopathy. *Ophthalmologica*. 2007; 221:78–94. [PubMed: 17380062]
6. Morales SA, Mareninov S, Prasad P, et al. Collagen gel contraction by ARPE-19 cells is mediated by a FAK-Src dependent pathway. *Exp Eye Res*. 2007; 85:790–798. [PubMed: 17915217]
7. Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol*. 2003; 19:397–422. [PubMed: 14570575]
8. Morales SA, Mareninov S, Wadehra M, et al. FAK activation and the role of epithelial membrane protein 2 (EMP2) in collagen gel contraction. *Invest Ophthalmol Vis Sci*. 2009; 50:462–469. [PubMed: 18469192]
9. Wadehra M, Sulur GG, Braun J, et al. Epithelial membrane protein-2 is expressed in discrete anatomical regions of the eye. *Exp Mol Pathol*. 2003; 74:106–112. [PubMed: 12710941]
10. Forbes A, Wadehra M, Mareninov S, et al. The tetraspan protein EMP2 regulates expression of caveolin-1. *J Biol Chem*. 2007; 282:26542–26551. [PubMed: 17609206]
11. Wadehra M, Goodglick L, Braun J. The tetraspan protein EMP2 modulates the surface expression of caveolins and glycosylphosphatidyl inositol-linked proteins. *Mol Biol Cell*. 2004; 15:2073–2083. [PubMed: 14978215]
12. Wadehra M, Iyer R, Goodglick L, et al. The tetraspan protein epithelial membrane protein-2 interacts with beta1 integrins and regulates adhesion. *J Biol Chem*. 2002; 277:41094–41100. [PubMed: 12189152]
13. Wadehra M, Mainigi M, Morales SA, et al. Steroid hormone regulation of EMP2 expression and localization in the endometrium. *Reprod Biol Endocrinol*. 2008; 6:15. [PubMed: 18400107]
14. Wadehra M, Su H, Gordon LK, et al. The tetraspan protein EMP2 increases surface expression of class I major histocompatibility complex proteins and susceptibility to CTL-mediated cell death. *Clin Immunol*. 2003; 107:129–136. [PubMed: 12763482]
15. Wang CX, Wadehra M, Fisk BC, et al. Epithelial membrane protein 2, a 4-transmembrane protein that suppresses B-cell lymphoma tumorigenicity. *Blood*. 2001; 97:3890–3895. [PubMed: 11389031]
16. Farrell HM Jr, Jimenez-Flores R, Bleck GT, et al. Nomenclature of the proteins of cows' milk—Sixth Revision. *J Dairy Sci*. 2004; 87:1641–1674. [PubMed: 15453478]

17. Nakagawa M, Refojo MF, Marin JF, et al. Retinoic acid in silicone and silicone-fluorosilicone copolymer oils in a rabbit model of proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci.* 1995; 36:2388–2395. [PubMed: 7591628]
18. Fastenberg DM, Diddie KR, Delmage JM, et al. Intraocular injection of silicone oil for experimental proliferative vitreoretinopathy. *Am J Ophthalmol.* 1983; 95:663–667. [PubMed: 6846457]
19. Fastenberg DM, Diddie KR, Dorey K, et al. The role of cellular proliferation in an experimental model of massive periretinal proliferation. *Am J Ophthalmology.* 1982; 93:565–572.
20. Eibl KH, Kook D, Priglinger S, et al. Inhibition of human retinal pigment epithelial cell attachment, spreading, and migration by alkylphosphocholines. *Invest Ophthalmol Vis Sci.* 2006; 47:364–370. [PubMed: 16384986]
21. Agrawal RN, He S, Spee C, et al. In vivo models of proliferative vitreoretinopathy. *Nat Protoc.* 2007; 2:67–77. [PubMed: 17401340]
22. Eibl KH, Lewis GP, Betts K, et al. The effect of alkylphosphocholines on intraretinal proliferation initiated by experimental retinal detachment. *Invest Ophthalmol Vis Sci.* 2007; 48:1305–1311. [PubMed: 17325177]
23. Lewis GP, Sethi CS, Linberg KA, et al. Experimental retinal reattachment: A new perspective. *Mol Neurobiol.* 2003; 28:159–175. [PubMed: 14576454]
24. Cook B, Lewis GP, Fisher SK, et al. Apoptotic photoreceptor degeneration in experimental retinal detachment. *Invest Ophthalmol Vis Sci.* 1995; 36:990–996. [PubMed: 7730033]
25. Fisher SK, Erickson PA, Lewis GP, et al. Intraretinal proliferation induced by retinal detachment. *Invest Ophthalmol Vis Sci.* 1991; 32:1739–1748. [PubMed: 2032796]
26. Lewis GP, Chapin EA, Byun J, et al. Muller cell reactivity and photoreceptor cell death are reduced after experimental retinal detachment using an inhibitor of the Akt/mTOR pathway. *Invest Ophthalmol Vis Sci.* 2009; 50:4429–4435. [PubMed: 19369237]
27. Zacks DN, Zheng QD, Han Y, et al. FAS-mediated apoptosis and its relation to intrinsic pathway activation in an experimental model of retinal detachment. *Invest Ophthalmol Vis Sci.* 2004; 45:4563–4569. [PubMed: 15557468]
28. Liu Y. Epithelial to mesenchymal transition in renal fibro-genesis: Pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol.* 2004; 15:1–12. [PubMed: 14694152]
29. Saika S, Kono-Saika S, Tanaka T, et al. Smad3 is required for dedifferentiation of retinal pigment epithelium following retinal detachment in mice. *Lab Invest.* 2004; 84:1245–1258. [PubMed: 15273699]
30. Saika S, Yamanaka O, Sumioka T, et al. Fibrotic disorders in the eye: Targets of gene therapy. *Prog Retin Eye Res.* 2008; 27:177–196. [PubMed: 18243038]
31. Lee H, O'Meara SJ, O'Brien C, et al. The role of gremlin, a BMP antagonist, and epithelial-to-mesenchymal transition in proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci.* 2007; 48:4291–4299. [PubMed: 17724219]
32. Kim IK, Arroyo JG. Mechanisms in proliferative vitreoretinopathy. *Ophthalmol Clin North Am.* 2002; 15:81–86. [PubMed: 12064085]
33. Harada C, Mitamura Y, Harada T. The role of cytokines and trophic factors in epiretinal membranes: Involvement of signal transduction in glial cells. *Prog Retin Eye Res.* 2006; 25:149–164. [PubMed: 16377232]
34. Forward KI, Yu AK, Morales SA, et al. Differential expression of epithelial membrane protein 2 in human epiretinal membranes. *Invest Ophthalmol Vis Sci.* 2010; 51 ARVO E-abstract 6088.

FIGURE 1.

Gel contraction assay demonstrates significant blocking by the anti-EMP2 polyclonal antibody versus pre-immune serum both at 1:10 dilutions. Each experiment included six replicates. A Student's *t*-test (unpaired, two-tail) was used and a $P < 0.0001$ was found.

FIGURE 2.

Rabbit PVR model graded according to the Fastenberg system (Table 1). Three rabbits were in each group: Control—rabbits that received ARPE19 cells transfected with the vector alone; Ribo—rabbits that received ribozyme transfected cells with lower expression of EMP2; EMP2—rabbits that received cells transfected to overexpress EMP2. The rabbits received both clinical (A) and gross (B) examinations. The rabbits that received cells over expressing EMP2 had increased rates of PVR formation whereas the animals that received the cells with lower levels of EMP2 expression by ribozyme knockdown (Ribo) had decreased degrees of PVR formation. Note that in Figure 2A a significant difference between EMP2-overexpressing groups and control groups was only noted at 3 weeks ($p = 0.02$). The gross examination results presented in 2B were obtained 5 weeks after cell injection significant differences found between the Ribo group and control eyes ($p = 0.05$). A strong trend was also noted between the over-expressors and the Ribo group ($p = 0.06$). Student *t*-test *p* values shown above compared groups. Rabbits receiving EMP2 over-expressing cells had a trend toward greater PVR formation than those receiving wild-type cells; however, this was not statistically significant ($p = 0.24$).

FIGURE 3.

Gross pathologic sections of globes isolated from the experiment shown in Figure 2. These are representative photos of three of the nine eyes examined. Degrees of PVR membrane formation can be seen in these images. One globe is shown from each group. As in Figure 2: Control—rabbits that received ARPE19 cells transfected with the vector alone; Ribo—rabbits that received ribozyme transfected cells with lower expression of EMP2; EMP2—rabbits that received cells transfected to overexpress EMP2. The rabbits that received cells over expressing EMP2 had increased rates of PVR membrane formation, whereas the animals that received the cells with lower levels of EMP2 expression by ribozyme knockdown (Ribo) had decreased degrees of PVR membrane formation. The results presented were obtained at 5 weeks after cell injection.

FIGURE 4.

Pre-retinal membranes were found to have significant EMP2 expression. This figure shows EMP2 expression (violet) from in a control rabbit (A) and from EMP2 over-expressing rabbit (B). Arrows indicate the pre-retinal PVR membranes in each image. A normal rabbit section is included for reference (C).

FIGURE 5.

A rabbit model of PVR shows inhibition of PVR membrane formation when cells were pre-incubated with anti-EMP2 polyclonal antibody. Four rabbits were in each group: Anti-EMP2—rabbits that received ARPE19 cells pre-incubated with polyclonal anti-EMP2 antibody; Wild type (Wt)—rabbits that received wild-type ARPE19 cells; Pre-immune—rabbits that received ARPE19 cells pre-incubated with polyclonal pre-immune (control) antibody. The results presented were obtained 5 weeks after cell injection. Student *t*-test *p* values shown above compared groups.

FIGURE 6.

EMP2 expression was found in pre-retinal membranes of rabbits in all treatment groups (12 eyes). As in Figure 4, this figure shows EMP2 expression (violet) in all the pre-retinal PVR membranes and not in the retinal tissue. Treatment groups included rabbits receiving ARPE19 cells that were either untreated (4 eyes) (A), treated with pre-immune isotype control serum (4 eyes) (B), or anti-EMP2 antibody (4 eyes) (C). Arrows indicate the pre-retinal membranes in each image.

TABLE 1

The Fastenberg classification of the five stages of preretinal proliferation.

Grade	Findings on retinal fundus exam
1	Intravitreal membrane
2	Focal traction; localized vascular changes; hyperemia; engorgement; dilation; blood vessel elevation
3	Localized detachment of medullary ray
4	Extensive retinal detachment; total medullary ray detachment; peripapillary retinal detachment
5	Total retinal detachment; retinal folds and holes