Eye (2002) 16, 393–403

www.nature.com/eye

CAMBRIDGE OPHTHALMOLOGICAL SYMPOSIUM

Pathobiology of epiretinal and subretinal membranes: possible roles for the matricellular proteins thrombospondin 1 and osteonectin (SPARC)

P Hiscott[1,2,3], S Hagan[1], L Heathcote[1], CM Sheridan[1], CP Groenewald[1,2], I Grierson[1,2], D Wong[1,2] and L Paraoan[1]

Abstract

Epiretinal and subretinal membranes are fibrocellular proliferations which form on the surfaces of the neuroretina as a sequel to a variety of ocular diseases. When these proliferations complicate rhegmatogenous retinal detachment (a condition known as proliferative vitreoretinopathy or PVR), the membranes often contain numerous retinal pigment epithelial (RPE) cells and a variety of extracellular proteins. The extracellular proteins include adhesive proteins like collagen, laminin and fibronectin. In addition, several matricellular proteins with potential counter-adhesive functions are present in the membranes. Two such matricellular proteins, thrombospondin 1 and osteonectin (or SPARC: Secreted Protein Acidic and Rich in Cysteine), tend to be co-distributed with the RPE cells in PVR membranes. By virtue of their counter-adhesive properties, thrombospondin 1 and SPARC may reduce RPE cell-matrix adhesion and so permit key RPE cellular activities (for example, migration or shape change) in periretinal membrane development. Furthermore, within a 'cocktail' containing other proteins such as the metalloproteinases and growth factors like the scatter factor/hepatocyte growth factor family, matricellular proteins may play a role in the RPE cell dissociation from Bruch's membrane, which characterises early PVR.
Eye (2002) **16,** 393–403. doi:10.1038/sj.eye.6700196

Keywords: epiretinal membrane; subretinal membrane; thrombospondin 1; osteonectin; SPARC; proliferative vitreoretinopathy; scatter factor/hepatocyte growth factor

Introduction

An epiretinal membrane (ERM) is a cellular or fibrocellular proliferation on the inner surface of the retina whereas a subretinal membrane (SRM) is a similar proliferation located between the outer neuroretina and the retinal pigment epithelial monolayer. The principal clinical importance of ERMs lies in their tendency to contract, and such contraction often results in distortion or detachment of the underlying retina. Likewise, SRMs are frequently contractile and SRM shortening typically causes elevation of the neuroretina. Thus contractile ERMs and SRMs both may have profound visual consequences. Furthermore, since contraction is a characteristic of scars generally and is deemed to be cell-mediated, membrane formation is considered to represent an anomalous wound repair process and the activities of component cells to be responsible for membrane contraction.[1] Nevertheless not all ERMs and SRMs are contractile. Non-contractile membranes generally are asymptomatic and are sometimes called 'simple' membranes (to contrast with 'complex' contractile proliferations).[2]

ERMs and SRMs complicate a wide range of ocular disorders, although the spectrum of

[1]Unit of Ophthalmology
Department of Medicine
University of Liverpool
Liverpool, UK

[2]St Paul's Eye Unit
Royal Liverpool University Hospital
Liverpool, UK

[3]Department of Pathology
University of Liverpool
Liverpool, UK

Correspondence:
P Hiscott
Unit of Ophthalmology
Department of Medicine
University Clinical Departments
Duncan Building
Daulby Street
Liverpool L69 3GA
UK
Tel: + 44 151 706 4912
Fax: + 44 151 706 5802
E-mail: paulsh@liv.ac.uk

diseases that give rise to SRMs differ to a certain extent from those which instigate ERM formation (Table 1).[3,4] One condition well recognised for causing contractile ERM and SRM development is the complication of rhegmatogenous retinal detachment and its surgery known as proliferative vitreoretinopathy (PVR).[5] Here we shall chiefly consider PVR, touching on other causes of ERM and SRM formation by means of comparison.

The morphology of epiretinal and subretinal membranes

To some degree, the aetiology of ERMs and SRMs (or 'periretinal membranes') is reflected in the histological appearance of the tissue.[4] Therefore, in the rare instances where the aetiology of the membrane is in doubt, histological information may provide clues to the cause of membrane formation. Microscopic examination (of ERMs in particular) can also provide information about the surgical dissection plane involved in removing the specimen.

Cellular components of periretinal membranes

A predominantly vascular ERM is usually a sequel to ischaemic retinopathy (for example, proliferative diabetic retinopathy (PDR) or after central retinal vein occlusion) whereas vascularised SRMs are found in age-related macular degeneration and conditions like presumed ocular histoplasmosis syndrome (Table 1). By contrast, PVR epiretinal and subretinal membranes typically have few if any blood vessels but many retinal pigment epithelial (RPE) cells (Table 1; Figure 1).[6–9] RPE cells readily change their shape and become fibroblast- or macrophage-like in periretinal membranes (Figure 1).[6,7] Fibroblastic cells are present in most contractile periretinal membranes. In PVR many of these cells are RPE cells which have undergone mesenchymal transdifferentiation whereas in PDR few RPE-derived fibroblastic cells are present (except in the case of PDR membranes which have formed in the presence of a retinal hole).[10] Many periretinal membranes contain retinal glial cells. However, glial cells are not usually the predominant cell type except in surface wrinkling retinopathy ERMs, in simple ERMs and SRMs, and in ERMs associated with macular holes or retinitis pigmentosa (Table 1).[2,11–13] Recent work suggests that neural elements found in PVR membranes may reflect outgrowth from the retina into the developing membrane, rather than representing retinal tissue avulsed during membrane excision.[14]

In ERMs, inflammatory cells are usually abundant only when the membrane complicates intraocular inflammation (Table 1). Thus established PVR membranes usually contain few macrophages or other inflammatory cells, most macrophage-like cells being transdifferentiated RPE cells.[15] The cellular composition of PVR membranes may, nevertheless, be altered by therapeutic interventions—especially when the membranes complicate, or recur after, retinal detachment surgery in which a tamponade agent is employed.[16,17] For example, macrophages may be the predominant cell type in ERMs forming after silicone oil tamponade and up to one third of the cells in PVR membranes arising with perfluorohexyloctane (F6H8) tamponade are macrophages (on the basis of CD68 positivity and cytokeratin negativity).[17–19] Indeed, it has been suggested that tamponade agent-induced macrophage influx may augment the PVR process by the periretinal accumulation of a wide range of macrophage-derived growth factors. Moreover, some tamponade agents, including F6H8, can induce a marked foreign body reaction in the developing membranes (Table 1, Figure 1) while most of these

Table 1 Common histological and aetiological features of periretinal membranes

Major histological feature	*Example of ERM aetiology*	*Example of SRM aetiology*
Prominent vascular component	Proliferative diabetic retinopathy Central retinal vein occlusion	Age-related macular degeneration Presumed ocular histoplasmosis syndrome
Avascular, retinal pigment epithelial cell-predominant	Proliferative vitreoretinopathy	Proliferative vitreoretinopathy
Chiefly glial cell layers	Simple ERM Surface wrinkling retinopathy Retinitis pigmentosa	Simple SRM
Numerous monocytic inflammatory cells	Intraocular inflammation Reaction to tamponade agent	

ERM = epiretinal membrane.
SRM = subretinal membrane.

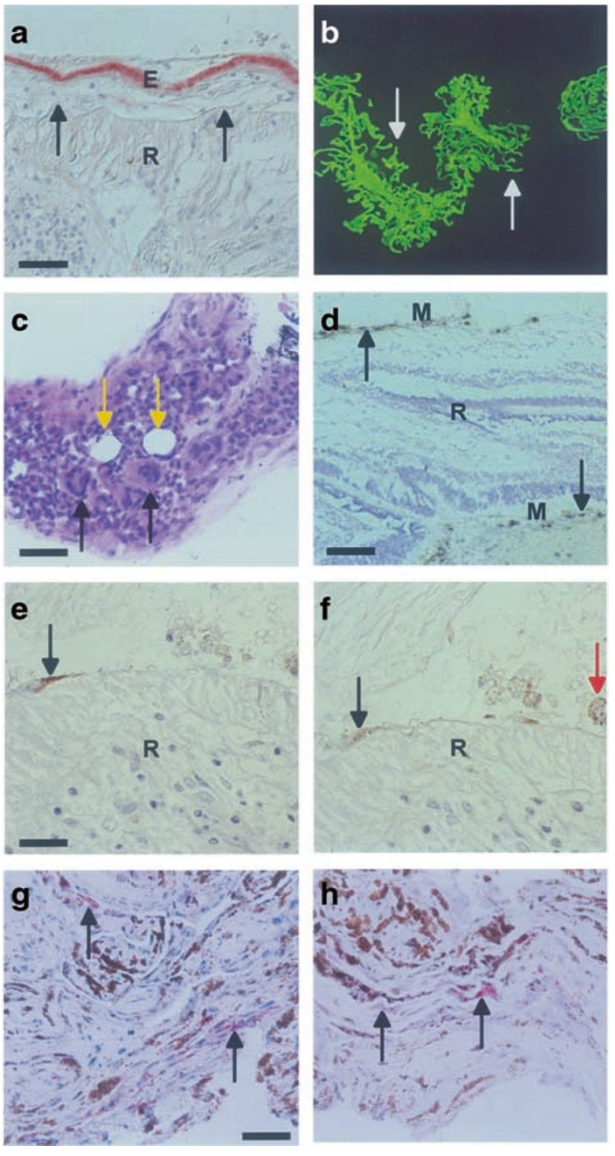

Figure 1 (a) Section through proliferative diabetic retinopathy epiretinal membrane (E) and underlying retina (R) stained with the immunoperoxidase technique for collagen II (haematoxylin counterstain). Note a prominent layer of collagen II (demonstrated red) within the epiretinal membrane. The vitreoretinal interface is labelled (arrows). (b) Section of a proliferative vitreoretinopathy (PVR) epiretinal membrane stained by the immunofluorescent technique for collagen I. Collagen I is abundant. Folded inner limiting lamina can be seen around the edge of the section (arrows). (c) Haematoxylin and eosin-stained section of a PVR epiretinal membrane which has formed in the presence of the tamponade agent perfluorohexyloctane (F6H8). Numerous macrophages are present. In addition, multinucleated giant cells (black arrows) are adjacent to spherical extracellular spaces (yellow arrows). These spaces are presumed to have contained F6H8.The inflammatory response may constitute a foreign body-type reaction to emulsified tamponade agent. (d) Autoradiograph of section through folded, tractionally-detached retina (R) with overlying epiretinal membrane (M top) and underlying subretinal membrane (M bottom). The section has been labelled by *in situ* hybridisation with an antisense radiolabelled probe for fibronectin mRNA (haematoxylin counterstain). The periretinal membranes contain many cells with abundant fibronectin mRNA (which appear black in the section from intense silver grain deposition over these cells—arrows), whereas cells in the retina exhibit only background labelling. (e) and (f) Sections through a peripheral retinectomy specimen stained with the immunoperoxidase method (brown reaction product, haematoxylin counterstain) for cytokeratins, an epithelial cell marker (e) or thrombospondin-1 (TSP-1) (f). In (e), a fibroblast-like retinal pigment epithelial (RPE) cell (black arrow) can be seen on the retina (R). In (f) this cell is noted to stain for TSP-1 (black arrow) while a macrophagic cell contains some of the glycoprotein as well (red arrow). Both cells contain melanin granules while other epiretinal elements also contain TSP-1. (g) and (h) Sections through a PVR subretinal membrane stained with the immunoperoxidase method (red reaction product, haematoxylin counterstain) for cytokeratins (g) or osteonectin/SPARC (h). The fibroblastic cells are chiefly of RPE cell origin as judged by their cytokeratin immunoreactivity (g) (arrows). In (h), which is a section deeper into the tissue, SPARC is present in many of the fibroblastic cells but extracellular SPARC is not seen. Scale bars represent (a) 75 μm; (b) magnification as in (a); (c) 75 μm; (d) 300 μm; (e) 35 μm; (f) magnification as in (e); (g) 75 μm; (h) magnification as in (g).

agents appear to be able to induce the formation of intracellular (and extracellular) spherical spaces of variable size.[19] These spaces, which are found not only in macrophages but also in other membrane cells including RPE cells, are presumed to represent droplets of tamponade agent incorporated in the evolving tissue.

Extracellular components of periretinal membranes

The extracellular composition of periretinal membranes also varies with the aetiology. For example, type II collagen is characteristically a prominent component of the fibrous part of epiretinal membranes complicating PDR. Conversely, type II collagen is mostly absent from, or only a minor element of, PVR epiretinal membranes. These differences presumably reflect the pathogenesis of the epiretinal membranes: PVR membranes usually arise after posterior vitreous detachment (and subsequent rhegmatogenous retinal detachment) whereas the capillaries of diabetic membranes are thought to use the vitreous cortex as a scaffold in which to propagate.[20] Thus in the latter, collagen type II-rich vitreous may become trapped in the developing membrane while the vitreous cortex generally is not available for the developing PVR membrane (Figure 1).[21] However, unlike PDR membranes, fragments of retinal inner limiting lamina are often found in PVR membranes and presumably indicate strong adhesion between the membrane and retinal surface (Figure 1).

A variety of other collagen subtypes, including I and

III, are common to most contractile membranes and, as in healing wounds, the collagenous element of periretinal membranes tends to increase with time.[1,22–26] Non-collagenous extracellular components of periretinal membranes include members of the elastic fibre family (though not usually mature elastic fibres) and a number of glycoproteins.[22,27–29]

The realisation that, in addition to a role as spacer elements, extracellular components can profoundly affect cell behaviour via cell-surface receptors such as the integrins has prompted considerable interest in wound healing proteins in general. The possibility that cell–matrix interactions could act as therapeutic targets in the control of reparative processes like periretinal membrane formation has led to a number of investigations concerning the relationship between cells, matrix and/or receptors in the membranes.[30]

Adhesive extracellular matrix proteins in periretinal membranes

In addition to collagen, other proteins that promote cell adhesion and are identified in periretinal membranes include laminin, vitronectin and fibronectin.[22,28,29] Fibronectins (a family of glycoproteins) have received notable attention in periretinal membranes. This attention may reflect the fact that fibronectin was among the first glycoproteins described in periretinal membranes,[22] that fibronectins have a multifunctional nature, or that they appear to be involved in wound healing from the earliest stages of repair (see Gailit and Clarke[31] for a brief review of extracellular matrix components at different phases of wound repair).

Fibronectin in periretinal membranes probably represents both plasma-derived and cellular fibronectins.[30] The former is thought to gain access to the retinal surface following breakdown of the blood-retinal barrier (eg after retinal detachment) or vitreous haemorrhage (as in PDR or trauma). Cellular fibronectin appears to originate from cells (including RPE cells) displaced to the retinal surfaces in the early membranes (Figure 1).[32–34] Soluble fibronectin is chemotactic to many cell types including RPE cells and hence may be involved in recruiting the cells to the retinal surfaces during early periretinal membrane formation.[35,36] Moreover, fibronectins promote cell–cell and cell–substrate adhesion. Indeed, fibronectin is implicated in the formation of a temporary scaffold at tissue surfaces involved in early repair and insoluble cellular fibronectin may form transmembrane links with contractile elements of cells. This latter characteristic has led to speculation that fibronectin is responsible for providing early structural integrity in periretinal membranes and the formation of a 'contractile unit'.[22] However, contraction is not the only cellular activity occuring in early membranes: for example, cell migration (which, in addition to cell recruitment, may generate tractional forces in the tissue) and proliferation are thought to be key to membrane development.[37]

Counter-adhesive proteins in periretinal membranes

Behaviour like proliferation, migration and shape change requires partial detachment of cells from their substrate. The matrix in wounds contains anti-adhesive proteins, often members of a group of proteins known as matricellular proteins, which may facilitate partial cell detachment and hence permit cell proliferation and migration.[38] In fact, matricellular proteins are defined as proteins which interact with many molecules in the extracellular environment as well as with a variety of cell surface receptors. In so doing, they are thought to produce multiprotein complexes comprising cell surface receptors, matricellular protein and extracellular molecules, thereby modifying diverse cell activities (and extracellular matrix structure).[39] Typically, matricellular proteins are highly expressed during tissue formative processes like wound repair and on the whole these proteins tend to be anti-adhesive both in solution and when part of a mixed substrate.[40] Matricellular proteins include tenascin, thombospondin 1 and 2, osteonectin and osteopontin. Tenascin, thrombospondin 1 and osteonectin have been described in PVR membranes and we have observed an association between thrombospondin 1 and RPE cells, and between osteonectin and RPE cells, in the membranes (Figure 1).[30,41–43]

The matricellular protein thrombospondin 1 and PVR membranes

Thrombospondin 1 (TSP1), once known just as thrombospondin, is a large protein (~420 kDa) and a member of a family of at least five secreted glycoproteins (TSP-1 to -4 and Cartilage Oligomeric Matrix Protein or TSP-5).[38,39,43] The glycoprotein is present in platelets and plasma, and is synthesised by a wide variety of cell types.[43] TSP1 binds to cells via several receptor types, such as integrins and CD36. TSP1 also has the ability to bind growth factors like platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β).[39] Intriguingly, binding to TSP1 activates latent TGF-β.[44] Conversely, peptide fragments of TSP1 tend to inhibit angiogenesis induced by basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) (reviewed

by Lawler[39]). Inhibition of bFGF and VEGF by these fragments may (partly) explain the natural anti-angiogenic property of TSP1 itself.

Growth factors are only one group of extracellular molecules which bind to, and are functionally modulated by, matricellular proteins like TSP1. Other groups include enzymes, such as the matrix metalloproteases, and adhesive proteins including fibronectin.[38] Interestingly, cellular fibronectin and TSP1 are often co-distributed in PVR and PDR periretinal membranes and a combination of the two proteins may provide an early matrix in PVR membrane formation.[45]

It has been suggested that, in mixed substrata akin to those found in periretinal membranes, matricellular proteins such as TSP1 antagonise the pro-adhesive effects of the adhesive proteins.[40] The 'de-adhesion' induced by the matricellular protein is postulated to reduce the focal adhesions and stress fibres typical of strongly adherent cells, producing a cell capable of activities like migration.[40] Thus TSP1 may counter the adhesive effects of matrix proteins like fibronectin and so permit or augment the types of cell behaviour necessary for the production of wound tissues, including PVR membranes. We have investigated the role of TSP1 in a model of PVR.[46] The model is that of RPE-populated collagen matrices, in which the activity of the cells causes the matrix to contract.[47] Although RPE cell-mediated matrix contraction does not appear to be altered by addition of blocking antibodies or peptide fragments to TSP1, the colocalisation of TSP1 with migratory cells in the model, and with RPE cells in PVR membranes, does support the concept that TSP1 may play a role in RPE cell migration.[30,46]

We have shown that RPE cells are capable of synthesising TSP1, TSP2, TSP3 and TSP4, and thus it seems likely that at least some of the TSP1 in PVR membranes is RPE cell-derived.[48,49] Indeed, since it is thought that a cell must actively synthesise TSP1 in order to bind the protein,[50] observations of TSP1 immunoreactive RPE cells in PVR membranes are in keeping with the idea that there is local TSP1 synthesis in the developing tissue. However, since TSP1 is also present in platelets and serum, haematogenous TSP1 might become involved in periretinal membrane formation in the same way as plasma fibronectin (see above). Also like fibronectin, TSP1 is present in both 'early' (less than 4 months clinical duration) and 'late' (greater than 4 months clinical duration) periretinal membranes. Persistence of TSP1 in 'late' membranes is further evidence that the nature of the repair process in PVR is prolonged and disorganised compared to that of cutaneous wound healing (in which TSP1 appears transiently in the early stages).[51]

The matricellular protein osteonectin/SPARC and PVR membranes

At approximately 43 kDa, osteonectin, also known as SPARC (Secreted Protein Acidic and Rich in Cysteine) and BM-40, is a much smaller molecule than TSP1.[52] Recently, ophthalmic interest in SPARC has been stimulated by the observation that SPARC-null mice develop severe, early cataract.[53] SPARC is related to several other proteins, including two neural glycoproteins called SC1/hevin and QR1, and appears to be the most strongly anti-adhesive of the matricellular proteins in that prolonged SPARC exposure causes cell rounding (an index of weak cell adhesion).[40] It is not known whether SPARC acts through a specific cell surface receptor or by interfering with adhesive interactions. Indeed, the precise cellular function(s) of SPARC are unclear and its biology is complicated by the rapid degradation of the glycoprotein which may occur once SPARC is released from the cell.[54] Some SPARC degradation products have properties opposite to the whole molecule: for example, SPARC itself is angiogenic but several of SPARC peptides are antiangiogenic (reviewed by Motamed and Sage 1997).[55]

The anti-adhesive properties of SPARC and the production of the glycoprotein in tissues of high cell proliferation rates (such as healing wounds)[51] led us to speculate that SPARC might play a role in PVR and other periretinal membranes. SPARC might modify cell-substrate adhesion and so augment RPE migration, proliferation and/or shape change, as is postulated for TSP1. We studied ERMs and SRMs from patients with PVR. Employing immunohistochemical methods, we were able to detect SPARC in both ERMs and SRMs (Figures 1 and 2). Furthermore, by examining peripheral retinectomies from patients with early PVR, we found SPARC in epiretinal cells before clinically detectable membranes were present (Figure 2). This SPARC immunoreactivity persists in established membranes and may be detected even in membranes of more than 4 months clinical duration (which we had previously termed 'late' membranes; see above).

There appears to be a distinct spatial distribution of SPARC in established periretinal membranes in that, although some extracellular protein can be seen, most staining appears to be intracellular (Figure 1). This pattern contrasts with TSP1 distribution, in which we have observed marked extracellular as well as intracellular accumulation in the membranes,[56] and may reflect the rapid extracellular breakdown of SPARC mentioned above. Finally, we were able to confirm that at least some of the PVR membrane cells, which contained SPARC, were of RPE origin.[30]

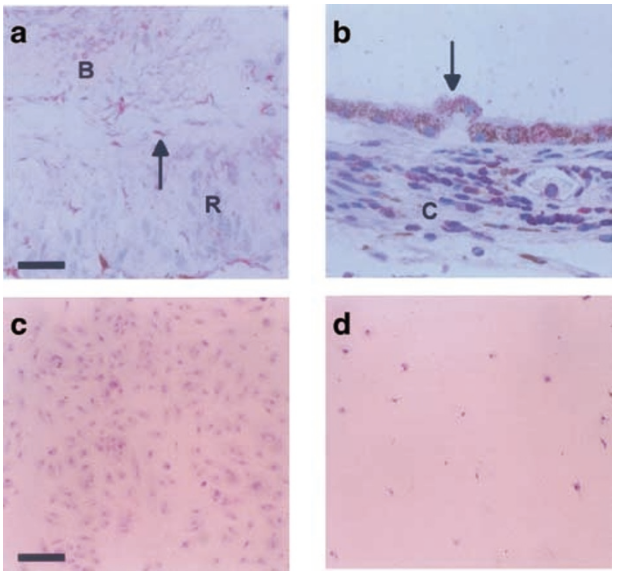

Figure 2 (1) PVR peripheral retinectomy specimen stained with the immunoperoxidase method (red reaction product, haematoxylin counterstain) for SPARC. The retina (R) and epiretinal blood (B) are marked. Epiretinal cells containing SPARC can be seen (arrow) and there is SPARC immunoreactivity in epiretinal blood. (b) A section showing choroid (C) and retinal pigment epithelial monolayer beneath an area of retinal detachment. The section has been stained with the immunoperoxidase method (red reaction product, haematoxylin counterstain) for SPARC. Note SPARC immunoreactivity in choroid and RPE monolayer, including a RPE cell apparently detaching from the monolayer (arrow). (c) and (d) RPE cells seeded at equal numbers onto a glass slide (to which RPE cells adhere) precoated with fibronectin (c) and SPARC (d). Note the far greater RPE cell attachment and spreading in the presence of fibronectin than in the presence of SPARC. Scale bars represent (a) 35 μm; (b) magnification as in (a); (c) 200 μm; (d) magnification as in (c).

The finding that SPARC colocalised with RPE cells in periretinal membranes raises the possibility that human RPE cells might produce the protein and that it might act in an autocrine-like way in influencing RPE behaviour during PVR development. We have investigated SPARC synthesis by RPE cells *in vitro*. Using a combination of methodologies, we were able to determine that RPE cells express the SPARC gene and make SPARC *in vitro*, secreting some of the protein.[57,58] The secreted protein was present in both the culture medium and the extracellular matrix of the cells (there is evidence that SPARC plays a role in the organisation of extracellular matrix generally).[58]

If SPARC were to play a role in cellular activities which typify PVR, such as RPE cell migration and proliferation, it might be expected to be produced in low density RPE cell cultures (where cells are migratory and dividing) rather than in high density cultures (where migration and proliferation is less marked). However, when we examined SPARC production in low and high density RPE cell cultures we observed that the opposite was the case. The proportion of SPARC mRNA and protein *increased* with increase in cell density.[58] In fact, this finding is consistent with observations that SPARC is antiproliferative for some other cell types.[52,53] Moreover, prolonged elevated levels of SPARC might be expected to inhibit cell motility both by rendering cell–matrix adhesion too weak for migration and by abrogating growth-factor mediated chemotaxis,[40,54] On the other hand, SPARC might still promote cell shape change. On balance, and counter to our original concept, the currently available information suggests that SPARC in PVR membranes tends to switch off the proliferative process. Indeed, partly because SPARC appears to have an antiproliferative role, the protein is being considered as key to the differentiation of a number of tissues in general.[53]

The concept that SPARC plays a role in cell differentiation is supported by a number of observations concerning the glycoprotein including that it emerges late in wound repair, and it binds and reduces the function of several growth factors which support cell proliferation (eg PDGF, VEGF and bFGF).[51–55] Furthermore, SPARC is produced in organs undergoing terminal differentiation (including the RPE monolayer) and SPARC knock-out causes developmental abnormalities in several species.[59] Conversely, although a certain amount of cell detachment may be necessary for cellular shape change in differentiating tissues (for example, the counteradhesive properties of SPARC may permit neuronal rearrangement *in vivo*),[53] SPARC's often strong counteradhesive effects on cells (including RPE cells; Figure 2) and SPARC-induced cell rounding might be expected to cause dedifferentiation in the RPE monolayer. After all, RPE cell rounding, as a prelude to RPE detachment from Bruch's membrane, is taken as an indicator of loss of RPE tertiary differentiation.[6,37]

A potential role for TSP1 and SPARC in early PVR membrane formation: functional interactions with Scatter Factor/Hepatocyte Growth Factor

RPE cell rounding and detachment from Bruch's membrane, along with extension of glia through the retinal surfaces, are thought to denote the earliest stage of PVR. Given their anti-adhesive properties, it is tempting to speculate that TSP1 and, perhaps to a greater extent, SPARC might play a role in this RPE cell-Bruch's membrane dissociation (in addition to the normal RPE layer, SPARC is prominent in cells apparently leaving Bruch's membrane in early PVR;

Figure 2). However, several other families of molecules are likely to be involved in controlling adhesive interactions between RPE cells and matrix, such as the matrix metalloproteinases (MMPs), and the metallo-disintegrin (ADAM) and the ADAM with thrombospondin repeats (ADAMTS) families.[60–63] Indeed, it is established that RPE cells are capable of synthesising many of these proteins,[60–63] and that MMPs are required for some RPE cell–collagen matrix interactions like those which are involved in collagen matrix contraction.[64,65]

In addition to separation from Bruch's membrane, RPE cells destined for the new PVR membranes have to detach from their neighbours. Matricellular proteins are attributed to have roles in cell–cell interactions[50] but, again, a variety of other molecules may be involved in such processes. For example, PDGF and interleukin-1 are both thought to be chemotactic to dedifferentiated human RPE cells and thus could both be involved in the early stages of PVR (reviewed by Burke[66] and Campochiaro[67]). Another growth factor family which has recently been shown to be motogenic to RPE cells has also been found to have an additional intriguing property: it causes epithelial sheets to dissociate. This family is known as Scatter Factor or Hepatocyte Growth Factor (HGF/SF).[68,69] Scatter factor is secreted by mesenchymal cells and is identical, or closely similar, to a plasma protein which causes hepatocytes to proliferate (hepatocyte growth factor).[68,69] A cytokine called macrophage stimulating protein is also thought to be a member of this growth factor family. HGF/SF has the ability to cause junctional breakdown and dissociation of epithelial cell sheets *in vitro*. Moreover, HGF/SF also brings about a phenotypic change in the epithelial cells so that the epithelial cells become fibroblast-like. Not surprisingly, the family has been implicated in the early stages of PVR.[70]

RPE cells express the receptor for HGF/SF (the receptor is known as c-met).[71,72] Moreover, the levels of HGF/SF are elevated in vitreous from patients with PDR and PVR.[73,74] Indeed, there is evidence that RPE cells can themselves produce HGF/SF.[71] However, as HGF/SF is secreted as an inactive single chain glycoprotein, its mere presence cannot be taken as an index of bioactivity: activation of HGF/SF is dependent on extracellular proteolytic cleavage of the precursor chain to an active heterodimer. Using a bioassay (based on mesenchymal transdifferentiation of cultured Madin-Darby canine kidney or MDCK cells to HGF/SF), we were able to detect *active* scatter factor in about 60% of vitreous samples (including PVR, PDR and retinal detachment vitreous).[75] In addition, the levels of total HGF/SF found in the vitreous and subretinal fluid of patients with PDR and PVR (up to 54 ng ml^{-1})[75] were well in excess of the levels required (~4 ng ml^{-1}) to produce a significant increase in migration and proliferation of RPE cells above control levels *in vitro* (Figure 3). Interestingly, the HGF/SF levels found in subretinal fluids were greater than those observed in vitreous samples (Table 2). Thus in established PVR, subretinal HGF/SF levels were double vitreous HGF/SF concentrations and, in patients with uncomplicated retinal detachments, subretinal HGF/SF levels were almost three times the vitreous concentrations (Table 2). Therefore, overall it seems likely that HGF/SF is at bioactive levels in periretinal fluids in the early stages of PVR and may be able to (partly) induce dissociative and phenotypic changes in the cells of the RPE monolayer. Since

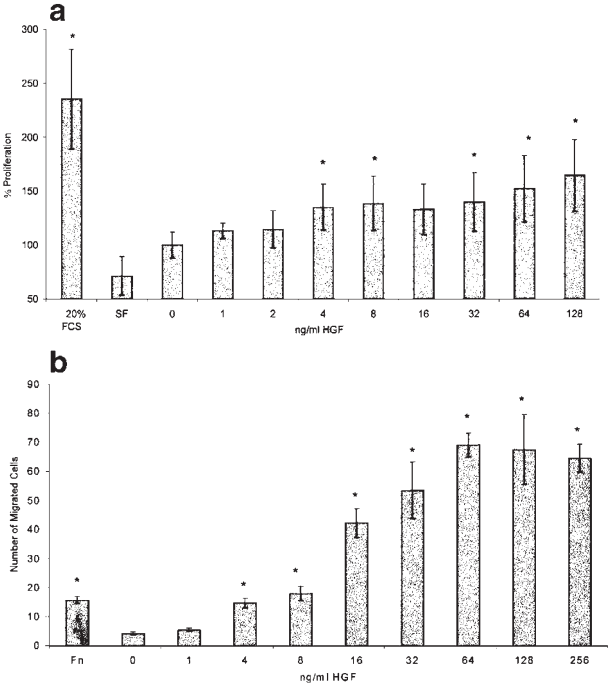

Figure 3 (a) Proliferative and (b) migratory response of retinal pigment epithelial (RPE) cells to Scatter Factor/Hepatocyte Growth Factor (HGF). (a) Proliferation of RPE cells grown in 20% fetal calf serum (20% FCS) in Ham's F10 (F10) which serves as a positive control, serum free (SF) F10 as a negative control, and increasing concentrations of HGF in 2% FCS/F10. Error bars represent SD. * = Statistically significant increase in proliferation over 2% FCS/F10 without added HGF (0) which was set to 100% (MTS assay, ANOVA with Bonferroni $P < 0.05$). (b) Cells migrating per high power field to the following agents: fibronectin (Fn: 10 μg ml^{-1} Fn in F10; positive control) and increasing concentrations of HGF in F10. Error bars represent SD. * = Statistically significant increase in numbers of migrated cells over F10 without added HGF (0) (Boyden migration chamber assay, ANOVA with Bonferroni $P < 0.05$). Note that HGF concentrations as low as 4 ng ml^{-1} produce a statistically significant increase in both RPE cell proliferation and migration.

Table 2 Levels of scatter factor/hepatocyte growth factor (HGF/SF) in the vitreous and subretinal fluid of patients with rhegmatogenous retinal detachment (RRD) and proliferative vitreoretinopathy (PVR)[a]

Anatomical site of sample	*Average HGF/SF level in RRD*	*Average HGF/SF level in PVR*
Vitreous	3.3 ng ml^{-1}	13.0 ng ml^{-1}
Subretinal	9.7 ng ml^{-1}	27.3 ng ml^{-1}

[a]Data from Grierson *et al* 2000; Briggs *et al* 2000.[70,75]

upregulation of c-met and SPARC have been linked in other proliferative processes,[76] we wonder whether a cocktail containing matricellular proteins like TSP1 and SPARC plus members of the HGF/SF family may be responsible for the changes in the RPE monolayer during the initial stages of PVR.

Summary

The presence of matricellular proteins in periretinal membranes and their ability to modify cell–matrix interactions suggests that this group of proteins may play a key role in the pathobiology of ERMs and SRMs. Matricellular proteins like TSP1 and SPARC may counter the adhesive characteristics of major matrix components (eg fibronectin, laminin, collagens) and so modulate periretinal cell activities such as migration or shape change.

With regard to PVR, the partial cell detachment induced by TSP1 and SPARC, the cell rounding which may occur as a sequel to prolonged SPARC exposure and the ability of HGF/SF to dissociate epithelial monolayers led us to speculate that matricellular proteins and members of the HGF/SF family might act in consort to initiate the separation of RPE cells from Bruch's membrane (Figure 4). Such a combination may also induce the characteristic phenotypic changes of RPE cells in early PVR and, as all of these proteins can be made by RPE cells, might reflect an autocrine-like effect. However, SPARC can also suppress other RPE cell activities associated with PVR such as migration and proliferation, although several of the peptide fragments of SPARC do support cellular proliferation. TSP1 and SPARC each bind to, and modify the actions of a number of growth factors, some of which in turn modify the cellular expression of these two matricellular proteins. Indeed, TSP1 binds to HGF/SF, inhibiting HGF/SF-induced chemotaxis of endothelial cells.[77] Moreover, there are a variety of other proteins and peptides which can modulate adhesion between RPE cells and matrix, and which are available to RPE cells during the early stages of PVR.

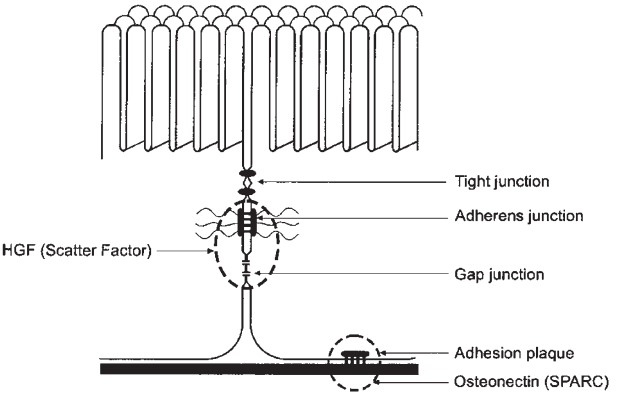

Figure 4 Diagrammatic representation of proposed sites of action of osteonectin (SPARC) and Scatter Factor/Hepatocyte Growth Factor (HGF) during the initial stages of PVR. The thick bottom line denotes Bruch's membrane upon which parts of two neighbouring retinal pigment epithelial (RPE) cells are depicted. It is suggested that, in consort with other molecules which counter cell adhesion (including other matricellular proteins such as TSP-1), prolonged elevated local SPARC levels act at sites of RPE-Bruch's membrane adhesion to loosen attachment of the cell to the membrane (dotted circle). At the same time, HGF may play a role in the breakdown of adhering and gap junctions between adjacent RPE cells (dotted oval). RPE cells would then be free to dissociate from the monolayer.

The role of matricellular proteins in the development of ERMs and SRMs appears complex and further investigations are needed to clarify how molecules like TSP1 and SPARC influence the process. Nevertheless, given the ostensibly pivotal role of matricellular proteins in the modulation of key cell–matrix interactions, such investigations may well lead to therapeutic gain in the management of periretinal membranes. Moreover, information concerning these proteins may be vital in reducing the risk of PVR as a complication of novel surgical procedures (such as RPE cell transplantation and retinal translocation) for the treatment of macular disease.

Acknowledgements

This work was supported by the R&D Support Fund of the Royal Liverpool & Broadgreen University Hospitals NHS Trust (grant Nos. 1617 and 1626), Guide Dogs For The Blind Association, North West Regional Health Authority R&D Directorate, St Paul's Foundation for the Prevention of Blindness, and two Fellowships from the Dunhill Medical Trust. Daniel Brotchie provided photographic assistance.

Presented at the XXXI Cambridge Ophthalmological Symposium, 4th and 5th September 2001.

References

1 Constable IJ, Tolentino FI, Donovan RH, Schepens CL. Clinico-pathologic correlation of vitreous membranes. In: Pruett RD, Regan DJ (eds). *Retinal Congress*. New York: Appleton-Century-Crofts: New York, 1974, pp 254–257.

2 Foos RY. Vitreoretinal juncture—simple epiretinal membranes. *Graefes Arch Clin Exp Ophthalmol* 1974; **189**: 231–250.

3 Clarkson JG, Green WR, Massof D. A histopathologic review of 168 cases of preretinal membrane. *Amer J Ophthalmol* 1977; **84**: 1–17.

4 Hiscott P, Wong D, Grierson I. Challenges in ophthalmic pathology: the vitreoretinal membrane biopsy. *Eye* 2000; **14**: 549–559.

5 The Retina Society Terminology Committee. The classification of retinal detachment with proliferative vitreoretinopathy. *Ophthalmology* 1983; **90**: 121–125.

6 Machemer R, Laqua H. Pigment epithelial proliferation in retinal detachment (massive periretinal proliferation). *Amer J Ophthalmol* 1975; **80**: 1–23.

7 Machemer R. Pathogenesis and classification of massive periretinal proliferation. *Brit J Ophthalmol* 1978; **62**: 737–747.

8 Hiscott P, Grierson I. Retinal detachment. In: Garner A, Klintworth GK (eds). *Pathobiology of Ocular Disease: a Dynamic Approach*, 2nd edn. Marcel Dekker: New York, 1994, pp 675–700.

9 Hiscott P, Morino I, Alexander R, Grierson I, Gregor Z. Cellular components of subretinal membranes in proliferative vitreoretinopathy. *Eye* 1989; **3**: 606–610.

10 Hiscott P, Gray R, Grierson I, Gregor Z. Cytokeratin-containing cells in proliferative diabetic retinopathy membranes. *Br J Ophthalmol* 1994; **78**: 219–222.

11 Roth AM, Foos RY. Surface wrinkling retinopathy in eyes enucleated at autopsy. *Trans Am Acad Ophthalmol Otolaryng* 1971; **75**: 1047–1058.

12 Heidenkummer HP, Kampik A. Morphologic analysis of epiretinal membranes in surgically treated idiopathic macular foramina. Results of light and electron microscopy. *Ophthalmologe* 1996; **93**: 675–679.

13 Szamier RB. Ultrastructural features of the preretinal membrane in retinitis pigmentosa. *Invest Ophtalmol Vis Sci* 1981; **21**: 227–236.

14 Sethi CS, Lewis GP, Leitner WP, Mann DL, Charteris DG, Fisher SK. Neuronal plasticity in complicated clinical and experimental retinal detachment (RD). [ARVO Abstract]. *Invest Ophthalmol Vis Sci* 2001; **42**: S445. Abstract 2401.

15 Chateris D, Hiscott P, Grierson I, Lightman S. Proliferative vitreoretinopathy: lymphocytes in epiretinal membranes. *Ophthalmology* 1992; **99**: 1364–1367.

16 Leaver PK, Grey RHB, Garner A. Silicone oil injection in the treatment of massive preretinal retraction. II. Late complications in 93 eyes. *Br J Ophthalmol* 1979; **63**: 361–367.

17 Heidenkummer HP, Kampik A. Comparative immunohistochemical studies of epiretinal membranes in proliferative vitreoretinal diseases. *Fortschr Ophthalmol* 1991; **88**: 219–224.

18 Heidenkummer HP, Messmer EM, Kampik A. Recurrent vitreoretinal membranes in intravitreal silicon oil tamponade. Morphologic and immunohistochemical studies. *Ophthalmologe* 1996; **93**: 121–125.

19 Hiscott P, Magee RM, Colthurst M, Lois N, Wong D. Clinicopathological correlation of epiretinal membranes and posterior lens opacification following perfluorohexyloctane (F6H8) tamponade. *Br J Ophthalmol* 2001; **85**: 179–183.

20 McLeod D. The vitreous and its disorders. In: Miller S (ed). *Clinical Ophthalmology*. Wright: Bristol, 1987, pp 258–274.

21 Schwartz SD, Alexander R, Hiscott P, Gregor ZJ. Recognition of vitreoschisis in proliferative diabetic retinopathy: a useful landmark in vitrectomy for diabetic traction retinal detachment. *Ophthalmology* 1996; **103**: 323–328.

22 Hiscott PS, Grierson I, McLeod D. The natural history of epiretinal membranes: a quantitative, immunohistochemical and autoradiographic study. *Br J Ophthalmol* 1985; **69**: 810–823.

23 Jerdan JA, Michels RG, Glaser BM. Diabetic preretinal membranes: an immunohistochemical study. *Arch Ophthalmol* 1986; **104**: 286–290.

24 Jerdan JA, Pepose JS, Michels RG, Hayashi H, de Bustros S, Sebag M, Glaser BM. Proliferative vitreoretinopathy membranes an immunohistochemical study. *Ophthalmology* 1989; **96**: 801–810.

25 Scheiffarth OF, Kampik A, Guenther H, v d Mark K. Proteins of the extracellular matrix in vitreoretinal membranes. *Graefe's Arch Clin Exp Ophthalmol* 1988; **226**: 357–361.

26 Morino I, Hiscott P, McKechnie N, Grierson I. Variation in epiretinal membrane components with clinical duration of the proliferative tissue. *Br J Ophthalmol* 1990; **74**: 393–399.

27 Alexander RA, Hiscott P, McGalliard J, Grierson I. Oxytalan fibres are a component of proliferative vitreoretinopathy membranes. *German J Ophthalmol* 1992; **1**: 382–387.

28 Rodrigues MM, Newsome DA, Machemer R. Further characterisation of epiretinal membranes in human massive periretinal proliferation. *Current Eye Res* 1981; **1**: 311–315.

29 Weller M, Weidemann P, Bresgen M, Heimann K. Vitronectin and proliferative intraocular disorders. I. A colocalisation study of the serum spreading factor, vitronectin, and fibronectin in traction membranes from patients with proliferative vitreoretinopathy. *Int Ophthalmol* 1991; **15**: 93–101.

30 Hiscott P, Sheridan C, Magee R, Grierson I. Matrix and the retinal pigment epithelium in proliferative retinal disease. *Prog Retinal Eye Res* 1999; **18**: 167–190.

31 Gailit J, Clark RAF. Wound repair in the context of extracellular matrix. *Curr Op Cell Biol* 1994; **6**: 717–725.

32 Hiscott P, Waller HA, Butler MG, Grierson I, Scott, DL. Local production of fibronectin by ectopic human retinal cells. *Cell Tissue Res* 1992; **267**: 185–192.

33 Hiscott P, Waller HA, Grierson I, Butler MG, Scott DL, Gregor Z, Morino I. Fibronectin synthesis in subretinal membranes of proliferative vitreoretinopathy. *Br J Ophthalmol* 1992; **76**: 486–490.

34 Hiscott P, Waller HA, Grierson I, Butler MG, Scott DL. The extracellular matrix of reparative tissue in the vitreous: fibronectin synthesis in diabetic retinopathy membranes. *Eye* 1993; **7**: 288–292.

35 Campochiaro PA, Jerdan JA, Glaser BM. Serum contains chemoattractants for human retinal pigment epithelial cells. *Arch Ophthalmol* 1984; **102**: 1830–1833.

36 Campochiaro PA, Jerdan JA, Glaser BM, Cardin AC,

Michels, RG. Vitreous aspirates from patients with proliferative vitreoretinopathy stimulate retinal pigment epithelial cell migration. *Arch Ophthalmol* 1985; **103**: 1403–1405.
37 Hiscott P, Sheridan C. The retinal pigment epithelium, epiretinal membranes and proliferative vitreoretinopathy. In: Marmor MF, Wolfensberger TJ (eds). *Retinal Pigment Epithelium—Function and Disease*. Oxford University Press: New York, 1998, pp 478–491.
38 Bornstein P. Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. *J Cell Biol* 1995; **130**: 503–506.
39 Lawler J. The functions of thrombospondin-1 and -2. *Curr Opin Cell Biol* 2000; **12**: 634–640.
40 Murphy-Ullrich JE. The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? *J Clin Invest* 2001; **107**: 785–790.
41 Immonen I, Tervo K, Virtanen I, Laatikainen L, Tervo T. Immunohistochemical demonstration of cellular fibronectin and tenascin in human epiretinal membranes. *Acta Ophthalmologica* 1991; **69**: 466–471.
42 Esser P, Weller M, Heimann K, Wiedemann P. Thrombospondin and its importance in proliferative retinal diseases. *Fortschr Ophthalmol* 1991; **88**: 337–340.
43 Lawler J. The structural and functional properties of thrombospondin. *Blood* 1986; **67**: 1197–1209.
44 Murphy-Ullrich JE, Poczatek M. Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. *Cytokine Growth Factor Rev* 2000; **11**: 59–69.
45 Hiscott P, Larkin G, Robey HL, Orr G, Grierson I. Thrombospondin as a component of the extracellular matrix of epiretinal membranes: comparisons with cellular fibronectin. *Eye* 1992; **6**: 566–569.
46 Sheridan CM, Hiscott P, Grierson I. The role of thrombospondin 1 in RPE migration and in human RPE induced collagen matrix contraction. [ARVO Abstract]. *Invest Ophthalmol Vis Sci* 2001; **42**: S811. Abstract 4348.
47 Mazure A, Grierson I. In vitro studies of the contractility of cell types involved in proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci* 1992; **33**: 3407–3416.
48 Larkin G, Hiscott P, Sheridah G, Occleston NL, Khaw PT, Grierson I. The production of thrombospondin and fibronectin by retinal pigment epithelial (RPE) cells. [ARVO Abstract]. *Invest Ophthalmol Vis Sci* 1994; **35**: S2039. Abstract 3638.
49 Carron JA, Hiscott P, Hagan S, Sheridan CM, Magee R, Gallagher JA. Cultured human retinal pigment epithelial cells differentially express thrombospondin-1, -2, -3, and -4. *Int J Biochem Cell Biol* 2000; **32**: 1137–1142.
50 Lahav J. The functions of thrombospondin and its involvement in physiology and pathophysiology. *Biochim Biophys Acta* 1993; **1182**: 1–14.
51 Reed MJ, Puolakkainen P, Lane TF, Dickerson D, Bornstein P, Sage HE. Differential expression of SPARC and thrombospondin 1 in wound repair: immunolocalization and in situ hybridisation. *J Histochem Cytochem* 1993; **41**: 1467–1477.
52 Lane TF, Sage EH. The biology of SPARC, a protein that modulates cell-matrix interactions. *FASEB J* 1994; **8**: 163–173.
53 Bradshaw AD, Sage EH. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *J Clin Invest* 2001; **107**: 1049–1054.
54 Motamed K. SPARC (osteonectin/BM-40). *Int J Biochem Cell Biol* 1999; **31**: 1363–1366.
55 Motamed K, Sage EH. Regulation of vascular morphogenesis by the matricellular protein SPARC. *Kidney Internat* 1997; **51**: 1383–1387.
56 Hiscott P, Armstrong A, Batterbury M, Kaye S. Repair in avascular tissues: fibrosis in the transparent structures of the eye and thrombospondin 1. *Histol Histopathol* 1999; **14**: 1309–1320.
57 Paraoan L, Grierson I, Maden BEH. Analysis of expressed sequence tags of retinal pigment epithelium: cystatin C is an abundant transcript. *Int J Biochem Cell Biol* 2000; **32**: 417–426.
58 Magee RM, Hagan S, Hiscott P, Carron JA, Sheridan CM, McGalliard J, Grierson I. Synthesis and secretion of osteonectin (SPARC) by human retinal pigment epithelial cells is modulated by cell density. *Invest Ophthalmol Vis Sci* 2000; **41**: 2707–2711.
59 Kim SY, Ondhia N, Vidgen D, Malaval L, Ringuette M, Kalnis VI. Spatiotemporal distribution of SPARC/Osteonectin in developing and mature chick retina. *Exp Eye Res* 1997; **65**: 681–689.
60 Alexander JP, Bradley JMB, Gabourel JD, Acott TS. Expression of matrix metalloproteinases and inhibitor by human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 1990; **31**: 2520–2528.
61 Hunt RC, Fox A, Al Pakalnis V, Sigel MM, Kosnosky W, Choudhury P, Black EP. Cytokines cause cultured retinal pigment epithelial cells to secrete metalloproteinases and to contract collagen gels. *Invest Ophthalmol Vis Sci* 1993; **34**: 3179–3186.
62 Webster L, Chignell AH, Limb GA. Predominance of MMP-1 and MMP-2 in epiretinal and subretinal membranes of proliferative vitreoretinopathy. *Exp Eye Res* 1999; **68**: 91–98.
63 Mckie JN, Bevitt DJ, Lorite MJ, Pimenides D, Clarke MP, Langton KP, Barker MD. RPE cells express several members of the metallo-disintegrin (ADAM) family of extracellular matrix modifying enzymes. [ARVO Abstract]. *Invest Ophthalmol Vis Sci* 2001; **42**: S222. Abstract 1197.
64 Sheridan CM, Hiscott P, Khaw PT, Grierson I. The role of matrix metalloproteinases (MMPs) in human retinal pigment epithelial-induced collagen matrix contraction and adhesion to collagen type-I. [ARVO Abstract]. *Invest Ophthalmol Vis Sci* 1999; **40**: S461. Abstract 2432.
65 Sheridan CM, Occleston NL, Hiscott P, Kon CH, Khaw PT, Grierson I. Matrix metalloproteinases: a role in the contraction of vitreo-retinal scar tissue. *Amer J Pathol* 2001; **159**: 1555–1566.
66 Burke JM. Cell interactions in proliferative vitreoretinopathy: do growth factors play a role? In: Heimann K, Wiedemann P (eds). *Proliferative Vitreoretinopathy*. Kaden: Heidelberg, 1989, pp 80–87.
67 Campochiaro PA. Growth factors in the retinal pigment epithelium and retina. In: Marmor MF, Wolfensberger TJ (eds). *Retinal Pigment Epithelium—Function and Disease*. Oxford University Press: New York, 1998, pp 459–477.
68 Stoker M, Gherardi E, Perryman M, Gray J. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature* 1987; **327**: 239–242.
69 Furlong RA, Takehara T, Taylor WG, Nakamura N, Rubin JS. Comparison of biological and immunochemical properties indicates that scatter factor and hepatocyte

growth factor are indistinguishable. *J Cell Sci* 1991; **100**: 173–177.
70 Grierson I, Heathcote L, Hiscott P, Hogg P, Briggs M. Hepatocyte growth factor/scatter factor in the eye. *Prog Retinal Eye Res* 2000; **19**: 779–802.
71 He PM, He S, Garner JA, Ryan SJ, Hinton DR. Retinal pigment epithelial cells secrete and respond to hepatocyte growth factor. *Biochem Biophys Res Commun* 1998; **249**: 253–257.
72 Lashkari K, Rahimi N, Kazlauskas A. Hepatocyte growth factor receptor in human RPE cells: implications in proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci* 1999; **40**: 149–156.
73 Katsura Y, Okano T, Noritake M, Kosano H, Nishigori H, Kado S, Matsuoka T. Hepatocyte growth factor in vitreous fluid of patients with proliferative diabetic retinopathy and other retinal disorders. *Diabetes Care* 1998; **21**: 1759–1763.
74 Mitamura Y, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y, Nishihira J. Hepatocyte growth factor levels in the vitreous of patients with proliferative vitreoretinopathy. *Am J Ophthalmol* 2000; **129**: 678–680.
75 Briggs MC, Grierson I, Hiscott P, Hunt JA. Active scatter factor (HGF/SF) in proliferative vitreoretinal disease. *Invest Ophthalmol Vis Sci* 2000; **41**: 3085–3094.
76 Porte H, Triboulet JP, Kotelevets L, Carrat F, Prevot S, Nordlinger B. Overexpression of stromelysin-3, BM-40/SPARC, and MET genes in human esophageal carcinoma: implications for prognosis. *Clin Cancer Res* 1998; **4**: 1375–1382.
77 Lamszus K, Joseph A, Jin L, Yao Y, Chowdhury S, Fuchs A. Scatter factor binds to thrombospondin and other extracellular matrix components. *Am J Pathol* 1996; **149**: 805–819.