



Mini review

The role of matricellular proteins in glaucoma

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ABSTRACT

Glaucoma is an optic neuropathy affecting approximately 60 million people worldwide and is the second most common cause of irreversible blindness. Elevated intraocular pressure (IOP) is the main risk factor for developing glaucoma and is caused by impaired aqueous humor drainage through the trabecular meshwork (TM) and Schlemm's canal (SC). In primary open angle glaucoma (POAG), this elevation in IOP in turn leads to deformation at the optic nerve head (ONH) specifically at the lamina cribrosa (LC) region where there is also a deposition of extracellular matrix (ECM) molecules such as collagen and fibronectin.

Matricellular proteins are non-structural secreted glycoproteins that help cells communicate with their surrounding ECM. This family of proteins includes connective tissue growth factor (CTGF), also known as CCN2, thrombospondins (TSPs), secreted protein acidic and rich in cysteine (SPARC), periostin, osteonectin, and Tenascin-C and -X and other ECM proteins. All members appear to play a role in fibrosis and increased ECM deposition. Most are widely expressed in tissues particularly in the TM and ONH and deficiency of TSP1 and SPARC have been shown to lower IOP in mouse models of glaucoma through enhanced outflow facility. The role of these proteins in glaucoma is emerging as some have an association with the pathophysiology of the TM and LC regions and might therefore be potential targets for therapeutic intervention in glaucoma.

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1. Introduction

1.1. Glaucoma

Glaucoma is the second leading cause of irreversible blindness worldwide, thought to affect 60 million people (Kelliher et al., 2006; Quigley and Broman, 2006). In the western world, glaucoma affects

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Aqueous humour outflow pathways

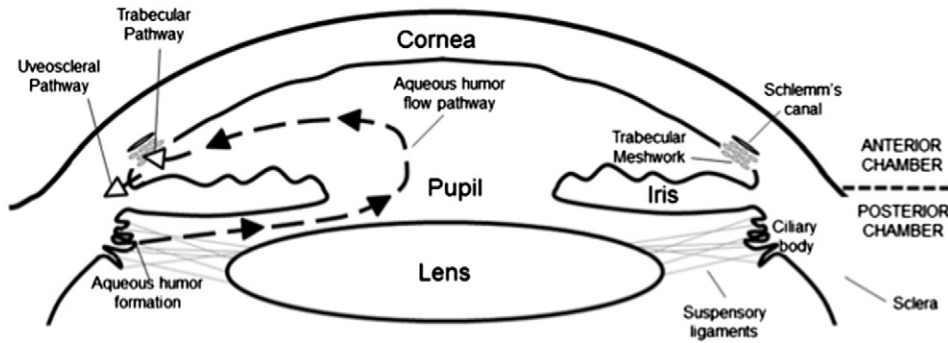


Fig. 1. Aqueous humor outflow pathways. The primary risk factor for onset and progression of glaucoma is raised intraocular pressure (IOP). IOP is generated in the anterior eye via the aqueous humor circulation system. The aqueous humor is secreted by the ciliary epithelium and flows to the anterior chamber to leave through the trabecular meshwork (TM) outflow pathways. In primary open angle glaucoma (POAG) the resistance to outflow increases in the TM, particularly in the juxtacanalicular connective tissue (JCT) region culminating in elevated IOP. The TM and Schlemm's canal (SC) provide the major route for the outflow of the aqueous humor from the eye and it is here responsible for the increased IOP associated with POAG due to increased outflow resistance. Changes in extracellular matrix (ECM) are thought to be involved in the increased outflow resistance in POAG. While the molecular events responsible for the impaired TM drainage and deposition at the ONH are not well understood, it is thought that ECM turnover in these regions and the proteins responsible may be contributory factors. Cellular contraction and relaxation of TM and SC cells are also important factors in the maintenance of normal aqueous humor outflow facility and agents that can alter contraction can change outflow rates. *Genetics and Environmental Stress Factor Contributions to Anterior Segment Malformations and Glaucoma* by Yoko A. Ito and Michael A. Walter, "Glaucoma – Basic and Clinical Aspects" edited by Shimon Rumelt, ISBN 978-953-51-1064-4, InTech, April 4, 2013.

1–2% of the population over the age of 40 and the prevalence rises to 5% of those aged 70 years and over. It is a chronic progressive optic neuropathy with characteristic extracellular matrix (ECM) changes in the optic nerve head (ONH) and subsequent visual field defects. The primary risk factor for onset and progression of glaucoma is raised intraocular pressure (IOP) (Anon., 1998a,b; Gordon et al., 2002; Leske et al., 2004; Spry et al., 2005). IOP is generated in the anterior eye via the aqueous humor circulation system. The aqueous humor is secreted by the non-pigmented ciliary epithelium and flows to the anterior chamber to leave through the trabecular meshwork (TM) outflow pathways (Tamm, 2009). The TM and Schlemm's canal (SC) provide the major route for the outflow of the aqueous humor from the eye and it is here responsible for the increased IOP associated with primary open angle glaucoma (POAG) due to increased outflow resistance (Moses, 1977; Maepea and Bill, 1992). In POAG the resistance to outflow increases (Johnson et al., 2002) in the TM, particularly in the juxtacanalicular connective tissue (JCT) region culminating in elevated IOP (Johnson, 2006) (Fig. 1). Changes in ECM are also thought to have a role in the increased outflow resistance of the TM in POAG. While the molecular events responsible for the impaired TM drainage and ECM deposition at the ONH are not well understood, it is thought that ECM turnover in these regions and the proteins responsible may be contributory factors. Cellular contraction and relaxation of TM and SC cells are important factors in the maintenance of normal aqueous humor outflow facility and agents that can alter contraction can change outflow rates (Epstein et al., 1987, 1999; Tian et al., 2000; Wiederholt et al., 2000; Rao et al., 2005; Tian and Kaufman, 2005; Yu et al., 2008).

Chronic elevation in IOP causes a deformation at the ONH specifically at the lamina cribrosa (LC) region in the ONH. Hernandez et al., showed that it is the LC which undergoes fibrosis and mechanical failure in POAG (Hernandez et al., 1990). The LC region of the ONH consists of perforated fibroelastic plates through which the unmyelinated retinal ganglion cell axons pass through before they converge as the optic nerve (Anderson, 1969). ONH astrocytes and LC cells are members of the glial cell population of the ONH and attach to the basement membrane. LC cells can be differentiated from astrocytes by their non-expression of glial fibrillary acid protein (Hernandez et al., 1988). These laminar plates contain ECM such as elastin and collagens I, III, V and VI and it is here the nerve axons degenerate in parallel with the apoptotic cell death of retinal ganglion cells and results in progressive visual field loss. Axonal degeneration may be caused by the blockade of

the anterograde and retrograde axonal transport systems at the level of the LC leading to a deprivation of neurotrophic signals (Quigley, 2011) and is accompanied by a local remodeling of the ECM in the ONH. The disturbed ECM remodeling at the ONH is particularly evident in the LC region (Burgoyne et al., 2005).

The ECM is a key component of multicellular organisms forming an intricate proteinaceous network that fills the extracellular spaces and provides structural support and tissue organization (Ozbek et al., 2010). Maintaining the integrity of the ECM is necessary for the normal structure and function of connective tissue. However, in fibrosis there is an excessive deposition of ECM to pathological rather than physiological levels. Alterations in the levels of modulators of ECM homeostasis such as matrix metalloproteinases (MMPs) – 2 – 3 and – 14 also occur in the POAG LC (Yan et al., 2000a; Yuan and Neufeld, 2001). MMPs are zinc-dependent endopeptidases that degrade ECM components such as collagen and fibronectin. MMPs have been described as important modulators of aqueous humor outflow through their ability to remodel the TM ECM and maintain a constant outflow resistance and ensuing IOP (De Groef et al., 2013). MMPs are therefore potential therapeutic targets for the treatment of glaucoma specifically their ability to modulate aqueous humor outflow (De Groef et al., 2013).

The connective tissue changes in POAG affect the TM and the LC and may result from a common defect in these cells. It has been proposed that the TM and the LC are biochemically similar tissues and that the cells cultured from the two are very similar (Hernandez et al., 1987; Rehnberg et al., 1987; Morrison et al., 1989; Yun et al., 1989; Wilson et al., 1993; Clark et al., 1994; Steely et al., 2000; Kirwan et al., 2009). The fibrotic phenotype associated with glaucoma in the LC and TM regions has been widely reported (Rehnberg et al., 1987; Hann et al., 2001; Kirwan et al., 2009). In glaucoma, the LC undergoes thickening (Yang et al., 2010) and posterior migration (Yang et al., 2011) in the early stages of the disease process, and later undergoes shearing and collapse of the LC plates finally leading to a thin fibrotic connective tissue structure/scar (Jonas et al., 2003) where we observe disturbed ECM metabolism (Burgoyne et al., 2005) and increased deposition of collagens and elastin (Hernandez and Pena, 1997). Similar to the LC, the TM of patients with POAG is characterized by the build-up of ECM material (Tektas and Lutjen-Drecoll, 2009) and this accumulation eventually results in increased outflow resistance with subsequent elevated IOP. Pseudoexfoliation (PXF) syndrome is currently the single most important identifiable risk factor for open-angle glaucoma (Schlotzer-

Table 1
Role of matricellular proteins in tissues affected in glaucoma.

Protein/ tissue	AqH/IOP levels	Trabecular meshwork	ONH/LC/astrocyte
SPARC	Expressed: Yan et al. (2000b), Rhee et al. (2003) Overexpression elevates IOP: Oh et al. (2013), SPARC $-/-$ mice have lower IOPs compared to WT: Haddadin et al. (2009)	Expressed: Rhee et al. (2003), Tomarev et al. (2003) Increased by TGF β 2: Kang et al. (2013), Bollinger et al. (2011) Increased by stretch: Vittal et al. (2005)	Increased in GLC: Kirwan et al. (2009) Induced by cyclical stretch of LC cells: Kirwan et al. (2005a)
CTGF	Increased in AqH from PXFG patients: Ho et al. (2005), Browne et al. (2011)	Expressed: Tomarev et al. (2003) Increased by stretch: Vittal et al. (2005) Induces ECM deposition: Junglas et al. (2009) Necessary for TGF β 2 induced ECM synthesis: Junglas et al. (2009) Induced by oxidative stress & AqH: Wallace et al. (2013)	Increased by TGF β 1: Kirwan et al. (2005b) Necessary for TGF β 2 ECM synthesis in astrocytes: Fuchshofer et al. (2005)
TSP1/2	TSP $-/-$ mice have lower IOPs compared to WT: Haddadin et al. (2012)	Expressed: Tomarev et al. (2003), Tripathi et al. (1991) TSP1 located to JCT region & TSP2 to uveoscleral meshwork: Hiscott et al. (1996) Increased by stretch: Vittal et al. (2005)	Increased in GLC: Kirwan et al. (2009) Induced by cyclical stretch of LC cells: Kirwan et al. (2005a) Increased in LC cells following exposure to TGF β 1: Kirwan et al. (2005b)
Periostin		Elevated in GTM: Liton et al. (2006) Increased by stretch: Vittal et al. (2005) Induced by TGF β 1/2: Zhao et al. (2004)	Induced by cyclical stretch of LC cells: Kirwan et al. (2005a) Increased in GLC: Kirwan et al. (2009),

AqH: Aqueous Humor, IOP: Intraocular Pressure, ONH: Optic Nerve Head, LC: Lamina Cribrosa, GLC: Glaucomatous LC, GTM: Glaucomatous TM, PXFG: Pseudoexfoliation Glaucoma, JCT: Juxtacanalicular Connective Tissue.

Schrehardt and Naumann, 2006). It is an age related generalized disorder of the ECM characterized by the production and progressive accumulation of fibrillar material (such as fibronectin and fibrillin-1) in ocular tissues and in the connective tissue portions of the various visceral organs (Schlotzer-Schrehardt et al., 1997, 2001). Therefore, there is substantial evidence that ECM production/turnover is integral to the pathophysiology of glaucoma and in glaucoma filtration surgery.

1.2. Matricellular proteins

Given the importance of ECM deposition and turnover in the TM and LC regions in glaucoma, it is not surprising that there is also an emerging role for matricellular proteins in this neuropathy. The term “matricellular proteins” was first used by Paul Bornstein to describe a group of proteins that serve as links between cells and ECM (Bornstein, 1995). This group of proteins is described as being non-structural secreted glycoproteins that enable cells to communicate with and control their surrounding ECM. The original family included, secreted protein and rich in cysteine (SPARC), thrombospondin-1 (TSP-1) and TSP-2, osteonectin and Tenascin-C, but has now grown to include connective tissue growth factor (CTGF) or CCN2, periostin, Tenascin-C and -X, SC1/hevin, the short fibulins, and other proteins. Interestingly a number of these matricellular proteins are expressed widely in ocular tissues including cornea, lens, retina, vitreous, aqueous and TM playing a specific defined role in each (Gilbert et al., 1999; Yan and Sage, 1999; Kantorow et al., 2000; Yan et al., 2000b; Berryhill et al., 2003; Rhee et al., 2003; Hiscott et al., 2006; Rhee et al., 2009) (see Table 1). Indeed, Tomarev et al., described CTGF and SPARC-like 1 as being part of the 50 most abundant cDNA clones in the human TM cDNA library (Tomarev et al., 2003). The focus of this review will be on the emerging role of matricellular proteins in glaucoma and glaucoma filtration surgery.

2. Biological roles of matricellular proteins in glaucoma

2.1. Connective tissue growth factor

Connective tissue growth factor (CTGF) is a member of the CCN family named after its three original members; cysteine rich protein 61 (Cyr61, CCN1), connective tissue growth factor (CTGF, CCN2) and nephroblastoma overexpressed protein (Nov, CCN3). Three additional Wnt-inducible secreted proteins were later identified (CCN4, CCN5, CCN6). Early studies suggested that these proteins were bona-fide

growth factors; however data now supports the role of CCN proteins as matricellular proteins that can bind to matrix and modulate cellular functions (Leask and Abraham, 2006; Yeager and Perbal, 2007; Chen and Lau, 2009) via the modification of signals from other molecules such as transforming growth factor beta (TGF β). Evidence proposes that CTGF is strongly associated with fibrosis (Brigstock, 2009), that CTGF is a TGF β inducible gene, and CTGF is significantly up-regulated in experimental models of fibrosis associated with aberrant ECM deposition (Ito et al., 1998; Lasky et al., 1998; Gupta et al., 2000; Sato et al., 2000; Lang et al., 2008). Wang et al., demonstrated that CTGF and TGF β 2 co-operate to promote fibrosis and that inhibition of CTGF alone can ameliorate the observed fibrotic phenotype (e.g. reduce the increased hydroxyproline:proline ratios) in a unilateral ureteral obstruction renal fibrosis model and an intratracheal bleomycin instillation model of pulmonary fibrosis (Wang et al., 2011a). CTGF mediates some TGF β -dependent effects on ECM production, including collagen and fibronectin (Crean et al., 2002; Weston et al., 2003). In astrocytes, the transfection of CTGF siRNA before TGF β 2 treatment inhibited the TGF β 2-induced up regulation of CTGF and fibronectin (Fuchshofer et al., 2005). Furthermore, CTGF is a critical mediator of the effects of TGF β 2 on ECM synthesis in human TM cells as siRNA knockdown of CTGF inhibited TGF β 2-induced up-regulation of fibronectin (Junglas et al., 2009). There appears to be a minimum critical threshold level of CTGF that is required to initiate/maintain fibrosis, and if CTGF levels are below this threshold, fibrogenesis is diminished. Interestingly, CTGF is up-regulated in both LC and TM cells under the conditions of cell stretching which are similar to conditions under elevated IOP (Kirwan et al., 2005a; Vittal et al., 2005).

There has been a significant interest in the role of TGF β 2 and CTGF in aqueous humor homeostasis associated with raised IOP and glaucoma (Tripathi et al., 1994a; Welge-Lussen et al., 2001). Junglas et al., have used a transgenic mouse model to show that CTGF expressed in the aqueous humor elevates IOP which is associated with TM actin cytoskeleton modification (Junglas et al., 2012). TGF β alters ECM production and turnover in both the LC and TM and has been shown in numerous studies to play a role in ocular wound healing (Cordeiro et al., 2000a; Picht et al., 2001) and its role in the pathogenesis of glaucoma is also well documented (Kottler et al., 2005; Fuchshofer, 2010; Fuchshofer and Tamm, 2011). Several studies have reported elevated aqueous humor levels of TGF β 2 in POAG (Tripathi et al., 1994b; Inatani et al., 2001) and TGF β 1 in PXFG patients (Schlotzer-Schrehardt et al., 2001). Data from our laboratory has shown that TGF β 1 has an effect on global gene expression

profiles, especially pro-fibrotic ECM genes in nerve head LC cells (Kirwan et al., 2005b) and that the CTGF level in the aqueous humor of patients with PXFG was significantly higher than that in both POAG and normal control subjects (Ho et al., 2005; Browne et al., 2011). It appears that coordinate expression of TGF β 1/2 and CTGF is a normal feature of wound healing. However, pathological fibrosis is often attributed to uncontrolled matrix deposition, perhaps mediated by a CTGF-enriched microenvironment. This has therefore focused attention on CTGF as a possible therapeutic target, which would avoid the pleiotropic effects of TGF β inhibition.

2.2. Thrombospondin

The aqueous humor of POAG patients contains significantly elevated levels of TGF β 2 (Tripathi et al., 1994b; Inatani et al., 2001). However, most of this TGF β 2 is present in the latent, inactive form and is unable to interact with its cellular receptors (Gleizes et al., 1997; Annes et al., 2003). This latent form can be activated *in vitro* and *in vivo* by the matricellular protein thrombospondin 1 (TSP1) (Schultz-Cherry et al., 1994; Murphy-Ullrich and Poczatek, 2000). TSP1 belongs to a small family of secreted glycoproteins (Adams, 2001) of which there are 5 members TSP1–5 (Adams and Lawler, 2004). TSP1 induces activation through conformational changes in the latent complex by binding to specific sequences in the type 1 repeats or thrombospondin repeats (TSRs) (Schultz-Cherry et al., 1994) (Schultz-Cherry and Murphy-Ullrich, 1993). Functionally, TSP1 and TSP2 play a role in continued tissue turnover (Bornstein, 2001) and are required for normal cutaneous wound healing. However, in fibrosis, wound healing and other circumstances where there is an excessive ECM production, TSP1 expression is increased (Uno et al., 2004; Suzuki et al., 2005; Hiscott et al., 2006). Blockade of TSP1 or loss of TSP1 expression can help reduce pathologic tissue remodeling (Poczatek et al., 2000; Hugo, 2003; Daniel et al., 2007). TSP1 is a potent activator of TGF β 1 and binds to and activates TGF β under physiological conditions (Murphy-Ullrich et al., 1992). This finding is substantiated by the fact that cultures of TSP1 null cell isolates revealed one eighth the amount of active TGF β 1 (Crawford et al., 1998). Nevertheless, it is thought that the principal role for TSP1 regulation of TGF β 1 activation is under the conditions of stress or injury as opposed to developmental circumstances (Yevdokimova et al., 2001; Wang et al., 2002; Zhou et al., 2006). TGF β 1 treatment has also been shown to increase TSP1 expression indicating a reciprocal relationship (Penttinen et al., 1988). Interestingly, in the TM, the administration of both TGF β 1 and TGF β 2 resulted in an upregulation of TSP1 (Flugel-Koch et al., 2004; Fleenor et al., 2006; Fuchshofer et al., 2007). TSP1 and TSP2 have been studied in both ocular and non-ocular tissues (Adams and Lawler, 2004; Hiscott et al., 2006) and are widely expressed throughout the eye. TSP1 is present in the TM in which it increases with age and is made by TM cells *in vitro* (Tripathi et al., 1991, 1997; Liu et al., 2003) and is more intense in one third of patients with POAG (Flugel-Koch et al., 2004) in agreement with the elevated levels of TGF β 2 in the aqueous of these patients (Tripathi et al., 1994b; Inatani et al., 2001). TSP1 has also been observed in the TM of human eyes (Tripathi et al., 1991) as well as corneal and stromal fibroblasts (Hiscott et al., 1996). TSP1 transcripts have been identified in a cDNA library generated from fresh TM (Tomarev et al., 2003). In the TM TSP1 is primarily located at the JCT region (anatomical location of outflow resistance) and TSP2 is localized at the uveoscleral meshwork (Tomarev et al., 2003; Hiscott et al., 2006), therefore TSP1 may help to regulate IOP via interactions between TM cells and their matrices (Liu et al., 2003). TSP1 is also up-regulated in response to stretch in TM cells (Vital et al., 2005) indicating an important role in IOP regulation. Studies from our laboratory investigating the role of LC cells in the ONH fibrotic response associated with glaucoma have also implicated TSP1 as being an important factor in ECM deposition/turnover. Gene expression analysis comparing differentially expressed ECM genes between human primary LC cells obtained from both normal and glaucomatous

donors showed an increase in expression of TSP1 in glaucomatous cells (Kirwan et al., 2009). This differential expression was also observed following mechanical stretch (mimicking elevated IOP) of LC cells (Kirwan et al., 2005a) and in response to exposure of LC cells to exogenous TGF β 1 (Kirwan et al., 2005b). These data indicate that the expression and secretion of TGF β mediated by TSP1 in LC cells may lead to progressive ECM accumulation and eventual damage.

IOPs of TSP1 null and TSP2 null mice have been measured in comparison to wild type (WT) mice. A study by Haddadin et al., showed that average IOPs of TSP1 null and TSP2 null were 10% and 7% ($P < 0.05$) less than that of the corresponding WT mice (Haddadin et al., 2012). Average IOPs of TSP1 null mice and WT mice were 14.2 \pm 2.0 and 15.8 \pm 1.5 mm Hg respectively and average IOPs of TSP2 null mice and WT mice were 16.8 \pm 2.0 and 18.1 \pm 1.6 mm Hg respectively. These lower IOP readings were thought to be due to the TSP1 and TSP2 null mice demonstrating enhanced aqueous drainage. TSP levels in the TM were analyzed by immunofluorescence experiments and suggested that there may be a possible synergistic effect between TSP1 and TSP2 as TSP1 null mice had reduced expression of TSP2 and similarly TSP2 null mice had reduced levels of TSP1. TSP1 can also suppress the activation of promatrix MMP9 while TSP1 null mice exhibit higher levels of activated MMP9 (Rodriguez-Manzanique et al., 2001) and TSP2 null mice exhibit significantly greater MMP2 levels (Kyriakides et al., 2001). Collagen fibril diameter in the JCT was also assessed and was altered in the TSP1 and TSP2-null mice, it is proposed that this may be either a direct or an indirect sign of altered matrix turnover and therefore affect outflow facility. These findings are suggestive of TSPs' role in aqueous humor outflow resistance through ECM alterations in the JCT region. It also appears reasonable that local increased levels of TSP in the TM could cause abnormally high levels of active TGF β in the TM leading to pathological increases in ECM deposition as studies have shown that the implantation of subcutaneous TSP1 soaked sponges increased the levels of active TGF β 1 (Sakai et al., 2003). It remains to be determined if future therapies targeting the reduction of TSP1 or TSP2 to lower IOP may prove to be an attractive option for the treatment of glaucoma.

2.3. Secreted protein acidic and rich in cysteine (SPARC)

Secreted protein acidic and rich in cysteine (SPARC) (osteonectin/BM-40) is responsible for the mediation of ECM organization and turn over in many human tissues. It was first described as the main noncollagenous constituent of bone (Termine et al., 1981; Brekken and Sage, 2000) and its functions include the regulation of cell function and tissue remodeling by exerting counteradhesive actions, by modulating growth factor signaling and by serving as a cell cycle inhibitor (Chlenski and Cohn, 2009). Growth factor-mediated effects appear to be implicated in the regulation of SPARC in injured tissues such as the TGF β family's capacity to induce SPARC in various cell populations such as corneal fibroblasts (Abe et al., 2004) and TM cells. SPARC is the most highly produced protein in TM cells following treatment with TGF β 2 (Bollinger et al., 2011; Kang et al., 2013). Increased SPARC expression is observed in chronic fibrotic conditions (Strandjord et al., 1999) suggestive of a pro-fibrotic role for this matricellular protein. In agreement SPARC-null mice have decreased the deposition of laminin and type I and IV collagens in the kidney in a diabetic nephropathy model (Taneda et al., 2003), yet again indicating that a primary function of this matricellular protein is the regulation of ECM production.

Similar to TSPs 1 and 2, SPARC is up-regulated in LC cells obtained from glaucomatous human donors as compared to normal cells and it is also increased following the cyclical stretch of LC cells (Kirwan et al., 2005a, 2009). SPARC is found in the aqueous humor and is highly expressed in the TM (Rhee et al., 2003; Tomarev et al., 2003), especially in the JCT region where aqueous humor outflow resistance is highest and believed to be the anatomic location of outflow resistance (Rhee et al., 2009). The cause of this compromised

aqueous drainage through the TM is not fully understood: however, it is well documented that patients with POAG have a much higher level of TGF β 2 in their aqueous humor as compared to age matched controls (Tripathi et al., 1994b; Inatani et al., 2001; Picht et al., 2001; Ochiai and Ochiai, 2002). Moreover, SPARC expression is significantly increased when TM cells are stretched in vitro (Vittal et al., 2005), stretch being an in vivo consequence of elevated IOP. These TM findings in conjunction with our finding that the cyclical stretch of primary human LC cells induces SPARC (Kirwan et al., 2005a) indicate that SPARC may have a regulatory role in IOP regulation. Statins have been shown to increase aqueous outflow and Lovastatin has recently been identified as an important pharmacological suppressor of SPARC expression in TM cells (Villarreal et al., 2014). Oh et al., described how the overexpression of SPARC in human TM increases IOP in perfused cadaveric human anterior segments and SPARC alters extracellular matrix specifically at the JCT, with a selective decrease of MMP-9 activity thought to be the likely mechanism (Oh et al., 2013). Abnormal accumulations of ECM within the JCT have been identified in eyes with POAG compared with age-matched controls (Rohen et al., 1993). Interestingly, SPARC also suppresses apoptosis in fibroblasts from patients with idiopathic pulmonary fibrosis (Chang et al., 2010) and defective clearance of these cells may be important in disease progression. SPARC has also been shown to be significantly increased in the iris of primary angle closure glaucoma patients, suggesting that it could play a role in the development of primary angle closure glaucoma by influencing the biomechanical properties of the iris through a change in ECM organization (Chua et al., 2008).

Haddadin et al., performed a study on IOP levels in SPARC-null mice compared to corresponding WT and heterozygous mice (Haddadin et al., 2009). SPARC-null mice were found to have lower IOPs than their corresponding WT mice with equal central corneal thickness; heterozygous SPARC mice had intermediate IOPs indicating that the transgenic deletion of SPARC significantly affects the aqueous humor outflow pattern. The collagen fibril diameter was significantly decreased in the JCT of SPARC-null mice reflecting the importance of SPARC in ECM processing and a potential mechanism by which IOP is reduced in SPARC-null mice. Significant changes in collagen fibril diameter in other tissues of SPARC-null mice have been reported in other studies (Bradshaw et al., 2002, 2003; Martinek et al., 2007). No significant morphological difference was detected between the TM of SPARC-null and WT tissues.

2.3.1. Matricellular proteins (SPARC and CTGF) in glaucoma surgery

Filtration surgery is used as a treatment for glaucoma when topical medications are ineffective. While it is one of the most effective methods for lowering IOP in the treatment of glaucoma, a major obstacle to successful outcome of this surgery is the post-operative wound healing response of the fistula (maintains aqueous humor outflow) due to excessive deposition of ECM (Reddick et al., 1985). Human Tenon's fibroblasts (HTFs) are the main effector cells involved in the excessive subconjunctival scarring observed following glaucoma surgery (Skuta and Parrish, 1987). Following the activation of HTFs by cytokines and growth factors (Khaw et al., 1994; Daniels et al., 1998; Cordeiro et al., 2000b) contained in the aqueous humor passing through the surgical wound (Costa et al., 1993) there is increased proliferation, migration, collagen contraction and ECM synthesis. To this end, anti-proliferative agents such as Mitomycin C and 5-fluorouracil (Kitazawa et al., 1991; Skuta et al., 1992) have been used in an attempt to prevent surgical failure/improve surgical outcome. The anti-fibrotic and anti-proliferative effects of Bevacizumab (O'Neill et al., 2010) and Paclitaxel (Choritz et al., 2009) on HTFs have also been investigated in vitro and while showing efficacy there still remains a significant surgical failure rate (Higginbotham et al., 1996).

Fuchshofer et al., described the presence of SPARC in the blood vessels of normal human Tenon's capsules, however, more intense SPARC

staining was observed in blood vessels and connective tissue in scarred capsules (Fuchshofer et al., 2011) and is also significantly increased in HTFs following treatment with either TGF β 1 or TGF β 2. As the fibrogenic effects of SPARC have been well-described, strategies targeting SPARC to reduce fibrosis and improve surgical outcome would seem an attractive therapeutic approach. Seet et al. (2010) demonstrated that SPARC-null conjunctival fibroblasts do not respond to TGF β 2 with increased expression of ECM proteins and possess a lower level of MMP2. In vivo they demonstrated that the deficiency of SPARC could maintain the surgically enhanced wound for a longer period of time than WT. At 14 days post-surgery, blebs were maintained in 87.5% of the operated SPARC-null mice as compared to 0% of the WT mice. Wounds in SPARC-null mice also have less collagen I relative to the corresponding WT mice and therefore have altered ECM composition. Collagen I and fibronectin were not enhanced in the SPARC-null mice by TGF β 2 treatment, whereas WT mice exhibited a TGF β 2-induced increase of both ECM proteins, implying that SPARC deficiency may alleviate the fibrotic phenotype by the reduction of collagen I and fibronectin in the presence of TGF β 2.

In vivo, bleb size was increased in SPARC-null mice as compared to WT mice. It has been long recognized that bleb size is a representative of filtering efficiency of the surgical wound and it appears that deficiency in SPARC results in more efficient aqueous filtration. Given the fact that the WT conjunctiva showed thick strands of fibrous material in the sub-conjunctival space in contrast to the SPARC-null mice in which the bleb appeared vacuous with thin wispy strands of matrix material, it is thought that the alteration of collagen deposition might occur in vivo, similar to observations in vitro. Additionally, SPARC and collagen I were present at significant levels at the sites of injury in comparison to normal conjunctiva. SPARC can also stimulate the contraction of collagen gels by HTF cells (Fuchshofer et al., 2011) and therefore may directly contribute to the scarring process. It can therefore be concluded that SPARC plays an integral role in ECM organization that is crucial to the maintenance of a healthy functioning filtration system and that bleb failure in WT eyes is most likely due to the obstruction of the aqueous outflow from increased ECM deposition in the subconjunctival space.

Cytokines play an important role in the scar formation of the bleb (Esson et al., 2004). Studies have shown that CTGF while working as the downstream factor of TGF β (Ruiz-Ortega et al., 2007) is one of the leading causes of bleb scarring (Liang et al., 2010). It can accelerate fibroblast proliferation, migration, adhesion and secretion of ECM and has been shown to be of importance for primary tenon fibroblast function (Seher et al., 2011). Yuan et al., demonstrated the role of CTGF in wound healing post filtering surgery with the overexpression of CTGF in the bleb (Yuan et al., 2009) in agreement with the reported higher expression levels of CTGF in a filtering bleb post surgery (Esson et al., 2004). Anti-CTGF anti-fibrotic therapies are a possible means of improving surgical outcome while avoiding the significant side effects of suppressing TGF β due to its spectrum of physiological functions (Blom et al., 2002). CTGF silencing through siRNA may prove to be an attractive therapeutic option as suppression of CTGF reduced HTF proliferation (Jing et al., 2013) and sub-conjunctival injection of CTGF antibody following filtration surgery in rabbits was found to maintain a larger bleb area and lower IOP post-surgery in a rabbit model (Wang et al., 2011b).

2.4. Periostin

Periostin, similar to other matricellular proteins, is upregulated in injured and remodeling tissues where it binds to extracellular matrix proteins. While it does not play a direct structural role, it does modulate cell phenotype and function through integrin-mediated interactions (Norris et al., 2008). Original studies suggested that periostin was expressed at very low levels in most adult tissues, however we now know that it is expressed in a wide variety of tissues (Jackson-Boeters et al., 2009).

Periostin is expressed at extremely high levels in collagen-rich connective tissue subjected to mechanical stretch *in vivo* such as the cornea (Norris et al., 2007) and lamina cribrosa (Kirwan et al., 2005a). Indeed many matricellular proteins including periostin are up-regulated in TM cells in response to mechanical stretch (Vittal et al., 2005) and in the ONH of a rat model of elevated IOP (Johnson et al., 2007). Studies from our group have shown that periostin is upregulated at the mRNA level (Kirwan et al., 2009) in LC cells obtained from glaucomatous human donors as compared to normal controls. Increased periostin expression associated with tissue injury, repair and remodeling may be due to the local activation of TGF β 1 and BMP signaling as both are potent inducers of periostin in cells including fibroblasts (Wen et al., 2010), smooth muscle cells (Li et al., 2002) and osteoblasts (Horiuchi et al., 1999). In some cases periostin appears to play an important role by enhancing profibrotic TGF β signaling (Sidhu et al., 2010). Periostin can bind directly to matrix proteins such as collagen types I and V, fibronectin and Tenascin-C (Takayama et al., 2006) and regulate the assembly of collagen fibrils and by doing so, modulate the biomechanical properties of connective tissues (Norris et al., 2007). These collagen fibrils are the ECM component allowing connective tissues to withstand tensile forces. As previously described matricellular proteins are particularly important in collagen assembly (Bornstein et al., 2000; Yang et al., 2000) with TSP2-null mice showing the disruption of collagen fibrillogenesis (Bornstein et al., 2000) and SPARC-null mice having significantly smaller collagen fibrils (Martinek et al., 2007), all of which may affect aqueous humor outflow facility and subsequently IOP.

3. Conclusion

It has been long recognized that there is a fibrotic pathology associated with glaucoma, specifically in the TM tissue at the anterior part of the eye and at the LC region of the ONH at the posterior eye. Elevated IOP is the most common risk factor for developing glaucoma (Anon., 1998a,b; Gordon et al., 2002; Leske et al., 2003; Spry et al., 2005; Leske et al., 2007). In POAG, the resistance of aqueous humor outflow at the TM increases due to alterations in ECM homeostasis, which is responsible for the elevation of IOP, deformation at the ONH, and subsequent nerve axon loss associated with progressive visual field loss. Current therapies focus solely on lowering IOP and do not address the pathogenic fibrotic processes in the TM and LC or direct protect the optic nerve axons. This is especially significant in a subset of patients that show disease progression while maintaining normal IOP. Changes in ECM deposition and turnover are speculated to play a vital role in outflow resistance in POAG and in the structural changes observed at the ONH. Such changes in ECM can be governed by matricellular proteins and there is a growing interest in the role of this family of proteins in the disease pathology associated with glaucoma.

Of particular interest to our group is the role of CTGF in glaucoma pathogenesis. In many ways, targeting CTGF is a far more attractive strategy than targeting TGF β as it avoids the pleiotropic effects associated with the latter. We have previously shown that elevated levels of CTGF are partnered to PXFG (Ho et al., 2005; Browne et al., 2011), whereas Junglas et al., demonstrated that CTGF expressed in the aqueous humor can elevate IOP correlated with TM actin cytoskeleton (Junglas et al., 2012). The use of anti-CTGF antibody technology such as FG-3019 may offer a realistic option for future maintenance of IOP as well as addressing fibrotic pathology (Wallace et al., 2013). Other matricellular proteins can also affect IOP readings. This knowledge provides a unique opportunity for the management/treatment of glaucoma. As mentioned, both TSP1 and SPARC-null mice exhibited lower IOP reading compared to their WT counterparts (Haddadin et al., 2009, 2012) through enhanced aqueous drainage. Therefore approaches at altering the aqueous humor outflow pattern through the modulation of TSP1 and SPARC expression could be a potential mechanism to lowering IOP in the disease state. Attenuation of the expression of matricellular

proteins such as SPARC could also be of extreme benefit to the success rate of glaucoma filtration surgery (Seet et al., 2010).

Undoubtedly, a lot still remains to be learned regarding the function of matricellular proteins in glaucoma, however targeting matricellular proteins represents an attractive strategy for the regulation of ECM deposition and turnover in the disease and surgical setting.

Abbreviations

IOP	intraocular pressure
POAG	primary open angle glaucoma
ONH	optic nerve head
LC	lamina cribrosa
TM	trabecular meshwork
JCT	juxtacanalicular connective tissue
ECM	extracellular matrix
MMP	matrix metalloproteinases
TGF β	transforming growth factor beta
PXF	pseudoexfoliation
PXFG	pseudoexfoliation glaucoma
CTGF	connective tissue growth factor
TSP	thrombospondin
SPARC	secreted protein, acidic and rich in cysteine
siRNA	small interfering Ribonucleic acid
HTF	Human Tenon Fibroblast.

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