The Thrombospondin1-TGF- β Pathway and Glaucoma

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Abstract

Glaucoma is characterized by abnormal remodeling of the extracellular matrix (ECM) in the trabecular meshwork and in the connective tissue beams of the lamina cribrosa (LC) at the optic nerve head (ONH), which is associated with axonal damage. Mechanical strain can stimulate ECM remodeling and increased expression of matricellular proteins. Thrombospondins 1 and 2 are induced by cyclic mechanical strain in the eye in both the trabecular meshwork and in the LC region of the ONH. TGF-betas 1 and 2 are increased in glaucoma and play a role in the pathologic remodeling of the ECM in the eye in glaucoma. In this study, we address the role of thrombospondin1 as a regulator of latent TGF-beta activation and discuss the potential therapeutic use of antagonists of the thrombospondin1-TGF-beta pathway for treatment of glaucoma.

GLAUCOMA IS THE MAJOR CAUSE of blindness and often associated with elevated intraocular pressure (IOP). IOP-related mechanical strain can be both a cause and a result of extracellular matrix (ECM) remodeling in the eye, at both normal and elevated levels of IOP. Abnormal remodeling of the ECM increases resistance to outflow of aqueous humor in the trabecular meshwork (TM), which results in elevated IOP, a primary risk factor for glaucoma.¹

In all forms of primary open-angle glaucoma, IOP also causes deleterious remodeling of the connective tissue beams of the lamina cribrosa (LC) at the optic nerve head (ONH), leading to a characteristic cupping and biomechanical alterations in the LC. These changes are associated with decreased axoplasmic flow and lead to retinal ganglion cell axonal damage, although the underlying mechanisms are not fully understood.^{2-8,9} These connective tissue beams are composed primarily of collagens I, III, V, and VI and elastin synthesized by astrocytes and by a unique nonglial myofibroblast-like cell type, the LC cell; in glaucoma, the composition and organization of the ECM are altered.¹⁰⁻¹⁴

Matricellular Proteins in Glaucoma

Astrocytes and LC cells at the ONH demonstrate upregulated expression of matricellular proteins involved in profibrotic remodeling, including thrombospondin 1 (TSP1), CCN2 (CTGF), SPARC, periostin, and tenascin-C, in glaucoma.¹⁵⁻¹⁸ Matricellular proteins are components of the ECM that are not primary structural elements of the ECM, but rather they interact with cells, other ECM components, and growth factors to regulate cellular functions, growth

factor activity, and assembly and structure of the ECM.^{19,20} Matricellular proteins play key roles in ECM remodeling in glaucoma.^{17,21} Interestingly, some matricellular proteins are induced by mechanical forces (TSP1, SPARC, tenascin-C, CCN2) on the trabecular meshwork and LC cells, such as cyclic mechanical strain/stretch, which are characteristic of glaucoma.^{15,22-24} Matricellular proteins also contribute to IOP, as genetic deletion of SPARC, TSP1, or TSP2 results in reduced IOP in mouse models.^{25,26}

TGF- β and Glaucoma

TGF- β is recognized as a central player in the pathology of glaucoma.²⁷⁻³³ Elevated TGF- β 2 levels are found in the aqueous humor of glaucomatous eyes.³⁴⁻³⁷ TGF- β 2 is also increased in the ONH and LC in glaucoma.³⁸ Similar observations were made in nonhuman primate models of glaucoma, which showed stronger staining of TGF- β 1 in the LC and increased TGF- β 2 in the glial cells around the LC.³² Local cells in the eye are the primary source of TGF- β 2. Mechanical strain increases TGF- β 2 protein expression by LC cells *in vitro*, and LC cells isolated from human glaucomatous eyes have increased transcript for TGF- β 1.^{14,15,39} TGF- β s, especially TGF- β 2, are associated with the pathologic remodeling of both the trabecular meshwork and the ONH; TGF- β 2 stimulates ECM protein expression by ONH astrocytes and LC cells, decreases ECM turnover, and it increases fibroblast contractility.²⁷⁻³¹ TGF- β 2 has also been shown to induce senescence-associated changes in TM cells, which could contribute to fibrotic remodeling in the eye, as has been shown in lung fibrosis.⁴⁰⁻⁴²

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TGF- β is widely expressed by most cell types and expression of its receptors is nearly ubiquitous. Levels of TGF- β protein far exceed levels required for homeostatic signaling; fortunately, TGF- β is produced by cells in a biologically inactive form and mechanisms that convert the latent molecule to a biologically active growth factor represent one level of control of this potent growth factor. Latent TGF- β comprised a 278 amino acid N-terminal prodomain called the latency-associated peptide (LAP), which remains noncovalently associated with the C-terminal 112 amino acids following intracellular processing by furin.⁴³ Interactions of specific regions of the LAP with the C-terminal region of mature TGF- β are required to mask the TGF- β receptor sites in the C-terminus and maintain latency.^{44–46}

There are diverse mechanisms by which latent TGF- β can be converted to its biologically active form; these mechanisms act by disrupting critical LAP interactions with the mature domain of TGF- β . The mechanism deployed varies with the cell type and the particular disease milieu. Latent TGF- β can be converted to the active form through proteolysis of LAP by matrix metalloproteinase (MMPs) or plasmin, through binding to integrins $\alpha_v\beta_5$, $\alpha_v\beta_8$, or $\alpha_v\beta_6$, by mechanical forces involving cytoskeletal–ECM interactions, through modifications of the latent complex by viral enzymes or oxidation, or by binding to the matricellular protein, TSP1.^{43,47–52}

TGF- β and TSP1 in Glaucoma

TSP1 is a complex, multifunctional matricellular ECM and secreted protein, abundant in platelets and widely expressed, especially in tissue injury and repair responses.^{53,54} TSP1 expression is induced by growth factors and cellular stresses. TSP1 is a homotrimer composed of distinct structural domains associated with specific receptors and binding partners and specific cellular functions. TSP1 has multiple receptors, including integrins, proteoglycans, calreticulin, CD36, CD47, EGFR (indirect), and the gabapentin receptor. TSP1 is a major regulator of latent TGF- β activation in a number of diseases.^{48,52} TSP1 has numerous TGF- β -independent functions in the hemostasis, cell adhesion, migration, and regulation of growth factor (EGF, VEGF, FGF) activity.⁵³ TSP1 is an endogenous inhibitor of tumor angiogenesis, primarily through its inhibitory effects on VEGF and FGF signaling.^{55,56} TSP1 binding to its receptors CD47 and CD36 attenuates nitric oxide signaling.^{57,58}

Plasma TGF- β 1 and TSP1 levels are increased in primary open-angle glaucoma patients and there is a linear correlation between total TGF- β and TSP1.⁵⁹ TGF- β 2 increases TSP1 mRNA in ONH astrocytes and in perfused organ cultures of the porcine anterior eye.^{27,60} TGF- β 1 and dexamethasone increase TSP1 in TM cells.⁶¹ Pertinent to the increased IOP in glaucoma, cyclic mechanical stress induces TGF- β 1 in the TM and LC, and it also induces TSP1 in LC cells.^{14,62} Mechanical forces (stretch, shear forces, cell contractility) similarly stimulate TSP1 expression in other tissues.^{63–66} TSP1 levels are also increased in glaucomatous versus normal LC cells.¹⁵ The reduced nitric oxide-soluble guanylate cyclase levels in glaucoma could also contribute to TSP1 upregulation as we have shown for diabetic conditions.⁶⁷ Although this remains to be tested directly, these data suggest that biomechanical factors in

glaucoma potentially upregulate a feedforward mechanism to amplify active TGF- β and glaucomatous ECM remodeling through enhancement of the activator TSP1.

It is not clear whether TSPs have TGF- β -independent roles in glaucoma through control of cell deadhesion, angiogenesis inhibition, nitric oxide inhibition, or collagen fibrillogenesis. Both TSP1 and TSP2 can stimulate cell deadhesion, nitric oxide inhibition, MMP inhibition, and angiogenesis inhibition, and TSP2 also regulates collagen fibrillogenesis.^{54,57,68–71} Since TSP2 null mice also have reduced IOP, it is possible that regulation of MMP activity and collagen fibrillogenesis by TSP1/2 also plays a role in remodeling in glaucoma.

TSP1 Activates Latent TGF- β s

TSP1 binds to the latent TGF- β complex to stimulate TGF- β activation at the cell surface or in the extracellular milieu.⁵⁰ Activation occurs through binding of the KRFLK sequence in the TSP1 type 1 repeats (TSRs) to LSKL in the LAPs of the TGF- β 1, 2, and 3 latent complexes. This interaction disrupts LAP-mature domain interactions to expose the receptor binding sequences on the mature domain, rendering TGF- β capable of signaling.^{44,72,73} The importance of these sequences has been confirmed by biochemical and structural studies.^{45,46} Peptides of these sequences can be used to antagonize TSP1-TGF- β activation (LSKL, GGWSHW) or stimulate activation (KRFLK, RKPK).⁵² Activation is specific for the TSP1 isoform as TSP2 lacks the RFLK sequence.⁷²

Multiple laboratories showed that TSP1 is a primary regulator of TGF- β bioactivity in different diseases (see table in Ref.⁵⁰).^{74,75} Although there are some developmental similarities between the TSP1 and TGF- β 1 null mice in the lungs and pancreas, integrin-mediated TGF- β activation is the predominant activation mechanism during development.^{76,77} Rather, as TSP1 is upregulated by factors associated with disease, TSP1 regulates TGF- β activation primarily in disease.

TSP1 antagonist as a therapeutic for glaucoma

TGF- β , specifically TGF- β 2, is a clinical target in glaucoma. TGF- β antagonists have been proposed as therapeutics to achieve better outcomes following surgical interventions and to prevent disease progression.^{78–82} These therapies are either monoclonal antibodies or antisense oligonucleotides to TGF- β 2. However, the clinical use of any TGF- β antagonist raises concerns about inflammation and carcinogenesis due to the loss of homeostatic TGF- β activity, which suppresses inflammation, epithelial hyperplasia, and carcinogenesis.^{83,84} Therefore, targeted approaches to selectively limit disease-related TGF- β would have a significant therapeutic benefit. One such targeted approach is to specifically block the mechanism by which TGF- β is activated in the glaucomatous eye.

We have developed a peptide antagonist LSKL, which competitively inhibits TSP1 binding to the LAP to block activation of latent TGF- β . This peptide inhibits TSP1-TGF- β activation to attenuate disease in numerous rat and mouse models of TGF- β -dependent disease.⁵⁰ We showed that the LSKL peptide is effective in reducing fibrosis and end-organ dysfunction in preclinical models of cardiac and renal diabetic complications in two species (rat, mouse).^{85,86}

Animals necropsied after 15 weeks of LSKL (30 m/kg 3×/week, i.p.) showed no inflammation or tumors in all major organs and no impairment of wound healing,⁸⁶ suggesting that blocking TSP1-TGF- β activation is safe.

Current lead compounds based on LSKL have improved pharmacokinetics and metabolic stability with both *in vivo* and *in vitro* activity (Murphy-Ullrich and Suto, unpublished data). As TSP1 can activate both latent TGF- β 1 and TGF- β 2, this antagonist would have utility against the major isoforms of TGF- β that play a role in glaucoma pathogenesis. The LAP region of TGF- β 2 does not have the RGD integrin-binding sequence; therefore, latent TGF- β 2 is not activated by integrins,⁵¹ restricting the utility of integrin antagonists for treatment of glaucoma. Another advantage of this targeted approach is that potentially beneficial functions of TSP1, such as its anti-VEGF activity, would not be blocked, as they could be of use by anti-TSP1 monoclonal antibody-based therapeutics.

Acknowledgment

The authors wish to thank the EyeSight Foundation of Alabama for support of the glaucoma-related studies.

Author Disclosure Statement

No competing financial interests exist.

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Received: January 26, 2015

Accepted: February 23, 2015

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