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Pathogenesis of glaucoma: Extracellular matrix dysfunction in the trabecular meshwork-A review

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Abstract

The trabecular meshwork regulates aqueous humour outflow from the anterior chamber of the eye. It does this by establishing a tunable outflow resistance, defined by the interplay between cells and their extracellular matrix (ECM) milieu, and the molecular interactions between ECM proteins. During normal tissue homeostasis, the ECM is remodelled and trabecular cell behaviour is modified, permitting increased aqueous fluid outflow to maintain intraocular pressure (IOP) within a relatively narrow physiological pressure. Dysfunction in the normal homeostatic process leads to increased outflow resistance and elevated IOP, which is a primary risk factor for glaucoma. This review delineates some of the changes in the ECM that lead to gross as well as some more subtle changes in the structure and function of the ECM, and their impact on trabecular cell behaviour. These changes are discussed in the context of outflow resistance and glaucoma.

Keywords

extracellular matrix; glaucoma; trabecular meshwork

1 | INTRODUCTION TO GLAUCOMA

Intraocular pressure (IOP) is maintained through a delicate balance between the production of aqueous humour (AH) by the ciliary muscle and its drainage through the trabecular meshwork (TM).[1] A major regulatory site of AH drainage in humans is believed to reside within the extracellular matrix (ECM) of the juxtacanalicular tissue (JCT) and the underlying inner wall of Schlemm's canal.[2] The role the ECM plays in regulating AH outflow is unknown. At first glance, it is reasonable to assume that the ECM in the JCT could act as a passive filter that restricts outflow across the TM by providing physical resistance to the movement of AH. However, for reasons discussed in several reviews,[3–6] current evidence suggests that subtle changes in ECM composition are more likely to affect

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CONFLICT OF INTEREST

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AH outflow by altering the organisation of the outflow resistance, the compliance of the TM, or the contractile properties of the trabecular cells in the TM.[4,7] In this article, we discuss the properties of the ECM and how it might control drainage of AH and hence regulate IOP.

1.1 | The conventional AH outflow pathway

There are two different pathways through which AH leaves the anterior chamber. Both are located in the irideocorneal angle of the eye (Figure 1). In humans, the major outlet for ~80% of AH is called the conventional outflow pathway, which consists of the TM and Schlemm's canal (TM/SC). AH outflow through this pathway is pressure-dependent and upon exiting the TM/SC, it drains directly to the episcleral veins. Molecules pass quickly through the conventional outflow pathway, in a minute or less.[1,8–10] Currently, Rhopressa® is the only drug that targets this pathway. It changes the contractile properties of the TM, which ultimately alters the properties of the ECM.[11] The second route is through the uveoscleral pathway, which is referred to as the unconventional pathway (Figure 1). In this pathway, AH exits the anterior chamber by diffusing through the intercellular spaces among ciliary muscle fibres. The level of aqueous outflow through this pathway varies with age and tends to be about 3%–14% in adults (>60 years of age).[8,12] The unconventional pathway is the target of several glaucoma drugs for example latanoprost, a prostaglandin F2α analog.

The TM consists of three distinct cell layers (Figure 2A).[13] The innermost layer is the uveal meshwork. It consists of a loose network of connective tissue covered by a monolayer of endothelial-like cells. This layer does not offer much resistance to AH outflow because intercellular spaces are too large. The corneoscleral meshwork is the next layer. This is a series of lamellae composed of collagen, glycoproteins, glycosaminoglycans (GAGs), and elastin, each of which are covered by a single layer of TM cells. Although the intertrabecular spaces are smaller than the uveal meshwork, the intercellular passages are still too large to account for any resistance to AH outflow. However, since cells in the corneo- and uveal meshworks are highly phagocytic, these regions contribute to outflow resistance by removing cellular debris that may clog the outflow channels.[14,15] In the deepest region of the tissue, adjacent to the inner wall of SC, is the JCT, or cribriform region.[16,17] In this region, the JCT cells are scattered throughout a dense ECM and are connected to one another by long cellular processes. These cellular processes also make attachments to SC cells and the elastin network extending from the anterior tendons of the ciliary muscle.[18–20] Adjacent to the JCT is the inner wall of SC. The inner wall consists of a monolayer of endothelial cells that sits upon a discontinuous basement membrane and separates the TM from the lumen of SC. The majority of the tissue resistance to outflow resides within an area 20 μm beneath the inner wall of SC.[3,21] Recently, this was further refined and hypothesised that the region of resistance may lie within 500 nm to 1 μm below the basal surface of SC cells.[2] The outflow resistance is likely the result of the organisation of specific ECM proteins found within this region (Figure 2B). However, evidence is accumulating that a portion of resistance also resides in collector channels and aqueous veins distal to Schlemm's canal.[22]

Trabecular outflow is not uniform around the circumference of the eye, which has given rise to a theory of segmental flow. 'Segmental theory' is based on a number of tracer studies

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using cationic ferritin, zymosan, latex microspheres or fluorescent Qdots, which demonstrate variable distributions of the tracer around the circumference of the eye.[23–29] This variable distribution has led to the concept that the TM has regions of relatively high outflow and areas that are underperfused, or low outflow.[29] The exact cause for this unknown, but changes in the composition of the ECM may be responsible.[23,30] The anatomic structure of SC could also contribute since TM high flow regions appear near SC giant vacuoles and collector channels.[31,32]

1.2 | Type of glaucoma associated with ECM

Glaucoma is a group of diseases with varying pathophysiological processes that share a common end-point of optic nerve head damage. It remains unclear why some eyes are more likely to develop glaucoma than others. Several possibilities exist, which may vary among patients, including the porosity of the JCT tissue and its ECM composition, the amount of pseudoexfoliative material present and/or the metabolic activities (including synthetic and proteolytic enzymes) of the TM. To date, several forms of glaucoma are reported to involve changes in the ECM: primary open-angle glaucoma (POAG), glucocorticoid-induced glaucoma (GIG), primary angle-closure glaucoma (PACG), primary congenital glaucoma (PCG) and pseudoexfoliation glaucoma (PEX). Other glaucoma subtypes can also involve ECM accumulation, but these have been less well studied and the specific ECM molecules involved have not been described in detail.

1.2.1 | POAG—In POAG, there is an open and normal appearing anterior chamber angle, and IOP can be elevated, which is a common risk factor for POAG.[33] The prevalence of POAG increases with age and hence age is also considered an important risk factor for the pathogenesis of POAG. Other risk factors include race, myopia, central corneal thickness and family history. Although there are similarities between the ageing eye and POAG, there are some significant differences. In particular, there is an increase in the ECM material adhering to the sheaths of elastic fibres in POAG patients. ECM proteins found in these sheaths include glycoproteins (fibronectin, vitronectin, laminin and tenascin), proteoglycans and GAGs (decorin, versican, hyaluronan [HA]), collagens (types I, III, IV, V and VI), elastic fibre components (MAGP-1, fibrillin) and the glaucoma-associated protein, myocilin.[34] In addition to changes in the ECM, histological examination of the eyes from POAG patients show narrowing of intertrabecular spaces and collector channels, and a closure of SC. Fewer trabecular endothelial cells are present in TM,[35] which could contribute to the narrowed spaces and cause TM beams to fuse and thicken. One of the factors believed to be responsible for these POAG-associated changes is an increase in TGFβ2,[36,37] which triggers the expression of many ECM proteins[38] and induces cellular senescence.[39,40]

1.2.2 | GIG—GIG is a secondary open-angle glaucoma following treatment with glucocorticoids (GCs) and is caused by a restriction in the movement of AH through the TM. Although only a small percentage of people develop elevated IOP in response to GC treatments, nearly 90% of POAG patients develop IOP elevation if treated with GCs.[41] In GIG, AH outflow is restricted due to an increase in ECM synthesis concurrent with a decrease in the ECM degradation. The increase in ECM material, especially type IV

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collagen and fibronectin, is found predominantly along the basement membrane of the inner wall of SC.[42,43] Decreased phagocytic activity of TM cells may also be partially responsible for elevated IOP.[44] GCs also induce re-organisation of the TM actin cytoskeleton into structures called cross-linked actin networks (CLANs).[45] These structures are especially upregulated by cellular interactions with fibronectin or type IV collagen via specific cell surface receptors called integrins.[46] GCs also increase myocilin expression, a glaucoma-associated ECM protein, which can lead to alterations in cell-ECM interactions and TM cell migration.[47–49] Finally, GCs can also cause an increase in the expression and activity of the αvβ3 integrin.[50] This integrin is a receptor for many ECM proteins altered in GIG and whose activity is associated with many of the phenotypic changes associated with GIG as well as with POAG.[46,50–52] Interestingly, αvβ3 integrin expression and activity is downregulated upon removal of GCs, which may explain why IOP returns to normal upon cessation of GC treatments. In summary, increased ECM material and changes in cell-matrix interactions, induced by GC treatment, can influence TM cellular properties and functions.

1.2.3 | PEX—PEX is one of the most common causes of glaucoma worldwide and is estimated to account for 25% of open-angle glaucomas.[53] It is characterised by the deposition of a protein-like material within the TM and on the anterior lens capsule. In PEX, there is a significant loss of TM cells and denuded lamellae appear to have fused.[42] TM cell loss is presumably due to these phagocytic cells being overloaded with pigment granules and detaching from the beams.[42] ECM material is also increased in the sub-endothelial region where the lamellae have fused. These changes are secondary to the pigment dispersion from the iris.[42] The accumulation of pseudoexfoliative material in the TM results in obstruction of the AH outflow pathway, thus elevating IOP. PEX material contains several ECM proteins including lysyl-like oxidase 1 (LOXL1), tropoelastin, elastin, fibrillin-1, LTBP-1 and LTBP-2, emilin, vitronectin and clusterin.[54] The recent identification of SNPs in *LOXL1* indicates that *LOXL1* gene variants are a strong risk factor for PEX.[55] Increased levels of connective tissue growth factor (CTGF), which regulate expression of ECM proteins, have also been identified in PEX patients.[56]

1.2.4 | Other glaucomas linked to ECM dysfunction—In primary angle-closure glaucoma (PACG) patients, the peripheral iris becomes opposed to the TM, thereby closing the irideocorneal angle and causing IOP elevation.[57] Angle closure can happen slowly (chronic), which is often asymptomatic, or can occur rapidly (acute) causing rapid eye pain and severe IOP elevation. PACG is more prevalent in women and in patients of Asian descent. Genetic analyses have revealed association with missense variants in the type XVIII (*COL18A1*) and XI (*COL11A1*) collagen genes.[57–59] For Glu185Lys in *COL18A1*, clinical measurements related to angle closure were affected in 10 PACG family members, but other parameters such as lens thickness, axial length, central corneal thickness and cup/disc ratios were not outside normal ranges. Thus, there was no clear-cut correlation between the genetic variant and morphological abnormalities. The *COL11A1* variant, rs3753841, which was identified in a large genome-wide association study (GWAS), causes a missense mutation Pro1323Leu.[59] This disrupts the Gly-Pro-Hyp triplet that is essential for correct folding of the collagen protomer. Thus, the PACG-associated Pro1323-Leu variant may disrupt folding of collagen molecules, and/or flexibility of the assembled fibrils.[60] This could contribute

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to biomechanical alterations that slowly culminate in iris-TM contact in the irideocorneal angle.

PCG primarily affects infants less than 4 years of age.[61] Most cases are sporadic, although about 10%–40% are familial. The irideocorneal angle develops abnormally, which hinders AH outflow leading to elevated IOP. Of the three genes associated with PCG, one is latent transforming growth factor beta binding protein-2 (LTBP2), an extracellular protein that functions to regulate TGFβ activity.[62] A LTBP2 mutation also causes PCG in cats.[63]

2 | ALTERATIONS TO ECM COMPONENTS AFFECTING TM STRUCTURE, ORGANISATION AND FUNCTION

Overall levels of ECM molecules affect structure and organisation of the outflow resistance, but other, more subtle changes in the ECM can also have dramatic effects on TM function. This section focuses on specific ECM proteins and describes how changes in their biomechanical properties, altered mRNA splicing, missense genetic variants, and specific proteolytic events, can contribute to the generation of dysfunctional matrices in the glaucomatous TM.

2.1 | ECM components

2.1.1 | Collagens—One of the major fibrillar ECM components found in the TM/SC outflow pathway is collagen. Collagen is a family of proteins that are highly conserved in their structure and function.[64] In vertebrates, there are at least 28 different types of collagens, all of which have a Glycine-X-Y sequence motif, where X is usually proline and Y is usually hydroxyproline. Each collagen type is composed of three polypeptide chains, called α-chains, which assemble to form a triple helical protomer. The protomers are either homo- or heterotrimers that assemble into unique supramolecular complexes. A number of collagens have been identified in the TM, but comparatively little is known about their molecular composition, relative abundance, or function within the uveal, corneoscleral, or JCT regions of the TM.

Type I collagen is a heterotrimer of two identical α1(I)-chains and one α2(I)-chain.[65] Each α-chain is staggered relative to a neighbouring molecule to create a striated collagen fibril exhibiting a 67-nm banding pattern. Type I collagen fibrils are found in the trabecular beam core, in the basement membrane along the beams, and throughout the JCT. Fibrillar collagens are responsible for providing tensile strength to the TM, which is an essential physical requirement given the biomechanical demands on the TM. Type I collagen is highly resistant to proteolytic degradation, but does contain a highly conserved collagenase-sensitive site between Gly775 and Ile776 residues in the α1-chain. Transgenic mice harbouring a mutation in this site, which renders type I collagen more resistant to degradation,[66] display progressive ocular hypertension as they age compared to wild-type littermates.[67] This suggests that type I collagen turnover is required to maintain normotensive IOP. Type I collagen is often co-assembled with types III and V collagen, which are also found in the center of the beams and the JCT. Co-assembly of type V collagen into type I collagen fibrils serves an important function in regulating the diameter

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of type I collagen fibres.[68] Type V collagen also serves as a point of attachment for microfilaments to type I collagen fibrils. Interestingly, the intronic SNP, rs7044529, in the *COL5A1* gene influences central corneal thickness, which is a risk factor for POAG.[69]

The network-forming type IV collagen is a major constituent in all basement membranes.[65,70] In the TM/SC outflow pathway, type IV collagen is found in the basement membranes covering the trabecular beams, underneath SC cells along the inner and outer walls, and in the sheath material around elastin fibres.[71] The structure of the type IV protomer is different from that of type I collagen. Each type IV α-chain consists of several triple helical regions that are interrupted by non-triple helical regions. This increases the flexibility of the type IV collagen protomer, but also makes it more susceptible to proteolytic digestion. Currently, six α (IV) chains (α1–α6) have been identified, which assemble into at least five different heterotrimeric isoforms of type IV collagen. Five of the six α(IV)-chains have been identified in the TM. The α3 chain has not been detected.[20] Interestingly, the expression of type IV collagen is not affected by TGFβ2,[20] but it is elevated by dexamethasone supporting the observation that an increase in basement membrane material is associated with GIG.[42,72] This raises the question as to what causes an increase in type IV collagen in the sheath material in POAG patients.[42,72]

Type IV collagen is crucial for the stability and function of basement membranes.[73] Differential expression of type IV protomers confers functional specificity to basement membranes and hence expression varies with the tissue. For example, in kidney, at least three distinct type IV collagens can be found: the [α1(IV)]2α2 (IV) and [α5(IV)]2α6 (IV) protomers are in Bowman's capsule basement membrane, while the glomerular basement membrane contains the α3(IV)α4(IV)α5(IV) protomer.[73] Whether there is any heterogeneity among the various basement membranes in the TM (e.g., lamellae vs. JCT) is not known, but clearly if there was, the identity of collagen IV protomers could play a role in imparting different functional properties to TM regions or segmental flow. Proteolytic fragments of type IV collagen can exist as biologically active fragments in tissues or in the circulation. These are known as matrikines. For instance, non-collagenous (NC1) domains of type IV collagen can regulate cell attachment, migration, proliferation and gene expression.[74] The specific function appears to depend on which α-chain the fragment was derived from. The NC1 domains from the α4(IV)-chain and the α1(IV) chains can control expression of matrix metalloproteinases (MMP)-14 and MMP-2, respectively.[74,75] Thus, fragments from these domains could be important in the regulation of AH outflow. Since the activities of these NC1-derived matrikines are only expressed after type IV collagen has undergone proteolytic degradation, this implies that their activities may only be exposed as the ECM is remodelled in response to pressure elevation. The release of these matrikines and additional upregulation of the MMP activity could be one way that matrix turnover and AH outflow are enhanced.

Another collagen that is important in the TM/SC outflow pathway is type VI collagen. Type VI collagen is a heterotrimer consisting of α1(VI), α2(VI) and α3(VI) chains.[76] The α3(VI) chain (COL6A3) is the largest chain and it is essential for heterotrimeric assembly of the type VI collagen monomer.[77] Type VI collagen interacts with several ECM proteins such as type I collagen, decorin, MAGP-1 and hyaluronan (HA).[76] Type VI collagen is

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localised to the basement membrane of the trabecular lamellae, in the sheath of elastic-like fibres in the JCT region and underlying SC endothelial cells.[72] Several lines of evidence have linked it to glaucoma. For instance, type VI collagen levels are increased in normal aged TM, and significantly increased in the plaque-like material present in glaucomatous TM.[78] Type VI collagen also shows differential segmental distribution and it is enriched in low outflow regions of normal eyes perfused at 2x pressure.[79] Other studies showed a potential genetic association between type VI collagen and glaucoma. In a GWAS, two SNPs in *COL6* were associated with elevated IOP.[80] One SNP in *COL6A3*, rs7599762, is intronic and the other SNP, rs2839082, is intergenic, lying between *COL6A1* and *COL6A2*. A missense *COL6A3* gene variant, rs112913396, was also identified in a large POAG family with elevated IOP.[81] This SNP causes an Asp563Gly change in one of the N-terminal von willebrand factor A domains. Since the α3(VI) chain directs assembly of the heterotrimeric type VI collagen protomer,[77] it is conceivable that this missense change affects the amount of type VI collagen in the TM and/or disrupts protein–protein interactions with microfibrils and elastin fibres in the ECM.

2.1.2 | Fibronectin—Fibronectin is another major ECM protein found in the TM/SC pathway.[82] It exists as a soluble globular protein and as an insoluble fibrillar network. Soluble fibronectin is found in AH. Some POAG patients have increased levels of fibronectin in AH, which may be related to elevated levels of TGFβ2 and CTGF.[83] However, it is most likely inactive in the AH since many of its biological domains are inaccessible within the soluble form.[84,85] This soluble fibronectin can be assembled into an insoluble fibrillar network. This process, unlike collagen fibrillogenesis, is a step-wise, cell surface receptor-mediated process that is regulated by the contractile properties of the cells.[86] Fibronectin in these fibrils have extended conformations with exposed biological domains and represent the active form of fibronectin. Fibronectin fibrils are localised to the core of the trabecular beams, in the sheath material surrounding the elastin tendons, in the amorphous fibrogranular material in the JCT, and along the inner wall of SC.[71] During ageing, and in some POAG patients, increased fibronectin is observed in the JCT and in the sheath material along the elastin tendons.[42,71,87] Fibronectin fibrils are one of the earliest ECM fibrils to be assembled and hence these fibrils mediate the incorporation of other proteins into the ECM by acting as a scaffold upon which additional ECM fibrils, proteins and growth factors are assembled into the ECM.[88,89] Thus, fibronectin plays an important role in ECM organisation and acts as a bioreservoir for many proteins and growth factors important in regulating AH outflow.

In humans, fibronectin exists as a dimer composed of two non-identical polypeptide chains that are disulphide-bonded at their carboxyl termini.[85] Each chain contains a series of homologous sequences called type I, II and III repeats that are arranged into discrete domains with biological activities important for TM biology including promoting cell adhesion, proliferation, differentiation, apoptosis, contractility and gene expression. During ECM turnover, proteolytic enzymes cleave between the domains to produce small bioactive fibronectin fragments, which would be available in the TM in vivo during ECM turnover. The Heparin II (HepII) domain is a good example of such a domain.[85] When isolated HepII domains were perfused into either human- or monkey cultured anterior segments, AH

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outflow facility was increased by 93% and IOP was significantly decreased.[90,91] Isolated HepII domains can also affect MMP expression,[92,93] and the availability of myocilin and vascular endothelial growth factor (VEGF) since it binds both these proteins. Myocilin is a GC response protein associated with glaucoma and regulates cell adhesion,[47] and MYOC mutations were associated with abnormally thickened TM resulting in fusion of the TM beams in a Japanese POAG family.[94] VEGF can increase outflow facility in ex vivo mouse eye cultures.[95–99] Hence, the proteolytic release of the HepII domain has the potential to affect outflow facility by numerous mechanisms.

Alternative mRNA splicing of three exons in the fibronectin gene yields 20 fibronectin isoforms.[85,100] The three exons are called EDA (or EIIIA), EDB (or EIIIB) and IIICS (or V). EDA and EDB exons each encode a single type III repeat and are spliced out entirely, whereas the V exon undergoes exon subdivision to generate a V1, V2 or V3 region.[101] The expression of these isoforms is age- and tissue-related and display changes during development and pathological processes. For instance, the EDA and EDB domains are found in fibronectin in both adult human anterior segments and in cultured TM cells derived from adult tissues, but are spliced out in soluble fibronectin found in AH.[102] Alternative splicing of the V2 region in the TM is affected by mechanical stretch generated by the contractile forces of the TM,[101] and hence could be absent in fibronectin in glaucomatous TM. Mechanical stretch does not significantly affect splicing of the EDA and EDB exons,[103] but growth factors such as TGF-β1 and TGF-β2 would promote the inclusion of EDA+ fibronectin.[102,104]

Fibronectin fibrils can be composed of a mixture of different isoforms and in the TM/SC fibrils could consist of at least three different fibronectin isoforms (EDB− EDA+, EDB+ EDA− and EDB+ EDA+). These isoforms confer different structural and functional properties to fibronectin fibrils.[105] For instance, EDA+ fibronectin matrices are highly branched, thin fibres that form small pores. In contrast, EDA− or EDB− fibronectin matrices form thicker, less branched fibrils with larger more elongated pores. Inclusion of the EDA+ fibronectin also promotes fibrosis and hence decreases outflow facility due to EDA interactions with the TLR4 receptor,[106,107] or integrins α4β1 or α9β1, which drive the myofibroblastic-like phenotype of cells.[108] EDB+ fibronectin in fibrils could enhance VEGF expression and phagocytosis.[109–111] EDB+/EDA+ fibronectin in fibrils could potently induce TGF-β mRNA expression,[112] which in turn, would create a positive feedback loop that further drives fibronectin expression.[104] Finally, the presence of EDA+ fibronectin in fibrils could enhance exposure of the RGD sequence in fibronectin[113] and hence binding to the α5β1 integrin, which would increase stress fibre formation and alter the contractile properties of TM cells.

2.1.3 | Laminins—

Laminins are glycoproteins that are found in all basement membranes and are important in maintaining their function.[114] Laminins are heterotrimeric proteins composed of three polypeptide chains, an α-chain, a β-chain and a γ-chain.[115] The three chains form a cross-like structure with long and short arms.[114] The long arms bind to specific cell surface receptors, which help anchor cells to the basement membrane, while the short arms bind ECM proteins (including itself), which enables laminin to form a sheet-like structure. There are 11 distinct laminin genes, leading to 15 heterotrimeric forms

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of laminin.[114] Like the collagens, the laminin molecules are named according to their chain composition. Thus, laminin-511 contains α5, β1 and γ1 chains.

We know very little about laminins in the TM/SC outflow pathway. Laminin-111 and -211 are in the basement membrane of the inner wall of SC as well as the uveal and corneoscleral trabecular meshwork, but here, immunostaining was sparser suggesting that laminins were present at lower levels.[71,95] Interestingly, the presence of laminin did not seem to vary between normal and glaucomatous tissues and was not affected by age.[116] Laminin levels were upregulated in dexamethasone treated HTM cultures,[117] suggesting that, like type IV collagen, it may be more involved in GIG, but not POAG. Genetic studies have associated some laminin chains with glaucoma. A missense gene variant in the *LAMB2* chain was associated with POAG and elevated IOP.[81] This Gln987Lys variant lies in the fifth EGF-like repeat of the short arm of the cross, a region that associates with other ECM proteins and is important for organisation and stability of the basement membrane.[114] Mutations in *LAMB2* are also associated with Pierson syndrome, a rare developmental disorder affecting eyes and kidneys.[118] Additional studies are clearly needed to determine what laminins are found throughout the TM, and how they contribute to the function of TM and SC cells.

2.1.4 | Elastin and fibrillin microfibrils—Elastic fibres are an essential component of the ECM in the TM/SC pathway.[16,119] They provide both structural integrity and elastic recoil upon contraction of the ciliary muscle. Alterations in the elastic fibre structure have been implicated in the pathogenesis of glaucoma, especially PEX.[53] Elastin fibres consist of bundles of elastin molecules cross-linked together by lysyl oxidases and encased by fibrillin-rich microfibrils.[34] These are found in the JCT of the TM and the uveal or corneoscleral meshwork, where they are attached to the core of the beams.[13] Fibrillin-1 is the major structural component of microfibrils and although microfibrils can form fibrous structures on their own, they are usually found surrounding the elastin core of elastic fibres.[120] The assembly of elastin and fibrillin into elastin fibres is a highly complex process involving several key accessory proteins including LOXL1, fibulin-4, fibulin-5, latent transforming growth factor β binding protein 4 (LTBP-4), and microfibril associated protein 4 (MFAP4).[119,121] MFAP-1 and -2 have been found been associated with the elastin fibre system in the TM/SC.[122]

Microfibrils have a major role in regulating TGFβ activation since they are an extracellular pool of latent TGFβ.[123,124] Latent TGFβ is activated through interactions with thrombospondin-1 or integrins, by proteolysis, or changes in tissue elasticity.[123,125,126] Because microfibrils bind many ECM proteins and activate TGFβ, this led to the 'microfibril hypothesis', which suggests that defective microfibrils may be an underlying cause of glaucoma.[120] Mutations in fibrillin-1 cause Marfan syndrome.[127] POAG is common in Marfan patients, as well as ectopia lentis and thin corneas, which can both contribute to POAG.[128]

2.2 | Matricellular proteins

Matricellular proteins do not play a structural role in the support of tissues, but rather modulate the function and organisation of the ECM.[129] Notable characteristics of

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matricellular proteins is that they display high levels of expression during development and/or tissue injury and they interact with many ECM regulators such as growth factors, cytokines and proteases.[130] Somewhat surprisingly, their genetic ablation in knockout mice does not lead to lethality, but it does cause more subtle phenotypes, some of which affect IOP regulation.

2.2.1 | Thrombospondins—

Thromobospondins are a family of five distinct gene products (TSP-1, -2, -3, -4, and COMP), which are expressed in many tissues in the body.[131] TSP-1 and -2 assemble into homotrimers, while TSP-3, -4 and COMP form pentameric molecules. TSP-1 has been reported to play major roles in glaucoma pathophysiology.[132] While TSP-1 immunoreactivity is seen consistently in the deepest portion of the JCT in normal TM, TSP-1 immunostaining in 16 POAG eyes reveals variable TM expression,[133] suggesting that the effect of TSP-1 in POAG is not solely dependent on expression levels. TSP-1 can influence TM cell function by modulating cell-matrix interactions.[134] It binds to 83 ligands including structural proteins (collagens), non-structural components (proteoglycans) and cell surface ECM receptors, some of which, for example integrins, can influence actin cytoskeletal rearrangement and hence contractile properties of the TM.[135] TSP-1 is a potent activator of TGFβ1 and TGFβ2,[125] which stimulates ECM synthesis. TSP-1 and -2 are critical in organising the ECM in the JCT and hence IOP regulation. In TSP knockout mice, the diameter of collagen fibrils were thinner and they display lowered IOPs than their wild-type littermates.[136]

In humans, a gene variant causing a Asn700Ser mutation in TSP-1 is associated with POAG in a large Oregon family,[81] as well as myocardial infarction.[137,138] The variant, Asn700Ser, is located in one of the TSP-1 calcium-wire domains.[139] Introduction of a Ser residue causes significant functional changes to the TSP-1. It disrupts calcium binding,[140] alters the stability of the adjacent EGF-like and calcium-wire domains[141,142] and makes the protein more susceptible to proteolytic digestion.[143] While these in vitro studies provide clues, the molecular consequences of the Asn700Ser variant in TM in situ are still to be discerned.

2.2.2 | Tenascin C—

Tenascin C is a large extracellular glycoprotein, which is primarily expressed during development, but has transient expression in diseased or injured adult tissues.[144] The mature tenascin C molecule is a hexamer, with each ‘arm’ composed of multiple domains that interact with numerous ECM components. Like fibronectin, tenascin C can undergo alternative splicing, with the insertion of up to nine extra type III modules.[103] Each of these modules confer additional binding sites for other ECM molecules and provide tenascin C with additional cell-type specific functions.[145] Mechanical stretching of TM cells not only induces tenascin C gene expression,[101] but mechanical stretching also influences alternative splicing with tenascin C transcripts progressively including more of the type III domain D.[68] Type III domain D has binding sites for α7β1 integrin as well as the F3/contactin protein, which activates the Notch signalling pathway.[146,147] Despite increases in transcription in response to mechanical stretch, tenascin C knockout mice do not show any difference in IOP measurements compared with wild-type littermates.[148,149]

2.2.3 | SPARC—

Secreted protein and rich in cysteine (SPARC) is another matricellular protein that is expressed in the TM and ciliary body.[150,151] The role of SPARC in

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outflow regulation was demonstrated using SPARC-null mice. While there was no gross morphological differences in the irideocorneal angle tissue between SPARC knockout and wild-type mice, SPARC-null mice had significantly lower IOPs (~3 mmHg) than wild-type littermates and higher outflow facility through the conventional pathway.[152,153] Fluorescent tracer studies showed a more uniform outflow around the circumference of the SPARC-null mouse eyes.[154] High segmental outflow regions exhibited a more open structure between TM beams, while collagen fibril diameters in the JCT region were significantly decreased. SPARC interacts with myocilin,[155] and its expression is regulated by various factors important to glaucoma including TGFβ2 and microRNA miR-29b.[156–158]

2.3 | Proteoglycans

Despite the large prevalence of numerous proteoglycans in the TM, we know very little of their role. Proteoglycans consist of a core protein with one or more types of GAG chain covalently attached to it. GAG chains consist of polymers of repeating disaccharides that contain hexosamines, galactose or hexuronic acid and they are sulphated. There are four major types of GAGs attached to a protein core: heparin sulphate, dermatan sulphate, chondroitin sulphate and keratan sulphate. Proteoglycans can be found in the ECM and as transmembrane proteins. Ultrastructural studies have indicated that chondroitin sulphate and dermatan sulphate proteoglycans are associated with collagen in both the trabecular beams and in the JCT. Heparan sulphate proteoglycans are associated mainly with the basal lamina of the trabecular beams and endothelial cells of Schlemm's Canal (Figure 2B).[159] During ageing, there is loss of chondroitin sulphate and dermatan sulphate proteoglycans that are associated with collagen. Furthermore, there is a decrease in the level of heparin sulphate proteoglycans associated with the basal lamina in the adult eye compared to an infant eye.[159] Changes in the levels of sulphated proteoglycans are likely to lead to tissue compression and decreased hydration in the meshwork as well as changes in the contractile and signalling properties of TM/SC cells.

A number of different types of proteoglycans have been detected in human trabecular meshwork cells.[6,150] They include chondroitin sulphate proteoglycans like versican,[30] transmembrane heparin sulphate proteoglycans such as syndecans 1–4,[160] and members of the small leucine-rich proteoglycan (SLRP) family such as decorin.[161]

2.3.1 | Versican—Versican is a chondroitin-substituted proteoglycan that is present in the corneoscleral meshwork as well as the JCT.[2] It interacts with the non-sulphated GAG chain, hyaluronan, at its N-terminus as well as many other ECM molecules. Versican undergoes complex alternative splicing of its central large exons,[103] which modulates protein isoform and influences the number of GAG attachment sites. Versican showed differences in its segmental distribution, where it was more highly expressed in low flow areas than in high segmental outflow regions. Versican is cleaved by A Disintegrin And Metalloproteinase with ThromboSpondin type 1 motifs-4 (ADAMTS-4). In response to 2x pressure in ex vivo perfusion cultures, ADAMTS-4 was highly increased in the JCT region, concomitant with loss of versican immunostaining, suggesting a role for versican turnover in IOP regulation.[162]

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2.3.2 | Syndecans—
Syndecans are a family transmembrane proteins carrying heparan sulphate and chondroitin sulphate, which interact with a large number of growth factors, cytokines, adhesion receptors, and other ECM proteins. They play important roles in inflammation,[163] tissue repair, and matrix assembly.[164] There are four members of this family and immunolabelling studies have shown that three of the four members are present in the TM/SC albeit at different levels.[160] Syndecan-3 and -4 were the most prevalent on TM cells lining the beams and in the JCT of the TM/SC pathway. Syndecan-2 was also present, but at lower levels. Although the role of these syndecans in the TM/SC is not known, it is tempting to speculate that they would play a role in modulating outflow since they closely associate with proteins that regulate contractility of the actomyosin network. Syndecan-4 in particular has been found to be a component of CLANs and interactions between laminin and syndecan-4 induces CLAN formation in HTM cells.[165,166]

2.3.3 | Decorin—
Decorin is a small proteoglycan found in the ECM that is a member of the SLRP family.[167] It binds to type I collagen fibrils and fibronectin in the ECM and plays a role in matrix assembly. It also binds to thrombospondin and has been shown to regulate the biological activity of TGFβ1 by binding it and preventing interaction between TGFβ1 and its receptor.[168] Decorin is found in AH and its levels are elevated in POAG. Using decorin deficient mice, the loss of decorin caused an increase in IOP due to increased TGFβ signalling.[161]

2.4 | Cell-matrix interactions

2.4.1 | Integrins—
Integrins are a family of cell surface receptors that bind directly to ECM proteins such as fibronectin, TSPs, laminin and collagens (Figure 3). They transmit signals that regulate a number of biological processes relevant to the functions of the TM and pathogenesis of glaucoma. Among the processes controlled by integrins are cell contractility, phagocytosis, cell growth, apoptosis, differentiation, gene expression and organisation of the ECM. Integrins also collaborate with growth factors to regulate a large number of signalling pathways including Ras-MAPK pathway, PI3K-Akt pathway and Rho family GTPases.[169–171] In fact, cells deprived of the cell-matrix signalling events are often refractory to growth factors.[172,173] and changes in integrin signalling due to changes in the composition of the ECMs can also alter the cell's responses to growth factors. For example, primary mammary epithelial cells treated with insulin are protected from cell death when they are plated on laminin, tenascin C or type IV collagen, but if the cells were plated on type I collagen, they underwent cell death in the presence of insulin.[174,175]

In vitro studies suggest that many of the changes associated with GIG and POAG involve activation of the αvβ3 integrin signalling pathway. αvβ3 integrin is the receptor used by CTGF, which is responsible for many of the changes during POAG.[176] αvβ3 integrin is also a receptor for TSP-1, which is upregulated in one-third of POAG patients, and in cultures treated with CTGF and TGFβ1.[133] In addition to binding directly to αvβ3 integrin, TSP-1 can activate αvβ3 integrin signalling by binding to a co-receptor called integrin-associated protein.[177] This interaction produces CLANs observed in glaucomatous tissues and glucocorticoid-treated cells.[46,178,179] Activation of αvβ3 integrin also induces expression of TGFβ2,[180] inhibits phagocytosis,[51] and increases the deposition of fibronectin

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matrices.[180,181] Finally, deletion of this integrin in the mouse eye causes a decrease in IOP, while activating αvβ3 increases IOP, further suggesting a role of αvβ3 integrin in controlling IOP.[52]

2.4.2 | Hyaluronan and CD44—Hyaluronan (HA) is a GAG chain composed of repeating disaccharide units. In the TM, HA represents ~20%–25% of the total GAGs,[182] and is located at higher levels in the anterior, non-filtering portion of the TM and in areas of the JCT that are closest to SC. The presence of HA might prevent adherence of debris/molecules contained in AH to prevent clogging of the outflow pathways.[183,184] HA concentrations are decreased in normal humans as they age, but there is a much greater loss in POAG TM.[124,185,186] It is not known whether this is due to decreased synthesis or increased degradation.

CD44 is a transmembrane protein that binds extracellular molecules (HA, collagen, versican, and fibronectin) (Figure 4). Furthermore, CD44 is connected to the intracellular actin cytoskeleton through actin-binding ezrin, radixin and moesin proteins.[187,188] CD44-HA interactions regulate cell phenotype and function. For instance, promoting HA synthesis causes a substantial increase in the number of cell surface filopodia, while inhibiting HA synthesis, by either drug treatment or by silencing HA synthase genes, reduces filopodia and reorganises F-actin into stress fibres.[189–192] Thus, changes in HA concentration can affect transmission of extracellular signals to intracellular cytoskeleton and lead to detrimental TM cell functions that are observed in glaucoma.

The extracellular portion of CD44, sCD44, which can be proteolytically shed from the full protein,[193] could also play a role in the pathogenesis of glaucoma. The level of sCD44 in the AH of POAG patients is 2.2-fold higher than normal AH.[194–196] sCD44 from POAG AH is hypophosphorylated and has decreased affinity for HA.[197] This increased level of sCD44 would compete for HA binding, thereby affecting signal propagation by cell membrane-bound CD44. It could also affect IOP since mice over-expressing sCD44 saw a significant increase in their IOP.[198]

CD44 also plays an important role in regulating the normal turnover of HA.[188,199] As HA is degraded into fragments by hyaluronidases, CD44 facilitates the uptake of HA into the cell where it is intracellularly degraded.[200–202] If HA fragments are too large, or are bound by other proteoglycans such as versican, then internalisation by CD44 is sterically inhibited. sCD44 may disrupt membrane-bound CD44-HA interactions, or competitively disrupt binding to other ECM components, and affect HA internalisation. Thus, decreased HA concentration and increased sCD44 observed in POAG eyes could be due to dysfunction in the CD44-HA degradation pathway. Interestingly, the CD44-HA pathway is being exploited for nanoparticle drug delivery (Figure 4). HA-coated nanoparticles are efficiently internalised by TM cells in culture and in anterior segment perfusion organ culture.[203] Thus, uptake of HA-coated nanoparticles via CD44 has properties of an attractive intracameral drug delivery system.

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3 | HOW ALTERED ECM COULD AFFECT OUTFLOW FACILITY/IOP

3.1 | Biomechanical properties of ECM

In normal eyes, cells in the TM/SC outflow pathway can sense changes in IOP and respond by temporally and spatially modifying the architecture of the ECM to change outflow resistance through the TM/SC.[2] Many of the major changes in the properties of the ECM occur specifically within the basement membrane in the inner wall of SC. As mentioned above (Section 1.1), there are regions of segmented flow, where the rate of AH outflow can vary significantly, thus creating regions of high and low flow.[23,25,26,204] Atomic force microscopy studies indicate that there are differences in the elastic modulus, a measurement of tissue stiffness, between these regions of high and low flow, which could explain the difference in flow rates.[79,205] Regions of high flow had a lower elastic modulus and thus appeared to be more compliant than low flow regions. In both GIG and POAG, the biomechanical properties of the ECM in the TM/SC are altered, and these tissues have a higher elastic modulus or rigidity.[205–207] Although the reason for these differences in the elastic modulus are still unknown, it is likely due to a combination of increased expression of ECM proteins as well as enhanced crosslinking of the ECM by tissue transglutaminases and glycation.[208–210] In addition to TM biomechanics, there is compelling evidence that altered corneal biomechanical properties influence glaucoma, and central corneal thickness and corneal hysteresis now considered risk factors for glaucoma.[211,212]

ECM stiffness generates a variety of different cellular responses in TM cells in vitro.[213] Cross-linked, stiffer matrices activate the actin-binding protein RhoA and affect β-catenin and YAP/TAZ signalling in human TM cells in vitro. Stiff substrates can also affect how cells respond to growth factor stimulation[214] and is associated with senescence in TM cell in culture.[215] For instance, increased stiffness sensitises epithelial cells to EGF and smaller EGF concentrations were need to override contact inhibition.[214] ECM stiffness also influences the TGFβ pathway in chondrocyte differentiation.[216]

Changes in the rigidity affects ECM synthesis and organisation.[217] On stiff substrates, fibronectin fibrils in TM cultures appear as an elaborate network of long fibrils, but on soft substrates the fibrils appear shorter and as individual unbranched fibrils.[217] Furthermore, SPARC expression is significantly upregulated when TM cells are grown on stiff substrates mimicking glaucomatous TM (75 kPa), whereas SPARC expression was decreased on soft substrates similar to normal TM tissue (5 kPa).[218] Substrate stiffness may affect alternative splicing of ECM genes. Versican is a large proteoglycan that undergoes alternative splicing of its two large central GAG domains.[219] Recent studies showed that when TM cells were cultured on 75 kPa substrates, the V1 isoform of versican, which has 12–15 GAG attachment sites, predominated.[2] However, on a soft substrate, expression of this isoform decreased and expression of the V2 isoform of versican, which has a lower number of GAG binding sites (5–8 sites), increased.[2] Since GAG chains are hydrophilic, versican splicing could influence hydration of the outflow channels. Thus, tissue biomechanics not only affects ECM gene expression, but may also produce more subtle changes such as altered alternative splicing. Finally, ECM rigidity in POAG patients is also likely to affect the structure and activity of fibronectin fibrils. A fibronectin fibril is very elastic and can stretch about four

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times its original length because the type III repeats lack disulphide bonds.[220,221] Thus, their conformation is sensitive to physical forces. This means that mechanical forces, such as those generated by contraction of the actomyosin network, can unravel the tertiary structure of the type III repeat and disrupt binding sites and/or expose novel cryptic sites in fibronectin with unique biological activities.[220,222] For example, a 30%–35% stretch applied to immobilised fibronectin revealed a cryptic site in the III1 repeat, which promoted assembly of fibronectin fibrils and stimulated cell growth and contractility.[222] This enhanced unfolding of fibronectin fibrils was associated with more rigid fibrils in ageing matrices and hence would be expected to occur in glaucomatous matrices.[223] Recent studies in HTM cultures support this idea.[181] Using cells that overexpress an activated αvβ3 integrin to mimic a glaucomatous phenotype, studies showed that a higher percentage of fibrils are positively immunolabelled with an antibody, mAb L8, which detects an epitope that is exposed when fibronectin is stretched in response to tension.[222] This suggests that fibronectin fibrils in these cultures may be more unfolded and would be expected to have different biochemical properties that would affect cell behaviour.[224]

Changes in TM cell contractility, or IOP-induced stretching of the TM tissue, could also expose, or mask, cryptic binding sites in fibronectin fibrils used by integrins or other ECM proteins (Figure 3). For example, the binding site for α5β1 integrin requires a specific conformation in the III9-III10 repeats so that both the RGD and PHRSN sequences, respectively, are accessible.[225,226] When fibronectin fibrils are stretched, the distance between these sites increases, preventing α5β1 integrin forming a high affinity bond with fibronectin.[121,226,227] Disruption of this α5β1 integrin binding site would affect ECM assembly since this is the major integrin involved in fibronectin fibril assembly.[86] Stretching of fibronectin fibrils could also affect incorporation of other proteins such as tenascin C,[228] CD44,[229] versican,[230] myocilin[231] and various growth factors into the ECM.[232]

Interestingly, not all fibrils exhibit the same level of stretch, which implies that fibronectin fibrils could have different activities in different regions of the TM and may explain segmental AH flow. High-resolution cryo-scanning electron microscopy studies on fibrils formed from plasma fibronectin in vitro that found that HepII epitopes were only detected when fibrils were straight.[233] This suggests that under high IOP conditions in the TM/SC outflow pathway, when fibronectin fibrils are more likely to be stretched, bioactive sites within HepII domain may be exposed within the matrix.

3.2 | Altered growth factor availability

Changes in ECM rigidity or composition may alter the availability of growth factors bound to fibronectin. Some of these growth factor-binding domains are cryptic and will only be exposed upon proteolytic processing, or when subject to tension force generated by cells. For example, there are two classes of VEGF binding sites on fibronectin.[234] One is constitutively available, while the other is only exposed when the conformation of fibronectin is altered via interactions with heparin. Other growth factors that could be affected by changes to fibronectin folding are IGF, FGF, TGF-β, HGF and PDGF.[89]

Changes in the levels of TGF-β bound to fibronectin fibrils are likely to have a major impact on the pathogenesis of glaucoma. Multiple studies have shown that TGFβ1 and

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TGFβ2 play a role in the development of PEX and POAG, respectively. Elevated levels of TGFβ2 have been found in AH in 50% of patients with POAG,[36,37,235] and this increase in TGFβ2 has been shown to cause a reduction in AH outflow and increased IOP in both mice and rats.[236–238] In contrast, significantly increased levels of TGFβ1, but not TGFβ2, have been detected in the AH of PEX eyes.[239] Elevated levels of TGFβ1 in PEX are believed to be responsible for the upregulation of fibrillin-1 in PEX extracellular material. CTGF, which acts downstream from TGFβ and induces synthesis of ECM fibrotic bio-markers and actin stress fibre formation, would also be affected. Over-expression of CTGF in transgenic mice, or by adenoviral delivery, increases IOP and causes glaucomatous-like damage to the posterior mouse eye.[240]

4 | CONCLUDING REMARKS

Controlling IOP remains the most effective treatment to preserving vision in glaucoma patients and doing that requires a better understanding of how the extracellular environment affects TM outflow resistance. The key challenge facing us is to identify which ECM molecules to therapeutically target in order to modify that extracellular environment, but to avoid unintended detrimental effects. As we gain a better understanding of the ECM molecules in the TM, we realise that changes in how ECM molecules interact with each other, and with cells, is just as important as the function of a single protein. Hence, gross changes, or the more subtle changes discussed above, are likely to have a significant impact on the structure and function of the TM. Over time, as deleterious changes accumulate, they would lead to a re-organisation of the ECM architecture that essentially alters the function of the TM/SC outflow pathway and contributes to elevated IOP in glaucoma.

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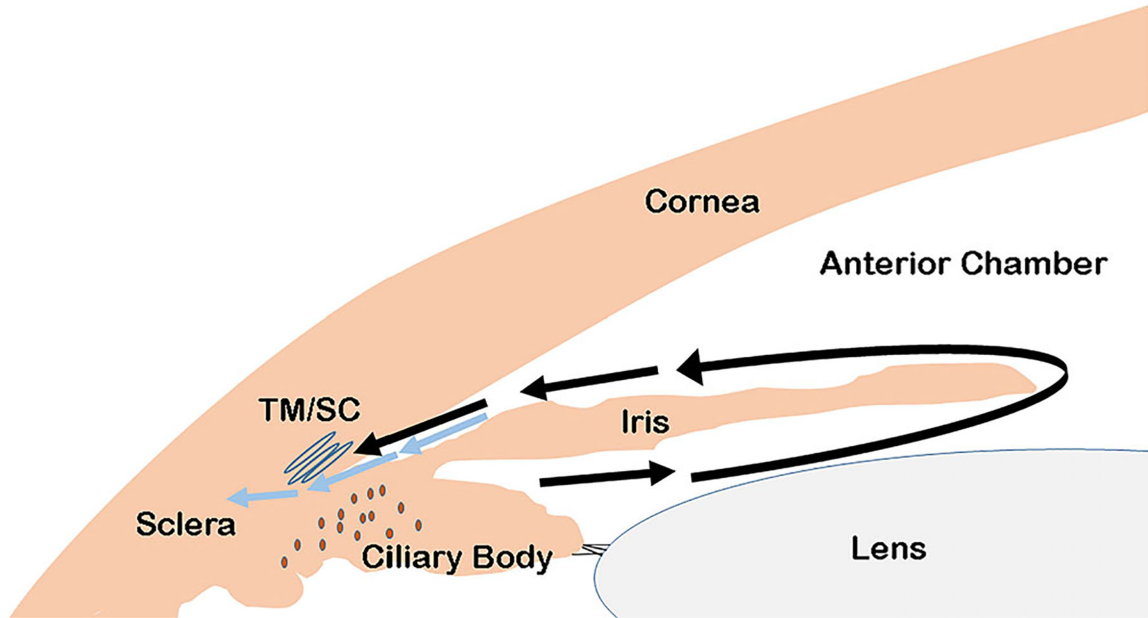

FIGURE 1.
The conventional and unconventional outflow pathway. Aqueous humour produced by the ciliary body flows into the anterior chamber between the lens and iris. In the conventional pathway, the majority of the aqueous fluid exits the anterior chamber through the trabecular meshwork/Schlemm's canal (TM/SC) system (black arrows). In the unconventional pathway, aqueous humour exits through the sclera (blue arrows)

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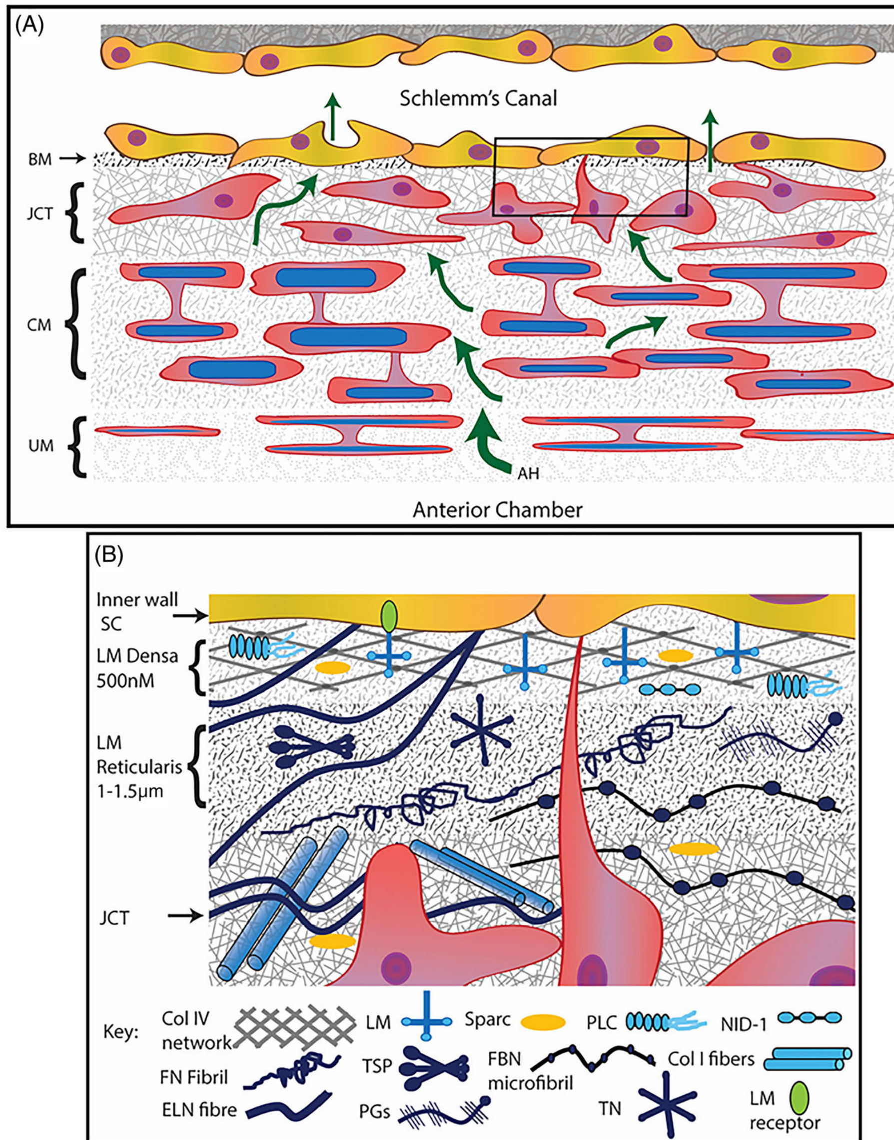

FIGURE 2.

The trabecular meshwork/Schlemm's canal outflow pathway. (A) Aqueous humour (AH; green arrows) flows through the uveoscleral meshwork (UM), corneoscleral meshwork (CM) and the juxtacanalicular tissue (JCT). It then crosses the basement membrane (BM) underlying the inner wall of Schlemm's Canal (SC) to exit either paracellularly and transcellularly into the lumen of SC. Box indicates placement of image show in panel B. (B) Diagram illustrates ECM proteins found in the two layers of the BM and the upper region of the JCT. The BM is divided into the lamina (LM) densa and the LM reticularis. Cellular protrusions extend between JCT cells, and between JCT and SC cells. These protrusions play important roles in detecting alterations in the ECM biomechanical environment and communicating signals between cells. Col; collagen, TSP; thrombospondin, FN, fibronectin; FBN, fibrillin; ELN, elastin; TN, tenascin; PGs,

proteoglycans (e.g., versican and hyaluronan); NID-1, nidogen; PLC, perlecan; LM, laminin. *Source*: Figure was modified from a previously published image Reference 82

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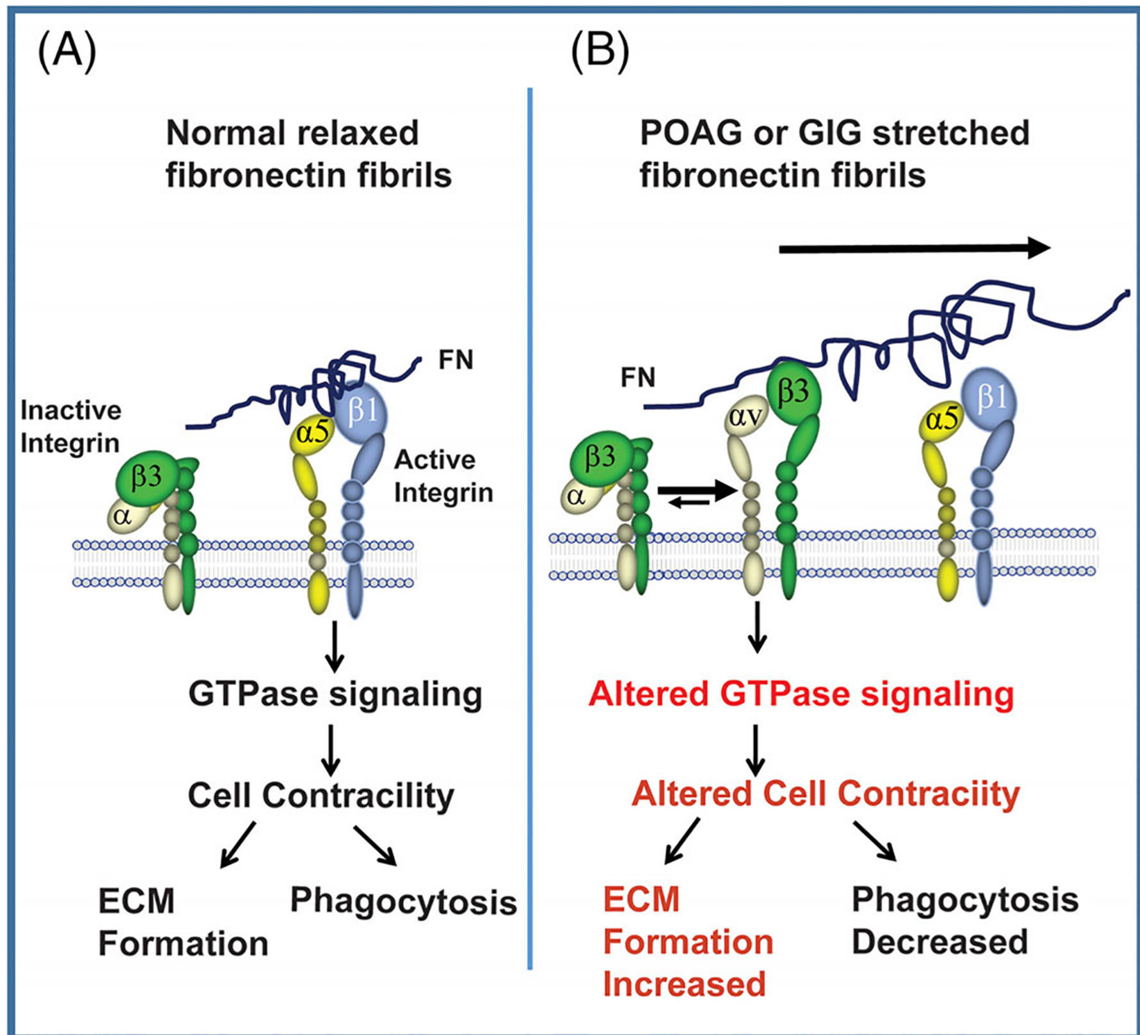

FIGURE 3.
Hypothetical scenario how conformation of fibronectin (FN) fibrils affect integrin signalling. (A) The α5β1 integrin can recognise its binding motif in a highly coiled FN fibril, which activates a GTPase signalling pathway that controls ECM formation and phagocytosis. (B) If the fibril is stretched, the α5β1 integrin binding motif in the fibril is conformationally altered preventing the α5β1 integrin from binding FN. The stretching of the fibril, however, reveals a binding motif for the αvβ3 integrin. Activation of the αvβ3 integrin alters the GTPase signalling pathway that controls ECM formation and phagocytosis

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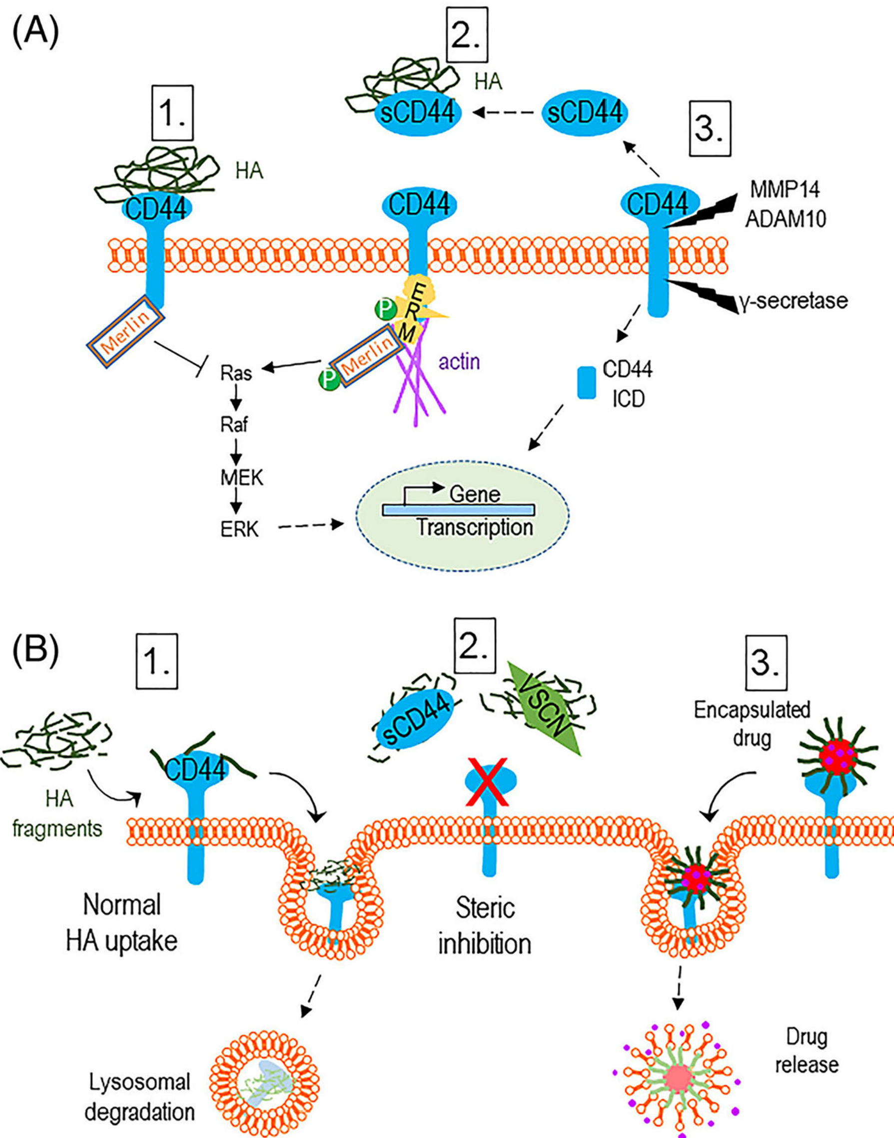

FIGURE 4.

CD44 and hyaluronan in trabecular meshwork cells. (A) Schematic showing CD44 interactions with hyaluronic acid (HA), intracellular signalling pathways and proteolytic processing. (1) CD44 binds to HA via its extracellular domain and dephosphorylated merlin on its intracellular domain (ICD). This inhibits the Ras–Raf–MEK–ERK signalling cascade. (2) If HA-CD44 interactions are disrupted, CD44 binds to phosphorylated Ezrin-Radixin-Moesin (ERM) complex and phosphorylated Merlin, which activates the Ras–Raf–MEK–ERK signalling cascade and ultimately alters gene transcription. Phosphorylated ERM also bind to actin filaments. (3) The extracellular domain of CD44 can be proteolytically cleaved by proteinases such as MMP14 and ADAM10, which leads to 'shed' CD44 (sCD44). sCD44 competes with membrane-bound CD44 for binding to HA. CD44 is also cleaved intracellularly by γ-secretase, which liberates the ICD. This small proteolytic fragment launches a signalling cascade, which ultimately alters gene transcription. (B)

HA internalisation by CD44. (1) HA is degraded into small fragments by extracellular hyaluronidases. HA fragments are internalised by CD44 in a clathrin-dependent endocytic mechanism and are degraded in the lysosome. (2) If HA fragments are bound to sCD44, or to other HA-binding proteoglycans (such as versican [VSCN]), uptake by CD44 is sterically hindered. (3) HA-coated nanoparticles can encapsulate a glaucoma drug and they can be internalised by CD44. Once the nanoparticles are degraded, the drug of interest is released intracellularly

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