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Review

Modulation of extracellular matrix turnover in the trabecular meshwork

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ABSTRACT

Intraocular pressure (IOP) is the most critical risk factor for primary open angle glaucoma (POAG). In most cases of POAG, IOP is increased because of an abnormally high aqueous humor outflow resistance in the juxtacanalicular region of the trabecular meshwork. A distinct structural change in the trabecular meshwork of patients with POAG is the increase in fibrillar extracellular matrix in the juxtacanalicular region of the trabecular meshwork. Our knowledge on the molecular factors that govern turnover of the extracellular matrix in the trabecular meshwork has increased considerably in recent years. It has become clear that quality and quantity of the extracellular matrix in the trabecular meshwork are regulated by several signaling molecules that interact with each other to promote its synthesis, degradation, or extracellular modification. Transforming growth factor-β1 and β2 (TGF-β1 and TGF-β2) which derive from the aqueous humor or may be locally expressed induce in cultured trabecular meshwork cells the expression of a variety of extracellular matrix molecules. The action of TGF-βs very likely requires local activation by thrombospondin-1 and is partly mediated by its downstream mediator connective tissue growth factor, both of which are constitutively expressed in the trabecular meshwork. Bone morphogenetic proteins (BMP)-7 and -4 effectively antagonize the effects of TGF-β2 on matrix deposition. The antagonizing effects of BMP-7 are mediated in trabecular meshwork cells through Smad7. Smad7 is a key molecular switch to inhibit TGF-β2 signaling in the trabecular meshwork.

1. Introduction

In primary open angle glaucoma (POAG), a major cause of blindness worldwide (Quigley, 1996), the critical risk factor for axonal damage at the optic nerve head is an intraocular pressure (IOP) which is too high for the health of the optic nerve head (Collaborative Normal-Tension Glaucoma Study Group, 1998a, 1998b; The AGIS Investigators, 2000; Gordon et al., 2002; Leske et al., 2003). IOP is increased in POAG when aqueous humor (AH) outflow resistance in the juxtacanalicular region (JCT) of the human trabecular meshwork (TM) is abnormally high (Grant, 1963; Johnson, 2006). The mechanisms that are responsible for the increase in TM outflow resistance in POAG are unclear (Johnson, 2006; Tamm et al., 2007). There is some evidence though that changes in the amount and quality of the TM extracellular matrix (ECM) are involved, as eyes with POAG show a significant increase in fibrillar ECM in the JCT outflow pathways (Lütjen-Drecoll et al., 1986; Rohen et al., 1993). Our knowledge on the molecular factors that govern ECM turnover in the TM has increased considerably in recent years. It has become clear that quality and quantity of ECM in the TM are regulated by several signaling molecules that interact with each other to promote synthesis, degradation, or extracellular modification of the ECM (Tamm and Fuchshofer, 2007). This article will review the growing list of molecules which act in a complex network to influence ECM homoeostasis in the TM.

2. Transforming growth factor-β

Transforming growth factor-β (TGF-β) is a member of a family of dimeric polypeptide growth factors. There are three isoforms of TGF-β: TGF-β1, TGF-β2, and TGF-β3, which are each encoded by a distinct gene. In the normal anterior eye, TGF-β2 appears to be the predominant isoform as it is found at relative high concentrations in the AH of normal eyes (Granstein et al., 1990; Jampel et al., 1990; Cousins et al., 1991). TGF-β2 is very likely secreted into the AH from the epithelial cells of ciliary body (Helbig et al., 1991) and lens (Allen et al., 1998; Gordon-Thomson et al., 1998; Wallentin et al., 1998). Immunohistochemical studies have shown immunoreactivity for TGF-β1 in the stroma of the ciliary processes, while TGF-β3 was not detected in structures of the anterior eye (Pasquale et al., 1993). The normal TM does not label with antibodies against the various TGF-β isoforms (Pasquale et al., 1993), but cultured TM cells are capable to secrete TGF-β2 and TGF-β1 (Tripathi et al., 1993b, 1994a), and express receptors for both factors (Borisuth et al., 1992; Tripathi

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et al., 1993a). The physiological role of TGF-β2 in the AH appears to be tightly linked with the immunosuppressive environment of the anterior chamber (Streilein et al., 1997; Streilein, 1999) and the phenomenon of anterior chamber associated immune deviation (ACAID).

Among the factors that modulate ECM turnover in the TM, TGF-β2 is the one which is most likely involved in the pathological ECM increase in POAG. Multiple groups have reported significantly higher levels of TGF-β2 in the aqueous humor collected from human POAG eyes as compared with AH from patients that underwent cataract operation (Tripathi et al., 1994b; Inatani et al., 2001; Picht et al., 2001). The factors which cause or contribute to the increase in TGF-β2 in the AH of patients with POAG are unclear. In addition, there is no information available on the nature of the ocular tissues which secrete higher than normal amounts of TGF-β2 in the AH, but because of the direction of AH flow, ciliary body and lens are more likely candidates than the TM. The increase in TGF-β2 appears to be typical for POAG and not to be directly caused by an increase in intraocular pressure, as patients with pseudoexfoliation glaucoma do not show elevated levels of TGF-β2 in their AH (Picht et al., 2001). In contrast, AH of patients with pseudoexfoliation syndrome or glaucoma contains elevated amounts of TGF-β1 and TGF-β3 (Schlötzer-Schrehardt et al., 2001; Yoneda et al., 2007).

In multiple disorders throughout the body, TGF-β signaling mediates a pathological increase in ECM secretion and deposition, and is causatively involved in fibrosis (Ihn, 2002b; Schnaper et al., 2003; Huggins and Sahn, 2004; Bataller and Brenner, 2005; Gressner and Weiskirchen, 2006; Liu, 2006; Willis and Borok, 2007). It is reasonable to assume that passage of higher than normal amounts of TGF-β2 through the TM will induce changes in TM gene expression that may result in an increase in TM ECM deposition. Experimental support for this assumption comes from experiments involving anterior eye segment perfusion cultures, in which perfusion with TGF-β2 promoted a focal accumulation of fine fibrillar extracellular material in the TM (Gottanka et al., 2004) and an increase in fibronectin synthesis (Bachmann et al., 2006; Fleenor et al., 2006), effects that were correlated with a reduction in outflow facility. Comparable results were obtained in experiments involving monkey anterior eye segment organ cultures, in which perfusion with TGF-β2 caused a decrease in outflow facility by ~40% compared to pretreatment baseline (Bhattacharya et al., 2009). The decrease in outflow facility correlated with an increase in cochlin, a secreted protein that comprises the major non-collagen component of the ECM of the inner ear, and is present in glaucomatous TM, but absent in the normal TM (Bhattacharya et al., 2005). In addition, numerous studies provided evidence that treatment of TM cells in monolayer cell culture leads to a substantial increase in the expression and synthesis of a broad variety of ECM proteins (collagens III, IV and VI, elastin, fibronectin, versican, laminin, and myocilin) that all contribute to the ECM of the TM *in situ* (Tamm et al., 1999; Li et al., 2000; Zhao et al., 2004; Zhao and Russell, 2005; Fleenor et al., 2006; Fuchshofer et al., 2006, 2007).

TGF-β2 treatment of cultured TM cells also stimulates the synthesis of proteins that modify turnover and deposition of ECM. Accordingly, TGF-β2 induces in TM cells the expression of tissue transglutaminase and its mRNA, as well as its action on irreversible and covalent cross-linking of fibronectin (Welge-Lüssen et al., 2000). Moreover, TGF-β2 leads to an increased TM synthesis of plasminogen activator inhibitor (PAI-1) which is a potent inhibitor of matrix metalloproteinases (MMPs) (Fuchshofer et al., 2003; Fleenor et al., 2006). In the TM, PAI-1 appears to primarily inhibit MMP-2 (Fuchshofer et al., 2003), an enzyme that degrades collagen type IV, the major structural component of basement membranes. The expression of matrix Gla protein (MGP) which is among the most highly expressed genes in fresh human TM (Tomarev et al., 2003), is significantly downregulated following treatment with TGF-β2 (Xue et al., 2007). In certain soft tissues, MGP is important to prevent ectopic calcification, and mice that are deficient in Mgp ($Mgp^{-/-}$) develop severe vascular atherosclerosis and calcification (Luo et al., 1997). While the available data on the morphology and pathology of human TM in POAG very clearly indicate that a major calcification process comparable to that in atherosclerosis is absent in the TM (Rohen and Witmer, 1972; Tripathi, 1972; Fine et al., 1981; Rohen et al., 1981, 1993; Alvarado and Murphy, 1992), it is certainly tempting to speculate that a more subtle mineralization of the TM ECM or a comparable process is involved in the structural changes of the TM in POAG, and that TGF-β signaling is causatively involved.

It is of interest to note that TGF-β signaling does not only effect ECM turnover in the TM, but also acts on the TM actin cytoskeleton, as treatment with TGF-β1 induces the expression of α-smooth muscle actin in cultured TM cells (Tamm et al., 1996). α-Smooth muscle actin is the isoform of actin that is expressed in smooth muscle cells and myofibroblasts, a cell type that predominates in connective tissues of healing wounds and scars (Wang et al., 2006). In myofibroblasts, the induction of α-smooth muscle actin by TGF-β1 substantially enhances cell traction force (Chen et al., 2007). In the normal TM *in situ*, some cells are immunoreactive for α-smooth muscle actin (de Kater et al., 1992; Flügel et al., 1992), while virtually all cells in the scleral spur region, close to the posterior attachment of the TM, express this actin isoform (Tamm et al., 1992). There is the distinct possibility that an increase in the activity of TGF-β signaling in the eye increases the number of α-smooth muscle actin-positive cells in the TM thereby increasing TM cell tone, an effect that has been shown to correlate with an increase in outflow resistance (Wiederholt et al., 2000).

3. TGF-β activation and thrombospondin-1

TGF-β signaling is not only involved in ECM turnover, but acts in other extremely important biological processes throughout the body and in the eye, including proliferation, apoptosis, and modulation of the immune system. Accordingly, defects in TGF-β function are not only associated with fibrosis, but also with other pathological states such as tumor cell growth and autoimmune diseases (Blobe et al., 2000; Gordon and Blobe, 2008). Because of their multifunctional role, the activity of TGF-βs *in vivo* needs to be subject to tight control mechanisms. TGF-βs are secreted as large latent complexes (LLC), which are unable to interact with cellular receptors, a mechanism that prevents uncontrolled activation of the TGF-β signaling pathway (Annes et al., 2003). LLC consist of the native TGF-β dimer which is noncovalently associated with its propeptide, the latency associated protein (LAP), and of a second gene product, the latent TGF-β-binding protein (LTBP) which binds covalently to LAP. Mammalian cells express four different LTBP isoforms, of which only three (LTBP-1, -3, and -4) can associate with TGF-βs (Saharinen and Keski-Oja, 2000). Following secretion, the LLC may be covalently linked to the ECM via the N-terminus of LTBP (Annes et al., 2003). Upon TGF-β activation, TGF-βs are liberated from both LAP and LTBP.

TGF-β2 in the AH of normal and POAG eyes is mostly found in its latent, inactive form (Tripathi et al., 1994b; Picht et al., 2001) and it seems reasonable to assume that its effects on TM biology will considerably increase, if it is locally activated in the TM. *In vitro*, active TGF-βs are generated by extremes of pH, heat, or chaotropic agents, mechanisms that are not likely to be of physiological relevance for TGF-β activation *in vivo*. The physiological mechanisms of TGF-β activation *in vivo* may involve proteolytic processing since *in vitro* studies have identified a number of proteases including plasmin, MMP-2 and MMP-9 as activators of latent TGF-β (Sato and