TGFβ2-Induced Changes in Human Trabecular Meshwork: Implications for Intraocular Pressure

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Purpose. Transforming growth factor (TGF)-β2 levels are elevated in glaucomatous human aqueous humor. TGFβ is a cytokine that alters extracellular matrix (ECM) metabolism, and excess ECM has been proposed to increase aqueous outflow resistance in the trabecular meshwork (TM) of glaucomatous eyes. This study was undertaken to investigate effects of TGFβ2 on secretion of fibronectin and the protease inhibitor plasminogen activator inhibitor (PAI)-1 from human TM cell cultures and perfused human ocular anterior segments.

Methods. Total RNA was isolated from pooled human TM cell monolayers and used for a gene microarray expression analysis. Supernatants from treated human TM cells were analyzed by ELISA for fibronectin or PAI-1 content. TGFβ2 effects on intraocular pressure (IOP) were evaluated in a perfused organ culture model using human anterior segments, and eluates were analyzed for fibronectin and PAI-1 content.

Results. Overnight treatment of TM cells with TGFβ2 upregulated multiple ECM-related genes, such as PAI-1. TGFβ2 also increased secretion of both fibronectin and PAI-1 from TM cells. TGFβ2 effects on TM cells were blocked by inhibitors of the TGFβ type 1 receptor. In perfused human anterior segments, TGFβ2 treatment elevated IOP and increased eluate fibronectin and PAI-1 content.

Conclusions. TGFβ2 effects on IOP may be transduced by TGFβ type-1 receptor-mediated changes in TM secretion of ECM-related factors such as fibronectin and PAI-1. Modulation of TGFβ2-induced changes in the ECM may provide a novel and viable approach to the management of glaucoma.

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The transforming growth factor (TGF)-β family of cytokines includes multifunctional proteins that regulate production of a wide variety of gene products, and thus control a wide variety of cellular processes. For example, TGFβ family members are involved in inflammation, wound healing, extracellular matrix accumulation, bone formation, tissue development, cellular differentiation, and tumor progression, among others.1–4 Three mammalian isoforms have been identified to date: TGFβ1, -β2, and -β3, and these isoforms are structurally similar, despite being encoded by different genes.5 Regardless of their similarity, effects of the various isoforms can vary, particularly in phenotypic effects during early development.6 Such diversity may be related to divergent affinities of the isoforms for the type I and -II TGFβ receptors.7 For example, TGFβ1 has been shown to be 100-fold more potent than TGFβ2 for inhibition of endothelial cell growth and migration, an effect that may be due to greater receptor specificity conferred by a specific region (amino acids [aa] 40-82) of the TGFβ1 isoform.8

Endogenous TGFβ elicits multiple effects in ocular tissues. These effects may be tissue-specific. For example, TGFβ has been reported to suppress the proliferation and increase the migration, of corneal epithelial cells during wound healing.9 Conversely, TGFβ stimulates the proliferation of corneal stromal fibroblasts.10 In addition, TGFβ1 and -β2 stimulate in vitro proliferation of human Tenon’s capsule fibroblasts derived from glaucoma patients, with both isoforms inducing migration and increased extracellular matrix (ECM) production.11 TGFβ2 treatment also enhances the expression of matrix components by cultured human optic nerve astrocytes and by the human lens epithelial cell line FHL124.12,13 Finally, elevated in vivo TGFβ levels have been correlated with the fibrotic response of proliferative vitreoretinopathy: intravitreal levels of TGFβ2 increased approximately threefold compared with patients without excess fibrosis.14 However, TGFβ effects on the anterior segment are not exclusively pathogenic. TGFβ2 in the aqueous humor (AH) is also responsible for anterior chamber-associated immune deviation, a mechanism that protects the eye from inflammation and immune-related tissue damage.15 TGFβ2 appears to be involved in the pathogenesis of certain forms of glaucoma.16 Ocular hypertension is one of the major risk factors for the development and progression of primary open-angle glaucoma (POAG), a leading cause of blindness. One potential causative factor for the POAG-associated increase in intraocular pressure (IOP) is compromised outflow facility of AH through the trabecular meshwork (TM). A disproportionate accretion of ECM occurs in the TM region of POAG eyes,17,18 and this buildup is believed to impart greater resistance to AH outflow, resulting in increased IOP.

Various groups have reported significantly higher levels of TGFβ2 in AH collected from human POAG eyes.19–23 Coupling this finding with the expression data from both human and nonhuman TM cells makes it tempting to speculate that a direct link exists between elevated TGFβ2 levels in AH with altered and/or increased ECM content and an ensuing increase in IOP. Indeed, Gottanka et al.24 demonstrated that ex vivo human anterior segments respond with reproducible decreases in outflow facility when perfused with TGFβ2.

Recent studies have also shown that in vitro treatment of cultured human TM cells with TGFβ2 leads to changes in gene expression, including genes that may contribute to ECM accumulation. Zhao et al.25 showed that treatment of human TM cells with either TGFβ1 or -β2 stimulates the expression of several ECM genes, including versican, elastin, collagens, fibrillin, laminin, and fibulin. In addition, TGFβ2-treated human TM cells altered the production of the enzymes promatrix metalloproteinase-2 and plasminogen activator inhibitor (PAI)-126 each of which likely play a role in ECM-remodeling by the TM. TGFβ2 has also been linked to increased production of such
ECM components as fibronectin from cultured porcine and bovine TM cells and of collagen from human and bovine TM cells.27–29 Furthermore, treatment of human TM cell cultures with either TGFβ1 or β2 leads to significant increases in fibronectin and tissue transglutaminase, an enzyme that co-valently cross-links ECM proteins, thereby conferring resistance to fibronectin degradation.30

In our studies, we confirmed the ability of TGFβ2 to alter TM expression of fibronectin and PAI-1 and correlated the secretion of both from TGFβ2-treated human TM cells and human anterior segments in perfusion organ culture. In addition, we identified pharmacological agents that significantly reduced the effects of TGFβ2 in human TM cell cultures. Identification of such agents may therefore provide a unique means for IOP-lowering in patients with POAG.

METHODS

Culture of Human TM Cells

Cultured transformed and nontransformed human TM cells were used in this study. Generation and characterization of the GTM-3 transformed cell line has been described.31 Other, nontransformed TM cells (Table 1) were also isolated and characterized according to published procedures.32–35 Maintenance growth medium consisted of Dulbecco’s modified Eagle’s medium with a dipeptide (L-alanyl-L-glutamine) supplement (Invitrogen-Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and antibiotic-free medium containing the appropriate test compound(s). Specimens were maintained in a humidified atmosphere of 5% CO2 until a stable baseline IOP was achieved, typically 2 to 4 days. Tissues with unstable IOP were discarded. Stable tissues were then perfused at constant pressure with serum-free Dulbecco’s modified Eagle’s medium. IOP was monitored every 5 seconds and averaged each hour. Perfused tissue was allowed to equilibrate at 37°C and 5% CO2 then perfused at constant pressure with serum-free Dulbecco’s modified Eagle’s medium. IOP was monitored every 5 seconds and averaged each hour. Perfused tissue was allowed to equilibrate at 37°C and 5% CO2 until a stable baseline IOP was achieved, typically 2 to 4 days. Tissues with unstable IOP were discarded. Stable tissues were then further perfused with medium containing the test compound(s) as indicated, and changes in IOP were recorded. Eluate samples were collected daily for ELISA analysis of fibronectin and PAI-1 content. Tissues were fixed and evaluated for viability and morphology by light and electron microscopy at the termination of each study. Data from unacceptable tissues were excluded from the results. Criteria for “unacceptable” tissues included findings such as excess debris in the TM region, denudation of TM beams, loss of TM and/or Schlemm’s canal, and breaks or collapse of Schlemm’s canal.40,41

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* Transformed cell line.
RESULTS

Detailed information on the human TM cell strains used in these studies is listed in Table 1. Cells were derived from both glaucomatous donors (GTM) and donors who had no known history of glaucoma (NTM). Furthermore, the cells used in these studies were generated from both male and female donors and from donors of an extensive age range (6 months to 94 years), to ascertain whether responses were gender- and/or age-dependent. Because of their fast growth rate and reproducible response(s), a transformed cell line (GTM-3) was used for most of the studies; however, nontransformed lines were used for gene expression analysis and also in selected studies to confirm GTM-3 results.

Overnight (16 hours) treatment of nontransformed GTM cells with TGFβ2 (5 ng/mL; 200 pM) resulted in measurable changes in expression for a large number of genes, as determined by gene microarray analysis (GeneChip; Affymetrix). TGFβ2-treated GTM cells exhibited a twofold or more upregulation of a variety of ECM-related genes, compared with untreated control cultures. Examples are listed in Table 2. The most striking change in expression (a 69-fold increase) was exhibited by PRG4 which encodes lubricin (proteoglycan 4), a secreted glycoprotein ordinarily associated with synovial fluid. The expression of another proteoglycan gene CSPG2 (versican) and of the glycosaminoglycan metabolism enzymes UGDH (UDP-glucose dehydrogenase) and HAS1 (hyaluronan synthase 1) also were induced by TGFβ2 treatment, as was the collagen gene COL4A1. Genes regulating ECM turnover, including SERPINE1 (PAI-1) and ADAMTS5 were stimulated as well. In addition, two genes regulating TGFβ activity, THBS1 (thrombospondin 1) and LTBP1 (latent TGFβ binding protein 1) were increased with TGFβ2 treatment.

Based on both published data and our own gene array results, we chose to focus on two known TGFβ2-regulated gene products: fibronectin and PAI-1. In our studies, a 24-hour exposure of GTM-3 cells to 5 ng/mL TGFβ2, -β2, or -β3, led to significant increases in both fibronectin and PAI-1 protein levels in supernatants from treated monolayers, compared with the untreated control (Fig. 1). These data suggest that the response is not isoform specific for either effect in GTM-3 cells, despite apparent differences in efficacy. For example, at 5 ng/mL, TGFβ2 elicited a lower PAI-1 response than did TGFβ1 and -β3. However, because published reports indicate that the TGFβ2 isoform is more likely to be elevated in POAG, the remainder of our studies focused on TGFβ2-mediated effects.

The time course of TGFβ2 (5 ng/mL) effect on soluble fibronectin and PAI-1 release from GTM-3 cells is shown in Figure 2. Significant (P < 0.05) and measurable changes in supernatant fibronectin content were detectable as early as 8-hours after administration (see inset; top panel); fibronectin levels continued to increase steadily at each subsequent tested time point (16, 24, 48 hours). No apparent diminution of fibronectin response occurred, even at 48 hours after treatment. In contrast, PAI-1 levels increased rapidly within the first 24 hours after treatment with TGFβ2, at which time the response appeared to reach a steady state, up to at least 48 hours (Fig. 2, bottom panel). Based on these results, and to avoid

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Effect of TGFβ isoforms (5 ng/mL, 24 hours) on GTM-3 cells. Fibronectin (top) and PAI-1 (bottom) levels in cell supernatants.
possible detrimental effects of extended serum-free assay conditions, all other studies with cultured cells were conducted in a 24-hour incubation with TGFβ2.

Figure 3 depicts the effects of TGFβ2 (24 hours) on GTM-3 cell supernatant levels of both fibronectin and PAI-1. Responses were dose dependent: exposure to increasing concentrations of TGFβ2 led to concomitant significant increases in the accumulation of both fibronectin (top panel) and PAI-1 (bottom panel) in the supernatants. TGFβ2 treatment exerted minimal effects on GTM-3 cell viability, as determined by neutral red uptake, with the exception of a moderate (15%) decrease in viability at the 25-ng/mL concentration (data not shown). Thus, all subsequent experiments were conducted using a standard 5-ng/mL concentration, a level that did not result in measurable effects on GTM-3 cell viability.

To verify that effects in the transformed GTM-3 cell line are predictive of effects in human TM cell cultures in general, the response to TGFβ2 was also evaluated in various nontransformed human TM cell lines. Table 3 demonstrates the effect of TGFβ2 (5 ng/mL, 24 hours) on fibronectin and PAI-1 content in supernatants from GTM-3 cells and from similarly treated nontransformed TM cell lines. Supernatant PAI-1 content in TGFβ2-treated GTM-3 cells was equivalent to that for nontransformed TM cells: supernatants from GTM-3 cells averaged 266 ± 8 ng/well compared with average PAI-1 levels of 256.5 to 315.9 ng/well in treated nontransformed TM cells.

Because PAI-1 has been demonstrated to regulate the levels of various ECM molecules, we tested PAI-1's effects on fibronectin production in TM cells. We found that, in addition to TGFβ2 treatment, a 24-hour exposure to recombinant human PAI-1 (rhPAI-1) itself also resulted in a concentration-depen-

### Figure 2
Fibronectin (top) and PAI-1 (bottom) levels in GTM-3 cell supernatants with or without treatment with TGFβ2 (5 ng/mL) for various time periods. Top inset: fibronectin levels at 0 and 8 hours. Data are expressed as the mean ± SEM. *Statistical significance (P < 0.05) versus the corresponding vehicle time point group, by Student's t-test.

### Figure 3
Concentration-dependent effect of TGFβ2 (24 hours) on levels of fibronectin (top) or PAI-1 (bottom) in GTM-3 cell supernatants. Data are expressed as the mean ± SEM. *Statistically significant (P < 0.05) versus the respective vehicle group, by one-way ANOVA followed by the Dunnett test.
dent increase in the fibronectin content of GTM-3 cell super-
natants (Fig. 4).

PAI-1 and fibronectin secretion from the transformed 
(GTM-3) cells, as well as from nontransformed TM cells, are 
probably signaled by activation of the type I TGFβ receptor 
(also called activin receptor-like kinase 5; ALK5), because the 
effect of TGFβ2 on both fibronectin and PAI-1 accumulation is 
dose-dependently blocked by type I receptor inhibitors. Figure 
5 indicates the effect of two inhibitors of type I TGF-
kinases: SB431542, which also inhibits ALK4 and ALK7 recep-
tors in addition to ALK5
and ALK5 inhibitor I ([3-(pyridin-2-
yl)-4-(4-quinonyl)]-1H-pyrazole). Both inhibitors potently and 
dose-dependently antagonized TGFβ2-induced (5 ng/mL, 24 
hours) increase in GTM-3 cell supernatant content of fibronec-
tin and PAI-1 (Fig. 5).

Finally, because the TM constitutes the region through 
which the bulk of aqueous outflow occurs, we further inves-
tigated the effects of TGFβ2 in an ex vivo outflow model using 
human donor ocular tissues. Continuous perfusion of human 
anterior segments with TGFβ2 (5 ng/mL) generated a time-
dependent increase in IOP versus that of the vehicle-treated 
control. Such increases became statistically significant within 
24 hours after the initiation of treatment and persisted for at 
least 4 days (Fig. 6). Based on the observed ability of TGFβ2 to 
increase soluble fibronectin and PAI-1 levels in cultured HTM 
cell supernatants, the content of each was measured in the 
perfusate from untreated versus TGFβ2-treated tissues. Consist-
ent with results from the cell cultures, TGFβ2 time-depend-
ently increased both fibronectin and PAI-1 levels in the per-
clusates from these tissues, compared with the vehicle-treated 
control (Fig. 7).

The increase in perfusate content was statistically signifi-
cant as early as day 2 for fibronectin and day 1 for PAI-1 after 

**TABLE 3. Effect of TGFβ2 on HTM Cell Secretion of Fibronectin and PAI-1**

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Data reflect mean ± SEM.
* 5 ng/mL TGFβ2, 24 hours.

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**FIGURE 4.** Effect of rhPAI (24 hours) on fibronectin levels in GTM-3 supernatants. The effect of TGFβ2 (5 ng/mL) is included for comparison. Data are the mean ± SEM. *Statistically significant (P < 0.05) versus the vehicle group, by one-way ANOVA followed by the Dunnett test.

**FIGURE 5.** Dose-dependent effects of TGFβ type 1 receptor kinase inhibitors on TGFβ2-induced (24 hours) fibronectin (top) or PAI-1 (bottom) increase in GTM-3 cell supernatants. Data are mean ± SEM.
the initiation of treatment. Similar to the effect of TGFβ2 on IOP, the elevation of both fibronectin and PAI-1 levels in the perfusates persisted for at least 4 days. Of interest, perfusate PAI-1 levels peaked rapidly and then appeared to reach a steady state, whereas fibronectin content was still increasing, even at the day-4 time point. These data are consistent with those of cultured TM cells, albeit with a slightly different time course.

**DISCUSSION**

TGFβ2 increased the expression of the ECM-associated molecules fibronectin and PAI-1 in all cultured human TM cells tested, regardless of whether the cells were derived from nonglaucomatous or glaucomatous, male or female, young or elderly donors. TGFβ2 also elevated IOP by increased outflow resistance within 24 hours of exposure in an ex vivo perfusion-cultured human anterior segment model. The increase in IOP was associated with the elaboration of fibronectin and PAI-1 content in the perfusate.

As predicted, TGFβ2-treated TM cell cultures exhibited upregulated mRNA expression levels of numerous genes, including many genes related to the ECM. The substantial (>69 fold) upregulation of PRG4 is both intriguing and puzzling. There are no known published reports to indicate production of lubricin by the TM or by any other ocular cells or tissues; therefore, the significance of this finding remains to be determined. In contrast, the upregulation of many other ECM-related gene products was not unexpected. For example, upregulation of thrombospondin 1 mRNA expression by HTM cells has been shown to occur in response to both TGFβ1 and β2.25,45 Our data also reveal increased thrombospondin 1 mRNA expression in the TGFβ2-treated TM cells. Similarly, our studies detected measurable increases in the expression of mRNA from genes encoding various proteoglycans (e.g., versican) and collagen (COL4A1), in agreement with previously published data.25 Our gene expression analysis further showed increased expression of PAI-1 (SERPINE1), consistent with our ELISA data.

The production of ECM components, such as fibronectin, by TM cells has been described previously. For example, we have shown that dexamethasone treatment upregulates both secreted and cell-associated fibronectin in cultured human TM.32 Exposure to high glucose concentrations in culture medium also induces fibronectin production by TM derived from both bovine and human tissues.46,47 although such increases are relatively modest (estimated at 31% and 27% for bovine and human, respectively). In addition, supplementation of culture medium with growth factors known to be present in aqueous humor increases porcine TM expression of both fibronectin mRNA and protein.48 In those studies, the mixture of growth factors used included, among others, both TGFβ1 and β2.

Several laboratories have reported the ability of TGFβ2 to increase fibronectin mRNA and/or protein levels in both non-
human (bovine, porcine) and human TM cell cultures. In our studies, we detected a less than twofold upregulation in fibronectin mRNA expression. However, the human fibronectin gene is capable of encoding 20 distinct polypeptides due to splicing variations of the primary transcript. Seven of these 20 potential splice variants contain RefSeq accessions in the National Center for Biotechnology Information (NCBI) database. Of the probe sets present on the gene microarray (U133A GeneChip; Affymetrix), only six actually target known variants of the human fibronectin gene: Five detect splice variants 1 to 6 (probes 210495_s_at, 211719_s_at, 212464_s_at, 214701_s_at, and 216442_x_at), one targets splice variant 7 (214702_x_at). Thus evaluation of the effects of TGFβ2 on TM fibronectin mRNA expression is complicated by the potential inability of the probe sets to detect other variants that may also be upregulated in these cells. Using a gene chip (U133A GeneChip; Affymetrix), Zhao et al. also failed to detect a more than twofold change in fibronectin mRNA expression by TGFβ2-treated human TM cells.

In contrast to gene expression results, our ELISA studies clearly showed the ability of TGFβ2 to enhance TM cell fibronectin protein expression, and we further demonstrated that the effect of TGFβ2 was mediated via type I TGFβ receptors, using the inhibitors SB431542 and [3-(pyridin-2-yl)-4-(4-quinonyl)-]1H-pyrazole. The effect on either fibronectin or PAI-1 levels did not appear to be isoform specific, in that three different isoforms of TGFβ (-1, -2, and -3) gave nearly equivalent results when evaluated under our test conditions. The TGFβ2 isoform, however, is increased in the AH of POAG eyes when compared with AH collected from non-POAG patients. Thus, we chose to focus the bulk of our experiment on the effects of the TGFβ2 isoform. In particular, we further directed our studies toward the effect of TGFβ2 on perfused human anterior segments in organ culture.

Human eyes perfused continuously with TGFβ2 developed decreased outflow facility and an increased accumulation of ECM materials. In our study, we also found that continuous perfusion of ex vivo human anterior segments with TGFβ2 produced a time-dependent elevation of IOP, although our overall change in outflow facility (a 45% decrease at 4 days) was greater than that reported by Gottanka et al. Furthermore, consistent with our results showing the stimulatory effect of TGFβ2 on the fibronectin level in human TM cell supernatants, we found that TGFβ2 time-dependently increased the fibronectin level in the perfusate from treated human ocular anterior segments. In addition, we discovered a dramatic time-dependent increase in perfusate PAI-1 content in response to TGFβ2 treatment. Of interest, PAI-1 levels have also been shown to be increased in aqueous humor from patients with glaucoma, compared with patients without glaucoma.

Fuchsofer et al. have reported a time-dependent increase in the expression of PAI-1 mRNA transcripts from HTM cell cultures incubated with 1 ng/mL TGFβ2; PAI-1 protein levels were also increased in the supernatants of treated cells. We have confirmed their results, using both transformed and nontransformed human TM cultures. In our hands, a 16-hour treatment of nontransformed GTM cells with 5 ng/mL TGFβ2 elicited a nearly sevenfold increase in PAI-1 gene expression. Furthermore, we also verified that TGFβ2 treatment induced significant increases in supernatant levels of PAI-1 from HTM cell lines and demonstrated that such increases were dose-dependent. Similar to results seen for fibronectin, the TGFβ2 effect appeared to be mediated through a type I TGFβ receptor, based on the ability of ALK5 inhibitors to antagonize PAI-1 release from treated cells.

The increase in PAI-1 may play an accessory role in the observed TGFβ2-mediated accumulation of fibronectin. PAI-1 inhibits the activity of both tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). Both tPA and uPA catalyze the conversion of plasminogen into plasmin, a key intermediate in the fibrinolytic cascade. Plasmin, in addition, is known to promote the conversion of certain pro matrix metalloproteinases (MMPs) into their active, ECM-degrading forms. Therefore, an increase in PAI-1 levels would be expected to lead to a reduction of activated MMP levels and, conceivably, accumulation of pro-MMPs within responsive tissues.

We, and others, have previously shown that human TM cells express significant levels of MMPs, such as stromelysin-I (MMP-3) on both a constitutive and stimulated level. Furthermore, exposure to TGFβ2 leads to enhanced accumulation of the pro form of MMP-3 by human TM cell cultures (Fleenor DL, unpublished observations, 2000). This finding appears to be consistent with the current results. The TGFβ2-mediated increase in PAI-1 probably provokes decreased conversion of pro MMP-3 to active MMP-3. A decrease in active MMP-3, therefore, would result in decreased proteolytic degradation and increased accumulation of ECM constituents, such as fibronectin, a known MMP-3 substrate. Indeed, in this study, exposure to rhPAI-1 led to significantly increased fibronectin content in treated HTM cell supernatants.

Elevated levels of AH TGFβ2 thus may have the dual effect of both a direct increase in TM production of ECM components (e.g., fibronectin) and an enhanced production of gene products inhibiting ECM degradation (e.g., PAI-1). However, in addition to potential effects on ECM accumulation, elevated PAI-1 levels may have other consequences that also contribute to the pathogenesis of glaucoma. It has been reported previously that a progressive loss of human TM cells occurs with normal aging. The rate of this loss appears to be accelerated in POAG eyes. The underlying mechanism(s) for this loss is not yet known although, in addition to a decrease in replicative potential, it has been attributed to a variety of factors that affect TM cell migration and/or adhesion, including increased phagocytosis, oxidative stress, myocilin, and the presence of chemotactants within the aqueous humor.

Our data may point to yet another explanation for the observed decline in meshwork cellularity. Incubation of cultured TM cells with TGFβ2 produces an overall decrease in the number of cells, and TGFβ2 also significantly inhibits growth factor-induced TM cell proliferation. In addition, elevated PAI-1 levels have been shown to be linked to both decreased adhesion and increased detachment of a variety of cell types. Such activities are apparently not mediated by the classic protease inhibitor activity associated with PAI-1, but rather with the ability of PAI-1 to modulate the association of factors such as vitronectin and urokinase-like plasminogen activator with adhesion receptors. It is thus tempting to speculate that PAI-1 triggers similar actions in the meshwork, thereby contributing to TM cell loss.

In conclusion, we have demonstrated that cultured human TM cells expressed and secreted both fibronectin and PAI-1 in response to stimulation with the cytokine TGFβ2, as well as to the isoforms TGFβ1 and -β3. The stimulatory effect was reproducible and quantifiable in all lines tested, regardless of donor age or history of POAG. These results are in good agreement with other published reports. Furthermore, despite the interstrain differences in basal levels, each of the various cell strains responded consistently to TGFβ2 with increased fibronectin and PAI-1. Finally, we also used a perfusion organ culture model to demonstrate that perfused human anterior segments respond to TGFβ2 with concomitant increases in IOP and fibronectin and PAI-1 secretion. It therefore seems reasonable...
to postulate that TGFβ2 effects on IOP may be transduced by changes in TM secretion of ECM-related factors such as fibronectin and PAI-1. Modulation of such TGFβ2-induced changes in the ECM may thus provide a novel and viable approach to the management of glaucoma.

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