Connective Tissue Growth Factor Is Increased in Pseudoexfoliation Glaucoma

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PURPOSE. Pseudoexfoliation (PXF) syndrome is a generalized disorder of the extracellular matrix (ECM) involving the trabecular meshwork (TM), associated with raised intraocular pressure, glaucoma, and cataract. The purposes of this study were to quantify aqueous humor connective tissue growth factor (CTGF) in PXF glaucoma, to determine the effect of CTGF on ECM production in TM cells, and to identify intracellular CTGF signaling pathways.

METHODS. Aqueous humor samples were obtained from patients undergoing routine cataract surgery or trabeculectomy. CTGF levels were quantified by ELISA. The effect of CTGF on fibrillin-1 expression in TM cells was investigated by real-time PCR. Western immunoblot analysis was used to investigate CTGF signaling. c-Jun/AP-1 activation was measured in CHO cells by ELISA after stimulation with CTGF.

RESULTS. PXF with glaucoma had the highest aqueous humor level of CTGF ($n = 18$; $5.15 \pm 0.79$ ng/mL [SEM]; $P < 0.01$) compared with PXF without glaucoma ($n = 15$; $2.76 \pm 0.64$ ng/mL), primary open-angle glaucoma (POAG; $n = 20$; $3.05 \pm 0.40$ ng/mL), and the control ($n = 21$; $2.60 \pm 0.29$ ng/mL). In vitro exposure of TM cells to CTGF resulted in a 50% upregulation of fibrillin-1, which was partially blocked with the MEK (mitogen-activated protein extracellular kinase) inhibitor PD98059. Western blot analysis demonstrated increased phosphorylation of p42/44 MAPK, p38 MAPK, and JNK pathways in response to CTGF. c-Jun/AP-1 activity was significantly increased in response to CTGF treatment.

CONCLUSIONS. Increased levels of CTGF in the aqueous humor of PXF patients likely has pathologic significance through increased production of fibrillin-1 by TM cells through activation of p42/44 MAPK, p38 MAPK, and JNK pathways. (Invest Ophthalmol Vis Sci. 2011;52:3660–3666) DOI:10.1167/iovs.10-5209

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Pseudoexfoliation syndrome (PXF) is an age-related disorder that manifests with abnormal fibrillar extracellular material (ECM) accumulation in ocular tissues. Fibrellar material similar to that in the eyes of PXF patients has more recently been detected in the skin and visceral organs of patients with PXF.2 In the eye, PXF is detected by pupil dilation and subsequent slit lamp examination. Deposits of fibrillar material are observed in the anterior segment, primarily on the pupillary border and anterior lens capsule. The disease usually has an insidious onset, unless complications occur such as cataract and glaucoma. PXF is reported to be responsible for more than half of the cases of open-angle glaucoma in Norway, Ireland, Greece, and Saudi Arabia.3 Patients with PXF have a 5.3% chance of developing glaucoma within 5 years, increasing to 15.4% within 10 years.4 PXF-associated secondary open-angle glaucoma is a relatively severe and progressive type of glaucoma with a worse prognosis and more extensive complications with surgery.5 Many factors contribute to the pathologic features of open-angle glaucoma in PXF, including obstruction of the trabecular meshwork (TM) area by the deposition of pseudoexfoliative material, juxtaocular endothelial cell dysfunction, and increased aqueous humor protein levels.6 The protein components of pseudoexfoliative material include both noncollagenous basement membrane components such as laminin, fibronectin,7 amyloid P, and vitronectin. Also contained in the pseudoexfoliative material are proteinaceous components of elastic fibers, such as elastin, tropoelastin, fibrillin-1, microfibril-associated glycoprotein-1, and latent TGF-β-binding proteins (LTBP-1 and -2).8,9 The elastic microfibril theory of PXF pathogenesis hypothesizes that PXF is a type of elastosis characterized by excessive synthesis of elastic microfibrillar components throughout the body. This theory was supported by molecular biological studies confirming the overexpression of fibrillin-1, LTBP-1, and LTBP-2 mRNA in most of the affected tissues and cell types.8,10 In 1991 Bradham et al.11 discovered human connective tissue growth factor (CTGF/CCN2) as a protein secreted by human umbilical vascular endothelial cells. CTGF is a member of a family of proteins with a similar structure known as the CCN family.12 Members of the CCN family have four distinct modules in their structure. These modules are an insulin-like growth factor (IGF)-binding protein–like module (IGF-BP), von Willebrand factor type C repeat (VWC), thrombospondin type-1 repeat (TSP1), and C-terminal module (CT). CTGF is a 36- to 38-kDa protein rich in cysteine13 and is expressed in a variety of cell types, including fibroblasts, vascular smooth muscle cells, endothelial cells, neuronal cells, and epithelial cells.14 CTGF and transforming growth factor (TGF)-β interact via a binding site located in the amino terminal von Willebrand factor domain of CTGF.15 CTGF expression is induced by TGFβ, and CTGF mediates some of the downstream effects of TGFβ on proliferation, migration, and ECM production.16,17 Pathologic fibrosis is often attributed to uncontrolled matrix deposition, perhaps mediated by CTGF.18


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CTGF was first identified as a profibrotic mediator that was upregulated in both in vivo and in vitro models of diabetic nephropathy, with a suppression subtractive hybridization (SSH) screen and characterized the mechanism through which elevated CTGF expression leads to matrix accumulation (for review, see Ref. 21). Current models of activity propose that CTGF largely functions as a matricellular protein, modulating and integrating the role of other growth factor signaling pathways, including those of the TGFβ superfamily. Signaling and regulatory insights have remained elusive with the observation that CTGF can bind to multiple receptors on the cell surface and activate divergent signaling pathways, including p42/p44 MAPK, PI3K, and p38 MAPK. Functional interplay of these signaling networks is proposed as the key mechanism controlling CTGF-mediated gene transcription.

The three isoforms of TGFβ, β1, β2, and β3 are found in the eye. TGFβ has been shown in numerous studies to play a role in ocular wound healing. TGFβ and the role in the pathogenesis of glaucoma is also well documented. Several studies have reported elevated aqueous humor levels of TGFβ2 in patients with POAG. On the other hand, the aqueous humor of patients with PXF glaucoma was found to have elevated levels of TGFβ1. In their study, Schlotzer-Schrehardt et al. showed that TGFβ1, in both the latent and intrinsically active forms, was significantly raised in PXF glaucoma eyes compared with POAG eyes. In contrast, both the latent and active forms of TGFβ2 were elevated in POAG eyes but not in PXF glaucomatous eyes. TGFβ1 had a more pronounced effect on ECM production on cultured human Tenon’s capsule from PXF subjects, whereas TGFβ2 was more effective in promoting cell migration and collagen contraction in cultures originating from POAG patients.

In a previous study, our group found that the CTGF level in aqueous humor of patients with PXF with glaucoma was significantly higher than in normal control subjects undergoing cataract surgery or patients with PXF without glaucoma, raising the possibility that CTGF plays a pathogenic role. We wanted to expand this work to include a group of POAG patients for comparison with a PXF glaucoma group. Second, we wanted to study the molecular effects of CTGF stimulation of TM cells, looking in particular at expression of ECM components (fibrillin-1) and the intracellular signaling pathways affected by CTGF in TM cells.

Materials and Methods

Patient Samples

Patients with the following conditions were recruited for the study: PXF (mean age, 78.5 ± 5.6 [SD] years), PXF with glaucoma (mean age, 79.5 ± 6.3 years), POAG (mean age, 73.6 ± 8.8), and cataract (mean age, 73.6 ± 10.9; control group). After informed consent was obtained, aqueous humor was collected from patients who were undergoing routine cataract or trabeculectomy surgery. This study was approved by the Research Ethics Committee of the Mater Misericordiae Hospital and adhered to the tenets of the Declaration of Helsinki. The removal of aqueous humor was performed via a clear corneal paracentesis before the addition of the CTGF. PD098059 is a specific p42/p44 kinase inhibitor at concentration of 25 ng/mL for the times indicated in the relevant figures. Cells were serum starved 24 hours before treatment. CTGF was used at a concentration of 2 ng/mL. The assay’s minimum detectable concentration was determined to be 2 ng/mL.

Cell Culture

Human TM cells were isolated from carefully dissected TM tissue explants derived from nonglaucomatous (NTM) donors, as described elsewhere, and cultured in DMEM (Invitrogen) supplemented with 2 mM l-glutamine, sodium pyruvate, 10% fetal bovine serum, 50 U/mL penicillin, and 50 µg/mL streptomycin (passages 6–9). The three donors were a 77-year-old Caucasian man with no history of glaucoma, a 92-year-old Caucasian woman with no history of glaucoma, and an 87-year-old Caucasian man with no history of glaucoma. Cells were provided at passage 6 and were generally used up to passage 9 only. The culture medium was replenished every 3 days. The cells were cultured for 24 hours before treatment. CTGF was used at a concentration of 25 ng/mL for the times indicated in the relevant figures. Cells were also preincubated in 10 ng/mL of PD098059 for 30 minutes before the addition of the CTGF. PD098059 is a specific p42/p44 kinase inhibitor.
MAPK pathway inhibitor that binds to inactive MEK and prevents its phosphorylation and activation by Ras.

**Western Blot Analysis**

TM cells were serum starved for 24 hours and exposed to various agents as indicated. Lysates were harvested in RIPA lysis buffer (20 mM Tris-HCl [pH 7.4], 50 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 5 mM NaF, 1 mM PMFS, 1 mM Na3VO4, 1 μM leupeptin, and 0.5 μM aprotinin). The lysates were centrifuged at 14,000 rpm for 20 minutes and the supernatants were normalized for total protein. Protein concentration in the cell lysates was determined with a Bradford Assay (Bio-Rad, Hemel Hempstead, UK). For Western blot analysis, 10 μg of TM protein extract was loaded onto each lane and separated by SDS-PAGE. The proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Watford, UK), blocked with 5% (wt/vol) nonfat dried milk in TBST for 1 hour at room temperature, and probed overnight at 4°C with antibodies raised against β-actin (1:10,000;Sigma-Aldrich), phospho p42/44 (1:1,000), total p42/44 (1:1,000), phospho p38 (1:1,000), total p38 (1:1,000), and phospho JNK (1:1,000) all Cell Signaling Technology, Danvers, MA). Membranes were incubated with HRP-conjugated secondary Abs (1:2000) for 1 hour at room temperature, and proteins were visualized by chemiluminescence. Unless otherwise stated, the antibodies used were supplied by Cell Signaling Technology. Images of developed photographic film were acquired with a CCD camera and captured with Image-acquisition and-analysis software (Visionworks LS; UVP, Upland, CA). Densitometric analysis of band intensity was performed (Image, ver. 4.0; Scion Corp., Frederick MD).

**Quantitative RT-PCR**

A real-time PCR genetic analysis assay (TaqMan; Applied Biosystems, Inc. [ABI], Foster City, CA) was used to quantify the relative gene expression levels of fibrillin-1. The primers and probe for fibrillin-1 (product number Hs00171191_ml) were supplied as a preoptimized single tube primer/probe (Gene Expression Assay; ABI). Probe design is based on Homo sapiens FBN1 sequences NM_000138.3. The probes for the target genes were labeled with the fluorescent dye, FAM (amine-reactive succinimidyl ester of carboxyfluorescein) on the 5′ end and a nonfluorescent quencher on the 3′ end. PCR reactions were set up with master mix (TaqMan Universal PCR Master Mix; ABI). cDNA was amplified on a sequence-detection system (7900HT; ABI) at default thermal cycling conditions: 2 minutes at 50°C, 10 minutes at 95°C for enzyme activation, and then 40 cycles of 15 seconds at 95°C for denaturation and 1 minute at 60°C for annealing and extension. The results were analyzed using the relative standard curve/delta Ct method of analysis.

**c-Jun/AP-1 Transcription Factor ELISA**

The c-Jun/AP-1 activation assay from Active Motif (cat. no. 46096; Carlsbad, CA) was performed according to the manufacturer’s instructions, with Chinese hamster ovary (CHO) cells, as these provide a readily available model for cells of a fibroblastic phenotype, and TM cells are not readily transfectable. Briefly, oligonucleotides corresponding to the consensus binding site specific for the transcription factor(s) of interest were coated on a 96-well plate, and nucleic extracts from CTGF and/or control treated cells were added to the wells. Activated transcription factors bind the oligonucleotide sequence at its consensus site and are then quantified by ELISA; a primary antibody specific for an epitope on the bound and active form of the transcription factor is then added followed by subsequent incubation with secondary antibody and developing solution. Absorbance was then read on a spectrophotometer (Synergy HT; BioTek, Winooski, VT) within 5 minutes at 450 nm, with a reference wavelength of 655 nm.

**Statistics**

Data of the immunoassays are expressed as the mean ± SEM. The group means were compared and analyzed by using Fisher’s protected least-significant difference (PLSD) test and the Bonferroni/Dunn test, after one-way analysis of variance (ANOVA) identified statistically significant differences (StatView; SAS Institute, Cary, NC). P < 0.05 was considered to be statistically significant for Fisher’s PLSD test, and likewise P < 0.01 for the Bonferroni/Dunn test. Data for the c-Jun/AP-1 activity assay were expressed as the normalized change ratio ± SEM. The groups were compared using a Student’s t-test. P < 0.05 was considered to be significant.

**Results**

**Levels of CTGF in the Aqueous of Patients with PXF Glaucoma**

The PXF-with-glaucoma group had the highest aqueous humor mean CTGF level (5.15 ± 0.79 [SEM] ng/mL), and the result was statistically significant (Fig. 1, P < 0.01; Table 1), when compared with PXF without glaucoma (2.76 ± 0.64 ng/mL), POAG (3.05 ± 0.40 ng/mL), and controls (2.60 ± 0.29 ng/mL). There was no significant correlation between CTGF levels and age or sex within the study group, combining PXF, PXF glaucoma, and POAG patients (Table 1).

**Effect of CTGF on ECM Expression in TM Cells**

Human primary TM cells of passages 6 to 9 were growth arrested in serum-free medium before the start of any experiment, and all exposures to CTGF were performed under serum-free conditions. The cells were exposed to 25 ng/mL CTGF for 6, 24, and 48 hours. RNA was extracted and cDNA was obtained by reverse transcription. Real-time PCR (TaqMan; ABI) was performed to examine the effect of CTGF on the expression of the ECM component fibrillin-1. CTGF stimulated approximately a 1.5-fold increase in fibrillin-1 mRNA as early as 6 hours after treatment (Fig. 2, P < 0.05, Student’s t-test). This increase was sustained at 24 hours, but had returned to basal by 48 hours. Results are representative of those in three separate experiments.

**Figure 1.** CTGF levels in aqueous humor (ELISA). The PXF with glaucoma group had the highest aqueous humor mean CTGF level of 5.15 ± 0.79 (SEM) ng/mL and this is statistically significant (P < 0.01) when compared with PXF without glaucoma (2.76 ± 0.64 ng/mL), POAG (3.05 ± 0.40 ng/mL), and controls (2.60 ± 0.29 ng/mL). The group means were compared and analyzed using Fisher’s (PLSD) test and Bonferroni/Dunn test after one-way analysis of variance (ANOVA) identified statistically significant differences.
TABLE 1. Patient Characteristics and Mean CTGF Levels

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients* n (%)</th>
<th>Mean CTGF (ng/mL)</th>
<th>P</th>
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<tbody>
<tr>
<td>Mean age, y</td>
<td>76 ± 8.04</td>
<td>3.38</td>
<td>0.98</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47.3 (35)</td>
<td>3.77</td>
<td>0.20</td>
</tr>
<tr>
<td>Female</td>
<td>52.7 (39)</td>
<td>3.03</td>
<td></td>
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<tr>
<td>PXF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With glaucoma</td>
<td>24.4 (18)</td>
<td>5.15</td>
<td>0.002</td>
</tr>
<tr>
<td>Without glaucoma</td>
<td>20.2 (15)</td>
<td>2.76</td>
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* n = 74.
† Univariate ANOVA.

FIGURE 2. Relative quantitation of fibrillin-1 by real-time PCR after CTGF treatment. TM cells were treated with CTGF (25 ng/mL) after 24 hours of serum starvation. Real-time PCR was performed to investigate changes in the levels of fibrillin-1 mRNA in TM cells treated with CTGF (25 ng/mL). P < 0.05, Student’s t-test; n = 5.

FIGURE 3. CTGF stimulates the expression of fibrillin-1 and the activation of p42/44 MAPK, p58 MAPK and c-Jun-N-terminal kinase pathways. (A) Real-time PCR for fibrillin-1 after CTGF treatment and addition of PD098059. Real-time PCR was performed to investigate changes in the levels of fibrillin-1 mRNA in TM cells treated with CTGF (25 ng/mL). Preincubation for 30 minutes with PD098059 (10 ng/mL) was used to investigate the role played by MAP kinase signaling in CTGF-driven changes in fibrillin-1 mRNA levels (P < 0.05, Student’s t-test; n = 3). (B) Western blot analysis of p42/44 MAPK, p58 MAPK, and JNK pathways. TM cells were treated with CTGF for up to 3 hours, and the phosphorylation status of p42/44 MAPK, p58 MAPK, and JNK determined by Western blot. Results are representative of three independent experiments. (C) Densitometric analysis of Western blots. Images were acquired using a CCD camera and captured with image-acquisition and -analysis software. Densitometric analysis of band intensity was then performed. Results are shown as arbitrary units ± SE. n = 3, *P < 0.05, **P < 0.01, ***P < 0.001, †P < 0.05, ††P < 0.01, †††P < 0.001, Student’s t-test.

Role of p42/44 MAPK in CTGF Induction of Fibrillin-1 in Human TM Cells

Having demonstrated that treatment of TM cells with CTGF resulted in upregulation of fibrillin-1, we wished to determine which signaling pathways are involved. Preincubation for 30 minutes with PD098059 (10 ng/mL) was used to investigate the role played by MAPK signaling in the CTGF induction of fibrillin-1 mRNA levels (Fig. 3A). A statistically significant downregulation of fibrillin-1 mRNA levels was observed after pretreatment with PD098059 (P < 0.05, Student’s t-test). The effect of CTGF treatment on the intracellular-mediated p42/44 MAPK pathway in TM cells was determined with phospho-specific antibodies. Lysates taken from TM cells exposed to CTGF (25 ng/mL for 0, 10, 30, and 180 minutes) resulted in time-dependent effects on p42/44 MAPK phosphorylation. The results showed a transient increase in phosphorylation of p42/44 MAPK, reaching a maximum at 10 minutes and declining to below the background levels within 180 minutes. Representative Western blot and densitometric analysis of phosphorylation status (phosphorylated/total) are shown in Figures 3B and 3C. Treatment of the TM cells with CTGF (25 ng/mL for 0, 10, 30, and 180 minutes) did not substantially alter total cellular content of p42/44 MAPK.

The Effect of CTGF on the p38 Signaling Pathway

The JNK and p38 pathways comprise other components of the MAPK cascade. The effect of CTGF treatment on the JNK and p38 pathways in TM cells was determined using phospho-specific antibodies. Lysates taken from TM cells exposed to CTGF (25 ng/mL for 0, 10, 30, and 180 minutes) resulted in time-dependent increases in p38 and JNK phosphorylation. There were increases in phosphorylation of both p38 and JNK, reaching a maximum at 30 minutes and declining thereafter, whereas levels of total p38 MAP and JNK remained essentially unchanged. Representative Western blot and densitometric analysis of phosphorylation status (phosphorylated/total) are shown in Figures 3B and 3C.
The Effect of CTGF on Activity of c-Jun/AP-1

To study the role of CTGF on the parallel activity of the JNK target c-Jun/AP-1, CHO cells were treated with CTGF (25 ng/mL) for 10, 30, and 60 minutes. CTGF stimulated a 1.38-fold increase at 10 minutes after treatment (P < 0.05 vs. 10-minute control, Student's t-test; Fig. 4) indicating a marked increase in transcription from the c-Jun/AP-1 promoter. These results suggest that phosphorylation of the JNK pathway downstream of the MAPK signaling cascade forms the basis of a potential intracellular signaling response to CTGF.

DISCUSSION

Previous studies have examined the aqueous humor CTGF levels in glaucoma patients compared with those in nonglaucomatous individuals.33 The levels of CTGF were higher in PXF than in the controls, and the increase in CTGF was related to the severity of the disease. The amount of PXF material in the juxtacanalicular tissue and the outflow filtration area correlated with intraocular pressure and optic nerve damage,40 suggesting that increased levels of CTGF are pathologically significant.

CTGF is a potent inducer of ECM protein expression, including fibrillar and basement membrane collagens.41 There appears to be a direct correlation between increased expression of CTGF and excessive accumulation and deposition of type-1 collagen in areas of fibrosis in diseased tissue from human and animal specimens and animal models of fibrosis.42 Fibrillin-containing fibrils increase in the extracellular component in PXF,8 and fibrillin is a component of the elastic microfibrils of the pseudoexfoliation material (PXM). Therefore, CTGF may increase deposition of the elastic microfibrillar components in PXM, resulting in an excessive ECM accumulation.

To elucidate the role of CTGF in the fibrotic process in PXF in vitro, we introduced rhCTGF into cultured primary human TM cells, and the induction stimulated an increase in the mRNA levels of fibrillin-1. Previous studies have shown increased extracellular deposition of fibrillin-containing fibrils in PXF, suggesting that enhanced expression of fibrillin or abnormal aggregation of fibrillin-containing microfibrils is involved in the pathogenesis of PXF.8

Previous work has demonstrated that CTGF can bind β3-integrin and activate signaling cascades that ultimately result in the upregulation of expression of extracellular matrix components.20,15 In our study, rhCTGF activated the MAPK cascade in human TM cells, as evidenced by increased phosphorylation of the p42/44 MAPK. The consequences of this increase are not clear; however, it is likely that the mechanism through which CTGF results in increased fibrillin expression in TM cells is similar to that of other cell types. The CTGF-induced phosphorylation of p42/44 MAPK was blocked by pretreating the cells with the MEK inhibitor PD098059. Signaling pathway cross talk is a common feature of matricellular growth and adhesion factors, including the CNN family, of which CTGF is the most prominent member.20 Previous work in our laboratory suggested transactivation of the stress-activated protein kinase cascade (SAPK) by CTGF signaling through the MAPK cascade.52 We investigated the phosphorylation status of the SAPK effector, c-Jun NH(2)-terminal kinase (JNKs), which phosphorylates and activates the ATP transcription factor and other cellular factors implicated in regulating altered gene expression. CTGF stimulated both phosphorylation of JNK and the activity of c-Jun/AP-1.

The sequence of events on binding of CTGF to multiple cell surface receptors remains somewhat controversial. Clearly, the activation and interplay of signaling pathways and networks ultimately has consequences for cell behavior and transcriptional activity. The timecourse of signaling events suggests a transient activation of pathways, perhaps reflecting the relatively short half-life of CTGF. Studies performed by our group and others have shown that much higher concentrations of CTGF lead to prolonged ERK signaling but ultimately have no bearing on outcomes such as cell migration and cell adhesion or, indeed, ECM production.70,22,24,44 The apparent increase in c-Jun/AP-1 activity after 10 minutes of treatment with CTGF reflects the view that transcription factor activation is regulated by these cascades, but artifically suggests that gene expression would be activated at these early time points, when the experimental reality is that increased fibrillin expression was only significantly increased at 6 hours. The addition of the MEK inhibitor PD098059 reduced fibrillin levels to a nonsignificant ratio to basal at all time points and was significantly different from CTGF treated only at 24 hours when induction of fibrillin-1 was at maximum. Many transcription factors control multiple genes, which are often expressed at different time points and levels, differing in induction threshold and expression capacity.

The key interaction between transcription factors and DNA is dependent on both DNA sequence and a change in the accessibility of the DNA. It is likely that these nucleosome-mediated alterations in DNA are necessary for transcription binding, and hence, availability of the active transcription factor itself is not sufficient for activation of gene expression. We currently do not clearly understand how interaction between transcription factors and promoters translates into quantitative gene expression profiles.15

Our previous findings suggested that CTGF contributes to ECM regulation in fibrotic diseases via interaction with integrins and subsequent recruitment and activation of Src kinase.20 We further characterized the divergent early signaling events including activation of p42/44 MAPK, PI3Kinase, and p38 MAPK.24 and proposed that the functional interplay of these networks is key to the regulation of CTGF-mediated gene transcription. These in vitro studies support this hypothesis by indicating activation of p42/44 MAPK, p38 MAPK, and JNK by CTGF in TM cells. Moreover, increased expression of fibrillin-1 in response to CTGF could be partially abrogated with the MEK inhibitor PD098059, suggesting a crucial role for p42/44 MAPK. Results of a recent study from the laboratory of Kolch (von Kriegsheim et al.40) propose a key role for growth factor-mediated ERK signaling in the determination of cell fate decisions, such as migration and matrix production. In these circumstances, the consequences of ERK activation in TM cells, because of increased levels of CTGF are likely to be profound.
In conclusion, we show raised CTGF concentrations in the aqueous of PXF glaucoma patients compared with POAG, PXF without glaucoma, and normal cataracts. This finding raises the intriguing possibility that stresses associated with increased intraocular pressure in glaucoma in combination with PXF result in altered pathophysiology. Given the established role for CTGF as a modulator of matrix production in many fibrotic diseases, we propose a pathophysiological significance for increased CTGF levels in PXF glaucoma. We further propose that activation of p14/44, p38 MAPK, and JNK pathways in TM cells leads to increased fibrillin-1 expression and that anti-CTGF therapies may prove beneficial in treating patients with PXF glaucoma.

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