Role of TGFβ/Smad Signaling in Gremlin Induction of Human Trabecular Meshwork Extracellular Matrix Proteins

Anirudh Sethi, Ankur Jain, Gulab S. Zode, Robert J. Wordinger, and Abbot F. Clark

PURPOSE. The bone morphogenetic protein (BMP) antagonist gremlin is elevated in glaucomatous trabecular meshwork (TM) cells and tissues and elevates intraocular pressure (IOP). Gremlin also blocks BMP4 inhibition of transforming growth factor (TGF)-β2 induction of TM extracellular matrix (ECM) proteins. The purpose of this study was to determine whether Gremlin regulates ECM proteins in cultured human TM cells.

METHODS. Human TM cells were treated with recombinant gremlin to determine the effects on ECM gene and protein expression. Expression of the ECM genes FN, COL1, PAI1, and ELN was examined in cultured human TM cells by quantitative RT-PCR and Western immunoblot analysis. TM cells were pretreated with TGFβR inhibitors (LY564947, SB431542 or TGFβR1/TGFβ2 siRNAs), inhibitors of the Smad signaling pathway (SIS3 or Smad2/3/4 siRNAs), or CTGF siRNA to identify the signaling pathway(s) involved in gremlin induction of ECM gene and protein expression.

RESULTS. All ECM genes analyzed (FN, COL1, PAI1, and ELN) were induced by gremlin. This gremlin induction of ECM genes and protein expression was blocked by inhibitors of TGFβR and the canonical Smad2/3/4 and CTGF-signaling pathways.

CONCLUSIONS. Gremlin employs canonical TGFβ2/Smad signaling to induce ECM genes and proteins in cultured human TM cells. Gremlin also induces both TGFβ2 and CTGF, which can act downstream to mediate some of these ECM changes in TM cells. (Invest Ophthalmol Vis Sci. 2011;52:5251-5259) DOI: 10.1167/iovs.11-7587

Glaucoma is a leading cause of irreversible visual impairment and blindness in the world, with primary open-angle glaucoma (POAG) being the major form of glaucoma. Elevated intraocular pressure (IOP) is a major risk factor for the development and progression of glaucoma. Ocular hypertension is due to increased aqueous humor outflow resistance in the trabecular meshwork (TM) and is associated with increased deposition of extracellular matrix (ECM) material within the TM. Transforming growth factor (TGF)-β2 levels are elevated in the aqueous humor and TM. The bone morphogenetic protein (BMP) antagonist gremlin may potentiate the profibrotic effects of TGFβ2 by blocking the BMP4 regulation of TGFβ2 activity. However, whether gremlin alone can induce fibrosis-like activities in cultured TM cells is currently unknown, and the potential signaling mechanisms involved have not been characterized. The purpose of the present study was to determine (1) whether gremlin induces ECM genes and proteins in cultured TM cells and (2) what signaling mechanisms are involved in gremlin induction of ECM genes and proteins.

METHODS

TM Cell Culture

Human TM cells were isolated from carefully dissected human TM tissue explants derived from patients with glaucoma or from normal donors and characterized as previously described. All donor tissues were obtained from regional eye banks and managed according to the guidelines in the Declaration of Helsinki for research involving human tissue. Isolated TM cells were cultured in Dulbecco’s modified Eagle medium with 10% fetal bovine serum.
Eagle’s medium (DMEM) containing 1-glutamine (0.292 mg/mL; Invitrogen Gibco), penicillin (100 units/mL)/streptomycin (0.1 mg/mL; Invitrogen Gibco), and 10% fetal bovine serum (all from Invitrogen Gibco, Grand Island, NY).

**TM Cell Treatments**

TM cells were grown to 100% confluence and then kept in serum-free medium for 24 hours before treatments, to avoid the effect of serum proteins. TM cells were incubated with fresh medium containing specific signaling inhibitors for 1 to 6 hours before the addition of various concentrations of recombinant gremlin protein (R&D System, Minneapolis, MN). The small molecule inhibitors LY364947 (5 μM; Tocris Biosciences, Ellisville, MO) and SB431542 (5 μM; Sigma-Aldrich, St. Louis, MO) were used to examine the effects of inhibition of TGFβ receptor-1/2. The Smad3 phosphorylation inhibitor SIS3 (10 μM; Sigma-Aldrich), the JNK inhibitor SP600125 (10 μM; Sigma-Aldrich), and the P-38 inhibitor SB203580 (5 μM; Tocris Biosciences) were used to examine the effects of inhibition on canonical Smad, JNK, and P-38 signaling pathways.

**Small Interfering RNA and Transfection**

siRNAs for Smad2, -3, and -4; TGFBR1; TGFBR2; and CTGF as well as nontargeting control siRNAs (SMARTpool) were purchased from Dharmacon (Lafayette, CO). siRNA transfection was performed as described previously.26–27 Three different TM cell strains were grown in 12-plate wells containing DMEM with 10% FBS. In one tube, 4 μL of transfection reagent (DharmaFECT 1; Dharmacon) was mixed gently with 200 μL of reduced-serum medium (Opti-MEM Invitrogen, Carlsbad, CA) and incubated for 5 minutes at room temperature. In separate tubes, siRNAs were mixed gently with 200 μL of the serum-reduced medium. These two tubes were combined, gently mixed, and incubated for 20 minutes at room temperature. After incubation, DMEM without FBS and antibiotics was added to obtain a final volume of 2 mL for each well (10 nM of test and control siRNAs). The cells were washed with sterile PBS, incubated with siRNA transfection solution for 24 hours at 37°C, washed with sterile PBS, and incubated with 10% FBS containing DMEM for 24 hours at 37°C. They were then washed with serum-free DMEM for 24 hours and treated with TGFβ2 in serum-free DMEM for an additional 48 hours. Cell lysates and conditioned medium were analyzed for various proteins by Western blot analysis (see Table 1 for list of the antibodies used).

**Immunoblot Studies**

Total cellular RNA was extracted from cultured TM cells (TRI Reagent RT extraction; MRC Inc., Cincinnati, OH), and a cDNA synthesis kit (Superscript VILO; Invitrogen) was used for first-strand cDNA synthesis. Primers for the various LOX genes were designed by using Primer3 software (http://frodo.wi.mit.edu/primer3 Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA). The primer pairs are listed in Table 2.

**Quantitative Real-Time PCR**

Real-time PCR was performed as described previously.19 Briefly, 2.5 μL of cDNA was used in a reaction consisting of 1.5 units per reaction of antibody-bond Tag enzyme (Jump Start; Sigma-Aldrich), 10× PCR buffer, 1.5 mM MgCl2, 200 nM dNTP mix, 100 nM PCR primers (Table 2), 2.5 μL green nucleic acid dye (EvaGreen; Biotium, Hayward, CA), as well as 30 nM passive reference dye (Rox; USB, Cleveland, OH) per 50-μL reaction. PCR was performed on a real-time thermal cycler (model Mx3000p; Stratagene, La Jolla, CA), with cycling parameters of initial denaturation at 95°C; 40 cycles of 95°C 30 seconds, 60°C 30 seconds, and 72°C 60 seconds, and a denaturation cycle for creation of a dissociation curves. Reactions for each sample were run in duplicate, cycle thresholds (Ct) were normalized to either β-actin or GAPDH expression as housekeeping genes, and comparative quantitation was performed (MxPro ver. 4.0 software, Stratagene). Only individual PCR samples with single-peak dissociation curves were selected for data analysis.

**Protein Extraction and Western Blot Analysis**

**Secreted Proteins.** ECM proteins secreted by TM cells were determined by Western immunoblot analysis. Conditioned medium was collected from human TM cells after 24-hour treatment with gremlin in serum-free medium containing 0.5 mg/mL BSA. Proteins were separated on a 10% denaturing polyacrylamide gel and transferred by electrophoresis to a PVDF membrane. Blots were blocked with 5% fat-free dry milk in Tris-buffered saline tween buffer (TBST) for 1 hour and then incubated overnight with primary antibodies (Table 1). The membranes were washed with TBST and processed with corresponding horseradish peroxidase–conjugated secondary antibodies (Table 1). The proteins were then visualized (Fluor Chem 8900 imager; Alpha Innotech, San Leandro, CA) using ECL detection reagent (SuperSignal West Femto Maximum Sensitivity Substrate; Pierce Biotechnology Rockford, IL).

**Cell-Associated Proteins.** Total cellular protein was extracted from the TM cells using mammalian protein extraction buffer (MPER; Pierce Biotechnology), containing protease inhibitor (Pierce Biotechnology) and phosphatase inhibitor (Pierce Biotechnology) cocktails. Protein concentration was determined using a DC protein assay system (Bio-Rad Laboratories, Hercules, CA). The cellular proteins were sepa-
rated on denaturing polyacrylamide gels and then transferred to PVDF membranes by electrophoresis. Blots were blocked with 5% fat-free dry milk in TBST for 1 hour and then incubated overnight with primary antibodies (Table 1). The membranes were washed with TBST and processed with corresponding horseradish peroxidase-conjugated secondary antibodies (Table 1). The proteins were then visualized (Fluor Chem 8900 imager; Alpha Innotech) using ECL detection reagent (SuperSignal West Femto Maximum Sensitivity Substrate; Pierce Biotechnology). To ensure equal protein loading, the same blot was subsequently developed for β-actin expression.

**Fibronectin ELISA**

Conditioned medium of human TM cells was collected and evaluated for gremlin effects on fibronectin production using a commercially available ELISA kit (Quantimatix Human Fibronectin; Chemicon International, Billerica, MA). We previously demonstrated that treatment of cultured human TM cells with TGFβ2 significantly increases fibronectin levels in the culture medium.10,19

**Statistical Analysis**

For comparing results between two groups, the Student’s t-test was performed. One-way ANOVA was used for comparison of results between more than two groups.

**RESULTS**

**Gremlin Induces ECM mRNA and Proteins in TM Cells**

Gremlin has been previously reported to antagonize BMP4 inhibition of TGFβ2-induction of ECM proteins like FN and PAI1 in human TM cells.19 However, it is not known whether gremlin alone can induce these ECM proteins in TM cells. Therefore, we determined the effect of gremlin on FN, COL1a, PAI1, and ELN expression in cultured human TM cells. Treatment with gremlin (1 μg/mL) for 24 hours significantly induced FN, COL1a, PAI1, and ELN mRNA expression (n = 3; P < 0.05; Fig. 1A), as well as ECM protein expression in cell lysates and conditioned medium (Fig. 1B). We also performed quantitative FN ELISA on the conditioned medium samples of the four TM strains used for the Western immunoblot assay (Fig. 1B), and gremlin significantly elevated the amount of secreted FN in the treated TM cells compared with the controls (n < 0.001; Fig. 1C).

**Gremlin Induces ECM Genes and Proteins in a Concentration- and Time-Dependent Fashion**

TM cell strains (n = 3) were treated with increasing concentrations of gremlin (0–5 μg/mL) for 24 hours. The mRNA and protein expression of FN, COL1a, PAI1, and ELN were determined using qRT-PCR and Western immunoblot, respectively. Gremlin induced expression of ECM mRNA (Fig. 2A), cell-associated (Fig. 2B), and secreted ECM proteins (Fig. 2C) in a concentration-dependent manner. Gremlin also significantly elevated the amount of secreted FN assessed by ELISA in a concentration-dependent manner (Fig. 2D). TM cells were treated with gremlin for 6, 12, and 24 hours to determine the time dependence of ECM mRNA induction. Gremlin significantly (P < 0.01) induced FN, PAI1, COL1, and ELN mRNA expression, although the time course of induction varied slightly for each gene (Fig. 3A). Similarly, TM cell strains (n = 3) were treated with gremlin (1 μg/mL) for 3, 12, 24, 48, and 72 hours to evaluate effects on ECM protein expression. Gremlin induced both cell-associated and secreted ECM proteins as early as 12 hours and maintained this induction for up to 72 hours (Figs. 3B, 3C). The gremlin induction of elastin mRNA peaked and then decreased after 24 hours of treatment, whereas levels of elastin protein were still increased after 72 hours. This apparent discrepancy may be due to the inherent stability and slow turnover of the elastin protein, which would persist even after levels of elastin mRNA have decreased. Gremlin also significantly elevated the amount of secreted FN in a time-dependent manner (Fig. 3D). Therefore, gremlin induction of ECM mRNA and proteins was both time and dose dependent.

**TGFβ Signaling in Gremlin Induction of ECM Proteins**

Gremlin was previously reported to antagonize the BMP4 inhibition of TGFβ2-induced ECM proteins in human TM cells,19 but the signaling mechanism(s) involved was not determined. We used various small molecule inhibitors to explore the involvement of TGFβ signaling pathway(s) in gremlin-mediated ECM induction. SB431542 is a selective TGFBR1 and TGFBR2 receptor inhibitor, whereas LY364947 is a relatively selective inhibitor for the TGFBR2 receptor.28 We pretreated TM cell strains (n = 3) for 1 hour, with or without 5 μM SB431542 or LY364947, followed by treatment with recombinant gremlin (1 μg/mL) for 24 hours. Gremlin elevated FN, COL1, PAI1, and ELN mRNA expression compared to untreated or inhibitor only–treated samples (P < 0.001). Pretreatment with either of the two inhibitors, LY364947 or SB431542, completely blocked gremlin-mediated mRNA induction in all the cell strains (P < 0.001; Fig. 4A).

We used the same strategy to evaluate the effects of these TGFBR inhibitors on gremlin induction of ECM proteins. Gremlin elevated cell-associated as well as secreted FN, COL1, PAI1, and ELN protein levels compared with untreated or vehicle-
treated samples. Each of the two inhibitors, LY364947 (Fig. 4B) and SB431542 (Fig. 4C), completely inhibited the gremlin-mediated ECM protein induction. Treatment with the inhibitors alone did not have any effect on the ECM proteins’ expression. We also analyzed the conditioned medium samples using FN ELISA. Gremlin treatment significantly elevated the amount of secreted FN, which was blocked by pretreatment with the two TGFBR inhibitors (Fig. 4D). The FN ELISA data agreed with our Western immunoblot data (Figs. 4B, 4C).

In addition to TGFBR1/2 inhibitors, we used siRNA-mediated TGFBR1 knockdown to confirm the role of TGFβ receptor signaling in gremlin induction of ECM proteins. Gremlin-treated TM cells were untransfected or transfected with a nontargeting siRNA control or TGFBR1 siRNA. As previously shown, gremlin induced ECM protein expression. Control siRNA did not affect endogenous TGFBR1 levels and did not affect gremlin induction of ECM protein expression. Consistent with the data with small molecule TGFBR1 or TGFBR2 inhibition, TGFBR1 siRNA knockdown inhibited gremlin induction of ECM proteins (Fig. 4E).

TM cells endogenously express TGFβ2 and gremlin proteins, and both TGFβ2 and gremlin (Figs. 1–3) induce ECM proteins in TM cells. We wanted to determine whether gremlin treatment alters endogenous levels of TGFβ2 and vice versa, which in turn may regulate gremlin’s ECM induction profile. TM cell strains (n = 3) were treated with increasing concentrations of gremlin (0–5 μg/mL) or TGFβ2 (0–10 ng/mL) for 24 hours. The mRNA and protein expression of TGFβ2 and gremlin were determined using qRT-PCR and Western immunoblot, respectively. Gremlin induced TGFβ2, and TGFβ2 induced gremlin mRNA (Fig. 5A) and protein (Fig. 5B) expression in a concentration-dependent manner.

We used siRNA-mediated TGFB2 knockdown to confirm the role of TGFβ2 in gremlin induction of ECM proteins. Gremlin-treated TM cells were either untransfected or transfected with a nontargeting siRNA control or TGFB2 siRNA. Gremlin in-

**Figure 2.** Concentration-dependent gremlin induction of ECM mRNA and proteins. Concentration-dependent induction of ECM gene mRNA (A), cell-associated protein (B), and secreted proteins (C) by 0 to 5 μg/mL gremlin in cultured TM cell strains (n = 3). qRT-PCR values (A) represent gremlin induction compared to controls and normalized to ACTB as the housekeeping gene. Three replicates of each sample were used. Concentration-dependent induction of cell-associated (B) and secreted FN proteins (C, D) by 0 to 5 μg/mL gremlin in cultured TM cell strains (n = 3). Western immunoblots (B, C) are representative of data obtained in three TM cell strains. Three replicates of each sample were used for the FN ELISA (D). One-way ANOVA was used for statistical analyses. *0.01 < P < 0.05, **0.0001 < P < 0.01, and ***P < 0.0001.

**Figure 3.** Time-dependent gremlin induction of ECM proteins. Time course induction (0–72 hours) of ECM mRNA (A), cell-associated proteins (B), secreted ECM proteins (C), and secreted FN proteins (D) after treatment of cultured TM cell strains with gremlin (1 μg/mL; n = 3). qRT-PCR values (A) represent the ratio of gremlin induction to controls and normalized to ACTB. Three replicates of each sample were used for qRT-PCR (A) and FN ELISA (D). Western immunoblots (B, C) are representative of data obtained in three TM cell strains. One-way ANOVA was used for statistical analyses. *0.01 < P < 0.05, **0.0001 < P < 0.01, and ***P < 0.0001.
duced both ECM proteins and TGFβ2. Control siRNA did not affect endogenous TGFB2 levels and did not alter gremlin induction of ECM protein expression. However, TGFB2 knockdown blocked gremlin induction of ECM proteins (Fig. 5C). Taken together, our results (Figs. 4, 5) strongly support the roles of TGFβ2 and TGFBR in gremlin induction of ECM protein expression.

Role of CTGF in Gremlin Induction of ECM Proteins

Connective tissue growth factor (CTGF) regulates several ECM proteins in cultured human TM cells and mediates TGFβ2 induction of FN, collagens I, II, and IV; and integrins.32 We wanted to determine whether CTGF is involved in gremlin induction of ECM proteins in TM cells. TM cells were either untransfected or transfected with a nontargeting siRNA control or CTGF siRNA before treatment with gremlin. Gremlin alone induced both ECM proteins and CTGF. Control siRNA did not affect endogenous CTGF levels and did not affect gremlin induction of ECM proteins expression. However, CTGF knockdown blocked gremlin induction of cell-associated and secreted ECM proteins FN and COL1 but not ELN or PAI1 (Fig. 6).

Gremlin Induces ECM Proteins Using Smad Signaling Pathway

The profibrotic cytokine TGFβ2 can activate both canonical Smad and noncanonical signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway in various cells and tissues.33–35 TGFβ signaling is complex because these different signal transduction pathways can interact with each other.36,37 We wanted to determine whether gremlin treatment activates any of the TGFβ signaling pathways and whether any of the TGFβ signaling mechanism(s) are involved in gremlin induction of ECM proteins in TM cells.

A previous study showed that TGFβ2 activates both canonical Smad and MAPK signaling in TM cells.38 We treated four primary TM cell strains with TGFβ2 (5 ng/mL) or gremlin (1 μg/mL) for 15, 30, 60, 120, and 240 minutes, and total and phosphorylated Smad2 and Smad3, as well as P38 and JNK1/2 MAPK proteins were evaluated by Western immunoblot. TGFβ2 phosphorylated both canonical (Smad2 and Smad3) and noncanonical (P38 and JNK1/2) pathways (Figs. 7A, 7B). In contrast, gremlin activated only the Smad pathway (Fig. 7A) but not the P38 or JNK1/2 pathways (Fig. 7B). There were no changes in total Smad2, Smad3, P38, and JNK1/2 levels.
Phosphorylated Smad2 and -3, together or individually, form a complex with co-Smad4 to regulate transcription of their target genes. To determine whether Smad3 transcriptionally regulates the ECM proteins, we used SIS3, a selective small molecule inhibitor of Smad3. Three TM cell strains were treated with SIS3 (10 μM) 6 hours before treating with recombinant gremlin for an additional 24 hours, to study mRNA and protein expression of FN, COL1, PAI1, and ELN. Untreated cells and SIS3-alone–treated cells served as negative controls. Gremlin induction of ECM mRNA and cell-associated protein expression was inhibited by SIS3 pretreatment (P < 0.01; Figs. 8A, 8B). SIS3 treatment also blocked gremlin induction of cell-associated and secreted ECM proteins as well as soluble FN in conditioned medium analyzed by Western immunoblot (Fig. 8B) and ELISA (Fig. 8C), respectively. These results concurred with our mRNA results (Fig. 8A). Therefore, gremlin induction of ECM mRNA and proteins is mediated by Smad3 signaling.

To confirm the role of Smad signaling in gremlin regulation of ECM protein expression, we used siRNA-mediated knock-down in TM cell strains.
Smad2/3/4 inhibition blocks gremlin induction of ECM proteins. Treatment of TM cells with Smad3 inhibitor SIS3 blocks gremlin induction of ECM mRNA (A), cell-associated protein (B), and secreted protein expression (B, C). (A) qRT-PCR analysis of the gremlin induction of ECM genes mRNA in the presence of specific inhibitor of Smad3 (SIS3). qRT-PCR values represent the ratio of induction of ECM mRNA normalized to ACTB in gremlin-treated samples compared with the controls (triplicates of three TM cell strains). One-way ANOVA was used for statistical analyses of qRT-PCR (A) and FN ELISA (C) results. #, differences between gremlin samples versus gremlin+ inhibitor samples; *, differences between gremlin treated versus the untreated cells; ** and ###P < 0.0001. (B) Western immunoblots of cell-associated and secreted ECM proteins after pretreatment with SIS3 (10 μM) followed by gremlin (1 μg/mL) treatment for 24 hours. Immunoblots are representative of three different TM cell strains. ACTB was used as a loading control. Untreated and SIS3-only–treated cells served as negative controls. (C) FN ELISA of secreted FN from gremlin + SIS3 treated TM cells (n = 3 strains). (D-F) Western immunoblots of ECM proteins in TM cells pretreated with Smad3 (D), Smad2 (E), or Smad4 (F) siRNAs followed by gremlin treatment. Control cells were transfected with nontargeting siRNA. Immunoblots are representative of results from three TM cell lines. Each Smad siRNA knocked down its target protein. SIS3 siRNA suppressed the gremlin induction of all four ECM proteins. Smad2 and -4 siRNAs consistently suppressed gremlin induction of FN and PAI1, but variably suppressed gremlin induction of ELN and COL1 proteins.

**DISCUSSION**

Interactive TGFβ/BMP signaling plays an important role in ECM homeostasis, and perturbation in the balance of this signaling is associated with fibrotic diseases, including glaucoma. TGFβ2 plays an important role in glaucoma pathogenesis. Aqueous humor levels of TGFβ2 are significantly elevated in POAG patients, and TGFβ2 is also elevated in glaucomatous TM cells and tissues (Tovar-Vidales, submitted for publication). TGFβ2 increases the expression of several ECM proteins in the TM and also elevates IOP in perfusion cultured anterior segments and rodent eyes. Trabecular meshwork cells and tissues express BMPs, BMP receptors, and BMP antagonists, and BMP4 and -7 inhibit TGFβ2 induction of ECM proteins. Inhibition of BMP signaling exacerbates the TGFβ2 effect on the TM ECM. Gremlin protein levels are higher in GTM cells, and gremlin blocks BMP suppression of TGFβ2 mediated effects on the TM ECM. In addition, gremlin treatment alone elevates IOP in perfusion cultured anterior segments, suggesting that perturbation of normal TGFβ2/BMP homeostasis can play a role in ocular hypertension.

To directly test this latter hypothesis, we examined the effect of gremlin on TM ECM expression. We found that gremlin increased ECM mRNA and protein expression. However, in contrast to TGFβ2, which activates both the Smad and non-Smad MAPK signaling pathways, gremlin activated only the canonical Smad2/3 pathway. Inhibition of Smad signaling blocked gremlin’s effect on TM ECM expression. Connective tissue growth factor is induced by TGFβ2 and acts as a downstream mediator of TGFβ signaling, regulating the induction of multiple ECM proteins including FN and collagen types I, II, IV.
and V1.\textsuperscript{32} Interestingly, our results show that gremlin induced CTGF and that the gremlin induction of FN and COL1 was dependent on CTGF. In contrast, gremlin induction of PAI1 and ELN were not dependent on CTGF. Others have also reported that CTGF does not induce PAI1 expression in human TM cells.\textsuperscript{32} We did not examine whether CTGF can also induce gremlin in a feed-forward loop and whether gremlin can act also as a mediator of CTGF signaling. These experiments are currently under investigation.

We suggest that gremlin increases TM cell ECM expression by inhibiting the balance between BMP and TGF\(\beta\)2 in the regulation of ECM metabolism (Fig. 9). Gremlin binds to BMP and inhibits BMP’s modulatory effect on TGF\(\beta\)2 induction of ECM proteins. Pretreatment of TM cells with specific siRNAs effectively knocked down endogenous TGF\(\beta\)2 and CTGF expression before treatment with gremlin. Therefore, the inhibition of BMP by gremlin has no effect on ECM expression because there is no longer endogenous TGF\(\beta\)2 or CTGF to enhance ECM expression.

Most studies of gremlin have been focused on its role in development of fibrotic diseases. It is not uncommon to find developmental genes re-expressed in several diseased conditions including several kinds of cancer. However, additional studies are needed to address this hypothesis in glaucoma.

It also appears that gremlin and TGF\(\beta\)2 are involved in a “feed-forward” pathogenic pathway. We have shown that gremlin increases TGF\(\beta\)2 expression, and TGF\(\beta\)2 increases gremlin expression in TM cells. This process would further exacerbate ECM deposition within the TM, potentially leading to increased aqueous humor outflow resistance and IOP elevation. Levels of both TGF\(\beta\)2 and gremlin are elevated in the anterior segment in glaucoma, but the primary cause of increased expression of these signaling molecules in glaucomatous eyes is currently unknown. Mechanical stress (i.e., cyclic stretch) and substrate elasticity have been shown to increase TGF\(\beta\)2 expression in the TM.\textsuperscript{41,42} The effects of these perturbations on gremlin expression have not been evaluated. It is plausible to hypothesize that elevated levels of gremlin in glaucoma patients leads to higher TGF\(\beta\)2 levels in the TM.

TGF\(\beta\) and gremlin also play a role in other fibrotic diseases. Gremlin has been associated with several fibrotic diseases of lungs\textsuperscript{43} and kidneys\textsuperscript{44,45} and in osteoarthritis.\textsuperscript{46} Gremlin was shown to induce expression of FN\textsuperscript{27} and several types of collagens.\textsuperscript{47} Gremlin has also been reported as a downstream mediator of TGF\(\beta\)3’s fibrotic effects in the kidney.\textsuperscript{48} Several growth factors like CTGF have been reported to induce ECM proteins like TGF\(\beta\)2 in several kinds of cells.\textsuperscript{48}

The potential relationship between the ECM proteins in regulating aqueous outflow in gremlin-induced ocular hypertension and POAG warrants further study. Do any of these ECM proteins play a direct role in gremlin-induced ocular hypertension? Which ECM proteins are more essential for normal TM homeostasis, and are any of these proteins directly involved in glaucoma pathogenesis? Do different gremlin signaling mechanisms regulate glaucoma-like changes in the TM and directly cause IOP elevation? Our current results provide a foundation to address these questions in future studies.

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References


Gremlin Induces Trabecular Meshwork ECM 5259.