Constitutive Expression and Release of TGF-β2 by Human Trabecular Meshwork Cells

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INTRODUCTION

Primary open-angle glaucoma (POAG) is a common cause of blindness worldwide, affecting nearly 2 million individuals 40 years of age and older in the US. Elevated intracocular pressure (IOP) is a primary risk factor for the development of POAG. In healthy eyes, normal IOP is sustained through balanced production and outflow of aqueous humor (AH). In adults, the majority (>50%) of AH exits the eye through a conventional outflow pathway involving the trabecular meshwork (TM). Resistance to AH outflow through the TM is mediated, in part, through enhanced cellular contractility and increased extracellular matrix deposition in the TM.

Whereas the causes of outflow resistance and elevated IOP in POAG patients remains unclear, pathways of POAG have been strongly correlated with aberrantly-elevated levels of a variety of soluble factors in the AH. Of particular significance, levels of biologically-active transforming growth factor (TGF)-β2 are known to be increased by 60-70% in the AH of POAG patients as compared to healthy control subjects. Despite a clear link between aberrantly-elevated levels of TGF-β2 and increased resistance to AH outflow through the TM, there remains a paucity of data on the mechanisms regulating constitutive TGF-β2 expression and release.

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METHODS

Primary Anterior Segment Perfusion: Anterior segment perfusion experiments are performed using fresh porcine eyes obtained from a local abattoir. Globes are perfused aseptically at the equator, and the iris, lens, and vitreous gently removed. The prepared anterior segment is continuously perfused at a constant flow rate of 4.5 µl/min with pre-warmed DMEM supplemented with antibiotics and antiprotease. Anterior segments are cultured under a humidified atmosphere of 5% CO2/95% air at 37°C and allowed to stabilize overnight. Following stabilization, media perfusing porcine anterior segments served as baseline media. All media (900 µl H12) or recombinant active human TGF-β2 (10 ng/ml; R&D Systems). In some cases, stabilized porcine anterior segments were perfused with either vehicle (0.05% DMSO) or the TGF-β2R inhibitor SB-431542 (1 µM). IOP was monitored and recorded every 3 minutes (PowerLab 8/35 with LabChart Pro).

Human TM Cell Culture: Primary human TM cells were harvested from discarded human cadavers. TM cells were enzymatically dissociated (Von Zee et al., 2012). An SV40-transformed human TM cell line (GTM3) derived from a male glaucomatous patient was a generous gift from Alcon Labs. All cultures were maintained at 37°C under an atmosphere of 5% CO2/95% air.

Treatment of Human TM Cells: Human TM cells were cultured in CDM and treated with (i) vehicle (0.05% ethanol) or chemically activated lovastatin (20 µM), (ii) vehicle (0.05% ethanol) or a biologically-active TGF-β2 inhibitor (GAPTI-298; 20 µM), or (iii) vehicle (0.05% DMSO) or C3 exoenzyme (10 µM).

siRNA-Targeted Knockdown: GTM3 cells were transfected with siRNA (100 nM) targeting RhoA, RhoB, or RhoC using Lipofectamine in a 1:1 mixture of OptiMEM and cell culture medium without serum (RhoA) or Lipofectamine in a 1:1 mixture of OptiMEM and cell culture medium without serum (RhoB and RhoC). Results are representative of 2 separate experiments.

Real-Time RT-PCR: Total RNA was extracted from human TM cells using TRIzol reagent, and 5 µg was reverse-transcribed using Super Script III First Strand Synthesis System. Human-specific TGF-β2 or GAPDH cDNA was amplified by qPCR on a MiniOpticon PCR detection system. For each sample, the specificity of the reaction product was determined by melt curve analysis. The expression of TGF-β2 mRNA was determined by the standard curve method. The relative fold-change in gene expression was normalized to GAPDH.

Statistical Analysis: Results are expressed as the means ± SEM of triplicate samples. Data were analyzed by Student’s t-test, one-way ANOVA with a Bonferroni’s multiple comparison post-hoc analysis, as indicated. In all cases, statistical significance was defined as p < 0.05.

RESULTS

TGF-β2 signaling facilitates elevated IOP in cultured porcine anterior segments

Inhibition of geranylation attenuates constitutive TGF-β2 expression and secretion

Lovastatin attenuates constitutive TGF-β2 mRNA expression and protein secretion

Summary: Inhibition of endogenous TGF-β1/ALK-5 signaling lowers IOP

Human TM cells express and secrete biologically-active TGF-β2

Rho GTPases facilitate constitutive TGF-β2 expression and secretion

siRNA-targeted knockdown of RhoA selectively attenuates constitutive TGF-β2 mRNA expression

CONCLUSION

• Human TM cells represent a source of elevated biologically-active TGF-β2 in the aqueous humor of patients with POAG
• Absent Rho GT-Pase signaling mediates TGF-β2 expression and secretion in human TM cells

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