TGF-β2 Attenuates Constitutive Expression of Bradykinin B2 Receptors in Human Trabecular Meshwork Cells

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INTRODUCTION

Glaucoma is a leading cause of blindness, projected to affect nearly 80 million people worldwide by the year 2020.1 In the US, it is estimated that nearly 2 million individuals age 45 years and older have primary open angle glaucoma (POAG), the most prevalent form of the disease.2 Current treatment options for patients with POAG are aimed at lowering chronically elevated intraocular pressure (IOP), a poorly-understood risk factor associated with POAG.3 In healthy eyes, normal IOP is maintained through balanced production and outflow of aqueous humor (AH).4 In adults, the majority (>50%) of AH exits the eye through the trabecular meshwork (TM).5 Increased resistance to AH outflow through the TM is considered to be a significant contributor to aberrant elevation of IOP in POAG patients. The mechanism by which this occurs remains poorly defined.

In the anterior chamber of POAG patients, the levels of transforming growth factor (TGF)-β2 in AH is aberrantly elevated compared to healthy eyes.6,7 Similarly, perfusion of TGF-β2 through cultured human, bovine, and porcine anterior segments significantly elevates IOP ex vivo by increasing outflow resistance through the TM.8,9 In vitro, exogenous addition of TGF-β2 has been shown to increase content of a number of factors associated with elevated IOP, including extracellular matrix components as well as endothelin-1.10-12 However, there remains a paucity of data on the complete mechanism by which TGF-β2 promotes aberrantly elevated IOP.

Activation of the bradykinin (Bk) receptor B2 has recently been shown to lower IOP in ocular hypertensive non-human primates.13 By comparison, TGF-β2 is known to modulate Bk-associated signaling pathways.14-16 However, the relationship between TGF-β2 and Bk signaling in TM cells remains undefined. In this study, we investigated the effects of TGF-β2 signaling on IOP and B2 receptor content in porcine anterior segments and human TM cells, respectively.

METHODS

Cell Culture: Primary human TM (NTM) cells were harvested from decellularized human瞳孔 chambre rims and cultured to confluence as approved by the Inner VA and Loyola University Institutional review boards. SV-transformed TM cells derived from a male glaucoma patient (GT3M) and a male nontype-glaucoma patient (NTM5) were a generous gift from Alcon laboratories. TM cell cultures were maintained at 37°C in an atmosphere of 5% CO2/95% air. Primary TM cells and SV-transformed TM cells were incubated for 24h upon reaching confluence. Following transfection, culture media was replaced with serum-free media and cells were subsequently treated with vehicle or recombinant human TGF-β2 (10 ng/ml) as we have previously described.17-19

siRNA-Targeted Knockdown: TM cells were transfected with siRNA directed against a scrambled siRNA sequence (25-100 nM; negative control), Smad2 (10 nM), or Smad3 (10 nM) using Lipofectamine in a 1:1 mixture of OptiMEM and cell culture medium without serum or antibiotics/stimulants. Primary TM cells and SV-transformed TM cells were transfected for 48h. Real-time RT-PCR: Total RNA was extracted from human TM cells using TRIzol reagent and reverse-transcribed as we have described previously.10 Human-specific B2 mRNA or GAPDH cDNA sequences were real-time transcribed as we have described previously.10 Real-time PCR: Real-time PCR was performed using 2x SYBR Green PCR Master Mix (ThermoFisher). Relative fold-changes in B2 mRNA were calculated by the 2^-ΔΔCq method and normalized to GAPDH as an internal control. Statistical Analysis: Results are expressed as mean ± SEM (N=3) from a single experiment. *p < 0.05, Student’s t-test.

RESULTS

Figure 1. TGF-β2 increases IOP in cultured porcine anterior segments

Figure 2. TGF-β2 attenuates constitutive B2 mRNA expression in human TM cells

Figure 4. Knockdown of receptor-associated Smads prevents TGF-β2 mediated decreases in B2 mRNA expression

Figure 5. Knockdown of RhoA prevents TGF-β2 mediated decreases in B2 mRNA expression

CONCLUSION

Elevated content of TGF-β2 in the aqueous humor of POAG patients may increase IOP, in part, by attenuating constitutive B2 receptor expression within the conventional outflow pathway.

REFERENCES


ACKNOWLEDGMENTS: The authors would like to acknowledge Don Donna M. Peters and Jennifer A. Faralli (University of Wisconsin-Madison) for their assistance with anterior segment perfusion experiments. This work was supported, in part, by grants from the Illinois Society for the Prevention of Blindness (SL), the Department of Veterans Affairs (C36SBR & 817766 (EBS), C7260M (CP)), the Midwest Eye-Banks, and the Richard A. Ferris Charitable Foundation.