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

Cynthia L. Pervan1,2, Jonathan D. Lautz1,3, Andrea L. Blitzer1,4, and Evan B. Stubbs, Jr.1,2,3

1Research Service (151), Edward Hines, Jr. VA Hospital, Hines, IL
2Ophthalmology, 3Program of Neuroscience, 4Stritch School of Medicine, Loyola University Chicago, Maywood, IL

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 intraocular pressure (IOP) is a primary risk factor for the initiation and progression of POAG. In healthy eyes, normal IOP is sustained through balanced production and outflow of aqueous humor (AH). In adults, the majority (~85%) 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 cause of outflow resistance and elevated IOP in POAG patients remains unclear, pathogenesis of POAG has 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 content 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 content in the eye.

Within the human anterior segment, TGF-β2 immunoreactivity has been localized to limbal and lens epithelial cells, the ciliary body, and the conjunctival stroma. In contrast, only TGF-β2 protein secretion has been localized to human TM cells.

In this study, we sought to investigate whether TM cells serve as a constitutive source of TGF-β2 in the human anterior segment, and determine the mechanisms underlying constitutive TGF-β2 expression and release.

METHODS

Porcine Anterior Segment Perfusion: Anterior segment perfusion experiments were performed using fresh porcine eyes obtained from a local abattoir. Globes were rinsed anatomically in heparinized saline (0.45 µl/min with pre-determined supplemented with antibiotics and anticoagulants. Anterior segments are cultured in modified M-199 containing 5% antibiotics/antimycotics.

Human TM Cell Culture: Primary human TM cells were harvested from discarded human laminectomy discs and cultured in accordance with our previously described (Kim Zee et al., 2012). An SHK2-transformed human TM cell line (HTEM2) derived from a male glucocorticoid patient was a generous gift from Michael Lab. All cultures were maintained at 37°C under an atmosphere of 5% CO2/95% air.

Treatment of Human TCM Cells: Human TM cells were cultured to confluence and treated with (A) 0.1% ethanol or (B) 0.1% ethanol or chemically activated lovastatin (10 µM), (B) 0.1% (w/v) DMSO or (C) GGTI-298 (20 µM). Relative GAPDH-normalized content of TGF-β2 mRNA (n=6) or (B, C) absolute content of secreted biologically-active TGF-β2 protein (n=6) in SHK2-transformed human cells incubated with vehicle (0.01% DMSO) or GGTI-298 (20 µM), *p < 0.05, Student’s t-test.

RESULTS

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

Inhibition of geranylgeranylation attenuates constitutive TGF-β2 expression and secretion

The purpose of this study was to investigate whether TM cells serve as a constitutive source of TGF-β2 in the human anterior segment, and determine the mechanisms underlying constitutive TGF-β2 expression and release.

Lovastatin attenuates constitutive TGF-β2 mRNA expression and protein secretion

TGF-β2 protein stability is unaltered by disrupting geranylgeranylation

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

SUMMARY

• Inhibition of endogenous TGF-β/RhoA 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
• Aberrant Rho GT-Pase signaling mediates TGF-β2 expression and secretion in human TM cells

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Charles Bouchard and Ms. Angelina Marino for helpful assistance. Supported, in part, by grants from the Department of Veterans Affairs, the Midwest Eye-Banks, the Illinois Society for the Prevention of Blindness, the Edward Hines Jr. VA Hospital IRM&D Pilot Research Program, and the Richard A. Peritt Charitable Foundation.