Elevated hydrostatic pressure selectively alters TGF-β2, ET-1, and CTGF gene expression in human trabecular meshwork cells

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Introduction
Glaucoma is a leading cause of blindness worldwide, projected to affect nearly 80 million people by the year 2020. 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.

The pathophysiology of POAG remains unclear. Elevated intraocular pressure (IOP), however, is considered a poorly understood hallmark of POAG. Within healthy eyes, IOP is maintained by a balance of aqueous humor (AH) production and outflow. Increased AH outflow resistance is considered a major contributor to IOP elevation.

Previous studies have shown that TGF-β2, a pro-fibrotic cytokine, enhances outflow resistance and increases IOP, in part, by inducing endothelin-1 (ET-1) and connective tissue growth factor (CTGF) expression and release within the TM.¹⁻³

The effect of elevated pressure itself, however, on TM cell responses is not well understood. Here, we show that human TM cells acutely exposed to elevated hydrostatic pressure exhibit marked increases in endogenous TGF-β1, ET-1, and TGF-β2 expression and release.

Methods

General Design: Primary hTM or hTM transformed human trabecular meshwork (hTM 2) cells were cultured in DMEM containing 1% FBS, 1% Pen/Strep, 1% HEPES, and 0.5% penicillin/streptomycin at an atmosphere of 5% CO2 / 95% air at 37°C. Cultured cultures were serum-starved x 24h, media replaced with fresh DMEM, and immediately seeded in a chamber under an atmosphere of 7% CO2 / 95% air at 37°C at an equal ambient pressure (1 atmosphere/37 mm Hg) or at an elevated pressure of +30 mm Hg above ambient for up to 24h.

E-Actin Staining: Cultured TM cells were fixed in freshly prepared glutaraldehyde (10% v/v, pH 7.4) for 4% formaldehyde (20% v/v). Fixed cells were then permeabilized with 0.1% Triton X-100, blocked with 3% BSA, and incubated with a 1:200 dilution of anti-α-actin primary antibody (BD Biosciences). The secondary antibody was a 1:200 AlexaFluor 546 conjugated lysophosphatidic acid (LPA) receptor, and imaged using a Leica TCS SPE confocal microscope and imaging suite.

Real-Time RT-PCR: Total RNA was extracted from treated cells using TRIzol reagent and 1 μg RNA was reverse transcribed using a first-strand cDNA synthesis kit (Invitrogen). RNA was amplified in a 20 μl reaction containing 0.2 μM of each primer (Table 1). PCR products were quantified by agarose gel electrophoresis and ethidium bromide staining.

Results

Figure 1: Hydrostatic Pressure Chamber

Figure 2: Effect of elevated hydrostatic pressure on TM cell viability

Figure 3: Elevated hydrostatic pressure increases F-actin

Figure 4: Elevated hydrostatic pressure selectively enhances expression of "POAG" associated genes

Figure 5: Elevated hydrostatic pressure enhances TGF-β2, ppET-1, and CTGF expression and release

Figure 6: Elevated hydrostatic pressure selectively alters TGF-β2 and ppET-1 expression in vitro

Figure 7: Elevated hydrostatic pressure alters TGF-β2, ppET-1, and CTGF expression independent of TRPV1 or TRPV4

Figure 8: Elevated hydrostatic pressure alters TGF-β2, ppET-1, and CTGF expression independent of oxidative stress

Summary/Conclusion
Cultured human TM cells acutely challenged with elevated hydrostatic pressure exhibit increases in F-actin as well as selective increases in TGF-β2, ET-1, TGF-β2, and Col Type VI expression. Elevated hydrostatic pressure mediated changes in gene expression occur by a mechanism that involves activation of the ETα1 receptor but is independent of TRPV 1, TRPV 4 or oxidative stress. Collectively, these results suggest that Pressure-dependent changes in TM gene expression represent a feed-forward mechanism that exacerbates TGF-β2 associated increases in TM cell contractility and altered ECM remodeling in affected POAG patients.

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