Glucocorticoid-induced cell-derived matrices modulate human trabecular meshwork cell behavior via transforming growth factor β2 signaling pathways

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PURPOSE. Aberrant trabecular meshwork (TM) extracellular matrix (ECM) remodeling including altered stiffness is an important causal risk factor for ocular hypertension in primary open angle or steroid-induced glaucoma. However, mechanism(s) that govern the specific consequence of a diseased TM ECM or its interaction with profibrotic cytokines on TM cell fate is unclear. Because transforming growth factor β2 (TGFβ2) signaling (Smads and non-Smads) is well-implicated in glaucoma, we determined whether glucocorticoid-induced cell-derived matrices (GIMs) alter this pathway in human TM (hTM) cells intrinsically or in concert with exogenous TGFβ2.

METHODS. Cultured primary hTM cells [n=4 donors; mean age±SD, 69.8±8.5 yr.] were treated with/without 100 nM dexamethasone for 4 weeks. Subsequently, cells were removed with an alkali buffer to obtain vehicle control (VehMs) and GIMs. A fresh batch of hTM cells from the same donor was seeded on VehMs and GIMs in media containing 1% serum in the absence/presence of 5 ng/mL TGFβ2 treatment with/without 5 µM type I TGFβ receptor (TGFβR1) inhibitor for either 24 hours (for protein expression) or 7 days (for target gene expression). Protein and gene expression were quantified by Western immunoblotting and RT-qPCR respectively.

RESULTS. 1.Smads: In the absence of exogenous TGFβ2/TGFβR1 inhibitor, total protein levels of Smad3 was significantly upregulated in hTM cells seeded on GIMs compared to VehMs. In the presence of exogenous TGFβ2, GIMs significantly downregulated Smad2 and Smad3 expression, while Smad4 was overexpressed. Phosphorylated forms of Smad2 and Smad3 were significantly increased by GIMs only in the presence of TGFβ2. 2.Non-Smads: Without exogenous TGFβ2, GIMs significantly increased basal levels of ERK while changes in P38, JNK and RhoA were not significant compared to VehMs. Addition of exogenous TGFβ2 to cells on GIMs resulted in overexpression of JNK while RhoA was markedly decreased. However, with the exception of pJNK, the phosphorylated forms of all these non-Smads were significantly increased in cells cultured on GIMs regardless of exogenous TGFβ2. 3.Target protein/gene expression: Significant overexpression of α-smooth muscle actin was observed on GIMs only with exogenous TGFβ2. GIMs intrinsically overexpressed structural ECM (fibronectin, myocilin, collagen I,IV and VI), matricellular (CTGF and SPARC), and crosslinking (LOX, LOXL1, LOXL2, LOXL3, LOXL4, and TGM2) genes significantly in TM cells. Further, GIMs markedly upregulated MMP14 and TIMP2 accompanied by significant downregulation of MMP1. In the presence of TGFβ2, the extent to which these genes were altered were further exaggerated significantly. Other ECM genes (TSP1, PAI, MMP2, MMP9, ADAMTS4 and TIMP1) that were unaltered intrinsically by GIM were significantly upregulated with the addition of exogenous TGFβ2. 4.TGFβR1 Inhibition: Regardless of exogenous TGFβ2, inhibition of TGFβR1 blocked GIM-induced protein expression of connective tissue growth factor significantly in TM cells to levels similar to that of VehMs.

CONCLUSIONS. Our data demonstrates that both Smad and non-Smad signaling molecules are altered in hTM cells on GIMs in the presence or absence of exogenous TGFβ2. Specifically, target genes that inhibit ECM turnover and induce ECM crosslinking are significantly upregulated, suggesting that dual pathological insults may contribute to increases in matrix stiffness to impair aqueous outflow. Thus, inhibiting TGFβ2 signaling pathways could be a viable strategy for ameliorating GIM- and/or TGFβ2-induced ocular hypertensive cell phenotypes.
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**Smad and non-Smad TGFβ2 signaling pathways**

- TGFβRII and TGFβRI
- Cell membrane
- JNK
- RhoA
- Smad2
- Smad3
- ERK
- P38
- Nucleus

- Actin cytoskeletal changes (αSMA)
- ECM changes (Structural, Matricellular, MMPs, TIMPs, and Crosslinking genes)