TGF-β-induced activation of Smad-independent signaling in human trabecular meshwork cells

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Purpose

Primary open angle glaucoma (POAG) is associated with increased aqueous levels of TGF-β2 and cytoskeletal alterations in trabecular meshwork cells [1,2]. The Smad proteins 2/3 and 4 are the classical intracellular signaling mediators downstream of TGF-β receptor activation [3]. However, TGF-β was shown to induce Smad-independent signaling as well to exert cell type-specific effects [4]. Therefore we assessed the influence of TGF-β on mitogen-activated protein kinases and the P38-AKT pathway.

Methods

Human trabecular meshwork cells were cultivated from donor cornea rings. Activation of Smad-, ERK-, and AKT-signaling pathways was assessed by Western Blot using phosphorylation-specific antibodies. Cytoskeletal structures and cell-cell adhesions were studied by Western Blot and confocal immunofluorescence microscopy. Kinase inhibitors were used to address the significance of distinct signaling pathways.

Results

In trabecular meshwork cells TGF-β activated ERK- and AKT- signaling as well as the Smad-2/3 signaling pathway to induce the expression of α-smooth muscle actin, β-catenin, OB- and N-cadherin. The MEK-1/2 inhibitors U0126 and PD184352 enhanced baseline F-actin and α-SMA expression and promoted TGF-β-induced α-SMA expression, but diminished TGF-β-induced effects on β-catenin and N-cadherin. Inhibition of the P38-AKT pathway by LY294002 or on AKT-inhibitor affected cell-cell contacts and inhibited TGF-β-induced N-cadherin, β-catenin and α-SMA expression.

Conclusions

The MEK/ERK and P38-AKT signaling pathways differentially modulate TGF-β-induced protein expression and localization in trabecular meshwork cells. In addition, MEK baseline activity appears to influence actin stress fiber formation, while AKT signaling promotes cell-cell adhesion. Activation of non-Smad signaling pathways by TGF-β may therefore have unexplored roles in the pathophysiology of POAG.

Literatures


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