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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 PI3K-AKT pathway.

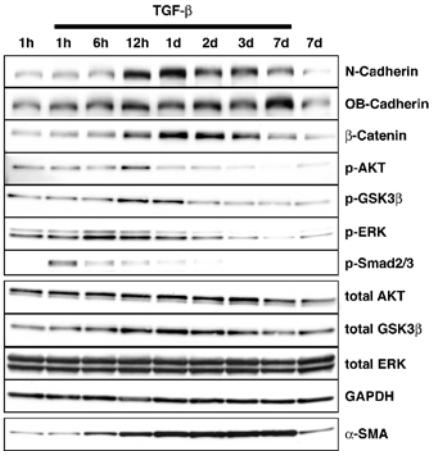

Fig.1: Effects of TGF-β2 on protein expression and phosphorylation in human trabecular meshwork cells.
Cells were treated with TGF-β2 [2ng/ml] for 1 to 72 hours. Protein expression and phosphorylation were assessed by Western Blot. N-Cadherin, OB-cadherin, β-catenin and α-smooth muscle actin (α-SMA) expression are enhanced following TGF-β2 stimulation. The Smad-2/-3, ERK and AKT pathways are transiently activated.

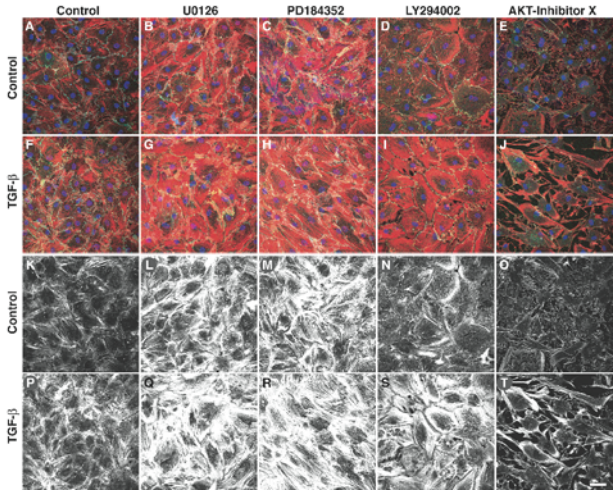

Fig.2: Effects of MEK-1/-2 or PI3K-AKT inhibitors on cytoskeletal features of human trabecular meshwork cells.
Cells were treated for two days with specific kinase inhibitors for MEK-1/-2 (U0126, 10μM; PD184352, 10μM), PI3K (LY294002, 20μM) or AKT-1/-2 (AKT-inhibitor X, 10μM) in the presence or absence of TGF-β2 [2ng/ml]. Subsequently, the cells were stained for F-actin (red in A-J, white in K-T), β-catenin (cyan, A-J) and DNA (blue, A-J) and observed by confocal microscopy. All images were acquired using identical settings. TGF-β-treatment or inhibition of MEK increased F-actin staining with additive effects when used in combination. Inhibition of PI3K or AKT diminished cell-cell contact (β-catenin in D,E,I,J) and affected TGF-β-induced stress fiber rearrangements.

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 PI3K-AKT pathway by LY294002 or an AKT-inhibitor affected cell-cell contacts and inhibited TGF-β-induced N-cadherin, β-catenin and α-SMA expression.

Conclusions

The MEK-ERK and PI3K-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.

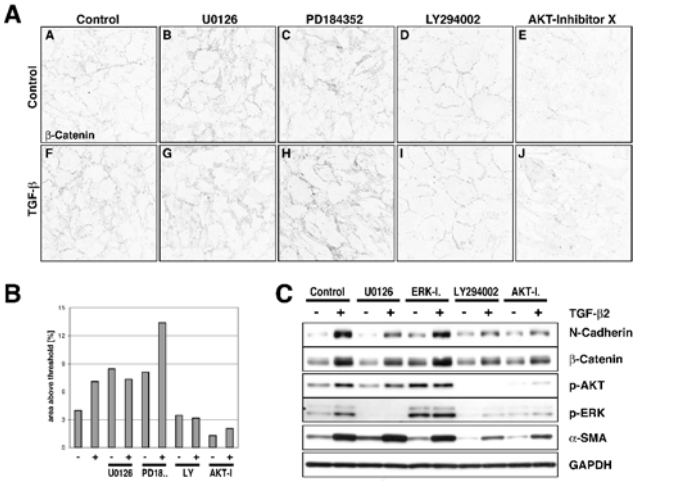

Fig.3: Effects of MEK-1/-2 or PI3K-AKT inhibitors on human trabecular meshwork cells.
Cells were treated with specific inhibitors for MEK (U0126, 10μM; PD184352, 10μM), ERK (ERK-inhibitor Calbiochem 328006, 25μM), PI3K (LY294002, 20μM) or AKT (AKT-inhibitor X, 10μM) in the presence or absence of TGF-β2 [2ng/ml] for 48h. (A) Localization of β-catenin, images inversed. (B) Quantization of β-catenin signal intensities in (A). (C) Western Blot depicts enhanced α-SMA expression with U0126 and attenuation of TGF-β-induced β-catenin, N-cadherin and α-SMA expression by PI3K-AKT inhibitors. An inhibitor preventing ERK-substrate interaction had no effect on α-SMA expression.

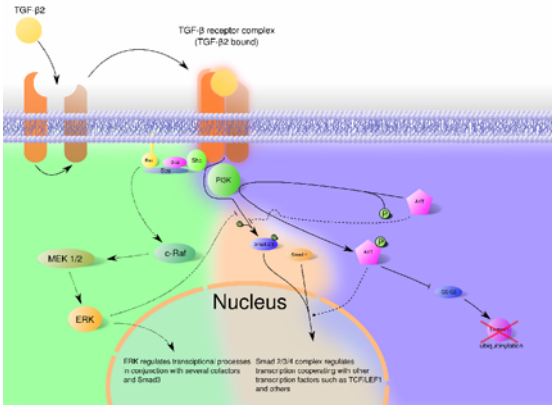

Fig. 4: Schematic illustration of the TGF-β2 signaling pathways discussed.
Besides the canonical pathway involving Smad-2/-3 and -4 (orange background) there are several non-Smad mediators involved in the transduction of the TGF-β2 signal from the outer membrane of the cell to the nucleus including PI3K/AKT (blue background) and MEK/ERK (green background).

Literature

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