

ROS-MAPK Pathway is Involved in TGF- β 1-induced Epithelial-Mesenchymal Transition in Renal Epithelial Cells

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Background : Epithelial-mesenchymal transition (EMT) plays an important role in renal tubulointerstitial fibrosis and TGF- β 1 is the key mediator of EMT. Smad pathway and mitogen-activated protein kinase (MAPK) pathway have been shown to mediate TGF- β 1-induced extracellular matrix (ECM) upregulation. Since reactive oxygen species (ROS) are upstream signaling molecules of MAPK and are involved in TGF- β 1 signaling, the present study examined the role of ROS in TGF- β 1-induced EMT and ECM remodeling in NRK-52E cells, rat proximal tubular epithelial cells.

Methods : Growth arrested and synchronized NRK-52E cells were cultured under TGF- β 1 (0-20 ng/ml) or H₂O₂ (0-500 μ M) for up to 96 hours. Antioxidants [N-acetylcystein (NAC) 5 mM or catalase 500 U/mL], NADPH oxidase inhibitors [diphenylene iodonium (DPI) 0.1 μ M or apocynin 100 μ M], and MAPK inhibitors (PD 98059, an MEK inhibitor, 50 μ M or p38 MAPK inhibitor 5 μ M) were employed to determine the role of ROS and MAPK pathway in TGF- β 1-induced EMT and ECM accumulation. Fibronectin and PAI-1 secreted into the media, E-cadherin and alpha-smooth muscle actin (α -SMA) protein expression in cells were analyzed by Western blot analysis and normalized by β -actin expression. Dichlorofluorescein-sensitive intracellular ROS was measured by FACS.

Results : TGF- β 1 increased cellular ROS, phosphorylated Smad2, p38 MAPK, and ERK, alpha-SMA expression, and fibronectin and PAI-1 secretion and decreased E-cadherin expression in a dose- and time-dependent manner. H₂O₂ also increased phosphorylated p38 MAPK and ERK, but not Smad2. H₂O₂ decreased E-cadherin expression and increased alpha-SMA expression, fibronectin and PAI-1 secretion in a dose- and time-dependent manner. TGF- β 1 and H₂O₂ induced phenotypic change in NRK-52E cells. NAC, catalase, DPI, and apocynin all effectively inhibited TGF- β 1-induced p38 MAPK and ERK activation, EMT, and fibronectin and PAI-1 secretion. Both PD 98059 and p38 MAPK inhibitor effectively inhibited TGF- β 1- or H₂O₂-induced EMT and fibronectin and PAI-1 secretion.

Conclusion : Our data suggest that TGF- β 1 induces EMT and increases ECM secretion by proximal tubular epithelial cells partly through ROS-MAPK pathway and that NADPH oxidase play a role in TGF- β 1-induced ROS generation in NRK-52 cells