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Reactive Oxygen Species Mediate TGF-β 1-induced Tubular Epithelial-mesenchymal Transition

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Background: Tubulointerstitial fibrosis is closely associated with progressive decline in renal function in diabetes. Epithelial-mesenchymal transition (EMT) plays an important role in tubulointerstitial fibrosis. TGF-β1 is the key mediator of extracellular matrix (ECM) remodeling in diabetic kidney. Since reactive oxygen species (ROS) mediate some of the cellular actions of TGF-β1 in non-renal cells, the present study examined if ROS also mediate TGF-β1-induced EMT and ECM secretion by tubular epithelial cells.

Methods: Growth arrested and synchronized proximal tubular epithelial cell lines, LLC-PK1 and NRK-52E cells, were cultured under various concentrations of TGF-β1 (0-20 ng/ml) for up to 5 days in the presence or absence of antioxidant N-acetylcystein (NAC:5 mM), catalase (500 U/ml), NADPH oxidase inhibitors (DPI 1 μM or apocynin 100 μM). H2O2 (0-500 μM) was also used to activate cells. Fibronectin and plasminogen activator inhibitor-1 (PAI-1) secreted into the media and HSP47 (a chaperon protein for collagen synthesis), E-cadherin and α-smooth muscle actin (α-SMA) protein expression in cells were analyzed by Western blot analysis and normalized by β-actin expression. DCF-sensitive intracellular ROS was measured by FACS.

Results: TGF-β1 decreased E-cadherin and increased α-SMA and HSP47 expression and PAI-1 and fibronectin secretion by NRK-52E cells in a dose- and time-dependent manner. These alterations were accompanied by phenotypic change in tubular cells. H2O2 also decreased E-cadherin expression, increased α-SMA and HSP47 expression, PAI-1 and fibronectin secretion by NRK-52E cells. TGF-β1 increased intracellular ROS in LLC-PK1 and NRK-52E cells. Both NAC and catalase effectively inhibited TGF-b1-induced EMT in NRK-52E cells. DPI and apocynin effectively inhibited TGF-β1-induced fibronectin upregulation in LLC-PK1 cells.

Conclusions: Our data demonstrate that TGF-β1 induces EMT and increases fibronectin secretion by proximal tubular epithelial cells partly through ROS and that NADPH oxidase plays a role in TGF-β1-induced ROS generation in tubular epithelial cells.