Glucose-evoked changes in Transforming Growth Factor Beta1 modulate cell-substrate binding in human proximal tubule derived epithelial cells.

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Aims: The tubular-basement membrane is a highly regulated microenvironment that facilitates numerous cell-matrix interactions critical in maintaining epithelial phenotype. Currently, we know little of how cell substrate and cell-cell interactions are modulated in diabetic nephropathy. This study identifies a role for glucose-evoked changes in TGF-β1, in modulation of interactions between proximal tubule derived epithelial cells and key components of the extracellular matrix.

Methods: HK2 cells were cultured in either 5mM-glucose +/- TGF-β1 (2-10ng/mL) or 25mM-glucose. Glucose evoked increases in TGF-β1 secretion were determined by ELISA. HK2-ECM interactions were assessed via ECM arrays.

Results: Cell culture supernatant of HK2-cells cultured in 25mM-glucose exhibited increased TGF-β1 secretion from 334pg/mL±4.1% to 994pg/mL ±4.3% as compared to 5mM-control (n=3 $P<0.01$). Incubation of HK2 cells on wells pre-coated with candidate substrates confirmed increased afffinity for Fibronectin>CollagenIV>Collagen I>Laminin as determined by an ECM assay. TGF-β1 treated HK2 cells (48hrs) evoked increased binding to Collagen I, Collagen IV and Laminin to 340±26%, 228±38% and 289±42% respectively, whilst binding to fibronectin was unaltered as compared to control (n=3 $P<0.01$). HK2 cells cultured in 25mM glucose exhibited increased binding to Collagen I, Collagen IV and Laminin to 183±4%, 157±3% and 175±20% respectively, whilst binding to fibronectin was reduced to 80±5% as compared to control (n=3 $P<0.001$).

Conclusions: The current study suggests that glucose-evoked changes in TGF-β1 are instrumental in reorganizing the extracellular-matrix and cell-substrate interactions in proximal tubule epithelial cells, changes that alter cell architecture, integrity and function, which can ultimately result in kidney damage ahead of overt renal failure in Diabetic-Nephropathy.

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