Transforming Growth Factor Beta 1 alters purinergic signalling and increases ATP release from epithelial cells derived from the human proximal tubule.

Price GW, Squires PE, Hills CE.
School of Life Sciences, University of Lincoln, Lincoln, LN6 7TS, UK

Aim: Connexins (Cx) form gap-junctions or hemi-channels through which messengers, including adenosine triphosphate (ATP) can propogate. This study investigated a role for Transforming Growth Factor-beta1 (TGF-b1), a major pro-fibrotic cytokine associated with glucose-evoked renal fibrosis, in regulating Cx-mediated cell-to-cell communication.

Methods: Expression of P2Y1, P2Y6, Cx26 and Cx43 in TGF-b1 (10ng/mL, 48hrs) treated human proximal (HK2) tubule cells were assessed by immunoblot analysis. Calcium microfluorimetry assessed changes in cytosolic calcium ($[Ca^{2+}]_i$) in response to increasing concentrations of ADP and ATP (0.01-10μM). Cells, preloaded with ATP (10mM for 10mins), were used to measure real-time ATP release via bio-sensing.

Results:

TGF-b1 (10ng/mL, 48hrs) significantly decreased expression of Cx26 (63.5±8.8%, p<0.01,n=5), Cx43 (51.1±2.9%, p<0.0001,n=6), P2Y1 (23.6±1.4%, p<0.0001,n=4) and P2Y6 (41.9±2.0%, p<0.01,n=3) as compared to control. Basal-to-peak ADP (10μM) and ATP (10μM) evoked changes in $[Ca^{2+}]_i$ were significantly reduced following TGF-b1 treatment (p<0.0001,n=3). Removing extracellular calcium opens hemi-channels and allows ATP release from HK2 cells. In TGF-b1 (10ng/mL) treated cells the amount of ATP release increased from 0.24μM± (control) to 2±0.47μM (p=0.0012, n=3).

Conclusions: TGF-b1 significantly decreased expression of Cx26, Cx43, P2Y1 and P2Y6 in HK2 cells and functionally reduced purinergic-evoked Ca2+-signalling. However, ATP bio-sensing suggests that the cytokine increases the amount of hemi-channel ATP release. Loss of connexin expression appears to impair GJ-mediated cell-communication but increases the amount of hemi-channel activity. This may represent a compensatory mechanism to maintain cell-to-cell communication in the diabetic kidney.

This work is supported by a grant from Diabetes UK (BDA: 11/0004215).