Background: Changes in the expression of connexins have been linked to renal damage in diabetes and both hemi-channels and gap junctions represent potential therapeutic targets for the treatment of diabetic nephropathy. In the current study, we utilize model epithelial cells from human renal proximal tubules (HK2), to demonstrate a role for glucose and its downstream beta1 isoform of the pro-fibrotic cytokine transforming growth factor (TGFβ1) on connexin expression and hemi-channel activity.
Methods: Connexin-26 and connexin-43 expression was assessed by immunoblot analysis in HK2 cells cultured in either low glucose (5mmol/L) +/- TGFβ1 (10ng/mL) or high glucose (25mmol/L) for 7days. ELISA was used to measure TGF-β1 secretion. Carboxyfluorescein uptake was used to measure hemi-channel activity in TGFβ1 treated HK2 cells at 7days, whilst ATP bio-sensing determined real time release of ATP.
Results: In response to high glucose (25mmol/L) treatment for 7days, HK2 cells increased TGFβ1 secretion to 994.4±43.6pg/ml compared to 5mmol/L glucose (334±14.9pg/ml; n=3; $P < 0.01$). Immunoblot analysis confirmed that TGFβ1 (10ng/mL) up-regulates expression of both connexin-26 and connexin-43 to 203.9±7.5% and 151.1±7.1% respectively as compared to control (n = 4; $P<0.001$). Dye uptake using carboxyfluorescein, demonstrated increased fluorescence in TGFβ1 treated (10ng/mL) cells at 7days compared to control (430±18% increase), whilst pre-incubation with the hemi-channel blocker carbenoxolone (200microM) significantly reduced uptake in both non-stimulated and TGFβ1 treated cells to 41±2.7% and 64±2.6% respectively (n=3 $P<0.001$). ATP bio-sensing confirmed that the TGFβ1 evoked increase in hemi-channel activity was paralleled by an increase in ATP release (1.99±0.47microM compared to control 0.29± 0.06microM; n=3 $P<0.05$).
Conclusion: Recent studies link increased hemi-channel mediated ATP release to the progression and development of fibrosis in multiple tissue types. Understanding the contribution of connexin-mediated paracrine cell-to-cell communication in the pathogenesis of tubulointerstial fibrosis will help identify potential candidate proteins/pathways in the diabetic kidney ahead of future therapeutic intervention.