Transforming growth factor beta 1 initiates activation of the NLRP3 inflammasome in human derived proximal tubule kidney cells.

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Aims: In diabetic nephropathy, tubulointerstitial fibrosis occurs when early injury activates secretion of inflammatory mediators. The inflammasome is a protein complex, which can be activated by Adenosine Tri Phosphate (ATP), triggering secretion of pro-inflammatory mediators including, interleukin-18 (IL-18) and (IL-6). Our previous data confirms that increased expression of Connexin-26 and Connexin-43 in biopsy material from patients with nephropathy, is paralleled by Transforming growth factor beta (TGF-β1) induced hemi-channel mediated ATP release. In this study we investigate a role for glucose-evoked changes in TGF-β1 in activation of the NLRP3-inflammasome in proximal tubule cells.

Methods: Human kidney tubular epithelial cells (HK2) were treated with TGF-β1 (2-10ng/ml) or ATPγS (100μM) for 48hours. Expression of NLRP3 inflamamsome, Caspase-1, Interleukin-6 and Interleukin-18 were determined by immunoblotting.

Results: Treatment with TGF-β1 (2-10ng/ml) evoked increased expression of inflammasome components Caspase-1 to 202±25%, 205±59% and 228±41% (n= $P<0.05$); and NLRP3 to 283±67%, 389±91% and 455±153% (n=3 $P<0.05$) respectively. Expression of IL-6 and IL-18 was increased to 157±8%, 181±38% and 231±143% (n=3 $P<0.05$) and 425±194%, 388±47% and 355±8% (n=3 $P<0.05$) respectively. Treatment with ATPγS (100μM) evoked increased expression of Caspase-1 to 193±1.5% (n= $P<0.001$) and IL-6 to 227±14% as compared to control (n=3 $P<0.05$).

Conclusions: In the current study, we confirm TGF-β1 and ATPγS induced activation of the inflammasome and downstream inflammatory mediators in human proximal tubule cells. Since elevated levels of ATP can trigger activation of the inflammasome, we hypothesise that TGF-β1 induced aberrant hemi-channel mediated ATP release can drive increased fibrosis of the proximal tubule through activation of the inflammasome.

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