**fibrosis-related MicroRNAs ARE DYSREGULATED BY Hyperglycaemia IN HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS**

Renal tubulointerstitial fibrosis (TIF) is the hallmark of chronic kidney disease and drives its progression to end-stage renal disease. Excessive deposition of extracellular matrix (ECM) in TIF causes thickening of basement membranes, disruption of normal cell-to-cell interactions and loss of tissue elasticity, thus leading to the destruction of the renal tubules. Diabetes can cause diabetic nephropathy, which is characterised by ECM accumulation in the glomerular mesangium and tubulointerstitium mediated by the key pro-fibrotic cytokine TGF-β1. The aim of this study is to identify fibrosis-related microRNAs (miRs) as potential therapeutic targets for prevention of TIF in diabetes.

Immortalised human renal proximal tubular epithelial cells (HK-2 cells) were exposed to glucose (5, 25, 30mM D-glucose or the osmotic control 5mM D-glucose+25mM L-glucose) for up to 72h. Glucose consumption, scratch wound healing, cell viability/proliferation, mitochondrial function analyses and TGF-β1 ELISA tests were undertaken. The expression of 84 fibrosis-related miRs was evaluated using qPCR arrays (Qiagen, UK) in HK-2 cells after 24h, 48h or 72h incubation with different concentrations of glucose.

The results demonstrated that the cell viability/proliferation was significantly decreased following exposure to 30mM D-glucose compared with 5mM D-glucose. Furthermore, reduced glucose consumption and wound healing were observed in response to 30mM D-glucose, whereas 25mM D-glucose accelerated wound healing. In contrast to the effect of 25mM D-glucose, TGF-β1 levels in media from cells exposed to 30mM D-glucose were significantly elevated. Although the mitochondrial energy (ATP) generation was decreased in cells exposed to either 25 or 30mM D-glucose, this effect was significant at 30mM D-glucose. qPCR array experiments demonstrated dysregulated expression of over 15 fibrosis-related miRs in response to hyperglycaemia. Upregulated miRs included miR-150-5p and miR-216a-5p, and amongst the downregulated miRs were miR-211-5p and miR-122-5p.

In conclusion, our data suggest that a critical threshold level of glucose (between 25mM and 30mM) increases TGF-β1 in HK-2 cells and is detrimental to cell proliferation, migration and metabolism. Furthermore, a number of fibrosis-related miRs are dysregulated by hyperglycaemia in HK-2 cells. The effects of these miRs need to be investigated further to identify potential therapeutic targets for the prevention of proximal tubular damage in diabetes.