MicroRNA-26a inhibits TGF-β-induced extracellular matrix protein expression in podocytes by targeting *CTGF* and is downregulated in diabetic nephropathy

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Abstract

Aims/hypothesis The accumulation of extracellular matrix (ECM) proteins, such as collagen, is one of the main hallmarks of diabetic nephropathy [1]. Evidence has shown that TGF-β plays a key role in these pathological changes [2]. Podocyte injury and loss are found in the very early stages of diabetic nephropathy and may be a major starting point of glomerular injury in the disease [3]. Several cytokines or growth factors, such as TGF-β and connective tissue growth factor (CTGF, also known as CCN2) [4,5], have been proposed to be involved in podocyte injury. The precise mechanisms of their role, however, remain unclear.

MicroRNAs (miRNAs) are small (approximately 22-nucleotide long), noncoding RNAs that downregulate gene expression by modulating the stability and/or translation of target mRNAs. Dysregulation of miRNAs is involved in numerous pathological conditions, including renal diseases [6,7]. Several miRNAs are reported to be involved in the TGF-β-dependent pathogenesis of diabetic nephropathy [8-10]. The mechanism of how they modulate the disease seem very complex and further investigations are required to clarify the role of these miRNAs in diabetic nephropathy [11]. We aimed to identify microRNAs (miRNAs) targeting *CTGF* on podocytes in diabetic nephropathy.

Methods We investigated miRNAs targeting *CTGF* on podocytes with miRNA array analysis and identified a candidate miRNA, miR-26a. Using overexpression and silencing of miR-26a in cultured podocytes, we examined changes of ECM and its host genes. We further investigated glomerular miR-26a expression in humans and in mouse models of diabetic nephropathy.

Results miR-26a, which was downregulated by TGF-β1, was expressed in glomerular cells including podocytes and in tubules by in situ hybridisation. Glomerular miR-26a expression was downregulated by 70% in streptozotocin-induced diabetic mice. Transfection of miR-26a mimics in cultured human podocytes decreased the CTGF protein level by 50%, and directly

inhibited *CTGF* expression in podocytes, as demonstrated by a reporter assay with the 3'-untranslated region of the *CTGF* gene. This effect was abolished by a mutant plasmid. miR-26a mimics also inhibited TGF-β1-induced collagen expression, SMAD-binding activity and expression of its host genes *CTDSP2* and *CTDSPL*. Knockdown of *CTDSP2* and *CTDSPL* increased collagen expression in TGF-β-stimulated podocytes, suggesting that host genes also regulate TGF-β/SMAD signalling. Finally, we observed a positive correlation between microdissected glomerular miR-26a expression levels and estimated GFR in patients with diabetic nephropathy.

Conclusions/interpretation The downregulation of miR-26a is involved in the progression of diabetic nephropathy both in humans and in mice through enhanced TGF-β/CTGF signalling.

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Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

KK, HY and MM designed the study, wrote the paper and approved its final version. KM, MK, TK, HI, AI, KPM, YK , SO, NT, KN and MY contributed to the study design, acquisition of data, interpretation of the article, revision of the manuscript and approved its final version. MAS and AS contributed materials and analysis and interpretation of data, revised the article's intellectual content and approved the final version. HY is responsible for the integrity of the work as a whole.

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