JAB1 Promotes High Glucose-Induced Inflammation and Extracellular Matrix Deposition in Glomerular Mesangial Cells by Regulating Angiopoietin-Like Protein 2

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Abbreviations: ANGPTL2 – angiopoietin-like protein 2; CSN5 – constitutive photomorphogenic-9 signalosome; GMCs – glomerular mesangial cells; IL-6 – interleukin 6; IL-1β – interleukin 1β; JAB1 – c-Jun activation domain-binding protein 1; MDA – malondialdehyde; MCP-1 – monocyte chemoattractant protein 1; ROS – reactive oxygen species; SOD – superoxide dismutase; TNF-α – tumour necrosis factor α; TGF-β1 – transforming growth factor β 1.

Abstract. Diabetic or hyperglycaemic conditions stimulate the inflammatory response, excessive accumulation of extracellular matrix, and result in glomerulosclerosis, a scarring process of diabetic nephropathy. c-Jun activation domain-binding protein 1 (JAB1) functions as a regulator of pathways involved in cellular apoptosis and proliferation. The role of JAB1 in diabetic nephropathy was investigated in this study. Firstly, glomerular mesangial cells (GMCs) were treated with high glucose, and high glucose conditions induced up-regulation of JAB1 in the GMCs. Moreover, IL-6, TNF-α, MCP-1, and IL-1β were also elevated in high glucose-induced GMCs. Secondly, silencing of JAB1 reduced the levels of IL-6, TNF-α, MCP-1, and IL-1β in high glucose-induced GMCs. In addition, silencing of JAB1 attenuated the high glucose-induced decrease of superoxide dismutase (SOD) and the increase of reactive oxygen species (ROS) and malondialdehyde (MDA). The increased TGF-β1, collagen I, collagen IV, and fibronectin levels in high glucose-induced GMCs were restored by knockdown of JAB1. Thirdly, angiopoietin-like protein 2 (ANGPTL2) expression was reduced by JAB1.

Over-expression of ANGPTL2 weakened the JAB1 silence-induced decrease of IL-6, TNF-α, MCP-1, IL-1β, TGF-β1, collagen I, collagen IV, and fibronectin. In conclusion, silencing of JAB1 reduced extracellular matrix deposition and suppressed inflammation in high glucose-induced GMCs through down-regulation of ANGPTL2.

Introduction

Diabetic nephropathy is one of the most important and common complications of diabetes mellitus, which is characterized by a persistent increase in urinary albumin protein (Samsu, 2021). Diabetic nephropathy, with increasing incidence, mortality and morbidity in diabetic patients, leads to end-stage renal disease (Lim, 2014). The main pathological features of diabetic nephropathy are renal interstitial fibrosis, renal myelitis, renal papillary necrosis, and renal arteriosclerosis (Alsaad and Herzenberg, 2007). Distinct risk factors, including dyslipidaemia, high blood pressure, elevated glucose levels, and long duration of diabetes, promote the progression and development of diabetic nephropathy (Rao et al., 2021).

Increasing evidence has shown that hyperglycaemia increases reactive oxygen species in renal tubule epithelial cells and glomerular membrane cells, resulting in renal tubule damage, inflammation and extracellular matrix changes (Fakhruddin et al., 2017). Moreover, excessive accumulation of extracellular matrix contributes to tubulointerstitial fibrosis, thickening of glomerular basement membrane and mesangial matrix, thus leading to progression of diabetic nephropathy (Dugbartey, 2017). Therefore, inhibition of extracellular matrix accumulation and inflammation is considered to be an effective strategy for management of diabetic nephropathy (Wang et al., 2021).

c-Jun activation domain-binding protein 1 (JAB1), fifth component of the constitutive photomorphogenic-9
signalosome (CSN5), has been shown to function as c-Jun coactivator or regulate other pathways involved in cellular proliferation and apoptosis (Shackleford and Clare, 2010), thus participating in the cell cycle control of cancers (Shackleford and Clare, 2010; Wang et al., 2016). For example, JAB1 bound to centromeric components to modulate the mitotic apparatus (Chun et al., 2013), and JAB1 negatively regulated p27 to promote cell proliferation of nasopharyngeal carcinoma (Pan et al., 2012). Inhibition of JAB1 induced breast cancer cell apoptosis and repressed cell proliferation (Xiao et al., 2019). JAB1 contributed to epithelial-mesenchymal transition and metastasis of renal cell carcinoma (Zhang et al., 2017). In palmitate-induced hepatocytes, JAB1 was up-regulated, and depletion of JAB1 promoted insulin sensitivity to attenuate insulin resistance (Zhao et al., 2020). However, the role of JAB1 in diabetic nephropathy remains unknown. JAB1 has been shown to promote ANGPTL2 expression and regulate carcinogenesis of thyroid carcinoma cells (Xie et al., 2021). ANGPTL2 modulated secretion of pro-inflammatory cytokines and exerted physiological and pathological functions in chronic diseases, such as atherosclerosis, diabetes, and cancers (Thorin-Trescases and Thorin, 2014). Therefore, JAB1 was hypothesized to regulate diabetic nephropathy through regulation of ANGPTL2.

The effects of JAB1 on inflammation and extracellular matrix accumulation of high glucose-induced GMCs were then investigated in this study.

Material and Methods

Cell culture and transfection

Human GMCs were purchased from Boster Biological Technology Co., Ltd. (Wuhan, China). Cells were cultured in DMEM containing 10% foetal bovine serum (Thermo Fisher Scientific Inc., Waltham, MA) at 37 °C. In the high glucose group, cells were maintained in medium containing 25 mM D-glucose (Sigma-Aldrich, St. Louis, MO). In the normal glucose group, cells were incubated with 5.5 mM glucose (Sigma-Aldrich). siRNA targeting JAB1 (si-JAB1; sense: 5'-CCAGACUAUU-CCACUUAATT-3’ and antisense: 5’-AUUAAGUGG AAUAGUCUGTT-3) and the negative control (si-NC) were synthesized by GenePharma (Shanghai, China). Full-length JAB1 or ANGPTL2 were constructed into the pcDNA3.1 vector (V79020, Invitrogen, Carlsbad, CA) for the over-expression of JAB1 or ANGPTL2. For cell transfection, GMCs (2 × 10⁶ cells/well) were seeded in a 96-well plate containing 25 mM D-glucose for 24 h, and then transfected with si-NC, si-JAB1, pcDNA-JAB1, or pcDNA-ANGPTL2. Two days later, the cells were examined by functional assays.

qRT-PCR

GMCs post normal or high glucose conditions were lysed in TRIZol (Invitrogen), and the extracted RNAs were transcribed into cDNAs by the Reverse Transcription System (Applied Biosystems, Carlsbad, CA). Analysis of JAB1 (NM006837) was done by SYBR Green Master (Roche, Mannheim, Germany) and normalized to GAPDH (BC083511). Primers: JAB1 (forward: 5’-TGTGCTGAGATGTGTAGC-3’ and reverse: 5’-CGGCAACTGTGTTGCATT-3’) and GAPDH (forward: 5’-GAAGCTGAGTCCGAGTC-3’ and reverse: 5’-GAAATGTTAGGGATTCGTT-3’) were used. The 2^-ΔΔCt method was used to detect the gene expression of JAB1.

ELISA

 Supernatants of cultured GMCs were collected, the contents of IL-6, TNF-α, MCP-1, IL-1β, MDA, and SOD were measured using commercial ELISA kits (CUSABIO, Wuhan, China) in at least triplicates.

Detection of ROS

The cultured GMCs with indicated treatment and transfections were harvested and treated with 10 μM 2’,7’-dichlorodihydrofluorescein diacetate (Sigma-Aldrich) for 30 min. Cells were then analysed by a Multi-Mode Microplate Reader (BioTek, Winooski, VT) to determine the mean fluorescence intensity of 2’,7’-dichlorofluorescein. The images of cells were captured by a fluorescence microscope (Olympus, Tokyo, Japan).

Western blot

Cell proteins were extracted in RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific Inc.) and the protein concentration was measured by a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific Inc.). Protein samples (30 μg) were separated by 10% SDS-PAGE and transferred onto nitrocellulose membranes. After blocking with 5% non-fat milk, the membranes were incubated with primary antibodies against p21 (ab207743) and JAB1 (ab12323; 1 : 2000), TGF-β1 (ab92486; 1 : 2500), collagen I (ab34710; 1 : 2500), collagen IV (ab6586; 1 : 3500), fibronectin (ab2413; 1 : 3500), GAPDH (ab8245; 1 : 2000). The membranes were then probed by incubating with peroxidase-conjugated secondary antibody (ab205718 and ab205719; 1 : 5000). ECL reagent (Millipore, Billerica, MA) was used to assess the bands. The expression of JAB1, TGF-β1, collagen I, collagen IV, and fibronectin were normalized to GAPDH. All antibodies were acquired from Abcam (Cambridge, UK).

Statistical analysis

All the data with at least triple replicates were expressed as mean ± SEM, and analysed by Student’s t test or one-way analysis of variance with SPSS software. A P value of < 0.05 was considered as statistically significant.
Results

**JAB1 was elevated in high glucose-induced GMCs and controlled p21 expression.**

Human GMCs were incubated with high glucose for 24 h. mRNA expression of JAB1 was up-regulated in high glucose-induced GMCs compared to the normal glucose conditions (Fig. 1A). High glucose also induced up-regulation of the JAB1 protein in GMCs compared to the normal glucose conditions (Fig. 1B). Exposure of GMCs to high glucose inhibited p21, and silencing of JAB1 restored the p21 expression (Fig. 2). These changes demonstrated the potential relation between JAB1 and diabetic nephropathy.

**JAB1 contributed to inflammation in high glucose-induced GMCs**

High glucose-induced GMCs were transfected with si-JAB1 for the down-regulation of JAB1 (Fig. 3A). Levels of IL-1β (Fig. 3B), IL-6 (Fig. 3C), TNF-α (Fig. 3D), and MCP-1 (Fig. 3E) were increased in GMCs post high glucose conditions. However, silencing of JAB1 reduced the levels of IL-1β (Fig. 3B), IL-6 (Fig. 3C), TNF-α (Fig. 3D), and MCP-1 (Fig. 3E) in high glucose-induced GMCs, suggesting that suppression of JAB1 reduced the inflammation in diabetic nephropathy.

**JAB1 contributed to oxidative stress in high glucose-induced GMCs**

High glucose induced up-regulation of ROS in GMCs (Fig. 4A and B). However, silencing of JAB1 reduced the level of ROS in high glucose-induced GMCs (Fig. 4A and B). Moreover, high glucose-induced up-regulation of MDA (Fig. 4C) and down-regulation of SOD (Fig. 4D) were reversed by knockdown of JAB1. JAB1 contributed to extracellular matrix deposition in high glucose-induced GMCs.

**JAB1 contributed to inflammation and extracellular matrix deposition in high glucose-induced GMCs through regulation of ANGPTL2**

Silencing of JAB1 reduced protein expression of ANGPTL2 in high glucose-induced GMCs (Fig. 6A),

Fig. 1. JAB1 was elevated in high glucose-induced GMCs
(A) mRNA expression of JAB1 was up-regulated in high glucose-induced GMCs compared to the normal glucose condition. (B) Protein expression of JAB1 was up-regulated in high glucose-induced GMCs compared to the normal glucose condition. Data were expressed as mean ± SEM. ** vs. normal glucose, P < 0.01.
NG – normal glucose; HG – high glucose.

Fig. 2. Silencing of JAB1 restored expression of p21 in high glucose-induced GMCs
High glucose (HG and HG+si-NC) reduced protein expression of p21 in GMCs, while silencing of JAB1 (HG+si-JAB1) restored p21 expression in high glucose-induced GMCs. ** vs. NC, P < 0.01. ## vs. si-JAB1, P < 0.01.

Protein expression of TGF-β1, collagen I, collagen IV, and fibronectin was enhanced in high glucose-induced GMCs (Fig. 5). Transfection with si-JAB1 reduced expression of TGF-β1, collagen I, collagen IV, and fibronectin (Fig. 5), indicating that knockdown of JAB1 suppressed extracellular matrix deposition in high glucose-induced GMCs.

Fig. 3. JAB1 contributed to inflammation in high glucose-induced GMCs
(A) mRNA expression of JAB1 was up-regulated in high glucose-induced GMCs compared to the normal glucose condition. (B) Protein expression of JAB1 was up-regulated in high glucose-induced GMCs compared to the normal glucose condition. Data were expressed as mean ± SEM. ** vs. normal glucose, P < 0.01.
NG – normal glucose; HG – high glucose.
Fig. 3. JAB1 contributed to inflammation in high glucose-induced GMCs
(A) High glucose-induced GMCs were transfected with si-JAB1 for the down-regulation of JAB1. (B) Silencing of JAB1 reduced levels of IL-1β in high glucose-induced GMCs. (C) Silencing of JAB1 reduced levels of IL-6 in high glucose-induced GMCs. (D) Silencing of JAB1 reduced levels of TNF-α in high glucose-induced GMCs. (E) Silencing of JAB1 reduced levels of MCP-1 in high glucose-induced GMCs.
Data were expressed as mean ± SEM. ** vs. NG, P < 0.01. ## vs. HG+si-NC, P < 0.01. si-JAB1 – siRNA targeting JAB1; si-NC – negative control of siRNA.

Fig. 4. JAB1 contributed to oxidative stress in high glucose-induced GMCs
(A) Silencing of JAB1 reduced the level of ROS in high glucose-induced GMCs. (B) Relative fluorescence intensity in high glucose-induced GMCs with si-NC or si-JAB1 transfections. (C) Silencing of JAB1 attenuated high glucose-induced up-regulation of MDA in GMCs. (D) Silencing of JAB1 attenuated high glucose-induced down-regulation of SOD in GMCs.
** vs. NG, P < 0.01. ## vs. HG+si-NC, P < 0.01.
while over-expression of JAB1 enhanced ANGPTL2 (Fig. 6A). Decreased levels of IL-1β, IL-6, TNF-α, and MCP-1 in high glucose-induced GMCs caused by silencing of JAB1 were restored by over-expression of ANGPTL2 (Fig. 6B). Over-expression of ANGPTL2 attenuated the JAB1 suppression-induced decrease of TGF-β1, collagen I, collagen IV, and fibronectin in high glucose-induced GMCs (Fig. 6C), revealing the regulatory role of JAB1/ANGPTL2 in inflammation and extracellular matrix deposition in high glucose-induced GMCs.

Discussion

Glomerular hyperfiltration and hypertrophy, deposition of extracellular matrix, cell apoptosis, and inflammation in glomeruli are common steps in the development of diabetic nephropathy (Sulaiman, 2019). JAB1 has been shown to be involved in palmitate-induced insulin resistance, and suppression of JAB1 promoted the insulin sensitivity of hepatocytes (Zhao et al., 2020). Moreover, JAB1 contributed to renal cell carcinoma cell proliferation and suppressed cell apoptosis (Zhang et al., 2017). Therefore, the role of JAB1 in diabetic nephropathy was investigated in this study.

Modifications of glomerular mesangial cells behaviour has been shown to be implicated in the pathogenesis of glomerulosclerosis (Bartlett et al., 2017). Excessive proliferation of glomerular cells induces glomerular hypertrophy and contributes to development of progressive glomerulosclerosis, thus leading to diabetic nephropathy (Han et al., 2017). Moreover, excessive accumulation of extracellular matrix in mesangial cells is also regarded as a critical determinant in the development of diabetic nephropathy (Han et al., 2017). Increasing evidence supports the finding that high glucose induces excessive proliferation of glomerular cells (Tung et al., 2018). Therefore, high glucose-induced GMCs have been widely used as an in vitro cell model of diabetic nephropathy (Yang et al., 2016). In this study, GMCs were treated with 25 mM glucose. In line with the previous study that JAB1 was up-regulated in palmitate-induced hepatocytes (Zhao et al., 2020), high glucose promoted expression of JAB1 in GMCs. Therefore, JAB1 was hypothesized to promote cell proliferation of GMCs, thus contributing to progression of diabetic nephropathy.

Excessive inflammatory responses induced by high glucose with up-regulation of IL-1β, IL-6, and TNF-α have been reported to result in renal injury during the development of diabetic nephropathy and are regarded as a critical regulator of diabetic nephropathy (Han et al., 2017). Anti-inflammatory agents showed benefit for the amelioration of diabetic nephropathy (Parveen et al., 2018). JAB1 was involved in oxidized low-density lipoprotein-induced release of pro-inflammatory cytokines in macrophages (Schwarz et al., 2017). This study demonstrated that high glucose-induced increase of pro-inflammatory cytokines, IL-1β, IL-6, TNF-α, and MCP-1, was reduced by knockdown of JAB1. Therefore, JAB1 suppression could reduce the inflammation in diabetic nephropathy.

Kidney fibrosis involving excess deposition of extracellular matrix is considered as the outcome of progressive kidney diseases, including diabetic nephropathy (Kanasaki et al., 2013). Inflammation is responsible for the abnormal deposition of extracellular matrix, and leads to tubulointerstitial fibrosis and glomerulosclerosis during the development of diabetic nephropathy.
High glucose induced an increase in the major components of extracellular matrix, such as collagen I, collagen IV, and fibronectin, in mesangial cells (Chen et al., 2018). TGF-β1, an important cytokine for fibrogenesis and deposition of extracellular matrix in diabetic nephropathy, was also up-regulated by high glucose conditions in the mesangial cells (Chen et al., 2018). The present study showed that high glucose stimulated expression of TGF-β1, collagen I, collagen IV, and fibronectin in GMCs, while knockdown of JAB1 reduced the expression of TGF-β1, collagen I, collagen IV, and fibronectin to attenuate deposition of extracellular matrix.

Reactive oxygen species are elevated in the renal tissues post high glucose conditions, and oxidative stress triggers synthesis of extracellular matrix and inflammation to aggravate the tissue damage in diabetic nephropathy (Bai et al., 2019). JAB1 was found to be related to oxidative stress in relapsed acute monocytic leukaemia (Zhou et al., 2017). The anti-oxidant effect of JAB1 inhibition could contribute to amelioration of glucose-induced GMC damage.

ANGPTL2 was up-regulated in diabetic glomerulopathy (Sun et al., 2007), and a high serum level of ANGPTL2 was considered to be predictor of diabetic nephropathy (Konishi et al., 2017). ANGPTL2 promot-
ed renal fibrosis in diabetic nephropathy (Huang et al., 2019), while inhibition of ANGPTL2 suppressed extracellular matrix accumulation and inflammatory cytokine secretion to attenuate diabetic nephropathy (Yang et al., 2017). JAB1 in this study promoted expression of ANGPTL2 in high glucose-induced GMCs, and overexpression of ANGPTL2 attenuated the JAB1 suppression-induced decrease of IL-6, TNF-α, MCP-1, IL-1β, TGF-β1, collagen I, and collagen IV.

In summary, knockdown of JAB1 reduced the inflammation and repressed extracellular matrix accumulation in high glucose-induced GMCs, possibly through downregulation of ANGPTL2. These results might provide a novel potential target for the prevention of diabetic nephropathy.

Competing interests
The authors state that there are no conflicts of interest to disclose.

Contribution of authors
P. Z. designed the study, supervised the data collection, Y. G. and C. L. analysed the data, interpreted the data, Y. W. prepared the manuscript for publication and read and approved the manuscript. All authors have read and approved the manuscript.

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