Activation of CXCL16/CXCR6 pathway by inflammation accelerates the progression of diabetic nephropathy

Ze Bo Hu, Kun Ling Ma, Yang Zhang, Yu Wu, Liang Liu, Jian Lu, Bi Cheng Liu. Institute of Nephrology, Zhongda Hospital, Southeast University. Corresponding author: Kun Ling Ma, Email：klma05@163.com

Background

It has been accepted that diabetic nephropathy (DN) is a chronic inflammatory disease and always accompanied with lipid disorder. Inflammation and lipid disorder play crucial roles in synergistically accelerating the progression of DN. This study aimed to investigate the potential mechanisms of inflammation with lipid disorder in tubulointerstitial injuries of DN via *in vivo* and *in vitro* studies.

Methods

Diabetic db/db mice were randomly divided into two groups: db/db, and db/db+casein for eight weeks. Casein was subcutaneously injected to induce chronic inflammation. In *in vitro* study, HK-2 cells were treated with IL-1β with high concentration of glucose. The morphological change of renal pathology and ultra-microstructure were checked by pathological staining and electron microscopy. Lipid accumulation in kidneys was observed by Filiping staining and quantitative assay of intracellular free cholesterol. The expression of CXCL16/CXCR6 pathway, inflammatory cytokine, and fibrosis related molecules were detected by immunohistochemistry or immunofluorescence staining, and Western blot. Reactive oxygen species (ROS) staining was performed by DCFH-DA and Semi-quantified by Flow Cytometry.

Results

1. Inflammation was established by casein injection in mice.

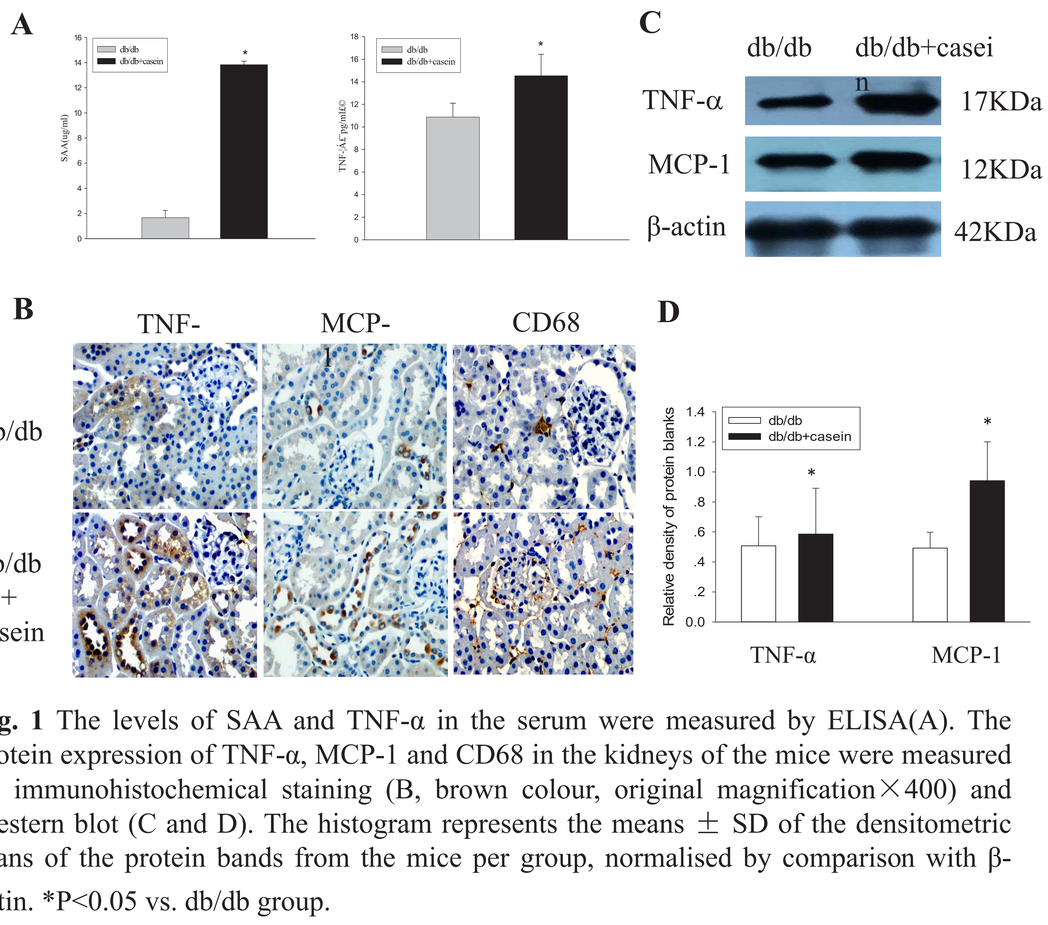

Fig. 1 The levels of SAA and TNF-α in the serum were measured by ELISA(A). The protein expression of TNF-α, MCP-1 and CD68 in the kidneys of the mice were measured by immunohistochemical staining (B, brown colour, original magnification×400) and Western blot (C and D). The histogram represents the means ± SD of the densitometric scans of the protein bands from the mice per group, normalised by comparison with β-actin. *P<0.05 vs. db/db group.

2. Inflammation exacerbated the damage to the kidney in db/db mice

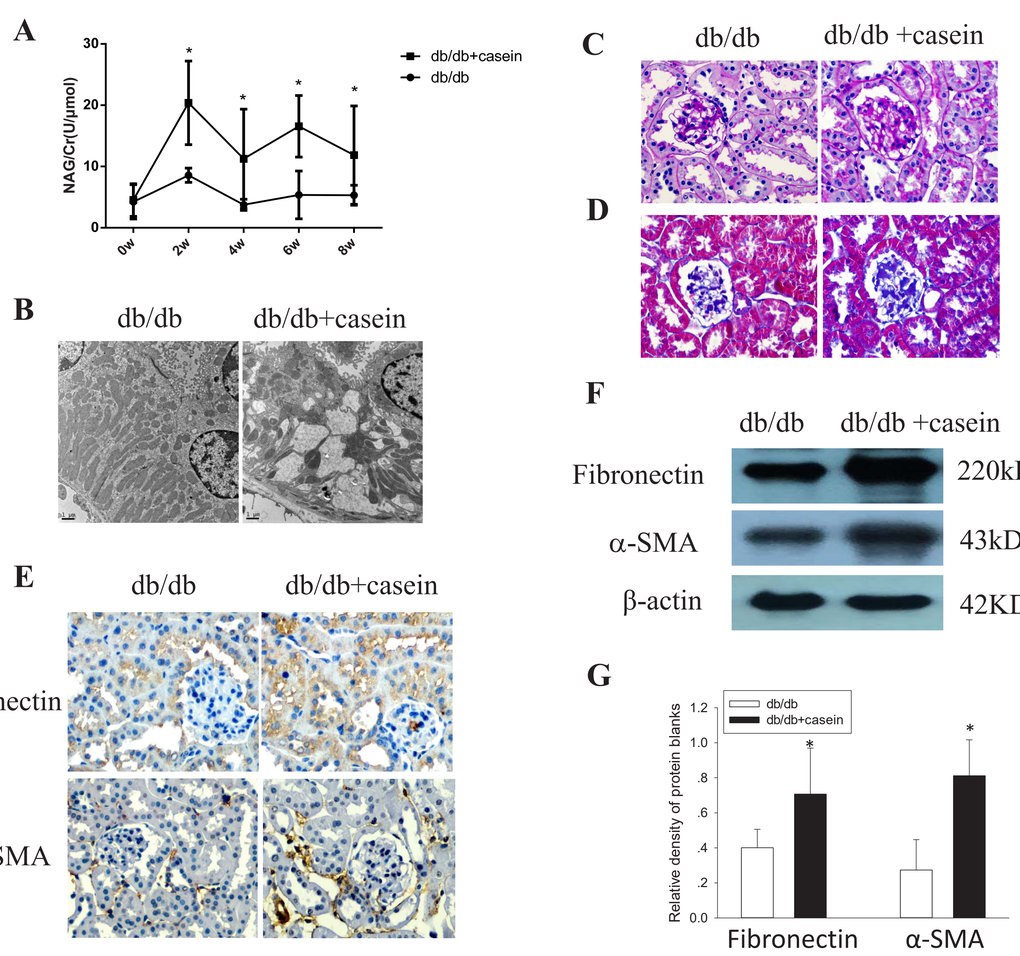

Fig.2 The quantitative analysis of the 24-hour urinary NAG each week in the mice was checked by the ELISA (A). The change in the glomerular ultramicrostructure was checked by electromicroscope (B) The histopathological changes were assessed using PAS staining (C, red colour, original magnification ×400). Masson's trichrome (D, blue colour, original magnification ×400). The protein expression of Fibronectin and a-SMA in the kidneys of the mice were measured by immunohistochemical staining (E, brown colour, original magnification×400) and Western blotting (F and G). The histogram represents the means ± SD of the densitometric scans of the protein bands from the mice per group, normalised by comparison with β-actin. *P<0.05 *vs*. db/db group.

3. Inflammation increased lipid accumulation in tubular epithelium in vivo and *in vitro*.

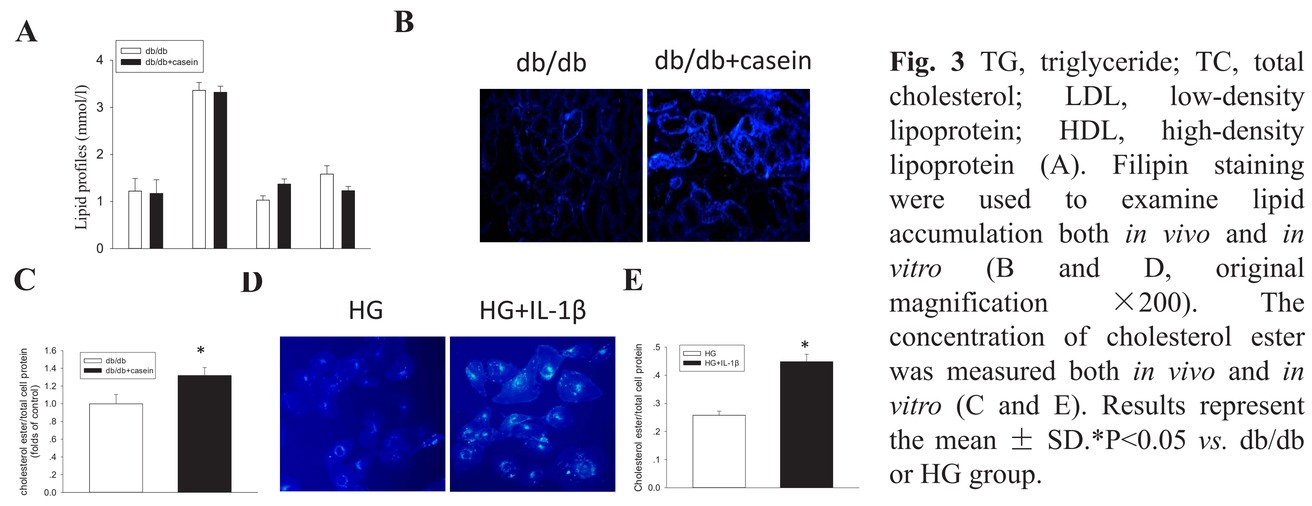

Fig. 3 TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein (A). Filipin staining were used to examine lipid accumulation both *in vivo* and *in vitro* (B and D, original magnification ×200). The concentration of cholesterol ester was measured both *in vivo* and *in vitro* (C and E). Results represent the mean ± SD.*P<0.05 *vs.* db/db or HG group.

4. Inflammation accelerated lipid-mediated tubular epithelium injuries *in vitro.*

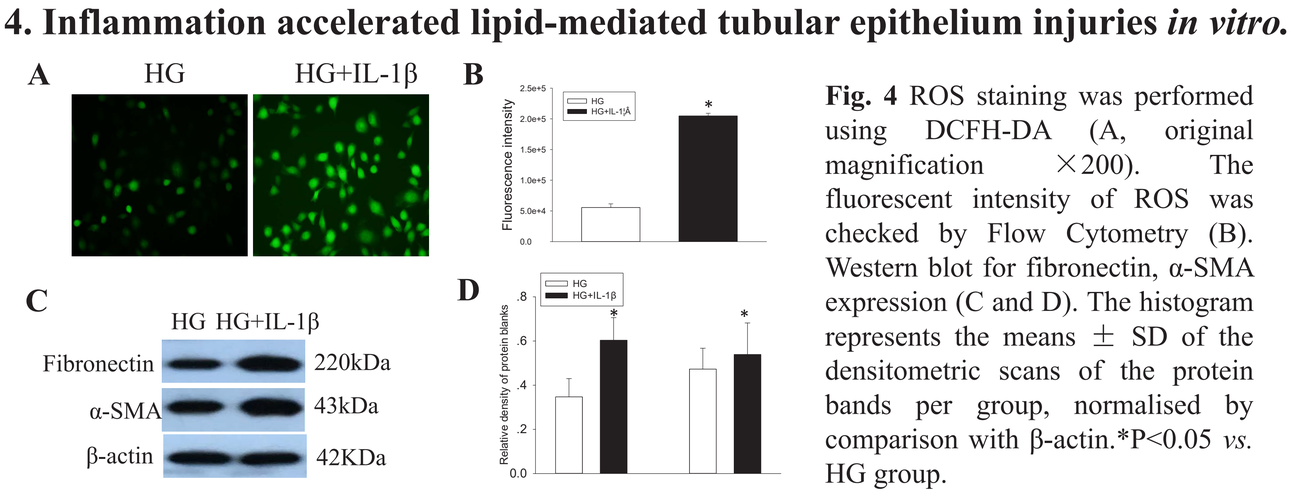

Fig. 4 ROS staining was performed using DCFH-DA (A, original magnification ×200). The fluorescent intensity of ROS was checked by Flow Cytometry (B). Western blot for fibronectin, α-SMA expression (C and D). The histogram represents the means ± SD of the densitometric scans of the protein bands per group, normalised by comparison with β-actin.*P<0.05 *vs.* HG group.

5. Inflammation increased protein expression of CXCL16 pathway *in vivo* and *in vitro.*

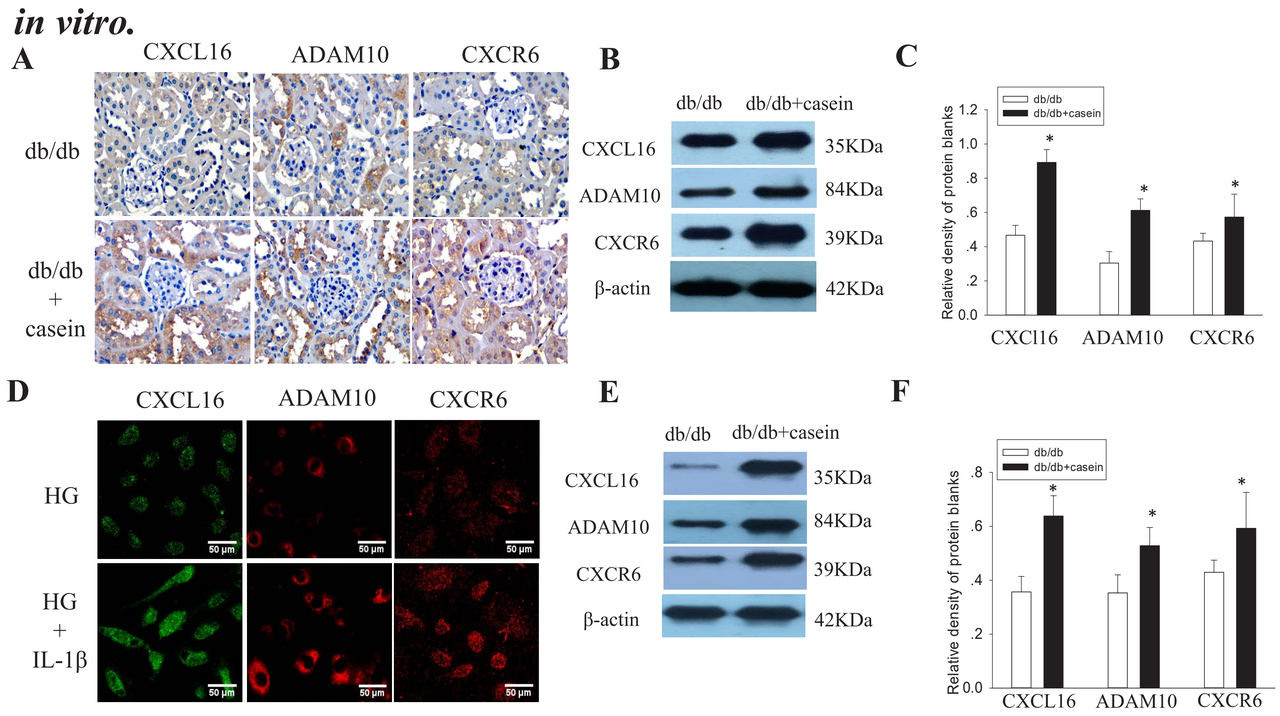

Fig. 4 The protein expression of CXCL16, ADAM10 and CXCR6 were measured by immunohistochemical staining *in vivo* (A, brown colour, original magnification×400) and Western blot (B and C). Immunofluorescence staining (D, original magnification×400) and Western blot (E and F) for CXCL16, ADAM10, CXCR6 *in vitro*. The histogram represents the means ± SD of the densitometric scans of the protein bands per group, normalised by comparison with β-actin. *P<0.05 *vs.* db/db or HG group.

6. Inhibition of CXCL16 pathway decreased lipid accumulation and alleviated tubular epithelium injuries.

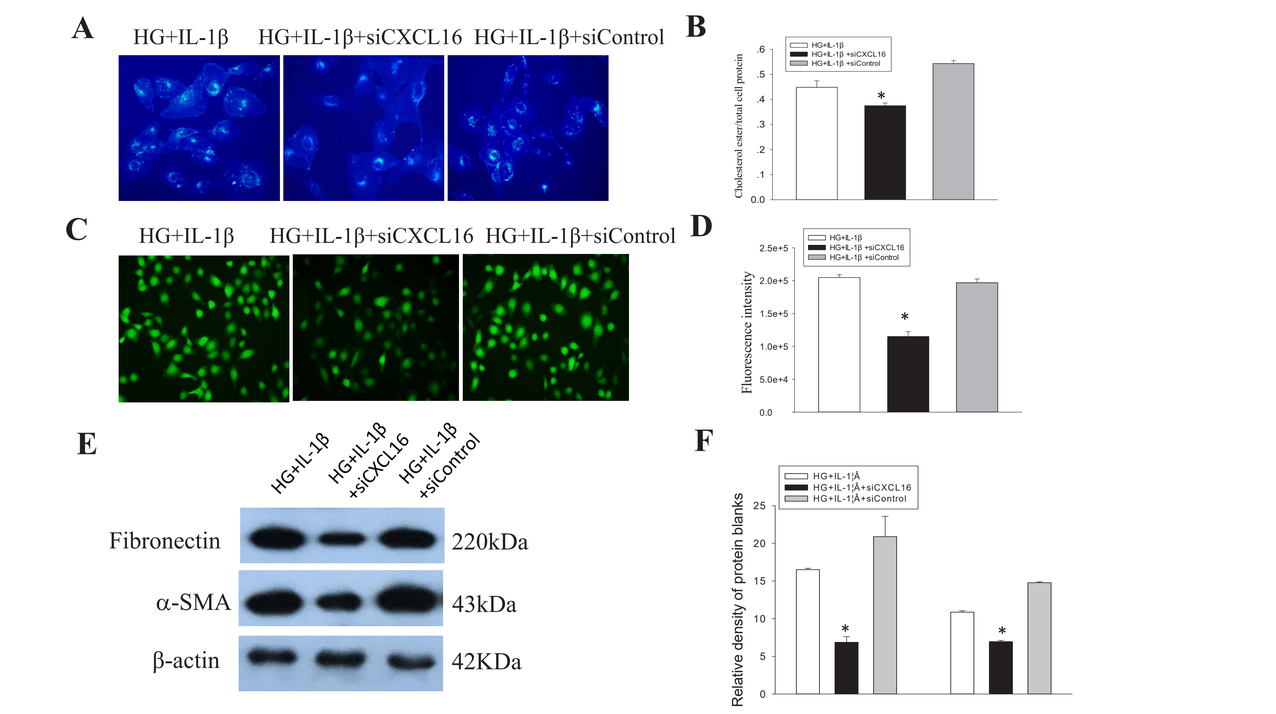

Fig. 4 Filipin staining were used to examine lipid accumulation in each group (A and B). ROS staining was performed using DCFH-DA (C, original magnification ×200). The fluorescent intensity of ROS was checked by Flow Cytometry (D). The protein expression of fibronectin and α-SMA were measured by Western blotting (E and F). The histogram represents the means ± SD of the densitometric scans of the protein bands per group, normalised by comparison with β-actin. *P<0.05 vs. HG group.

Conclusion

Inflammation accelerates tubulointerstitial injury in DN partly through the activation of CXCL16/CXCR6 pathway, which may facilitate lipid accumulaton in tubular epithelial cells.

DOI: 10.3252/pso.eu.53era.2016