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Transmembrane protein 72, expressed in the distal convoluted tubule, may play a potential role in diabetic kidney disease

Weiting He, Jianteng Xie, Min Lu, Danfeng Liu[2], Runli Jia, Wenjian Wang
Department of Internal Medicine-Nephrology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, China

Objectives : Transmembrane protein 72 (TMEM72) is highly expressed in tubules of the kidney in patients with diabetic nephrology. This study is designed to explore the role and the potential mechanism of TMEM72 in the development of diabetic tubulopathy.
Methods : Serum TMEM72 concentration was tested in health control, patients with diabetes mellitus (DM) and diabetic kidney disease (DKD). The variation trend of TMEM72 was determined by immunohistochemistry on kidney tissues from patients in different stage of DKD. Immunofluorescence staining was performed with TMEM72, SGLT2, NKCC2 and AQP2 to identify the expression site of TMEM72 in mice renal tubules. To investigate the potential cellular pathway that TMEM72 was involved, an immunofluorescence test was performed with TMEM72, LAMP1, Mito-tracker and Calnexin in cultured distal convoluted tubule (DCT) epithelial cells. Western blot was used to detect the activity of TMEM72 in HK2 cells following HG treatment.
Results : The concentration of serum TMEM72 was lower in DM and DKD groups compared to the health control ($P<0.001$). The expression of TMEM72 also decreased gradually in human kidney tissue of different stage of DKD following the progression of disease. Co-localization of TMEM72 and NKCC2 in immunofluorescence staining indicated that TMEM72 was mainly expressed in the lysosomes of distal convoluted tubule. In vitro, TMEM72 was markedly down-regulated in mouse tubular cell and HK-2 cells treated with high glucose. Overexpression or knock down of TMEM72 demonstrated a significant increased or decreased mitophagy in high glucose treated HK2. In vivo, tubules specific knock-out of TEME72 exhibited a significantly tubule injury evidenced by marked increase of KIM-1/Cr, NGAL/Cr, Albumin/Cr β-micro globin/Cr in the urine of mouse.
Conclusions : Our current study has revealed that TMEM72 may act as a novel participator in DKD by being involved in the renal tubular injury.

Fig. 1.jpg

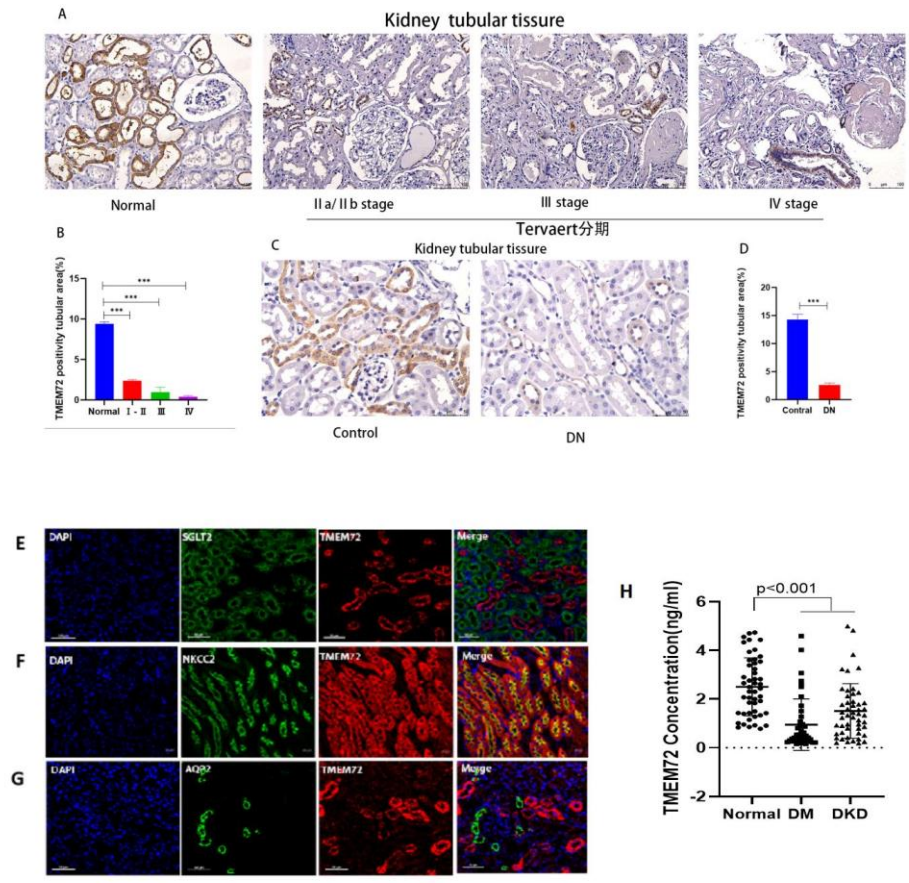

Fig. 1 A-D. The expression of TMEM72 in human kidney tissue. (A) The expression of TMEM72 decreased gradually in kidney tissue of different stage of DKD followed the progression of disease. (B) Semi-quantitative analysies of the immunohistochemically stained TMEM72 positive tubule area to fig. A. (C) TMEM72 immunohistochemical staining in kidney tubular sections from mouse with healthy control and DN. (D) Semi-quantitative analysies of the immunohistochemically stained TMEM72 positive tubule area to fig. C. DN, diabetic nephrolopathy. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$; ns, no significance. **E-G. Localization of TMEM72 shown by immunofluorescence staining in mouse kidney tissue.** (**E**) TMEM72(red) and SGLT2(green) did not show co-localization, DAPI(blue). (**F**) TMEM72(red) co-localized with NKCC2(green) as representative immunofluorescent staining shown, DAPI(blue). (**G**) TMEM72(red) and AQP2(green) did not show co-localization, DAPI(blue). **H.** Serum TMEM72 concentration in health control (Normal, n=49), patients with diabetes mellitus (DM, n=44) and diabetic kidney disease (DKD,n=50). $p < 0.001$ *vs.* Normal.

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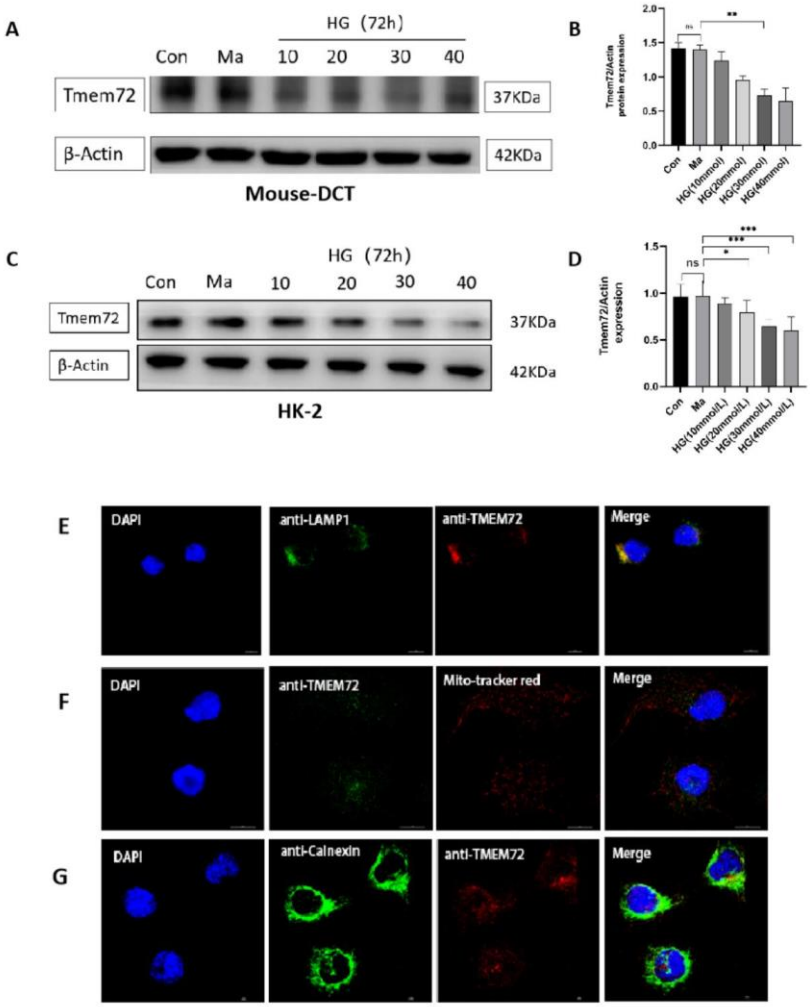

Fig.2 A-D. Expression of TMEM72 in cultured cells stimulated by different glucose concentrations in vitro. (**A and B**) TMEM72 was down-regulated as the glucose concentration gradient rised in cultured mouse DCT cells. Densitometric analysis using Western blot by three independent experiments. (**C and D**) TMEM72 was down-regulated as the glucose concentration gradient rised in cultured human kidney-2(HK-2) cells. Densitometric analysis using Western blot by three independent experiments. **E-G. Localization of TMEM72 shown by immunofluorescence staining in cultured mouse DCT cells.** (E) TMEM72(red) co-localized with LAMP1(green) as representative immunofluorescent staining shown, DAPI(blue). (**F**) TMEM72(red) and Mito-tracker(green) did not show co-localization, DAPI(blue). (**G**) TMEM72(red) and Calnexin(green) did not show co-localization, DAPI(blue).