RENAL TUBULE-SPECIFIC NRF2 DELETION ATTENUATES GLOMERULAR HYPERFILTRATION AND KIDNEY INJURY VIA DOWN-REGULATION OF SGLT2 AND ANGIOTENSINOGEN EXPRESSION IN DIABETIC MICE

WCN 24-AB-1234

John S.D. Chan, Ke Su, Shuiling Zhao, Wen-Xia Yang, Junzheng Peng, Kana Miyata, Janos Filep, Julie R. Ingelfinger, Shao-Ling Zhang

Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM), Montreal, QC, Canada

Abstract

Introduction Nuclear factor erythroid 2-related factor 2 (NRF2) functions as a master regulator of redox balance, conferring cellular cytoprotective responses. The effects of NRF2 activation are, however, controversial in animals and humans with diabetes. We reported previously that overexpression of NRF2 in renal proximal tubular cells (RPTCs) increases expression of sodium-glucose co-transporter 2 (SGLT2) and angiotensinogen (AGT, the sole precursor of angiotensins), exacerbates dysglycemia and progression of diabetic kidney disease (DKD) in type 1 diabetic (T1D) Akita *Nrf2*$^{-/-}$/*Nrf2*^{RPTC} transgenic mice (Diabetes 2021). The physiological role of renal NRF2 in the progression of DKD is not completely understood. We studied the impact of *Nrf2* deletion specifically in renal tubule (RT) of Akita mice on progression of DKD.

Methods Akita RT-specific *Nrf2* knock-out (Akita *Nrf2*^{RT} KO) mice were generated by cross-breeding Akita with *Nrf2*^{RT} KO mice using Pax8-Cre (male *Nrf2* floxed mice were crossbred with female RT-specific Cre deleter (Pax8-Cre) mice). Renal functional and morphological changes were assessed in male Akita *Nrf2*^{RT} KO mice vs. Akita *Nrf2*$^{lox/lox}$, non-diabetic *Nrf2*$^{lox/lox}$ and *Nrf2*^{RT} KO mice at 10 to 20 weeks of age. Immunostaining on kidney sections, Western blot (WB) and real-time qPCR (RT-qPCR) were used to assess protein and gene expression in isolated renal proximal tubules (RPTs).

Results Glomerular filtration rate (GFR) (estimated with fluorescein isothiocyanate inulin) was increased in Akita mice but was normalized in Akita *Nrf2*^{RT} KO mice. Fasting blood glucose (FBG), systolic blood pressure (SBP, monitored with a BP-2000 tail-cuff pressure monitor), kidney hypertrophy, glomerular tuft volume, RPTC volume, tubular luminal dilatation, tubular injury score, podocyte loss (assessed by p57 and WT-1 immunofluorescence staining) and urinary albumin-creatinine ratio were significantly increased in Akita mice vs. nondiabetic *Nrf2*$^{lox/lox}$ and *Nrf2*^{RT} KO mice. These abnormalities were greatly attenuated in Akita *Nrf2*^{RT} KO mice, except for FBG and SBP. Fractional excretion of glucose was increased in Akita mice vs. non-diabetic *Nrf2*$^{lox/lox}$ and *Nrf2*^{RT} KO mice and increased further in Akita *Nrf2*^{RT} KO mice. Treatment with a selective A1 adenosine receptor inhibitor (A1aRi; blockade of tubuloglomerular feedback (TGF)) of Akita and Akita *Nrf2*^{RT} KO mice resulted in increases in GFR vs. untreated Akita and Akita *Nrf2*^{RT} KO mice, respectively. The increases in GFR were significantly greater in Akita *Nrf2*^{RT} KO mice than in Akita mice. SGLT2 and AGT expression in RPTs were significantly lower in Akita *Nrf2*^{RT} KO mice vs. Akita mice.

Conclusion Our data demonstrated that selective RT-*Nrf2* deletion ameliorates glomerular hyperfiltration and kidney injury in Akita mice, indicating that renal NRF2 signaling plays an important role in modulating GFR and DKD progression, at least in part, via the regulation of SGLT2 and AGT expression in RPTs and TGF in diabetic mice.

Introduction

Nuclear factor erythroid-2 related factor 2 (NRF2) expression is increased in kidneys of mice and patients with diabetes. We have reported that overexpression of NRF2 in renal proximal tubular cells (RPTCs) increases sodium-glucose co-transporter 2 (SGLT2) and angiotensinogen (AGT) expression and exacerbates dysglycemia and progression of nephropathy in type 1 diabetic (T1D) Akita Nrf2-/-/Nrf2RPTC transgenic (Tg) mice (Diabetes 2021). However, the pathophysiological role of renal NRF2 in the progression of diabetic kidney disease (DKD) is not well understood. We now report the impact of Nrf2 deletion specifically in renal tubules (RT) of Akita mice on the expression of SGLT2 and AGT in RPTCs in the setting of hyperglycemia and kidney injury.

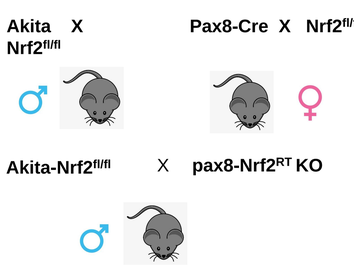
Hypothesis

Renal Tubule-Specific NRF2 Deletion Down-Regulates SGLT2 and Angiotensinogen Expression and Ameliorates GFR and Kidney Injury in Akita Mice

Methods

Generation of Akita Nrf2^{RT}KO mice

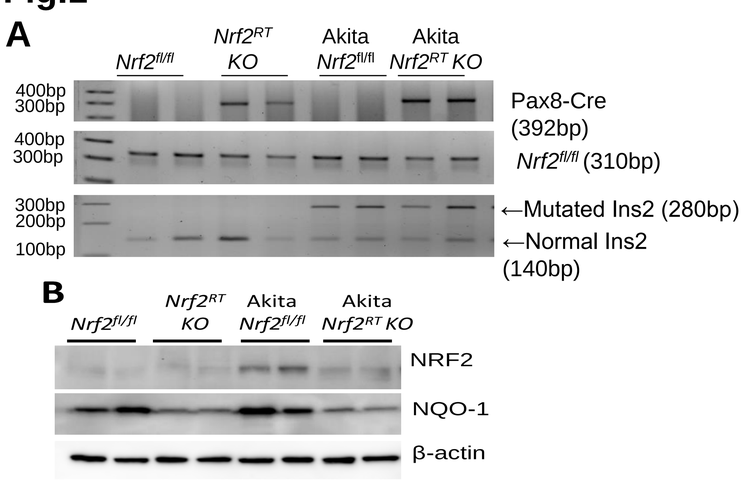
- Akita RT-specific Nrf2 knock-out (Akita *Nrf2*^{RT} *KO*) mice were generated by crossbreeding Akita with Nrf2RT KO mice using Pax8-Cre (through crossbreeding male Nrf2 floxed mice with female RT-specific Cre deleter (Pax8-Cre) mice). Physiological and kidney morphological changes were assessed in male Akita *Nrf2*^{RT} *KO*, Akita *Nrf2*$^{fl/fl}$, non-diabetic *Nrf2*$^{fl/fl}$ and *Nrf2*^{RT} *KO* mice at the age of 10 to 20 weeks.



A1aRi were administered by ip for 1 weeks

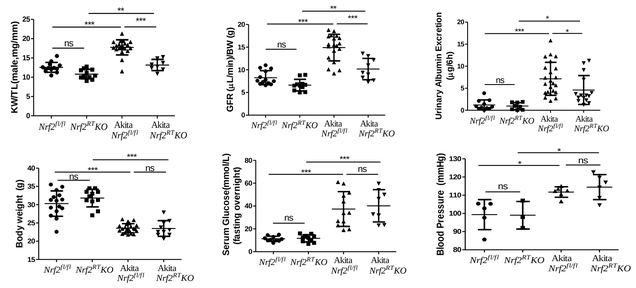
Results

Fig.1



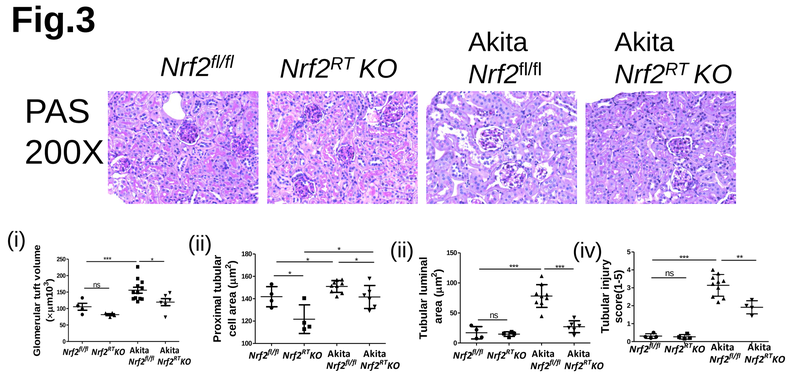
***RT-Nrf2* Knockout in Akita mice abolished NRF2expression in RPTs**

Fig.2



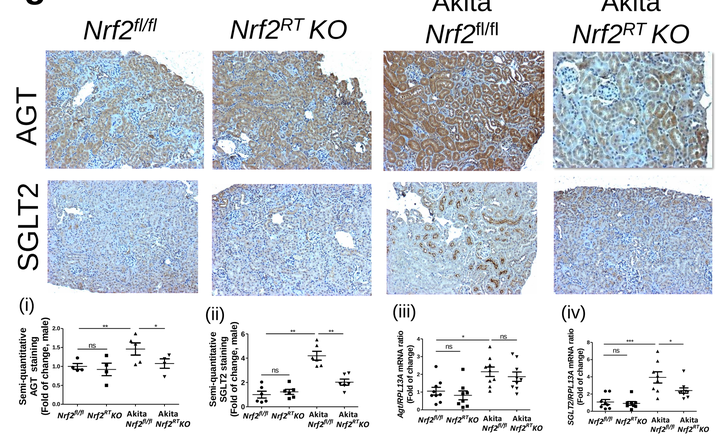
RT-*Nrf2* Knockout in Akita mice significantly attenuated kidney weight (KW)/tibial length (TL), glomerular filtration rate (GFR) and urinary albumin excretion and did not significantly affect body weight, fasting blood glucose and systolic blood pressure (SBP) vs. Akita mice.

Fig.3



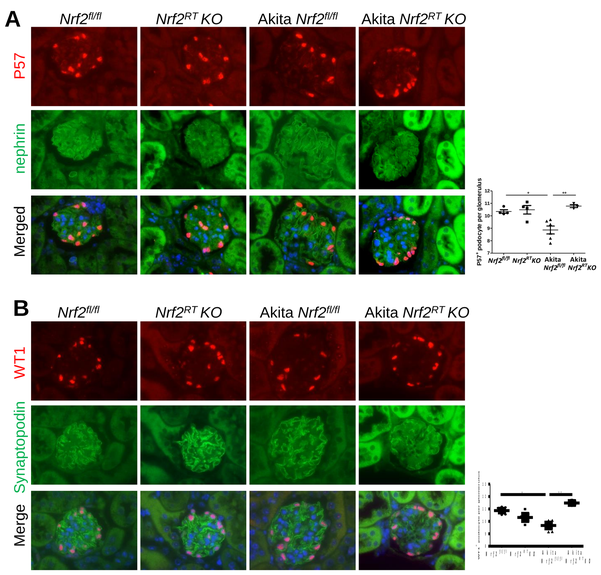
RT-*Nrf2* Knockout in Akita mice significantly attenuated glomerular tuft volume, proximal tubular cell size, tubular luminal dilatation with accumulation of cell debris and tubular injury score vs. Akita mice.

Fig. 4



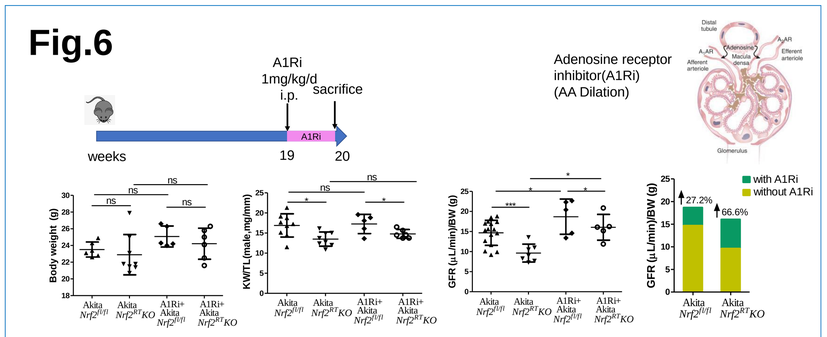
RT-*Nrf2* Knockout in Akita mice significantly attenuated AGT and SGLT2 protein and mRNA expression in RPTs vs. Akita mice.

Fig. 5



Podocyte numbers (p57/nephrin and WT1/synaptopodin IF staining) were significantly decreased in Akita mice and normalized in RT-*Nrf2* Knockout Akita mice.

Fig.6



A1Ri administration significantly attenuated KW/TL and GFR in Akita mice and its effect was further enhanced in RT-*Nrf2* Knockout Akita mice but not BW.

Conclusions

RT-Nrf2 deletion ameliorates GFR and kidney injury in Akita mice, indicating renal NRF2 is important in tubuloglomerular feedback via down-regulation of intrarenal SGLT2 and AGT expression.

Acknowledgements

Supported by Canadian Institutes of Health Research (CIHR), Kidney Foundation of Canada (KFOC) and China Scholarship Council