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Ablation of carbohydrate-responsive element-binding protein improves kidney injury in streptozotocin-induced diabetic mice

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Abstract. – OBJECTIVE: Carbohydrate-responsive element-binding protein (ChREBP) has been reported to regulate glucose and lipids metabolism in the liver. However, its role in the complicated pathophysiology of diabetic nephropathy is not understood.

MATERIALS AND METHODS: C57BL/6 mice treated with streptozotocin (STZ) or vehicle control to induce diabetic models. The mRNA and protein levels of ChREBP in kidneys of control and diabetic mice were determined by real-time PCR or Western Blot, respectively. The expression of inflammatory and endoplasmic reticulum stress markers in ChREBP deficient or Wild-type mice was also determined by real-time PCR or Western Blot. Urine was collected over 16 hours on the day prior to sacrifice, and albuminuria and urine creatinine were determined by Elisa or Creatinine Assay Kit.

RESULTS: We found that expression of ChREBP and its downstream target genes were up-regulated in C57BL/6 mice with diabetic nephropathy. Subsequently, we demonstrated that ChREBP knockout mice were protected against the development of diabetic nephropathy induced by streptozotocin (STZ), showing less albuminuria, inflammation and glomerular hypertrophy as compared to diabetic wild-type mice. Reduced expression of inflammatory and endoplasmic reticulum stress markers were also observed in ChREBP deficient mice.

CONCLUSIONS: Our data indicate that ablation or inhibition of ChREBP might improve kidney injury in streptozotocin-induced diabetic animals.

Key Words:
Carbohydrate-responsive element-binding protein, Diabetic nephropathy, Kidney injury, Diabetic mice.

Introduction

Diabetic nephropathy (DN), a serious complication of type 2 diabetes, is one of the most common causes of chronic kidney disease globally[1-5]. Clinically, DN is characterized by macroalbuminuria and decline in glomerular filtration rate (GFR), hypertension, and a high risk of cardiovascular morbidity and mortality[6,7]. However, its molecular mechanisms remain largely unexplored.

Excess carbohydrate or glucose has been shown to regulate de novo lipogenesis by inducing genes expression of liver pyruvate kinase (LPK) and stearoyl-CoA desaturase-1 (SCD-1)[8,9]. In this process, a protein named carbohydrate response element binding protein (ChREBP) play a critical role[10]. ChREBP could bind to the carbohydrate response element (ChoRE) in the promoter regions of LPK and SCD-1, to activate their mRNA transcription[11,12]. In the liver, the expression and activity of ChREBP are regulated by fasting and feeding[13,14]. During feeding, xylulose-5-phosphate in the hexose monophosphate pathway activates protein phosphatase 2A, which dephosphorylates ChREBP and activates its transcriptional activity[13,14]. As a result, ChREBP knockout mice exhibited reduced liver triglycerides and glycogen contents, compared to wild-type mice[15,16]. Besides, ChREBP was markedly up-regulated in diabetic livers and knockdown of ChREBP in obese mice improves metabolic disorders, including insulin resistance, glucose intolerance and liver steatosis[17,18].

In the present study, we examined the expression levels of ChREBP and its target genes in the kidney of streptozotocin-induced diabetic mice and further investigated the roles of ChREBP in diabetic nephropathy.

Materials and Methods

Animals

Male C57BL/6 mice were purchased from the Shanghai Laboratory Animal Company (SLAC,

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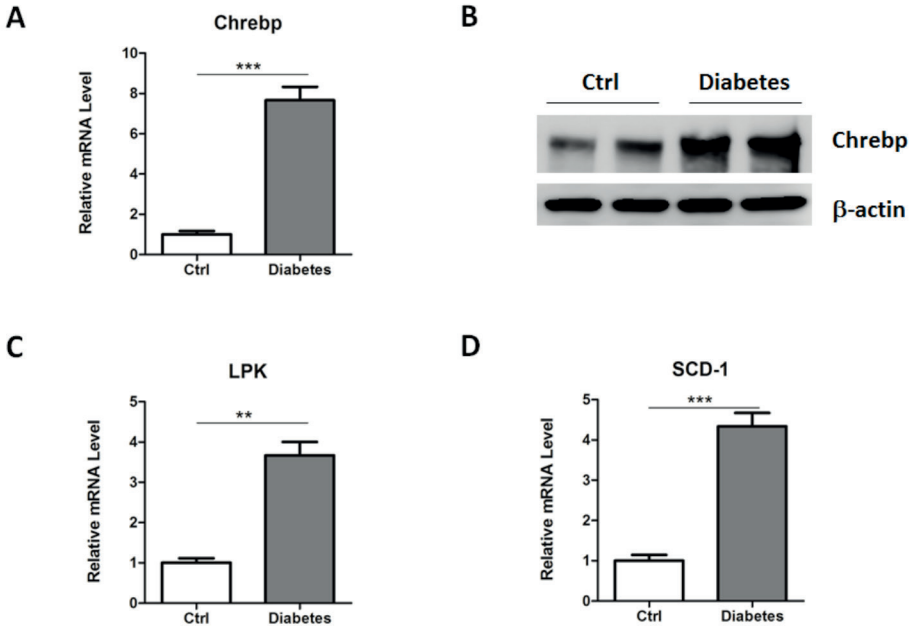

Figure 1. Expression of Chrebp and its target genes was upregulated in the early diabetic kidney in WT mice. ***(A-B)*** Relative mRNA and representative protein levels of Chrebp in wild-type mice after diabetes induction. ***(C-D)*** Relative mRNA levels of LPK and SCD-1 were determined.

Shanghai, China). ChREBP knockout mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and backcrossed to C57BL/6 background for 5 generations. All animal experiments were performed with the approval of the Animal Ethics Committee of the Jining No. 1 People's Hospital, China.

Animal Experiments

Mice were fasted for 4 hours; then, they were injected intraperitoneally with 60 mg/kg STZ (Sigma-Aldrich, St. Louis, MO, USA) or vehicle control for 5 consecutive days. Mice with a blood glucose level over 16 mmol/L were considered to be diabetic. Animals were sacrificed at week 6, week 12 and week 24, respectively.

Sample Harvest and Quantification of Albuminuria and Urine Creatinine

Urine was collected over 16 hours on the day prior to sacrifice. Blood and kidney tissues were harvested at sacrifice. Tissue slices were fixed with 10% neutral-buffered formalin for paraffin embedding, frozen in OCT compound (Sakura Finetek Inc., Torrance, CA, USA) or snap frozen in liquid nitrogen for mRNA extraction. Urine albumin was quantified using the Mouse Albumin ELISA Quantitation Set according to the manufacturer's instructions (Bethyl Laboratories, Montgomery, TX, USA). Urine creatinine was measured enzymatically by Creatinine Assay Kit (ab65340, Abcam, Cambridge, MA, USA).

Real-Time RT-PCR

Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was amplified in Universal Master Mix (Applied Biosystems, Foster City, CA, USA) with gene-specific primers. Quantitative real-time PCR was performed by using an Applied Biosystems 7300 Real-time PCR System and a TaqMan Universal PCR Master Mix. Expression levels of the target genes were normalized to that of the β-actin.

Western Blots

Tissues samples were harvested and lysed with ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4, 100 mM β-Mercaptoethanol, 2% w/v SDS, 10% glycerol). Proteins in the supernatants were quantified by bicinchoninic acid assay (BCA), and an equally amount of proteins were separated by 12% SDS-polyacrylamide gel electrophoresis (PAGE). Immunoblots

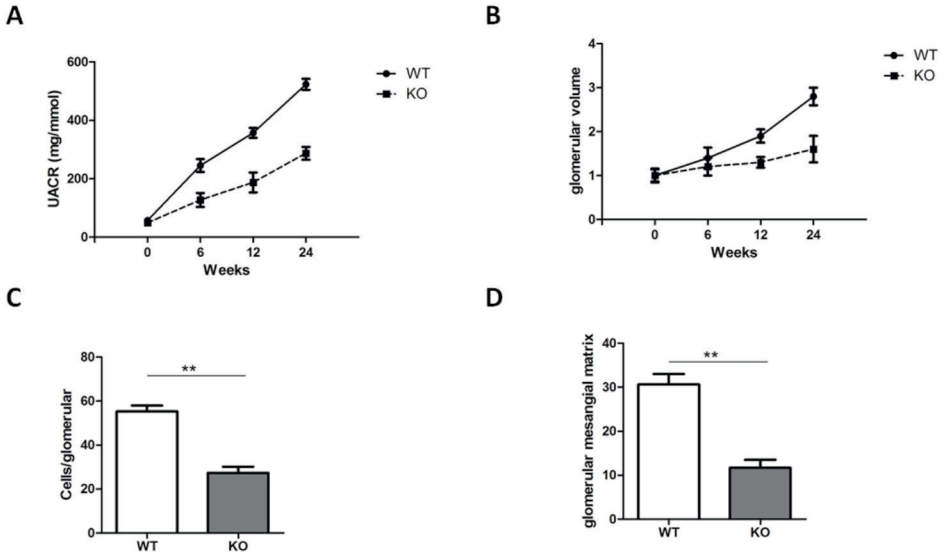

Figure 2. Chrebp deficiency attenuated albuminuria in DN compared to WT mice. ***(A-D)*** UACR, glomerular volume, glomerular cellularity and glomerular mesangial matrix was determined in WT and KO mice.

were performed using primary antibodies targeting ChREBP, p65, p-p65, IRE1a, p-IRE1a, pERK, p-pERK, and CHOP. All antibodies were purchased from Abcam (Cambridge, MA, USA). Protein levels were normalized to total β-actin, using a rabbit anti-β-actin antibody (Abcam, Cambridge, MA, USA). The proteins were then visualized by a Millipore Immobilon Western Chemiluminescent HRP Substrate (Merck KGaA, Darmstadt, Germany).

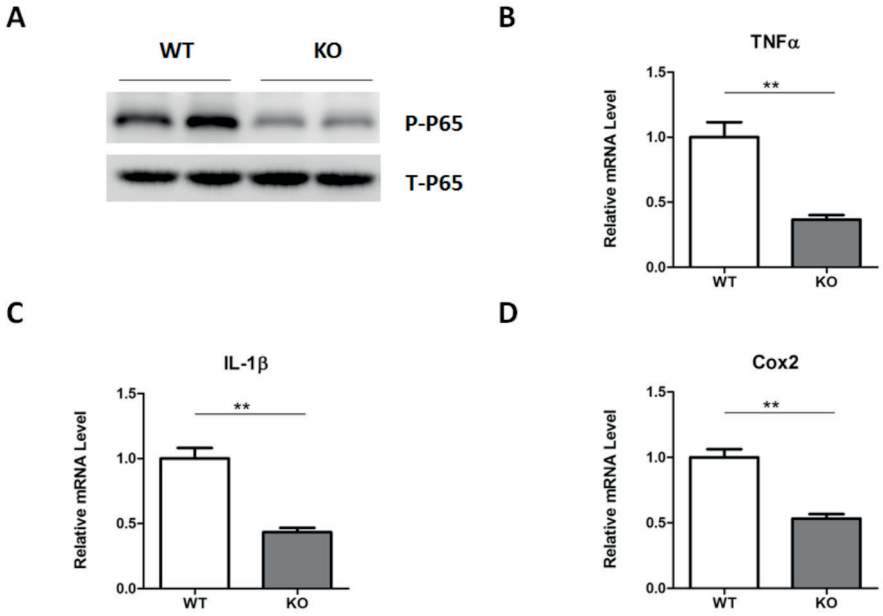

Figure 3. Expression of pro-inflammatory cytokines. ***(A)*** Representative protein levels of phosphorylated p65 in two groups of mice. ***(B)*** Relative mRNA levels of TNFα, IL-1β and Cox2 in two groups of mice

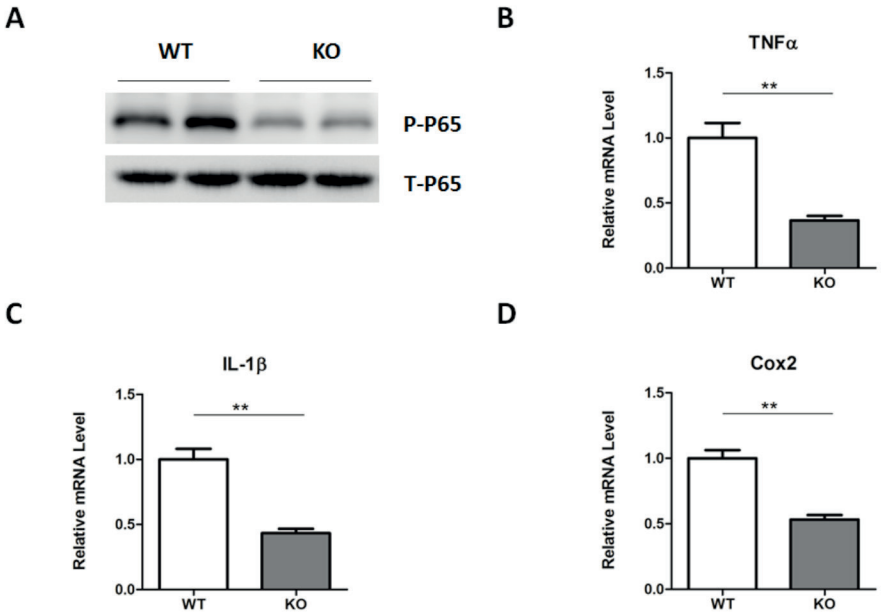

Figure 4. Expression of endoplasmic reticulum stress-makers. ***(A-B)*** Representative protein levels of phosphorylated IRE1a and PERK in two groups of mice

Statistical Analysis

All data are presented as mean ± SEM. The differences between two groups were analyzed by Student *t*-tests. *p*-values less than 0.05 were considered statistically significant.

Results

Expression of ChREBP were Upregulated in Kidneys of Diabetic Mice

Firstly, to examine the expression levels of ChREBP in diabetic mice, C57BL/6 mice were treated with streptozotocin (STZ) or vehicle control to induce diabetic models. 10 weeks later, mice were sacrificed and kidneys were removed to analyze genes expression. As a result, mRNA and protein levels of ChREBP were significantly up-regulated in the kidneys of diabetic mice (Figure 1A and 1B). Besides, mRNA expression of its target genes, LPK and SCD-1, were also up-regulated (Figure 1C and 1D).

ChREBP Deficiency Attenuated Albuminuria

The up-regulation of ChREBP in kidneys of diabetic mice suggested that ChREBP might play a role in the development of DN. Therefore, wild-type (WT) and ChREBP knockout mice were treated with STZ for several weeks. As a result, urine albumin-to-creatinine ratio (UACR), glomerular volume, glomerular cellularity and glomerular mesangial matrix were all reduced in ChREBP knockout mice (Figure 2A-2D).

Expression of Inflammatory Molecules was Reduced in Kidneys of ChREBP Knockout Mice

Aberrant activation of inflammation and endoplasmic reticulum stress play an important role in the development of DN[19,20]. Therefore, the expression of inflammatory markers, including phosphorylated p65 and mRNA levels of pro-inflammatory cytokines were determined. As shown in the Figure 3A-3D, phosphorylated p65 was attenuated in ChREBP knockout mice (Figure 3A). Besides, expression of inflammatory molecules was also reduced (Figure 3B-3D). Moreover, phosphorylated IRE1α and PERK were also reduced in ChREBP knockout mice (Figure 4A-4B). In agreement, expression of CHOP, markers of cell apoptosis, was significantly decreased (Figure 5A-5B).

Discussion

Although the role of ChREBP in the regulation of hepatic glycolysis and de novo lipogenesis has

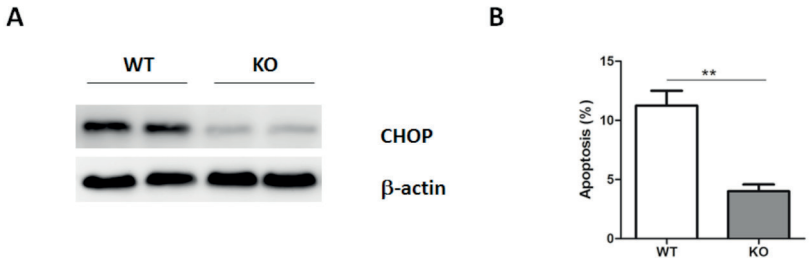

Figure 5. Cell apoptosis in two groups of mice. ***(A)*** Relative mRNA levels of CHOP in two groups of mice. ***(B)*** Cell apoptosis was determined by TUNEL.

been well-established, its expression and function in the development of DNA remain incompletely understood. Notably, a recent study investigated the role of ChREBP in mesangial cells in diabetic nephropathy[21]. They found that treatment with high glucose increased cellular O-GlcNAc and O-GlcNAcylated ChREBP in mesangial cells compared with normal glucose[21]. Here, our *in vivo* results showed that expression of ChREBP was increased in mice treated with hyperglycemia. We speculate that these results were consistent, suggesting that up-regulation of ChREBP activity and (or) expression might be an important event in the development of DN.

To further confirm the role of ChREBP, wild-type and ChREBP knockout mice were used. We treated these two groups of mice with STZ to induce hyperglycemia and diabetes. As a result, urine albumin-to-creatinine ratio (UACR), glomerular volume, glomerular cellularity and glomerular mesangial matrix were all reduced in ChREBP knockout mice, suggesting ablation of ChREBP protects diabetic mice from kidney injury. Moreover, at the molecular level, we found that expression of inflammatory and endoplasmic reticulum stress were also attenuated in ChREBP knockout mice, further confirm the roles of ChREBP in the development of DN. However, whether ChREBP could directly or indirectly regulate inflammation or endoplasmic reticulum stress in kidneys remains to be determined in future studies.

Conclusions

Our studies identified ChREBP as a key mediator in DN. Given the involvement of ChREBP signaling in these pivotal phases of diabetic nephropathy, strategies to down-regulate or suppress the expression or activity of ChREBP should be explored to target DN.

Conflict of Interest

The authors declare no conflicts of interest.

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