PACS-2 deficiency in tubular cells aggravates lipid-related kidney injury in diabetic kidney disease

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Abstract

Background: Lipid accumulation in tubular cells plays a key role in diabetic kidney disease (DKD). Targeting lipid metabolism disorders has clinical value in delaying the progression of DKD, but the precise mechanism by which molecules mediate lipid-related kidney injury remains unclear. Phosphofurin acidic cluster sorting protein 2 (PACS-2) is a multifunctional sorting protein that plays a role in lipid metabolism. This study determined the role of PACS-2 in lipid-related kidney injury in DKD.

Methods: Diabetes was induced by a high-fat diet combined with intraperitoneal injections of streptozotocin (HFD/STZ) in proximal tubule-specific knockout of Pacs-2 mice (PT-Pacs-2−/− mice) and the control mice (Pacs-2fl/fl mice). Transcriptomic analysis was performed between Pacs-2fl/fl mice and PT-Pacs-2−/− mice.

Results: Diabetic PT-Pacs-2−/− mice developed more severe tubule injury and proteinuria compared to diabetic Pacs-2fl/fl mice, which accompanied with increasing lipid synthesis, uptake and decreasing cholesterol efflux as well as lipid accumulation in tubules of the kidney. Furthermore, transcriptome analysis showed that the mRNA level of sterol O-acyltransferase 1 (Soat1) was up-regulated in the kidney of control PT-Pacs-2−/− mice. Transfection of HK2 cells with PACS-2 siRNA under high glucose plus palmitic acid (HGPA) condition aggravated lipid deposition and increased the expression of SOAT1 and sterol regulatory element-binding proteins (SREBPs), while the effect was blocked partially in that of co-transfection of SOAT1 siRNA.

Conclusions: PACS-2 has a protective role against lipid-related kidney injury in DKD through SOAT1/SREBP signaling.

Keywords: Diabetic kidney disease, PACS-2, SOAT1, SREBP

Background

Diabetic kidney disease (DKD) is chronic kidney disease caused by diabetes, and is highly prevalent across the globe (Hussain et al. 2021). The main histological features of the disease are glomerular and tubular basement membrane thickening, mesangial expansion and mesangiolysis, arteriolar hyalinosis and tubulointerstitial fibrosis (Thomas 2021). Recently there is clear evidence showing that tubular injury plays a pivotal role in the pathogenesis of DKD (Vallon 2011), and it is superior to glomerular pathology as a predictor of CKD progression (Bohle et al. 1987; Risdon et al. 1968). The main mechanisms of tubular injury in DKD include a series of injury pathways caused by hyperglycemia, oxygen metabolism disorders, inflammation, fibrosis and apoptosis (Duan et al. 2021), but new molecules related to tubular injury in DKD still need to be identified.
Lipid accumulation in tubular cells is a common phenomenon in patients with DKD (Chen et al. 2019; Herman-Edelstein et al. 2014). Proximal tubular cells have high levels of baseline energy consumption and fatty acids are the preferred substrate for proximal tubule ATP generation under normal circumstances (Guder et al. 1986; Nieth and Schollmeyer 1966). Therefore, proximal tubule cells are more susceptible to lipid metabolism disorders. Moorhead et al. (1982) first proposed that lipid accumulation contributes to chronic kidney injury in 1982, increasing evidence supports that excess lipid in kidney could lead to a series of kidney injuries by endoplasmic reticulum (ER) stress, mitochondrial dysfunction, reactive oxygen species (ROS) production, cell apoptosis, etc. (Declèves et al. 2014; Hosokawa et al. 2019; Khan et al. 2014; Weinberg 2006; Wu et al. 2021c). Studies by our group and others have shown that renal lipid accumulation is positively correlated with the tubulointerstitial damage in DKD (Chen et al. 2019; Han et al. 2021; Yang et al. 2018). Proposed mechanisms leading to lipid accumulation in proximal tubule include increased uptake of lipid, increased synthesis of lipid, diminished ω-oxidation and cholesterol efflux (Kang et al. 2015; Procotor et al. 2006; Sun et al. 2002). Our previous studies also show that disulfide-bond A oxidoreductase-like protein protects against lipid-related kidney damage in DKD by increasing triglyceride hydrolysis and decreasing cholesterol synthesis (Chen et al. 2019) and identify that the deficiency of lipophagy, a process by which lipid droplets are degraded via autophagy, also plays an important role in ectopic lipid accumulation in DKD (Han et al. 2021). However, other molecules related to lipid-related kidney injury still need to be further identified.

Phosphofurin acidic cluster sorting protein 2 (PACS-2) is a member of PACS family expressed in skeletal muscle, brain, heart and kidney, and it plays important roles in membrane trafficking, autophagy and apoptosis (Li et al. 2020). Recently, the role of PACS-2 in lipid metabolism has attracted attention. Simmen et al. (2005) suggested that PACS-2 controlled the formation of ER lipid-synthesizing centers found at mitochondria-associated endoplasmic reticulum membranes (MAMs) and the knockdown of PACS-2 significantly reduced the amount of long chain fatty acid CoA ligase 4 (FACL4) and phosphatidylserine synthase 1 (PSS1) in the MAM fraction. Besides, Pacs-2 knockout mice have elevated liver sirtuin 1 (SIRT1) activity and are protected from diet-induced obesity (Krzysiak et al. 2018). Moreover, reduction of Pacs-2 levels also results in improved insulin sensitivity and improved liver steatosis in ob/ob mice (Arruda et al. 2014). In addition, a recent study from our laboratory found that PACS-2 was mainly expressed in renal tubules and was decreased in kidney of STZ-induced diabetic mice and patients with DKD (Li et al. 2022). But the effects of PACS-2 on renal lipid metabolism and lipid-related kidney injury of DKD remain unclear.

In this study, we demonstrated that tubule-specific deletion of Pacs-2 gene in high fat diet/streptozotocin (HFD/STZ)-induced diabetic mice aggravated lipid accumulation and lipid related kidney injury. The precise mechanisms involve the promotion of lipid synthesis and inhibition of cholesterol efflux by inducing the expression of sterol O-acyltransferase 1 (SOAT1) and then activating its downstream sterol regulatory element-binding proteins (SREBPs).

Materials and methods

Animal experiments and procedures

Proximal tubule-specific Pacs-2 knockout (PT-Pacs-2−/−) mice were generated by the Cre-LoxP recombination strategy as previously described (Li et al. 2022). Cre-negative littermate wild-type mice (Pacs-2fl/fl) were used as controls. PT-Pacs-2−/− mice and their control group Pacs-2fl/fl mice were fed with a high-fat diet (HFD) (45% fat, ResearchDiets, USA) for 1 month at 4 weeks of age, followed by intraperitoneal injection of 100 mg/kg streptozotocin (STZ) (Sigma-Aldrich, USA). After the injection, mice with random blood glucose levels >12 mmol/l were included for the experiment. They were named diabetic PT-Pacs-2−/− mice and diabetic Pacs-2fl/fl mice, respectively. Then the mice were maintained for another 20 weeks of HFD feeding. Mice fed with a standard fat diet (SFD) were used as non-diabetic controls, called control Pacs-2fl/fl mice and control PT-Pacs-2−/− mice, respectively. The mice were randomly assigned to four groups. At the end of the last week, mice were sacrificed and anesthetized with sodium pentobarbital. Urine and kidney tissues were collected for various experiments. The sample size was estimated from the effect of the study. All the experiments were approved by the Medical Ethics Committee of Central South University and followed the NIH guidelines for the care and use of laboratory animals.

Assessment of physiological features and renal function

Body weight and blood glucose were measured every 2 weeks. Urine albumin was measured by a Mouse MAU (Microalbuminuria) ELISA Kit (Sangon Biotech, China) according to the specification of this kit. Urine creatinine levels were measured in the same samples using Cical-liquid-N CRE (Kanto Chemical Co Inc, Japan). Urine N-acetyl-β-d-glucosaminidase (NAG) levels were measured with a NAG assay kit (Jiancheng Bioengineering, China) following the manufacturer's protocol.
Morphological analysis
Kidney tissues from mice were fixed with 4% paraformaldehyde (PFA) for 14–16 h, embedded in paraffin, sectioned, and stained with hematoxylin–eosin (HE), periodic acid Schiff (PAS), Masson staining, and observed under light microscopy. Tubular damage and glomerular damage were assessed using a semiquantitative scoring system, as described previously (Sun et al. 2011).

Transcriptome analysis
The transcriptome analysis was conducted by Seqhealth Technology Co., LTD (Wuhan, China). Total RNA from the renal cortex of control Pt-Pacs-2/− mice and control Pt-Pacs-2+/− mice was extracted using RNAiso Plus (TaKaRa, Japan) according to the manufacturer’s instructions. The RNA concentration was determined by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and the integrity was tested by 1.0% agarose gel electrophoresis. 5 μg total RNAs were used for mRNA sequencing library preparation. After the library quality inspection is qualified, ~300 bp products were sequenced in HiSeq X10 system (Illumina, San Diego, CA, USA). For the RNA-seq data analysis, the data was aligned to the mouse reference genome to obtain comprehensive transcript information and differentially expressed genes (DEGs) as described previously (Ma et al. 2021).

Immunohistochemistry (IHC)
Paraffin-embedded renal sections were deparaffinized in xylene, rehydrated in ethanol and antigen repaired in citrate buffer. After blocking endogenous peroxidase activity with peroxidase blocking solution, the sections were exposed to 5% bovine serum albumin (BSA) and sequentially incubated with primary antibody, anti-Adipophilin (1:200, proteintech, 15294-1-AP, China), treated with dianaminobenzidine, and counterstained with hematoxylin. Finally the sections were dehydrated, soaked in xylene, air-dried, and sealed. Ten images under brightfield were randomly taken for each group in a blind fashion. Images were obtained in a blinded manner and analyzed with ImageJ software as described previously (Gao et al. 2020).

Oil Red O staining
Oil Red O staining was performed in a common manner as previously described (Pei et al. 2016; Yuan et al. 2017). Briefly, the mouse kidney tissues were fixed in 4% PFA for 24–48 h and was dehydrated in 30% sucrose solution overnight. Then the tissues were embedded in O.C.T. compound and cryocut cross-sections (5 mm) were prepared. The frozen sections were air-dried at room temperature and then stained with Oil Red O dye (Servicebio, China) according to the manufacturer’s instructions. HK-2 cells were fixed in 4% PFA for 30 min. After washing with PBS, HK-2 cells were stained with Oil Red O dye for 30 min and then dipped into 60% isopropanol three times. The deposition of lipid droplets in tissue or HK-2 cells was observed under a Nikon microscope. Images were obtained in a blinded manner and analyzed with ImageJ software.

Cell culture and treatments
The human proximal tubular cell line (HK-2 cells) was obtained from ATCC and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM)/F12 supplemented with 10% FBS and 100 U/mL penicillin plus 0.1 mg/mL streptomycin. One day before transfection, cells were grown to ~70% confluence. Then HK-2 cells were transfected with siRNAs or plasmids by Lipofectamine 3000 (Invitrogen, USA) in accordance with the manufacturer’s instructions. pcDNA3 hPACS-2 Flag plasmid was a gift from Professor Gary Thomas (Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, USA). PACS-2 siRNA was purchased from Ribobio Co., Ltd (China), SOAT1 siRNA were purchased from Tsingke Biotechnology Co., Ltd (China). Scrambled siRNAs (Tsingke Biotechnology Co., Ltd, China) and empty vector pcDNA 3.1 vector were applied to parallel cultures as negative controls. Subsequently, cells were stimulated with high glucose plus palmitate (HGPA; final concentration 30 mmol/L glucose and 300 μmol/L of the saturated free fatty acid palmitate [16:0]) for 24 h then harvested (Wu et al. 2021a; Sun et al. 2021; Lin et al. 2022; Wu et al. 2021b). The control group used mannitol and fatty acid free BSA (Solarbio, China) as the isotonic solvent control group.
Bodipy staining
HK-2 cells were washed using PBS and stained with BODIPY493/503 (Invitrogen, USA) 1000-fold diluted for 30 min according to the manufacturer’s instructions. Cell nuclei were counterstained hoechst and images acquired by laser confocal microscopy.

Cholesterol and free fatty acid content determination
Total cholesterol (TC), free cholesterol (FC) and free fatty acid (FFA) content in HK-2 cells was determined using the Micro Total Cholesterolenone (TC) Content Assay Kit (Solarbio, China), Micro Free Cholesterolene (FC) Content Assay Kit (Solarbio, China) and Free fatty acid detection kit (Solarbio, China) respectively, following the manufacturer’s protocol. Amount of cholesteryl ester (CE) were determined by subtracting the amount of free cholesterol from total cholesterol as described previously (Huang et al. 2018).

Real time qRT-PCR
The total RNA extracted from the renal cortex and cells using RNAiso Plus was reverse transcribed into cDNA using PrimeScriptTM Reagent Kit (TaKaRa, Japan) with Bio-Rad iCycler system (Bio-Rad, Hercules, CA). Real time qRT-PCR was performed using TB GreenTM Premix Ex Taq II reagent (TaKaRa, Japan) with 7300 Real-Time PCR System (Applied Biosystems). The sequences of the primers are listed in Additional file 2: Table S1. The expression showing that PACS-2 was knocked out in renal cortex.

Western blot analysis
Western blot analysis was performed as described previously (Xu et al. 2016). In brief, protein from mice renal cortex as well as cell pellets was extracted using RIPA buffer (CWBio, China) containing protease and phosphatase inhibitor cocktail (CWBio, China). BCA protein assay (Thermo Fisher Scientific, USA) was used for the quantification of protein concentrations. Equal amounts of proteins were used in the Western blot analysis. Primary antibodies specific for PACS2 (1:1000, proteintech, 19508-1-AP, China), FN (1:1000, abcam, ab2413, USA), α-SMA (1:3000, abcam, ab32575, USA), SOAT1 (1:1000, abclonal, A6311, China), SREBF1 (1:500, santa cruz, sc-13551, USA), SREBF2 (1:500, santa cruz, sc-13552, USA), Beta Actin (1:5000, proteintech, 60008-1-lg, China) were used for western blot.

Statistical analysis
Data are expressed as mean±SD. Statistical analyses were performed with GraphPad Prism (version 8.0). The values were analyzed by one-way ANOVA followed by post hoc Tukey’s test or two-way ANOVA followed by post hoc Tukey’s test. Significance was defined as *p<0.05, **p<0.01, ***p<0.001.

Results
Tubule-specific Pacs-2 deletion aggravates kidney injury in HFD/STZ-induced mice
The PT-Pacs-2−/− mice were generated by a Cre-LoxP recombination system in our group as previously described (Li et al. 2022), which was confirmed by western blot analysis of extracted renal cortex. And there was no significant difference in the expression of PACS-2 in other organs between Pacs-2fl/fl mice and PT-Pacs-2−/− mice (Additional file 1: Figure S1). Mice were induced to develop DKD with HFD/STZ treatment. The protein expression of Pacs-2 was lower in diabetic Pacs-2fl/fl mice compared to that of control Pacs-2fl/fl mice, and this decrease was further aggravated in diabetic PT-Pacs-2−/− mice (Fig. 1A, B). In addition, the body weight of diabetic mice was lower than that of non-diabetic mice after the injection of STZ (Fig. 1C), and the opposite pattern was observed for the blood glucose level (Fig. 1D). Besides, diabetic Pacs-2fl/fl mice had a higher kidney weight/body weight (KW/BW) ratio than that of control Pacs-2fl/fl mice, and the level was further increased in diabetic PT-Pacs-2−/− mice (Fig. 1E), indicating the development of more severe hypertrophy in diabetic kidneys when tubular Pacs-2 was deleted. Diabetic PT-Pacs-2−/− mice also showed higher urinary albumin and NAG excretion compared to their controls (Fig. 1F). Moreover, histopathological analysis showed that kidney injury was further aggravated in diabetic PT-Pacs-2−/− mice compared with diabetic Pacs-2fl/fl mice, including notable tubular epithelial disruption, tubular basement membrane thickening, tubulointerstitial fibrosis, hypertrophy of glomeruli and increased mesangial matrix (Fig. 1G–I). These results indicate that deletion of Pacs-2 in proximal tubular cells promotes the renal injury and progression of DKD.

Pacs-2 gene deficiency contributes to lipid accumulation in tubular cells of diabetic mice
Compared to diabetic Pacs-2fl/fl mice, diabetic PT-Pacs-2−/− mice had more lipid droplets and neutral lipids.
Fig. 1 (See legend on previous page.)
deposition in kidney tubules, as assessed by immunohistochemistry of Adipophilin and Oil Red O staining (Fig. 2A–C). Next, we investigated which molecules are associated with perturbed lipid metabolism underlying lipid accumulation in the kidney of diabetic PT-Pacs−2−/− mice. As shown in Fig. 2D, compared to control Pacs−2fl/fl mice, a notable increase of the mRNA levels of Srebp1 and its downstream acetyl-CoA carboxylase alpha (Acaca) and fatty acid synthase (Fasn), controlling de novo fatty acid synthesis in the kidney of diabetic Pacs−2fl/fl mice were observed, which accompanied with increased expression of Srebp2 with its downstream low density lipoprotein receptor (Ldlr) and 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr), which mediated cholesterol intake and biosynthesis, respectively, and the levels were further increased in diabetic PT-Pacs−2−/− mice. In addition, the mRNA level of ATP binding cassette subfamily a member 1 (Abca1) controlling cholesterol efflux was decreased in diabetic Pacs−2fl/fl mice compared to control Pacs−2fl/fl mice, and the levels were further decreased in diabetic PT-Pacs−2−/− mice. However, there was no significant difference in the mRNA levels of peroxisome proliferator activated receptor alpha (Ppara) between diabetic Pacs−2fl/fl mice and diabetic PT-Pacs−2−/− mice. Since lipid accumulation was found to accelerate renal tubule fibrosis (Gai et al. 2019), we detected the expression of fibronectin (FN) and smooth muscle alpha-actin (α-SMA). As expect, the expression levels of FN and α-SMA in the renal cortex of diabetic PT-Pacs−2−/− mice were higher than those of diabetic Pacs−2fl/fl mice (Fig. 2E, F).

Identification of SOAT1 as a downstream molecule of PACS-2 and the expression of SOAT1 was increased in the kidney of diabetic mice

Next we explored the mechanism by which PACS-2 regulated lipid metabolism in the diabetic kidney. By transcriptome analysis of renal cortex, 219 differentially expressed genes (DEGs) were identified between renal cortex of control Pacs−2fl/fl mice and control PT-Pacs−2−/− mice, of which 127 genes were up-regulated while 92 genes were down-regulated in control PT-Pacs−2−/− mice (Fig. 4A). The heat map of DEGs involved in lipid metabolism showed that Soat1, an ER resident protein converting FC to CE, was notably upregulated in control PT-Pacs−2−/− mice compared with control Pacs−2fl/fl mice (Fig. 4B), and this finding was further confirmed by real time qRT-PCR, immunofluorescence and western blot analysis, respectively (Fig. 4C, D, F and Additional file 1: Figure S2). Immunofluorescence images delineated that the expression of SOAT1 was mainly localized in tubular cells but not in glomeruli. And the increased fluorescence intensity of SOAT1 was observed in diabetic Pacs−2fl/fl mice compared to control Pacs−2fl/fl mice. Furthermore, Pacs−2 deletion in tubular cells further increased the expression of SOAT1 (Fig. 4D, E). Similar results were found by western blot analysis (Fig. 4F, G). Besides, by western blot analysis, the intensity of the bands reflected as the expression of N-terminal cleavage product of SREBP1 and SREBP2 (n-SREBP1 and n-SREBP2), which represented the activation of SREBP1 and SREBP2, was notably increased in diabetic PT-Pacs−2−/− mice compared to diabetic Pacs−2fl/fl mice. This data indicated that deletion of Pacs−2 may activate SREBP1 and SREBP2, the key transcription factors of lipid metabolism, by increasing the expression of SOAT1 in diabetic conditions.

Treatment of HK-2 cells with PACS-2 and SOAT1 siRNA regulates the expression of n-SREBPs and lipid accumulation

Since SOAT1 may play a critical role in the process of PACS-2 modulating the lipid accumulation in tubular cells under DKD condition, next we further detected the effect of silencing PACS-2 and SOAT1 by siRNA treatment in vitro. Results showed that gene silencing of PACS-2 increased expression of SOAT1 and activation of SREBPs in HK-2 cells exposed to HGPA ambiance compared to cells treated with control siRNA. While the effect was blocked partially in HK-2 cells co-transfected with SOAT1 siRNA (Fig. 5A, B). By real time qRT-PCR
Fig. 2 (See legend on previous page.)
Fig. 3  Overexpression of PACS-2 ameliorates lipid accumulation in HK-2 cells induced by HGPA. A Gene overexpression efficiency of PACS-2 by western blot analysis in PACS-2 plasmid transfected HK-2 cells. B Representative Oil Red O staining images of HK-2 cells in different groups. Scale bar: 20 μm. C Oil Red O staining area. D Cholesteryl ester content. E Free cholesterol content. F Total cholesterol content. G Free fatty acid content. All data are presented as means ± SD; *p < 0.05, **p < 0.01, ***p < 0.001. n = 4.

Fig. 4 Identification of Soat1 as a downstream molecule of Pacs-2. A Volcano plot of DEGs in kidney cortex of control Pacs-2flo/flo mice and control PT-Pacs-2−/− mice. Splashes represent different genes, and the gray splashes mean genes without significant different expression. The red splashes mean significantly up-regulated genes in control PT-Pacs-2−/− mice, and the blue splashes mean significantly down-regulated genes in control PT-Pacs-2−/− mice. B Heat map of top eight DEGs involved in lipid metabolism; n = 3 mice per group. C Real-time qRT-PCR showing the relative mRNA expression of Soat1. n = 6 mice per group. D Representative immunofluorescence images of SOAT1 (green) in kidney tissues from each group. The nuclei were delineated by DAPI (blue). n = 6 mice per group. Bars = 50 μm. E Semiquantification analysis of protein expression. F Representative western blot bands of SOAT1, n-SREBP1, n-SREBP2 expression in the kidney cortex, n = 6 mice per group. G Relative band density. All data are presented as means ± SD; *p < 0.05, **p < 0.01, ***p < 0.001.
analysis, a notably increased expression of SREBP1, ACACA, FASN, SREBP2, HMGCR and decreased expression of ABCA1 were found in HK-2 cells treated with HGPA plus PACS-2 siRNA, but the effect was reversed partially by treating with SOAT1 siRNA. In addition, an increased tendency of the mRNA level of LDLR was seen.
despite no statistical difference (Fig. 5C). Furthermore, silencing of PACS-2 promoted intracellular lipid deposition and increased the levels of CE, TC and FFA in HK-2 cells exposed to HGPA ambiance, however this effect was partially blocked by treatment with SOAT1 siRNA (Fig. 5D–F, H and I). No change was seen in the content of FC (Fig. 5G). These data indicated that reduced PACS-2 could activate SREBPs and promote lipid accumulation by SOAT1 pathway.

Discussion

This study was designed to explore the role of PACS-2 in lipid-related kidney injury of DKD condition. We found that tubule-specific Pacs-2 deletion markedly aggravated lipid accumulation in tubular cells by increasing the expression of SOAT1 and then regulating lipid synthesis, uptake and efflux by SOAT1/SREBPs signaling, eventually resulting in worsening albuminuria excretion and kidney injury. These results also highlighted the importance of lipid metabolism abnormality in the pathogenesis of DKD, and PACS-2 may be a potential therapeutic target of DKD.

Recent studies have shown that Pacs-2 is associated with insulin resistance and liver steatosis in the liver of obese mice through regulating glucose and lipid metabolism (Arruda et al. 2014; Krzysiak et al. 2018). Arruda et al. (2014) found that the expression of PACS-2 was up-regulated in the liver of ob/ob mice and HFD-induced obese mice, and silencing Pacs-2 improved insulin sensitivity and liver steatosis by decreasing MAM integrity and improving glucose metabolism, rather than regulating genes involved in de novo lipogenesis or cholesterol metabolism. Krzysiak et al. (2018) also suggested that the loss of Pacs-2 protected HFD-induced obese mice from both hepatic steatosis and whole-body adiposity by elevating liver SIRT1 activity and its downstream fatty acid oxidation genes. Besides, a recent study showed that the expression of PACS-2 decreased in the kidneys of type 1 and type 2 diabetes mice and Pacs-2 knockout mice with diabetes displayed deterioration of kidney function (Xue et al. 2021). However the role of PACS-2 in tubular cells under diabetic condition remains to be further elucidated. Here, we found that the expression of PACS-2 was decreased in tubular cells of HFD/STZ-induced diabetic mice, which accompanied with renal injury (Fig. 1). Furthermore tubule-specific Pacs-2 deletion contributed to lipid accumulation in tubular cells under diabetic condition by increasing FFA and cholesterol synthesis, cholesterol uptake and decreasing cholesterol efflux (Fig. 2). In contrast, overexpression of PACS-2 in HK-2 cells reduced the lipid accumulation and content of CE, TC and FFA, while gene silencing of PACS-2 by siRNA had the opposite effect (Figs. 3 and 5). As we all know, the triglycerides themselves serve primarily a storage function with toxicity deriving mainly from FFA, FC, CE, ceramides, etc. (Listenberger et al. 2003; Nakamichi et al. 2021; Tang et al. 2010), indicating PACS-2 may have a renoprotective role from lipid-related kidney injury in DKD. Besides, control PT-Pacs-2+/− mice did not show renal lipid accumulation and kidney injury, suggesting that the effects of Pacs-2 knockout could be tolerated or compensated in the non-DKD state. Similar observations were previously noted in the liver where knockout of Pacs-2 in mice maintained on a control diet did not result in the change of whole-body adiposity, hepatic steatosis and insulin resistance (Krzysiak et al. 2018).

SOAT1 is a key molecule in lipid metabolism which can convert free cholesterol to cholesteryl esters to prevent overaccumulation of free cholesterol at cellular membranes (Chang et al. 2009). Liu et al. (2020) found that Soat1 deficiency in diabetic mice reduced cholesteryl ester content in kidney cortex and protected from disease progression. Here, by transcriptome analysis we identified the mRNA level of SOAT1 was up-regulated in the kidney of PACS-2 deficiency mice and found the expression of SOAT1 was increased in tubular cells of diabetic kidney which accompanied with the up-regulation of SREBPs (Fig. 4). SREBPs are key transcription factors regulating lipid metabolism (Shimano and Sato 2017). Geng et al. (2016) found that inhibition of SOAT1 suppressed glioblastoma growth via blocking SREBP-1-mediated lipogenesis. The activity of SREBPs is tightly regulated by a negative feedback loop triggered by ER membrane cholesterol (Goldstein et al. 2006). When cholesterol builds up in ER membranes, SREBPs remain bound to membranes of the ER and are therefore inactive. While cells are depleted of sterols, the SREBPs move to the Golgi complex where two proteases release the active portions of the SREBPs (n-SREBPs), which then enter the nucleus and activate transcription of target genes such as FASN, ACACA, ABCAI1, et al. (Goldstein et al. 2002). In addition, when ER cholesterol increases, cells can esterify it with fatty acid to form CE and sequester them into

Fig. 5 Treatment of HK-2 cells with PACS-2 and SOAT1 siRNA regulates lipid metabolism. A and B Representative western blot and quantification of PACS-2, SOAT1, n-SREBP1, n-SREBP2 in HK-2 cells. C Measurement of the mRNA expression of selected markers. D Representative images of cells stained with Bodipy (green); nuclei stained with Hoechst (blue). E Quantification of the Bodipy staining. F Cholesteryl ester content. G Free cholesterol content. H Total cholesterol content. I Free fatty acid content. All data are presented as means ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.
Fig. 5 (See legend on previous page.)
lipid droplets through the activity of SOAT1 (Chang et al. 2006). That’s probably the reason why the activity of SREBPs remain high in cells even though lipids like cholesterol are also high (Geng et al. 2016). To confirm that PACS-2 regulates lipid-related kidney injury through SOAT1/SREBPs pathway, HK-2 cells were transfected with siRNA and exposed to HGPA. PACS-2 siRNA increased the mRNA levels of SREBP1, ACACA, FASN, SREBP2, HMGCR, SOAT1 and the protein levels of SOAT1, n-SREBP1 and n-SREBP2 as well as lipid droplets. However these changes were partially reversed by cotreatment with SOAT1 siRNA (Fig. 5). These data indicated that PACS-2 protected lipid-related kidney injury in DKD via the PACS-2/SOAT1/SREBPs pathway. However, the mechanism of how PACS-2 regulates the expression of SOAT1 in DKD is unclear. Further studies will be required to advance our understanding of PACS-2 modulation of lipid metabolism in DKD.

Conclusions
In summary, this study shows that PACS-2 is an important molecular participant in lipid-related kidney injury in DKD. PACS-2 gene deficiency in tubular cells can further aggravate renal injury by upregulating the expression of SOAT1, and then, enhancing the activity of SREBPs, eventually leading to lipid accumulation and lipid related kidney injury. Pharmacological targeting of PACS-2 and its related signaling pathways may provide a novel therapeutical target for the development of DKD.

Abbreviations
PACS-2: Phosphofurin acidic cluster sorting protein 2; HFD: High fat diet; STZ: Streptozotocin; SOAT1: Sterol O-acyltransferase 1; HGPA: High glucose plus palmitic acid; SREBPs: Sterol regulatory element-binding protein; DKD: Diabetic kidney disease; ROS: Reactive oxygen species; MAMs: Mitochondria-associated endoplasmic reticulum membranes; FACL4: Long chain fatty acid CoA ligase 4; PSS1: Phosphatidylserine synthase 1; SIRT1: Sirtuin 1; SFD: Standard fat diet; NAG: N-Acetyl-B-D-glucosaminidase; DEGs: Differentially expressed genes; HE: Hematoxylin–eosin; PAS: Periodic acid Schiff; BSA: Bovine serum albumin; TC: Total cholesterol; FC: Free cholesterol; FFA: Free fatty acid; CE: Cholesteryl ester.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s10020-022-00545-x.

Additional file 1: Fig. S1. The expression of PACS-2 in other organs of two groups of Mice. (A and B) Western blot and quantification of PACS-2 in the heart, skeletal muscle and liver of Paccs-2fl/fl mice and PT-Pacs-2-/- mice, ns, not significant. n = 4. Fig. S2. Gene silencing of PACS-2 increases the expression of SOAT1 in HK-2 cells. (A and B) A representative western blot and quantification of SOAT1 and PACC-2 in HK-2 cells under control environment. *p < 0.05, **p < 0.01. n = 4.

Additional file 2: Table S1. The sequences of primer pairs.

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Author contributions
CYZ performed the experiments and wrote the manuscript. LL, CRL, CYT, JC, YL, JFY, YYX, MY, NJ provided technical support for this study and participated in discussions about this study. YCH, YL, SLL, LX and LS designed this study and edited this manuscript. LS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
All the experiments were approved by the Medical Ethics Committee of Central South University and followed the NIH guidelines for the care and use of laboratory animals.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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