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Liraglutide promotes autophagy by regulating the AMPK/mTOR pathway in a rat remnant kidney model of chronic renal failure

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Abstract

Background We aimed to determine whether the glucagon-like peptide-1 receptor (GLP-1R) agonist liraglutide (LRG) could ameliorate renal function through promoting autophagy via regulating the AMPK/mTOR pathway in a rat remnant kidney model of chronic renal failure.

Methods Rats were divided into four groups ($n = 10$ per group) as follows: (1) sham, (2) nephrectomy (NPX), (3) LRG control (LRG control), and (4) LRG treatment (LRG). Except for rats in the sham group, all rats underwent 5/6 nephrectomy surgery to establish a remnant kidney model of chronic renal failure. In addition, rats in LRG group received LRG as a subcutaneous injection at a dose of 10 mg/kg (once daily) for 4 consecutive weeks, whereas rats in the LRG control group received treatment similar to that of rats in the LRG group, except saline was used instead of LRG. After 4 weeks of treatment, serum creatinine (Scr), blood urea nitrogen (BUN), and urinary albumin excretion were determined. Immunofluorescence assay, immunoprecipitation assay, and Western blot analysis were performed to evaluate the AMPK/mTOR pathway expression of proteins.

Results Nephrectomized rats (including rats in the NPX, LRG control, and LRG groups) showed higher levels of the Scr, BUN, and urinary albumin excretion, as well as down-regulation of GLP-1R, LC3-II, and AMPK phosphorylation, and up-regulation of mTOR phosphorylation when compared with rats in the sham group. However, those changes were blocked by liraglutide.

Conclusion Liraglutide may promote autophagy through regulating the AMPK/mTOR pathway to exert renoprotective effects in a rat remnant kidney model of chronic renal failure.

Keywords Autophagy · AMPK/mTOR pathway · Chronic kidney disease · Liraglutide · Remnant kidney

Background

Chronic kidney disease (CKD), a leading cause of mortality and morbidity, caused 1.19 million deaths around the world, which has increased by 28.8% from 2006 [1]. This made CKD the 11th leading cause of death in 2016, compared with 13th and 27th in 2013 and 1990, respectively [1]. The latest survey from the Global Burden of Disease Study indicated that in the past decade, the total disability-adjusted life years of CKD increased significantly from 29,200 to 35,000, which far outdistanced many neurological disorders, including dementia and Parkinson's disease, as well as chronic liver disease [1, 2]. To slow down CKD progression, major advancements have been made in pharmacological treatment; however, many patients still progress to end-stage renal disease (ESRD) and are required to undergo renal replacement therapy or wait for renal transplantation, which places a significant burden on society and families involved. Therefore, studying the underlying mechanism of CKD and identifying promising or novel therapeutic targets against CKD progression are of utmost importance.

Autophagy is a conserved self-digestion process of the cell that is involved in cellular homeostatic quality control and regeneration, and the cellular stress response mechanism. Defective autophagy or deregulated autophagic activities have been found in many complex human diseases, such as Alzheimer's disease [3], Parkinson's disease [4], and cancer [5, 6]. Additionally, autophagy is closely related with various pathophysiological changes of the kidney, such as renal fibrosis [7], renal injury [8],

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renal ischemia–reperfusion injury [9], and renal glomerular hypertrophy [10]. In previous studies, it has been shown that in different kidney diseases, autophagy has both a renoprotective and a pathogenic role [11, 12]. However, studies that focus on autophagy or if activation of autophagy plays an important role in the occurrence and development of CKD are limited. Currently, there are no standard clinical measurements for autophagy. The 2016 guidelines for monitoring of autophagy suggest that for the assessment autophagy, one should use transmission electron microscopy, autophagy-related gene 8 (Atg8)/microtubule-associated protein-1 light chain 3 (LC3) quantification, related LC3 binding protein turnover assays, amp-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) levels, as well as additional autophagy-related protein markers, such as p62 and ATG5 [13]. Among these autophagy-related proteins, LC3 is most frequently used for monitoring autophagy function, and AMPK and mTOR are important regulators of autophagy. In the previous studies, it has been suggested that a glucagon-like peptide-1 (GLP-1) analog, liraglutide (LRG) could enhance autophagy to decrease lipid accumulation in hepatocytes that are involved in the AMPK/mTOR pathway [14]. Data from another study indicated that LRG increased level of the lipid-modified microtubule-associated protein 1 light chain 3β (LC3-II) in the left ventricles of mice to improve cardiac function [15]. Thus, it is believed that GLP-1 and its receptor GLP-1R are implicated in autophagy, and suggest that GLP-1 or GLP-1R may be promising targets for autophagy-related diseases. In our study, we hypothesized that the LRG might promote autophagy through mediating AMPK-mTOR signaling in a rat remnant kidney model of chronic renal failure to alleviate renal injury.

Materials and methods

Animals

Male Sprague–Dawley rats (6–8 weeks) weighing 200–250 g were purchased from Animal Experiment Center of Institute of Radiation Medicine of the Chinese Academy of Medical Sciences. Rats were fed food and water under specific pathogen-free (SPF) conditions at approximately at 23 ± 2 °C and $50 \pm 10\%$ humidity with a 12-h light–dark cycle and adapted to the environment for 1 week before starting experiments. Protocols involving the use of animals for conducting this study were approved by the Institutional Animal Ethics Committee of Chinese Academy of Sciences (Beijing, China). All efforts were made to minimize suffering of the animals as much as possible.

5/6 nephrectomy

The procedure for 5/6 nephrectomy in rats was performed according to a previous study [16, 17]. Briefly, the left renal artery was temporarily occluded under anesthesia; then 2/3 of the left kidney was ligated and excised. Bleeding was controlled by hemostatic thrombin reagent. Subsequently, the abdominal incision was closed. One week later, the entire right kidney was ligated and excised. Animals were allowed to recover in their cages. Our pilot experiments showed that a duration of 4 weeks was sufficient for the development of CKD.

Groupings

Rats were randomly divided into four groups as follows ($n = 10$ per group): Sham group: rats underwent the same operations as nephrectomized (NPX) rats, except for 5/6 nephrectomy; NPX group: rats underwent 5/6 nephrectomy; LRG control (LRG control) group: rats underwent 5/6 nephrectomy and received a subcutaneous injection with an equal dose of LRG in normal saline (once daily) for 4 weeks; LRG treatment (LRG) group: rats underwent 5/6 nephrectomy and received a daily subcutaneous injection with 10 mg/kg LRG (Victoza, NovoNordisc, Bagsvaerd, Denmark) for 4 consecutive weeks.

Assessment of renal function

Serum creatinine (Scr), urinary creatinine, and blood urea nitrogen (BUN) were determined using an automatic biochemical analyzer (Olympus2700, Tokyo, Japan). Urinary albumin was measured using an automatic analyzer (BN II, Siemens, Marburg, Germany). The urinary albumin excretion was expressed as the ratio of urinary albumin/urinary creatinine.

Immunofluorescence assay

Nephrectomized rats were anesthetized using pentobarbital sodium (40 mg/kg intraperitoneal injection), and intracardially perfused with normal saline (100 mL) followed by 4% paraformaldehyde (100 mL). The remnant kidneys were removed, fixed overnight in 4% paraformaldehyde, and transferred to 30% sucrose solutions for cryoprotection. Frozen Sects. (10-μm thick) were cut using a freezing microtome (Leica, Germany). Sections were washed with phosphate-buffered saline (PBS), and then blocked with bovine serum albumin solution (0.5%) for 1 h at room temperature. Next, sections were incubated overnight at 4 °C with mouse anti-GLP-1R (1:100, Abcam, USA), a mixture of mouse anti-GLP-1R (1:100) and rabbit anti-AMPK (1:50, Abcam, USA), or a mixture of mouse anti-GLP-1R (1:100) and rabbit anti-mTOR (1:50, Cell Signaling Technology Inc, USA). After washing with PBS, sections were incubated for 1 h with goat anti-rabbit IgG (H + L) FITC–conjugated second antibody (1:50, Proteintech, USA) or mixture of goat anti-mouse IgG(H + L) FITC-conjugated second antibody (1;50) and goat anti-mouse IgG (H + L) TRITC-conjugated second antibody (1:50, Proteintech, USA). Excess secondary antibody was washed off with PBS. Subsequently, sections were incubated in diamidine phenylindole (DAPI) for 5 min at room temperature, and washed three times with PBS. Then, sections were sealed using a coverslip and the staining was observed under a fluorescence microscope (Nikon 1R, Tokyo, Japan).

Western blot analysis

After 4 weeks of treatment, rats were anesthetized with pentobarbital sodium (40 mg/kg intraperitoneal injection) and remnant kidneys were removed, and frozen at – 80 °C for future Western blot analysis according to previous studies [18]. Briefly, samples were thawed on ice, and homogenized using a motor-driven glass tissue homogenizer in ice-cold RIPA solution (Dingguo, China), containing protease and phosphatase inhibitors (Dingguo, China). Homogenized samples were centrifuged at 14,000*g* for 20 min at 4 °C and supernatant was collected. Then, the supernatant was mixed with loading buffer (Dingguo, China) and boiled for 5 min. The protein concentration was determined using a BCA protein kit (Dingguo, China) according to the manufacturer's instructions. Equal amounts of protein were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. PVDF membranes were blocked with non-fat dried milk for 1 h, and membranes were incubated overnight at 4 °C with rabbit anti-GLP-1R (1:1000, Abcam, USA), rabbit anti-AMPK (1:1000, Abcam, USA), rabbit anti-phosphorylated (p)-AMPK antibody (Thr172, 1:1000, Abcam, USA), rabbit anti-mTOR (1:1000, Cell Signaling Technology Inc, USA), rabbit anti-p-mTOR (Ser2448, 1:1000, Cell Signaling Technology Inc, USA), rabbit anti-LC3 (1:500, Cell Signaling Technology Inc, USA), rabbit anti-p62 (1:1000, Abcam, USA), rabbit anti-ATG5 (1:1000, Abcam, USA) or rabbit anti-GAPDH (1:3000, Proteintech, USA). After washing with Tris-buffered saline, containing Tween-20 (TBST), PVDF membranes were incubated with goat anti-rabbit IgG (H + L) HRP-conjugated second antibody (1:3000, Proteintech, USA) for 1 h at room temperature. Finally, the membrane was developed using an enhanced chemiluminescence assay (Beyotime, Beijing, China) and reactive proteins were visualized and standardized to GAPDH.

Immunoprecipitation analysis

Immunoprecipitation analysis was performed as described previously [19]. Briefly, remnant kidneys were thawed on ice, and homogenized using a motor-driven glass tissue homogenizer in ice-cold RIPA solution (Dingguo, China), containing protease and phosphatase inhibitors (Dingguo, China). Next, homogenized samples were centrifuged at 14,000*g* for 20 min at 4 °C, and the supernatant was collected. Next, the supernatant was incubated with rabbit anti-GLP-1R antibody or normal rabbit IgG (negative control, Abcam, USA) overnight at 4 °C with agitation. To each immune complex, Protein A + G Sepharose beads (Abcam, USA) were added and the lysate-bead mixture was incubated for 4 h at 4 °C with rotary agitation. Subsequently, Western blot analysis was performed for additional protein separation and detection.

Statistical analysis

Data were analyzed using SPSS 12.0 software and are expressed as the mean ± standard deviation (SD). One-way ANOVA followed by post hoc Tukey's test was used to test the significance of differences between two groups. $P < 0.05$ was considered statistically significant.

Results

GLP-1R interrelates with the AMPK/mTOR pathway in a rat remnant kidney model of chronic renal failure

To determine whether GLP-1R interrelates with the AMPK/mTOR pathway in a rat remnant kidney model of chronic renal failure, immunoprecipitation analyses and immunofluorescence were performed. First, immunofluorescence assay was performed to determine whether GLP-1R was expressed in the kidney. As shown in Fig. 1a, GLP-1R was widely distributed in the cell membrane and cytoplasm of kidney cells. As shown in Fig. 1a, GLP-1R clearly interacted with AMPK/mTOR in the kidney, which was further supported by the double immunofluorescent staining that showed GLP-1R and AMPK/mTOR co-localized in the cells of kidney (Fig. 1b–d).

Liraglutide ameliorates renal function in a rat remnant kidney model of chronic renal failure

Physical and biochemical data of experimental animals before liraglutide treatment and at 4 weeks after 5/6 nephrectomy are shown in Table 1. Nephrectomized rats (NPX group) showed higher Scr, BUN and urinary

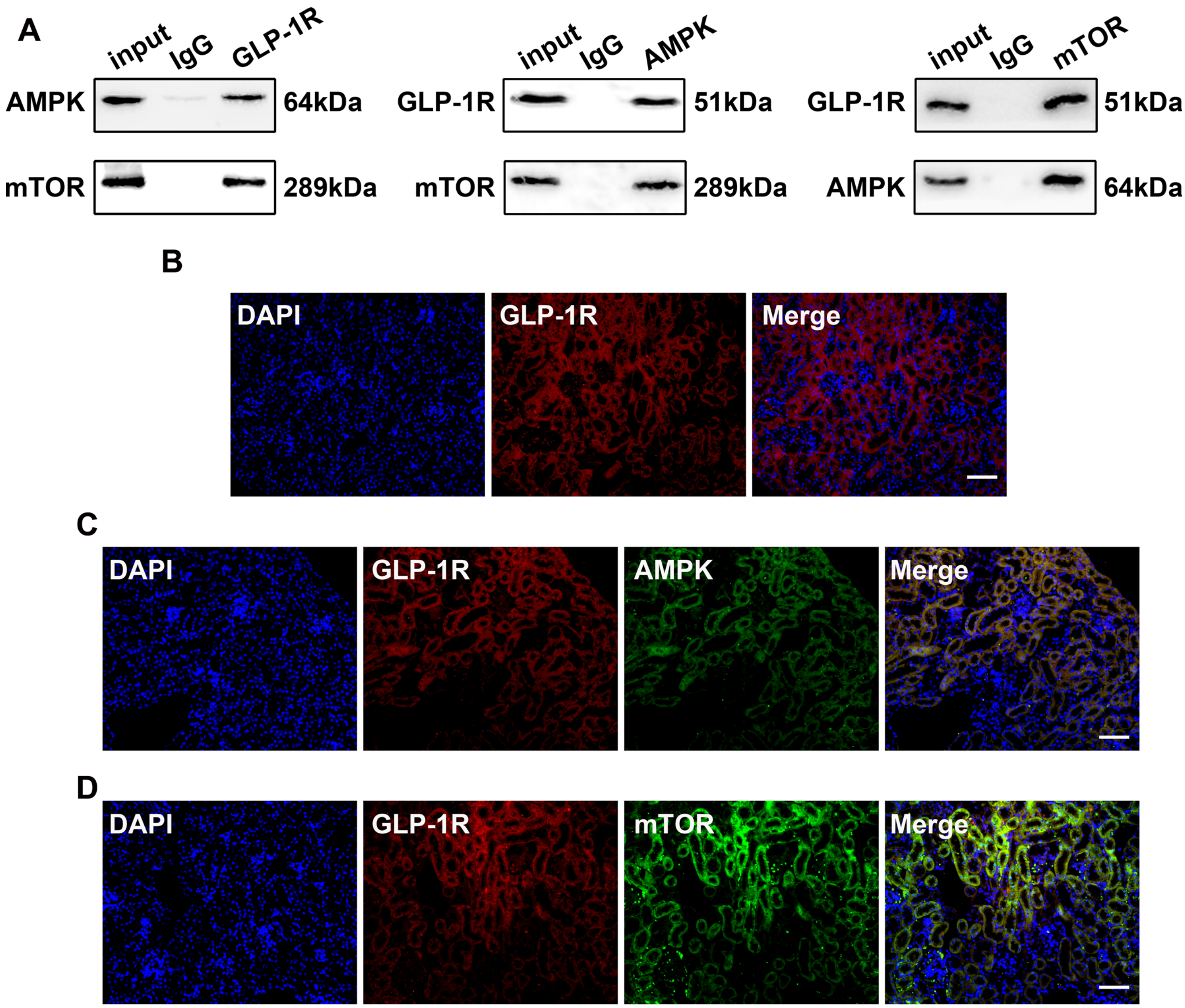

Fig. 1 GLP-1R interrelates with the AMPK/mTOR pathway **a** Immunoprecipitation showing representative immunoblot bands indicating a positive interaction between GLP-1R and AMPK/mTOR. **b** Immunofluorescence analysis indicating that GLP-1R was widely distributed in kidney cells. Scale bar = 50 μm. **c** Double immunofluorescence assay staining indicating that GLP-1R co-localized with AMPK in the kidney. Scale bar = 50 μm. **d** Double immunofluorescence assay staining indicating that GLP-1R co-localized with mTOR in the kidney. Scale bar = 50 μm

Table 1 Renal function in all groups of rats before liraglutide treatment and at 4 weeks after 5/6 nephrectomy $n = 10$ per group

Variable	Sham	NPX	LRG control	LRG
Body weight (g)	274.3 ± 15.2	249.2 ± 9.9*	245.4 ± 10.3	252.6 ± 7.5
Scr (μmol/L)	38.52 ± 1.77	63.24 ± 4.63*	66.12 ± 5.53	61.98 ± 0.93
BUN (mmol/L)	6.53 ± 0.63	10.02 ± 0.38*	9.68 ± 1.02	9.93 ± 0.94
Urinary albumin excretion (mg/g)	0.52 ± 0.48	25.57 ± 2.08*	23.97 ± 1.78	22.74 ± 0.93

*$P < 0.05$ vs sham group

albumin excretion and low body weight when compared with rats in the sham group (Table 1, $P < 0.05$,). Those indexes in NPX group, LRG control group and LRG group had no statistics significance (Table 1, $P > 0.05$). Physical and biochemical data of experimental animals at 4 weeks after liraglutide treatment are shown in Table 2.

Table 2 Renal function in all groups of rats at 4 weeks after liraglutide treatment $n = 10$ per group

Variable	Sham	NPX	LRG control	LRG
Body weight (g)	309.7 ± 11.2	226.1 ± 8.2*	219.7 ± 15.9	267.8 ± 9.1#
Remnant kidney weight/Body weight	/	4.6 ± 0.8	4.5 ± 1.2	3.4 ± 0.4#
Scr (μmol/L)	34.90 ± 1.87	68.10 ± 3.89*	67.00 ± 4.96	49.80 ± 1.12#
BUN (mmol/L)	6.61 ± 0.26	9.28 ± 0.11*	9.35 ± 0.09	7.74 ± 0.14#
Urinary albumin excretion (mg/g)	0.45 ± 0.01	28.40 ± 1.44*	31.1 ± 1.60	0.87 ± 0.10#

*$P < 0.05$ vs sham group, #$P < 0.05$ vs LRG control group

Nephrectomized rats (NPX group) showed higher Scr, BUN and urinary albumin excretion and low body weight when compared with rats in the sham group (Table 2, $P < 0.05$,). Those indexes in NPX group and LRG control group had no statistics significance (Table 2, $P > 0.05$), which suggested that saline treatment could not ameliorate renal function. However, the levels of remnant kidney weight/body weight, Scr, BUN and urinary albumin excretion were statistically decreased and body weight was statistically increased in LRG group compared with LRG control group (Table 2, $P < 0.05$), which suggested that liraglutide treatment could ameliorate renal function.

Liraglutide up-regulates GLP-1R expression in a rat remnant kidney model of chronic renal failure

Western blot analysis was performed to determine the changes in GLP-1R expression after LRG treatment. As shown in Fig. 2, a lower level of GLP-1R was determined in nephrectomized rats (NPX group) when compared with rats in the sham group ($P < 0.05$). In addition, GLP-1R expression in rats in the NPX group and LRG control group did not reach statistical significance (Fig. 2, $P > 0.05$), indicating that saline treatment did not alter GLP-1R expression. However, GLP-1R expression was significantly increased in rats in the LRG group when compared with rats in the LRG control group (Fig. 2, $P < 0.05$), indicating that LRG treatment up-regulated GLP-1R expression.

Liraglutide regulates AMPK/mTOR pathway in a rat remnant kidney model of chronic renal failure

Western blot analysis was employed to determine changes in expression of the AMPK/mTOR pathway after LRG treatment. As shown in Fig. 3, lower levels of p-AMPK/AMPK and higher levels of p-mTOR/mTOR were observed in nephrectomized rats (NPX group) when compared with rats in the sham group ($P < 0.05$). Levels of the p-AMPK/AMPK and p-mTOR/mTOR in rats in the NPX group and LRG control group did not show statistical significance (Fig. 3, $P > 0.05$), suggesting that saline treatment did not alter levels of the AMPK/mTOR pathway. However, levels of p-AMPK/AMPK were statistically increased and the levels of p-mTOR/mTOR were statistically decreased in rats in the LRG group when compared with rats in the LRG control group (Fig. 3, $P < 0.05$), thereby suggesting that LRG treatment regulated the AMPK/mTOR pathway.

Liraglutide promotes autophagy in a rat remnant kidney model of chronic renal failure

As a marker of the autophagosome, the protein level of LC3-II represents the amount of autophagosome. Therefore, the ratio of LC3-II/LC3-I was compared among the sham group, NPX group, LRG control group, and the LRG group. As shown in Fig. 4, we found that the ratio of LC3-II/LC3-I was decreased in nephrectomized rats (NPX group) when compared with rats in the sham group ($P < 0.05$). Moreover, the ratio of LC3-II/LC3-I in

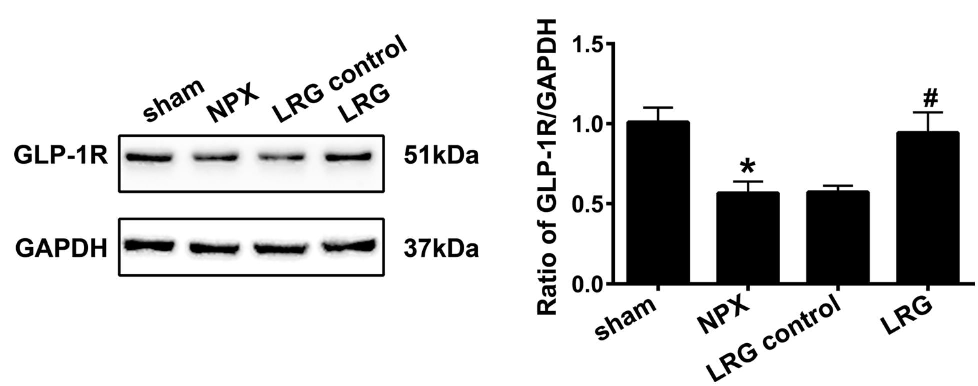

Fig. 2 Liraglutide up-regulates GLP-1R expression Western blot analysis for GLP-1R in different groups ($n = 6$ per group). *$P < 0.05$ vs the sham group; #$P < 0.05$ vs the LRG control group

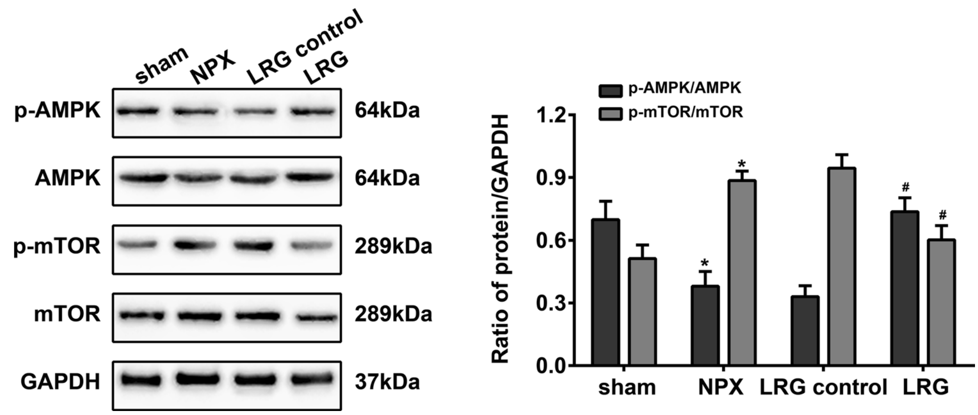

Fig. 3 Liraglutide regulates the AMPK/mTOR pathway Western blot analysis for p-AMPK, AMPK, p-mTOR, and mTOR in different groups ($n=6$ per group). $^{*}P<0.05$ vs the sham group; $^{\#}P<0.05$ vs the LRG control group

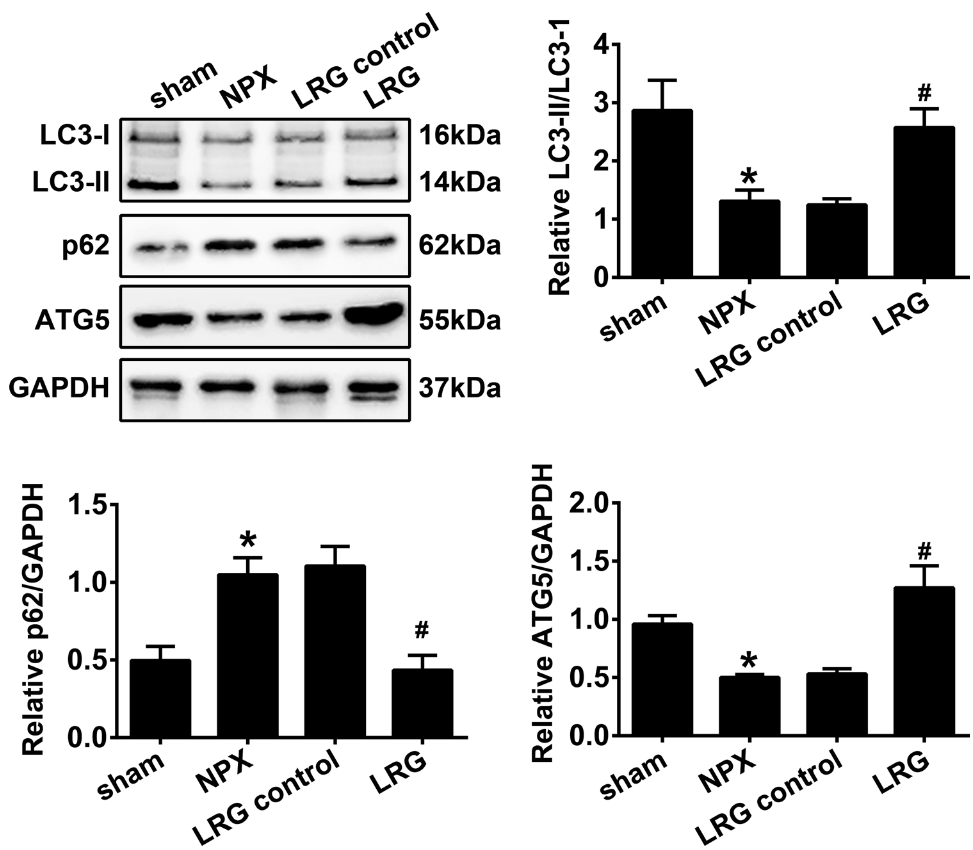

Fig. 4 Liraglutide promotes autophagy Western blot analysis for LC3, p62 and ATG5 in different groups ($n=6$ per group). $^{*}P<0.05$ vs the sham group; $^{\#}P<0.05$ vs the LRG control group

rats in the NPX group and LRG control group did not show statistical significance (Fig. 4, $P>0.05$). However, the ratio of LC3-II/LC3-I was statistically increased in rats in the LRG group when compared with rats in the LRG control group (Fig. 4, $P<0.05$). Additionally, we also detected the effects of liraglutide on the autophagy by assessing the expression of other autophagy marker, such as p62 and ATG5. As shown in Fig. 4, the expression of p62 was increased in nephrectomized rats (NPX group) when compared with rats in the sham group ($P<0.05$), and the expression of p62 was statistically decreased in rats in the LRG group when compared with rats in the LRG control group ($P<0.05$). The expression of ATG5 was increased in nephrectomized rats (NPX group) when compared with rats in the sham group ($P<0.05$), and the expression of ATG5 was statistically decreased in rats in the LRG group when compared with rats in the LRG control group ($P<0.05$). Also, there was no statistical significance in the expression of p62 and ATG5 between NPX group and LRG control group (Fig. 4, $P>0.05$). Those data indicated that liraglutide promoted the expression of LC3-II and ATG5 and p62 degradation.

Discussion

In the present study, we showed that LRG markedly reduced the levels of Scr, BUN, and urinary albumin excretion in a rat remnant kidney model of chronic renal failure. We also demonstrated that LRG up-regulated levels of GLP-1R and AMPK phosphorylation, down-regulated levels of mTOR phosphorylation, and promoted autophagy in a rat remnant kidney model of chronic renal failure. Moreover, we found that GLP-1R co-localized and interacted with the AMPK/mTOR pathway in the kidney. Together, these data indicated that LRG promoted autophagy through regulation of the AMPK/mTOR pathway and exerted renoprotective effects in a rat remnant kidney model of chronic renal failure.

Podocytes have been identified as the most fragile cell types of the glomerulus, and a decrease in the number or dysfuction of glomerular podocytes and glomerular endothelial cells directly correlated with disease progression in several renal diseases, including diabetic nephropathy, IgA nephropathy, HIV-associated nephropathies, or obstructive nephropathy, focal and segmental glomerulosclerosis [20, 21]. Recent evidence has indicated that podocytes have a high level of basal autophagy, and podocyte-specific deletion of Atg5 resulted in proteinuria, lipofuscin accumulation, an increase in oxidized proteins, loss of podocytes, and aging-related glomerulosclerosis, suggesting that autophagy contributed to podocyte maintenance [22]. Except for podocytes, autophagy of other cell types is also altered in several renal diseases. Indeed, Singh et al. reported that loss of the essential autophagy gene Atg7 in endothelial cells could lead to an impaired autophagic flux that was accompanied by endothelial–mesenchymal transition, loss of endothelial cells, up-regulation of key pro-fibrotic genes, and collagen accumulation, thereby suggesting that endothelial autophagy is a potential therapeutic target to limit kidney fibrosis [23]. As another example, Jiang and colleagues demonstrated that renal proximal tubule-specific Atg7 knockout mice were markedly more sensitive to cisplatin-induced acute kidney injury as indicated by renal functional loss, tissue damage, and apoptosis, thus suggesting a renoprotective role of tubular cell autophagy in acute kidney injury [24]. Combined, these data indicated that autophagy has protective roles in many cell types and may represent an effective therapeutic target for various kidney diseases.

AMPK and mTOR play a significant role in regulating autophagy. AMPK antagonizes the autophagy-inhibitory effect of amino acids (at the level of phagophore assembly) by phosphorylating proteins that are involved in the mTOR pathway, thereby leading to the induction of autophagy [13, 25]. AMPK and mTOR regulate autophagy through coordinated phosphorylation of protein kinase ULK1. Under glucose starvation, AMPK promotes autophagy by directly activating ULK1 through phosphorylation. Under conditions of nutrient sufficiency, high mTOR activity prevents ULK1 activation by phosphorylating alternate ULK1 residues, thereby disrupting the interaction between ULK1 and AMPK, which results in a reduction of autophagy [13, 25]. In our study, we demonstrated that decreased autophagy was accompanied by down-regulation of AMPK phosphorylation and up-regulation of mTOR phosphorylation. The AMPK/mTOR pathway is mediated by many upstream molecules. In the previous studies, it has been reported that GLP-1 or its receptor GLP-1R played an important role in regulation of the AMPK/mTOR pathway [26, 27]. In the present study, we demonstrated by immunofluorescence staining and immunoprecipitation that in the kidney GLP-1R co-localized and interacted with the AMPK/mTOR pathway. Thus, these data indicated that GLP-1R is involved in regulation of the AMPK/mTOR pathway in the kidney.

GLP-1R belongs to the G-protein coupled receptor (GPCR) family, which activates downstream pathways including cAMP/protein kinase A (PKA), cAMP/guanine-nucleotide exchange factor (Epac) or phosphatidylinositol-3 kinase/PKC pathways [28, 29]. With the development of experimental techniques, GLP-1R was found to be highly expressed not only in β-cells of the pancreas and lungs, but also in parietal cells of the stomach, pylorus, adipose tissue, heart, kidney, pituitary glands, and the brain [30, 31]. For kidney, GLP-1Rs have been demonstrated in the renal vasculature; however, there has been no complete agreement about in which part of the nephron GLP-1Rs are located [32]. Generally, the receptor is not found in distal tubules; however, in several studies, the GLP-1R was found in the proximal tubules and the glomerulus at both mRNA and protein level [32]. In line with previous studies, we used immunofluorescence staining to determine that GLP-1R was widely located in glomerular endothelial cells [33]. In many studies, it has been suggested that GLP-1 or GLP-1R might protect kidney from many types of injuries through various mechanisms. In a study by Yin et al., for example, it was reported that GLP-1 exhibited renoprotective effects by alleviating tubulointerstitial injury via inhibiting phosphorylation of MAPK and NF-κB [34]. Similarly, we found that up-regulation of GLP-1R by LRG markedly reduced the Scr, BUN, and urinary albumin excretion in a rat remnant kidney model of chronic renal failure, indicating the renoprotective effects of LRG on nephrectomized rats. Moreover, treatment with LRG also up-regulated the level of AMPK phosphorylation, down-regulated the level of mTOR phosphorylation, and enhanced the expression of LC3-II and ATG5 and pP62 degradation in a rat remnant kidney model of chronic renal failure. Together, these data indicated that up-regulation of

GLP-1R by LRG promoted autophagy through regulation of the AMPK/mTOR pathway.

Conclusions

In summary, our findings demonstrated that LRG relieved renal function autophagy in a rat remnant kidney model of chronic renal failure by promoting autophagy and regulating the AMPK/mTOR pathway, thereby providing a novel and promising therapeutic strategy for CKD. Although the abnormal levels of GLP-1R and AMPK/mTOR pathway were detected in a rat remnant kidney model of chronic renal failure, further research is needed to determine which cells GLP-1R and AMPK/mTOR pathway are involved.

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Compliance with ethical standards

Conflict of interests The authors declare no conflict of interests.

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