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LABORATORY STUDY

Apelin-13 exerts protective effects against acute kidney injury by lysosomal function regulation

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ABSTRACT

Background: Recent studies suggest that the loss of lysosomal function is associated with acute kidney injury (AKI), potentially leading to impaired autophagy. Apelin has been known to regulate autophagy processes in cardiovascular and pulmonary diseases. We sought to explore its potential contribution in lysosomal function and autophagy modulation during AKI.

Methods: Apelin-13 (30 μg/kg) or a vehicle control was administered to mice intraperitoneally 24 h prior to and at 0 h, 24 h, and 48 h following renal ischemia-reperfusion (I/R) injury or a sham procedure. Kidney and serum samples were collected for analysis 24 or 72 h postoperatively.

Results: Our findings indicate that apelin-13 significantly mitigated renal damage and inhibited apoptosis post-AKI. Flow cytometry analysis revealed that apelin-13 treatment modulates the macrophages polarization within the kidney from M1 to M2 phenotype. Additionally, apelin-13 was found to reduce the expression of the (pro)renin receptor, restore lysosomal membrane permeability, augment lysosomal biogenesis, and enhance autophagic flux in the kidney following AKI.

Conclusions: Our study elucidates novel mechanisms underlying the protective effects of apelin in AKI through modulating lysosomal function and autophagy.

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KEYWORDS
Apelin; acute kidney injury; lysosome; autophagy

Introduction

Organelle damage is a critical factor in the development of acute kidney injury (AKI). Pathogenic endoplasmic reticulum stress and mitochondrial dysfunction are well-documented contributors to AKI pathogenesis [1]. Our research, in concert with others, has demonstrated that enhancing the activity of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) can mitigate mitochondrial dysfunction and renal injury post-AKI [2,3]. Further findings indicate that this effect was believed to be mediated through autophagy and lysosomal biogenesis regulated by the transcription factor EB (TFEB) [4,5], which suggests that the lysosome may represent a novel therapeutic target for AKI.

Lysosomes are pivotal in the degradation of damaged organelles, aggregated proteins, and pathogens. They act as signaling hubs essential for energy and nutrient sensing, signal transduction, and the autophagy regulation. Their cellular positioning is dynamic and responsive to various stimuli, including cellular stress and pH changes [6]. Dysfunction and depletion of lysosomes can lead to impaired autophagy, which has been linked to a spectrum of kidney diseases, such as crystalline nephropathy [7], diabetic nephropathy [8], and septic AKI [9]. Furthermore, lysosomal enzyme leakage, aberrant intracellular localization, increased lysosomal pH, and lysosomal membrane permeabilization can all contribute to lysosomal dysfunction, thereby worsening renal injury [10]. Although the importance of lysosomal health is widely recognized, there remains a notable deficiency in therapeutic strategies specifically targeting lysosomal dysfunction in AKI.

Apelin, an endogenous ligand of the apelin receptor, has been identified in the glomeruli, vascular epithelial cells, and tubular epithelial cells of the kidney [11]. Its reno-protective properties have been observed in various AKI models, where apelin has been shown to suppress apoptosis, alleviate inflammation, and reduce TGF-β1 levels [12–14]. To date, emerging studies investigating the impact of apelin on lysosomal function have primarily centered on its role in autophagy. Interestingly, apelin exhibits context-dependent dual effects on autophagy-either promoting [15,16] or inhibiting [17,18] it -depending on cell types, pathological conditions and signaling pathways involved. However, its specific role in addressing lysosomal function and autophagy in the kidney during AKI remains elusive. In this study, we aimed to explore the potential effects of apelin treatment to modulate lysosomal function and autophagy within the kidney in the context of AKI.

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Materials and methods

Animals

Male C57BL/6 mice, aged between 8 to 10 weeks and weighing between 22 to 26 grams, were procured from the Animal Experimental Center at the Tongji Medical College of Huazhong University of Science and Technology. Throughout the experimental period, mice were housed in a standardized, pathogen-free environment with a regulated 12-h light/dark cycle. Food and sterile water were provided to them *ad libitum*. The care and treatment of all animals adhered to the Guidance Suggestions for the Care and Use of Laboratory Animals, as prescribed by the Ministry of Science and Technology of the People's Republic of China.

Study protocol

The mice were randomly assigned to six distinct groups. Renal ischemia/reperfusion (I/R) injury was induced using atraumatic vascular clamps placed on both renal pedicles for a period of 45 min. Mice in the sham operated group underwent identical surgical procedures but without the application of vascular clamps. Following the operation, the animals were provided with warm saline for hydration and were kept on a heating pad set to 40 °C to maintain a stable body temperature of 37 °C until they fully recovered from anesthesia. In the treatment group, apelin-13 (MCE, USA) was administered *via* intraperitoneal injection at a dosage of 30 µg/kg, 24 h prior to the renal I/R procedure, and at 0 h, 24 h, and 48 h post-renal I/R. The mice in the sham group received a single intraperitoneal injection of the vehicle (saline solution) at corresponding time points.

A total of 36 mice were humanely euthanized at 24 h or 72 h post-sham or I/R operation, with six mice assigned to each group. Immediately after euthanasia, kidney and serum samples were collected for subsequent analysis. The investigators remained blinded to the group allocation throughout the study.

Renal function examination

The creatinine and urine nitrogen levels in the serum were assessed by a commercially available assay kit (Nanjing Jiancheng Biological Engineering Institute, Jiangsu, China) according to the manufacturer's guidelines.

Histological examination

For histological analysis, renal tissues were postfixed with a 4% paraformaldehyde solution and subsequently embedded in paraffin. Sections of the renal tissue, 4-micrometer thick, were prepared. The sections were stained with periodic acid Schiff (PAS) and then examined under light microscopy (Olympus, Japan).

Tubular injury was scored semiquantitatively on a 0 to 5 scale by two experienced blinded pathologists who examined ≥10 cortical fields (×200 magnification) of PAS-stained sections ($n = 3–4$) as previously described [19], according to the percentage of the outer medulla area affected by tubular dilation, tubular atrophy, tubular cast formation, vacuolization, degeneration, and sloughing off of tubular epithelial cells or loss of the brush border and thickening of the tubular basement membrane (0 = no lesion, 1 = <25%, 2 = >25% to 50%, 3 = >50% to 75%, 4 = >75% to <100%, 5 = 100%).

TUNEL assay

The TUNEL assay was performed to identify apoptotic cells in renal tissues using commercial reagent (Roche, Germany) according to the manufacturer's protocol, Images were taken by fluorescence microscopy (Olympus, Japan). For quantification, 10 representative fields were selected from each section and the number of TUNEL positive cells/mm^2 was evaluated.

Flow cytometry analysis of macrophage polarization

To create a single-cell suspension, mice kidney tissues were aseptically dissected into pieces in phosphate-buffered saline (PBS), digested with 0.25% trypsin in RPMI 1640 medium, and filtered through a 40 µm cell strainer. The cells were then incubated with antibodies against F4/80, iNOS, and Arginase 1 (all from eBioscience, USA) at 4 °C overnight. After three washes with PBS, the cells were gated and analyzed using a flow cytometer (ACEA Novocyte, USA), with data processed through FlowJo software (version 10.0.7).

Western blot analysis

Mice kidneys protein samples were subjected to SDS-PAGE, transferred onto polyvinylidene fluoride (PVDF) membranes and incubated at 4 °C overnight with primary antibodies specific for the (pro)renin receptor (PRR, 1:1000, Abcam, USA), ATP6V0d2 (1:1000, Abcam, USA), lysosomal-associated membrane protein 1 (LAMP1, 1:1000, Abcam, USA), TFEB (1:1000, Abcam, USA), LC3B (1:1000, Abcam, USA), phosphatidylinositol-4 kinase type 2α (PI4K2A, 1:500, Santa Cruz Biotechnology, USA), and GAPDH (1:1000, Abcam, USA). The membranes were then incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:50000 dilution, Invitrogen, USA) at 25 °C for 1.5 h. Chemiluminescent bands were visualized and quantified using a chemiluminescence detection system and ImageJ software.

Transmission electron microscopy (TEM)

Kidney tissues were fixed in cold 2.5% glutaraldehyde (0.1 mol/L) in phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated through graded ethanol and embedded in epoxy resin. Ultrathin sections (60-80 nm) were cut and stained with 2% aqueous uranyl acetate and lead citrate. The electron microscope (FEI Tecnai G2 12, Netherlands) equipped with Gatan 832 CCD camera (Gatan, Pleasanton, USA) was used to imaging ultrastructure.

Statistical analysis

All data were presented as the mean ± SEM. Data that were normally distributed were performed using two-way ANOVA followed by Tukey's post-hoc test. Data analysis was conducted using GraphPad Prism version 8.0, with statistical significance determined at a *P*-value threshold of < 0.05.

Results

Apelin-13 protects renal function during AKI

Serum creatinine and urine nitrogen levels were utilized to evaluate renal function. As shown in Figure 1A,B, when compared to the AKI group, mice in the Apelin-13 group exhibited

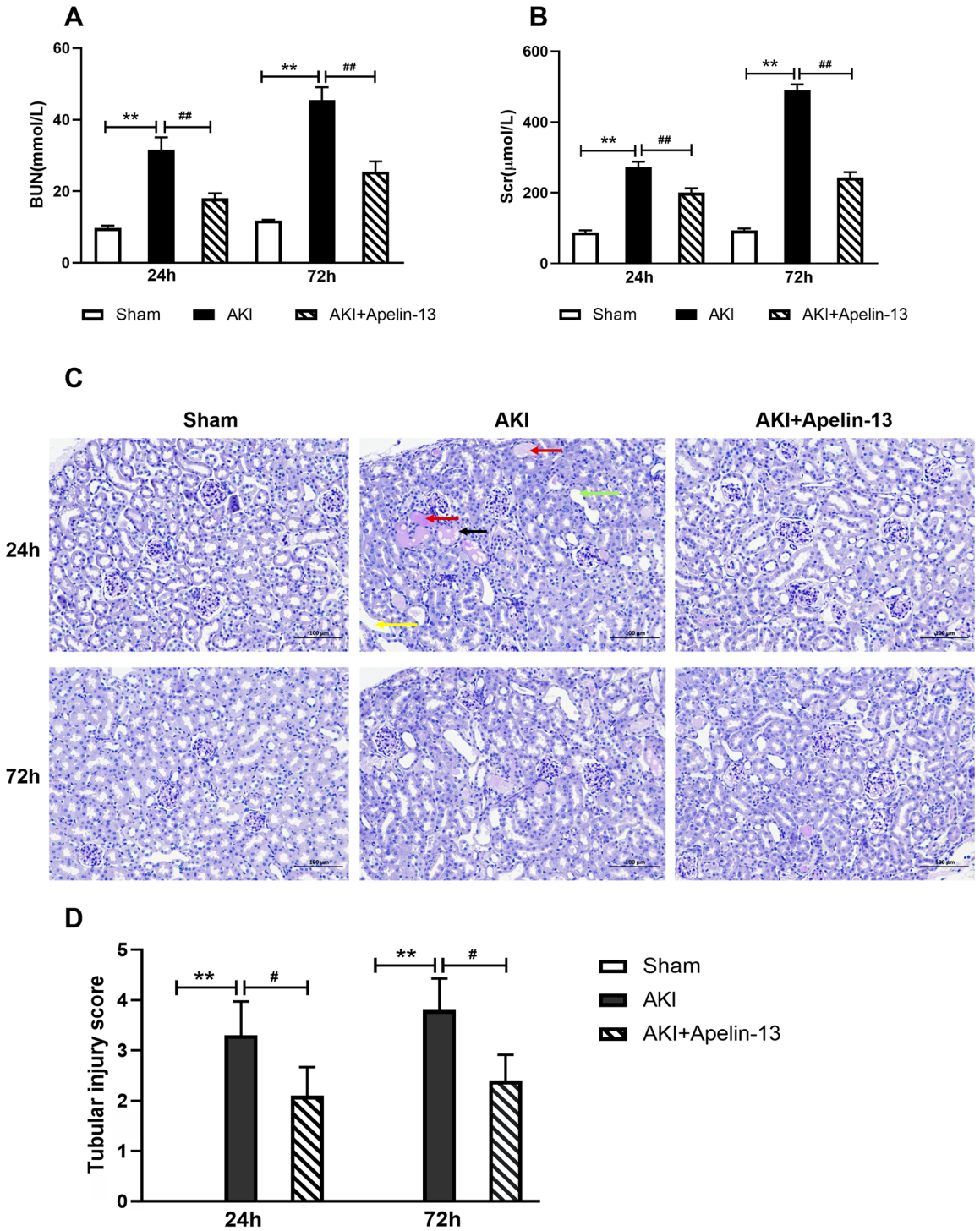

Figure 1. Apelin-13 treatment alleviated kidney function and renal tubulointerstitial damage. (A) The level of serum urine nitrogen in different groups. (B) The level of serum creatinine in different groups. (C) Histological changes of kidney in each group by PAS staining (×200). (D) Tubular injury score in different groups. Black arrow: vacuolar degeneration. Red arrow: cast. Green arrow: widespread tubular necrosis. Yellow arrow: tubular dilation. Scale bar, 100 μm. $^{**}p < 0.01$ versus sham group; $^{\#\#}p < 0.01$ versus AKI group.

significantly reduced levels of urine nitrogen (for 24h, 18.03 ± 0.81 versus 31.57 ± 2.02 mmol/L, $p < 0.01$; for 72h, 25.49 ± 1.64 versus 45.46 ± 2.09 mmol/L, $p < 0.01$) and serum creatinine (for 24h, 200.5 ± 7.22 versus 272.8 ± 8.63 μmol/L, $p < 0.01$; for 72h, 243.1 ± 8.88 versus 490.3 ± 9.56 μmol/L, $p < 0.01$) at both 24 h and 72 h post-AKI, suggesting improved renal function.

Apelin-13 ameliorates kidney histopathological changes

To assess histopathological alterations in the kidney, PAS staining was employed across different experimental groups. Upon examination at 24h and 72h post-AKI, the AKI group exhibited pronounced histopathological changes, including vacuolar degeneration, cast formation, widespread tubular necrosis, tubular dilation, and a marked loss of the brush border, the tubular injury score was risen to 3.30 ± 0.31 at 24h and 3.80 ± 0.82 at 72h respectively, as depicted in Figure 1C,D. In stark contrast, mice that received Apelin-13 treatment exhibited milder histopathological alterations with lower tubular injury score (2.10 ± 0.18 at 24h and 2.40 ± 0.16 at 72h, both $p < 0.05$), indicating a potential protective effect of Apelin-13 on renal tissue damage.

Apelin-13 inhibits apoptosis in the kidney after AKI

We evaluated apoptosis using TUNEL staining. As depicted in Figure 2A,B, a minimal presence of TUNEL-positive cells was discernible in the kidney tissue of the sham-operated group. However, following the induction of AKI, there was a notable increase in the number of these positive cells (for 24h, 19.19 ± 0.87% versus 3.47 ± 0.53%, $p < 0.01$; for 72h, 23.99 ± 1.13% versus 3.52 ± 0.38%, $p < 0.01$). Intriguingly, subsequent treatment with Apelin-13 led to a decrease in the count of TUNEL-positive cells in the kidney (10.82 ± 0.98% at 24h and 14.52 ± 0.62% at 72h, both $p < 0.01$).

Apelin-13 affects M1/M2 macrophages polarization in the kidney after AKI

To further investigate the impact of Apelin-13 on the M1/M2 polarization of macrophages following AKI, flow cytometry was employed to quantify macrophage populations. We detected the levels of polarization biomarkers, including iNOS for M1-type and Arginase 1 for M2-type. As shown in Figure 3, the number of M1 macrophages increased at 24h post-AKI (8.19 ± 0.42% versus 2.57 ± 0.02%, $p < 0.01$) and

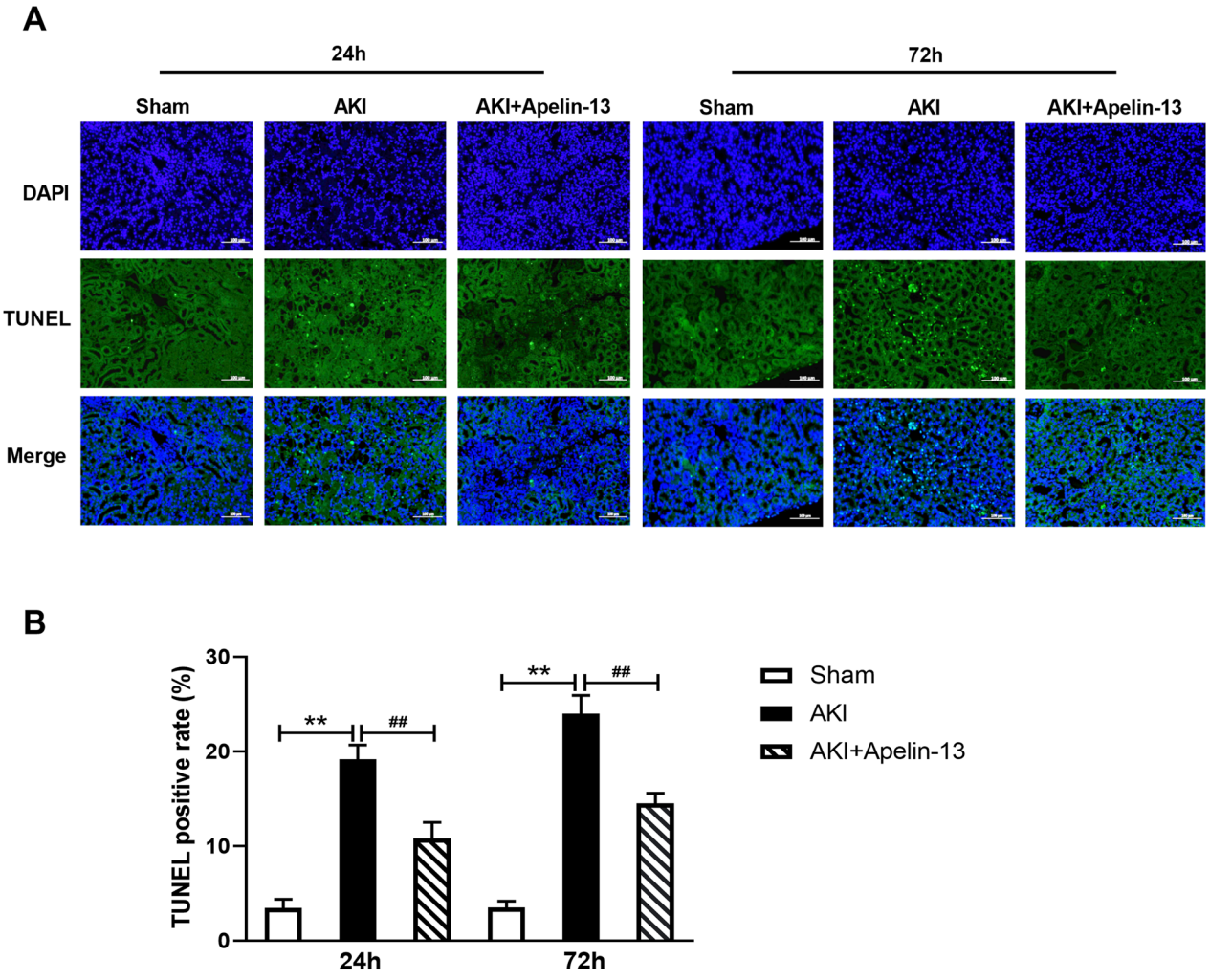

Figure 2. Apelin-13 treatment inhibits apoptosis during AKI. (A) Representative photographs show TUNEL staining in different groups. Scale bar, 100 μm. (B) Quantitative data show the difference of TUNEL-positive tubular cell numbers in different groups. $^{**}p < 0.01$ versus sham group; $^{\#\#}p < 0.01$ versus AKI group.

further rose at 72h (13.80 ± 0.20% versus 2.29 ± 0.06%, $p < 0.01$) post-AKI. However, Apelin-13 treatment significantly reduced the number of M1 macrophages at both 24h (4.82 ± 0.14%, $p < 0.05$) and 72h (8.13 ± 0.07%, $p < 0.01$), indicating that Apelin-13 inhibits the differentiation of M1 macrophages. Conversely, the number of M2 macrophages

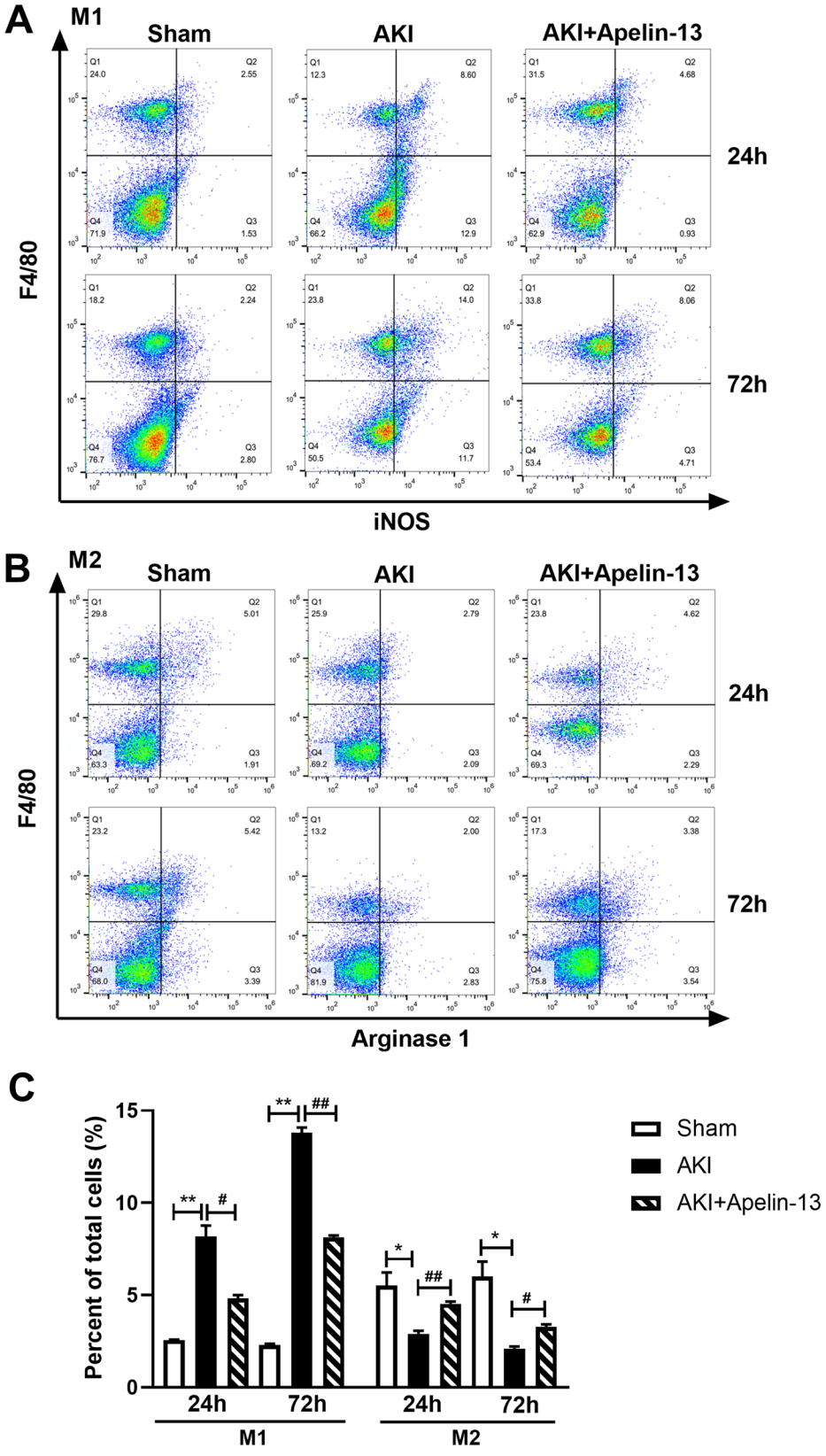

Figure 3. Apelin-13 treatment modulates macrophage M1/M2 polarization during AKI. (A) Flow cytometry analysis of M1 macrophage at 24h and 72h post-AKI in different groups. (B) Flow cytometry analysis of M2 macrophage at 24h and 72h post-AKI. (C) Quantification of M1/M2 macrophage in each group. $^{*}p < 0.05$ versus sham group; $^{**}p < 0.05$ versus sham group; $^{\#}p < 0.05$ versus AKI group; $^{\#\#}p < 0.01$ versus AKI group.

decreased at 24h post-AKI(2.91 ± 0.12% versus 5.52 ± 0.51%, $p < 0.05$) and was even lower at 72h post-AKI(2.09 ± 0.09% versus 6.01 ± 0.58%, $p < 0.05$). Apelin-13 treatment significantly increased the number of M2 macrophages at both 24h (4.54 ± 0.09%, $p < 0.01$) and 72h (3.28 ± 0.11%, $p < 0.05$) post-AKI, suggesting that Apelin-13 promotes the differentiation of M2 macrophages.

Apelin-13 inhibits PRR expression in kidney after AKI

As presented in Figure 4A,B, we observed a significant upregulation of PRR expression in the kidney at 24h post-AKI, with a further increase at 72h, which was entirely mitigated by Apelin-13 treatment.

Apelin-13 protects renal lysosome function after AKI

To assess kidney lysosome function post-AKI, we evaluated the expression levels of lysosomal markers, including ATP6V0d2, LAMP1, and TFEB. As shown in Figure 4C,D, ATP6V0d2 levels were elevated at 24h post-AKI and further increased at 72h. In contrast, LAMP1 and TFEB levels were reduced at 24h post-AKI and continued to decrease at 72h. However, Apelin-13 treatment significantly restored the

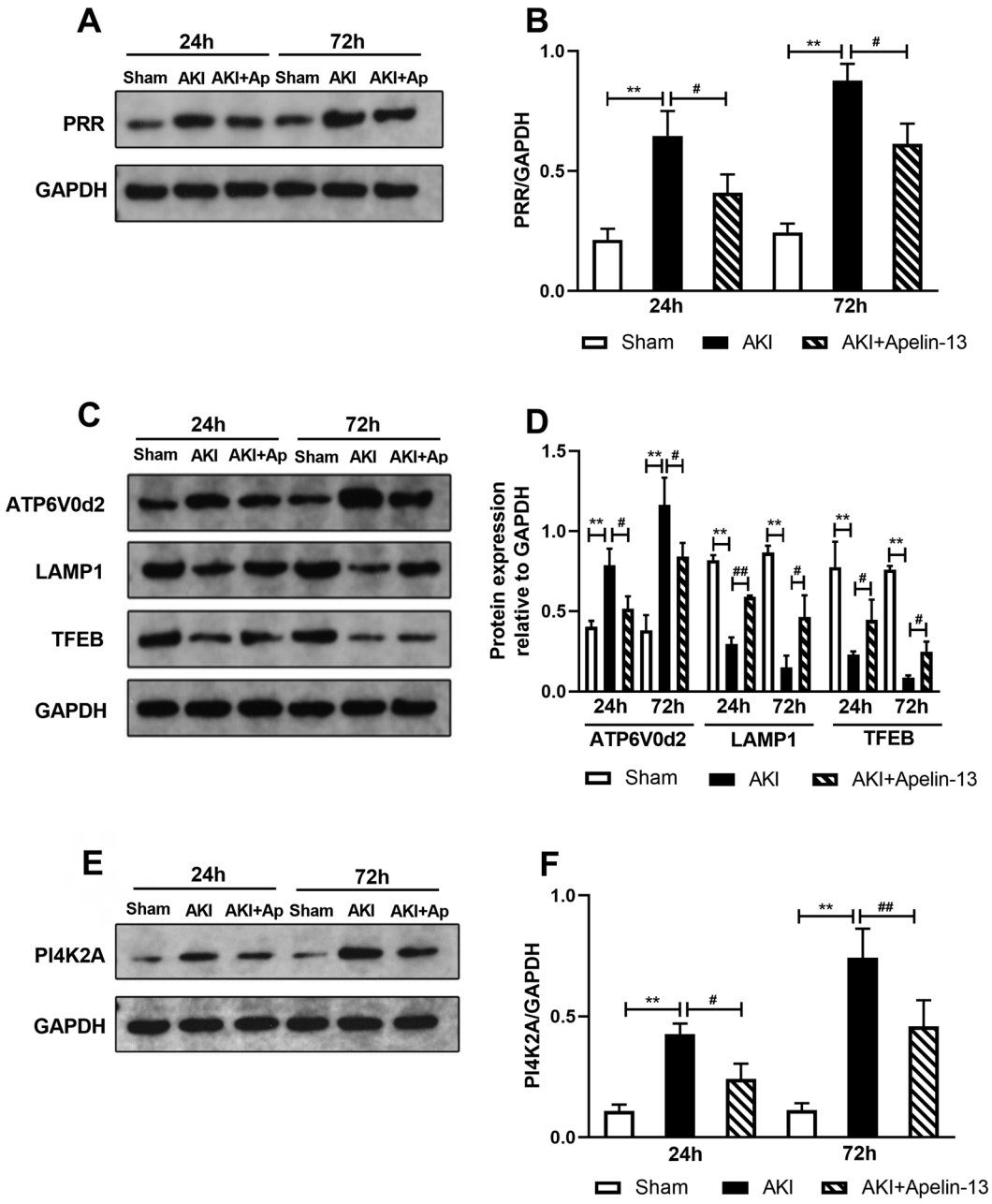

Figure 4. Apelin-13 treatment protects lysosome function after AKI. (A) Western blotting and quantification (B) of PRR expression in each group. (C) Western blotting and quantification (D) of lysosomal markers (ATP6V0d2, LAMP1, TFEB) expression in each group. (E) Western blotting and quantification (F) of PI4K2A expression in each group. $^{**}p < 0.05$ versus sham group; $^{\#}p < 0.05$ *versus* AKI group; $^{\#\#}p < 0.01$ versus AKI group.

expression of these lysosomal markers, indicating improved lysosomal function.

Apelin-13 decreases PI4K2A expression in the kidney after AKI

To explore the effect of Apelin-13 on lysosome repair, we assessed the expression of PI4K2A. We observed an increased expression of PI4K2A at 24h post-AKI, with a further rise at 72h, which was attenuated by Apelin-13 treatment (Figure 4E,F).

Apelin-13 enhances autophagic flux in the kidney after AKI

Autophagy was assessed among different groups. As shown in Figure 5A,B, the ratio of LC3B II to LC3B I was increased at 24h but decreased at 72h post-AKI. Apelin-13 treatment

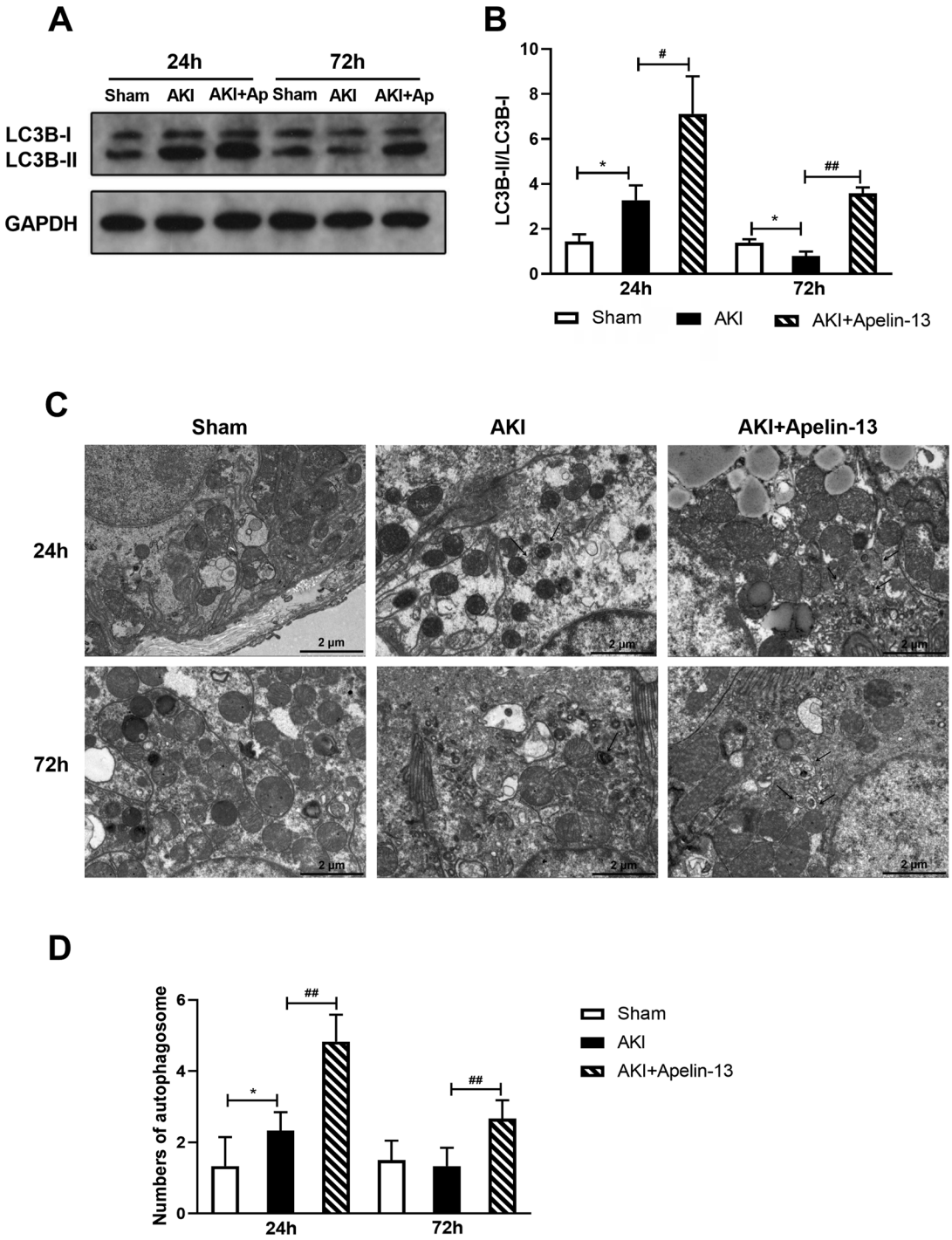

Figure 5. Apelin-13 treatment promotes autophagic flux in kidney. (A) Western blotting and quantification (B) of LC3B expression in each group. (C) Representative TEM photos show autophagosome in each group. (D) Comparison of the number of autophagosomes. $n = 3$/group. Black arrow: autophagosome. Scale bar, 2 μm. $^{*}p < 0.05$ versus sham group; $^{\#}p < 0.05$ versus AKI group; $^{\#\#}p < 0.01$ versus AKI group.

increased this ratio, indicating an enhancement of autophagic flux. We also observed the autophagosomes in each group by TEM (Figure 5C). The trend was further corroborated by our quantitative analysis of the autophagosomes, as illustrated in Figure 5D.

Discussion

Apelin is expressed as a 77-amino acid precursor, which is further cleaved into shorter segments, including apelin-13, apelin-17, apelin-36, and [Pyr1] apelin [20]. Due to its potent activity, apelin-13 is the predominant isoform within the apelin family [21]. Therefore, we selected apelin-13 for this study. The dosage of apelin-13 was based on previous studies with minor modifications, which demonstrated efficacy and safety in similar mice renal I/R injury model [12–14]. To align more closely with clinical practice, the administration timing was chosen to span the entire process from preoperative to postoperative. The 24h and 72h timepoints were chosen here based on the typical pathological progression (early injury and repair phase) of AKI. Herein, we report for the first time the effects of Apelin-13 at this specific dosage (30 μg/kg) on renal lysosomal function and autophagy during this phase following AKI. Consequently, we discovered that apelin-13 effectively modulates macrophage M1/M2 polarization, inhibits PRR expression, restores lysosomal function, and increases autophagic activity in the kidney following AKI.

We initially observed the protective effect of apelin-13 on the kidney post-AKI through renal function tests, PAS staining and TUNEL assay, which corroborates previous findings. It has been reported that distinct macrophage phenotypes are present in kidney tissues at different stages of AKI. For instance, M1 macrophages infiltrate the kidney within 48h following I/R injury, whereas M2 macrophages predominate at later time points [22]. During the renal repair phase, M1 macrophages can be transition into the M2 phenotype, shifting their function from promoting inflammation and tissue damage to facilitating renal tubular repair [23]. In this study, we found that apelin-13 exerted an inhibitory effect on M1 macrophages and a promoting effect on M2 macrophages from the early stage of AKI, particularly during the repair phase, suggesting its potential role in renal repair. Indeed, apelin has been documented to play crucial roles in the induction of macrophage physiological functions and to modulate macrophage activity to alleviate inflammation and fibrosis in several pro-inflammatory disease models, such as atherosclerosis, myogenesis, and angiogenesis [24]. Here, we identified for the first time that apelin-13 is a novel modulator of macrophage M1/M2 polarization during AKI, the specific regulatory mechanism, as well as the potential involvement of multiple signaling pathways, such as NF-κB, STAT1, PI3K/Akt, Nrf2, AMPK/mTOR, TGF-β/Smad and HIF-1α, requires further investigation.

PRR, a transmembrane receptor for renin and prorenin, is intricately involved in the regulation of organ development, physiology, and pathophysiology across multiple systems, either dependently or independently of the renin-angiotensin system (RAS) [25]. In the current study, we observed an increase in PRR expression at the onset of AKI and increased rapidly with the progression of AKI, which was believed can lead to inflammation, intrarenal RAS elevation, and fibrosis [26]. Concurrently, apelin expression has been found decreased previously [11]. Notably, emerging evidence suggests that apelin and PRR may reciprocally inhibit each other's expression, exerting opposing effects on intrarenal RAS, which significantly impacts blood pressure regulation, kidney injury, and urine concentrating ability [27]. Here, in an AKI model, we also found apelin-13 infusion significantly decreased PRR expression. Hence, in the present study, despite the lack of clarity regarding the precise mechanism, it is conceivable that the imbalance in apelin and PRR expression may serve as an initial factor contributing to subsequent kidney injury following AKI. Further studies are warranted to validate this hypothesis.

PRR is also known as ATP6AP2 (ATPase, H+-transporting, lysosomal accessory protein 2), which is essential for normal V-ATPase activity. V-ATPase is recognized for its role in regulating lysosomal acidification by pumping H+ions into lysosomes, thereby lowering intraluminal pH to the acidic range necessary for activating hydrolases with acidic pH optima [28,29]. Some reports have indicated that prorenin treatment may enhance PRR and V-ATPase expression, promoting renal fibrosis *in vivo* [30,31]. ATP6V0d2, a subunit of V-ATPase, is specifically expressed at the apical pole of intercalated cells in both cortical and medullary collecting ducts and has been shown to interact closely with ATP6AP2 based on STRING research [32,33]. In our study, ATP6V0d2 expression was found to be enhanced in the kidney following AKI but was decreased with apelin-13 administration, indicating apelin-13 can improve renal tubular cell lysosomal acidification and maintain intracellular pH homeostasis, potentially attributed to its inhibitory effects on PRR expression. More studies are still necessary to elucidate the exact mechanisms.

LAMP1, a major protein component of the lysosomal membrane, is commonly used as a lysosome marker. In this study, we observed a decrease in LAMP1 expression following AKI, indicating increased lysosomal membrane permeability, but was reversed with apelin-13 administration, suggesting apelin-13 restores lysosomal membrane permeability. Increased lysosomal membrane stability may lead to the leakage of lysosomal cysteine proteolytic enzymes, such as Cathepsin B, D, and L, into the cytoplasm or tissue space. This leakage can trigger local or systemic inflammation by activating M1 macrophages [34] and the NLRP3 inflammasome [35] in AKI. Therefore, the regulatory effects of apelin-13 on macrophage M1/M2 polarization observed in our study can be partially attributed to the preservation of lysosomal membrane permeability.

TFEB is a critical transcription factor for lysosomal biogenesis, homeostasis, and autophagy by regulating genes containing the CLEAR motif. Increasing number of studies have shown that TFEB activates the intracellular clearance of pathogenic factors by enhancing autophagy and lysosomal function in various kidney diseases, including cystinosis,

acute kidney injury, and diabetic nephropathy [36]. In this study, we found that TFEB expression was decreased following AKI but was restored with apelin-13 administration, indicating apelin-13 stimulates TFEB transcription to increase lysosomal biogenesis and improve their capacity for degrading lysosomal substrates post-AKI. Multiple studies have demonstrated that TFEB localization and activity are regulated through phosphorylation. Phosphorylated TFEB is sequestered in the cytoplasm and translocated to the nucleus upon dephosphorylation in response to nutrient deprivation, with signaling molecules such as mTORC1, ERK2, and Akt pathways involved in this process [37]. Further investigation is required to explore the detailed mechanisms of how apelin activates TFEB expression and nuclear translocation during AKI.

Interestingly, we observed an increase in PI4K2A expression over time following AKI. PI4K2A, recently reported to be enriched on damaged lysosomes, mediates lysosome repair within minutes of lysosomal membrane permeabilization [38,39]. Elevated PI4K2A expression may serve as an indicator of lysosomal damage. Therefore, the decreased PI4K2A expression observed with apelin-13 treatment in this study further supports the notion that apelin-13 may mitigate renal lysosomal injury post-AKI.

Autophagy is a cytoprotective mechanism that relies on lysosomes to eliminate and recycle damaged macromolecules and organelles, thus maintaining cellular homeostasis. In numerous AKI models, autophagy deficiency in proximal tubules exacerbates renal impairment, while the activation of autophagy has been shown to have protective effects, underscoring the protective role of autophagy in the kidney [40]. Recent studies have indicated that apelin induces autophagy and promotes protective effects in cardiomyocytes [41], transplanted mesenchymal stem cells [42], and human umbilical vein endothelial cells [16]. In our study, apelin-13 treatment was found to increase renal autophagy whether in the early or repair stage of AKI, as evidenced by increased LC3B-II expression and autophagosome formation. As mentioned earlier, TFEB plays a crucial role in autophagy. Recent studies found that apelin-13 could promote autophagy in different type cells *via* TFEB and mTOR pathway [43,44], highlighting the central role of TFEB in autophagy activation by apelin. Thus, the increased autophagy effect of apelin-13 observed in our study may also be associated with TFEB upregulation. The detailed mechanism through which apelin-13 regulates autophagy remains to be further clarified.

It should be noted that there are several limitations in our study. Firstly, the current study is a preliminary observational investigation based on animal models. While the findings provide initial evidence, the underlying molecular pathways and causal relationships remain unclear. Future studies should employ transgenic animal models and *in vitro* systems to dissect the specific signaling mechanisms. Techniques such as CRISPR/Cas9-mediated gene editing or pharmacological inhibitors could help clarify whether observed effects are mediated directly by apelin or secondary to compensatory pathways. Secondly, our study solely examined a single apelin concentration, limiting its translational potential. Dose-dependent effects are critical for therapeutic applications. Future work should systematically explore a range of apelin concentrations in both animal models and human-derived renal cells to establish a therapeutic window. Parallel pharmacokinetic and pharmacodynamic studies are warranted to determine optimal dosing regimens. Thirdly, the temporal scope of our study focused exclusively on early-phase AKI and initial repair stages (24–72 h post-AKI), leaving its long-term impact unexplored. Future investigations should extend observation periods to longer-term intervals (e.g., weeks to months) to evaluate apelin's impact on the dynamics of lysosomal-autophagy pathways in AKI-to-CKD transition.

Although more work is warranted, our results reveal that apelin-13 is instrumental in the restoration of lysosomal function and autophagic activity within the kidney following AKI. Our study contributes novel insights into the protective mechanisms of apelin in AKI and the lysosome represents a viable therapeutic target for AKI.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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