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Autophagy attenuates tubulointerstitial fibrosis through regulating transforming growth factor- β and NLRP3 inflammasome signaling pathway

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Abstract

Renal fibrosis is the final common pathway of various renal injuries and it leads to chronic kidney disease. Autophagy is a cellular process of degradation of damaged cytoplasmic components and regulates cell death and proliferation. Cellular response during unilateral ureteral obstruction (UUO) is tubular segment specific. Thus the role of autophagy on renal tubulointerstitial fibrosis (TIF) after UUO may be different according to segment of nephron. The role of autophagy during UUO remains unclear especially in distal tubules. In this study, we investigated the role of autophagy in distal tubules on renal TIF using conditional knockout mice in which Atg7 was genetically ablated specifically in distal tubular epithelial cell (TEC). In green fluorescent protein (GFP)-LC3 transgenic mice, GFP-LC3 puncta was highly expressed in distal tubular cells of the obstructed kidneys after UUO. Genetic deletion of Atg7 specifically in distal TEC increased renal tubulointerstitial fibrosis and epithelial-mesenchymal transition-like phenotype change after UUO through Smad4-dependent transforming growth factor (TGF)- β pathway. Distal tubule-specific autophagy-deficient mice increased the accumulation of damaged mitochondria and SQSTM1/p62-positive aggregates in the obstructed kidney and resulted in increased expression of NLRP3 inflammasome, interleukin (IL) 1- β and caspase-1. Distal TEC-specific Atg7 deletion enhanced apoptosis of TECs after UUO. In summary, our data showed that autophagy in distal TEC plays a protective role in development of renal tubulointerstitial fibrosis through regulating the expression of TGF- β and IL1- β after UUO.

Introduction

Renal fibrosis is the final common pathway of various renal injuries and leads to chronic kidney disease and end-stage renal disease^{1,2}. Renal fibrosis is characterized by excessive production and progressive accumulation of extracellular matrix (ECM) protein, such as collagen I and fibronectin¹. The matrix-producing fibroblasts in the

renal interstitium are considered to be the main source of increased ECM protein during renal fibrosis^{3,4}. Renal tubular epithelial cells contribute to the pathogenesis of renal fibrosis by modulating their apoptosis and proliferation, or by secretion of cytokines inducing inflammation and the formation of fibroblast in response to various injuries¹.

Autophagy is an evolutionarily conserved, lysosomal-mediated cellular process of degradation of damaged organelles, protein aggregates, and other macromolecules in the cytoplasm and regulates cell death under normal physiological conditions as well as pathological conditions^{5,6}. Autophagy is involved in renal diseases, including

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acute kidney injury, glomerular diseases, and aging of kidney⁷⁻⁹.

Autophagy has been reported to regulate renal fibrosis but its role on renal fibrosis remains unclear¹⁰⁻¹³. We previously reported that autophagy has a protective role in renal fibrosis induced by Unilateral ureteral obstruction (UUO)¹⁰. A previous study demonstrated that autophagy regulates the expression of transforming growth factor (TGF)- β and suppress renal tubulointerstitial fibrosis in UUO model using LC3^{-/-} mice and beclin 1 heterozygous mice¹¹. In contrast, another study reported that the persistent activation of autophagy promotes renal tubulointerstitial fibrosis (TIF) during UUO in proximal tubular cell-specific Atg7 knockout mice⁹. This discrepancy is may be due to the different role of autophagy for TIF according to the different cell types or the diverse cross talk among the cells¹⁴. In addition, cellular response after UUO differs between proximal and distal nephron¹⁵. While the distal nephron is an important component in UUO injury, the mechanism of distal nephron injury after UUO still remains unclear.

NLRP3 (NOD-like receptor, pyrin domain-containing 3) is a member of the NOD-like receptors (NLRs) involving innate immune response¹⁶. NLRP3 forms a protein complex, the inflammasome, which induces caspase-1 activation that results in the maturation and secretion of pro-inflammatory cytokines such as (interleukin) IL-1 β and IL-18¹⁷. Thus, NLRP3 inflammasome signaling pathway regulates a variety of host innate immune defense pathways in response to pathogen or damage-associated molecular patterns by microbial and non-microbial stimuli¹⁷. In renal injury, a previous study demonstrated that absence of NLRP3 attenuated tubular injury, inflammation, and fibrosis after UUO¹⁷. There is growing evidence that autophagy regulates NLRP3 inflammasome signaling pathway¹⁶. Thus, we hypothesized that the autophagy may regulate the renal TIF after

UUO through the regulation of NLRP3 inflammasome signaling pathway.

In this study, we demonstrated that autophagy deficiency in distal tubular epithelial cells (TECs) resulted in an increase of damaged mitochondria and oxidative stress, which activated NLRP3 inflammasome/caspase-1/IL-1 β signaling pathway and induced apoptosis of TECs. These data provide an insight for regulating autophagy as a therapeutic option for CKD.

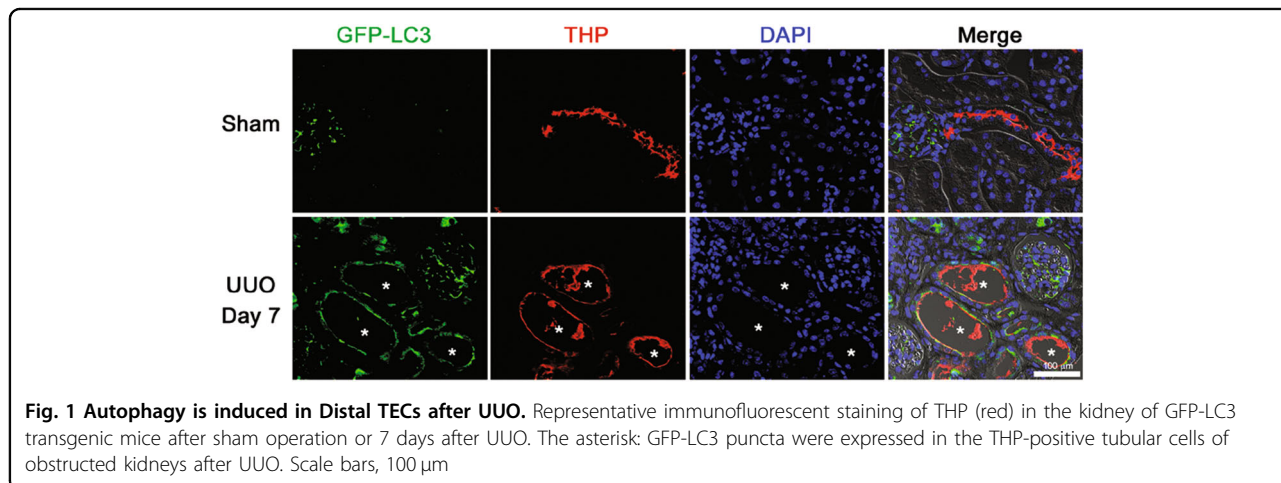
Results

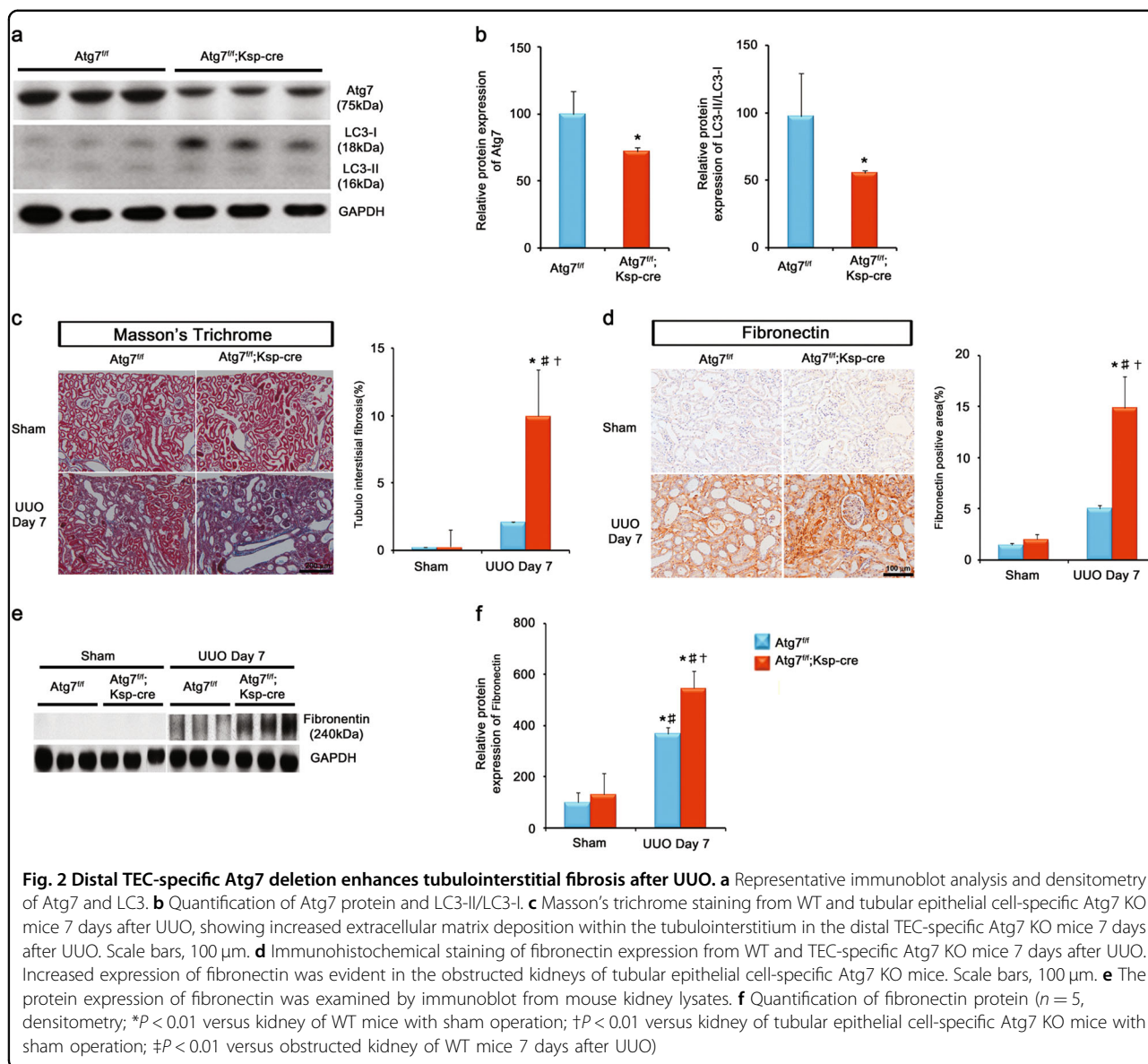
Autophagy is induced in Distal TECs after UUO

First, to determine the induction of autophagy in renal distal tubular epithelial cells after UUO, we used green fluorescent protein (GFP)-LC3 transgenic mice. In sham operation, GFP-LC3 puncta were rarely observed in renal TECs. After UUO, the expression of GFP-LC3 puncta was increased and GFP-LC3 puncta were co-localized with Tamm-Horsfall protein (THP)-positive cells (Fig. 1). Western blot analysis revealed that LC3-II/LC3-I was significantly increased after UUO in wild-type (WT) mice (Supplementary Fig. 1a). These data suggest the induction of autophagy in renal distal TECs after UUO.

Distal TEC-specific Atg7 deletion enhances tubulointerstitial fibrosis after UUO

To investigate the functional role of autophagy-induced renal distal TECs in the obstructed kidney after UUO on renal TIF, we generated conditional knockout mice in which Atg7 is genetically ablated specifically in distal TECs (Atg7^{fllox/fllox};Ksp-Cre⁺). The protein expression of Atg7 from western blot analyses of whole-kidney lysates was significantly decreased in kidneys of Atg7^{fllox/fllox};Ksp-Cre⁺ mice compared with those of WT mice (Fig. 2a and b). LC3-II/LC3-I was significantly decreased in Atg7^{fllox/fllox};Ksp-Cre⁺ mice (Fig. 2a and b). These data confirm an efficient deletion of Atg7 in TECs. No obvious





histologic phenotype was not observed in $Atg7^{fllox/fllox}; Ksp-Cre^{+}$ mice (Supplementary Fig. 1b).

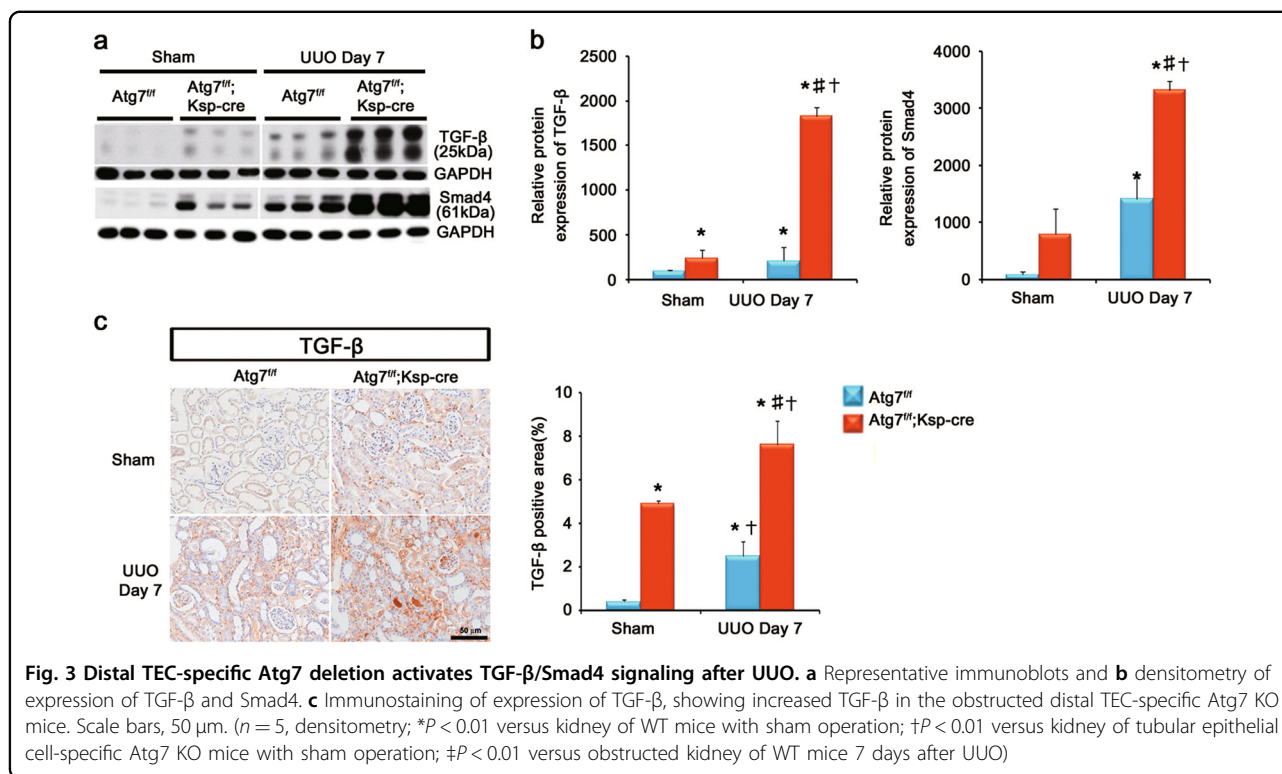
We next examined the effect of distal TEC-specific deletion of Atg7 in renal TIF induced by UUU. Masson's trichrome staining revealed that increased extracellular matrix deposition within the tubulointerstitium at 7 days after UUU in WT mice compared with sham-operated kidneys, which was substantially increased in the obstructed kidneys of $Atg7^{fllox/fllox}; Ksp-Cre^{+}$ mice (Fig. 2c). Consistently, similar data were obtained by immunohistochemical staining and the western blot analyses for fibronectin (Fig. 2d–f). Plasminogen activator inhibitor 1 (PAI-1) regulates fibrinolysis and the plasmin-mediated ECM matrix metalloproteinase activation.^{18,19} PAI-1 contributes to renal fibrosis by promoting migration of

profibrotic cells through a protease-independent mechanism.¹⁹ In this study, immunohistochemical staining and western blot analyses revealed upregulation of PAI-1 in obstructed kidneys of $Atg7^{fllox/fllox}; Ksp-Cre^{+}$ mice after UUU (Supplementary Fig. 1c and d).

Taken together, these data indicate that distal TEC-specific Atg7 deletion increases ECM protein and enhances renal TIF after UUU.

Distal TEC-specific Atg7 deletion activates TGF- β /Smad4 signaling and enhances tubular EMT-like phenotype change after UUU

TGF- β is a major cytokine mediating renal TIF by inducing the production of ECM proteins and may be regulated by autophagy degradation^{11,20}. TGF- β /Smad



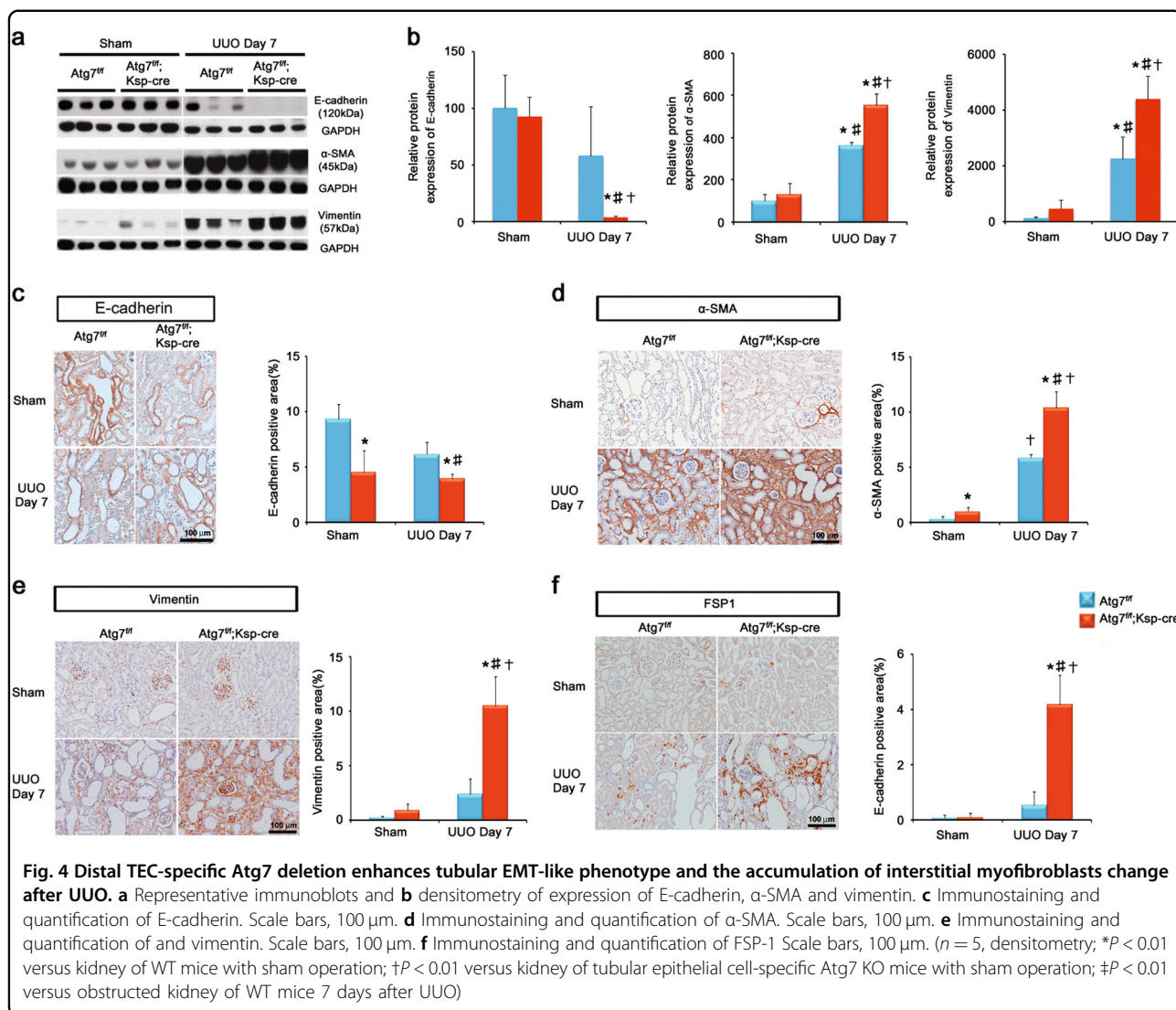
signaling is a major pathway leading to renal TIF²⁰. Thus, we investigated the effect of distal TEC-specific Atg7 deletion on the expression of the TGF-β/Smad pathway after UUU. The protein expression of TGF-β and Smad4 markedly increased in obstructed kidneys of Atg7^{flox/flox}; Ksp-Cre⁺ mice compared with those of WT mice, as demonstrated by western blot analyses (Fig. 3a and b). Immunohistochemical staining revealed upregulation of TGF-β in the interstitium of the obstructed kidneys of Atg7^{flox/flox};Ksp-Cre⁺ mice after UUU (Fig. 3c).

TGF-β may induce epithelial to mesenchymal transition (EMT)-like phenotype changes during the development of renal fibrosis²⁰. Thus, we examined the phenotype markers of EMT including E-cadherin, α-smooth muscle antibody (SMA), vimentin and fibroblast-specific protein (FSP)-1 as a marker of fibroblasts. E-cadherin is an epithelial cell marker and loss of E-cadherin represents the earliest step during TGF-β-induced EMT-like phenotype changes²¹. Western blot analyses showed that the protein expression of E-cadherin was decreased in WT mice at day 7 after UUU, which was substantially decreased in Atg7^{flox/flox};Ksp-Cre⁺ mice (Fig. 4a and b). The expression of α-SMA, which is an important marker of myofibroblast²¹, and vimentin, which is a cytoskeleton protein and a specific marker for mesenchymal cells, markedly upregulated in obstructed kidneys of Atg7^{flox/flox};Ksp-Cre⁺ mice compared with obstructed kidneys of WT mice after UUU (Fig. 4a and b). Consistently, immunohistochemical staining revealed

similar data (Fig. 4c–e). FSP-1-positive cells were substantially increased in the renal interstitium in Atg7^{flox/flox}; Ksp-Cre⁺ mice compared to those of WT mice after UUU (Fig. 4f). These data indicate that autophagy regulates Smad4-dependent TGF-β pathway and mediates TGF-β-induced tubular EMT-like phenotype changes and the accumulation of interstitial myofibroblasts after UUU.

Distal TEC-specific Atg7 deletion resulted in the accumulation of p62/SQSTM1 and damaged mitochondria through the oxidative DNA damage

p62/SQSTM1 is an ubiquitin-binding scaffold protein that is degraded by autophagy²². In previous studies, p62/SQSTM1-associated protein aggregates accumulates in Atg7 autophagy-deficient mouse liver and targeted deletion of p62/SQSTM1 prevents the accumulation of these protein aggregates^{23,24}. In this way the levels of p62/SQSTM1 accumulation serves as a good measure of defects in selective autophagy^{23,24}. In this study, the accumulation of p62/SQSTM1 was rarely observed at 7 days after UUU in WT mice, which was abundantly increased in the obstructed kidneys of Atg7^{flox/flox};Ksp-Cre⁺ mice (Fig. 5a). Furthermore, p62/SQSTM1 was colocalized with THP which is a targeted-autophagy-deficient tubular cells of Atg7^{flox/flox};Ksp-Cre⁺ mice (Fig. 5b). These finding indicates a selective and an efficient inhibition of autophagy in distal tubular cells of Atg7^{flox/flox};Ksp-Cre⁺ mice.



We next investigated the effect of autophagy deletion on ultrastructural mitochondrial alteration in renal tubular cells after UUU. EM showed the accumulation of damaged mitochondria with spherical shape and cristolysis and lipid inclusions were abundantly observed in outer medulla of kidneys of $Atg7^{flox/flox};Ksp-Cre^{+}$ mice (Fig. 5c). Mitochondria are the main source of reactive oxygen species (ROS) generation and mitochondrial DNA also be a main target of ROS^{25,26}. In this study, the increased immunoreactivity of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was observed in $Atg7^{flox/flox};Ksp-Cre^{+}$ mice after UUU (Fig. 5d).

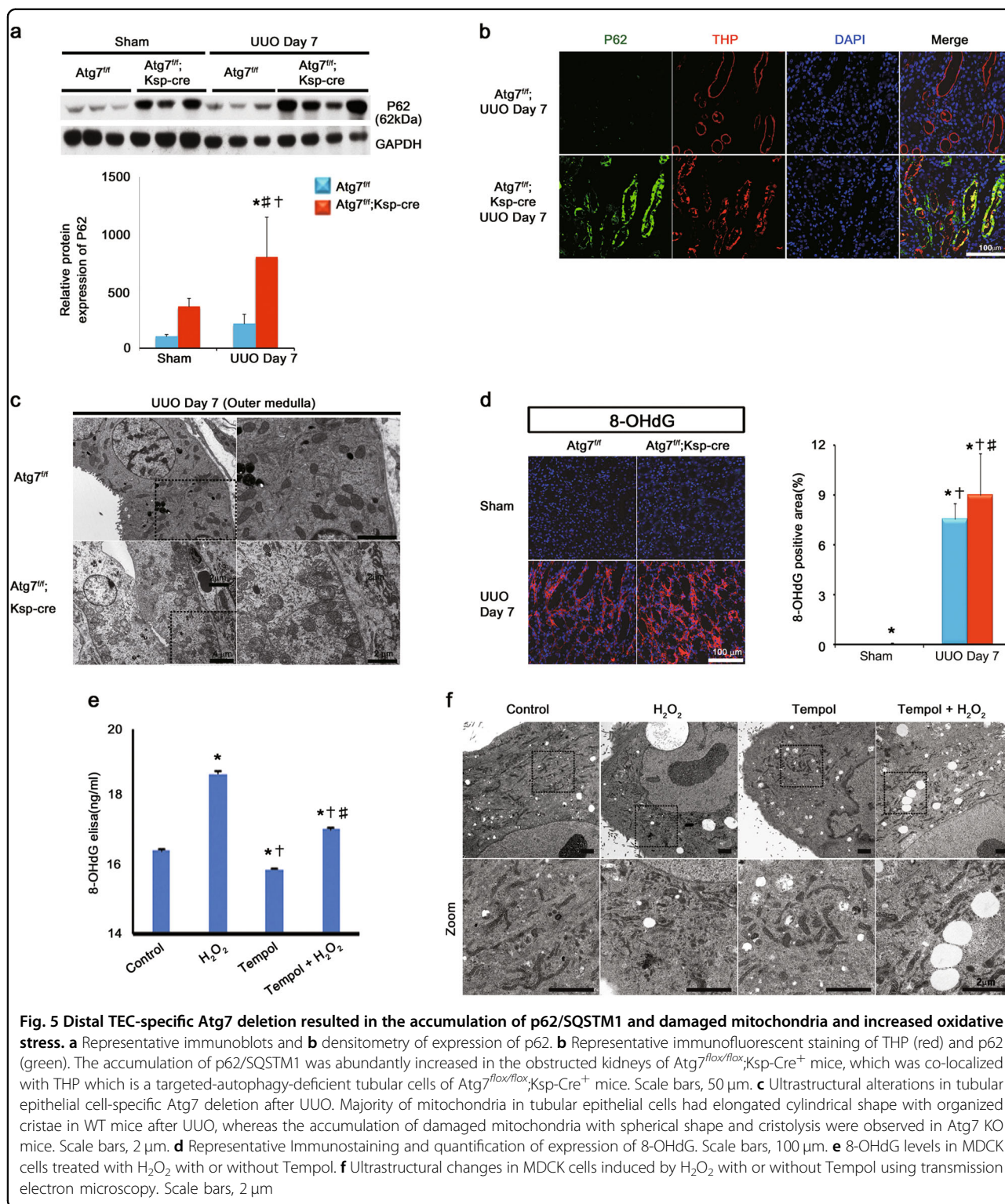
To determine the interaction between oxidative DNA damage and the accumulation of the mitochondria, we treated Madin-Darby Canine Kidney (MDCK) cells with hydrogen peroxide (H_2O_2) in vitro and examined the levels of 8-OHdG. H_2O_2 treatment of confluent cells increased the level of 8-OHdG, which was prevented by

Tempol (an antioxidant as a superoxide dismutase mimetic agent) treatment (Fig. 5e). TEM analyses showed the accumulation of damaged mitochondria with fragmented and spherical shape and cristolysis were increased by H_2O_2 treatment, which was recovered by Tempol treatment (Fig. 5f). These findings indicate that the oxidative DNA damage results in the accumulation of damaged mitochondria.

Taken together, our data suggest that autophagy deficiency accumulates the damaged mitochondria through the exacerbation of the oxidative DNA damage after UUU.

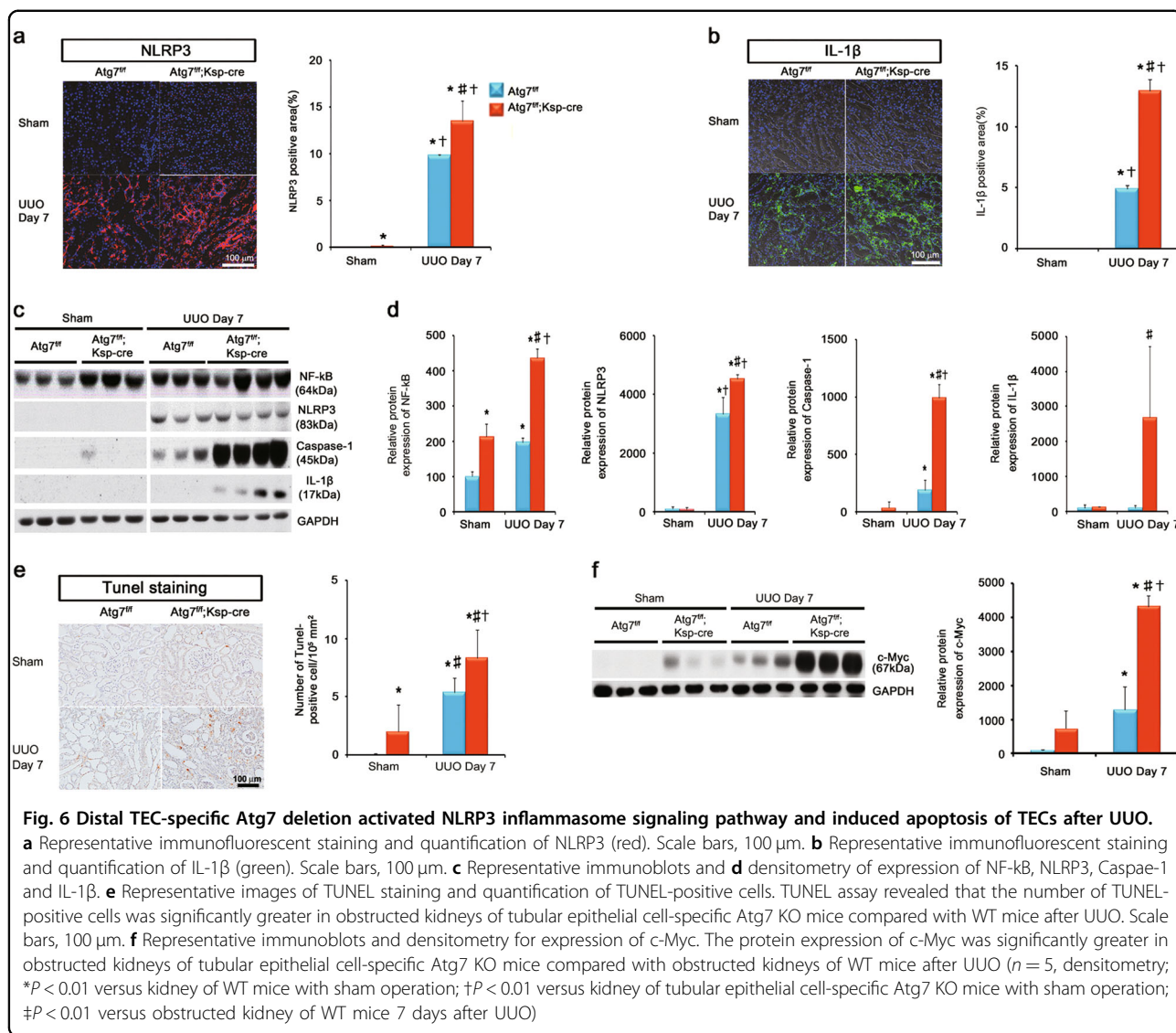
Distal TEC-specific Atg7 deletion activated NLRP3 inflammasome signaling pathway and induced apoptosis of TECs after UUU

The accumulation of damaged mitochondria can induce autophagy as well as inflammasome signaling pathway in innate immunity¹⁶. In this study, we investigated the effect



of autophagy deficiency on NLRP3 inflammasome signaling pathway. Immunohistochemical staining revealed the ablation of Atg7 in distal TEC resulted in the activation of NLRP3 inflammasome and its downstream, IL-1 β ,

after UUO (Fig. 6a and b). Immunoblot assay revealed that Atg7 deficiency in distal TEC induced NF-kB/NLRP3/Caspae-1/ IL-1 β signaling pathway after UUO (Fig. 6c and d).



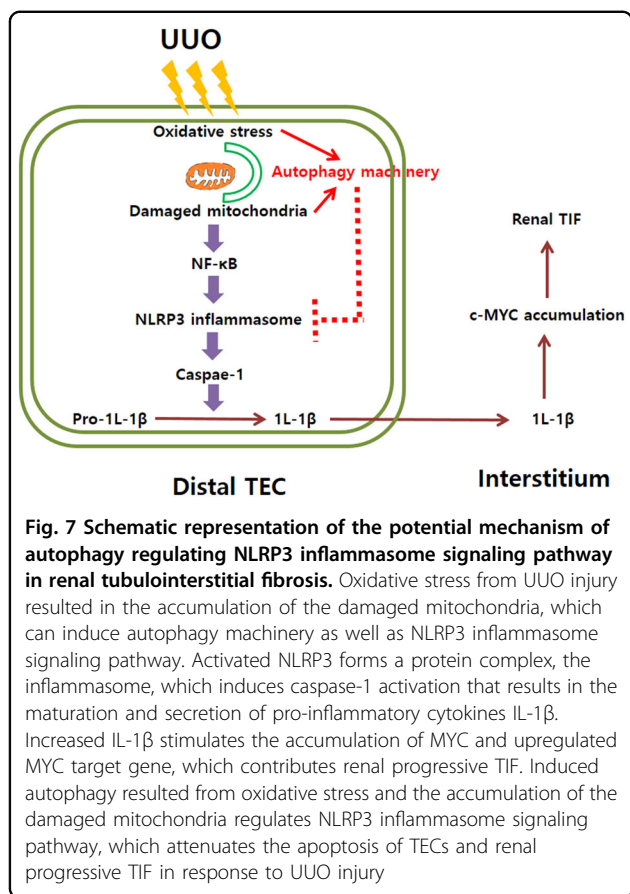
NLRP3 inflammasome signaling pathway in the context of autophagy regulates cellular apoptosis in response to various forms of cellular injury^{10–12,16,25,27}. Thus, we investigated the effect of autophagy on apoptosis of renal TECs after UUU. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay revealed that the number of TUNEL-positive cells was significantly greater in obstructed kidneys of distal TEC-specific autophagy-deficient mice compared with WT mice after UUU (Fig. 6e). These findings indicated a protective role of autophagy in apoptosis of renal TECs after UUU. Taken together, these data suggest that autophagy in distal TECs regulates apoptosis of renal TEC through the NLRP3/Caspase-1/IL-1β signaling pathway in response to UUU. A recent study demonstrated that the accumulation of MYC and upregulated MYC target gene resulted from IL-1β stimulation was necessary for renal progressive

TIF²⁸. In this study, the protein expression of c-MYC was substantially increased in the distal TEC-specific autophagy-deficient mice after UUU. These findings suggested that upregulated NLRP3/Caspase-1/IL-1β signaling pathway in autophagy-deficient mice after UUU resulted in the accumulation of c-MYC, which may enhance renal TIF and tubular injury (Fig. 7)

Materials and methods

Animals

Atg7^{fllox/fllox} mice were crossed with Ksp-Cre mice (Jackson Laboratories, West Grove, PA, USA) to generate distal tubule-specific Atg7 knockout mice (Atg7^{fllox/fllox}; Ksp-Cre mice). Atg7^{fllox/fllox} littermates served as controls. All mice were crossed on a C57BL6 background and only male mice were used in the study. UUU was performed as described previously¹¹. Briefly, mice were anesthetized



with zoletil and the left ureter was exposed via a left dorsal incision. The mid-ureter was then obstructed using a two-point ligation with silk sutures. The sham-operated mice underwent the same procedure with the exception of obstruction of the left ureter and used as controls. Mice were killed at 3, 7, and 14 days after UUO. The kidneys were briefly perfused with phosphate-buffered saline (PBS, pH 7.4) to rinse away any remaining blood. This was followed by perfusion with periodate-lysine-2% paraformaldehyde solution for 10 min. After perfusion, the kidneys were removed and cut into 1–2 mm thick slices, which were further fixed by immersion in the same fixative overnight at 4 °C. After fixation, the kidney slices were rinsed in PBS and dehydrated in a graded series of ethanol solutions and embedded in paraffin. All the experimental procedures were performed according to the animal care and ethics legislation and the study was approved by the Animal Care Committee of Bucheon Saint Mary's Hospital.

Cell culture

Madin-Darby canine kidney cells (MDCK, American Type Culture Collection) were cultured in MEM with 10% FBS (Mediatech Inc.) with streptomycin/penicillin. After becoming confluent, cells were treated with 0.01%

hydrogen peroxide (H₂O₂) (Sigma, St. Louis, MO) with or without Tempol 0.5 mM for 1 h.

8-hydroxy-2'-deoxyguanosine (8-OHdG) assay

The MDCK cells were seeded in 6-well plates at 2×10^5 cells/well. The supernatant of the culture medium and cytoplasmic fraction was collected following exposure to H₂O₂ and/or Tempol for 1 h. To determine the occurrence of oxidative DNA damage, the OxiSelect™ Oxidative DNA Damage ELISA kit (Cell Biolabs, Inc., San Diego, CA, USA) was used for the detection and quantification of 8-OHdG.

Antibodies

The antibodies used in this study were as follows: Atg7 (Sigma-Aldrich, St. Louis, MO, USA), LC3B (anti-LC3B; Sigma-Aldrich, St. Louis, MO, USA), P62 (PROGEN Biotechnik GmbH, Heidelberg, Germany), fibronectin (DAKO, Glostrup, Denmark), TGF- β (R&D systems, Minneapolis, Minnesota, USA), E-cadherin (BD Transduction Laboratories, Lexington, KY, USA), α -SMA (Sigma-Aldrich, St. Louis, MO, USA), vimentin (Santa Cruz Biotechnology, California, USA), PAI-1 (Santa Cruz Biotechnology, California, USA), NLRP3 (Adipogen, San Diego, USA), caspase-1 (Santa Cruz Biotechnology), FSP1 (Thermo scientific, Fremont, USA), IL- β (Cell signaling technology, Inc. Danvers, MA, USA), NF- κ B (Abcam, Cambridge, UK), 8-OHdG (JaICA, haruoka, Fukuroi, Shiizuoka, Japan) and GAPDH (Santa Cruz Biotechnology) were used. Apoptosis was detected using an Apop-Tag Peroxidase In Situ Apoptosis Detection Kit (Millipore, Billerica, MA, USA).

Immunohistochemical analysis

Some kidney sections were processed and stained with periodic acid-Schiff (PAS) or Masson's trichrome stain. Other sections were processed for post-embedding immunohistochemistry analysis. After deparaffin, the sections were hydrated and incubated with 0.5% Triton X-100/PBS solution for 30 min and then they were washed with PBS three times. The non-specific binding sites were blocked with normal donkey serum diluted 1:10 in PBS for 1 h, and then the sections were incubated for overnight at 4 °C in a primary antibody. After rinsing in PBS, the sections were incubated in peroxidase-conjugated anti-mouse or anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h. For coloration, the sections were incubated with a mixture of 0.05% 3,3'-diaminobenzidine that contained 0.01% H₂O₂ at room temperature until a brown color was visible and they were then washed with Tris buffer (pH 7.6), counterstained with hematoxylin and observed under light microscopy. The sections were scanned and automatically digitized using a (Leica SCN400), and then they were analyzed using the software (Tissuemorph/DP, Visiopharm, Denmark).

Western blot analysis

The kidney was homogenized in boiling lysis buffer (1% SDS, 1 mM sodium orthovanadate, and 10 mM Tris, pH 7.4) and the protein concentration was determined with the BCA Protein assay kit (Pierce Biotechnology Inc., Rockford, IL, USA). Equal amounts of the protein were separated on SDS–polyacrylamide gel. The gel was transferred onto a NC membrane. For immunodetection, the blots were incubated overnight in PBS that containing 0.1% Tween-20 and 5% skim milk with the primary antibody. The blots were washed and then incubated with a secondary antibody conjugated to horseradish peroxidase (Jackson Immuno Research Laboratories) and the blots were visualized using a western blotting luminol reagent kit (Santa Cruz Biotechnology, Santa Cruz, CA.).

Electron microscopic (EM) analysis

For observing the autophagy and ultrastructural changes of mitochondria, we performed a conventional transmission EM study. Kidney block samples and MDCK cells were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer for overnight at 4 °C. After washing in 0.1 M phosphate buffer, the samples were postfixed with 1% osmium tetroxide in the same buffer for 1 h at 4 °C. Next the samples were dehydrated with a series of the graded ethyl alcohol solution, exchanged through acetone, and the samples were next embedded in Epon 812.

Ultrathin sections (70~80 nm) were obtained by an ultramicrotome (Leica Ultracut UCT, Germany). Ultrathin sections were double stained with uranyl acetate and lead citrate and they were examined in a transmission electron microscope (JEM 1010, Japan) at 60 kV. For the quantitative determination, 20 field of low magnification ($\times 6000$) were randomly selected from each section of the cortex and the amount of autophagosomes per $100 \mu\text{m}^2$ was evaluated.

Statistics

Values are presented as the mean \pm SD. Data were compared between groups using an Mann–Whitney test or Kruskal–Wallis test as appropriate. *P*-values less than 0.05 were considered significant. All statistical analyses were performed using SPSS 16.0 software (Chicago, IL, USA).

Discussion

In this study, we demonstrated the role and its mechanisms of autophagy in renal tubulointerstitial fibrosis. We showed that genetic deficiency of Atg7 in a distal TECs-specific fashion upregulated the expression of TGF- β /Smad4 signaling pathway, which in turn induced EMT-like phenotype changes and led to accelerated renal

tubulointerstitial fibrosis after UO. We also showed the Atg7 deficiency in distal TECs-induced apoptosis through activating NLRP3 inflammasome signaling pathway after UO. Our results established that autophagy in distal TECs plays a pivotal role in development of renal tubulointerstitial fibrosis and induction of apoptosis in TECs.

Previous studies investigated the mechanisms of autophagy in development of renal tubulointerstitial fibrosis. Using LC3 $^{-/-}$ mice or Beclin1 haploinsufficient mice Ding et al. showed that autophagy deficiency resulted in increased levels of mature TGF- β and was associated with more severe fibrosis and renal tubular epithelial cell apoptosis in the obstructed kidney after UO¹¹. They suggest the induction of autophagy by TGF- β itself promotes mature TGF- β degradation, which subsequently reduce secretion of TGF- β and attenuates renal interstitial fibrosis¹¹. In addition, using distal TECs-specific Atg7 knockout mice, we suggest that autophagy machinery regulates the Smad-dependent TGF- β signaling pathway and subsequent EMT-like phenotype changes in renal tubulointerstitial fibrosis.

Myofibroblast has been recognized the main effector cell producing ECM protein during renal fibrosis¹. The precise origin of myofibroblast is still remains controversial¹. EMT has been considered to contribute to the myofibroblast pool during renal fibrosis, but recent fate tracing studies revealed the limited contribution on the myofibroblast pool^{21,29,30}. Furthermore, in vivo studies, EMT was not observed mice with overexpression of TGF- β 1 in renal tubules or autophagy-deficient mice, such as LC3 $^{-/-}$ mice or Beclin1 haploinsufficient mice^{11,30}. Thus, autophagy may be unlikely to induce EMT during renal fibrosis. Unexpectedly, this study showed the EMT-like phenotype changes in distal TECs-specific Atg7 knockout mice after UO. We cautiously suggest that autophagy may regulate renal fibrosis through EMT-like phenotype changes or partial EMT although its contribution to renal fibrosis may be limited. Nevertheless, the direct evidences with cell fate tracing are still needed to clarify the contribution of autophagy in EMT during renal fibrosis.

One of the interesting points of this study is the role of autophagy in activation of NLRP3 inflammasome signaling pathway during renal fibrosis. In innate immunity, autophagy has been reported to play a number of roles in regulating inflammasome activation and IL-1 family cytokine secretion by the removal of inflammasome-activating endogenous signals or the sequestration and degradation of inflammasome components¹⁶. This role for autophagy in NLRP3 inflammasome signaling pathway is not limited to immune cells. In diabetic retinopathy, a study showed that inhibition of autophagy in retinal pigment epithelial cells induced IL-1 β release via ROS

mediated NLRP3 inflammasome activation under high glucose condition¹⁶. Our data suggested that autophagy deficiency in distal TECs resulted in an increase of damaged mitochondria and oxidative stress, which activated NLRP3 inflammasome/caspase-1/IL-1 β signaling pathway and induced apoptosis of TECs. Activated IL-1 β stimulates the accumulation of MYC and upregulated MYC target gene, which contributes renal progressive TIF. Induced autophagy resulted from oxidative stress and the accumulation of the damaged mitochondria regulates NLRP3 inflammasome signaling pathway, which attenuates the apoptosis of TECs and renal progressive TIF in response to UUO injury (Fig. 7). Our data suggest the linking mechanism of autophagy with renal tubular apoptosis and fibrosis after UUO.

Conclusions

In conclusion, we have demonstrated that induction of autophagy in distal TECs after UUO has a protective role in renal tubulointerstitial fibrosis through the regulation of TGF- β /Smad4 signaling pathway and NLRP3 inflammasome/caspase-1/IL-1 β signaling pathway. Thus, precise regulation of autophagy may be a therapeutic option for CKD.

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Competing interests

The authors declare that they have no conflict of interest.

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