Hypertension

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Cathepsin K Deficiency Prevented Kidney Damage and Dysfunction in Response to 5/6 Nephrectomy Injury in Mice With or Without Chronic Stress

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BACKGROUND: Chronic psychological stress is a risk factor for kidney disease, including kidney dysfunction and hypertension. Lysosomal CatK (cathepsin K) participates in various human pathobiologies. We investigated the role of CatK in kidney remodeling and hypertension in response to 5/6 nephrectomy injury in mice with or without chronic stress.

METHODS: Male 7-week-old WT (wild type; $CatK^{+/+}$) and CatK-deficient ($CatK^{-/-}$) mice that were or were not subjected to chronic stress underwent 5/6 nephrectomy. At 8 weeks post-stress/surgery, the stress was observed to have accelerated injury-induced glomerulosclerosis, proteinuria, and blood pressure elevation.

RESULTS: Compared with the nonstressed mice, the stressed mice showed increased levels of TLR (Toll-like receptor)-2/4, $p22^{phox}$, $gp91^{phox}$, CatK, MMP (matrix metalloproteinase)-2/9, collagen type I and III genes, PPAR-γ (peroxisome proliferator-activated receptor-gamma), NLRP-3 (NOD-like receptor thermal protein domain associated protein 3), p21, p16, and cleaved caspase-8 proteins, podocyte foot process effacement, macrophage accumulation, apoptosis, and decreased levels of Bcl-2 (B cell lymphoma 2) and Sirt1, as well as decreased glomerular desmin expression in the kidneys. These harmful changes were retarded by the genetic or pharmacological inhibition of CatK. Consistently, CatK inhibition ameliorated 5/6 nephrectomy–related kidney injury and dysfunction. In mesangial cells, CatK silencing or overexpression, respectively, reduced or increased the PPAR-γ and cleaved caspase-8 protein levels, providing evidence and a mechanistic explanation of CatK's involvement in PPAR-γ/caspase-8–mediated cell apoptosis in response to superoxide and stressed serum.

CONCLUSIONS: These results demonstrate that CatK plays an essential role in kidney remodeling and hypertension in response to 5/6 nephrectomy or stress, possibly via a reduction of glomerular inflammation, apoptosis, and fibrosis, suggesting a novel therapeutic strategy for controlling kidney injury in mice under chronic psychological stress conditions. **(*Hypertension*. 2022;79:1713–1723. DOI: 10.1161/HYPERTENSIONAHA.122.19137.)** • **Supplemental Material**

Key Words: animals ■ cathepsin K ■ fibrosis ■ inflammation ■ proteinuria

The organic response to chronic psychological stress (CPS) involves 2 major components of the stress system: the hypothalamic pituitary-adrenal axis and the sympathetic nervous system.[1] Activation of both of these systems stimulates the secretion of adrenal catecholamines and glucocorticoid to the respective systemic hormonal and inflammatory responses.[2–4] CPS has been linked to disorder of the cardiovascular-renal

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NOVELTY AND RELEVANCE

What Is New?

Chronic stress accelerates kidney injury and hypertension in an animal model induced by 5/6 nephrectomy surgery. The role of lysosomal cathepsin K in the development of kidney dysfunction and hypertension under chronic stress conditions is unclear. We obtained evidence that cathepsin K inhibition exerts a protective effect against stress/injury-related kidney dysfunction and hypertension through a reduction of glomerular inflammation, apoptosis, and fibrosis in kidney.

What Is Relevant?

Cathepsin K is a widely expressed cysteine protease that has gained attention because of its enzymatic and nonenzymatic function signaling. However, the roles of cathepsin K in the pathogenesis of kidney injury, dysfunction, and hypertension in animals and humans under chronic psychological stress conditions are not clear.

Clinical/Pathophysiological Implications?

To our knowledge, this is the first study demonstrating that cathepsin K is a critical molecule for the development of the kidney remodeling and hypertension in response to 5/6 nephrectomy and stress. Our findings suggest that the targeting of the cathepsin K–mediated PPAR-γ (peroxisome proliferator-activated receptor-gamma)-caspase-8 activation pathway may provide a therapeutic route for managing the chronic stress–related proteinuria and hypertension caused by oxidative stress–induced and inflammation-induced apoptosis and glomerular fibrosis.

Nonstandard Abbreviations and Acronyms

5/6Nx	5/6 nephrectomy
AT1Rα	angiotensin receptor 1 alpha
CatK	cathepsin K
CPS	chronic psychological stress
ECM	extracellular matrix
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
MCP-1	monocyte chemoattractant protein-1
MMP	matrix metalloproteinase
PPAR-γ	peroxisome proliferator-activated receptor-gamma
TLR	Toll-like receptor
TNF-α	tumor necrosis factor-alpha

system.[5–7] As stress is known to induce inflammation, oxidative stress, and apoptosis, leading to the onset of kidney dysfunction and hypertension,[8] it is important to understand how stressors activate inflammation and cell apoptosis.

Laboratory studies documented over the past 10 years explored the CPS-mediated acceleration of metabolic and inflammatory disorders.[9] It was reported that chronic variable stress accelerated diet-induced vascular senescence and atherosclerotic plaque growth and instability.[10–12] Our recent study using a mouse model demonstrated that 2-week immobilization promoted experimental neointimal hyperplasia in response to injury.[13] We have also observed that chronic stress exerted a stimulatory effect on iron chloride$_3$–induced carotid artery thrombus formation in mice.[14,15] Although recent clinical studies indicate that the kidney with chronic disease is sensitive to various life stressors,[16–18] the precise mechanisms by which stress causes kidney damage and dysfunction remain largely unknown.

Cysteine protease cathepsins have traditionally been considered lysosomal restricted proteases that modulate the proteolysis of unwanted proteins.[19] A number of experimental and clinical investigations obtained several important findings that suggested a pivotal role of CatK (cathepsin K) in tissue regeneration and cardiorenal disease.[20] In mice, CatK activity is required for Notch-1 activation during hypoxia-induced neovascularization.[21] CatK expression increased by TGF-β (transforming growth factor-beta) promotes kidney tubular epithelial cell nuclear membrane importer importin-β expression, Smad-2/3 activation, and ECM (extracellular matrix) production, leading to kidney fibrosis.[22] Chronic stress increases the CatK expression in atherosclerotic plaques and injured arterial tissues.[15] Mice lacking CatK are resistant to injuries under immobilized stress conditions.[14] Clinical findings have shown that increased plasma CatK levels are associated with both coronary calcification and the clinical outcome in patients with chronic kidney disease.[23]

Given that the 5/6 nephrotomy (5/6Nx) model that is used as a model of very severe chronic kidney disease[24] is often used to screen drugs and explore the molecular mechanisms of chronic kidney disease, in the present study, we used a murine model of 5/6Nx plus chronic stress to explore the role(s) of CatK in the pathogenesis of chronic stress–mediated acceleration of kidney damage and dysfunction in mice with chronic kidney disease. We used CatK$^{-/-}$ (CatK knockout) and WT (wild type; CatK$^{+/+}$) mice that had been subjected to 5/6Nx surgery alone or the combination of this surgery with chronic stress for 2 months; all of the mice then received

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a vehicle or a specific CatK inhibitor (CatKII). To investigate the underlying molecular mechanisms, we subjected mesangial cells to superoxide- and stressed serum–induced apoptosis assays and a biological analysis after the silencing or overexpression of CatK, respectively.

METHODS

All data, analytical methods, and study materials are available in the Supplemental Material and from the corresponding author on reasonable request.

Statistical Analysis

All results are presented as the mean±SEM. Before applying statistical methods, we evaluated whether the data fit a normal distribution by conducting a Pearson normality test. Student *t* tests (for comparisons of 2 groups) or a 1-way ANOVA (for comparisons of ≥3 groups) followed by Tukey post hoc tests were used for the statistical analyses. The blood pressure data were subjected to a 2-way repeated measures ANOVA and Bonferroni post hoc tests. A P value <0.05 was considered significant. All of the morphometric measurements were performed by 2 observers in a blind manner, and the values they obtained were averaged. The authors had full access to and take full responsibility for the data. All authors have read and agree to the article as written.

RESULTS

Chronic Stress Promoted Elevated Systolic Blood Pressure and Increased Urinary Protein and Glomerular Damage in 5/6Nx Mice

For the investigation of the impacts of stress on renal function and hypertension after kidney injury, we subjected CatK$^{+/+}$ mice to 5/6Nx surgery combined with chronic variable stress or a sham operation. The systolic blood pressure (SBP) changes were evaluated in the nonstressed and stressed CatK$^{+/+}$ mice within 2 months after the 5/6Nx or sham surgery (Figure 1A). The results revealed that the SBP levels were greatly increased in the 5/6Nx mice compared with the sham-operated mice. Compared with the sham mice, stress accelerated SBP elevation in the 5/6Nx mice from 4 to 8 weeks after the 5/6Nx or sham surgery.

The results revealed that the 5/6Nx surgery increased the protein content in urine compared with the content in the sham mice, and stress caused an increase in the levels of proteinuria compared with 5/6Nx mice that were not subjected to stress (Figure 1B). We also observed representative microscopy images of desmin-stained sections of CatK$^{+/+}$ kidneys. The quantitative data showed that stress increased the area of glomerular damage and the number of cells positive for CatK$^{+/+}$ caused by the 5/6Nx surgery (Figure 1C through 1E), indicating that the expression of CatK is related to kidney injury and stress.

CatK Deletion Ameliorated the 5/6Nx- or Stress-Related Podocyte Injury, Glomerulosclerosis, Proteinuria, Hypertension, and Kidney Injury in Mice

As shown in Figure S1, the quantitative polymerase chain reaction data with the related specific primers (Table S1) demonstrated that the expression levels of the targeted oxidative stress genes (gp91^{phox} and p22^{phox}), inflammation genes (TLR [Toll-like receptor]-2/4), and proteolysis-related genes (MMP [matrix metalloproteinase]-2/9) were lower in the kidneys of the S-CatK$^{-/-}$+5/6Nx mice compared with those in the kidneys of the CatK$^{+/+}$+5/6Nx mice. In addition, at 8 weeks, the CatK deletion mitigated the elevation of SBP during the follow-up period, the protein content in urine, and the inflammatory factors IL (interleukin)-17 and TNF-α (tumor necrosis factor-alpha) in the blood of the 5/6Nx mice with or without chronic stress (Figure 2A through 2D; Figure S2A through S2D).

At 8 weeks, CatK$^{-/-}$ had mitigated the harmful changes in the levels of Sirt1, NLRP-3 (NOD-like receptor thermal protein domain associated protein 3), PPAR-γ (peroxisome proliferator-activated receptor-gamma), Bcl-2 (B cell lymphoma 2), C-cas-8, p21, and p16 (Figure 2E and 2F) in the kidneys of the 5/6Nx mice that were subjected to chronic stress. CatK deficiency also reduced the levels of gp91^{phox}, NLRP-3, PPAR-γ, C-Cas-8, p-p38MAPK, and p-Erk1/2 (extracellular regulated protein kinase1/2), as well as the levels of the investigated genes (TLR-2/4, TNF-α, MCP-1 [monocyte chemoattractant protein-1], gp91^{phox}, p22^{phox}, collagen type I/III, CatS, CatK, CatK, MMP-2/9, IL-1, and AT1Rα [angiotensin receptor 1 alpha]) and blood creatinine and blood urea nitrogen in the 5/6Nx-alone mice (Figure S2E through S2I; Table S2). However, there were no significant differences in SBP or other parameters between the two genotypes of mice that underwent the sham operation (Figure 2; Figure S2). In contrast to the stressed mice, we observed that CatK deletion exerted no effect on the expressions of p16 and Sirt1 in the kidneys of 5/6Nx mice (Figure S2G through S2I).

To evaluate glomerular morphological changes among the 4 experimental groups, we used electron microscopy and performed staining by using periodic acid-Schiff, Masson trichrome, desmin, single-stranded DNA, and macrophages in the kidneys. The electron microscopy analysis showed that compared with the S-CatK$^{+/+}$+5/6Nx mice, the S-CatK$^{-/-}$+5/6Nx mice had a significantly increased number of podocyte processes, indicating that CatK$^{-/-}$effectively prevented the disappearance of stress-related podocyte processes (Figure 3A and 3B). Moreover, the quantitative data of the desmin, periodic acid-Schiff, and Masson staining showed that CatK$^{-/-}$ prevented glomerular injury and fibrosis glomerulosclerosis (Figure 3A through 3C). The immunostaining analysis

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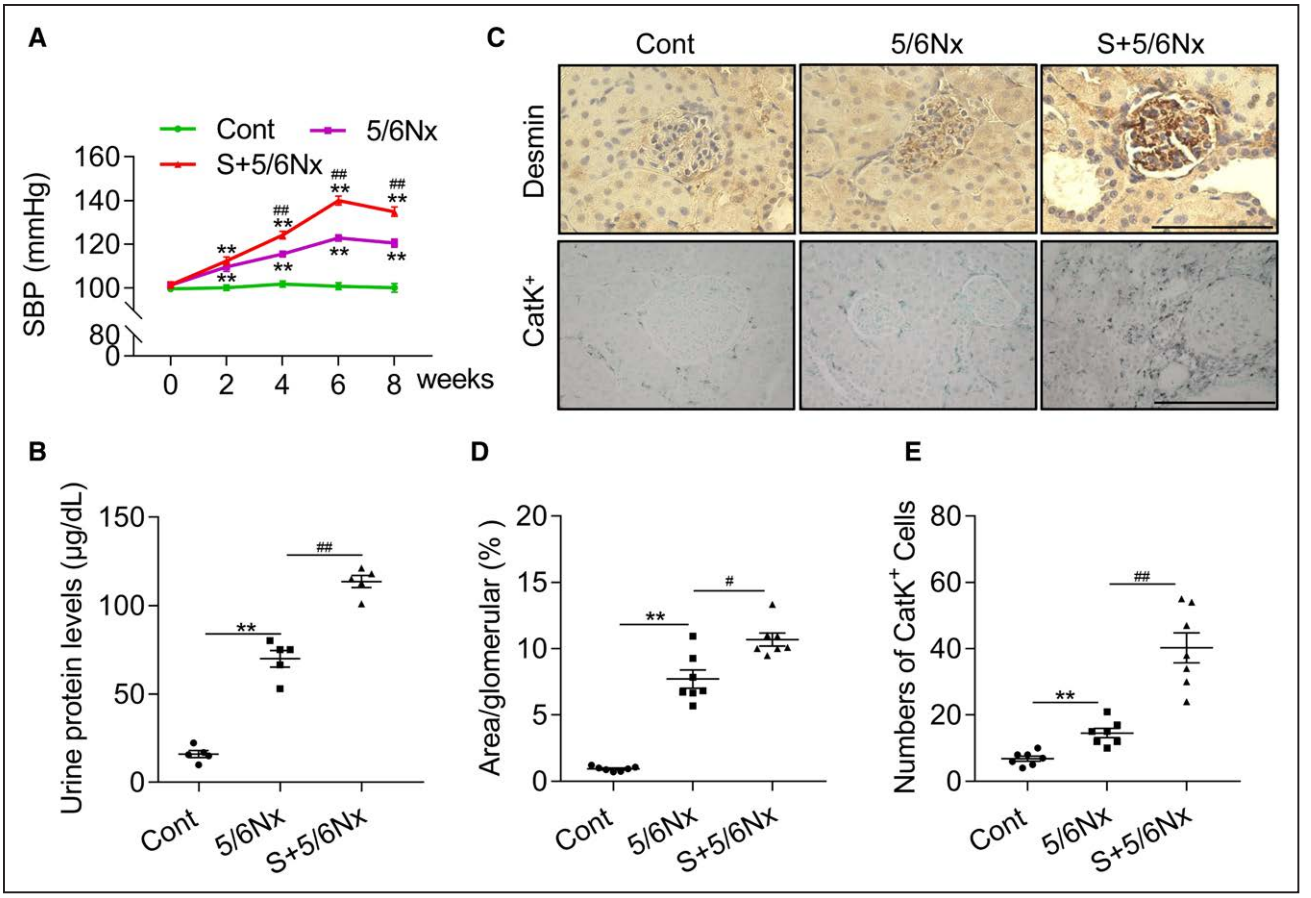

Figure 1. Chronic stress accelerated kidney dysfunction and systolic blood pressure (SBP) elevation in response to 5/6 nephrectomy (5/6Nx).

A, SBP in the 3 experimental groups (n=6–8). **B**, Urine protein results (n=5). **C** through **E**, Representative immunostaining images (**C**) and quantitative date for desmin (**D**) and CatK (cathepsin K)$^{+}$ (**E**) expressions in the kidney groups. Scale bar, 75 μm. Data are mean±SEM (n=5–8). S+5/6Nx indicates a 5/6Nx mouse that received chronic stress. *P<0.05, **P<0.01 vs day 0; #P<0.05, ##P<0.01 vs corresponding 5/6Nx-alone mice by 2-way repeated measures ANOVA and Bonferroni post hoc tests (**A**) or ANOVA and Tukey post hoc tests (**B**, **D**, and **E**). Cont indicates CatK$^{+/+}$sham.

revealed that the numbers of macrophages and single-stranded DNA^{+} apoptotic cells were lower in the glomeruli of the 5/6Nx-stressed CatK$^{-/-}$ mice compared with those of the 5/6Nx-stressed CatK$^{+/+}$ mice (Figure 3A and 3D). Likewise, CatK$^{-/-}$ exerted a renoprotective effect in the 5/6Nx-alone mice (Figure S3). However, there were no significant differences in these glomerular microchanges between the 2 genotypes of mice that underwent the sham surgery (Figure 3).

Pharmacological Inhibition of CatK Prevented 5/6Nx- and Stress-Related Podocyte Injury, Glomerulosclerosis, Proteinuria, and Hypertension

We further investigated whether the pharmacological inhibition of CatK with a CatKII inhibitor exerts a renoprotective effect in 5/6Nx- and stress-related renal injury. CatKII markedly reduced the elevated SBP from 6 to 8 weeks and the urine protein content at 8 weeks in both CatK$^{+/+}$+5/6Nx mice (Figure S4A and S4B) and S-CatK$^{+/+}$+5/6Nx mice (Figure S6A and S6B). The quantitative real-time polymerase chain reaction data demonstrated that CatKII lowered the levels of TLR-2, TNF-α, MCP-1, collagen types I and III, and MMP-2 and -9 genes in the 5/6Nx kidney tissues (Figure S4C and S4D). Moreover, CatKII mitigated the harmful changes in the levels of PPAR-γ, Bcl-2, and C-Cas-8 in the 5/6Nx-stressed kidneys (Figure S6C and S6D).

As shown in Figure 4 and Figure S5, CatK inhibition had a renoprotective effect on podocyte foot process effacement, macrophage infiltration, glomerular injury, apoptosis, and fibrosis, as well as glomerulosclerosis in S-CatK$^{+/+}$+5/6Nx and CatK$^{+/+}$+5/6Nx mice.

Genetic Modification of CatK Modified the Oxidative Stress–Induced and Stress Serum–Induced Mesangial Cell Apoptosis

To investigate whether a genetic modification of CatK would modify the oxidative stress–induced and stress serum–induced mesangial cell apoptosis, we stimulated

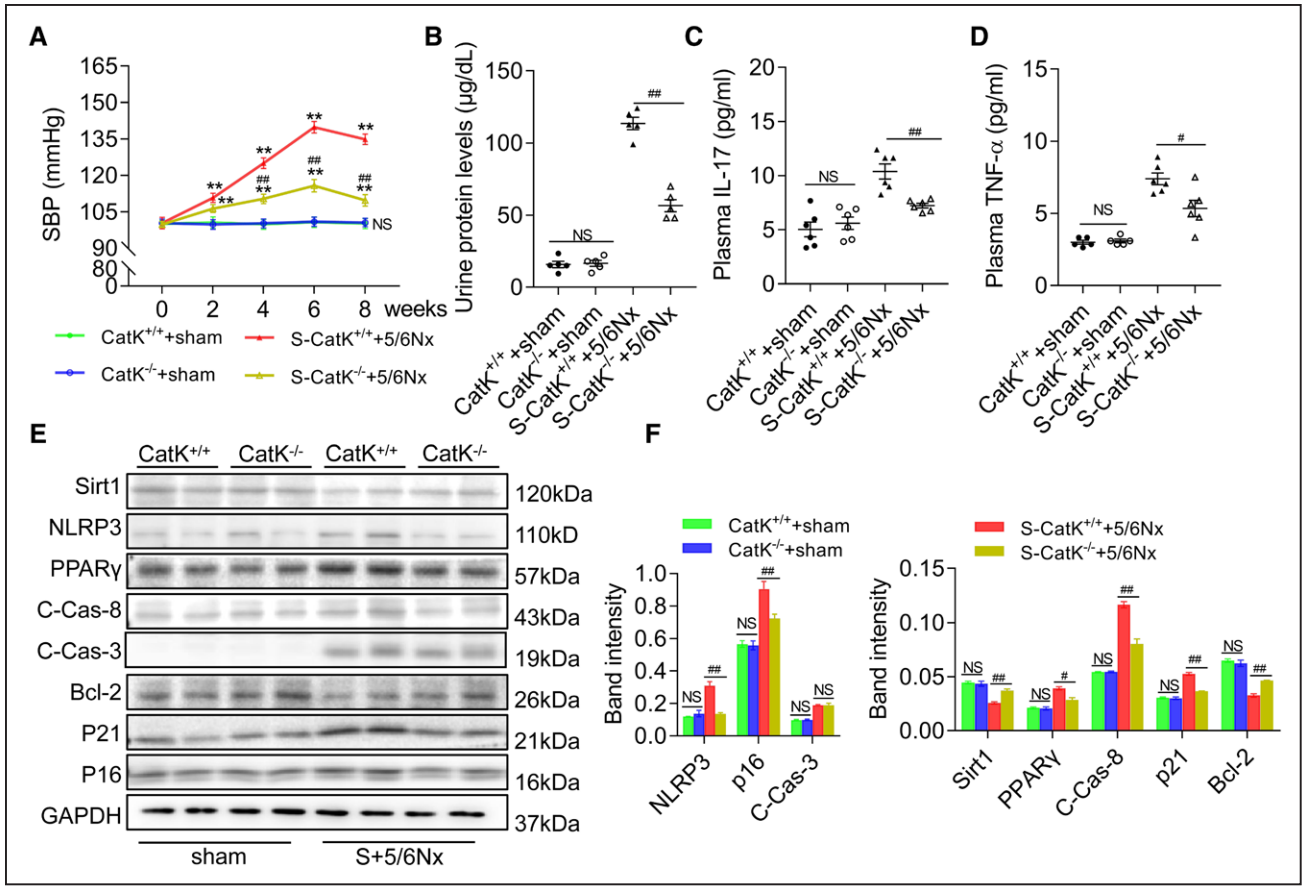

Figure 2. CatK (cathepsin K) deletion ameliorated systolic blood pressure (SBP) elevation and proteinuria in 5/6 nephrectomy (5/6Nx) mice subjected to chronic stress.

A, SBP recordings in the 4 experimental groups measured at the indicated time points (n=8 for each group). **B**, Urine protein test results of mice in the 4 groups at 8 weeks after stress. **C** and **D**, ELISA results showing the levels of plasma IL (interleukin)-17 and TNF-α (tumor necrosis factor-alpha) at 8 weeks after stress. **E** and **F**, Representative immunoblot images (**E**) and quantitative data (**F**) showing the target protein levels (Sirt1 [sirtuin1], NLRP-3 [NOD-like receptor thermal protein domain associated protein 3], PPAR-γ [peroxisome proliferator-activated receptor-gamma], Bcl-2 [B cell lymphoma 2], C-Cas-8, p21, and p16) in the kidneys of the 4 experimental groups. Data are mean±SEM (n=3–8). NS indicates not significant. *P<0.05, **P<0.01 vs day 0; #P<0.05, ##P<0.01 vs corresponding S-CatK$^{+/+}$+5/6Nx-alone mice by 2-way repeated measures ANOVA and Bonferroni post hoc tests (**A**) or ANOVA and Tukey post hoc tests (**B**–**D**, **F**).

mesangial cells with 0-, 50-, and 100-μM H_2O_2 for 24 hours. Quantitative real-time polymerase chain reaction data showed that the CatK gene expression and the PPAR-γ, C-Cas-8, and Bcl-2 protein expressions were sensitive to H_2O_2 stimulation in a dose-dependent manner (Figure S7A through S7C).

As anticipated, CatK silencing suppressed the CatK gene expression (Figure S8A). We observed that the levels of PPAR-γ and C-Cas-8 proteins were higher in the lysates of the mesangial cells treated with oxidative stress serum and 5% stress serum, and these changes were prevented by CatK silencing (Figure 5A and 5B; Figure S8B and S8C). Terminal deoxynucleotidyl transferase dUTP nick end labeling staining showed that CatK silencing prevented oxidative stress serum and 5% stress serum–induced mesangial cell apoptosis (Figure 5C and 5D; Figure S8D and S8E), suggesting that CatK$^{-/-}$ has a protective effect on oxidative stress–induced and stressed serum–induced mesangial cell apoptosis. In contrast, the levels of CatK mRNA were significantly increased in mesangial cells by transfection with the CatK plasmid (Figure S9A). The overexpression of CatK resulted in increases in the levels of PPAR-γ and C-Cas-8 proteins and a decrease in the level of Bcl-2 in response to the H_2O_2 and stressed serum (Figure 6A and 6B; Figure S9B and S9C). The oxidative stress serum and 5% stressed serum promoted mesangial cell apoptosis (Figure 6C and 6D; Figure S9D and S9E), thus providing a mechanistic explanation of CatK involvement in glomerular apoptosis.

DISCUSSION

Identifying novel targets to suppress glomerular injury, fibrosis, proteinuria, and hypertension will contribute to therapeutic strategies for patients with chronic kidney disease who are also experiencing CPS. The critical nontraditional and traditional roles of lysosomal cysteinyl

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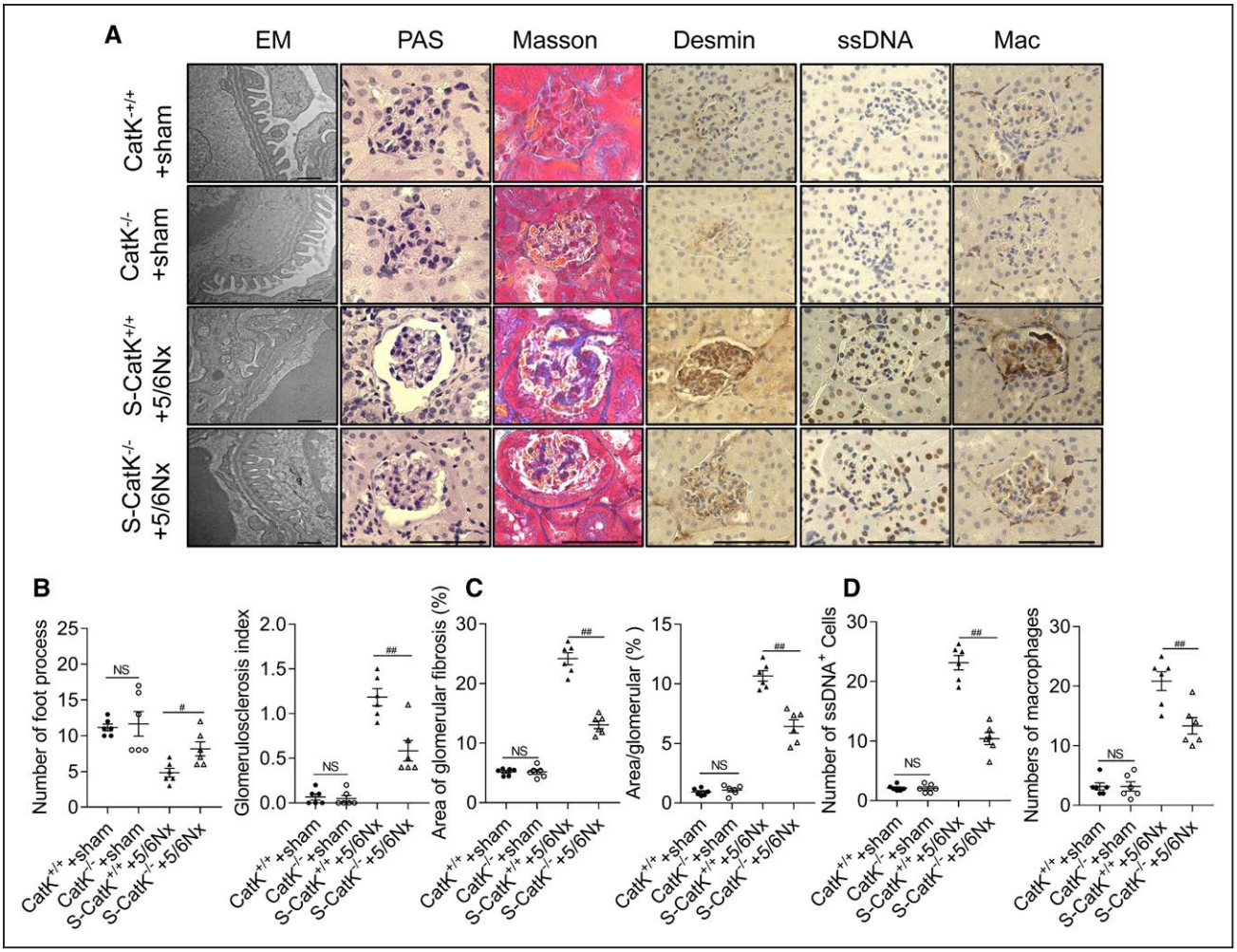

Figure 3. CatK (cathepsin K) deficiency mitigated 5/6 nephrectomy (5/6Nx)/chronic stress–related podocyte injury and glomerular microchanges.

A through **D**, Representative images (**A**) combined with quantitative data obtained by electron microscopy and periodic acid-Schiff (**B**), Masson trichrome and desmin (**C**), single-stranded DNA (ssDNA) and macrophage (Mac; **D**) staining show the levels of podocyte foot process, glomerulosclerosis, glomerular fibrosis, ssDNA$^+$ apoptosis, and Mac infiltration. TEM scale bar, 500 nm. Scale bar for others, 75 μm. Data are mean±SEM (n=5–8). #P<0.05, ##P<0.01 vs the corresponding S-CatK$^{+/+}$+5/6Nx mice by 1-way ANOVA and Tukey post hoc tests. EM indicates electron microscopy; and TEM, transmission electron microscope.

CatK in various physiological and pathological conditions have been highlighted by a comprehensive review article.[25] Although many studies uncovered proteolysis-dependent and independent mechanisms underlying atherosclerotic cardiovascular disease and regeneration,[14,21] a very limited number of laboratory data have indicated that in rats fed a high-salt diet and mice that underwent unilateral ureteral obstruction surgery, the injured kidney tissues have increased CatS and CatK mRNA and protein.[22,26] To the best of our knowledge, the present study is the first to report that genetic and pharmacological interventions targeting CatK confer renal protection against chronic stress with 5/6Nx mechanical injury.

Experimental and clinical evidence has established that oxidative stress plays an important role in cytokine/chemokine secretions and podocyte/mesangial cell injury, leading to glomerular fibrosis and kidney failure.[26,27] Our present findings demonstrated that the 5/6Nx-injured kidney tissues with and without chronic stress had increased levels of p22^{Phox} and gp91^{Phox} genes and proteins, as well as oxidative stress production. p22^{Phox} and gp91^{Phox} are important membrane-type subunits of NADPH oxidase.[28] NADPH oxidases are the main source of reactive oxygen species, and it has been demonstrated that genetic and pharmacological inhibitions of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase subunits ameliorated the development of renal failure in several kidney injury models.[29,30]

Because chronic stress accelerated kidney injury and dysfunction under our present experimental conditions, we propose that the enhancement of stress-induced oxidative stress may contribute to kidney remodeling and failing in mice that had been subjected to 5/6Nx injury. A significant finding of our present work is that CatK deletion and its pharmacological inhibition prevent glomerular apoptosis, podocyte foot process effacement, glomerulosclerosis,

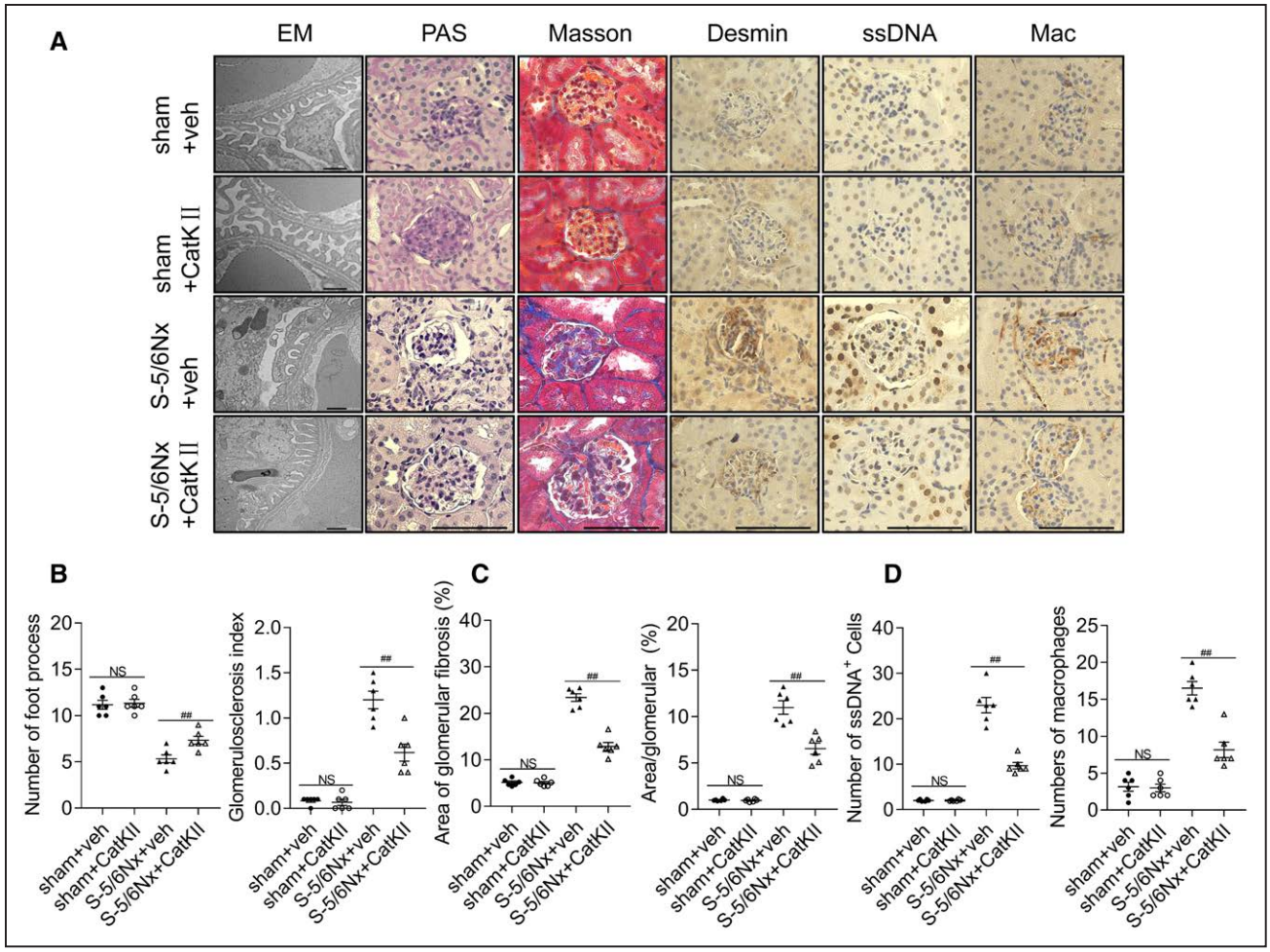

Figure 4. CatK (cathepsin K) inhibition alleviated podocyte injuries and glomerular fibrosis in 5/6 mice under stress conditions. **A** through **D**, Representative images (**A**) and quantitative data of electron microscopy and periodic acid-Schiff (PAS; **B**), Masson trichrome and desmin (**C**), and single-stranded DNA (ssDNA) and macrophage (Mac; **D**) staining show the levels of podocyte foot process, glomerulosclerosis, glomerular fibrosis, ssDNA$^+$ apoptosis, and Mac infiltration. TEM scale bar, 500 nm. Scale bar, 75 μm. Data are mean±SEM (n=5–8). 5/6Nx indicates 5/6 nephrectomy. #P<0.05, ##P<0.01 vs the corresponding S-5/6Nx+vehicle (veh) mice by 1-way ANOVA and Tukey post hoc tests.

fibrosis, and proteinuria, accompanied by the mitigation of gp91^{Phox} and p22^{Phox} expression, as well as apoptosis-related protein expressions (C-Cas-8, Bcl-2, and PPAR-γ) or senescence-related protein expression (p16 and p21) in the 5/6Nx kidney lesions of mice with and without chronic stress. This is further supported by the cellular experimental data that CatK silencing exerted a beneficial effect on the mesangial cell apoptosis and harmful changes in the levels of those apoptosis-related proteins (PPAR-γ, C-Cas-8, and Bcl-2) in response to H_2O_2 and stressed serum. Our findings thus provide evidence that the renal protective actions of CatK inhibition by genetic and pharmacological approaches occur, at least in part, through the amelioration of PPAR-γ/Cas-8–mediated and Bcl-2–mediated glomerular apoptosis and senescence that are mediated by NADPH oxidase inactivation in 5/6Nx mice under our experimental conditions.

Accumulating evidence indicates that chronic stress can cause inflammatory and metabolic cardiovascular diseases (eg, thrombosis, hypertension, and atherogenesis).[10–14] Our present findings demonstrated that stress elevated blood pressure and increased the accumulation of macrophages in the glomerulus and perivascular tissues compared with those of nonstressed 5/6Nx-alone mice. The stressed kidneys had elevated inflammatory gene expressions (ie, TLR-2, TLR-4, IL-1β, TNF-α, ICAM-1 [intercellular adhesion molecule-1], MCP-1, and AT1Rα). Proinflammatory effects of these molecules on the development of renal injury and failure and hypertension have been demonstrated by a number of studies from our group and others.[15,31] Thus, the chronic stress that was applied in the present study promoted the development of injury-induced glomerulosclerosis and dysfunction and hypertension by enhancing inflammatory actions.

In addition, inflammatory cytokines modulate the expression and activity of CatK in inflammatory macrophages and renal cells (ie, podocytes, mesangial cells, and

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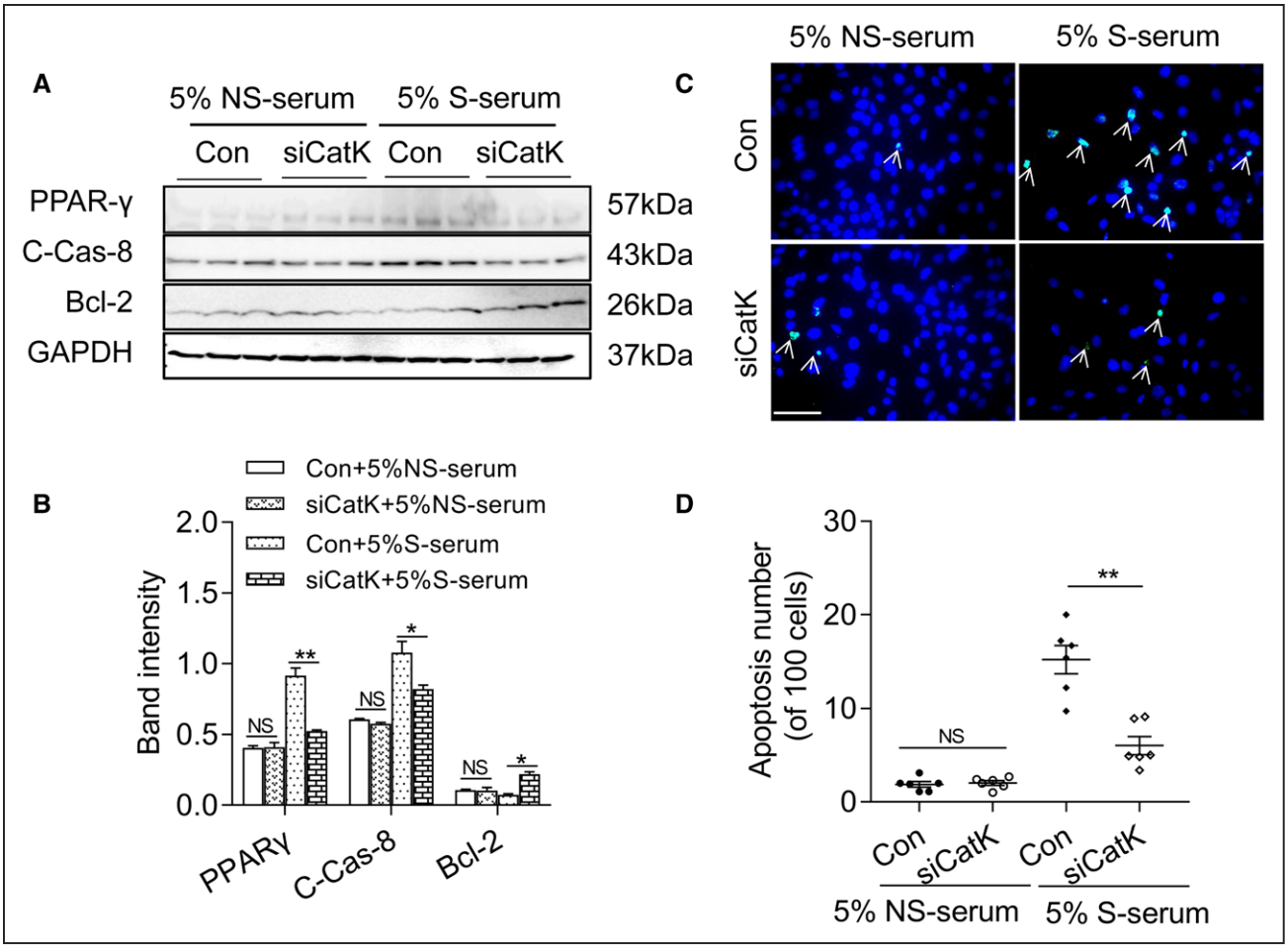

Figure 5. CatK (cathepsin K) silencing mitigated target apoptosis-related protein changes in glomerular mesangial cells in response to stressed serum.

A and **B**, Representative Western blotting images and quantitative data of the investigated molecular protein levels (PPAR-γ [peroxisome proliferator-activated receptor-gamma], C-Cas-8, and Bcl-2 [B cell lymphoma 2]) in mesangial cells treated with nonstress (NS) and stress (S) serum. **C** and **D**, Representative Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining images and number of apoptotic cells induced by both serum stimulations in mesangial cells. Scale bar, 75 μm. Data are mean±SEM (n=6). *P<0.05, **P<0.01 by 1-way ANOVA and Tukey post hoc tests.

tubular epithelial cells).[22,26] Our present observations show that the inhibition of CatK by genetic and pharmacological approaches mitigated the elevation in blood pressure and the kidney inflammation, remodeling, and dysfunction in 5/6Nx and stressed 5/6Nx mice. These results thus demonstrate the ability of CatK inhibition to lower blood IL-17 and TNF-α levels and macrophage infiltration, and they suggest that the production of inflammatory cytokines exerts salutary effects on the injured kidney by a reduction of the TLR-2/4 signaling pathway, thereby improving kidney damage, dysfunction, and hypertension under 5/6Nx-alone and 5/6Nx mice combined with chronic stress conditions.

It is also notable that 2-week immobilized stress elevated the blood pressure of mice, and this change was only mildly rectified by the genetic and pharmacological inhibitions of CatK and CatS, although both interventions significantly inhibited systemic and vascular inflammation.[14,15] These findings suggest that the CatK$^{-/-}$-mediated blood pressure reduction in mice under our experimental conditions might be attributable not to inflammation reduction but rather to mitigating kidney damage and dysfunction. However, further study is necessary to clarify this issue.

The ability of chronic stress to enhance CatK expression and activity probably contributed to kidney remodeling under our experimental conditions. In agreement with an animal study in which CatK deletion reduced unilateral ureteral obstruction–induced kidney fibrosis in mice,[22] we observed that the mice lacking CatK or loaded with the CatK inhibitor were resistant to the 5/6Nx surgery and to the 5/6Nx surgery plus chronic stress. We have observed that a renoprotective effect of pitavastatin (a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor) was associated with the reduction of CatK expression and activity in Dahl salt-sensitive rats fed a high-salt diet.[26] CatK has been shown to modulate cardiovascular wall ECM protein metabolism and fibrosis in response to various injuries.[32] Our present

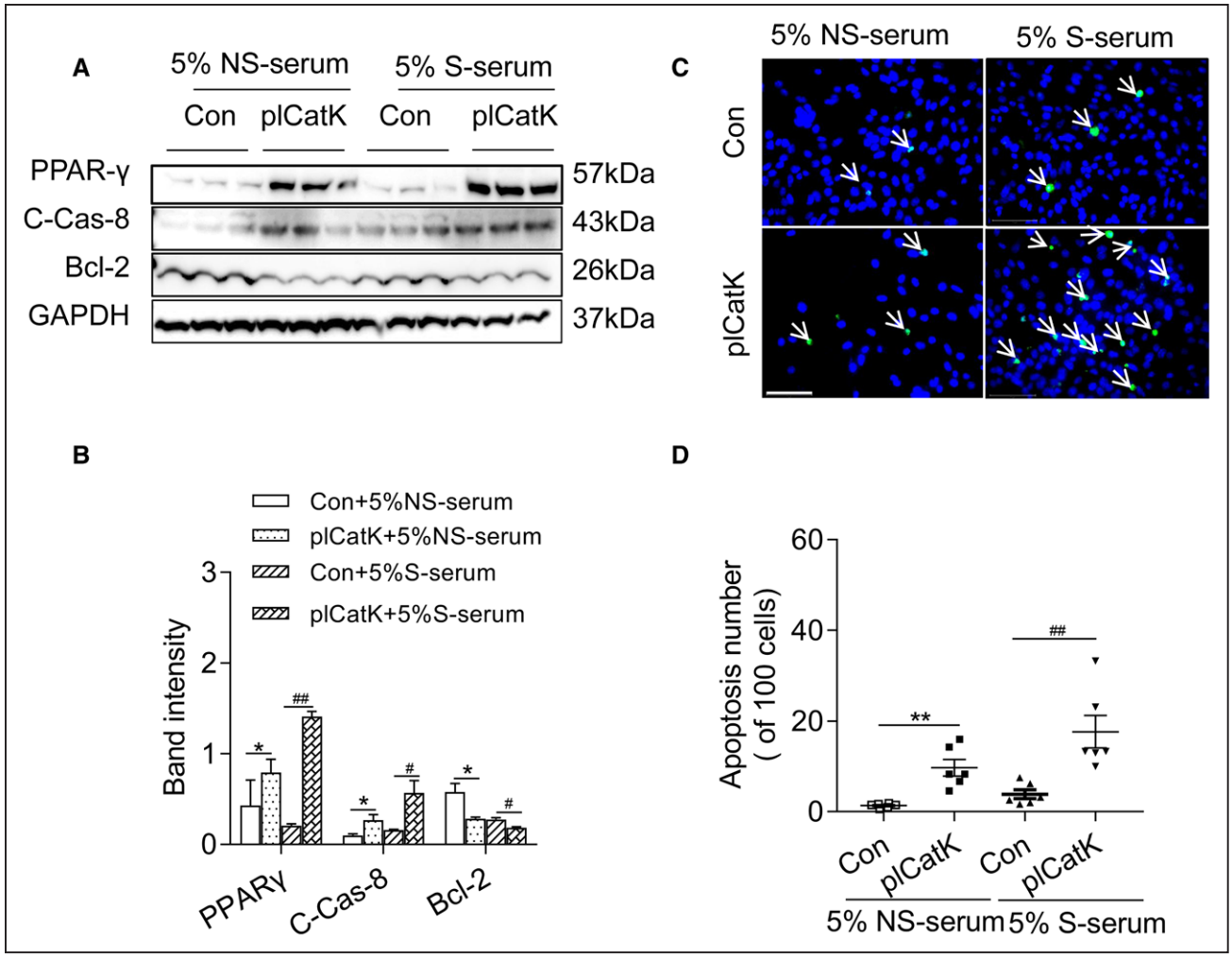

Figure 6. CatK (cathepsin K) overexpression by plasmid-CatK (plCatK) accelerated stressed serum–induced cell apoptosis. **A** and **B**, Representative images and quantitative data of targeted molecular protein levels in mesangial cells in response to nonstress (NS) and stress (S) serum. **C** and **D**, Representative Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining images combined with the quantitative data show the number of apoptotic mesangial cells induced by NS- and S-serum. Scale bar, 75 μm. Data are mean±SEM. **P*<0.05, ***P*<0.01 vs control/non-treated (Con)+5%NS- serum; #*P*<0.05, ##*P*<0.01 vs the corresponding Con+5%S-serum by 1-way ANOVA and Tukey post hoc tests.

experiments revealed that the genetic and pharmacological inhibition of CatK mitigates both 5/6Nx injury and the combination of 5/6Nx and chronic stress–induced type I and III collagen gene expressions and glomerular fibrosis. Thus, the upregulation of CatK expression and activity could represent a common proteolysis-related mechanism in 5/6Nx injury alone or 5/6Nx/chronic stress–related remodeling in CatK$^{+/+}$ mice under our experimental conditions.

Investigations by our group and others demonstrated close interactions between ATR1 (angiotensin receptor 1) and the TLR-2/4 axis in the regulation of the expressions of MMP-2/9 and CatK in macrophages and cardiovascular cells by several intracellular signaling pathways.[33,34] Collectively, these findings raise the possibility that chronic stress can accelerate kidney remodeling and dysfunction in response to 5/6Nx injury via the enhancement of CatK in cooperation with MMP-2/9–dependent proteolytic mechanisms that may be mediated by ATR1- and TLR-2/4 signaling activations.

A comprehensive review documented the roles of cathepsins and their endogenous inhibitor (cystatin C) system in proliferative diseases.[25] One of our recent studies demonstrated that CatK links smooth apoptosis and proliferation via the modulation of proliferin-1 growth signaling during injury-related vascular remodeling and neointimal hyperplasia.[35] Our present results show that CatK inhibition ameliorated 5/6Nx injury–related glomerular fibrosis compared with the control mice. p38MAPK/Erk1/2 signaling has been shown to participate in kidney matrix protein synthesis and fibrosis in cardiorenal syndrome.[36] In the present study, 5/6Nx injury resulted in an increase in the levels of p-p38MAPK and p-Erk1/2 proteins, as well as TLR-2 and 4 genes compared with the corresponding control mice; these harmful effects were rectified by CatK deletion and pharmacological inhibition.

Several lines of investigations demonstrated that TLR-2 and TLR-4 participate in cardiovascular and renal remodeling and fibrosis via p38MAPK/Erk1/2 signaling activation.[36,37] Thus, CatK inhibition may rectify the alterations in the expressions of TLR-2 and TLR-4 genes, resulting in p38MAPK/Erk1/2 signaling overactivation, contributing to the mitigation of kidney fibrosis in 5/6Nx mice without chronic stress. It should be noted that CatK$^{-/-}$ lowered the levels of the senescence-related proteins p16 and Sirt1 in the kidney tissues of 5/6Nx mice. However, there were no differences in these targeted protein levels between the CatK$^{+/+}$ and CatK$^{-/-}$ 5/6Nx kidneys. Taking these results together with accumulating evidence that CPS accelerates various organs' aging processes,[10–12] suggesting that the CatK deletion–mediated mitigation of 5/6Nx kidney damage and dysfunction and hypertension might be partially due to the retarding of chronic stress–related kidney senescence in mice under our experimental conditions.

There are several study limitations. First, the chronic variable stress model used herein is an animal stress model that cannot completely mimic human psychological stress. The study was also not designed to clarify the role of CatK in hypertension under the control of hypertensive cytokines. It remains unclear whether the cytokine-dependent blood pressure elevation is mediated by CatK, and we did not evaluate the heart weights and plasma glucocorticoid levels of the mice. Lastly, we could not recalculate the blood pressure levels obtained in all of the in vivo experiments by radiotelemetry. Further research is necessary to investigate these issues.

PERSPECTIVES

The expressions of the CatK gene and protein were increased in the 5/6Nx kidney tissues of mice with and without chronic stress. CatK deletion alleviated chronic stress–related glomerular damage, fibrosis, and proteinuria in mice in response to the injury or stress. The pharmacological inhibition of CatK mimics the renoprotective effects of genetic CatK deletion. A selective CatK inhibitor might have potential utility in the management of kidney damage and failure in patients with both chronic kidney disease and CPS.

ARTICLE INFORMATION

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Author Contributions

X. Yue researched the biological and histological data and wrote the first draft of the manuscript. L. Piao, Z. Huang, Y. Wan, S. Takeshi, and K. Nakamura researched the morphological data and assisted with the 5/6 nephrectomy injury mouse models. X. Meng, A. Inoue, and S. Xu researched the real-time polymerase chain reaction data and mouse genotyping. G.P. Shi reviewed the manuscript, contributed to the discussion, and provided the transgenic mice. T. Murohara and M. Kuzuya edited the manuscript and planned the study. X.W. Cheng designed the study and handled the funding and supervision. We thank K. Shimizu for the technical assistance.

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Disclosures

None.

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