Am J Transl Res 2022;14(5):3419-3429
www.ajtr.org /ISSN:1943-8141/AJTR0139430

Original Article

SIRT1 ameliorates renal ischemia-reperfusion injury through suppressing endoplasmic reticulum stress-mediated autophagy

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Received September 26, 2021; Accepted April 7, 2022; Epub May 15, 2022; Published May 30, 2022

Abstract: Background: Renal ischemia-reperfusion (IR) injury is a therapeutic challenge for surgeons. Sirtuin 1 (SIRT1) is an NAD^+-dependent deacetylase that plays a vital role in modulating cellular senescence and aging. In this study, we determined whether SIRT1 upregulation could alleviate renal IR injury and the underlying mechanism. Methods: A renal IR model was induced in male C57BL/6 mice. Blood urea nitrogen and serum creatinine were evaluated as markers of kidney function, and renal injury was assessed by pathological examination. The inflammatory milieu was analyzed by real-time RT-PCR and myeloperoxidase immunofluorescence assays. Western blotting was used to quantify SIRT1 protein expression, endoplasmic reticulum stress, and autophagy. Results: SIRT1 was upregulated in renal tissue after IR. Blood analysis and histopathologic examination demonstrated that SIRT1 preserved renal function and reduced renal damage. Further evaluation illustrated that IR induced autophagy and endoplasmic reticulum stress, while SIRT1 upregulation reduced endoplasmic reticulum stress-mediated autophagy levels. Conclusions: SIRT1 upregulation protects the kidney against IR-induced injury by inhibiting endoplasmic reticulum stress-mediated autophagy.

Keywords: SIRT1, ischemia-reperfusion injury, endoplasmic reticulum stress, autophagy, renal transplantation

Introduction

Renal transplantation is one of the preferred treatments for patients with advanced-stage kidney disease, but ischemia and reperfusion injury are inevitable problems associated with kidney transplantation [1]. Ischemia induces hypoxia and subsequently results in cell death, tissue damage, and functional effects on renal tubules. Paradoxically, this injury is distinctly enhanced during reperfusion or the restoration of blood flow [2]. Kidney IR injury not only causes acute kidney injury (AKI) but also contributes significantly to delayed graft function in kidney transplantation. There are currently no effective treatment strategies for this type of injury [3]. The mechanisms underlying IR injury are complex and involve multiple factors, including free radical production, calcium overload, and increases in inflammatory cells [2]. Given the adverse events caused by renal IR damage, there is an urgent need to find novel, improved, and sustainable therapeutic methods.

Renal IR injury causes endoplasmic reticulum (ER) stress, which is a cellular stress response involved in protein synthesis, folding, and translocation, lipid synthesis, and calcium homeostasis. ER stress, also called the unfolded protein response (UPR), is sequentially stimulated by the PRKR-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6). Nonetheless, excessive ER stress may result in tissue injury, and substantial evidence has shown that ER stress induces autophagy, which is an evolutionarily conserved cytoprotective process of lysosome-mediated self-digestion and recycling [4]. Autophagy helps maintain cellular homeostasis and achieves the metabolic needs of cells and the renewal of various organelles [5].

However, paradoxically, some studies have reported that autophagy also promotes renal IR injury. Therefore, autophagy might play dual roles in renal IR injury, mediating protective and detrimental effects [6, 7].

Sirtuin 1 (SIRT1), a NAD^+-dependent deacetylase, exerts crucial effects on many pathophysiologic processes, such as inflammation, oxidative stress, apoptosis, proliferation, and aging [8]. Numerous studies have shown that the susceptibility of various tissues to ischemia is regulated by aging mechanisms [9]. SIRT1 downregulation under ischemic conditions increases the susceptibility of some organs to ischemic damage, while SIRT1 upregulation alleviates ischemic damage [10]. Additionally, increasing evidence has demonstrated that SIRT1 delays senescence by enhancing autophagy and alleviating ischemia-induced apoptosis [11, 12]. On the contrary, some studies have also shown that SIRT1 overexpression inhibits autophagy in the kidneys [13, 14]. Therefore, the relationship between autophagy and SIRT1 in kidney IR injury remains to be elucidated.

In this study, a murine renal IR injury model was established to examine whether SIRT1 upregulation could protect the kidney from reperfusion injury, aiming to provide novel insights into its mechanisms and potential therapeutic strategy in renal IR injury.

Materials and methods

Animals and the induction of renal IR injury

Male C57BL/6 mice (22-25 g) were purchased from Joint Ventures Sipper BK Experimental Animal Company. The mice were randomly divided into different groups. We used the unilateral renal IR injury model. First, the mice underwent a midline laparotomy to create a clear view of the surgical area. Then, the intestines were gently pushed towards the contralateral side to expose the left renal artery and vein, and the left kidney was subjected to ischemia by applying a microvascular clamp to the renal artery and vein for 30 minutes. Ischemia in the kidney was observed as gradual uniform darkening. Mice in the control group did not undergo vascular occlusion of the left kidney. Specimens were collected for further analysis at the indicated time after reperfusion. All animals received human care in compliance with the guidelines of the National Institute of Health for the Care and Use of Laboratory Animals (No. 85-23, revised 1985) and approved by the Scientific Investigation Board of Navy Medical University, Shanghai, China.

Drug treatments

SRT1720 (50 mg/kg; #HY-10532) [15], selisistat (10 mg/kg; #HY-15452) [16], 4-phenyl butyric acid (4-PBA) (10 mg/kg; #HY-A0281) [17], rapamycin (5 mg/kg; #HY-10219) [18] and 3-methyladenine (3-MA; 30 mg/kg; #HY-19312) [19] were purchased from MedChemExpress (Monmouth Junction, NJ, USA) and injected before renal IR injury based on previous reports and the manufacturer's protocols.

Western blotting

First, equivalent amounts of protein extracted from kidney tissue were loaded and separated on 10% SDS-PAGE gels and transferred to a polyvinylidene fluoride membrane (Millipore, Shanghai, China). Next, the membrane was blocked with 5% nonfat milk for 1 h and then incubated overnight at 4°C with the following specific primary antibodies: polyclonal rabbit anti-mouse SIRT1, BiP, CHOP, p-PERK, PERK, p-IRE1α, IRE1α, ATF6, Atg-7, Beclin-1, LC3B, and GAPDH Cell Signaling Technology, Danvers, MA, USA. After three washes with Tris-buffered saline-Tween (TBST) buffer, the membranes were incubated with HRP-conjugated secondary antibodies (Cell Signaling Technology) for 1 h at room temperature. The protein bands were then visualized using the ECL Plus system (GE Healthcare, Little Chalfont, UK). The intensity of the bands was quantified with ImageJ (NIH, Bethesda, MD, USA).

Biochemical assays of kidney function

Kidney function was assessed by measuring blood urea nitrogen (BUN) and serum creatinine (SCr) concentrations at 0, 6, 12, 24, and 48 h after ischemia according to the manufacturer's instructions at the Translational Medicine Center of Changzheng Hospital.

Kidney histopathological analysis

The mice were sacrificed at 24 h after ischemic insult. Renal tissue was fixed in 4% paraformal-

dehyde for 24 h and embedded in paraffin. Paraffin-embedded tissue samples were cut into sections and stained with hematoxylin-eosin (H&E). Six areas (20×) per section were randomly chosen for tissue sectioning. Morphologic assessments were performed to examine histopathological injury. Renal tubules with the following histopathological changes were considered damaged: tubular dilation and disruption, cast formation, sloughing off of tubular epithelial cells, and cell lysis. Tissue injury was scored based on the percentage of damaged tubules: 0 points for less than 5%, 1 point for 5%-25%, 2 points for 25%-50%, 3 points for 50%-75%, and 4 points for damage greater than 75%.

Real-time RT-PCR analysis

Total RNA was isolated from mouse kidney tissues using TRIzol reagent (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocol. Subsequently, cDNA was synthesized with oligo d(T) (Applied Biosystems, Waltham, MA, USA) and the Superscript III Reverse Transcriptase Kit (Invitrogen). A StepOne Real-Time PCR System (Applied Biosystems) and the SYBR RT-PCR kit (Takara, Tokyo, Japan) were used to perform real-time RT-PCR analysis. The expression of each sample was normalized to that of GAPDH. The primer pairs used were as follows: TNF-α: forward, 5'-AAG CCT GTA GCC CAC GTC GTA-3', reverse, 5'-GGC ACC ACT AGT TGG TTG TCT TTG-3'; IL-6: forward, 5'-ACA ACC ACG GCC TTC CCT ACT T-3', reverse, 5'-CACGAT TTC CCA GAG AAC ATG TG-3'; IL-10: forward, 5'-GCT TTA CTG ACT GGC ATG AG-3', reverse, 5'-CGC AGC TCT AGG AGC ATG TG-3'; and GAPDH: forward, 5'-TGA CCA CAG TCC ATG CCA TC-3', reverse, 5'-GAC GGA CAC ATT GGG GGT AG-3'.

Immunofluorescence assay

Kidney sections were de-paraffinized, rehydrated, and handled according to a standard protocol. Then the sections were incubated with MPO (Abclonal, Wuhan, China) and LC3 (Cell Signaling Technology) overnight. The slides were then washed three times with PBS and immunostained with secondary antibodies (Abclonal) at room temperature for 2 h. Finally, the sections were visualized with a fluorescence microscope.

Immunohistochemical staining

The expression of CHOP and BiP was analyzed by immunohistochemistry. Kidney samples were embedded in paraffin and subsequently cut into sections. Then, the sections were deparaffinized and incubated with sodium citrate (pH = 6.0) at 65°C. Primary antibodies against CHOP (Abcam, Cambridge, MA, USA) and BiP (Abcam) were added and incubated overnight. The slides were then washed three times with PBS and incubated with HRP-conjugated secondary antibodies (Abcam) at room temperature for 1 h. Antigen-antibody complex signals were detected according to the manufacturer's protocol. For quantification, six fields were randomly captured from each tissue section and photographed with a digital camera under a microscope.

Statistical analysis

The results are expressed as the mean ± standard deviation (SD). The data obtained from two groups were analyzed using an unpaired Student's t tests or a Mann-Whitney test (two tailed). ANOVA followed by Bonferroni's tests were used to determine the statistical significance among multiple groups. Statistical analysis was conducted using GraphPad Prism 7 (La Jolla, CA, USA). In every test, $P < 0.05$ was considered significant.

Results

SIRT1 is upregulated during the progression of renal IR injury

SIRT1 expression in renal tissues after ischemia was investigated using western blotting. The protein levels of SIRT1 were significantly higher in the renal IR group than in the sham group, and the expression of SIRT1 increased in a time-dependent manner (**Figure 1A** and **1B**). However, there was no difference between the expression of SIRT1 at 2 h and 4 h postischemia. These findings suggest that SIRT1 may play a vital role in IR injury.

SIRT1 improves renal function and ameliorates renal IR injury

To assess the protective effects of SIRT1 on renal damage, the SIRT1 agonist SRT1720 and the SIRT1 inhibitor selisistat were administered

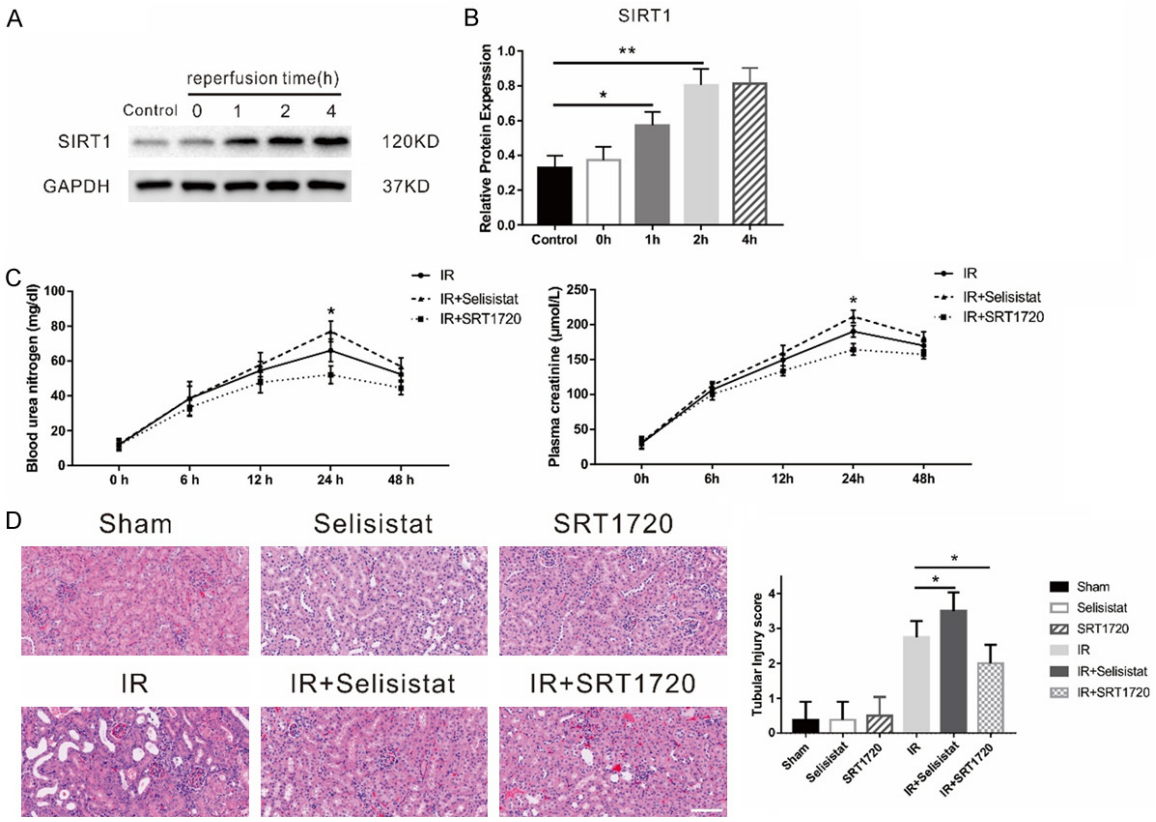

Figure 1. SIRT1 upregulation preserves renal function in kidneys with IR injury. A. SIRT1 protein levels were measured by western blot analysis at different reperfusion time points. B. Statistical analysis of SIRT1 expression. C. Serum BUN and SCr levels in the IR, IR+SRT1720, and IR+selisistat groups at 0, 6, 12, 24, and 48 h postischemia. D. H&E staining of kidney tissues 24 h postreperfusion (20×) and tubular injury scores were assessed. Scale bar represent 20 μm. The data are shown as the mean ± SD. N = 6 for each group. *P < 0.05, **P < 0.01.

to the mice. The levels of BUN and SCr were significantly higher in the IR group than in the IR+SRT1720 group at 24 h postreperfusion, and these two indicators were further elevated in the IR+selisistat group (**Figure 1C**). In addition, IR-injured renal tissue was stained by H&E, and representative images of renal pathological changes in the mice are shown in **Figure 1D**. Sections from mice in the IR+selisistat group showed the worst histopathological features with tubular dilation and disruption, sloughing of tubular epithelial cells, and cell lysis; therefore, the renal damage score was highest in the IR+selisistat group. In contrast, SRT1720 pretreatment significantly attenuated the extent of renal injury and reduced kidney damage scores compared to those of the IR group. These results suggest that SIRT1 plays a protective role in the progression of renal IR-induced injury, while SIRT1 suppression might harm renal function.

SIRT1 activation reduces IR-induced inflammation in the kidney

Given the fact that cytokines play a critical role in IR-induced liver injury, we therefore compared their expressions following IR injury. It was found that renal IR injury resulted in significant increases of TNF-α and IL-6 and a decrease in the expression of IL-10, which are indicators of the inflammatory response (**Figure 2A**). SRT1720 pretreatment significantly inhibited the expression of proinflammatory factors after IR, while selisistat pretreatment increased the expression of proinflammatory factors after IR. Immunofluorescence labeling of MPO was used to evaluate the severity of neutrophil infiltration in kidney tissues in all groups of mice 24 h after reperfusion (**Figure 2B** and **2C**). Quantitative analysis revealed that mice subjected to IR had higher neutrophil infiltration than those in the sham-surgery group,

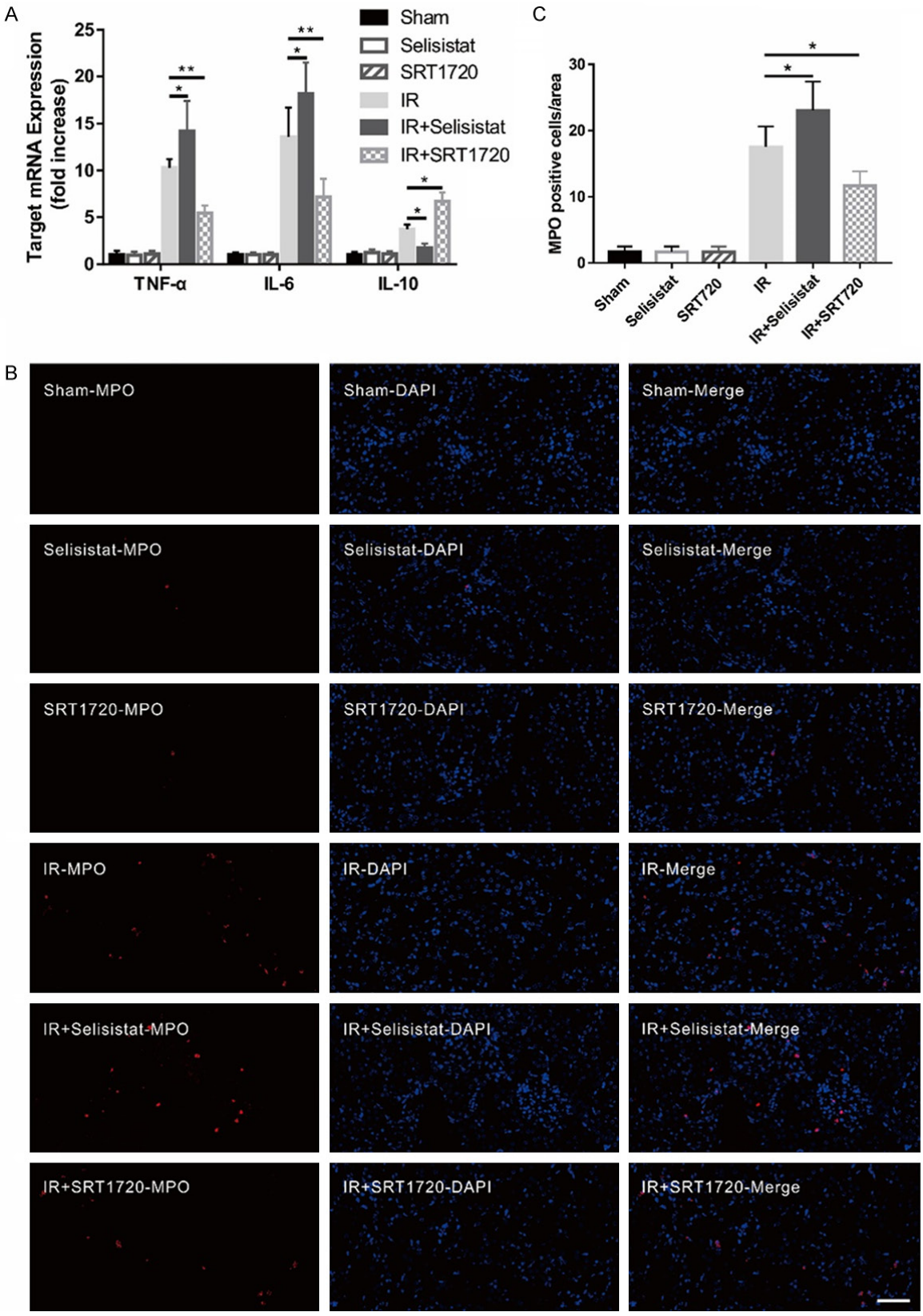

Figure 2. SIRT1 activation reduces the IR-induced inflammatory milieu. A. Histograms of the mRNA expression levels of TNF-α, IL-6, and IL-10. B. Representative immunofluorescence of MPO-stained and DAPI-stained kidney sections. C. Analysis of MPO-positive cells. Scale bar represents 20 μm. The data are expressed as the mean ± SD. N = 6 for each group. *P < 0.05, **P < 0.01.

and this higher neutrophil infiltration was attenuated by SRT1720. In contrast, the amount of MPO-positive cells in the selisistat-pretreated group was higher than that in IR group mice. These findings suggest that SIRT1 activation can ameliorate the inflammatory response and that SIRT1 suppression enhances inflammation during renal IR injury.

SIRT1 enhancement attenuates IR-induced ER stress

ER stress results from the dysfunction of ER homeostasis and can exacerbate many diseases. The state of ER stress in postischemic renal tissues was assessed using two vital UPR molecules: BiP and CHOP. After IR, immunohistochemical staining showed an increase in BiP levels in renal tubular cells. Compared to the IR group, BiP levels decreased in the IR+SRT1720 group and increased in the IR+selisistat group (**Figure 3A**). Western blot (WB) examination further verified the sustained BiP expression in the various groups of mice after IR (**Figure 3B**). Additionally, high levels of CHOP were observed in the renal cortex after IR. Notably, CHOP expression was decreased in the IR+SRT1720 group, while a significant increase in CHOP occurred in mice treated with selisistat before IR injury (**Figure 3A** and **3C**). WB further showed that the phosphorylation levels of PERK and IRE1α and the level of ATF6 were markedly increased in the IR group compared to the sham group, and the increase was much more distinct in the IR+selisistat group, while the expression of these proteins was decreased in the IR+SRT1720 group (**Figure 3D**). In summary, these results suggest that ER stress is induced and sustained in renal tissues after IR injury and that SIRT1 upregulation can attenuate ER stress.

SIRT1 upregulation reduces renal ER stress-induced autophagy in the context of IR injury

First, to assess whether ER stress activates autophagy in renal tissues after IR, the ER stress inhibitor 4-PBA was administered prior to IR. Moreover, the well-known autophagy activator rapamycin and the autophagy inhibitor 3-MA were administered before IR. The expression of autophagy and ER stress indicators was measured by western blotting. As shown in **Figure 4A**, increased expression of CHOP, BiP, Atg-7, Beclin-1, and LC3 II was observed in the IR group compared with the sham group, and the increase was more pronounced in the IR+selisistat group. Conversely, the expression of these proteins was decreased in the IR+selisistat+4-PBA group, and this decline was also seen in the IR+SRT1720 group, which indicated that SRT1720 not only inhibited ER stress but also suppressed autophagy. However, rapamycin pretreatment significantly increased the expression of Atg-7, Beclin-1, and LC3 but not CHOP or BiP compared with the mice in the IR group; moreover, only the expression of Atg-7, Beclin-1, and LC3 decreased in the IR+3-MA mice compared with those in the IR group, suggesting that 3-MA specifically controlled autophagy without significantly affecting ER stress (**Figure 4D-F**). These results indicate that autophagy is regulated by ER stress in renal tissues during IR, while the upregulation of autophagy does not inhibit ER stress. Moreover, SIRT1 activation might inhibit autophagy.

Further experiments were performed to examine the influence of SIRT1 on autophagy in the context of renal IR. **Figure 4G** shows that an increase in LC3-positive cells was observed in the IR group compared to the sham group, which paralleled the increased levels of ER stress. Consistently, the number of LC3-positive cells was lower in the IR+SRT1720 group than in the IR group, while the number of LC3-positive cells was increased in the IR+selisistat group compared to the IR group. Taken together, these findings illustrate that SIRT1 upregulation can reduce renal ER stress-induced autophagy to alleviate IR injury.

Discussion

Renal ischemia-reperfusion (IR) injury is an inevitable challenge in clinical medicine. As one of the most common acute kidney injuries, renal IR injury is commonly observed in patients with trauma, shock, and kidney transplantation [20, 21]. In renal transplantation, IR injury may result in a molecular and cellular inflammatory response and reduce long term graft survival. Therefore, there is growing interest in developing novel protective stimuli that can be applied at the onset of renal IR to limit subsequent injury. In this study, we provided substantial evidence supporting that SIRT1 exerts a reno-

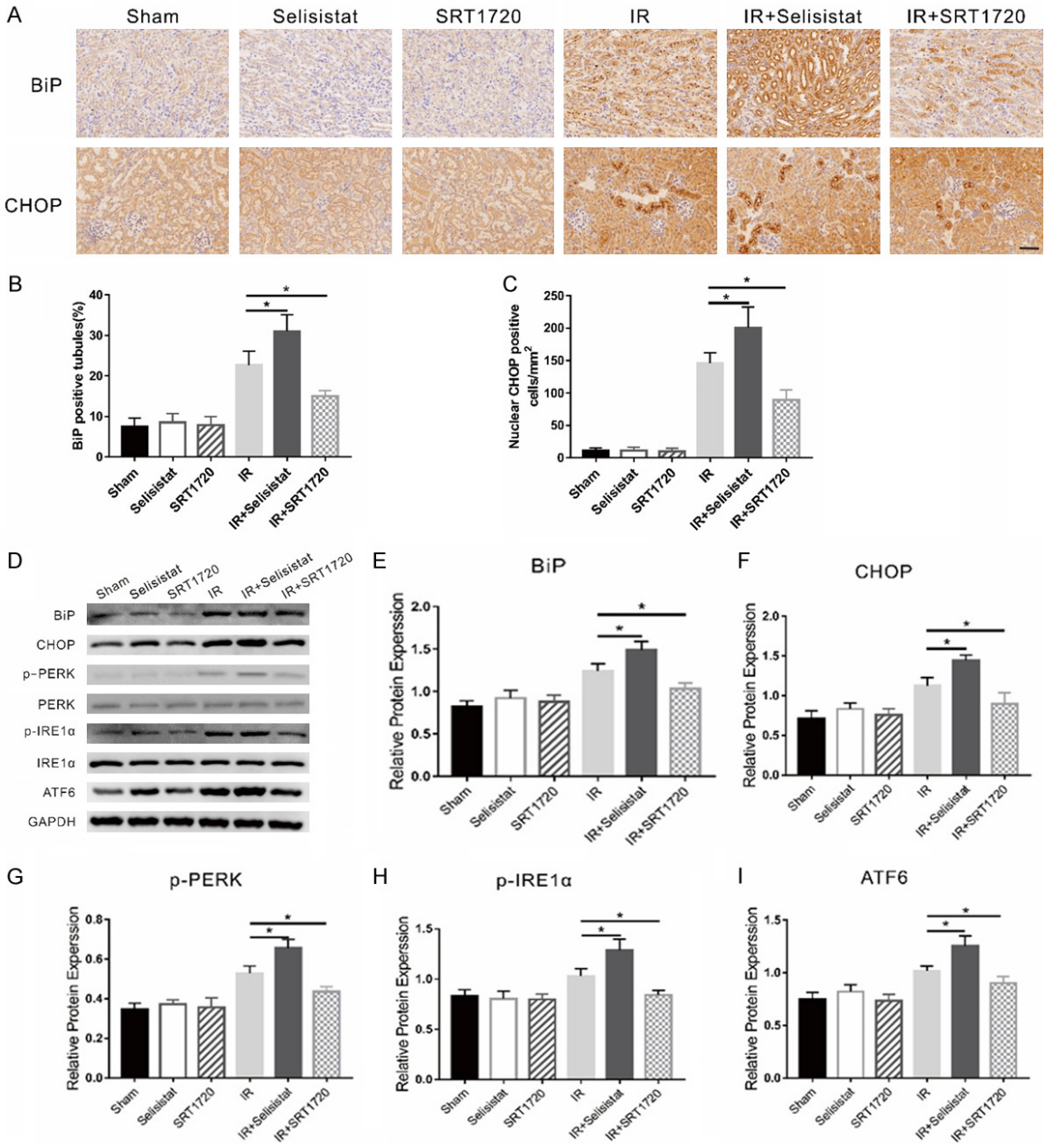

Figure 3. SIRT1 enhancement attenuates IR-induced ER stress. A. Representative photographs of immunohistochemical staining of BiP and CHOP. B. Quantitative analysis of BiP staining. C. Quantitative analysis of CHOP staining. D. Protein expression levels of BiP, CHOP, p-PERK, PERK, p-IRE1α, IRE1α, ATF6, and GAPDH were measured by western blotting. E-I. Quantitative statistical analysis of protein expression. Scale bar represents 50 μm. The data are presented as mean ± SD. N = 6 for each group. *P < 0.05.

protective role of SIRT1 in renal IR injury by suppressing ER stress-mediated autophagy.

SIRT1 is reported as a potential therapeutic target for several diseases, such as cancer, diabetes, and atherosclerosis [22]. In view of this, there has been a growing interest in developing SIRT1 as a novel protective stimuli applied in different stressful conditions [11, 12, 23]. However, the role of SIRT1 in renal IR injury has remained unclear. In this study, we found that the expression of SIRT1 increased in a time-dependent manner following renal IR injury, which indicated that the protective capacity of SIRT1 may strengthen with the enhencement of its expression during renal IR injury. The

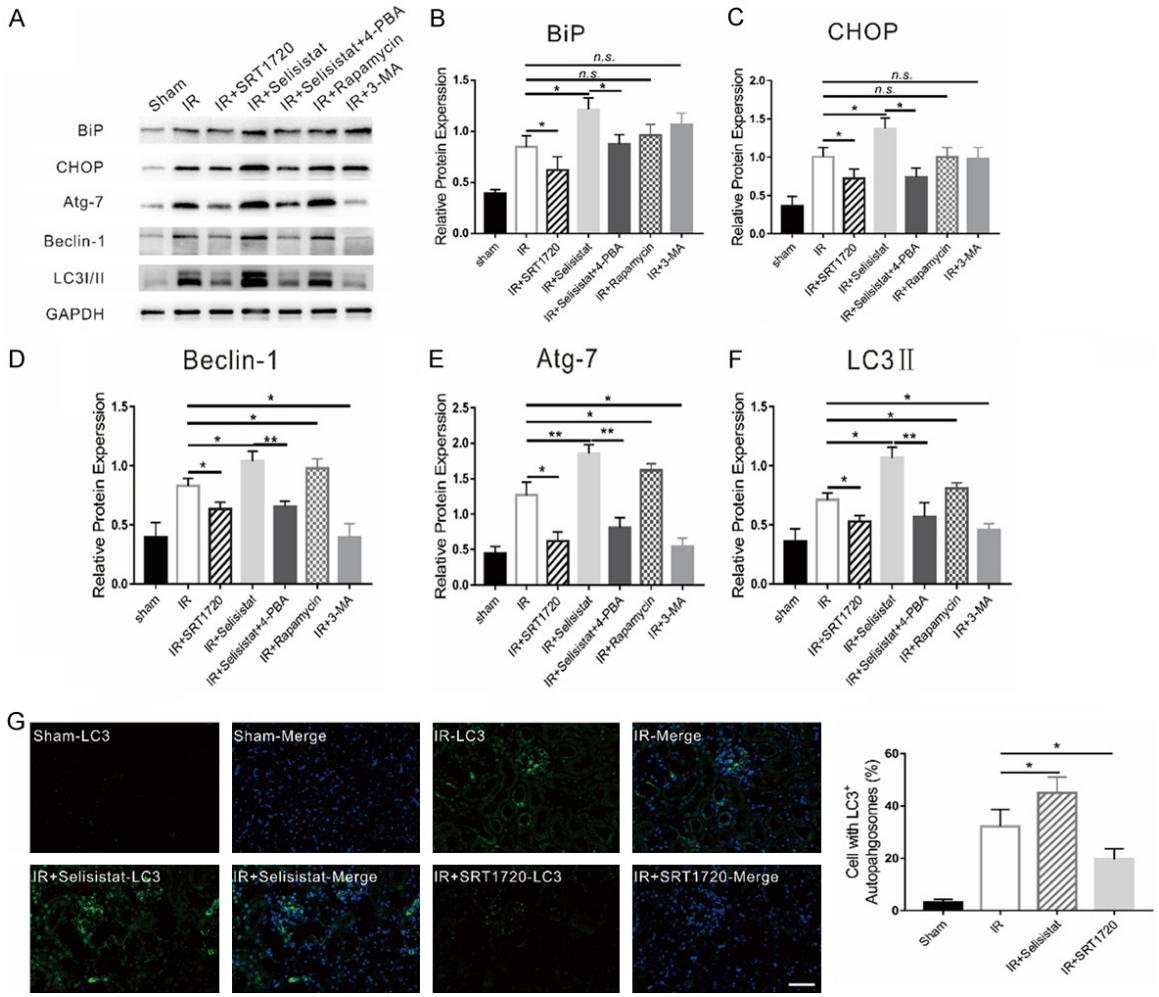

Figure 4. SIRT1 upregulation reduces renal ER stress-induced autophagy. A. The expression levels of BiP, CHOP, Atg-7, Beclin-1, LC3, and GAPDH were assessed by western blotting. B-F. Quantitative analysis of BiP, CHOP, Atg-7, Beclin-1, and LC3 II. G. Representative immunofluorescence images of LC3-stained sections and statistical analysis of LC3-positive cells. Scale bar represents 20 μm. The data are expressed as mean ± SD. N = 6 for each group. *P < 0.05, **P < 0.01, *n.s.*>0.05.

pathology of renal IR injury is characterized by a complex cascade of cellular and biochemical events that contribute to renal tissue damage over time, and various processes are involved in IR damage, including leucocyte infiltration and endothelial cell injury, which may affect kidney function and ultimately lead to kidney transplant failure [24]. Therefore, we first examined the protective and anti-inflammatory effects of SIRT1 following renal IR. With respect to SIRT1 modulators, we employed SRT-1720 (a SIRT1 agonist) and selisistat (a SIRT1 inhibitor) in this study. The reduced levels of pathologic injury, BUN, and SCr demonstrated that upregulation of SIRT1 significantly preserved renal function. In addition, the MPO results and decline in proinflammatory cytokines confirmed the anti-inflammatory role of SIRT1, suggesting that SIRT1 upregulation may ameliorate renal IR injury by reducing the inflammatory response and vice versa.

The ER is an intracellular organelle that plays an essential role in protein homeostasis by reducing protein synthesis, enhancing protein folding capacity by upregulating the transcription of molecular chaperones, and activating ER-associated protein degradation [25]. ER stress is pivotal to the pathogenesis of renal IR injury and is activated when ER homeostasis is disturbed by various stimuli, such as ischemia, hypoxia, Ca^{2+} overload, and reactive oxy-

gen species [25, 26]. Consistent with previous reports, we found that ER stress was triggered after renal IR-induced injury and could be suppressed by the upregulation of SIRT1, as evidenced by the decreases in p-PERK, p-IRE1α, ATF6, BiP, and CHOP [18]. Immunohistochemical staining was used to evaluate the levels of ER stress in kidney tissues by measuring the expression of BiP and CHOP, and the results showed that the expression of BiP and CHOP decreased in the IR+SRT1720 group compared to the IR group, further suggesting that SIRT1 exerted protective effects against renal IR.

It is well-documented that autophagy is essential in various physiological and pathological processes, including starvation adaptation, intracellular organelle clearance, aging, and antigen presentation. Accumulating evidence suggests that autophagy exerts both protective and detrimental effects in renal IR injury [27]. On one hand, autophagy plays a protective role in IR injury through providing an alternative source of energy and organelle via its constitutive function. On the other hand, excessive autophagy may result in type II programmed cell death, which is also known as autophagic cell death. Substantial evidence has demonstrated that the protective effects of SIRT1 are strongly related to the stimulation of autophagy in most organs [28, 29]. However, several lines of evidence indicate that autophagy also promotes renal IR damage [5, 30]. After confirming that ER stress inhibition was involved in SIRT1 related renal protection, we next utilized 4-PBA (an ER stress inhibitor), rapamycin (an autophagy activator) and 3-MA (an autophagy inhibitor) to determine whether autophagy was involved in the SIRT1-related renal protection and to identify the exact role during injury. Intriguingly, we found that IR mice pretreated with selisistat exhibited much higher autophagy levels than control IR mice, while 4-PBA pretreatment completely reversed autophagy. This result suggests that the protective effect of SIRT1 is autophagy-dependent as regulated by the ER stress pathway following IR injury. Previous studies have shown that the ER serves as a Ca^{2+} store inside the cell, and intracellular Ca^{2+} signal is essential for mTOR-dependent autophagy [31]. When ER stress is enhanced, it leads to Ca^{2+} release from the ER to the cytosol, which results in activation of autophagy. By employing western blotting, we further confirmed this hypothesis with the observation that either upregulation of autophagy levels by rapamycin or downregulation of autophagy levels by 3-MA had no significant on ER stress, suggesting that autophagy was downstream of ER stress.

A previous study found that deacetylation of histone mark H4K16 by SIRT1 leads to upregulation of various autophagy-related genes such as ATG, and subsequently promotes autophagy [32]. This differing effect of SIRT1 regarding autophagy revealed that the precise interaction between SIRT1 and autophagy is still not well understood. To our knowledge, the difference in the types and severity of injuries may produce different outcome of autophagy. Besides, regulation of SIRT1 may have different effects according to context.

According to the results of this study, we revealed that upregulation of SIRT1 could bring about a reduction in ER stress, whereas ER stress suppression could subsequently inhibit harmful cellular autophagy and protect renal function. Therefore, we believe that our findings provide novel insights into the molecular mechanisms-as well as possible therapeutic benefits-of SIRT1 in renal IR injury.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (81801590 and 81671577). The funder provided financial support to the study, but was not involved in the design of the study, the collection, analysis, and interpretation of data, or in the writing of the manuscript.

Disclosure of conflict of interest

None.

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References

[1] Requiao-Moura LR, Durao Junior Mde S, Matos AC and Pacheco-Silva A. Ischemia and reperfu-

sion injury in renal transplantation: hemodynamic and immunological paradigms. Einstein (Sao Paulo) 2015; 13: 129-135.
[2] Zhao H, Alam A, Soo AP, George AJT and Ma D. Ischemia-reperfusion injury reduces long term renal graft survival: mechanism and beyond. EBioMedicine 2018; 28: 31-42.
[3] Panah F, Ghorbanihaghjo A, Argani H, Asadi Zarmehri M and Nazari Soltan Ahmad S. Ischemic acute kidney injury and klotho in renal transplantation. Clin Biochem 2018; 55: 3-8.
[4] Decuypere JP, Ceulemans LJ, Agostinis P, Monbaliu D, Naesens M, Pirenne J and Jochmans I. Autophagy and the kidney: implications for ischemia-reperfusion injury and therapy. Am J Kidney Dis 2015; 66: 699-709.
[5] Ravanan P, Srikumar IF and Talwar P. Autophagy: the spotlight for cellular stress responses. Life Sci 2017; 188: 53-67.
[6] Li L, Wang ZV, Hill JA and Lin FM. New autophagy reporter mice reveal dynamics of proximal tubular autophagy. J Am Soc Nephrol 2014; 25: 305-315.
[7] Li YJ, Lei YH, Yao N, Wang CR, Hu N, Ye WC, Zhang DM and Chen ZS. Autophagy and multidrug resistance in cancer. Chin J Cancer 2017; 36: 52.
[8] Gong L, He J, Sun X, Li L, Zhang X and Gan H. Activation of sirtuin1 protects against ischemia/reperfusion-induced acute kidney injury. Biomed Pharmacother 2020; 125: 110021.
[9] Chen C, Zhou M, Ge Y and Wang X. SIRT1 and aging related signaling pathways. Mech Ageing Dev 2020; 187: 111215.
[10] Wang L, Quan N, Sun W, Chen X, Cates C, Rousselle T, Zhou X, Zhao X and Li J. Cardiomyocyte-specific deletion of Sirt1 gene sensitizes myocardium to ischaemia and reperfusion injury. Cardiovasc Res 2018; 114: 805-821.
[11] Ou X, Lee MR, Huang X, Messina-Graham S and Broxmeyer HE. SIRT1 positively regulates autophagy and mitochondria function in embryonic stem cells under oxidative stress. Stem Cells 2014; 32: 1183-1194.
[12] Liu T, Ma X, Ouyang T, Chen H, Lin J, Liu J, Xiao Y, Yu J and Huang Y. SIRT1 reverses senescence via enhancing autophagy and attenuates oxidative stress-induced apoptosis through promoting p53 degradation. Int J Biol Macromol 2018; 117: 225-234.
[13] Xu C, Wang L, Fozouni P, Evjen G, Chandra V, Jiang J, Lu C, Nicastri M, Bretz C, Winkler JD, Amaravadi R, Garcia BA, Adams PD, Ott M, Tong W, Johansen T, Dou Z and Berger SL. SIRT1 is downregulated by autophagy in senescence and ageing. Nat Cell Biol 2020; 22: 1170-1179.
[14] Jiang Y, Botchway BOA, Hu Z and Fang M. Overexpression of SIRT1 inhibits corticosterone-induced autophagy. Neuroscience 2019; 411: 11-22.
[15] Luo GP, Jian Z, Zhu Y, Zhu Y, Chen BC, Ma RY, Tang FQ and Xiao YB. Sirt1 promotes autophagy and inhibits apoptosis to protect cardiomyocytes from hypoxic stress. Int J Mol Med 2019; 43: 2033-2043.
[16] Wang X, Buechler NL, Yoza BK, McCall CE and Vachharajani VT. Resveratrol attenuates microvascular inflammation in sepsis via SIRT-1-Induced modulation of adhesion molecules in ob/ob mice. Obesity (Silver Spring) 2015; 23: 1209-1217.
[17] Zeng M, Sang W, Chen S, Chen R, Zhang H, Xue F, Li Z, Liu Y, Gong Y, Zhang H and Kong X. 4-PBA inhibits LPS-induced inflammation through regulating ER stress and autophagy in acute lung injury models. Toxicol Lett 2017; 271: 26-37.
[18] Liu H, Dong J, Song S, Zhao Y, Wang J, Fu Z and Yang J. Spermidine ameliorates liver ischaemia-reperfusion injury through the regulation of autophagy by the AMPK-mTOR-ULK1 signalling pathway. Biochem Biophys Res Commun 2019; 519: 227-233.
[19] Yang J, Liu H, Han S, Fu Z, Wang J, Chen Y and Wang L. Melatonin pretreatment alleviates renal ischemia-reperfusion injury by promoting autophagic flux via TLR4/MyD88/MEK/ERK/mTORC1 signaling. FASEB J 2020; 34: 12324-12337.
[20] Bagheri Y, Barati A, Nouraei S, Jalili Namini N, Bakhshi M, Fathi E and Montazersaheb S. Comparative study of gavage and intraperitoneal administration of gamma-oryzanol in alleviation/attenuation in a rat animal model of renal ischemia/reperfusion-induced injury. Iran J Basic Med Sci 2021; 24: 175-183.
[21] Changizi-Ashtiyani S, Hafazeh L, Ghasemi F, Najafi H, Babaei S, JalallyMashayekhi F, Hoseini SJ and Bastani B. The effect of adipose-derived mesenchymal stem cells on renal function and histopathology in a rat model of ischemia-reperfusion induced acute kidney injury. Iran J Basic Med Sci 2020; 23: 999-1006.
[22] Zheng Y, Kou J, Wang P, Ye T, Wang Z, Gao Z, Cong L, Li M, Dong B, Yang W, Li Q, Li H, Wang R and Yang L. Berberine-induced TFEB deacetylation by SIRT1 promotes autophagy in peritoneal macrophages. Aging (Albany NY) 2021; 13: 7096-7119.
[23] Singh V and Ubaid S. Role of silent information regulator 1 (SIRT1) in regulating oxidative stress and inflammation. Inflammation 2020; 43: 1589-1598.
[24] Luo G, Jian Z, Zhu Y, Zhu Y, Chen B, Ma R, Tang F and Xiao Y. Sirt1 promotes autophagy and inhibits apoptosis to protect cardiomyocytes from hypoxic stress. Int J Mol Med 2019; 43: 2033-2043.

[25] Chino H and Mizushima N. ER-Phagy: quality control and turnover of endoplasmic reticulum. Trends Cell Biol 2020; 30: 384-398.
[26] Obert DP, Wolpert AK, Grimm NL and Korff S. ER stress preconditioning ameliorates liver damage after hemorrhagic shock and reperfusion. Exp Ther Med 2021; 21: 248.
[27] Zhu Y, Ding A, Yang D, Cui T, Yang H, Zhang H and Wang C. CYP2J2-produced epoxyeicosatrienoic acids attenuate ischemia/reperfusion-induced acute kidney injury by activating the SIRT1-FoxO3a pathway. Life Sci 2020; 246: 117327.
[28] Shah SA, Khan M, Jo MH, Jo MG, Amin FU and Kim MO. Melatonin stimulates the SIRT1/Nrf2 signaling pathway counteracting lipopolysaccharide (LPS)-induced oxidative stress to rescue postnatal rat brain. CNS Neurosci Ther 2017; 23: 33-44.
[29] Yang QB, He YL, Zhong XW, Xie WG and Zhou JG. Resveratrol ameliorates gouty inflammation via upregulation of sirtuin 1 to promote autophagy in gout patients. Inflammopharmacology 2019; 27: 47-56.
[30] Li L, Wang ZV, Hill JA and Lin F. New autophagy reporter mice reveal dynamics of proximal tubular autophagy. J Am Soc Nephrol 2014; 25: 305-315.
[31] Ma X, Lu JY, Moraru A, Teleman AA, Fang J, Qiu Y, Liu P and Xu T. A novel regulator of ER Ca2+ drives Hippo-mediated tumorigenesis. Oncogene 2020; 39: 1378-1387.
[32] Wang W, Wang Q, Wan D, Sun Y, Wang L, Chen H, Liu C, Petersen RB, Li J, Xue W, Zheng L and Huang K. Histone HIST1H1C/H1.2 regulates autophagy in the development of diabetic retinopathy. Autophagy 2017; 13: 941-954.