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SIRT3 inactivation promotes acute kidney injury through elevated acetylation of SOD2 and p53

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ABSTRACT

Background: The deactivation of SIRT3, a novel deacetylase located in mitochondria, can aggravate multiple organ dysfunction. However, the role of SIRT3 and its downstream targets in ischemia/reperfusion (I/R)-induced acute kidney injury (AKI) remain unknown.
Materials and methods: I/R was reproduced in a rat model using a clamp placed on the left and right renal pedicles for 40 min. The rats were intraperitoneally injected with either the vehicle or a selective SIRT3 inhibitor (3-TYP) and scarified at different time points (4, 8, and 24 h after I/R). A portion of the renal tissue was extracted for histological analysis, and another portion was collected for the isolation of renal tubular epithelial cells for Western blotting, SOD2 and SIRT3 activity, cell apoptosis, and the determination of oxidative stress.
Results: The I/R-induced AKI model was successfully reproduced and SIRT3 activity was considerably reduced than control (sham operated) group, accompanied by increased acetylation of SOD2 and p53, as well as their elevated physical interaction in extracted mitochondrial protein (all P values < 0.05). Moreover, SIRT3 suppression by 3-TYP treatment (comparing with the vehicle treatment group) aggravated AKI, as evidenced by increased indicators of oxidative stress (increased mitochondrial red fluorescence MitoSOX and decreased reduced glutathione/oxidized glutathione ratio, all P values < 0.01).
Conclusions: The elevation of SOD2 and p53 protein acetylation in the mitochondria of renal tubular epithelial cells is an important signaling event in the pathogenesis of I/R-induced AKI. Thus, deacetylase SIRT3 may be an upstream regulator of both SOD2 and p53, and the SIRT3 deactivation may aggravate AKI.

Introduction

Acute kidney injury (AKI) is a common complication of ischemia/reperfusion (I/R), which is induced by trauma, hemorrhage, or major surgery.[1,2] To date, the mechanism of I/R-induced AKI is not fully understood. It is noteworthy that mitochondrial dysfunction is a vital role in the pathogenesis of AKI.[3]

Recently, some mitochondria-associated molecules have been found to play an important role in AKI.[3] Manganese-

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dependent superoxide dismutase (MnSOD) (also known as SOD2) is a homotetrameric enzyme located in the mitochondrial matrix.[4] Thus, the induction of SOD2 can exert a protective effect by interrupting the mitochondrial vicious circle between electron leakage from the electron transport chain, the formation of superoxide, and further mitochondrial damage.[4] Recently, SOD2-mediated antioxidative stress was found to exert a protective effect in sepsis-induced AKI[5,6] and hemorrhagic shock-induced small intestine injury.[7] In addition to SOD2 induction, p53 suppression also demonstrated a protective role in I/R-induced AKI.[8-11] Our previous work also demonstrated that increased cytoplasmic-to-mitochondrial translocation of p53 could accelerate apoptosis.[12]

Interestingly, SOD2 and p53 were found to physically interact inside mitochondria. Moreover, p53 activation has been found to reduce SOD2 activity in some tumor disease models.[13] In cisplatin-induced AKI cells, elevated p53 expression is associated with decreased SOD2 expression.[14] In the mitochondria of JB6 skin epidermal cells, p53 interacts with the primary antioxidant enzyme, MnSOD, consistent with the reduction of its superoxide scavenging activity and a subsequent decrease of mitochondrial membrane potential after the tumor progression.[15]

Of note, both SOD2 and p53 have been shown to be regulated by acetylation/deacetylation,[7,14,16] indicating that these two molecules might share a common upstream signaling pathway. Mitochondrial NAD-dependent deacetylase sirtuin-3 (also known as SIRT3) is a member of the mammalian sirtuin family of proteins, which are homologs to the yeast Sir2 protein.[17] SIRT3 is a soluble protein located in the mitochondrial matrix that activates or deactivates mitochondrial target proteins by deacetylating key lysine residues.[17,18] However, the regulatory effect of SIRT3 on p53 and the interaction between SOD2 and p53 in I/R-induced AKI have not been reported. Thus, we hypothesize that SIRT3 is a key upstream signaling molecule of both p53 and SOD2 protein expression, and the deactivation of SIRT3 aggravates I/R-induced AKI. In this study, we try to explore the precise downstream molecular mechanism of the deacetylase, SIRT3, and to provide the basis for the development of potential drugs that target SIRT3 for the future clinical treatment of I/R-induced AKI.

Materials and methods

Reagents and antibodies

MitoSOX was purchased from Molecular Probes (Invitrogen, CA). The mitochondrial/cytosolic protein extraction kit was purchased from Best Bio Co (Beijing, China). Percoll gradient density centrifugation was purchased from GE healthcare (Chicago, IL, USA). Antibodies against SOD2, p53, SIRT3, Bcl-2, Bcl-xl, Bad, Bax, cleaved-caspase 3, cox-IV, and β-actin were purchased from ABclonal (Wuhan, China). The SIRT3 activity assay kit was obtained from Abcam (Cambridge, UK). The fluorescein isothiocyanate (FITC) Annexin V apoptosis detection kit was purchased from BioLegend (San Diego, CA, USA). SOD2 activity kit was purchased from Dojindo Co (Shanghai, China). The secondary polyclonal rabbit anti-rat immunoglobulin/FITC and immunoprecipitation kit was purchased from Proteintech Co (Chicago, IL). Antibodies against acetylated lysine (ac-lys) and the polyvinylidene fluoride (PVDF) membrane were purchased from Millipore (Billerica, MA). 3-(1H-1,2,3-triazol-4-yl) pyridine (3-TYP), a selective SIRT3 inhibitor, was synthesized and characterized by the School of Pharmaceutical Sciences at Southern Medical University (Guangzhou, China) based on the work of Pi *et al.*[19] All other chemicals were obtained from Sigma (St. Louis, MO).

I/R model establishment

The present study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health, Bethesda, MD, USA). The study protocol was approved by the Committee on Ethics in Animal Experiments of Southern Medical University.

In total, 64 specific pathogen-free Sprague–Dawley rats (male and female, obtained from the laboratory animal center of Southern Medical University) weighing between 180 and 220 g were used in this study. The rats were housed in plastic cages with a controlled temperature of 25°C, humidity of 50%-55%, and a 12-h light/dark cycle. All the animals had free access to food and water. All rats were anesthetized and maintained with isoflurane (RWD Life science, Shenzhen, China). Then the rats were subjected to 40-min I/R by placing a clamp on the left and right renal pedicles. For the I/R experiment, after the initial clamping of the renal pedicle, the abdominal incision was closed for the 40-min duration to ensure that the entire kidney was maintained at 37°C. The kidneys were monitored for adequate reflow upon clamp release, and the rats recovered on a warming table until they regained consciousness.[20] For studying dynamic molecule expression and determining acetylation, some rats (8 per group; 32 in total) were euthanized by cervical dislocation at different time points (control [0 h], 4, 8, and 24 h after I/R). Finally, the time point of 8 h following renal I/R was confirmed to be a successful rat model of AKI and was applied for subsequent study (Supplemental Fig. 1).

Moreover, to determine the role of SIRT3 in I/R-induced AKI, other rats (8 per group, 32 in total) were randomly divided into (1) the control group, in which the rats were anesthetized and underwent surgery without any other treatment; (2) the I/R + vehicle group, in which the rats were given a vehicle (0.3 mL, 0.1% dimethyl sulfoxide, and 99.9% normal saline) and then subjected to renal I/R after 30 min; and (3) the I/R + 3-TYP group, in which the rats were given 3-TYP (dissolved in the equal amount of dimethyl sulfoxide as vehicle group), a selective inhibitor of SIRT3 (0.3 mL, 5 mg/kg) and then subjected to renal I/R after 30 min.

Histological analysis and pathological scoring

To evaluate the histological changes, a portion of the renal tissue samples was extracted from the rats euthanized in the previous experiment. Each sample (8 per group) was fixed by immersion in a 4% formaldehyde solution. The samples were then embedded in paraffin, sliced into 5-μm sections, and stained with hematoxylin and eosin to perform blinded histological assessments. The degree of congestion,