MEDICAL SCIENCE MONITOR BASIC RESEARCH

MOLECULAR BIOLOGY

eISSN 2325-4416
© Med Sci Monit Basic Res, 2016; 22: 165-174
DOI: 10.12659/MSMBR.901909

Received: 2016.10.11
Accepted: 2016.11.24
Published: 2016.12.16

Sirtuin1 (SIRT1) Regulates Tumor Necrosis Factor-alpha (TNF-α-Induced) Aquaporin-2 (AQP2) Expression in Renal Medullary Collecting Duct Cells Through Inhibiting the NF-κB Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Qinqin Lin
BCDE 1 Yuanwen Geng
BCDEF 2 Shuaishuai Lin
ABCDEFG 3 Zhenjun Tian

1 College of Physical Education, Yanshan University, Qinhuangdao, Hebei, P.R. China
2 Department of Education, Beijing Sport University, Beijing, P.R. China
3 Institute of Sports and Exercise Biology, Shaanxi Normal University, Xi'an, Shaanxi, P.R. China

Corresponding Author: Qinqin Lin, e-mail: linqinqin@ysu.edu.cn
Source of support: Supported by the National Natural Science Foundation of China (No. 31300978, 31371199), the Science and Technology Program of Hebei Province of China (No. 12277652), and the Outstanding Youth fund of Higher Education of Hebei Province of China (No. YQ2014004)

Background: Aquaporin-2 (AQP2) plays a major role in water reabsorption in the renal collecting duct, and is involved in a variety of renal disease. Recent studies have indicate that sirtuin1 (SIRT1) exerts renoprotective properties against kidney diseases. This study aimed to determine the potential role of SIRT1 in AQP2 expression induced by tumor necrosis factor-alpha (TNF-α) and to disclose the underlying mechanism in renal inner medullary collecting duct (IMCD) cells.

Material/Methods: Quantitative real-time PCR and Western blotting were respectively identified mRNA and protein expression. Immunofluorescence staining was used to detect the localization of AQP2. Small-interfering RNA (siRNA) was carried out for mechanism study.

Results: Results showed that AQP2 was clearly increased in the plasma membrane and decreased in the cytoplasm of IMCD cells treated with AVP. TNF-α treatment in IMCD cells significantly reduced SIRT1 and AQP2 expression, and increased acetylated NF-κBp65 protein level in time- and concentration-dependent manners. Moreover, SIRT1 overexpression or the activator SRT1720 augmented AQP2 expression and reduced the acetylation of NF-κBp65, which was reversed by SIRT1 siRNA or the inhibitors Ex527 and sirtinol in TNF-α-induced IMCD cells. Knockdown of NF-κBp65 or NF-κBp65 inhibition by pyrrolidine dithiocarbamate (PDTC) enhanced AQP2 expression in IMCD cells exposed to TNF-α. Importantly, knockdown of NF-κBp65 augmented the up-regulation of SIRT1 on AQP2 expression in IMCD cells induced by TNF-α.

Conclusions: These findings indicate that SIRT1 increases AQP2 expression in TNF-α-induced IMCD cells via the NF-κB-dependent signalling pathway, which might provide novel insight to understanding the renoprotective effects of SIRT1 in kidney diseases.

MeSH Keywords: Acute Kidney Injury • Aquaporin 2 • Kidney Tubules, Collecting • Sirtuin 1

Full-text PDF: http://www.basic.medscimonit.com/abstract/index/idArt/901909

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Sirtuin 1 (SIRT1), a member of the evolutionarily conserved family of NAD^+-dependent histone deacetylase, is implicated in a wide range of cellular functions involved in the pathogenesis of diseases including cardiovascular diseases, kidney disease [1,2]. Recently, emerging evidences has suggested that SIRT1 is a novel target for prevention of kidney diseases [2–4]. Kidney-specific overexpression of SIRT1 rescues cisplatin-induced acute kidney injury (AKI) in transgenic mice [5], while SIRT1 knockout in diabetes and AKI mice shows aggravation of renal changes occurring [6,7]. It is well known that AKI is characterized by structural alterations in renal tubule epithelia, and concomitant with impaired urinary concentration and sodium and water reabsorption [8,9]. Aquaporins (AQPs; a family of water channel proteins), especially AQP2, plays a major role in water reabsorption in the renal inner medullary collecting duct (IMCD). AQP2 has been shown to be decreased markedly in the proximal tubule and IMCD in rats with AKI induced by ischemia/reperfusion injury or sepsis [9,10]. However, there is no direct evidence to demonstrate the relationship between SIRT1 and AQP2. Therefore, this study was conducted to explore the possible effect of SIRT1 on AQP2 expression in renal IMCD cells induced by TNF-α. Furthermore, it's well accepted that the renoprotective properties of SIRT1 are closely related to the regulation of nuclear transcription factor (NF-κB) [11,12]. It has been shown that NF-κB is involved in AQP2 expression in renal collecting duct principal cells [13], in mouse cortical collecting duct cells [14], and kidneys of rats with sepsis-induced acute renal failure [15]. So, we also investigated whether NF-κB pathway contributed to the regulation of SIRT1 on AQP2 expression in renal IMCD cells.

Material and Methods

Reagents and cell transfection

TNF-α was ordered from PeproTech (Rocky Hill, NJ, USA). SRT1720 and Ex527 were obtained from Selleck Chemicals (Houston, TX, USA). Sirtinol and pyrrolidine dithiocarbamate (PDTC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). SIRT1 siRNA, NF-κBp65 siRNA, and the scrambled negative controls were purchased from GenePharma (Shanghai, China). Recombinant plasmid pcDNA3.1(+) was sponsored by Dr. Bu. To overexpress SIRT1, the cells cultured in 6-well plates were transfected with recombinant plasmid pcDNA3.1(+)-SIRT1. To knockdown endogenous SIRT1 and NF-κB, IMCD cells were transfected with 100 nM SIRT1 siRNA or NF-κBp65 siRNA. Transfection was carried by using Lipofectamine 2000 (Invitrogen, NY, USA) according to the manufacturer's protocol. The cells were harvested 48 h after transfection to assess the expression of SIRT1 or NF-κBp65.

Cell culture

Primary cultures enriched in IMCD cells were prepared from male Sprague-Dawley rats (120–140 g body wt), as previously described [16]. All experimental protocols were approved by the Review Committee for the Use of Human or Animal Subjects of Yanshan University. Both kidneys were rapidly removed from rats under light diethyl ether inhalation anaesthesia. After kidney excision, the renal inner medullas including papilla were rapidly removed, cut into small pieces, and digested in phosphate-buffered saline (PBS) containing 1 mg/ml hyaluronidase and 2.2 mg/ml collagenase type CLS-II at 37°C under continuous agitation for 90 min. After centrifugation, the pellet was washed in prewarmed culture medium (High glucose Dulbecco's modified Eagle's medium containing 100 mM NaCl, 100 mM urea, 1% non-essential amino acids, 1% ultroser, 500 μM DBcAMP, 20 U/ml nystatin and 0.25 μg/ml gentamicin). The IMCD cell suspension was then seeded in collagen type IV-coated chamber slides for immunocytochemical analysis or in a 60-mm culture dish for immunoblot analysis. After 24 h, wash cells twice with 600 mosmol hypertonic culture medium (High glucose Dulbecco's modified Eagle's medium containing 100 mM NaCl and 100 mM urea) and add fresh medium at 37°C in 5% CO_2 and 95% air for 3 days and then in medium without DBcAMP and nystatin for 24 h before starting the experiment on day 6.

Immunofluorescence staining

IMCD cells were fixed in 10% paraformaldehyde-PBS for 30 min. The fixed cell membrane was fenestrated with 0.5% Triton X-100 in PBS for 5 min, and then blocked with 5% goat serum for 30 min. The cells were incubated with polyclonal anti-rabbit AQP2 antibody (sc-28629, 1: 100, Santa Cruz, CA, USA) overnight at 4°C, and then incubated with a TRITC-conjugated goat anti-rabbit IgG polyclonal antibody (1: 100 dilutions) for 2 h. After the nuclei were stained with 4'-6-diamidino-2-phenylindole (DAPI) dye (1: 800 dilutions) for 15 min, the stained cells were investigated using a confocal laser scanning microscopy (Nikon, Tokyo, Japan).

Western blot analysis

Cell samples were collected and lysed at 4°C in RIPA buffer with the protease inhibitor cocktail (Roche, Mannheim, Germany). The protein was separated on 12% SDS PAGE gels and then transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 5% non-fat milk for 1 h and then incubated at 4°C overnight with rabbit polyclonal anti-SIRT1 (sc-15404, 1: 500, Santa Cruz, CA, USA), anti-AQP2 (sc-28629, 1: 100, Santa Cruz, CA, USA), anti-acetyl-NF-κBp65 (Lys 310) (Ac-p65) (ab19870, 1: 400, Abcam, Cambridge, MA, UK), anti-NF-κBp65 (ab16502, 1: 400, Abcam), and anti-GAPDH (TDY052, 1: 5000, TDY biotech, Beijing, China) respectively. GAPDH was used as internal standard. Then, the

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

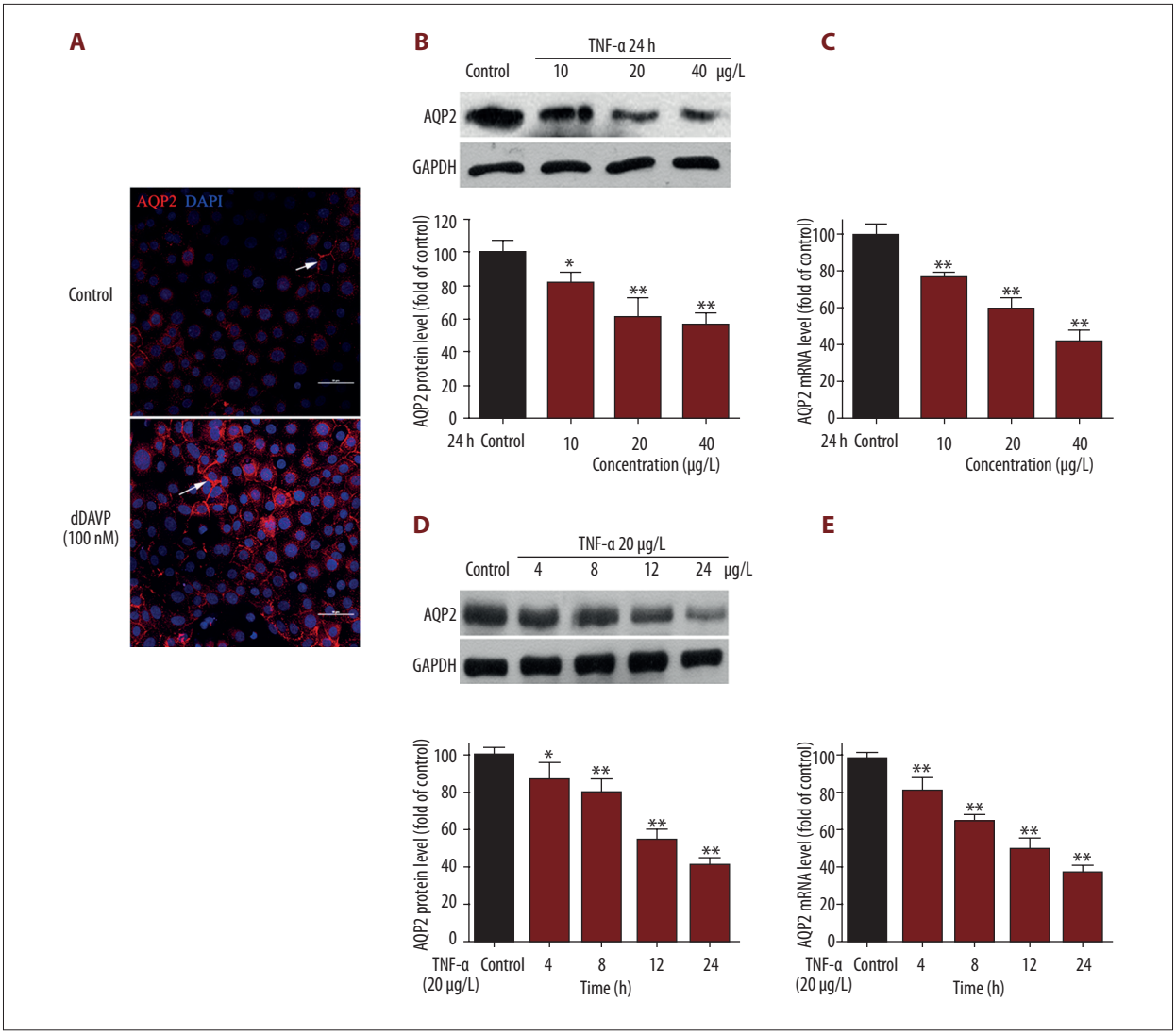


Figure 1. TNF-α decreased AQP2 expression in IMCD cells. (**A**) Immunofluorescence microscopy of aquaporin 2 (AQP2) in primary IMCD cells. IMCD cells were left under control conditions or stimulated with 100 nM AVP for 30 min at 37°C. AQP2 was stained with anti-AQP2 antibody (coloured in red/indicated by the arrow). The nuclear protein was stained with DAPI (blue). Merge represents the combined image of AQP2 fluorescence and nuclear staining. Images of cells were generated on a microscope at 200× magnification, bar=50μm (n=5). (**B, C**) The cells were treated with different concentrations of TNF-α (10, 20 and 40 μg/L) for 24 h, and the protein and mRNA expression of AQP2 was examined by Western blotting and RT-qPCR, respectively. (**D, E**) The cells were stimulated with TNF-α (20 μg/L) for the indicated time, and the protein and mRNA expression of AQP2 was examined by Western blotting and RT-qPCR, respectively. * $P<0.05$ and ** $P<0.01$ *vs.* control group. The data are presented as the mean ± standard deviation from 3 independent experiments.

membranes were incubated with HRP-conjugated secondary antibodies (S004, 1: 10000, TDY biotech) for 45 min. Protein signals were subsequently detected using the enhanced chemiluminescence (Pierce, Rockford, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from IMCD cells using TRIzol reagent (Life Technologies, Foster, CA, USA). Complementary DNA was synthesized using a PrimeScript RT reagent kit (TaKaRa, Dalian, China). qRT-PCR was performed using the SYBR Green PCR Master Mix (TaKaRa) on a Bio-Rad CFX connect real-time system (Hercules, CA, USA) according to the manufacturer's instructions. All primers were as follows: SIRT1 forward 5'-CAGTTCCAGCCATCTCTGTG-3'and reverse 5'-GCAACCTGC TCCAAGGTATC-3'; AQP2 forward 5'-TTCCTTCGAGCTGCCTTCTA-3'and reverse 5'-TTGTGGAGAGCATTGACAGC-3'; GAPDH forward 5'-ACAGCAACAGGGTGG TGGAC-3'and reverse 5'-TTTGAGGGTGCAGCGAACTT-3'. GAPDH was used as an internal

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

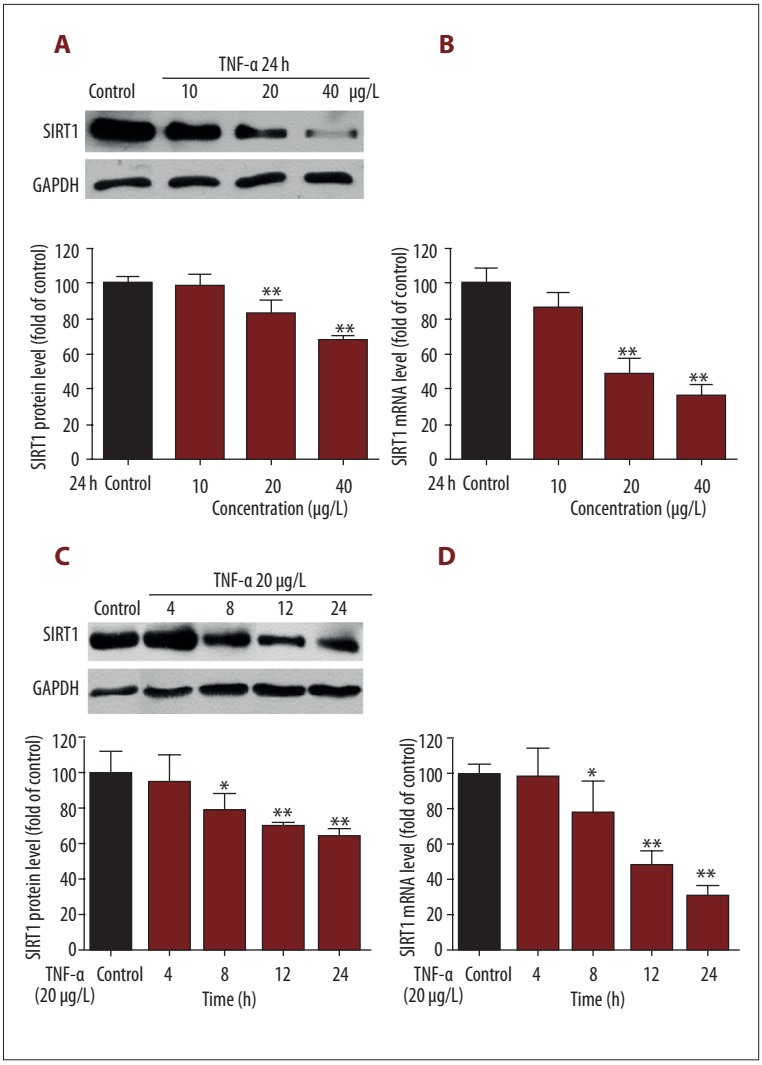


Figure 2. TNF-α reduced SIRT1 expression in IMCD cells. (**A, B**) The cells were treated with different concentrations of TNF-α (10, 20 and 40 μg/L) for 24 h, and the protein and mRNA expression of SIRT1 was examined by Western blotting and RT-qPCR, respectively. (**C, D**) The cells were stimulated with TNF-α (20 μg/L) for the indicated time, and the protein and mRNA expression of SIRT1 was examined by Western blotting and RT-qPCR, respectively. * $P<0.05$ and ** $P<0.01$ *vs.* control group. The data are presented as the mean ± standard deviation from 3 independent experiments.

control. Quantification of relative gene expression was calculated with $2^{-\Delta\Delta Ct}$ method as described by the manufacturer.

Statistical analysis

Data were expressed as the mean ± standard deviation. Data were analysed by one-way ANOVA, followed by a post hoc test. $P<0.05$ was considered statistically significant.

Results

TNF-α decreased AQP2 expression in IMCD cells

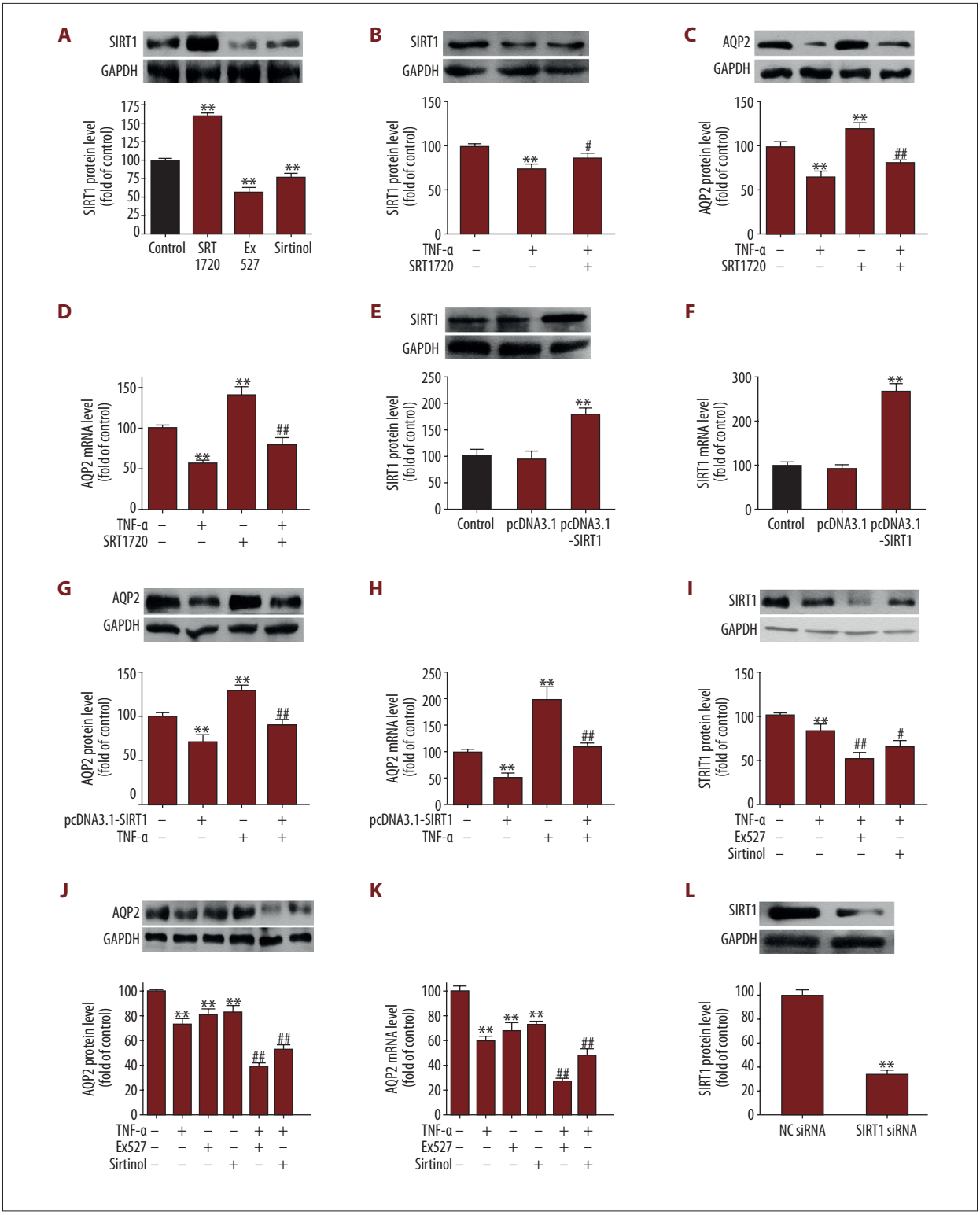
AQP2 immunofluorescent labeling was largely dispersed along the cytoplasm of primary cultured IMCD cells in the absence of 1-desamino-8-D-arginine vasopressin (dDAVP) stimulation in control group. In contrast, treatment with 100 nM AVP for 30 clearly increased the AQP2 expression in the plasma membrane and decreased it in the cytoplasm (Figure 1A), indicating that the IMCD cells were correctly cultured.

To study the effect of TNF-α on AQP2 expression, the IMCD cells were treated with TNF-α (10, 20 and 40 μg/L) for 24 h, and the expression of AQP2 protein and mRNA was determined by Western blot analysis and qRT-PCR, respectively. The results showed that TNF-α significantly decreased the expression of AQP2 mRNA and protein in a concentration-dependent manner (Figure 1B, 1C). We further investigate the time courses of AQP2 stimulated with TNF-α (20 μg/L) for 4, 8, 12 and 24 h. As shown in Figure 1D,1E, TNF-α time-dependently reduced the expression of AQP2 mRNA and protein in IMCD cells.

TNF-α down-regulated SIRT1 expression in IMCD cells

We next investigated the effect of TNF-α on SIRT1 expression in IMCD cells, the IMCD cells were treated with TNF-α (10, 20 and 40 μg/L) for 24 h. The results showed that TNF-α

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]



Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

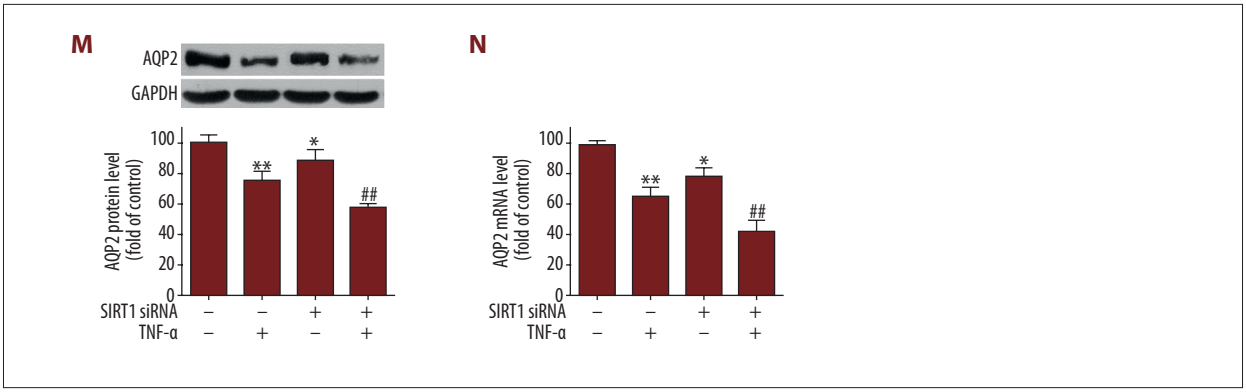


Figure 3. SIRT1 increased AQP2 expression in TNF-α-induced IMCD cells. (**A**) The cells were treated with SRT1720 (10 μM), Ex527 (1 μM) or sirtinol (10 μM) for 24 h, and the expression of SIRT1 was examined by Western blotting. ** $P<0.01$ *vs.* control group. (**B–D**) The cells were pretreated with SRT1720 (10 μM) for 1 h prior to stimulation with TNF-α (20 μg/L) for 24 h, and the expression of SIRT1 and AQP2 was examined. ** $P<0.01$ *vs.* control group; ## $P<0.01$ *vs.* TNF-α group. (**E, F**) The IMCD cells were transfected with pcDNA3.1(+)-SIRT1 for 48 h, and the protein and mRNA expression of SIRT1 was examined by Western blotting and RT-qPCR, respectively. ** $P<0.01$ *vs.* pcDNA3.1 group. (**G, H**) The SIRT1-overexpressing cells were stimulated with TNF-α (20 μg/L) for 24 h, and the protein and mRNA expression of AQP2 was examined by Western blotting and RT-qPCR, respectively. ** $P<0.01$ *vs.* pcDNA3.1 group; ## $P<0.01$ *vs.* pcDNA3.1 + TNF-α group. The data are presented as mean ± standard deviation from 3 independent experiments. (**I–K**) The cells were pretreated with Ex527 (1 μM) or sirtinol (10 μM) for 1 h prior to stimulation with TNF-α (20 μg/L) for 24 h, and the expression of SIRT1 and AQP2 was examined. ** $P<0.01$ *vs.* control group; # $P<0.05$ and ## $P<0.01$ *vs.* TNF-α group. (**L**) The IMCD cells were transfected with negative control siRNA (NC siRNA) or SIRT1 siRNA for 48 h, and the protein expression of SIRT1 was detected by Western blotting. ** $P<0.01$ *vs.* NC siRNA group. (**M, N**) The SIRT1-knockdown cells were stimulated with TNF-α (20 μg/L) for 24 h, and the protein and mRNA expression of AQP2 was examined by Western blotting and RT-qPCR, respectively. * $P<0.05$ and ** $P<0.01$ *vs.* NC siRNA group; ## $P<0.01$ *vs.* NC siRNA + TNF-α group. The data are presented as the mean ± standard deviation from 3 independent experiments.

down-regulated SIRT1 expression at protein and mRNA level in IMCD cells in a concentration-dependent manner (Figure 2A, 2B). To study the time courses of SIRT1 expression, the IMCD cells were treated with TNF-α (20 μg/L) for 4, 8, 12 and 24 h. The results revealed that TNF-α inhibited the protein and mRNA expression of SIRT1 in a time-dependent manner (Figure 2C, 2D).

SIRT1 increased AQP2 expression in TNF-α-induced IMCD cells

To identify the role of SIRT1 in the expression of AQP2 in TNF-α-induced IMCD cells, we overexpressed the expressions of SIRT1 in IMCD cells. After treatment with the SIRT1 activator SRT1720 (10 μM) or the pcDNA3.1(+)-SIRT1 plasmid, increases in SIRT1 expression were observed in IMCD cells compared with the control group (Figure 3A, 3E, 3F). Then the IMCD cells were pretreated with SRT1720 for 1 h or pcDNA3.1(+)-SIRT1 for 48 h, and then stimulated with TNF-α (20μg/L) for 24 h. SIRT1 activator SRT1720 obviously increased the expression of SIRT1 and AQP2 in TNF-α-induced IMCD cells (Figure 3B–3D). Overexpression of SIRT1 is further supported the results by inducing expression of AQP2 compared with the TNF-α group (Figure 3G, 3H). Then, we silenced the expression of SIRT1 in IMCD cells. Treatment of IMCD cells with the SIRT1 inhibitors Ex527 (1 μM) and sirtinol (10 μM) or SIRT1 siRNA strikingly reduced the expression of SIRT1 (Figure 3A, 3L). Moreover, the IMCD cells were pretreated with Ex527 or sirtinol for 1 h or SIRT1 siRNA for 48 h, and then stimulated with TNF-α (20 μg/L) for 24 h. The results showed that SIRT1 inhibitors Ex527 and sirtinol decreased the expression of SIRT1 and AQP2 in TNF-α-induced IMCD cells (Figure 3I–3K). In addition, knockdown of SIRT1 remarkably decreased AQP2 mRNA and protein expression in IMCD cells exposed to TNF-α (Figure 3M, 3N).

NF-κB pathway inhibited AQP2 expression in TNF-α-induced IMCD cells

To assess the acetylation of NF-κBp65 in TNF-α-induced IMCD cells, Western blot was performed. The results showed that treatment of IMCD cells with TNF-α (10, 20 and 40 μg/L) for 24 h significantly increased Ac-p65 protein expression in a concentration-dependent manner (Figure 4A). We further studied the time courses of Ac-p65 expression in IMCD cells by treating with TNF-α (20 μg/L) for 4, 8, 12 and 24 h. The results showed that TNF-α up-regulated Ac-p65 protein expression in a time-dependent manner (Figure 4B).

To further assess whether NF-κB pathway was associated with the expression of AQP2 in TNF-α-induced IMCD cells, we silenced NF-κB pathway. Pretreatment with the NF-κB inhibitor PDTC (10 μM) or NF-κBp65 siRNA strikingly decreased Ac-p65 protein expression (Figure 4C, 4F). Then the IMCD cells

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

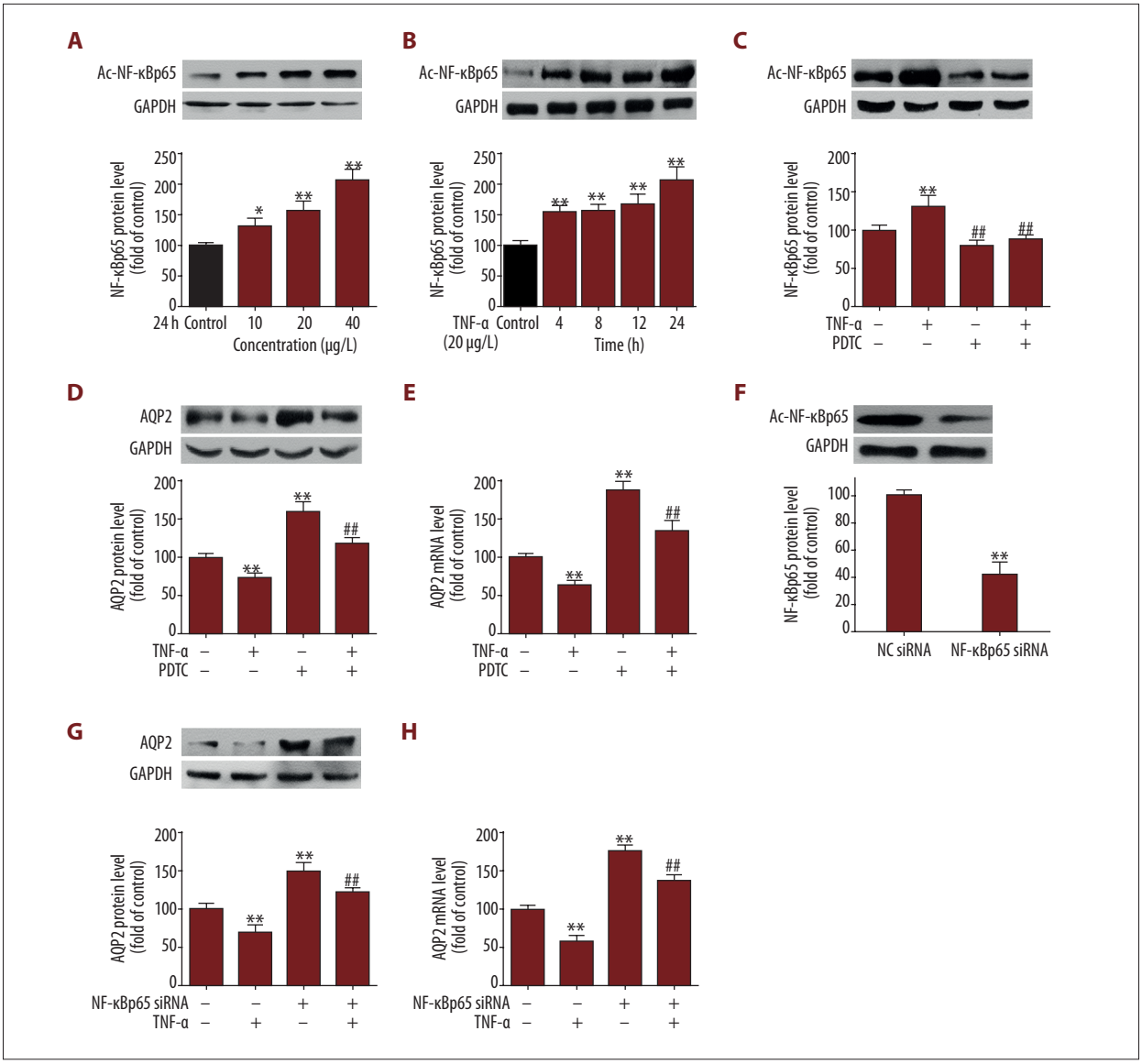


Figure 4. NF-κB pathway inhibited AQP2 expression in TNF-α-induced IMCD cells. (**A**) The cells were treated with different concentrations of TNF-α (10, 20 and 40 μg/L) for 24 h, and the expression of Ac-NF-κBp65 (Ac-p65) was examined. * $P<0.05$ and ** $P<0.01$ *vs.* control group. (**B**) The cells were stimulated with TNF-α (20 μg/L) for the indicated time, and the expression of Ac-NF-κBp65 (Ac-p65) was examined. ** $P<0.01$ *vs.* control group. (**C–E**) The IMCD cells were pretreated with PDTC (10 μM) for 1 h prior to stimulation with TNF-α (20 μg/L) for 24 h, and the expression of AQP2 and Ac-p65 was examined. ** $P<0.01$ *vs.* control group; ## $P<0.01$ *vs.* TNF-α group. (**F**) The IMCD cells were transfected with negative control siRNA (NC siRNA) or NF-κBp65 siRNA for 48 h, and the protein expression of Ac-p65 was detected by Western blotting. ** $P<0.01$ *vs.* NC siRNA group. (**G, H**) The NF-κBp65-knockdown cells were stimulated with TNF-α (20 μg/L) for 24 h, and the protein and mRNA expression of AQP2 was examined by Western blotting and real-time PCR, respectively. ** $P<0.01$ *vs.* NC siRNA group; ## $P<0.01$ *vs.* NC siRNA + TNF-α group. The data are presented as the mean ± standard deviation from 3 independent experiments.

were treated with PDTC for 1 h or NF-κBp65 siRNA for 48 h, and then stimulated with TNF-α (20μg/L) for 24 h. The results showed that PDTC visibly increased AQP2 mRNA and protein expression in TNF-α-induced IMCD cells (Figure 4D, 4E). Knockdown of NF-κBp65 was further supported the above results by increasing the expression of AQP2 in IMCD cells induce by TNF-α (Figure 4G, 4H).

SIRT1 increased AQP2 expression via NF-κB pathway in TNF-α-induced IMCD cells

To prove the relationship between SIRT1 and NF-κB in IMCD cells, the expression of Ac-p65 was assessed. We found that the SIRT1 activator SRT1720 significantly reduced Ac-p65 expression, which was reversed by the SIRT1 inhibitors Ex527 and

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

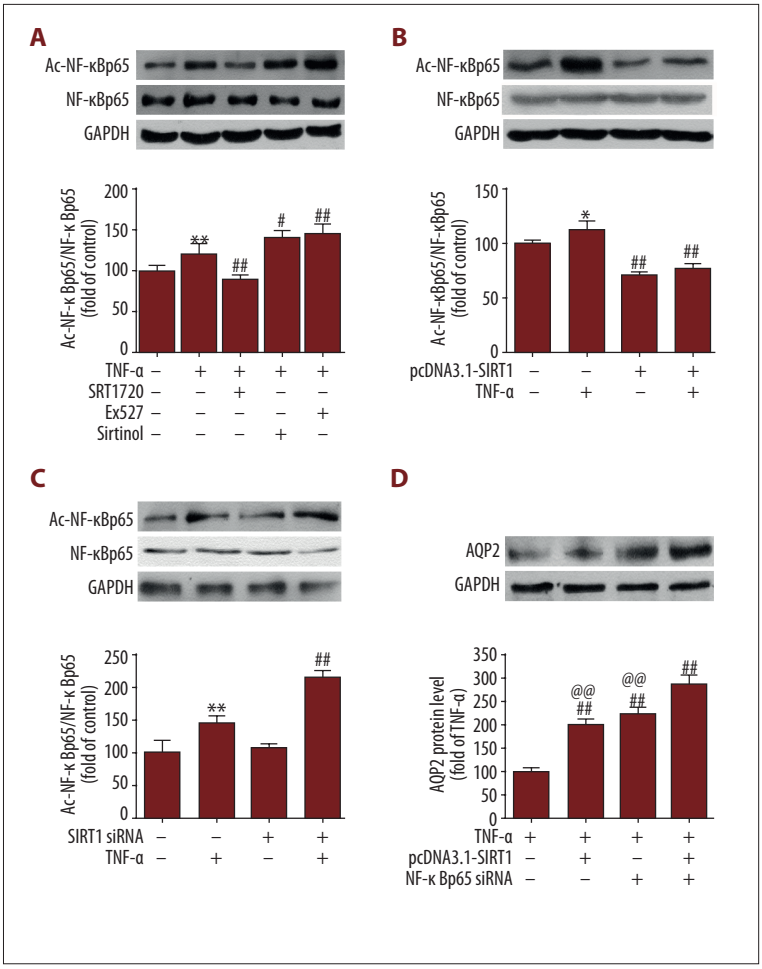


Figure 5. SIRT1 increased AQP2 expression via NF-κB pathway in TNF-α-induced IMCD cells. (**A**) The cells were treated with SRT1720 (10 μM), Ex527 (1 μM) or sirtinol (10 μM) 1 h prior to stimulation with TNF-α (20 μg/L) for 24 h, and the expression of Ac-NF-κBp65 (Ac-p65) was examined. ** *P*<0.01 *vs.* control group; ## *P*<0.01 *vs.* TNF-α group. (**B**) SIRT1 overexpressing cells were stimulated with TNF-α (20 μg/L) for 24 h, and the expression of Ac-p65 was examined. * *P*<0.05 *vs.* pcDNA3.1 group; ## *P*<0.01 *vs.* pcDNA3.1 + TNF-α group. (**C**) SIRT1 knockdown cells were stimulated with TNF-α (20 μg/L) for 24 h, and the expression of Ac-p65 was examined. ** *P*<0.01 *vs.* NC siRNA group, ## *P*<0.01 *vs.* NC siRNA + TNF-α group. (**D**) NF-κBp65 siRNA cells were transfected with pcDNA3.1(+)-SIRT1 for 48 h, and subsequently treated with TNF-α (20 μg/L) for 24 h, and the protein expression of AQP2 was examined by Western blotting. ## *P*<0.01 *vs.* TNF-α group; @@ *P*<0.01 *vs.* NF-κBp65 siRNA + pcDNA3.1(+)-SIRT1 + TNF-α group. The data are presented as the mean ± standard deviation from 3 independent experiments.

sirtinol in TNF-α-induced IMCD cells (Figure 5A). Moreover, the SIRT1 overexpressing or knockdown cells were used to further verify the above results. The results revealed that SIRT1 overexpression significantly decreased TNF-α-induced Ac-p65 expression (Figure 5B), while knockdown of SIRT1 obviously increased Ac-p65 expression in IMCD cells expose to TNF-α (Figure 5C).

To further clarify the underlying mechanism involved in SIRT1-regulated AQP2 expression, NF-κB signaling was included. The results revealed that the expression of AQP2 was correspondingly increased after preconditioning of NF-κBp65 knockdown cells with pcDNA3.1(+)-SIRT1, compared with NF-κBp65 siRNA or pcDNA3.1(+)-SIRT1 single treatment in TNF-α-induced IMCD cells (Figure 5D). These results imply that SIRT1 can regulate AQP2 expression in IMCD cells stimulated with TNF-α via suppression of NF-κB signalling.

Discussions

Emerging evidence has suggested that SIRT1 expresses in medullary tubular cells, and protects and maintains kidney cell function [17]. Our present study found that treatment of IMCD cells with TNF-α significantly decreased SIRT1 expression at mRNA and protein levels in a time-and concentration-dependent manner. It has also revealed that high glucose obviously decreased SIRT1 mRNA and protein expression in rat mesangial cells [18]. Other studies have reported that SIRT1 expression was decreased in rats with gentamicin or cisplatin-induced nephrotoxicity [19,20]. Moreover, reduced SIRT1 expression has been found in mouse cortical collecting duct cells [14], in human kidney proximal tubule epithelial cells [21], and in rats with AKI induced by severe burns [22] and renal ischaemia-reperfusion injury [23]. Therefore, these findings imply that the decrease in SIRT1 expression is involved in renal injury processes *in vivo* and *in vitro*.

It has been shown that the abundance of AQP2 is decreased in collecting duct cells in a variety of water balance disorders as demonstrated in many studies [24]. The present study found that the expression of AQP2 mRNA and protein was time- and concentration- dependently decreased in IMCD cells in response to TNF-α. Hasler et al. revealed that LPS decreased AQP2 expression in cultured collecting duct principal cells [13].

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

In addition to *in vitro* experiment, the *in vivo* study also found that AQP2 expression was significantly reduced in the renal cortex and papillas in rats with sepsis-induced acute renal failure [15]. Conversely, AQP2 mRNA expression was significantly up-regulated in the kidneys of cirrhotic rats [25]. These different results imply that AQP2 might play different roles in types of renal-related disease in response to different stimuli. More importantly, the present study found that SIRT1 overexpression or activation by SRT1720 increased AQP2 expression in IMCD cells treated with TNF-α. The corresponding changes were also observed when silencing SIRT1 expression by SIRT1 siRNA or he inhibitors Ex527 and sirtinol. Our results suggest that SIRT1 can regulate AQP2 expression in IMCD cells. This is consistent with the results of the latest study showing that resveratrol increased the expression of AQP2 in mouse cortical collecting duct cells induced by 4-hydroxy-2-hexenal, a inducer for tubular injury, by activating SIRT1 pathway [14]. These results indicate that SIRT1 increased AQP2 expression which was involved in water balance disorders-associated diseases.

It is well recognized that the renoprotective effect of SIRT1 is due to deacetylating its target proteins, such as NF-κB, a master transcription factor involved in the development of renal disease [11,26]. The transcriptional activity of NF-κB requires acetylation at lysine 310 [27,28]. In the present study, TNF-α significantly increased the acetylation of NF-κBp65 (Lys 310) expression, which was reversed by SIRT1 overexpression or the activator SRT1720, and augmented by SIRT1 siRNA or the inhibitors Ex527 and sirtinol in IMCD cells. Other study further supported that TNF-α increased the acetylation of NF-κB at lysine 310 in fibroblasts, while this effect was inhibited by resveratrol via a SIRT1-dependent manner [29]. These findings show that SIRT1 plays renoprotective role in antagonizing inflammatory renal injury in IMCD cells through deacetylation of NF-κBp65 at lysine 310. Furthermore, it has been revealed that activation of NF-κB observed in renal diseases most likely accounts for the observed decrease in AQP2 expression [30]. The present study found that knockdown of NF-κB or the NF-κB inhibitor PDTC remarkably antagonized the downregulation of AQP2 induced by TNF-α in IMCD cells. Our results further found that knockdown of NF-κBp65 significantly augmented the effect of SIRT1 on increased expression of AQP2 in IMCD cells exposed to TNF-α. Similarly, SIRT1 activator resveratrol significantly increased AQP2 expression by suppressing the nuclear localization of the NF-κBp65 subunit *in vitro* [14]. Taken together, these findings suggest that SIRT1-mediated rescuing of AQP2 expression was due, at least in part, to inhibited NF-κB signalling in IMCD cells.

Conclusions

Our results provide a crucial role of SIRT1 in the regulation of AQP2 expression. Activation of SIRT1 efficiently inhibits water balance disorders. These findings might be important to understand the potential role of SIRT1 activation in water balance disorders-related diseases.

Conflicts of interest

The authors have no conflicts of interest.

References:

1. Bindu S, Pillai VB, Gupta MP: Role of sirtuins in regulating pathophysiology of the heart. Trends Endocrinol Metab, 2016; 27: 563–73
2. Yacoub R, Lee K, He JC: The role of SIRT1 in diabetic kidney disease. Front Endocrinol (Lausanne), 2014; 5: 166
3. Wakino S, Hasegawa K, Itoh H: Sirtuin and metabolic kidney disease. Kidney Int, 2015; 88: 691–98
4. Guclu A, Erdur FM, Turkmen K: The emerging role of Sirtuin 1 in cellular metabolism, diabetes mellitus, diabetic kidney disease and hypertension. Exp Clin Endocrinol Diabetes, 2016; 124: 131–39
5. Hasegawa K, Wakino S, Yoshioka K et al: Kidney-specific overexpression of Sirt1 protects against acute kidney injury by retaining peroxisome function. J Biol Chem, 2010; 285: 13045–56
6. Hasegawa K, Wakino S, Simic P et al: Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. Nat Med, 2013; 19: 1496–504
7. Gao R, Chen J, Hu Y et al: Sirt1 deletion leads to enhanced inflammation and aggravates endotoxin-induced acute kidney injury. PLoS One, 2014; 9: e98909
8. Cha SA, Park BM, Jung YJ et al: Regional heterogeneity of expression of renal NPRs, TonEBP, and AQP-2 mRNAs in rats with acute kidney injury. Peptides, 2015; 69: 33–39
9. Kwon TH, Frokiaer J, Fernandez-Llama P et a l: Reduced abundance of aquaporins in rats with bilateral ischemia-induced acute renal failure: Prevention by alpha-MSH. Am J Physiol, 1999; 277: F413–27
10. Rodrigues CE, Sanches TR, Volpini RA et al: Effects of continuous erythropoietin receptor activator in sepsis-induced acute kidney injury and multiorgan dysfunction. PLoS One, 2012; 7: e29893
11. Liu R, Zhong Y, Li X et al: Role of transcription factor acetylation in diabetic kidney disease. Diabetes, 2014; 63: 2440–53
12. Lee JH, Song MY, Song EK et al. Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor-kappaB signaling pathway. Diabetes, 2009; 58: 344–51
13. Hasler U, Leroy V, Jeon US et al: NF-kappaB modulates aquaporin-2 transcription in renal collecting duct principal cells. J Biol Chem, 2008; 283: 28095–105
14. Bae EH, Joo SY, Ma SK, Lee J, Kim SW: Resveratrol attenuates 4-hydroxy-2-hexenal-induced oxidative stress in mouse cortical collecting duct cells. Korean J Physiol Pharmacol, 2016; 20: 229–36
15. Hocherl K, Schmidt C, Kurt B, Bucher M: Inhibition of NF-kappaB ameliorates sepsis-induced downregulation of aquaporin-2/V2 receptor expression and acute renal failure *in vivo*. Am J Physiol Renal Physiol, 2010; 298: F196–204
16. Faust D, Geelhaar A, Eisermann B et al: Culturing primary rat inner medullary collecting duct cells. J Vis Exp, 2013; (76)
17. Dong YJ, Liu N, Xiao Z et al: Renal protective effect of sirtuin 1. J Diabetes Res, 2014; 2014: 843786

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

18. Du YG, Wang LP, Qian JW et al: Panax notoginseng saponins protect kidney from diabetes by up-regulating silent information regulator 1 and activating antioxidant proteins in rats. Chin J Integr Med, 2015 [Epub ahead of print]
19. He L, Peng X, Zhu J et al: Protective effects of curcumin on acute gentamicin-induced nephrotoxicity in rats. Can J Physiol Pharmacol, 2015; 93: 275–82
20. Ugur S, Ulu R, Dogukan A et al: The renoprotective effect of curcumin in cisplatin-induced nephrotoxicity. Ren Fail, 2015; 37: 332–36
21. Wang XL, Wu LY, Zhao L et al: SIRT1 activator ameliorates the renal tubular injury induced by hyperglycemia *in vivo* and *in vitro* via inhibiting apoptosis. Biomed Pharmacother, 2016; 83: 41–50
22. Bai XZ, He T, Gao JX et al: Melatonin prevents acute kidney injury in severely burned rats via the activation of SIRT1. Sci Rep, 2016; 6: 32199
23. Zhao L, Xu L, Tao X et al: Protective effect of the total flavonoids from Rosa laevigata Michx fruit on renal ischemia-reperfusion injury through suppression of oxidative stress and inflammation. Molecules, 2016; 21(7): pii: E952
24. Nielsen S, Frokiaer J, Marples D et al: Aquaporins in the kidney: From molecules to medicine. Physiol Rev, 2002; 82: 205–44
25. Huang YY, Sun JY, Wang JY et al: Terlipressin resolves ascites of cirrhotic rats through downregulation of aquaporin 2. J Int Med Res, 2012; 40: 1735–44
26. Jung YJ, Lee JE, Lee AS et al: SIRT1 overexpression decreases cisplatin-induced acetylation of NF-kappaB p65 subunit and cytotoxicity in renal proximal tubule cells. Biochem Biophys Res Commun, 2012; 419: 206–10
27. Tak PP, Firestein GS: NF-kappaB: A key role in inflammatory diseases. J Clin Invest, 2001; 107: 7–11
28. Chen LF, Mu Y, Greene WC: Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. EMBO J, 2002; 21: 6539–48
29. Zhu X, Liu Q, Wang M et al: Activation of Sirt1 by resveratrol inhibits TNF-alpha induced inflammation in fibroblasts. PLoS One, 2011; 6: e27081
30. Hasler U, Leroy V, Martin PY, Feraille E: Aquaporin-2 abundance in the renal collecting duct: New insights from cultured cell models. Am J Physiol Renal Physiol, 2009; 297: F10–18

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]