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Original Research Article

TRIM8 regulates contrast-induced renal cell injury via the TLR4/MyD88/ NF-kB pathway

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Abstract

Purpose: *To investigate the effect of tripartite motif-containing protein 8 (TRIM8) on contrast media-induced injury on kidneys in mice and on human renal tubular epithelial cells.*
Methods: *Contrast media-induced nephropathy (CIN) model was constructed by injecting meglumine diatrizoate in mice. Small interference technology was used to knock down the expression of TRIM8 in HK-2 cells, while quantitative real-time polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA) were used to determine the expression levels of the inflammatory factors: TNF-α, TGF-β1, IL-1β, and IL-4. Cell counting kit-8 (CCK-8) assay was employed to determine the cell viability of each group, and TUNEL staining applied to detect apoptosis morphology using a fluorescence microscope. Flow-type Annexin V-FITC/PI double staining and Caspase-3 activity colorimetry were used to observe the changes in apoptosis. Expression of apoptosis factors, including Bax, Caspase3, Caspase9, and anti-apoptotic factor Bcl-2, was determined by qRT-PCR, while expressions of the TLR4/MyD88/NF-kB pathway were determined using Western blot and qRT-PCR.*
Results: *In CIN group, the kidney tissues significantly expressed IL-1β and Caspase3, while the contents of BUN, SCr, and UP all increased ($p < 0.05$). At the same time, expression of TRIM8 in the kidney tissues was up-regulated. After knocking down TRIM8, HK-2 cells inhibited the contrast agent-induced apoptosis and inflammation and as well as TLR4/MyD88/NF-kB pathway ($p < 0.05$).*
Conclusion: *TRIM8 regulates contrast-induced renal cell damage via TLR4/MyD88/NF-KB pathway. The results of the current study may provide new insights for the development of new treatment strategies for CIN.*

Keywords: *Contrast-media induced nephropathy, Tripartite motif-containing protein 8, Inflammation, Apoptosis, TLR4/MyD88/NF-kB pathway*

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INTRODUCTION

Contrast-media-induced nephropathy (CIN) refers to acute kidney injury caused by contrast agents, also known as contrast-induced nephropathy, which often occurs in patients undergoing interventional diagnosis and treatment techniques. It is characterized by long hospital stays, high complications, and high mortality. Contrast-induced nephropathy seriously affects patients' quality of life [1]. In recent years, with the rapid development of

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imaging and interventional technology, and the significant increases in the number of patients with cardiovascular and cerebrovascular diseases, the use of contrast agents has become more widely used in clinics. Therefore, CIN has become a common cause of hospital-acquired acute kidney injury besides insufficient renal perfusion and nephrotoxic drugs. At present, the pathophysiological mechanism of CIN is not yet clear [2]. However, most studies believe that it is related to changes in renal hemodynamics, oxidative stress injury [3], apoptosis [4], and release of inflammatory factors [5].

Tripartite motif-containing protein 8 (TRIM8) is a member of the TRIM protein family and plays an important role in various pathological processes such as innate immunity and inflammatory responses [6]. Studies have shown that the gain-function mutant of TRIM20 forms inflammatory bodies with ASC and Caspase1, which activate Caspase1 in mice [7]. In addition, some other TRIMs are related to the regulation of inflammatory bodies. For example, TRIM16, which is a homologue very close to TRIM20, lacks a PYD domain and enhances the production of IL-1β caused by non-classical secretion [8]. In addition, studies have reported that TRIM8 increases myocardial hypertrophy by mediating inflammation and insulin resistance [9]. However, there are relatively few studies on TRIM family members in the kidney. Hence, this study will explore the effect of TRIM8 on contrast agent nephropathy.

Toll-like receptors (TLRs) were initially recognized as sentinels that recognized exogenous pathogens, and they play a key role in the body's defense system. TLRs are expressed in various cells, including macrophages and monocytes [10]. In the TLR4/MyD88/NF-κB signaling pathway, NF-kB is the key molecule at the lower end and plays an important role in regulating the immune response and cell proliferation [11], inflammatory response, and apoptosis [12]. However, it is unclear whether the molecular mechanism of TRIM8 affecting contrast agent-induced apoptosis and inflammation is related to the TLR4/MyD88/NF-kB signaling pathway.

EXPERIMENTAL

Animals and model preparation

One hundred and twenty C57BL/6 mice, weighing 20 - 25 g, were provided by the Experimental Animal Center of Jilin University. This study was approved by the Animal Ethics Committee of Taizhou People's Hospital Animal Center (approval no. TZ20#3). All procedures were conducted in accordance with the 'Animal Research: Reporting *in vivo* Experiments guidelines 2.0' [13]. The mice were kept in metabolic cages, water-free, and fasting for 24 h. Except for the control group, the remaining groups were injected with 25 % glycerol solution 10 mL/kg in the hind limb muscle. The control group was injected with an equal volume of sodium chloride injection in the hind limb muscle. After the injection, free water and feed were restored. Twenty-four hours after glycerin injection, each group was given intravenous injection. After 1 h, the model group was intravenously injected with 76 % meglumine diatrizoate (World RM, Dongguan, China) 10 mL/kg, and the control group was intravenously injected with an equal volume of sodium chloride injection. Mics are placed in metabolic cages. After intravenous injection of 76 % meglumine diatrizoate 24 h, urine was collected from 0 to 24 h.

Determination of serum levels of creatinine (SCr), urea nitrogen (BUN) and urine protein (UP)

Urine and blood were collected from 0 to 24 h and placed in a refrigerator at 4 °C for no more than 2 h, centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was kept at -80 °C for later use following the instructions of the SCr, BUN and UP kits (Jian Cheng, Nanjing, China).

Immunohistochemical staining

Kidney tissue paraffin sections were placed in sodium citrate buffer for antigen repair, and 5% sheep serum was used for blocking. Kidney tissue sections were incubated with primary antibody (IL-1β, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; Caspase3, Abcam, Cambridge, MA, USA, Mouse, 1:1000) on the same day, overnight at 4 °C. The second day, horseradish peroxidase-labeled secondary antibody (Yifei Xue, Nanjing, China, 1:3000) was added dropwise, DAB reagent (Yifei Xue, Nanjing, China) was used for development, and hematoxylin counterstained and mounted. Observed the positive expression of the tissue under the light microscope (Thermo Fisher Scientific, Waltham, MA, USA). And use Image-pro Plus 6.0 pathological image analysis software to analyze the average optical density of positive targets for semi-quantitative analysis.

Cell culture

HK-2 cells (Cell Culture Center, Shanghai, China) were cultured in Gibco Dulbecco's

Modified Eagle Medium: F-12 (DMEM/F-12; Life Technology, Wuhan, China) complete medium containing 10 % fetal bovine serum (FBS; Life Technology, Wuhan, China) in a 37 °C, 5 % CO_2 incubator, and passaged every 48 h.

Cell transfection and treatment

SiRNA-TRIM8 was transfected with Lipofectamine^{2000} as the transfection reagent (Camilo Biological, Nanjing, China) at a concentration of 50 nmol/L. The control group was treated with Lipofectamine^{2000}, the siRNA-TRIM8 group was treated with Lipofectamine^{2000}+siRNA-TRIM8, and the siRNA-NC group was treated with Lipofectamine^{2000}+siRNA-NC. The expression of TRIM8 mRNA was detected 24 h after transfection. 24 h after transfection, HK-2 cells were stimulated with 100 mg/mL meglumine septalate for 24 h, and cells in the control group were cultured with normal medium for another 24 h. After transfection of HK-2 cells for 24 h, MyD88 inhibitor TJ-M2010-5 (World RM, Dongguan, China) was used to intervene HK-2 cells at a concentration of 20 uM for 2 h, followed by contrast agent for 24 h.

Cell counting kit-8 (CCK-8) assay

The cells were seeded in 96-well plates at 5 × 10^3/100 µL per well, and after 24 h of culture, they were replaced with serum-free medium for synchronization for 24 h. Each well was replaced with serum-free medium or 100 µL of pre-provisioned meglumine diatrizoate. After the above treatment was completed, each well was replaced with a 100 µL incubator without FBS medium containing 10 % CCK-8 reagent (Kaiji, Nanjing, China) for 2 h, and the microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) measured the absorbance value at a wavelength of 450 nm. Sample concentration was calculated based on the standard curve function.

Enzyme-linked immunosorbent assay (ELISA)

The plasma and cell supernatants of mice in each group were collected, and centrifuged at 4 °C for 15 min, and the supernatant was taken. The expression levels of inflammatory factors TNF-α, TGF-β_1, IL-1β, and IL-4 were detected according to the instructions of Elisa kit (Thermo Fisher Scientific, Waltham, MA, USA).

Annexin V-PI double staining method

After taking the cell supernatant of each group in a 15 ml centrifuge tube, the cells were digested in each well, dispersed using a pipette, transferred to centrifuge tubes containing the supernatant of the original cells, and centrifuged at 1000 rpm for 5 min. Then the supernatant was discarded, the cells were resuspended in cold PBS and washed twice, and then centrifuged at 1000 rpm for 5 min at 4 °C. The supernatant was discarded and resuspended in 200 µL of binding buffer, and 10 µL Annexin V was added and incubated at room temperature in the dark for 20 min. 5 uL PI was then added and incubated at room temperature in the dark for 10 min, so as to determine the rate of apoptosis by flow cytometry.

Caspase-3 activity detection

Each group of cells was collected, and 200 µL of ice-cold Lysis Buffer (Elabscience, Wuhan, China) was added to the precipitated cells for extraction of total protein. Then, the BCA method (Jian Cheng, Nanjing, China) was used to determine the concentration of each group. 100 µL of cell lysis supernatant containing 500 µg protein was pipetted into a 96-well plate. 100 µL of 2 × Reaction Buffer and 10 µL Caspase-3 substrate (Elabscience, Wuhan, China) were added to each well and incubated at 37 °C in the dark for 4 h. The microplate reader measures the absorbance value at a wavelength of 405 nm.

Tunel staining

Cells in each group were fixed with 4 % paraformaldehyde and operated according to the instructions on the TUNEL kit (Elabscience, Wuhan, China). The positive cells were examined under a fluorescent microscope (Thermo Fisher Scientific, Waltham, MA, USA). The ratio of the number of positive nuclei to the total number of nuclei was calculated as the apoptosis index.

Western blotting

Forty microgram protein samples were taken from each group, and separated using sodium dodecyl sulfate-polyacrylamide gel. Then, the dispersed protein was then transferred to a polyvinylidene difluoride (PVDF, Thermo Fisher Scientific, Waltham, MA, USA) membrane for 1 h at room temperature and incubated with a primary antibody (TLR4, Abcam, Cambridge, MA, USA, Rabbit, 1:2000; NF-kB, Abcam, Cambridge, MA, USA, Mouse, 1:2000; GAPDH, Abcam, Cambridge, MA, USA, Mouse, 1:5000) at 4 °C overnight. PBST was used to wash the membrane, and then it was incubated with a secondary antibody (Yifei Xue Biotechnology, Nanjing, China, 1:2000) at room temperature for

1 h. After washing the membrane again, immuno-reactive bands were visualized by enhanced chemiluminescence (ECL) detection kit (Amersham Biosciences, Foster City, CA, USA). The gray value was analyzed using ImageJ software (Version 1.38; National Institutes of Health, Bethesda, MA, USA).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was reverse-transcribed into cDNA using the PrimeScript RT reagent kit (TaKaRa Biotechnology Co., Ltd., Dalian, China), and according to the manufacturer's protocol. qPCR amplification (primer sequences are shown in Table 1), GADPH was used as an internal reference, using $2^{-\Delta\Delta Ct}$ method to calculate the mRNA expression of the target gene.

Statistical analysis

The test data are expressed in mean ± standard deviation (SD), and the SPSS statistical analysis software (version 26.0) (IBM, Armonk, NY, USA) is used for data analysis. The difference between groups when compared with Student's *t*-test. $P < 0.05$ indicates that the difference is significant.

RESULTS

Contrast agent caused changes in kidney structure and function, and increased the expression of TRIM8

First, this study examined the expression of IL-1β and Caspase3 in kidney tissue, and found that the control group had an intact kidney structure, normal glomerular size, no interstitial edema, or low expression of IL-1β and Caspase3 (Figure 1 A and B). The difference was that the glomerular volume of the CIN group increased significantly, the renal tubular epithelial cells showed edema, and the IL-1β and Caspase3 expression increased significantly. From this, this study speculated that the CIN group kidney cells undergo apoptosis and inflammation. Next, the results of BUN, SCr, and 24 h UP were detected, and BUN, Scr, and 24 h UP in the CIN group were obviously increased, indicated that the renal function of the CIN group was also obviously reduced (Figure 1 C, D, and E). At the same time, this study checked the expression of TRIM8 in both groups. qRT-PCR confirmed that TRIM8 was obviously increased in the CIN group, indicating that TRIM8 may be involved in the development of CIN (Figure 1 F).

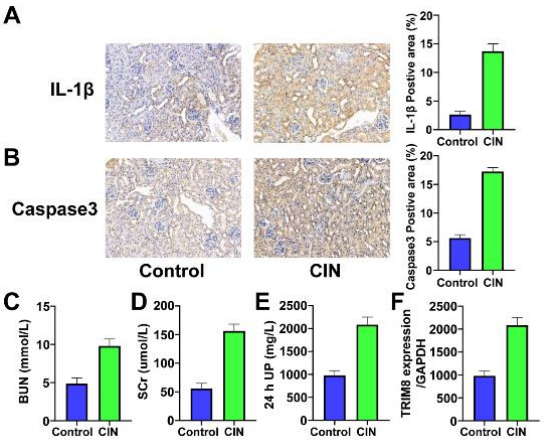

Figure 1: Contrast agent causes changes in kidney structure and function and increased expression of TRIM8. (A and B) Immunohistochemical staining showing expressions of IL-1β and Caspase3 in renal tissue, and semi-quantitative analysis. (C and D) Detection of serum BUN, SCr content. (E) Detection of urine 24 h UP content. (F) The expression levels of TRIM8 in renal tissue. (*indicates that compared with the control group $p < 0.05$)

Table 1: Real-time PCR primers used

Gene	Forward (5'>3')	Reverse (5'>3')
TRIM8	CCAAGCAGGCAATGGAC	CGTGGAGGAATGGAGAAGA
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
TGF-β1	TGGGGACTTCTTGGCACT	ATAGGGGCGTCTGAGGAAC
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL-4	TTTTGAACGAGGTCACAGG	GGCACATCCATCTCCGT
Bax	CAGTTGAAGTTGCCATCAGC	CAGTTGAAGTTACCATCAGC
Bcl-2	GACTGAGTACCTGAACCGGCATC	CTGAGCAGCGTCTTCAGAGACA
Caspase3	TGGAACAAATGGACCTGTTGACC	AGGACTCAAATTCTGTTGCCACC
Caspase9	AGCGATTCTGCCTTTCAC	TGGAGATTTTGTGGTCAGC
MyD88	TCATGTTCTCCATACCCTTGGT	AAACTGCGAGTGGGGTCAG
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

Knockdown TRIM8 improved contrast medium-induced inflammation of HK-2 cells

Next, this study knocked down the expression of TRMI8 in HK-2 cells by small interference technology, and verified the interference efficiency by qRT-PCR technology (Figure 2 A). Then, the expressions of inflammatory cytokines TNF-α, TGF-β_1, IL-1β and IL-4 were detected by qRT-PCR (Figure 2 B to E). The results showed that the expressions of TNF-α, TGF-β_1, IL-1β and IL-4 in the meglumine diatrizoate group was increased compared with the control group, while the expressions of TNF-α, TGF-β_1, IL-1β and IL-4 in the meglumine diatrizoate+si-TRIM8 group was effectively inhibited. At the same time, the Elisa method detected similar inflammatory factors and obtained similar results (Figures 2 F to I). Knockdown TRIM8 effectively inhibited the contrast agent-induced inflammation of HK-2 cells.

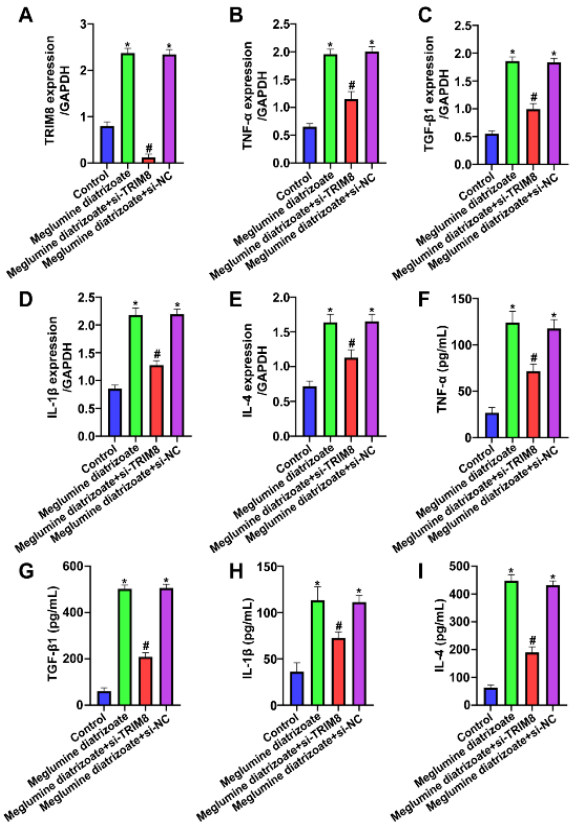

Figure 2: Knockdown TRIM8 improved contrast medium-induced inflammation of HK-2 cells. (A) expression levels of TRIM8 in HK-2 cells. (B-E) expression levels of TNF-α, TGF-β_1, IL-1β, IL-4 in HK-2 cells. (F-I) The content of TNF-α, TGF-β_1, IL-1β, IL-4 in HK-2 cells supernatant. ("*" indicates that compared with the control group, "#" indicates that compared with the Meglumine diatrizoate group $p < 0.05$)

Knockdown TRIM8 improved contrast medium-induced apoptosis of HK-2 cells

The results showed that after 24 h of meglumine diatrizoate treatment of HK-2 cells, the cell activity was obviously reduced, but after knocking down TRMI8 expression, the cell activity increased obviously, but it was still lower than that of the control group (Figure 3 A). In addition, TUNEL staining results showed that compared with the control group, the apoptosis index of the meglumine diatrizoate group was obviously increased, but after knocking down TRMI8 expression, the apoptosis index was obviously reduced (Figure 3 B). At the same time, the Annexin V-FITC/PI double staining method also achieved similar results (Figure 3 C). In addition, we detected Caspase3 activity and found that the meglumine diatrizoate can obviously promote the increased of Caspase3 activity. Conversely, when we knocked down TRMI8 expression, Caspase3 activity was effectively inhibited (Figure 3 D). Next, the qRT-PCR technology detected the expression of apoptotic factors Bax, Caspase3, Caspase9 and ant-apoptotic factors Bcl-2 (Figure 3 E to H). We found that the expression of Bax, Caspase3 and Caspase9 in the meglumine diatrizoate group was obviously increased, and the expression of Bcl-2 was obviously reduced, which was consistent with previous studies. After knocking down TRMI8 expression, Bcl-2 expression increased obviously, and Bax, Caspase3 and Caspase9 expression were effectively suppressed. The above results suggest that knocking down TRIM8 effectively inhibited the contrast-induced apoptosis of HK-2 cells.

TLR4/MyD88/NF-KB pathway was involved in the regulation of HK-2 cell damage by contrast agents

The results showed that the treatment of HK-2 cells with meglumine diatrizoate increased the expression of TLR4 and NF-kB protein. In the meglumine diatrizoate+si-TRIM8 group, TLR4 and NF-kB protein expressions reduced. Western blot results suggested that TLR4/MyD88/NF-kB was indeed involved in the process of contrast agent damage to HK-2 cells, and the knockdown of the TRMI8 expression effectively inhibited the activation of this pathway (Figure 4 A). qRT-PCR results showed that MyD88 mRNA also increased in the meglumine diatrizoate group and decreased in the meglumine diatrizoate+si-TRIM8 group (Figure 4 B). Next, we specifically bind to the TIR domain of MyD88 through the small molecule compound TJ-M2010-5, which interferes with the function of the TIR domain of MyD88. The ELISA results

proved that the expression of inflammatory factors TNF-α, TGF-β_1, IL-1β and IL-4 in the meglumine diatrizoate+si-TRIM8+TJ-M2010-5 group were lower than those in the meglumine diatrizoate+si-TRIM8+DMSO group (Figure 4 C to F).

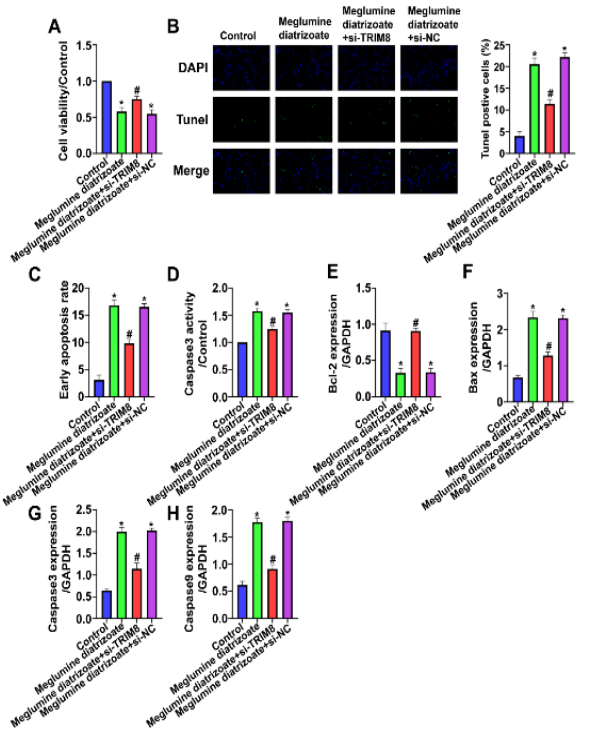

Figure 3: Knockdown TRIM8 improved contrast medium-induced apoptosis of HK-2 cells. (A) Detection of HK-2 cell activity. (B) Tunel staining was used to detect the apoptosis of HK-2 cells, and positive rate analysis. (C) Annexin V-FITC/PI Double Staining method was used to detect the early apoptosis rate of HK-2 cells. (D) Detection of Caspase-3 activity. (E-H) The expression levels of Bcl-2, Bax, Caspase3, Caspase9 in HK-2 cells. (*indicates that compared with the control group, #indicates that compared with the Meglumine diatrizoate group $p < 0.05$)

DISCUSSION

Contrast nephropathy refers to the acute impairment of renal function caused by the clinical application of X-ray contrast agents, in addition to other factors that cause changes in renal function. Many different contrast animal models of nephropathy have been reported in the literature. In order to have an ideal decrease in renal function, most methods have done basic renal injury before injecting contrast media. Based on previous research reports, the glycerol induction model was chosen [14], in addition to the mechanism where glycerol as a hypertonic substance causes kidney damage by damaging the renal tubules.

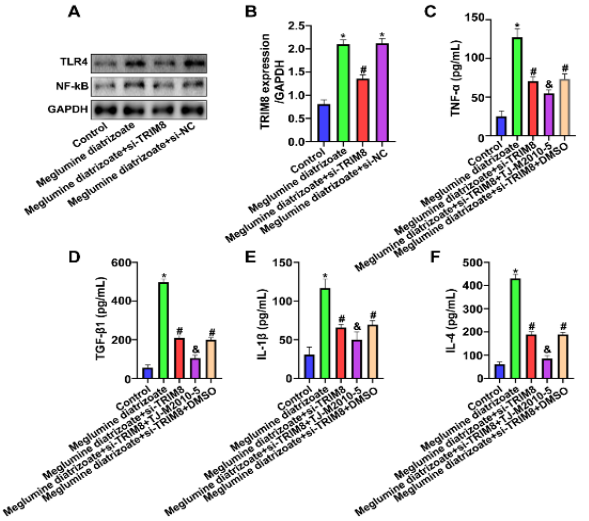

Figure 4: TLR4/MyD88/NF-kB pathway is involved in the regulation of HK-2 cell damage by contrast agents. (A): Western blot was used to detect the expression of TLR4, NF-kB in HK-2 cells; (B): The expression levels of MyD88 in HK-2 cells. (*indicates that compared with the control group, #indicates that compared with the Meglumine diatrizoate group $p < 0.05$); (C-F): The content of TNF-α, TGF-β_1, IL-1β, IL-4 in HK-2 cells supernatant. (*indicates that compared with the control group, #indicates that compared with the Meglumine diatrizoate group, #indicates that compared with the Meglumine diatrizoate+si-TRIM8+DMSO group $p < 0.05$)

On the one hand, due to its release of renal toxic substances, such as myoglobin and hemoglobin, the resultant effect was the contraction of renal blood vessels and glomerular blood vessels, further resulting in decreases in renal blood flow and glomerular filtration rate. On the other hand, a large amount of myoglobin and hemoglobin cannot be reabsorbed by the renal tubule after filtration, resulting in blockage of the renal tubule. One day after modeling, The BUN, SCr and 24 h UP levels in urine were detected. The levels of BUN, SCr and 24 h UP in urine were significantly increased in CIN group. SCr, BUN and UP are indicators that reflect kidney function damage. These metabolites are involved in the progress of kidney disease, which can indicate whether the CIN model constructed was successful.

Observation under the light microscope revealed vacuolar degeneration of renal tubular epithelial cells and a pathological enlargement of the glomeruli in the CIN group. At the same time, the expressions of IL-1β and Caspase3 in the tissues were obviously increased, and the results of qRT-PCR found TRIM8 expression in the CIN group was obviously increased. This suggested that TRIM8 may be involved in the contrast agent-induced apoptosis and inflammation of renal cells. To further explore the role of TRIM8 in CIN, HK-2 cells were treated with meglumine

diatrizoate. Previous studies have found that overexpression of TRIM8 positively regulated TNF-α and IL-1β signaling pathways, thereby activating the NF-kB pathway [15]. The NF-kB pathway has been shown to regulate natural rabbit disease, adaptive immunity, the cell migration [16], and even apoptosis [17,18]. Therefore, we used qRT-PCR and ELISA technology to detect the expression of inflammatory factors TNF-α, TGF-β_1, IL-1β and IL-4. Our results not only confirmed that knockdown TRIM8 expression can inhibit the increased expression of TNF-α and IL-1β, but also found that knockdown TRIM8 expression inhibited the increase of TGF-β_1 and IL-4 expression. In addition, the study found that TRIM8 activated oxidative stress response by inhibiting the Nrf2 pathway, thereby increasing myocardial cell apoptosis induced by high glucose [19]. Apoptosis is a complex pathophysiological process. In recent years, continuous research has found that B cell lymphoma-2 family members Bax, Bad, Bcl-2 and cysteine protease family members Caspase3, Caspase6, Caspase8. Caspase9 are involved in the regulation of apoptotic signals [20]. Among them, Caspase3 is a key protease in the cysteine protease family activated by various apoptosis stimulating factors, and it is the only way for the apoptosis protease cascade reaction. It also plays an extremely important role in the process of early initiation and execution of apoptosis. Currently, there are relatively few studies on the effects of TRIM8 on renal cell apoptosis caused by contrast agents. Therefore, after the expression of TRIM8 was knocked down, we confirmed that the cysteine protease induced apoptosis of HK-2 cells by CCK8 assay, TUNEL staining, Annexin V-FITC/PI double staining and Caspase-3 activity detection, and the interference with TRIM8 expression obviously inhibited apoptosis.

During the occurrence of CIN, many endogenous or exogenous components undergo structural changes due to the release of cellular enzymes, and thus react with TLRs to initiate the TLR4/MyD88/NF-kB pathway. With the activation of NF-kB, it is translocated into the nucleus, causing further expressions of inflammatory factors [21]. We found that the meglumine diatrizoate activate the TLR4/MyD88/NF-kB pathway, thereby activating downstream factors, and after knock down TRIM8, the TLR4/MyD88/NF-kB pathway was inhibited. At the same time, previous studies have found that MyD88 is the core molecule of this pathway, so we interfere with the function of MyD88's TIR domain through the specific binding of small molecule compound TJ-M2010-5, prevented MyD88 from forming homodimers and made MyD88 unable to be activated, thus blocking the transduction of MyD88/NF-kB [22]. The results showed that when TJ-M2010-5 was used to bind the TIR domain of MyD88, the expressions of inflammatory factors TNF-α, TGF-β_1, IL-1β and IL-4 were obviously reduced. These results confirmed that TRIM8 regulated contrast-induced renal injury through the TLR4/MyD88/NF-kB pathway.

CONCLUSION

TRIM8 mediates contrast-induced renal injury through thevia TLR4/MyD88/NF-kB pathway. The findings of this study may provide new insights for the development of treatment strategies for CIN.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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