Mechanism of miRNA-141-3p in Calcium Oxalate-Induced Renal Tubular Epithelial Cell Injury via NLRP3-Mediated Pyroptosis

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Keywords

Renal calculi · Renal tubular epithelial cells · Calcium oxalate · Pyroptosis · miR-141-3p

Abstract

Background/Aims: Renal calculi represent a prevalent disorder associated with mineral deposition in renal calyces and the pelvis. Aberrant microRNA (miRNA) expression is implicated in renal injury. This study investigated the mechanism of miR-141-3p in calcium oxalate (CaOx) crystal-induced renal tubular epithelial cell (RTEC) injury. **Methods:** Human RTECs HK-2 cells were treated with CaOx crystals to induce RTEC injury. Cell viability was evaluated using Cell Counting Kit-8 assay, and apoptosis was measured using flow cytometry. The contents of lactate dehydrogenase (LDH), malondialdehyde (MDA), superoxide dismutase (SOD), interleukin (IL)-1 β , and IL-18 were measured using enzyme-linked immunosorbent assay kits. The expressions of NLRP3, cleaved caspase-1, and GSDMD-N were detected using Western blot. miR-141-3p and NLRP3 expressions were determined using reverse transcription quantitative polymerase chain reaction. The binding of miR-141-3p and NLRP3 was validated using a dual-luciferase assay. The role of NLRP3 in the protection of miR-141-3p on RTEC injury was verified using func-

tional rescue experiments. **Results:** CaOx crystals induced RTEC injury, manifested as attenuated cell viability, enhanced apoptosis, elevated intracellular LDH and MDA levels, and decreased SOD level. Pyroptosis of RTECs was enhanced by CaOx crystal induction, evidenced by elevated expressions of cleaved caspase-1, GSDMD-N, IL-1 β , and IL-18. miR-141-3p expression was reduced in CaOx crystal-induced RTECs. miR-141-3p overexpression alleviated CaOx crystal-induced RTEC injury and suppressed pyroptosis of RTECs. miR-141-3p bound to NLRP3 and thereby repressed NLRP3 expression. NLRP3 overexpression reversed the protective effect of miR-141-3p overexpression on RTECs. **Conclusion:** miR-141-3p repressed NLRP3-mediated pyroptosis by suppressing NLRP3 expression, thus protecting CaOx crystal-induced RTEC injury.

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Introduction

Renal calculi are mineral depositions in renal calyces and pelvis that are free or attached to renal papillae [1]. Although many renal calculi are asymptomatic for a long period, the problems of pain, infection, and obstruction are increasing, and over 50% of patients have recurrent

renal calculi after 5 years [2]. Despite the considerable advances in the surgical treatment of removing renal calculi, the progress in preventing renal calculi recurrence is limited [3]. The high recurrence rate and a heavy surgical burden have increased the risks of renal function reduction and chronic renal injury in these patients [4]. Thus, exploring potent therapeutic targets to mitigate renal injury in patients with renal calculi remains an urgent issue.

Approximately 80% of renal calculi are initiated by the deposition of calcium oxalate (CaOx) crystals [5]. CaOx stone matrix contains numerous protein molecules, which can facilitate the aggregation, nucleation, and growth of CaOx crystals within tubular cells and finally result in the formation of renal calculi [6]. The critical mechanism of renal calculi formation is the adhesion of CaOx crystals to renal tubular epithelial cells (RTECs) [7]. The interaction between CaOx crystals and RTECs stimulates the generation of reactive oxygen species, secretes massive inflammatory cytokines, and activates an inflammatory cascade in RTECs, leading to intrarenal inflammation and RTEC injury [8]. It has been demonstrated that CaOx crystals-triggered renal inflammation is mediated by the Nod-like receptor protein 3 (NLRP3) inflammasome [9]. NLRP3 inflammasome is a well-studied cytoplasmic protein complex that can activate caspase-1, which causes the maturation of interleukin (IL)-1 β and IL-18 and then induces a proinflammatory form of cell death, known as pyroptosis [10]. NLRP3 inflammasome-mediated pyroptosis in RTECs contributes to the progression of diverse kidney diseases [11]. Nevertheless, the exact effects and molecular mechanism of NLRP3-mediated pyroptosis in RTECs in renal calculi have not been elucidated before.

microRNAs (miRNAs) are a class of small endogenous noncoding RNAs (18–25 nt in length) that modulate gene expression at the posttranscriptional level [12]. Microarray analysis has identified several miRNAs with differential expression profiles in CaOx crystal-induced RTECs [13]. miR-141-3p has been reported to support the epithelioid morphology of RTECs and repress renal interstitial fibrosis [14]. However, the role of miR-141-3p in CaOx crystal-induced RTECs remains unknown. Intriguingly, miR-141-3p overexpression can also suppress the expressions of pyroptosis-associated proteins in high glucose-induced cardiomyocytes [15]. Hence, this study sought to determine whether miR-141-3p can play a role in CaOx crystal-induced RTECs by mediating NLRP3-mediated pyroptosis, hoping to confer novel insights for the treatment of renal calculi with miRNA as a molecular target.

Materials and Methods

Cell Culture and Treatment

Human RTECs HK-2 were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium/F12 containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (Gibco, 100 IU/mL penicillin and 100 μ g/mL streptomycin) at 37°C with 5% CO₂.

Cell injury was induced by CaOx crystals as described in the previous literature [16, 17]: the cells were cultured in the complete medium containing 0, 0.1, 1, 2, and 4 mM CaOx crystals. After 24 h, the cells and supernatants were collected for further analysis.

Cell Transfection

The expression of miR-141-3p or NLRP3 in cells was upregulated by miR-141-3p mimic (miR-mimic) or oe-NLRP3. miR-mimic, oe-NLRP3, and their corresponding negative controls (miR-NC and oe-NC) were provided by GenePharma (Shanghai, China) and transiently transfected into HK-2 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in line with the manufacturer's instructions. The cells were collected 48 h after transfection for further analyses.

Cell Counting Kit-8 Assay

Cell viability was evaluated using the Cell Counting Kit-8 assay kit (KeyGEN, Nanjing, China). Briefly, HK-2 cells were seeded into 6-well plates, and each well was added with 10 μ L Cell Counting Kit-8 solution, followed by 2-h incubation at 37°C. The optical density at 450 nm was measured. The experiment was repeated 3 times independently.

Flow Cytometry

The apoptosis level was evaluated using flow cytometry. The CaOx-treated and transfected HK-2 cells were rinsed with phosphate-buffered saline, suspended in annexin V-FITC binding buffer (Beyotime, Shanghai, China), and incubated with annexin V-FITC (Beyotime) and propidium iodide (Beyotime). Finally, the apoptosis was analyzed using a flow cytometer (Beckman Coulter, Inc., Chaska, MN, USA).

Reverse-Transcription Quantitative Polymerase Chain Reaction

The total RNA was extracted from HK-2 cells using TRIzol reagent (Invitrogen) and reverse transcribed into cDNA using the reverse transcription kit (R&D Systems, Minneapolis, MN, USA). miR-141-3p was detected using the Taqman Advanced microRNA Assay (Thermo Fisher Scientific Inc., Waltham, MA, USA). mRNA was detected using the Universal SYBR Green PCR kit (Takara, Kyoto, Japan). PCR was performed using the 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA), with U6 as the internal reference of miR-141-3p and GAPDH as the internal reference of NLRP3. The relative expression of genes was calculated using the 2^{- $\Delta\Delta$ Ct} method. The experiment was repeated 3 times independently. Table 1 shows the primer sequences.

Western Blot

The total protein was isolated from cells using the PRO-PREPTM protein extract (iNtRON Biotechnology, Seongnam, South Korea), and the protein concentration was determined us-

Table 1. RT-qPCR primers

	Forward primer (5'–3')	Reverse primer (5'–3')
miR-141-3p	CCTCGTCTTGAGCTGAGAGC	AGGGCTCCCTGAAGGTTACT
NLRP3	GCAAGCACCCGCTGCAAGC	CTACCAAGAAGGCTCAAAGA
GAPDH	GATGCTGGCGCTGAGTACG	GCTAAGCAGTTGGTGGTGC
U6	CTCGCTTCGGCAGCACATA	AACGATTACGAATTTGCGT

RT-qPCR, reverse transcription quantitative polymerase chain reaction; miR-141-3p, miRNA-141-3p; NLRP3, Nod-like receptor protein 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

ing the bicinchoninic acid kit (R&D SYSTEMS). The protein samples were separated by SDS-PAGE, then transferred onto the polyvinylidene difluoride membranes, and blocked with 5% skim milk (BD Biosciences, Sparks, MD, USA) for 2 h. Afterward, the diluted primary antirabbit monoclonal antibody cleaved caspase-1 (4199, 1:1000, CST, Boston, USA), rabbit monoclonal antibody GSDMD-N (ab215203, 1:1000, Abcam, Cambridge, UK), and rabbit polyclonal antibody NLRP3 (ab214185, 1: 1000, Abcam) were added for incubation overnight. Next, the secondary antibody goat anti-rabbit IgG H&L (HRP) (ab6721, 1:2000, Abcam) was supplemented for 1-h incubation. Finally, the membranes were developed using enhanced chemiluminescence solution (EMD Millipore, Billerica, MA, USA). The intensity of each band in Western blot images was brightened using Image Pro Plus 6.0 (Media Cybernetics, San Diego, CA, USA). β -Actin was used as the internal reference. The experiment was repeated 3 times independently.

Enzyme-Linked Immunosorbent Assay

The levels of lactate dehydrogenase (LDH), malondialdehyde (MDA), superoxide dismutase (SOD), IL-1 β , and IL-18 were measured using enzyme-linked immunosorbent assay kits (Protein-tech, Chicago, IL, USA).

Bioinformatics

The binding site of miR-141-3p and NLRP3 was predicted through the TargetScan database (http://www.targetscan.org/vert_72/) [18].

Dual-Luciferase Assay

The synthesized NLRP3 gene fragment containing miR-141-3p binding site (NLRP3-WT) and NLRP3-MUT mutated according to the binding site was constructed into pmirGLO-reporter plasmids (Huayueyang Biotechnology, Beijing, China). The constructed plasmids were cotransfected with mimic-NC or miR-141-3p-mimic into HK-2 cells. After 48 h, cells were collected and lysed. Luciferase activity was measured using the Firefly Luciferase Assay kit (K801-200; Biovision, Mountain View, CA, USA). All plasmids were supplied by GenePharma (Shanghai, China). The experiment was repeated 3 times independently.

Statistical Analysis

Data analysis and map plotting were introduced using the SPSS 21.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). The measurement data are expressed as mean $\pm$ standard deviation. Compari-

sons among multiple groups were performed using one-way or two-way analysis of variance (ANOVA), following Tukey's multiple comparison test. A value of $p < 0.05$ meant a significant difference.

Results

CaOx Crystals -Induced RTEC Injury and miR-141-3p Were Poorly Expressed in CaOx-Induced RTEC Injury

In this study, we established an RTEC injury model by treating HK-2 cells with different concentrations of CaOx crystals (0.1, 1, 2, and 4 mM) and found that CaOx crystals elevated the apoptosis level of HK-2 cells ($p < 0.05$; Fig. 1a) and reduced the cell viability ($p < 0.05$; Fig. 1b). The alterations of LDH, MDA, and SOD in HK-2 cells were also measured, and our results demonstrated that CaOx crystals notably elevated the contents of LDH and MDA, but reduced the content of SOD ($p < 0.05$; Fig. 1c–e). To determine whether miR-141-3p was implicated in the regulation of renal tubular epithelial injury, we detected miR-141-3p expression in cells and found that miR-141-3p expression was reduced significantly after CaOx crystal treatment ($p < 0.05$, Fig. 1f). Briefly, CaOx crystals induced the injury of RTECs in a concentration-dependent manner and downregulated miR-141-3p expression in cells. According to our results, CaOx crystals at a concentration of 2 mM were selected for the subsequent experiments.

miR-141-3p Overexpression Alleviated CaOx Crystal-Induced RTEC Injury

To explore the regulatory effect of miR-141-3p on RTEC injury induced by CaOx crystals (2 mM), we overexpressed miR-141-3p in HK-2 cells ($p < 0.05$, Fig. 2a). Overexpression of miR-141-3p significantly reduced the apoptosis level ($p < 0.05$, Fig. 2b) and enhanced cell viability ($p < 0.05$, Fig. 2c). After overexpression of miR-141-

Fig. 1. CaOx-induced RTEC injury and miR-141-3p were poorly expressed in CaOx-induced RTEC injury. Different concentrations of CaOx crystals (0.1, 1, 2, and 4 mM) were used to induce RTEC injury. **a** Apoptosis level was measured using flow cytometry. **b** Cell viability was evaluated using the CCK-8 assay. **c–e** The contents of LDH, MDA, and SOD were detected using ELISA. **f** miR-141-3p expression was determined using RT-qPCR. The cell

experiment was repeated 3 times independently. Data are presented as mean $\pm$ standard deviation and analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test, $*p < 0.05$, $**p < 0.01$. RT-qPCR, reverse transcription quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; CCK-8, Cell Counting Kit-8.

3p, the contents of LDH and MDA in cells were decreased ($p < 0.05$, Fig. 2d, e) and the content of SOD was increased ($p < 0.05$, Fig. 2f). In short, overexpression of miR-141-3p reduced RTEC injury induced by CaOx crystals and improved cell viability.

miR-141-3p Overexpression Suppressed CaOx Crystal-Induced Pyroptosis of RTECs

CaOx crystals can trigger inflammasomes to activate pyroptosis, resulting in epithelial cell injury [19]. Therefore, we speculated whether miR-141-3p alleviated CaOx crystal-induced RTEC injury by inhibiting the pyroptosis of RTECs. To test this conjecture, we first determined the NLRP3 protein level in HK-2 cells and found that the NLRP3 protein level was decreased notably after overexpression of miR-141-3p (Fig. 3a). The protein levels of cleaved caspase-1 and GSDMD-N were decreased with the upregulation of miR-141-3p ($p < 0.05$, Fig. 3a). After overexpression of miR-141-3p, the levels of IL-1 β and IL-

18 were also reduced notably ($p < 0.05$, Fig. 3b). All these indicated that miR-141-3p overexpression suppressed CaOx crystal-induced pyroptosis of RTECs.

miR-141-3p Repressed NLRP3 Expression

Inhibiting the activation of the NLRP3 inflammasome pathway reduces the release of inflammatory mediators, thereby improving CaOx crystal-induced renal tissue injury [20]. Through the TargetScan database (http://www.targetscan.org/vert_72/) prediction, it was found that there was a binding site between miR-141-3p and NLRP3 (Fig. 4a). The dual-luciferase assay validated the binding of miR-141-3p and NLRP3 ($p < 0.05$, Fig. 4b). Then, NLRP3 expression in RTECs was detected, and the results exhibited that CaOx crystals significantly elevated NLRP3 expression, while NLRP3 expression was notably declined after overexpression of miR-141-3p ($p < 0.05$, Fig. 4c). Briefly, miR-141-3p repressed NLRP3 expression by binding to NLRP3 in CaOx crystal-induced RTECs.

Fig. 2. miR-141-3p overexpression alleviated CaOx crystal-induced RTEC injury. RTEC injury was induced by CaOx crystals with a concentration of 2 mM. miR-mimic was transfected into the CaOx crystal-induced cells to upregulated miR-141-3p expression, with mimic-NC as the control. **a** miR-141-3p transfection efficiency was verified using RT-qPCR. **b** Apoptosis level was measured using flow cytometry. **c** Cell viability was evaluated using the CCK-

8 assay. **d-f** The contents of LDH, MDA, and SOD were detected using ELISA. The cell experiment was repeated 3 times independently. Data are presented as mean $\pm$ standard deviation and analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test, ** $p < 0.01$. RT-qPCR, reverse transcription quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; CCK-8, Cell Counting Kit-8.

NLRP3 Overexpression Reversed the Protective Effect of miR-141-3p Overexpression on RTECs

To further verify that NLRP3 was involved in the protective effect of miR-141-3p on RTECs, we overexpressed NLRP3 in cells ($p < 0.05$, Fig. 5a) and then conducted a combined experiment with miR-141-3p overexpression. Overexpression of NLRP3 significantly enhanced the apoptosis level and reduced cell activity ($p < 0.05$, Fig. 5b, c), elevated the contents of LDH and MDA, and decreased

the content of SOD ($p < 0.05$, Fig. 5d). In addition, after the combined treatment of NLRP3 overexpression and miR-141-3p overexpression, the protein levels of cleaved caspase-1 and GSDMD-N in cells were increased notably ($p < 0.05$, Fig. 5e), and the inflammation-related factors IL-1 β and IL-18 were also elevated ($p < 0.05$, Fig. 5f). Briefly, NLRP3 overexpression reversed the protective effect of miR-141-3p overexpression on RTECs.

Fig. 3. miR-141-3p overexpression suppressed CaOx crystal-induced pyroptosis of RTECs. RTEC injury was induced by CaOx crystals with a concentration of 2 mM. miR-mimic was transfected into the CaOx crystal-induced cells to upregulated miR-141-3p expression, with mimic-NC as the control. **a** The protein levels of NLRP3, cleaved caspase-1, and GSDMD-N were detected using

Western blot. **b** The levels of IL-1 β and IL-18 were detected using ELISA. The cell experiment was repeated 3 times independently. Data are presented as mean $\pm$ standard deviation and analyzed using two-way ANOVA, followed by Tukey's multiple comparisons test, $**p < 0.01$. ELISA, enzyme-linked immunosorbent assay.

Fig. 4. miR-141-3p repressed NLRP3 expression. **a** The binding site between miR-141-3p and NLRP3 was predicted through the TargetScan database (http://www.targetscan.org/vert_72/). **b** The binding of miR-141-3p and NLRP3 was verified using a dual-luciferase assay. **c** NLRP3 expression was determined using RT-qPCR. The cell experiment was repeated 3 times independently. Data are presented as mean $\pm$ standard deviation. Data in panel B were analyzed using two-way ANOVA, and data in panel (c) were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test, $**p < 0.01$. RT-qPCR, reverse transcription quantitative polymerase chain reaction.

Discussion

Renal calculi are prevalent in all population groups with a rising incidence rate and often become lifelong trouble [2]. Abnormal aggregation of CaOx crystals in the kidney is one of the major causes of renal calculi [16, 17]. Recently, an analysis of miRNA expression profiles in CaOx-induced renal calculi suggests the implication of miRNAs in the development of renal calculi [12]. It has been reported that miR-141-3p expression is reduced in RTEC injury [14]. This study elucidated that miR-141-3p overexpression alleviated CaOx crystal-induced RTEC injury by targeting NLRP3-mediated pyroptosis.

Renal calculi are predominantly composed of CaOx and exist in the form of monohydrate or dihydrate [1]. In the current study, we established a cell model of RTEC injury by treating HK-2 cells with different concentrations of CaOx crystals. The exposure of RTECs to CaOx can lead to a signaling cascade that causes apoptosis via the p38 mitogen-activated protein kinase pathway [21]. We found that CaOx crystal treatment notably enhanced the apoptosis of RTECs and reduced cell viability. CaOx-induced oxidative stress can result in RTEC injury and renal calculi formation [22]. We determined the contents of oxidative stress-related indicators including LDH, MDA, and SOD in CaOx crystal-induced HK-2 cells and found that CaOx crystals elevated the contents of LDH and MDA but reduced the content of SOD. Briefly, these results indicated that CaOx crystals induced RTEC injury.

miRNAs are a class of posttranscriptional gene modulators, which are implicated in the alteration of gene expression in renal cells induced by CaOx crystals [13]. miR-141-3p expression is downregulated in the HK-2 cell model of renal fibrosis [14]. Nevertheless, the role of miR-141-3p in CaOx crystal-induced RTEC injury remained unknown. We were the first to demonstrate that miR-141-3p expression was reduced in CaOx crystal-induced RTECs. To investigate the exact function of miR-141-3p in CaOx crystal-induced RTECs, we overexpressed miR-141-3p in CaOx crystal-induced HK-2 cells. miR-141-3p overexpression notably reduced the apoptosis level, enhanced cell viability, decreased LDH and MDA contents, and increased SOD content. Briefly, miR-141-3p overexpression alleviated CaOx crystal-induced RTEC injury.

Pyroptosis is a form of proinflammatory cell death, leading to the release of cytokines to activate proinflammatory mediators [23]. Recent evidence has provided the mechanism underlying pyroptosis in CaOx crystal-induced renal calculi [24]. miR-141-3p can suppress high glucose-induced cardiomyocyte pyroptosis [15]. We speculated that miR-141-3p might alleviate CaOx crystal-induced RTEC injury by suppressing pyroptosis of RTECs. Pyroptosis is a functional result of NLRP3 inflammasome activation, which is mediated by the membrane pore-forming activity of GSDMD N-terminal domain secreted by caspase-1 cleavage, thereby releasing massive proinflammatory cytokines such as IL-1 β and IL-18 [23]. Hence, we detected the pyroptosis-related factors in CaOx crystal-induced HK-2 cells and found that CaOx crystals elevated the NLRP3 expression, enhanced the contents of IL-1 β and IL-18, and increased the levels of cleaved caspase-1 and GSDMD-N proteins in HK-2 cells,

while miR-141-3p overexpression notably reversed the above trends, suggesting that miR-141-3p overexpression suppressed CaOx crystal-induced pyroptosis of RTECs.

Thereafter, we focused on the downstream mechanism of miR-141-3p in CaOx crystal-induced RTEC injury. miR-141-3p was predicted to have a binding site with NLRP3, and the dual-luciferase assay validated the binding of miR-141-3p and NLRP3. CaOx crystals trigger renal inflammation via NLRP3-mediated IL-1 β secretion [25]. NLRP3-deficient mice suffering from chronic oxalate nephropathy have reduced progressive renal failure and mortality [26]. Our results exhibited that CaOx crystals elevated NLRP3 expression, while miR-141-3p overexpression reduced NLRP3 expression, suggesting that miR-141-3p targeted NLRP3 in CaOx crystal-induced RTECs. Then, we overexpressed NLRP3 in HK-2 cells to verify that NLRP3 was involved in the protective effect of miR-141-3p on CaOx crystal-induced RTECs. NLRP3 inhibition can suppress the secretion of inflammatory mediators, thereby mitigating CaOx crystal-induced renal injury and crystal deposits in HK-2 cells [20]. Consistently, our results demonstrated that NLRP3 overexpression notably enhanced the apoptosis level, reduced cell viability, and increased the pyroptosis of RTECs. Briefly, NLRP3 overexpression reversed the protective effect of miR-141-3p overexpression on RTECs.

Conclusion

To sum up, miR-141-3p represses NLRP3 expression by directly binding to NLRP3, thereby suppressing NLRP3-mediated pyroptosis of RTECs. This finding may provide a theoretical basis for the clinical treatment of hyperoxaluria or idiopathic kidney stones. However, we merely verified this mechanism in cell experiments and failed to validate it in animal experiments. To explore the clinical significance of this mechanism in the diagnosis of hyperoxaluria or idiopathic kidney stones, we should further verify this mechanism in animal models (such as rats/mice). There are great differences between animal models (rats/mice) and humans in physiological structure, so more preclinical and clinical studies are needed. Hence, we will further verify the role of the miR-141-3p/NLRP3 axis in hyperoxaluria or idiopathic kidney stones in animal models in future experiments and conduct clinical experiments according to the research results to explore the application value of this mechanism in hyperoxaluria or idiopathic kidney stones. Moreover, there still lacked in-depth study on the upstream mechanism of miR-141-3p and the downstream mechanism of NLRP3.

Fig. 5. NLRP3 overexpression reversed the protective effect of miR-141-3p overexpression on RTECs. RTEC injury was induced by CaOx crystals with a concentration of 2 mM. CaOx crystal-induced RTECs were transfected with oe-NLRP3 and then co-treated with miR-141-3p overexpression for a combined experiment. **a** NLRP3 expression was determined using RT-qPCR and Western blot. **b** Cell viability was evaluated using the CCK-8 assay. **c** Apoptosis level was measured using flow cytometry. **d** The contents of LDH, MDA, and SOD were detected using ELISA. **e** The protein levels of NLRP3, cleaved caspase-1, and GSDMD-N were detected using Western blot. **f** The levels of IL-1 β and IL-18 were detected using ELISA. The cell experiment was repeated 3 times independently. Data are presented as mean $\pm$ standard deviation. Data in panels (**a–d**) were analyzed using one-way ANOVA, and data in panels (**e, f**) were analyzed using two-way ANOVA, followed by Tukey's multiple comparisons test, $^{**}p < 0.01$. RT-qPCR, reverse transcription quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; CCK-8, Cell Counting Kit-8.

In the future, we will also further explore the upstream genes of miR-141-3p and the downstream genes of NLRP3 in CaOx crystal-induced RTEC injury.

Statement of Ethics

The study does not involve the use of animals or human subjects.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Xiu-Guo Gan has given substantial contributions to the conception and the design of the manuscript and Zhi-Hao Wang and Hai-Tao Xu contributed to acquisition, analysis, and interpretation of the data. All authors have participated to drafting the manuscript and Xiu-Guo Gan revised it critically. All authors read and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.