Knockdown of KCNQ1OT1 Alleviates the Activation of NLRP3 Inflammasome Through miR-17-5p/TXNIP Axis in Retinal Müller Cells

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Research Article

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Abstract

Diabetic retinopathy (DR) is one of the most severe and common complications caused by diabetic mellites. Inhibiting NLRP3 inflammasome activation displays a crucial therapeutic value in DR. Studies have shown that KCNQ1OT1 plays a critical role in regulating NLRP3 inflammasome activation and participates in the pathogenesis of diabetic complications. The present study aims to explore the role, and the potential mechanism of KCNQ1OT1 in regulating the activation of NLRP3 inflammasome in DR. The expression of KCNQ1OT1 and the activation of NLRP3 inflammasome were increased in experimental DR models. KCNQ1OT1 knockdown alleviated NLRP3 inflammasome-associated molecules expression. In addition, KCNQ1OT1 was found to be localized mainly in the cytoplasm of Müller cells and facilitated TXNIP expression by acting as a miR-17-5p sponge. KCNQ1OT1 promoted the activation of NLRP3 inflammasome through miR-17-5p/TXNIP axis. Moreover, the clinical samples of patients with DR showed that the expression of KCNQ1OT1 and the activation of NLRP3 inflammasome were all increased, further supporting the hypothesis that the KCNQ1OT1 dysregulation may be the molecular mechanism of the pathogenesis of DR. Therefore, KCNQ1OT1 may serve as a new therapeutic target for DR.

1. Introduction

Diabetic retinopathy (DR), one of the most severe and common complications caused by diabetic mellites, can eventually lead to irreversibly visual impairment and is the leading cause of blindness in working-age populations[1]. Our previous study has found that pro-Epigallocatechin-3-gallate (EGCG) inhibited hyperglycemia-induced proliferation and pro-angiogenic cytokine production in Müller cells, which was mediated by inhibiting thioredoxin-interacting protein (TXNIP)/NLRP3 inflammasome axis, suggesting that NLRP3 inflammasome activation plays a vital role in the pathogenesis of DR.

TXNIP is an early response gene induced by hyperglycemia and diabetes[2], which highly induces and mediates the expression of inflammatory cytokine interleukin-1 beta (IL-1β) and glial fibrillary acidic protein in the retina of diabetic mice [3]. In addition, TXNIP has been found involved in the activation of NLRP3 inflammasome and the promotion of caspase-1 expression[4]. The N-terminal pyridine segment of NLRP3 serves as a scaffold that nucleated ASC to recruit pro-caspase-1 into the inflammasome sensing pathogen-associated molecular patterns and danger-associated molecular patterns. Then, the active caspase-1 can lead to the conversion of IL-1β precursors (pro-IL-1β) into mature IL-1β. We have reported that inhibiting the activation of NLRP3 inflammasome protected Müller cells from gliosis activation and probably inhibited the development of DR, however the underlying mechanisms remain unknown.

Long non-coding RNAs (LncRNAs) are non-coding RNAs with more than 200 nucleotides in length which have no or limited protein-coding potential. The abnormal expression of lncRNAs has been found involved in several biological processes[5]. As one of the most common lncRNAs, KCNQ1 overlapping transcript 1 (KCNQ1OT1) plays a crucial role in regulating NLRP3 inflammasome activation and is involved in the pathogenesis of diabetic complications. KCNQ1OT1 was highly expressed in cardiac

tissue and high glucose (HG)-induced cardiomyocytes from diabetic patients and diabetic mice. Silencing of KCNQ1OT1 attenuated NLRP3 assembly and caspase-1 activation as well as IL-1β expression and maturation by targeting miR-214/caspase-1 axis[6, 7]. Downregulation of KCNQ1OT1 inhibited HG-induced oxidative stress, inflammation, and NLRP3 activation in renal tubular epithelial cells by upregulating the expression of miR-506-3p[8]. Moreover, in diabetic corneal endothelial keratopathy, KCNQ1OT1 knockdown decreased the expression of NLRP3 and cleaved caspase-1 and IL-1β maturation[9]. However, no studies have focused on the ability of KCNQ1OT1 to regulate NLRP3 inflammasome activation in Müller cells during DR. Therefore, in the present study, we aimed to evaluate the role of KCNQ1OT1 in the activation of NLRP3 inflammasome and to explore its potential mechanism.

2. Material And Methods

2.1. Mice model of DR

Eight-week-old male C57BL/6J mice (wild-type, WT) were obtained from the Laboratory Animal Center of Soochow University and were fed adaptively for one week. The DR model was induced by a high-fat diet and intraperitoneal injection of streptozotocin 50 mg/d for five consecutive days. The control group was fed a normal diet and injected intraperitoneally with sodium citrate buffer. All mice were maintained under standard laboratory conditions within a 12-hour light-dark cycle. Mice with tail vein blood glucose greater than 16.7 mmol/L were diagnosed with diabetes and were selected for the subsequent studies. After eight weeks of successful modeling, the mice were subjected to the corresponding experiments. All animal experiments were approved by the Animal Research Ethics Committee of Soochow University and conformed to the Chinese National Standard.

2.2. Cell culture and treatment

Rat retinal Müller cells rMC-1 were obtained from the American Type Culture Collection (ATCC). The cells were cultured in DMEM medium (Gibco, USA) supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml) (Gibco), and 10% fetal bovine serum (FBS, Gibco). The cells were incubated in a humidification incubator with 5% CO_2 at 37℃. Müller cells were cultured in 5 mM (average glucose) or 30 mM D-glucose (high glucose) (Sigma, USA) for 24 h to stimulate the diabetic environment before or after the specific experiments.

2.4. Intravitreal injection

Intravitreal injections were performed before the diabetes induction. After anesthesia, approximately 1μL of adeno-associated virus with KCNQ1OT1-shRNA or scrambled-shRNA was injected into the mouse vitreous cavity with a 33G needle. Two weeks later, a diabetic mice model was built as previously mentioned.

2.5. Cell transfection

KCNQ1OT1 siRNA, miR-17-5p mimic, TXNIP siRNA, and their negative controls were obtained from RiboBio (Guangzhou, China), and KCNQ1OT1 overexpression plasmids were purchased from GenePharma (Suzhou, China). The plasmids and siRNAs were transfected with Lipofectamine 2000 transfection reagent (Invitrogen, USA).

2.6. Quantitative real-time PCR (qRT-PCR)

TRIzol® reagent (Invitrogen, USA) was used to extract total RNA of the mice retina, the Müller cells, and the peripheral blood mononuclear cells (PBMCs). 1μg of total RNA was reverse transcribed into cDNA with Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). qRT-PCR was conducted with the powerUP SYBR Green Master Mix (Thermo Fisher Scientific, USA). qRT-PCR was also performed[10]. The primers used in this study is showed in the Supplemental table 1. The relative expression of genes was calculated by the $2^{-\Delta\Delta CT}$. The levels of KCNQ1OT1, TXNIP, NLRP3, ASC, Caspase1, and IL-1β were normalized to GAPDH while miR-17-5p was normalized to U6.

2.7. Western blot

The western blot analysis was conducted [10]. In brief, the cultured Müller cells and the mice retina were collected and lysed with protein lysate. BCA assay kit (Beyotime, China) was used to detect the concentration of the protein. Equivalent samples were separated by SDS-PAGE and then transferred to PVDF membranes. Then the membranes were hybridized with primary antibodies against NLRP3 (Abcam, USA), ASC (Santa Cruz Biotechnology, USA), Caspase1 (Abcam; for cleaved caspase-1 and pro-caspase-1), IL-1β (Abcam; cleaved IL-1β and pro-IL-1β), and TXNIP (Abcam) at 4°C overnight. After washing, secondary antibodies were incubated the next day. Image J was used to quantify the intensities of the bands and GAPDH was used as a loading control.

2.8. Enzyme-linked immunosorbent assay (ELISA)

Protein extraction was purified from the retina of mice and culture medium of Müller cells. The concentration of inflammatory cytokine IL-1β was detected by a commercial ELISA Kits (Jiancheng, China) according to the instructions of the manufacturers.

2.9. Immunofluorescence

Immunofluorescence was performed. Müller cells were prepared [11]. Primary antibodies against Caspase-1 (Proteintech, China) were incubated with the slides overnight at 4℃. After washing, they were incubated with secondary antibodies and then stained with 4', 6-diamidino-2-phenylindole (DAPI) (Solarbio). The fluorescence was visualized by confocal microscope (SP8, Leica, Germany).

2.10. Immunohistochemical

Briefly, the eye sections of the mice were washed with phosphate-buffered saline (PBS) and then incubated with the primary antibodies against caspase-1 (Proteintech) overnight at 4℃. The primary antibodies were diluted in PBS and supplemented with 0.5% Triton X-100. After washing with PBS, the sections were incubated with biotinylated mouse anti-rabbit IgG (Proteintech) at room temperature. After

30 min, the slides were incubated with avidin-biotin-peroxidase complex using ABC kit. Then 3,3' -diaminobenzidine tetrahydrochloride was used to observe the color reaction. After washed in water, all sections were counterstained with hematoxylin. The results were obtained with a microscope (Leica).

2.11. Fluorescence in Situ Hybridization (FISH)

The FISH assay was conducted [12]. Cy3-labeled probes KCNQ1OT1, 18S, and U6 were obtained from RiboBio (Guangzhou, China). After prehybridization, Müller cells were incubated with the probes overnight at 37℃. The cell nuclei were stained with DAPI and image results were obtained with a confocal microscope (SP8, Leica, Germany).

2.12. Luciferase reporter assay

Luciferase reporters containing wild-type (WT) or mutated KCNQ1OT1 and TXNIP were cloned to the downstream of the firefly luciferase gene. The miR-17-5p mimic and the negative control were co-transfected into Müller cells respectively. The dual-luciferase reporter assay system (Promega, Madison, WI) was used to measure the luciferase activities according to the instructions of manufacturers.

2.13. Clinical Specimens

Patients with type 2 diabetic mellites or non-diabetic controls attending Lixiang Eye Hospital were recruited for this study from July 2020 to April 2021. Fluorescein fundus angiography was conducted to assess DR and the fundus photographs were taken by ultra wide field digitalretinal image by Optos 200TX. According to the Diabetic Retinopathy Disease Severity Scale, diabetics were divided into three groups: non-obvious retinopathy (NDR), non-proliferative diabetic retinopathy (NPDR), and PDR[13]. Patients who had other diabetic complications, had received intravitreal treatments or photocoagulation in the first three months, or had other ocular diseases (such as keratitis, uveitis and retinal detachment) were excluded. All experimental protocols in this study followed the guidelines of the Declaration of Helsinki and were approved by the Human Ethics Committee of Lixiang Eye Hospital, Soochow University.

2.14 PBMCs isolation

The peripheral blood was collected from the vein of patients in non-diabetic controls, NDR, NPDR, and PDR (n = 5/group). The fresh peripheral blood was collected in an anticoagulant centrifuge tube and then centrifuged at 1800g for 5 minutes to remove the supernatant. An equal amount of PBS was added to the tube and then mixed by inversion. In another 15 ml centrifuge tube, separation of lymphocyte from Ficoll peripheral blood at room temperature (Solarbio, China) was added in advance. Then the diluted blood was slowly added along the wall of the tube. After centrifugation for 20 min, the liquid in the centrifuge tube was stratified, and the white film in the middle was carefully absorbed by the pipettor and added into another 15ml centrifuge tube. PBS 2 ml was added, and the supernatant was removed by centrifuging at 1800 g for 5 min. The procedure was repeated three times to wash the PBMCs.

2.15. Statistical Analysis

All data are presented as Mean ± SEM. The experiments in this study were conducted in at least triplicates. Statistical analysis was performed by Student's t-test (2 groups comparisons) or one-way ANOVA followed by Tukey's multiple comparison post-test (multi-group comparisons). $P < 0.05$ was considered statistically significant.

3. Results

3.1. The promotion of KCNQ1OT1 on the activation of the NLRP3 inflammasome in the retinas of mice with DR

To explore the effect of KCNQ1OT1 on DR, KCNQ1OT1 was knocked down by KCNQ1OT1-shRNA intravitreal injection in the STZ-induced DR model. The expression of KCNQ1OT1 was increased in the retinas of mice with DR and decreased by using KCNQ1OT1-shRNA, (Fig. 1A). Then, to identify the effect of KCNQ1OT1 on the activation of NLRP3 inflammasome, the protein levels of NLRP3 inflammasome-related factors, including NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β and cleaved IL-1β were detected, which were increased in the retinas of mice with DR and inhibited by KCNQ1OT1 knockdown (Figs. 1B-1G). In addition, caspase-1 immunoreactivity was also increased in mice with DR which was suppressed by KCNQ1OT1 knockdown, indicating that KCNQ1OT1 probably be involved in NLRP3 inflammasome activation.

3.2. KCNQ1OT1 knockdown alleviates the activation of the NLRP3 inflammasome in HG-treated Müller cells

We then detected the expression of KCNQ1OT1 in vitro after HG-treated. The results indicated that KCNQ1OT1 was significantly increased in HG-treated Müller cells and decreased by KCNQ1OT1 siRNA transfection (Fig. 2A). Moreover, consistent with the results of in vivo studies, the protein levels of NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β and cleaved IL-1β were upregulated but each was inhibited by KCNQ1OT1 knockdown (Figs. 2B-2H).

3.3. KCNQ1OT1 promotes the activation of the NLRP3 inflammasome by acting as a ceRNA in Müller cells

To explore the potential mechanism of the lncRNA in Müller cells, a FISH assay was performed to explore the subcellular localization of KCNQ1OT1. The results suggested KCNQ1OT1 was mainly localized in the cytoplasm in Müller cells (Fig. 3A), indicating that KCNQ1OT1 probably function in the cytoplasm. Hence, it was speculated that KCNQ1OT1 acted as a miRNA sponge. With Starbase 3.0 database, it was found that miR-17-5P had two binding sites for KCNQ1OT1(Fig. 3D). Firstly, the expression of miR-17-5P was decreased in the retinas of mice with DR and HG-treated Müller cells, contrary to the expression of KCNQ1OT1 (Figs. 3B and 3C). The luciferase reporter gene assay showed that miR-17-5p mimic reduced the luciferase activity in cells transfected with wild-type KCNQ1OT1 sequence compared with the NC group (miR-17-5p mimic negative control). Co-transfection of miR-17-5p mimic and mutant sequence on

one of the two binding sites (mut-KCNQ1OT1-1 or mut-KCNQ1OT1-2) inhibited the luciferase activity. In contrast, miR-17-5p mimic stimulation had no effect on the luciferase activity elicited by the double mutations (mut-KCNQ1OT1-3) (Fig. 3D). To further investigate the effect of miR-17-5P in Müller cells, miR-17-5P mimic was co-transfected with pcDNA-KCNQ1OT1. We found that miR-17-5P mimic decreased the protein levels of NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β and cleaved IL-1β, which was reversed by pcDNA-KCNQ1OT1 co-transfection (Figs. 3E and 3I).

3.4. KCNQ1OT1/miR-17-5P/TXNIP is involved in regulating the activation of NLRP3 inflammasome in Müller cells

To verify the specific mechanism of KCNQ1OT1 in regulating the activation of NLRP3 inflammasome in HG-treated Müller cells, Starbase 3.0 and Targetscan were used to predict the target gene of miR-17-5p. It should be noted that TXNIP have two binding sites with miR-17-5p (Fig. 4C) and is closely related to NLRP3 inflammasome. Firstly, the expression of TXNIP was increased in the retinas of mice with DR and HG treated Müller cells (Figs. 4A-4B). Then luciferase reporter gene assay was conducted to further verified the relationship between miR-17-5p and TXNIP. The cells were co-transfected with miR-17-5p mimic and luciferase vectors carrying the wild-type TXNIP sequence or mutant sequence on two binding sites (mutTXNIP-1, mutTXNIP-2, or mutTXNIP-3). As shown in Fig. 4C, miR-17-5p mimic significantly inhibited the luciferase activity of wild-type TXNIP, mutTXNIP-1, or mutTXNIP-2 and had no effect on the luciferase activities of mutTXNIP-3 transfection (Fig. 4C).

To explore whether KCNQ1OT1 exerted a facilitative effect on NLRP3 activation through KCNQ1OT1/miR-17-5p/TXNIP axis, a series of experiments were performed. As expected, the protein levels of NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β and cleaved IL-1β were inhibited by miR-17-5p mimic and increased by pcDNA-TXNIP co-transfection (Figs. 4D-4H). Similarly, KCNQ1OT1 overexpression promoted the activation of NLRP3 inflammasome but was reversed by TXNIP knockdown (Figs. 4I-4M). In conclusion, KCNQ1OT1 acted as a miR-17-5p sponge and promoted the activation of NLRP3 inflammasome in DR through enhancing TXNIP expression.

3.5. Clinical relevance of KCNQ1OT1 dysregulation in DR

We then investigated the clinical significance of KCNQ1OT1/miR-17-5P/TXNIP signaling. The PBMCs were collected from the veins of patients with non-diabetic controls, NDR, NPDR, and PDR (n = 5/group). Figure 1A showed the representative results of the fundus photographs in each group.

qRT-PCR showed that the expression of KCNQ1OT1 was increased in the PBMCs of patients with NPDR and PDR and its expression was correlated with disease severity (Fig. 5B). In contrast, miR-17-5P expression was downregulated in patients with NPDR and PDR (Fig. 5C). Additionally, the levels of NLRP3 inflammasome-related factors, including NLRP3, ASC, caspase-1, IL-1β were also upregulated, which further supported the hypothesis that the KCNQ1OT1 is in involved in the activation of NLRP3 inflammasome and that KCNQ1OT1 dysregulation may be the molecular mechanism of the pathogenesis of DR.

4. Discussion

LncRNAs have been reported to be involved in the pathogenesis of DR[14–16]. In the present study, the activation of NLRP3 inflammasome in Müller cells is confirmed to be pivotal in HG-induced sterile inflammation. KCNQ1OT1 is an essential starting point in the process of NLRP3 inflammasome activation and KCNQ1OT1 knockdown inhibited HG-induced the activation of NLRP3 inflammasome in Müller cells, which expands the understanding of the pathogenesis of DR. Furthermore, KCNQ1OT1 regulates the activation of NLRP3 inflammasome via miR-17-5p/TXNIP axis in vitro and in vivo, indicating the potential of KCNQ1OT1 for the prevention and reversal of DR.

Recent studies have confirmed that KCNQ1OT1 plays a vital role in diabetic complications, such as diabetic cardiomyopathy[6], diabetic nephropathy[8], and diabetic ocular disease[9, 17]. KCNQ1OT1 and NLRP3 inflammasome are triggered in the corneal endothelium of diabetic patients and mice, as well as in HG-treated corneal endothelial cells. KCNQ1OT1 regulates the expression of caspase-1 and IL-1β by acting as a ceRNA and competitively binding miR-214 in corneal endothelial cells[9]. The expression of KCNQ1OT1 is elevated in HG-treated human retinal endothelial cells (hRECs) and in the aqueous humor of patients with DR compared with the normal control group. KCNQ1OT1 promotes DR progression by the regulation of miR-1470 and epidermal growth factor receptor (EGFR)[17]. However, whether KCNQ1OT1 is involved in regulating NLRP3 inflammasome activation of Müller cells in DR remains unknown. Our study represents the first report involving the expression and function of KCNQ1OT1 in diabetic retinal Müller cells. In the present study, we found that KCNQ1OT1 is highly expressed in the retinas of diabetic mice, HG-treated Müller cells and the PBMCs of patients with DR. And inhibiting KCNQ1OT1 ameliorates the activation of NLRP3 inflammasome both in vitro and in vivo, which is mediated via miR-17-5p/TXNIP axis. Thus, KCNQ1OT1 is expected to be an innovative therapeutic target for DR.

TXNIP, an endogenous inhibitor of thioredoxin (TRX), has been reported as an essential protein for activation of NLRP3 inflammasome[18]. TXNIP interacts with TRX and fails to activate NLRP3 in resting cells. Under oxidative stress, TXNIP is released from TRX and binds directly to the leucine-rich region of NLRP3, which results in inflammasome assembly [19]. Previous studies have revealed that TXNIP plays a crucial role in the pathological process of many retinal cells and is involved in the progression of DR[20]. TXNIP is one of the highest genes induced by HG and diabetes in retinal microvascular endothelial cells (RMECs). HG treatment increases the production of ROS and promotes pyroptotic cell death through TXNIP/NLRP3 axis[21, 22]. Therefore, HG increases the TXNIP expression at the mRNA and protein levels. The upregulation of TXNIP is associated with mitochondrial membrane depolarization, fragmentation and mitophagic flux, while TXNIP knockdown prevents mitochondrial fragmentation, mitophagic flux and lysosome enlargement under HG environment.[23]. HG sustains TXNIP expression in the Müller glia of the retina in mice, and TXNIP is involved in Müller glia activation, ER stress, oxidative stress, and sterile inflammation under chronic hyperglycemia[3]. TXNIP knockout prevents the HG-induced mitochondrial damage and mitophagy in mice Müller cells. TXNIP is also significantly upregulated in the retina of diabetic mice in vivo and induced the expression of GFAP and LC3BII puncta, which are reversed by injection intravitreally of TXNIP siRNA [24]. We have reported previously that the protein levels of NLRP3

inflammasome-related molecules (including TXNIP, NLRP3, ASC, cleaved caspase-1, pro-caspase-1, cleaved IL-1β, and pro-IL-1β) are upregulated by HG treatment and downregulated by TXNIP knockdown. The interaction between TXNIP and NLRP3 was increased under HG condition, implying the crucial role of TXNIP in NLRP3 inflammasome activation in Müller cells under HG condition[25]. However, the regulatory mechanism of TXNIP remains unclear. In the present study, we find that the expression of TXNIP is increased in HG-stimulated Müller cells and in the retina of diabetic mice, which is consistent with previous studies. TXNIP plays a key role in promoting NLRP3 inflammasome activation in vitro and in vivo of DR models, which is regulated by KCNQ1OT1 through binding to miR-17-5pcompetitively. Furthermore, whether the effect of TXNIP in regulating oxidative stress, glial cell activation, endoplasmic reticulum stress and autophagy is regulated by KCNQ1OT1 remains to be further investigated.

NLRP3 inflammasome is compromised by NLRP3 associated with adapter protein, apoptosis-associated speck-like protein containing a caspase recruitment segment (ASC) and pro-caspase-1[26]. During NLRP3 inflammasome activation, the oligomerization of pro-caspase-1 proteins induces autoproteolytic cleavage to cleaved caspase-1. Cleaved caspase-1 cleaves pro-IL-1β to IL-1β [27, 28]. Studies have demonstrated that the activation of NLRP3 inflammasome plays a crucial role in the pathogenesis of DR[29]. Jinhua Gan et al. found that HG-induced the activation of NLRP3/caspase-1/GSDMD signaling pathway, which resulted in the loss of retinal pericytes in vitro model of DR[30]. In HG-treated human retinal pigment epithelium (RPE), overexpressed MELLT3 decreases NLRP3 inflammasome activation by targeting miR-25-3p/phosphatase and tensin homolog (PTEN) /proteinserine/threonine kinase (PSKs) axis[31]. DR is now discussed as a probable chronic inflammatory disease and displays a crucial therapeutic value on inhibition of NLRP3 inflammasome activation in the treatment of DR. Our results reveals that HG promotes NLRP3 inflammasome assembly and facilitates the activation of caspase-1. Cleaved caspase-1 then matures the inflammatory cytokines IL-1β in retinal Müller cells. KCNQ1OT1 knockdown inhibits the NLRP3/caspase-1 inflammasome signaling, which is mediated by miR-17-5p/TXNIP axis. This study extends our understanding on the role of NLRP3 inflammasome and KCNQ1OT1 in retinal Müller cells, providing potential biomarkers or therapeutic targets for DR.

In conclusion, it was found in this study that KCNQ1OT1 promoted the activation of NLRP3 inflammasome in vitro and in vivo of DR models, which was mediated by miR-17-5p/TXNIP axis. Moreover, in the clinical samples of patients with DR, the expression of KCNQ1OT1 and the activation of NLRP3 inflammasome were all increased, further supporting the hypothesis that the KCNQ1OT1 dysregulation may be the molecular mechanism of the pathogenesis of DR. Therefore, KCNQ1OT1 might be an effective interference target for the prevention and treatment of DR.

Declarations

AUTHOR'S CONTRIBUTION

Y. L, G. P. C, A. M. S and M. H. Z designed the research and wrote the manuscript; L. L. D, L. L. L., Y. P. D., A. H. X., X. J. C., Y. L, G. P. C, and M. H. Z performed the experiments; Y. L and M. H. Z collected and analyzed

the data.

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DATA AVAILABILITY

Not applicable

Ethical Approval. All mice studies were conducted according to protocols approved by the Animal Ethics Committee of Soochow University.

Consent for Publication. All authors have reviewed the manuscript and have given consent for publication.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Figures

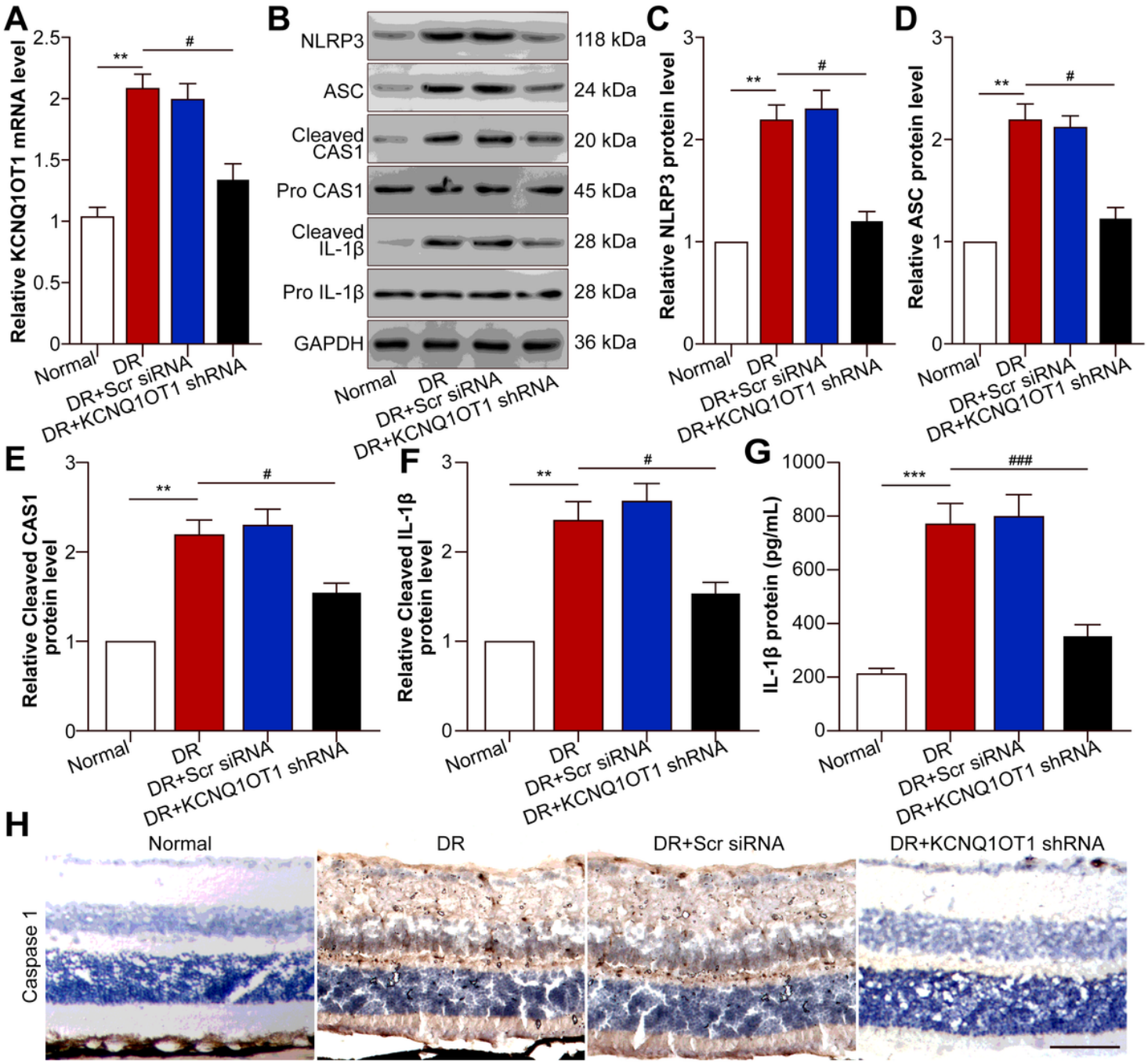

Figure 1

The promotion of KCNQ1OT1 on the activation of the NLRP3 inflammasome in the retinas of mice with DR (A). qRT-PCR was used to detect the expression of KCNQ1OT1, **P<0.01 versus Normal group, #P<0.05 KCNQ1OT1 shRNA versus DR group; (B). Western blot was conducted to detect the expressions of NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β, cleaved IL-1β in the retinas of mice with DR. (C-F). The relative protein expression of NLRP3 inflammasome-related molecules compared with the loading control GAPDH is shown. **P<0.01 versus Normal group, #P<0.05 KCNQ1OT1 shRNA versus DR group; (G). ELISA was performed to detect IL-1β protein level in the retinas of mice with DR. ***P<0.001

versus Normal group, ###P<0.001 KCNQ1OT1 shRNA versus DR group; (H). Immunohistochemical was conducted to detect the expression of caspase-1 in the retina of mice. Scale bar=50 μm. n=3/group.

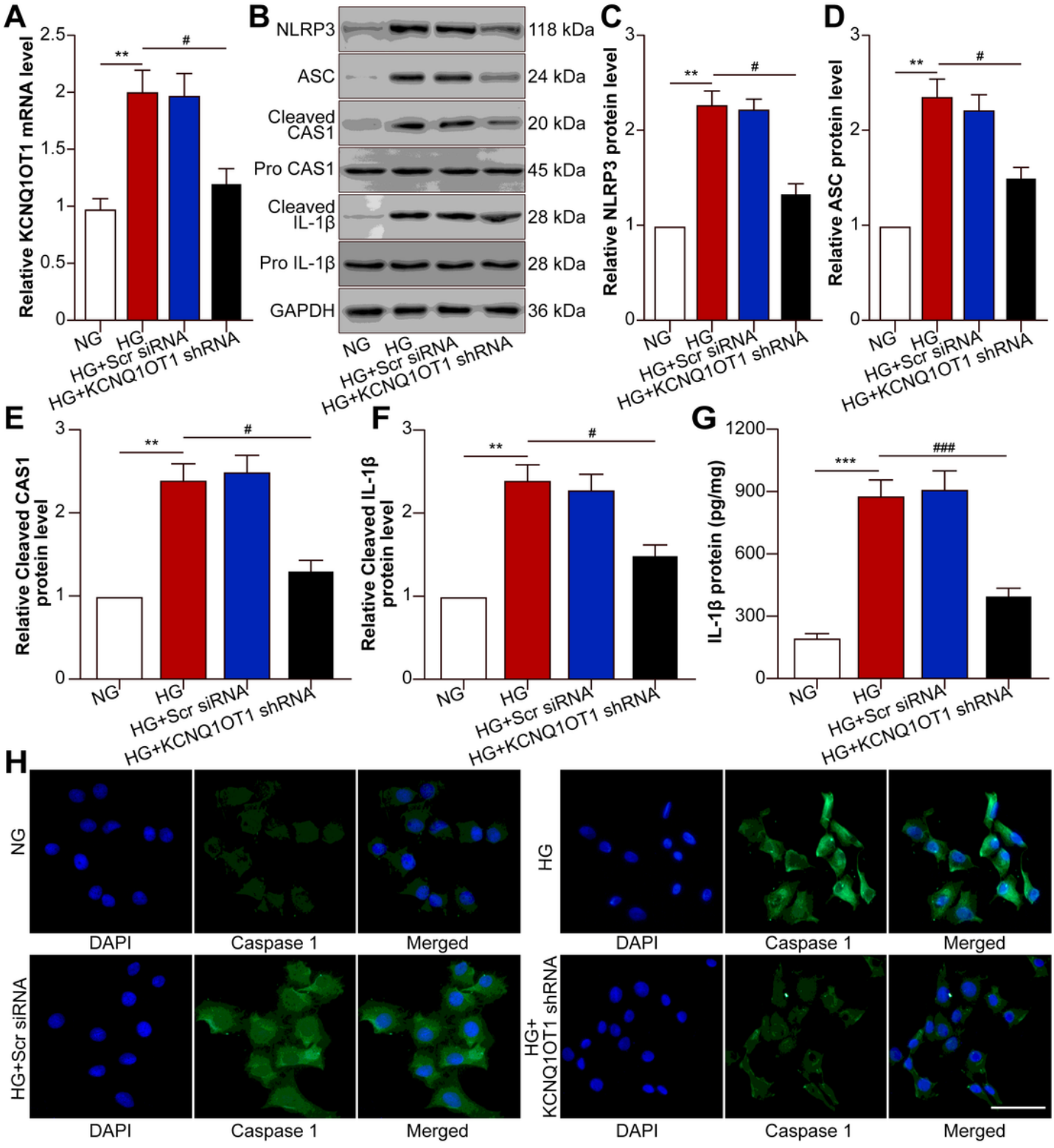

Figure 2

KCNQ1OT1 knockdown alleviates the activation of the NLRP3 inflammasome in HG-treated Müller cells (A). qRT-PCR was conducted to detect the expression of KCNQ1OT1 in HG-treated Müller cells. **P<0.01 versus NG group, #P<0.05 KCNQ1OT1 siRNA versus HG group; (B). Western blot was conducted to detect

the expression of NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β, cleaved IL-1β in Müller cells. (C-F). The relative protein expression of NLRP3 inflammasome-related molecules compared to the loading control GAPDH is shown. **P<0.01 versus NG group, #P<0.05 KCNQ1OT1 siRNA versus HG group; (G). ELISA was performed to detect the amount of IL-1β in Müller cells. ***P<0.001 versus NG group, ###P<0.001 KCNQ1OT1 siRNA versus HG group; (H). Immunofluorescence was performed to detect the expression of caspase-1 in Müller cells. Scale bar=30 μm. n=3/group.

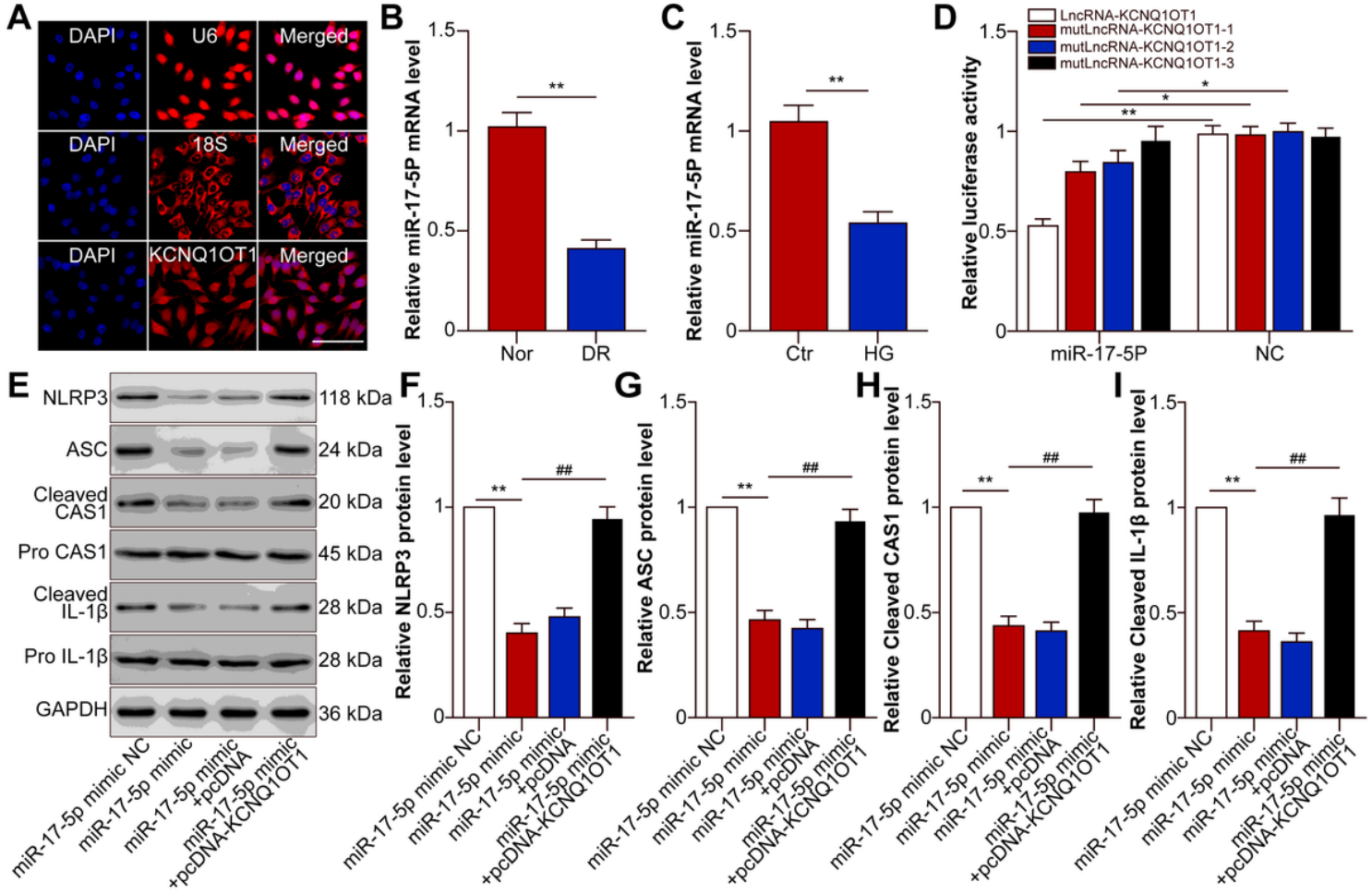

Figure 3

KCNQ1OT1 promotes the activation of the NLRP3 inflammasome by acting as a ceRNA in Müller cells. (A). The FISH assay was conducted to detect the localization of KCNQ1OT1 in Müller cells. 18S and U6 were taken as cytoplasmic and nuclear reference respectively, Scale bar: 50 μm. (B). qRT-PCR was performed to detect the mRNA level of miR-17-5p in the retinas of mice with DR. **P<0.01 versus Normal group (Nor); (C). qRT-PCR was performed to detect the mRNA level of miR-17-5p in Müller cells. **P<0.01 versus control group (Ctr); (D). The luciferase activity in Müller cells co-transfected with KCNQ1OT1-WT/Mut and miR-17-5p. *P<0.05, **P<0.01 versus miR-17-5p transfection. (E). Western blot was conducted to detect the expression of NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β, cleaved IL-1β in Müller cells. (F-I). The relative protein expression of NLRP3 inflammasome-related molecules compared to the loading control GAPDH is shown. **P<0.01 versus miR-17-5p mimic NC group, ##P<0.01 miR-17-5p mimic plus pcDNA-KCNQ1OT1 versus miR-17-5p mimic group. n=3/group.

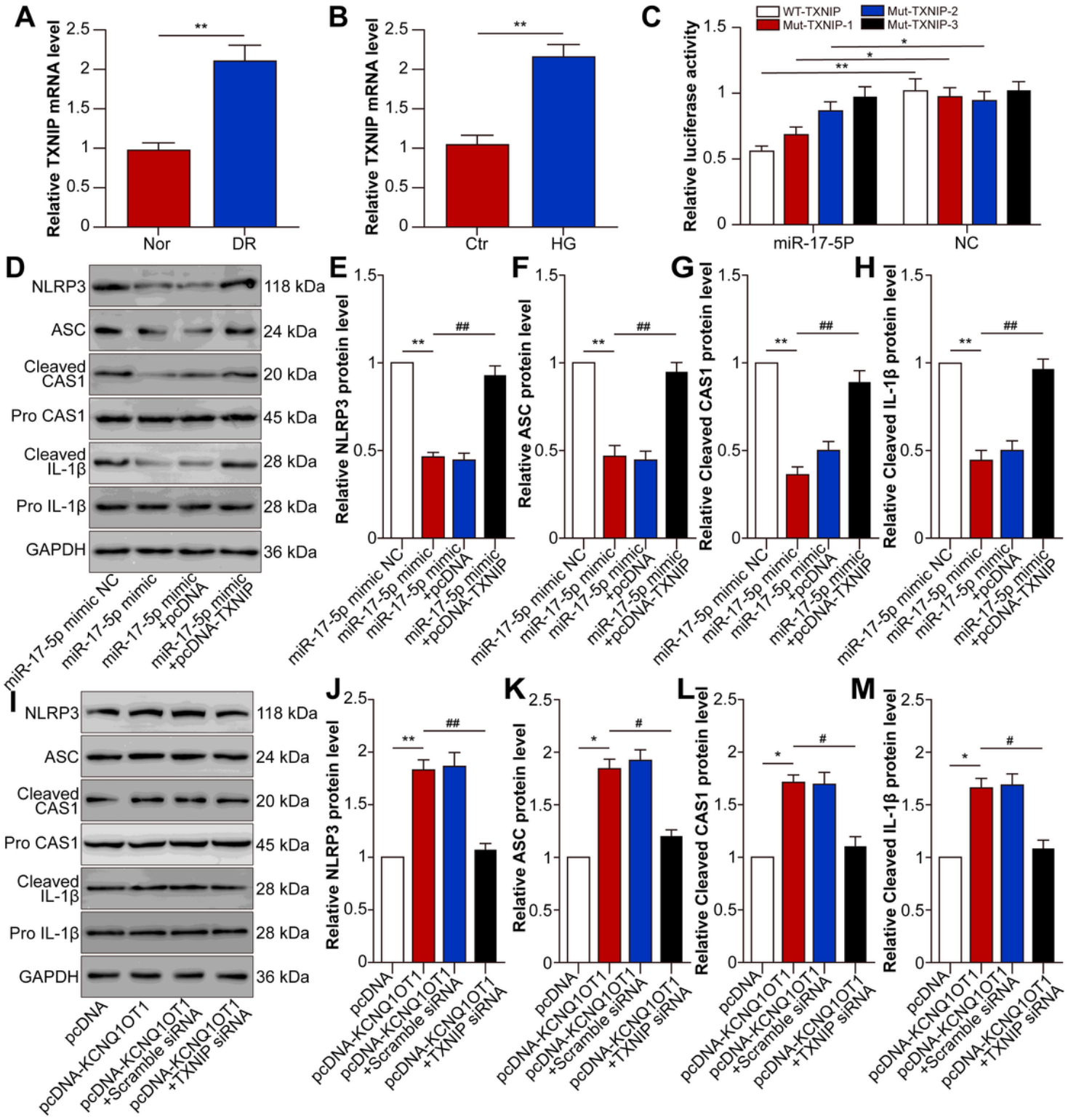

Figure 4

KCNQ1OT1/miR-17-5P/TXNIP is involved in regulating NLRP3 inflammasome activation in Müller cells (A). qRT-PCR was conducted to detect the mRNA level of TXNIP in the retinas of mice with DR. *P<0.01 versus Nor; (B). qRT-PCR was performed to detect the mRNA level of TXNIP in Müller cells. **P<0.01 versus Nor; (C). The luciferase activity of co-transfected with TXNIP-WT/Mut and miR-17-5p mimic in Müller cells. *P<0.05, **P<0.01 versus miR-17-5p. (D). Müller cells are co-transfected with miR-17-5p

mimic and TXNIP overexpression plasmid or their negative control (NC), and then treated with HG for 24h. Western blot was performed to detect the protein level NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β, cleaved IL-1β in Müller cells. (E-H). The relative protein expression of NLRP3 inflammasome-related molecules compared to the loading control GAPDH is shown. **P<0.01 versus miR-17-5p mimic NC group, ##P<0.01 miR-17-5p mimic plus pcDNA-TXNIP versus miR-17-5p mimic group. (I). Müller cells were co-transfected with KCNQ1OT1 overexpression plasmid and TXNIP siRNA or the negative control (NC) and then treated with HG for 24h. Western blot was performed to detect the protein level NLRP3, ASC, pro-caspase-1, cleaved caspase-1, pro-IL-1β, cleaved IL-1β in Müller cells. (J-M). The relative protein expression of NLRP3 inflammasome-related molecules compared to the loading control GAPDH is shown. *P<0.05, **P<0.01 versus pcDNA group, #P<0.05, ##P<0.01 miR-17-5p mimic plus pcDNA-TXNIP versus miR-17-5p mimic group. n=3/group.

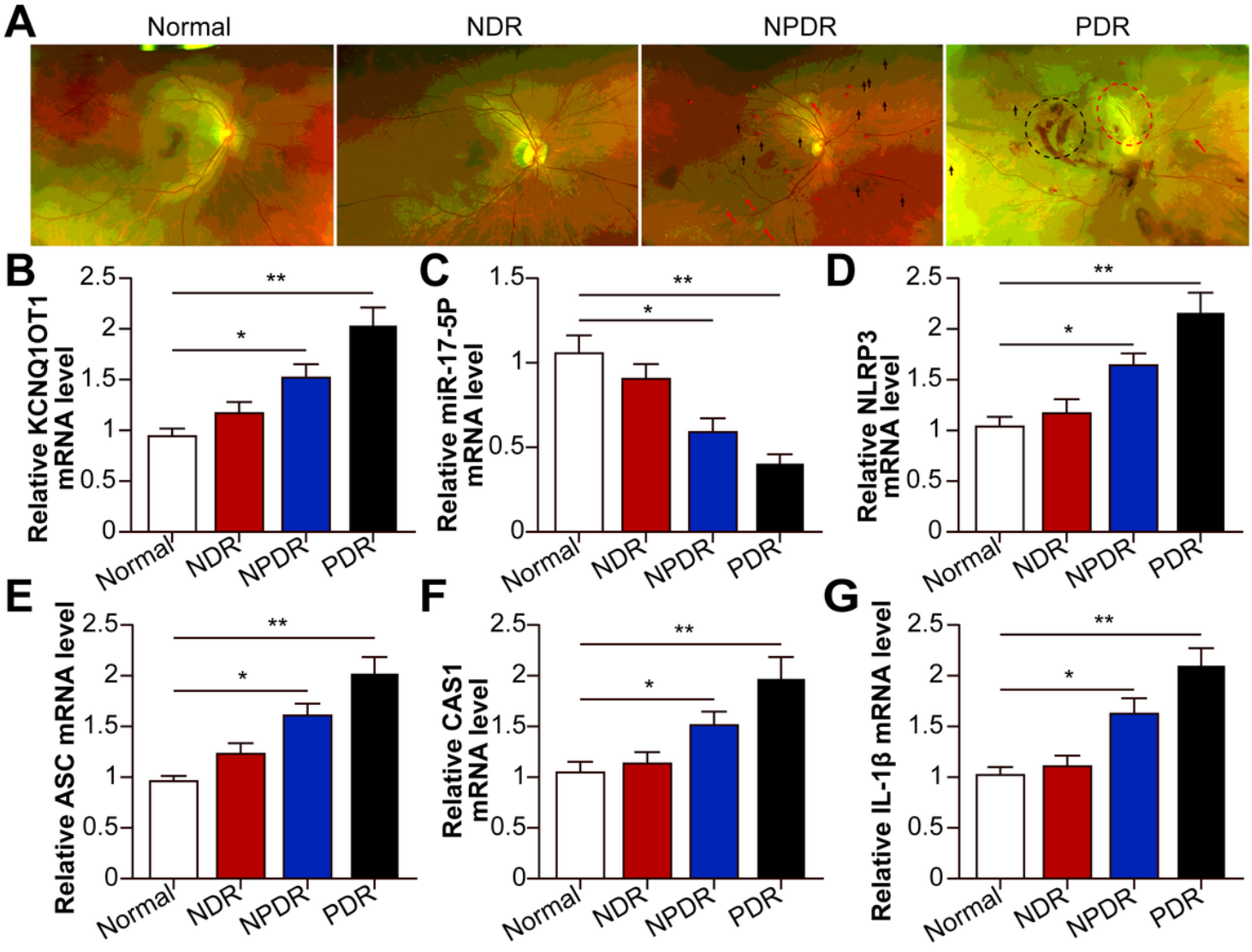

Figure 5

Clinical relevance of KCNQ1OT1 dysregulation in DR. (A). The representative fundus photographs normal control, NDR, NPDR, and PDR were showed. The black arrows point to the microaneurysm, the red arrows point to cotton-wool spots and the "*" marks bleeding blots. In the PDR group, the preretinal bleeding is

circled in black and neovascularization is circled in red. (B). qRT-PCR was conducted to measure the mRNA expression of KCNQ1OT1 in PBMCs, *P<0.05, **P<0.01 versus normal group. (C). qRT-PCR was conducted to measure the mRNA expression of miR-17-5P in PBMCs, *P<0.05, **P<0.01 versus normal group. (D). qRT-PCR was conducted to measure the mRNA expression of NLRP3 in PBMCs, *P<0.05, **P<0.01 versus normal group. (E). qRT-PCR was conducted to measure the mRNA expression of ASC in PBMCs, *P<0.05, **P<0.01 versus normal group. (F). qRT-PCR was conducted to measure the mRNA expression of caspase-1 in PBMCs, *P<0.05, **P<0.01 versus normal group. (G). qRT-PCR was conducted to measure the mRNA expression of IL-1β in PBMCs, *P<0.05, **P<0.01 versus normal group. n=3/group.

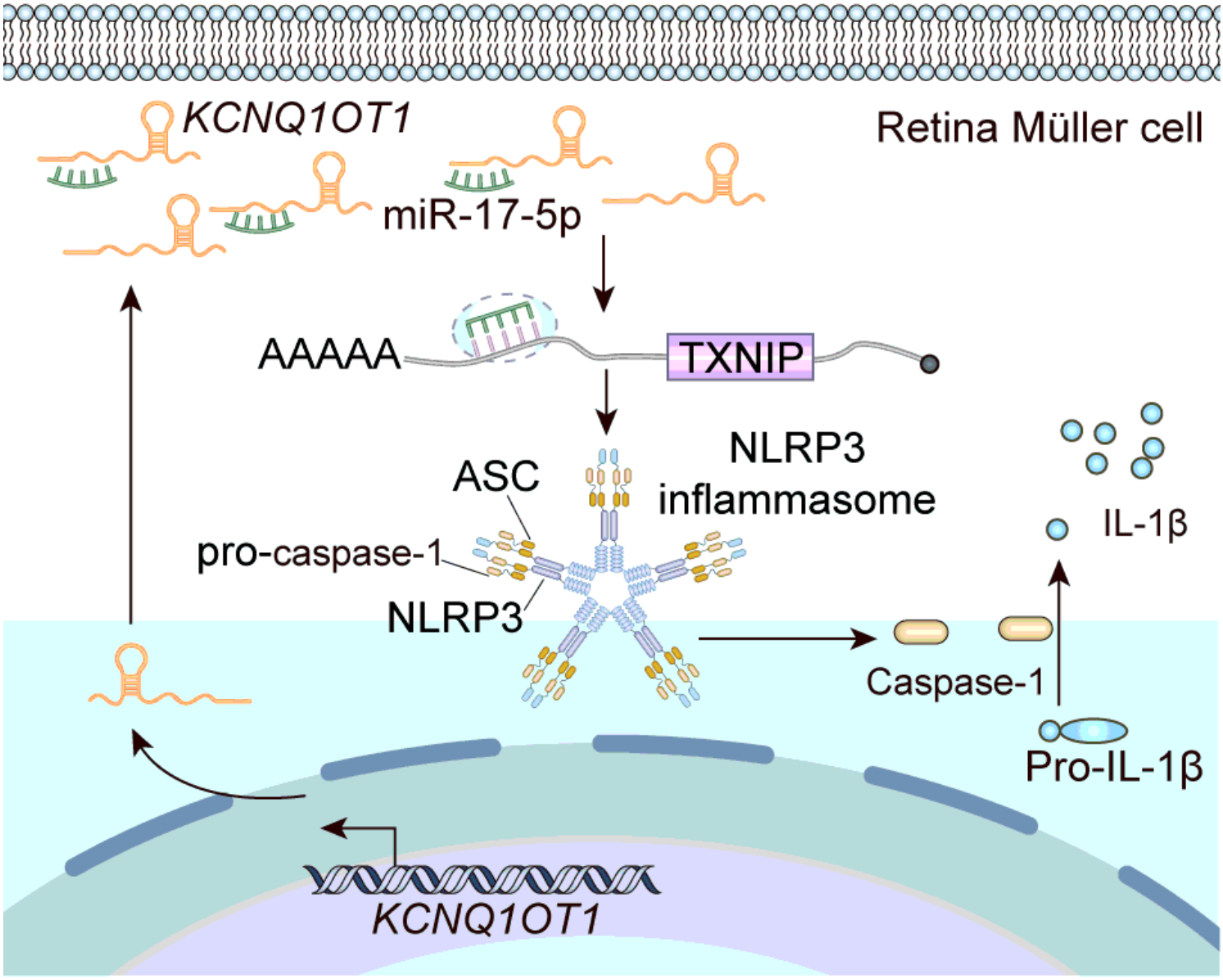

Figure 6

The Schematic diagram of the mechanism of KCNQ1OT1 in Müller cells. Under HG environment, the expression of KCNQ1OT1 is increased. KCNQ1OT1 then promotes the expression of TXNIP by acting as a

miR-17-5P sponge. TXNIP promotes the activation of NLRP3 inflammasome, and the oligomerization of pro-caspase1 proteins induces autoproteolytic cleavage into cleaved caspase-1. Cleaved caspase-1 then cleaves pro-IL-1β to matured IL-1β.

Supplementary Files

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- supplementaltable1.docx