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Research article

LncRNA MEG3 promotes glaucomatous retinal ganglion cell apoptosis in acute glaucoma mice via up-regulating *miR-106* target gene *caspase-8*

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Abstract

Background: *MiR-106b* and *caspase-8* played a key role in the development of acute glaucoma. Increasing evidence has indicated that long non-coding *RNA (lncRNA) maternally expressed gene 3 (MEG3)* participated in regulating pathophysiological processes. However, the association among *MEG3, miR-106b* and *caspase-8* remained unclear.

Methods: We employed the mouse model of acute glaucoma and oxygen and glucose deprivation (OGD)/reoxygenation cellular model for *in vivo* and *in vitro* experiments. The miRNA inhibitor and small interfering RNA (siRNA) were transfected into primary retinal ganglion cells (RGCs) for miRNA and lncRNA knockdown. The interaction among *MEG3, miR-106b* and *caspase-8* was assessed by RNA immunoprecipitation, RNA pull down and luciferase reporter assay. The changes in gene expression were assessed by quantitative Real-Time PCR (qRT-PCR) and western blot. Cell apoptosis analysis was performed using flow cytometry.

Results: *MEG3* expression was increased in the mouse model of acute glaucoma and OGD-treated RGCs. *MEG3* knockdown alleviated RGC apoptosis following OGD. RNA immunoprecipitation and RNA pull down displayed that *MEG3* directly targeted *miR-106b*, and luciferase reporter assay confirmed the interaction between *miR-106b* and *caspase-8*. *MEG3* silencing significantly relieved RGC apoptosis via downregulating *miR-106b* target gene *caspase-8*.

Conclusion: *MEG3* increased the apoptosis of glaucomatous RGC via *miR-106b/caspase-8 axis*.

Keywords: acute glaucoma; retinal ganglion cells; *MEG3; miR-106b; caspase-8*

INTRODUCTION

It is generally known that acute glaucoma is one of the leading causes of permanent vision loss and irreversible blindness worldwide, which is characterized by a rapid increase of intraocular pressure (IOP) resulting from a blockage around drainage canals and consequent retinal ischemia, leading to progressive damage to retinal ganglion cells (RGCs)[1,2]. Despite intensive medical treatment, increasing evidence has suggested that acute glaucoma continues progressing to blindness in quite a few patients[3]. Until recently, elevated IOP has been considered to be a major risk factor for the pathogenesis of RGC death in acute glaucoma[4]. Nevertheless, the detailed mechanisms by which elevated IOP ultimately led to RGC apoptosis were largely unknown.

In the past years, emerging evidence has showed that the caspase aspartate-specific cysteine protease family are involved in programmed cell death in eukaryotes[5]. Several studies have reported that caspase family consists of at least 14 members in mammalian cells. *Caspase-8* is synthesized as a pro-enzyme and comprises a large N-terminal prodomain as well as a C-terminal catalytic domain, playing a crucial role in triggering death receptor-mediated apoptosis[6]. Recently, accumulating evidence has strongly implied that as an initiator caspase, *caspase-8* has been implicated in acute glaucoma. For instance, Chi et al found that substantial rise in IOP induces Toll-like receptor 4 (TLR4)/*caspase-8* signaling pathway activation, thereby leading to retinal ischemic injury and RGC death[7]. Furthermore, a recent report has indirectly revealed the apoptotic functions of *caspase-8*, demonstrating that high-mobility group box 1 (HMGB1) promotes the activation of *caspase-8* via NF-κB pathway, resulting in inflammatory response[8].

MicroRNAs (miRNAs) are a class of small single-strand-

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Table S1. SiRNA sequences used for transfection.

SiRNA	Sequence
Si-MEG3	Sense 5'-AACAGCAAAUGGCACAGGAAGAGACGC-3'
	Anti-sense 5'-GCGUCUUCCUGUGCCAUUUGCUGUU-3'
miR-106b mimic	Sense 5′-UAAAGUGCUGACAGUACAGUGCAGAU-3′
	Anti-sense 5′-AUUUCACGACUGUCACGACUA-3′
miR-106 inhibitor	5′-AUUUCACGACUGUCACGACUA-3′

ed (~22-nucleotide-long) non-coding RNAs that play important roles in physiopathologic processes by negatively regulating target genes. A number of dysregulated miRNAs have been actively involved in diverse biological processes. For example, Jamie et al reported that the miRNA cluster *caspase-8~25* promotes the proliferation of self-renewing neural stem/progenitor cell (NSPC) and the generation of new neurons under the condition of self-differentiation[9]. Hari et al have found the significant downregulation of *caspase-8* in glaucomatous retinae[10]. Nonetheless, the *caspase-8*-related molecular mechanisms were not well understood.

MEG3, an lncRNA, acts as a tumor suppressor in various cancers by influencing the apoptosis and proliferation of tumor cells, such as neuroblastomas and gliomas[11,12]. Previous reports have shown that *MEG3* expression is positively correlated with the progression of patients with retinoblastoma and inhibits tumor growth via *Wnt/β-catenin* pathway activation[13]. Besides the antineoplastic effect, other studies also indicated that the activation of *MEG3* triggers ischemic neuronal death[14]. However, little was known about the molecular mechanisms and biological roles of *MEG3* in acute glaucoma. Therefore, our study was designed to reveal whether *MEG3* affects acute glaucoma progression and ascertain the potential regulatory mechanism.

MATERIALS AND METHODS

Mouse model of acute glaucoma

Animal experiments performed in this study were approved by the Animal Ethics Committee of the Affiliated Hospital of Inner Mongolia University for the Nationalities and qualify to the ARVO guidelines of animal use in eye research. Prior to *in vivo* experiment, a total of 30 adult male C57BL/6 mice obtained from Inner Mongolia University for the Nationalities were anesthetized by an intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine. The anterior chamber of the right eye was cannulated with a 30-gauge needle connected to a syringe filled with normal saline to maintain an IOP around 120 mmHg for 1h. Retinal ischemia was verified by the whitening of the iris and loss of the red reflex. After withdrawal of the needle, reperfusion occurred as IOP was normalized within 5 min measured by a noncontact tonometer (Nidek Co., Ltd., Aichi, Japan). The contralateral left eye as a control eye carried on sham-operated procedure. All mice were then subjected to 6, 24, 48, or 72 h of reoxygenation before euthanasia. Retinal tissues were collected for the following procedure.

Isolation and culture of primary RGC

Retinal tissues were isolated from enucleated eyeballs of 12-day-old newborn C57BL/6 mice and maintained in calcium/magnesium-free Hank's balanced salt solution (Life Technologies, Carlsbad, CA, USA) containing 16.5 U/mL of papain (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at room temperature. Primary RGCs were purified from the collected retinal cell suspension using two-step immunopanning (TSI) method as previously

Table S2. The primer sequences used for qRT-PCR.

Gene	Primer sequence
MEG3	Forward, 5′-CTGCCCATCTACACCTCACG-3′
	Reverse 5′-CTCTCCGCCGTCTGCGCTAGGGGCT-3′
GAPDH	Forward 5′-GTCAACGGATTTGGTCTGTATT-3′
	Reverse 5′-AGTCTTCTGGGTGGCAGTGAT-3′
U6	Forward 5′-CTCGCTTCGGCAGCACA-3′
	Reverse 5′-AACGCTTCACGAATTTGCGT-3′
Caspase8	Forward 5′-TTCCTACCGAGATCCTGTGAATGG-3′
	Reverse 5′-AGAGCTTCTTCCGTAGTGTGAAGG-3′
miR-106b	Forward CTGCTGGGACTAAAGTGCTGAC
	Reverse GCAGCAAGTACCCACAGTGC

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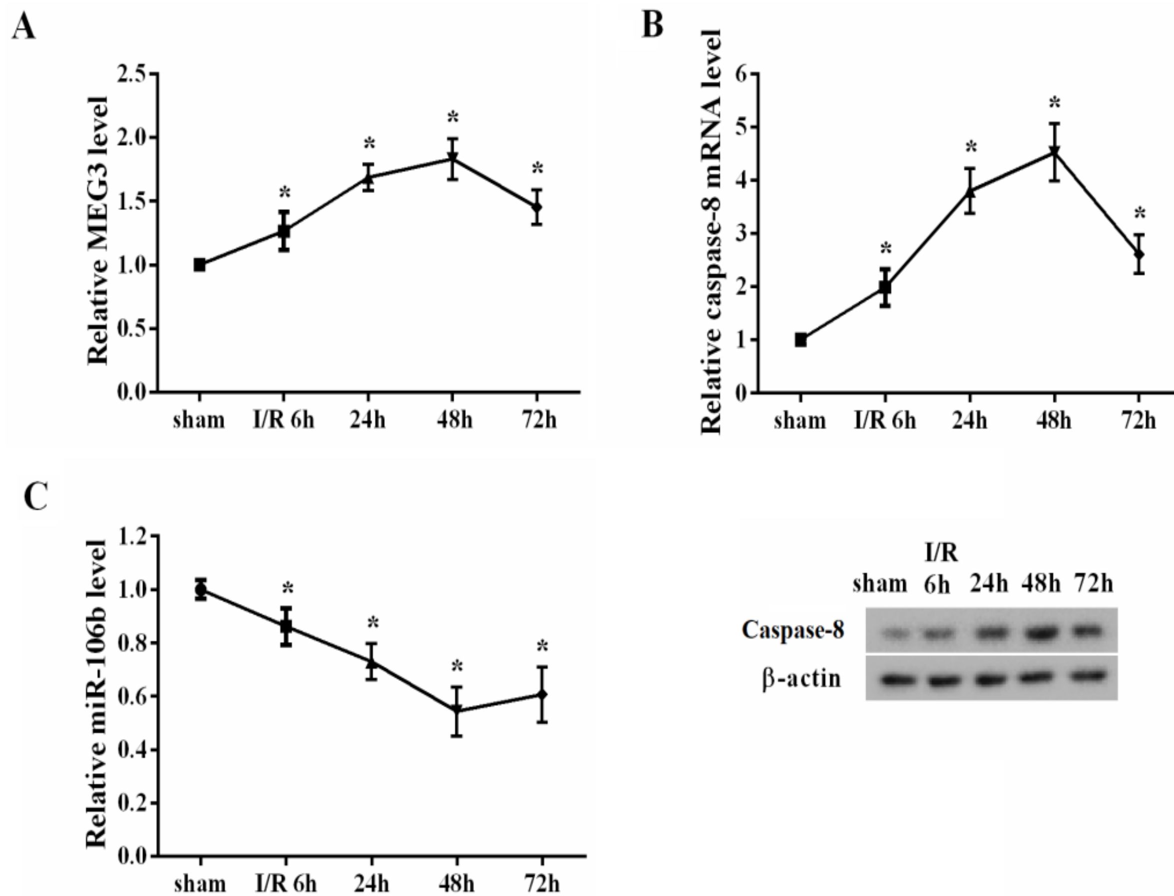

Figure 1. Changes of gene expression in IOP-induced mouse model of acute glaucoma. (A)Relative MEG3 expression in ischemic retina at different time points (6, 24, 48 and 72h) after reperfusion and sham-operated retinal tissues using qRT-PCR. (B)The relative caspase-8 expression at mRNA and protein levels in ischemic retina at different time points (6, 24, 48 and 72h) after reperfusion and sham-operated retinal tissues using qRT-PCR and western blot, respectively. (C)The relative mRNA expression of miR-106b in ischemic retina at different time points (6, 24, 48 and 72h) after reperfusion and sham-operated retinal tissues using qRT-PCR. n= 6. *P<0.05 compared with the sham group.

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described[15] by incubation with rabbit-anti-mouse macrophage antibody (1:50; Fitzgerald Industries International, Concord, MA, USA) for 5 min and goat-anti-rabbit IgG antibody (1:200; Southern Biotechnology Associates, Birmingham, AL, USA) for 30 min at room temperature. All adherent RGCs were harvested by incubation with trypsin solution (Gibco, Carlsbad, CA, USA) and cultured in Dulbecco's modified eagle medium/Ham's F12 (DMEM/F12; Life Technologies) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin (Gibco), and 100 μg/ml streptomycin (Gibco) at 37 °C in humidified 5% CO_2 and 95% air.

Oxygen and glucose deprivation (OGD) cellular model

Primary RGCs were seeded on poly-L-ornithine and laminin precoated coverslips in 24-well plate with 2.5×10^5 cells per well and incubated at 37°C in humidified 5% CO2 and 95% air. Twenty-four hours after seeding, cells were washed twice with phosphate-buffered saline (PBS), cultured in glucose-free Roswell Park Memorial Institute (RPMI) 1640 medium containing L-glutamine and incubated in 5% CO_2/95% N_2 in an anaerobic chamber at 37°C for 4h. Subsequently, cells were then grown in DMEM/F12 containing glucose and returned to a normoxic environment (5% CO_2 and 95% air) for another 12h at 37°C. In addition, RGCs exposed to normal culture media in a normoxic incubator were used as controls. OGD treated cells and controls were collected to qRT-PCR and western blotting analysis for *MEG3, caspase-8 and caspase-8* expression.

RNA interference

To investigate the biological role of *MEG3* in cellular ischemia/reperfusion (I/R) injury, primary RGCs were randomly divided into 4 groups: control, OGD, OGD+siRNA control (si-Ctrl) and OGD+siRNA-*MEG3* (si-*MEG3*). Briefly, cells were cultured in 96-well plates at 1×10^4 cells/well overnight, transfected with si-*MEG3* or si-Ctrl

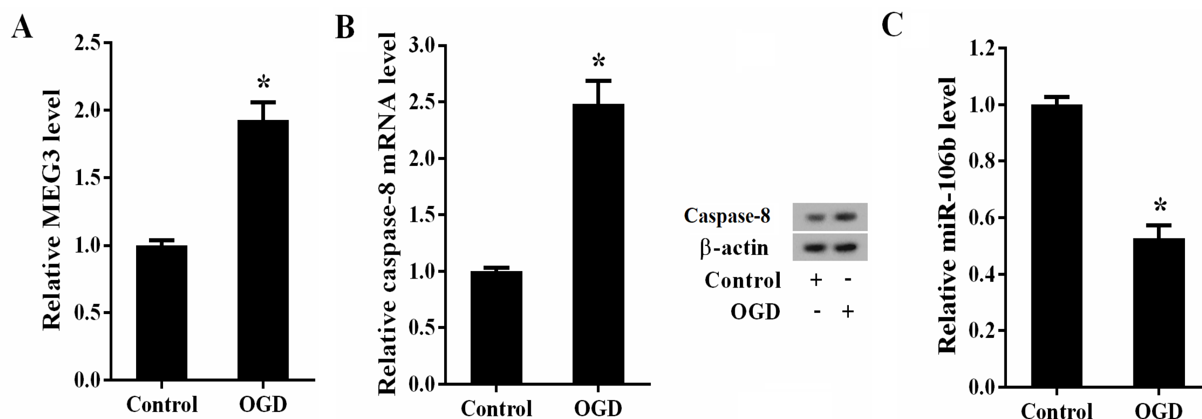

Figure 2. Changes of gene expression in OGD/reoxygenation induced I/R injury in vitro. (A)Relative MEG3 expression in RGC cells following OGD/reoxygenation treatment or normal culture (control). (B)The relative caspase-8 expression at mRNA and protein levels in RGC cells following OGD/reoxygenation treatment or normal culture (control). (C) The relative mRNA expression of miR-106b in RGC cells following OGD/reoxygenation treatment or normal culture (control). *P<0.05 compared with the control group.

using X-tremeGENE siRNA Transfection Reagent (Roche Applied Science, Mannheim, Germany) following the manufacturer's instructions and exposed to OGD treatment for 4h after 24h transfection. The sequence of si-*MEG3* was shown in the Table S1. The mRNA expression of *MEG3* was determined by qRT-PCR and cell apoptosis analysis was performed after 12h reoxygenation.

RNA immunoprecipitation

DIANA tools (http://carolina.imis.athena-innovation.gr/) were used to predict the potential interaction of *MEG3* and *caspase-8*. RIP assay was performed using Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. RGCs at 80% density (approximately 1.0×10^7 cells) were washed with cold PBS and lysed with RIP lysis buffer at 4°C for 30min. Cell extracts were incubated with protein A/G sepharose beads conjugated to anti-Ago2 antibody (Millipore) or normal IgG at 4 °C and washed with lysis buffer for five times. Immunoprecipitated RNAs and total RNA from the whole cell lysates (input controls) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for western blotting or extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol for qRT-PCR analysis.

RNA pull-down

The interaction between *MEG3* and *caspase-8* was further examined by RNA pull-down using a Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. Protein extracts from RGCs (approximately 1.0×10^7 cells) were mixed with 50 pmol of biotinylated *MEG3* (or its negative control LOC) and incubated with 50μL of streptavidin magnetic beads 4� for 1h. The associated RNA-protein complex was isolated using Biotin Elution Buffer and boiled in SDS buffer for 10 min. The retrieved protein was detected using western blot analysis for Ago2 protein levels, while *caspase-8* mRNA levels were measured by qRT-PCR.

Luciferase reporter assay

Targetscan online bioinformatics software (http://www.targetscan.org) was used to identify the underlying binding sites of *caspase-8* and *caspase-8*. To verify the interaction between them, luciferase reporter assay was performed in RGCs. The *caspase-8* recombinant plasmids containg the wild type binding site of *caspase-8* (*caspase-8*-WT) or mutated binding site of *caspase-8* (*caspase-8*-Mut) were constructed, and then respectively co-transfected with *caspase-8* mimic or inhibitor or their corresponding negative controls into RGCs using Lipofectamine 2000 (Invitrogen, USA). Cells were harvested 24h post-transfection and were incubated with passive lysis buffer at room temperature for 10 minutes. Luciferase activity was measured using the Dual Luciferase Assay kit (Promega, Madison, WI, USA) following the manufacturer's instructions.

Cell transfection

To further explore the molecular mechanism and biological function of *MEG3* in OGD-induced RGCs ischemic injury, primary RGCs were then randomized to 6 groups as follows: control, OGD, OGD+si-Ctrl, OGD+si-*MEG3*, OGD+si-*MEG3*+NC and OGD+si-*MEG3*+*caspase-8* inhibitor. Before transfection, cells were seeded in 6-well plates at a density of 4×10^5 cells/ml for one day. When cell con-

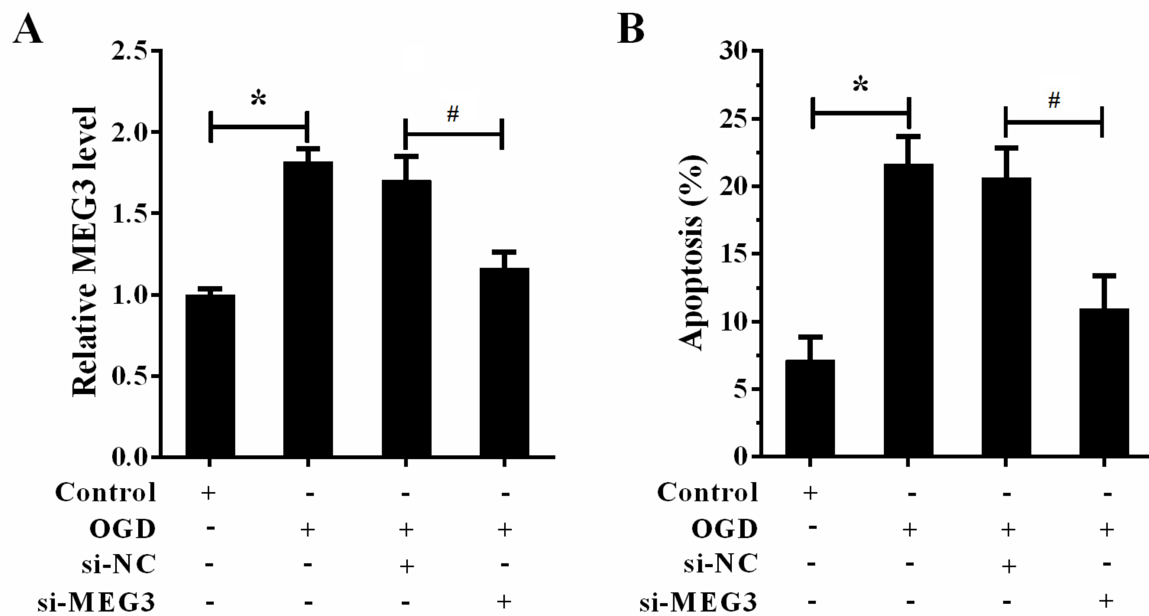

Figure 3. Effect of MEG3 on RGC apoptosis. (A)Relative MEG3 expression of RGCs in the group of control, OGD, OGD+si-Ctrl and OGD+si-MEG3. (B)The percentage of apoptotic RGCs in the group of control, OGD, OGD+si-Ctrl and OGD+si-MEG3 detected by flow cytometry. *P<0.05 compared with the control group; #P<0.05 compared with OGD+si-Ctrl group.

fluence reached more than 70%, the miRNA inhibitor and small interfering RNA (siRNA) as well as negative controls (NC or si-Ctrl) purchased from GenePharma (Shanghai, China) were transfected into cells using a genefectine transfection reagent (Sigma-Aldrich). The sequences of *caspase-8* mimic and inhibitor were shown in the Table S2. After 24h transfection, cells were subjected to OGD/reoxygenation treatment followed by the subsequent experiments including *MEG3*, *caspase-8* and *caspase-8* expression and cell apoptosis.

QRT-PCR analysis

Total RNA was extracted from retinal tissues and RGCs using Trizol reagent and reverse-transcribed to cDNA using a PrimeScript RT Reagent Kit (TaKaRa, Dalian, China) according to the manufacturer's protocol. The relative mRNA expression levels of *MEG3*, *caspase-8* and *caspase-8* were normalized to GAPDH, U6 and GAPDH snRNA expression, respectively, determined using SYBR Premix Ex Taq (TaKaRa) on an ABI 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) and calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for qRT-PCR were shown in the Table S2.

Western blot

For analysis of *caspase-8* protein expression in retinal samples and RGCs, total protein was extracted using Radio-Immunoprecipitation Assay (RIPA) buffer (Beyotime, Shanghai, China), centrifuged with 14,000 rpm for 15 min at 4°C. Protein extracts and Prestained Protein Marker (Beyotime, China) were run on 10% SDS, transferred onto PVDF membranes and blocked with Tris buffered saline tween (TBST) containing 5% skim milk at room temperature for 2h. Blots were probed with rabbit anti-mouse *caspase-8* polyclonal antibody (1:1000; Cell Signaling Technology, Boston, MA, USA) or β-actin mouse monoclonal antibody (1:1000; Beyotime, China) at 4°C overnight, incubated with horseradish-peroxidase (HRP)-coupled goat anti-rabbit IgG (1:2000; Abcam, Cambridge, UK) at room temperature for 1~2h and visualized on a Molecular Imager ChemiDoc XRS System (Bio-Rad Laboratories, Hercules, CA, USA) using an ECL Plus Western Blotting Substrate (Thermo Scientific, Shanghai, China).

Cell apoptosis assay

Cell apoptosis was analyzed by flow cytometry using Annexin V-FITC apoptosis detection kit (BD Biosciences; San Jose, CA, USA) according to the manufacturer's protocols. Cells following transfection and OGD/reoxygenation treatment were collected, washed with cold PBS and stained with binding buffer containing Annexin V-FITC and propidine iodide (PI) at 4°C under darkness for 15 min. Finally, cells were recorded using flow cytometry

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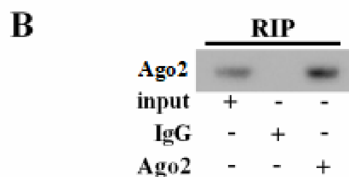

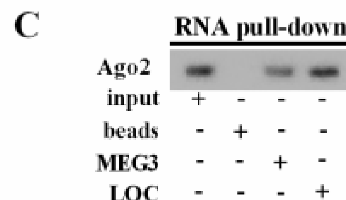

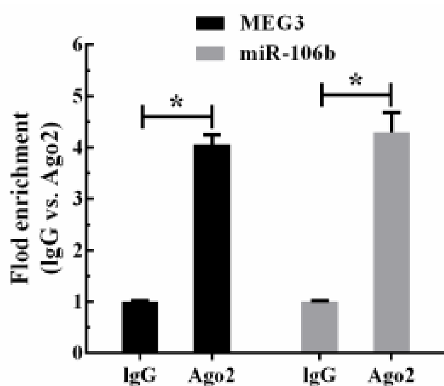

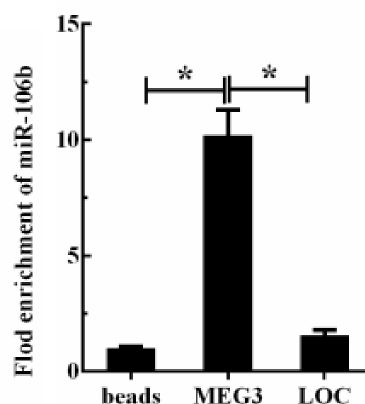

Figure 4. MEG3 targeted miR-106b. (A)The binding sites on the MEG3 and miR-106b predicted by DIANA tools. (B) RIP experiment with anti-Ago2, IgG as negative control or 10% input as a positive control from RGC extracts using western blotting and qRT-PCR. *P<0.05 compared with IgG group. (C) Ago2 expression levels and enrichment of miR-106b expression after RNA pull-down experiment with RGC extracts in different groups. *P<0.05 compared with beads and LOC group.

(Beckman Coulter, Fullerton, CA, USA).

Statistical analysis

The data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). Significant differences between groups were analyzed using two-sided Student's t test, and P<0.05 was considered to be statistically significant.

RESULTS

MEG3 and *caspase-8* was upregulated while *caspase-8* was downregulated in mouse model of acute glaucoma and OGD-treated RGCs

To investigate the underlying role of *MEG3* in acute glaucoma, we examined *MEG3* as well as *caspase-8* and *caspase-8* expression in 6 paired high IOP-induced ischemic retinal tissues with different reperfusion time points (6, 24, 48 and 72h) and sham-operated contralateral tissues by qRT-PCR and western blot. Our data revealed that the expression of *MEG3* (Fig. 1A, P<0.05) and *caspase-8* (Fig. 1B, P<0.05) were elevated while *caspase-8* expression (Fig. 1C, P<0.05) was decreased in ischemic retina at different time points after reperfusion relative to the sham-operated controls. Conformably, higher levels of *MEG3* (Fig. 2A, P<0.05) and *caspase-8* (Fig. 2B, P<0.05) were observed, whereas, *caspase-8* was significantly downregulated (Fig. 2C, P<0.05) in OGD/reoxygenation treated primary RGCs as compared with the normal cultured controls. Taken together, these data indicated that *MEG3*, *caspase-8* and *caspase-8* might be involved in the development of acute glaucoma.

MEG3 knockdown suppressed OGD-induced RGC apoptosis

To evaluate the biological functions of *MEG3*, the *MEG3* expression levels and apoptosis rate of RGCs following OGD and/or *MEG3* knockdown with siRNA transfection were analyzed by qRT-PCR and flow cytometry. Following OGD, the mRNA expression of *MEG3* (Fig. 3A, P<0.05) and the percentage of apoptotic RGCs (Fig. 3B, P<0.05)

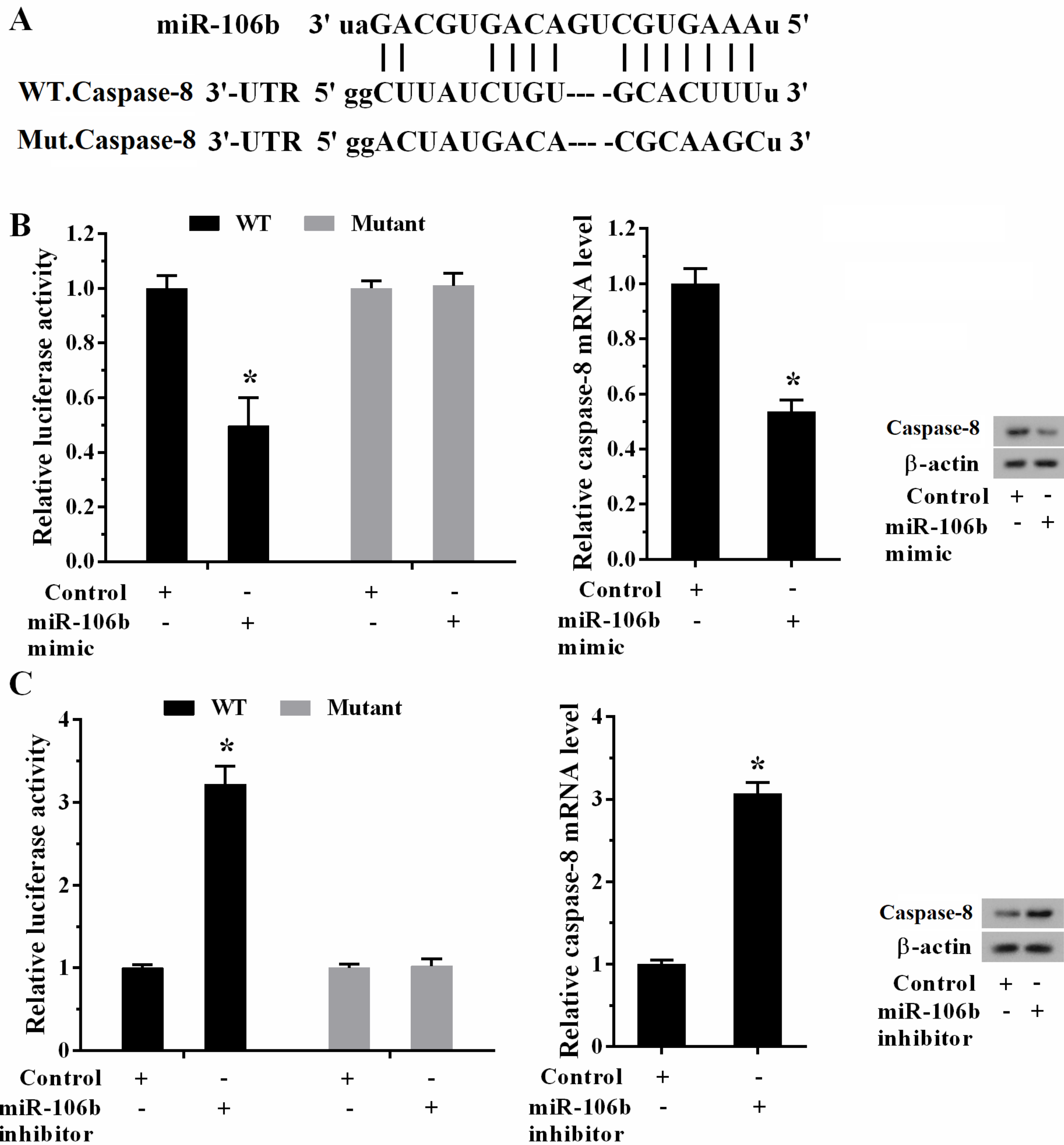

Figure 5. Effect of miR-106b on caspase-8 expression. (A) The potential gene target of miR-106b predicted by mircoRNA.org online database. (B) Relative luciferase activity of RGCs co-transfected with miR-106b mimic/pre-NC and caspase-8-3'UTR-WT or caspase-8-3'UTR-MUT plasmid. The mRNA and protein expression levels of caspase-8 in RGCs transfected with miR-106b mimic or pre-NC (control). (C) Relative luciferase activity of RGCs co-transfected with miR-106b inhibitor/NC and caspase-8-3'UTR-WT or caspase-8-3'UTR-MUT plasmid. The mRNA and protein expression levels of caspase-8 in RGCs transfected with miR-106b inhibitor or NC (control). *P<0.05 compared with the control group.

were markedly enhanced as compared with those of the normal control group. However, the knockdown of *MEG3* expression dramatically reduced the *MEG3* mRNA expression levels (Fig. 3A, P<0.05) and the apoptosis rate of RGCs (Fig. 3B, P<0.05) following OGD treatment. These findings demonstrated that *MEG3* inhibition reduced the apoptotic rate of RGCs in vitro.

MEG3 targeted *caspase-8*

MEG3 was reported to play important roles in post-transcriptional regulation in various cancers[16]. However, the specific downstream regulators involved in the abnormal expression of *MEG3* in acute glaucoma still remained unknown. Previously, our study has found that *MEG3* harbored one putative binding site for *caspase-8*

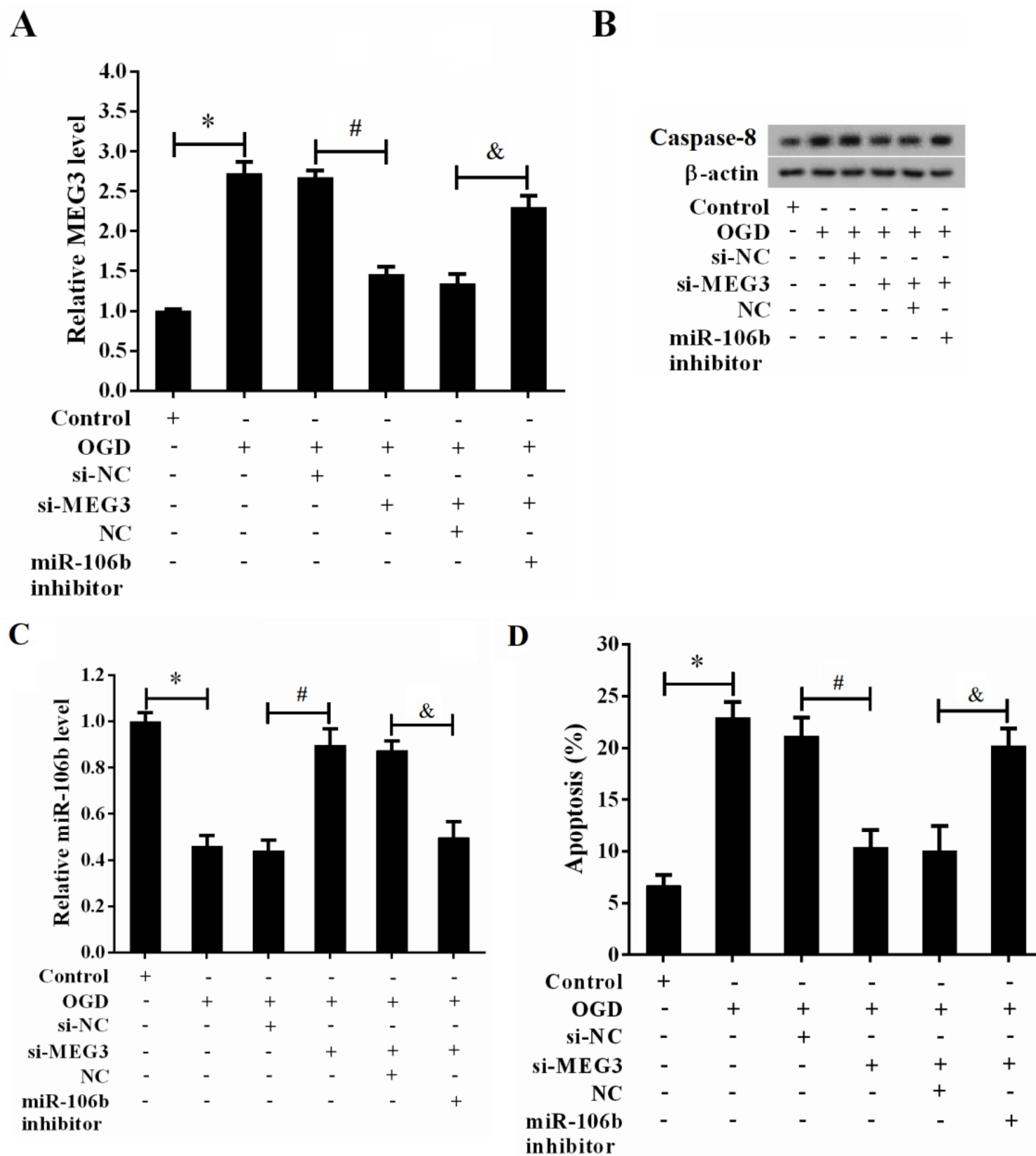

Figure 6. The molecular regulation and biological functions of MEG3 in OGD-induced I/R injury. (A)Relative MEG3 expression of RGCs in the group of control, OGD, OGD+si-Ctrl, OGD+si-MEG3, OGD+si-MEG3+NC and OGD+si-MEG3+miR-106b inhibitor. (B) The relative caspase-8 expression at protein levels of RGCs in the group of control, OGD, OGD+si-Ctrl, OGD+si-MEG3, OGD+si-MEG3+NC and OGD+si-MEG3+miR-106b inhibitor. (C) The relative mRNA expression of miR-106b of RGCs in the group of control, OGD, OGD+si-Ctrl, OGD+si-MEG3, OGD+si-MEG3+NC and OGD+si-MEG3+miR-106b inhibitor. (D) The percentage of apoptotic RGCs in the group of control, OGD, OGD+si-Ctrl, OGD+si-MEG3, OGD+si-MEG3+NC and OGD+si-MEG3+miR-106b inhibitor. *P<0.05 compared with the control group; #P<0.05 compared with OGD+si-Ctrl group; &P<0.05 compared with OGD+si-MEG3+NC group.

predicted by the online DIANA tools (Fig. 4A). The RNA immunoprecipitation and RNA pull-down assay were applied to confirm the potential binding protein. As shown in Fig. 4B, *MEG3* and *caspase-8* enrichment was observed in Ago2-RNA precipitates, while less enrichment was found in IgG precipitates (P<0.05). Furthermore, RNA pull down assay revealed that the expression levels of *caspase-8* in *MEG3* pulled down pellet was higher than those of beads and loading control (Fig. 4C, P<0.05). Together, these results demonstrated that *MEG3* targets *caspase-8*.

Caspase-8 negatively regulated *caspase-8*

It was well known that *caspase-8* was a carcinogenic

miRNA, in contrast, *caspase-8* acted as a tumor suppressor in cancers[17,18]. To explore whether *caspase-8* was a direct target of *caspase-8*, luciferase reporter assay was performed, since Targetscan software (http://www.targetscan.org) has predicted the interaction between *caspase-8* and *caspase-8* (Fig. 5A). The results revealed that the *caspase-8* mimic decreased the luciferase activity (Fig. 5B, P<0.05), while the *caspase-8* inhibitor elevated the luciferase activity (Fig. 5C, P<0.05) in *caspase-8*-WT co-transfected system, conversely, this *caspase-8*-MUT scarcely responded to neither *caspase-8* mimic nor *caspase-8* inhibitor. In addition, *caspase-8* overexpression led to a decrease in the expression of *caspase-8* at mRNA and protein levels (Fig. 5B, P<0.05), on the contrary, *caspase-8* inhibitor transfection reversed this trend (Fig. 5C, P<0.05), suggesting that the direct binding existed between *caspase-8* and *caspase-8*.

MEG3 exacerbated RGC apoptosis through *caspase-8*/*caspase-8* axis

We further gained insights into the molecular mechanism by which *MEG3* knockdown inhibited RGC apoptosis. As expected, OGD treatment upregulated the expression of *MEG3* (Fig. 6A, P<0.05) and *caspase-8* protein (Fig. 6B, P<0.05), downregulated the mRNA expression of *caspase-8* (Fig. 6C, P<0.05) and increased RGC apoptosis rate (Fig. 6D, P<0.05), respectively. Nevertheless, *MEG3* knockdown resulted in a marked reduction in the expression of *MEG3* (Fig. 6A, P<0.05) and *caspase-8* (Fig. 6B, P<0.05) as well as the percentage of apoptotic RGCs (Fig. 6D, P<0.05), but caused an elevated expression of *caspase-8* (Fig. 6C, P<0.05), however, co-transfection of si-*MEG3* and *caspase-8* inhibitor led to an opposite effect. Conjointly, our results manifested that *MEG3* deteriorated cell apoptosis of RGCs through regulating *caspase-8*/*caspase-8* axis in acute glaucoma.

DISCUSSION

Acute glaucoma is a momentously sight-threatening cause of non-reversible blindness worldwide featured with a sudden and extensive IOP increase, which in turn led to RGC apoptosis[19]. Increasing number of studies have demonstrated the fact that *MEG3* is aberrantly expressed in the pathogenesis and development of some tumors and functions as a novel tumor suppressor[20,21]. Hence, we speculated that *MEG3* might also play an underlying role in the development of acute glaucoma. In this study, we found that *MEG3* was upregulated in increased IOP-induced ischemic retinae following the mouse model of acute glaucoma as compared with the sham controls, which was consistent with a previous study showing that *MEG3* is expressed with higher levels following ischemia in adult mice[14]. Besides, it has recently been shown that the abnormal expression of *caspase-8* and *caspase-8* are observed in retinal ischemic injury[7,10], suggesting that they were the vital regulators in occurrence and progression of acute glaucoma. In our study, we also found a marked increase and decrease in the expression of *caspase-8* and *caspase-8*, respectively, in glaucomatous retinae. In the further in vitro experiments, we discovered that OGD treatment increased *MEG3* expression and *caspase-8* mRNA and protein expression while downregulated *caspase-8* mRNA expression in addition to facilitating RGC apoptosis.

To investigate the biological roles of *MEG3* in acute glaucoma, primary RGCs were transfected with si-*MEG3* or si-Ctrl following OGD/ reoxygenation treatment. Our current study showed that the knockdown of *MEG3* significantly resulted in a decrease of the percentage of apoptotic RGCs following OGD treatment in vitro, manifesting that *MEG3* might exert pro-apoptotic effect in glaucomatous RGCs. The aforementioned evidence has elucidated that *caspase-8* as well as *caspase-8* also have important roles in RGC apoptosis. Recently, evidence is emerging that lncRNAs are involved in regulation of downstream target miRNAs[22]. Therefore, investigations regarding the interaction between lncRNAs and miRNAs can deepen our understanding of the mechanisms underlying acute glaucoma. Therefore, we further explored the possible molecular mechanisms of *MEG3* action in RGCs. Interestingly, The RIP and RNA pull-down assay both confirmed that *MEG3* might be directly bind with *caspase-8* as expected. Our study further examined the interaction between *caspase-8* and *caspase-8* owing to existence of underlying binding sites of *caspase-8* and *caspase-8* predicted by Targetscan bioinformatics software. Luciferase reporter assay illustrated that *caspase-8* was a downstream target gene of *caspase-8*. What's more, the overexpression of *caspase-8* suppressed *caspase-8* expression at mRNA and protein levels, while *caspase-8* knockdown upregulated *caspase-8* in RGCs. Since *MEG3* downregulated *caspase-8* by direct targeting in vitro, we assumed that *MEG3* might regulate *caspase-8* through *caspase-8*.

To detect the correlation between *MEG3* and *caspase-8* in RGC apoptosis regulation, siRNA and miRNA inhibitor have been transfected into RGCs to knock down *MEG3* and *caspase-8*, respectively. We confirmed that *MEG3* and *caspase-8* were upregulated, whereas, *caspase-8* expression was inhibited by both *MEG3* and *caspase-8* knockdown after OGD treatment. In addition, the apoptosis of RGCs was promoted and the protein level of *caspase-8* was up-regulated. These data indicated that *MEG3* promoted RGC apoptosis following ischemia/reperfusion (I/R) injury induced by OGD/reoxygenation via negatively regulating *caspase-8*, which in turn directly targeted *caspase-8*.

There are still two limitations in the current study. First,

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although we have detected the expression of *MEG3*, *caspase-8* and *caspase-8* in the mouse model of glaucoma and OGD/reoxygenation induced cell model, we have no data about their expressions in the retinas of patients with primary open-angle glaucoma (POAG). Second, although we have demonstrated that *MEG3* promoted the apoptosis of OGD/ reoxygenation-induced RGC by regulating *caspase-8*/*caspase-8* pathway in vitro, the function of *MEG3* in acute glaucoma have not be verified in vivo. Therefore, in the future, we will perform more in-depth study to improve the two shortfalls and make our study more clinically significant.

In summary, the present study authenticated for the first time that the interaction might exist among the lncRNA *MEG3*, *caspase-8* and *caspase-8* in increased IOP-induced acute glaucoma. *MEG3* exacerbated ischemic RGC apoptosis via directly regulating *caspase-8*/*caspase-8* axis. Our research would deepen the understanding of the pathogenesis of acute glaucoma and provide a novel insight into seeking for the treatment strategy of it.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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