Original paper

CTRP9 mitigates the apoptosis and unfolded protein response of OGD/R-induced retinal ganglion cells by regulating the AMPK pathway

Xiaofan Yang[1], Yuling Niu[2]

[1]Department of Ophthalmology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), Hangzhou, China
[2]Department of Ophthalmology, The People's Hospital of Baoan Shenzhen, Shenzhen, China

C1q/TNF-related protein-9 (CTRP9) has been reported to play roles in several types of retinal diseases. However, the role and the potential mechanism of CTRP9 in glaucoma are still incompletely understood. The expression of CTRP9 in OGD/R-induced retinal ganglion cells (RGCs) was detected by quantitative real-time polymerase chain reaction and western blot assay.

Cell proliferation was identified by cell counting Kit-8 assay. Flow cytometry, enzyme-linked immunosorbent assay and western blot assay were performed to assess cell apoptosis. Unfolded protein response (UPR), endoplasmic reticulum (ER) stress and the AMPK pathway were evaluated by western blot assay.

The data showed that the expression of CTRP9 was significantly downregulated in OGD/R-induced 661W cells. OGD/R treatment reduced cell viability, promoted cell apoptosis and activated the UPR and ER stress. The overexpression of CTRP9 reversed the effects of OGD/R on 661W cell viability, apoptosis, the UPR and ER stress, as well as the AMPK pathway. However, Compound C, an inhibitor of AMPK signaling, reversed the protection of CTRP9 overexpression against injury from OGD/R in 661W cells.

In summary, the results revealed that CTRP9 abated the apoptosis and UPR of OGD/R-induced RGCs by regulating the AMPK pathway, which may provide a promising target for the treatment of glaucoma.

Key words: C1q/TNF-related protein-9, glaucoma, OGD/R, AMPK, unfolded protein response.

Introduction

Glaucoma encompasses a range of chronically progressive disorders affecting the optic nerve, characterized by the expansion of optic nerve physiology sag and the progressive degeneration of retinal ganglion cells (RGCs) [1]. Retinal ganglion cells apoptosis and optic nerve fiber loss are the pathological basis of glaucoma blinding, and blindness caused by glaucoma is usually irreversible [2]. Studies have found that elevated intraocular pressure is the main pathogenic factor of glaucoma, and reducing intraocular pressure is the main clinical treatment. However, the causes of glaucoma are complex, so simply lowering intraocular pressure cannot completely prevent the development of glaucoma [3–5]. The retinal ganglion cells are neurons of the central nervous system and they can receive signals, process them, and transmit them in axons through the optic nerve to further centers in the brain [6]. The apoptosis of RGCs is

considered to be closely related to the pathogenesis of glaucoma, which is affected by a variety of factors, including interrupting nutritional factors, abnormal blood supply, increased glutamate and nitric oxide and gene mutations [7]. Thus, it is of critical importance for us to explore the molecular mechanisms involved in the apoptosis of RGCs and how to prevent the loss of RGCs in glaucoma.

C1q/TNF-related protein-9 (CTRP9) is a newly discovered adipose tissue-derived cytokine, which is involved in the clearance of apoptotic cells by phagocytes, regulation of the inflammatory response and immune tolerance [8]. A previous study revealed that CTRP9 is associated with regulation of the biological roles of the retina [9]. CTRP9 is reported to attenuate retinal inflammation, protect the blood-retinal barrier, and alleviate the vascular leakage in the early stage of diabetic retinas [10]. CTRP9 was significantly downregulated in HG-induced ARPE-19 cells and attenuated HG-induced oxidative stress and prevented apoptosis in human retinal pigment epithelial cells *via* activation of the AMPK/Nrf2 pathway [11]. Li *et al*. also reported that CTRP9 was lowly expressed in retinal tissues from mice with diabetes mellitus and CTRP9 overexpression attenuated retinal inflammation and protected the blood-retinal barrier in db/db mice [12]. In addition, CTRP9 protected against acute cardiac ischemia-reperfusion injury *via* an AMPK-dependent mechanism [13]. CTRP9 also improved neurofunctional behavior and inhibited the neuronal apoptosis in mice subjected to experimental intracerebral hemorrhage [14]. Although an important role for CTRP9 in the retina and neurons has been researched, molecular mechanisms regulated by CTRP9 during glaucoma remain unclear. Therefore, we aimed to explore the role of CTRP9 in oxygen-glucose deprivation/reperfusion (OGD/R)-induced RGCs and how CTRP9 influences the process of glaucoma.

Material and methods

Cell culture and treatment

The 661W photoreceptor cells, which were derived from a murine retinal tumor and have similar cellular and biochemical characteristics to cone photoreceptor neurons, were provided by Dr. Muayyad Al-Ubaidi (University of Oklahoma Health Sciences Center) [15–17]. 661W cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humid incubator with 5% CO_2. To establish an *in vitro* OGD/R model, 661W cells were cultured with glucose-free DMEM in an oxygen-free incubator with 5% CO_2 and 95% N_2 at 37°C for 2 hours. After hypoxia, the cells were transferred to normal medium in an atmosphere with 95% air and 5% CO_2 for 12 hours. To explore the relationship between CTRP9 and the AMPK signaling pathway, Compound C (10 μM), an inhibitor of AMPK, was adopted to treat 661W cells for 30 min.

Cell transfection

CTRP9-specific pcDNA overexpression vector (Oe-CTRP9) and corresponding negative control (Oe-NC) were synthesized by GenePharma (Shanghai, China). These recombinants were transfected into 661W cells using Lipofectamine 2000 reagent (Invitrogen, USA) according to the manufacturer's instructions. After 48 h, cells were harvested for the following assays.

Cell counting Kit-8 assay

661W cells were transfected with Oe-CTRP9 and exposed to OGD/R. After that, the cells were seeded into 96-well plates at a density of 5×10^4 cells/ml and cultured in DMEM with 10% FBS for 24, 48 and 72 hour. After the time points, 10 μl of cell counting Kit-8 (CCK-8) solution (Beyotime, Haimen, China) was added to each well and the plates were incubated at 37°C for 2 hours, and then the absorbance at 450 nm was measured with Microplate Reader (Bio-Rad, La Jolla, CA, USA).

Flow cytometry

Cell apoptosis was detected using FITC Annexin V/PI Apoptosis Detection Kit I (Ribobio, Guangzhou, China) according to the manufacturer's protocol. Cells were transfected with Oe-CTRP9 and exposed to OGD/R. Subsequently, cells were collected, washed with precooled phosphate buffer saline and re-suspended in binding buffer. Cells were incubated with 5 μl of Annexin V-FITC at normal temperature for 15 min, followed by incubation with 10 μl of propidium iodide (PI, 10 mg/ml) in a dark room for 5 min. Flowjo software (Tree Star, Ashland, OR, USA) was used for apoptosis analysis.

Enzyme-linked immunosorbent assay

The contents of cleaved caspase 3 in 661W cells were determined with enzyme-linked immunosorbent assay (ELISA) kits according to the instructions. The color absorbance was measured at 450 nm using a BioTek microplate reader (Winooski, VT, USA).

Quantitative real-time polymerase chain reaction

Total RNAs were extracted from 661W cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The quality and concentration of RNA were detected using a NanoDrop 2000 (Quawell, San Jose, CA, USA) at 260 and 280 nm. A cDNA Synthesis Kit

(TaKaRa, Japan) was used to reverse transcribe 2 μg of RNA into cDNA. Amplification of the cDNA was performed by quantitative real-time polymerase chain reaction (qRT-PCR) using the SYBR Premix Ex Taq II Kit (Takara, Shiga, Japan). The relative mRNA level was normalized with GAPDH by the $2^{-\Delta\Delta Ct}$ method.

Western blot assay

The total proteins were extracted from 661W cells using RIPA buffer (Auragene, Changsha, China). The BCA Protein Assay Kit (Dingguo, Beijing, China) was used to detect the protein concentration according to the manufacturer's instructions. Protein samples were separated by 10% SDS-PAGE and electrophoretically transferred onto PVDF membranes. Next, the membrane was blocked in 5% nonfat milk for 1.5 hour and then incubated with primary antibodies (Bax, Bcl-2, p-PERK, PERK, p-eIF2α, eIF2α, GRP78, ATF6, CHOP, Caspase 12, XBP1, p-AMPK, AMPK, GAPDH) overnight at 4°C. Then, the membranes were incubated with anti-mouse or anti-rabbit secondary antibodies. The immunoreactive protein bands were visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions. The density of the band was determined using ImageJ software (Version 1.49; NIH, Bethesda, MD, USA).

Statistical analysis

All statistical analyses were conducted using statistical analysis software (SPSS, Version 23.0). All data are presented as the mean ±SD (standard deviation) from at least three independent experiments. Student's *t*-test was used for the comparison between two groups. Results were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test for multiple comparisons. Values of $p < 0.05$ were considered to be statistically significant.

Results

CTRP9 was downregulated in OGD/R-induced 661W cells, and upregulation of CTRP9 increased cell viability

To investigate the role of CTRP9 in the glaucoma development, we detected the expression of CTRP9 in OGD/R-induced 661W cells. As shown in Figures 1A, B, the mRNA expression and protein level of CTRP9 in OGD/R-induced 661W cells were significantly lower than those in untreated 661W cells. Then we transfected Oe-CTRP9 to upregulate CTRP9 in OGD/R-induced 661W cells and the transfection efficiency was detected by qRT-PCR and western blot analysis (Figs. 1C, D). CCK-8 assay showed that the capacity of cell proliferation was remarkably inhibited after treatment of OGD/R and CTRP9 overexpression increased the cell proliferation in OGD/R-induced 661W cells (Fig. 1E).

Upregulation of CTRP9 inhibited OGD/R-induced apoptosis of 661W cells

To explore the biological roles of CTRP9 in OGD/R-induced 661W cells, we measured the cell apoptosis. As shown in Figures 1A, B, the apoptosis rate of OGD/R-induced 661W cells was noticeably increased while CTRP9 overexpression alleviated the apoptosis rate of 661W cells (Figs. 2A, B). In addition, ELISA results showed that OGD/R increased the level of cleaved caspase 3 and CTRP9 overexpression reduced the increased cleaved caspase 3 level (Fig. 2C). Western blot assay revealed that OGD/R led to an increased Bcl-2 level and decreased Bax level, while upregulation of CTRP9 reversed the levels of Bcl-2 and Bax (Fig. 2D).

CTRP9 overexpression repressed OGD/R-induced unfolded protein response and endoplasmic reticulum stress in 661W cells

Then, we investigated the effects of CTRP9 overexpression on unfolded protein response (UPR) and endoplasmic reticulum (ER) stress in OGD/R-induced 661W cells. As shown in Figure 3, OGD/R treatment significantly enhanced the protein levels of p-PERK, p-eIF2α, GRP78, ATF6, CHOP, Caspase 12 and XBP1 in 661W cells. However, CTRP9 overexpression reversed the levels of these proteins in OGD/R-induced 661W cells.

AMPK inhibition reversed the protective effect of CTRP9 overexpression on OGD/R-induced 661W cells

To investigate the potential mechanism by which CTRP9 overexpression regulates glaucoma, we detected the expressions of the AMPK signaling in 661W cells. As shown in Figure 4A, the level of p-AMPK was inhibited by OGD/R and CTRP9 overexpression rehabilitated the decreased p-AMPK level. CCK-8 assay revealed that the inhibitor of AMPK Compound C decreased the cell proliferation of OGD/R-induced 661W cells (Fig. 4B). Flow cytometry showed that the inhibition of AMPK increased the rate of cell apoptosis (Figs. 4C, D). In addition, Compound C treatment elevated the protein levels of cleaved caspase 3 and Bax while it reduced the Bcl-2 level (Figs. 4E, F). Moreover, Compound C promoted the protein expression of p-PERK, p-eIF2α, GRP78, ATF6, CHOP, caspase 12 and XBP1 in OGD/R-induced 661W cells transfected with Oe-CTRP9 (Fig. 5).

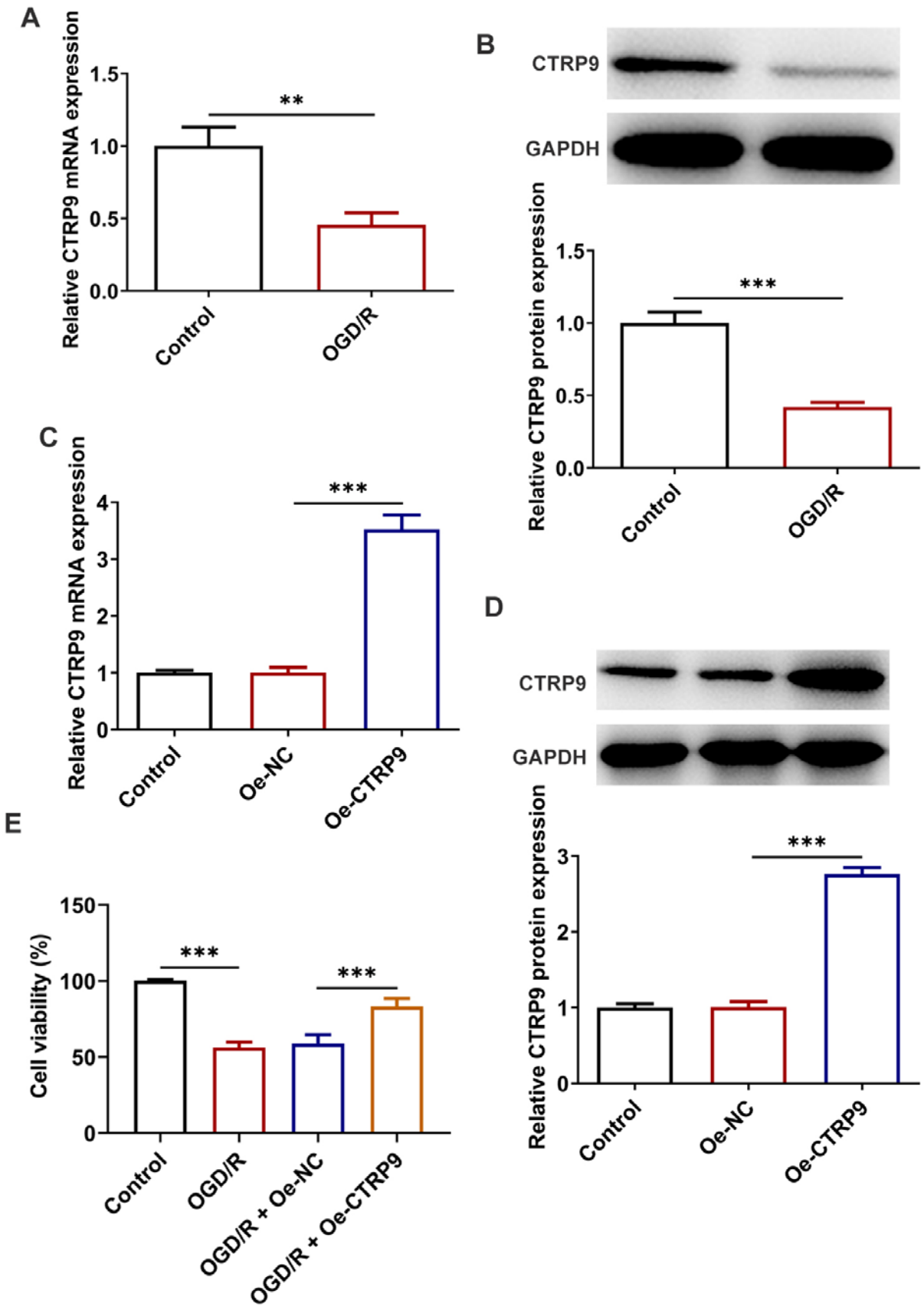


Fig. 1. C1q/TNF-related protein-9 (CTRP9) is downregulated in OGD/R-induced 661W cells, and upregulation of CTRP9 increases the cell viability. mRNA expression (A) and protein level (B) of CTRP9 in OGD/R-induced 661W cells were detected by quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting. mRNA expression (C) and protein level (D) of CTRP9 in OGD/R-induced 661W cells transfected with Oe-CTRP9 were detected by qRT-PCR and western blotting. E) Cell proliferation was evaluated by cell counting Kit-8 assay

Results are the mean ±SD.

***$p < 0.01$, ***$p < 0.001$*

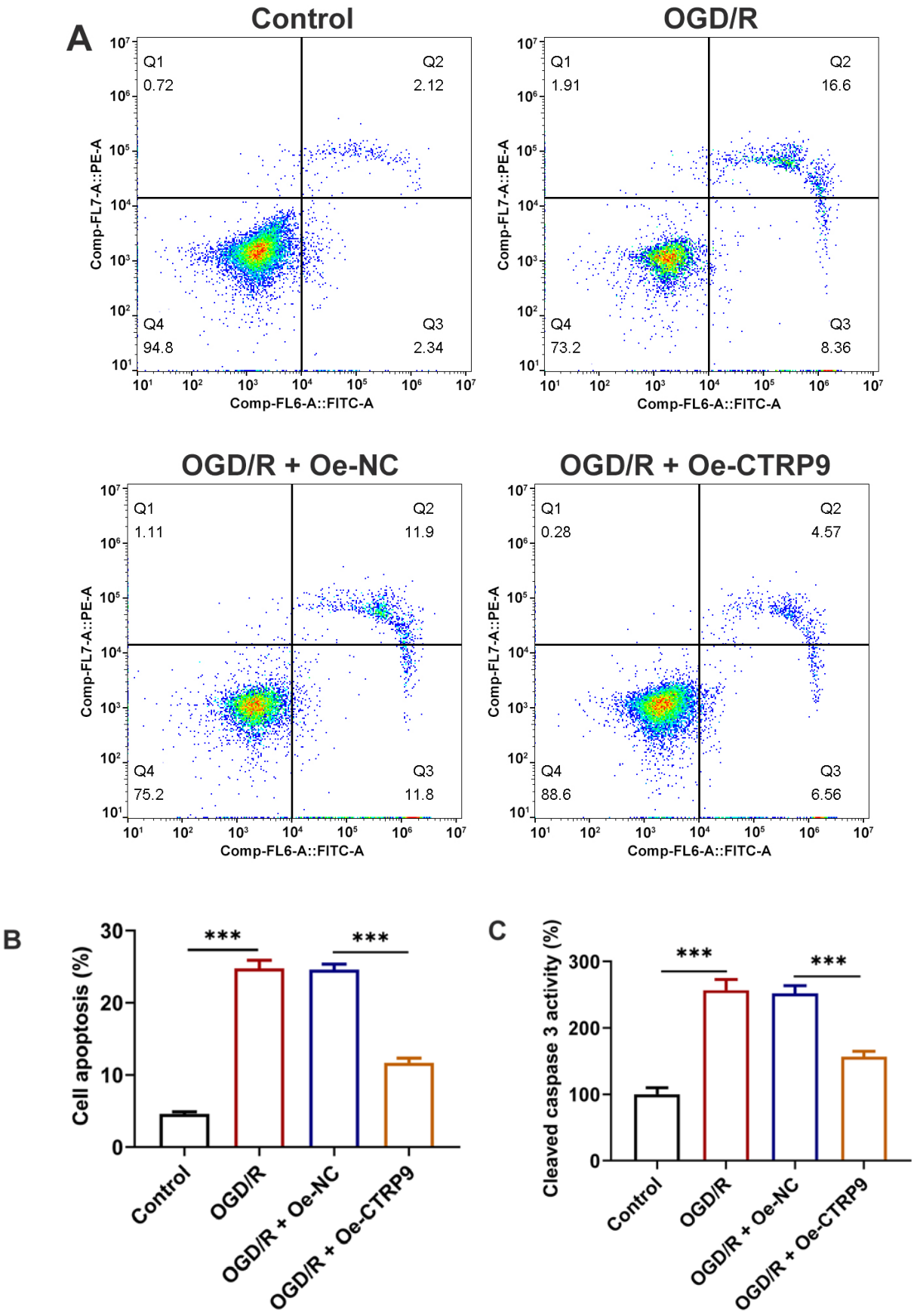


Fig. 2. Upregulation of C1q/TNF-related protein-9 inhibits OGD/R-induced apoptosis of 661W cells. A, B) Cell apoptosis was detected by flow cytometry. C) The level of cleaved caspase 3 was detected by enzyme-linked immunosorbent assay

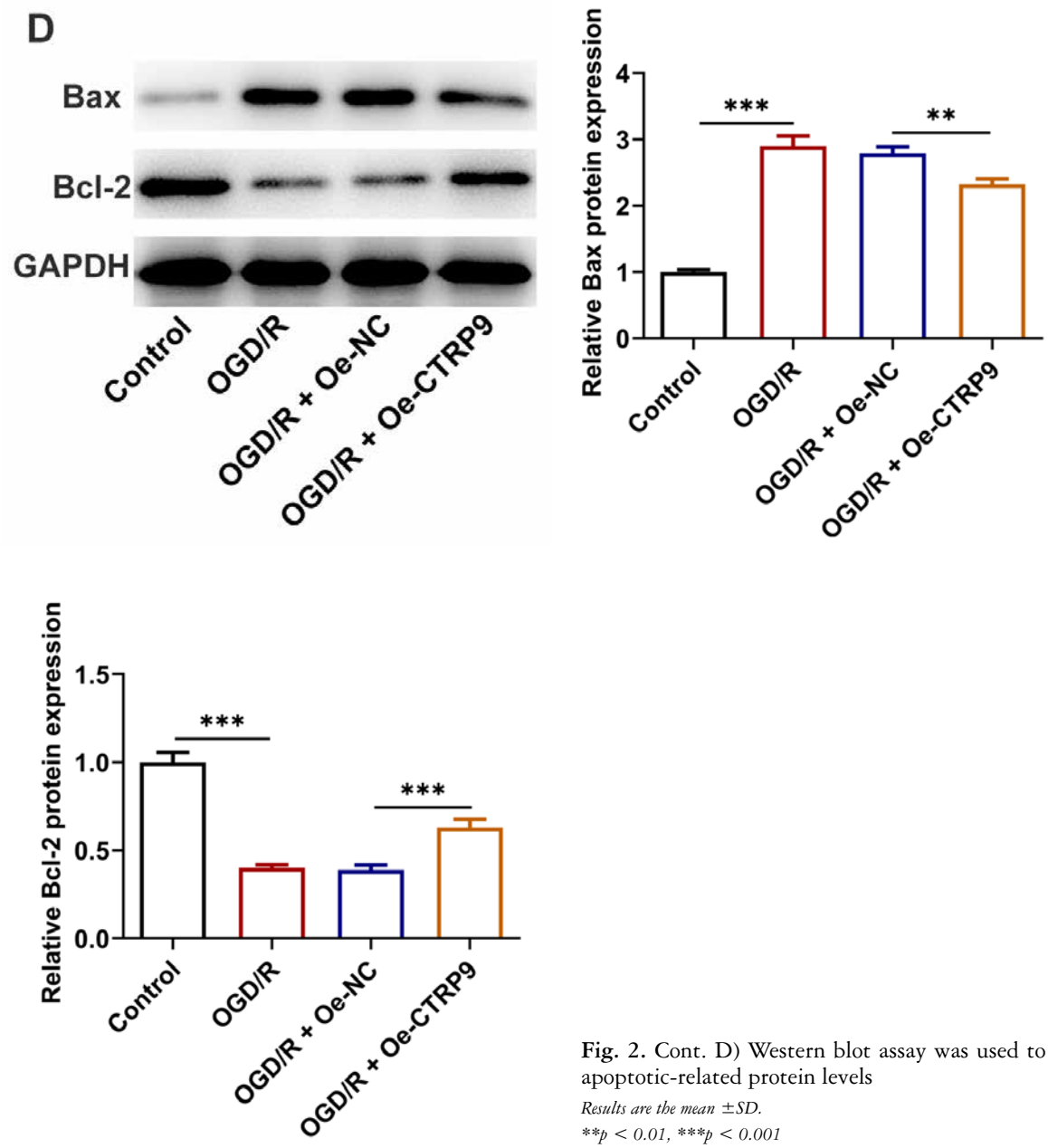


Fig. 2. Cont. D) Western blot assay was used to evaluate apoptotic-related protein levels

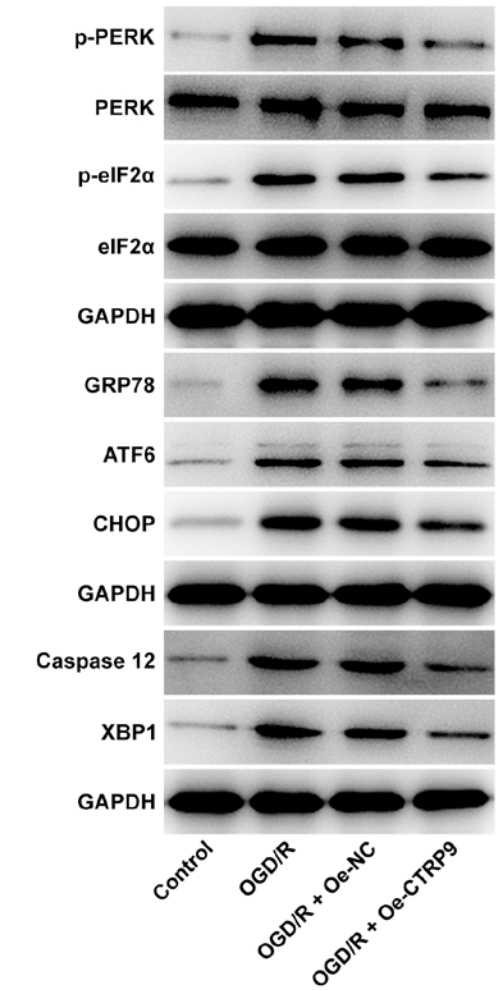
Results are the mean ±SD.

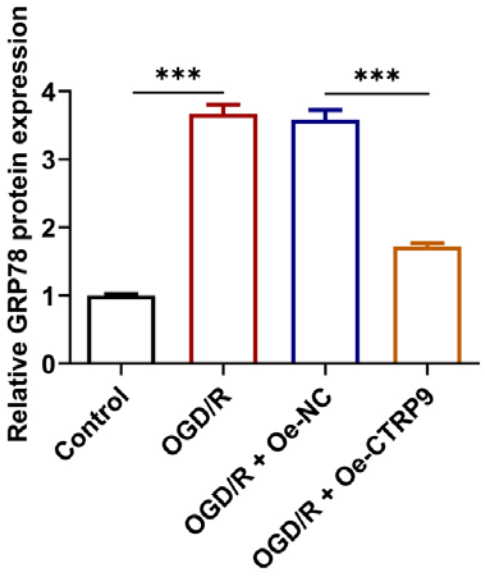
***p < 0.01, ***p < 0.001*

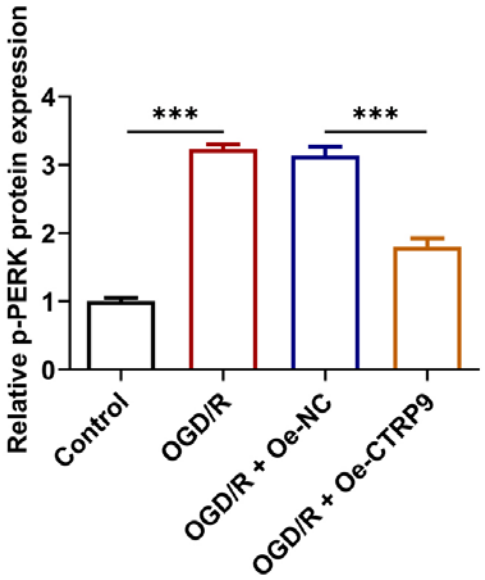
Discussion

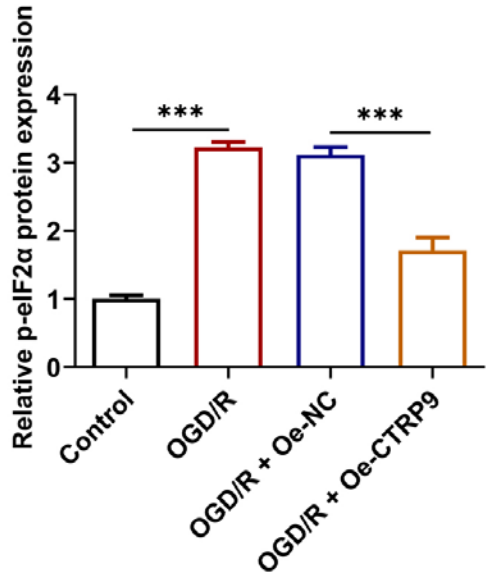
Glaucoma is a complex eye disease characterized by loss of RGCs and damage to the optic nerve, resulting in impaired vision [18]. Currently, the only effective clinical treatment for glaucoma is to prevent vision loss by reducing eye pressure with drugs or surgery, but it cannot prevent the effects of retinal ganglion cell loss on visual function [19–21]. Studies have shown that RGC apoptosis and optic nerve axon loss are the fundamental causes of optic nerve injury in glaucoma [22, 23]. Thus, it is crucial to protect the function of RGCs and inhibit cell apoptosis. In this study, CTRP9 expression was downregulated in OGD/R-induced RGCs. Overexpression of CTRP9 restrained cell apoptosis, UPR and ER stress. In addition, CTRP9 overexpression inhibited AMPK signaling and AMPK inhibition reversed the protective effect of CTRP9 overexpression on OGD/R-induced 661W cells.

CTRPs, as bioactive cardiokines, are secreted by adipose tissue and cardiac endothelial cells [24]. Further molecular identification of the CTRP family has shown various 15 constituents, including CTRP1 to 15, which are adiponectin paralogs [25]. CTRP9 has been reported to play regulatory roles in multiple types of ischemia/reperfusion injury [13, 26]. A study showed that cardiac-derived CTRP9 protected against myocardial ischemia/reperfusion injury by calreticulin-dependent inhibition of apoptosis [27]. Another study revealed that CTRP9 suppressed ER stress to protect diabetic rat hearts from isch-









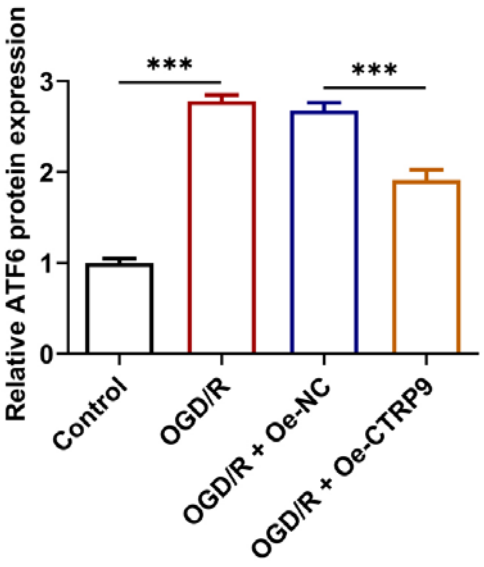


Fig. 3. C1q/TNF-related protein-9 overexpression represses OGD/R-induced unfolded protein response and endoplasmic reticulum stress in 661W cells. Western blot assay was used to evaluate the protein levels of p-PERK, PERK, p-eIF2α, eIF2α, GRP78, ATF6, CHOP, caspase 12 and XBP1 in OGD/R-induced 661W cells

Results are the mean ±SD.
****p < 0.001*

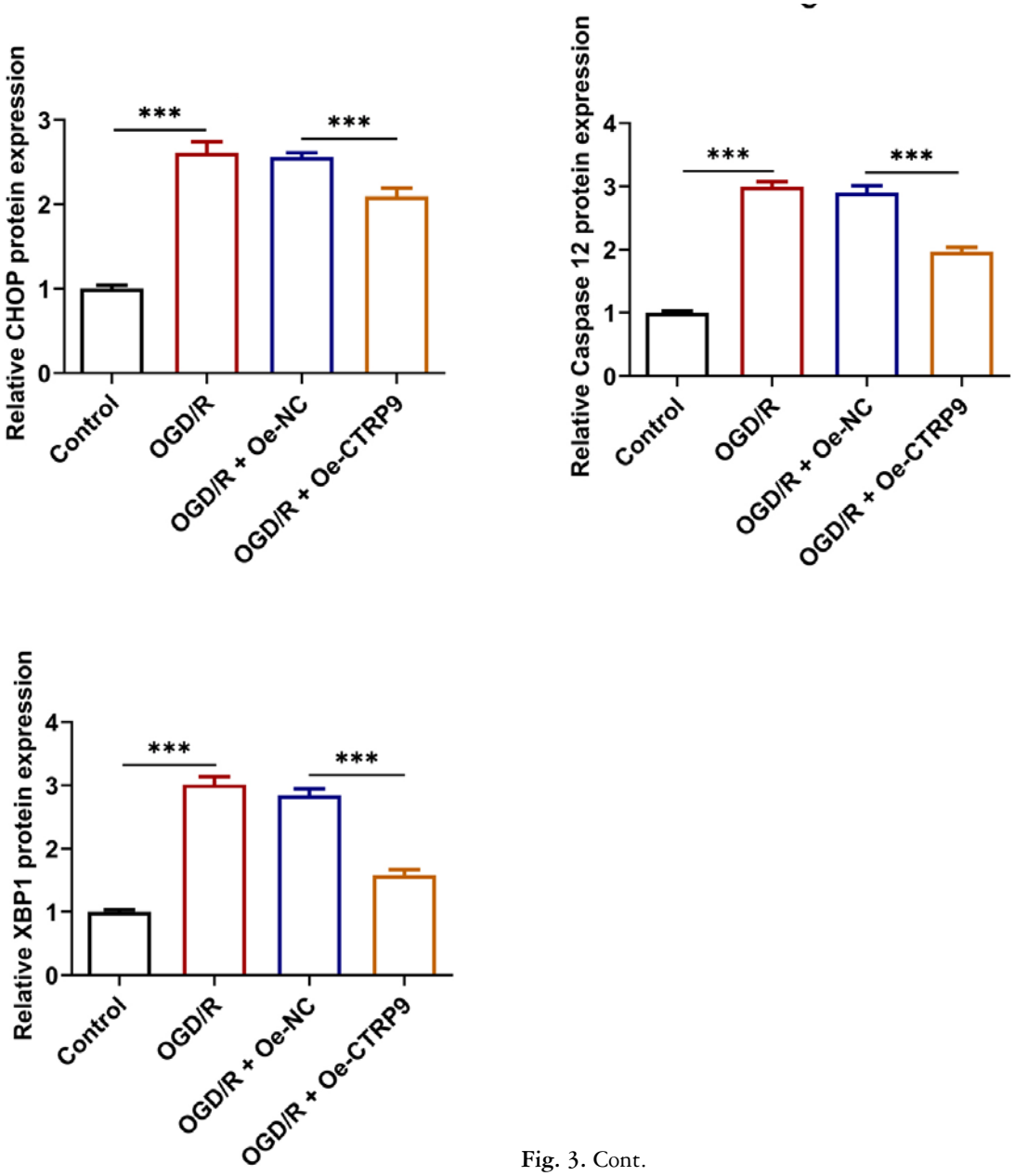


Fig. 3. Cont.

emia/reperfusion injury through increasing the disulfide-bond A oxidoreductase-like protein [28]. In addition, CTRP9 can improve cell senescence through PGC-1α/AMPK signaling in mesenchymal stem cells, which is expected to enhance the therapeutic effect of stem cells in post-stroke brain repair [29]. In intracerebral hemorrhage mice, the activation of AdipoR1 with CTRP9 can improve neural function and maintain blood-brain barrier integrity through the APPL1/AMPK/Nrf2 signaling pathway [30]. In the current study, CTRP9 was lowly expressed in OGD/R-induced 661W cells and CTRP9 overexpression elevated the OGD/R-induced decreased cell viability, and inhibited OGD/R-induced cell apoptosis, UPR and ER stress, which was consistent with previous reports. However, we only performed *in vitro* experiments and did not conduct animal or clinical trials. The expression and functional roles of CTRP9 in either an *in vivo* system or patient samples with retinal I/R should be explored in a further study.

AMPK is the central regulator of both lipid and glucose metabolism and is considered to be a potential therapeutic target for various diseases, including eye disorders [31]. Li *et al*. reported that metformin eye drops prevented fibrosis after glaucoma filtering surgery in rats by activating the AMPK/Nrf2 signaling pathway [32. Kim *et al*. reported that apelin-13 inhibited methylglyoxal (MGO) induced UPR and endothelial dysfunction by regulating the AMPK pathway, and MGO signaling-induced endothelial cell apoptosis was mediated by ER stress, which led to activation of the UPR [33]. Moreover, the CXCR3

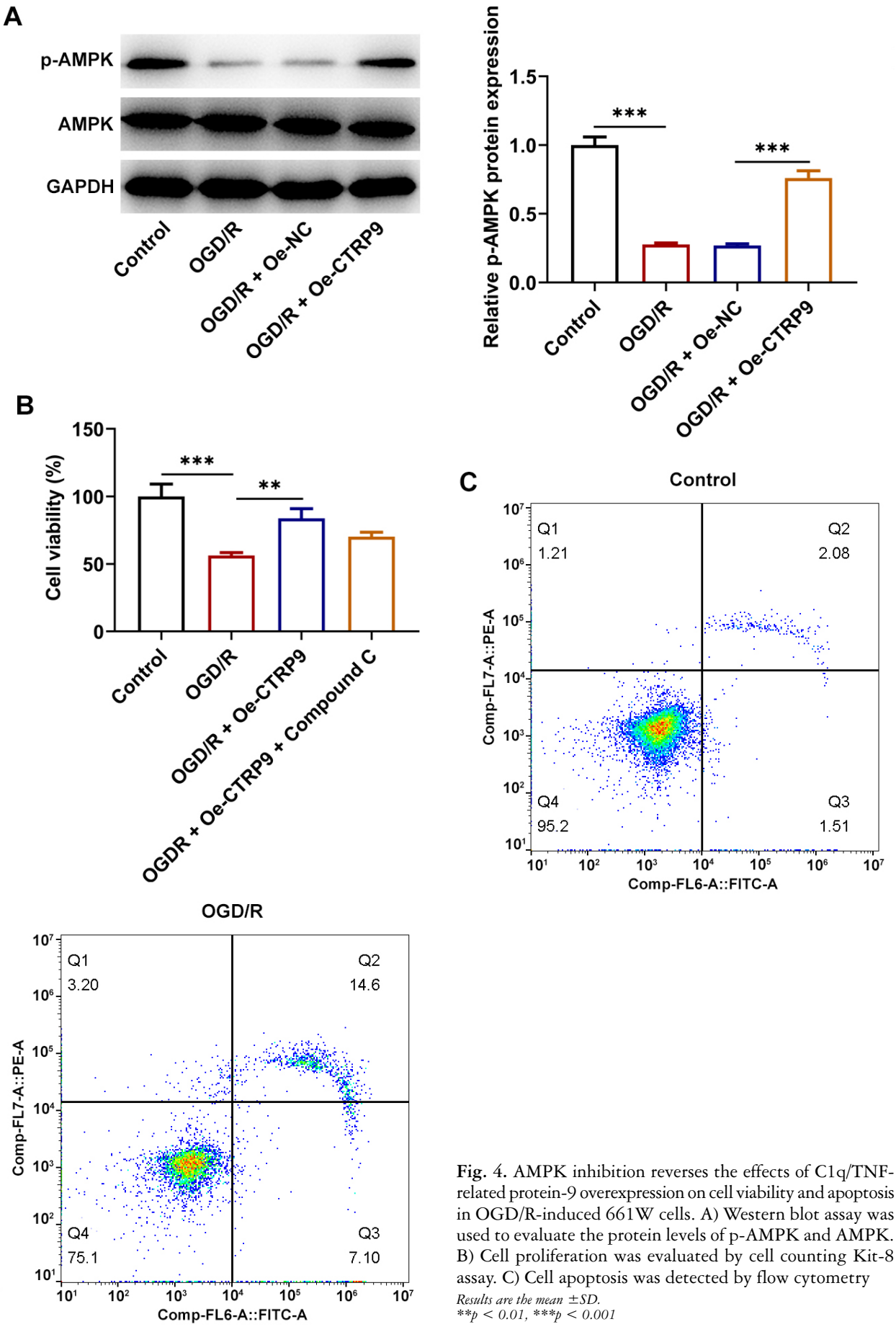


Fig. 4. AMPK inhibition reverses the effects of C1q/TNF-related protein-9 overexpression on cell viability and apoptosis in OGD/R-induced 661W cells. A) Western blot assay was used to evaluate the protein levels of p-AMPK and AMPK. B) Cell proliferation was evaluated by cell counting Kit-8 assay. C) Cell apoptosis was detected by flow cytometry

Results are the mean ±SD.
***p < 0.01, ***p < 0.001*

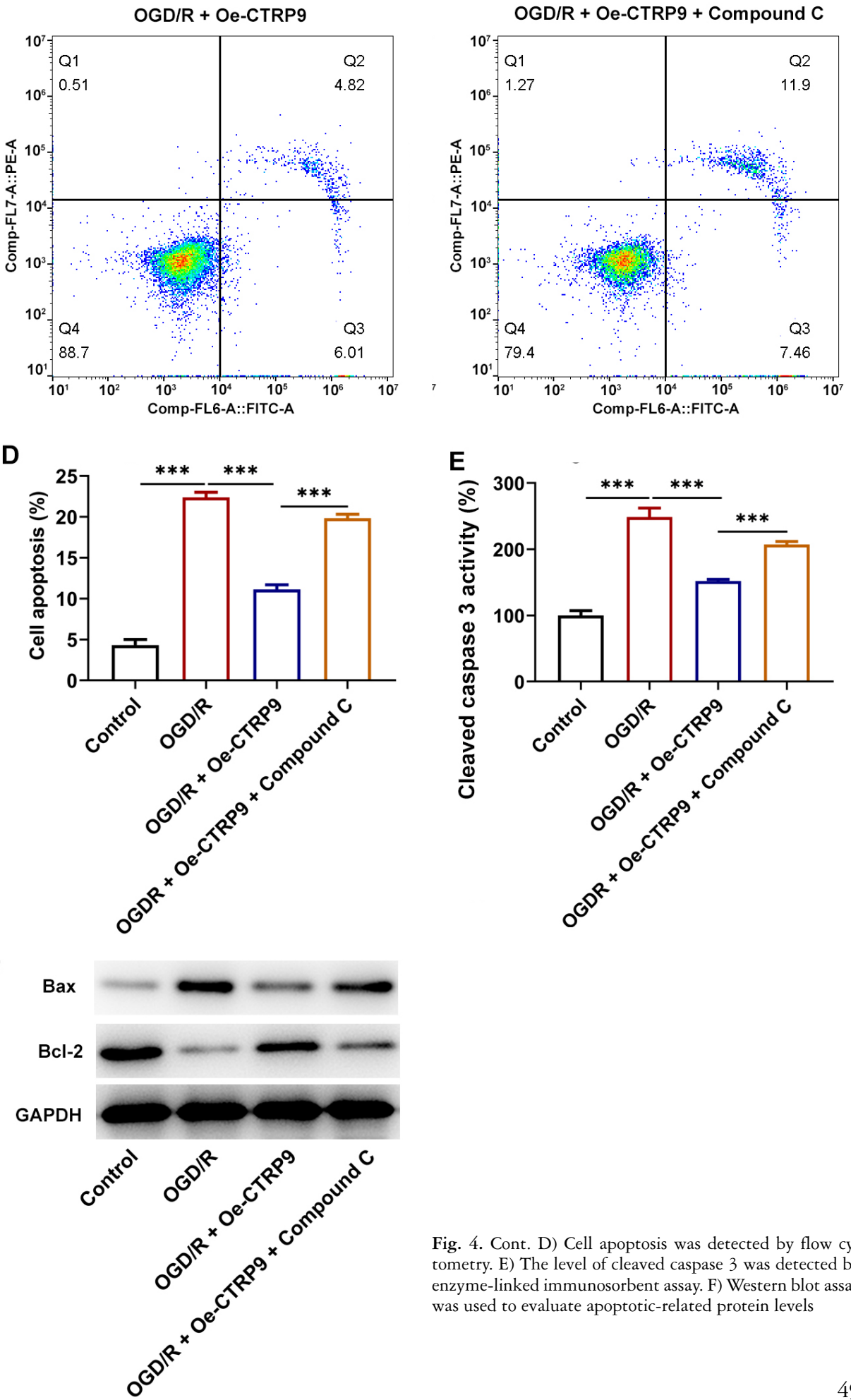


Fig. 4. Cont. D) Cell apoptosis was detected by flow cytometry. E) The level of cleaved caspase 3 was detected by enzyme-linked immunosorbent assay. F) Western blot assay was used to evaluate apoptotic-related protein levels

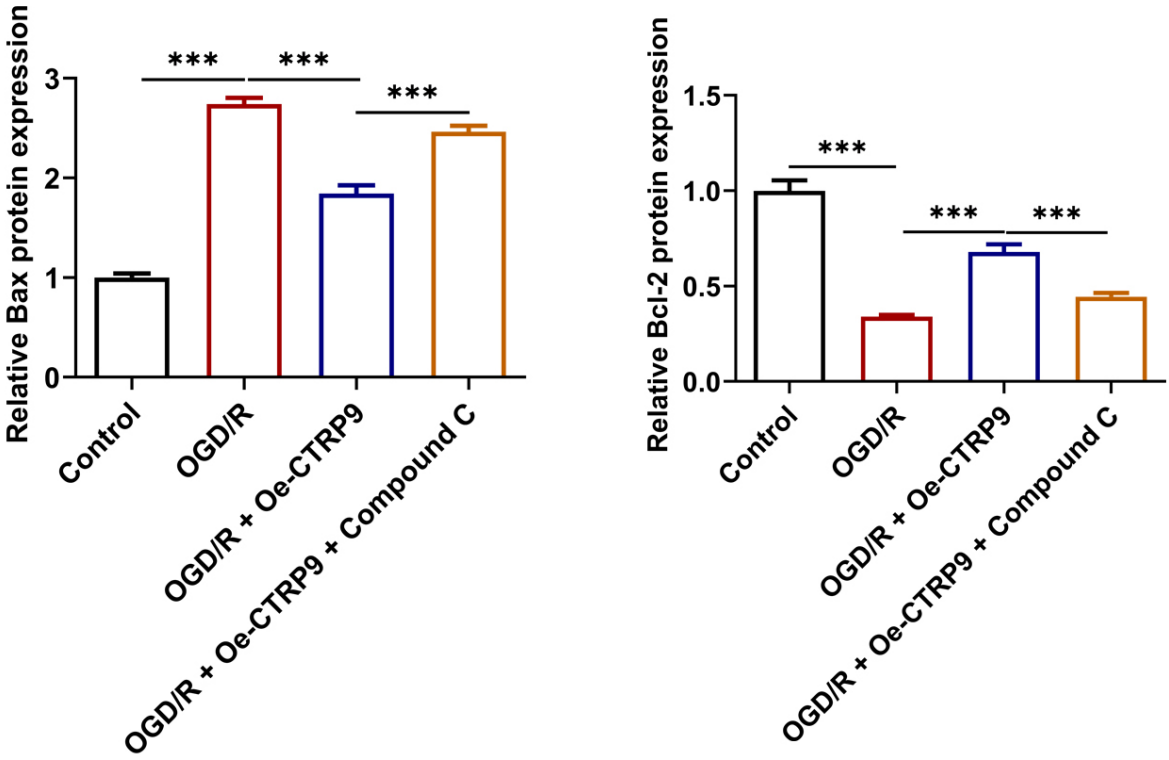


Fig. 4. Cont. F) Western blot assay was used to evaluate apoptotic-related protein levels

pathway regulated by ER stress was found to mediate inflammation and neuronal damage in acute glaucoma [34]. Chitranshi *et al*. demonstrated that sustained Src homology region 2-containing protein tyrosine phosphatase 2 (Shp2) upregulation induced RGC degeneration and silencing of Shp2 in glaucoma reversed the inner retinal degenerative phenotype in glaucoma [35]. In addition, CTRP9 was reported to suppress high glucose-induced oxidative stress and apoptosis in retinal pigment epithelial cells through activation of the AMPK/Nrf2 signaling pathway [11]. In this study, CTRP9 overexpression activated the AMPK signaling in OGD/R-induced 661W cells. When the inhibitor of AMPK signaling was added, the effects of CTRP9 overexpression on cell viability, apoptosis, UPR and ER stress were reversed, indicating that CTRP9 regulated OGD/R-induced RGCs by mediating the AMPK signaling. However, we did not add the inhibitor of CTRP9 because CTRP9 was downregulated in OGD/induced 661W cells and CTRP9 overexpression may show the roles of CTRP9 better, which will be considered in our further study.

Conclusions

In summary, the study indicated the functions of CTRP9 in OGD/R-induced RGC cell viability, apoptosis, UPR and ER stress by regulating AMPK signaling, suggesting that CTRP9 could be a promising therapeutic strategy for glaucoma.

The authors declare no conflict of interest.

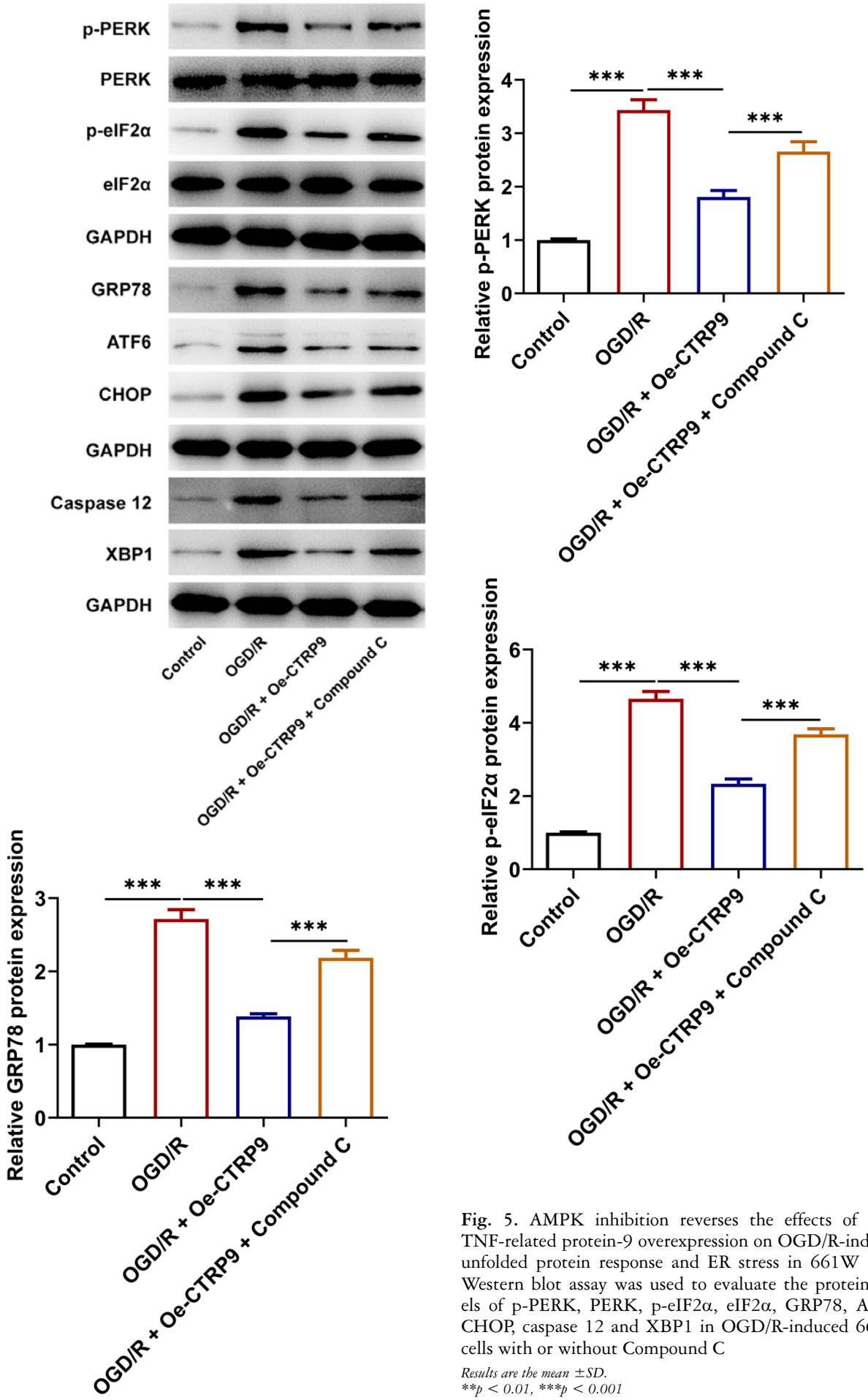


Fig. 5. AMPK inhibition reverses the effects of C1q/TNF-related protein-9 overexpression on OGD/R-induced unfolded protein response and ER stress in 661W cells. Western blot assay was used to evaluate the protein levels of p-PERK, PERK, p-eIF2α, eIF2α, GRP78, ATF6, CHOP, caspase 12 and XBP1 in OGD/R-induced 661W cells with or without Compound C

Results are the mean ±SD.
***p < 0.01, ***p < 0.001*

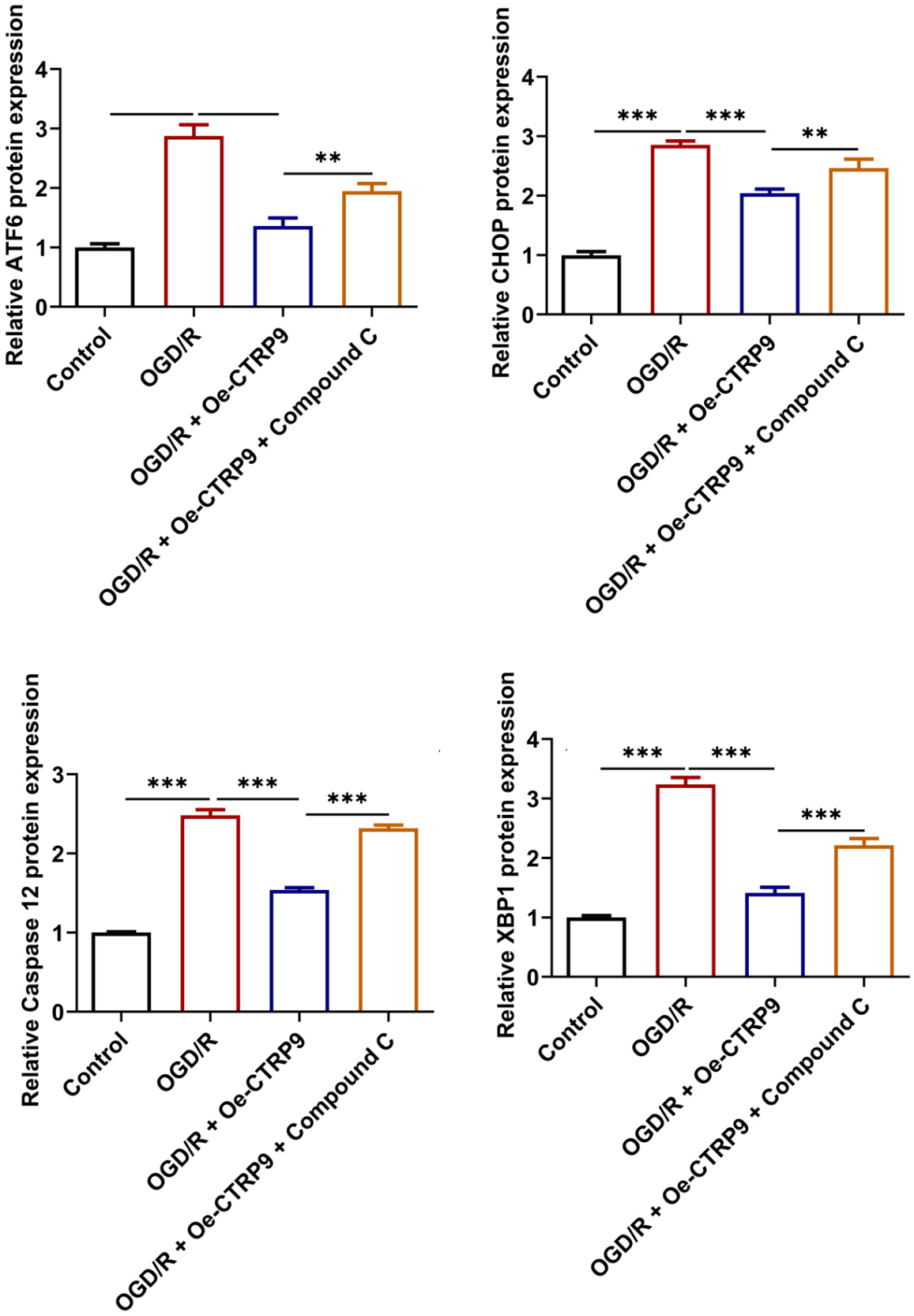

Relative ATF6 protein expression
0
1
2
3
4
**
Control
OGD/R
OGD/R + Oe-CTRP9
OGD/R + Oe-CTRP9 + Compound C
Relative CHOP protein expression
0
1
2
3
4
***
***
**
Control
OGD/R
OGD/R + Oe-CTRP9
OGD/R + Oe-CTRP9 + Compound C
Relative Caspase 12 protein expression
0
1
2
3
***
***
***
Control
OGD/R
OGD/R + Oe-CTRP9
OGD/R + Oe-CTRP9 + Compound C
Relative XBP1 protein expression
0
1
2
3
4
***
***
***
Control
OGD/R
OGD/R + Oe-CTRP9
OGD/R + Oe-CTRP9 + Compound C

Fig. 5. Cont.

References

1. Kang JM, Tanna AP. Glaucoma. Med Clin N Am 2021; 105: 493-510.
2. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. Jama 2014; 311: 1901-1911.
3. He S, Stankowska DL, Ellis DZ, Krishnamoorthy RR, Yorio T. Targets of neuroprotection in glaucoma. J Ocular Pharmacol Ther 2018; 34: 85-106.
4. Thau A, Lloyd M, Freedman S, et al. New classification system for pediatric glaucoma: implications for clinical care and a research registry. Curr Opin Ophthalmol 2018; 29: 385-394.
5. Graham KL, McCowan C, White A. Genetic and biochemical biomarkers in canine glaucoma. Vet Pathol 2017; 54: 194-203.
6. Detwiler PB. Phototransduction in retinal ganglion cells. Yale J Biol Med 2018; 91: 49-52.
7. Wei X, Cho KS, Thee EF, Jager MJ, Chen DF. Neuroinflammation and microglia in glaucoma: time for a paradigm shift. J Neurosci Res 2019; 97: 70-76.
8. Lee J, Yoo JH, Kim HS, et al. C1q/TNF-related protein-9 attenuates palmitic acid-induced endothelial cell senescence *via* increasing autophagy. Mol Cell Endocrinol 2021; 521: 111114.
9. Inooka D, Omori Y, Ouchi N, et al. Ablation of Ctrp9, ligand of AdipoR1, and lower number of cone photoreceptors in mouse retina. Invest Ophthalmol Vis Sci 2022; 63: 14.
10. Zhou P, Liu L, Gao W. Association between serum CTRP9 levels and diabetic retinopathy in patients with type 2 diabetes mellitus. J South Med Univ 2021; 41: 459-463.
11. Cheng Y, Qi Y, Liu S, et al. C1q/TNF-related protein 9 inhibits high glucose-induced oxidative stress and apoptosis in retinal pigment epithelial cells through the activation of AMPK/Nrf2 signaling pathway. Cell Transplant 2020; 29: 963689720962052.
12. Li W, Ma N, Liu MX, et al. C1q/TNF-related protein-9 attenuates retinal inflammation and protects blood-retinal barrier in db/db mice. Eur J Pharmacol 2019; 853: 289-298.
13. Kambara T, Ohashi K, Shibata R, et al. CTRP9 protein protects against myocardial injury following ischemia-reperfusion through AMP-activated protein kinase (AMPK)-dependent mechanism. J Biol Chem 2012; 287: 18965-18973.
14. Zhao L, Zhang JH, Sherchan P, et al. Administration of rCTRP9 attenuates neuronal apoptosis through AdipoR1/PI3K/Akt signaling pathway after ICH in mice. Cell Transplant 2019; 28: 756-766.
15. Zhou C, Luo D, Xia W, et al. Nuclear factor (Erythroid-Derived 2)-like 2 (Nrf2) contributes to the neuroprotective effects of histone deacetylase inhibitors in retinal ischemia-reperfusion injury. Neuroscience 2019; 418: 25-36.
16. Xu YP, Han F, Tan J. Edaravone protects the retina against ischemia/reperfusion-induced oxidative injury through the PI3K/Akt/Nrf2 pathway. Mol Med Rep 2017; 16: 9210-9216.
17. Lee D, Kunimi H, Negishi K, Kurihara T. Degeneration of retinal ganglion cells in hypoxic responses: hypoxia-inducible factor inhibition, a new therapeutic insight. Neural Regen Res 2022; 17: 2230-2231.
18. McMonnies C. Reactive oxygen species, oxidative stress, glaucoma and hyperbaric oxygen therapy. J Optom 2018; 11: 3-9.
19. Storgaard L, Tran TL, Freiberg JC, Hauser AS, Kolko M. Glaucoma clinical research: trends in treatment strategies and drug development. Front Med 2021; 8: 733080.
20. Fea AM, Novarese C, Caselgrandi P, Boscia G. Glaucoma treatment and hydrogel: current insights and state of the art. Gels 2022; 8: 510.
21. Matsou A, Anastasopoulos E. Investigational drugs targeting prostaglandin receptors for the treatment of glaucoma. Exp Opin Investig Drugs 2018; 27: 777-785.
22. Almasieh M, Wilson AM, Morquette B, Cueva Vargas JL, di Polo A. The molecular basis of retinal ganglion cell death in glaucoma. Prog Ret Eye Res 2012; 31: 152-181.
23. Geyer O, Levo Y. Glaucoma is an autoimmune disease. Autoimmun Rev 2020; 19: 102535.
24. Zhang H, Zhang-Sun ZY, Xue CX, et al. CTRP family in diseases associated with inflammation and metabolism: molecular mechanisms and clinical implication. Acta Pharmacol Sin 2023; 44:710-725.
25. Kong M, Gao Y, Guo X, Xie Y, Yu Y. Role of the CTRP family in tumor development and progression. Oncol Lett 2021; 22: 723.
26. Yamaguchi S, Shibata R, Ohashi K, et al. C1q/TNF-related protein 9 promotes revascularization in response to ischemia *via* an eNOS-dependent manner. Front Pharmacol 2020; 11: 1313.
27. Zhao D, Feng P, Sun Y, et al. Cardiac-derived CTRP9 protects against myocardial ischemia/reperfusion injury *via* calreticulin-dependent inhibition of apoptosis. Cell Death Dis 2018; 9: 723.
28. Bai S, Cheng L, Yang Y, et al. C1q/TNF-related protein 9 protects diabetic rat heart against ischemia reperfusion injury: role of endoplasmic reticulum stress. Oxid Med Cell Longev 2016; 2016: 1902025.
29. Li Q, Zhu Z, Wang C, et al. CTRP9 ameliorates cellular senescence *via* PGC-1α/AMPK signaling in mesenchymal stem cells. Int J Mol Med 2018; 42: 1054-1063.
30. Zhao W, Kong F, Gong X, et al. Activation of AdipoR1 with rCTRP9 preserves BBB integrity through the APPL1/AMPK/Nrf2 signaling pathway in ICH mice. Oxid Med Cell Longev 2021; 2021: 2801263.
31. Carling D. AMPK signalling in health and disease. Curr Opin Cell Biol 2017; 45: 31-37.
32. Li X, Leng Y, Jiang Q, et al. Eye drops of metformin prevents fibrosis after glaucoma filtration surgery in rats *via* activating AMPK/Nrf2 signaling pathway. Front Pharmacol 2020; 11: 1038.
33. Kim S, Kim S, Hwang AR, et al. Apelin-13 inhibits methylglyoxal-induced unfolded protein responses and endothelial dysfunction *via* regulating AMPK pathway. Int J Mol Sci 2020; 21: 4069.
34. Ha Y, Liu H, Xu Z, et al. Endoplasmic reticulum stress-regulated CXCR3 pathway mediates inflammation and neuronal injury in acute glaucoma. Cell Death Dis 2015; 6: e1900.
35. Chitranshi N, Dheer Y, Mirzaei M, et al. Loss of Shp2 rescues BDNF/TrkB signaling and contributes to improved retinal ganglion cell neuroprotection. Mol Ther 2019; 27: 424-441.

Address for correspondence

Yuling Niu
The People's Hospital of Baoan Shenzhen
No.118 Longjing Second Road, Bao'an District
Shenzhen, 518101, China
e-mail: niuyuling_1122@163.com