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Lycium barbarum polysaccharides alleviate hydrogen peroxide-induced injury by up-regulation of miR-4295 in human trabecular meshwork cells

Yuxia Liu[a,*], Yan Zhang[b]

[a] Department of Ophthalmology, Zhoukou Central Hospital, Zhoukou, Henan 466000, China
[b] Department of Pathology, Huaihe Hospital of Henan University, Kaifeng, Henan 475000, China

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ABSTRACT

Glaucoma is a chronic neurodegenerative disease which produces damage to the optic nerve and causes sightlessness. Current remains lack of effective method for glaucoma. *Lycium barbarum polysaccharides* (LBPs) have pleiotropic effects on various diseases. However, the effect of LBPs on glaucoma remains unclear. The study aimed to clarify the protective effect of LBPs against hydrogen peroxide (H_2O_2)-induced oxidative damage in human trabecular meshwork (HTM) cells. HTM cells were exposed to H_2O_2 (0–400 μM) for 24 h to construct an oxidative damage model. Then, the different concentrations of LBPs (0–500 μg mL^{-1}) were used to pre-treated HTM cells, and cell viability, apoptosis, protein levels of pro−/cleaved-caspase-3 and pro−/cleaved-caspase-9, and reactive oxygen species (ROS) generations were detected. MicroRNA (miR)-4295 inhibitor and its control were transfected into HTM cells, and the biological functions of miR-4295 were assessed in H_2O_2 and LBPs treated cells. Phosphatidylinositol 3-kinase (PI3K)/protein Kinase B (AKT) and extracellular regulated protein kinases (ERK) pathways were determined by western blot assay. LBPs significantly promoted cell viability, reduced apoptosis, declined cleaved-caspase-3/-9 and ROS level in HTM cells after H_2O_2 administration. MiR-4295 expression was up-regulated in H_2O_2 and LBPs treated cells. The protective effect of LBPs on H_2O_2-injured HTM cells was obviously reversed by miR-4295 inhibition. LBPs activated PI3K/AKT and ERK signaling pathways through up-regulation of miR-4295 in H_2O_2-injured HTM cells. These data demonstrated that LBPs alleviated H_2O_2-induced injury by up-regulation of miR-4295 in HTM cells, indicating the protective effect of LBPs on HTM cells against oxidative damage.

1. Introduction

Glaucoma is a genetic heterogeneous neurodegenerative disease characterized by the loss of retinal ganglion cells and the degeneration of axons in the optic nerve (Schlamp et al., 2006). High intraocular pressure is a common feature in the different types of glaucoma, which is a major risk factor for the development of glaucoma (Ha Thanh, 2014). Previous studies have clarified that high intraocular pressure can lead to the optic nerve dead and retinal ganglion cells apoptosis, resulting in the impairment of visual function (Demer et al., 2017; Yao et al., 2015). The reason of high intraocular pressure is due to the destruction of the dynamic balance of aqueous humor circulation (Tamm et al., 2015). The trabecular meshwork (TM) is the principal pathway for the drainage of aqueous humor, and also an important part of physiological or pharmacological regulation of aqueous humor outflow (Kelley et al., 2015; Ueda et al., 2018). Recent study demonstrated that TM cells and its extracellular matrix play an active role in the aqueous outflow mechanism (Hernandez et al., 2018). Injured TM cells are closely related to produce the high intraocular pressure (Stamer et al., 2011). Therefore, searching for effective drugs to relieve TM cells injury in glaucoma patients is of great importance for the clinical treatment of glaucoma.

Lycium barbarum is a traditional Chinese medicine (TCM) with many physiological and pharmacological activities, including neuro-protective, anti-oxidative, anti-aging and immunomodulatory effects (Gao et al., 2017; Tang et al., 2012). *Lycium barbarum polysaccharides* (LBPs) are compounds extracted from the dry fruit of *Lycium barbarum*, which are the main substance that *Lycium barbarum* exhibited the biological effects (Yang et al., 2013). Several studies have found that LBPs could protect many kinds of cells or tissues against oxidative damage (Li et al., 2011b; Luo et al., 2006; Qi et al., 2014; Zhou et al., 2016). Niu et al. uncovered the protective effect of LBPs on oxidative damage in skeletal muscle of exhaustive exercise rats (Niu et al., 2008). Additionally, one study from Huang et al. reported that LBP could inhibit H_2O_2-induced

* Corresponding author at: Department of Ophthalmology, Zhoukou Central Hospital, No.26, East Section of Renmin Road, Zhoukou, Henan 466000, China.
E-mail address: yuxialiu0001@sina.com (Y. Liu).

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testicle cell damage, indicating the significant protective action to germ cells (Huang et al., 2003). Further, Qi et al. found that LBPs could protect lens epithelial cells against oxidative stress-induced cell apoptosis and senescence (Qi et al., 2014). However, it is still unknown whether LBPs could protect human trabecular meshwork (HTM) cells against oxidative stress damage.

The current study was aimed to investigate the protective effect of LBPs on HTM from hydrogen peroxide (H_2O_2)-induced oxidative damage. Cell viability, apoptosis, apoptosis-associated factors and the production of reactive oxygen species (ROS) were examined in HTM cells after administration with H_2O_2. The molecular mechanisms of LBPs on H_2O_2-induced oxidative damage in HTM cells were explored through examining phosphatidylinositol 3-kinase (PI3K)/protein Kinase B (AKT) and extracellular regulated protein kinases (ERK) signaling pathways. The study might provide a new idea for the treatment of glaucoma.

2. Materials and methods

2.1. Cell culture

HTM cells used in this study were obtained from Sciencell Research Laboratories (Carlsbad, CA, USA). Cells were cultured in trabecular meshwork cell medium (TMCM, Cat. No. 6591) containing with 2% fetal bovine serum (FBS), 1% trabecular meshwork cell growth supplement (TMCGS) and 1% penicillin/streptomycin solution (P/S, all from ScienCell, Carlsbad, USA) at 37 °C with 95% air and 5% CO_2.

2.2. Cell treatment

HTM cells were stimulated with different concentrations of H_2O_2 (50–400 μM, Sigma-Aldrich Co., LCC, St Louis, MO, USA) for 24 h for constructing an oxidative damage model in HTM cells. LBPs was extracted from L. *barbarum* (Ningxia, China) according the method of Yang et al. and Yu et al. described (Yang and Li, 2014; Yu et al., 2005). In brief, the dried fruit of L. *barbarum* (100 g) was boiled in the deionized water (6 L) for 3 h, and removed the impurities by a filter paper. Subsequently, the extract was concentrated to the volume under vacuum at 40 °C and diluted to deionized water. Then, the extract was precipitated with 95% ethanol, and was centrifuged to remove the supernatant. The extracts were freeze-dried into powder form for storage. The freeze-dried powder of LBPs was freshly diluted with TMCM, and adjusted to the concentrations in 100, 200,300, 400 and 500 μg mL^{-1} for the experiments. At the beginning of following study, the five concentration gradients of H_2O_2 (50, 100, 200, 300 and 400 μM) were used to stimulate HTM cells for 24 h.

2.3. Cell Counting Kit-8 (CCK-8) assay

HTM cells were cultured in 96-well plate with 5000 cells/well for 24 h. After incubation, cells were then treated with LBPs for 1 h and exposed to H_2O_2 for another 24 h. After stimulation, 10 μL CCK-8 solution (Dojindo Molecular Technologies, Gaithersburg, MD) was added to the each well of 96-well plate, and were cultured for 1 h at 37 °C under a moist environment with 95% air and 5% CO_2. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

2.4. Cell apoptosis assay

After treatment with H_2O_2 and LBPs, cell apoptosis was performed by using propidium iodide (PI) and fluorescein isothiocynate (FITC)-conjugated Annexin V staining (Invitrogen Corporation, Carlsbad, CA, USA). The treated cells were washed in phosphate buffered saline (PBS, Sigma-Aldrich) and re-suspended with 1 × Binging Buffer (Sigma-Aldrich). Subsequently, these treated cells were stained with 5 μL Annexin V-FITC and 10 μL PI for 1 h at room temperature in the dark. After staining, the percent of apoptotic cells was detected by using flow cytometry assay (Beckman Coulter, Fullerton, CA, USA), and these data were analyzed by using FlowJo software (Tree Star, San Carlos, CA, USA).

2.5. ROS assay

The ROS production was measured by using flow cytometry with 2, 7-dichlorofluorescein diacetate (DCFH-DA, Serva, Heidelberg, Germany). Briefly, cells were cultured in a 6-well plate for 24 h and then treated with LBPs for 1 h, and exposed to H_2O_2 for another 24 h. After this, cells were incubated with 10 μM DCFH-DA for 20 min at 37 °C in the dark. After this, cells were washed three times with PBS, and digested by 0.25% trypsin-EDTA for collecting these samples. After centrifugation, the supernatants were removed and cells were re-suspended to 500 μL PBS. The fluorescent intensities were measured using a flow cytometer at wavelengths of 488 nm excitation and 521 nm emission.

2.6. Cell transfection

The plasmids of miR-4295 inhibitor (sequence: 5′-AAG GAA AAC AUU GCA CUG-3′) and the negative control (NC, sequence: 5′-UCA CAA CCU CCU AGA AAG AGU AGA-3′) were synthesized by GenePharma Co. (Shanghai, China). All cell transfections were conducted by using Lipofectamine 3000 reagent (Invitrogen) based on the manufacturer's protocol.

2.7. Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was extracted from cells treated with H_2O_2 and LBPs using Trizol reagent (Life Technologies Corporation, Carlsbad, CA, USA) following the manufacturer's instructions. The cDNA was synthesized by using a PrimeScript™ 1st Strand cDNA Synthesis kit (TaKaRa, Dalian, China). The Taqman Universal Master Mix II with the TaqMan MicroRNA Assay (Applied Biosystems, Foster City, CA, USA) was used to determine the expression level of miR-4295 in these treated cells. U6 snRNA acted as an internal control in the experiment, and the sequence of specific primer was as forward: 5′-CTT CGG CAG CAC ATA TAC T-3′ and Revers: 5′-AAA ATA TGG AAC GCT TCA CG-3′. The data was detected by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.8. Western blot assay

After treatment with H_2O_2 and LBPs, the proteins from these treated cells were extracted by using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China). A commercial assay kit (BCA™ Protein Assay Reagent Kit, Pierce, Rockford) based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total proteins. The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Afterward, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis analysis was performed to separate the sample proteins. The proteins were transferred to polyvinylidene fluoride (PVDF) membranes, and the membranes were then blocked in 5% non-fat milk for 1 h at room temperature. Primary antibodies of pro-caspase-3 (ab32499), cleaved-caspase-3 (ab32042), pro-caspase-9 (ab32068), cleaved-caspase-9 (ab2324), phospho (p)-PI3K (ab182651), t-PI3K (ab191606), phospho (p)-AKT (ab81283), t-AKT (ab18785), p-ERK (ab32537), t-ERK (ab131438) and β-actin (ab8227, from Abcam, Cambridge, UK) were incubated with the membranes at 4 °C overnight. After washing, the membranes were incubated with the horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (ab205718, 1:2000, Abcam) for 1 h at room temperature. The western bots were visualized by using a standard enhanced chemiluminescence (ECL) Western

blotting detection reagent (GE Healthcare, Braunschweig, Germany). The intensity of the bands was quantified by using the ImageJ software (Bio-Rad, Shanghai, China).

2.9. Statistical analysis

The results from multiple experiments are presented as the mean ± standard deviation (SD). Statistical analyses were performed using Graphpad statistical software (GraphPad, San Diego, CA, USA). *P*-values were calculated by using student's *t*-test or a one-way analysis of variance (ANOVA) following by Tukey post-hoc analysis. $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered to indicate statistically significant results. All experiments were carried out three repeats.

3. Results

3.1. H_2O_2 induced oxidative damage in HTM cells

Cell viability, apoptosis and ROS level were investigated in HTM cells after stimulation with H_2O_2. Results showed a significant decrease tendency in HTM cells with the increase concentrations of H_2O_2 ($P < 0.05$, $P < 0.01$ or $P < 0.001$, Fig. 1A). Then, 200 μM H_2O_2 was selected as the optimum concentration working on the subsequent experiments. The results in Fig. 1B showed that H_2O_2 treatment significantly induced cell apoptosis compared with control group ($P < 0.01$). Moreover, the protein levels of cleaved-caspase-3 and cleaved-caspase-9 were activated in HTM cells after treatment with H_2O_2 (Fig. 1C). Further, we observed that the ROS level was prominently increased after treatment with 200 μM H_2O_2 ($P < 0.01$, Fig. 1D). All these results stated that H_2O_2 treatment could suppress cell viability, induce cell apoptosis and enhance the level of ROS in HTM cells, which indicating that H_2O_2-induced oxidative damage model was successfully constructed in HTM cells.

3.2. LBPs weakened H_2O_2-induced oxidative damage in HTM cells

Cell viability of HTM cells after treatment with different concentrations of LBPs was examined. As shown in Fig. 2A, cell viability was presented after treatment with 400 or 500 μg mL^{-1} LBPs ($P < 0.05$). There was no significant change at the concentrations of 100–300 μg mL^{-1} LBPs. In order to uncover the protective effect of LBPs on H_2O_2-induced oxidative damage in HTM cells, cell viability in HTM cells co-treated with H_2O_2 and LBPs was determined. The results in Fig. 2B presented that LBPs significantly prevented the reduction of cell viability induced by H_2O_2 at a dose-dependent manner ($P < 0.05$ or $P < 0.01$). The maximum promoting effect of LBPs on cell viability was exhibited at the concentration of 300 μg mL^{-1}, thus this concentration of LBPs was selected to treat HTM cells in the following experiments. Flow cytometry and western blot assays revealed that LBPs (300 μg mL^{-1}) treatment significantly reduced cell apoptosis and inhibited the activation of cleaved-caspase-3 and cleaved-caspase-9 in HTM cells after administration of H_2O_2 ($P < 0.05$, Fig. 2C and D). The production of ROS was analyzed by using DCFH-DA staining, and the result displayed that LBPs (300 μg mL^{-1}) significantly declined the level of ROS in H_2O_2-stimulated HTM cells. Summing up the above results suggested that LBPs could protect HTM cells against oxidative damage induced by H_2O_2.

3.3. MiR-4295 was up-regulated by LBPs in H_2O_2-exposed HTM cells

Expression level of miR-4295 in H_2O_2 and LBPs treated cells was analyzed by using RT-qPCR. The results in Fig. 3 exhibited that H_2O_2 stimulation significantly decreased miR-4295 expression level in HTM cells compared with no treated cells ($P < 0.01$). After stimulation with

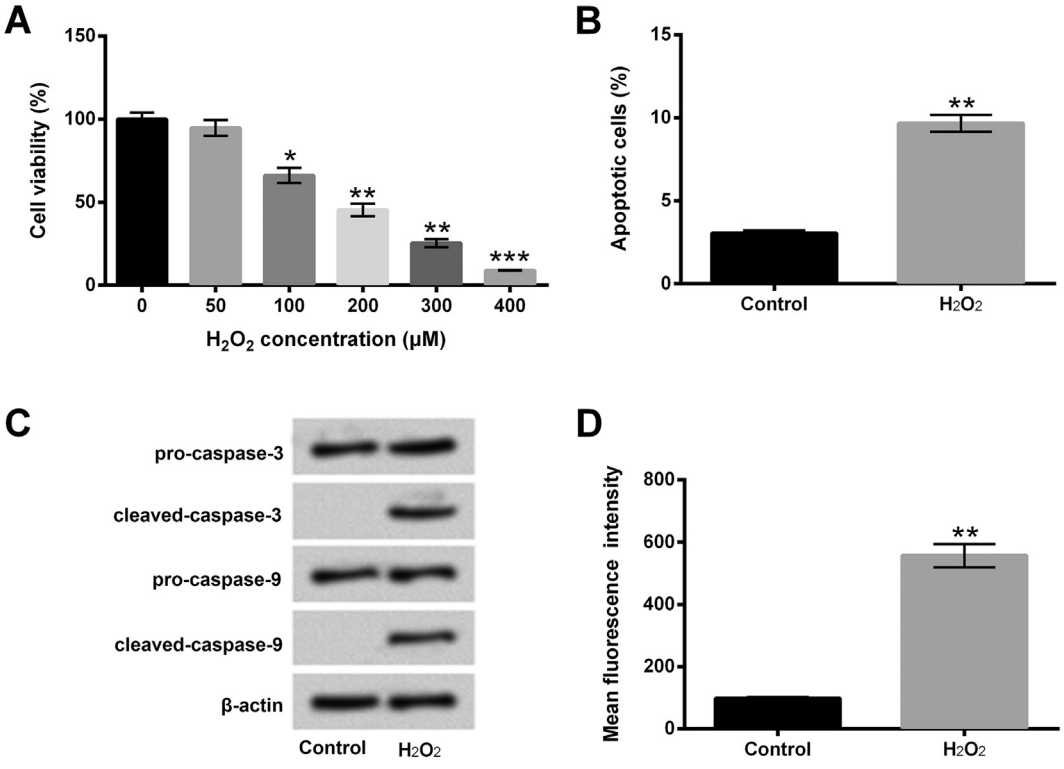

Fig. 1. H_2O_2 induced oxidative damage in HTM cells.
HTM cells were cultured with the different concentrations of H_2O_2 (50, 100, 200, 300 and 400 μM) for 24 h, and non-treated cells acted as a control. (A) Cell viability was examined by Cell Counting Kit-8 (CCK-8) assay; (B) Cell apoptosis was assessed by flow cytometry assay; (C) Protein levels of pro−/cleaved-caspase-3 and pro−/cleaved-caspase-9 were determined by western blot assay; (D) The reactive oxygen species (ROS) level was detected by 2, 7-dichlorofluorescein diacetate (DCFH-DA) staining; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

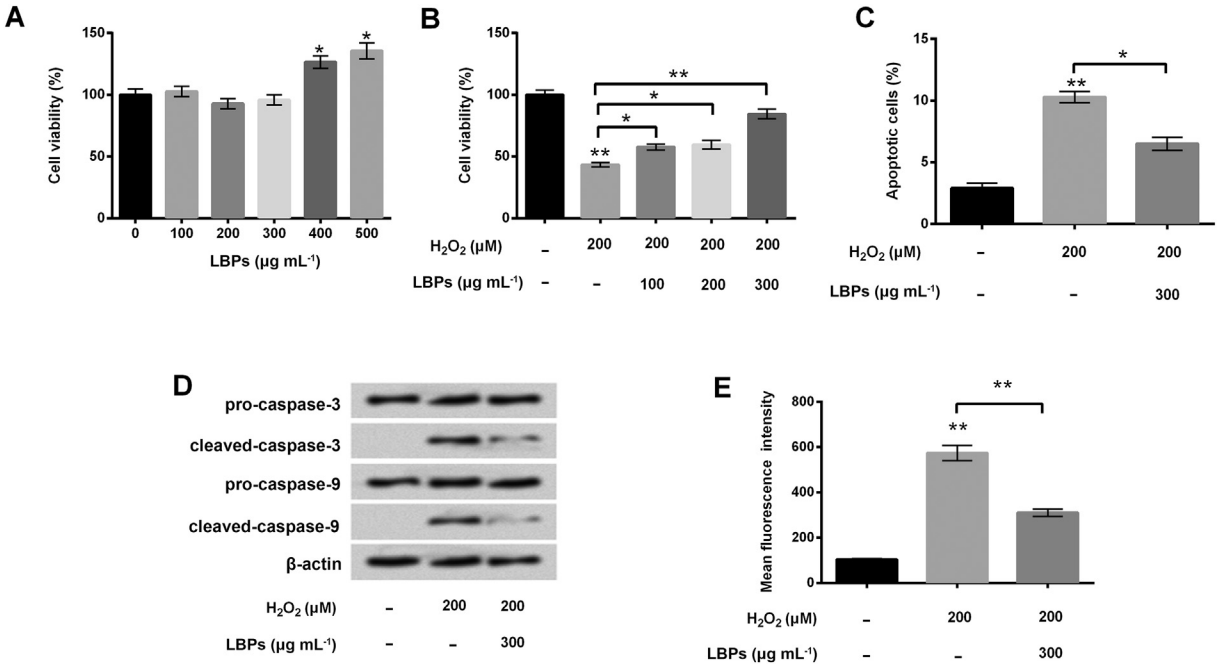

Fig. 2. LBPs attenuated H_2O_2-induced oxidative damage in HTM cells.
(A) HTM cells were cultured with the different concentrations of LBPs (100, 200, 300, 400 and 500 μg mL^{-1}) for testing cell cytotoxicity by using Cell Counting Kit-8 (CCK-8) assay; (B) HTM cells were cultured with LBPs (100, 200 and 300 μg mL^{-1}) for 24 h before exposed to 200 μM H_2O_2, and then cell viability was examined in these treated cells by using CCK-8 assay; after treatment with 200 μM H_2O_2 and 300 μg mL^{-1} LBPs, (C) cell apoptosis, (D) protein levels of pro−/cleaved-caspase-3 and pro−/cleaved-caspase-9, and (E) the reactive oxygen species (ROS) level were assessed by using flow cytometry, western blot and 2, 7-dichlorofluorescein diacetate (DCFH-DA) staining, respectively; *, $P < 0.05$; **, $P < 0.01$.

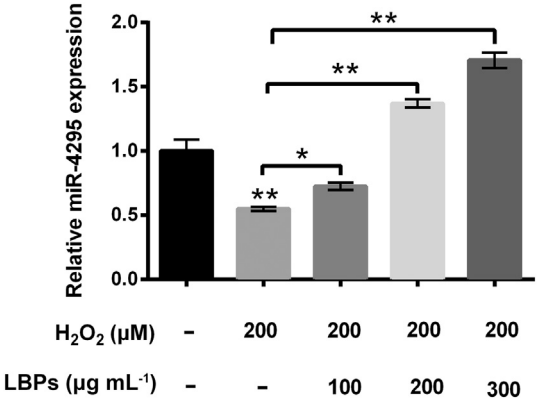

Fig. 3. LBPs elevated the expression level of miR-4295 in H_2O_2-injured HTM cells.
HTM cells were cultured with LBPs (100, 200 and 300 μg mL^{-1}) for 24 h before exposed to 200 μM H_2O_2, and the expression level of miR-4295 was then determined by using reverse transcription-quantitative PCR (RT-qPCR) assay; *, $P < 0.05$; **, $P < 0.01$.

LBPs, the expression level of miR-4295 was obviously increased at a dose-dependent manner ($P < 0.05$ or $P < 0.01$). This result indicated that LBPs could up-regulate miR-4295 expression in H_2O_2-exposed HTM cells.

3.4. MiR-4295 suppression reversed the protective effect of LBPs on H_2O_2-injured HTM cells

HTM cells were transfected with miR-4295 inhibitor and NC, and the expression levels of miR-4295 in these transfected cells were analyzed by RT-qPCR. Results in Fig. 4A showed that miR-4295 expression was significantly declined in HTM cells after transfection with miR-4295 inhibitor compared with its control group ($P < 0.01$). Subsequently, we further examined the regulatory effect of miR-4295 on H_2O_2 and LBPs treated HTM cells. As shown in Fig. 4B–D, we observed that miR-4295 suppression significantly declined cell viability, elevated apoptosis and promoted the activation of cleaved-caspase-3 and cleaved-caspase-9 in HTM cells after administration with H_2O_2 and LBPs ($P < 0.01$). Besides, the level of ROS was markedly enhanced by miR-4295 suppression in H_2O_2 and LBPs treated cells ($P < 0.01$). The above results hinted that miR-4295 suppression could decline the protective effect of LBPs on HTM cells against H_2O_2-induced oxidative damage.

3.5. LBPs activated PI3K/AKT and ERK signaling pathways in H_2O_2-injured HTM cells through up-regulation of miR-4295

For clarification of the molecular mechanisms of LBPs on H_2O_2-induced oxidative damage in HTM cells, the regulatory effects of LBPs on PI3K/AKT and ERK signaling pathways were determined. In Fig. 5A and B, we found that H_2O_2 stimulation down-regulated the protein levels of p-PI3K and p-AKT ($P < 0.05$ or $P < 0.01$), while LBPs regained the activation of PI3K/AKT signaling pathway ($P < 0.001$). When miR-4295 inhibitor was transfected into HTM cells, the promoting effects of LBPs on PI3K/AKT signaling pathway were significantly declined ($P < 0.001$). Similarly, the protein level of p-ERK was notably down-regulated by H_2O_2 administration ($P < 0.05$), LBPs obviously enhanced p-ERK protein level in H_2O_2-injured HTM cells ($P < 0.05$). However, miR-4295 suppression inhibited the protein level of p-ERK in LBPs and H_2O_2 treated cells ($P < 0.05$, Fig. 5C and D). As a consequence, LBPs exerted the protective effect on H_2O_2-injured HTM cells might through activation of PI3K/AKT and ERK signaling pathways *via* up-regulation of miR-4295 expression.

4. Discussion

Glaucoma is a chronic neurodegenerative disease which produces damage to the optic nerve and results in sightlessness (Kumar et al.,

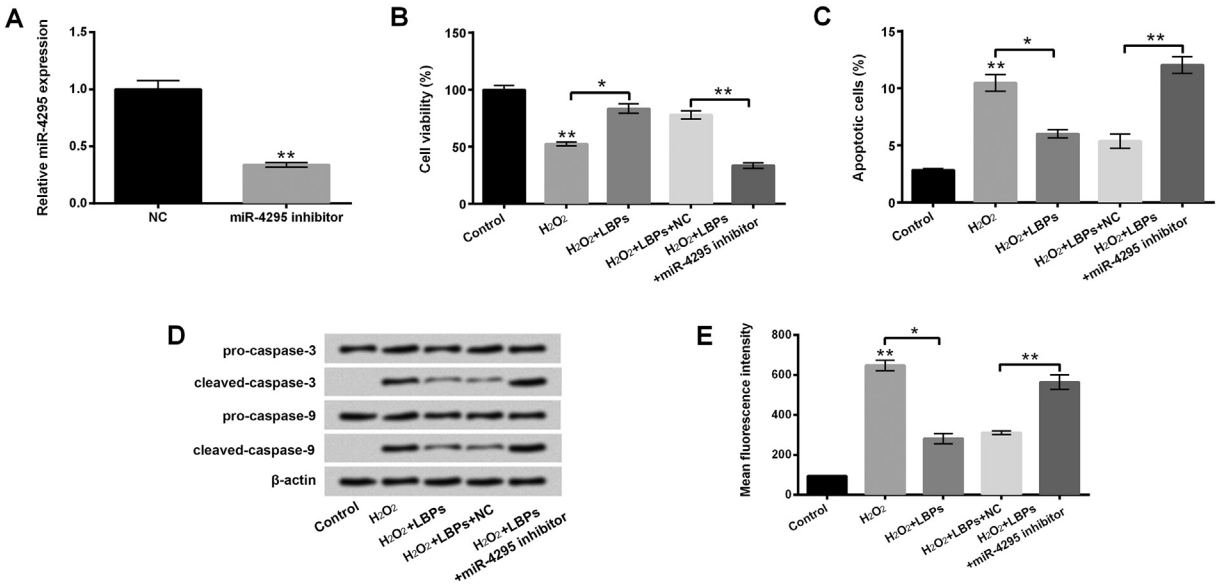

Fig. 4. LBPs exerted the protective effect on H_2O_2-injured HTM cells through up-regulation of miR-4295.
HTM cells were transfected with miR-4295 inhibitor and its negative control (NC), (A) the expression level of miR-4295 in these transfected cells was analyzed by using reverse transcription-quantitative PCR (RT-qPCR) assay; after transfection with miR-4295 inhibitor and treatment with H_2O_2 and LBPs, (B) cell viability, (C) apoptosis, (D) protein levels of pro−/cleaved-caspase-3 and pro−/cleaved-caspase-9, and (E) the reactive oxygen species (ROS) level were assessed by using Cell Counting Kit-8 (CCK-8), flow cytometry, western blot and 2, 7-dichlorofluorescein diacetate (DCFH-DA) staining, respectively; *, $P < 0.05$; **, $P < 0.01$.

2018). However, effective methods for the treatment glaucoma are still lacked. Recently, increasing evidences confirmed that TCMs have achieved good curative effect for the treatment of glaucoma. However, the effect of LBPs on glaucoma is still unclear. In this study, we aimed to uncover whether LBPs exerted the protective effect on HTM cells against H_2O_2-induced oxidative damage. We found that LBPs significantly up-regulated the expression level of miR-4295 in H_2O_2-treated cells. MiR-4295 inhibition reversed the protective effect of LBPs on H_2O_2-injured HTM cells. Finally, we observed that LBPs activated PI3K/AKT and ERK signaling pathways through up-regulation of miR-4295 in H_2O_2-injured HTM cells.

Medically, studies have showed that oxidative stress is one of the important mechanisms leading to glaucoma, and the occurrence of glaucoma is associated with the imbalance of ROS metabolism (Aslan et al., 2013; Pinazo-Duran et al., 2015). It is generally known that H_2O_2 is an important ROS, which can induce cell oxidative damage in various diseases (Kumar and Khanum, 2013; Zheng et al., 2010). Recently, Lv et al. used H_2O_2 to construct an oxidative stress damage model in RGC-5 cells to mimic the development of glaucoma *in vitro*, and indicated the protective effect of Crocin on H_2O_2-injured RGC-5 cells (Lv et al., 2016). Similarly, Wang et al. used different concentrations of H_2O_2 (100–800 μM) to treat RGC-5 cells, and explored the protective effect of protocatechuic acid (PCA) on H_2O_2-injured RGC-5 cells (Wang et al., 2015). In this study, 200 μM H_2O_2 was chosen for the following study (Wang et al., 2015). Based on these studies, we also successfully constructed a model of H_2O_2-indced oxidative damage in HTM cells.

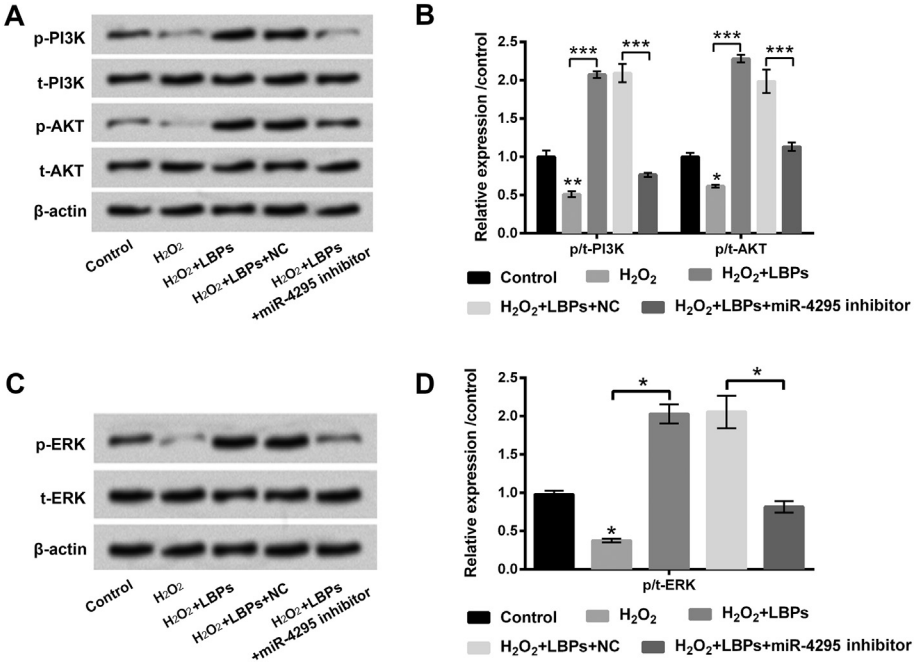

Fig. 5. LBPs activated PI3K/AKT and ERK signaling pathways in H_2O_2-injured HTM cells through up-regulation of miR-4295.
After transfection with miR-4295 inhibitor and treatment with H_2O_2 and LBPs, the protein levels of (A and B) p/t-phosphatidylinositol 3-kinase (PI3K), p/t-protein Kinase B (AKT) and (C and D) p/t-extracellular regulated protein kinases (ERK) were determined by western blot assay; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Similar with Wang et al. research, 200 μM H_2O_2 as the optimum concentration was used in the subsequent experiments.

LBPs are water-soluble polysaccharides extracted from *Lycium barbarum*, which are well known for the pleiotropic effects, including antioxidant activity (Yang et al., 2015). The antioxidant functions of LBPs have been confirmed in a large number of experiments. Studies demonstrate that LBPs can inhibit the action of 1, 1-diphenyl-2 picrylhydrazyl radical (DPPH) and 2, 2-azino-bis-(3-ethylbenzothiazoline6-sulfonic acid) (ABTS), and have the significant properties of reducibility and scavenging free radicals and superoxide anion (Li and Zhou, 2007; Li et al., 2007; Lin et al., 2009). One study from Pan et al. found that LBPs could reduce H_2O_2-indced oxidative damage in myocardial cells (Pan et al., 2017). Another study from Luo et al. reported the protective effect of LBPs on H_2O_2-indced DNA damage in mouse testicular cells (Luo et al., 2006).

However, it is still unclear whether LBPs can protect HTM cells against H_2O_2-indced oxidative damage. In the present study, we found that LBPs significantly promoted cell viability, reduced apoptosis and inhibited the production of ROS in H_2O_2-injured HTM cells, indicating the protective effect of LBPs against oxidative damage in HTM cells. These data hinted that LBPs might be a new antioxidant agent that could be used as a potential implication for the treatment of glaucoma.

Increasing evidence demonstrates that miRNAs are important mediators in neural proliferation, differentiation, and neuro-degeneration (Hébert and De, 2009). Study from Li et al. reported that miR-204 could induce cell apoptosis, inhibit viability, increase accumulation of oxidized protein after treatment with H_2O_2 in HTM cells (Li et al., 2011a). Another study from Luna et al. demonstrated that miR-29b could negatively regulate the expression of multiple genes involved in the synthesis and deposition of ECM in TM cells under chronic oxidative stress (Coralia et al., 2009). Further, miR-450 was reported to be involved in various biological processes bearing relevance for glaucoma (Izzotti et al., 2015). Therefore, we have reason to believe that miRNAs are involved in the pathogenesis of glaucoma. MiR-4295 is a novel oncogenic miRNA, which has been investigated in various cancers (Shao et al., 2015; Yuan et al., 2018). However, whether miR-4295 is involved in regulating the protective effect of LBPs on H_2O_2-induced oxidative damage in HTM cells remains unclear. Our study uncovered that LBPs up-regulated miR-4295 expression after H_2O_2 treatment, miR-4295 suppression reversed the protective effect of LBPs on H_2O_2-induced oxidative damage in HTM cells. These data indicated that miR-4295 might be a key regulator in the pathogenesis and development of glaucoma.

PI3K/AKT and ERK are important signaling pathways, which are closely associated with oxidative stress response in different diseases (Kučera et al., 2016; Umoh et al., 2014). Recent study demonstrated that PI3K-AKT and ERK signaling pathways are primary oxidative stress response pathways in TM cells, and inhibitions of PI3K-AKT and ERK decreased TM cell viability under oxidative stress (Awai-Kasaoka et al., 2013). Study from Xiao et al. reported that LBPs could protect rat liver from non-alcoholic steatohepatitis-induced injury through mediating PI3K/AKT and MEK/ERK pathways (Xiao et al., 2013). Based on these previous studies, we suppose that whether PI3K/AKT and ERK signaling pathways are involved in the regulation of the protective effect of LBPs on HTM cells. In our study, we found that LBPs activated PI3K/AKT and ERK signaling pathways in H_2O_2-injured HTM cells through up-regulation of miR-4295. These data indicated that PI3K/AKT and ERK signaling pathways might be involved in regulating the protective effect of LBPs on H_2O_2-induced oxidative damage in HTM cells.

5. Conclusion

Taken together, our data demonstrated that LBPs had ability to protect HTM cells against oxidative damage caused by H_2O_2 through activating PI3K/AKT and ERK signaling pathways by up-regulation of miR-4295. These findings indicated that LBPs might be a novel antioxidant agent that could be used as a potential implication for the treatment of glaucoma. This study might provide a new reference for the treatment of glaucoma clinically. Further studies are still needed to explore the extensive role of LBPs in glaucoma.

Competing interests

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2018.12.007.

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