Downregulation of microRNA-181a attenuates hydrogen peroxide-induced human lens epithelial cell apoptosis *in vitro*

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Received September 5, 2016; Accepted July 7, 2017

DOI: 10.3892/mmr.2018.8608

Abstract. Apoptosis of human lens epithelial (HLE) cells is a process closely associated with cataract formation. The aim of the present study was to explore the effects of microRNA (miR)-181a against hydrogen peroxide (H_2O_2)-induced apoptosis in HLE cells *in vitro*. The recombinant lentiviral plasmid pLKO. 1-puro-miR-181a was constructed and used to transfect human HLE-B3 cells with the short hairpin (sh)RNA to silence the expression of miR-181a. The apoptotic rate of both HLE-B3 cells in which miR-181a expression was stably silenced and in untransfected HLE-B3 cells was assessed in the presence of H_2O_2 using flow cytometry. The mRNA expression levels of the apoptosis-related genes caspase-3 (*CASP3*) and B-cell lymphoma-2-associated X protein (*BAX*), and of the potential target genes for miR-181a, *c-MET*, cyclooxygenase 2 (*COX-2*) and snail family transcriptional repressor 2 (*SNAI2*) were measured using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) levels were assessed using ELISA. RT-qPCR analysis revealed that miR-181a expression was downregulated in HLE-B3 cells following transfection with miR-181a-shRNA. Treatment with H_2O_2 significantly reduced the viability of HLE-B3 cells, whereas miR-181a knockdown was revealed to attenuate the effects on cell viability following H_2O_2 treatment. In addition, the downregulation of miR-181a expression significantly decreased H_2O_2-induced cell apoptosis, which was accompanied by a downregulation in *CASP3* and *BAX* and *COX-2* expression. Furthermore, the levels of MDA were decreased, whereas the levels of SOD and CAT were increased following miR-181a silencing. The present findings suggested that miR-181a knockdown may protect HLE-B3 cells against H_2O_2-induced apoptosis *in vitro*. The molecular mechanisms involved in the protective effects of miR-181a silencing may involve the suppression of *CASP3*, *BAX* and *COX-2* expression, and the inhibition of MDA generation.

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Key words: cataract, lentivirus-based RNA interference, cell apoptosis, human lens epithelial cells, miRNA-181a

Introduction

Cataract is a major ophthalmological disorder that is characterized by the opacification of the eye lens (1). Cataract is an age-related disorder, and is one of the main causes of severe visual impairment or blindness in the aging population (2). Age-related cataract can lower the quality of life and affect the global economy, rendering the disease a significant public health issue (3). The causes of cataract are numerous and complex, and include drug-induced alterations, developmental abnormalities, ultraviolet radiation exposure, trauma and metabolic disorders (4). Previous studies have reported that the apoptosis of human lens epithelial (HLE) cells contributed to all types of cataract, with the exception of congenital disorders (5,6). Furthermore, oxidative stress and the formation of oxygen free radicals are also involved in cataract, and have been identified as a major risk factor for cataract development (4,7). Previous studies have demonstrated that hydrogen peroxide (H_2O_2) could induce the apoptosis of HLE cells (8,9); however, the molecular mechanisms implicated in H_2O_2-induced HLE cell apoptosis have yet to be fully elucidated.

MicroRNAs (miRNAs) have attracted attention in the efforts to elucidate the pathophysiological mechanisms implicated in various diseases or cancers (10). miRNAs are a class of small non-coding RNA molecules, 19-22 nucleotides long, which can target the 3'untranslated region of mRNAs to induce translational repression or mRNA degradation (11). miRNAs have been reported to participate in the regulation of several physiological and pathological processes, including cell proliferation, apoptosis, senescence and stress response (12). Previous studies have suggested that the aberrant expression of miRNAs may be implicated in the pathogenesis of age-related diseases, including the progression of cataract (3,13). One study reported that miRNA (miR)-125b was able to inhibit the apoptosis of HLE cells in age-related cataract by targeting p53 (14). Another study demonstrated that the let-7b miRNA precursor induced HLE cell apoptosis by directly regulating the expression of leucine-rich repeat-containing G protein-coupled receptor 4 in cataract (15). However, the information available on miRNA expression in cataract-related tissues is limited.

Previous studies have revealed that the downregulation of miR-181a was able to significantly inhibit the H_2O_2-induced apoptosis of cardiomyocytes (16), that miR-181a could inhibit the migration, proliferation and epithelial-mesenchymal transition (EMT) of lens epithelial cells (11). Therefore, it may be hypothesized that miR-181a serves a crucial role in the apoptosis of HLE cells induced by H_2O_2 and in the development of cataract in humans.

In the present study, RNA interference (RNAi) was performed using short hairpin (sh)RNA-based stable gene knockdown (17) to silence the expression of miR-181a in human HLE-B3 cells. The effects of miR-181a knockdown were evaluated on HLE-B3 proliferation and apoptosis in the presence of H_2O_2. In addition, the molecular mechanisms underlying the effects of miR-181a downregulation on HLE-B3 cell apoptosis were investigated. Elucidation of the functional roles of miR-181a may offer novel insight to decipher the complex regulatory mechanisms underlying the pathogenesis of cataract.

Materials and methods

Cell culture. Human HLE-B3 lens epithelial cells was were purchased from Jennio Biotech Co., Ltd. (Guangzhou, China) and the human 293T cell line was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HLE-B3 and 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (all from both from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Cell cultures were maintained in a humidified incubator at 37˚C in a 5% CO_2 atmosphere.

Generation of lentivirus-based RNAi plasmid. The hsa-miR-181a-5p sequence was obtained from the miRBase database (http://www.mirbase.org). The lentivirus-based RNAi transfer plasmid pLKO. 1-puro-miR-181a (miR-181a-shRNA) targeting miR-181a (5'-AACAUUCAACGCUGUCGG UGAGU-3') and the control plasmid pLKO. 1-puro were prepared using the pLKO. 1-puro plasmid obtained from the State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Shanghai Jiao Tong University (Shanghai, China). To generate the pLKO. 1-puro-miR-181a, a miR-181a sponge was constructed using annealed oligonucleotides for tandem miR-181a-binding sites, as previously described (18). Briefly, the miR-181a sponge oligonucleotides (forward, 5'-CCGGACTCACCGACACGCATGAATGTT CCGGACTCACCGACACGCATGAATGTTCCGGACTCA CCGACACGCATGAATGTTTTTTTT-3' and reverse 5'-AAT TAAAAAAAACATTCATGCGTGTCGGTGAGTCCGGA ACATTCATGCGTGTCGGTGAGTCCGGAACATTCATG CGTGTCGGTGAGT-3') were annealed using 50 pmol forward and reverse oligonucleotides, and 10X polymerase chain reaction (PCR) buffer (Takara Bio, Inc., Otsu, Japan), and cloned after the U6 promoter in the *Age*I/*Eco*RI-digested pLKO.1-puro vector for the construction of pLKO.1-puro-miR-181a. The annealing conditions were as follows: 94˚C for 3 min; followed by 55 cycles at 80˚C for 30 sec with -1˚C/cycle. The constructs were verified prior to use by sequencing by Sangon Biotech Co., Ltd. (Shanghai, China).

Lentiviral production. Lentivirus production and infection of the targeted cells were performed as previously described (19). Briefly, prior to transfection, $25x10^6$ 293T cells were plated onto 60x15 mm Petri dishes and grown to 80% confluence. The cells were then co-transfected with 3 μg psPAX2 packaging plasmid and 1 μg pMD2.G envelope plasmid (both from Invitrogen; Thermo Fisher Scientific, Inc.), along with 4 μg either pLKO.1-puro empty vector or pLKO.1-puro-miR-181a plasmid using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) as the transfection reagent, according to the manufacturer's protocol. Cells were cultured in serum-free DMEM for 6 h, following which the medium was replaced with DMEM supplemented with 10% FBS. The culture supernatants containing the lentiviral particles were collected at 48 and 72 h post-transfection. The supernatants were mixed, filtered by 0.45-μm filter and concentrated by ultracentrifugation at 4,000 x g for 20 min at 4˚C. Viral supernatants were then aliquoted and stored at -80˚C as a viral stock.

Stable transduction with shRNA-encoding lentivirus. HLE-B3 cells were divided into the following 3 experimental groups: Normal control, negative control and shRNA-transfected groups. A total of $1x10^5$ cells were seeded in complete DMEM medium in a 24-well plate 24 h prior to transduction. Untreated HLE-B3 cells were used as the normal control group. The supernatant of the lentiviral particles containing the empty pLKO.1-puro vector was used to transduce the negative control cells for 24 h at 37˚C, followed by the addition of fresh complete DMEM medium, HLE-B3 cells were infected with the supernatant of the recombinant lentivirus containing the miR-181a-shRNA for 24 h at 37˚C, followed by the addition of fresh complete DMEM medium. Cells stably expressing the shRNA were obtained by puromycin selection at ~72 h using DMEM containing 2 μg/ml puromycin until the HLE-B3 cells all died in the control group. Confirmation of miR-181a knockdown was performed by reverse transcription-quantitative PCR (RT-qPCR).

Cell proliferation assay. Cell proliferation was evaluated by the colorimetric water-soluble tetrazolium salt assay, Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan), according to the manufacturer's protocol immediately following confirmation of successful knockdown (20). Briefly, control or stably transfected HLE-B3 cells were seeded ($2x10^4$ cells/well) in 96-well round bottom plates immediately following transfection. Following overnight incubation at 37˚C in serum-free medium, the cells were challenged with or without 200 μM H_2O_2 for 24 h at 37˚C. Subsequently, 10 μl CCK-8 solution was added to each well and cells were incubated for another 4 h at 37˚C. The number of viable cells was assessed by measuring the optical density values of the samples at 450 nm using a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Flow cytometric analysis of apoptosis. Flow cytometry was used to assess cell apoptosis using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (BD Biosciences, San Jose, CA, USA), according to the manufacturer's protocol. Briefly, control and stably transfected HLE-B3 cells were seeded ($4x10^5$ cells/well) in 6-well

plates. Cells were cultured in serum-free medium overnight at 37˚C and subsequently challenged with or without 200 μM H_2O_2 for an additional 24 h at 37˚C. Cells were trypsinized, collected by centrifugation at 500 x g at 4˚C for 6 min, washed with phosphate-buffered saline (PBS) and resuspended in 1X binding buffer. For apoptosis detection, 5 μl annexin V-FITC and 5 μl PI were added to a culture tube containing 100 μl cell suspension and incubated for 15 min in a dark container at room temperature (25˚C). Subsequently, 400 μl 1X binding buffer was added to each culture tube and the samples were assessed using a FACSCalibur flow cytometer with CellQuest software (version 5.1; BD Biosciences) within 1 h. Apoptotic cell populations were detected by flow cytometric analysis and the fractions of cell population were analyzed in different quadrants through the quadrant statistics, with the results being calculated as percentages of apoptotic cells.

RT-qPCR. Total RNA from normal and stably transfected HLE-B3 cells (5x10^6 cells), with or without H_2O_2 treatment, was isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The purity and concentration of RNA were determined using a NanoDrop spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). RT-qPCR was performed to evaluate the expression of the apoptosis-related genes caspase-3 (*CASP3*) and B-cell lymphoma-2-assciated X protein (*BAX*), and of the potential target genes for miR-181a *c-MET*, cyclooxygenase 2 (*COX-2*) and snail family transcriptional repressor 2 (*SNAI2*). β-actin was used as the internal control to normalize gene expression levels. Sequences of the specific primers used in the present study are presented in Table I. All primers were designed and synthesized by Takara Biotechnology Co., Ltd. (Dalian, China).

First-strand cDNA was synthesized using the PrimeScript First-Strand cDNA Synthesis kit (Takara Biotechnology Co., Ltd.) with Oligo-dT primers in a 20 μl reaction mixture containing 0.5 μg DNase-treated RNA, 4 μl 5X PrimeScript RT Master Mix and RNase-free water to a total volume of 20 μl, according to the manufacturer's protocol. The reaction was incubated at 37˚C for 15 min and at 85˚C for 5 sec. qPCR was performed on cDNA using Power SYBR Green PCR Master Mix in a 7500 Fast Real-Time PCR system (both from Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The PCR reaction volume was 20 μl, containing 10 μl 2X SYBR Premix Ex Taq, 10 μM of each primer, and diluted cDNA to a total volume of 20 μl. The thermocycling conditions were as follows: Initial denaturation at 50˚C for 3 min and at 95˚C for 30 min, followed by 40 cycles at 95˚C for 10 sec and annealing at 60˚C for 30 sec. The specificity was verified by melting curve analysis (60-95˚C) following the 40 cycles of amplification. Each sample was analyzed in triplicate. The quantification cycle (Cq) was set within the exponential phase of the PCR and relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method (21).

For quantification of miRNAs, a TaqMan MicroRNA reverse transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used according to the manufacturer's protocol. The primers used for miR-181a were as follows: miR-181a forward, 5'-GCGGCGAACATTCAACGATG-3' and reverse, 5'-GTGCAGGGTCCGAGG-3'. Relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method (21).

Table I. Sequences of the primers used for reverse transcription-quantitative polymerase chain reaction.

Gene	Primer sequence (5'-3')
CASP3	F: GTGCTATTGTGAGGCGGTTGT
	R: TCCATGTATGATCTTTGGTTC
c-MET	F: AGAAGGCTAAAGGAAACGAA
	R: GGACCGTCAAGAAGTAAATAAA
COX-2	F: CCCTGAGCATCTACGGTTTG
	R: CAGTATTAGCCTGCTTGTCT
SNAI2	F: ATTTATGCAATAAGACCTATTCT
	R: AGGCTCACATATTCCTTGTCACA
BAX	F: CTGACGGCAACTTCAACTGGG
	R: GGAGTCTCACCCAACCACCCT
β-actin	F: AGCGGGAAATCGTGCGTG
	R: CAGGGTACATGGTGGTGGTGCC

BAX, B-cell lymphoma-2-associated X protein; *CASP3*, caspase-3; *COX*, cyclooxygenase; F, forward; R, reverse; *SNAI2*, snail family transcriptional repressor 2.

Measurement of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) levels. Control and stably transfected HLE-B3 cells were seeded (4x10^5 cells/well) in 6-well plates. Cells were cultured in serum-free medium overnight at 37˚C and subsequently challenged with 200 μM H_2O_2 or with nothing for an additional 24 h at 37˚C. Cells were harvested and lysed by two rounds of sonication in 100 μl PBS (3 times for 10 sec) in an ice-water bath, incubated at -80˚C for 30 min in between. Concentrations of MDA, SOD and CAT were measured using commercially available MDA ELISA kits (cat no. ml027131), SOD ELISA kit (cat no. ml026976), and CAT ELISA kit (cat no. ml026352) (all from Shanghai Enzyme-linked Biological Technology, Co., Ltd., Shanghai, China), according to the manufacturer's protocol. The absorbance of each sample was measured at 450 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Statistical analysis. All statistical analyses were performed using SPSS software version 17.0 (SPSS, Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation of 3 independent experiments. The statistical significance of the differences between groups was assessed using Student's t-test for pair-wise comparisons or one-way analysis of variance followed by the Student-Newman-Keuls post hoc test for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Lentiviral knockdown of miR-181a in HLE-B3 cells. Knockdown of miR-181a expression in HLE-B3 cells was

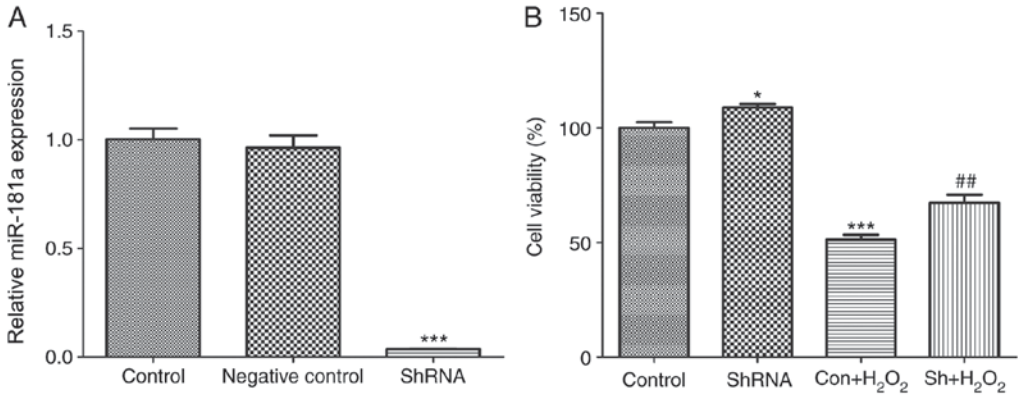

Figure 1. Effects of miR-181a silencing in HLE-B3 cells. (A) miR-181a expression levels in the normal control, the shRNA-negative control and the miR-181a-shRNA group. Data are expressed as the mean ± standard deviation; ***P<0.001 vs. control and negative control. (B) Effects of miR-181a knockdown with or without H_2O_2 co-treatment on the proliferation of HLE-B3 cells. Data are expressed as the mean ± standard deviation; *P<0.05 and ***P<0.001 vs. con; $^{\#\#}$P<0.01 Sh+H_2O_2 vs. con+H_2O_2. H_2O_2, hydrogen peroxide; con, control; HLE, human lens epithelial; miR, microRNA; sh, short hairpin RNA.

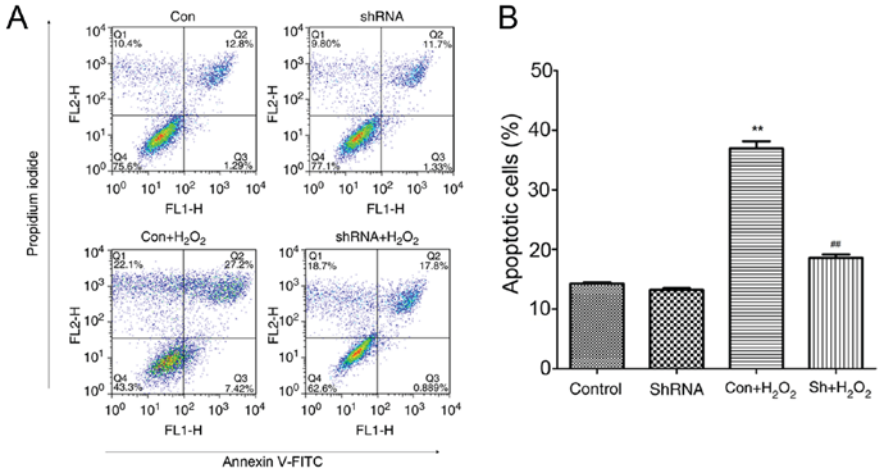

Figure 2. Analysis of HLE-B3 cell apoptosis. (A) HLE-B3 cell apoptosis was assessed using flow cytometry following staining with annexin V/FITC and PI. (B) Quantification of flow cytometry results. **P<0.01 vs. con, $^{\#\#}$P<0.01 vs. Sh+H_2O_2 vs. con+H_2O_2. Con, control; FITC, fluorescein isothiocyanate; H_2O_2, hydrogen peroxide; HLE, human lens epithelial; miR, microRNA; PI, propidium iodide; shRNA, short hairpin RNA.

performed using pLKO.1-puro-miR-181a lentiviral particles that express a miR-181a-targeting shRNA, whereas an empty pLKO.1-puro vector was used as a negative control. To test the knockdown efficiency, the expression of miR-181a was examined using RT-qPCR 48 h post-infection. The expression levels of miR-181a in miR-181a-shRNA-transfected cells were significantly decreased compared with expression levels in the normal control and negative control cells (P<0.001; Fig. 1A). These findings indicated that the construction of the HLE-B3 cell line in which miR-181a expression was stably silenced was successful.

miR-181a knockdown enhances HLE-B3 cell proliferation following H_2O_2 treatment. To assess the effects of shRNA-mediated miR-181a silencing on cell proliferation and survival following H_2O_2 treatment, the growth of HLE-B3 cells was investigated *in vitro*. miR-181a knockdown significantly enhanced the proliferation of HLE-B3 cells compared with normal control cells (P<0.05; Fig. 1B). HLE-B3 cells treated with H_2O_2 (200 μM) exhibited a decrease in viability compared with untreated control cells (P<0.001; Fig. 1B). Notably, knockdown of miR-181a expression partially rescued H_2O_2-treated HLE-B3 cell viability, as compared with the untransfected H_2O_2-treated group (P<0.05; Fig. 1B); however, the proliferation of shRNA-transfected H_2O_2-treated cells remained significantly reduced compared with the control group (P<0.01; Fig. 1B). These findings suggested that shRNA-mediated miR-181a silencing may partially protect the viability of HLE-B3 cells against oxidative stress-induced compromise.

miR-181a knockdown counteracts H_2O_2-induced apoptosis in HLE-B3 cells. Decreased cell viability is closely associated with cell apoptosis (22); therefore, the present study examined whether the downregulation of miR-181a expression in HLE-B3 cells may exert a protective effect against H_2O_2-induced apoptosis. HLE-B3 cells infected with miR-181a-shRNA and treated with H_2O_2 appeared to be less susceptible to H_2O_2-induced damage, as indicated by the lower apoptotic rates compared with untransfected H_2O_2-treated cells (P<0.05; Fig. 2). However, no significant difference in the apoptotic rate was detected between the control and the shRNA-treated groups (P>0.05). These findings suggested that

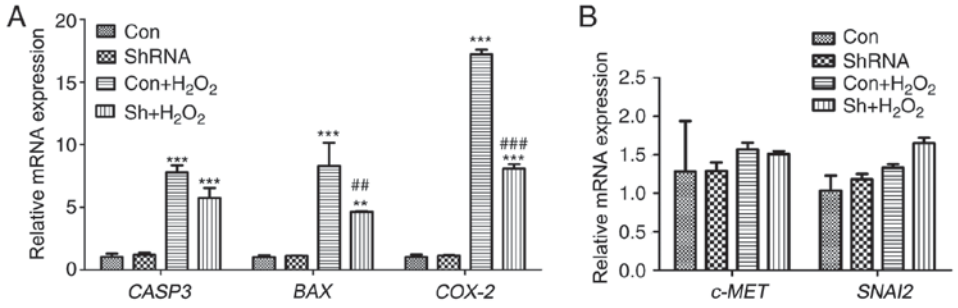

Figure 3. Alterations in gene expressions following miR-181a knockdown in HLE-B3 cells were assessed using reverse transcription-quantitative polymerase chain reaction. (A) Relative mRNA expression of the apoptosis-associated genes *CASP3* and *BAX*, and of the potential miR-181a target gene *COX-2*. (B) Relative mRNA expression of the potential miR-181a target genes *c-MET* and *SNAI2*. Data are expressed as the mean ± standard deviation; **P<0.01, ***P<0.001 vs. con; $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 Sh+H_2O_2 vs. con+H_2O_2. *BAX*, B-cell lymphoma-2-associated X protein; *CASP3*, caspase-3; con, control; *COX-2*, cyclooxygenase 2; H_2O_2, hydrogen peroxide; HLE, human lens epithelial; miR, microRNA; sh, short hairpin; *SNAI2*, snail family transcriptional repressor 2.

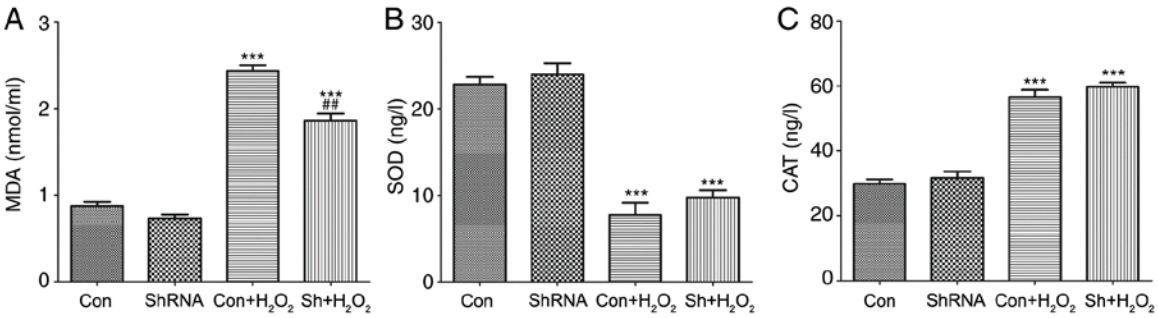

Figure 4. Levels of MDA, SOD and CAT were measured in HLE-B3 cells using ELISA kits. Intracellular expression levels of (A) MDA, (B) SOD and (C) CAT were measured in cells treated with or without miR-181a-shRNA and with or without H_2O_2 co-treatment. Data are expressed as the mean ± standard deviation; ***P<0.001 vs. con; $^{\#\#}$P<0.01 vs. con + H_2O_2. CAT, catalase; con, control; H_2O_2, hydrogen peroxide; HLE, human lens epithelial; miR, microRNA; MDA, malondialdehyde; sh, short hairpin; SOD, superoxide dismutase.

the downregulation of miR-181a expression in HLE-B3 cells may exert a protective effect against H_2O_2-induced apoptosis.

Alterations in gene expression following miR-181a knockdown in HLE-B3 cells. To assess the potential effects of shRNA-mediated miR-181a silencing on gene expression, mRNA expression levels of the apoptosis-associated genes *CASP3* and *BAX*, and of the potential target genes for miR-181a *c-MET*, *COX-2* and *SNAI2* were detected. The results demonstrated that there were no significant differences in shRNA vs. con without treatment. The present results demonstrated that H_2O_2 exposure significantly increased the expression of *CASP3*, *BAX* and *COX-2* compared with expression levels in the normal control group (P<0.001; Fig. 3A). Notably, following knockdown of miR-181a expression in H_2O_2-treated cells, the mRNA expression levels of *BAX* and *COX-2* were significantly suppressed compared with in untransfected H_2O_2-treated cells (P<0.01 and P<0.001 respectively; Fig. 3A). Conversely, cells treated with miR181a-shRNA with or without H_2O_2 co-treatment exhibited no significant effects on the mRNA expression levels of *c-MET* or *SNAI2* (P>0.05; Fig. 3B). These results suggested that shRNA-mediated miR-181a silencing may affect the expression of apoptosis-associated genes *CASP3* and *BAX*, and of the putative *COX-2* miR-181a target gene.

Effects of miR-181a silencing on MDA, SOD and CAT expression levels. MDA is a product of lipid peroxidation and its intracellular levels are indicative of oxidative damage (14), whereas SOD and CAT are endogenous antioxidative enzymes (23). The levels of MDA, SOD and CAT in HLE-B3 cells from the various experimental groups are presented in Fig. 4. The results demonstrated that there were no significant differences in shRNA vs. con without treatment. The results demonstrated that in the presence of H_2O_2, the intracellular levels of MDA and CAT were significantly increased in HLE-B3 cells compared with expression in untreated control cells (P<0.001; Fig. 4A and C, respectively). Conversely, SOD expression levels were significantly reduced in cells following H_2O_2 treatment compared with untreated control cells (P<0.001; Fig. 4B). Notably, the levels of MDA were significantly suppressed in H_2O_2-treated cells following miR-181a knockdown compared with in untransfected H_2O_2-treated cells (P<0.01; Fig. 4A). Conversely, miR-181a-shRNA treatment exhibited no significant effects on the intracellular levels of SOD and CAT in the H_2O_2 co-treated group compared with the expression levels in the untransfected H_2O_2-treated cells (P>0.05; Fig. 4B and C, respectively). These results suggested that miR-181a knockdown may suppress the production of MDA following exposure to oxidative stress *in vitro*.

Discussion

Cataract is among the leading causes of blindness worldwide, and 90% of cataract-related cases of blindness occur in

developing countries (4). miRNAs have garnered attention as a prominent class of gene expression regulators, and mounting evidence supports the implication of miRNAs in the pathophysiology of human cataract (12). The results of the present study suggested that the downregulation of miR-181a expression using RNAi-mediated suppression may protect or rescue HLE-B3 cells *in vitro* from undergoing apoptosis following H_2O_2 exposure. The molecular mechanisms underlying the effects of miR-181a knockdown in human lens cells may involve the suppression of *CASP3*, *COX-2* and *BAX* expression, and the inhibition of MDA production.

Oxidative stress serves a crucial role in the regulation of several cellular events, including oxidative damage and cell death (24). Reactive oxygen species, including H_2O_2, may induce irreversible oxidative modifications to nucleic acids, lipids and proteins, and may lead to cellular damage and cell necrosis or apoptosis (25). A previous study observed a significant decrease in HLE-B3 cell viability in the presence of 200 μM H_2O_2 compared with control untreated cells (22); therefore. 200 μM H_2O_2 was used in the present study to treat HLE-B3 cells and to investigate the effects of silencing miR-181a expression. The present results demonstrated that following transduction with a lentiviral vector encoding miR-181a-shRNA, the expression of miR-181a was successfully silenced. Notably, miR-181a was revealed to partially rescue the compromise in HLE-B3 cell viability following oxidative stress exposure compared with viability in H_2O_2-treated cells.

Compromises in cell viability are closely associated to cell apoptosis, and the present study examined whether miR-181a downregulation in HLE-B3 cells following H_2O_2 exposure affected apoptotic signaling pathways. CASP3 mediates apoptosis thorough the regulation of several crucial events during apoptosis (26). During apoptosis, BAX proteins undergo conformational changes and translocate to the mitochondrial outer membrane, thus allowing the release of proapoptotic factors from the intermembrane space (27). A previous study reported that Grx2 was able to protect cells against H_2O_2-induced compromises in cell viability and against apoptosis through the inhibition of proapoptotic signaling, including BAX activation and CASP3 release (22). Similarly, the present results demonstrated that the downregulation of miR-181a suppressed the mRNA expression of *BAX* and *CASP3* in HLE-B3 cells. These results suggested that miR-181a may serve a significant role in the pathogenesis of cataract, through the regulation of *BAX* and *CASP3* expression in lens cells. Furthermore, the intracellular levels of MDA, SOD and CAT were investigated; MDA levels are indicative of oxidative damage (16), whereas SOD and CAT are antioxidative enzymes (23). The present results revealed that miR-181a knockdown suppressed the generation of MDA, thus suggesting that miR-181a silencing may inhibit oxidative damage associated with apoptosis in lens cells.

Several potential target genes have been identified for miR-181a: For example, a previous study demonstrated that miR-181a-5p was downregulated in hepatocellular cancer and suggested that miR-181a-5p may inhibit tumor motility and invasion by directly targeting the expression of *c-Met* (28). Another study suggested that miR-181a may suppress salivary adenoid cystic carcinoma metastasis by targeting the extracellular signal-regulated kinase/SNAI pathway (29). Furthermore, miRNA-181a has been reported to inhibit the proliferation, migration and EMT of lens epithelial cells by directly targeting *SNAI2* and *COX-2* expression (11). Therefore, the present study investigated the expression of three potential target genes for miR-181a, namely *c-MET*, *COX-2* and *SNAI2* in HLE-B3 cells. The results demonstrated that miR-181a knockdown using RNAi significantly downregulated the mRNA expression of *COX-2* in H_2O_2-treated HLE-B3 cells compared with in untransfected H_2O_2-treated cells. However, H_2O_2 treatment and miR-181a silencing did not exert a significant effect on the mRNA expression levels of *c-MET* and *SNAI2*. The present data suggested that the downregulation of miR-181a expression may inhibit HLE cell apoptosis by targeting *COX-2* expression. Further studies are required to fully elucidate the molecular mechanisms and the target genes that are implicated in the protective effects of miR-181a knockdown.

In conclusion, the results of the present study suggested that the downregulation of miR-181a expression may protect or rescue HLE-B3 cells from undergoing apoptosis following H_2O_2 exposure *in vitro*. The molecular mechanisms underlying the protective effects of miR-181a silencing may involve the downregulation of *CASP3*, *BAX* and *COX-2* expression, and the inhibition of lipid peroxidation. Further studies are required to fully elucidate the functional roles of miR-181a and may provide the foundation for a better understanding of the miRNA-mediated regulation of cataract development in the eye with eventual implications in the diagnosis and treatment of cataract.

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