Long non-coding RNA nuclear paraspeckle assembly transcript 1 protects human lens epithelial cells against H_2O_2 stimuli through the nuclear factor kappa b/p65 and p38/mitogen-activated protein kinase axis

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Background: Long non-coding RNA (lncRNA) nuclear paraspeckle assembly transcript 1 (NEAT1) plays a regulatory role in many biological processes; however, its role in cataracts has yet to be illuminated. This study aimed to investigate the protective role of NEAT1 in hydrogen peroxide (H_2O_2)-treated human lens epithelial cells (HLECs) and its underlying molecular mechanism.

Methods: HLECs (SRA01/04) were treated with 300 μM H_2O_2 to mimic cataract *in vitro*. Cell viability was detected by performing an MTT assay and EdU staining. Flow cytometry was carried out to detect apoptosis of HLECs. DNA damage was examined using γ-H2A histone family member X staining. and reactive oxygen species (ROS) production was measured using 2',7'dichlorofluorescin diacetate staining. The expression levels of lncRNA and proteins were detected with quantitative real-time polymerase chain reaction and western blot, respectively.

Results: The expression of NEAT1 was observed to be increased in H_2O_2-treated HLECs and age-related cataract (ARC) tissues. Knockdown NEAT1 strongly protected against H_2O_2-induced cell death and also regulated the expression of cleaved caspase-3, B-cell lymphoma 2, and Bcl-2-associated X protein. Further, knockdown NEAT1 also significantly suppressed H_2O_2-induced intracellular ROS production and malondialdehyde (MDA) content, but elevated the glutathione (GSH) activity of H_2O_2-treated cells. Also, it is demonstrated that si-NEAT1 greatly inhibited H_2O_2-induced phosphorylation of NF-κB p65 and p38 MAPK.

Conclusions: This study confirmed that knockdown NEAT1 attenuated H_2O_2-induced damage in HLECs, and inhibited the oxidative stress and apoptosis of HLECs via regulating nuclear factor-kappa B (NF-κB) p65 and p38 MAPK signaling. It may provide a potential target for clinical treatment of cataracts.

Keywords: Cataract; hydrogen peroxide (H_2O_2); long non-coding RNA (lncRNA); apoptosis; oxidative stress

Introduction

Cataract is a common ophthalmic disease that is characterized by partial or total loss of lens transparency (1). If left untreated, a cataract can lead to loss of vision and eye function. A decrease of light transmission to the retina is the main cause of vision loss during aging, especially in patients with age-related cataracts (ARCs) (2,3). At present, no treatment to prevent the formation of cataracts exists. Although surgical extirpation and intraocular lens implantation are effective methods for treating cataracts,

Page 2 of 12

Zhou et al. Role of long non-coding RNA in ophthalmic disease

they are associated with a huge financial burden for patients, as well as serious postoperative complications, such as vitreous prolapse and corneal abrasions (4). Apoptosis of human lens epithelial cells (HLECs) has long been considered to be the cytological basis of non-congenital cataracts (5). Therefore, determining the pathogenesis of cataract is crucial to reducing the incidence this disease.

Usually, there is a homeostasis of free radicals and antioxidants present in the aqueous humor and lens. An imbalance in these molecules leads to the occurrence of oxidative stress (OS) (6). OS mediated by reactive oxygen species (ROS) disrupts the integrity and function of cells by attacking lens proteins, which is the main contributor to cataract formation (7,8). Increased ROS production or decreased levels of antioxidants exacerbate OS (9), alter the cellular environment, and trigger HLEC apoptosis, which is considered to be an early event of cataract development (10). Hydrogen peroxide (H_2O_2) is a non-free radical derived from the ROS family that can easily penetrate the lipid membrane and produce toxicity in the lens (11). Previous studies have shown that ROS production is stimulated by H_2O_2-induced epithelial cell injury and protein degradation, similar to human cataract damage (12). Therefore, H_2O_2 has been widely used to induce apoptosis of HLECs in order to simulate cataract formation in cell models (13).

Long non-coding RNAs (lncRNAs) are a class of transcripts exceeding 200 nucleotides in length that have little or no protein coding function (14). LncRNAs regulate gene expression at the transcriptional and posttranscriptional levels (15), and their abnormal expression in diseases is a topic that has been extensively researched. Abnormal expression of lncRNAs, such as lncRNA taurine up-regulated 1 (TUG1), has been found to play a role in the development of cataracts (16). Recent studies have reported that lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) has a protective effect on cells, and a lack of NEAT1 has been observed to increase the susceptibility to stress-induced cell death (17). Furthermore, the up-regulation of NEAT1 can help to alleviate doxorubicin-induced cardiac injury (18). To our knowledge, no report to date has explored the effect of NEAT1 on H_2O_2-stimulated HLECs. In this study, we studied the preventive role of NEAT1 in H_2O_2-treated HLECs (SRA01/04), and attempted to further elucidate the molecular mechanisms involved in this protective effect. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-20-7365).

Methods

Clinical pathology specimens

Between May 2018 and March 2020, tissue samples from 45 patients with ARC were obtained from the Affiliated Hospital of Nantong University, and all patients signed a written informed consent before taking part. The ARC cohort included 15 cases of age-related cortical cataract (ARCC), 15 cases of age-related nuclear cataract (ARNC), and 15 cases of age-related posterior subcapsular cataract (ARPC). The patients in this cohort were aged from 50 to 60 years and had no history of eye damage. A control group comprising 10 cases (aged 36–60 years old, with no known ocular or systemic diseases) who underwent anterior retinal vitrectomy was also enrolled. The 45 ARC patients were screened using the lens opacity classification system III (LOCS III) as the criteria (19). All procedures were approved by the ethics committee of Nantong University (No.NT20180513) and carried out in accordance with the Declaration of Helsinki (2013 version).

Cell culture

HLECs SRA01/04 were obtained from China Center for Type Culture Collection (CCTCC, Wuhan, China). The SRA01/04 cells were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island, USA) with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Sigma, St. Louis, USA) in a humidified incubator at 37 ℃ with 5% CO_2.

Cell viability assay

SRA01/04 cells were cultured in 96-well plates at a density of 1×10^6 cells/well, as previously described (20). The cells were exposed to H_2O_2 (Sigma) at different concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, or 500 μM) for 24 hours. Subsequently, 50 mL MTT reagent (Abcam, Cambridge, UK) was used to culture cells at 37 ℃ for 3 hours. Then, culture plates with 150 mL MTT solvent added were shaken on an orbital shaker for 15 minutes. A microplate reader (Bio-Rad, California, USA) was used to measure the optical density at 590 nm.

γH2AX immunofluorescent staining

DNA damage was performed according to the methods

previously described (21). H_2O_2-induced SRA01/04 cells grown on glass cover slides were fixed in 4% formaldehyde at 4 ℃ overnight, and then the membrane was permeated with phosphate-buffered saline (PBS) containing 0.2% Triton X-100 for 15 minutes. Next, the above cells were sealed with 5% bovine serum albumin (BSA) at room temperature for 1.5 hours, and then incubated with γ-H2A histone family member X (γ-H2AX) antibody (Abcam) overnight at 4 ℃. Following that, they were stained with Alexa Fluor488-labeled (Thermo Fisher, Waltham, USA) secondary antibody for 1 hour at room temperature. Images were obtained with a Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan).

Flow cytometry

Apoptosis of SRA01/04 cells was determined using an Annexin V-FITC/PI Apoptosis Detection Kit (Sigma). SRA01/04 cells were digested with trypsin (Beyotime) and collected. The cells were washed twice with ice-cold PBS and resuspended in 1× Binding Buffer (300 μL, Beyotime) according to the manufacturer's instructions. Subsequently, the cells were double-stained with fluorescein isothiocyanate (FITC)-Annexin V (5 μL) and propidium iodide (PI, 5 μL) in the dark at room temperature for 15 minutes. Finally, a flow cytometer (Thermo Fisher) was used to analyze the fluorescence signals accompanied by light scattering.

Generation of OS

SRA01/04 cells were plated into a 6-well plate at a density of 2×10^5/well and incubated in a 5% CO_2 incubator at 37 ℃. When the cells reached 70–80% confluency, the upper culture medium was removed and the plate was washed with PBS. The cells were stained with 20 μM 2',7'-dichlorofluorescin diacetate (DCFDA) solution from a DCFDA/H2DCFDA-Cellular ROS Assay Kit (ab113851, Abcam) for 30 minutes at 37 ℃, according to the manufacturer's instructions. Then, the living cells were imaged with a filter group suitable for FITC. Additionally, the content of malondialdehyde (MDA) and activity of glutathione (GSH) were examined using the corresponding assay kits (Beyotime, Haimen, China).

Cell transfection

Low-expressed lncRNA NEAT1 was obtained in SRA01/04 cells using small interfering RNA (siRNA) against NEAT1 (5'-GAGCAATGACCCCGGTGACG-3') and a non-targeting siRNA as a normal control (NC, 5'-TAGATACCCCCAGGCCTACC-3'), which were synthesized by Sangon Biotech (Shanghai, China). SRA01/04 cells were transfected with si-NEAT1 or si-NC using Lipofectamine™ 3000 Transfection Reagent (Invitrogen, CA, USA), according to the manufacturer's instructions. After that, SRA01/04 cells were treated with H_2O_2 (300 μM).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from SRA01/04 cells and ARC tissues using TRIzol reagent (Invitrogen), and cDNA was synthesized using a PrimeScript™ RT reagent kit (Takara, Otsu, Japan), according to the manufacturer's instructions. DNA was amplifiied through qRT-PCR using SYBR Green Mix Kit (Takara). The expression of NEAT1 was analyzed using the $2^{-\Delta\Delta Ct}$ method (22), and was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers of NEAT1 and GAPDH used are listed below:

NEAT1: F 5'-CTTCCTCCCTTTAACTTATCCATT CAC-3',

R 5'-CTCTTCCTCCACCATTACCAACAATAC-3';
GAPDH: F 5'-AGGTCGGTGTGAACGGATTTG-3',
R 5'-TGTAGACCATGTAGTTGAGGTCA-3'.

5-Ethynyl-2'-deoxyuridine (EdU) assay

To evaluate the influence of NEAT1 on SRA01/04 cell proliferation, EdU staining (Invitrogen, Waltham, USA) was conducted. SRA01/04 cells were inoculated into a 6-well plate (1×10^4 cells/well) and incubated at 37 ℃ with 5% CO_2 for 24 hours, then treated with 50 μM EdU labeling solution for 2 hours in the dark, according to the manufacturer's instructions. After that, the cells were fixed in 4% paraformaldehyde for 15 minutes at 37 ℃, and stained with 100 μL Hoechst 33342 (ApexBio, Houston, USA) at 37 ℃ for 10 minutes in the dark. Finally, images of EdU positive cells were captured with an inverted fluorescence microscope (Olympus, Tokyo, Japan).

Western blot (WB) analysis

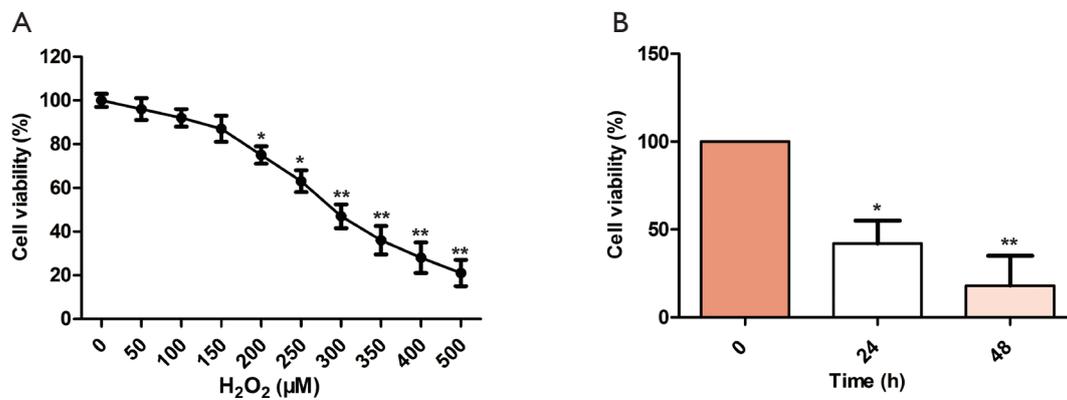Total protein from SRA01/04 cells was extracted using

Page 4 of 12

Zhou et al. Role of long non-coding RNA in ophthalmic disease

Figure 1 Effect of H_2O_2 on the cell viability of HLECs. (A) SRA01/04 cells were treated with H_2O_2 at different concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, or 500 µM) for 24 hours. (B) Subsequently, SRA01/04 cells were exposed to 300 µM H_2O_2 at different time points (0, 24, or 48 hours). Data from three independent procedures are presented as the mean ± SD. *, P<0.05; **, P<0.01 *vs.* control. H_2O_2, hydrogen peroxide; HLECs, human lens epithelial cells.

RIPA lysis buffer (Beyotime) and separated with 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel, after which the protein was transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad). The PVDF membranes were sealed in 5% nonfat milk at room temperature for 2 hours, and then incubated with the primary antibodies against B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), cleaved caspase-3, nuclear factor-kappa B (NF-κB) p65, Phospho-NF-κB p65, p38 mitogen-activated protein kinase (p38 MAPK), and Phospho-p38 MAPK at 4 ℃ overnight. On the second day, the appropriate secondary antibodies bound to horseradish peroxidase (Beyotime) were incubated at room temperature for 1 hour. Finally, the strips were exposed with electrochemiluminescence (ECL) reagent (Pierce, Rockford, USA), and quantitative analysis was performed using image laboratory software (Bio-Rad). All antibodies were obtained from Cell Signaling Technology (CST, Beverly, USA).

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and analyzed with one-way analysis of variance (Significant differences between three or more groups) or Student's *t*-test (significant differences between two groups). Statistical analyses were conducted with SPSS Statistics 19.0 (IBM, Armonk, USA) and Prism 7 (GraphPad, San Diego, USA) software. A P value of <0.05 was considered to show a statistically significant difference.

Results

H_2O_2 inhibited the cell viability of HLECs

To examine the cytotoxic effects of H_2O_2 on HLECs, SRA01/04 cells were treated with different concentrations of H_2O_2 (from 0–500 µM) for 24 hours. As shown in *Figure 1A*, the viability of SRA01/04 cells was significantly reduced under treatment with 200, 250, 300, 350, 400, and 500 µM H_2O_2. Moreover, with 300 µM H_2O_2, cell viability was remarkably decreased at different time points (0, 24, and 48 h; *Figure 1B*). Therefore, H_2O_2 at a concentration of 300 µM was used for the subsequent experiments.

H_2O_2 induced HLEC oxidative injury

After stimulation with H_2O_2 (300 µM), the number of cells that stained positive for γ-H2AX was increased (*Figure 2A*) and p53 protein expression was remarkably up-regulated (*Figure 2B,C*) compared with the controls. As shown in *Figure 2D,E*, the percentage of apoptotic cells in the H_2O_2 group was dramatically higher than that in the control group. And H_2O_2 induced the up-regulation of Bax and down-regulation of cleaved caspase-3 and Bcl-2. Furthermore, the average fluorescence intensity of ROS and MDA content were increased after the exposure of SRA01/04 cells to H_2O_2. Similarly, H_2O_2 inhibited GSH activity in SRA01/04 cells (*Figure 2F,G,H*). Together, these results indicated that H_2O_2 induced HLEC oxidative injury.
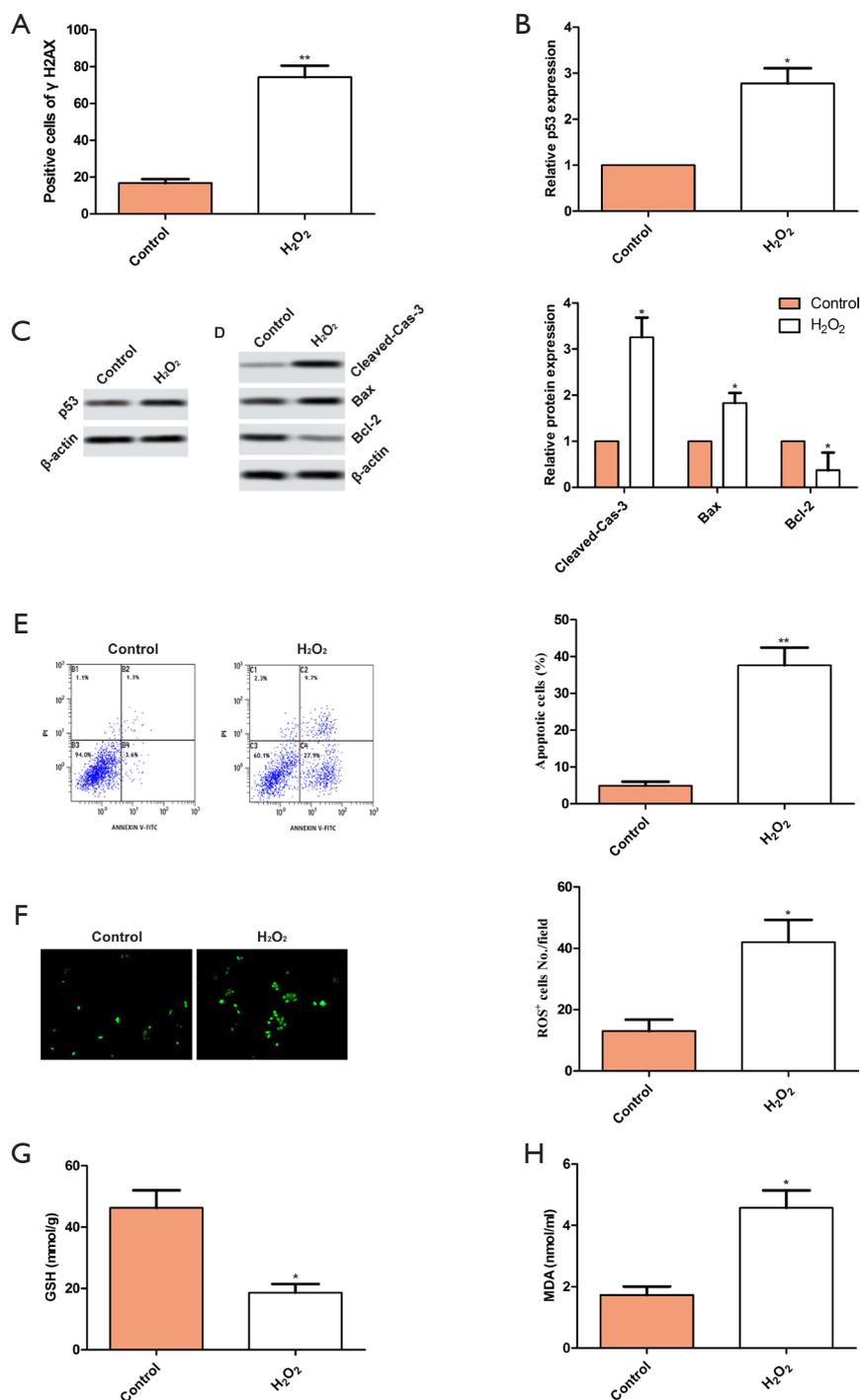
Figure 2 Effect of H_2O_2 on cell injury in HLECs. (A) The DNA level of SRA01/04 cells was examined by performing γ-H2AX immunofluorescent staining. (B,C) The relative protein expression of p53 was examined with western blot. (D) The relative protein expression of cleaved caspase-3, Bax, and Bcl-2 were examined by western blot. (E) The apoptosis rate of SRA01/04 cells treated with H_2O_2 was measured using flow cytometry. (F) ROS⁺ production was determined through DCFDA staining (magnification, 100×). (G) MDA content and (H) GSH activity were detected using commercial assay kits. Data from three independent procedures are presented as the mean ± SD. *, P<0.05; **, P<0.01 *vs.* control. H_2O_2, hydrogen peroxide; HLECs, human lens epithelial cells; Bax, Bcl-2-associated X; Bcl-2, B-cell lymphoma-2; ROS, reactive oxygen species; DCFDA, 2',7'-dichlorofluorescin diacetate; MDA, malondialdehyde; GSH, glutathione.
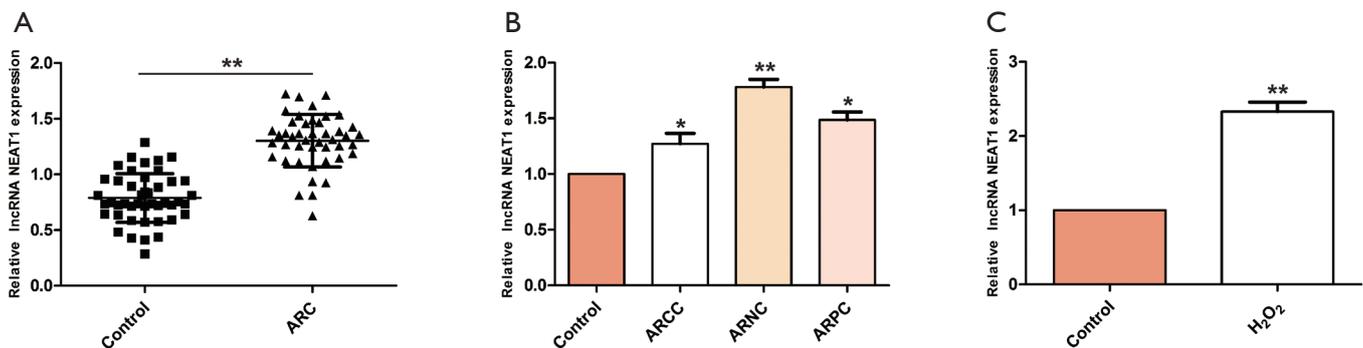
Page 6 of 12

Zhou et al. Role of long non-coding RNA in ophthalmic disease

Figure 3 NEAT was down-regulated in ARC tissues and H_2O_2-treated HLECs. (A) The mRNA level of NEAT in normal tissues and ARC tissues was detected by qRT-PCR. (B) The mRNA levels of NEAT in tissue samples from the ARC subgroup (ARCC, ARNC, and ARPC) and the control group were detected by qRT-PCR. (C) The mRNA levels of NEAT in SRA01/04 cells with or without exposure to H_2O_2 was detected by qRT-PCR. Data from three independent procedures are presented as the mean ± SD. *, P<0.05; **, P<0.01 *vs.* control. NEAT, nuclear paraspeckle assembly transcript 1; ARC, age-related cataract; H_2O_2, hydrogen peroxide; HLECs, human lens epithelial cells; mRNA, messenger RNA; ARCC, age-related cortical cataract; ARNC, age-related nuclear cataract; ARPC, age-related posterior subcapsular cataract; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

NEAT1 was upregulated in ARC tissues and H_2O_2-treated HLECs

As shown in *Figure 3A*, qRT-PCR confirmed that NEAT1 expression in ARC tissues was increased compared to that in the control tissue samples. ARC subgroup analysis was performed to determine which subtype of ARC had the highest expression NEAT1. The expression of NEAT1 was found to be most significantly increased in the ARCC group (*Figure 3B*). Moreover, after treatment with 200 µM H_2O_2 for 24 hours, the expression of NEAT1 was significantly increased compared to the control group (*Figure 3C*).

NEAT1 knockdown improved H_2O_2-induced cell proliferation

qRT-PCR analysis (*Figure 4A*) showed that the expression of NEAT1 in SRA01/04 cells was increased by H_2O_2 treatment, while cells transfected with si-NEAT1 exhibited lower NEAT1 expression, which indicated high transfection efficiency. MTT detection found that H_2O_2 significantly reduced the cell viability of SRA01/04 cells at 0, 24, 48, and 72 hours, while a low expression of NEAT1 could partially attenuate the inhibitory effect of H_2O_2 on cell viability (*Figure 4B*). Furthermore, the data from the EdU assay demonstrated that while H_2O_2 triggered the inhibition of cell proliferation, si-NEAT1 transfection

significantly weakened the inhibitory effect of H2O2 on cell proliferation (*Figure 4C*).

NEAT1 knockdown suppressed H_2O_2-induced apoptosis

To verify the effect of NEAT1 on H_2O_2-induced apoptosis of SRA01/04 cells, flow cytometry and western blot assay were performed. Annexin V FITC/PI double staining revealed that si-NEAT1 transfection markedly decreased the apoptosis rate of SRA01/04 cells, compared with the H_2O_2 group (*Figure 5A*). Also, western blot revealed that H_2O_2 treatment up-regulated the expression levels of cleaved caspase-3 and Bax, and down-regulated Bcl-2 expression. However, these changes were reversed by transfection with si-NEAT1 (*Figure 5B*).

NEAT1 knockdown alleviated H_2O_2-induced OS

The antioxidant defense system-related processes of SRA01/04 cells, such as ROS production, MDA, and GSH activity, was also examined. The average fluorescence intensity of intracellular ROS production was determined using a DCFHDA method (*Figure 6A*). After H_2O_2 treatment, the average fluorescence intensity of ROS in SRA01/04 cells was increased, while ROS content was significantly decreased after transfection with si-NEAT1. Compared to the control group, GSH activity (*Figure 6B*)
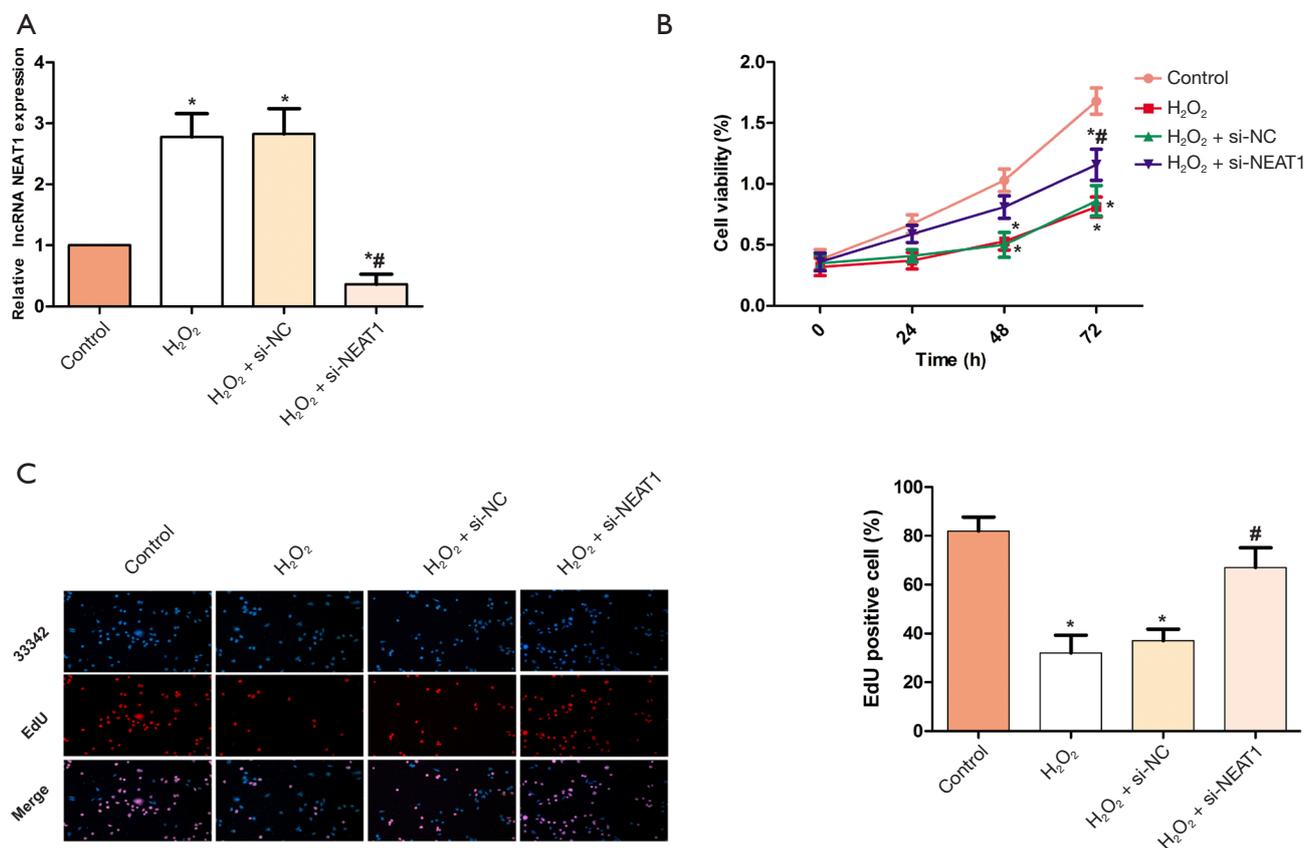
Figure 4 Effect of NEAT on H_2O_2-induced cell proliferation of HLECs. SRA01/04 cells were transfected with si-NC or si-NEAT. (A) The mRNA levels of NEAT in SRA01/04 cells with or without exposure to H_2O_2 were detected by qRT-PCR. (B) Cell viability of SRA01/04 cells was examined with 300 μM H_2O_2 at different time points (0, 24, 48, or 72 h). (C) Cell proliferation was evaluated by EdU staining (magnification, 20×). Data from three independent procedures are presented as the mean ± SD. *, P<0.05 vs. control; #, P<0.05 vs. H_2O_2. NEAT, nuclear paraspeckle assembly transcript 1; H_2O_2, hydrogen peroxide; HLECs, human lens epithelial cells; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; 5-Ethynyl-2'-deoxyuridine.

was decreased and MDA content (*Figure 6C*) was increased in SRA01/04 cells after exposure to H_2O_2, and transfection with si-NEAT1 reversed these effects (*Figure 6B,C*).

NEAT1 protected against H_2O_2 stimuli through the NF-κB p65/p38 MAPK pathways

To explore the molecular mechanism of the protective effect of NEAT1 against H_2O_2 stimulation, we assessed the effects of NEAT1 on the expression of p65 and p38 MAPK, which play an important role in the regulation of intracellular metabolism (23). Our data revealed that stimulation with H_2O_2 resulted in a significant increase in p65 and p38 MAPK expression. As expected, the increases in p65 and p38 MAPK expression were significantly reduced by si-

NEAT1 (*Figure 7A*). To further confirm the inhibitory effect of NEAT1, SRA01/04 cells exposed to H_2O_2 were treated with p65 (PDTC) or p-38 MAPK (LY2228820) inhibitors. Compared to those transfected with si-NEAT1 alone, H_2O_2-treated SRA01/04 cells transfected with si-NEAT1 combined with PDTC or LY2228820 exhibited much higher cell viability (*Figure 7B*) and GSH activity (*Figure 7C*), and a much lower MDA content (*Figure 7D*).

Discussion

Cataract is a multifactorial ophthalmic disease characterized by opacity or a loss of transparency in the normally clear lens of the eye (1). Surgical intervention and replacement of the lens is the only clinical treatment for cataract patients.
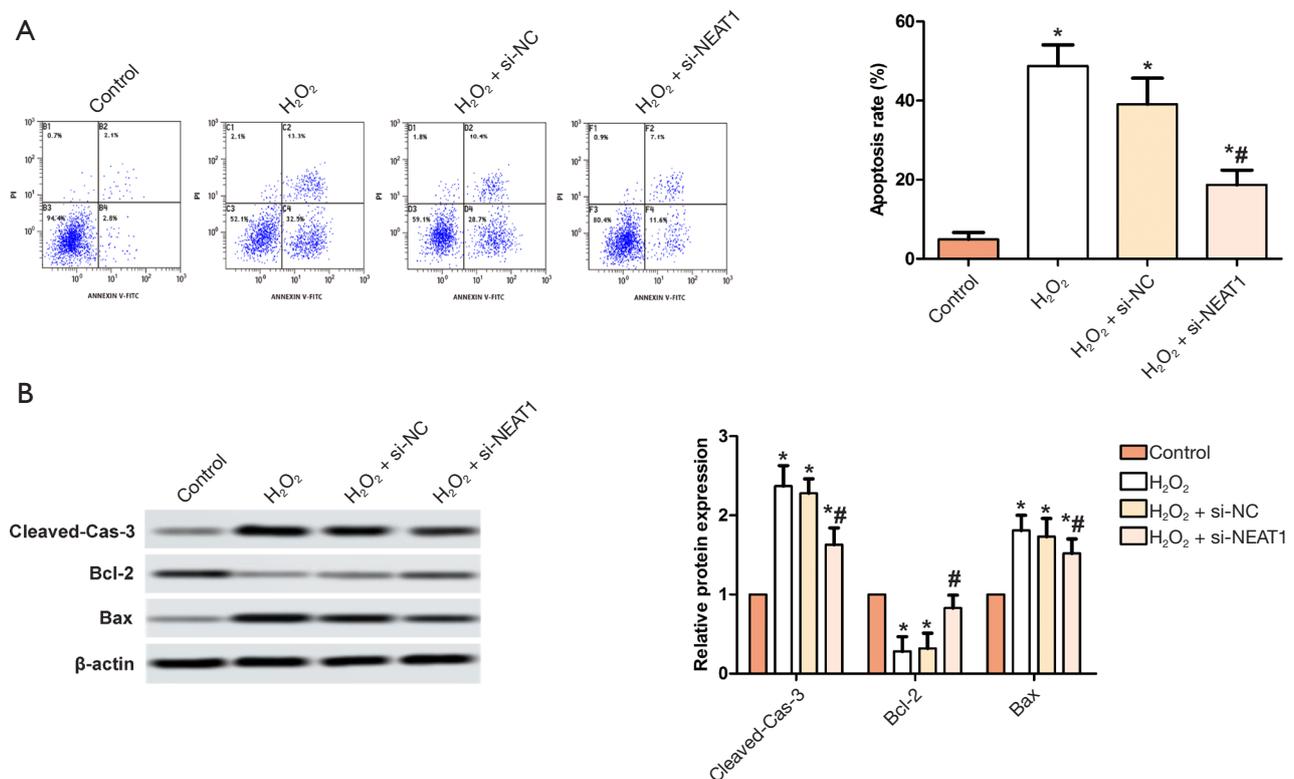
Page 8 of 12

Zhou et al. Role of long non-coding RNA in ophthalmic disease

Figure 5 Effect of NEAT on H_2O_2-induced apoptosis of HLECs. SRA01/04 cells were transfected with si-NC or si-NEAT. (A) The apoptosis rate was detected by flow cytometry. (B) The relative protein expressions of cleaved caspase-3, Bax, and Bcl-2 were examined through western blot. Data from three independent procedures are presented as the mean ± SD. *, P<0.05 *vs.* control; #, P<0.05 *vs.* H_2O_2. NEAT, nuclear paraspeckle assembly transcript 1; H_2O_2, hydrogen peroxide; HLECs, human lens epithelial cells; Bax, Bcl-2-associated X; Bcl-2, B-cell lymphoma-2.

Therefore, the study of cataracts should focus on the exploration of new therapeutic targets. Apoptosis of lens epithelial cells is universally acknowledged to be closely related to the formation of cataracts, and OS induced by ROS has been considered as a major contributor to apoptosis of lens epithelial cells (12,24). In this study, H_2O_2 was used to construct a cell model of oxidative damage in HLECs. We found that H_2O_2 significantly suppressed cell viability in a dose- and time-dependent manner, and promoted OS and apoptosis of SRA01/04 cells, which was consistent with previous reports (25). Additionally, H_2O_2 treatment was observed to significantly increase the expression of NEAT1 in HLECs. Also, pathological examination showed that NEAT1 expression was up-regulated in ARCC, ARNC and, ARPC patient specimens compared to the control group.

Apoptosis of HLECs is a common cellular basis for non-congenital cataracts (5,10). Under multiple stresses, including H_2O_2 stimulation, lower levels of p53 proteins are activated through subsequent cell cycle arrest or apoptosis pathways (26). In this study, we found that H_2O_2 reduced cell viability of HLECs in a concentration- and time-dependent manner (*Figure 1A,B*), suggesting that H_2O_2 is cytotoxic to HLECs. To quantitatively assess the potential protective effect of NEAT1 for H_2O_2-induced cell death, we detected the percentage of apoptotic SRA01/04 cells by performing a V/PI double-staining assay. A significant increase was observed in the total number of apoptotic cells after exposure to 300 μM H_2O_2 (*Figure 5*). Compared with H_2O_2 alone, a low expression of NEAT1 led to an obvious decrease in the number of apoptotic cells. Caspases are recognized as the main promoters and executors of apoptosis (27). Caspase-3 is a key effector of apoptosis, and activated caspase-3 is involved in the mitochondrial-mediated pathway. Therefore, we examined the expression levels of cleaved caspase-3, Bax, and Bcl-2 through western blot (*Figure 5B*). Notably, si-NEAT1 significantly inhibited the down-regulation of Bcl-2, and up-regulation of cleaved
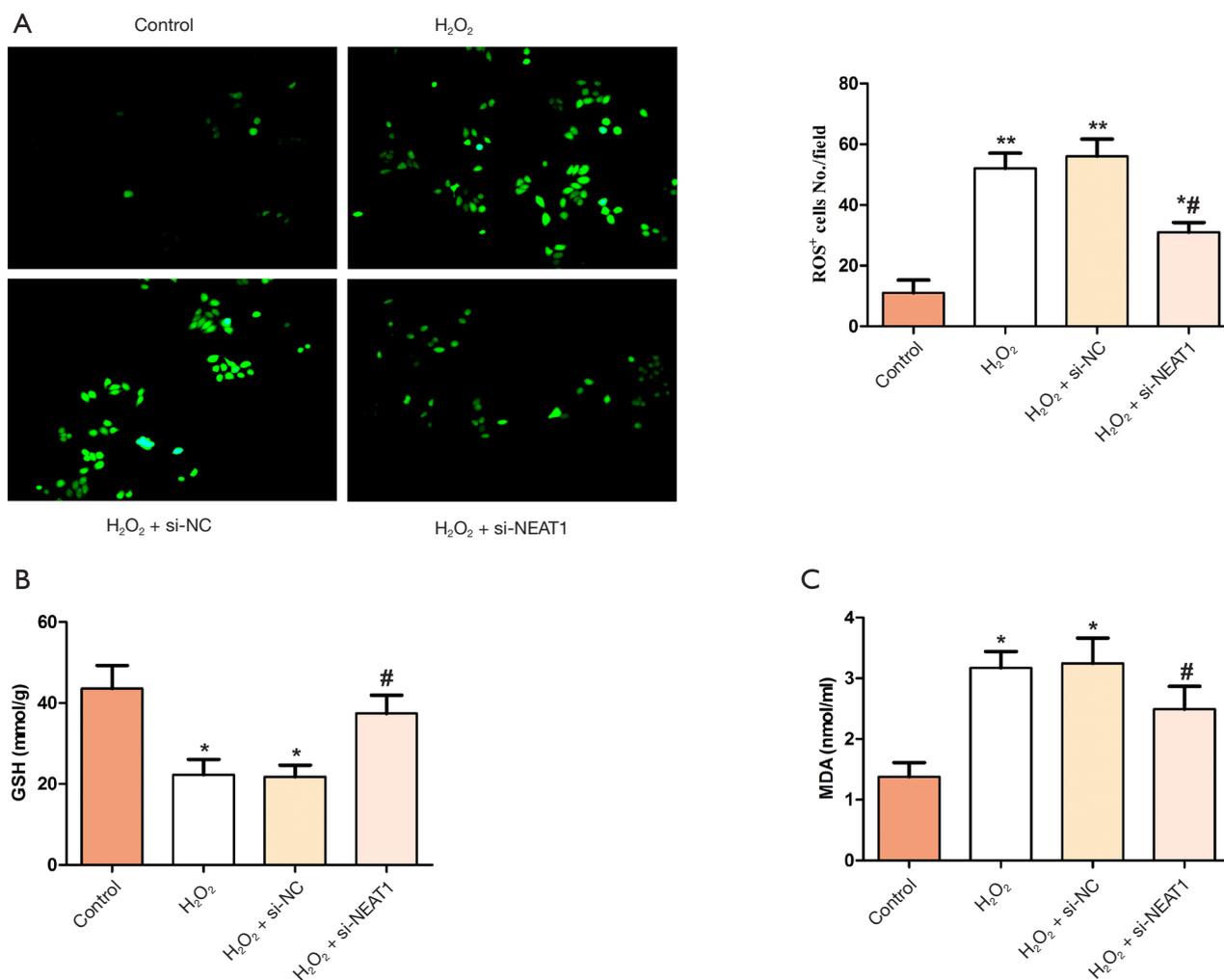
Figure 6 Effect of NEAT on H_2O_2-induced oxidative stress in HLECs. SRA01/04 cells were transfected with si-NC or si-NEAT. (A) ROS+ production was measured through DCFDA staining (magnification, 100×). (B) MDA content and (C) GSH activity were detected using commercial assay kits. Data from three independent experiments are presented as the mean ± SD. *, $P<0.05$; **, $P<0.01$ vs. control; #, $P<0.05$ vs. H_2O_2. NEAT, nuclear paraspeckle assembly transcript 1; H_2O_2, hydrogen peroxide; HLECs, human lens epithelial cells; ROS, reactive oxygen species; DCFDA, 2',7'-dichlorofluorescin diacetate; MDA, malondialdehyde; GSH, glutathione.

caspase-3 and Bax. Overall, these results suggested that a low expression of NEAT1 protected against H_2O_2-induced HLEC apoptosis.

OS is a loss of balance between oxidants and antioxidants due to increased free radical production or reduced free radical scavenging capacity (28). H_2O_2 is the main component of ROS. Stimulation by H_2O_2 destroys the lens' natural antioxidant defense system, resulting in the decrease of SOD and GSH activity, and the increase of MDA content (29). We observed that H_2O_2 induced the production of intracellular ROS in HLECs, while si-

NEAT1 pretreatment significantly inhibited ROS production of intracellular free radicals (*Figure 6A*). Meanwhile, we also found that, compared with the control group, GSH activity was significantly decreased in H_2O_2-treated HLECs, while MDA content was increased, suggesting that H_2O_2 induced the OS of HLECs. Transfection with si-NEAT1 significantly increased GSH activity and reduced MDA content (*Figure 6B,C*). These results indicated that NEAT1 knockdown had antioxidant activity in H_2O_2-treated HLECs (13,24,25).
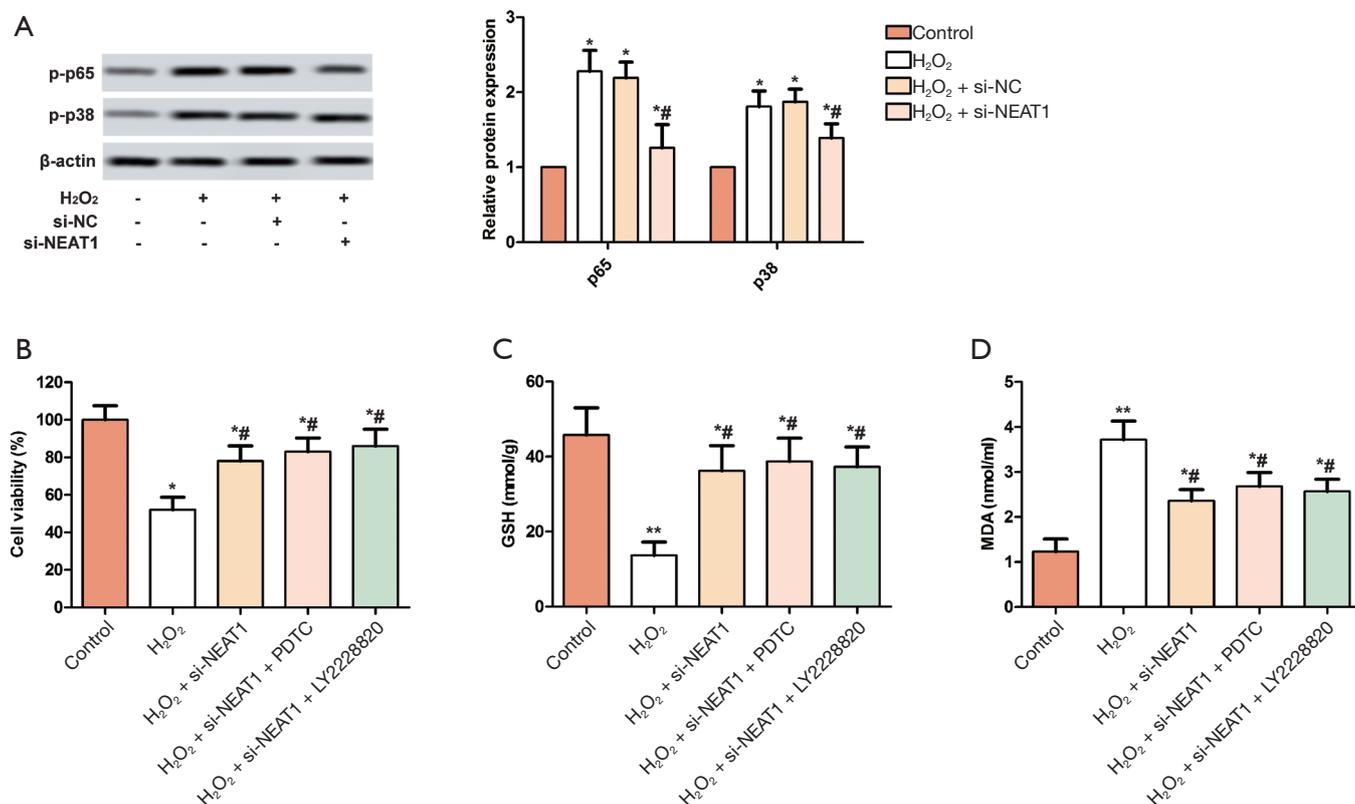
To explore the molecular mechanism of NEAT1 on

Page 10 of 12

Zhou et al. Role of long non-coding RNA in ophthalmic disease

Figure 7 NEAT inhibited *NF-κB/p65* and *p38/MAPK* signaling in H_2O_2-treated HLECs. SRA01/04 cells were transfected with si-NC or si-NEAT. (A) The relative protein expressions of p65, and p38 were examined through western blot. SRA01/04 cells were pre-treated with si-NEAT, p65 inhibitor (PDTC, 2 μM), or p-38 inhibitor (LY2228820, 1.25 μM), or combination of si-NEAT and PDTC or LY2228820 for 2 hours. (B) Cell viability was then measured using the MTT method. Data three independent experiments are presented as the mean ± SD from. (C) GSH activity and (D) MDA content was detected using commercial assay kits. *, $P<0.05$; **, $P<0.01$ *vs.* control; #, $P<0.05$ *vs.* NEAT, nuclear paraspeckle assembly transcript 1; H_2O_2. H_2O_2, hydrogen peroxide; HLECs, human lens epithelial cells; PDTC, pyrrolidinedithiocarbamate ammonium.

H_2O_2-induced HLEs, we assessed the potential role of NEAT1 on NF-κB p65 and p38 MAPK expression, which plays an important role in the regulation of intracellular metabolism and response to stress (30,31). NF-κB belongs to the family of evolutionarily conserved transcription factors, which regulate cell survival, apoptosis, and inflammatory response (32). Previous research has shown that H_2O_2 could activate NF-κB pathways in HLECs, activated p65 transferred from the cytoplasm to the nucleus (33). However, the inactivated p65 effectively attenuated the cytotoxicity of HLECs exposed to H_2O_2 (34). We found that the expression of phosphorylated p65 was significantly increased in H_2O_2-induced HLECs (*Figure 7A*). OS can specifically activate p38 MAPK signaling pathways under different conditions. Inactivation of p38 phosphorylation reduces H_2O_2-induced apoptosis and ROS production in

HLECs (35). Our data showed that H_2O_2 treatment led to a significant increase in p38 phosphorylation. As expected, si-NEAT1 significantly interfered with this increase in p38 phosphorylation (*Figure 7A*). To further confirm the role of p65 and p38 MAPK signaling pathways, HLECs exposed to H_2O_2 being treated with p65 inhibitors (PDTC) or p38 inhibitors (LY2228820). Compared to those transfected with si-NEAT1 alone, SRA01/04 cells treated with si-NEAT1 in combination with either PDTC or LY2228820 showed higher cell viability (*Figure 7B*). Similarly, after treatment with si-NEAT1 combined with PDTC or LY2228820, more significant changes were observed in GSH activity (*Figure 7C*) and MDA level (*Figure 7D*). Taken together, these results demonstrated that NEAT1 exerts protective effects against H_2O_2-induced cell injury in HLECs through mediating p65 and p38 MAPK signaling pathways (36).

Conclusions

In conclusion, we constructed an *in vitro* model of oxidative damage by exposing HLECs (SRA01/04) to H_2O_2. The present study showed for the first time that NEAT1 knockdown could protect HLECs against H_2O_2-induced cell damage by inactivating the phosphorylation of p65 and p38 MAPK. Our study has provided a potential therapeutic target for the treatment of cataracts, but further *in vivo* studies are needed to fully elucidate NEAT1 functional role.

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Footnote

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Page 12 of 12

Zhou et al. Role of long non-coding RNA in ophthalmic disease

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