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Original Research Article

Down-regulation of NRIP1 alleviates pyroptosis in human lens epithelial cells exposed to hydrogen peroxide by inhibiting NF-κB activation

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Abstract

Purpose: *To investigate the role of nuclear receptor-interacting protein 1 (NRIP1) in oxidative stress-induced apoptosis and pyroptosis in cataract disease.*
Methods: *Human lens epithelial cells (HLE-B3 cells) were exposed to hydrogen peroxide (H_2O_2). NRIP1 expression in hydrogen peroxide (H_2O_2)-treated HLE-B3 cells was determined by western blotting and quantitative reverse transcription polymerase chain reaction (qRT-PCR). CCK8 and EdU staining were used to assess cell viability. Flow cytometry and western blotting were used to assess pyroptosis.*
Results: *NRIP1 was significantly up-regulated in HLE-B3 cells post-H_2O_2 incubation ($p < 0.01$). Hydrogen peroxide incubation reduced cell viability and proliferation of HLE-B3 cells, while NRIP1 knockdown enhanced cell viability and proliferation. NRIP1 silencing attenuated the H_2O_2-induced increase in NLRP3, N-terminal domain of gasdermin D, caspase-1, interleukin (IL)-1β, and IL-18 in HLE-B3 cells, but suppressed the pyroptosis of H_2O_2-treated HLE-B3 cells. Hydrogen peroxide incubation down-regulated protein expression of cytoplasmic NF-κB and up-regulated nuclear NF-κB, while the expression of cytoplasmic NF-κB was increased and nuclear NF-κB was decreased in HLE-B3 cells by HLE-B3 interference.*
Conclusion: *NRIP1 down-regulation represses apoptosis and pyroptosis of H_2O_2-treated human lens epithelial cells by inhibiting NF-κB activation, thus, providing a potential strategy to treat cataract disease.*

Keywords: *NRIP1, Oxidative stress, Apoptosis, Pyroptosis, Human lens epithelial cells, NF-κB, Cataract*

INTRODUCTION

A cataract is characterized by opacity of the lens, results in severe visual impairment, and is the leading cause of blindness and visional disability worldwide [1]. Although surgical intervention is effective in preventing cataract lens formation, the recurrence of cataracts after surgery suggests that cataracts remain a major public health problem [2]. Therefore, developing new strategies to treat cataracts is urgent.

Environmental insult induces dysregulated metabolism in the lens, a single layer of epithelial cells, and contributes to the pathogenesis of cataracts [3]. Emerging evidence has shown that oxidative stress contributes to aggregation, oxidation, and degradation of lens proteins, leading to apoptosis and pyroptosis of lens epithelial cells and the development of a cataract [4]. Suppression of oxidative stress-induced lens epithelial cell apoptosis is considered a potential strategy to prevent cataract formation [5]. Pyroptosis is implicated in the pathogenesis of distinct diseases [6]. Oxidative stress also induces pyroptosis of lens epithelial cells, and pyroptosis inhibition has shown promising effects in preventing cataract formation [7].

Nuclear receptor-interacting protein 1 (NRIP1, also known as RIP140) was first identified in cancer cells and participates in physiological processes of tumors by interacting with other transcription factors [8]. NRIP1 has been shown to promote gastric cancer progression [9]. Oxidative stress-induced senescence is suppressed by NRIP1 deletion [10], and NRIP1 down-regulation protects HK-2 against high glucose-induced inflammation and apoptosis [11]. Moreover, NRIP1 was highly expressed in the lens of cataract patients compared with normal lens, demonstrated by oligonucleotide microarray hybridization experiments [12]. However, the role of NRIP1 in oxidative stress-induced pyroptosis of lens epithelial cells has not been reported yet.

In this study, human lens epithelial cells (HLE-B3 cells) were exposed to hydrogen peroxide (H_2O_2) to establish an *in vitro* cell model of cataract formation. The effects of NRIP1 on pyroptosis of H_2O_2-treated HLE-B3 cells were then investigated.

EXPERIMENTAL

Cell culture and treatment

Human HLE-B3 cells were purchased from Procell Life Science and Technology Co., Ltd. (Wuhan, China) and were cultured in RPMI-1640 medium (HyClone, Logan, UT, USA) containing 1 g/L of glucose, 10 % fetal bovine serum (HyClone) (Gibco, Carlsbad, CA, USA), and 1 % penicillin/streptomycin (Gibco). The cells were incubated with 25, 50, or 100 μM H_2O_2 (Sigma-Aldrich, St. Louis, MO, USA) for 24 h.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The RNAs were extracted from HLE-B3 cells post H_2O_2 exposure with TRIzol (Invitrogen, Carlsbad, CA, USA). The RNAs were transcribed into cDNAs and then were used for qRT-PCR analysis of NRIP1. The primers are shown in Table 1, and NRIP1 expression was normalized to GAPDH expression.

CCK8 and EdU staining

HLE-B3 cells were seeded into 96-well plates, incubated with 50 μM H_2O_2 for 24 h, and then transfected with shRNA targeting NRIP1 (shNRIP1) or negative control (shNC) by Lipofectamine 3000 (Invitrogen). Two days later, the cells were cultured in RPMI-1640 medium for another 4, 8, 12, or 24 h before incubation with CCK-8 solution (Beyotime, Beijing, China). Absorbance at 450 nm was measured using a microplate reader (BioTek, Winooski, VT, USA). For EdU staining, the transfected HLE-B3 cells were incubated with 50 μM EdU from an EdU cell proliferation detection kit (Sigma-Aldrich). The cells were then fixed in 4 % paraformaldehyde and incubated with a specific antibody against EdU (Abcam, Cambridge, UK). The nuclei were stained with DAPI (Sigma-Aldrich), and the cells were observed with a fluorescence microscope (Nikon, Tokyo, Japan).

Flow cytometry

After HLE-B3 cell incubation with H_2O_2 and transfection, cells were harvested using trypsin and resuspended in phosphate-buffered saline. The cells were then labeled with propidium iodide (Beyotime, Beijing, China) and analyzed by flow cytometry (Becton Dickinson Biosciences, San Jose, CA, USA).

Table 1: Sequences of primers used

Gene		Primer
PHLDA2	Forward Reverse	5’-GTGTCGACATGACTCATGGAGAAGAGCTT-3’ 5’-GTGGGCCCTTATTCTGATTCTTTCTTTATCG-3’
GAPDH	Forward Reverse	5’-GAAGGTGAAGGTCGGAGTC-3’ 5’- GAAGATGGTGATGGGATTTC-3’

Western blotting

HLE-B3 cells were lysed in RIPA lysis buffer (Beyotime) and then centrifuged at 12000 g to harvest the supernatants. The PARIS™ Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to isolate the cytoplasmic and nuclear proteins. The protein concentrations of the supernatants were determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). The samples were then separated using 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked with 5 % bovine serum albumin and probed with the following specific antibodies: anti-NRIP1 and anti-NLRP3 (1:2000; Abcam), anti-GSDMD-N (N-terminal domain of gasdermin D) and anti-caspase-1 (1:2500; Abcam), anti-IL (interleukin)-1β and anti-IL-18 (1:3000; Abcam), anti-NF-κB and anti-β-actin (1:3500; Abcam), anti-cytoplasmic NF-κB and anti-β-tubulin (1:4000; Abcam), and anti-nuclear NF-κB and anti-Histone H3 (1:4500; Abcam). Following incubation with horseradish peroxidase-conjugated secondary antibody (1:5000; Abcam) and tetramethylbenzidine, the protein bands were visualized using chemiluminescence (Sigma-Aldrich).

Statistical analysis

The data were expressed as means ± standard error of the mean (n = 3) and analyzed using Student's t test or one-way analysis of variance and SPSS software 19.0 (Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

NRIP1 expression is elevated in H_2O_2-treated HLE-B3 cells

HLE-B3 was incubated with H_2O_2 to establish a cell model of cataracts. NRIP1 mRNA levels were up-regulated in HLE-B3 cells post H_2O_2 incubation (Figure 1 A). Moreover, protein expression of NRIP1 was also increased in H_2O_2-treated HLE-B3 cells in a dose-dependent manner (Figure 1 B), suggesting a possible relationship between NRIP1 and H_2O_2-induced oxidative stress in HLE-B3 cells.

Down-regulation of NRIP1 promotes cell proliferation of H_2O_2-treated HLE-B3 cells

Hydrogen peroxide-treated HLE-B3 cells were transfected with shNRIP1 to investigate the effect of NRIP1 on cataracts. Transfection with shNRIP1 reduced the protein expression of NRIP1 in H_2O_2-treated HLE-B3 cells (Figure 2 A). Hydrogen peroxide treatment decreased cell viability in HLE-B3 cells (Figure 2 B) and reduced the number of EdU-positive HLE-B3 cells (Figure 2 C). However, NRIP1 knockdown increased the viability of H_2O_2-treated HLE-B3 cells (Figure 2 B) and promoted cell proliferation (Figure 2 C). These results suggest a pro-proliferative effect of NRIP1 down-regulation in H_2O_2-treated HLE-B3 cells.

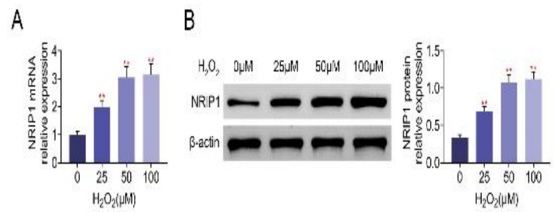

Figure 1: NRIP1 is elevated in H_2O_2-treated HLE-B3 cells. (A) Expression of NRIP1 mRNA was up-regulated in HLE-B3 cells post-H_2O_2 incubation in a dose-dependent manner. (B) The expression of NRIP1 protein was increased in H_2O_2-treated HLE-B3 cells in a dose-dependent manner. $^{**}P < 0.01$, compared with 0 μM H_2O_2

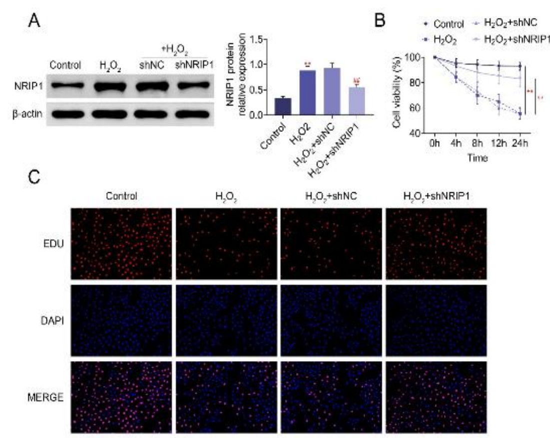

Figure 2: Down-regulation of NRIP1 promotes cell proliferation of H_2O_2-treated HLE-B3 cells. (A) Transfection with shNRIP1 reduced protein expression of NRIP1 in H_2O_2-treated HLE-B3 cells. (B) Knockdown of NRIP1 increased cell viability of H_2O_2-treated HLE-B3 cells. (C) Knockdown of NRIP1 promoted cell proliferation of H_2O_2-treated HLE-B3 cells. $^{**}P < 0.01$, compared with the control; $^{\#\#}p < 0.01$, compared with shNC

Down-regulation of NRIP1 suppresses pyroptosis in H_2O_2-treated HLE-B3 cells

Protein expression of NLRP3, GSDMD-N, caspase-1, IL-1β, and IL-18 in HLE-B3 cells were enhanced by H_2O_2 treatment (Figure 3 A). NRIP1 silencing decreased the expression levels of NLRP3, GSDMD-N, caspase-1, IL-1β, and IL-18 in H_2O_2-treated HLE-B3 cells to suppress pyroptosis (Figure 3 A). Moreover, H_2O_2 increased pyroptosis in HLE-B3 cells (Figure 3 B), while transfection with shNRIP1 reduced pyroptosis (Figure 3 B). These results revealed that NRIP1 contributed to pyroptosis in H_2O_2-treated HLE-B3 cells.

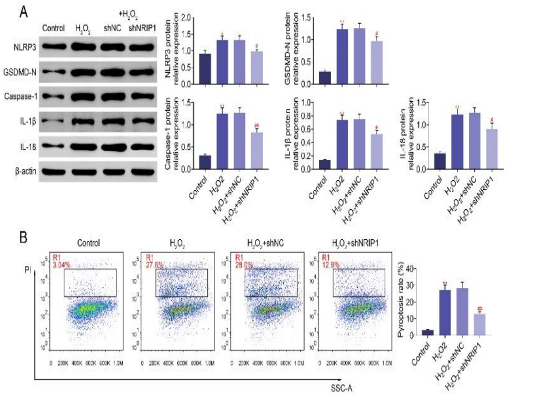

Figure 3: Down-regulation of NRIP1 suppresses pyroptosis in H_2O_2-treated HLE-B3 cells. (A) NRIP1 silencing reduced NLRP3, GSDMD-N, caspase-1, IL-1β, and IL-18 expression in H_2O_2-treated HLE-B3 cells. (B)Transfection with shNRIP1 reduced pyroptosis in H_2O_2-treated HLE-B3 cells. $^{**}P < 0.01$, compared with the control; $\#p < 0.05$ and $\#\#p < 0.01$, respectively, compared with shNC

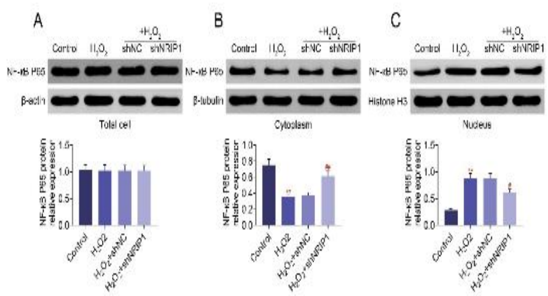

Figure 4: Down-regulation of NRIP1 suppresses activation of NF-κB signaling. (A) Protein expression of NF-κB was not affected by H_2O_2 incubation or shNRIP1 transfection in HLE-B3 cells. (B) Incubation with H_2O_2 reduced the protein expression of cytoplasmic NF-κB in HLE-B3 cells, while NRIP1 silencing enhanced cytoplasmic NF-κB expression in H_2O_2-treated HLE-B3 cells. (C) Incubation with H_2O_2 enhanced the protein expression of nuclear NF-κB in HLE-B3 cells, while NRIP1 silencing reduced nuclear NF-κB expression in H_2O_2-treated HLE-B3 cells. $^{**}P <$ 0.01, compared with the control; $\#p < 0.05$ and $\#\#p <$ 0.01, respectively, compared with shNC

Down-regulation of NRIP1 suppresses activation of NF-κB signaling

Protein expression of NF-κB was not affected by H_2O_2 incubation or shNRIP1 transfection (Figure 4 A). However, H_2O_2 incubation reduced protein expression of cytoplasmic NF-κB in HLE-B3 cells (Figure 4 B), while up-regulating nuclear NF-κB expression (Figure 4 C). Furthermore, the expression of cytoplasmic NF-κB in H_2O_2-treated HLE-B3 cells was up-regulated (Figure 4 B), but nuclear NF-κB expression was down-regulated (Figure 4 C), by NRIP1 knockdown, indicating that NRIP1 contributed to activation of NF-κB signaling in H_2O_2-treated HLE-B3 cells.

DISCUSSION

Emerging evidence indicates that NRIP1, a versatile transcriptional co-regulator, regulates peroxisome proliferator-activated receptor gamma, coactivator 1 alpha to affect mitochondrial biogenesis and oxidative metabolism [13]. Because oxidative stress plays a crucial role in the initiation and progression of cataracts [5], NRIP1 may regulate oxidative stress during cataract progression.

Hydrogen peroxide stimulates the generation of hydroxyl radicals, damages ion pump activity, and depletes glutathione to promote oxidative stress in lens epithelial cells, leading to lens epithelium damage and contributing to cataract formation [14]. In the present study, HLE-B3 cells were incubated with H_2O_2, leading to cytotoxicity in HLE-B3 cells with decreased cell viability and proliferation. A previous study showed that NRIP1 is up-regulated in the lens of cataract patients compared with normal lens [12]. We showed that NRIP1 expression was also enhanced in H_2O_2-treated HLE-B3 cells. Moreover, NRIP1 knockdown increased cell viability of H_2O_2-treated HLE-B3 cells and promoted cell proliferation, attenuating the cytotoxic effect of H_2O_2 on lens epithelial cells.

Pyroptosis, an inflammatory type of cell death, is implicated in cataract formation [9]. Levels of pyroptosis markers, including NLRP3, GSDMD-N, caspase-1, IL-1β, and IL-18, are increased in both cataract patients and irradiation-treated lens epithelial cells [15]. Pyroptosis suppression is considered a potential strategy to prevent cataract formation [15]. Additionally, H_2O_2 has also been shown to induce activation of NLRP3/caspase-1 and promote the release of inflammatory factors (IL-1β and IL-18) in lens epithelial cells [7]. Furthermore, an inhibitor of caspase-1 reduced H_2O_2-induced pyroptosis in lens epithelial cells [7]. Our study showed that NRIP1 silencing attenuated H_2O_2-induced increased expression of NLRP3, GSDMD-N, caspase-1, IL-1β, and IL-18 in HLE-B3 cells and suppressed pyroptosis in H_2O_2-treated HLE-B3 cells. Therefore, NRIP1 down-regulation may prevent cataract formation by suppressing pyroptosis.

NF-κB signaling is activated by IL-1β and contributes to the production of inflammatory cytokines, and activation of NF-κB signaling is implicated in the pathogenesis of cataract formation [16]. Suppression of NF-κB nuclear translocation ameliorated irradiation-induced damage to lens epithelial cells [17]. Nuclear receptor-interacting protein 1 is a coactivator of NF-κB via its interaction with p65, promoting the release of downstream inflammatory cytokines [18]. We showed that NRIP1 interference up-regulated cytoplasmic NF-κB expression and down-regulated nuclear NF-κB expression, suppressing the nuclear translocation of NF-κB in H_2O_2-treated HLE-B3 cells.

In summary, NRIP1 was involved in H_2O_2-induced pyroptosis in human lens epithelial cells. NRIP1 silencing promoted cell proliferation of H_2O_2-treated lens epithelial cells and suppressed pyroptosis by inactivating NF-κB signaling. Therefore, NRIP1 may be a promising target to prevent cataract formation. However, the effects of NRIP1 on H_2O_2-induced apoptosis, inflammation, and oxidative stress in lens epithelial cells warrant further investigation. Animal models of cataracts should also be designed to investigate the role of NRIP1 in cataract formation.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hongyan Yao and Shanjun Wu designed the study and supervised the data collection. Bifei Lan analyzed and interpreted the data. Li Cai and Zefeng Li prepared the manuscript for publication and reviewed the draft of the manuscript. All the authors have read and approved the manuscript.

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