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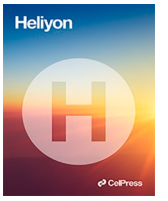

Research article

Silencing of TXNIP attenuates oxidative stress injury in HEI-OC1 by inhibiting the activation of NLRP3 and NF-κB

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ABSTRACT

Sensorineural hearing loss (SNHL) is the most common type of hearing loss worldwide. The primary mechanism is oxidative injury to the cochlea as a result of oxidative stress. Therefore, exploring antioxidant strategies is particularly important in addressing SNHL.Thioredoxin-interacting protein (TXNIP) is an upstream target of oxidative stress-induced damage, and the NOD-like receptor protein 3 (NLRP3) and NF-κB pathways may be the main downstream molecular pathways, but this has not been reported in SNHL. Therefore, we investigated the molecular mechanism and role of TXNIP in oxidative stress injury induced by H_2O_2 in the HEI-OC1 auditory cells. To induce oxidative stress, HEI-OC1 cells were treated with H_2O_2. The TXNIP expression was measured by western blotting and Immunofluorescence. Intracellular TXNIP was knocked down using small interfering RNAs (siRNAs). Cell viability was measured by CCK8, total intracellular reactive oxygen species (ROS) by DCFH-DA, mitochondrial ROS by Mito-SOX, NLRP3, pro-caspase-1, total p65 NF-κB, and phospho-p65 NF-κB expression were measured by western blotting. Statistical analyses were performed using one-way analysis of variance, and $p < 0.05$ was considered statistically significant. We found that H_2O_2 treatment induced oxidative stress injury in HEI-OC1 cells, as evidenced by decreased cell viability and increased total intracellular and mitochondrial ROS levels ($p < 0.05$). TXNIP expression was elevated, and NLRP3 and NF-κB were activated ($p < 0.05$). Moreover, siRNA-TXINIP co-treatment reversed these changes and protected HEI-OC1 cells from oxidative stress ($p < 0.05$). We concluded that H_2O_2-induced oxidative stress in HEI-OC1 cells was alleviated by TXNIP inhibition. The finding may provide new insight into the prevention and treatment of SNHL.

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1. Introduction

Hearing loss is the most common sensory impairment disease, which affects people of all ages. The World Health Organization has predicted that more than 5 % of the world's population currently suffers from hearing loss, and this is estimated to rise to 10 % of the world's population by 2050 [1]. The most commom type of hearing loss is sensorineural hearing loss (SNHL), which includes hereditary, drug-induced, and noise, and age-related hearing loss. The main pathological feature of SNHL is damage to hair cells, which are sensors of the cochlear conduction pathway that cannot regenerate after damage [2]. Due to its high metabolic demand, the cochlea is particularly susceptible to oxidative stress induced by reactive oxygen species (ROS) [3]. ROS generation contributes to multiple pathways of apoptosis and necrotic cell death within auditory tissues and is the main cause of most types of SNHL [4]. The main structures damaged by ROS are the organs of Corti (OC), the stria vascularis, and spiral ganglia. The most important cells in the OC are hair cells, which are crucial for converting sound from mechanical motion signals to recognizable electrical signals in the brain. a large number of experiments focusing on oxidative stress have drawn attention to the protective function of antioxidant defense in SNHL [5], However there is still a lot of room for exploration in this area.

Thioredoxin-interacting protein (TXNIP) is a multifunctional protein, also known as thioredoxin binding protein-2 or vitamin D3 upregulated protein 1, which is a crucial negative modulator in the redox system, binds to thioredoxin (TRX) to inhibit the antioxidant activity of TRX, thereby causing cellular oxidative stress [6]. TXNIP is associated with oxidative stress and inflammation in many diseases, such as diabetes and its complications [7], neurodegenerative diseases [8], cancers [9], and cerebrovascular diseases [10]. However, TXNIP has rarely been associated with SNHL. Cisplatin-induced NOD-like receptor protein 3 (NLRP3) inflammasome activation-mediated pyroptosis has been reported to be alleviated by the downregulating of TXNIP in marginal cells, suggesting that TXNIP positively regulates NLRP3 inflammasome activation [11]. Nuclear factor-kappa B (NF-κB) is a pro-inflammatory transcription factor that can be used by TXNIP to promote inflammatory gene expression through chromatin modification and nuclear translocation of NF-κB [12]. In diabetic retinopathy, TXNIP activation leads to NF-κB activation, which in turn increases the production of inflammatory factors. Suppression of the TXNIP/NF-κB pathway inhibits inflammatory factors and restores tight connections between endothelial cells [13]. However, neither the TXNIP/NLRP3 nor the TXNIP/NF-κB pathway has been reported in SNHL.

Currently, there is no evidence suggesting that TXNIP is a therapeutic target for combating oxidative stress-induced damage in hair cells. In this study, we simulated oxidative stress damage in HEI-OC1 cells by treatment with H_2O_2. We used small interfering RNAs (siRNAs) to regulate the expression of TXNIP, and we assayed the expression of related molecules to explore the role and mechanism of TXNIP in hair cell degeneration under oxidative stress. Our findings may provide new insights into the molecular mechanisms and potential therapeutic strategies for SNHL.

2. Methods and materials

2.1. Cell culture and hydrogen peroxide treatment of HEI-OC1 cells

The inner ear cell line HEI-OC1 was purchased from the UCLA Technology Development Group (Los Angeles, CA, United States) and cultured in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum (Gibco, United States) and 50 μg/ml ampicillin (Sangon Biotech, Shanghai) at 33 °C in a humidified 5 % CO_2 atmosphere. Upon reaching 70–80 % confluence after 24 h of culture, H_2O_2 was added to the culture medium to induce oxidative stress.

2.2. Cell vitality assay

Each well of a 96-well plate contained five cultured HEI-OC1 cell clones. Following cell attachment, the HEI-OC1 cells were subjected to several treatments. As directed by the manufacturer, cells were treated and then incubated for 2 h at 37 °C with the Cell Counting Kit 8 (CCK-8) reagent (MCE, HY-K0301, Shanghai, China). The absorbance was recorded at 450 nm by microplate reader (Bio-Rad). Values were standardized to 100 % of untreated blank control cells.

2.3. Western blot analysis

HEI-OC1 cell samples were lysed using RIPA lysis buffer (Epizyme, PC101, shanghai, China), protease inhibitor (Epizyme, GRF101, shanghai, China) and phosphatase inhibitor (Epizyme, GRF102, shanghai, China) for 30 min at 4 °C to extract total protein. Each protein concentration was assayed using the BCA Protein Quantification Kit (Epizyme, ZJ102, shanghai, China). Equal quantities of protein samples were separated by 10 % or 12.5 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Epizyme, PG212 and PG213, shanghai, China), then transferred onto nitrocellulose membranes (Pall, 66485, USA). The membranes were blocked for 1 h at room temperature with 1xTBST containing 5 % non-fat milk (Sangon Biotech, Shanghai), then incubated with primary antibody (TXNIP, Cell Signaling Technology Cat# 14715, RRID:AB_2714178; NLRP3, ServiceBio Cat# GB114320, RRID:AB_3073726; Caspase-1, Santa Cruz Biotechnology Cat# sc-56036, RRID:AB_781816; NF-kB p65, Cell Signaling Technology Cat# 8242, RRID:AB_10859369; Phospho-NF-kB p65, Cell Signaling Technology Cat# 3033, RRID:AB_331284; GAPDH, Proteintech Cat# 60004-1-Ig, RRID:AB_2107436; Beta Actin, Proteintech Cat# 66009-1-Ig, RRID:AB_2687938) at 4 °C overnight. After washing three times with 1 × TBST buffer, the membranes were incubated with secondary antibodies (HRP Goat Anti-Rabbit IgG(H + L), ABclonal Cat# AS014, RRID:AB_2769854; HRP Goat Anti-Mouse IgG(H + L), ABclonal Cat# AS003, RRID:AB_2769851) for 2 h at room temperature. Finally, the membranes were developed using Omni-ECL™ Femto Light Chemiluminescence Kit (Epizyme, SQ201, shanghai, China) and

photographed using the ChemiDocXRS imaging system (Bio-Rad, CA, USA). The images were analyzed using ImageJ software (Bethesda, USA).

2.4. Immunofluorescence microscopy

HEI-OC1 cells were fixed in paraformaldehyde (4 %) for 20 min, then washed in PBS two times. After permeabilizing the cell specimens in 0.5 % Triton X-100 solution for 1 h, they were blocked with 10 % normal goat serum for 1 h at room temperature. The samples were first incubated with the primary anti-TXNIP antibody (ABclonal, A9342, China) for 12 h at 4 °C, washed three times with PBS, and then incubated with a fluorescent secondary antibody Goat Anti-Rabbit IgG H + L (Alexa Fluor 488), (Abcam, ab150077, China) for 1 h at 37 °C in the dark. Finally, the cells were incubated with DAPI in the dark and immediately imaged.

2.5. siRNA transfection

HEI-OC1 cells were transfected with TXNIP-siRNAs (RiboBio, Guangzhou, China) using Lipofectamine™ 3000 (Invitrogen, L3000001, USA), in accordance with the manufacturer's instructions. The transfection control was HEI-OC1 cells transfected with siRNA-negative control (siRNA-NC). The control group consisted of untreated HEI-OC1 cells. The transfection process takes 24 h.

2.6. ROS measurement

Confocal Petri dishes were inoculated with HEI-OC1 cells. Once the cells were attached to the dishes, they were cultured for 20 min in the dark in 10 μmol/L DCFH-DA (Beyotime, S0033S, China) to detect total ROS and 500 nmol/L Mito-SOX Red (Thermo Fisher, M36007, USA) to mitochondrial ROS. The cells were incubated with Hoechst reagent (Beyotime, C1028, China) for 10 min, and images were acquired using a fluorescence microscope and processed using ImageJ software.

2.7. JC-1

Cells were cultured in six-well plates, and each well was treated differently. Appropriate amount of JC-1 (200 μM) (MCE, HY-K0601, Shanghai, China)was brought to room temperature, 10 μL of JC-1 (200 μM) was added to the medium of each well at a

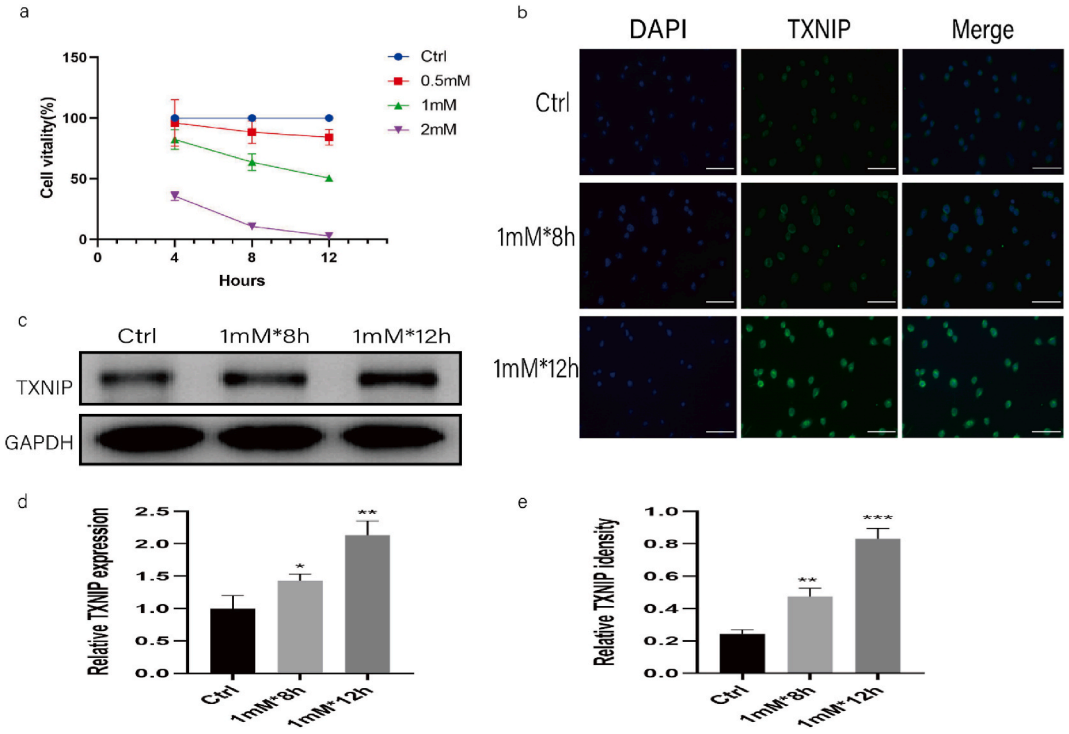

Fig. 1. Intracellular expression of TXNIP in HEI-OC1 at different times of 1 mM H_2O_2 stimulation. (a) HEI-OC1 cell viability at 4, 8, and 12 h following stimulation with gradient concentrations of H_2O_2 (0.5, 1, and 2 mM). (b) Immunofluorescence staining of TXNIP (green), and DAPI (blue) revealed the expression of TXNIP in HEI-OC1 cells at different stimulation times. (c) Western blotting showed the expression of TXNIP in different groups. Quantitative analysis of (d) Western blot and (e) immunofluorescence showed that 1 mM H_2O_2 stimulation made a time-dependent increase in the intracellular content of TXNIP. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the control group. Scale bars = 50 μm.

final concentration of 2 μM, and the cells were incubated at 37 °C in a cell culture incubator for 15–20 min. After incubation at 37 °C, the supernatant was aspirated, and the cells were washed with PBS (1 ×) for two times. Add 500 μL of PBS (1 ×) and observe with a fluorescence microscope. The images were analyzed using ImageJ software (Bethesda, USA).

2.8. ELISA

Culture medium supernatants of six groups of cells with different treatments were collected, and IL-1β and IL-18 were detected in culture medium supernatants by ELISA kits according to the instructions (MULTI SCIENCES, EK201BHS/EK218-AW1, China). Dual-wavelength detection was performed using microplate reader (Bio-Rad) to determine the OD value at the 450 nm maximum absorption wavelength and the 630 nm reference wavelength. The calibrated OD value was the measured value at 450 nm minus the measured value at 630 nm.

2.9. Statistical analysis

The data are presented as mean and the standard deviation (SD), with each experiment repeated at least three times. Comparisons three or more groups were performed using one-way analysis of variance and Dunnett's multiple comparison test. $P < 0.05$ indicates a statistically significant difference. SPSS software (version 22.0; IBM Corp, USA) was used for the statistical analysis and GraphPad Prism software (version 8.0.2, GraphPad Software Inc., USA) for constructing figure.

3. Results

3.1. Expression of TXNIP was upregulated in H_2O_2-induced HEI-OC1 cells

We measured the vitality of HEI-OC1 cells after stimulation with different concentrations of H_2O_2 at various times using CCK8 reagent (Fig. 1a), and we found that a concentration of 1 mM was appropriate; therefore, we used cells treated with 1 mM H_2O_2 for 8 and 12 h. The expression of TXNIP was detected by immunofluorescence microscopy (Fig. 1b), and western blotting (Fig. 1c), showing a time-dependent increase compared to its expression in the untreated group ($p < 0.05$, Fig. 1d and e), suggesting that TXNIP is involved in oxidative stress in H_2O_2-induced HEI-OC1 cells.

3.2. TXNIP silencing ameliorated H_2O_2-induced cell damage

To investigate whether silencing TXNIP could ameliorate cellular damage in an oxidative stress state, siRNA was used to knockdown TXNIP, as assessed by western blotting (Fig. 2a). CCK8 was used to assay cell viability (Fig. 2b). We observed a significant attenuation of the H_2O_2-induced decrease in cell viability after pretreatment of cells with siRNA-TXNIP ($p < 0.01$, Fig. 2b). TXNIP silencing ameliorated oxidative damage in HEI-OC1 hair cells.

3.3. Silencing TXNIP attenuated oxidative stress and mitochondrial dysfunction in H_2O_2-induced HEI-OC1

TXNIP positively regulates ROS production. To investigate whether TXNIP silencing could reduce H_2O_2-induced total and mitochondrial ROS accumulation in HEI-OC1 cells, we used DCFH-DA to probe total ROS in the cells (Fig. 3a) and Mito-SOX to probe mitochondrial ROS (Fig. 3b). The phenomenon was that H_2O_2 elevated the intracellular ROS ($p < 0.001$) and mitochondrial ROS ($p < 0.001$) levels, and silencing of TXNIP inhibited the increase of intracellular ROS ($p < 0.001$, Fig. 3c), which was also observed after incubation of the cells with Mito-SOX ($p < 0.05$, Fig. 3d). TXNIP silencing ameliorated cellular damage by attenuating oxidative stress

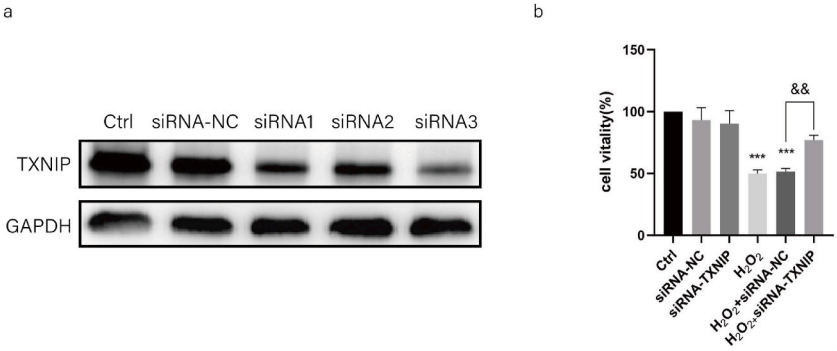

Fig. 2. Cell viability in different treatment groups. (a) Results of screening three potential siRNAs for knocking down TXNIP. (b) Effect of TXNIP knockdown on the vitality of HEI-OC1 cells treated with 1 mM H_2O_2 for 12 h ***$p < 0.001$ versus the control group. && $p < 0.01$ versus the H_2O_2+siRNA-NC group.

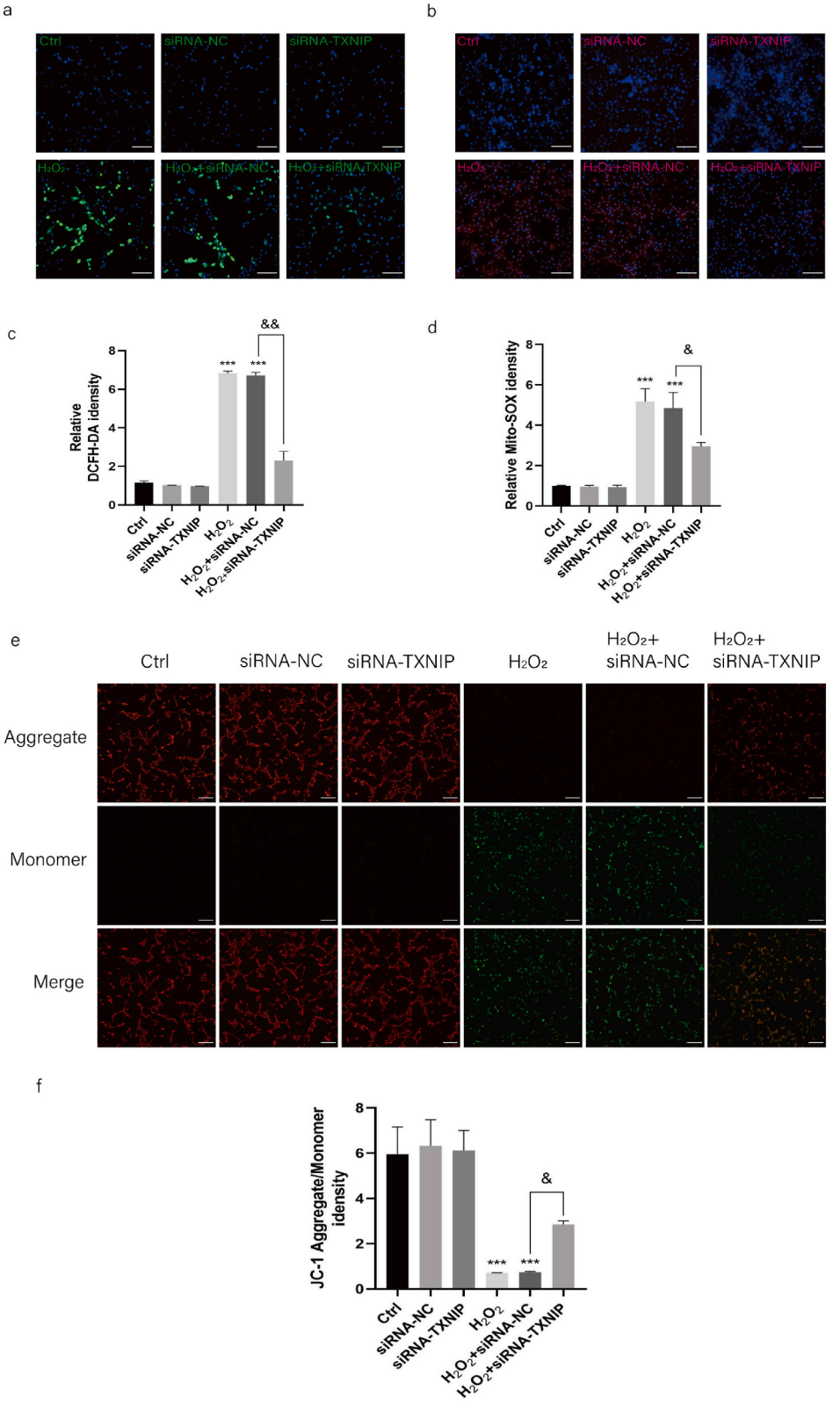

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Fig. 3. Effect of TXNIP knockdown on H_2O_2-induced ROS and MMP. (a) DCFH-DA-labelled total intracellular ROS of different groups (green). (b) Mito-SOX-labelled mitochondrial ROS of different groups (red). Quantitative analysis of (c) DCFH-DA and (d) Mito-SOX showing that TXNIP knockdown significantly reduced H_2O_2-stimulated intracellular and mitochondrial ROS accumulation. (e) JC-1 labelled MMP of different groups ***p < 0.001 versus the control group. & p < 0.05, && p < 0.01 versus the H_2O_2+siRNA-NC group. Scale bars = 200 μm.

and mitochondrial malfunction in HEI-OC1 cells.

To further illustrate the effect of silencing TXNIP on mitochondrial dysfunction, the JC-1 kit was used to assay mitochondrial membrane potential (MMP) (Fig. 3e). The MMP was significantly lower in the H2O2 group compared with the ctrl group ($p < 0.001$), whereas the MMP was higher in the H2O2+siRNA-TXNIP group compared with the H2O2+siRNA-NC group ($p < 0.05$) (Fig. 3f). So silencing TXNIP ameliorated the H2O2-induced decrease in MMP.

3.4. Silencing TXNIP inhibited H_2O_2-induced NLRP3 inflammasome activation

TXNIP is involved in ROS-induced activation of NLRP3 inflammatory vesicles. To verify this, we examined NLRP3、pro-caspase-1、Cleaved-caspase-1 and pro-IL-18 levels by western blotting (Fig. 4a). NLRP3 (Fig. 4b) was significantly elevated in the H_2O_2 ($p < 0.01$) and H_2O_2 (siRNA-NC) groups ($p < 0.01$), and its levels in the H_2O_2 (siRNA-NC) groups were higher than those in the H_2O_2-treated group with siRNA-TXNIP ($p < 0.05$). However, the opposite trend in pro-caspase-1 ($p < 0.05$, Fig. 4c) was related to the conversion of the precursor proteins into the activator form after stimulation. The trend of Cleaved-caspase-1 was similar to that of NLRP3 ($p < 0.05$ Fig. 4d) and the trend of pro-IL-18 ($p < 0.05$ Fig. 4e) was similar to that of pro-caspase-1. In addition, H2O2 led to elevated IL-18 ($p < 0.001$) and IL-1β($p < 0.001$), and silencing TXNIP inhibited this trend ($p < 0.05$) (Fig. 4f and g). Taken together, these results demonstrate that TXNIP silencing ameliorates oxidative damage in HEI-OC1 cells by inhibiting NLRP3 inflammatory vesicle activation.

3.5. Silencing TXNIP inhibited H_2O_2-induced NF-κB activation

To study the involvement of TXNIP in NF-κB activation, we measured the expression of the NF-KB's subunit p65 by western blotting (Fig. 5a). The results showed that the total p65 content remained unchanged (Fig. 5b), but phosphorylated p65 was significantly higher

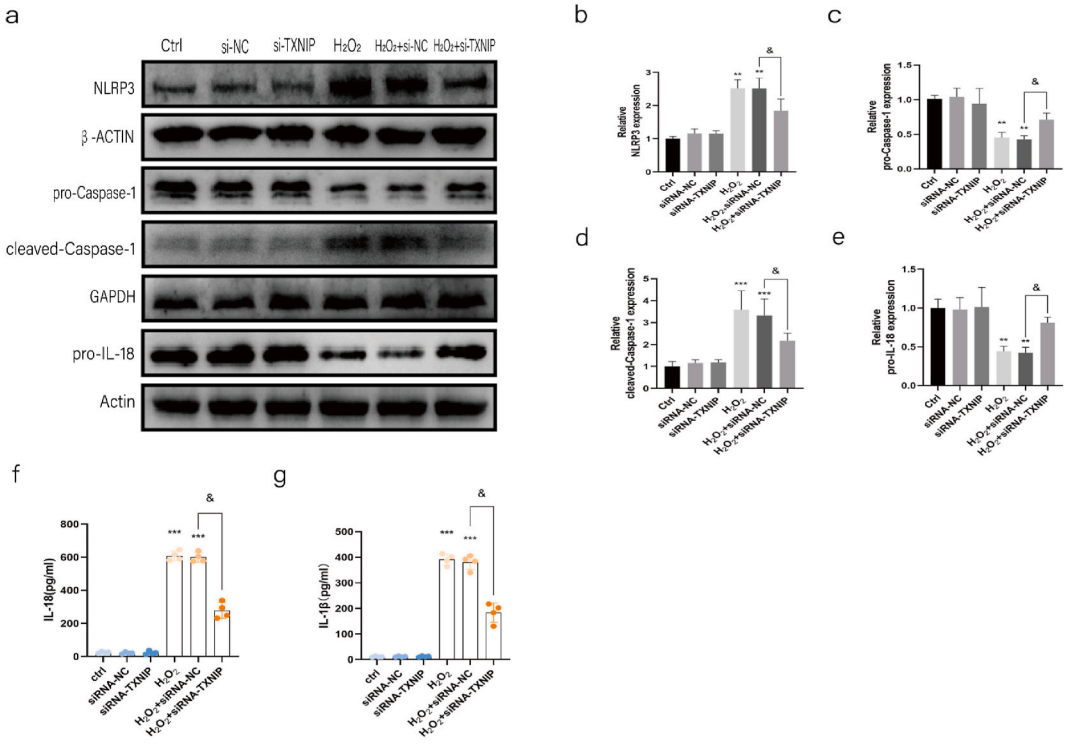

Fig. 4. Activation of NLRP3 inflammatory vesicles in different groups of HEI-OC1 cells. (a) Expression content of NLRP3、pro-caspase-1、Cleaved-caspase-1 and pro-IL-18 in different groups. Quantitative analysis of (b) NLRP3, (c) pro-caspase-1, (d) cleaved-caspase-1 and (e) pro-IL-18 showed that TXNIP knockdown inhibited H_2O_2-induced NLRP3 inflammatory vesicle activation. (f) The difference in the IL-18 concentrations in the supernatant of each group. (g) The difference in the IL-1β concentrations in the supernatant of each group. **p < 0.01, ***p < 0.001 versus the control group. & p < 0.05 versus the H_2O_2+siRNA-NC group.

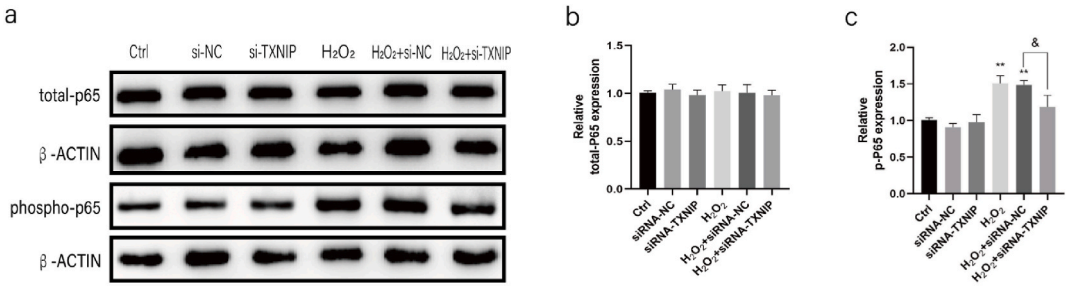

Fig. 5. NF-κB activation in different groups of HEI-OC1 cells. (a) Intracellular expression of total-p65 and phospho-p65 in different groups. Quantitative analysis of (b) total-p65 and (c) phospho-p65 showed that TXNIP knockdown inhibited H_2O_2-induced NF-κB activation. **$p < 0.01$ versus the control group. & $p < 0.05$ versus the H_2O_2+siRNA-NC group.

in the H_2O_2-treated group ($p < 0.001$) and the H_2O_2-treated groups with negative control siRNA ($p < 0.01$) than in the control group and was lower in the siRNA-TXNIP pretreated H_2O_2 group than that in the siRNA-NC pretreated H_2O_2 group ($p < 0.05$, Fig. 5c). We deduced that TXNIP silencing ameliorated oxidative damage caused by HEI-OC1 cells by inhibiting NF-κB activation.

4. Discussion

In this study, we confirmed the role of TXNIP oxidative stress model in vitro. An imbalance between the generation of ROS and the capacity of the antioxidant defence system results in oxidative stress. In recent years, more and more studies on the regulation of TXNIP and ROS have been conducted. Multiple stimuli can induce intracellular ROS overproduction [14], including H_2O_2, and excessive ROS stimulate TXNIP to dissociate from TRX, causing elevation of TXNIP in the cell [15], TXNIP in turn positively regulates ROS [16]. In our study, accumulation of total and mitochondrial ROS in HEI-OC1 cells was observed after H_2O_2 administration. It was also found that the increase in TXNIP content in HEI-OC1 cells treated with H_2O_2 was time-dependent compared to the control group. After silencing TXNIP with siRNA targeting, ROS elevation and MMP decrease was inhibited and oxidative damage to hair cells was reduced. Our findings provide reliable preliminary research evidence for the scientific study that TXNIP may be a future target for the prevention and treatment of SNHL.

ROS-induced oxidative stress is a significant factor in multiple systemic and cochlear injuries. Previous research has shown that 40 min of low perfusion results in an average 10-fold increase in the concentration of hydroxyl radicals in the lymphatic vessels of the mouse cochlea, and that high levels of hydroxyl radicals can persist for 40–80 min after reperfusion [17]. The accumulation of superoxide and lipid peroxidation products can lead to apoptosis of cochlear cells, whereas vasoactive lipid peroxidation products can impair blood flow in the microcirculation, which further increases the production of ROS. This ultimately results in hair cell death via apoptosis and/or necrosis [18]. Mitochondria are the main sources of ROS, including superoxide anions, hydroxyl radicals, H_2O_2 and singlet oxygen [19]. Excessive production of mitochondrial ROS in the cochlea can lead to oxidative degradation of major mitochondrial constituents, resulting in mitochondrial dysfunction and the inability of mitochondria to meet the high-energy needs of cochlear cells, leading to inadequate cellular bioenergy [20]. Inner and outer hair cells are the two most important sensory cells in the cochlea, and are important pathological targets in the occurrence of SNHL. Outer hair cells (OHCs) can endure prolonged high-energy requirements and mechanically induced stimuli, and they are inherently more susceptible to oxidative damage. The metabolic activity in the inner hair cells, stria vascularis, and spiral ganglion cells is also high, making them equally susceptible to mitochondrial dysfunction. Furthermore, dysfunctional mitochondria promote additional production of mitochondrial ROS through a positive feed-back pathway, eventually activating the cell apoptotic pathway and resulting in hearing loss [3].

TXNIP is involved in pro-inflammatory responses, such as activation of NLRP3 inflammasome and NF-κB. Under non-stress conditions, TXNIP binds to TRX, leading to the inactivation of NLRP3 inflammatory vesicles because of the absence of a mutual interaction between TXNIP and NLRP3. Nevertheless, during oxidative stress, ROS production induces TRX-TXNIP dissociation, which enhances NLRP3-TXNIP interaction [21]. The NLRP3 inflammasome is a crucial element of the human immune system, participates in multiple inflammatory pathways, mediating the activation of caspase-1 and the secretion of the pro-inflammatory cytokine IL-1β/IL-18 during microbial infections and in response to cellular damage [22]. Nevertheless, Abnormal activation of the NLRP3 inflammasome has been implicated in many inflammatory diseases [23].

Several studies on the inner ear have demonstrated the involvement of NLRP3 inflammatory vesicles in SNHL. Studies have found that NLRP3 expression is significantly higher in older mice than in younger mice [24], that noise exposure significantly up-regulates NLRP3 in the porcine cochlea [25], and that Pou4f3 knockout and exocytosis ameliorate cisplatin-induced ototoxicity in mice by reducing NLRP3 [26]. Similarly, in HEI-OC1, NLRP3 was found to be involved in cellular damage, in which BDE-47 impaired HEI-OC1 cellular activity by upregulating NLRP3 [27]. Although the important effects of NLRP3 in SNHL have been clarified, the studies on its upstream and downstream mechanisms are still few, and further refinement of the mechanism of NLRP3 occurrence in SNHL may help us to solve the disease. In our study, NLRP3、pro-caspase-1、Cleaved-caspase-1 and pro-IL-18 expression levels were evaluated in cells,IL-18、IL-1βin cells secretion, following various treatments. After H_2O_2 treatment, the significant increase in NLRP3、Cleaved-caspase-1、IL-18 and IL-1βexpression suggest the activation of NLRP3 inflammasomes in HEI-OC1 cochlear hair cells under oxidative stress, whereas the simultaneous decrease in pro-caspase-1 and pro-IL-18 was due to the conversion of precursor proteins

into active peptides under H_2O_2 stimulation [28]. After TXNIP silencing, the expression of NLRP3、Cleaved-caspase-1、IL-18 and IL-1β decreased, whereas that of pro-caspase-1 and pro-IL-18 increased, indicating that the downregulation of TXNIP in HEI-OC1 cells inhibited the activation of NLRP3 inflammasomes induced by oxidative stress. Previous studies have found that knock down of TXNIP expression inhibits activation of the NLRP3 inflammasome in cochlear marginal cells treated with cisplatin, thereby alleviating oxidative stress damage to cochlear marginal cells [11]. Therefore, our results add data from a hair cell model on the targeting effects of TXNIP in cochlea. However, the direct binding of TXNIP to NLRP3 requires further investigation.

In the cochlea, ROS also interact with the NF-κB signalling pathway in a variety of ways, and the level of NF-κB activity is also affected by the level of ROS, which can produce pro-inflammatory mediators by activating the NF-κB signalling cascade [29]. Overexpression of NF-κB has been detected in in vivo models of noise-induced [30], and drug [31], and age-related hearing loss [32]. Previous studies have found that Asiaticoside ameliorates high glucose-induced oxidative stress damage in HEI-OC1 cells by inhibiting NF- κB [33]. The p65 subunit is an important component of NF-κB, and phosphorylation of p65 into the nucleus represents the activation of the NF-κB pathway. Total p65 levels are usually unchanged, whereas phosphorylated p65 levels increase upon NF-κB activation [34]. We examined the expression of total and phosphorylated p65 in each group of cells and found that the total p65 was essentially unchanged and phosphorylated p65 was significantly elevated after H_2O_2 treatment. Simultaneous silencing of TXNIP prevents H_2O_2-stimulated p65 phosphorylation. This suggests that the NF-κB pathway is also activated after oxidative stress in HEI-OC1 cells, and that the inhibition of TXNIP overexpression prevents the activation of this pathway and exerts antioxidant and anti-inflammatory effects.

In conclusion, we addressed such ROS/NF-κB and ROS/NLRP3 pathway mechanisms in HEI-OC1 cells using TXNIP as an entry point, which may be a further refinement of the pathogenesis of SNHL. The results of the present study suggest that TXNIP is involved in oxidative damage in HEI-OC1 cochlear hair cells and that blocking TXNIP exerts anti-oxidative stress and anti-inflammatory effects by inhibiting both NLRP3 and NF-κB pathways. However, based on preliminary explorations with in vitro cell line models, further in vivo animal models are needed to validate whether targeted modulation of TXNIP is a possible new strategy in the field of antioxidant defence against SNHL.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Ning Ma: Writing – original draft, Methodology. **Liang Xia:** Formal analysis, Data curation. **Zhong Zheng:** Validation, Investigation. **Xiaoyan Chen:** Validation, Formal analysis. **Weiwei Xing:** Writing – review & editing, Project administration, Conceptualization. **Yanmei Feng:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yanmei Feng reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37753.

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