The E3 Ligase RNF157 Inhibits Lens Epithelial Cell Apoptosis by Negatively Regulating p53 in Age-Related Cataracts

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Purpose. Age-related cataract (ARC) is a major cause of vision impairment worldwide. The E3 ubiquitin ligase RING finger protein 157 (RNF157) is involved in regulating cell survival and downregulated in human cataractous lens samples. However, the function of RNF157 in cataracts remains unclear. This study aimed to determine the role of RNF157 in ARC.

Methods. Real-time polymerase chain reaction (PCR) and Western blotting were used to analyze the expression of RNF157 in clinical lens capsules, rat cataract models, and oxidative stress cell models. Western blot analysis and flow cytometry were used to evaluate cell apoptosis. Co-IP assay, protein stability assay, and ubiquitination assay were used to detect the interaction between RNF157 and its substrate p53.

Results. The expression of RNF157 was downregulated in human cataract samples, UVB-induced rat cataract model, and H_2O_2-treated human lens epithelial cells (LECs). Ectopic expression of RNF157 protected LECs from H_2O_2-induced apoptosis. In contrast, knockdown of RNF157 enhanced oxidative stress-induced apoptotic cell death. Moreover, silence of RNF157 in the rat ex vivo lens model exacerbated lens opacity. Mechanistically, RNF157 causes ubiquitination and degradation of the tumor antigen p53. Overexpression of p53 eliminated the antiapoptotic effects of RNF157, whereas p53 knockdown rescued RNF157 silencing-induced cell death.

Conclusions. Our findings revealed that reduced RNF157 expression promoted LEC apoptosis by upregulating p53 in cataracts, suggesting that the regulation of RNF157 expression may serve as a potential therapeutic strategy for cataracts.

Keywords: Cataract, RNF157, apoptosis, p53, ubiquitination

Age-related cataract (ARC) is the leading cause of blindness and vision impairment in adults aged 50 years and older. Because of the growing aged population, the number of people affected by cataracts is increasing.[1] Surgery is the only efficacious treatment of cataracts thus far. However, it is often accompanied by postoperative complications and high costs.[2]

Lens epithelial cells (LECs) are the primary source of metabolic activity in the lens and play a vital role in lens homeostasis.[2] Although the mechanisms of ARC have not been fully understood, oxidative stress-induced LEC apoptosis is considered a common cellular basis for noncongenital cataractogenesis.[3–5] The tumor antigen p53 triggers cell cycle arrest and apoptosis in response to cellular stress and is thought to play a prominent role in ARC.[6–8] Some evidence suggests that p53 is significantly upregulated in LECs of patients with cataract and in an H_2O_2-induced cataract model.[7,9,10]

The ubiquitin-proteasome system (UPS) is an essential regulatory system for cell signal transduction and protein homeostasis.[11] Both apoptosis and oxidative stress modulation are known to be under the control of the UPS.[12–14] The UPS regulates not only lens differentiation but also Ca^{2+} homeostasis and oxidative stress in the lens.[15–17] Among the UPS components, the highly conserved E3 ubiquitin ligases mediate the transfer of ubiquitin to substrates, which is a decisive component of the UPS cascade for its high substrate specificity.[18] Several recent studies uncovered that E3 ubiquitin ligases are involved in lens development and cataractogenesis. The E3 ubiquitin ligase Parkin eliminates LEC mitochondria depolarized under oxidative stress conditions and might play a part in lens transparency.[17] In addition, small ubiquitin-like modifier (SUMO) E3 ligases, which mediate the transfer of SUMO to substrate proteins, also participate in cataractogenesis.[5,19] For example, SUMO E3 ligase PIAS1 upregulates proapoptotic factor expression and promotes oxidative stress-induced apoptosis, which is implicated in lens pathogenesis.[5]

The E3 ubiquitin ligase RING finger protein 157 (RNF157) is characterized as a pro-survival factor in neurons and

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as a downstream target of PI3K/MAPK signaling for regulating the cell cycle.[20,21] International Mouse Phenotyping Consortium (IMPC) data show that Rnf157$^{-/-}$ mice display a cataract phenotype in early adulthood.[22] In addition, the RNF157 expression in human cataractous lens samples was significantly lower than that in transparent lens samples ($P < 0.001$), according to the sequencing data from Wu et al.[23] Although these clues suggest a potential role of RNF157 in cataracts, how RNF157 regulates cataracts remains elusive.

In this study, we showed that the expression of the E3 ubiquitin ligase RNF157 was downregulated in cataracts. RNF157 overexpression attenuates H_2O_2-induced HLE-B3 cell apoptosis, whereas knockdown of RNF157 exacerbates cell apoptosis under oxidative stress conditions. Furthermore, we demonstrated that RNF157 mediated HLE-B3 cell survival by inducing ubiquitination-dependent degeneration of p53, revealing a novel mechanism of cataractogenesis.

Materials and Methods

Human Samples

This study was approved by the First Affiliated Hospital of Xi'an Jiaotong University Ethics Committee in accordance with the Declaration of Helsinki. All the patients signed written informed consent forms for the use of their samples. We obtained five fresh anterior lens capsules from patients with age-related cataract with no other eye diseases during cataract surgery at the First Affiliated Hospital of Xi'an Jiaotong University. Three normal anterior lens capsules were obtained from the Eye Bank of the First Affiliated Hospital of Xi'an Jiaotong University. Age did not differ between the two groups (55.5 ± 7.92 vs. 55.71 ± 3.52 years). Specimens were snap-frozen immediately and stored at −80°C.

Rats and Cell Lines

Sprague Dawley (SD) rats weighing 180 to 200 g with a sex ratio of 1:1 were purchased from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China), which adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The human lens epithelial B3 cell line (HLE-B3) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in modified eagle media (MEM) with 10% fetal bovine serum (FBS) in a humidified incubator at 37°C with 5% CO_2.

In Vivo and Ex Vivo Assays

UVB-Induced Rat Cataract Model. Construction of the rat UVB-induced cataract model was performed as described.[24] Briefly, the rats' pupils were dilated, and then the animals were anesthetized by pentobarbital sodium. Next, the right eyes were exposed to UVB (302 nm, 9 kJ/m^2, UVLM-26, UVP) once a day for 7 days. The left eyes served as a control. The rats were euthanized, and the lenses were immediately dissected by a posterior approach under sterile conditions.

Rat Lens Ex Vivo Assays. Lenses were extracted from the rats by a posterior approach under sterile conditions and cultured in DMEM containing 3% FBS in a 37°C humidified incubator with 5% CO2. After 24 hours, transparent lenses were chosen for further treatment. The lenses were treated with or without 200 µM H_2O_2 for 4 days to establish an H_2O_2-induced ex vivo cataract model. For knockdown of RNF157 in rat lens cultures, lens cultures were transfected with 25 nmol/L siRNAs and Lipofectamine RNAiMAX (Invitrogen) for 48 hours. Then, 200 µM H_2O_2 was added to the medium, and the lenses were cultured for an additional 4 days. The H_2O_2 was replenished every 12 hours. The lenses were imaged every 3 days. A mixture of three siRNAs was used to knockdown rat RNF157 (#1: CCATTATCAACCGTCACAA; #2 CAGAAACCGTGCACTACAA; and #3: CAAGTCCATTTCCCAGAAT).

Rat Lens Epithelial Explants. Rat lenses were dissected and rinsed. Carefully make a small incision at the capsule posterior side, it was peeled off, and the capsule was secured to the base of the culture dish as described.[25] The epithelial explants were treated with or without 200 µM H_2O_2 for 48 hours. Then, the explants were harvested for further experiments.

Immunohistochemistry and Hematoxylin and EosinStaining

Immunohistochemistry (IHC) and hematoxylin and eosin (H&E) staining were performed as described.[26] The slides were subjected to IHC analysis using RNF157 (Bioss; bs9226R, 1:100 dilution) and p53 (Proteintech; 1:100 dilution) specific antibodies. Slides were imaged through a Nikon Microscope System.

Quantitative Real Time Polymerase Chain Reaction

Total RNA of rat lens epithelial cells and HLE-B3 cells was isolated using TRIzol reagent (Tiangen, China) according to the manufacturer's instructions. The cDNA was then synthesized using the Transcriptor First Strand cDNA Synthesis Kit (04897030001; Roche). Quantitative real time polymerase chain reaction (qRT-PCR) was performed by a QuantiNov SYBR Green PCR Kit (Qiagen) in the CFX Connect Real-time PCR Detection System (Bio-Rad). Relative mRNA levels were calculated using the $2^{\wedge-\Delta\Delta Ct}$ method. The primers were as follows: human β-Actin (forward, 5′-CACCATTGGCAATGAGCGGTTC-3′; and reverse, 5′-AGGTCTTTGCGGATGTCCACGT-3′); rat β-Actin (forward, 5′-CACCATTGGCAATGAGCGGTTC-3′; and reverse, 5′-AGGTCTTTGCGGATGTCCACGT-3′); human RNF157 (forward, 5′- CTCACCTTGTCGTCATCTGGAG-3′; and reverse, 5′- AGACGGTGTCAGTGCTGATCTG-3′); rat RNF157 (forward, 5′- GGCGTCAGCTACCTTCTTCA-3′; and reverse, 5′-CATCCTCTGCCACCTTGGAA-3′); and human p53 (forward, 5′- CCTCAGCATCTTATCCGAGTGG-3′; and reverse, 5′-TGGATGGTGGTACAGTCAGAGC-3′). β-Actin was used as a housekeeping gene.

Western Blot Analysis

HLE-B3 cell lysates were harvested, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked using 1% bovine serum albumin (BSA) for 1 hour and then incubated with primary antibodies at a 1:1000 dilution overnight at 4°C. Then, the membranes were incubated with a 1:3000 dilution of secondary antibodies for 1 hour at room temperature (RT), and protein signals were detected using an enhanced

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chemiluminescence Western blotting detection kit (Bio-Rad). Antibodies against RNF157 (WH0114804M1) and Flag (F1804) were purchased from Sigma. Anti-caspase3 (#9662), anti-cleaved-caspase3 (#9664), and anti-Myc tag (#2276) antibodies were purchased from Cell Signaling (Danvers, MA). Anti-ubiquitin (sc-8017) and anti-β-actin (sc-8432) antibodies were obtained from Santa Cruz Biotechnology. Anti-p53 (10442-1-AP) was purchased from Proteintech (Wuhan, China). Goat anti-rabbit IgG secondary antibody (401315) and goat anti-mouse IgG secondary antibody (401215) were obtained from Millipore. Western blots were quantified using Image J (National Institutes of Health [NIH]).

Annexin V/PI Apoptosis Detection

HLE-B3 cells were washed twice with phosphate-buffered saline (PBS) and resuspended in Annexin V binding buffer. Then, the cells were stained cells using the Annexin-V/PI Kit (#640932; BioLegend) according to the manufacturer's protocol. After staining, cells were detected by flow cytometry on a CytoFLEX system (Beckman Coulter). The data were analyzed with CytExpert software (Beckman Coulter).

Cell Counting Kit-8 Assay

HLE-B3 cell proliferation activity was assessed with the cell counting kit-8 (CCK-8) assay, as described by the manufacturer (Beyotime Biotechnology, China). HLE-B3 cells were seeded into 96-well plates with 3 replicate wells per group. After treatment, 10 µL of kit reagent was added, and then the samples were incubated for 1 hour at 37°C. The absorbance at 450 nm was determined on a multi-well plate reader (Benchmark Plus; Bio-Rad, Tokyo, Japan).

Immunoprecipitation and Ubiquitination Assay

HLE-B3 cells transfected with the indicated plasmids were incubated with MG132 (10 µM) for 6 hours before being harvested at 48 hours post-transfection. Cells were lysed with IP lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP40, 1 mM EDTA, and protease inhibitor cocktail [Roche]) for 20 minutes on ice. Cell lysate was spun down by a centrifuge at 12,000 rpm at 4°C for 15 minutes; then, the supernatant was collected and incubated with anti-Myc tag antibody (#2276; CST) or anti-Flag antibody (F1804; Sigma-Aldrich) at 4°C overnight with gentle rocking. The next day, the samples were incubated with Protein A/G agarose beads (Santa Cruz Biotechnology) for another 4 hours at 4°C. After that, the beads were washed twice with lysis buffer. Then, the cell lysate was boiled at 100°C for 10 minutes in 2X Laemmli sample buffer (Bio-Rad) containing 5% 2-mercaptoethanol. The proteins were analyzed using SDS/PAGE followed by Western blotting, and goat anti-mouse IgG light chain specific secondary antibody served as a negative control (Abclonal; AS062). For the ubiquitination assay, 20 mM NEM (Selleck; S3692) was added to the IP lysis buffer.

Mass Spectrometry Analysis

HLE-B3 cells were transfected with the Flag-RNF157 or Flag empty vector. After 48 hours, the cells were lysed with IP buffer and immunoprecipitated with Flag (Sigma-Aldrich; F1804) antibodies as described. Then, the bound proteins were separated by SDS-PAGE and digested with trypsin. The peptides were subjected to a nanospray ionization (NSI) source followed by tandem mass spectrometry (MS/MS) in Q Exactive Plus (Thermo) coupled to an EASY-nLC 1000 UPLC system (Thermo). The resulting MS/MS data were processed using Proteome Discoverer 1.3. Tandem mass spectra were searched against the human UniProt database. Proteins with P values < 0.05 and a fold change > 2 were considered significantly different between the two sample groups. Gene ontology (GO) bioinformatic analysis was performed using R Studio, and the false discovery rate (FDR) method was used to adjust the P value.

HLE-B3 DNA and siRNA Transfection

Plasmid transfection was performed using jetPRIME (Polyplus) with a 1:3 DNA to jetPRIME ratio (w/v) according to the manufacturer's instructions. In the knockdown assay, HLE-B3 cells were transfected with 12.5 nmol/L siRNA and Lipofectamine RNAiMAX (Invitrogen). The sequences for siRNAs were as follows: human RNF157 siRNAs (#1: CCATCACCATCTATTACCA; #2: CCGAGAAGTTTACCCTCTA; and #3: CTGGCAGGCTGATGACAAT); human p53 siRNA (#1: CAGUCUACCUCCCGCCAUA; and #2: GAGGUUGGCUCUGACUGUA); and negative control (NC) siRNA (UUCUCCGAACGUGUCACGU). After verifying the knockdown efficiency, siRNF157 #1 and siRNF157 #2 were mixed and transfected into cells.

Protein Stability Assays

HLE-B3 cells were treated with cycloheximide (CHX, 25 µg/mL; Sigma-Aldrich) or MG132 (10 µM) for the indicated times after transfection with siRNAs or DNA plasmid for 48 hours. Then, the cells were lysed with Laemmli sample buffer and analyzed by Western blotting.

Immunofluorescence Assay

Rat Lens Epithelial Explants. Rat lens epithelial explants treated with or without H_2O_2 were fixed for 30 minutes with 4% paraformaldehyde and permeabilized in 0.1% Triton X-100 for 3 minutes. The explants were then immunostained using RNF157 (Bioss; 1:100 dilution) and p53 antibodies (Proteintech; 1:100 dilution). Images were acquired using an Olympus fluorescence microscope system.

HLE-B3 Cells. HLE-B3 cells were fixed in 4% formaldehyde for 20 minutes and permeabilized in 0.1% Triton X-100 for 3 minutes. Then, the cells were incubated with antibodies against RNF157 (Bioss; 1:200 dilution) and p53 (Proteintech; 1:1000 dilution) at 4°C overnight, followed by incubating with fluorescein-conjugated IgG (Alexa Fluor 488 goat anti-rabbit IgG or Alexa Fluor 594 goat anti-mouse IgG; Invitrogen; 1:2000) secondary antibody. The nucleus was counterstained by DAPI. Images at 400 times magnification were captured using a fluorescence microscope system (Olympus, Tokyo, Japan).

Statistical Analysis

All data are expressed as the mean ± standard deviation using GraphPad software (Prism 7). The Student's two-tailed t-test was used for two-group comparisons. Multiple groups were compared using one-way ANOVA followed by Tukey's test, and Dunnett's test was used for comparisons of multiple experimental groups to the control group. The P values

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< 0.05 were considered statistically significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Results

RNF157 is Downregulated in Cataracts

To explore the role of RNF157 in cataracts, we first measured the RNF157 expression in human normal lens and cataract lens samples. As shown in Figure 1A, RNF157 mRNA expression was reduced in the cataract lens samples compared with normal lens samples. Ultraviolet radiation is linked to cataract development,[27] so we then used UVB irradiation to construct a rat cataract model. The normal lens was transparent with epithelial and fiber cells in alignment, whereas the UVB-irradiated group lens appeared opaque with disordered epithelial structure and swollen fiber cells (Fig. 1B). Consistently, the RNF157 mRNA level was decreased approximately 40% in UVB-induced cataract rat lenses (Fig. 1C). IHC assays indicated that RNF157 was mainly expressed in LECs and was reduced in rat cataract lenses (Supplementary Fig. S1A). Additionally, we generated an H_2O_2induced ex vivo rat cataract model, as shown in Figure 1D. H_2O_2 treatment induced rat lens obvious opacification. The qRT-PCR and IHC assays showed that both the mRNA and protein levels of RNF157 were decreased in the H_2O_2-induced ex vivo rat cataract model (Fig. 1E and Supplementary Fig. S1B). Moreover, we generated a rat lens epithelial explant model to investigate RNF157 expression in the model. The immunofluorescence results were highly consistent with those of the UVB-induced in vivo cataract model and H_2O_2-induced ex vivo model findings, showing that RNF157 was downregulated (Supplementary Fig. S1C).

Next, H_2O_2 was used to establish an HLE-B3 cell oxidative stress model, and the cell apoptosis rate was markedly increased, along with cleaved caspase-3 levels (Figs. 1F–I).

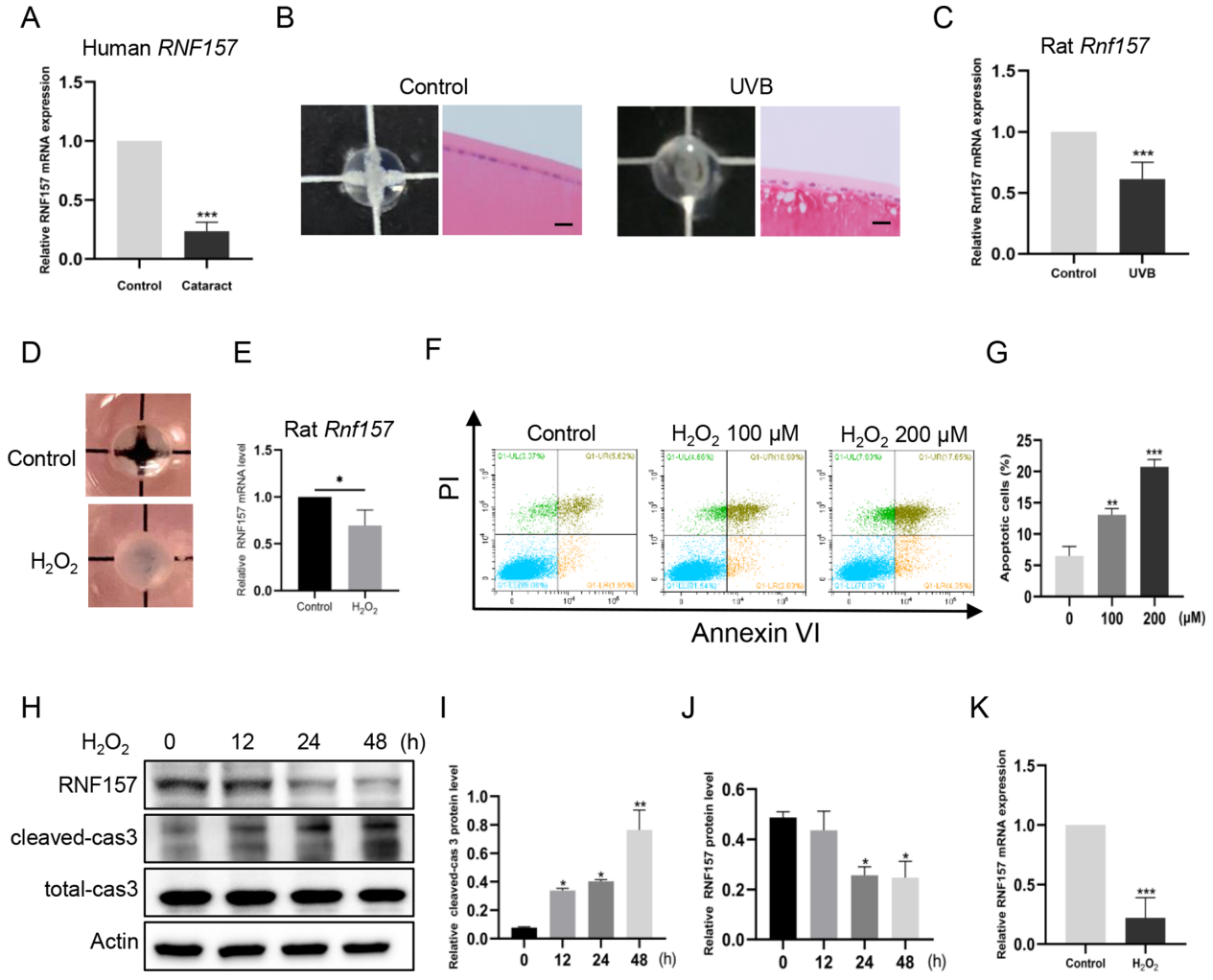

Figure 1. RNF157 expression is downregulated in cataracts. (**A**) The qRT-PCR analysis of RNF157 expression in human lens capsules. The values shown are mean ± standard deviation ($n = 5$). *** $P < 0.001$. (**B**) Representative picture and corresponding H&E staining of transparent rat lenses and UVB-irradiated rat lenses. Scale bar = 20 µm. (**C**) RNF157 mRNA levels in the lenses of the normal and UVB-induced cataract rat groups were detected by qRT-PCR. The values shown are mean ± standard deviation ($n = 3$). *** $P < 0.001$. (**D**) Representative picture of H_2O_2-induced ex vivo rat cataract model. (**E**) The mRNA levels of RNF157 in the H_2O_2-induced ex vivo rat cataract model. (**F, G**) Flow cytometry analysis of H_2O_2-induced apoptosis. (**H**) Western blotting of RNF157 and caspase-3 protein expression in HLE-B3 cells treated with 200 µM H_2O_2 for different durations. (**I, J**) Quantification of Western blotting shown in **H**. (**K**) RNF157 mRNA expression in HLE-B3 cells treated with 200 µM H_2O_2 for 24 hours. The values shown are mean ± standard deviation ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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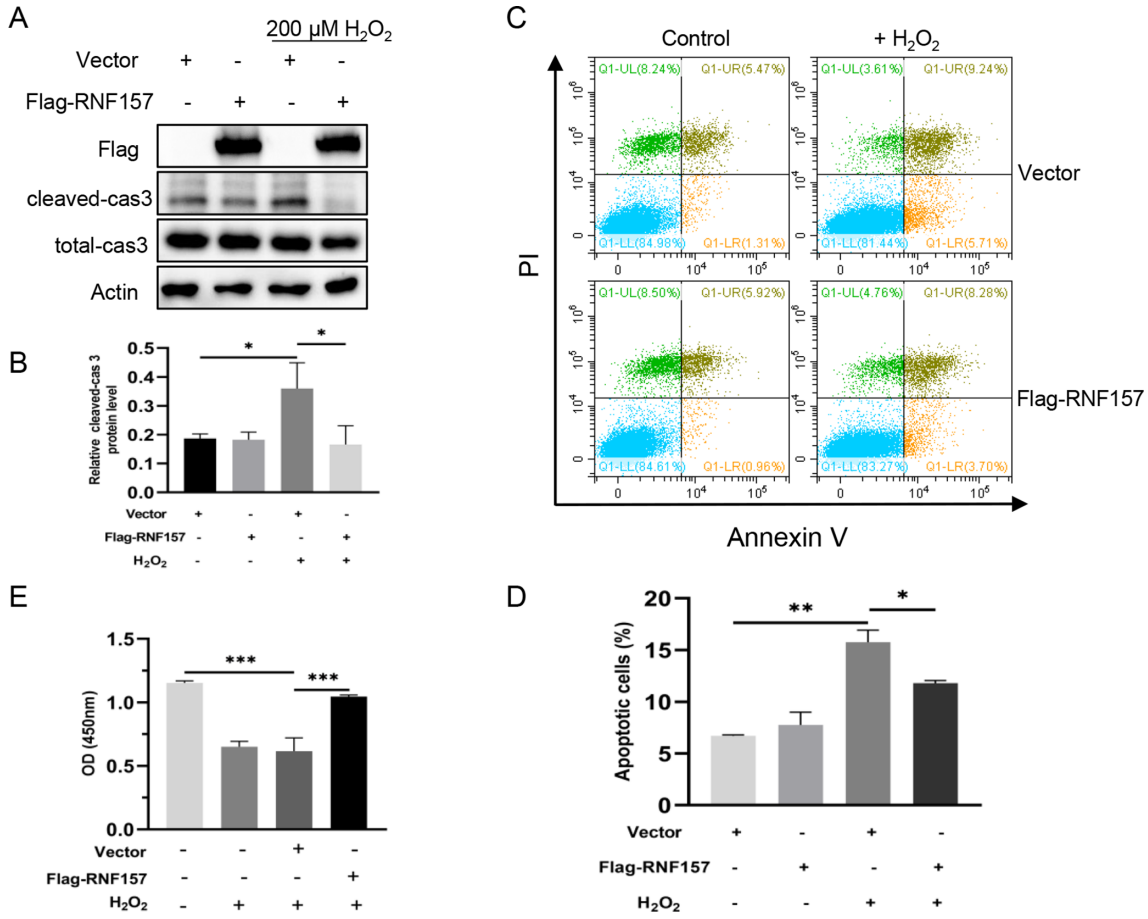

Figure 2. Overexpression of RNF157 protects HLE-B3 cells against H_2O_2-induced apoptosis. HLE-B3 cells were transfected with Flag-RNF157 or Flag vector for 24 hours and then were treated with 200 μM H_2O_2 for another 24 hours. (**A**) Western blotting of Flag-RNF157, cleaved caspase-3, and total caspase-3 in treated HLE-B3 cells. (**B**) Quantification of cleaved caspase-3 proteins by Western blotting. (**C, D**) Flow cytometry analysis of cell apoptosis after treatment. (**E**) Post-treatment cell viability detected via the CCK-8 assay. The values shown are mean ± standard deviation ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

However, RNF157 protein and mRNA levels were significantly reduced under oxidative stress conditions (Figs. 1H, J, K). Incubation of HLE-B3 cells with H_2O_2 at a concentration of 200 μM for 24 hours had a significant influence on apoptosis and RNF157 expression. Therefore, treatment with 200 μM H_2O_2 for 24 hours was used in subsequent experiments. Overall, these results suggested that RNF157 was downregulated in oxidative stress-induced cataracts.

RNF157 Protects HLE-B3 Cells From Apoptosis

To assess the function of RNF157 in oxidative stress damage, we examined the effect of RNF157 overexpression on H_2O_2-induced cell apoptosis. We first tested the expression of Flag-RNF157 by Western blotting (Fig. 2A). HLE-B3 cells transfected with the Flag-RNF157 plasmid showed an attenuated H_2O_2-induced increase in the apoptosis rate and cleaved caspase-3 protein level compared to those of empty vector-transfected cells (see Figs. 2A–D). In addition, overexpression of RNF157 resulted in enhanced cell viability compared with that of the control group under H_2O_2 treatment (Fig. 2E). These results supported that RNF157 protects HLE-B3 cells from oxidative stress-induced apoptosis and promotes HLE-B3 cell survival.

RNF157 Knockdown Aggravated H_2O_2-induced HLE-B3 Cell Apoptosis

We next performed an siRNA approach to determine the function of RNF157 in HLE-B3 cells. As shown in Figures 3A and 3B, RNF157 siRNA #2 and RNF157 siRNA #3 exhibited the greatest knockdown efficiency, so these two siRNAs were mixed to knockdown endogenous RNF157 in the follow-up experiments. We found that knockdown of RNF157 with siRNA elevated caspase-3 cleavage and the proportion of apoptotic HLE-B3 cells under H_2O_2 treatment (Figs. 3C–F). These results revealed that cells with a reduction in RNF157 displayed notable sensitivity to oxidative stress, and H_2O_2-induced HLE-B3 cell apoptosis was promoted.

RNF157 Knockdown Induces Lens Opacity Ex Vivo

To verify the function of RNF157 in the lens, we used an ex vivo cataract model as indicated.[24] First, we analyzed the rat Rnf157 siRNA knockdown efficiency, and the RNF157 expression level was significantly reduced by using siRNAs (Fig. 4A). As shown in Figure 4B, the rat lens was still transparent after ex vivo culture, whereas 200 μM H_2O_2 caused opacification of the rat lens at 6 days. Interestingly,

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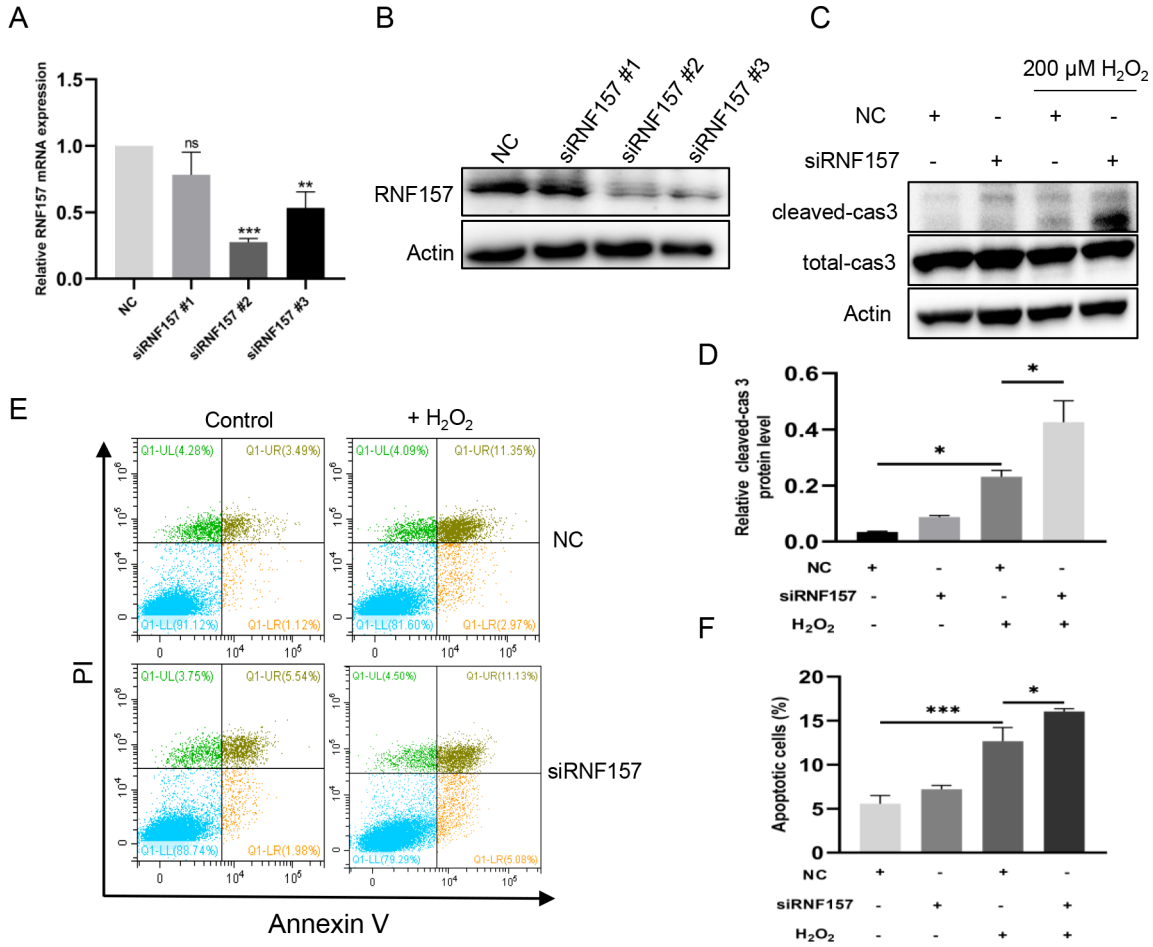

Figure 3. RNF157 knockdown sensitives HLE-B3 cells to H_2O_2-induced apoptosis. (**A, B**) RNF157 siRNA transfection efficiency detected by qRT-PCR and western blotting. The values shown are mean ± standard deviation ($n = 3$). ** $P < 0.01$, *** $P < 0.001$. ns means not significant. HLE-B3 cells were transfected with RNF157 siRNAs or NC siRNA for 24 hours and then were treated with 200 μM H_2O_2 for another 24 hours. (**C, D**) Western blotting and quantification of caspase-3 proteins in treated HLE-B3 cells. (**E, F**) Flow cytometry analysis of cell apoptosis after the indicated treatment. The values shown are mean ± standard deviation ($n = 3$). * $P < 0.05$, *** $P < 0.001$.

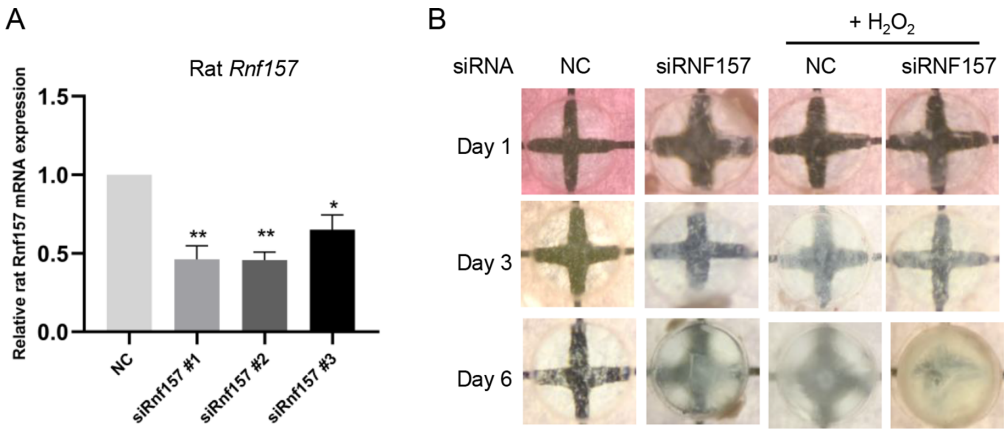

Figure 4. RNF157 knockdown facilitates oxidative stress-induced lens opacity ex vivo. (**A**) Rat RNF157 siRNA knockdown efficiency in the rat lenses. The values shown are mean ± standard deviation ($n = 3$). * $P < 0.05$, ** $P < 0.01$. (**B**) The lenses were kept in medium and photographed by a dissecting microscope.

transfection of RNF157 siRNAs aggravated the lens opacification induced by treatment with H_2O_2 (see Fig. 4B), suggesting that decreased RNF157 levels exacerbated H_2O_2-induced lens opacity.

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The E3 Ligase RNF157 Promotes p53 Degradation

To identify the proteins that potentially interact with RNF157, we used an immunoprecipitation mass spectrometry (Co-IP/MS) approach to identify RNF157-interacting proteins in HLE-B3 cells transfected with Flag empty vector versus Flag-RNF157 vector. A total of 787 proteins were detected within the Flag-RNF157 IP group. Enrichment analysis revealed that the identified proteins were involved in several biological processes (Supplementary Fig. S3). We focused on apoptotic signaling for further study. The pro-apoptotic factor p53, which is connected to cataracts, was found in the positive regulation of intrinsic apoptotic signaling pathway-related proteins IP by Flag-RNF157 (Fig. 5A).

We next investigated the p53 protein level in rat cataract models. As shown in Supplementary Figure S2, the p53 protein level was upregulated in the UVB-induced cataract model, H_2O_2-induced the ex vivo cataract model, and the H_2O_2 treated rat lens epithelial explants. Then, we detected the effects of RNF157 on p53 expression. Ectopic expression of RNF157 significantly decreased p53 protein (endogenous or exogenous) expression (Figs. 5B, 5C), and knockdown of RNF157 with siRNA upregulated p53 protein levels, whereas qRT-PCR results showed that RNF157 did not influence p53 mRNA levels (Figs. 5D, 5E). However, the proteasome inhibitor MG132 ameliorated RNF157-mediated downregulation of p53 (Fig. 5F). Moreover, the expression of ectopic RNF157 notably accelerated p53 degradation,

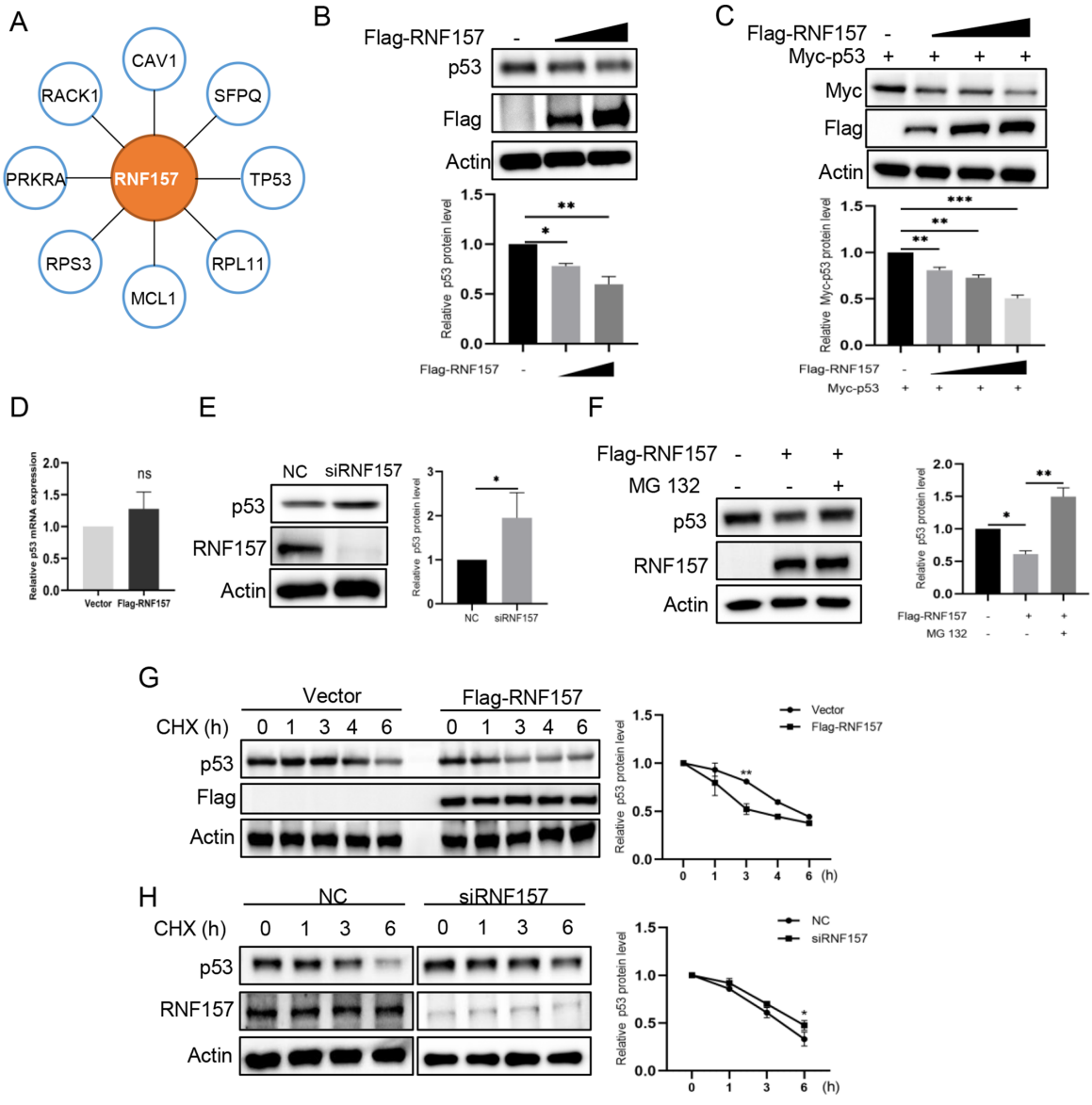

Figure 5. The E3 ligase RNF157 facilitates p53 degradation through the proteasome pathway. (**A**) The proteins were enriched in the positive regulation of intrinsic apoptotic signaling pathway. (**B, C**) Influence of RNF157 on endogenous or exogenous p53 protein expression. (**D**) The qRT-PCR was performed to detect the effects of exogenous RNF157 on p53 mRNA expression. The values shown are mean ± standard deviation ($n = 3$). ns means not significant. (**E**) Effect of RNF157 knockdown on the p53 protein level. (**F**) HLE-B3 cells were transfected with empty vector or Flag-RNF157 vector for 36 hours and then were treated with 10 µM MG132 for 6 hours before Western blotting. (**G**) HLE-B3 cells transfected with empty vector or Flag-RNF157 vector were treated with CHX (25 µg/mL) for the indicated durations and analyzed by Western blotting. (**H**) HLE-B3 cells transfected with NC siRNA or RNF157 siRNA were treated with CHX (25 µg/mL) for the indicated duration before Western blot analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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whereas silencing of RNF157 prolonged the p53 half-life in response to cycloheximide (CHX) treatment in HLE-B3 cells (Figs. 5G, 5H). Together, these results provide important insights regarding the notion that RNF157 could facilitate p53 protein degradation via the proteasome pathway.

RNF157 Promotes the Ubiquitination of p53

To demonstrate whether RNF157 is an E3 ligase for p53, we next tested the protein complex in vivo by a co-immunoprecipitation assay. As shown in Figure 6A, ectopic expression of Flag-RNF157 formed a complex with ectopic Myc-p53 in HLE-B3 cells. Moreover, an immunofluorescence staining assay showed that RNF157 and p53 colocalized in the cytoplasm in HLE-B3 cells (Fig. 6B). We further investigated the role of RNF157 in p53 protein ubiquitination in vivo, and the results showed that knockdown of RNF157 decreased the polyubiquitination of p53, whereas ectopic expression of RNF157 facilitated p53 protein polyubiquitination (Figs. 6C, 6D). Overall, these results indicated that the E3 ligase RNF157 could interact with p53 and promote ubiquitination of the p53 protein.

p53 Mediates the Effects of RNF157 on H_2O_2-Induced Cell Apoptosis

We then investigated whether p53 is involved in the RNF157-induced antiapoptotic effects. Overexpression of p53 significantly reversed the RNF157-mediated downregulation of the apoptosis rate and cleaved caspase-3 levels under oxida-

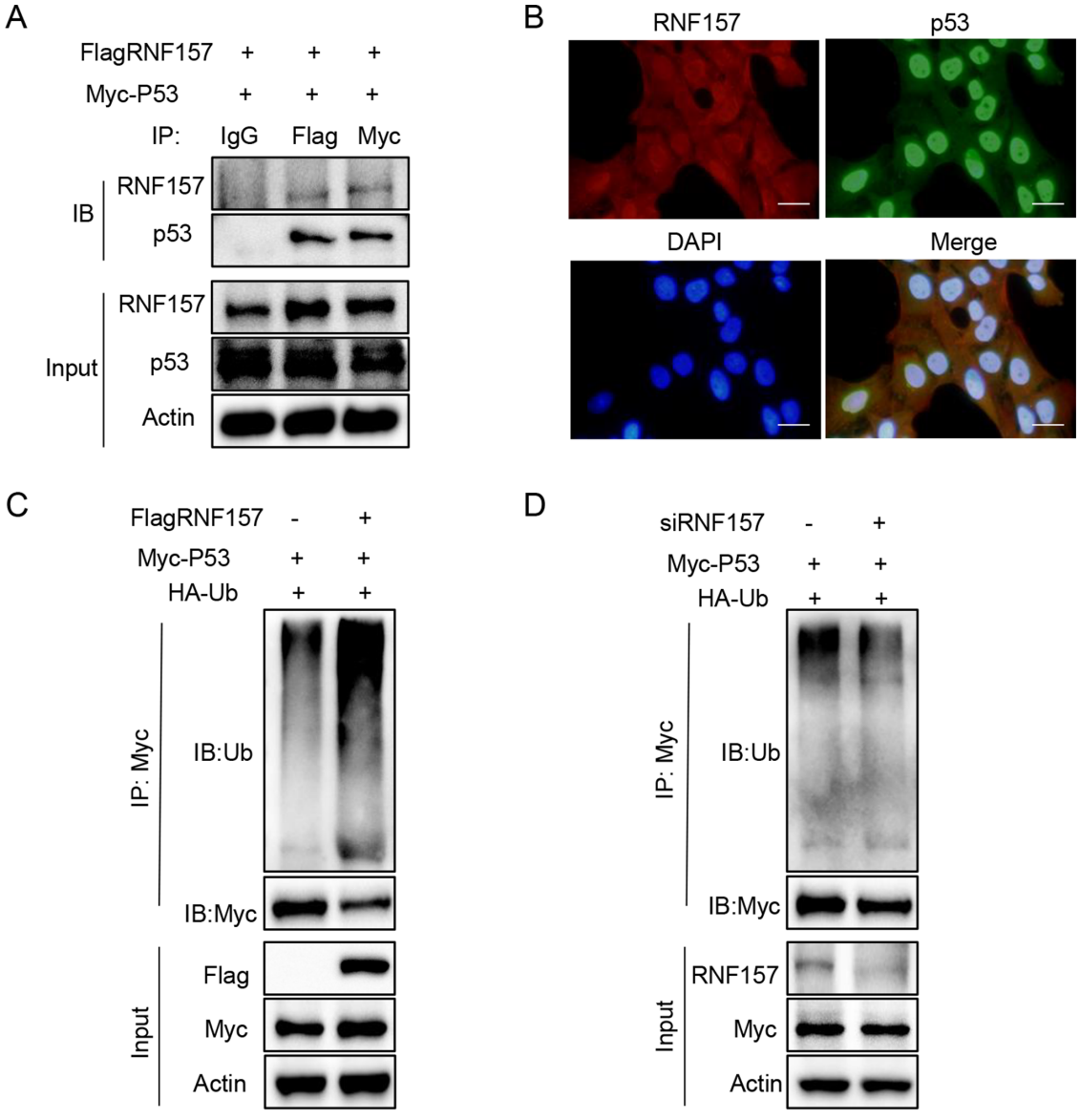

Figure 6. RNF157 interacts with p53 and promotes the ubiquitination of p53. (**A**) HLE-B3 cells were co-transfected with Flag-RNF157 and Myc-p53 plasmids and then treated with MG132 (10 μM) for 6 hours before immunoprecipitation (IP) followed by western blot analysis. IgG served as a negative control. (**B**) Representative fluorescence images of RNF157 and p53 in HLE-B3 cells. Scale bar = 10 μm. (**C**) HLE-B3 cells were co-transfected with HA-ubiquitin, Myc-p53, and Flag-RNF157 or empty plasmids for 36 hours. Cells were then treated with 10 μM MG132 for 6 hours, followed by IP and Western blot analyses. (**D**) HLE-B3 cells were co-transfected with HA-ubiquitin, Myc-p53 plasmids, and RNF157 siRNA or NC siRNA for 48 hours and then treated with 10 μM MG132 for 6 hours. IP and Western blot analyses were performed using the indicated antibodies.

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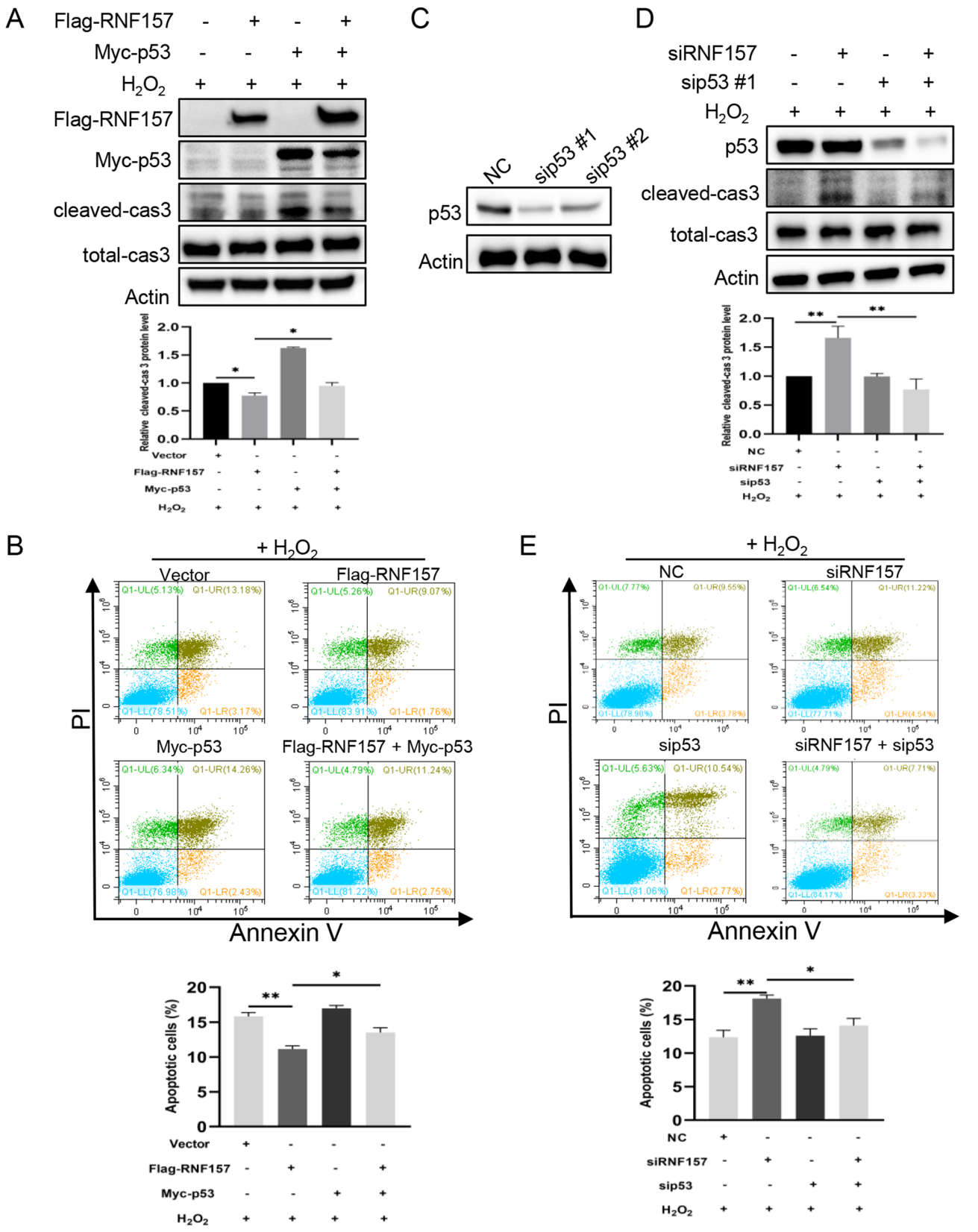

Figure 7. The p53 mediates the effects of RNF157 on H_2O_2-induced cell apoptosis. (**A, B**) HLE-B3 cells were transfected with Flag-RNF157 plasmid, Myc-p53 plasmid, or empty vector, caspase-3 activity was measured by Western blotting, and the apoptosis rate was tested via flow cytometry analysis. The values shown are mean ± standard deviation ($n = 3$). * $P < 0.05$, ** $P < 0.01$. (**C**) Knockdown efficiency of p53 siRNAs in HLE-B3 cells. (**D, E**) Effects of double knockdown of RNF157 and p53 on the activation of caspase-3 and the cell apoptosis rate upon H_2O_2 treatment. The values shown are mean ± standard deviation ($n = 3$). * $P < 0.05$, ** $P < 0.01$.

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tive stress conditions (Figs. 7A, 7B). Furthermore, silencing of p53 rescued the RNF157 siRNA-induced HLE-B3 cell apoptosis effects upon H_2O_2 treatment (Figs. 7C–E). Taken together, these results indicate that RNF157 protects HLE-B3 cells from apoptosis by altering p53 protein expression.

Discussion

In this study, we showed that RNF157 plays an anti-apoptotic role in human lens epithelial cells under oxidative stress conditions. First, we found that RNF157 expression was downregulated in lens samples of patients with cataract and in different cataract models when compared with normal controls. Second, RNF157 overexpression alleviated H_2O_2-induced HLE-B3 cell apoptosis, whereas knockdown of RNF157 resulted in increased susceptibility of HLE-B3 cells to H_2O_2-induced apoptosis. Moreover, Co-IP/MS assay revealed that RNF157 could interact with p53. Mechanistically, RNF157 enhanced polyubiquitination and subsequent degradation of p53 via the proteasome pathway, leading to resistance to oxidative stress-induced apoptosis in HLE-B3 cells.

Studies have shown that oxidative stress occurs during the early stage of cataracts and that H_2O_2 levels are significantly higher in the aqueous humor of patients with cataract than in those of people without cataracts[28,29] In addition, patients with ARC have notable higher levels of LEC apoptosis than healthy people.[30] Thus, oxidative stress-induced apoptosis of LECs has been regarded as an important contributor to the pathogenesis of cataracts.[3,28,31] LEC apoptosis leads to loss of homeostasis, accumulation of crystallin proteins, and ultimately cataracts. Unraveling the molecular basis of LEC apoptosis will increase our understanding of cataractogenesis and provide insights into the development of therapeutic strategies for cataracts.

It has been well established that the E3 ubiquitin ligase RNF157 is a regulator of survival in cultured neurons.[20] Recently, Taner Dogan and coworkers suggested that knockdown of endogenous RNF157 led to late S phase and G2/M arrest and apoptosis in tumor cells.[21] Another study demonstrated that RNF157$^{-/-}$ mice displayed a cataract phenotype in early adulthood,[22] whereas the underlying mechanism of RNF157 in cataract remains unclear. In our study, we discovered that RNF157 is an important apoptosis regulator in LECs. Notably, RNF157 depletion exacerbated H_2O_2-induced rat lens opacity in the ex vivo model. These findings suggest that RNF157 plays antiapoptotic role in LECs and downregulate of RNF157 expression might contribute to the development of cataracts.

The p53 protein is kept at low levels by continuous ubiquitination and degradation by the proteasome under unperturbed conditions. Conversely, p53 ubiquitination is inhibited in response to various cellular stresses, leading to stabilization and activation of the p53 protein.[32,33] The p53 protein is a target of numerous E3 ligases, and the largest group among them is the RING (Really Interesting New Gene) domain E3 ligases.[33–35] Inhibition of the p53 pathway significantly attenuates LEC apoptosis.[10] Therefore, development of drugs that target p53 ubiquitination is a promising cataract treatment strategy.

The RING-type E3 ligase RNF157 includes a RING domain and exhibits ubiquitin ligase activity by directly binding to ubiquitin-conjugating enzymes.[20,36] The results of the current study indicate that RNF157 is a novel E3 ligase that targets p53 for ubiquitination and proteasomal degradation in LECs. The ortholog of RNF157 in *Drosophila*, CG9941, could interact with the CG5334 protein (whose human homolog is MKRN1), and MKRN1 has been described as an E3 ligase for p53 and p21.[21,37] Further research is needed to evaluate if RNF157 and MKRN1 could potentially cooperate to play a role in p53 ubiquitination. The Co-IP and ubiquitination assay results imply the existence of an intracellular interaction between RNF157 and p53 in LECs. However, whether these two molecules interact directly with each other needs further investigation. Overexpression of RNF157 inhibited H_2O_2-induced apoptosis, which was rescued by simultaneous expression of p53. In contrast, knockdown of p53 restored RNF157 depletion-induced susceptibility of HLE-B3 cells to oxidative stress. Future in vivo experiments will help explain these observations. Nevertheless, these results indicate that the interaction between RNF157 and p53 is critical for LEC survival.

In summary, our findings demonstrate that deficiency of RNF157 expression might aggravate oxidative stress-induced LEC apoptosis through p53 signaling in cataracts, suggesting that modulating the expression of RNF157 is a potentially useful therapeutic approach for cataract.

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