

SMP30 Attenuates Lens Epithelial Cells Pyroptosis of Cataract via the Downregulation of p-STAT3

Yongshun Liang¹, Qingqiao Gan¹, Xin Zhong¹, Tian Lan¹, Yingqin Yang², Lixia Lin¹, Chengye Tang¹, Hao Liang¹

¹Department of Ophthalmology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China;

²Department of Ophthalmology, Affiliated Hospital of Guilin Medical University, Guilin, Guangxi, People's Republic of China

Correspondence: Hao Liang, Department of Ophthalmology, The First Affiliated Hospital of Guangxi Medical University, No. 6 Shuangyong Road, Qingxiu District, Nanning, Guangxi Zhuang Autonomous Region, People's Republic of China, Email liangh@stu.gxmu.edu.cn

Background: To determine the effect of senescence marker protein 30 (SMP30) regulation on the levels of p-STAT3 in the pathophysiology of lens epithelial cells (LECs) pyroptosis in cataracts.

Methods: Initially, cataracts were induced in rats using ultraviolet B (UVB) irradiation. Transmission electron microscopy was utilized to observe morphological changes in rat LECs, and RT-qPCR was utilized to quantify SMP30 and pyroptosis-related marker genes (GSDMD, Caspase-1, NLRP3, IL-1 β , and IL-18). Subsequently, SMP30-AAV2 vectors were injected into the vitreous cavity to overexpress SMP30 in rat lenses. Proteomic analysis identified differential proteins associated with pyroptosis post-SMP30 overexpression. Stable SMP30-overexpressing human LECs (SRA01/04 cells) were established via lentiviral transfection. Western blot, ELISA, and RT-qPCR were used to investigate the role of SMP30 in the pyroptosis of LECs treated with H₂O₂. Additionally, rescue experiments with p-STAT3 agonists and inhibitors elucidated SMP30's molecular mechanisms in H₂O₂-induced LECs pyroptosis.

Results: After UVB irradiation, SMP30 expression significantly decreased in rat lens capsules, while pyroptosis-related marker gene expression markedly increased. Ten crucial pyroptosis-related proteins were identified by proteomic analysis following SMP30 overexpression, with STAT3 receiving the highest score. SMP30 overexpression during H₂O₂-induced pyroptosis in SRA01/04 cells significantly decreased the expression of pyroptosis-related markers (GSDMD, Caspase-1, NLRP3, IL-1 β , and IL-18). The p-STAT3 agonist Colivelin weakened the anti-pyroptotic effect of SMP30, while the p-STAT3 inhibitor Stattic enhanced the anti-pyroptotic effect of SMP30.

Conclusion: The expression levels of SMP30 were downregulated in cataract cell pyroptosis. When overexpressed, SMP30 can reduce lens epithelial cell pyroptosis by downregulating the expression of p-STAT3. Thus, SMP30 demonstrates promising potential in preventing and treating cataracts.

Keywords: pyroptosis, SMP30, p-STAT3, cataract, lens epithelial cells

Introduction

Cataracts are a primary contributor to blindness and vision loss in adults globally. According to the Global Burden of Disease Study 2021 (GBD 2021; <https://ghdx.healthdata.org/gbd-2021>), the global prevalence of cataracts reached 100.57 million cases in 2021. China accounted for 19.79 million of these cases, representing approximately 19.7% of the global burden.¹ The incidence of cataracts is rising annually due to the increasing world's aging population, posing a serious threat to public health.^{2,3} Research revealed that various factors, including genetics, age, oxidative stress, inflammation, and ultraviolet radiation, are linked to the occurrence of cataracts.² However, its pathogenesis remains undetermined. Some studies suggest that oxidative stress-induced lens epithelial cells (LECs) apoptosis is a pathological mechanism of cataract formation.⁴ However, current research indicates that cataract occurrence and development are mediated by several types of cell death, including ferroptosis,⁵ autophagy,⁶ and pyroptosis.⁷ Among these, pyroptosis has attracted increasing attention from researchers.

Pyroptosis is a recently discovered type of programmed cell death (PCD),⁸ it is defined by inflammasome activation, cell membrane permeabilization, membrane rupture, and the development and release of pro-inflammatory cytokines IL-1 β and IL-18.⁹ It is triggered by caspases-1 through a classical pathway. Inflammasomes activate caspase-1, which then cleaves GSDMD, releasing the N-terminal domain (GSDMD-N). The release of this domain creates holes in the membrane of the cell. When this occurs, the cell membrane becomes compromised, allowing sodium ions to enter along with water molecules, leading to increased osmotic pressure inside the cell and causing it to swell. Eventually, the cell membrane ruptures, releasing cellular contents and triggering an inflammatory response.¹⁰ Furthermore, pro-IL-1 β and pro-IL-18 are concurrently cleaved into their active forms by activated caspase-1, IL-1 β , and IL-18, which are subsequently released through the pores made by GSDMD to induce inflammation. Another mode of pyroptosis activation is through the non-classical pathway. This pathway is mediated by caspase-4/5/11. The direct binding of lipopolysaccharides (LPS) to caspase-4/5/11 can activate these enzymes, cleaving GSDMD and causing pyroptosis.¹¹ Moreover, pyroptosis is closely associated with oxidative stress and inflammation, with studies reporting that reactive oxygen species (ROS) are crucial to the process.¹²

The identification of senescence marker protein 30 (SMP30) dates back to 1978. A study revealed that SMP30 constitutes rat liver protein and its expression decreased with age in the rats.¹³ From this study, it can be observed that SMP30 is linked with the aging process. In human cataract patients aged above 60, the expression levels of SMP30 were observed to be decreased when compared to individuals aged below 60,¹⁴ thus implicating its function in the aging process. With a molecular weight of 34 kDa, SMP30 is broadly expressed in the liver, kidneys, lungs, brain, thymus, heart, testicles, ovaries, and eyeballs, with higher expression levels in the liver and kidneys. SMP30 has multiple biological functions; it contributes to the synthesis of vitamin C, helps protect against oxidative damage, inhibits proliferation and apoptosis, and influences the synthesis of DNA and RNA.^{15,16} Additionally, it regulates important cellular processes like proliferation, antioxidation, and anti-apoptosis, which protect against cataracts.^{17–19} Ishikawa et al²⁰ discovered that SMP30-knockout mice were more prone to the development of cataracts following UVB exposure than wild-type mice. SMP30 was revealed to be expressed within the anterior capsule's LECs in human lenses.¹⁴ According to recent studies, SMP30 has also been linked to the regulation of proteins associated with pyroptosis.²¹ However, the relationship between SMP30 and cell pyroptosis in the development of cataracts has not been established and requires further investigation.

In our investigation, we used ultraviolet B (UVB) irradiation to induce cataracts in rats, and we discovered that pyroptosis was activated in the LECs. Subsequently, we constructed a rat cataract model with overexpressed SMP30 and identified the downstream protein STAT3 of SMP30 through proteomic analysis. In H₂O₂-induced pyroptosis of the human LECs SRA01/04, we found that SMP30 alleviates pyroptosis by reducing the levels of p-STAT3. Consequently, targeting SMP30 may be a useful strategy for treating and preventing cataracts.

Methods and Materials

Experimental Animals

A total of 55 Wistar rats, aged 2 months and weighing approximately 200 g (27 males and 28 females), were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (License number, SCXK (Jing) 2021–0006). The rats were maintained in an SPF-grade laboratory and fed standard food under a cycle of 12 hours of light and darkness. All animal studies were approved by the Guangxi Medical University Animal Research Committee (Approval number, 202107443) and conducted in accordance with the ARRIVE Guidelines 2.0. The animal experimental procedures of this study were strictly in accordance with the Guidelines for the Ethical Review of Animal Welfare (GB/T 35892–2018). Three groups of rats were established, the normal control (NC) group, the ultraviolet B irradiation (UVB) group, and the overexpression SMP30+UVB irradiation (SMP30+UVB) group.

Injection of Adeno-Associated Virus

The SMP30-AAV2 virus (GeneChem, Shanghai, China) was intravitreally injected into the SMP30+UVB group following a week of adaptive feeding. First, the rats were anesthetized with isoflurane gas (Ruwode, China). Their pupils were

dilated using compound tropicamide eye drops (Santen, Japan). Then, proparacaine hydrochloride eye drops (Alcon, USA) were applied for surface anesthesia. A 33G injection needle was used to make a small hole 1mm posterior to the corneal limbus. A Hamilton microsyringe (Hamilton Company, USA) was then used to slowly inject 2 μ L of the SMP30-AAV2 virus (1×10^{12} v.g./mL) into the vitreous cavity. The microsyringe was carefully removed 60 seconds after administering the SMP30-AAV2 virus. Ultimately, tobramycin-dexamethasone eye ointment (Alcon, USA) was used to treat the eyes. Four weeks after the virus injection, UVB irradiation was performed to establish the cataract model.

UVB-Induced Rat Cataract Model

An intraperitoneal injection of 7% chloral hydrate was used to sedate the rats. Following sedation, the pupils were dilated with compound tropicamide eye drops for approximately 5 minutes. The eyes were then exposed to a 308 nm ultraviolet lamp, adjusted to an appropriate height to achieve an irradiation intensity of 1×10^3 μ W/cm². The eyes were irradiated for 15 minutes daily for 7 consecutive days, with a total irradiation dose of 9 KJ/m². After irradiation, ofloxacin eye ointment (Xingqi, China) was applied to prevent corneal infection, and the rats were allowed to wake up naturally.

Cell Culture and Transfection

The SRA01/04 cell line was obtained from Shanghai Zhong Qiao Xin Zhou Biotechnology Co.,Ltd. and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum and 1% antibiotics (streptomycin and penicillin). Cells were maintained in an incubator at 37°C with 5% CO₂. For experiments, cells were exposed to 500 μ mol/L H₂O₂ for 24 hours. Cells were transfected with SMP30 overexpression lentivirus (GenePharma, Shanghai, China), and stable cell lines were selected using 1 μ g/mL puromycin. The manufacturer's recommendations were followed when using the STAT3 agonist Colivelin (HY-P1061, MCE, USA) and inhibitor Stattic (HY-13818, MCE, USA).

Transmission Electron Microscope

The rats were euthanized. After being quickly fixed at 4 °C for the entire night, the eyes were washed using phosphate buffer solution. After being fixed in a 1% osmium acid solution in the dark, the samples were once more rinsed with phosphate buffer solution. Following dehydration using a series of ethanol and acetone solutions at varying concentrations, the samples underwent treatment with acetone and 812 embedding resin at 37 °C, followed by infiltration and embedding in pure 812 resin. Polymerization was conducted in an oven at 60 °C for 48 hours. Next, the polymerized samples were sliced into 60–80 nm thick pieces using an ultramicrotome, and then they were put onto 150-mesh square copper grids. The slices were first incubated in a saturated alcoholic uranyl acetate solution before being stained with a lead citrate solution. Following staining, the sections were washed, dried, and then examined and studied using a transmission electron microscope.

4D-FastDIA Proteomics

4D-FastDIA proteomics were conducted by PTM BIO CO., Ltd. (Hangzhou, China). Proteomic analysis was performed on rat lens capsules from the NC group, UVB group, and SMP30+UVB group. Six rat lens capsules were pooled into a single sample because the low protein content in a single rat lens capsule makes it unsuitable for proteomic analysis. Three pooled samples were available to each group for biological replicate detection. The raw data for every sample was obtained using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The DIA-NN (v.1.8) search engine was used to process the data. *Rattus_norvegicus_10116_PR_20230103.fasta* (47945 items) concatenated with the reverse decoy database was searched against tandem mass spectra.

Analysis of Pyroptosis-Related Differential Proteins

For protein mass spectra data analysis, we used the limma package²² to identify the differentially expressed proteins (DEPs) between the two groups. The comparison groups were UVB/NC and SMP30+UVB/UVB. The screening criteria were established as follows, significant upregulation was defined as a P value < 0.05 and at least 1.2-fold change (FC >

1.2), while significant downregulation was defined as an FC < 1/1.2. A volcano plot was constructed to display the results of the differential expression using the R package ggplot2 (version 3.3.6).

For the acquisition of pyroptosis-related proteins (PRPs), we searched the GeneCards database (<http://www.genecards.org/>)²³ using the keyword “Pyroptosis”. Genes classified as “Protein Coding” were defined as PRPs. To identify pyroptosis-related differentially expressed proteins (PRDEPs) associated with SMP30, we intersected DEPs with PRPs and visualized the results using a Venn diagram drawn with the R package ggplot2. Additionally, the differential analysis results were presented with a heatmap created using the R package ComplexHeatmap.²⁴

Gene Ontology (GO) Analysis

We conducted GO annotation analysis on PRDEPs using the R clusterProfiler package.²⁵ Results were considered statistically significant if the adjusted p-value (p.adj) was less than 0.05. Additionally, we corrected the p-values using the Benjamini-Hochberg (BH) method. We used the R ggplot2 tool to display the GO enrichment analysis results for better comprehension and interpretation of the data.

Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis

We performed annotation analysis on PRDEPs using the KEGG pathway database; a P value < 0.05 was regarded as statistically significant. The results are presented in bubble plots using the R package ggplot2 (version 3.3.6).

Protein-Protein Interaction (PPI) Analysis

For PPI analysis on the PRDEPs, we used the STRING database (STRING v11.0) (<https://string-db.org/>).²⁶ Using Cytoscape (version 3.9.1), DEPs with a minimum interaction score greater than 0.4 were displayed. Additionally, the PRDEPs' maximal clique centrality (MCC) scores were determined using the CytoHubba plugin. The top 10 highest-scoring proteins were identified as hub proteins and were subsequently visualized using Cytoscape software.

Moreover, a heatmap was used to show the pairwise protein expression correlations of the hub proteins, which were investigated using a Spearman correlation analysis.

Cell Viability Assay

The vitality of SRA01/04 cells was assessed under different H₂O₂ stimulation levels using the Cell Counting Kit-8 (CCK-8) reagent (HYCEZMBIO, Wuhan, China) according to the manufacturer's protocol.

Real-Time Quantitative PCR (RT-qPCR) Assay

Total RNA was isolated using the QIAGEN, Germany-produced RNeasy Plus Mini Kit. For reverse transcription, HiScript[®] III RT SuperMix for qPCR (+gDNA wiper) (Vazyme, Nanjing, China) was utilized. The qPCR procedure was carried out using the ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). The 2^{-ΔΔCt} technique was utilized to determine the relative mRNA expression of target genes, with the housekeeping gene ACTB acting as an internal reference control. [Supplementary File 1, Table S1](#) contains a list of primer sequences.

Western Blot Assay

Following the manufacturer's protocol, sample protein concentrations were calculated using the BCA Protein Concentration Assay Kit (NCM Biotech, Suzhou, China). [Supplementary File 2, Table S2](#) contains a list of the primary antibodies utilized in this investigation, together with information on their suppliers and dilutions. HRP-labeled goat anti-mouse and anti-rabbit IgG antibodies (diluted 1:10,000) were obtained from Abmart (Shanghai, China). ECL chemiluminescence (Tanon, Shanghai, China) was used to visualize protein bands.

ELISA Assay

Using the ELISA kits (UpingBio, Zhejiang, China), the levels of IL-18 and IL-1β in the SRA01/04 cells' growth media were measured.

Statistical Analysis

The expression for quantitative data is presented as mean \pm standard deviation ($\bar{x} \pm S$). An independent samples *T*-test was used to compare two groups; a one-way ANOVA was used to compare more than two groups; and the Tukey method was used for post-hoc analysis. Correlations between various compounds were computed using Spearman correlation analysis. Every *P* value was two-sided, and a value of $P < 0.05$ was viewed as statistically significant. R and GraphPad Prism 9.0 were used for statistical analysis and visualization.

Results

Pyroptosis and Down-Regulation of SMP30 Expression Were Observed in UVB-Induced Cataracts in Rats

Based on the UVB-induced cataract rat model, we first investigated the variations in pyroptosis and SMP30 expression levels at the tissue level. A successful rat model of cataract was developed following 1 week of UVB irradiation (Figure 1A). According to the results from transmission electron microscopy, the cell membrane of the LECs in the NC group (Figure 1B) was intact and the overall structure of the cells was relatively normal. There was uniform chromatin, an oval nucleus (N), and a clear double nuclear membrane. Furthermore, mitochondria (M) were abundant, with intact membrane, uniform matrix in the membrane, and parallel ridges. The matrix was light in certain LECs, and there was no obvious expansion of rough endoplasmic reticulum (RER). Moreover, ribosome attachment could be observed on the surface. Lastly, there was no obvious hyperplasia and hypertrophy of the Golgi apparatus (Go). In the UVB group, LECs exhibited relatively severe damage. The cell membranes appeared incomplete and the concentration of the intracellular matrix had a high electron density. Notably, there was a high accumulation of significantly swollen and vacuolated organelles. Additionally, there was nuclear (N) pyknosis, the heterochromatin was increased, and the nuclear membrane was significantly expanded. The mitochondria appeared swollen, the nuclear gap was widened, the matrix in the membrane was dissolved, and the ridges had disappeared with altered vacuoles. There were some severe cases where the membrane was damaged. The RER appeared enlarged, degranulated, and vacuolated. From these findings, it was observed that the cells in the UVB group showed the induction of pyroptosis (organelle swelling, vacuolar degeneration, and incomplete cell membrane). The UVB group had substantially higher mRNA expression of NLRP3, Caspase-1, GSDMD, IL-18, and IL-1 β in comparison to the NC group (Figure 1C), and the UVB group had substantially lower mRNA and protein levels of SMP30 (Figure 1D–F). From these results, we can surmise that pyroptosis contributes to the occurrence of cataract, and SMP30 may be involved in regulating this process.

SMP30 Was Down-Regulated in H₂O₂-Induced Pyroptosis of SRA01/04 Cells

Next, we investigated the expression of SMP30 in human LECs and whether its expression changed in H₂O₂-induced pyroptosis. The findings show a progressive decline in cell viability with increasing H₂O₂ concentration over time as depicted in Figure 2A.

The cells were treated for 24 and 48 hours with H₂O₂ at a concentration of 500 μ mol/L when the cell viability was decreased by 50% (which was the closest to the half inhibition rate (IC₅₀) of the cells). To improve the modeling efficacy, we selected 500 μ mol / L H₂O₂ treatment for 24 hours as the follow-up modeling standard. Following H₂O₂ treatment, SMP30's mRNA and protein expression levels were significantly decreased (Figure 2B–D). Figure 2E shows a significant increase in the mRNA expression of genes related to pyroptosis, including NLRP3, Caspase-1, GSDMD, IL-18, and IL-1 β . These findings suggest that SMP30 may participate in cataract pyroptosis.

SMP30 Overexpression Alleviated H₂O₂-Induced Pyroptosis in LECs

To gain additional insight into the function of SMP30 in pyroptosis, we screened stable SRA01/04 cell lines overexpressing SMP30. The results showed that, when compared to the LV-Control and NC groups, the LV-SMP30 group successfully overexpressed SMP30 (Figure 3A–C). After overexpressing SMP30, cell viability increased (Figure 3D), and the expression of NLRP3, Caspase-1, GSDMD, IL-18, and IL-1 β genes was significantly decreased (Figure 3E). Moreover, NLRP3, Caspase-1 p20 and GSDMD-N protein levels (Figure 3F and G), as well as the concentrations of IL-18 (Figure 3H) and

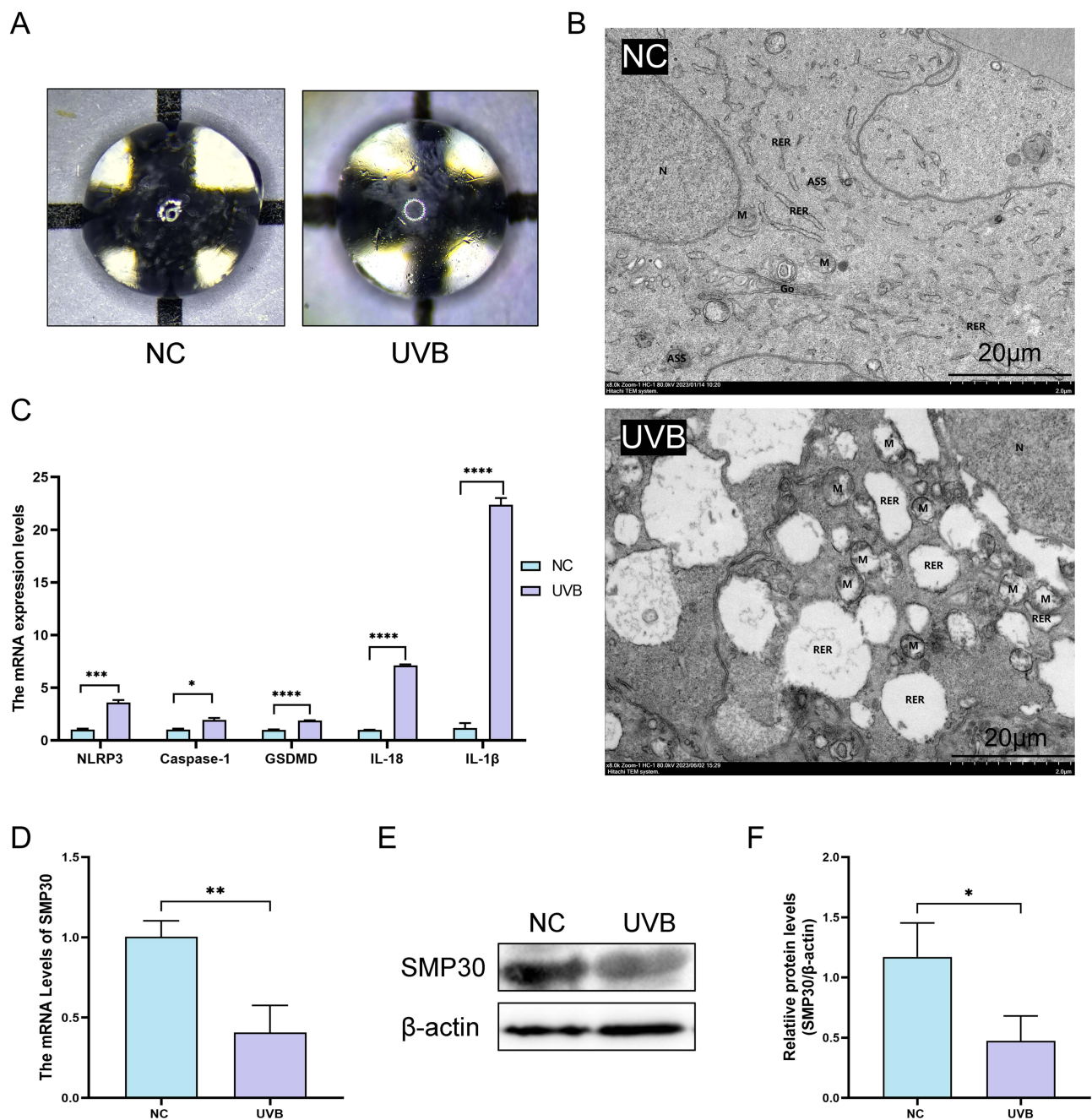


Figure 1 Pyroptosis and decreased SMP30 levels in the lens capsule of UVB-induced cataract in rats. **(A)** Cataract formation in rats induced by ultraviolet B irradiation. **(B)** The morphology of LECs in two groups under transmission electron microscope. **(C)** RT-qPCR analysis of the NLRP3, Caspase-1, GSDMD, IL-18, IL-1 β and SMP30. **(D)** mRNA expression in the lens capsule of rats in the NC and UVB groups. **(E and F)** Quantification of densitometry and Western blot investigation of SMP30 expression in the lens capsule. In comparison to the NC group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

Abbreviations: M, mitochondria; N, nucleus; RER, rough endoplasmic reticulum; Go, Golgi apparatus; ASS, autolysosome.

IL-1 β (Figure 3I), were all considerably lower in the cell culture supernatant. This suggests that the overexpression of SMP30 mitigates the degree of pyroptosis in H₂O₂-induced SRA01/04 cells.

Molecular Mechanism of SMP30 in UVB-Induced Pyroptosis of Cataract in Rat Based on Proteomic Analysis

We first overexpressed SMP30 levels in rat LECs by intravitreal injection of the AAV2 virus to establish the molecular mechanism by which SMP30 mitigates cell pyroptosis in cataracts.²⁷ Then, we ran a 4D-FastDIA proteomic analysis on

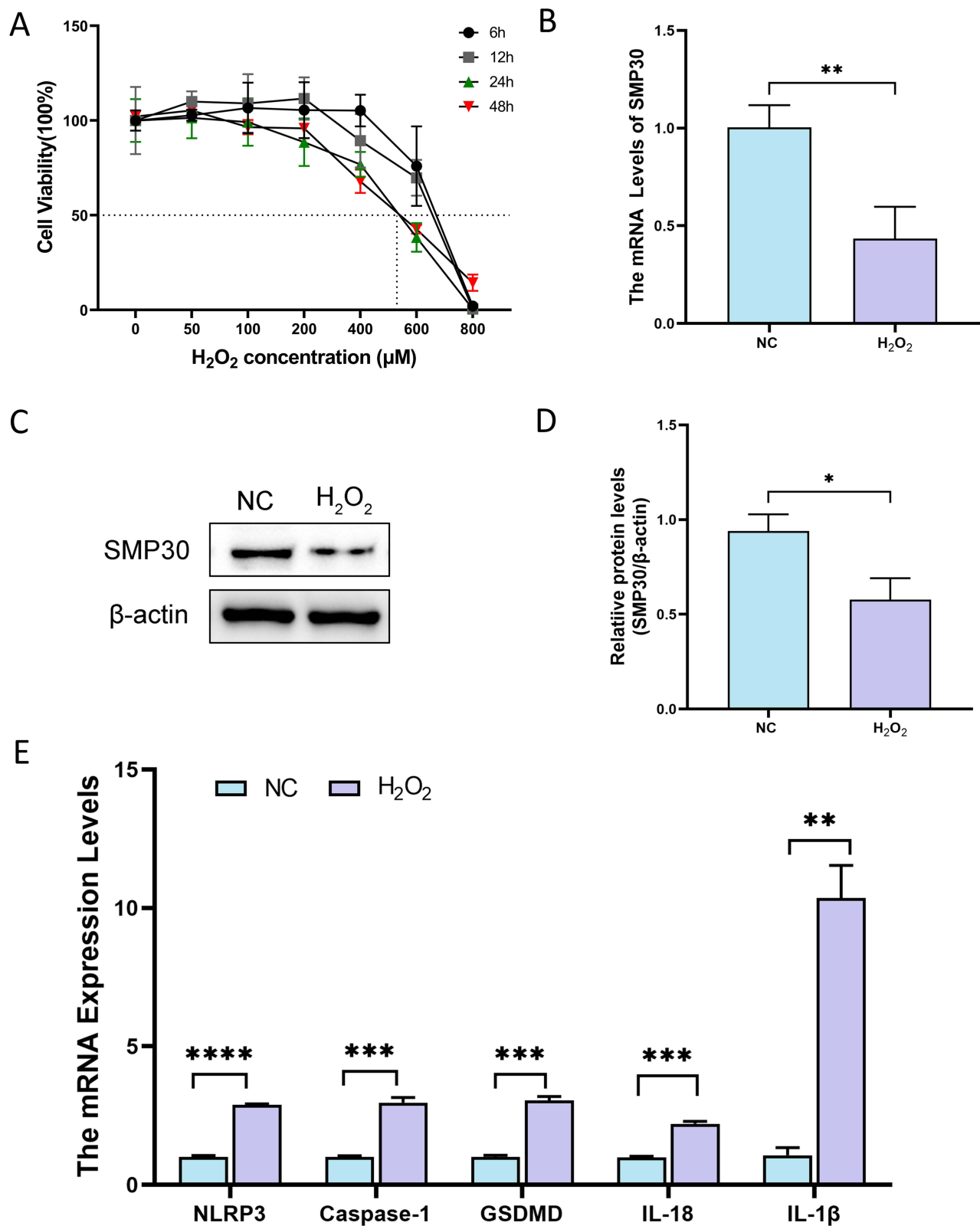


Figure 2 Levels of SMP30 decreased during pyroptosis. H₂O₂ stimulated SRA01/04 cells. **(A)** The CCK8 assay was used to determine cell viability. Different concentrations (0, 50, 100, 200, 400, 600, and 800 μmol/L) of H₂O₂ were applied to SRA01/04 cells for 6, 12, 24, and 48 hours, respectively. **(B)** The mRNA level of SMP30. **(C and D)** Protein level of SMP30. **(E)** The mRNA levels of NLRP3, Caspase-1, GSDMD, IL-18 and IL-1β. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 and *****p* < 0.0001.

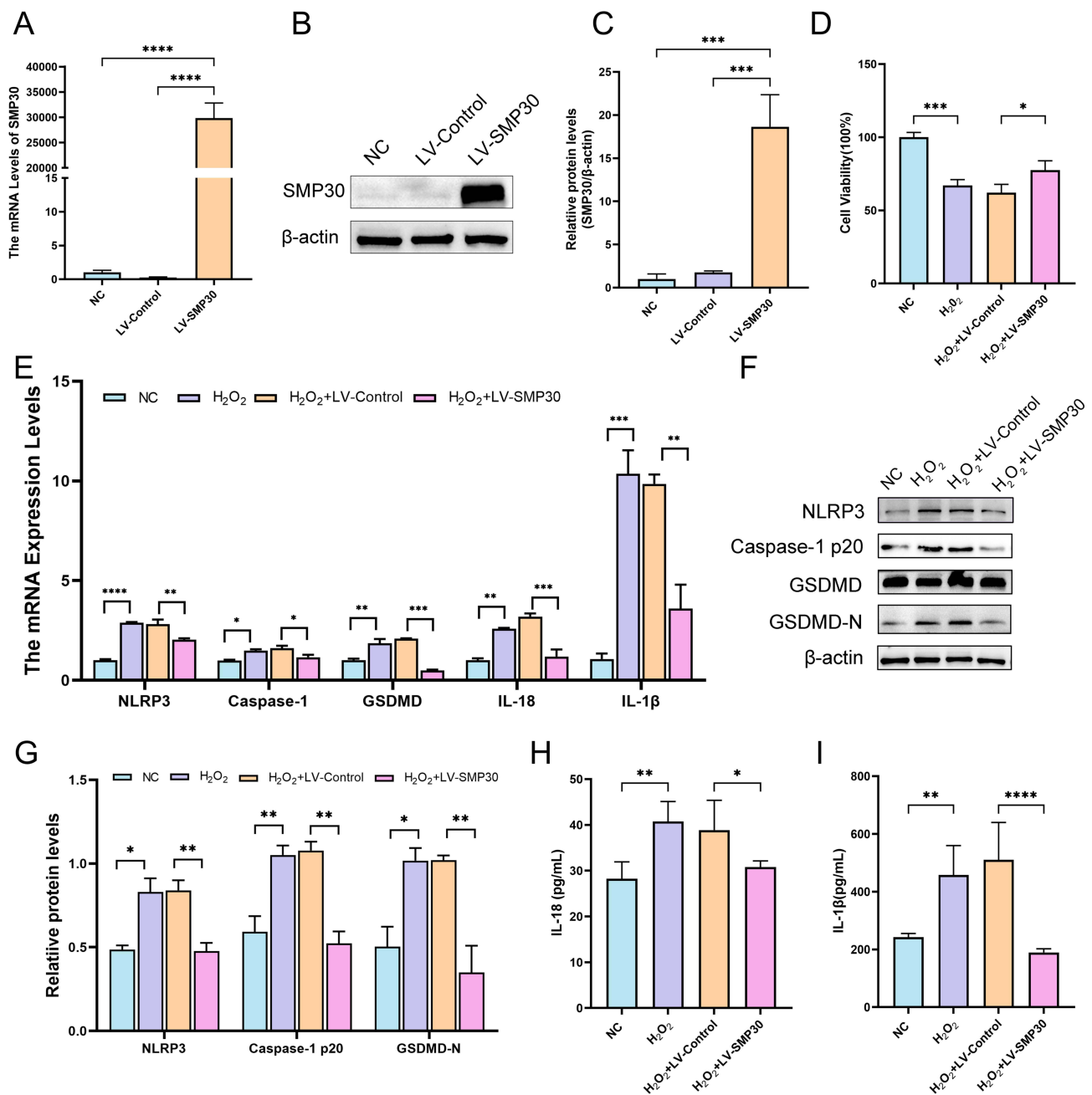


Figure 3 SMP30 overexpression alleviated H₂O₂-induced pyroptosis in SRA01/04 cells. (**A–C**) RT-qPCR and Western blot were utilized to determine the efficacy of f SMP30 overexpression in SRA01/04 cells. (**D**) The CCK-8 assay was used to evaluate how SMP30 overexpression affected the viability of H₂O₂-induced cells. (**E**) The expression of NLRP3, caspase-1, GSDMD, IL-18, and IL-1β genes was assessed using RT-qPCR. (**F** and **G**) The expression of NLRP3, caspase-1 p20, and GSDMD-N was assessed by Western blot. The concentration of IL-18 (**H**) and IL-1β (**I**) in cell supernatant. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 and *****p* < 0.0001.

the lens capsules of rats from the UVB, SMP30+UVB, and NC groups. A total of 2060 DEPs with fold change (FC) greater than 1.2 or less than 1/1.2 and a *p*-value less than 0.05 were found in both the UVB and NC groups. The UVB group showed downregulation of 1070 proteins and upregulation of 990 proteins. In contrast, 2861 DEPs satisfying the same threshold criteria were found in the SMP30+UVB and UVB group. In the SMP30+UVB group, there were 1343 upregulated and 1518 downregulated proteins. Volcano plots illustrating the results of the DEP analysis are presented in [Figure 4A](#) and [B](#). Furthermore, a search in the Gencard database for “Pyroptosis” yielded 425 PRPs. To obtain PRDEPs, the intersection of DEPs between the UVB/NC groups and the SMP30+UVB/UVB groups with PRPs was calculated,

resulting in a total of 38 PRDEPs. A Venn diagram (Figure 4C) and a heatmap (Figure 4D) were constructed to illustrate these findings. The results demonstrated a distinct clustering pattern of these 38 PRDEPs across different groups.

For these 38 PRDEPs, we ran GO and KEGG enrichment analyses to analyze their functions in molecular functions, biological pathways, biological processes, and cellular components. As demonstrated by GO analysis results (Figure 4E), they were primarily associated with biological processes, including aging, pyroptosis, and the regulation of apoptotic signaling pathways. Furthermore, they are involved in molecular processes like heat shock protein binding, transcription factor binding, RNA polymerase II-specific DNA binding, and protein serine/threonine kinase activity, as well as cell components like microtubules, nuclear envelopes, and polarized growth sites. In the KEGG enrichment analysis (Figure 4F), we found a total of 90 KEGG signaling pathways, mainly enriched in NOD-like receptor signaling pathway, PI3K-Akt signaling pathway, IL-17 signaling pathway, cell senescence, and apoptosis signaling pathways.

Utilizing the STRING database, we conducted a PPI analysis on 38 PRDEPs to acquire hub proteins. We constructed a PPI network with 28 nodes and 70 edges, using a minimum correlation coefficient greater than 0.4 as the criteria. The PPI network diagram was generated using Cytoscape software (Figure 5A). Then, we utilized the CytoHubba plug-in to compute the Maximum Cluster Centrality (MCC) score and identify the top 10 hub proteins. The results showed that the top 10 proteins from high to low were STAT3, EGFR, PARP1, GSK3B, MAPK14, CASP8, CDK1, HDAC2, NFKB1, DNMT1 (Figure 5B), indicating that these 10 proteins may be the key targets of SMP30 regulating pyroptosis. Notably, STAT3 emerges as one of the most prominently upregulated proteins upon SMP30 overexpression. Additionally, we created a heat map (Figure 5C) and used Spearman to conduct correlation analysis to establish the correlation between 10 hub proteins.

The results revealed significant correlations among various proteins. A notable negative correlation was observed between STAT3 and EGFR ($r = -0.943$, $P = 0.0048$), as well as between STAT3 and NFKB1 ($r = -0.886$, $P = 0.0188$).

Overexpression of SMP30 Decreased the Expression Level of p-STAT3

To corroborate the proteomic analysis findings, we analyzed how the overexpression of SMP30 affected STAT3. Following H₂O₂ exposure, STAT3 mRNA expression was significantly decreased; however, following SMP30 overexpression, STAT3 mRNA expression was significantly increased (Figure 6A). The expression of total STAT3 (T-STAT3) protein significantly decreased after H₂O₂ exposure, and significantly increased after overexpression of SMP30 (Figure 6B and C). However, the active form of STAT3, phosphorylated STAT3 (p-STAT3), showed an opposite trend to T-STAT3 (Figure 6B and D). The findings suggested that SMP30 and p-STAT3 might have a negative regulatory relationship.

SMP30 Alleviated H₂O₂-Induced Pyroptosis in SRA01/04 Cells via Regulating p-STAT3

We performed a rescue experiment to determine if SMP30 reduces H₂O₂-induced pyroptosis in SRA01/04 cells through the regulation of the level of p-STAT3. Colivelin is a STAT3 agonist that increases p-STAT3 expression in cells, while Stattic is a STAT3 inhibitor that decreases p-STAT3 levels. We respectively added Colivelin (50 nM) and Stattic (2 μM) to the cells in co-culture to observe if the protective effect of SMP30 on cell pyroptosis changes.

The findings showed that p-STAT3 expression increased in response to the addition of Colivelin (Figure 7A and B). The H₂O₂+LV-SMP30+Colivelin group exhibited substantially elevated expression levels of NLRP3, GSDMD, Caspase-1, IL-1β, and IL-18 when compared to the H₂O₂+LV-SMP30+DMSO group (Figure 7C–G). This suggests that Colivelin reduced the protective effect of SMP30 in H₂O₂-induced pyroptosis. After adding Stattic, the expression of p-STAT3 decreased (Figure 8A and B). Compared with the H₂O₂+LV-SMP30+DMSO group, the expression levels of NLRP3, GSDMD, Caspase-1, IL-1β, and IL-18 in the H₂O₂+LV-SMP30+Colivelin group were significantly decreased (Figure 8C–G). This suggests that Stattic enhanced the protective effect of SMP30 against H₂O₂-induced pyroptosis.

Discussion

SMP30, a newly discovered anti-aging agent, possesses antioxidant and anti-inflammatory characteristics. Our previous research has demonstrated the beneficial effects of SMP30 in preventing oxidative stress-induced damage and anti-apoptosis in cataract.^{14,17,28–30} The mechanism of SMP30 is currently being thoroughly investigated by a number of

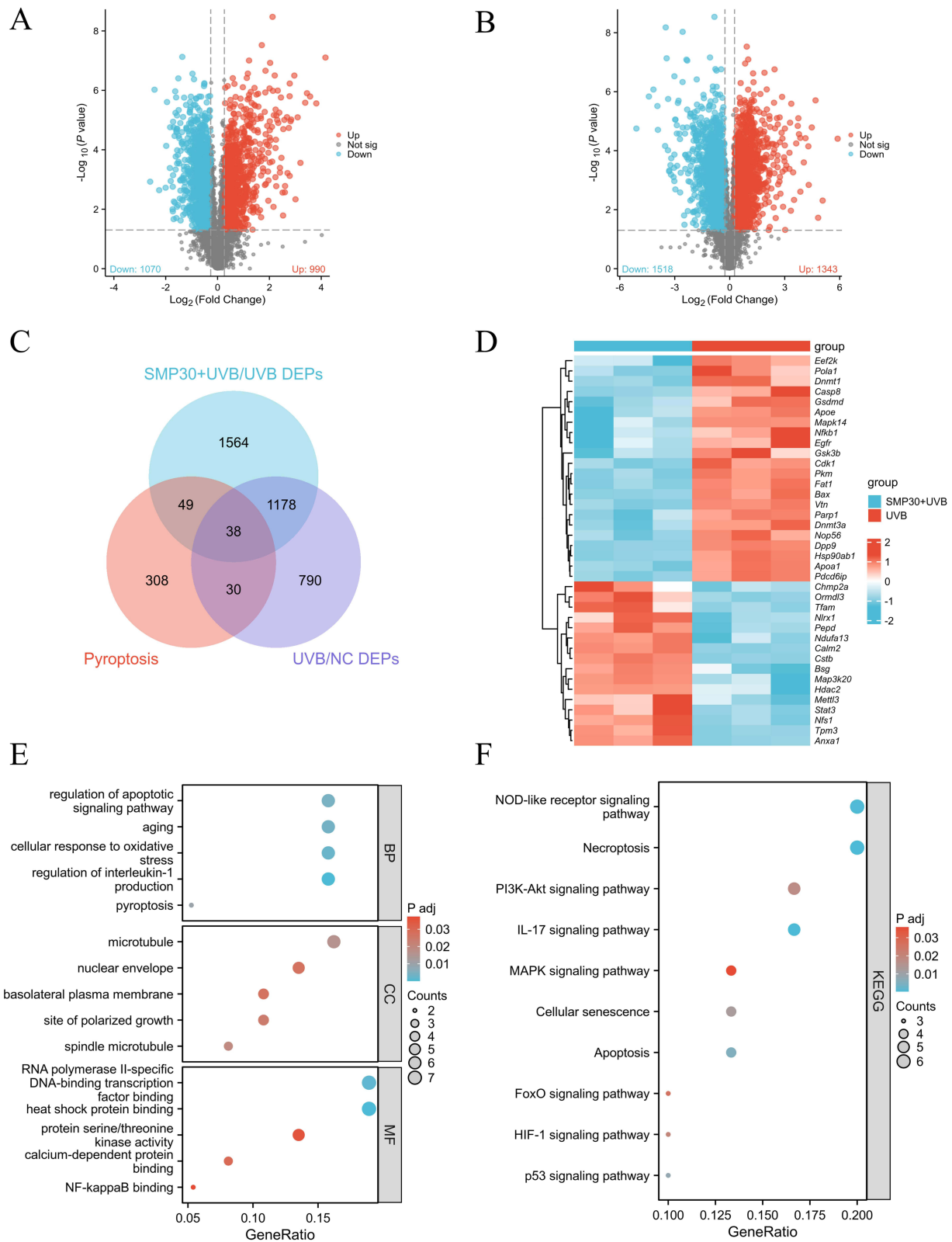


Figure 4 Analysis of differentially expressed proteins. **(A)** volcano plots illustrating the DEPs for the NC and UVB groups. **(B)** volcano plots illustrating the differences in DEPs between the UVB group and SMP30+UVB group. **(C)** Venn diagram depicting the intersection between DEPs and PRPs. **(D)** Heatmap of the expression levels of 38 PRDEPs in SMP30+UVB and UVB group rats. **(E)** Bubble chart illustrating GO enrichment analysis. **(F)** Bubble chart depicting KEGG enrichment analysis.

Abbreviations: DEPs, differentially expressed proteins; PRP, pyroptosis-related proteins; PRDEPs, Pyroptosis-related differentially expressed proteins; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

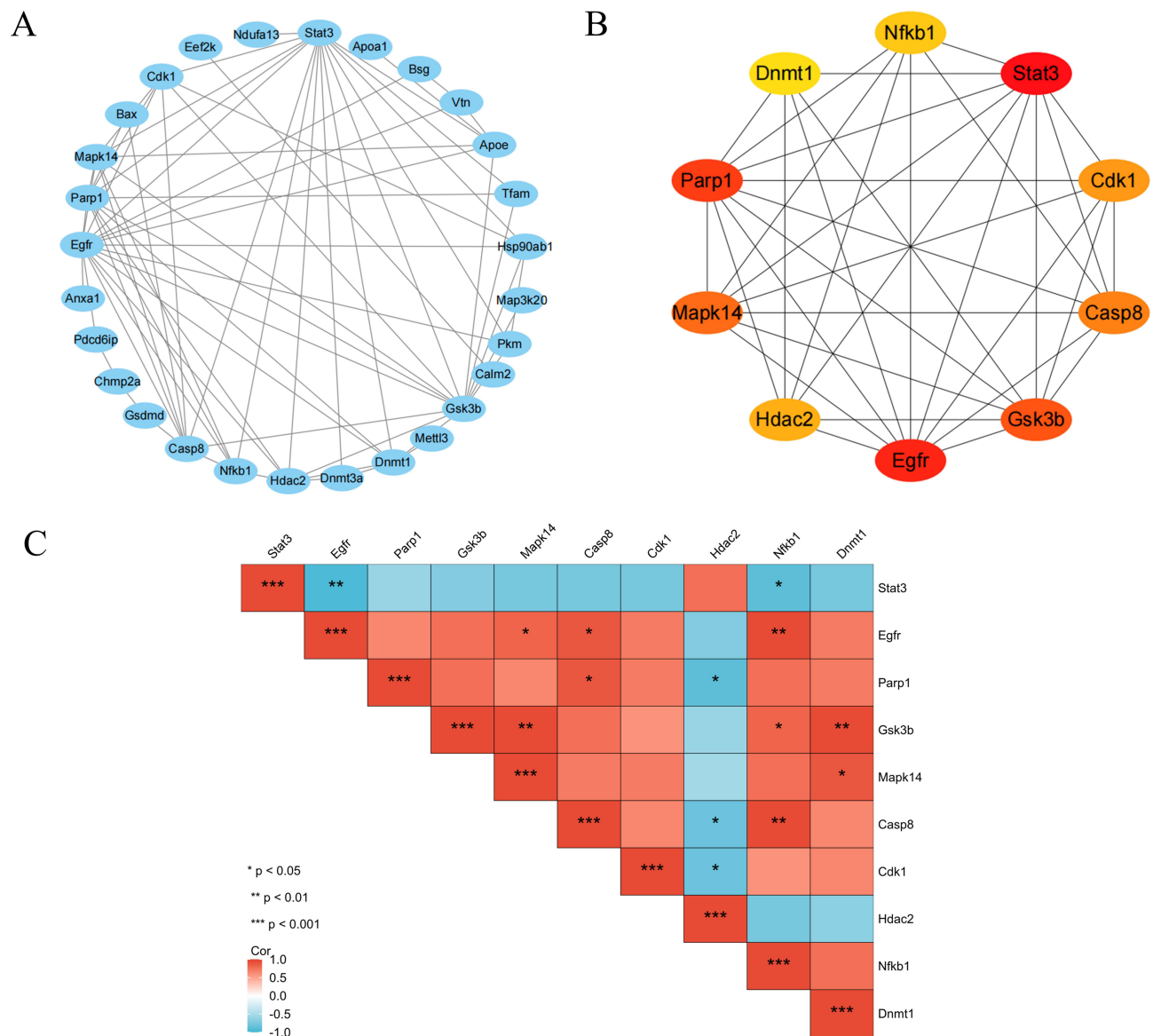


Figure 5 Screening of hub proteins. **(A)** The PPI network of 38 PRDEPs. **(B)** The PPI network of 10 hub proteins, the deeper the red, the higher the MCC score. **(C)** A heatmap illustrating the correlation analysis among the ten hub proteins, where positive and negative correlations are represented by the colors red and blue, respectively. **Abbreviations:** PPI, protein-protein interaction; PRDEPs, Pyroptosis-related differentially expressed proteins; MCC, maximum cluster centrality.

research teams in an effort to provide fresh approaches to the treatment of cataracts and associated conditions. In the study by Teng et al,³¹ they induced oxidative stress in human LECs using 4-hydroxynonenal (4-HNE). Their findings indicated that SMP30 overexpression significantly suppressed the accumulation of mitochondrial ROS and restored mitochondrial function. SMP30 protects LECs from damage caused by oxidative stress through the Nrf2/Keap1 signaling pathway. According to a different study, SMP30 protects retinal ganglion cells from apoptosis induced by high glucose, oxidative stress, and inflammatory damage by increasing Nrf2 expression via the Akt/GSK-3 β pathway.³² These studies indicate that SMP30 can inhibit the accumulation of ROS in mitochondria and exhibit anti-inflammatory effects. Oxidative stress is intricately linked to cell pyroptosis, where elevated ROS levels can trigger activation of the NLRP3/caspase-1 complex. This activation ultimately leads to cell pyroptosis by promoting the release of IL-1 β and IL-18.³³

Using SMP30 knockout mice, a recent study examined the impact of SMP30 on the NLRP3/Caspase-1 inflammatory pathway and oxidative stress damage following myocardial infarction.²¹ SMP30 is involved in the regulation of oxidative

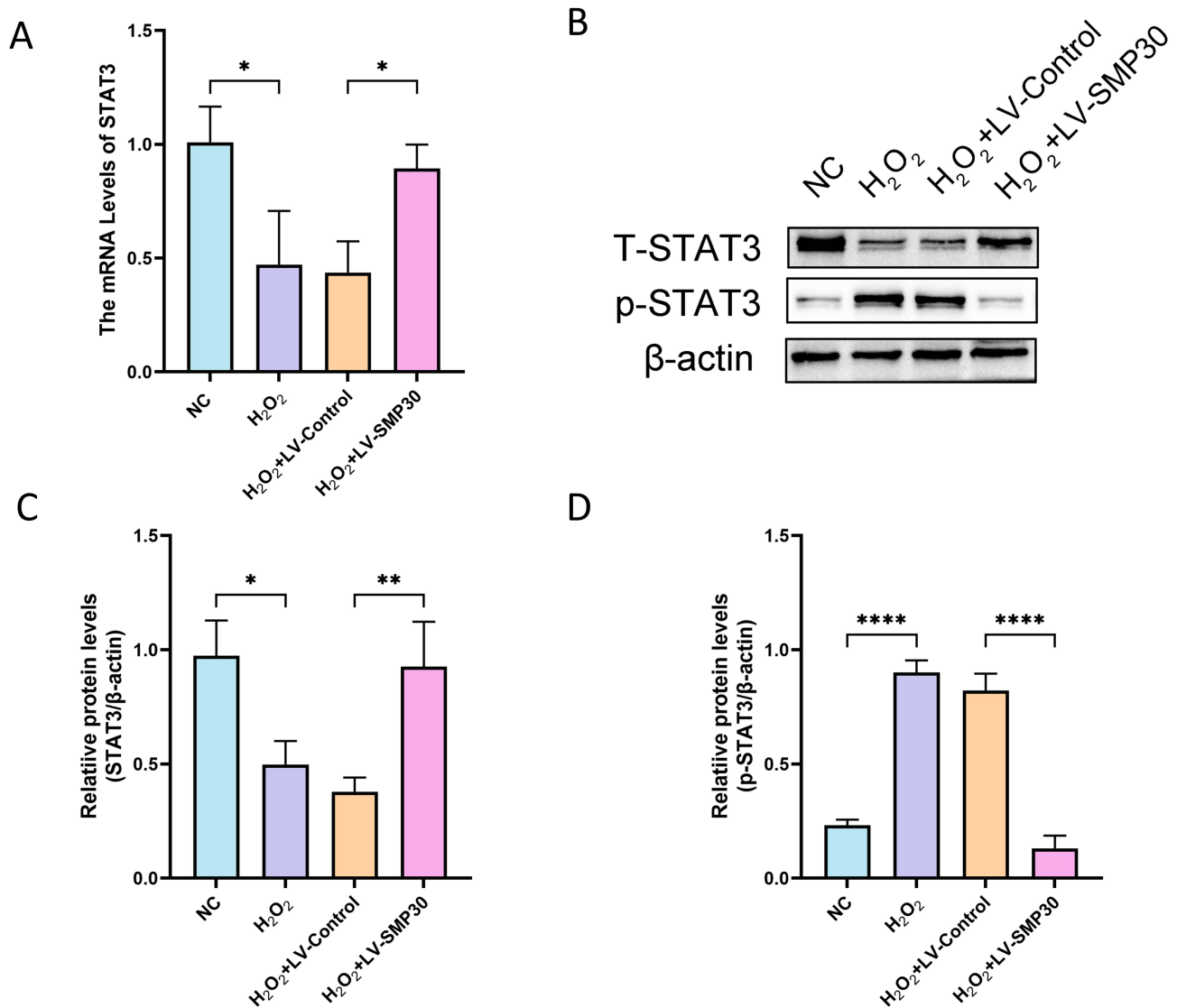


Figure 6 The effect of SMP30 overexpression on STAT3 in H₂O₂-induced SRA01/04 cells. **(A)** The effect of SMP30 overexpression on STAT3 mRNA in H₂O₂-induced SRA01/04 cells was determined via RT-qPCR. **(B–D)** The effect of overexpressing SMP30 on the levels of T-STAT3 and p-STAT3 proteins. n=3, *p < 0.05, **p < 0.01, ****p < 0.0001.

stress and pyroptosis-related proteins, as evidenced by the results, which showed that SMP30 knockout mice had more severe oxidative stress damage, increased MDA levels, significantly reduced SOD activity, downregulated antioxidant stress markers SOD2 and NQO1, upregulated pro-oxidant stress marker gp91^{phox}, and elevated pyroptosis-related markers Cleaved-caspase-1 and NLRP3.²¹ The relationship between SMP30 and the pyroptosis effector protein GSDMD was not investigated in this experiment. Recent studies have revealed that pyroptosis also contributes to the initiation and progression of cataracts.⁷ However, whether SMP30 exerts anti-pyroptotic effects in cataracts remains unclear. In this work, we used UVB to induce cataracts in rats, and we showed that these conditions were associated with lens epithelial cell pyroptosis, as well as decreased SMP30 mRNA and protein levels in the cataract capsular tissue. To further investigate the role of SMP30 in cataract cell pyroptosis, we overexpressed SMP30 in rat LECs by injecting an AAV2 virus overexpressing SMP30 into the vitreous cavity.²⁷ Using 4D-FastDIA proteomics to detect protein expression changes, we found that overexpression of SMP30 reduced the expression of GSDMD, indicating that SMP30 might have an anti-pyroptotic effect, though its specific mechanism is unclear. Consequently, we used GO, KEGG, and PPI to further analyze PRDEPs and discovered that STAT3 expression increased following SMP30 overexpression. To verify the role of

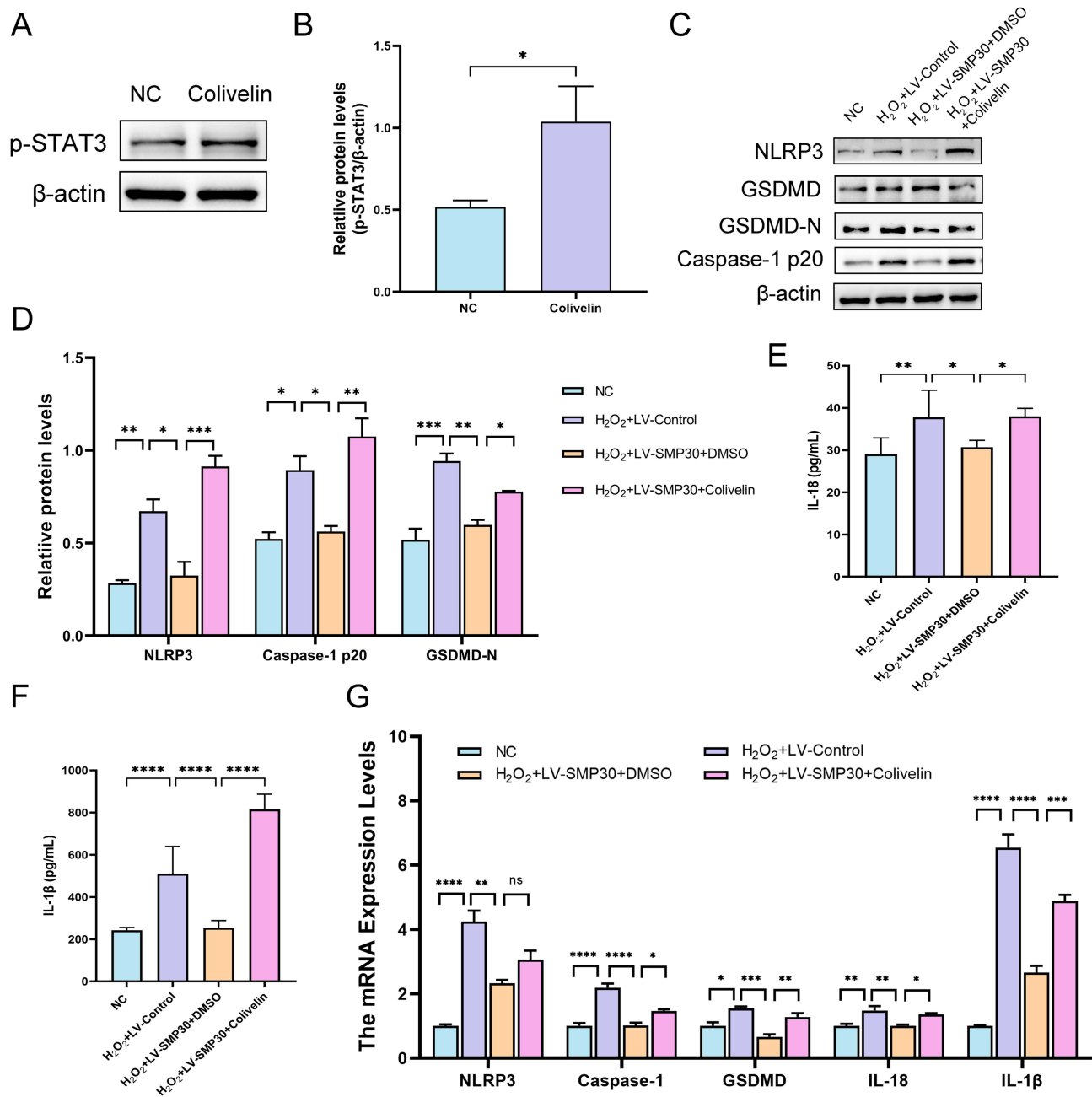


Figure 7 Colivelin attenuates the protective role of SMP30 in H₂O₂-induced pyroptosis in SRA01/04 cells. SRA01/04 cells transfected with SMP30 were co-cultured with or without the p-STAT3 agonist Colivelin with H₂O₂ for 24h. (A and B) The protein expression level of p-STAT3 was elevated following the addition of Colivelin. (C and D) The protein expression levels of NLRP3, GSDMD-N, and Caspase-1 p20 detected with Western Blot. The concentration of IL-18 (E) and IL-1β (F) in cell supernatant. (G) The mRNA levels of NLRP3, GSDMD, Caspase-1, IL-1β, and IL-18 were detected by RT-qPCR. n=3, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

SMP30 in pyroptosis and its correlation with STAT3, we constructed lentiviruses overexpressing SMP30 and transfected them into SRA01/04 cells to obtain stable cell lines overexpressing SMP30. The results showed that upon exposure to H₂O₂, SRA01/04 cells exhibited elevated expression levels of GSDMD, NLRP3, Caspase-1, IL-1β, and IL-18. Conversely, overexpression of SMP30 mitigated H₂O₂-induced pyroptosis in SRA01/04 cells, proving that SMP30 has an anti-pyroptotic effect. Furthermore, after overexpression of SMP30, the phosphorylation level of STAT3 decreased, suggesting that SMP30 alleviates cell pyroptosis possibly through downregulating the phosphorylation of STAT3.

The 1994 discovery of the signal transducer and activator of the transcription (STAT) family is crucial for the transduction of cytokine signals and interferon-mediated antiviral effects.³⁴ By facilitating signal transmission from the

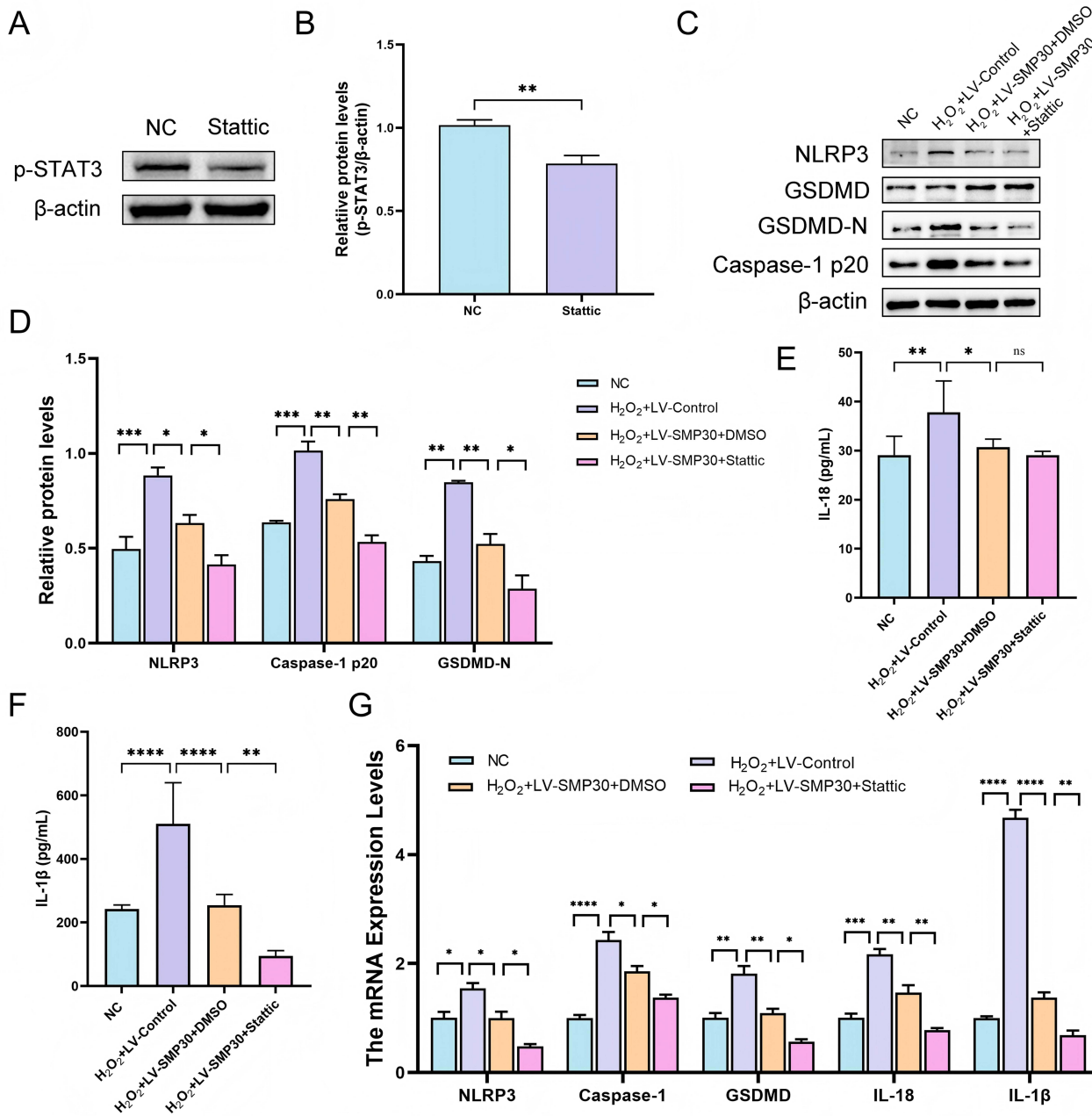


Figure 8 Stat3 enhances the protective role of SMP30 in H₂O₂-induced pyroptosis in SRA01/04 cells. SRA01/04 cells transfected with SMP30 were co-cultured with or without the p-STAT3 inhibitor Stat3 with H₂O₂ for 24h. (A and B) The protein expression level of p-STAT3 was elevated following the addition of Stat3. (C and D) The protein expression levels of NLRP3, GSDMD-N, and Caspase-1 p20 detected with Western Blot. The concentration of IL-18 (E) and IL-1β (F) in cell supernatant. (G) The mRNA levels of NLRP3, GSDMD, Caspase-1, IL-1β, and IL-18 were detected by RT-qPCR. n=3, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

cell membrane to the nucleus, these proteins help trigger the transcription of genes downstream. Seven STAT genes (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) are found in the human genome. They are essential for immune system regulation, angiogenesis, tumorigenesis, apoptosis, and cell proliferation.³⁵ STAT3 is the most extensively researched of the genes. The STAT3 signaling pathway has become a therapeutic target for a variety of cancer types because STAT3 is essential in regulating antitumor immune responses.³⁶ Additionally, STAT3 is also associated with pyroptosis, autophagy, and ferroptosis.^{37–39} Several investigations have revealed how crucial the STAT3 signaling pathway is to pyroptosis.^{40–43} However, there are differing views on whether STAT3 promotes or inhibits pyroptosis. In a study involving breast cancer mice treated with polydatin, it was found that polydatin could increase the levels of

pyroptosis-related genes by inhibiting p-JAK2 and p-STAT3. This suggests that the JAK2/STAT3 pathway is related to pyroptosis, and the reduction of p-STAT3 increased pyroptosis.⁴² However, another study found that p-STAT3 could promote the transcription of NLRP3, thereby mediating pyroptosis through caspase-1.⁴¹ Furthermore, p-STAT3 interacted with programmed death-ligand 1 (PD-L1) during TNF α -induced apoptosis, facilitating PD-L1 nuclear translocation and thereby enhancing GSDMC gene transcription. This mechanism leads to caspase-8 specific cleavage of GSDMC, producing its N-terminal domain, which creates holes in the cell membrane and ultimately initiates pyroptosis.⁴³ The relationship between SMP30 and STAT3 in cataracts has not yet been the subject of any research. This study demonstrated that STAT3 inhibition (using Stattic) reduced p-STAT3 expression, which consequently attenuated pyroptosis and enhanced SMP30's anti-pyroptotic effects. Conversely, STAT3 activation (via Colivelin) increased p-STAT3 levels and compromised SMP30's protective function. These findings collectively establish that SMP30 alleviates H₂O₂-induced LECs pyroptosis through downregulation of STAT3 phosphorylation.

Conclusions

In summary, this preclinical study (rat models and LECs) demonstrates that SMP30 attenuates cataract progression by regulating p-STAT3 to suppress pyroptosis. However, it must be emphasized that these conclusions are based on preclinical models, and their translational potential requires further validation through human sample studies.

Abbreviations

BH, Benjamini-Hochberg; CCK-8, Cell Counting Kit-8; DEPs, differentially expressed proteins; FC, fold change; Go, Golgi apparatus; GO, Gene ontology; 4-HNE, 4-hydroxynonenal; IC₅₀, inhibition rate; KEGG, Kyoto encyclopedia of genes and genomes; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LECs, lens epithelial cells; LPS, lipopolysaccharides; M, mitochondria; MCC, maximal clique centrality; N, nucleus; NC, normal control; PCD, programmed cell death; p-STAT3, phosphorylated STAT3; PRPs, pyroptosis-related proteins; PPI, protein-protein interaction; PRDEPs, pyroptosis-related differentially expressed proteins; RER, rough endoplasmic reticulum; ROS, reactive oxygen species; RT-qPCR, Real-time quantitative PCR; STAT, signal transducer and activator of transcription; SMP30, senescence marker protein 30; T-STAT3, total STAT3; UVB, ultraviolet B.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The Guangxi Medical University Animal Research Committee gave its approval to all animal studies (Approval number, 202107443).

Consent for Publication

All authors have agreed to publish this manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by grants from the National Natural Science Foundation of China (grant number 81960174 and 81360146) and Self-funded project of Western medicine of Health Commission of Guangxi Zhuang Autonomous Region (Z-A20240430).

Disclosure

The authors declare no conflict of interests.

References

1. L L, L Y, J G, et al. Global, regional, and national burden of cataract, a comprehensive analysis and projections from 1990 to 2021. *PLoS One*. 2025;20(6). doi:10.1371/journal.pone.0326263
2. Asbell PA, Dualan I, Mindel J, Brocks D, Ahmad M, Epstein S. Age-related cataract. *Lancet*. 2005;365(9459):599–609. doi:10.1016/S0140-6736(05)17911-2
3. Lee CM, Afshari NA. The global state of cataract blindness. *Curr Opin Ophthalmol*. 2017;28(1):98–103. doi:10.1097/ICU.0000000000000340
4. Long AC, Colitz CMH, Bomser JA. Apoptotic and necrotic mechanisms of stress-induced human lens epithelial cell death. *Exp Biol Med*. 2004;229(10):1072–1080. doi:10.1177/153537020422901012
5. Ma D-Y, Liu J-X, Wang L-D. GSK-3 β -dependent Nrf2 antioxidant response modulates ferroptosis of lens epithelial cells in age-related cataract. *Free Radic Biol Med*. 2023;204:161–176. doi:10.1016/j.freeradbiomed.2023.04.022
6. Huang J, Yu W, He Q, et al. Autophagy facilitates age-related cell apoptosis—a new insight from senile cataract. *Cell Death Dis*. 2022;13(1):37. doi:10.1038/s41419-021-04489-8
7. Jin X, Jin H, Shi Y, Guo Y, Zhang H. Pyroptosis, a novel mechanism implicated in cataracts. *Mol Med Rep*. 2018;18(2):2277–2285. doi:10.3892/mmr.2018.9188
8. Zhang Y, Jiao Y, Li X, et al. Pyroptosis, a new insight into eye disease therapy. *Front Pharmacol*. 2021;12:797110. doi:10.3389/fphar.2021.797110
9. Gong W, Shi Y, Ren J. Research progresses of molecular mechanism of pyroptosis and its related diseases. *Immunobiology*. 2020;225(2):151884. doi:10.1016/j.imbio.2019.11.019
10. Ding J, Wang K, Liu W, et al. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature*. 2016;535(7610):111–116. doi:10.1038/nature18590
11. Yu P, Zhang X, Liu N, Tang L, Peng C, Chen X. Pyroptosis, mechanisms and diseases. *Signal Transduct Target Ther*. 2021;6(1):128. doi:10.1038/s41392-021-00507-5
12. Zheng D, Liu J, Piao H, Zhu Z, Wei R, Liu K. ROS-triggered endothelial cell death mechanisms, focus on pyroptosis, parthanatos, and ferroptosis. *Front Immunol*. 2022;13:1039241. doi:10.3389/fimmu.2022.1039241
13. Yamaguchi M, Yamamoto T. Purification of calcium binding substance from soluble fraction of normal rat liver. *Chem Pharm Bull*. 1978;26(6):1915–1918. doi:10.1248/cpb.26.1915
14. Lai W, Tan S, Li X, et al. Expression of senescence marker protein 30 in lens epithelial cells of cataract patients of different ages. *Chin J Experiment Ophthalmol*. 2014;32(06):521–524.
15. Senmaru T, Yamazaki M, Okada H, et al. Pancreatic insulin release in vitamin C-deficient senescence marker protein-30/gluconolactonase knockout mice. *J Clin Biochem Nutr*. 2012;50(2):114–118. doi:10.3164/jcbs.11-52
16. Yamaguchi M, Murata T. Involvement of regucalcin in lipid metabolism and diabetes. *Metabolism*. 2013;62(8):1045–1051. doi:10.1016/j.metabol.2013.01.023
17. Han Z, Li S, Li Y, et al. The anti-oxidant of senescence marker protein 30 on human lens epithelial in the early satage of acute oxidative stress. *New Adv Ophthalmol*. 2019;39(02):109–112.
18. Li S, Chen X, Han Z, et al. Effect of overexpression of senescence marker protein 30 on oxidative stress induced by high calcium in human lens epithelial cell line SRA01 / 04. *J Sun Yat-Sen Univ*. 2019;40(01):54–61.
19. Li S, Han Z, Aint TTW, et al. The effect of low expression of senescence marker protein 30 on the proliferation and oxidation of human lens epithelial cells under high calcium condition. *Int J Ophthalmol*. 2020;20(02):217–223.
20. Ishikawa Y, Hashizume K, Kishimoto S, et al. Effect of vitamin C depletion on UVR-B induced cataract in SMP30/GNL knockout mice. *Exp. Eye Res*. 2012;94(1):85–89. doi:10.1016/j.exer.2011.11.010
21. Jiang L, Zhang B, Zhu H, et al. Effect of senescence marker protein 30 on myocardial injury after myocardial infarction in mice through NLRP3 / Caspase1 inflammatory signaling pathway. *J Air Force Med Univ*. 2023;44(8):706–711. doi:10.13276/j.issn.2097-1656.2023.08.004
22. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. doi:10.1093/nar/gkv007
23. Rebhan M, Chalifa-Caspi V, Prilusky J, Lancet D. GeneCards, integrating information about genes, proteins and diseases. *Trends Genet*. 1997;13(4):163. doi:10.1016/S0168-9525(97)01103-7
24. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*. 2016;32(18):2847–2849. doi:10.1093/bioinformatics/btw313
25. Yu G, Wang LG, Han Y, He QY. clusterProfiler, an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284–287. doi:10.1089/omi.2011.0118
26. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11, protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607–D613. doi:10.1093/nar/gky1131
27. Liang Y, Lan T, Gan Q, Liang H. Successful transduction of target gene mediated by adeno-associated virus 2 into lens epithelial cells in rats. *J Virol Meth*. 2023;321:114792. doi:10.1016/j.jviromet.2023.114792
28. Chen X, Li SM, Li YW, Han ZH, Liang H. Effect of senescence marker protein 30 on the proliferation and apoptosis of human lens epithelial cells SRA01/04. *Int J Ophthalmol*. 2018;11(4):553–558. doi:10.18240/ijo.2018.04.03
29. Li S, Chen X, Lai W, et al. Downregulation of SMP30 in senescent human lens epithelial cells. *Mol Med Rep*. 2017;16(4):4022–4028. doi:10.3892/mmr.2017.7106
30. Li S, Wang J, Mahamane DD, et al. Expression and role of senescence marker protein 30 in lens epithelial cells of rats at different ages. *J Guangxi Med Univ*. 2021;38(04):689–693.
31. Teng H, Hong Y, Cao J, et al. Senescence marker protein30 protects lens epithelial cells against oxidative damage by restoring mitochondrial function. *Bioengineered*. 2022;13(5):12955–12971. doi:10.1080/21655979.2022.2079270

32. Zhang L, Zhu T, He F, Li X. Senescence marker protein 30 (SMP30) protects against high glucose-induced apoptosis, oxidative stress and inflammatory response in retinal ganglion cells by enhancing Nrf2 activation via regulation of Akt/GSK-3 β pathway. *Int Immunopharmacol.* **2021**;101(Pt B):108238. doi:10.1016/j.intimp.2021.108238
33. Alfonso-Loeches S, Ureña-Peralta JR, Morillo-Bargues MJ, Oliver-De La Cruz J, Guerri C. Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells. *Front Cell Neurosci.* **2014**;8:216. doi:10.3389/fncel.2014.00216
34. Wegenka UM, Lütticken C, Buschmann J, et al. The interleukin-6-activated acute-phase response factor is antigenically and functionally related to members of the signal transducer and activator of transcription (STAT) family. *Mol Cell Biol.* **1994**;14(5):3186–3196. doi:10.1128/mcb.14.5.3186-3196.1994
35. Verhoeven Y, Tilborghs S, Jacobs J, et al. The potential and controversy of targeting STAT family members in cancer. *Semin Cancer Biol.* **2020**;60:41–56. doi:10.1016/j.semcancer.2019.10.002
36. Zou S, Tong Q, Liu B, Huang W, Tian Y, Fu X. Targeting STAT3 in Cancer Immunotherapy. *Mol Cancer.* **2020**;19(1):145. doi:10.1186/s12943-020-01258-7
37. Y L, W Z, L H, et al. The role of STAT3 in autophagy. *Autophagy.* **2015**;11(5). doi:10.1080/15548627.2015.1017192
38. Zhang W, Gong M, Zhang W, et al. Thiostrepton induces ferroptosis in pancreatic cancer cells through STAT3/GPX4 signalling. *Cell Death Dis.* **2022**;13(7):630. doi:10.1038/s41419-022-05082-3
39. Yi S, Yang Y. Melatonin attenuates low shear stress-induced pyroptosis and endothelial cell dysfunction via the ROR α /miR-223/STAT-3 signalling pathway. *Exp Ther Med.* **2021**;22(6):1392. doi:10.3892/etm.2021.10828
40. Feng Y, Li W, Wang Z, et al. The p-STAT3/ANXA2 axis promotes caspase-1-mediated hepatocyte pyroptosis in non-alcoholic steatohepatitis. *J Transl Med.* **2022**;20(1):497. doi:10.1186/s12967-022-03692-1
41. Jiang Q, Tang G, Zhong XM, Ding DR, Wang H, Li JN. Role of Stat3 in NLRP3/caspase-1-mediated hippocampal neuronal pyroptosis in epileptic mice. *Synapse.* **2021**;75(12):e22221. doi:10.1002/syn.22221
42. Liu M, Li Y, Kong B, Zhang G, Zhang Q. Polydatin down-regulates the phosphorylation level of STAT3 and induces pyroptosis in triple-negative breast cancer mice with a high-fat diet. *Ann Transl Med.* **2022**;10(4):173. doi:10.21037/atm-22-73
43. Hou J, Zhao R, Xia W, et al. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. *Nat Cell Biol.* **2020**;22(10):1264–1275. doi:10.1038/s41556-020-0575-z

Journal of Inflammation Research

Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/journal-of-inflammation-research-journal>

Dovepress
Taylor & Francis Group