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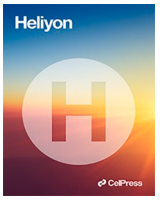

Research article

NS8593 inhibits sodium nitroprusside-induced chondrocyte apoptosis by mediating the STING signaling pathway

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ABSTRACT

Articular cartilage damage and chondrocyte apoptosis are among the distinguishing features of osteoarthritis. (R)-N-(benzimidazol-2-yl)-1,2,3,4-tetrahydro-1-naphtylamine (NS8593) is a transient receptor potential cation channel subfamily M member 7 (TRPM7) channel inhibitor and was initially considered a potent inhibitor of small-conductance Ca^{2+}-activated K^{+} channels(SK1-3 or KCa2.1–2.3 channels). Since SK is one of the targets for atrial fibrillation therapy, several studies have been conducted using NS8593 and it has been shown to be effective in improving atrial fibrillation in rats, dogs and horses. Recently, inhibition of TRPM7 has been reported to alleviate articular cartilage destruction. However, the role and mechanism of NS8593 on articular chondrocyte damage is unknown. The purpose of this study was to investigate the effect and mechanism of NS8593 on sodium nitroprusside (SNP)-induced chondrocyte apoptosis *in vitro*. The results showed that SNP decreased cell viability and induced chondrocyte apoptosis. NS8593 dose-dependently inhibited the SNP-induced decrease in cell viability and reduced chondrocyte apoptosis. In addition, SNP stimulation significantly increased the phosphorylation level of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING), and NS8593 treatment partially reversed the alteration of STING phosphorylation level. Treatment with the STING inhibitor H-151 inhibited SNP-induced chondrocyte apoptosis. These results suggest that NS8593 may inhibit SNP-induced chondrocyte apoptosis by suppressing the STING signaling pathway.

1. Introduction

Osteoarthritis is a common, non-inflammatory, degenerative joint disease that manifests as stiffness and pain in the early stages, which worsens after prolonged activity, and as localized swelling in the later stages, leading to joint deformity in severe cases. Osteoarthritis is more common in people over 50 years of age, with a higher prevalence in women than in men, and affects the quality of life of patients to varying degrees [1]. In addition, osteoarthritis is more likely to occur in joints that are frequently used and heavily

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loaded, and the main pathological changes are degeneration and loss of cartilage, as well as the formation of osteophytes. The pathogenesis of osteoarthritis is the combined result of multiple factors such as subchondral bone sclerosis, synovial inflammation, osteophyte formation and cartilage erosion, of which the most significant is the impact of articular cartilage degeneration [2]. Articular cartilage is mainly divided into three zones, the superficial zone, the intermediate zone, and the deep zone, and its main function is to provide a smooth and lubricating surface for low friction joints while transmitting heavy loads to the underlying subchondral bone [3]. Chondrocytes are the only cell type in articular cartilage and are responsible for maintaining cartilage homeostasis by synthesizing and secreting extracellular matrix, which is essential for preventing and delaying osteoarthritis [4]. It has also been suggested that excessive chondrocyte death is one of the main causes of osteoarthritis pathogenesis [5]. It is well known that chondrocyte apoptosis is involved in the pathogenesis of osteoarthritis and that inhibition of chondrocyte apoptosis significantly delays osteoarthritis progression. Therefore, it is important to develop new effective therapeutic medicines or targets to prevent the apoptosis of articular chondrocytes.

NS8593 (Fig. 1a) was identified as a potent inhibitor of small-conductance Ca^{2+}-activated K^{+} channels (SK1-3 or KCa2.1–2.3 channels), suppressing SK current with an IC_{50} of ≈5 μmol/L, without affecting Na^{+}, Ca^{2+}, or other K^{+} currents [6]. NS8593 exerts its inhibitory effect by decreasing the Ca^{2+} sensitivity of SK channels, rather than directly blocking their pore. In an *in vivo* rat model of paroxysmal atrial fibrillation with hypertension-induced atrial remodeling, inhibition of SK channels by NS8593 exhibited antiarrhythmic therapeutic effects [7]. It has also been reported that inhibition of SK channels by use of NS8593 protects against atrial fibrillation [8]. In addition, NS8593 is a transient receptor potential cation channel subfamily M member 7 (TRPM7) channel inhibitor. Chubanov et al. found that unlike other TRPM7 inhibitors, NS8593 targets and inhibits TRPM7 channels in a magnesium-dependent manner and does not affect other TRP channels [9]. TRPM7 expression is increased in the kidneys of unilateral ureteral obstruction (UUO) mice, and treatment with NS8593 attenuates renal atrophy and inhibits cell proliferation of tubular epithelial and mesangial cells in the kidneys of UUO mice, and reduces TRPM7 expression to normal levels [10]. Of interest, it has been reported that knockdown of TRPM7 or use of the TRPM7 inhibitor NS8593 increases tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in triple-negative breast cancer (TNBC) cells [11]. However, the role of NS8593 in osteoarthritis chondrocyte apoptosis is still unclear.

The experimental team of Barber et al. discovered the stimulator of interferon genes (STING, also known as STING1, TMEM173, MITA or MPYS), key proteins that regulate innate immunity, and established that sting localizes to the endoplasmic reticulum membrane and activates the nuclear factor kappa-B (NF-κB) and interferon regulatory factor 3 (IRF3) downstream signaling pathway, which plays a key role in antiviral immunity [12]. In 2014, two publications first reported that activation of the STING pathway in caspase-deficient cells promoted apoptosis and finally participated in the inflammatory response [13,14]. Recent studies have shown that STING not only regulates cell death but also plays a role in recognizing and amplifying immune responses induced by dying cells. Caspase12 is the caspase responsible for initiating endoplasmic reticulum stress-induced apoptosis. Based on multiple studies, it has been found that the mechanism of STING-mediated apoptosis is mainly related to endoplasmic reticulum stress, although it remains unclear whether STING regulates the activation of caspase12 [15]. In addition to endoplasmic reticulum stress, STING-mediated apoptosis may require activation of the IRF3 pathway under certain circumstances. Mechanistically, STING-mediated IRF3 phosphorylation triggers the formation of IRF3-BAX complexes, leading to BAX activation, cytochrome *c* release, and apoptosis [16].

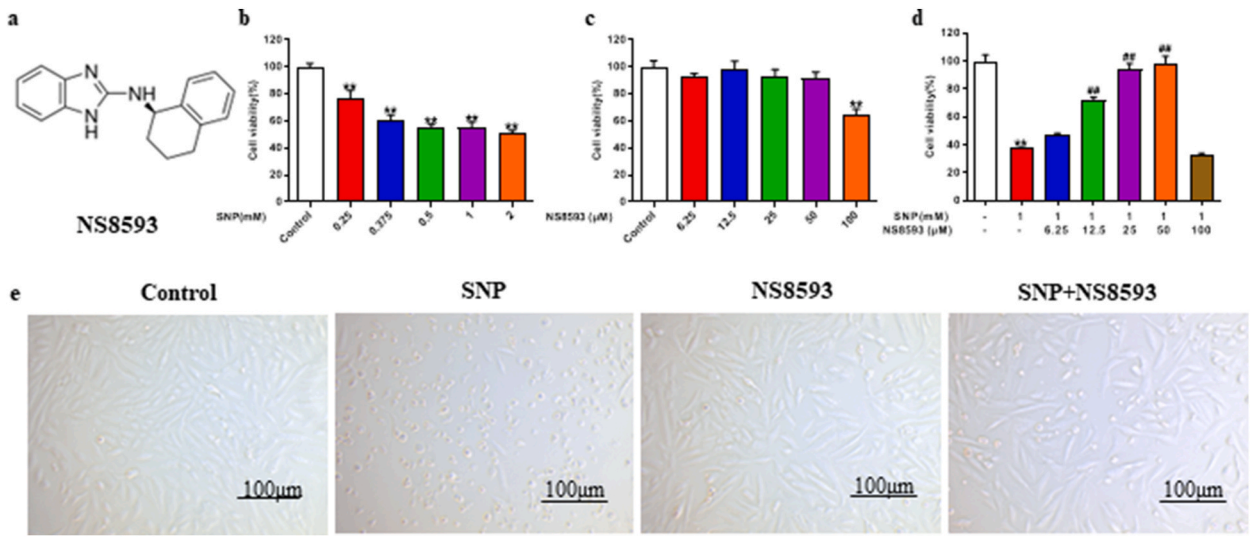

Fig. 1. NS8593 weakened the effect of SNP on C28/I2 cell viability and cell morphology. (a) Chemical structure of NS8593. (b) MTT experiment was used to determine the cell viability of C28/I2 cells treated with different concentrations of SNP for 24 h. (c) Effect of NS8593 on C28/I2 cell viability. (d) NS8593 alleviated the toxic effect of SNP on C28/I2 cells. (e) C28/I2 cell morphology under 100 × magnification microscope after the interaction of NS8593 and SNP. The results are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ SNP versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ SNP + NS8593 versus SNP.

Recent studies have shown that STING activation promotes apoptosis in T lymphocytes, myeloid cells and hepatocytes [17]. However, the role of STING in chondrocyte apoptosis is unclear.

Consequently, the purpose of this study was to investigate the protective effect of NS8593 on articular chondrocytes and its possible mechanism, and to further observe the effect of the STING signaling pathway in the protective effect of NS8593 on chondrocytes. The effects of NS8593 on sodium nitroprusside (SNP)-induced chondrocyte apoptosis and the STING signaling pathway as well as the effects of STING inhibition on SNP-induced chondrocyte apoptosis were observed by biochemical/molecular biological analysis, flow cytometry and cell damage assay. Our study demonstrates that NS8593 attenuates SNP-induced chondrocyte apoptosis by inhibiting the activation of the STING signaling pathway.

2. Materials and methods

2.1. Cell culture

Human chondrocyte C28/I2 cells (Millipore, USA) were cultured in Dulbecco's modified eagle medium (DMEM, Hyclone, Logan, UT, USA) containing 10 % fetal bovine serum (FBS, Wisent Inc., St Bruno, Quebec, Canada) and 1 % penicillin-streptomycin (Beyotime Institute of Biotechnology, Jiangsu, China) in a sterile humidified atmosphere containing 5 % CO_2 at 37 °C. The culture medium was changed every 2–3 days. Toluidine blue and type II collagen staining were used to identify chondrocytes. When they reached 80 % confluence in the culture flask, the cells were trypsinized and passaged. To induce apoptosis-like biological changes, the human C28/I2 cells were stimulated with SNP for 24 h as previously described [18].

2.2. MTT assay

Cell viability was evaluated by the MTT assay. Human chondrocyte C28/I2 cells were seeded at a density of 5×10^3 cells/well into 96-well plates with DMEM containing 10 % FBS, cultured for 24 h, and then treated with SNP (0.25–2 mM) for 24 h, or with NS8593 (6.25–100 μM) for 24 h. C28/I2 cells were subjected to combined treatment with SNP (1 mM) and NS8593 (6.25–100 μM) for 24 h. C28/I2 cells were treated with the STING inhibitor H-151 (0.125–2 μM) for 24 h, or combined treatment with SNP (1 mM) and H-151 (0.125–2 μM) for 24 h. Then 10 μL of 5 mg/mL MTT (Beyotime) dissolved in DMEM was added to each well of a 96-well plate and the cells were placed in an incubator at 37 °C for 4 h. Subsequently, 150 μL DMSO was added to dissolve formazan crystals. After a 10 min incubation period, absorbance values of living cells were read at 490 nm. Cell viability was expressed as a percentage relative to the control group.

2.3. Mitochondrial membrane potential analysis

Mitochondrial membrane potential ($\Delta\Psi m$) was detected by JC-1 or Rhodamine123 (Rh123) staining. Human chondrocyte C28/I2 cells were seeded at a density of 5×10^5 cells/well in 6-well plates, cultured for 24 h, and then treated with SNP (1 mM) and NS8593 (25 μM) for 12 h. Next, the C28/I2 cells were washed twice in PBS, JC-1 (Beyotime) (1:200) was directly added to the culture plates, reacted in the dark for 30 min, and washed twice in PBS, then examined under a fluorescence microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). Following treatment, the C28/I2 cells were washed twice in PBS, treated with Rh123 (Beyotime) (1:1000), reacted in the dark for 30 min, and observed under a fluorescence microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) after washing twice in PBS.

2.4. Flow cytometry assay

An Annexin V-FITC apoptosis detection kit (BestBio, China) was used to assess apoptosis rates. Cells were seeded into 6-well plates at a density of 2×10^6 cells/well and cultured for 24 h, then treated with SNP (1 mM) and NS8593 (25 μM) for 12 h. Collected cells were washed twice in PBS and re-suspended in binding buffer. Annexin V-FITC (2.5 μL) was added to samples and incubated for 15 min in the dark, followed by addition of 6 μL PI for 5 min in the dark. Apoptosis was measured using a flow cytometer (Beckman Coulter, Brea, CA, USA). According to the apoptosis detection kit instructions, Annexin V-FITC labels early apoptotic cells, while late apoptotic cells are labeled by both Annexin V-FITC and PI. Correspondingly, the upper right and lower right corners of the apoptosis flow result plots indicate early apoptotic cells and late apoptotic cells, respectively.

2.5. Measurement of intracellular reactive oxygen species (ROS)

To analyze intracellular ROS, cells were seeded at a density of 2×10^6 cells/well into 6-well plates and cultured for 24 h, then treated with SNP (1 mM) and NS8593 (25 μM) for 12 h. Intracellular ROS relative expression was assessed via flow cytometry. FBS-free DMEM was used for dilution of DCFH-DA (1:1000). The C28/I2 cells were digested with trypsin, and the cell suspension was centrifuged at 1500 rpm for 5 min at 4 °C. Then the collected cells were suspended in diluted DCFH-DA and placed in a cell incubator at 37 °C for 20 min. The cell suspension was collected by centrifugation at 4 °C and 1500 rpm for 5 min, and then washed three times in FBS-free DMEM. The labeled cells were analyzed on a flow cytometer (Beckman Coulter, Brea, CA, USA). Data were processed using FlowJo software.

2.6. TUNEL assay

TdT-mediated dUTP Nick-End Labeling (TUNEL) assays of human chondrocyte C28/I2 cells were performed using the One Step TUNEL Apoptosis Assay Kit (Beyotime), following the manufacturer's instructions. Briefly, cells were washed three times with PBS, then treated with the TUNEL reaction mixture in the dark at 37 °C for 30 min. The cells were again washed three times with PBS, and images were captured under a fluorescence microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

2.7. Western blot

Treated C28/I2 chondrocytes were washed twice in PBS and lysed on ice with RIPA buffer (Beyotime) with 1 % proteinase inhibitors. The homogenate was centrifuged at 13200 rpm for 30 min to remove insoluble materials, followed by the addition of SDS

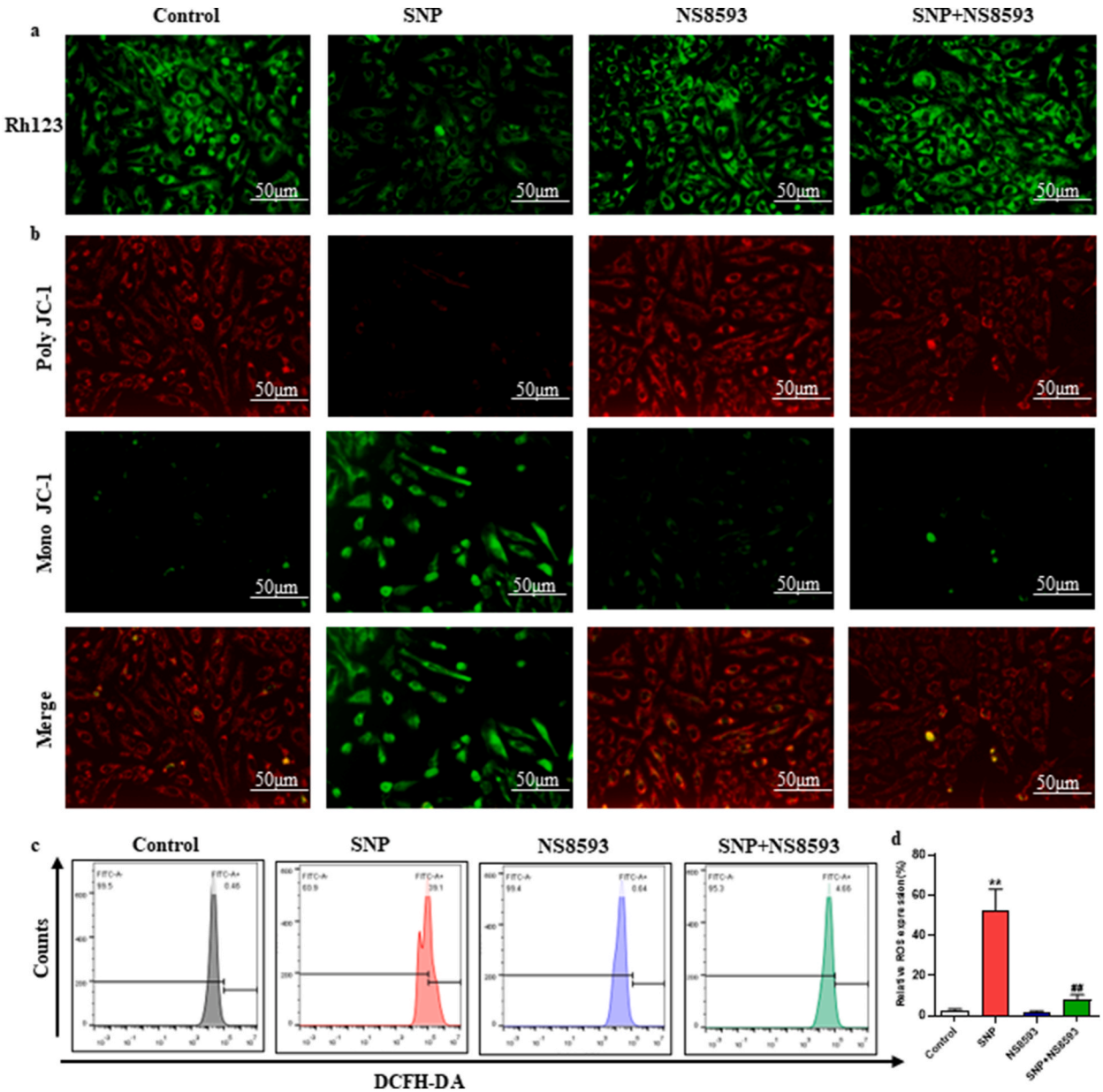

Fig. 2. NS8593 affected the level of oxidative stress and mitochondrial membrane potential induced by SNP in C28/I2 cells. (The following fluorescence pictures were taken under an inverted fluorescence microscope with 200 × magnification). (a–b) ΔΨm was detected by Rh123 and JC-1 staining in each group. (c–d) A scatter diagram of the DCFH-DA result showed the level of ROS in C28/I2 cells through flow cytometry. The results are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ SNP versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ SNP + NS8593 versus SNP.

loading buffer. The total protein concentration was determined by the BCA protein quantification method (Beyotime). An equal amount of sample was loaded onto a 10 % SDS gel and transferred to a polyvinylidene difluoride (PVDF) membrane via electrophoresis. After blocking with 5 % non-fat milk, membranes were incubated with specific anti-β-actin (Abcam, Cambridge, MA, USA; 1:1000), anti-PARP (Cell Signaling Technology, Danvers, MA, USA; 1:1000), anti-Bcl-2 (Cell Signaling Technology; 1:1000), or anti-Phospho-STING (Ser366) (Thermo Fisher Scientific, Waltham, MA, USA; 1:500) overnight at 4 °C. Membranes were subsequently washed with Tris-buffered saline (TBS) containing 0.24 % Tween-20 and incubated with horseradish peroxidase-conjugated secondary antibody for 60 min. The protein bands were visualized using an ECL solution (Affinity Biosciences Ltd., Cincinnati, OH, USA) and

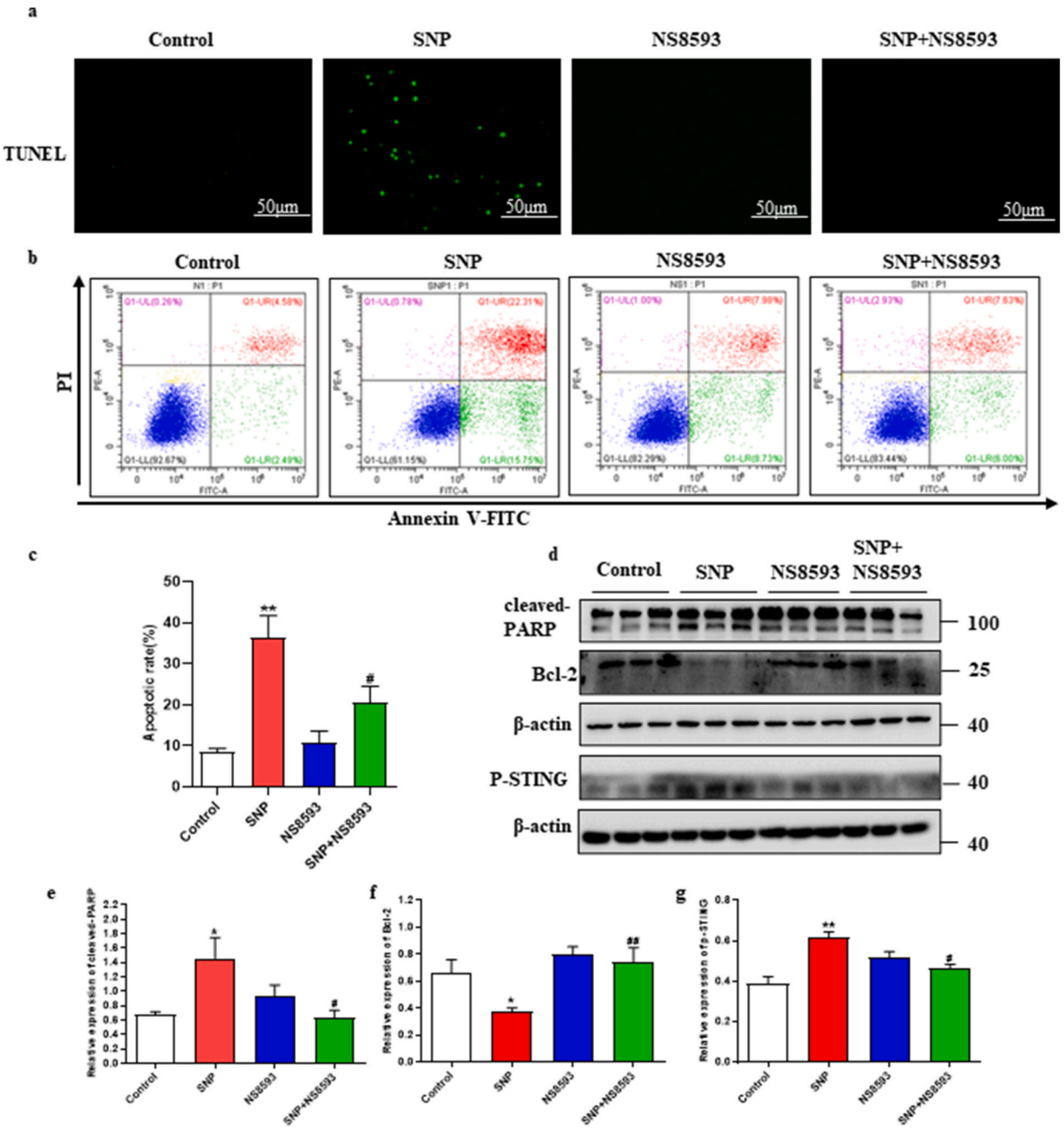

Fig. 3. NS8593 protects C28/I2 cells from sodium nitroprusside-induced apoptosis by mediating STING signaling pathway. (The following fluorescence pictures were taken under an inverted fluorescence microscope with 200 ×magnification.) (a) The apoptosis of C28/I2 cells were detected by TUNEL staining. (b–c) The apoptosis rate of C28/I2 cells was detected by Annexin V-FITC/PI apoptosis detection kit. The apoptosis rate was expressed by percentage. (d–f) The levels of apoptosis related proteins (cleaved-PARP and Bcl-2) were detected by Western blotting (n = 3). (d, g) The protein levels of p-STING was detected by Western blotting (n = 3). The results are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ SNP versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ SNP + NS8593 versus SNP.

detected with an automatic chemiluminescence imaging analysis system (Tanon). Western blot bands were quantified using Image J software.

2.8. Statistical analysis

Statistical comparisons were conducted using GraphPad Prism 8. All experiments were performed using at least three independent biological repeats, and data are presented as means ± standard deviation (SD) or means ± standard error of the mean (SEM). To compare the means between all measured variables, a one-way analysis of variance (ANOVA) followed by Dunnett's test or unpaired Student's *t*-test was used as appropriate. *P* values less than 0.05 were considered significant.

3. Results

3.1. NS8593 reverses SNP-induced changes in viability and morphology of C28/I2 cells

To explore the effect of NS8593 on SNP-induced chondrocyte apoptosis, cell viability was measured by MTT assay. As shown, cell viability decreased after treatment with different concentrations of SNP for 24 h, and 1 mM was selected as the final concentration to induce apoptosis (Fig. 1b). The results showed that 6.25–50 μM NS8593 did not affect cell viability after 24 h (Fig. 1c). As shown in Fig. 1d, NS8593 inhibited the SNP-induced decrease in chondrocyte viability in a concentration-dependent manner. Cell viability was further examined for 48 h of dosing treatment, and the results were consistent with the expectation that neither NS8593 (25 μM) nor SNP (1 mM) + NS8593 (25 μM) treatment for 48 h had a significant effect on chondrocyte viability (Supplementary Fig. 1). In addition to the determination of cell activity, we observed the morphological changes of cells treated with 25 μM NS8593 and 1 mM SNP under a microscope. The cells in the control group were spindle shaped. After treatment with 1 mM SNP for 24 h, the cells shrank and some dead cells were detached. Compared with the SNP group, the morphological changes of the cells treated with 25 μM NS8593 were reversed (Fig. 1e). Therefore, these results suggest that NS8593 protects C28/I2 cells from SNP-induced cell death.

3.2. The effects of NS8593 on ROS and mitochondrial membrane potential in SNP-stimulated C28/I2 cells

To investigate the effect of NS8593 on SNP-induced mitochondrial membrane potential in C28/I2 cells, we used two different staining methods for the assay. As shown in Fig. 2a, NS8593 rescued the SNP-induced decrease in mitochondrial membrane potential detected by Rh123 staining. Identically, the JC-1 staining method was used to detect the effect of NS8593 on the regulation of ΔΨm in chondrocytes. An increase in the red–green fluorescence ratio indicated higher polarization of the mitochondrial membrane. JC-1 staining results showed that the red–green fluorescence ratio of the SNP treatment group decreased, indicating that the absolute value of ΔΨm in the cells decreased (Fig. 2b). Interestingly, NS8593 treatment abrogated this effect, suggesting that NS8593 inhibited the SNP-induced reduction of ΔΨm in chondrocytes. In addition to this, in order to investigate whether the protective effect of NS8593 on SNP-stimulated C28/I2 cells is related to oxidative stress, we evaluated intracellular ROS levels. We used a DCFH-DA ROS fluorescent probe to detect ROS. The results of flow cytometry showed that the intracellular ROS level increased significantly after SNP stimulation. Compared with the SNP group, the ROS level in the NS8593 and SNP combined treatment groups decreased (Fig. 2c and d). The above results suggest that NS8593 may exert a protective effect by reducing ROS levels in SNP-stimulated C28/I2 cells.

3.3. NS8593 inhibits SNP-induced C28/I2 cell apoptosis

To investigate whether NS8593 reduces SNP-induced apoptosis in C28/I2 cells, we measured apoptosis using TUNEL staining, an Annexin V-FITC/PI apoptosis detection kit and western blotting. TUNEL staining of C28/I2 cells showed different degrees of apoptotic morphological changes in the nuclei of the SNP group (Fig. 3a). NS8593 alone had no significant effect on chondrocytes, but NS8593 in the combination group significantly reduced the morphological changes of SNP-induced apoptotic cells and exerted a protective effect on apoptotic chondrocytes (Fig. 3a). Only about 10 % of cells were apoptotic in the control group, while about 50 % of cells were apoptotic in the SNP group. However, the combination of NS8593 and SNP effectively reduced the apoptosis rate to about 18 %, and also reduced the proportion of late apoptotic cells (Fig. 3b and c). In addition, Western blot results showed that after SNP treatment, cleaved-PARP protein expression increased and Bcl-2 protein expression decreased, which could be reversed by NS8593 (Fig. 3d–f). To determine whether the STING signaling pathway is involved in the protective effect of NS8593 on apoptotic chondrocytes, we treated C28/I2 cells with SNP and examined the phosphorylation levels of STING protein by Western blot. Our data showed that SNP upregulates the phosphorylation level of STING, while NS8593 inhibits STING phosphorylation (Fig. 3dand g). These results suggest that NS8593 attenuates SNP-induced chondrocyte apoptosis, which may be related to the inhibition of STING signaling pathway activation.

3.4. Involvement of the STING signaling pathway in an NS8593-mediated reduction of articular chondrocyte apoptosis induced by SNP

To confirm whether the STING signaling pathway is involved in the protective effect of NS8593 against SNP-induced chondrocyte apoptosis, we treated C28/I2 cells with the STING inhibitor H-151 (Fig. 4a). MTT assay showed that H-151 restored the SNP-induced decrease in cell viability, and H-151 alone had no effect on chondrocyte viability within the experimental concentration range (Fig. 4b and c). We also observed the morphological changes of cells upon treatment with 1 μM H-151 and 1 mM SNP by TUNEL staining and found that H-151 inhibited SNP-induced chondrocyte apoptosis (Fig. 4d). Consistent with the TUNEL staining results, flow cytometry

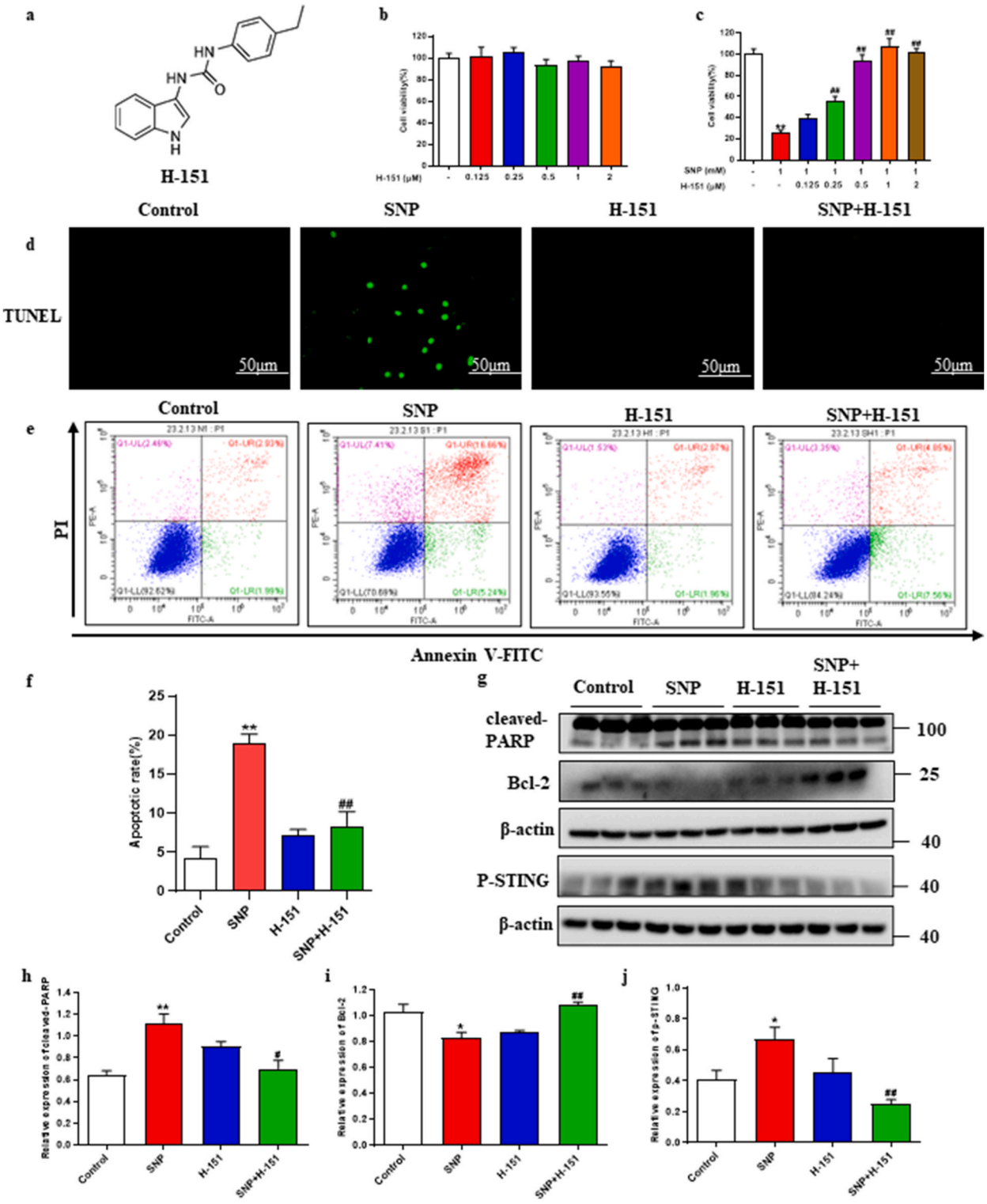

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Fig. 4. H-151 (inhibitor of STING signaling pathway) protects C28/I2 cells from sodium nitroprusside-induced apoptosis. (The following fluorescence pictures were taken under an inverted fluorescence microscope with 200× magnification.) (a) Chemical structure of H-151. (b) MTT experiment was used to determine the cell viability of C28/I2 cells treated with different concentrations of H-151 for 24 h. (c) H-151 alleviated the toxic effect of SNP on C28/I2 cells. (d) TUNEL staining found that H-151 inhibited SNP-induced chondrocyte apoptosis. (e–f) The apoptosis rate of C28/I2 cells was detected by Annexin V-FITC/PI apoptosis detection kit. The apoptosis rate was expressed by percentage. (g–i) The levels of apoptosis related proteins (cleaved PARP and Bcl-2) were detected by Western blotting (n = 3). (g, j) The protein levels of p-STING was detected by Western blotting (n = 3). The results are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ SNP versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ SNP + H-151 versus SNP.

also showed that H-151 restored SNP-induced chondrocyte apoptosis (Fig. 4e and f). In addition, Western blot results showed that cleaved-PARP protein and STING phosphorylated protein expression increased and Bcl-2 protein expression decreased after SNP treatment, and H-151 reversed this effect (Fig. 4g–j). These results suggest that the STING signaling pathway may be closely related to

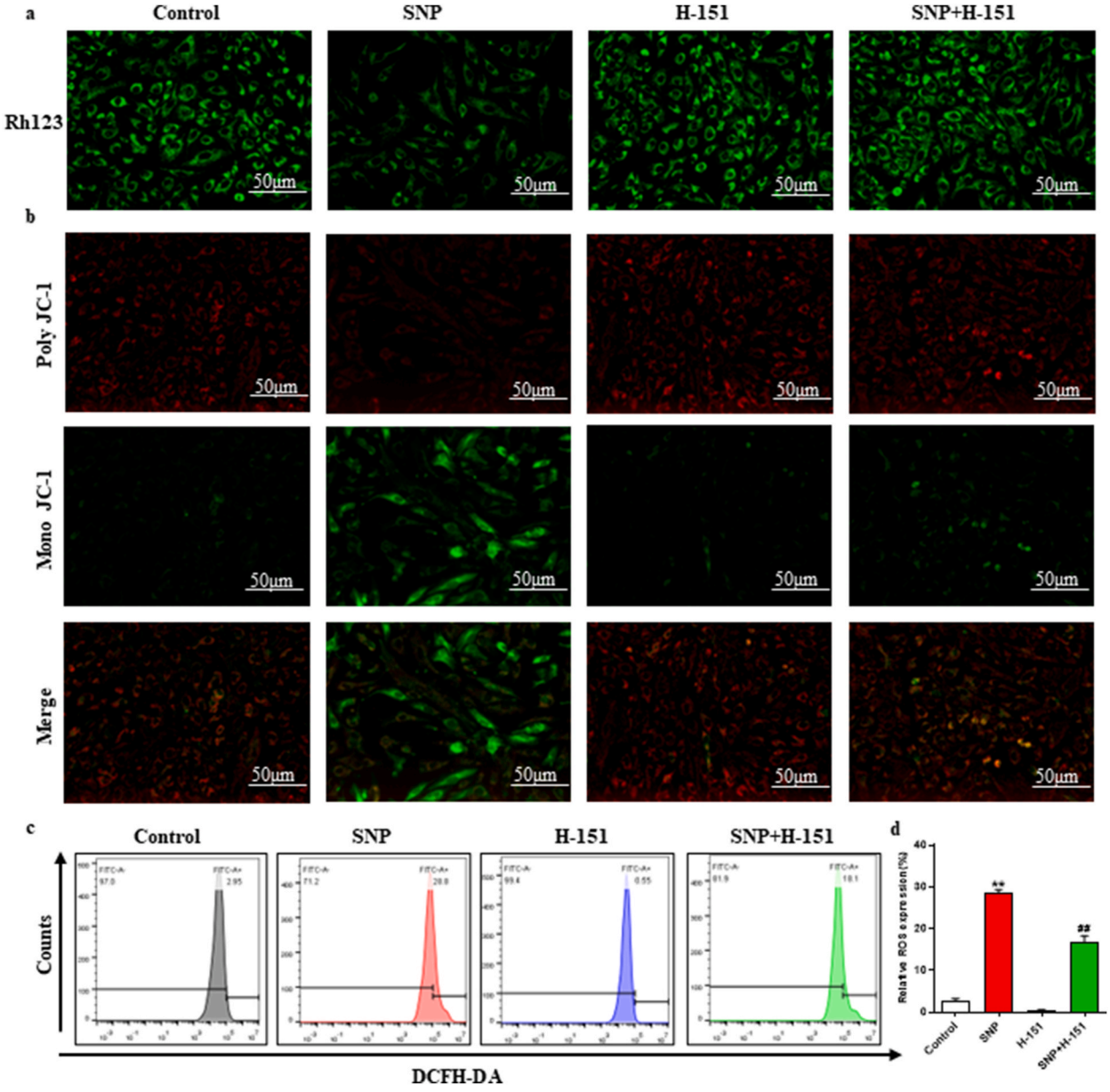

Fig. 5. H-151 (inhibitor of STING signaling pathway) affected the level of oxidative stress and mitochondrial membrane potential induced by SNP in C28/I2 cells. (The following fluorescence pictures were taken under an inverted fluorescence microscope with 200 × magnification.) (a–b) ΔΨm was detected by Rh123 and JC-1 staining in each group. (c–d) A scatter diagram of the DCFH-DA result showed the level of ROS in C28/I2 cells through flow cytometry. The results are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ SNP versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ SNP + H-151 versus SNP.

the protective effects of NS8593 on chondrocytes against apoptosis induced by SNP.

4. The role of the STING signaling pathway in the effect of NS8593 on ROS and mitochondrial membrane potential in SNP-stimulated C28/I2 cells

In order to study whether the STING signaling pathway is involved in the effect of NS8593 on mitochondrial membrane potential, we analyzed the level of ΔΨm in cells after adding H-151. Rh123 and JC-1 results showed that mitochondrial membrane potential decreased when SNP was used alone, but recovered when H-151 was used in combination (Fig. 5a and b). In addition, as shown in Fig. 5c and d, SNP leads to an increase in intracellular ROS, which can be reversed to some extent by H-151. The above results suggest that the STING signaling pathway may be closely related to the reduction of SNP-induced intracellular ROS levels and mitochondrial membrane potential in chondrocytes by NS8593.

5. Discussion

Articular cartilage degeneration is an important hallmark of osteoarthritis, and as the only cell type in articular cartilage, articular chondrocyte death is one of the main factors contributing to articular cartilage degeneration [19]. Therefore, the active development of drugs or methods that inhibit articular chondrocyte death is of great significance for the treatment of osteoarthritis. Apoptosis is caspase-dependent programmed cell death. According to relevant studies, apoptosis can lead to a variety of diseases, including osteoarthritis. However, the specific mechanism leading to the apoptosis of osteoarthritis articular chondrocytes is limited, and the role of STING signaling in this process is not fully understood. In this study, we first established an SNP-induced apoptosis model in human chondrocyte C28/I2 cells to simulate osteoarthritis articular cartilage degeneration, and found that 1 mM SNP significantly reduced chondrocyte viability, while the TRPM7 channel inhibitor NS8593 reversed this effect. Apamine is a highly selective inhibitor of SK channels, and in order to distinguish the role of SK channels from TRPM7, we treated chondrocytes with Apamine (0.0625–4 μM) and SNP (1 mM) + Apamine (0.0625–4 μM), respectively, for 24 h, and examined cell viability (Supplementary Fig. 2). In addition, the effect of SNP (1 mM) + Apamine (0.0625–4 μM) on cell morphology after treatment of chondrocytes was observed by bright field (Supplementary Fig. 2). It was found that Apamine (0.0625–0.5 μM) had no significant effect on cell viability, which decreased after 24 h of Apamine (1–4 μM) treatment of chondrocytes. In addition, treatment of chondrocytes with SNP (1 mM) and SNP (1 mM) + Apamine (0.0625–4 μM), respectively, revealed that Apamine could not rescue the SNP-induced decrease in cell viability. Consistent with the above results, brightfield found that Apamine could not rescue the SNP-induced morphological changes in chondrocytes. The above results provide preliminary evidence that the inhibitory effect of NS8593 on SNP-induced apoptosis in chondrocytes in this study was not through inhibition of SK channels. Furthermore, we found that NS8593 significantly inhibited SNP-induced apoptosis in C28/I2 cells, reduced intracellular ROS and increased mitochondrial membrane potential. Mechanistically, NS8593 attenuated chondrocyte apoptosis by inhibiting STING signaling, suggesting that inhibition of STING-mediated chondrocyte apoptosis is a promising target for the prevention or even treatment of osteoarthritis (Fig. 6).

In recent years, the prevalence and incidence of osteoarthritis have significantly increased in the middle-aged and elderly, and osteoarthritis is the main cause of disability in the elderly. Osteoarthritis is a common joint disease characterized predominantly by

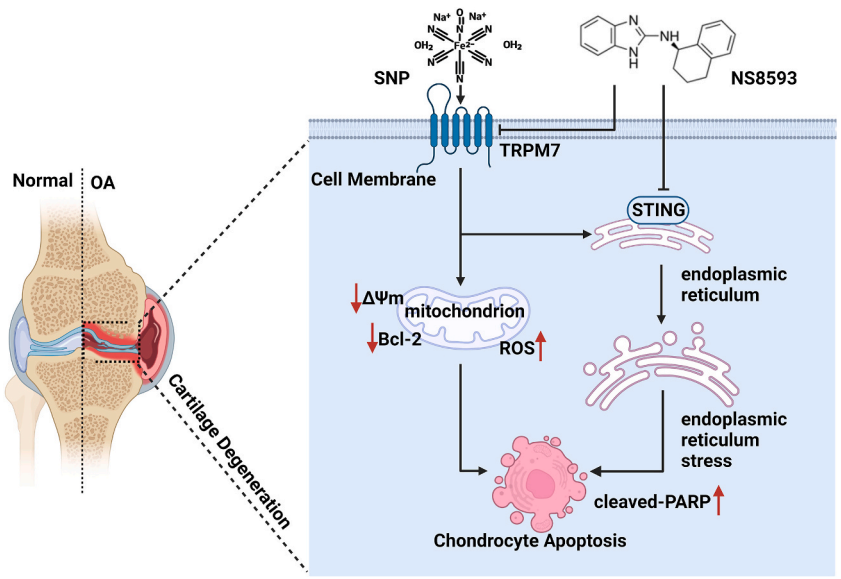

Fig. 6. Schematic diagram of NS8593 alleviating oxidative stress-induced osteoarthritis chondrocyte apoptosis by mediating STING signaling pathway.

articular cartilage degeneration and involving the entire joint tissue, including the subchondral cortex, calcified cartilage, synovium, trabecular bone, and joint capsule tissue [20]. Osteoarthritis preferentially affects weight-bearing joints such as the knee, hip, and lumbar spine. Patients present with joint pain, stiffness, hypertrophy, and limited mobility [21]. In the development of osteoarthritis, articular cartilage is severely degraded, and microscopically, chondrocyte apoptosis is the main change. At present, more and more studies have analyzed the hallmark molecular signals of chondrocyte apoptosis in osteoarthritis cartilage and found morphological and molecular characteristics of chondrocyte apoptosis, suggesting that chondrocyte apoptosis plays a key role in the pathogenesis of osteoarthritis [22].

The discovery of transient receptor potential (TRP) channels has been a major advance in the field of neurobiological research in recent years. The TRPM family, one of the subfamilies of the TRP cation channel family, is ubiquitous in almost all cell types and participates in physiological and pathological processes of the human body. The TRPM family comprises a total of eight members, TRPM1–TRPM8, and each TRPM channel has a conserved N-terminal MHR domain to distinguish it from other TRP channels. At the C-terminus, TRPM channels contain a TRP helix and a helical domain, and in addition, TRPM2, TRPM6, and TRPM7 have functional enzymatic domains at the C-terminus [23]. TRPM7, also known as LTRPC7 (Long TRP Channel 7), contains a cation channel and an α-kinase [24]. TRPM7 has six transmembrane segments (S1–S6); segments S1–S4 are receptors, and cation channels exist between segments S5–S6 [25]. TRPM7 channels are highly permeable to intracellular divalent cations, such as Ca^{2+}, Mg^{2+}, and Zn^{2+}, among others [26]. Early literature reported that Ca^{2+} inhibitors have anti-inflammatory and analgesic effects [27], while in addition, our group early demonstrated that intracellular Ca^{2+} is involved in RA chondrocyte apoptosis [28]. Of more concern, our group successively found that (1) 2-APB, a TRPM7 inhibitor, alleviated cartilage damage in AA rats by inhibiting chondrocyte apoptosis through regulating the IHH signaling pathway both *in vitro* and *in vivo* [29]; (2) TRPM7 mediates calcium influx and promotes chondrocyte ferroptosis through the PKCα–NOX4 signaling axis, ultimately leading to RA [30]. However, whether TRPM7 is involved in regulating apoptosis of osteoarthritis articular chondrocytes and the specific underlying regulatory mechanism is still unclear. NS8593 is a TRPM7 channel inhibitor, initially identified as a potent inhibitor of SK1-3 or KCa2.1–2.3 channels. SK is one of the targets for the treatment of atrial fibrillation, and many studies have now demonstrated that NS8593 can effectively ameliorate atrial fibrillation in rats, dogs, and horses [31]. However, there is no report on the use of NS8593 to treat osteoarthritis. To explore the possibility of treating osteoarthritis with NS8593, we established an SNP-induced apoptosis model using the chondrocyte cell line C28/I2 and found that NS8593 significantly reversed SNP-induced expression of apoptotic marker proteins in C28/I2 cells as well as the apoptotic rate. Taken together, these findings indicate that NS8593 inhibits SNP-induced chondrocyte apoptosis.

STING is a newly-discovered intracellular sensor of foreign and endogenous DNA and is thought to play a key role in host defense, autoimmune diseases and tumor immunity [32]. In 2021, Guo et al. found that the expression level of STING was increased in articular cartilage of osteoarthritis patients and a mouse model with destabilization of the medial meniscus (DMM)-induced osteoarthritis compared with normal controls [33]. To explore the possible mechanism of action of NS8593 in the treatment of osteoarthritis, we established an SNP-induced chondrocyte C28/I2 apoptosis model and found that NS8593 significantly inhibited SNP-induced chondrocyte C28/I2 p-STING protein expression. Furthermore, H-151, a commonly-used STING antagonist, is known to block STING activation-induced palmitoylation and to exhibit significant inhibitory effects on STING signal transduction *in vivo* and *in vitro* [34]. The present study found that H-151 inhibited the secretion of proinflammatory cytokines (IL-23, IL-17, and IL-6) and the infiltration of M1 macrophages [35]. Treatment of SNP-induced C28/I2 cells with H-151, a STING inhibitor, resulted in significant inhibition of SNP-induced expression of apoptotic marker proteins as well as the apoptotic rate in the chondrocyte cell line C28/I2. Therefore, we can speculate that the inhibition of SNP-induced chondrocyte apoptosis by NS8593 is associated with STING signaling.

6. Conclusion

In conclusion, our study found that the TRPM7 inhibitor NS8593 significantly inhibited SNP-induced chondrocyte apoptosis. Meanwhile, we also found that the STING signaling pathway was a key regulatory target for NS8593 to inhibit SNP-induced chondrocyte apoptosis, and that inhibition of STING signaling protected chondrocytes from apoptosis. Therefore, inhibition of STING signaling may be a possible therapeutic target for osteoarthritic articular cartilage degeneration.

Data availability statement

Raw data supporting the conclusions of this paper will be provided by the authors on request, without undue reservations.

CRediT authorship contribution statement

Rendi Zhu: Writing – original draft, Methodology, Investigation, Data curation. **Wenjuan Hao:** Writing – original draft, Validation, Methodology. **Shufang Li:** Methodology. **Yong Chen:** Methodology. **Fuli Zhou:** Methodology. **Renpeng Zhou:** Writing – review & editing, Funding acquisition. **Wei Hu:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that might 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.e31375.

References

[1] J. Martel-Pelletier, et al., Osteoarthritis, Nat Rev Dis Primers 2 (2016) 16072.
[2] Yunus, M.H.M., A.A.-O. Nordin, and H. Kamal,Pathophysiological Perspective of Osteoarthritis. LID - 10.3390/medicina56110614 [doi] LID - 614. (1648-9144 (Electronic))..
[3] Carballo, C.B., et al.,Basic Science of Articular Cartilage. (1556-228X (Electronic))..
[4] Liu, Y., K.M. Shah, and J. Luo, Strategies for Articular Cartilage Repair and Regeneration.(2296-4185 (Print))..
[5] Y. Rellmann, E. Eidhof, R. Dreier, Review: ER stress-induced cell death in osteoarthritic cartilage, Cell. Signal. 78 (2021) 109880.
[6] X.Y. Qi, et al., Role of small-conductance calcium-activated potassium channels in atrial electrophysiology and fibrillation in the dog, Circulation 129 (4) (2014) 430–440.
[7] J.G. Diness, et al., Effects on atrial fibrillation in aged hypertensive rats by Ca(2+)-activated K(+) channel inhibition, Hypertension 57 (6) (2011) 1129–1135.
[8] J.G. Diness, et al., Inhibition of small-conductance Ca2+-activated K+ channels terminates and protects against atrial fibrillation, Circ Arrhythm Electrophysiol 3 (4) (2010) 380–390.
[9] V. Chubanov, et al., Natural and synthetic modulators of SK (Kca2) potassium channels inhibit magnesium-dependent activity of the kinase-coupled cation channel TRPM7, Br. J. Pharmacol. 166 (4) (2012) 1357–1376.
[10] S. Suzuki, R. Penner, A. Fleig, TRPM7 contributes to progressive nephropathy, Sci. Rep. 10 (1) (2020) 2333.
[11] C. Song, et al., Suppression of TRPM7 enhances TRAIL-induced apoptosis in triple-negative breast cancer cells, J. Cell. Physiol. 235 (12) (2020) 10037–10050.
[12] Ishikawa, H. and G.N. Barber, STING Is an Endoplasmic Reticulum Adaptor that Facilitates Innate Immune Signalling.(1476-4687 (Electronic))..
[13] Rongvaux, A., et al., Apoptotic Caspases Prevent the Induction of Type I Interferons by Mitochondrial DNA. (1097-4172 (Electronic))..
[14] White, M.J., et al., Apoptotic Caspases Suppress mtDNA-Induced STING-Mediated Type I IFN Production. (1097-4172 (Electronic))..
[15] Zhang, R., R. Kang, and D. Tang, The STING1 Network Regulates Autophagy and Cell Death.(2059-3635 (Electronic))..
[16] Qiao, J.T., et al., Activation of the STING-IRF3 Pathway Promotes Hepatocyte Inflammation, Apoptosis and Induces Metabolic Disorders in Nonalcoholic Fatty Liver Disease.(1532-8600 (Electronic))..
[17] S. Liu, W. Guan, STING signaling promotes apoptosis, necrosis, and cell death: an Overview and Update, Mediators Inflamm 2018 (2018) 1202797.
[18] Quan, Y.Y., et al., Dominant Roles of Fenton Reaction in Sodium Nitroprusside-Induced Chondrocyte Apoptosis (1873-4596 (Electronic))..
[19] Xu, K., et al.,SIRT3 Ameliorates Osteoarthritis via Regulating Chondrocyte Autophagy and Apoptosis through the PI3K/Akt/mTOR Pathway. (1879-0003 (Electronic))..
[20] Goldring, M.B. and S.R. Goldring, Articular Cartilage and Subchondral Bone in the Pathogenesis of Osteoarthritis.(1749-6632 (Electronic))..
[21] Hall, M., et al.,How Does Hip Osteoarthritis Differ from Knee Osteoarthritis? (1522-9653 (Electronic))..
[22] Hwang, H.S. and H.A. Kim,Chondrocyte Apoptosis in the Pathogenesis of Osteoarthritis. (1422-0067 (Electronic))..
[23] Shi, R., et al.,Cell Death Modulation by Transient Receptor Potential Melastatin Channels TRPM2 and TRPM7 and Their Underlying Molecular Mechanisms. (1873-2968 (Electronic))..
[24] Inoue, K., Z.G. Xiong, and T. Ueki, The TRPM7 Channel in the Nervous and Cardiovascular Systems.(1875-5550 (Electronic))..
[25] Zou, Z.A.-O., et al.,TRPM7, Magnesium, and Signaling. LID - 10.3390/ijms20081877 [doi] LID - 1877. (1422-0067 (Electronic))..
[26] Oh, H.G. and S. Chung,Activation of Transient Receptor Potential Melastatin 7 (TRPM7) Channel Increases Basal Autophagy and Reduces Amyloid β-peptide. (1090-2104 (Electronic))..
[27] Park, R. and J.D. Ji,Calcium Channels: the Potential Therapeutic Targets for Inflammatory Bone Destruction of Rheumatoid Arthritis. (1420-908X (Electronic))..
[28] Wu, X., et al., The Role of Ca(2+) in Acid-Sensing Ion Channel 1a-Mediated Chondrocyte Pyroptosis in Rat Adjuvant Arthritis.(1530-0307 (Electronic))..
[29] Ma, G., et al., Blockade of TRPM7 Alleviates Chondrocyte Apoptosis and Articular Cartilage Damage in the Adjuvant Arthritis Rat Model through Regulation of the Indian Hedgehog Signaling Pathway.(1663-9812 (Print))..
[30] Zhou, R., et al., TRPM7 Channel Inhibition Attenuates Rheumatoid Arthritis Articular Chondrocyte Ferroptosis by Suppression of the PKCα-NOX4 axis.(2213-2317 (Electronic))..
[31] Diness, J.G., et al., Inhibition of Small-Conductance Ca2+-Activated K+ Channels Terminates and Protects against Atrial Fibrillation.(1941-3084 (Electronic))..
[32] H. Ishikawa, G.N. Barber, STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling, Nature 455 (7213) (2008) 674–678.
[33] Guo, Q., et al., STING Promotes Senescence, Apoptosis, and Extracellular Matrix Degradation in Osteoarthritis via the NF-Kb Signaling Pathway.(2041-4889 (Electronic))..
[34] Haag, S.M., et al., Targeting STING with Covalent Small-Molecule Inhibitors.(1476-4687 (Electronic))..
[35] Pan, Y., et al., The STING Antagonist H-151 Ameliorates Psoriasis via Suppression of STING/NF-κB-mediated Inflammation.(1476-5381 (Electronic))..