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Protective effects of ginsenoside Rg3 on TNF-α-induced human nucleus pulposus cells through inhibiting NF-κB signaling pathway

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ABSTRACT

This work aims to evaluate the effect of ginsenoside Rg3 on the apoptosis, proliferation, extracellular matrix (ECM) metabolism and oxidative stress-induced damage of human nucleus pulposus cells (NPCs) induced by TNF-α. The human NPCs were divided into Control, TNF-α, TNF-α + low Rg3, TNF-α + medium Rg3 and TNF-α + high Rg3 groups. Annexin V-FITC/PI, CCK-8 and flow cytometry were used to detect the apoptosis, proliferation, and cell cycle of NPCs, respectively. The expressions of ECM-related molecules were determined by qRT-PCR, ELISA and Western blotting. NF-κB p65 pathway and apoptosis-related proteins were evaluated by Western blotting, and the production of reactive oxygen species (ROS) was detected by DCFH-DA assay. Compared with Control group, NPCs in the TNF-α group had elevated proportion of apoptotic cells with up-regulation of Bax and Caspase-3 and down-regulation of Bcl-2. Besides, TNF-α inhibited proliferation and arrested cell cycle at G1 of NPCs. Moreover, human NPCs induced by TNF-α presented the increase in the expressions of ECM degrading genes (MMP3 and ADAMTS5), the content of ROS and malondialdehyde (MDA), and the expression of NF-κB/p65 in nucleus, but showed the decrease in the expression of ECM synthesis genes (Aggrecan and COL2A1) and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX). However, NPCs treated by both TNF-α and Rg3 demonstrated a certain degree of reversal in the above indexes, which became increasingly evident with the up-regulation of Rg3 concentration. Ginsenoside Rg3 may exert the effect of attenuating TNF-α-induced NPCs impairment via blocking the NF-κB signaling pathway.

1. Introduction

As one of major causes for intervertebral disc degenerative diseases, intervertebral disc degeneration (IDD) has been suggested as a significant contributor to back, neck, shoulder, and radicular pains, attracting more and more attention of both physicians and investigators [1,2]. A number of scholars thought that IDD was a complex health problem caused by multiple factors, including aging, genetic predisposition, mechanics, nutrition, immunity and so on, but the specific mechanism has not been clearly elucidated [3,4]. There was evidence reported that intervertebral disc, the important mechanical structure, could be stimulated by exogenous factors, like TNF-α (a strong inflammatory cytokine), which acted on the membrane receptor of intervertebral disc cells to activate the signal transduction pathway and influence the gene expression, as well as break the synthesis and metabolic balance of the key components (proteoglycan and type-II collagen) of intervertebral disc, eventually leading to IDD [5,6]. Therefore, it is of great significance to investigate the key link of IDD pathogenesis in human nucleus pulposus cells (NPCs) induced by TNF-α.

Ginseng has been widely accepted and used by many Asian and European countries as a Chinese medicinal herb [7,8]. Ginsenoside (GS) is the major pharmacologically active ingredient extracted from ginseng [9], which has long history dealing with human health and has been utilized as a traditional Chinese medicine extensively for officinal or recuperative purposes owing to its numerous pharmacological and physiological functions, such as anti-inflammatory, anti-stress, anti-oxidation, anti-tumor and immunomodulatory effects [10,11]. At present, approximately over 100 saponin monomers have been extracted and identified from ginseng, among which Rg3 (with the molecular

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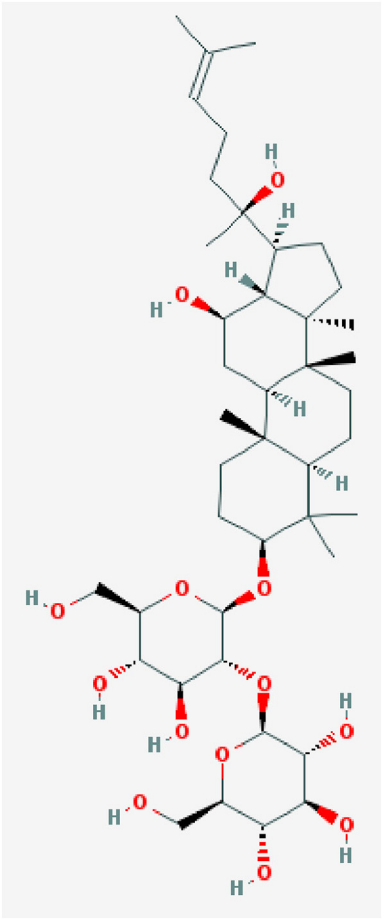

Fig. 1. The chemical structural formula of ginsenoside Rg3.

formula $C_{42}H_{72}O_{13}$) (Fig. 1) is regarded as the one of the most bioactive extracts from ginsenosides mainly found in red ginseng [12]. In recent years, accumulating studies have pointed out that Rg3 played its therapeutic role in diseases via mediating the NF-κB signaling pathway. For example, Wang J et al. found that Rg3 could increase the sensitivity of hypoxia-induced lung cancer cells to cisplatin via inhibiting NF-κB-mediated epithelial-mesenchymal transition (EMT)[13]. Also, Surh YJ et al. revealed that Rg3 can inhibit NF-κB to alleviate the ear edema induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) [14]. Notably, a study highlighted the essential role of NF-κB signaling pathway in IDD, exhibiting a therapeutic target for mitigating disc degenerative diseases [15]. Additionally, TNF-α can also modulate the NF-κB pathway to affect the angiogenic activity in intervertebral disc [16]. Moreover, through interfering with the NF-κB pathway, 17β-estradiol could ameliorate TNF-α-induced premature senescence of rat NPCs [6], but it is still unknown whether Rg3 can mediate NF-κB signaling pathway in TNF-α-induced NPCs. Thus, the primary human NPCs induced by TNF-α were selected in this study and treated with different concentrations of Rg3, to investigate the effects of ginsenoside Rg3 on the apoptosis, proliferation, ECM metabolism and oxidative stress-induced damage of NPCs via mediating NF-κB pathway.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethics Committee of our hospital and conformed to the protocols in the Declaration of Helsinki of the World Medical Association (WMA) [17]. The written informed consent form was obtained from every subject prior to the study.

2.2. Separation and primary culture of NPCs

Nucleus pulposus was collected and placed into the culture dish during the operation under aseptic conditions. Next, nucleus pulposus was isolated under a stereomicroscope, washed with Hanks' solution for three times, and then cut into pieces (1 mm^3) before centrifugation at 37 °C at the rate of 1500 rpm for 8 min. After that, the supernatant was removed and 0.2% type-II collagen was added. When all tissue pieces dissolved, the solution was filtered into the centrifuge tube, in which serum was added to terminate the digestion reaction. After centrifugation at 1500 rpm, the supernatant was taken out and added with culture medium for cell suspension. Then, 3 ml culture medium was added and the density of cells was calculated. Subsequently, human NPCs (5×10^5/ml) were inoculated into the culture flask of 25cm^2 and incubated at 37 °C in 5%CO_2. The culture medium was replaced every two or three days. When cell confluence reached 80–90%, cells should be cryopreserved and resuscitated in a routine fashion before the preparation of cell slides and the identification of NPCs.

2.3. TNF-α induction and grouping of human NPCs

The human NPCs (5×10^5/ml) collected at the logarithmic growth phase were inoculated to the culture flask (containing 10% FBS and 5 ml DMEM/F12) and incubated for 24 h at 37 °C in 5% CO_2. Next, the culture flask was added with TNF-α (10 ng/ml) for cell induction [6], while human NPCs without any treatment were set as the Control group. According to the different concentrations of ginsenoside Rg3 (25–100 μg/ml), which has been reported to affect the progressions of various diseases in vitro [18–22], NPCs induced by TNF-α were divided into 4 groups: TNF-α group (NPCs only induced by TNF-α), TNF-α + low Rg3 group (TNF-α-induced NPCs with 25 μg/ml of Rg3), TNF-α + medium Rg3 group (TNF-α-induced NPCs with 50 μg/ml of Rg3), and TNF-α + high Rg3 group (TNF-α-induced NPCs with 100 μg/ml of Rg3). Rg3 was bought from Thermo Fisher Scientific (Waltham, MA, USA).

2.4. Cell apoptosis detected by Annexin V-FITC/PI staining

Human NPCs were digested with 0.25% trypsin, washed by PBS buffer twice, and centrifuged for 5 min at the rate of 1000 rpm. Then, the culture medium was removed and pre-cooled binding buffer was added for cell suspension (1×10^6/ml). Next, Annexin V-FITC (5 μl, BD Company, USA) was added for incubation at 4 °C for 15 min, followed by the addition of propidium iodide (PI, 10 μl) for 5 min of incubation at 4 °C in the dark environment. Finally, cell suspension was transferred to the tube for detection in the flow cytometer.

2.5. Cell proliferationdetectedbyCCK8 and EdU assay

Cells were seeded onto 96-well plates (10^4 cells/well), which was incubated in 5% CO_2 at 37 °C. At the 12 h, 24 h, 48 h, and 72 h of incubation, plates were taken out to add CCK8 (10 μl/well, Beijing Solarbio Technology Co., Ltd., Beijing, China) for another 2 h of incubation. Next, the Microplate reader was used to read the optical density (OD) value at the wavelength of 490 nm, and the cell survival curve was drawn accordingly. The Cell Light™ 5-ethynyl-2-deoxyuridine (EdU) DNA (deoxyribonucleic acid) Kit (RiboBio, PRC) was applied to confirm the result of cell proliferation using CCK8 assay. EdU-labeling index was referred to the amount of EdU-stained nuclei divided by the amount of DAPI (4′,6-diamidino-2-phenylindole)-stained nuclei, which was calculated after randomly selecting five visual fields.

2.6. Cell cycle evaluated flow cytometry

Cells (1×10^6 cells/ml) were digested with 0.25% trypsin and inoculated to 6-well plates for incubation. When cell confluence reached

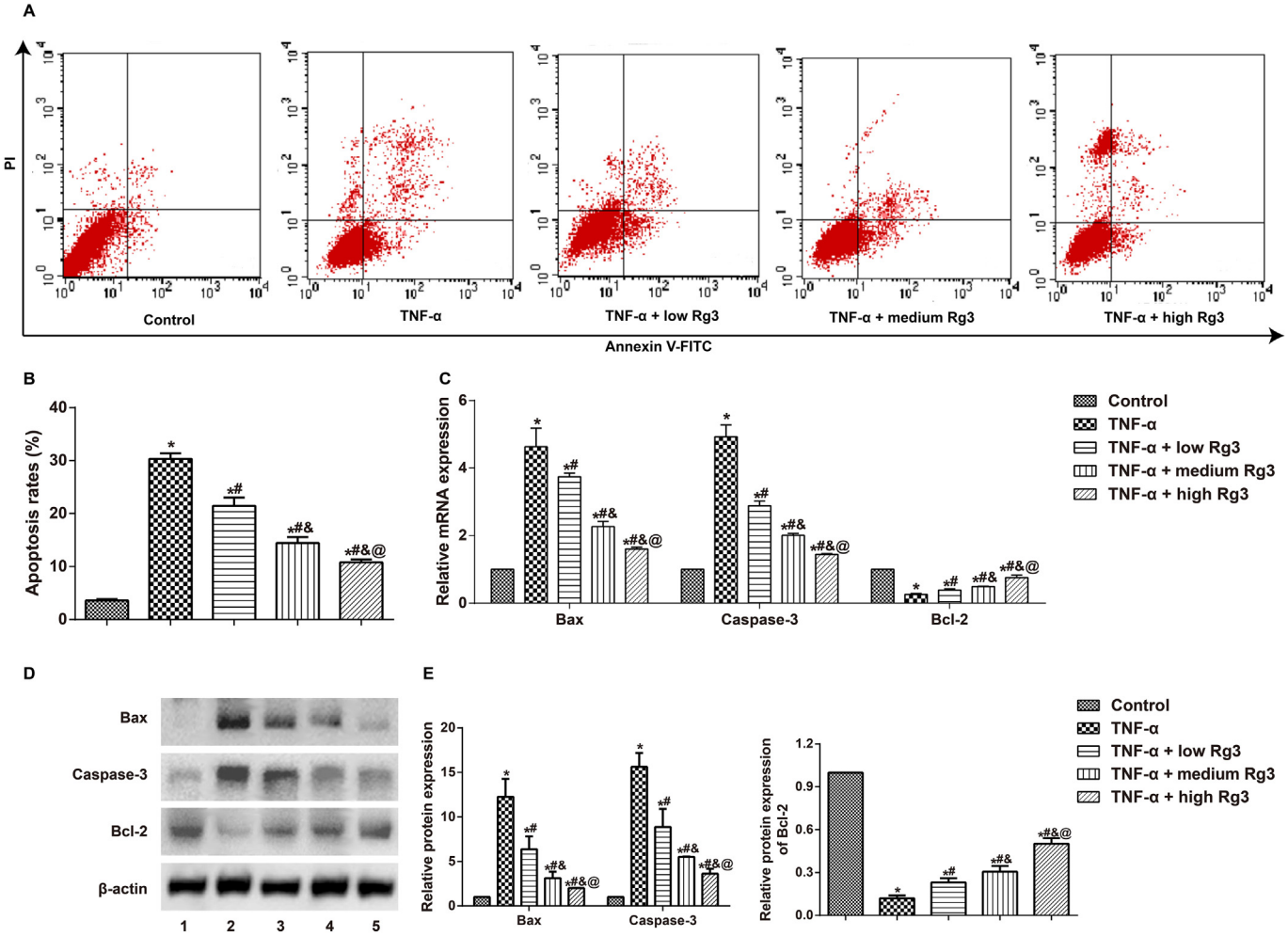

Fig. 2. Rg3 inhibits the apoptosis of human NPCs induced by TNF-α.
Note: A–B, The apoptosis of human NPCs detected by Annexin V-FITC/PI staining; C, The expression of apoptosis-related genes (*Bax*, *Caspase-3* and *Bcl-2*) in human NPCs detected by qRT-PCR; D–E, The expression of apoptosis-related proteins (Bax, Caspase-3 and Bcl-2) in human NPCs detected by Western blot; 1, Control group; 2, TNF-α group; 3, TNF-α + low Rg3 group; 4, TNF-α + medium Rg3 group; 5, TNF-α + high Rg3 group; *, $P < 0.05$ compared with Control group; $^{\#}$, $P < 0.05$ compared with TNF-α group; $^{\&}$, $P < 0.05$ compared with TNF-α + low Rg3 group; $^{@}$, $P < 0.05$ compared with TNF-α + medium Rg3 group.

80–90%, they were treated and grouped as described above. Next, cells were cultured in an incubator (setting: 37 °C, 5%CO_2) for 48 h. Then, cells were digested by 0.25% trypsin, made into single-cell suspension (1–5 × 10^6 cells/ml), washed with PBS buffer twice, and added with 1 ml pre-cooled 70% alcohol after removing the supernatant. Next, cells were placed at 4 °C for overnight, washed with PBS buffer, centrifuged for 5 min at the rate of 1000 rpm, filtered with 400-mesh screen mesh, and centrifuged again for 5 min at the rate of 1000 rpm. After discarding the PBS, cells were re-suspended with 0.5 ml PBS, added with 1 ml of PI dye, and placed at 4 °C for 30 min in avoidance of light. Finally, the flow cytometer was used to evaluate the cell cycle.

2.7. qRT-PCR

Total RNA was extracted from human NPCs cells by using TRIzol extraction kit (Invitrogen Ltd., Paisley, UK), and its purity and concentration were determined with an UV spectrometer (UV-1800, Japan), and observed for RNA intactness by using the agarose gel electrophoresis. Primers were designed with the software Primer 5.0 (Premier Biosoft, Palo Alto, CA, USA) and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The reverse transcription of total RNA into cDNA was realized by using the Primescript™ RT reagent Kit (Takara, Japan). SYBR® premix Ex Taq TM kit (Takara Biomedical Technology Co., Ltd., Dalian, China) was used for qRT-PCR. The relative expression of target genes was calculated using $2^{-\triangle\triangle Ct}$ method with GAPDH as the internal reference gene.

2.8. ELISA

The supernatant after centrifugation was collected and stored at −20 °C for later analysis. The expression levels of COL2A1, Aggrecan, ADAMTS-5 and MMP-3 in human NPCs of each group were determined by the ELISA kits (R&D Systems, Minneapolis, MN, USA).

2.9. Measurement of oxidative stress-related indexes

The production of reactive oxygen species (ROS) was measured by 2′,7′-dichlorofluorescein diacetate (DCFH-DA) assay (Molecular Probes, Inc., Eugene, OR, USA). DCFH-DA (10 μmol/l) was added into the plate for 60 min of cell incubation at 37 °C, followed by the washing with PBS and digestion with 0.25% trypsin. Subsequently, cells were centrifuged for 5 min at the rate of 1000 rpm, washed with PBS, and collected into the tube for detection in the flow cytometer (excitation wavelength of 488 nm and the emission wavelength of 525 nm). The content of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) in NPCs of each group was measured by detection kits provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

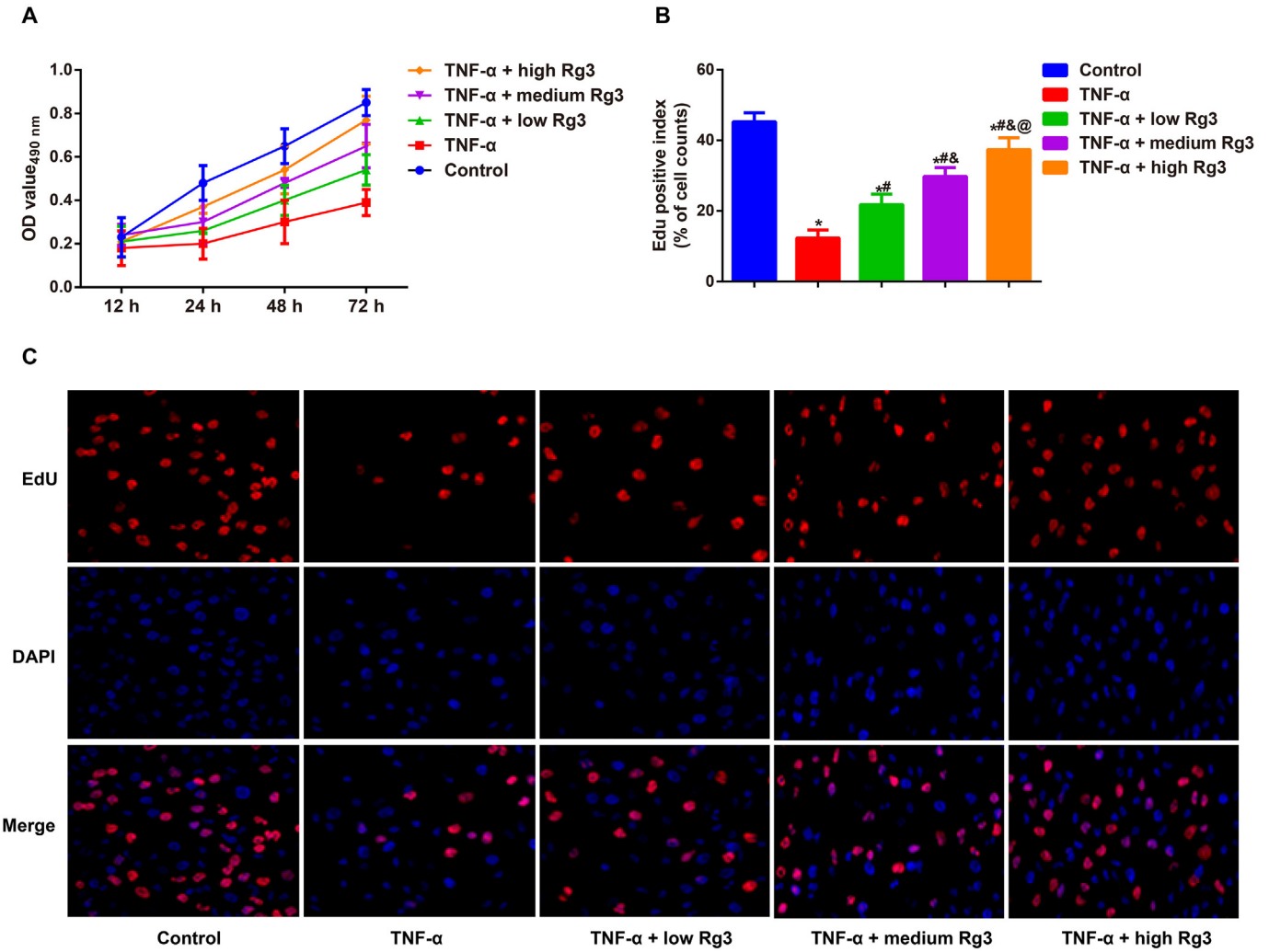

Fig. 3. Effect of Rg3 on the proliferation of TNF-α-induced human NPCs evaluated byCCK-8 (A) and EdU (B–C) assays.
Note: *, $P < 0.05$ compared with Control group; #, $P < 0.05$ compared with TNF-α group; &, $P < 0.05$ compared with TNF-α + low Rg3 group; @, $P < 0.05$ compared with TNF-α + medium Rg3 group.

2.10. Western blot

The nuclear and cytoplasmic proteins from the cultured human NPCs were separated by using the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Biotech, Jiangsu, China) following the manufacturer's protocol. The proteins were extracted and centrifuged for 15 min at the rate of 12,000 rpm before collecting the supernatant for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins separated by electrophoresis were transferred to the nitrocellulose filter by electric transfer, and the filter was blocked for 1 h in PBS solution with 5% skimmed milk powder. Next, primary antibodies were added for overnight reaction at 4 °C. The filter was washed with PBS buffer before the 1 h incubation at room temperature with horseradish peroxidase (HRP) conjugated secondary antibodies. Then, the filter was washed with PBS buffer before development by enhanced chemiluminescence (ECL). The gray value ratio of target bands to the reference band was regarded as the relative expression level of proteins, with Lamin B1, a-tubulin and β-actin as the loading control for proteins sample.

2.11. Statistical methods

Each experiment in this study was done three times, and the mean value was presented by mean ± standard deviation (SD). Data analysis was conducted with the software SPSS 22.0 (Chicago, IL, USA). Besides, the comparison among multiple groups was performed by using One-way ANOVA with least significant difference (LSD) test. Differences were indicated significant when $P < 0.05$.

3. Results

3.1. Rg3 inhibits the apoptosis of TNF-α-induced human NPCs

Compared with NPCs without any treatment, TNF-α-induced NPCs had elevated proportion of apoptotic cells with up-regulation of Bax and Caspase-3 and down-regulation of Bcl-2 (all $P < 0.05$). However, Rg3 resulted in the reduced *Bax* and *Caspase-3* expressions, the increased *Bcl-2* expression, and the inhibited cell apoptosis in TNF-α-induced NPCs (all $P < 0.05$), and these changes became more evident with the increase of Rg3 concentrations (Fig. 2A–C). Besides, Western blot analysis demonstrated a similar expression pattern of apoptosis-related proteins (Bax, Caspase-3, and Bcl-2) with mRNA levels (Fig. 2D–E).

3.2. Rg3 promotes the proliferation and improves cell cycle of TNF-α-induced human NPCs

CCK-8 and EdU assays were used to evaluate the proliferation of

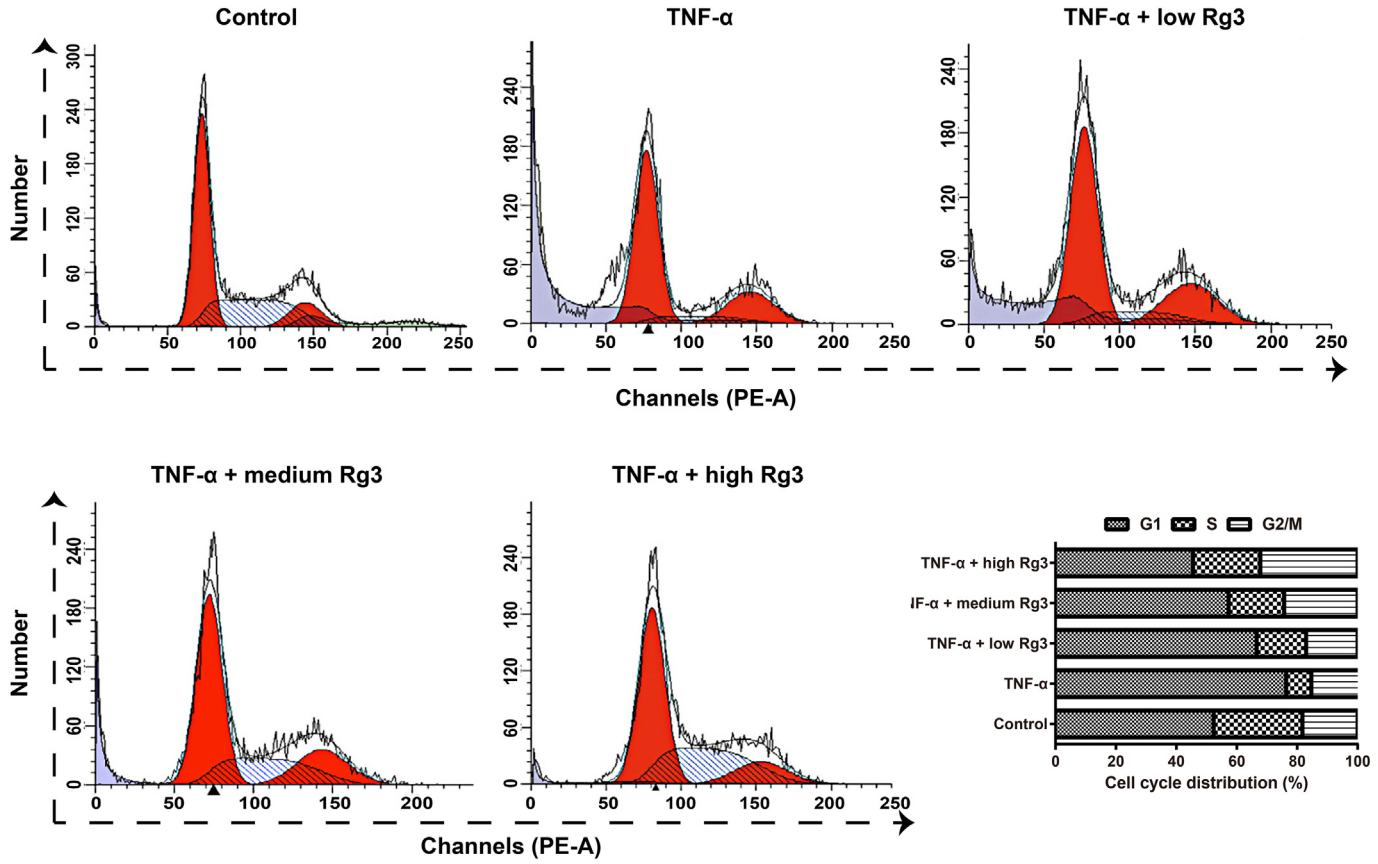

Fig. 4. Effect of Rg3 on the cell cycle of TNF-α-induced human NPCs assessed by flow cytometry.

human NPCs (Fig. 3). According to the results, NPCs in four TNF-α groups were inhibited in the proliferative ability compared with those in Control group ($P < 0.05$). Among four TNF-α-treated groups, those groups concurrently treated with Rg3 had the higher proliferation rate than the group treated with TNF-α-only (all $P < 0.05$). Besides, as shown by flow cytometry (Fig. 4), human NPCs induced by TNF-α were arrested the cell cycle progression at G1 phase with the reduced proportion in S phase; however, this phenomenon was effectively alleviated after treated by Rg3, and the proportion of cells entering S phase increased accordingly with the up-regulation of Rg3 concentration.

3.3. Rg3 inhibits the oxidative stress-induced damage of TNF-α-induce human NPCs

As displayed by DCFH-DA assay in Fig. 5A–B, the MFI of ROS was higher in the TNF-α group than the Control group ($P < 0.05$), but it was decreased in the TNF-α-induced human NPCs treated with the Rg3 in a concentration-dependent manner (all $P < 0.05$). Besides, TNF-α treatment led to the apparent increase in the MDA content and the reduction of SOD and GSH-PX activities, which could be reversed by the treatment of Rg3. Besides, the MDA content declined but the activities of SOD and GSH-PX was enhanced with the increase concentration of Rg3 (all $P < 0.05$, Fig. 5C–D).

3.4. Rg3 improves the ECM metabolism in TNF-α-induced human NPCs

The human NPCs only treated by TNF-α contributed to the up-regulation of ECM degrading genes (MMP3 and ADAMTS5) and the down-regulation of ECM synthesis genes (Aggrecan and COL2A1) in NPCs (all $P < 0.05$), as shown in Fig. 6. However, those NPCs with both TNF-α and Rg3 treatments led to the reduction of ECM degrading genes and the elevation of ECM synthesis genes in an Rg3-concentration-dependent manner (all $P < 0.05$).

3.5. Rg3 blocks the NF-κB signaling pathway in TNF-α-induced NPCs

On the basis of Western blot analysis, the expression of NF-κB/p65 in TNF-α-induced NPCs was decreased in the cytoplasm but was increased in the nucleus. However, as regards NPCs treated by TNF-α and Rg3 at the same time, the expression of NF-κB/p65 was elevated in cytoplasm but reduced in the nucleus after treated with Rg3 in TNF-α-induced NPCs, and these changes became more obvious with the increased concentration of Rg3 (Fig. 7).

4. Discussion

As reported previously, TNF-α has been found expressed in the aged and degenerative intervertebral disc degenerations from both humans and animal models [23]. In this study, the human NPCs induced by TNF-α were constructed, presenting obvious increase in oxidative stress-induced damage and apoptosis with the apparent reduction of cell proliferation and disordered ESM metabolism, which was consistent with a previous study [24]. Besides, TNF-α has been suggested to modulate the NF-κB signaling pathway and up-regulate the expression of MMPs to promote the extracellular matrix (ECM) degradation in intervertebral disc cells, thus aggravating the degeneration of intervertebral disc [25]. Therefore, we hypothesized that TNF-α may act as the key inflammatory factor of degeneration to be participated in the progression of IDD via regulation of NF-κB signaling pathway.

To our knowledge, cellular apoptosis has been demonstrated to play an important role in IDD. In the current research, we found that ginsenoside Rg3 could inhibit the apoptosis of TNF-α-induced NPCs owing to the anti-inflammatory effect of Rg3, since Rg3 could accelerate the inflammation resolution to reduce the apoptosis of NPCs [26,27].

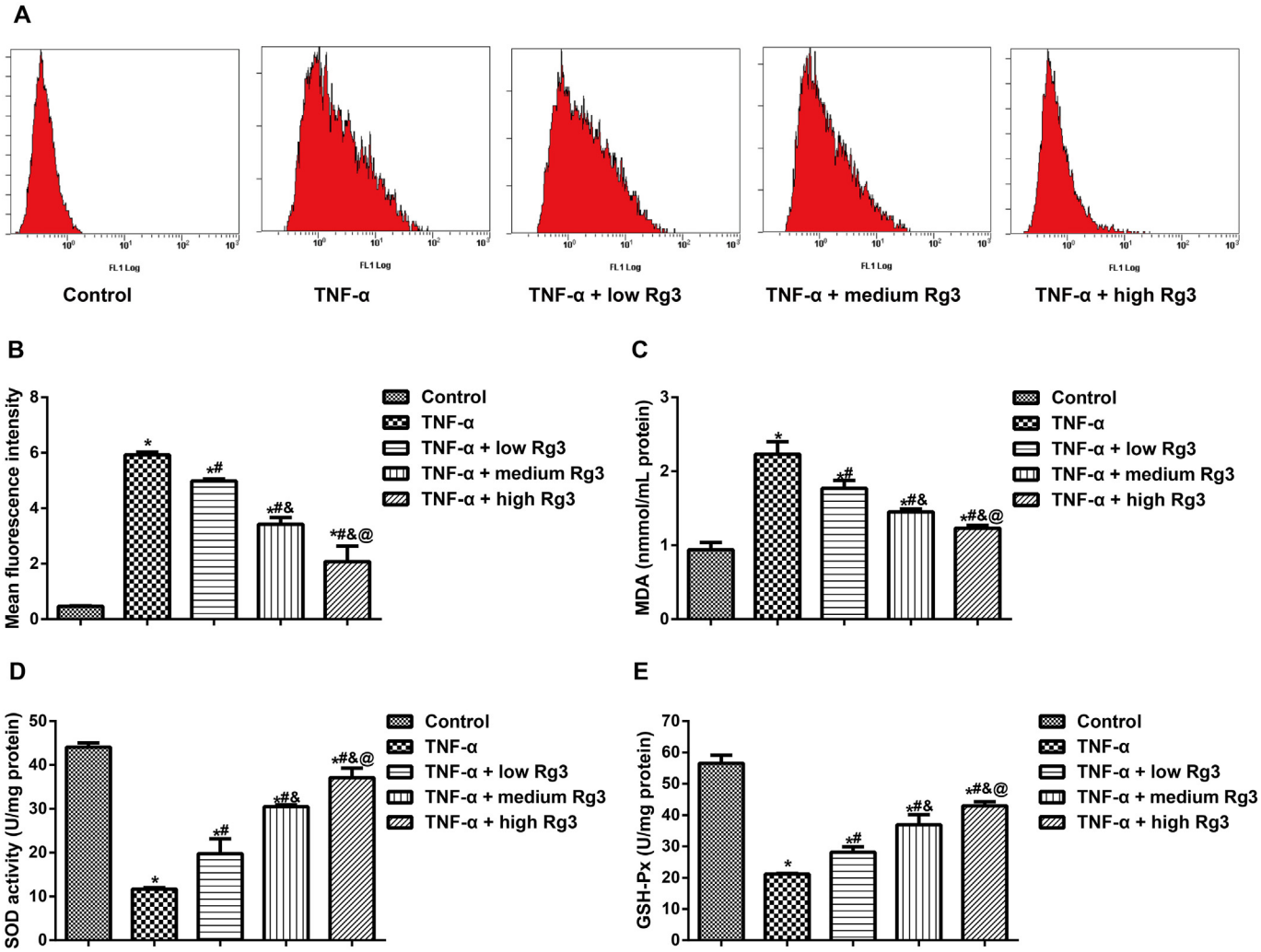

Fig. 5. Effect of Rg3 on the oxidative stress-induced damage of TNF-α-induce human NPCs determined by DCFH-DA assay.
Note: A–B, The production of ROS in each group determined by DCFH-DA assay; C–E, Comparison of the content of MDA (C) and the activities of SOD (D) and GSH-PX (E) in each group; *, $P < 0.05$ compared with Control group; #, $P < 0.05$ compared with TNF-α group; &, $P < 0.05$ compared with TNF-α + low Rg3 group; @, $P < 0.05$ compared with TNF-α + medium Rg3 group.

Similarly, Rg3 may activate the PI3K/AKT signaling pathway to inhibit inflammation responses and attenuate hepatocyte necrosis and apoptosis, thus playing a protective role in acetaminophen-induced liver injury [28]. On the other hand, Bcl-2 and Bax are two members of the Bcl-2 gene family and Bcl-2 is known as the anti-apoptosis gene due to its function of promoting cell survival and inhibiting cell apoptosis [29]. Under the stimulation of apoptosis-inducing factors, Bcl-2 and Bax would compete with each other to form homologous or heterogeneous dimers, which can modulate mitochondria to ignite caspase cascade, eventually leading to the production of caspase-3 and inducing cell apoptosis [30,31]. In our study, we found that Rg3 treatment could inhibit the expression of Bax and Caspase-3 and enhance the expression of Bcl-2 in NPCs, further indicating Rg3 can effectively attenuate the apoptosis of NPCs induced by TNF-α. Besides, evidence reported that Rg3 inhibited cell apoptosis and improved proliferation by altering the cell cycle in HT22 murine hippocampal neuronal cells induced by *N*-methyl-d-aspartic acid (NMDA) [32]. In this report, Rg3 could enhance the proliferative ability and ameliorate cell cycle of TNF-α-induced NPCs, thereby increasing the number of cells entering the S phase (DNA synthesis phase).

Accumulating previous studies have supported that oxidative stress, referring to the over production of ROS beyond the capacity of cellular antioxidant systems, played an essential role in causing cell apoptosis, including human NPCs [33–35]. As we know, the normal cells possess antioxidant defense systems, including SOD, catalase (CAT) and GSH-PX, which can effectively scavenge the surplus ROS to maintain the cell homeostasis [36]. In this work, we found the increase content of ROS in TNF-α-induced human NPCs, which were consistent with the finding in the study of Li Pet al. [6]. The consistent accumulation of ROS in intervertebral disc can activate the oxidative stress response, thereby inducing the aging-related IDD [37], while the reduced activities of SOD and GSH-PX suggested that TNF-α can cause the oxidative stress of human NPCs. Moreover, MDA, one of the final products of polyunsaturated fatty acids peroxidation in the cells, is induced by the lipid peroxidation of the cellular membrane, which has been accepted as the index of lipid-peroxidation [38]. With different concentrations of Rg3 treatment in TNF-α-induced NPCs in our investigation, the intracellular MDA content declined and the activities of SOD and GSH-PX went up with the increase of Rg3 concentrations, suggesting that Rg3 would inhibit the oxidative stress-induced damage in TNF-α-induced human NPCs. In line with our findings, Rg3 could attenuate ROS and maintain GSH pools in the sepsis models in the study of Xing W et al. in vitro [39]. In addition, Rg3 also could reduce the ROS level in prostatic stromal cells and decrease the transcriptional activity of IL-8 promoter by deactivating the transcription factors C/EBP β and p65 binding to IL-8 promoter.[40]. After detecting the expression of NF-κB p65 in

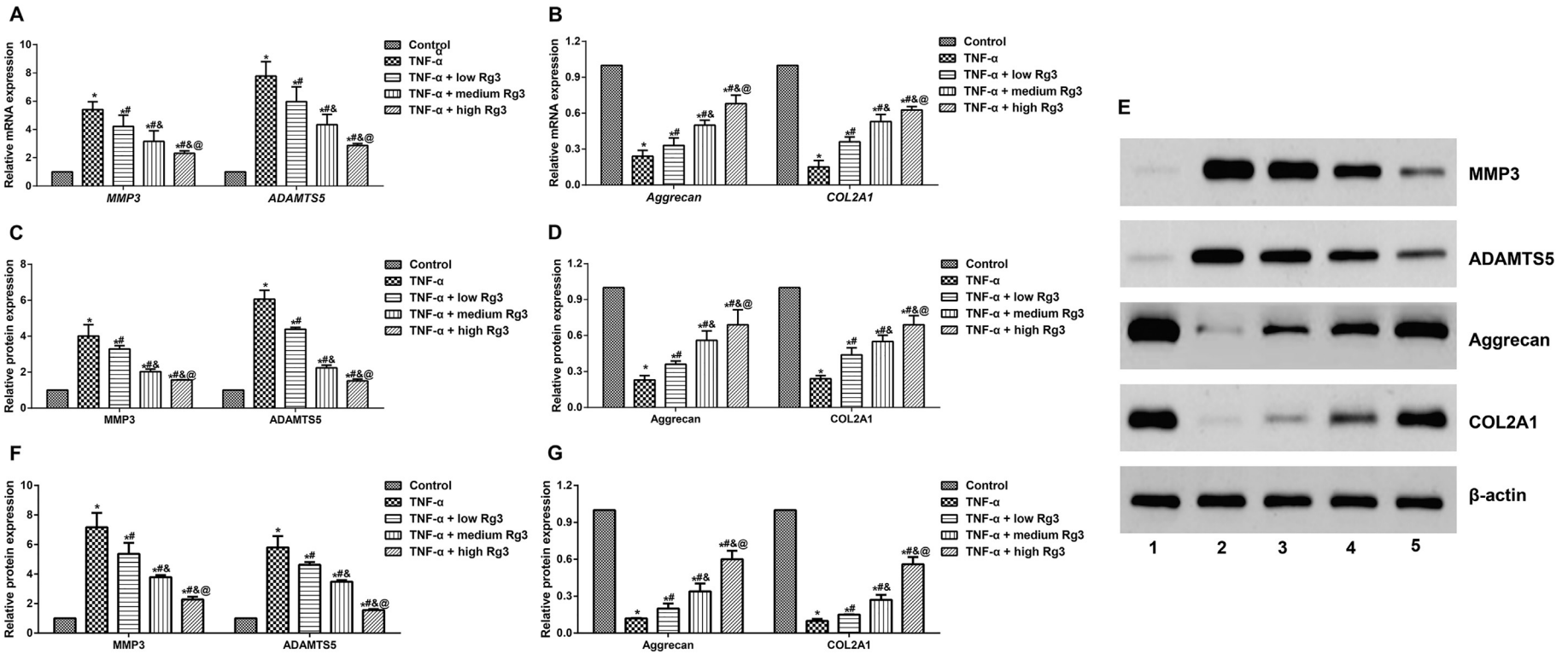

Fig. 6. Effect of Rg3 on the expressions of ECM degrading and synthesis genes in TNF-α-induced human NPCs.

Note: A–B, The mRNA levels of ECM degrading genes (*MMP3* and *ADAMTS5*) (A) and ECM synthesis genes (*Aggrecan* and *COL2A1*) (B) in each group detected by qRT-PCR;C-D, The protein expressions of ECM degrading genes (MMP3 and ADAMTS5) and ECM synthesis genes (Aggrecan and COL2A1) in each group detected by ELISA (C–D) and Western blot (E–G); 1, Control group; 2, TNF-α group; 3, TNF-α + low Rg3 group; 4, TNF-α + medium Rg3 group; 5, TNF-α + high Rg3 group; *, $P < 0.05$ compared with Control group; $^{\#}$, $P < 0.05$ compared with TNF-α group; $^{\&}$, $P < 0.05$ compared with TNF-α + low Rg3 group; $^{@}$, $P < 0.05$ compared with TNF-α + medium Rg3 group.

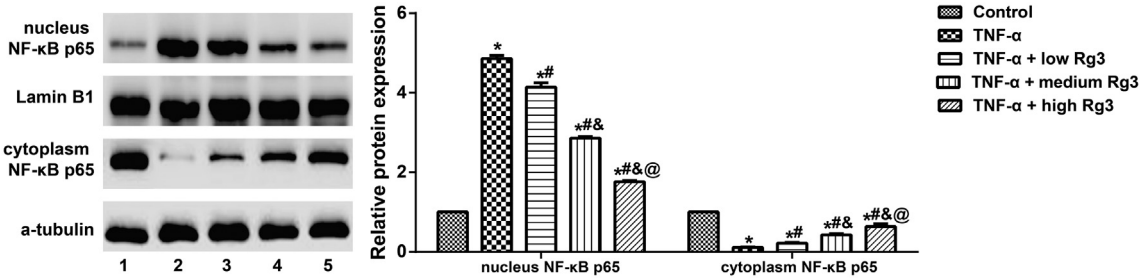

Fig. 7. The expression of NF-κB/p65 in the cytoplasm and nucleus in each group measured by Western blot.
Note: *, $P < 0.05$ compared with Control group; #, $P < 0.05$ compared with TNF-α group; &, $P < 0.05$ compared with TNF-α + low Rg3 group; @, $P < 0.05$ compared with TNF-α + medium Rg3 group.

cytoplasm and nucleus in TNF-α-induced NPCs, we indeed observed that Rg3 could decrease NF-κB p65 expression in the cytoplasm but increase its levels in the nucleus in a concentration-dependent manner, suggesting that Rg3 may play its anti-oxidation role via mediating the NF-κB signaling pathway [6]. Importantly, evidence identified that NF-κB was closely involved in the TNF-α-dependent expression of MMP-3 in NPCs [41]. Further, a number of studies noted that reducing oxidative stress-activated NF-κB could inhibit the cell apoptosis [42,43], which further added weight to the hypothesis that Rg3 may block the NF-κB signaling pathway to alleviate the oxidative stress-induced damage and apoptosis of human NPCs treated by TNF-α. No matter which factors triggered, IDD is ultimately marked by the imbalanced ECM metabolism in the nucleus pulposus of intervertebral discs, specifically, leading to the enhancement of ECM degradation and the reduction of ECM, like proteoglycan and collagen, thus aggravating the pathogenic progression of IDD [44,45]. Finally, this study also found that treatment with both TNF-α and Rg3 in NPCs resulted in lower expressions of ECM degrading genes (MMP3 and ADAMTS5) and higher expressions of ECM synthesis genes (Aggrecan and COL2A1) in an Rg3-concentration dependent manner than TNF-α-only treatment. As a matter of fact, SIRT6 can block the degradation of ECM nucleus pulposus cells in vitro by inhibiting the NF-κB-dependent transcription activity [44], suggesting that Rg3 can promote the synthesis of ECM and play the protective role in IDD via regulation of NF-κB signaling pathway.

To sum up, we found that ginsenoside Rg3 may block the NF-κB signaling pathway to inhibit the oxidative stress-induced damage, ameliorate cell cycle distribution, reduce cell apoptosis, and promote ECM metabolism in human NPCs induced by TNF-α, which offers a promising scientific clue for the clinical treatment of IDD.

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Disclosure of conflict of interest

None.

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