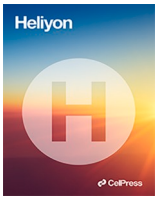
Heliyon 10 (2024) e37877

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Diacetoxy-6-gingerdiol protects the extracellular matrix of nucleus pulposus cells and ameliorates intervertebral disc degeneration by inhibiting the IL-1β-mediated NLRP3 pathway

Huifeng Xi [a,1], Yuesong Weng [b,1], Youmao Zheng [a], Lizhi Wu [a], Dawei Han [a,*]

[a] Department of Orthopedics, Taizhou Hospital Affiliated to Wenzhou Medical University, Linhai, Zhejiang, China
[b] Linhai Hospital of Traditional Chinese Medicine Healthcare Service Community, Linhai, Zhejiang, China

ARTICLE INFO

Keywords:
Gingerdiol
NLRP3
Intervertebral disc degeneration
Nucleus pulposus cells

ABSTRACT

Intervertebral disc degeneration (IDD) is a common cause of low back pain, causing a huge emotional and economic burden on patients and society. Reduction of nucleus pulposus cells (NPC) and extracellular matrix (ECM) is the main feature of IDD, and NPC is the main source of ECM. Thermal apoptosis is a newly discovered form of cell death in recent years that differs significantly from apoptosis in terms of molecular mechanisms and cellular morphological changes. Diacetoxy-6-gingerdiol(D-6-G), a type of gingerol, has anti-inflammatory and antioxidant effects, but whether it has an inhibitory effect on cellular pyroptosis is not clear. Therefore, in the present study, we investigated the effect of D-6-G on the ECM of the nucleus pulposus oblongata under IL-1β treatment, as well as the mechanism of its effect on NLRP3 inflammasome and cellular focal death. In vitro cellular experiments demonstrated that D-6-G could bind to and inhibit the activity of NLRP3 inflammasome, and interestingly, D-6-G could also inhibit cellular pyroptosis and protect the nucleus pulposusry cellular microenvironment by activating the Nrf2/HO-1 axis. In conclusion, we found that D-6-G could inhibit NLRP3 inflammatory vesicle activity as well as cellular pyroptosis in NPCs and protect the ECM, suggesting the potential of D-6-G to delay IDD.

1. Introduction

Low back pain (LBP) is currently the leading cause of adult disability [1]. In North America, more than 80 % of people have experienced varying degrees of LBP in their lifetime, and the annual economic expenditure caused by LBP exceeds 10 billion US dollars [2]. Although the cause of LBP remains to be further elucidated, it is often associated with Intervertebral disc degeneration (IDD) [3]. IDD is a degenerative disease that occurs in the intervertebral disc and can affect the intervertebral joints and nervous system. The intervertebral disc has a certain degree of elasticity and toughness, and plays a buffering and load-bearing role during spinal activity [4]. The number of cells is reduced, the function is abnormal, and the components of extracellular matrix (ECM) are reduced and the proportion is changed. The synthesis of ECM mainly depends on cells in the intervertebral disc [5]. A deeper understanding of the

* Corresponding author. Department of Orthopedics at the Taizhou Hospital which is affiliated with the Wenzhou Medical University in Linhai, Zhejiang, China.
E-mail address: brucehd123@126.com (D. Han).
[1] Huifeng Xi and Wenjun Pan contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e37877
Received 7 September 2024; Accepted 11 September 2024
Available online 18 September 2024

physiological properties of cells within the intervertebral disc will help find new strategies for treating IDD. It is generally believed that nucleus pulposus cells (NPCs) are responsible for the biosynthesis of ECM in the nucleus pulposus region, major synthesis of collagen II (COL II) and aggrecan (ACAN) [5], The ECM is degraded by Matrix Metalloproteinase 3 (MMP3) and A Disintegrin and Metalloproteinase with Thrombospondin motifs 4 (Adamts4), which belong to the family of protein hydrolases [6], and this region is the first to show degenerative changes in the process of IDD. Exploring the causes of abnormal ECM synthesis and the underlying molecular biological mechanisms will facilitate the search for new targets for early intervention in the pathological process of IDD.

Regular consumption of fresh fruits and vegetables can reduce the prevalence and prognosis of many diseases, according to numerous studies [7]. Ginger rhizome is a spice and herb that is loved all over the world. It's great to know that this contains gingerols, which are a group of pungent phenols. These compounds have various biological activities, such as anti-inflammatory, antioxidant, and even anticancer properties [8,9]. Therefore, gingerols are of great interest as potential therapeutic agents for the prevention and/or treatment of various diseases and for their safety for human use. Diacetoxy-6-gingerdiol (D-6-G) is one of the gingerols extracted from Dracocephalum heterophyllum [10]. Previous studies have shown that Nrf2 is mainly regulated by Kelch-like ECH-related protein 1 (Keap1), and that the presence of phytochemicals in food specifically reacts with the cysteine residues of Keap1, resulting in a conformational change that reduces the antioxidant effect of Nrf2 [11]. In this case, the addition of gingerol increases the expression of Nrf2 [12] and its downstream genes, such as heme oxygenase 1 (HO-1) [9], down-regulated NOD-, LRR- and pyrin domain-containing protein 3 (NLR3) expression [13,14].

2. Method

2.1. Reagents and antibodies

D-6-G (purity >99 %) and IL-1β (purity >99 %) were purchased from MCE (Shanghai, China). Primary antibodies against ADAMTS4 (#ab179475, 1:1000), MMP3 (#ab76110, 1:1000) were from Abcam (Cambridge, MA. USA). The secondary Antibodies Alexa Fluor 488(#HA1121, 1:200) and Alexa Fluor 594(#HA1122, 1:200) were products from Huabio (Hangzhou, China). The antibody Collagen II (#K009364P, 1:500) was purchased from Solarbio (Beijing, China). the abtibody Aggrecan (#PA1-1746, 1:1000) was obtained from Invitrogen (Beverly, MA, USA). The Anti-rabbit IgG, HRP Antibody (#S0001, 1:20000) were products from Affbiotech (Changzhou, China).

2.2. Cell culture

Rat nucleus pulposus cells were donated by Professor Di Chen from Rush University in the United States. Cells were cultured at 37 °C, 5 % CO2 and 94 % DMEM medium, 5 % fetal bovine serum and 1 % penicillin-streptomycin double antibody, and the medium was changed daily.

2.3. High-density cell culture

10 μl of NPCs at 10^7 cells/mL were inoculated into 24-well plates for 6h. After the cells were fully attached, cell culture medium and appropriate drugs were added and the medium was changed every other day for one week. The cells were then fixed with paraformaldehyde and stained according to the instructions of the toluidine blue staining kit. Finally, the 24-well plates were scanned and photographed using an EPSON V600 photo scanner (Japan).

2.4. Cell viability assay

Viability of D-6-G-treated NPCs was detected using the CCK-8 Cell Proliferation/Cytotoxicity Assay Kit. Following the kit

Table 1
Primer.

Gene	Primer sequences (5-3)	
ADAMTS 4	Forward	CGCTGAGTAGATTCGTGGAGAC
	Reverse	AGTTGACAGGGTTTCGGATGC
COL II	Forward	TGACCTGACGCCCATTCATC
	Reverse	TTTCCTGTCTCTGCCTTGACCC
MMP3	Forward	TGAGCAGCAACCAGGAATAGG
	Reverse	TGAGCAGCAACCAGGAATAGG
Aggrecan	Forward	AGGTGTCGCTCCCCAACTAT
	Reverse	CTTCACAGCGGTAGATCCCAG
NLRP3	Forward	CAGCCCCATGAGTTCCCTT
	Reverse	GTCTCCCCGCACAGCCT
GSDMD	Forward	CCAACATCTCAGGGCCCCAT
	Reverse	TGGCAAGTTTCTGCCCTGGA
GAPDH	Forward	ACCCAGAAGACTGTGGATGG
	Reverse	ACCCAGAAGACTGTGGATGG

instructions, $2x10^3$ NPCs were treated with serial dilutions of D-6-G (0–100 nM) for 24 or 48 h. 100 μl of DMEM containing CCK-8 solution was added to each well, and incubated at 37 °C for 90 min. Finally, the absorbance of each well was measured using a Multiskan FC microwell photometer (Thermo Fisher Scientific, Waltham, MA).

2.5. Real-time PCR analysis

Total RNA was extracted using the RN28-EASYspin Plus Tissue/Cellular Rapid RNA Extraction Kit (#RN2802) purchased from Aidlab Biotechnologies Co.,Ltd and samples were tested for purity and concentration. Then, using a HiFiScript cDNA Synthesis kit (Cwbiotech, Beijing, China), total RNA was reverse transcribed into cDNA. qRT-PCR was carried out on an ABI 7500 Real-Time PCR Detection System (Applied Biosystems, Foster City, CA, USA). Table 1 shows all the primers for detecting the target genes.

2.6. Western blot

Total proteins from NPCs were lysed and extracted using radioimmunoprecipitation buffer (RIPA) (Bioscia, China) containing phenylmethylsulfonyl fluoride (PMSF) (100:1).Protein samples were centrifuged at 4°C and 12,000 rpm for 15 min, and then protein concentration was determined using the BCA Protein Assay Kit (AMEKO, Shanghai, China). Gels were prepared according to the instructions of the PAGE Gel Fast Preparation Kit (Epizyme, Shanghai, China), and protein samples were separated and then transferred to PVDF membranes (Bio-Rad, Hercules, CA, United States). The membrane was then sealed for 50 min at room temperature using blocking buffer (Biosharp, Hefei, China). The PVDF membrane was incubated overnight at 4°C with primary antibody and then incubated with secondary antibody for 1h at room temperature. Antibody reactivity was detected using an ultra-sensitive ECL chemiluminescence kit (NCM Biotechnology, Suzhou, China) and ImageQuant LAS 500 (GE Health Care, Fairfield, CT, United States), and the intensity of the bands was quantified by ImageJ software.

2.7. Immunofluorescence

Samples of NPCs after 48 h of IL-1β (20 ng/ml) or G-6-D stimulation were fixed with 4 % paraformaldehyde (PFA) for 15 min and then permeabilized with 0.5 % TritonX-100 for 30 min.It was then blocked with 1 % bovine serum albumin (BSA) for 30 min and incubated overnight with anti-NLRP3 antibody or anti-Nrf2 antibody.The next day, the cells were incubated with fluorescently labeled secondary antibody for 30 min. Finally, NP cells were counterstained with DAPI for 10 min, observed and photographed under the immunofluorescence microscope.

2.8. Protein-ligand molecular docking

The crystal structure of the target protein NLRP3 (PDBID: 2naq and 7mx3) was obtained from the protein database RCSB, and the structure of diacetoxy-6-gingerdiol was obtained from the Pubchem database. Separation of Primary Ligand and Protein Structures, Dehydration and Removal of Organic Matter with PyMOL Software. Hydrogenation was performed with AutodockTools, charge was checked, and the docking grid box for the protein structure was constructed, in addition, the Root of the small molecule ligand was determined, and the ligand's torsionally reversible bond was selected in AutodockTools. Lastly, the protein structure and small molecule ligand format was converted from ".PDB" to ".PDBQT" in AutodockTools. Docking was performed using Vina, then protein-compound docking scores were calculated and the results were analyzed and visualized in 3D and 2D using the PLIP online website and Discovery Studio software.

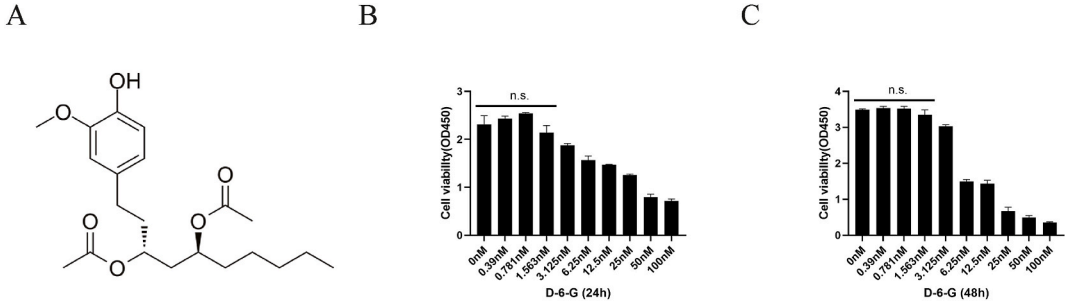


Fig. 1. The effect of D-6-G on the viability and proliferation of NPCs. (A) The chemicalstructure of Td. (B) Cell counting Kit-8 assay results of NPCs stimulated with with different equiproportionate concentrations (0–100 μM) of D-6-G at 24h. (C) Cell counting Kit-8 assay results of NPCs stimulated with with different equiproportionate concentrations (0–100 μM) of D-6-G at 48h. All data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, n = 3.

2.9. Statistical analysis

All experiments were independently repeated three times. Quantitative results analyzed by GraphPad Prism (USA) are expressed as mean ± standard deviation (SD). A p-value of less than 0.05 was regarded as statistically significant.

3. Result

3.1. Cytotoxicity and potential cytoprotective effects of G-6-D

Fig. 1A is a schematic diagram of the chemical structure of D-6-G. To test the toxic and protective effects of D-6-G on NPCs, we incubated equal amounts of NPCs with different concentrations of D-6-G for 24h and 48h.We found that D-6-G was not positively cytotoxic to NPCs at concentrations of 1 nM or lower(Fig. 1B and C), and therefore we used concentrations of 1 nM and 0.5 nM for NPCs in all subsequent experiments.

3.1.1. D-6-G may target NLRP3 for action in NPCs

To demonstrate that D-6-G can play an important role in NPCs, we molecularly docked D-6-G with NLRP3.The results showed that the average binding energies between the target protein NLRP3 and compound D-6-G were −7.0 kcal/mol Quote Table 2, indicating that the active ingredient had a strong affinity activity with the target (Fig. 2A-B). We then used qPCR and Western blot to verify again. As shown, D-6-G plays an important role for NLRP3 in NPCs, and the results were analyzed to conclude that in the IL-1β-induced IDD model, D-6-G was able to play a protective role by decreasing the expression of NLRP3 in NPCs (Fig. 2C-E).

3.2. D-6-G promotes anabolism and inhibits catabolism of ECM in NPCs

In order to investigate whether D-6-G has a protective effect against IDD, we first performed qPCR and west blot experiments, and we found that D-6-G promoted the expression of the anabolic markers Col-2 and Aggrecan, and decreased the expression of the catabolic markers ADAMTS-4 and MMP3, in an in vitro model of IDD stimulated by IL-1β(Fig. 3A-C). Subsequently, we revalidated the marker Col-2 using cytofluorescence assay and showed that D-6-G significantly promoted the expression of the marker Col-2(Fig. 3D-E). All of which indicated that D-6-G could play a protective role against nasopharyngeal carcinoma in an in vitro IDD model.

3.3. D-6-G inhibits pyroptosis in NPCs

Subsequently we investigated the effect of D-6-G on pyroptosis in NPCs.First, we found by qPCR that in the IL-1β-induced IDD model, the expression of the pyroptosis marker GSDMD was significantly suppressed by G-6-D(Fig. 4A)We then similarly verified by Western blot that D-6-G inhibited the expression of the pyroptosis pathway marker proteins GSDMD, Caspase-1 and IL-1β in the IL-1β-induced IDD model(Fig. 4B-C).These results suggest that D-6-G inhibits pyroptosis in NPCs.

3.4. D-6-G functions through Nrf2/HO-1/NLRP3 axis

However, we found by Western blot that D-6-G promoted the expression of Nrf2 and HO-1 in the IL-1β-induced IDD model(Fig. 5A-B).We examined Nrf2 by cytofluorescence under the same conditions, and D-6-G similarly promoted Nrf2 expression(Fig. 5C-D).Taken together with previous experiments, we suggest that D-6-G may act through Nrf2/HO-1/NLRP3 in NPCs to mitigate the progression of IL-1β-induced IDD models.

4. Discussion

Our study revealed that D-6-G is an inhibitor of NLRP3 inflammasome. In NP cells, D-6-G inhibited IL-1β-induced NLRP3 inflammasome activation. Interestingly, D-6-G could inhibit Nrf2 and its downstream HO-1 expression by upregulating Nrf2 and its

Table 2
Average binding energies.

mode	affinity (kcal/mol)	dist from best mode	dist from best mode
Diacetoxy-6-gingerdiol -NLRP3			
1	6.6	0.000	0.000
2	6.4	1.702	3.878
3	6.4	2.216	7.503
4	6.2	2.646	5.643
5	6.2	1.621	3.126
6	6.1	2.868	6.591
7	6.1	2.353	8.162
8	6.0	1.923	3.550
9	5.9	1.841	6.759

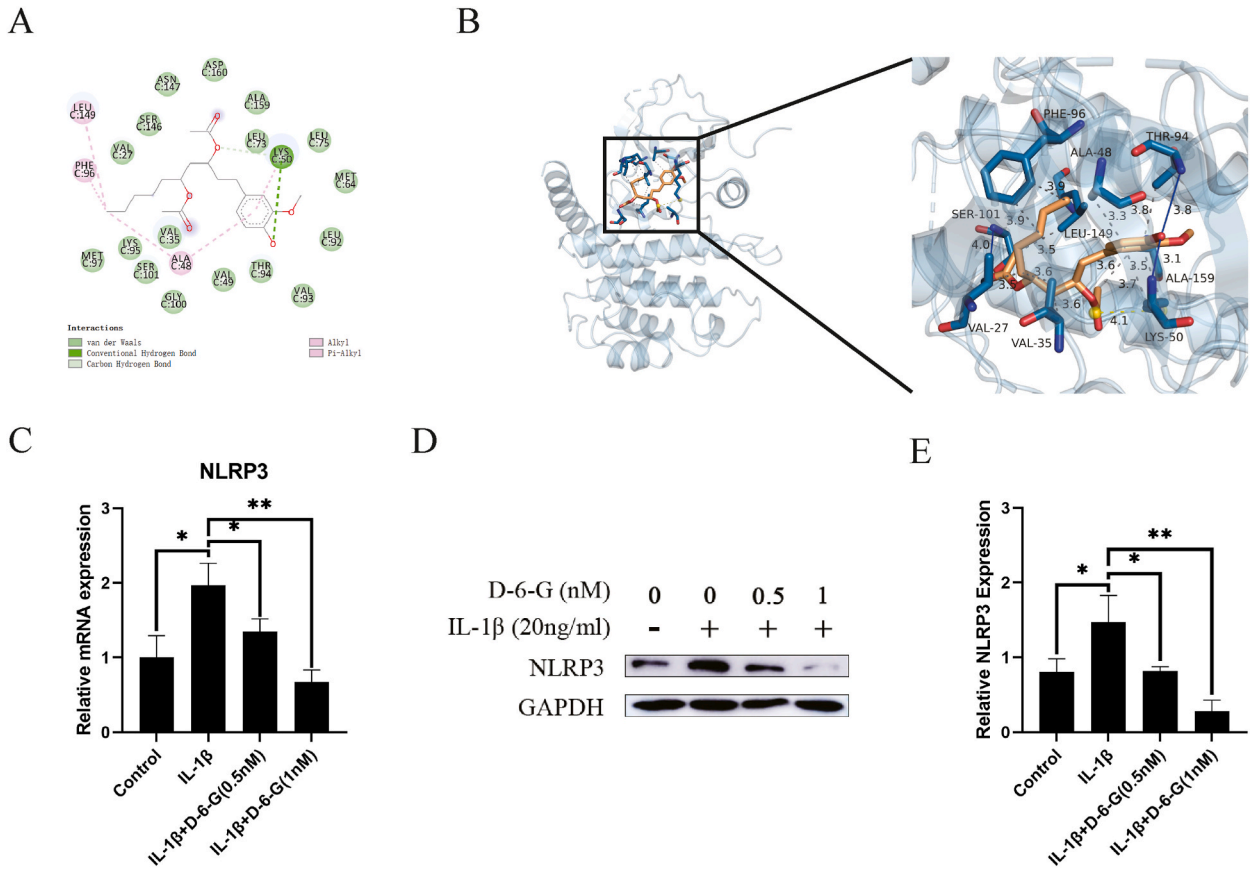


Fig. 2. D-6-G targets NLRP3 in NPCs. (A) Results of D-6-G force analysis of 2D images. (B) The 3D structure image of D-6-G interacting with NLRP3 in the image. (C) The gene expression NLRP3 was detected by qPCR in the NPCs, treated with or without the administration of IL-1β for 24h. (D,E) Western blot analysis of NLRP3 and GAPDH expression in NPCs stimulated with IL-1β (20 ng/ml). The protein expression ware evaluated by Image J. All data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, n = 3.

downstream HO-1 expression (Fig. 6).

Balancing the homeostasis of the ECM environment in NPCs after inflammatory stimuli is extremely important, especially for the regulation of anabolism and catabolism [15]. Anabolism mainly consists of COLL II and ACAN, and COLL II is an important component of the nucleus pulposus, which provides the main structural support of the nucleus pulposus [16]. In addition, COL II is involved in cell adhesion and migration processes, providing a suitable microenvironment for cell growth and differentiation [17]. Aggrecan, on the other hand, is an important component of the ECM, which is a macromolecular polysaccharide protein formed by the combination of core proteins with glycosaminoglycans (GAGs) [18]. Aggrecan is able to bind tightly with proteins such as COL II, and together they form the complex network structure of the nucleus pulposus [19]. This structure not only provides pressure resistance and elasticity to the nucleus pulposus, but also absorbs and disperses external forces to protect the spine from injury [20]. MMP3 and ADAMTS4 are closely related to enzymes that act as the ECM, and have an important influence on the balance of the extracellular matrix of the nucleus pulposus [21]. MMP3 is a key protease for degrading articular cartilage, and it is able to break down proteoglycans, laminin, fibronectin, cartilage linker proteins, fibronectin, and various collagens, etc [22]. ADAMTS4 has a catalytic structural domain, a deintegrin structural domain, and a carboxy-terminal structural domain with a TSP-1 motif, which gives it multiple biological functions [23,24]. These functions include degradation of specific components of the ECM and participation in the regulation of extracellular matrix homeostasis in the medulla [25]. Our study demonstrated that D-6-G can contribute to the maintenance of ECM homeostasis and stabilize the intravertebral disc environment by increasing the expression of COL2 and ACAN and inhibiting the expression of MMP3 and adamts4 in IL-1β-treated NPCs.

In this experiment, we also explored the effect and mechanism of action of D-6-G on IL-1β-mediated NPCs pyroptosis and found that D-6-G exerted a protective effect on NPCs pyroptosis by up-regulating Nrf2. D-6-G is one of the gingerol extracted from ginger, and gingerol have been reported to have the effect of delaying regression and ameliorating inflammation [9], but the potential mechanism of action still needs to be further investigated. Cellular pyroptosis belongs to a type of inflammatory cell death, so we previously hypothesized that D-6-G may have a potential role in inhibiting cellular pyroptosis. In this study, treatment of NPCs with D-6-G for 24 h along with IL-1β treatment effectively alleviated the up-regulation of scorch death-related proteins in NPCs, revealing the protective effect of D-6-G against IL-1β-induced scorch death of NPCs in degenerated intervertebral discs. Recent studies have shown a close

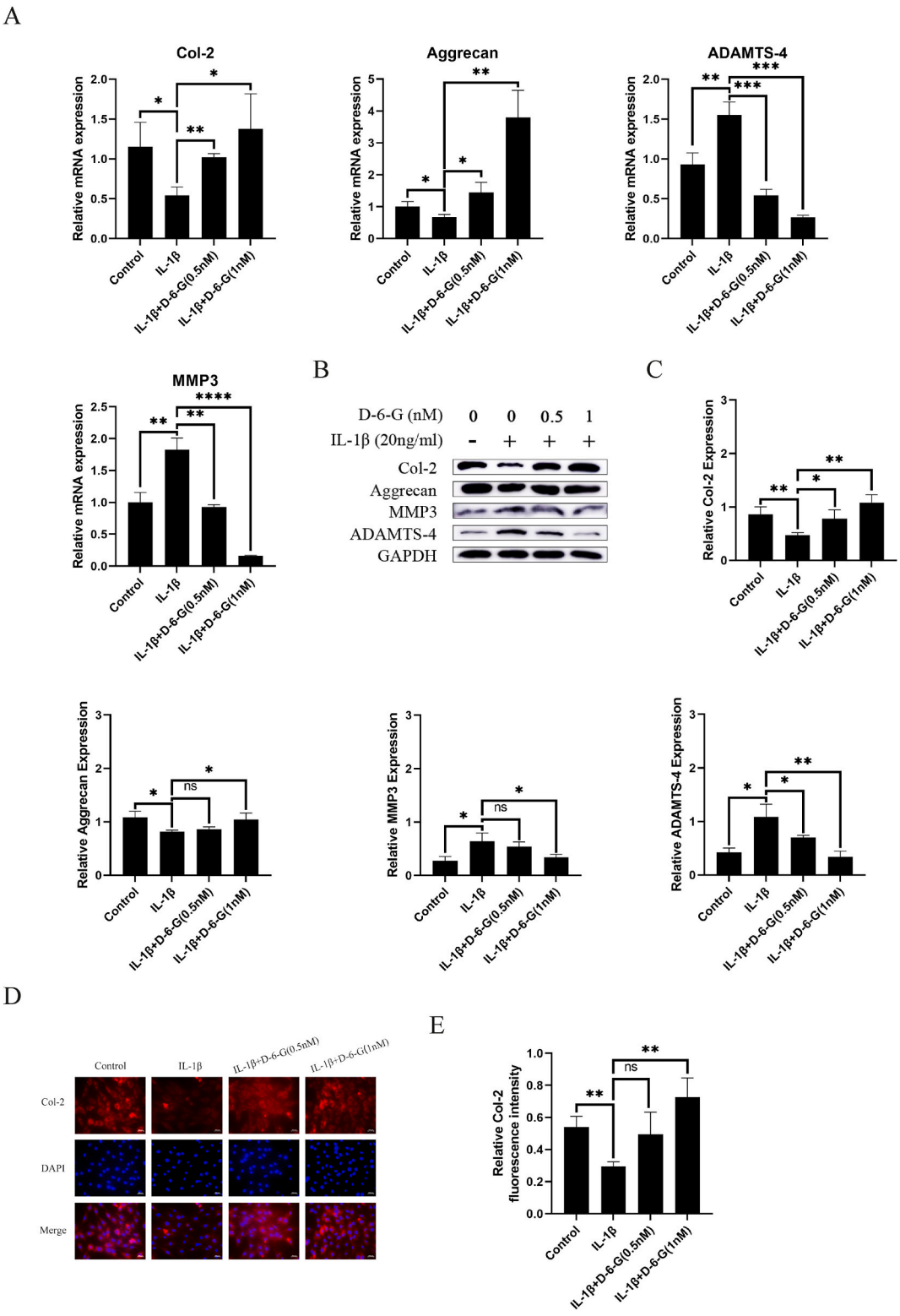


Fig. 3. Effect of D-6-G on ECM secretion by NPCs. (A) The gene expression Col-2, Aggrecan, ADAMTS-4 and MMP3, were detected by qPCR in the NPCs, treated with or without the administration of IL-1β for 24h. (B,C) Western blot analysis of Col-2, Aggrecan, ADAMTS-4,MMP3 and GAPDH expression in NPCs stimulated with IL-1β (20 ng/ml). The protein expressionware evaluated by Image J. (D,E) The protein expression of Col-2 was evaluated by cell immunofluorescence (scale bar: 100 μm) and Image J. All data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, n = 3.

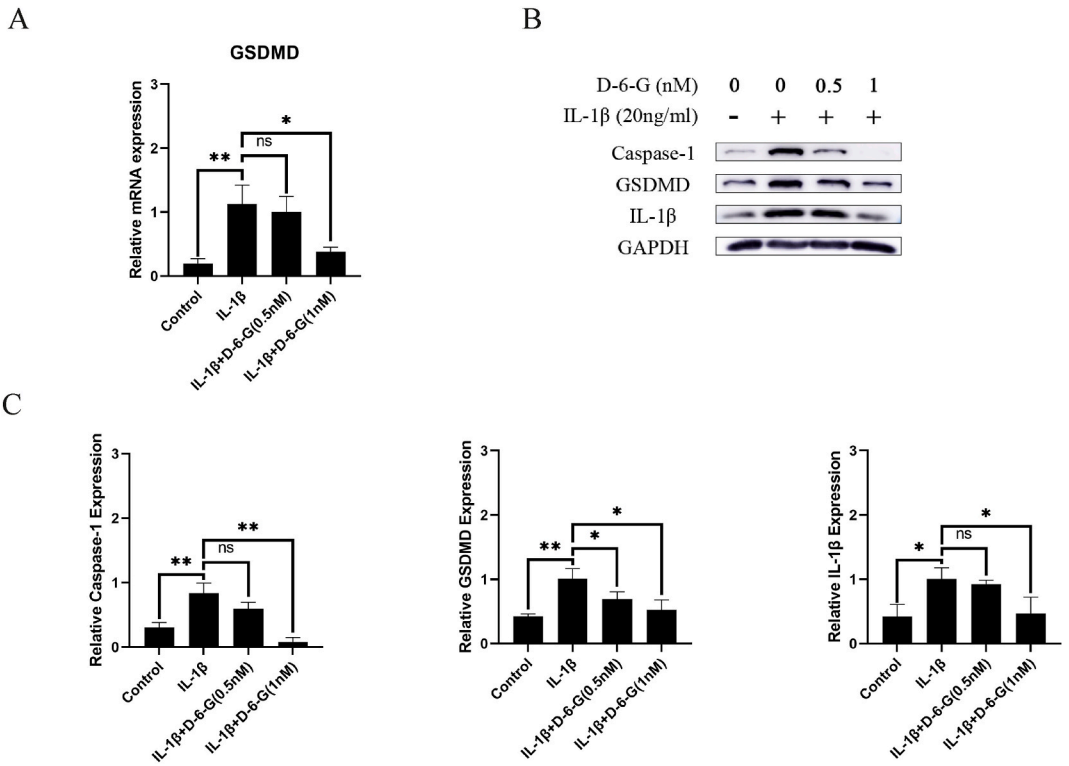


Fig. 4. Effect of D-6-G on pyroptosis in NPCs. (A) The gene expression GSDMD was detected by qPCR in the NPCs, treated with or without the administration of IL-1β for 24h. (B,C) Western blot analysis of Caspase-1,GSDMD,IL-1β and GAPDH expression in NPCs stimulated with IL-1β (20 ng/ml). The protein expression ware evaluated by Image J. All data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, n = 3.

relationship between Nrf2 and pyroptosis [26,27], Nrf2 inhibits the activation of NLRP3 inflammasome through several mechanisms. First, Nrf2 upregulates the expression of antioxidant genes, such as HO-1, and these antioxidant proteins are capable of scavenging reactive oxygen species (ROS) produced intracellularly [28]. Since ROS is one of the important triggers for the activation of NLRP3 inflammasome, Nrf2 can indirectly inhibit the activation of NLRP3 inflammasome by reducing the production of ROS [29]. Second, Nrf2 can also directly inhibit the expression of constituent proteins of NLRP3 inflammasome. For example, Nrf2 can inhibit the expression of inflammatory vesicle-associated proteins such as NLRP3, ASC (apoptosis-associated speck-like protein) and Caspase-1, thereby preventing the assembly and activation of inflammasome [30]. In addition, Nrf2 can inhibit the activation of NLRP3 inflammasome by regulating downstream signaling pathways. For example, Nrf2 can activate the PI3K/Akt signaling pathway, which inhibits the activation of NLRP3 inflammasome and promotes the production of anti-inflammatory factors [31]. In summary, Nrf2 inhibits the activation of NLRP3 inflammasome and exerts anti-inflammatory effects through various mechanisms such as reducing ROS production, inhibiting the expression of inflammatory vesicle-associated proteins, and modulating the downstream signaling pathways, etc. Therefore, in-depth studies on the relationship between Nrf2 and pyroptosis are very important for the understanding of the regulatory mechanisms of cell death and inflammatory responses and the development of new therapeutic strategies.

Our experiment still has some limitations, firstly this experiment mainly focuses on the study of nucleus pulposus (NP) cells, while the influence of disc degeneration is multifaceted, the annulus fibrosus (AF) and cartilaginous endplates contain a large number of COLL II and chondrocytes [32,33], and the occurrence of clefts in the annulus fibrosus leads to extrusion of the NPs, and allows the nociceptors to grow inward, and blood vessels are formed in the internal AF and NPs, which leads to discogenic back pain [34]. Subsequently, our experiments lacked the in vivo evidence that the mouse model of aging is the most consistent with the physiological changes of disc degeneration [35], so our next phase of experiments will focus on the changes in the progression of intervertebral disc degeneration in the aging model under the treatment of D-6-G, as well as the effects on the various structures of the intervertebral discs.

Currently, the mainstream drug therapy for early and middle stage IDD is based on symptom control drugs such as non-steroidal anti-inflammatory analgesics and glucocorticosteroids [36], which not only fail to control the progression of IDD, but also bring many adverse effects such as gastrointestinal bleeding and cardiovascular accidents in the long term [37]. Therefore, the development of new drugs for the treatment of IDD is a hotspot of current research in the field of orthopedics. Chinese medicine has a long history of treating cartilage degeneration. In recent years, Chinese and foreign scholars have carried out a series of studies on the therapeutic effects of active ingredients in TCM on cartilage degeneration. Han et al. found that ginger extract 6- gingerol increased the expression of Bcl-2, decreased the expression of Bax and caspase-3, attenuated hydrogen peroxide-induced apoptosis of myeloid-derived mesenchymal stem cells, protected against ECM invasiveness, and delayed the progression of IDD [38]. Wang et al. demonstrated

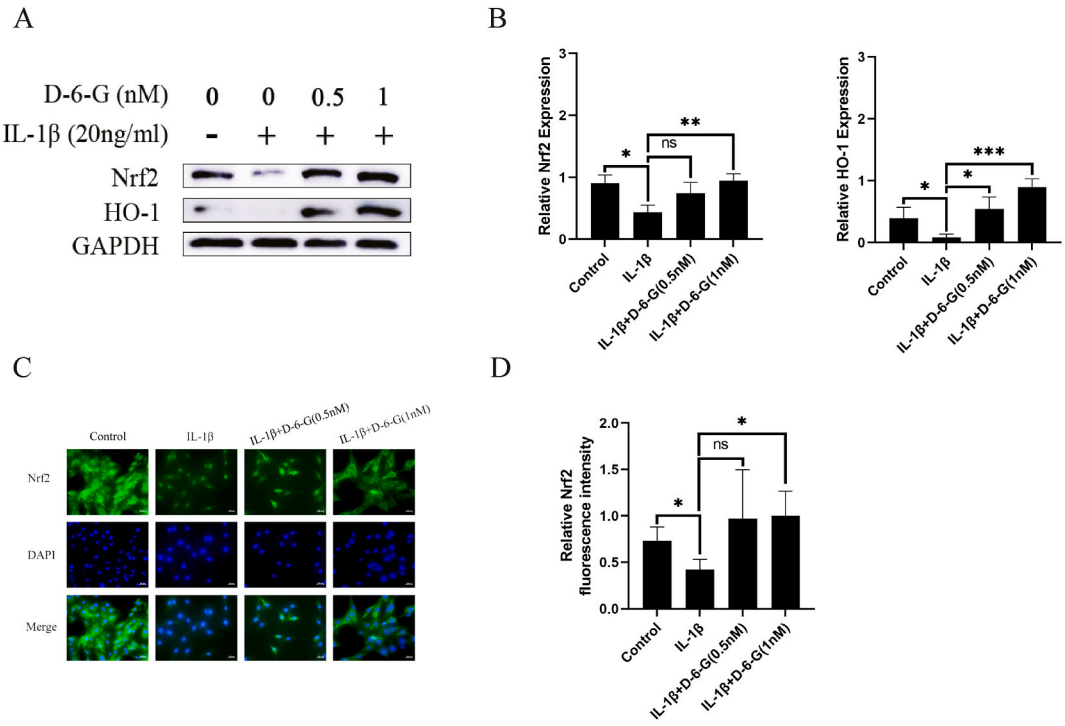


Fig. 5. D-6-G may act through the Nrf2/HO-1/NLRP3 axis. (A,B) Western blot analysis of Nrf2 , HO-1 and GAPDH expression in NPCs stimulated with IL-1β (20 ng/ml). The protein expression ware evaluated by Image J. (C,D) The protein expression of Nrf2 was evaluated by cell immunofluorescence (scale bar: 100 μm) and image J. All data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, n = 3.

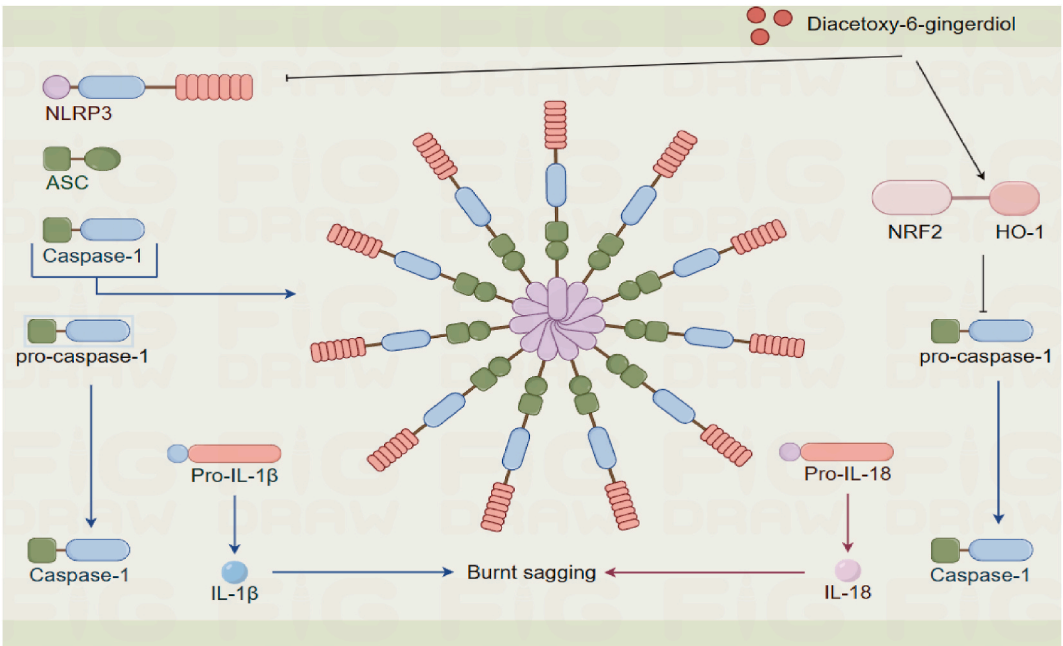


Fig. 6. Schematic representation of D-6-G inhibiting IDD. D-6-G inhibits pyroptosis in NPCs by promoting the activation of Nrf2/HO-1/NLRP3 signaling.

that Weilingxian saponin maintains cartilage extracellular matrix homeostasis and delays degenerative changes in articular cartilage by inhibiting IL-1β-induced MMP-3, MMP-13, and proteoglycanase epi [39]. Hwang et al. found that 6-gingerol inhibited IL-1-induced osteoclast differentiation by reducing PGE_2 levels to suppress RANKL expression in osteoblasts [40], A variety of active ingredients extracted from traditional Chinese medicines have been shown to have anti-inflammatory and antioxidant effects.D-6-G is a gingerol extracted from Dracocephalum heterophyllum, and current studies on D-6-G are limited to the discovery of anti-inflammatory and antioxidant effects. Our experiments firstly demonstrated that D-6-G could effectively protect NPCs from cellular damage from scorched death.

In summary, our study revealed for the first time the therapeutic effects of D-6-G on NPCs, which delayed the progression of IDD by upregulating NRF2 expression, activating downstream HO-1, scavenging intracellular oxygen radicals, and inhibiting the activation of NLRP3 inflammasome as well as cellular pyroptosis. The modulatory effect of D-6-G on NLRP3 inflammasome activation makes D-6-G a novel candidate for the future treatment of IDD.

Funding

Science and Technology Plan Project of Taizhou (24ywb15). Basic Public Welfare Research Project of Zhejiang Province (LGF21H060008).

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, Zhangfu Wang and Zhi Lin, upon reasonable request.

Ethics approval

This is an observational study. The Taizhou Hospital Research Ethics Committee has confirmed that no ethical approval is required.

CRediT authorship contribution statement

Huifeng Xi: Writing – review & editing. **Yuesong Weng:** Software, Supervision. **Youmao Zheng:** Data curation, Formal analysis, Investigation. **Lizhi Wu:** Validation, Writing – original draft. **Dawei Han:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] T. Vos, A.D. Flaxman, M. Naghavi, et al., Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010, Lancet (London, England) 380 (9859) (2012) 2163–2196, https://doi.org/10.1016/s0140-6736(12)61729-2.
[2] J.N. Katz, Lumbar disc disorders and low-back pain: socioeconomic factors and consequences, J. Bone Jt. Surg. Am. Vol. 88 (Suppl 2) (2006) 21–24, https://doi.org/10.2106/jbjs.E.01273.
[3] S. Baliga, K. Treon, N.J. Craig, Low back pain: current surgical approaches, Asian spine journal 9 (4) (2015) 645–657, https://doi.org/10.4184/asj.2015.9.4.645.
[4] S. Kirnaz, C. Capadona, T. Wong, et al., Fundamentals of intervertebral disc degeneration, World neurosurgery 157 (2022) 264–273, https://doi.org/10.1016/j.wneu.2021.09.066.
[5] S.M. Rider, S. Mizuno, J.D. Kang, Molecular mechanisms of intervertebral disc degeneration, Spine surgery and related research 3 (1) (2019) 1–11, https://doi.org/10.22603/ssrr.2017-0095.
[6] H. Ishino, I. Sakonju, Expression of metalloproteinases and their inhibitors in degenerated and extruded intervertebral disks in chondrodystrophic dogs, J. Vet. Med. Sci. 83 (4) (2021) 637–642, https://doi.org/10.1292/jvms.20-0317.
[7] B. Zhou, P. Perel, G.A. Mensah, et al., Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension, Nat. Rev. Cardiol. 18 (11) (2021) 785–802, https://doi.org/10.1038/s41569-021-00559-8.
[8] Y. Gao, Y. Lu, N. Zhang, et al., Preparation, pungency and bioactivity of gingerols from ginger (Zingiber officinale Roscoe): a review, Crit. Rev. Food Sci. Nutr. (2022) 1–26, https://doi.org/10.1080/10408398.2022.2124951.
[9] R.M.T. DE Lima, A.C. Dos Reis, A.P.M. DE Menezes, et al., Protective and therapeutic potential of ginger (Zingiber officinale) extract and [6]-gingerol in cancer: a comprehensive review, Phytother Res. : PTR 32 (10) (2018) 1885–1907, https://doi.org/10.1002/ptr.6134.
[10] J. Dang, Y. Lv, C. Li, et al., Integrated chromatographic approach for the discovery of gingerol antioxidants from Dracocephalum heterophyllum and their potential targets, Analytical methods : advancing methods and applications 14 (41) (2022) 4133–4145, https://doi.org/10.1039/d2ay01282k.
[11] X. Duan, J. Li, W. Li, et al., Antioxidant tert-butylhydroquinone ameliorates arsenic-induced intracellular damages and apoptosis through induction of Nrf2-dependent antioxidant responses as well as stabilization of anti-apoptotic factor Bcl-2 in human keratinocytes, Free Radic. Biol. Med. 94 (2016) 74–87, https://doi.org/10.1016/j.freeradbiomed.2016.02.009.
[12] S.M.U. Ahmed, L. Luo, A. Namani, et al., Nrf2 signaling pathway: pivotal roles in inflammation, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1863 (2) (2017) 585–597, https://doi.org/10.1016/j.bbadis.2016.11.005.
[13] Q. Cui, Z. Zhang, X. Tian, et al., Bifidobacterium bifidum ameliorates DSS-induced colitis in mice by regulating AHR/NRF2/NLRP3 inflammasome pathways through indole-3-lactic acid production, J. Agric. Food Chem. 71 (4) (2023) 1970–1981, https://doi.org/10.1021/acs.jafc.2c06894.
[14] S. Chen, X. Wu, Y. Lai, et al., Kindlin-2 inhibits NLRP3 inflammasome activation in nucleus pulposus to maintain homeostasis of the intervertebral disc, Bone research 10 (1) (2022) 5, https://doi.org/10.1038/s41413-021-00179-5.

[15] H. Xing, Z. Zhang, Q. Mao, et al., Injectable exosome-functionalized extracellular matrix hydrogel for metabolism balance and pyroptosis regulation in intervertebral disc degeneration, J. Nanobiotechnol. 19 (1) (2021) 264, https://doi.org/10.1186/s12951-021-00991-5.
[16] X. Zhou, N. Shen, Y. Tao, et al., Nucleus pulposus cell-derived efficient microcarrier for intervertebral disc tissue engineering, Biofabrication 15 (2) (2023), https://doi.org/10.1088/1758-5090/acb572.
[17] S. Chen, P. Fu, H. Wu, et al., Meniscus, articular cartilage and nucleus pulposus: a comparative review of cartilage-like tissues in anatomy, development and function, Cell Tissue Res. 370 (1) (2017) 53–70, https://doi.org/10.1007/s00441-017-2613-0.
[18] C. Kiani, L. Chen, Y.J. Wu, et al., Structure and function of aggrecan, Cell Res. 12 (1) (2002) 19–32, https://doi.org/10.1038/sj.cr.7290106.
[19] Z. Yang, X.J. Gao, X. Zhao, CDMP1 promotes type II collagen and aggrecan synthesis of nucleus pulposus cell via the mediation of ALK6, Eur. Rev. Med. Pharmacol. Sci. 24 (21) (2020) 10975–10983, https://doi.org/10.26355/eurrev_202011_23581.
[20] F. Lv, V.Y. Leung, S. Huang, et al., In search of nucleus pulposus-specific molecular markers, Rheumatology 53 (4) (2014) 600–610, https://doi.org/10.1093/rheumatology/ket303.
[21] Y. Zhang, F. He, Z. Chen, et al., Melatonin modulates IL-1β-induced extracellular matrix remodeling in human nucleus pulposus cells and attenuates rat intervertebral disc degeneration and inflammation, Aging 11 (22) (2019) 10499–10512, https://doi.org/10.18632/aging.102472.
[22] Y. Zhang, S. Li, P. Jin, et al., Dual functions of microRNA-17 in maintaining cartilage homeostasis and protection against osteoarthritis, Nat. Commun. 13 (1) (2022) 2447, https://doi.org/10.1038/s41467-022-30119-8.
[23] M.D. Tortorella, A.G. Tomasselli, K.J. Mathis, et al., Structural and inhibition analysis reveals the mechanism of selectivity of a series of aggrecanase inhibitors, J. Biol. Chem. 284 (36) (2009) 24185–24191, https://doi.org/10.1074/jbc.M109.029116.
[24] N.H. Lim, M. Kashiwagi, R. Visse, et al., Reactive-site mutants of N-TIMP-3 that selectively inhibit ADAMTS-4 and ADAMTS-5: biological and structural implications, Biochem. J. 431 (1) (2010) 113–122, https://doi.org/10.1042/bj20100725.
[25] N.V. Vo, R.A. Hartman, T. Yurube, et al., Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and degeneration, Spine J. : official journal of the North American Spine Society 13 (3) (2013) 331–341, https://doi.org/10.1016/j.spinee.2012.02.027.
[26] Y. Lin, T. Luo, A. Weng, et al., Gallic acid alleviates gouty arthritis by inhibiting NLRP3 inflammasome activation and pyroptosis through enhancing Nrf2 signaling, Front. Immunol. 11 (2020) 580593, https://doi.org/10.3389/fimmu.2020.580593.
[27] C.G. Hsu, C.L. ChávEZ, C. Zhang, et al., The lipid peroxidation product 4-hydroxynonenal inhibits NLRP3 inflammasome activation and macrophage pyroptosis, Cell Death Differ. 29 (9) (2022) 1790–1803, https://doi.org/10.1038/s41418-022-00966-5.
[28] C. Duan, H. Wang, D. Jiao, et al., Curcumin restrains oxidative stress of after intracerebral hemorrhage in rat by activating the Nrf2/HO-1 pathway, Front. Pharmacol. 13 (2022) 889226, https://doi.org/10.3389/fphar.2022.889226.
[29] Y.C. Cheng, L.W. Chu, J.Y. Chen, et al., Loganin attenuates high glucose-induced schwann cells pyroptosis by inhibiting ROS generation and NLRP3 inflammasome activation, Cells 9 (9) (2020), https://doi.org/10.3390/cells9091948.
[30] C. Zhang, M. Zhao, B. Wang, et al., The Nrf2-NLRP3-caspase-1 axis mediates the neuroprotective effects of Celastrol in Parkinson's disease, Redox Biol. 47 (2021) 102134, https://doi.org/10.1016/j.redox.2021.102134.
[31] Z. Gao, H. Zhan, W. Zong, et al., Salidroside alleviates acetaminophen-induced hepatotoxicity via Sirt1-mediated activation of Akt/Nrf2 pathway and suppression of NF-κB/NLRP3 inflammasome axis, Life Sci. 327 (2023) 121793, https://doi.org/10.1016/j.lfs.2023.121793.
[32] D. Stein, Y. Assaf, G. Dar, et al., 3D virtual reconstruction and quantitative assessment of the human intervertebral disc's annulus fibrosus: a DTI tractography study, Sci. Rep. 11 (1) (2021) 6815, https://doi.org/10.1038/s41598-021-86334-8.
[33] N. Newell, J.P. Little, A. Christou, et al., Biomechanics of the human intervertebral disc: a review of testing techniques and results, J. Mech. Behav. Biomed. Mater. 69 (2017) 420–434, https://doi.org/10.1016/j.jmbbm.2017.01.037.
[34] F.J. Lyu, H. Cui, H. Pan, et al., Painful intervertebral disc degeneration and inflammation: from laboratory evidence to clinical interventions, Bone research 9 (1) (2021) 7, https://doi.org/10.1038/s41413-020-00125-x.
[35] K. Vincent, S. Mohanty, R. Pinelli, et al., Aging of mouse intervertebral disc and association with back pain, Bone 123 (2019) 246–259, https://doi.org/10.1016/j.bone.2019.03.037.
[36] W. Liu, Z. Ma, Y. Wang, et al., Multiple nano-drug delivery systems for intervertebral disc degeneration: current status and future perspectives, Bioact. Mater. 23 (2023) 274–299, https://doi.org/10.1016/j.bioactmat.2022.11.006.
[37] Z. Krut, G. Pelled, D. Gazit, et al., Stem cells and exosomes: new therapies for intervertebral disc degeneration, Cells 10 (9) (2021), https://doi.org/10.3390/cells10092241.
[38] L. Nan, F. Wang, Y. Liu, et al., 6-gingerol protects nucleus pulposus-derived mesenchymal stem cells from oxidative injury by activating autophagy, World J. Stem Cell. 12 (12) (2020) 1603–1622, https://doi.org/10.4252/wjsc.v12.i12.1603.
[39] Z. Wang, J. Huang, S. Zhou, et al., Anemonin attenuates osteoarthritis progression through inhibiting the activation of IL-1β/NF-κB pathway, J. Cell Mol. Med. 21 (12) (2017) 3231–3243, https://doi.org/10.1111/jcmm.13227.
[40] Y. Hwang, T. Kim, R. Kim, et al., The natural product 6-gingerol inhibits inflammation-associated osteoclast differentiation via reduction of prostaglandin E_2 levels, Int. J. Mol. Sci. 19 (7) (2018), https://doi.org/10.3390/ijms19072068.