Network Pharmacological Dissection of the Mechanisms of Eucommiae Cortex-Achyranthis Radix Combination for Intervertebral Disc Herniation Treatment

Ho-Sung Lee¹², In-Hee Lee¹, Kyungrae Kang², Minho Jung², Seung Gu Yang³, Tae-Wook Kwon² and Dae-Yeon Lee¹²

Abstract

Eucommiae cortex (EC) and Achyranthis radix (AR) are herbal medicines widely used in combination for the treatment of intervertebral disc herniation (IDH). The mechanisms of action of the herbal combination have not been understood from integrative and comprehensive points of view. By adopting network pharmacological methodology, we aimed to investigate the pharmacological properties of the EC-AR combination as a therapeutic agent for IDH at a systematic molecular level. Using the pharmacokinetic information for the chemical ingredients of the EC-AR combination obtained from the comprehensive herbal drug-associated databases, we determined its 31 bioactive ingredients and 68 IDH-related therapeutic targets. By analyzing their enrichment for biological functions, we observed that the targets of the EC-AR combination were associated with the regulation of angiogenesis; cytokine and chemokine activity; oxidative and inflammatory stress responses; extracellular matrix organization; immune response; and cellular processes such as proliferation, apoptosis, autophagy, differentiation, migration, and activation. Pathway enrichment investigation revealed that the EC-AR combination may target IDH-pathology-associated signaling pathways, such as those of cellular senescence and chemokine, neurotrophin, TNF, MAPK, toll-like receptor, and VEGF signaling, to exhibit its therapeutic effects. Collectively, these data provide mechanistic insights into the pharmacological activity of herbal medicines for the treatment of musculoskeletal diseases such as IDH.

Keywords

herbal medicines, network pharmacology, intervertebral disc herniation, molecular mechanisms, multicomponent-multitarget drug

Introduction

Intervertebral disc herniation (IDH) is one of the most common musculoskeletal and orthopedic disorders affecting 1-3% of the worldwide population; it negatively impacts the quality of life (QoL) and health status of patients, and additionally places a significant economic burden on society.¹ IDH occurs when the intervertebral disc (IVD) components are displaced from the usual anatomical disc space; they protrude through the spinal canal and compress the spinal cord and nerve roots, resulting in pain in the arms, back, and legs, causing sciatica, tingling, numbness, and muscle weakness.² Pathomechanisms for IDH are suggested to be biological processes of disc cells and relevant materials, such as disc degeneration, angiogenesis, inflammation, degradation of extracellular matrix (ECM) components and collagen, and oxidative stress.²⁻⁶ Presently, non-anti-inflammatory analgesics, and muscle relaxants are used as conservative and non-operative therapies for IDH to enhance mobility and function, alleviate pain, and improve the QoL of patients.⁷⁻⁸ However, the pharmacological strategies for IDH treatment remain limited.⁷⁻⁸ Therefore, herbal medicines are increasingly being considered as effective non-operative therapies for IDH.

¹The Fore, 87 Ogeum-ro, Songpa-gu, Seoul 05542, Republic of Korea
²Forest Hospital, 129 Ogeum-ro, Songpa-gu, Seoul 05549, Republic of Korea
³Kyunghee Naro Hospital, 67, Dolma-ro, Bundang-gu, Seongnam 13586, Republic of Korea

Corresponding Author:
Dae-Yeon Lee, Forest Hospital, 129 Ogeum-ro, Songpa-gu, Seoul 05549, Republic of Korea.
Email: foresthrnd@gmail.com
prevention and treatment due to their potent pharmacological activity and low toxicity.9

Eucommiae cortex (EC) and Achyranthis radix (AR) are herbal medicines widely used in a combination for the treatment of IDH.9,10 Clinical studies have shown that the use of EC, AR, and herbal formulas containing EC and/or AR may enhance the total effective rate, cure rate, and alleviate pain intensity and relevant symptoms in patients with IDH.9,11 It has been reported that EC and AR may exhibit diverse pharmacological effects such as analgesic, anti-inflammatory, antioxidant, anti-spasmodic, bone protective, immunomodulatory, nerve regenerative and functional restorative, and neuroprotective activities.9,12–18 However, the systematic mechanisms underlying the pharmacological activity of the EC-AR combination for IDH treatment remain unexplored.

As the pharmacological effects of herbal medicines are conferred via the complex regulation of multiple therapeutic targets by various chemical ingredients, network pharmacology has been increasingly recognized as an effective and efficient methodology to investigate their therapeutic features in a systematic and comprehensive way.19–23 This research method integrates biomedicine, pharmacology, and computational network science and explores the multiple ingredient-multiple target mechanisms of herbal medicines in a network perspective.19–23 Network pharmacology analysis investigates bioactive chemical ingredients and their targets that play major roles in the therapeutic effects of herbal medicines, constructs herbal medicine-related networks based on the relevant data, and explores their functional and topological characteristics.19–23 This methodology is widely applied to studies regarding the mechanistic investigation of herbal medicines prescribed for various musculoskeletal diseases.24–29 The goal of the present study was to dissect the systematic mechanisms of the EC-AR herbal combination for IDH treatment.

**Materials and Methods**

**Determination of Active Chemical Ingredients in the EC-AR Combination**

The absorption, distribution, metabolism, and excretion (ADME) pharmacokinetic parameters (such as oral bioavailability, drug-likeness, and Caco-2 permeability), key considerations used for drug design and development, of the chemical ingredients of EC and AR were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP),30 Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM)31 and Traditional Chinese Medicine Integrated Database (TCMID).32 The chemical ingredients with oral bioavailability ≥30%, Caco-2 permeability ≥0.4, and drug-likeness ≥0.18 were determined as bioactive ingredients, as suggested previously.21 Oral bioavailability is the ratio of the amount of orally administered drug entering the systemic circulation and that reaching target tissues and organs; those with oral bioavailability ≥30% are considered to have effective absorptive capacity in the body.30,33 Caco-2 permeability is a measure used to examine the permeability, diffusion, and absorption rates of a compound in human Caco-2 intestinal cells; a compound with Caco-2 permeability ≥0.4 is generally determined to be effectively permeable in the intestinal epithelium.34,35 Drug-likeness is an indicator for qualitatively evaluating the suitability of a compound as a drug, considering its physical, chemical, and structural features using Tanimoto coefficients;30,36 a compound with drug-likeness ≥0.18 is regarded a potential drug because the mean value of drug-likeness of all available drugs is 0.18.30,36

**Identification of Targets of Active Chemical Ingredients in the EC-AR Combination**

The simplified molecular-input line-entry system (SMILES) notation for the active chemical ingredients of the EC-AR combination was obtained from the PubChem database.37 Then, we imported the obtained SMILES information into the following tools and databases used for the analysis of chemical-protein interactions: Similarity Ensemble Approach (SEA),38 PharmMapper,39 SwissTargetPrediction40, and Search Tool for Interactions of Chemicals (STITCH)5 51 In this way, we obtained the human targets of the bioactive ingredients of the EC-AR combination. The IDH-related human targets were retrieved from the databases, including the Therapeutic Target Database,42 DisGeNET,43 Online Mendelian Inheritance in Man,44 GeneCards,45 Comparative Toxicogenomics Database,46 and Pharmacogenomics Knowledgebase,47 as well as from previous relevant literature.27,48–54

**Construction of Herbal Medicine-Related Networks**

The herbal medicine-bioactive chemical ingredient-target (H-C-T) network was generated by linking the herbal medicines to their bioactive ingredients and the ingredients with their therapeutic targets. The herbal medicine-bioactive chemical ingredient-target-pathway (H-C-T-P) network was generated by linking the targets in the H-C-T network to their enriched disease-related pathways. The protein-protein interaction (PPI) network for the targets of herbal medicines was produced using the STRING database (interaction confidence score ≥0.7).55 The generated networks were visualized and their properties were investigated using Cytoscape.56 In a network, nodes describe herbal medicines, chemical ingredients, targets, or pathways, and links (or edges) describe the interaction relationships among them.57 The number of links that a node has is called the degree.57

**Functional Enrichment Analysis**

The g:Profiler58 and Kyoto Encyclopedia of Genes and Genomes database59 were used for the gene ontology (GO) and pathway enrichment analyses, respectively.
Molecular Docking Analysis

The structural information of the active chemical ingredients of the EC-AR combination and their therapeutic targets were investigated from the PubChem37 and RCSB Protein Data Bank,60 respectively. The information was imported into Autodock Vina,61 and the molecular docking scores of chemical ingredient-therapeutic target pairs were calculated. A chemical ingredient-target pair is considered to have potent binding activity if it exhibits a docking score £-5.0.62,63

Results

Bioactive Chemical Ingredients in the EC-AR Combination and Their Targets

The ADME pharmacokinetic parameters of the chemical ingredients of the EC-AR combination were obtained from TCMSP,30 BATMAN-TCM,31 and TCMD32 databases (Supplementary Table S1), and the ingredients with oral bioavailability ≥30%, drug-likeness ≥0.18, and Caco-2 permeability ≥-0.4 were determined to be bioactive. Some chemical ingredients that did not fulfill the aforementioned criteria were also considered bioactive because of their presence in large amount in the EC-AR combination and their therapeutic effects.64–72 Consequently, 49 bioactive chemical ingredients were identified in the EC-AR combination (Supplementary Table S2).

The targets of the bioactive chemical ingredients of the EC-AR combination were identified from a variety of tools and databases used to analyze protein-chemical interactions (see Materials and Methods). As a result, 279 targets were determined for the EC-AR combination where 68 targets of 31 chemical ingredients were IDH-related (Supplementary Table S3).

Network Pharmacological Investigation of EC-AR Combination

To carry out network pharmacological dissection of the therapeutic mechanisms of the EC-AR combination for IDH treatment, we generated an herbal medicine-bioactive chemical ingredient-target (H-C-T) consisting of 101 nodes (two herbal medicines, 31 bioactive chemical ingredients, and 68 IDH-related targets) and 190 edges (Figure 1 and Supplementary Table S3). Among the chemical ingredients in the network, quercetin (degree = 25), berberine (degree = 14), and kaempferol (degree = 11) had a large number of targets (Figure 1 and Supplementary Table S3), suggesting that they are potent contributors to the pharmacological effects of the EC-AR combination. Moreover, all chemical ingredients of the EC-AR combination shared one or more common targets, and 41 out of 68 targets interacted with two or more chemical ingredients (Figure 1), suggesting multiple ingredient-multiple target characteristics of herbal drugs.

To explore the interaction mechanisms underlying the targets of the EC-AR combination, we constructed a PPI network (60 nodes and 274 edges) where the targets served as the nodes (Figure 2). Further, based on the degree distribution of nodes in the network, we identified hubs, the high-degree nodes that play key biological roles and tend to be effective pharmacological targets.73,74 As described previously, nodes whose number of links is ≥two times the mean value of the degree of all the nodes in the network were defined as hubs.75,76 Therefore, IL6 (degree = 32), MAPK1 (degree = 29), AKT1 (degree = 26), TP53 (degree = 25), EGFR (degree = 21), MMP9 (degree = 20), IL1B (degree = 19), and CCL2 (degree = 19) were regarded as hubs (Figure 2). These nodes may serve as crucial targets conferring the therapeutic effects of the EC-AR combination for IDH treatment.

Figure 1. The herbal medicine-bioactive chemical ingredient-target network of the eucommiae Cortex-achyranthis radix combination. Green nodes, herbal medicines; red nodes, bioactive chemical ingredients; blue nodes, intervertebral disc herniation-related targets.
Thus, the results suggest the polypharmacological mechanisms of the EC-AR combination.

**Functional Enrichment Analysis of the Therapeutic Mechanisms of the EC-AR Combination**

To explore the therapeutic mechanisms of the EC-AR combination at the molecular level, we investigated the GO terms enriched with the targets of the EC-AR combination. The results showed that the targets of the EC-AR combination were involved in modulating important biological functions such as angiogenesis, cytokine and chemokine activity, oxidative and inflammatory stress responses, ECM organization, immune response, and cellular processes such as proliferation, apoptosis, autophagy, differentiation, migration, and activation (Supplementary Figure S1); this was consistent with the pathomechanisms of IDH and molecular activities of the herbal medicines reported in previous studies. 2,5,6,9,16,77–90 Pathway enrichment analysis further demonstrated that the targets of the EC-AR combination were enriched in diverse signaling pathways implicated in the IDH pathology; these pathways include “cellular senescence”, “chemokine signaling pathway”, “FoxO signaling pathway”, “growth hormone synthesis”, “HIF-1 signaling pathway”, “IL-17 signaling pathway”, “leukocyte transendothelial migration”, “MAPK signaling pathway”, “mTOR signaling pathway”, “neurotrophin signaling pathway”, “osteoclast differentiation”, “PI3K-Akt signaling pathway”, “prolactin signaling pathway”, “Ras signaling pathway”, “TNF signaling pathway”, “toll-like receptor signaling pathway”, “VEGF signaling pathway”, and “JAK-STAT signaling pathway” (Figure 3 and Supplementary Figure S1).

Together, the results of functional enrichment analysis suggested the comprehensive molecular and signaling mechanisms for the EC-AR combination for the treatment of IDH.

**Molecular Docking Analysis for EC-AR Combination**

To verify the binding activities among the bioactive chemical ingredients of the EC-AR combination and the targets, we carried out molecular docking analysis. The results indicated that the chemical ingredients and their hub targets may exhibit docking scores £-5.0 (Figure 4), suggesting their potential binding interactions.

**Discussion**

EC and AR are herbal medicines frequently used in combination as therapeutics for various musculoskeletal diseases such as osteoarthritis and IDH, with good efficacy and low toxicity. 9,24–26 Previous studies have analyzed their system-level
mechanisms for the treatment of osteoarthritis, however, their therapeutic properties in the case of IDH, from a view of network perspective, remain unexplored. This study aimed to dissect the comprehensive regulatory mechanisms of the EC-AR combination for IDH treatment based on the integrative network pharmacology methodology. By evaluating the pharmacokinetic characteristics of the chemical ingredients in the EC-AR combination, we determined 31 bioactive chemical ingredients and identified 68 IDH-related targets by investigating the protein-chemical binding interactions and performing network analysis. The 68 targets of the EC-AR combination were associated with the regulation of diverse biological activities, including angiogenesis, cytokine and chemokine activity, oxidative and inflammatory stress responses, ECM organization, immune response, and cellular processes such as proliferation, apoptosis, autophagy, differentiation, migration, and activation. These results were consistent with the previously reported pathological processes of IDH and the pharmacological mechanisms of the EC-AR herbal combination.

Pathway enrichment analysis indicated that the targets of the EC-AR combination may be enriched in diverse pathways implicated in IDH pathology, such as those of cellular senescence, chemokine, neurotrophin, TNF, MAPK, toll-like receptor, and VEGF signaling.

The key hub targets of the EC-AR combination were associated with IDH-related pathological processes and may act as pharmacological targets for the disease treatment. Interleukin (IL)-6 (encoded by \( \text{IL6} \)) is a proinflammatory cytokine crucially involved in the IDH-associated pathogenesis and pain development; the expression, activity, and polymorphisms of IL-6 are correlated with the risk, progression, and clinical prognosis of IDH and its inhibition may result in the alleviation of pain. Extracellular signal-regulated kinase (ERK)-2 (encoded by \( \text{MAPK1} \)) activates proinflammatory cytokines that may contribute to IDH progression by promoting inflammation and apoptosis of IVD cells. Inhibition of ERK2 activity may block the degeneration of IVDs and restore the disc function. Akt1 (encoded by \( \text{AKT1} \)) is a key regulator of the proliferation of IVD cells and its transcript level is associated with IDH progression and deterioration. Abnormal regulation of matrix metalloproteinase (MMP)-9 activity plays a role in IDH development and progression by

Figure 3. The herbal medicine-bioactive chemical ingredient-target-pathway network of the eucommiae Cortex-achyranthis radix combination. Green nodes, herbal medicines; red nodes, active chemical ingredients; blue nodes, intervertebral disc herniation-related targets; orange nodes, signaling pathways.

Figure 4. Molecular docking analysis of the bioactive chemical ingredients of eucommiae Cortex-achyranthis radix combination and their targets. (A) Baicalein-AKT1 (score = −6.2). (B) Berberine-AKT1 (score = −5.4). (C) Quercetin-AKT1 (score = −6.3). (D) Wogonin-CCL2 (score = −5.2). (E) Quercetin-EGFR (score = −7.9). (F) Aucubin-IL1B (score = −5.9). (G) Geniposide-IL6 (score = −6.3). (H) Berberine-MAPK1 (score = −7.1). (I) Baicalein-MMP9 (score = −6.5). (J) Caffeic acid-MMP9 (score = −6.4). (K) Quercetin-MMP9 (score = −6.3). (L) Wogonin-MMP9 (score = −5.9). (M) Berberine-TP53 (score = −6.8).
inducing disc matrix degradation and collagen loss; it is further related to the prognosis and disease severity in patients with IDH.\textsuperscript{102–109} p53 (encoded by \textit{TP53}) is a mediator of senescence, oxidative stress response, apoptosis, and inflammation in IVD cells and is implicated in neovascularization and infiltration associated with IDH pathogenesis; furthermore, p53 may have potential as a therapeutic target for IDH treatment.\textsuperscript{110–117} Aberrant activity of epidermal growth factor receptor (encoded by \textit{EGFR}) is reported in degenerative IVDs and its inhibition may suppress the degeneration of IVDs and ameliorate IDH.\textsuperscript{118,119} IL-1\(\beta\) (encoded by \textit{IL1B}) is an IDH-associated proinflammatory cytokine, and its genetic variability may influence the clinical outcome of patients with IDH.\textsuperscript{120} The chemokine (C-C motif) ligand 2 (CCL2; encoded by \textit{CCL2}) is associated with the persistence and prolongation of IDH-induced pain and pain intensity in patients with IDH.\textsuperscript{121,122}

Various pathways targeted by the EC-AR combination are reportedly involved in the pathophysiology of IDH. Cellular senescence of IVD cells is the most important pathophysiological mechanism underlying IVD degeneration leading to IDH, and the antisenescence-based pharmacological strategies may be effective in preventing or attenuating the disease onset and progression.\textsuperscript{123–125} The chemokine pathway is an inflammatory pathomechanism associated with IDH development and its activity is related to the generation, persistence, and severity of neuropathic and radicular pain induced by IDH; additionally, targeting this pathway may hold therapeutic potential.\textsuperscript{122,125–127} The Fork head box O (FoxO) pathway has an antioxidant effect that can protect IVDs against their degeneration induced by oxidative stress.\textsuperscript{146} The synthesis, expression, and activity of various growth factors and hormones, cytokines, and chemokines associated with osteoclast differentiation in IVD cells are related to IDH severity.\textsuperscript{141} Hypoxia inducible factor (HIF)-1 pathway is a key regulator of the resorption of herniated discs and survival and apoptotic cell death of disc cells in IVDs.\textsuperscript{142} The autoimmune- and inflammation-associated interleukin (IL)-17 pathway acts as a key mediator of IVD deterioration and IDH progression, and its higher activity is observed in the IVD tissues of patients with IDH.\textsuperscript{143,144} Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway acts as an inflammation regulator and causes IDH-associated symptoms that are important for the degeneration of herniated IVDs.\textsuperscript{145} The migration and infiltration of leukocytes into IVD tissues, accompanied by angiogenesis, neovascularization, and appearance of nerve fibers, may accelerate the inflammatory IVD degeneration and IDH-related disease processes.\textsuperscript{97} Mitogen activated protein kinase (MAPK) and Ras pathways are involved in the development and persistence of radicular pain and motor dysfunction by modulating the activities of pro-inflammatory, autophagic, and apoptotic cytokines and regulators, and targeting them can alleviate such pathological responses and further induce resorption of IVDs.\textsuperscript{146–152} The repression of the mammalian target of rapamycin (mTOR) pathway can attenuate IDH-induced pain and radiculopathies.\textsuperscript{153} The activity of the neurotrophin pathway is related to inflammatory IVD degeneration and its associated pain responses.\textsuperscript{154–157} Deregluation of the phosphatidylinositol 3-kinase (PI3 K)-Akt signaling pathway is implicated in IVD degeneration and IHD pathogenesis; its pharmacological intervention reduced IDH-associated pain by inhibiting inflammation responses.\textsuperscript{155,156} The prolactin pathway may prevent the progression of IVD degeneration by suppressing inflammation and apoptosis of IVD cells via the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) axis.\textsuperscript{159} Improper regulation of the tumor necrosis factor (TNF) pathway may correlate with an increased susceptibility to severity of IVD degeneration and herniation by modulating inflammation, disc cell apoptosis, disc resorption, macrophage infiltration, radiculopathy, onset, persistence, and chronicity of pain.\textsuperscript{160–172} The toll-like receptor (TLR) pathway may promote the upregulation of inflammatory cytokines and signaling pathways and also macrophage infiltration, thereby contributing to inflammation, apoptosis, oxidative stress, and degeneration of IVDs.\textsuperscript{173–179} Proangiogenic vascular endothelial growth factor (VEGF) signaling is involved in neovascularization, ECM degradation, and resorption of herniated IVDs.\textsuperscript{4,5,180,181}

Previous studies have shown the regulatory activities of the bioactive chemical ingredients of the EC-AR combination in the disc components and their pharmacological effects on IDH and disc degeneration. Aucubin suppresses the degradation of ECM in disc cells of IVDs, thereby inhibiting their degeneration.\textsuperscript{182} Baicalein exerts anti-inflammatory effects on disc cells by inactivating inflammation-inducing cytokines and other mediators and alleviates disc degeneration.\textsuperscript{183} Chlorogenic acid may block oxidative stress-induced apoptosis of disc cells and protect against degeneration of IVDs.\textsuperscript{184} Berberine suppresses ECM degradation, oxidative stress, inflammatory processes, and apoptotic cell death of disc cells by inhibiting matrix-degrading enzymes, oxidative stress- and inflammation-associated cytokines and regulators, and by inducing autophagy; these pharmacological effects may lead to the attenuation of IDH and IVD degeneration.\textsuperscript{185–187} Kaempferol alleviates the IVD degeneration by inhibiting ECM degradation and inflammation, increasing the viability of bone-marrow-derived mesenchymal stem cells (BMSCs), and reducing the lipid accumulation and adipogenesis in BMSCs.\textsuperscript{188} Quercetin exerts inhibitory effects on various senescence-related mechanisms and ameliorates IVD degeneration via regulating the nuclear factor erythroid 2-related factor 2 (Nrf2)/NF-kB axis.\textsuperscript{189} Wogonin reduces the activities of Nrf2/ARE and MAPK signaling, matrix-degrading proteases, and inflammatory mediators to upregulate key disc components, while suppressing ECM loss and progression of disc degeneration.\textsuperscript{190} The overall findings of this study present experimental evidence for the therapeutic effects of the EC-AR combination for IDH.

The present study has certain limitations. The study lacked experimental validation regarding the molecular regulatory mechanisms of the EC-AR combination and the contribution of its individual bioactive ingredients to the therapeutic effects. Future studies are warranted to address the
aforementioned issues and assess the efficacy and safety of the administration of EC-AR combined with IDH therapeutics such as acetaminophen, ibuprofen, muscle relaxants, and opioids.7,8

To conclude, this network pharmacology-based study revealed the systematic mechanisms of the EC-AR herbal combination for IDH treatment based on network and functional enrichment analyses. The overall findings offer a comprehensive and integrative basis and evidence for the pharmacological effects of the EC-AR herbal combination against musculoskeletal diseases.

Acknowledgments
Not applicable.

Author Contributions
Conceptualization: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee.
Methodology: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee.
Data collection: Ho-Sung Lee, In-Hee Lee, Kyungrae Kang, Minho Jung, Seung Gu Yang, Tae-Wook Kwon.
Data analysis and investigation: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee.
Writing: Ho-Sung Lee, In-Hee Lee, Dae-Yeon Lee.
All authors read and approved the final manuscript.

Data Statement
All data generated or analyzed during this study are included in this published article and its Supplemental materials file.

Declaration of Competing Interests
The authors declare that there is no conflict of interest.

Funding
The author(s) received no financial support for the research, authorship and/or publication of this article.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship and/or publication of this article.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

ORCID iD
Dae-Yeon Lee https://orcid.org/0000-0002-3198-9881

Supplemental material
Supplemental material for this article is available online.

References
1. Omidi-Kashani F, Hejrati H, Arianamnes S. Ten important tips in treating a patient with lumbar disc herniation. Asian Spine J. 2016;10(5):955-963.
2. Amin RM, Andrade NS, Neuman BJ. Lumbar disc herniation. Curr Rev Musculoskelet Med. 2017;10(4):507-516.
3. Borrelli E, Alexandre A, Ilakis E, Alexandre A, Bocci VJJ. Disc herniation and knee arthritis as chronic oxidative stress diseases: the therapeutic role of oxygen ozone therapy. J Arthritis. 2015;4(3):1-6.
4. Haro H, Kato T, Komori H, Osada M, Shinomiya K. Vascular endothelial growth factor (VEGF)-induced angiogenesis in herniated disc resorption. J Orthop Res. 2002;20(3):409-415.
5. Kato T, Haro H, Komori H, Shinomiya K. Sequential dynamics of inflammatory cytokine, angiogenesis inducing factor and matrix degrading enzymes during spontaneous resorption of the herniated disc. J Orthop Res. 2004;22(4):895-900.
6. Koike Y, Uzuki M, Kokubun S, Sawai T. Angiogenesis and inflammatory cell infiltration in lumbar disc herniation. Spine (Phila Pa 1976). 2003;28(17):1928-1933.
7. Chou R. Pharmacological management of low back pain. Drugs. 2010;70(4):387-402.
8. Cohen SP, Griffith S, Larkin TM, Villena F, Larkin R. Presentation, diagnoses, mechanisms of injury, and treatment of soldiers injured in operation Iraqi freedom: an epidemiological study conducted at two military pain management centers. Anesth Analg. 2005;101(4):1098-1103.
9. Zhang B, Xu H, Wang J, Liu B, Sun G. A narrative review of non-operative treatment, especially traditional Chinese medicine therapy, for lumbar intervertebral disc herniation. BioMed Trends. 2017;11(4):406-417.
10. Zhu L, Yu C, Zhang X, et al. The treatment of intervertebral disc degeneration using traditional Chinese medicine therapy, for lumbar intervertebral disc herniation. Evid Based Complement Alternat Med. 2020;2020(2381462):1-11.
11. Xiong Z, Yi P, Zhang I, Ma H, Li W, Tan M. Efficacy and safety of modified duhuo jisheng decoction in the treatment of lumbar disc herniation: a systematic review and meta-analysis. Evid Based Complement Alternat Med. 2020;2020(2381462):1-11.
12. Cheng Q, Jiang C, Wang C, et al. The Achyranthes bidentata poly-peptide k fraction enhances neuronal growth in vitro and
promotes peripheral nerve regeneration after crush injury in vivo. Neural Regen Res. 2014;9(24):2142-2150.

13. Wu J, Chen H, Li H, et al. Antidepressant potential of chlorogenic acid-enriched extract from Eucommia ulmoides oliver bark with neuron protection and promotion of serotonin release through enhancing synapsin I expression. Molecules. 2016;21(26):1-17.

14. Zhang R, Hu SJ, Li C, Zhang F, Gan HQ, Mei QB. Achyranthes bidentata root extract prevents OVX-induced osteoporosis in rats. J Ethnopharmacol. 2012;139(1):12-18.

15. Zhang R, Liu ZG, Li C, et al. Du-Zhong (Eucommia ulmoides oliv.) cortex extract prevents OVX-induced osteoporosis in rats. Bone. 2009;45(3):553-559.

16. Cho HK, Kim S-Y, Choi MJ, Baek SO, Kwak SG, Ahn SH. The effect of GCSB-5 a new herbal medicine on changes in pain behavior and neuroglial activation in a rat model of lumbar disc herniation. J Korean Neurosurg Soc. 2016;59(2):1-8.

17. Ida Y, Satoh Y, Katsumata M, et al. Two novel oleanolic acid sapogenins having a sialyl Lewis X mimic structure from Achyranthes fauriei root. Bioway Med Chen Lett. 1998;8(18):2555-2558.

18. Lee DY, Jo J-H, Kim W-S, et al. Antioxidant activity and standardization of extraction solvents of SJ004. J Kor Med Rehabil. 2020;30(2):67-75.

19. Poornima P, Kumar JD, Zhao Q, Blunder M, Efferth T. Network pharmacology of cancer: from understanding of complex interactomes to the design of multi-target specific therapeutics from nature. Pharm Res. 2016;11:290-302.

20. Lee WY, Lee CY, Kim YS, Kim CE. The methodological trends of traditional herbal medicine employing network pharmacology. Biomedicine. 2019;8(8):1-15.

21. Lee HS, Lee IH, Park SI, Lee DY. Network pharmacology-based investigation of the system-level molecular mechanisms of the hematopoietic activity of samul-tang, a traditional Korean herbal formula. Evid Based Complement Alternat Med. 2020;2020(9048089):1-17.

22. Zhang SQ, Xu HB, Zhang SJ, Li XY. Identification of the active compounds and significant pathways of Artemisia annua in the treatment of non-small cell lung carcinoma based on network pharmacology. Med Sci Monit. 2020;26(923624).

23. Lee HS, Lee IH, Kang K, Park SI, Kwon TW, Lee DY. An investigation of the molecular mechanisms underlying the analgesic effect of jakyag-gamche decoction: a network pharmacology study. Evid Based Complement Alternat Med. 2020;2020(6628641).

24. Chen Z, Wu G, Zheng R. A systematic pharmacology and in vitro study to identify the role of the active compounds of Achyranthes bidentata in the treatment of osteoarthritis. Med Sci Monit. 2020;26(925545):1-9.

25. Iqbal GH, Su BZ, Zhou WJ, Xiong H. Application of network pharmacology and molecular docking to elucidate the potential mechanism of Eucommia ulmoides-radix achyranthis bidentatae against osteoarthritis. BioData Min. 2020;13(12):1-18.

26. Zhang L, Shi X, Huang Z, et al. Network pharmacology approach to uncover the mechanism governing the effect of radix achyranthis bidentatae on osteoarthritis. BMC Complement Med Ther. 2020;20(121):1-12.
and education in fundamental biology, biomedicine, biotechnology and energy. Nucleic Acids Res. 2019;47(D1):D464-D474.

61. Trott O, Olson AJ. Autodock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multi threading. J Comput Chem. 2010;31(2):455-461.

62. Zhuang Z, Wen J, Zhang L, et al. Can network pharmacology identify the anti-virus and anti-inflammatory activities of shuangguanglian oral liquid used in Chinese medicine for respiratory tract infection? Eur J Integr Med. 2020;37(101139):1-12.

63. Zhang M, Yuan Y, Zhou W, et al. Network pharmacology analysis of chathu lizhong tang treating non-alcoholic fatty liver disease. Complement Biol Chem. 2020;86(107248):1-9.

64. Zhang ND, Han T, Huang BK, et al. Traditional Chinese medicine formulas for the treatment of osteoporosis: implication for antiosteoporotic drug discovery. J Ethnopharmacol. 2016;189:61-80.

65. Zhou YX, Zhang RQ, Rahman K, Cao ZX, Zhang H, Penc G. Diverse pharmacological activities and potential medicinal benefits of geniposide. Evid Based Complement Alternat Med. 2019;2019(4925682):1-16.

66. Dong Q, Yan J, Zheng M, Hua H, Tan J. Chemical constituents from seeds of Achalectis bidentata blume. J Trop Subtrop Bot. 2010;18(5):569-572.

67. Wei H-L, Li Y-J, Chen J, Li P. Triterpenoid saponins in roots of Epimedium grandiflorum var. puberulum Blume. J Integr Med. 2020;18(5):569-572.
76. Zhong J, Liu Z, Zhou X, Xu J. Synergic anti-pruritus mechanisms of action for the radix Sophorae flavescens and Fructus cnidii herbal pair. *Molecules.* 2017;22(1465):1-13.

77. Cheng Q, Shen Y, Cheng Z, et al. *Acbryanthus hiodentata* poly peptide k suppresses neuroinflammation in BV2 microglia through Nrf2-dependent mechanism. *Ann Transl Med.* 2019;7(20):1-12.

78. Cunha C, Silva AJ, Pereira P, Vaz R, Goncalves RM, Barbosa MA. The inflammatory response in the regression of lumbar disc herniation. *Arthritis Res Ther.* 2018;20(251):1-9.

79. Hu CX, Hu KY, Wang JF. Potential role of the compound Eucommia bone granules in patients with osteoarthritis and osteonecrosis: a retrospective study. *World J Clin Cases.* 2020;8(1):46-53.

80. Kadw T, Sowa G, Vo N, Kang JD. Molecular basis of intervertebral disc degeneration and herniations: what are the important translational questions? *Clin Orthop Relat Res.* 2015;473(6):1903-1912.

81. Kepler CK, Ponnappan RK, Tannoury CA, Risbud MV, Anderson DG. The molecular basis of intervertebral disc degeneration. *Spine J.* 2013;13(3):318-330.

82. Kim BH, Park KS, Chang JM. Elucidation of anti-inflammatory potencies of *Eucommia ulmoides* bark and *Plantago asiatica* seeds. *J Med Food.* 2009;12(4):764-769.

83. Kwon SH, Ma SX, Hwang JY, et al. The anti-inflammation activity of *Eucommia ulmoides* oliv. Bark involves NF-kappaB suppression and Nrf2-dependent HO-1 induction in BV-2 microglial cells. *Biomed Ther (Seoul).* 2016;24(3):268-282.

84. Mo Z, Li D, Zhang R, Chang M, Yang B, Tang S. Comparisons of the effectiveness and safety of tainu, acupuncture, traction, and Chinese herbs for lumbar disc herniation: a systematic review and network meta-analysis. *Evid Based Complement Alternat Med.* 2019;2019(6821310).

85. Wang JY, Chen XJ, Zhang L, Pan YY, Gu ZX, Yuan Y. Anti-inflammatory effects of *Eucommia ulmoides* oliv. Male flower extract on lipopolysaccharide-induced inflammation. *Clin Med J (Engl).* 2019;132(3):319-328.

86. Wang JY, Yuan Y, Chen XJ, et al. Extract from *Eucommia ulmoides* oliv. Ameliorates arthritis via regulation of inflammation, synovioyte proliferation and osteoclastogenesis in vitro and in vivo. *J Ethnopharmacol.* 2016;169(4):609-616.

87. Yuan D, Hussain T, Tan B, Liu Y, Ji P, Yin Y. The evaluation of antioxidant and anti-inflammatory effects of *Eucommia ulmoides* flavones using diquat-challenged piglet models. *Oxid Med Cell Longev.* 2017;2017(81409620):1-10.

88. Zhang F, Zhao X, Shen H, Zhang C. Molecular mechanisms of cell death in intervertebral disc degeneration (review). *Int J Mol Med.* 2016;37(6):1439-1448.

89. Hong N-D, Rho Y-S, Kim J-W, Won D-H, Kim N-J, Cho B-SJKJoP. Studies on the general pharmacological activities of *Eucommia ulmoides* olivier. *Korean J Pharmacogn.* 1988;19(2):102-110.

90. Vetrichelvan T, Jegadesan M. Effect of alchoholic extract of *Acbryanthus hiodentata* blume on acute and sub acute inflammation. *Ind J Pharmacol.* 2002;34(2):115-118.

91. Deng X, Zhao F, Kang B, Zhang X. Elevated interleukin-6 expression levels are associated with intervertebral disc degeneration. *Exp Ther Med.* 2016;11(4):1425-1432.

92. Haddadi K, Abediankenari S, Alipour A, et al. Association between serum levels of interleukin-6 on pain and disability in lumbar disc herniation surgery. *Asian J Neurosurg.* 2020;15(3):494-498.

93. Huang X, Chen F, Zhao J, et al. Interleukin 6 (IL-6) and IL-10 promoter region polymorphisms are associated with risk of lumbar disc herniation in a northern Chinese Han population. *Genet Test Mol Biomarkers.* 2017;21(1):17-23.

94. Sainoh T, Orita S, Miyagi M, et al. Interleukin-6 and interleukin-6 receptor expression, localization, and involvement in pain-sensing neuron activation in a mouse intervertebral disc injury model. *J Orthop Res.* 2015;33(10):1508-1514.

95. Wuertz K, Haglund L. Inflammatory mediators in intervertebral disc degeneration and discogenic pain. *Global Spine J.* 2013;3(3):175-184.

96. Niu CC, Lin SS, Yuan LJ, et al. Hyperbaric oxygen treatment suppresses MAPK signaling and mitochondrial apoptotic pathway in degenerated human intervertebral disc cells. *J Orthop Res.* 2013;31(2):204-209.

97. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol.* 2014;10(1):44-56.

98. Wang H, Tian Y, Wang J, et al. Inflammatory cytokines induce NOTCH signaling in nucleus pulposus cells: implications in intervertebral disc degeneration. *J Biol Chem.* 2013;288(23):16761-16774.

99. Pasku D, Soufla G, Katonis P, Tsarouhas A, Vakis A, Spandilos DA. Akt/PKB isoforms expression in the human lumbar herniated disc: correlation with clinical and MRI findings. *Eur Spine J.* 2011;20(10):1676-1683.

100. Pratsinis H, Constantinou V, Pavlakis K, Sapkas G, Kletzas D. Exogenous and autocrine growth factors stimulate human intervertebral disc cell proliferation via the ERK and Akt pathways. *J Orthop Res.* 2012;30(6):958-964.

101. Pratsinis H, Kletzas D. PDGF, bFGF and IGF-I stimulate the proliferation of intervertebral disc cells in vitro via the activation of the ERK and Akt signaling pathways. *Eur Spine J.* 2007;16(11):1858-1866.

102. Jing R, Liu Y, Guo P, et al. Evaluation of common variants in matrix metalloproteinase-9 gene with lumbar disc herniation in Han Chinese population. *Genet Test Mol Biomarkers.* 2018;22(10):622-629.

103. Levine JM, Ruaux CG, Bergman RL, Coates JR, Steiner JM, Williams DA. Matrix metalloproteinase-9 activity in the cerebrospinal fluid and serum of dogs with acute spinal cord trauma from intervertebral disk disease. *Am J Vet Res.* 2006;67(2):283-287.

104. Li PB, Tang WJ, Wang K, Zou K, Che B. Expressions of IL-1alpha and MMP-9 in degenerated lumbar disc tissues and their clinical significance. *Eur Rev Med Pharmacol Sci.* 2017;21(8):4007-4013.

105. Nagano S, Fujiki M, Tokunaga S, Misumi K. Analysis of cartilage matrix metalloprotease-9 in cerebrospinal fluid of miniature dachshund with intervertebral disc herniation. *Res Vet Sci.* 2012;93(3):1487-1492.

106. Nagano S, Kim SH, Tokunaga S, Arai K, Fujiki M, Misumi K. Matrix metalloprotease-9 activity in the cerebrospinal fluid and...
spinal injury severity in dogs with intervertebral disc herniation. Res Vet Sci. 2011;91(3):482-485.

107. Tanaka A, Kumagai S, Kawashiri S, et al. Expression of matrix metalloproteinase-2 and -9 in synovial fluid of the temporomandibular joint accompanied by anterior disc displacement. J Oral Pathol Med. 2001;30(3):59-64.

108. Xu YQ, Zhang ZH, Zheng YF, Feng SQ. Dysregulated miR-133a mediates loss of type II collagen by directly targeting matrix metalloproteinase 9 (MMP9) in human intervertebral disc degeneration. Spine (Phila Pa 1976). 2016;41(12):E717-E724.

109. Zagonis A, Alexiou GA, Batistatou A, Voulgaris S, Kyrtissis AP. The role of matrix metalloproteinase 9 in intervertebral disc degeneration. J Clin Neurol. 2011;18(10):1424-1425.

110. He J, Xue R, Li S, et al. Identification of the potential molecular targets for human intervertebral disc degeneration based on bioinformatic methods. Int J Mol Med. 2015;36(6):1593-1600.

111. Li Y, Cao I, Li J, et al. Influence of microgravity-induced intervertebral disc degeneration of rats on expression levels of p53/p16 and proinflammatory factors. Exp Ther Med. 2019;17(2):1367-1373.

112. Liu XW, Kang J, Fan XD, Sun LF. Expression and significance of VEGF and p53 in rat degenerated intervertebral disc tissues. Asian Pac J Trop Med. 2013;6(9):404-406.

113. Lu XY, Ding XH, Zhong LJ, Xia H, Chen XD, Huang H. Expression and significance of VEGF and p53 in degenerate intervertebral disc tissue. Asian Pac J Trop Med. 2013;6(1):79-81.

114. Xiong X, Dai L, Liang W, et al. Protective effect of p53 on the viability of intervertebral disc nucleus pulposus cells under low glucose condition. Biochem Biophys Res Commun. 2017;490(4):1414-1419.

115. Yang M, Peng Y, Liu W, Zhou M, Meng Q, Yuan C. Sirtuin 2 expression suppresses oxidative stress and senescence of nucleus pulposus cells through inhibition of the p53/p21 pathway. Biochem Biophys Res Commun. 2019;513(3):616-622.

116. Zhang Z, Lin J, Nisar M, et al. The Sirt1/P53 axis in diabetic intervertebral disc degeneration pathogenesis and therapeutics. Oxid Med Cell Longev. 2019;2019.

117. Zhou N, Lin X, Dong W, et al. SIRT1 Alleviates senescence of degenerative human intervertebral disc cartilage end-plate cells via the p53/p21 pathway. Sci Rep. 2016;6:22628.

118. Huang BR, Chen TS, Bau DT, et al. EGFR Is a pivotal regulator of thrombin-mediated inflammation in primary human nucleus pulposus culture. Sci Rep. 2017;7(1):8578.

119. Pan Z, Sun H, Xie B, et al. Therapeutic effects of gefitinib-encapsulated thermosensitive injectable hydrogel in intervertebral disc degeneration. Biomaterials. 2018;160:56-68.

120. Moon A, Schistad EI, Rygh LJ, Roe C, Gjerstad JR. Role of IL1A rs1800587, IL1B rs1143627 and IL1RN rs2234677 genotype regarding development of chronic lumbar radicular pain; a prospective one-year study. PLoS One. 2014;9(9):e107301.

121. Peng ZY, Chen R, Fang ZZ, Chen B, Wang ZH, Wang XY. Increased local expressions of CX3CL1 and CCL2 are related to clinical severity in lumbar disc herniation patients with sciatic pain. J Pain Res. 2017;10:157-165.

122. Zhu X, Cao S, Zhu MD, Liu JQ, Chen JJ, Gao YJ. Contribution of chemokine CCL2/CCR2 signaling in the dorsal root ganglion and spinal cord to the maintenance of neuropathic pain in a rat model of lumbar disc herniation. J Pain. 2014;15(5):516-526.

123. Chen J, Xie JJ, Jin MY, et al. Sirt6 overexpression suppresses senescence and apoptosis of nucleus pulposus cells by inducing autophagy in a model of intervertebral disc degeneration. Cell Death Dis. 2018;9(5):1-13.

124. Feng C, Liu H, Yang M, Zhang Y, Huang B, Zhou Y. Disc cell senescence in intervertebral disc degeneration: causes and molecular pathways. Cell Cycle. 2016;15(13):1674-1684.

125. Gao C, Ning B, Sang C, Zhang Y. Rapamycin prevents the intervertebral disc degeneration via inhibiting differentiation and senescence of annulus fibrosus cells. Aging (Albany NY). 2018;10(1):131-143.

126. Jiang I, Zhang X, Zheng X, et al. Apoptosis, senescence, and autophagy in rat nucleus pulposus cells: implications for diabetic intervertebral disc degeneration. J Orthop Res. 2013;31(5):692-702.

127. Le Maitre CL, Freemont AJ, Hoyland JA. Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. Arthritis Res Ther. 2007;9(3):R45.

128. Liang D, Hong D, Tang F, et al. Upregulated IncHRK2.1 prompts nucleus pulposus cell senescence in intervertebral disc degeneration. Mol Med Rep. 2020;22(6):5251-5261.

129. Liu C, Liu L, Yang M, et al. A positive feedback loop between EZH2 and NOX4 regulates nucleus pulposus cell senescence in age-related intervertebral disc degeneration. Cell Div. 2020;15(2):1-15.

130. Patil P, Niedernofer JI, Robbins PD, Lee J, Sowa G, Vo N. Cellular senescence in intervertebral disc aging and degeneration. Curr Mol Biol Rep. 2018;4(4):180-190.

131. Sudhir G, Balasubramaniam S, Jayabalan V, Sundaram S, Kumar V, Kailash K. Does type II diabetes induce early senescence and degeneration in human intervertebral discs? A tissue biomarker evaluation. Int J Spine Surg. 2020;4(3):341-346.

132. Wang F, Cai F, Shi R, Wang XH, Wu XT. Aging and age related expression of cytokines and chemokines in herniated nucleus pulposus resorption. Spine (Phila Pa 1976). 2019;2019.

133. Wang J, Huang C, Lin Z, et al. Polymatin suppresses nucleus pulposus cell senescence, promotes matrix homeostasis and attenuates intervertebral disc degeneration in rats. J Cell Mol Med. 2018;22(11):5720-5731.

134. Zhang Y, Yang B, Wang J, et al. Cell senescence: a nonnegligible cell state under survival stress in pathology of intervertebral disc degeneration. Osteoarthritis Cartilage. 2016;24(3):398-408.

135. Zhang J, Huang C, Lin Z, et al. Polydatin suppresses nucleus pulposus cell senescence, promotes matrix homeostasis and attenuates intervertebral disc degeneration in rats. J Cell Mol Med. 2018;22(11):5720-5731.

136. Ahn SH, Cho YW, Ahn MW, Jang SH, Sohn YK, Kim HS. mRNA expression of cytokines and chemokines in herniated lumbar intervertebral discs. Spine (Phila Pa 1976). 2002;27(9):911-917.

137. Haro H, Shinomiya K, Komori H, et al. Upregulated expression of chemokines in herniated nucleus pulposus resorption. Spine (Phila Pa 1976). 1996;21(14):1647-1652.
Kawaguchi S, Yamashita T, Katahira G, Yokozawa H, Torigoe T, Zhu Y, Liu JT, Yang LY, et al. P38 mitogen-activated protein kinase inhibition modulates nucleus pulposus cell apoptosis in spontaneous resorption of herniated intervertebral discs: an experimental study in rats. *Med Mol Rep*. 2016;13(3):4001-4006.

Li S, Hua W, Wang K, et al. Autophagy attenuates compression-induced apoptosis of human nucleus pulposus cells via MEK/ERK/NRF1/Ag7 signaling pathways during intervertebral disc degeneration. *Exp Cell Res*. 2018;370(1):87-97.

Liu Y, Li J, Li H, et al. AMP-activated protein kinase activation in dorsal root ganglion suppresses mTOR/p70S6K signaling and alleviates painful radiculopathies in lumbar disc herniation rat model. *Spine (Phila Pa 1976)*. 2019;44(15):E865-E872.

Garcia-Cosamalon J, del Valle ME, Calavia MG, et al. Intervertebral disc, sensory nerves and neurotrophins: who is who in discogenic pain? *J Anat*. 2010;217(1):1-15.

Li H, Liu H, Zhang N, Zhu Z. Involvement of the G-protein-coupled receptor 4 in the increased expression of RANK/RANKL/OPG system and neurotrophins by nucleus pulposus cells under the degenerated intervertebral disc-like acidic microenvironment. *Bone and Res Int*. 2020;2020(1):328436:1-12.

Ohata K, Tsujino H, Yamanaka H, et al. Expression of neurotrophic factors in the dorsal root ganglion in a rat model of lumbar disc herniation. *Pain*. 2002;99(1-2):121-132.

Purmessur D, Freemont AJ, Hoyland JA. Expression and regulation of neurotrophins in the nondegenerate and degenerate human intervertebral disc. *Arthritis Res Ther*. 2008;10(4):R99.

Xu W, Ding W, Sheng H, Lu D, Xu X, Xu B. Dexamethasone suppresses radial pain through targeting the L-PGDS/PI3 K/Akt pathway in rats With lumbar disc herniation. *Pain Pract*. 2020;21(1):64-74.

Wu X, Liu Y, Guo X, et al. Prolactin inhibits the progression of intervertebral disc degeneration through inactivation of the NF-kappaB pathway in rats. *Cell Death Dis*. 2018;9(8):1-11.

Andrade P, Hoogland G, Teernstra OP, et al. Elevated levels of tumor necrosis factor-alpha and TNFRI in recurrent herniated lumbar discs correlate with chronicity of postoperative sciatic pain. *Spine J*. 2016;16(2):243-251.

Andrade P, van Aalst J, Bauwens M, et al. Radionuclide tumor necrosis factor-alpha activity in herniated lumbar disc correlates with severe leg pain. *Surg Neurol Int*. 2020;11(344):1-3.

Cooper RG, Freemont AJ. TNF-alpha blockade for herniated intervertebral disc-induced sciatica: a way forward at last? *Rheumatology (Oxford)*. 2004;43(2):119-121.

Genevay S, Fincek A, Payer M, et al. Elevated levels of tumor necrosis factor-alpha in periradicular fat tissue in patients with radiculopathy from herniated disc. *Spine (Phila Pa 1976)*. 2008;33(19):2041-2046.

Gorth DJ, Shapiro IM, Risbud MV. Transgenic mice overexpressing human TNF-alpha experience early onset spontaneous intervertebral disc herniation in the absence of overt degeneration. *Cell Death Dis*. 2018;10(7):1-14.

Haro H, Crawford HC, Fingleton B, Shinomiya K, Spengler DM, Martrisan LM. Matrix metalloproteinase-7-dependent release of tumor necrosis factor-alpha in a model of herniated disc resorption. *J Clin Invest*. 2000;105(2):143-150.

Iwabuchi S, Ito M, Chikanishi T, Azuma Y, Haro H. Role of the tumor necrosis factor-alpha, cyclooxygenase-2, prostaglandin E2,
and effect of low-intensity pulsed ultrasound in an in vitro herniated disc resorption model. J Orthop Res. 2008;26(9):1274-1278.

167. Murata Y, Nanmark U, Rydevik B, Takahashi K, Olmarker K. The role of tumor necrosis factor-alpha in apoptosis of dorsal root ganglion cells induced by herniated nucleus pulposus in rats. Spine (Phila Pa 1976). 2008;33(2):155-162.

168. Murata Y, Onda A, Rydevik B, Takahashi K, Olmarker K. Distribution and appearance of tumor necrosis factor-alpha in the dorsal root ganglion exposed to experimental disc herniation in rats. Spine (Phila Pa 1976). 2004;29(20):2235-2241.

169. Ohtori S, Inoue G, Eguchi Y, et al. Tumor necrosis factor-alpha-immunoreactive cells in nucleus pulposus in adolescent patients with lumbar disc herniation. Spine (Phila Pa 1976). 2013;38(6):459-462.

170. Olmarker K, Nutu M, Storkson R. Changes in spontaneous behavior in rats exposed to experimental disc herniation are blocked by selective TNF-alpha inhibition. Spine (Phila Pa 1976). 2003;28(15):1635-1641; discussion 1642.

171. Yoshida M, Nakamura T, Sai A, Kikuchi T, Matsukawa A. Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model. Spine (Phila Pa 1976). 2005;30(1):55-61.

172. You C, Zhu K, Liu X, et al. Tumor necrosis factor-alpha-dependent infiltration of macrophages into the dorsal root ganglion in a rat disc herniation model. Spine (Phila Pa 1976). 2013;38(23):2003-2007.

173. Bi F, Liu W, Wu Z, Ji C, Chang C. Antiaging factor klotho retards the progress of intervertebral disc degeneration through the toll-like receptor 4-NF-kappaB pathway. Int J Cell Biol. 2020;2020(8319516):1-11.

174. Ge J, Chen I, Yang Y, Lu X, Xiang Z. Sparstolonin B prevents lumbar intervertebral disc degeneration through toll like receptor 4, NADPH oxidase activation and the protein kinase B signaling pathway. Mol Med Rep. 2018;17(1):1347-1353.

175. Krock E, Rosenzweig DH, Currie JB, Bisson DG, Ouellet JA, Haglund L. Toll-like receptor activation induces degeneration of human intervertebral discs. Sci Rep. 2017;7(1):17184.

176. Li Z, Wang X, Pan H, et al. Resistin promotes CCL4 expression through toll-like receptor-4 and activation of the p38 MAPK and NF-kappaB signaling pathways: implications for intervertebral disc degeneration. Osteoarthritis Cartilage. 2017;25(2):341-350.

177. Qin C, Zhang B, Zhang L, et al. MdD88-dependent toll-like receptor 4 signal pathway in intervertebral disc degeneration. Exp Ther Med. 2016;12(2):611-618.

178. Rajan NE, Bloom O, Maillhof R, et al. Toll-like receptor 4 (TLR4) expression and stimulation in a model of intervertebral disc inflammation and degeneration. Spine (Phila Pa 1976). 2013;38(16):1343-1351.

179. Zhang Q, Weng Y, Jiang Y, Zhao S, Zhou D, Xu N. Overexpression of miR-140-3p inhibits lipopolysaccharide-induced human intervertebral disc inflammation and degeneration by down-regulating toll-like receptor 4. Onco Rep. 2018;40(2):793-802.

180. Jia CQ, Zhao JG, Zhang SF, Qi F. Stromal cell-derived factor-1 and vascular endothelial growth factor may play an important role in the process of neo-vascularization of herniated intervertebral discs. J Int Med Res. 2009;37(1):136-144.

181. Tolonen J, Gronblad M, Viridi J, Seitsalo S, Rytomaa T, Karaharju EO. Platelet-derived growth factor and vascular endothelial growth factor expression in disc herniation tissue: and immunohistochemical study. Eur Spine J. 1997;6(1):63-69.

182. Yang S, Li I, Zhu I, et al. Aucubin inhibits IL-1beta- or TNF-alpha-induced extracellular matrix degradation in nucleus pulposus cell through blocking the miR-140-5p/CREB1 axis. J Cell Physiol. 2019;234(8):13639-13648.

183. Jin H, Wang Q, Wu J, et al. Baicalein inhibits the IL-1beta-induced inflammatory response in nucleus pulposus cells and attenuates disc degeneration in vivo. Inflammation. 2019;42(3):1032-1044.

184. Xie RH, Yin M, Yin CC, et al. Mechanism of chlorogenic acid on apoptosis of rat nucleus pulposus cells induced by oxidative stress. Zhong Yao Cai. 2014;37(3):465-469.

185. Chen Y, Zheng Z, Wang J, et al. Berberine suppresses apoptosis and extracellular matrix (ECM) degradation in nucleus pulposus cells and ameliorates disc degeneration in a rodent model. Int J Biol Sci. 2018;14(6):682-692.

186. Lu L, Jia X, Jin H, et al. Berberine prevents human nucleus pulposus cells from IL1beta induced extracellular matrix degradation and apoptosis by inhibiting the NFkappaB pathway. Int J Mol Med. 2019;43(4):1679-1686.

187. Luo R, Liao Z, Song Y, et al. Berberine ameliorates oxidative stress-induced apoptosis by modulating ER stress and autophagy in human nucleus pulposus cells. Life Sci. 2019;228:85-97.

188. Zhu J, Tang H, Zhang Z, et al. Kaempferol slows intervertebral disc degeneration by modifying LPS-induced osteogenesis/adipogenesis imbalance and inflammation response in BMSCs. Int Immunopharmacol. 2017;43:236-242.

189. Hao Z, Wang B, Shi Y, et al. Senolytic agent quercetin ameliorates intervertebral disc degeneration via the Nrf2/NF-kappaB axis. Osteoarthritis Cartilage. 2020;29(3):413-422.

190. Fang W, Zhou X, Wang J, et al. Wogonin mitigates intervertebral disc degeneration through the Nrf2/ARE and MAPK signaling pathways. Int Immunopharmacol. 2018;65:539-549.