# The mechanism of Bushen Huoxue decoction in treating intervertebral disc degeneration based on network pharmacology

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**Background:** This study aimed to explore the mechanism of Bushen Huoxue decoction (BHD) in treating intervertebral disc degeneration using the network pharmacology method.

**Methods:** Using of oral bioavailability >30% and drug-likeness >0.18 as the screening standards, the effective components and targets of BHD were retrieved from the TCMSP database and the BATMAN-TCM database. The disease targets of intervertebral disc degeneration were retrieved from the GeneCards database. The Wayne map of the interaction targets of the effective components of BHD and intervertebral disc degeneration were drawn using R software. The protein-protein interaction (PPI) network of common targets was constructed using STRING software. The network map of the interaction targets of the effective components of BHD-intervertebral disc degeneration was drawn using Cytoscape3.7.2 software. The GO and KEGG enrichment analysis of the common targets of BHD and intervertebral disc degeneration was performed using R software and the related plug-ins to screen the potential pathways and analyze its mechanism.

**Results:** This study screened 164 effective components of BHD, 131 interaction targets, 626 targets for degenerative disc disease, and 31 common interaction targets. IL6, VEGFA, CASP3, EGFR, ESR1, and MAPK8 appeared more frequently. These were mainly enriched in the AGE-RAGE, TNF, PI3K Akt, and MAPK signaling pathways.

**Conclusions:** BHD mainly intervenes in intervertebral disc degeneration through IL6, VEGFA, CASP3, EGFR, ESR1, and MAPK8. The mechanism of the intervention of BHD on intervertebral disc degeneration may be related to AGE-RAGE, TNF, PI3K Akt, MAPK, and other signal pathways.

**Keywords:** Network pharmacology; tonifying kidney and activating blood soup; intervertebral disc degeneration; the network diagram; common target protein interaction network

## Introduction

Intervertebral disc degeneration (IDD) is a chronic progressive disease caused by many factors. Its pathological changes are characterized by the decrease in proliferation activity, the quantity of nucleus pulposus cells, and the decrease in extracellular matrix synthesis (1,2). IDD is one of the main causes of chronic low back pain and the basis of various spinal diseases such as intervertebral disc herniation and
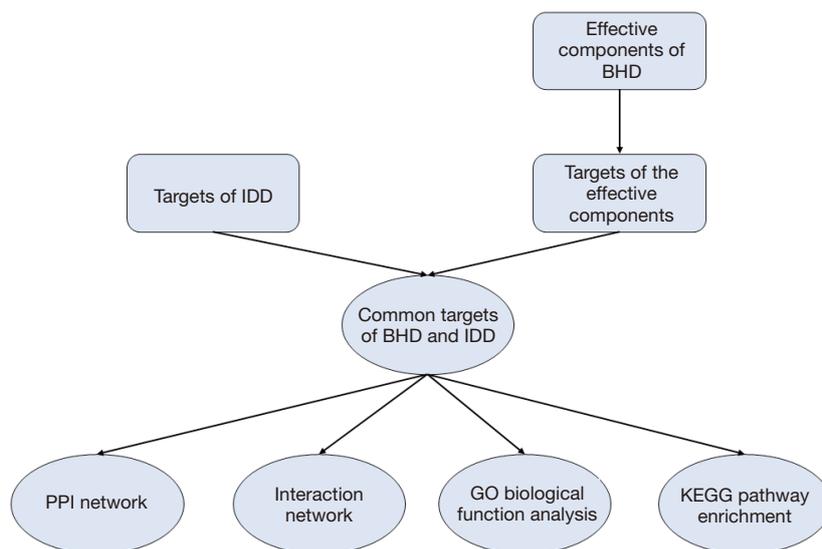
**Figure 1** The study flow chart. BHD, bushen huoxue decoction; IDD, intervertebral disc degeneration; PPI, protein-protein interaction.

spondylolisthesis. It seriously affects patients' quality of life and labor ability and imposes a great burden on the social economy.

Traditional Chinese medicine believes that improving renal function can effectively delay low back pain caused by IDD. Bushen Huoxue decoction (BHD) was recorded in the Great Achievement of Traumatology, written by Zhao Lian, a physician in the Qing Dynasty. It can effectively improve renal function and promote blood circulation. It is a commonly used prescription in the treatment of degenerative diseases of intervertebral discs in traditional Chinese medicine, with excellent clinical effect (3). Previous studies revealed that BHD could inhibit the adipogenic differentiation of bone marrow mesenchymal stem cells (BMSCs), promote their osteogenic differentiation and migration *in vitro*, and regulate the expression of IL-1β (4), TGF-β1 (5,6), and p38MAPK proteins (7) in degenerative discs. BHD can also regulate multiple targets and multiple mechanisms. Although we have uncovered part of the mechanism of BHD in the treatment of degenerative disc disease, this is just the tip of the iceberg. More complex regulatory mechanisms need to be explored and reviewed.

Previous studies on the regulation mechanism of BHD on IDD are generally one-sided, lacking macroscopic and overall understanding. Therefore, based on network pharmacology technology, this study explored the corresponding relationship between drug components and disease targets, predicted all possible regulatory mechanisms, and provided

a theoretical basis for the follow-up experimental study of BHD in the treatment of IDD. The study flow chart is shown in *Figure 1*. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/apm-20-2586).

## Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of Affiliated Hospital of Hunan Academy of Chinese Medicine (No. LLBH-201910310001) and informed consent was taken from all the patients.

### Effective components and the selection of targets of BHD

The TCMSP database (http://lsp.nwu.edu.cn/tcmsp. php) and BATMAN-TCM database (http://bionet. ncpsb.org/batman-tcm/) were used to retrieve all the effective components of the raw materials of BHD: Radix Rehmanniae, Eucommia ulmoides Oliv, Fructus Lycii, Fructus Psoraleae, Dodder, Angelicae Sinensis Radix, myrrh, Fructus corni, safflower, Angelica, and Cistanche deserticola. The preliminarily screened literature was read according to the criteria of oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18. The primarily screened literature was re-screened, thus, obtaining the effective components of BHD. Subsequently, through the TCMSP and BATMAN-TCM database, all the targets of each

component of BHD were retrieved. The targets and the effective components of BHD were then inputted into the Perl plug-ins.

### The screening of targets for degenerative disc disease

The disease targets of disc degeneration were searched through the GeneCards database (https://www.genecards.org/) using the search term "Intervertebral disc degeneration". The inclusion criterion was a relevance score of ≥17.0. After eliminating the repeated targets, the disease targets of disc degeneration were standardized through the UniProt database (https://www.uniprot.org).

### The screening of common targets of active ingredients, disease, and PPI network construction

To clarify the interaction between the related targets of IDD and the potential targets of BHD, the R language (https://www.r-project.org/) software and the Perl language program were used to intersect the disease targets and drug targets. After, the Wayne map was drawn. The STRING (https://string-db.org/) plug-in was used to build a common target protein-protein interaction (PPI) network. The protein type was set to "Homo sapiens", and other parameters remained as the default settings to obtain the PPI network. The Count. R plug-in was used to obtain the frequency of common protein targets.

### The construction of the interaction network of the effective component-disc degeneration-target

The Merge function of the Cytoscape 3.7.1 software was used to construct the interaction network of the effective component-disc degeneration-target. In the network diagram, "Node" represented the effective components and targets, and "edge" represented the relationship between the effective component-BHD-target.

### Gene Ontology (GO) biological function analysis and the KEGG pathway enrichment

In the Bioconductor database (http://www.bioconductor.org/) after the data download, R software was used (https://www.r-project.org/). GO enrichment analysis and KEGG enrichment analysis were carried out automatically for drug and disease interaction targets, and Perl plug-in was used to transform and output the results. GO analysis is mainly used to describe the function of gene products, including cell function, molecular function, and biological function. KEGG analysis mainly describes the location and role of the target in related biological signal pathways.

### Statistical analysis

All the GO and KEGG enrichment analysis results were selected by P<0.05 as the critical criterion, and the first 20 pathways were screened out.

## Results

### The screening of the active components of BHD

In the present study, using the TCMSP and BATMAN-TCM database, OB ≥30% and DL ≥0.18 were used as the inclusion criteria for screening to search the active components of 11 Chinese herbs in BHD. Then, 192 active components were obtained. Among these, Eucommia had 28 active components, radix angelicae tuhuo nine, wolfberry 45, angelica 28, safflower 22, myrrh 45, Cistanche deserticola six, Cornus 20, prepared Radix Rehmanniae two, Dodder 11, and Psoralea had two. After eliminating the repeated active components, 164 active components were obtained. A total of 10,442 targets of BHD were retrieved from the TCMSP and BATMAN-TCM database, and the Perl plug-in was used to screen the targets of the active ingredients of BHD. After eliminating the repeated parts, 131 targets were obtained.

### The screening targets for degenerative disc disease

In the present study, the disease targets of IDD were retrieved from the GeneCards and OMIM database. The relevance score of ≥17.0 was used as the inclusion criterion. After eliminating the repeated targets, 630 therapeutic targets of IDD were obtained.

### Common target screening and the construction of the PPI network

The R language software and Venny plug-in program were used to screen the common targets from 661 targets of intervertebral disc degenerative disease and 131 active drug components. A Wayne map was drawn (*Figure 2*). Then, 31 common targets were input in the STRING database (https://string-db.org/), the human genome was chosen, and

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the PPI network was constructed in the mode of "medium confidence: 0.4" (*Figure 3*). The Count. R plug-in was used to count the top 30 protein targets. The findings showed that IL6, VEGFA, CASP3, EGFR, ESR1, and MAPK8 appeared more frequently (*Figure 4*).

### The construction of the interaction network of the active components of BHD-intervertebral disc degeneration-target

The Cytoscape 3.7.1 software was used to obtain 77 nodes and 384 edges from the interaction network diagram of
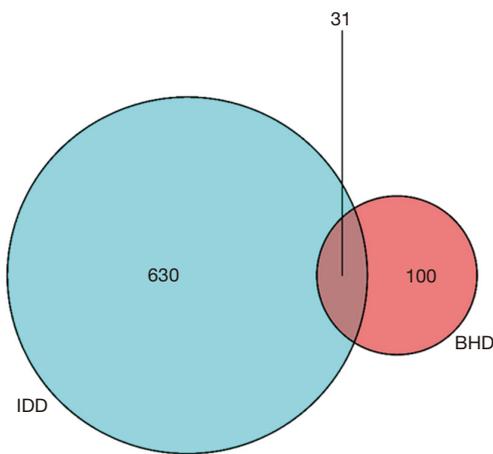


Figure 2 Venny diagram of components target of BHD and target of IDD. BHD, bushen huoxue decoction; IDD, intervertebral disc degeneration.
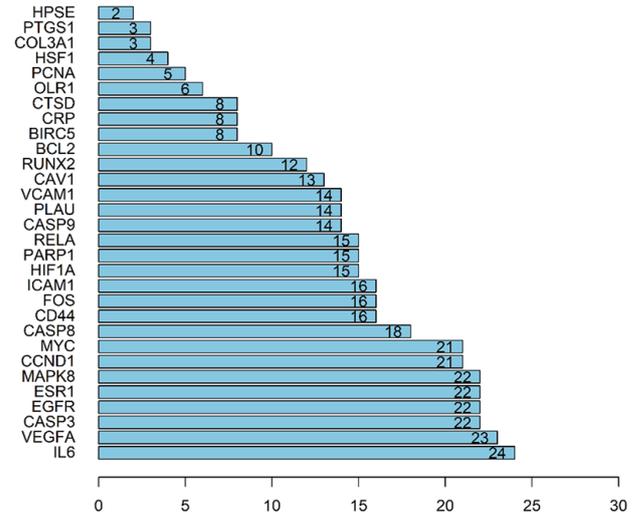


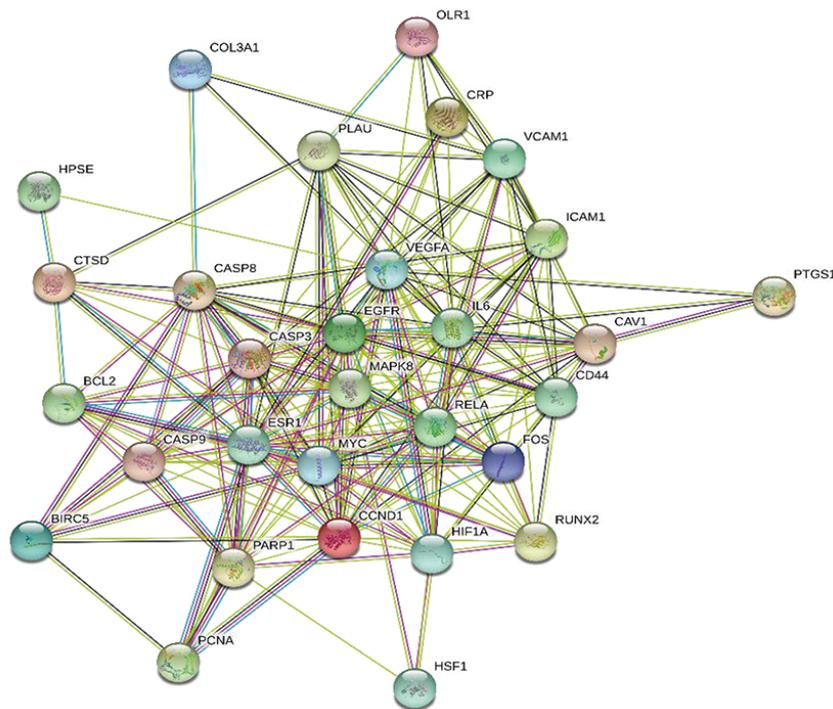Figure 4 Frequency of common protein targets.



Figure 3 PPI network of BHD and IDD. BHD, bushen huoxue decoction; IDD, intervertebral disc degeneration.
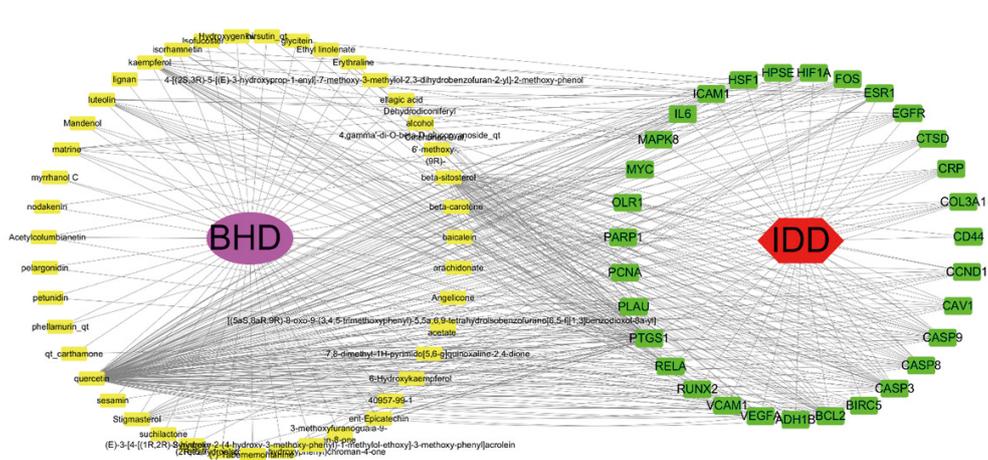
Figure 5 Active constituents-IDD-target interaction network. In the picture, purple is BHD, red is the disease disc degeneration, yellow is the active ingredient of BHD, and green is the common target of BHD and IDD. BHD, bushen huoxue decoction; IDD, intervertebral disc degeneration.
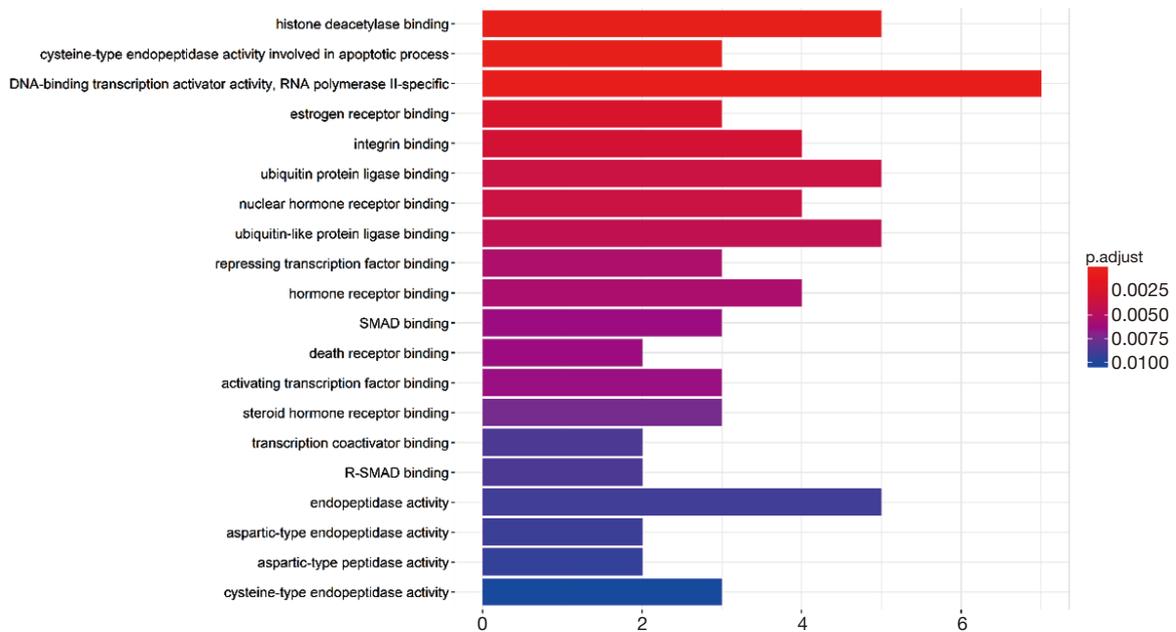


Figure 6 Histogram of GO analysis of common target.

active component-intervertebral disc degeneration-target (*Figure 5*). In *Figure 5*, purple is the drug BHD, red is the disease disc degeneration, yellow is the active component of BHD, and green is the common targets of BHD and IDD.

*The enrichment analysis of BHD in the treatment of intervertebral disc degeneration*

The Gene Ontology (GO) enrichment analysis of the

molecular function of the common targets of BHD and IDD was performed. *Figures 6* and *7* show the top 20 molecular functions. DNA binding transcription activator activity, RNA polymerase II specific, ubiquitin-protein ligase binding, histone deacetylase binding, and ubiquitin-like protein ligase binding are ranked in the top five positions. The Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathway enrichment analysis of the common targets was also carried out. *Figures 8* and *9*
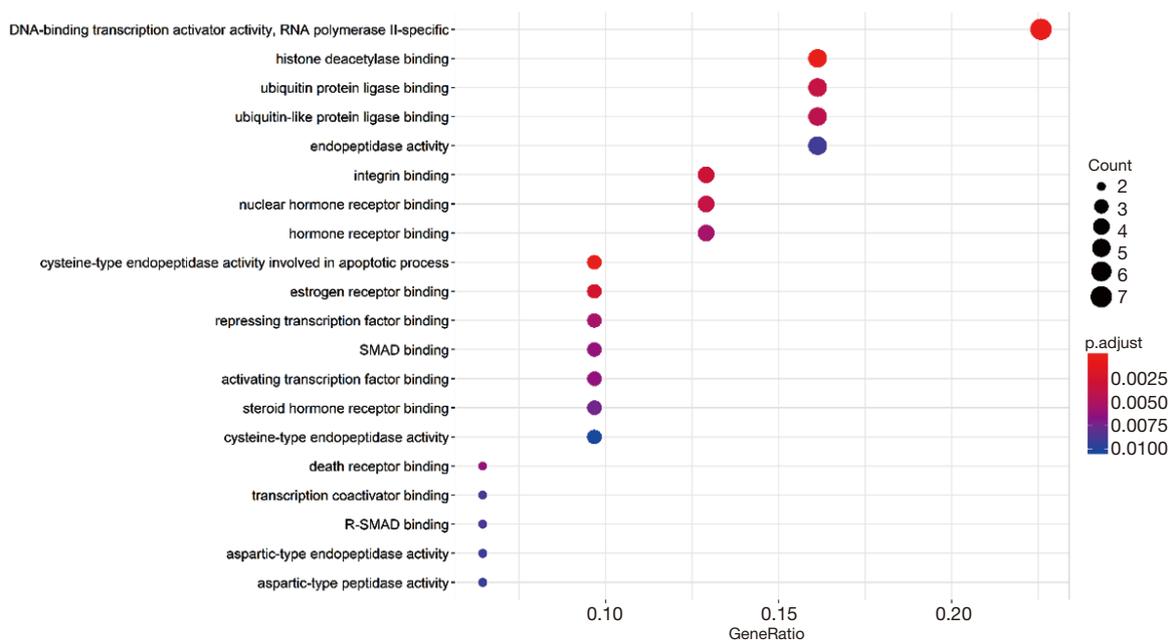
**Figure 7** Bubble chart of GO analysis of common target.

show the top 20 biological pathways. The results revealed that the top four enrichment frequency of biological pathways of interaction targets of BHD on IDD were Kaposi sarcoma-associated herpesvirus infection (KSHV), hepatitis B, Epstein-Barr virus infection, and proteoglycans in cancer. The results of the present study revealed that the targets of BHD intervention on IDD are mainly enriched in the AGE-RAGE signaling pathway, TNF signaling pathway, PI3K Akt signaling pathway, MAPK signaling pathway, Relaxin signaling pathway, NF kappa B signaling pathway, HIF-1 signaling pathway, prolactin signaling pathway, p53 signaling pathway, toll-like receptors signaling pathway, gyroid hormone signaling pathway, Estrogen signaling pathway, JAK-STAT signaling pathway, and node-like receptor signaling pathway.

## Discussion

In the present study, 164 effective components and 131 targets of BHD and 626 targets of IDD were screened, and 31 common interaction targets were obtained. IL6, VEGFA, CASP3, EGFR, ESR1, and MAPK8 appeared more frequently, which were mainly enriched in the AGE-RAGE, TNF, PI3K Akt, and MAPK signaling pathways.

The specific mechanism of IDD remains unknown. The biological characteristics of its degeneration are tissue

fibrosis caused by the progressive decrease of proteoglycan, dehydration of type II collagen, and an increase in type I collagen (8-10). During the process, interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), and other pro-inflammatory factors affect the balance of the extracellular matrix by stimulating the production of NO, matrix metalloproteinase, and proteoglycans while resulting in the degradation of the extracellular matrix and the degeneration of the pulpiform nucleus and fibrous rings (11-13). At present, research in traditional Chinese medicine puts the intervertebral disc into the category of muscles and bones, which is closely related to the liver and kidneys. BHD is from "The Great Achievement of Traumatology", written by Lian Zhao in the Qing Dynasty. During the decoction intervention, prepared Rehmannia and Eucommia are considered as sovereign medicine. These tonify the liver and kidneys and strengthen the muscles and bones. Cornus Officinalis, wolfberry, Cistanche deserticola, Psoralea, and Cuscuta are ministerial drugs that warm Yang and tonify the kidneys. Angelicae Sinensis Radix, myrrh, and safflower are conducting drugs that clear the channels and remove blood stasis. Angelica eliminates wind and dampness, disperses arthralgia, and relieves pain. The decoction tonifies the liver and kidneys, removes rheumatism, strengthens the body's resistance, eliminates pathogens, clears channels, and removes blood stasis. Drugs are used according to
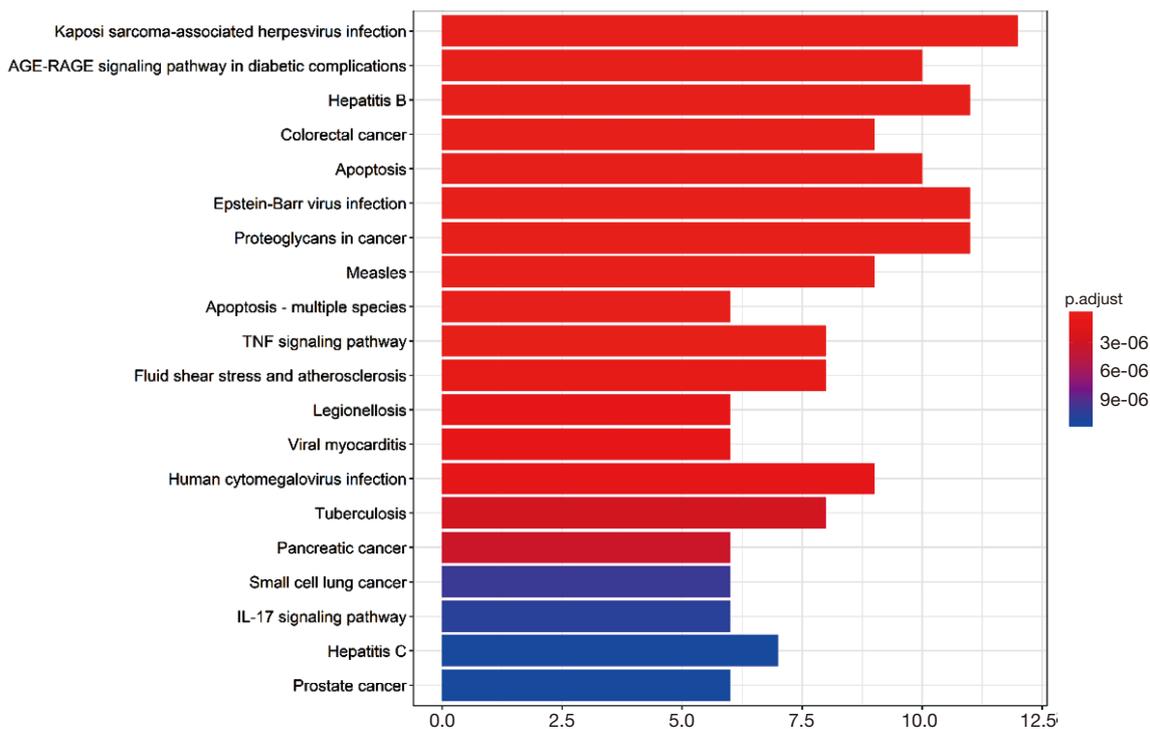
       

**Figure 8** Histogram of KEGG pathway analysis.

these symptoms, while both the symptoms and the roots are simultaneously considered. Satisfactory effects have been obtained in the clinical application of the decoction intervention. The investigators' previous study revealed that BHD could reduce the expression of various pro-inflammatory factors and key protein molecules in the degenerative intervertebral disc of rats while simultaneously controlling the degeneration of the intervertebral disc.

In the present study, TCMSP, BATMAN-TCM, GeneCards, STRING, and other biological information databases were used preliminarily to explore the mechanism of BHD-regulating IDD. The study screened 131 interaction targets of the effective components of BHD, and 31 were targeted at degenerative disc disease. Of these 31 targets, IL6, VEGFA, and CASP3 appeared most frequently. IL6 is confirmed to be involved in the degradation of the extracellular matrix in IDD (14). It is one of the most studied cytokines in the study of IDD. VEGFA may be co-expressed with the p53 gene in degenerative disc tissue and co-acts in neovascularization and infiltration to accelerate the degeneration of intervertebral disc tissues (15). CASP3 is mainly involved in the initiation of apoptosis in the process of IDD (16).

From the interaction network diagram of the active component-intervertebral disc degeneration-target, 77 nodes and 384 edges were obtained. These reflected the complex network characteristics of multiple targets and multiple mechanisms of BHD in the intervention of IDD. The results of the GO enrichment analysis of the molecular function of the common targets of BHD and IDD also revealed that these top-ranked components were mainly molecules with the function of protein binding. The enrichment analysis of the KEGG biological pathway revealed that the signal pathways with the most target enrichment of BHD intervention on IDD were the AGE-RAGE signaling pathway, TNF signaling pathway, PI3K Akt signaling pathway, and MAPK signaling pathway. The TNF and MAPK signaling pathways often play an important role in the process of IDD by promoting the secretion of inflammatory factors, the apoptosis of pulpiform nucleus cells, and the degradation of the extracellular matrix through synergic effects (17-19). The PI3K Akt signaling pathway can delay the degeneration of the intervertebral disc by regulating the expression of MMP-3 (20) while inhibiting the apoptosis of pulpiform nucleus cells (21). The results from domestic and foreign
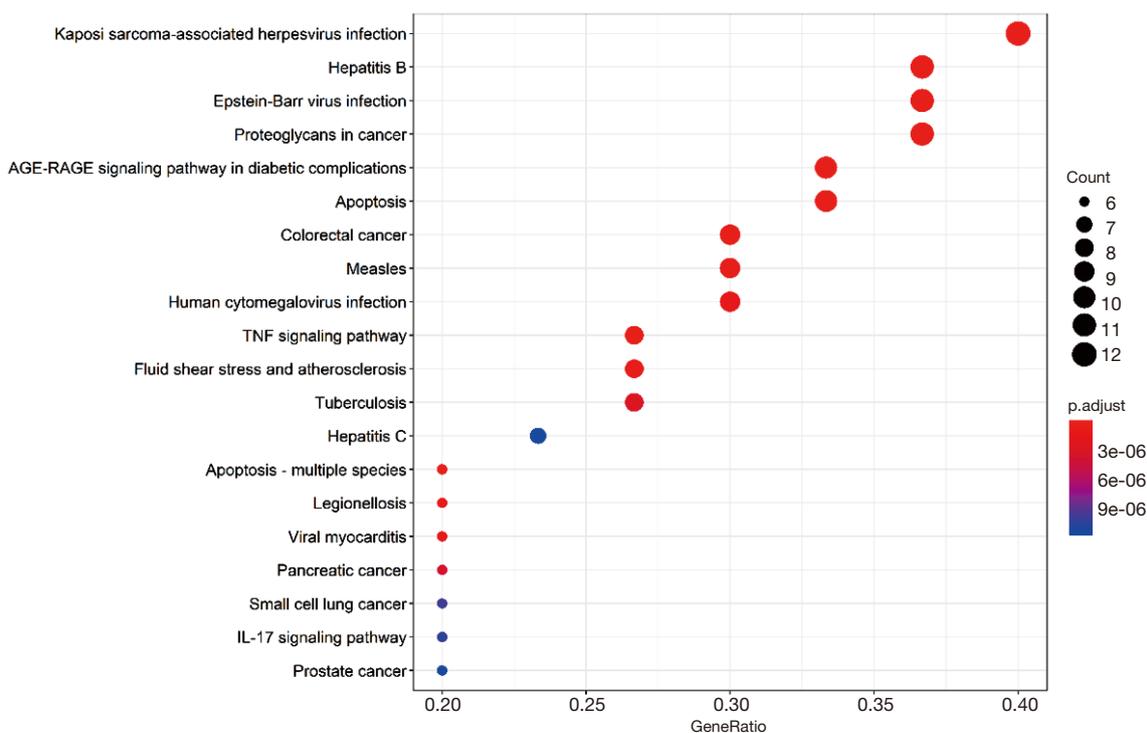
**Figure 9** Bubble chart of KEGG pathway analysis.

literature revealed that there are fewer studies on the correlation between the AGE-RAGE signaling pathway and IDD. Xunlu *et al.* (21,22) revealed, through integrating biological information, that the AGE-RAGE signaling pathway may play an important role in the process of IDD. This suggests the next direction of study on the mechanism of the development of IDD.

In summary, BHD intervenes in 31 targets in the process of IDD through 164 active ingredients and regulates various molecular functions and signal pathways through these targets while accordingly delaying the degeneration of the intervertebral disc. These findings provide a theoretical basis for further experimental studies on BHD in treating degenerative disc disease. The results of the KEGG enrichment analysis also revealed that the intervention targets of BHD on IDD frequently appeared in Kaposi's sarcoma-related herpes virus infection, hepatitis B, Epstein-Barr virus infection, and tumor proteoglycan. Whether the active components in BHD can regulate these pathological processes needs further study.

However, the regulatory targets and mechanisms of BHD on IDD summarized in this study are based on the current bioinformatics database, and the research on the

pathogenesis of IDD lacks innovative findings. In addition, this study is based on the database information analysis and prediction of BHD intervention mechanisms related to IDD, which belongs to theoretical research. Further experiments are needed to verify these theoretical results.

## Conclusions

This study screened the effective components, targets, and mechanisms of BHD in treating IDD using the network pharmacological technology while providing a theoretical basis for further experimental studies on BHD in treating degenerative disc disease.

## Acknowledgments

## Footnote

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at http://dx.doi.org/10.21037/

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*Data Sharing Statement:* Available at http://dx.doi.org/10.21037/apm-20-2586

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-2586). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of Affiliated Hospital of Hunan Academy of Chinese Medicine (No. LLBH-201910310001) and informed consent was taken from all the patients.

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