Research Article

Investigation of Molecular Mechanism of Banxia Xiexin Decoction in Colon Cancer via Network Pharmacology and In Vivo Studies

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Received 10 May 2022; Accepted 9 June 2022; Published 1 July 2022

Objective. Banxia Xiexin decoction (BXD) is widely used in the treatment of gastrointestinal and other digestive diseases. This study is based on network pharmacology to explore the molecular mechanism of BXD in the treatment of colon cancer. Methods. The bioactive components and potential targets of BXD were obtained from public database. The protein-protein interaction (PPI) network of the potential targets of BXD for colon cancer was constructed based on the STRING database, cytoscape software, gene ontology (GO), and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of the PPI network. Finally, we established a xenograft nude mouse model to verify the effect of BXD in colon cancer treatment. Results. We have acquired a total of 55 bioactive components and 136 cross-targets of BXD. The results of enrichment analysis suggested that the oxidative stress and diet were the key factors of colon cancer occurrence, and AGE-RAGE signaling pathway plays an essential role in the treatment of colon cancer with BXD. Animal experiments revealed that BXD could suppress tumor growth and induce tumor cell apoptosis in the xenograft nude mouse model with HCT116 cells. Conclusion. This study uncovered that BXD inhibits the malignant progression of colon cancer that may be related to multiple compounds (berberine, quercetin, baicalein, etc.), multiple targets (Bcl2, Bax, IL6, TNFα, CASP3, etc.), and multiple pathways (human cytomegalovirus infection, AGE-RAGE signaling pathway in diabetic complications, etc.).

1. Introduction

Global cancer statistics analysis shows 1,096,601 new cases and 551,269 deaths from colon cancer in 2018 [1]. Colon cancer is the third most common cancer in the world, and several factors, such as changes in lifestyle, are considered to be responsible [2]. Because of the exposure of screening technology and high-risk factors, the incidence of colon cancer has decreased. However, the different side effects of chemotherapy drugs and the smallest selection of effective drugs limit the treatment of colon cancer [3]. Currently, the available treatments for colon cancer include laparoscopic colectomy, radiotherapy, and chemotherapy, however, these treatments may have side effects on patients, such as a loss of appetite, hair loss, constipation, and vomiting [4]. Therefore, it is necessary to find an efficient drug for the treatment of colon cancer.

Banxia Xiexin decoction (BXD) [5] is derived from “Treatise on Febrile Diseases” written by Zhang Zhongjing in the Eastern Han Dynasty. It is commonly used in the treatment of digestive system diseases in modern times. BXD consists of seven herbs, such as Pinellia ternata (Thunb.) Makino (Ban-Xia), Zingiber officinale Roscoe (Gan-Jiang), Coptis chinensis Franch. (Huang-Lian), Scutellaria baicalensis Georgi (Huang-Qin), Panax ginseng C.A.Mey. (Ren-Shen), Ziziphus jujuba Mill. (Da-Zao), and Glycyrrhiza uralensis Fisch. (Gan-Cao). BXD has antioxidant, anti-inflammatory, antidiabetic, and anti-tumor properties. Clinical study has shown that BXD has a good therapeutic effect on colon cancer and can significantly inhibit the transition
from colitis to colon cancer [6]. In addition, Yan’s study has proved that BXD inhibits tumor growth in colon cancer cell transplanted nude mice [7]. However, its material basis and action mechanisms have not been systematically elucidated.

A traditional Chinese medicine formula may be composed of multiple components, and one component may correspond to multiple targets. Therefore, it is difficult to fully clarify its mechanism of action [8]. Network pharmacology is an emerging discipline based on systems biology, bioinformatics, and high throughput histology [9, 10]. It provides the biological process and pathway of the action of Chinese medicine by analyzing the targets related to the ingredients and diseases, and it helps one analyze the action mechanism of Chinese medicine in treating diseases [11].

In the present study, the potential compounds and targets of BXD against colon cancer are analyzed by network pharmacology. Then, a tumor xenograft mouse model was constructed to verify the effect of BXD on tumor growth and cell apoptosis, detect the expression levels of potential targets and inflammatory factors, and provide a practical basis for future medical clinical experiments and theoretical research.

2. Methods

2.1. Colon Cancer-Related Targets Screening. GeneCards database (https://www.genecards.org/) and DisGeNET database (https://www.disgenet.org) were used to search the colon cancer-related targets with “colon cancer” as the search word. The targets with a twofold median score were retained. Then, disease targets were combined, and the duplicate targets were removed.

2.2. The Collection of the Active Compounds and Targets. In Traditional Chinese Medicine Integrated Database (TCMID, http://www.megabionet.org/tcmid/), Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://ibts.hkbu.edu.hk/LSP/tcmsp.php), and Herb Ingredients’ Targets (HIT, http://lifecenter.sgst.cn/hit/) database, the authors searched the active compounds of BXD and eliminated compounds without target information. The authors searched for all the targets of the effective active ingredients of traditional Chinese medicine compounds in the database of TCMID, TCMSP, HIT, and Search Tool for Interacting Chemicals (STITCH, http://stitch.embl.de). Then, they took the target with a compound-target association score above 400 in the STITCH database.

2.3. Preliminary Screening of Drug-Like Properties. The AMDE (absorption, distribution, metabolism, and excretion) properties are the main indicators for evaluating the drug properties of the compound. Comparing the physicochemical characteristics of the compound with the characteristics of the marketed drug can effectively evaluate the drug-like properties of the compound. The quantitative estimate of drug-likeness (QED) proposed by Bickerton [12] was used to quickly evaluate the drug-like properties of active components. According to the QED value of DrugBank (https://www.drugbank.ca/)–listed drugs, the authors selected 0.3 as the threshold to screen compounds.

2.4. Rescreening of Chemical Composition Based on Binomial Statistical Model. One target may interact with multiple compounds, so that this target can be considered the main target of the formula. The enrichment scoring algorithm was based on the binomial statistical model to screen the main targets of BXD. The binomial statistical model (Equation 1) [13, 14] is as follows:

\[ P_i(X \geq k) = \sum_{m=k}^{n} \binom{n}{m} p^m (1 - p)^{n-m}, \]

and it indicates the probability that the target gene \( i \) is simultaneously acted on by at least \( k \) active components. \( n \) is the total number of compounds in the formula. When \( P < 0.0001 \), it is a small probability event to prove that the target gene is simultaneously acted on by at least \( k \) active compounds, and the target gene is considered to be the main target gene of the formula. Calculate the compound containing the main action target gene as the main action compound.

2.5. Protein-Protein Interaction (PPI) Network. To clarify the interaction between BXD active component targets and colon cancer disease targets, Venny 2.1 (https://bioin.fogp.cnb.csc.es/tools/venny/) was used to screen the potential targets related to both “component targets” and “disease targets.” Subsequently, the PPI network analysis was performed using the STING platform (https://string-db.org/). Then, the authors downloaded and imported it into Cytoscape 3.8.0 software. Finally, they used the Cytohubba plug-in of Cytoscape to calculate the “degree, betweenness, centerness,” and other scores of each target in the network, and they got the top 10 genes as the hub targets.

2.6. Biological Function Analysis. The obtained potential targets of BXD against colon cancer were analyzed by gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis. The hypergeometric distribution model (equation 2) was used to estimate the association between the annotation terms and the query gene.

\[ P = 1 - \sum_{i=0}^{k-1} \frac{\binom{\Delta M}{i} \binom{\Delta N - M}{\Delta n - i}}{\binom{\Delta N}{\Delta n}}, \]

where \( N \) is the total number of genes from reference terms, \( M \) is the number of annotated genes in a certain pathway or GO, \( n \) is the target of BXD waiting to be analyzed, and \( k \) is the number of shared genes between BXD targets and the reference set. The \( P \) value adjusted by the Bonferroni method and that less than 0.01 indicated that the correlation was significant.
Figure 1: The potential targets of Banxia Xiexin decoction (BXD) in colon cancer treatment. (a) Venn diagram showing BXD-related targets (204) intersected with colon cancer-related targets (1816), yielding a total of 136 overlapping targets. (b) The protein-protein interaction (PPI) network of 136 overlapping genes.

Table 1: The main action targets of Banxia Xiexin Decoction (BXD) against colon cancer.

| ID  | Name | ID  | Name | ID  | Name | ID  | Name |
|-----|------|-----|------|-----|------|-----|------|
| 3725| JUN  | 1234| CCR5 | 3106| HLA-B| 5320| PLA2G2A|
| 19  | ABCA1| 983 | CDK1 | 3107| HLA-C| 5328| PLAU |
| 5243| ABCB1| 1131| CHRM3| 3135| HLA-G| 5444| PON1 |
| 43  | ACHE | 1268| CNR1 | 3162| HMOX1| 5465| PPARA|
| 9570| ADIPOQ| 1277| COL1A1| 3356| HTR2A| 5468| PPARG|
| 148 | ADRA1A| 1385| CREB1| 3383| ICAM1| 5578| PRKCA|
| 154 | ADRB2| 1499| CTNNB1| 3586| IL10 | 5716| PSMID10|
| 183 | AGT  | 3576| CXCL8| 3553| IL1B | 5715| PSMID9|
| 231 | AKR1B1| 7852| CCR4 | 3569| IL6  | 10197| PSMEM3|
| 207 | AKT1 | 54205| CYCS | 3630| INS  | 5728| PTPN |
| 213 | ALB  | 1543| CYP1A1| 3667| IRS1 | 5742| PTGS1|
| 216 | ALDH1A1| 1544| CYP1A2| 9170| LPAR2| 5743| PTGS2|
| 217 | ALDH2| 1559| CYP2C9 | 5594| MAPK1 | 5770| PTPN2 |
| 301 | ANXA1| 1565| CYP2D6 | 1432| MAPK14| 5970| RELA |
| 335 | APOA1| 1571| CYP2E1 | 5595| MAPK3 | 6233| RPS27A|
| 338 | APOB | 1576| CYP3A4 | 5599| MAPK8 | 6256| RXRA |
| 348 | APOE | 1581| CYP7A1 | 4312| MMP1 | 6288| SAA1 |
| 351 | APP  | 10800| CYSLTR1| 4313| MMP2 | 6319| SCD |
| 367 | AR   | 1738| DLD  | 4318| MMP9 | 5054| SERPINE1|
| 551 | AVP  | 1906| EDN1 | 4353| MPO  | 6532| SLC6A4|
| 567 | B2M  | 1909| EDNRA| 2475| MTR  | 8140| SLC7A5|
| 581 | BAX  | 1910| EDNRB| 4552| MTRR | 6648| SOD2 |
| 590 | BCHE | 1956| EGFR | 4780| NFE2L2| 6667| SP1 |
| 596 | BCL2 | 2099| ESR1 | 4792| NFKBIA | 6714| SRC |
| 811 | CALR | 2147| F2   | 4843| NOS2 | 6817| SULT1A1 |
| 836 | CASP3| 2149| F2R  | 4846| NOS3 | 6863| TAC1 |
| 841 | CASP8| 2194| FASN | 1728| NQO1 | 7040| TGBF1 |
| 842 | CASP9| 2353| FOS  | 8856| NR1I2 | 7054| TH |
| 847 | CAT  | 2520| GAST | 2908| NR3C1 | 7099| TLR4 |
| 885 | CCK  | 2641| GCG  | 4923| NTSR1 | 7124| TNF |
| 887 | CCKBR| 2932| GSK3B| 4953| ODC1 | 8795| TNFRSF10B |
| 6347| CCL2 | 3061| HCRTR1| 142 | PARP1 | 7157| TP53 |
| 595 | CCND1| 3091| HIF1A| 5290| PIK3CA| 7299| TYR |
| 1232| CCR3 | 3105| HLA-A| 5319| PLA2G1B| 7422| VEGFA |
Table 2: The primary active components of BXD.

| ChemName         | QED   | ChemName            | QED   |
|------------------|-------|---------------------|-------|
| Oroxylin a       | 0.88622| Stigmasterol        | 0.45993|
| Wogonin          | 0.88622| Methionine          | 0.45058|
| Berberine        | 0.82454| Myristic acid       | 0.44896|
| Chrysin          | 0.82057| Oleamolic acid      | 0.44599|
| Apigenin         | 0.74033| Ursolic acid        | 0.44328|
| Ferulic acid     | 0.69573| Beta-sitosterol     | 0.43538|
| Stigmasterol     | 0.45993| Oleanolic acid      | 0.44599|
| Methionine       | 0.45058| Beta-sitosterol     | 0.43538|
| Wogonin          | 0.88622| Methionine          | 0.45058|
| Berberine        | 0.66329| Istdina             | 0.42068|
| p-coumaric acid | 0.65362| Hexadecanoic acid/palmitic acid | 0.41328|
| Gingerol/6-gingerol | 0.64652| L-valin             | 0.41201|
| Kaempferol       | 0.63723| PENTADECYLIC ACID   | 0.40593|
| Phenylalanine    | 0.61258| Gamma-aminobutyric acid | 0.39803|
| Hexanoic acid    | 0.56874| Gamma-aminobutyric acid/gamma-aminobutyric acid | 0.39803|
| Oleanolic acid   | 0.56781| Succinic acid       | 0.38636|
| Phenylalanine    | 0.56642| Succinic acid       | 0.38636|
| Succin acid      | 0.53025| Baicalin            | 0.36847|
| CMP              | 0.52108| Baicalin            | 0.36174|
| (s)-Tyrosine     | 0.51102| Alanine             | 0.35618|
| DTY              | 0.51102| L-alanine           | 0.35618|
| Quercetin        | 0.50642| LPG                 | 0.35618|
| Adenosine/adenine nucleoside | 0.49534| Choline             | 0.34046|
| Catechol         | 0.49463| Glycine             | 0.33765|
| Hydroquinone     | 0.49463| Linoleic acid       | 0.3335 |
| DBP              | 0.47523| Linolenic acid      | 0.33261|
| Myristic acid    | 0.47259| Zoomaric acid       | 0.32561|
| Niacin/nicotinic acid | 0.47152| GUP                 | 0.30455|
| Leucine          | 0.46862| Stearic acid        | 0.30168|
| Leucinum         | 0.46862|                    |       |

Figure 2: The herb-compound-target network with 55 bioactive compounds. The pink square in the figure is the key target of colon cancer by the compound. The circle represents the core compound with degree value ≥ 30. Different colors represent the compounds contained in different Chinese medicines. The green circles represent the compounds contained in various Chinese medicines, and the red prisms represent different Chinese medicines. The size of the graph in the figure represents the degree of the network in the size of the value.
Figure 3: Gene Ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of 136 overlapping genes. (a) Top 10 significantly enriched molecular functions (MF). (b) Top 15 significantly enriched biological processes (BP). (c) Top 10 significantly enriched cellular components (CC). (d) Top 15 significantly enriched pathways.

| ID     | Description                                           | p.adjust | Count |
|--------|-------------------------------------------------------|----------|-------|
| hsa05163 | Human cytomegalovirus infection                      | 8.6E-27  | 38    |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection       | 3.6E-26  | 35    |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications  | 1.3E-24  | 27    |
| hsa05170 | Human immunodeficiency virus 1 infection              | 8.6E-18  | 29    |
| hsa04926 | Relaxin signaling pathway                             | 8.6E-18  | 24    |
| hsa05142 | Chagas disease (American trypanosomiasis)            | 8.6E-18  | 22    |
| hsa05161 | Hepatitis B                                           | 8.6E-18  | 26    |
| hsa05418 | Fluid shear stress and atherosclerosis                | 5.4E-16  | 23    |
| hsa05215 | Prostate cancer                                       | 7.9E-16  | 20    |
| hsa04668 | TNF signaling pathway                                 | 8.0E-16  | 21    |
| hsa05122 | Endocrine resistance                                  | 8.0E-16  | 20    |
| hsa05210 | Colorectal cancer                                     | 9.9E-16  | 19    |
| hsa04657 | IL-17 signaling pathway                               | 5.4E-15  | 19    |
| hsa04066 | HIF-1 signaling pathway                               | 9.1E-14  | 19    |
| hsa05169 | Epstein-Barr virus infection                          | 1.1E-13  | 24    |

2.7. Cell Culture and Animal Model. The human colon cancer HCT116 cell lines were purchased from Shanghai Institutes for Biological Sciences. HCT116 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (Gibco, USA), 100 U/ml of penicillin, and 100 mg/ml streptomycin (Solvbio, China). Cells were maintained at 37°C with 5% CO₂.

The male BALB/c nude mice (5-6-week-old, 18–20 g weight) were purchased from the Institute of Laboratory Animal Science. The mice were housed in a specific pathogen-free condition with a 12-hour light/dark cycle at 25±2°C and had free access to food and water. Each mouse was subcutaneously injected with 0.2 mL of 1×10⁷/mL HCT116 cells into the right armpit to establish the colon
The tumor size was measured every 3 days. After 21 days, the serum was collected and the mice were sacrificed with carbon dioxide. Xenograft tumors were excised and weighed. Tumor inhibition rate (IR): IR = (average tumor weight in the model group − average tumor weight in the experimental group)/average tumor weight in the model group × 100%. All animal experiments were performed in accordance with the institutional guidelines of the Animal Care and Use Committee of Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University.

2.8. TUNEL Assay. The apoptosis of the tumor tissue was detected according to the instructions of a TUNEL Kit (Abcam, UK). Briefly, tumor sections were permeabilized with 0.1% TritonX-100 for 2 min. Then, incubate the permeabilized section with TUNEL reaction solution at 37°C for 1 hour. The slides were stained with FITC-conjugated rabbit anti-mouse IgG (1:100, Abcam) and 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, USA). Five areas were arbitrarily selected for observation under the microscope (Olympus, Japan).

2.9. Immunohistochemical Analysis. The expression of Ki67 in tumor tissues was detected using the previously reported method [15]. Briefly, tumor tissue sections were routinely sampled.
dewaxed, hydrated, and placed in hot citrate buffer (pH 6.0) for antigen retrieval for 2 min, followed by the addition of 3% H2O2 solution for 10 min to quench endogenous peroxidase activity. After blocking with 10% goat serum for 10 min, sections were incubated with Ki67 antibody (1:200, Abcam, ab16667) overnight at 4°C, followed by secondary antibody (1:200, Abcam) incubation at 37°C for 30 min. Subsequently, sections were stained with 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma) for 10 min at room temperature without light and counterstained with hematoxylin for 3 min. Finally, sections were visualized with light microscopy (Olympus).

2.10. Western Blot analysis. RIPA lysis buffer (Beyotime, China) was used to extract total protein from tumor tissues. Then, the same amount of protein was added to sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membrane, and sealed in 5% skim milk for 1 h. It was then incubated with anti-PI3K (1:1000, Cell Signaling Technology (CST), USA, #4257S), anti-p-PI3K (1:1000, CST, #17366S), anti-ERK1/2 (1:1000, Abcam, ab17942), anti-p-ERK1/2 (1:1000, Abcam, ab278538), anti-Bcl2 (1:1000, Abcam, ab196495), anti-Bax (1:1000, CST, #2772S), and anti-GAPDH (1:1000, Abcam, ab181603) at 4°C overnight. After washing three times with phosphate buffered saline (PBS), the membrane was incubated with secondary antibody (1:1000, Abcam) for 1 h. Finally, the enhanced chemiluminescent reagents were used to observe the protein bands.

2.11. qRT-PCR Analysis. Total RNA from the tumor tissue was obtained by Trizol reagent (Takara, Japan) and reverse transcribed to cDNA following PrimeScript RT-PCR Kit (Takara) instructions. The qRT-PCR was performed by 7500 real-time PCR system (Applied Biosystems, USA), follow with 95°C for 3 min, 40 cycle of 95°C for 12 s, and 62°C for 40 s. The relative expression of genes was calculated using the 2−ΔΔCT method and normalized to GAPDH. The primer sequences are shown in Table S1.

2.12. ELISA Assay. The authors detected the expression of inflammatory factors (CASP3, IL6, TNFα) in serum according to the instructions of the ELISA kit manufacturer (Beyotime, China).

2.13. Statistical Analysis. All data were expressed as mean with standard deviation (SD). Statistical analysis was done by one-way ANOVA, followed by Bonferroni test using the GraphPad Prism software. P < 0.05 was considered to be a statistically significant level.
3. Results

3.1. Screening of Colon Cancer Therapy Targets. A total of 1,320 and 1,243 colon cancer therapy targets were collected from GeneCard and DisGeNET database, respectively. Then, they merged the targets from the two database and deleted the duplicate targets, resulting in a total of 1,952 colon cancer treatment targets.

3.2. The Collection of the Active Compounds and Targets. From TCMID, TCMSP, and HIT database, a total of 588 active compounds were collected. In addition to the above data source, the authors have harvested 7,550 compound-targets from the STITCH database. Based on the QED value of DrugBank-listed drugs, 0.3 was selected as the threshold to screen compounds, and a total of 444 compounds with drug-like components were obtained. Subsequently, according to the binomial statistical model, we retrieved 340 compound-targets and 387 primary active compounds.

3.3. Screening the Overlapping Gene and Constructing PPI Network. The Venn diagram results show that BXD has 136 overlapping targets in the treatment of colon cancer and constructed a PPI network using Cytoscape 3.8.0 software (Figure 1, Table 1). Through these overlapping targets, 214 main active compounds were identified, among which 55 active compounds with degree greater than 30 were selected (Table 2). Moreover, we constructed a herb-compound-target network with the 55 active compounds by Cytoscape 3.8.0 software (Figure 2).

3.4. Biological Function Analysis. To explore the various mechanisms of the BXD against colon cancer, GO analysis and KEGG pathway analysis were performed of the 136 overlapping targets. The number of the GO annotation terms associated with 136 overlapping targets for MF, BP, and CC was 67, 1525, and 46 ($P < 0.01$), respectively. We showed the top 15 terms in Figure 3(a)–3(c). The MF terms were associated with amide binding, receptor agonist activity, peptide binding, heme binding, tetrapyrole binding, and...
others. The BP terms may relate to a response to oxidative stress, response to nutrient levels, response to the molecule of bacterial origin, response to lipopolysaccharide, aging, and so on. The CC terms are primarily involved in membrane microdomain, membrane raft, membrane region, vesicle lumen, early endosome, etc.

As a result, 135 key pathways were found to be markedly associated with BXD therapeutic colon cancer, followed by adjusted \( P \) value < 0.01. The result of top 15 terms was shown in Figure 3(d) and Table 3. The top 15 KEGG pathways were as follows: human cytomegalovirus infection, Kaposi sarcoma-associated herpesvirus infection, AGE-RAGE signaling pathway in diabetic complications, Hepatitis B, chagas disease (American trypanosomiasis), relaxin signaling pathway, human immunodeficiency virus 1 infection, fluid shear stress and atherosclerosis, prostate cancer, endocrine resistance, TNF signaling pathway, colorectal cancer, IL-17 signaling pathway, HIF-1 signaling pathway, and Epstein-Barr virus infection. The distribution of key targets in colorectal cancer is shown in Figure 4. The results indicated that the action targets of main bioactive components of BXD were distributed in different signaling pathways.

3.5. Screening the Hub Gene and Constructing Drug-Disease-Target-Pathway Network. Based on the 136 overlapping targets of the PPI network, hub genes were selected by CytoHubba. The results showed that VEGFA, AKT1, ALB, CASP3, INS, MAPK3, PTGS2, TNF, TP53, and IL6 were the top 10 hub nodes in the 136 overlapping targets of the PPI network. The PPI network of the hub genes was present in Figure 5. In addition, to elucidate the interrelationships of BXD, colon cancer, targets, and the top 20 pathways, we constructed a drug-disease-target-pathway network (Figure 6).

3.6. BXD Inhibited Tumor Growth and Induced Apoptosis in Tumor Tissues. To detect the antitumor effects of BXD, we constructed a xenograft tumor model with the HCT116 cell. Compared with the model group, BXD treatment, especially high-dose BXD, significantly inhibited tumor growth (Figure 7(a) \( P < 0.01 \), Figure 7(b), 7(c)). Moreover, the tumor IR result suggested that the tumor growth was signally suppressed by low-, middle-, and high-dose BXD and was dose-dependent \( (P < 0.01 \), Figure 7(d)).
In addition, the TUNEL and Ki67 staining results show that BXD inhibited tumor cell proliferation and induced tumor cell apoptosis (Figure 8).

3.7. Effects of BXD on Potential Targets in Xenograft Tumor Model. In this study, we selected PI3K, ERK1/2, Bcl2, and Bax as the potential targets of BXD based on the network pharmacology. Western blotting and qRT-PCR analysis results showed that Bcl2 protein levels decreased, Bax protein levels increased, and PI3K and ERK1/2 protein levels remained unchanged in the BXD-treated group compared with the model group. Moreover, western blotting results also showed that compared with the model group, the protein levels of p-PI3K and p-ERK1/2 in the BXD treatment group significantly decreased ($P < 0.05$, Figure 9).

3.8. Effects of BXD on CASP3, IL6, and TNFα. It has been reported that tumor-related inflammation is the seventh feature of tumors, and smoldering inflammation contributes to the proliferation and survival of the malignant cell [16]. Therefore, the authors determined the content of CASP3, TNFα, and IL6 inflammatory factors in the serum of the xenograft tumor model. The results showed that BXD treatment reduced the content of TNFα and IL6 and increased CASP3 ($P < 0.05$, Figure 10).
Colon cancer is one of the common digestive system diseases in China, and it is also the leading cause of cancer deaths in the world. At present, studies show that BXD can effectively alleviate the occurrence of colon cancer [7,17], however, its molecular mechanism is still unclear. This study revealed the potential targets and molecular mechanism of BXD against colon cancer through network pharmacology and constructed a tumor xenograft mouse model for experimental verification.

The results of network analysis showed that the bioactive compounds of BXD mainly included berberine, quercetin, baicalein, and so on. Berberine, an isoquinoline alkaloid, has been shown to suppress the colon cancer cell growth [18–20]. Prak’s study suggested that berberine-induced AMPK activation inhibits the metastatic potential of colon cancer cells [21]. Quercetin is a natural flavonoid compound with anti-inflammatory and antitumor properties. Ozsoy’s research showed that quercetin may induce the apoptosis of primary colon cancer cells and also trigger the senescence of colon cancer cells [22]. Study has shown that baicalein can significantly inhibit intestinal inflammation and induce cancer cell death [23]. In addition, studies have shown that inhibiting autophagy can enhance the apoptosis of colon cancer cells induced by baicalein [24].

In the present study, GO and KEGG pathway enrichment analyses were applied to further illustrate the mechanism of BXD in colon cancer treatment. The GO enrichment analysis showed that the potential genes mainly function in response to oxidative stress and nutrient levels. In a clinical research report, it was pointed out that oxidative stress is a key factor in the development of solid malignant tumors. The production of ROS/RNS in the colon causes oxidative stress and may make individuals susceptible to colon cancer [25]. Yang’s research pointed out that the recurrence level and length of exposure of colon cancer in a mouse model induced by a new Western diet are linked to the relatively dangerous nutrient colon cancer in humans [26]. KEGG pathway enrichment analysis showed that human cytomegalovirus infection and AGE-RAGE signaling pathway in diabetic complications were involved in the mechanism of BXD anticolon cancer. Human cytomegalovirus infection has been shown to be an oncogenic factor closely associated with colorectal cancer, which facilitates the spread and spread of tumors [27,28]. AGE/RAGE signaling pathway activates intracellular and downstream HIF-1α and PI3K/AKT signaling pathways to promote tumor cell proliferation, migration, invasion, clonning, and spheroidization, thereby inhibiting cell apoptosis and activating the epithelial-mesenchymal transition process [29]. Epithelial-mesenchymal transition plays an important role in the occurrence and development of colorectal cancer [30].

Finally, we constructed a tumor xenograft mouse model with HCT116 cells to verify the effect of BXD in colon cancer treatment. The TUNEL and Ki67 staining result indicated that BXD could suppress tumor cell growth. Furthermore, the western and qRT-PCR results suggested PI3K, ERK1/2, Bcl2, and Bax were the potential targets in colon cancer treatment. In addition, ELISA experiment results show that BXD could inhibit the expression of IL6 and TNFα proinflammatory factors and enhance the expression of CASP3 apoptotic protein.

4. Discussion

This study found that the occurrence of colon cancer was related to oxidative stress and eating habits. The BXD treatment of colon cancer may be related to berberine, quercetin, baicalein, and other active compounds. Bcl2, Bax, IL6, TNFα, CASP3, and other potential targets are related, and they may inhibit tumor growth and induce tumor cell apoptosis through AGE/RAGE and other signaling pathways. Importantly, our findings provide a potential drug for colon cancer clinical treatment and partially reveal the molecular mechanism of colon cancer treatment. At the same time, there are shortcomings in our research, such as the lack of support from clinical data.

5. Conclusion

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.
Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments
This work was supported by the study on the Mechanism of Banxia Xiexin Decoction Regulating Autophagy in the Treatment of Colorectal Cancer through lncRNA HOTAIR Mediated MAPK/mTOR Pathway (grant no. 20201203B202).

Supplementary Materials
Table S1: the sequences of primers used for qRT-PCR. (Supplementary Materials)

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