Anti-Tumor Effect and Mechanism of Bletilla Rhizoma Based on Network Pharmacology

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Abstract. Bletilla Rhizoma is a traditional Chinese medicine used for hemostasis. Modern research shows that Bletilla Rhizoma has anti-tumor effect, but its mechanism is not clear. We obtained the active chemical components and targets of Bletilla Rhizoma through the systematic pharmacology database of Traditional Chinese Medicine (TCMSP), and established the data set of the intersection of active components and disease targets. The protein-protein interaction network (PPI) was used to analyze the protein interaction network of Bletilla Rhizoma active components target and tumor target. The biological information annotation database (DAVID) was used to perform gene ontology (GO) function enrichment analysis and based on Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis to predict related mechanisms of anti-tumor effects of Bletilla Rhizoma. 15 active components were obtained from TCMSP, and 40 potential targets were screened out through the evaluation of the network topology. The DAVID database was used to perform gene GO functional enrichment analysis and KEGG pathway enrichment analysis on 40 potential target targets to screen for participation. The first 20 biological processes and signaling pathways involved in the anti-tumor effect of Bletilla Rhizoma were screened out. Through network pharmacology, we have drawn the conclusion that the anti-tumor effect of Bletilla Rhizoma is mainly regulated by pathways such as insulin resistance, legionelosis and proteoglycans in cancer, among which the proteoglycans in cancer pathway has a strong correlation with cancer, and its anti-cancer pathway is closely related to TNF, a key target.

1. Introduction

Bletilla Rhizoma is the tuber of Bletilla striata (Thunb.) Reichb.f., which has the functions of astringent, hemostasis, detoxification, tonic deficiency. It is commonly used in the treatment of traumatic bleeding, ulcer bleeding, hematemesis [1]. Modern pharmacological studies have found Bletilla Rhizoma has anti-tumor, anti-inflammatory, anti-gastric ulcer activities [2-4]. In recent years, Bletilla Rhizoma has been used in the treatment of tumors, including uterine fibroids, liver cancer and esophageal cancer [5]. Bletilla Rhizoma can inhibit the growth and proliferation of human gastric cancer cells (SGC), human ovarian cancer cells (A2780), hepatocarcinoma cells (HepG2) [6-7], and
can significantly induce A549 human lung adenocarcinoma cell cycle to block in the G0/G1 phase, which has potential activity in the treatment of lung cancer [8]. In addition, Bletilla Rhizoma can significantly inhibit S180 sarcoma and prolong the survival time of H22 ascites hepatoma mice [9]. At present, the anti-tumor effect of Bletilla Rhizoma is mainly based on animal and in vitro cell experiments, and the treatment and mechanism of human tumor still need to be further explored.

Tumors have multi-factor, multi-link pathogenic mechanisms, and the multi-target, multi-link, and various regulation methods of traditional Chinese medicine can be applied to multiple links of tumor occurrence and development. Traditional Chinese medicine has the characteristics of multi-component and multi-target, so it is difficult to define the material basis and mechanism of efficacy. Traditional research methods are difficult to carry out systematic research from the level of molecule, cell, tissue and organ to the whole [10]. Network pharmacology is different from the concept of "one drug, one target, one disease" in traditional pharmacology research methods. It constructs a multi-level network by retrieving relevant databases, combining high-throughput group data analysis and computer simulation. Based on the "disease gene target drug" interaction network, we can predict drug targets as a whole, and comprehensively and systematically study the law and mechanism of drug body interaction. Therefore, this article used the network pharmacology method to excavate the main anti-tumor active ingredients and action targets of Bletilla Rhizoma, so as to provide a theoretical basis for the in-depth development of experimental research and guidance of clinical rational drug use.

2. Materials and Methods

2.1. Screening of active ingredients
By using the Traditional Chinese Medicine Taiwan Database (TDT, http://tcm.cmu.edu.tw/), the BATMAN-TCM Database, the Traditional Chinese Medicine Comprehensive Database (TCMID, http://www.megabionet.org/tcmid/search/) and the Traditional Chinese Medicine System Pharmacological analysis platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php), retrieves all the chemical components contained in Bletilla Rhizoma. According to the TCMSP database [8], ADME parameters (OB≥30% and DL≥0.18) were used as the standard [9], or the Bletilla Rhizoma components reported in the literature were used to screen the active chemical components.

2.2. Establish target data set
By the comprehensive analysis of TCMSP database, Chinese medicine target database and BATMAN-TCM database [10], the targets of Bletilla Rhizoma active components were screened, and the component targets data set was established. Based on the similarity comparison, the active ingredients corresponding to the screening target were passed through the Chinese herbal medicine active ingredient database (HIT, http://lifecenter.sgst.cn/hit/) and the therapeutic target database (TTD, http://bidd.nus.edu.sg/group/cjttld/) to screen potential targets for active ingredients. A potential target data set of Bletilla Rhizoma and active components was established, and genes and protein targets related to tumors were screened through the GeneCards [11] database, and a disease-drug common target data set was established.

2.3. Build PPI network
The selected targets were imported into string (https://string-db.org/) to construct PPI, the protein type was set as "homin sapiens (human)", the confidence degree was 0.4, and the PPI was obtained. The PPI was saved as TSV file, imported into Cytoscape 3.6.1, analyzed with its "network analyzer" plug-in, and the core anti-tumor targets of Bletilla Rhizoma were determined.

2.4. KEGG signal pathway and GO biological process enrichment analysis
The common targets of disease and drug were introduced into the human genome annotation database David 6.8 (https://david.ncifcrf.gov/) for GO enrichment analysis and KEGG signal pathway analysis,
and $P<0.05$ was used as the critical value of significant function and pathway to screen the target genes.

3. Results

3.1. Active ingredients
15 active chemical components and 318 potential targets were selected, and the results are shown in Table 1.

Table 1. Bletilla Rhizoma active compounds and target number.

| No. | Component                                                                 | Target |
|-----|---------------------------------------------------------------------------|--------|
| 1   | PHB                                                                       | 15     |
| 2   | protocatechuic acid                                                      | 27     |
| 3   | physcion                                                                 | 25     |
| 4   | HBA                                                                       | 4      |
| 5   | caffeate                                                                  | 22     |
| 6   | blespirol                                                                | 2      |
| 7   | cinnamic acid                                                            | 9      |
| 8   | sitosterol                                                               | 46     |
| 9   | 2, 4, 7 – trimethoxy - 9, 10 - dihydrophenanthrene                        | 58     |
| 10  | 2, 3, 4, 7 - tetramethoxyphenanthrene                                    | 9      |
| 11  | 1 - (4 - hydroxybenzyl) - 4 - methoxy - 9, 10 - dihydrophenanthrene - 2, 7 - diol | 21     |
| 12  | 1, 3 – dimethoxy -5 - [2- (3 - methoxyphenyl) ethyl] benzene              | 28     |
| 13  | 1, 3 - dimethoxy - 5 - (2 - phenylethyl) benzene                         | 25     |
| 14  | 4, 7 - dihydroxy - 1 - p - hydroxybenzyl - 2 - methoxy - 9, 10 - dihydrophenanthrene | 21     |
| 15  | 3, 7 - dihydroxy - 2, 4 - dimethoxyphenanthrene - 3 - O - glucoside      | 6      |

3.2. Screening for disease and drug targets
With "tumour" as the key word, the tumor-related target genes were retrieved in Genecards. A total of 2568 related target genes were searched in OMIM, which were matched with related targets of Bletilla Rhizoma active components and Venn diagram was drawn, as shown in Figure 1. There are 40 common targets (TNF, EGFR, CASP3, ESR1, AR, NFKB1, PPARG, etc.) involved in the common system of white and active components—anti-tumor, which are key targets, as shown in Figure 2.

Figure 1. Venn diagram of matching Bletilla Rhizoma and disease target genes.
Figure 2. Top 10 proteins with strong associations.

Table 2. Topological parameters related to the direct target of Bletilla Rhizoma and its anti-tumor active components.

| UniProt ID | Protein names name                     | Degree | Closeness Centrality | Betweenness Centrality |
|------------|----------------------------------------|--------|----------------------|------------------------|
| P01375     | tumor necrosis factor TNF              | 20     | 0.6923               | 0.2305                 |
| P00533     | epidermal growth factor receptor EGFR  | 20     | 0.6792               | 0.1995                 |
| P42574     | Caspase - 3 CASP3                      | 19     | 0.6667               | 0.1538                 |
| P03372     | estrogen receptor ESR1                 | 15     | 0.6102               | 0.0445                 |
| P10275     | androgen receptor AR                   | 15     | 0.6000               | 0.0579                 |
| P19838     | nuclear factor NF - kB p105 subunit NFKB1 | 15     | 0.6102               | 0.0419                 |
| P37231     | peroxisome proliferator-activated receptor PPARG | 14     | 0.5806               | 0.0425                 |
| P04150     | glucocorticoid receptor NR3C1          | 14     | 0.5902               | 0.0221                 |
| Q15788     | nuclear receptor coactivator 1 NCOA1   | 12     | 0.5000               | 0.0198                 |
| P35869     | aryl hydrocarbon receptor AHR          | 11     | 0.5455               | 0.0137                 |

3.3. Go biological function enrichment analysis
GO enrichment analysis was performed on 40 common targets of Bletilla Rhizoma and tumor-related targets. The top 3 enriched targets include transcription initiation from RNA polymerase II promoter, positive regulation of transcription from RNA polymerase II promoter and signal transduction. The yellow circle represents the biological process of the adjusted $P<0.0001$, and the size of the circle indicates the enrichment of related targets in the pathway. The darker the color of the circles, the stronger the enrichment effect, reflecting that the anti-tumor mechanism of white and Bletilla Rhizoma may involve multiple biological processes in vivo, and suggesting that Bletilla Rhizoma and active ingredients may exert anti-tumor effects by regulating these biological processes.

3.4. KEGG pathway enrichment analysis
By using the software of Cytoscape 3.6.1, 40 common genes, diseases and components were visualized and analyzed, and *Bletilla* Rhizoma and anti-tumor interactive network was constructed, as shown in Figure 3. After selecting the corresponding interactive proteins and visualizing them with different colors and shapes, we can intuitively see the network relationship between the active chemical components and the target. There are 15 in red, representing the active components of the drug, and 40 in green, representing the active targets. The hexagon color represents the disease, and the purple color represents the drug. The larger the connectivity, the larger the shape, as shown in Figure 4.

**Figure 3.** Bubble chart of enrichment analysis of KEGG pathway.

**Figure 4.** Component-target-disease interaction network.
4. Discussion

*Bletilla* Rhizoma has a good therapeutic effect against liver cancer, esophageal tumor, cardiac cancer and uterine fibroids [11], but the anti-tumor mechanism is still unclear. Pharmacology prediction shows that through the network, *Bletilla* Rhizoma main chemical composition are protocatechuic acid, physcion, cinnamic acid, 3,7 dihydroxy-2,4-dimethoxyphenanthrene -3-O-glucoside, these a few classes have anti-tumor effects. For example, physcion has a strong inhibitory effect on Hela cell growth of human cervical cancer [12]; cinnamic acid has obvious inhibitory effect on lung adenocarcinoma cancer cell proliferation is A-5491 lung adenocarcinoma cancer cell effective inhibitors [13]. These anti-tumor active components are mainly related to the genes of TNF, prkca, EGFR, CASP3 and ESR1 on proteoglycans in cancer, pathways in cancer and hepatitis C pathway.

In this study, the TNF gene Degree, Closeness Centrality, and Betweenness Centrality were larger in the key target screening, ranking first in the comprehensive ranking, and is one of the key targets for white and anti-tumor. TNF often binds to TNF receptors present on the surface of tumor cells to cause tumor cell death, or directly inhibits cell growth mediated by specific receptors. KEGG map showed that in the Proteoglycans in cancer pathway, the EGFR gene was activated by Decorin, which indirectly promoted the apoptosis of tumor cells through Casp3. In addition, Decorin (proteoglycan) can activate TNF gene after the expression of MAPK/Erk and p38 singaling, and finally make tumor cells Growth suppression. Therefore, *Bletilla* Rhizoma may play an anti-tumor effect by promoting tumor cell apoptosis or inhibiting its growth. This article provides relevant evidence for the later research on the anti-tumor mechanism of *Bletilla* Rhizoma, but the specific anti-tumor mechanism of *Bletilla* Rhizoma needs to be further studied and verified.

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