Study on Anti-Tumor Network Mechanism of Miao National Herbs Periploca Forrestii

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Abstract. In this study, tumor disease targets and periploca forrestii(PF) associated targets were determined through the use of databases for the identification of putative therapeutic targets and then combined. After the interaction network of therapeutic targets against tumor was established, and the core targets against tumor were determined by degree analysis. For all putative therapeutic targets, analyses of biological function were performed to optimize the biological processes and key signaling pathways of PF in tumor treatment. The top 5 therapeutic targets were identified, including AKT1, TP53, PTGS2, AR and AHR. The biological processes associated with the antitumor activity of PF were mainly associated with positive regulation of cell proliferation, trypsis response, transcription of DNA templates and positive regulation of transcription from RNA polymerase II promote regulation; and signaling pathways associated with the antitumor activity of PF are mainly associated with influenza A signaling pathway, PI3K-Akt signaling pathway, and HTLV-I infection signaling pathway.

1. Introduction
Tumor are caused by various carcinogenic factors, and the cells of local tissues lose the normal regulation of growth at the gene level, leading to abnormal lesions formed by clonality abnormal proliferation [1-2]. According to the latest national cancer statistics released by the national cancer center in January 2019, cancer mortality accounts for 23.91% of all the deaths of residents. In some cities, tumor has surpassed cardiovascular disease and become the “first killer” of human death [3-4]. With the modernization and international development of traditional Chinese medicine (TCM), which has made certain achievements in the basic research and clinical treatment of anti-tumor, TCM has become one of the effective adjuvant therapies after tumor surgery. TCM can act on multiple stages of tumorigenesis and development, due to its multi-component, multi-target, multi-layered, high-efficiency and low-toxicity characteristics. At the same time, it has low toxic and side effects, which can improve the body’s immunity and is not prone to resistance advantages, its anti-tumor mechanism is by inducing cell differentiation, promoting apoptosis, killing tumor cells and regulating related gene expression. Therefore, TCM has always been an important source of drug development and has unique advantages in the prevention and treatment of tumors [5-7].
The PF is derived from dried roots or whole plants of Asclepiadaceae Periploca Linn, also known as “iron bone”, is one of the top ten seedling medicines in Guizhou, which mainly distributed in the southwestern region [8]. Started is contained in “Southern Yunnan Materia Medica”, it has bitter, spicy, mellow throat, and has a small poison. It has the effect of relaxing muscles and activating blood, removing wind and dehumidifying, so the miao people are often used to treat rheumatic pain, amenorrhea, bruises and other diseases [9]. PF contains chemical components such as flavonoids, triterpenes, steroids, and volatile oils [10]. It has anti-inflammatory, analgesic, immunosuppressive, antitumor and other pharmacological effects [11-12]. It has been reported in literature that the active anti-tumor component of the PF is periplocin, and the inhibition rate of this component reaches 97.48% when the concentration is 10 g·ml-1 [13]. The compounds isolated from chloroform layer of ethanol extract from rhizome of PF have strong inhibitory effect on A549 cells of lung cancer [14], indicating that PF has good anti-tumor activity. However, the mechanism of anti-tumor action of PF is unclear. The research about PF is mainly concentrated in the fields of extraction technology, chemical composition, quality control, pharmacological effect, and clinical application. There are very few systematic studies on the molecular mechanisms of multiple pathways. Therefore, this article the network pharmacology method to analyze and predict the potential mechanism of action of PF by using, which provides a theoretical basis for the further development and clinical application of the medicine PF.

In recent years, the research on the mechanism of TCM in network pharmacology has been a hot topic. Network pharmacology is based on biological theories, analyzing the network of biological systems, and select specific signal Nodes for multi-target drug molecular design is a new subject. It integrates drug and biological database, analyzes by means of bioinformatics, and constructs the prediction model of “drug-gene-target protein-disease network”, which is suitable for TCM to study the targets and mechanism of action from a “holistic perspective”. Network pharmacology emphasizes the multi-pathway regulation of signaling pathways, improves the therapeutic effect of drugs, and reduces toxic and side effects, so as to improve the success rate of clinical trials of new drugs and save drug development costs [16].

2. Methods

2.1. Data

The database used in the article is shown in the following table:

| database | website |
|----------|---------|
| TCMSP    | [http://lsp.nwu.edu.cn/tcmspsearch.php](http://lsp.nwu.edu.cn/tcmspsearch.php) |
| UniProt  | [http://www.uniprot.org](http://www.uniprot.org) |
| DAVID    | [https://david.ncifcrf.gov/summary.jsp](https://david.ncifcrf.gov/summary.jsp) |
| STRING   | [https://STRING-db.org](https://STRING-db.org) |
| Omicshare| [http://omicsshare.com/](http://omicsshare.com/) |
| Genecards| [http://www.genecards.org/](http://www.genecards.org/) |
| CAS      | [http://www.ichemistry.cn/](http://www.ichemistry.cn/) |
| OmicShare| [http://www.omicsshare.com/tools/](http://www.omicsshare.com/tools/) |
| CNKI     | [https://www.cnki.net/](https://www.cnki.net/) |

2.2. PF screening of the active chemical constituents and acquisition of the targets of action

The chemical constituents of PF were collected using databases such as CNKI, Weipu, Wanfang, etc. The collected chemical components were searched for chemical sources in the CAS database and “CAS” was selected through the TCMSP database to obtain bioavailability (OB ≥ 30%) and the drug-like property (DL≥0.18) is the standard. The active chemical constituents of the PF are selected and
molecular ID name corresponding to the corresponding active chemical components are preserved. And use the TCMSP database to search for target proteins corresponding to all active chemical components in PF, and the collected target protein names were converted into gene ID names by the protein database Uniprot, it is limited to human.

2.3. Acquisition of Potential Targets of PF
With “Tumor” as the key word, the Genecards database was used to obtain the target of the disease, the target of the active ingredient of the PF and the target of the disease were intersected as the common potential targets of the disease and the drug.

2.4. Construction of “Drug-Composition-Disease” Target Network Diagram of PF
The potential targets of diseases and drugs were introduced into Cytoscape3.6.1 software to construct the network diagram of drug-components-disease target genes, and the network diagram was used to demonstrate the interaction among the active components; diseases and targets.

2.5. Establishment of a network for the interaction between PF and disease target proteins
The species was set as “Homo sapiens” in the STRING database to obtain the protein interaction relationship of Network, and the software Cytoscape3.6.1 was imported. The “Network analyzer” plug-in of the software was used to analyze the node degree value of the above Network for the protein interaction Network.

2.6. Gene ontology (GO) enrichment analysis and KEGG pathway enrichment analysis
Import targets into the DAVID database for KEGG pathway analysis and GO biological process analysis; screening to obtain significantly enriched (P <0.01) pathways, and sorting according to the number of genes from top to bottom, which selects the top 20 pathways with gene number. Using the OmicShare website to draw pathway bubble diagrams, and use the KEGG Mapper function in the KEGG signal pathway database to mark the signal pathways most closely related to anti-tumor.

3. Results
3.1. Screening of chemical components and active components of PF
A total of 41 chemical components of PF were collected from databases such as CNKI, Weipu, Wanfang, etc. A total of 6 active chemical components of PF were screened according to ADME parameters, through CAS database and TCMSP database, bioavailability (OB≥30%) and class medicine (DL≥0.18). The results are shown in table 2.

| CAS number | Mol ID    | chemical component | targets | OB(%) | DL  |
|------------|-----------|--------------------|---------|-------|-----|
| 514-39-6   | MOL005658 | periplogenin       | 1       | 36.61 | 0.74|
| 64997-52-0 | MOL000358 | cupreol            | 37      | 36.91 | 0.75|
| 520-18-3   | MOL000422 | kaempferol         | 61      | 41.88 | 0.24|
| 117-39-5   | MOL000098 | meletin            | 152     | 46.43 | 0.28|
| 465-99-6   | MOL000296 | hederagenin        | 22      | 36.91 | 0.75|
| 474-58-8   | MOL001525 | daucosterol        | 2       | 36.91 | 0.75|

3.2. Target prediction of PF and construction of active ingredient-disease target network
The TCMSP database was used to screen active ingredients to obtain 275 targets. Genecards database was used to screen for 1275 disease targets. 48 common targets for drugs and diseases were obtained. One duplicate was eliminated to obtain 47 potential targets. 47 targets were imported into Cytoscape 3.6.1 software to build a PF active ingredient-disease target network diagram. After visualizing it with
graphics of different colors and shapes, the network relationship between “drug - ingredient - target - disease” could be intuitively seen, as shown in figure 1.

Figure 1. “drug - ingredient - target -disease” interactive network diagram of the anti-tumor effect of PF

3.3. Anti-tumor protein interaction network of PF

47 targets that may be related to anti-tumor activity of PF were introduced into the STRING database protein-protein correlation relationship, the protein network of PF was formed through Cytoscape3.6.1 software, as shown in figure 2. In figure 3, the node size is formed according to the Degree value. The larger the Degree value, the larger the node, indicating that the node is more important in the network, such as PTGS2, AKT1, TP53, IL10 and so on.

Figure 2. relationship diagram of protein interactions of potential targets for the antitumor effect of PF
3.4. GO biological function analysis and KEGG pathway analysis

KEGG pathway and GO biological processes were analyzed by DAVID database, and 50 biological processes were obtained by GO enrichment analysis. Screened (P <0.001) the top 20 pathways with gene numbers, and used the OmicShare website to draw pathway bubble diagrams (Figure 4), which mainly involved positive regulation of cell proliferation, transcription of DNA templates, positive regulation of RNA polymerase II promoter transcription, biological processes, inflammatory response and so on.

Figure 4. The first 20 entries of bioprocess enrichment

KEGG pathway enrichment analysis obtained 64 signal pathways, enumerating the top 20 signal pathways, which includes Pathways in cancer, PI3K-Akt signaling pathway, Pancreatic cancer, Prostate cancer and Hepatitis B, etc., see Figure 5. At the same time, using the KEGG Mapper function in the KEGG signal pathway database, 47 target proteins related to the antitumor signal of PF
were marked on the closest pathway in the antitumor signal pathway. The results showed that 11 target proteins participate in PI3K-Akt relevant regulation of the signaling pathway, see Figure 6.

Figure 5. Results of the first 20 enrichment analysis of the KEGG signal pathway

Figure 6. Labeling diagram of potential targets of the active components of PF on the PI3K-Akt signaling pathway

4. Discussion
As the internationalization of TCM has been enhanced, the standardization of anti-tumor research of TCM has also been deepened, and which will make certain contributions to the development of human treatment of cancer. It has been reported the components of the PF in the literature, such as
periplogenin, β-sitosterol, carotenoside, kaempferol, quercetin, hederagenin and so on have inhibitory effects on a variety of tumor cells [17], but the anti-tumor mechanism and target have been unclear. With the establishment and development of network pharmacology and the application of biological information analysis technology, the research on gene regulation mechanism of drug therapy has provided favorable conditions.

In this paper, network pharmacology method was adopted to construct a network based on active chemical components, proteins, signaling pathways and other databases, and targets function were enriched to systematically and comprehensively analyze the intervention and influence mechanism of drugs on diseases. The main active components of PF include periplogenin, β-sitosterol, carotenoside, kaempferol, quercetin and hederagenin, which are involved in the anti-tumor mechanism of PF. Zhongwei Chen et al [18] experimental results show that quercetin-specific silencing (Prohibitin) gene expression can inhibit nasopharyngeal cancer cell proliferation activity and living cell aging, distinguishing cancer cells with different degrees of apoptosis, and promote cancer cell apoptosis[19]; Quercetin has an inhibitory effect on the growth of a variety of tumor cells, and it has an important role in tumor chemoprevention [20]. β-sitosterol has anti-tumor effects on H22 tumor-bearing mice by regulating the expressions of IL-6, IFN-γ and VEGF, as well as it also promotes autophagy and apoptosis of cancer cells [21-22]. kaempferol can promote the apoptosis of human inflammatory breast cancer SUM190 cell line, which is related to down-regulate the phosphorylation level of PI3K, AKT and GSK-3β and inhibit the PI3K/AKT/ GSK-3β signaling pathway [23]. Yubo Han et al [24] used MTT method to determine the toxic effect of periplogenin on tumor cells cultured in vitro in male mice. The experimental results showed that glucoside could significantly prolong the survival time of ascites tumor bearing mice and inhibit the growth of solid tumors. Daucosterol has a significant inhibitory effect on migration of breast cancer cells and liver cancer cells [25]. Hederagenin can reduce the phosphorylation level of STAT3 in cancer cells and inhibit the activation of STAT3 signaling pathway. STAT3 signaling pathway inhibitor and hederagenin jointly inhibit the proliferation of cervical cancer cells, which may play an anti-cervical cancer cell role through the STAT3 signaling pathway [26]. The above studies have shown that the 6 active chemical components of PF from different targets and signaling pathways in inhibit the proliferation and metastasis of tumor cells.

According to the analysis of the protein interaction relationship of potential targets for the anti-tumor effect of PF, the key anti-tumor targets of PF may be AKT1, TP53, PTGS2, RELA and other targets. The activation of AKT1 can promote the proliferation of breast and prostate cancer cells and inhibit the migration and invasion of tumor cells. However, the growth of breast cancer and prostate cancer cells will be inhibited and their metastasis ability will be enhanced [27]. Currently, TP53 gene has been found to be the tumor suppressor gene with the highest correlation with tumors. The mutated TP53 gene can promote the proliferation, migration, survival and invasion of tumor cells, enhance the drug resistance of tumor cells, destroy the physiological structure of normal tissues, and promote the metabolism of tumor cells [28]. PTGS2 can promote tumor angiogenesis, epithelial cell dedifferentiation, enhance tumor cell invasion and metastasis potential, inhibit cell depletion and other biological processes [29]. Some researchs have reported that the decrease in RelA expression suggests that FFXY may inhibit the expression of the anti-apoptotic gene RelA by interfering with the activation of the NF-κb pathway, thereby promoting the apoptosis of PLGC cells, returning cell growth to normal, and further reversing the gastric mucosal damage. It has pathological biochemical indicators, protects and repairs gastric mucosal damage, and blocks the development of PLGC [30].

The results of KEGG pathway enrichment analysis indicated that the anti-tumor effect of PF may play a therapeutic role in signal pathways such as influenza A, PI3K-Akt signaling pathway, and HTLV-I infection. Relevant studies have shown that influenza A is prone to mutation and an acute infectious disease. The virus gene mutation can infect humans and accompanied by pneumonia. In severe cases, it may cause death due to heart, kidney and other organ failure, with a high death rate [31]. The PI3K-Akt signaling pathway is a signal transduction pathway mediated by enzyme-linked receptors, which further up-regulates Bcl-2 protein expression and down-regulates Bax protein
expression, thereby inhibiting myocardial cell apoptosis. In addition, the PI3K-Akt signaling pathway and cells the regulation of proliferation, differentiation, and glucose transport various cell functions and plays an important role in apoptosis, survival, and regulation of cell energy metabolism [32]. HTLV-I virus can induce the occurrence of cancer by activating host cell retrovirus, and it mainly has three mechanisms, including oncogene inducing carcinogenesis, t-lymphocytic leukemia line and insertion mutation [33].

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