Mechanisms of Sinomenine Nanoparticles in the Treatment of Breast Cancer Based on Network Pharmacology

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Abstract. This article mainly discusses the mechanism of SINOMENINE nanoparticles in the treatment of breast cancer by using the network pharmacology method. TCMPSP and Swiss Target Prediction databases were searched for the target of SINOMENINE. The "breast cancer" target was searched through the GeneCards database. The common targets of both SINOMENINE and breast cancer were considered as the targets of SINOMENINE for treating breast cancer. The software Cytoscape 3.7.1 was used for topological analysis and visualization. The DAVID databases were used for GO enrichment analysis. A total of 57 key targets for the treatment of breast cancer were obtained, of which AKT1, MTOR, EGFR, NOS3, MAPK8, MMP9, etc. The results of pathway enrichment and functional annotation show that SINOMENINE mainly involves protein phosphorylation and positive regulation of nitric oxide biosynthetic process by regulating Sphingolipid signaling pathway, Pathways in cancer, Measles, Smallcell lung cancer, Focal adhesion, and Insulin resistance. There are 10 major biological processes, including protein phosphorylation, signal transduction, negative regulation of apoptotic process, and protein autophosphorylation. This study not only initially revealed potential molecular mechanism of SINOMENINE, but also provided a reference for the in-depth development of SINOMENINE nanoparticles for the treatment of breast cancer.

1. Introduction
Breast cancer is a highly malignant tumor, endangering the health of women all over the world[1]. According to the statistics, breast cancer is one of the most common cancers in women in the United States, accounting for about 14% of all malignant tumor deaths in women[2]. In northern Europe and other countries, It also shows a high incidence[3]. In China, the incidence of breast cancer ranks first among all cancers in women, and the main cause of death among cancer patients over 45 years of age is breast cancer[4-5]. Therefore, breast cancer is still one of the most difficult cancer diseases in clinical practice. Common treatments for breast cancer include surgery, radiation therapy, chemotherapy, endocrine therapy, biological therapy[6]. The current treatment of breast cancer is mainly a comprehensive treatment based on surgery. In order to prolong the survival rate, reduce recurrence and metastasis, chemotherapy is currently the commonly used treatment option, but some patients cannot tolerate the complications of chemotherapy and receive effective treatment. Therefore, the development of natural medicines are easily tolerated by patients.
SINOMENINE is an alkaloid monomer extracted from the plant of caulis Sinomenii[7]. Its effects include immunosuppressive, analgesic, anti-inflammatory, antihypertensive and anti-arrhythmic[8]. With the gradual deepening of research on SINOMENINE, it has been found that SINOMENINE can inhibit a variety of malignant tumor cells. The has obvious inhibitory effects on cervical cancer, lung cancer, breast cancer, liver cancer, gastric cancer and other tumors. However, SINOMENINE has low bioavailability and poor targeting. We prepared it into SINOMENINE chitosan nanoparticles, which greatly improved its anti-tumor effect, thus revealing the mechanism of its anti-breast cancer through the method of network pharmacology.

Network pharmacology is based on the theory of systems biology, starting from the holistic and systemic nature of the interaction between drugs, targets and diseases, and using complex biological network models to reveal the complex overall biological network between drugs-genes-targets-disease. Through such an analysis process, it can predict the pharmacological mechanism of the drug[9-12]. This study, based on the analysis method of network pharmacology, comprehensively explores the mechanism of action of SINOMENINE nanoparticles in the treatment of breast cancer from the perspective of overall and system biology. Therefore, it provide a reference for the pharmacological mechanism of SINOMENINE nanoparticles and clinical application.

2. Materials and Methods

2.1. SINOMENINE-Related Targets
The TCMSP database and SwissTargetPrediction (http://www.swishtargetprediction.ch/) database were used to search the targets of SINOMENINE action and establish the active ingredient target data set.

2.2. Breast Cancer-Related Targets
With "breast cancer" as the key word, our screening criteria are "Gene" and "Homo sapiens". Disease targets are searched through the GeneCards database (http://www.genecards.org/).

2.3. Network Construction
The drug and the disease target are intersected. The target is the predicted of SINOMENINE in the treatment of breast cancer. PPI network was constructed by the String database (https://string-db.org/) Cytoscape 3.7.1 software constructed a "drug-disease" intersection network and visually analyzed. Each node satisfied the median of the topological parameters "Degree", "Betweenness centrality", and "Closeness centrality" value is screened. Select nodes that simultaneously satisfy more than 3 card values, As the core target of SINOMENINE in the treatment of breast cancer. At the same time, cytoscape 3.7.1 software is used to construct the "ingredient-core target-disease" network.

2.4. Bioinformatic Analysis
We use DAVID (https://david.ncifcrf.gov/tools.jsp) to perform gene ontology (GO) biological process and pathway enrichment analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) on the predicted target.

The biological processes, cell composition, molecular functions and signal pathways with extremely significant differences were screened out with P≤0.05 (as the screening criteria) by R language tools.

2.5. Pathway Mapper Construction
Use the KEGG Mapper function in the KEGG (https://www.genome.jp/kegg/) database to map the core targets on the relevant signal pathways.
3. Results

3.1. Target Prediction
All the targets retrieved from the TCMSP and Swiss TargetPrediction databases. The duplicate targets were also deleted. Finally, a total of 62 target corresponding to SINOMENINE was obtained.

In the GeneCards database, with the keyword "Breast Cancer", 15207 breast cancer-related disease targets were retrieved and integrated.

3.2. Screening of Core Targets
Take the intersection of the drug target and the disease target to get 57 intersection targets, and draw the Venn diagram. The results are shown in Figure 1. Enter the intersection target into the String database (https://string-db.org/) to construct the PPI network, and the result is shown in Figure 2. Use cytoscape 3.7.1 software to visually analyze the PPI network, the median values of the three topological parameters of all nodes are 6 (Degree), 0.0040 (Betweenness centrality), 0.4206 (Closeness centrality), and we select to satisfy more than three topologies. the node with the median value of the parameter is used as the core target. The results are shown in Table 1.

![Figure 1. Drug-disease intersection target Venn diagram](image-url)
Figure 2. The protein interaction network of drug-disease intersection target

Table 1. Related topological parameters of the core target

| UniProt CID | Gene name | Protein name | Degree | Closeness Centrality | Betweenness Centrality |
|------------|-----------|--------------|--------|----------------------|------------------------|
| P31749     | AKT1      | AKT Serine/Thrreonine Kinase 1 | 24     | 0.6125               | 0.2761                 |
| P29474     | NOS3      | Nitric Oxide Synthase 3        | 15     | 0.5158               | 0.1905                 |
| P35372     | OPRM1     | Opioid Receptor Mu 1           | 11     | 0.4757               | 0.1316                 |
| P45983     | MAPK8     | Mitogen-Activated Protein Kinase 8 | 18    | 0.5506               | 0.1010                 |
| P14780     | MMP9      | Matrix Metallopiptidase 9      | 16     | 0.5000               | 0.0971                 |
| P42345     | MTOR      | Mechanistic Target Of Rapamycin Kinase | 19 | 0.5213               | 0.0893                 |
| O14757     | CHEK1     | Checkpoint Kinase 1            | 11     | 0.4667               | 0.0771                 |
| P00533     | EGFR      | Epidermal Growth Factor Receptor | 18   | 0.5326               | 0.0743                 |
| P42336     | PIK3CA    | Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha | 13  | 0.5000               | 0.0605                 |
| P30411     | BDKRB2    | Bradykinin Receptor B2         | 11     | 0.4537               | 0.0506                 |
| P41145     | OPRK1     | Opioid Receptor Kappa 1        | 9      | 0.4579               | 0.0469                 |
| P30542     | ADORA1    | Adenosine A1 Receptor          | 9      | 0.4712               | 0.0307                 |
| P25106     | ACKR3     | Atypical Chemokine Receptor 3  | 9      | 0.4579               | 0.0278                 |
| P35354     | PTGS2     | Prostaglandin-Endoperoxide Synthase 2 | 13  | 0.4804               | 0.0275                 |
| P24864     | CCNE1     | Cyclin E1                      | 7      | 0.4375               | 0.0141                 |
| O60674     | JAK2      | Janus Kinase 2                 | 11     | 0.4579               | 0.0115                 |
| Q04759     | PRKCQ     | Protein Kinase C Theta         | 6      | 0.4224               | 0.0091                 |
| P98170     | XIAP      | X-Linked Inhibitor Of Apoptosis | 10  | 0.4495               | 0.0042                 |
3.3. Drug-Disease-Core Target Network Construction
The "drug-disease-core target" network was constructed by cytoscape 3.7.1 software, and the results are shown in Figure 3.

![Figure 3. Drugs-Diseases-Core Targets Network](image)

3.4. GO Biological Process Enrichment Analysis
These targets are subjected to GO enrichment analysis and Visualization by DAVID. Retain the results of $P \leq 0.05$, and perform visual analysis by R language tools. According to the smaller the Term P-value and the higher the reliability of Term, 134 biological processes were screened. The top 10 biological processes, cell composition, and molecular functions are listed. The results are shown in Figure 4. The results indicate that the anti-breast cancer effect of SINOMENINE may be produced through protein phosphorylation, positive regulation of nitric oxide biosynthetic process, protein autophosphorylation, negative regulation of apoptotic process, peptidyl-serine phosphorylation and other biological processes.

![Figure 4. Top 10 biological process enrichment results](image)

3.5. KEGG Pathway Enrichment Analysis
DAVID (https://david.ncifcrf.gov/tools.jsp) was used to perform KEGG pathway enrichment analysis, and the results of $P \leq 0.05$ were retained by R language tools. A total of 60 signal pathways were
enriched. The results are shown in Figure 5. The main signaling pathways include Sphingolipid signaling pathway, Pathways in cancer, Measles, Small cell lung cancer, Focal adhesion, Insulin resistance, etc. It is suggesting that the anti-breast cancer effect of SINOMENINE may be the result of the participation of multiple signaling pathways.

Figure 5. The 60 pathways enriched by major hubs

3.6. Pathway Annotation Diagram of SINOMENINE's Anti-Breast Cancer Effect
Enter the intersection target into the KEGG Mapper function of the KEGG database, and mark the number of the target in each relevant signal pathway. 15 target proteins are involved in regulating Pathways in cancer, revealing the multi-target and multi-pathway anti-cancer effects of SINOMENINE.

4. Discussion
SINOMENINE has a wide range of pharmacological effects. It is mainly used clinically for anti-inflammatory, immunosuppressive, antihypertensive, antiarrhythmic, analgesic, and sedation[13]. In recent years, the anti-tumor activity of SINOMENINE has also been confirmed in many ways, and a large number of results indicate that SINOMENINE has a good inhibitory effect on breast cancer, lung cancer, liver cancer, and other cancers. The network pharmacological analysis results show that AKT1, MTOR, EGFR, NOS3, MAPK8, and MMP9 may be the potential of SINOMENINE in breast cancer treatment. AKT1 is a serine/threonine protein kinase that participates in a variety of biological
processes, including metabolism, proliferation, cell survival, growth and angiogenesis. They are all mediated through a series of downstream substrates of serine/threonine phosphate.

GO biological process enrichment analysis shows that the anti-breast cancer effect of SINOMENINE involves biological processes such as apoptosis, signal transduction, biosynthesis, and inflammation. Among them, protein phosphorylation, signal transduction, negative regulation of apoptotic process, protein autophosphorylation, etc. Intracellular signal transduction may be the main process of SINOMENINE’s anti-breast cancer effect. Enrichment analysis of KEGG pathway showed that SINOMENINE mainly regulates Pathways in cancer, PI3K-Akt signaling pathway, Sphingolipid signaling pathway, Neuroactive ligand-receptor interaction, Measles, Focal adhesion, Proteoglycans in cancer, Small cell lung cancer, and Insulin resistance, Toxoplasmosis and other pathways. These targets play a very important role. In the PI3K/AKT signal pathway, phosphorylated PI3K and Akt will become important carcinogenic mediators, and mTOR downstream of Akt signal transduction is involved in the regulation of cell autophagy[14]. MDA-MB-231 breast cancer cells of production of ROS and the high expression of COX2 will increase the migration and invasion ability of cells[15]. SINOMENINE may inhibit the production of ROS and inflammatory signal transduction in breast cancer, thereby reducing metastasis.

5. Conclusions
A total of 57 potential targets were obtained through the online database, of which 18 are the core targets for SINOMENINE to inhibit breast cancer. GO biological process enrichment analysis and KEGG pathway enrichment analysis revealed 10 biological processes, cell composition, molecular functions and 60 pathways are closely related to the occurrence and development of inhibiting breast cancer, mainly involving Pathways in cancer, PI3K-Akt signaling pathway, Sphingolipid signaling pathway, Neuroactive ligand-receptor interaction, Measles, Focal adhesion, Proteoglycans in cancer and other molecular events. This study explored the mechanism of SINOMENINE nanoparticles on breast cancer, provided new ways and ideas for elucidating the mechanism of SINOMENINE nanoparticles, and also provided a theoretical reference for its application.

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