An Exploration on Mechanisms of Andrographolide Nanoemulsifying in Treating Gastric Cancer Based on Network Pharmacology

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Abstract. This article preliminarily revealed the mechanism of andrographolide (AD) nanoemulsifying inhibiting gastric cancer(GC). The targets of AD was searched by TCMSP and SwissTargetPrediction database, we entered the keyword “Gastric Cancer” in GeneCards database to find all targets related to GC. The intersection of AD and GC is taken as an important targets for AD to act on GC, Cytoscape 3.7.1 was used for topological analysis of the target, so as to obtain all important targets for AD to inhibit GC. DAVID database was used to perform biological process analysis on all potential targets, mainly through its analysis function. It involves Cell composition, molecular function and signal pathway enrichment analysis. Finally, we obtained a total of 53 potential targets related to GC, among which IL6, MAPK8, CREBBP, CDK2, MAPK14, AR and MAP2K1 may be important targets for inhibiting GC. The effect of AD on GC is mainly through Influenza A, Pathways in cancer, Sphingolipid signaling pathway, Progesterone-mediated oocyte maturation, Herpes simplex infection and other important pathways which are closely related to 10 biological processes such as protein phosphorylation, signal transduction, peptidyl-serine phosphorylation, positive regulation of gene expression, and positive regulation of transcription from RNA polymerase II promoter Sex. This study can provide guidance for the development of anti-GC drugs.

1. Introduction
On a global scale, Gastric Cancer (GC) is malignant gastrointestinal tumor. GC has a high incidence rate in my country[1], and the mortality rate ranks first among many cancers[2]. The clinical treatment of GC is mainly chemotherapy, but the toxic and side effects of chemotherapy drugs have brought much pain to patients, so we need to find a natural drug that is effective and side effects to relieve the pain of the patient.

Andrographolide (AD) is mainly isolated from Andrographis Paniculata plant[3]. Previous studies have shown that andrographolide has antibacterial, anti-inflammatory, antiviral, and immune regulation functions[4]. In recent years, the research of AD in the field of anti-tumor has become a hot spot. AD has a significant inhibitory effect on breast cancer, lung cancer, prostate cancer and other tumor cells[5-7], However, AD has low bioavailability and poor targeting, We prepared it into AD...
self-nanoemulsifying, which greatly improved its anti-tumor effect, thus revealing the mechanism of its anti-GC through the method of network pharmacology.

Network pharmacology is an emerging discipline that combines pharmacology, biology, statistics, chemistry, network analysis technology and other sciences. Recently, we mainly applied network pharmacology in new drug development[8], drug mechanism research[9], and disease marker discovery[10]. This study used bioinformatics methods to predict the mechanism of AD acting on GC, providing a reference for finding new anti-GC drugs and targets.

2. Materials and Methods

2.1. AD Targets

We employed the TCMSP and SwissTargetPrediction (www.swisstargetprediction.ch) database to search the targets of AD and collect data sets of AD targets.

2.2. GC Targets

In the GeneCards database (www.genecards.org), entered the keyword "gastric cancer", GC-related targets are obtained, and data sets of disease targets are established.

2.3. Network Construction

Drug and disease targets intersect, and the targets in the intersection are the important targets for AD to inhibit GC. String database (https://string-db.org/) analysis function is used to construct PPI network, cytoscape 3.7.1 software is used to visually analyze PPI network, and Degree, Betweenness centrality and Closeness centrality of values is determined. Select nodes that satisfy more than 3 card values at the same time as the core of targets.

2.4. Bioinformatic Analysis

In the DAVID (https://david.ncifcrf.gov/tools.jsp) database, genetic ontology (GO) is used to analyze biological processes(BP), cell composition(CC) and molecular functions(MF). Kyoto Encyclopedia of Genes and Genomes (KEGG) is used for pathway enrichment analysis.

The R language tool performs visual analysis, $P \leq 0.05$ as the screening criteria to screen out biological processes, cell compositions, molecular functions and signal pathways with extremely significant differences.

2.5. Pathway Mapper Construction

KEGG Mapper function was used to in the KEGG (https://www.genome.jp/kegg/) database map the core targets on the closely related signal pathways.

3. Results

3.1. Targets Obtained

In the TCMSP and Swiss Target Prediction databases, 61 AD targets were obtained, and in the GeneCards database, 12,202 GC targets were obtained.

3.2. Screening of Core Targets

Cytoscape 3.7.1 software was used for visual analysis, and the core target was selected that satisfies the median value of more than three topological parameters at the same time. The median values of the three topological parameters are 4 (Degree), 0.0065 (Betweenness centrality), 0.4144 (Closeness centrality). The results are shown in Table 1.
Figure 1. Drug-disease intersection target Venn diagram

Figure 2. The PPI network diagram
Table 1. Core targets related information

| UniProt CID | Gene name | Protein name                        | Degree | Closeness Centrality | Betweenness Centrality |
|------------|-----------|-------------------------------------|--------|-----------------------|------------------------|
| P05231     | IL6       | Interleukin 6                       | 25     | 0.6571                | 0.4840                 |
| P45983     | MAPK8     | Mitogen-Activated Protein Kinase 8  | 15     | 0.5610                | 0.0672                 |
| Q92793     | CREBBP    | CREB Binding Protein                | 14     | 0.5679                | 0.1243                 |
| P24941     | CDK2      | Cyclin Dependent Kinase 2           | 14     | 0.5412                | 0.1207                 |
| Q16539     | MAPK14    | Mitogen-Activated Protein Kinase 14 | 13     | 0.5287                | 0.1163                 |
| P10275     | AR        | Androgen Receptor                   | 12     | 0.5543                | 0.1254                 |
| Q02750     | MAP2K1    | Mitogen-Activated Protein Kinase 1  | 12     | 0.5169                | 0.0554                 |
| P06493     | CDK1      | Cyclin Dependent Kinase 1           | 11     | 0.4510                | 0.0343                 |
| P23458     | JAK1      | Janus Kinase 1                      | 10     | 0.4842                | 0.0168                 |
| Q05655     | PRKCD     | Protein Kinase C Delta              | 10     | 0.4510                | 0.0459                 |
| P06401     | PGR       | Progesterone Receptor               | 9      | 0.5227                | 0.0109                 |
| P53779     | MAPK10    | Mitogen-Activated Protein Kinase 10 | 9      | 0.4894                | 0.0151                 |
| P45984     | MAPK9     | Mitogen-Activated Protein Kinase 9  | 9      | 0.4792                | 0.0082                 |
| P50750     | CDK9      | Cyclin Dependent Kinase 9           | 8      | 0.5000                | 0.0237                 |
| Q00535     | CDK5      | Cyclin Dependent Kinase 5           | 7      | 0.4299                | 0.0082                 |
| O60674     | JAK2      | Janus Kinase 2                      | 7      | 0.4792                | 0.0080                 |
| Q02156     | PRKCE     | Protein Kinase C Epsilon            | 7      | 0.4220                | 0.0082                 |

3.3. Interactive network

We used cytoscape 3.7.1 software to draw a "drug-disease-core target" interactive network diagram, as shown in Figure 3.

![Figure 3. Drugs-Diseases-Core Targets interactive network diagram](image-url)
3.4. GO Analysis
A total of 82 biological processes were screened, and the main ten BP, CC, and MF were presented, as shown in Figure 4.

![GO Analysis results](image)

Figure 4. GO Analysis results

3.5. KEGG Analysis
57 pathways were obtained through KEGG pathway enrichment analysis, as shown in Figure 5. The most important pathways are as follows: Influenza A, Pathways in cancer, Sphingolipid signaling pathway, Progesterone-mediated oocyte maturation and Herpes simplex infection. AD may inhibit GC through multiple pathways.
4. Discussion

Through the research of this subject, the effect of AD on GC may be related to the targets of IL6, MAPK8, CREBBP, CDK2, MAPK14, and AR. IL6 is interleukin-6, which is a lymphokine produced by activated T cells and fibroblasts. It can promote the growth and differentiation of primitive bone marrow-derived cells and enhance the analysis function of natural killer cells. Studies have confirmed that IL-6/STAT3 signaling is related to tumors. Metastasis is closely related. Up-regulation of IL-6 gene expression can induce phosphorylation of STAT3 and promote tumor cell invasion, migration and EMT[11].

GO analysis results show that the biological processes involved in AD acting on GC include apoptosis, signal transduction, biosynthesis, and inflammation, among which protein phosphorylation, signal transduction, peptidyl-serine phosphorylation. The most important biological processes involved in AD acting on GC may include positive regulation of gene expression, Positive regulation of transcription from RNA polymerase II promoter.

KEGG analysis shows that AD mainly acts on GC through the following pathways, such as pathways in cancer, Influenza A, Sphingolipid signaling pathway, Progesterone-mediated oocyte maturation, Herpes simplex infection and other pathways. In the JAK2/STAT3 pathway,
phosphorylated JAK2 and STAT3 can promote the proliferation of cancer cells. Studies have confirmed that up-regulation of ROS can block the JAK2/STAT3 signaling pathway and inhibit the growth of a variety of tumor cells[12].

5. Conclusions
53 GC-related targets were obtained through the online database, of which 17 are the core targets for AD to inhibit GC. GO biological process enrichment analysis and KEGG pathway enrichment analysis revealed 10 biological processes, cell composition, molecular functions and 57 pathways are closely related to the occurrence and development of inhibiting breast cancer, mainly involving Pathways in cancer, Influenza A, Sphingolipid signaling pathway, Progesterone-mediated oocyte maturation, Herpes simplex infection. Our research predicted the mechanism of AD nanoemulsifying on GC, and provided guidance for the development of anti-GC drugs. However, the specific mechanism of AD nanoemulsifying in inhibiting GC needs the further study.

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