Analyzing the Multi-target Pharmacological Mechanism of Folium Artemisiae Argyi Acting on Breast Cancer: A Network Pharmacology Approach

Jinlu Wang

Tumor Hospital of Harbin Medical University

Xiuli Wang

The seventh Hospital in Qiqihaer

Han Zhang

Tumor Hospital of Harbin Medical University

Minghui Lu

Tumor Hospital of Harbin Medical University

Xingjian Niu

Tumor Hospital of Harbin Medical University

Lei Yin

Harbin Medical University

Yiran Wang

Harbin Medical University

Yucui Gu

Tumor Hospital of Harbin Medical University

Dongbo Li

Tumor Hospital of Harbin Medical University

Hongfei Ji

Harbin Medical University

Qingyuan Zhang (✉ zqyHMU1965@163.com)

Department of Medical Oncology, Harbin Medical University Cancer Hospital, Harbin Medical University, Harbin 150081, Heilongjiang, China

Research

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Abstract

Background: Folium Artemisiae Argyl (FAA) is a traditional Chinese herb medicine widely used in clinic. However, the underlying mechanisms of its anticancer effects have not been fully understood.

Methods: In this study, we applied a network pharmacology approach to dig out the potential mechanisms of FAA against breast cancer.

Results: We obtained 9 active ingredients and 236 potential targets from FAA in total to construct a network, which showed that quercetin served as the major ingredient in FAA. AKT1 (RAC-alpha serine/threonine-protein kinase), MYC (Myc proto-oncogene protein), CASP3 (Caspase-3), EGFR (Epidermal growth factor receptor), JUN (Transcription factor AP-1), CCND1 (G1/S-specific cyclin-D1), VEGFA (Vascular endothelial growth factor A), ESR1 (Estrogen receptor), MAPK1 (Mitogen-activated protein kinase 1) and EGF (Pro-epidermal growth factor) were identified as key targets of FAA in the treatment of breast cancer. The protein protein interaction (PPI) cluster demonstrated that AKT1 was the seed in this cluster, indicating that AKT1 played a crucial role in connecting other nodes in the PPI network. This enrichment demonstrated that FAA was highly related to signal transduction, endocrine system, replication and repair, cell growth and death. The enrichment results also verified that the underlying mechanisms of FAA against breast cancer might be attributed to coordinated regulation of several cancer-related pathways, such as MAPK signaling pathway, mammalian target of rapamycin (mTOR) signaling pathway, among of others.

Conclusions: To summarize, this study aimed to identify the potential targets and pathways of FAA as a treatment against breast cancer using the network pharmacology approach, and systematically elucidate the mechanisms of FAA in the treatment of breast cancer.

Introduction

Breast cancer is the most frequent malignancy occurring in women with 1.38 million new cases each year and nearly 0.46 million related deaths globally [1, 2]. According to current projections, there will be approximately 3.2 million new cases per year by 2050 [3]. As we all know, breast cancer is a heterogeneous disease with differences in occurrence, development, treatment and prognosis [4]. At present, appropriate comprehensive adjuvant treatments containing chemotherapy, radiotherapy, endocrine and targeting HER2 therapy are widely put into use, according five major molecular subtypes [5]. However, these treatments are costly and usually make a series of short- and long-term side effects, such as febrile neutropenia [6], alopecia [7], peripheral neuropathy [8] and cardiotoxicity [9], all of which significantly decrease the patient's quality of life. Not to mention those older patients in the terminal stages, the more intolerant these adverse reactions.

Folium artemisiae argyi (FAA), commonly called wormwood, is a traditional Chinese herb medicine which has antipyretic, analgesic and hemostatic effects [10]. It is used internally for warming channels, arresting bleeding, dispelling cold and relieving pain, while externally for eliminating dampness and

relieving itching [11] for thousands of years. Recently, growing attention has been paid to the role of Traditional Chinese medicine in the prevention and treatment of cancer because of various limitations of Western medicine, such as the toxicity and adverse side effects [12]. At the same time, Chinese herbs can target multiple points to achieve synergistic actions [13, 14]. According to pharmacology research, FAA contains multiple active chemical constituents, such as flavonoids, terpenoids, phenolic acids and volatile oils [15, 16] and exhibits a variety of effects, such as anticancer, anti-inflammatory and anti-oxidative [17, 18]. For example, Shafi et al. suggested that FAA inhibited proliferation and promoted apoptosis in breast cancer cells through Bcl-2 family proteins and the MEK/ERK pathway [10]. It was also reported that FAA had dose-dependent inhibitory effect on hepatoma cells [11]. However, although many studies verified that FAA showed remarkable antitumor functions, the underlying mechanisms have not yet been absolutely understood.

It is widely known that herbal medicines include multi-component, multi-target and multi-pathway features [19, 20]. Traditional Chinese medicine network pharmacology is a systematic research method based on the interaction network of herbs, compounds, targets, diseases and genes [21]. This approach lays emphasis on the integration of bio-informatics, systems biology and pharmacology, which not only explains the complex interactions between herbs and diseases at a systematic level, but also conforms to the systematic and holistic perspective of the Traditional Chinese medicine theory [22, 23]. Thus, we utilized a network pharmacology approach to explore the pharmacological mechanisms of FAA as a treatment for breast cancer in this study. At first, we screened for active ingredients of FAA by estimating their oral bio-availability (OB) and drug-likeness (DL) [24]. Next, we picked out the common targets shared by the FAA compound targets and the breast cancer related targets via two databases (Genecards and the Online Mendelian Inheritance in Man database (OMIM)) and then constructed the network by investigating the potential interactions between the various target nodes. In addition, protein-protein interaction (PPI) data were obtained from the Search Tool for the Retrieval of Interacting Genes (STRING) database, and enrichment analyses (gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)) were performed to explore the potential mechanisms of FAA against breast cancer. To summarize, this study aimed to identify the potential targets and pathways of FAA as a treatment against breast cancer using the network pharmacology approach, and systematically elucidate the mechanisms of FAA in the treatment of breast cancer.

Materials And Methods

2.1. Data Preparation

2.1.1. Active Ingredients and Targets against Breast Cancer in FAA

The ingredients in FAA were acquired from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP). It serves as a systematic platform to study herbs, including the identification of compounds and the screening of compound targets [25]. In addition, to identify the corresponding targets of FAA compounds against breast cancer, the TCMSP database was used to dig out potential targets.

Finally, 9 active herbal ingredients of FAA were selected (Table 1) by linking the active ingredients of FAA to the targets against breast cancer. A total of 236 targets of FAA compounds were obtained in total (specific targets were not shown).

2.1.2. Pharmacokinetic Predictions

In pharmaceutical study, ADME (absorption, distribution, metabolism and excretion) is a critical pattern to identify [24]. Therefore, we employed two major ADME-related properties, namely, the OB and DL to search the potential bio-active compounds of FAA in this study. Ingredients with $OB \geq 30\%$ and $DL \geq 0.18$ were considered as suggested drug screening criteria. The detailed information for all ingredients before screening was listed in Table S1.

2.1.3. Potential Target Genes of Breast Cancer

The data for the breast cancer related target genes were gathered from Genecards and the OMIM. The species was set to *Homo sapiens*. Genecards is an extensive platform that provides insight into predicted and annotated human genes. All of the gene-centric data are collected from 150 web resources, including genetic, genomic, proteomic, transcriptomic, and functional information [26]. Search strategy: Set the keyword as “breast cancer” and the score >30 after logging in to Genecards. The detailed information was listed in Table S2. The OMIM is a comprehensive, authoritative, and timely knowledgebase which links and catalogues all known diseases with a genetic component and provides further references to genomic analyses of catalogued genes [27]. Search strategy: Choose gene map at the website, and then set the keyword as “breast cancer”. The detailed information was listed in Table S3.

2.1.4. PPI Data

We acquired the PPI data from the STRING database. It defines PPI with confidence ranges for data scores (high >0.7 ; medium >0.4 ; low >0.15) [28]. In our study, we selected a confidence score of >0.4 to construct our PPI network.

2.2. Network Construction

The PPI network has been widely applied to show lots of different interactions of proteins in the context of complex diseases [23, 29], including breast cancer, prostate cancer, lung cancer, gastric cancer, etc. In this study, we constructed the network as follows: (1) we acquired the targets shared by the FAA compound targets and the breast cancer related targets, (2) We put these targets into STRING database and obtained the FAA against breast cancer targets PPI network, and (3) we exported the PPI results as a simple tabular text output (.tsv) and then imported the .tsv file into Cytoscape (version 3.6.1) to reconstruct the network in order to achieve better visualization and understanding for further analysis [30].

2.3. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

In this study, we used the ClusterProfiler package of R3.5.2 to perform GO enrichment analysis of the targets. The higher the score, the greater the importance of the genes represented in the list [31]. The ClusterProfiler package of R3.5.2 was also used to analyze KEGG pathway enrichment of overlapping target genes. KEGG analysis was used to explore the biological pathways and potential biological functions on the basis of the enrichment analysis of functional items [32].

Results And Discussion

3.1. Active Ingredients

In this study, we acquired a total of 9 active ingredients of FAA after ADME identification. Detailed information was shown in Table 1 (all Mol IDs could be tracked in the TCMSP database). All the FAA compounds before screening were presented in Table S1.

3.2. FAA Compound-Target Network

To further uncover the potential pharmacological mechanisms of FAA against breast cancer, target genes common to both active ingredients of FAA and breast cancer were selected in different databases. A total of 75 genes (Table S4) belonging to both the FAA target gene and breast cancer target gene networks were screened via Venn analysis (Figure 1a). The compound-target network was presented in Figure 1b, including 82 nodes and 171 edges, with a network density of 0.051 and a network diameter of 3. The detailed information of this network was depicted in Table 2. As could be seen from figure 1b, quercetin was the most critical component of FAA, which was connected to the most targets. It was one of the flavonoids from natural plant and exhibited a variety of activities such as antioxidant, anti-inflammatory, antiviral and antimicrobial through multiple signal transduction pathways [33, 34]. Several studies had validated that quercetin could inhibit various tumor progression, including breast cancer [35], prostate cancer [36], gastric cancer [37], ovarian cancer [38] and colorectal cancer [39]. Moreover, there were also some literatures reported that it not only had a synergistic effect when combined with chemotherapeutic or radiotherapy agents, but also could reduce an expected adverse side effect and a toxic reaction [40].

In addition, the results showed that many targets were affected by two or more compounds. For instance, Prostaglandin G/H synthase 1 (PTGS1) and Prostaglandin G/H synthase 2 (PTGS2) were both modulated by quercetin, stigmasterol, mandenol, etc. Constitutive PTGS1 and inducible PTGS2 belonged to two isozymes of PTGS which had pivotal effects both as a peroxidase and a dioxygenase [41]. Other study suggested that PTGS2 might inversely control the breast cancer metastasis and chemoresistance through the regulation of EMT, apoptosis and senescence [42]. And nuclear receptor coactivator 2 (NCOA2), a member of the p160 family, performed key roles in many different physiological and pathological processes, including cell growth, energy metabolism, endocrine regulation, and circadian rhythm [43]. More importantly, the expression of the NCOA2 gene played crucial roles in the development, progression, and metastasis of malignant tumors, such as breast cancer [44]. In prostate cancer patients, high expression of NCOA2 was more likely to relapse after androgen-deprivation therapy [45].

Similarly, Beta-2 adrenergic receptor (ADRB2), Gamma-aminobutyric acid receptor subunit alpha-1 (GABRA1), Heat shock protein HSP 90, Progesterone receptor (PGR) and Sodium channel protein type 5 subunit alpha (SCN5A) could also be regulated by more than two active ingredients. Not only was obtained an approximate observation of the relationship between these active ingredients and targets, but also discovered the potential pharmacological effects of FAA from this network of Figure 1b.

3.3. PPI Network

To explore the underlying mechanisms of FAA as a therapy against breast cancer, a PPI network of the FAA compound targets against breast cancer was constructed by connecting the targets of FAA compound and the breast cancer. First, we obtained a total of 75 target genes which belonged to both the FAA target gene and breast cancer target gene and got targets symbol names by uniprot. Next, all of these 75 target genes were imported into the STRING database to generate the PPI results (settings: *Homo sapiens* and confidence >0.4). The original STRING PPI network was presented in Figure S1. Then, we imported the PPI data generated in STRING into Cytoscape (version 3.6.1).

As Figure 2a showed, this PPI network included 75 nodes and 1247 edges, with a network diameter of 3, a clustering coefficient of 0.733 and an average number of 33.253 neighbors. The average node degree was 33.3 (both the different colors and the size of the circles indicated the degree). The detailed information of this network was displayed in Table 3. All target degrees were calculated using this network. In Figure 2b, the 10 targets with the greatest degrees were AKT1 (degree = 67), MYC (degree = 65), CASP3 (degree = 63), EGFR (degree = 62), JUN (degree = 61), CCND1 (degree = 60), VEGFA (degree = 60), ESR1 (degree = 59), MAPK1 (degree = 57) and EGF (degree = 55). As shown in Figure 3c, the cluster consisted of 68 nodes and 1155 edges. The average node degree was 34 and the clustering coefficient was 0.77. The red diamond in Figure 3c, AKT1, was the seed in this cluster and interacted with other FAA targets. This figure intuitively indicated that AKT1 played an important role in connecting other nodes in this PPI network. It was well-known that the serine/threonine kinase AKT1, one of the three isoforms in the Akt family, had emerged as a downstream effector of PI3K [46]. AKT inhibited apoptosis by suppressing the actions of BAD and caspase-9 [47]. In breast cancer, AKT1 activation accelerates cell proliferation, whereas Akt1 inhibition promotes Epithelial-to-Mesenchymal Transition [48].

3.4. GO Enrichment

To further discuss the multiple mechanisms of FAA as a treatment against breast cancer, we conducted GO enrichment analysis on the 75 common targets shared by the FAA compound targets and the breast cancer related targets [31]. To be more specific, the top 30 targets are as follows (Figure 2b): AKT1, MYC, CASP3, EGFR, JUN, CCND1, VEGFA, ESR1, MAPK1, EGF, IL6, PTGS2, ERBB2, FOS, MMP9, CXCL8, MMP2, BCL2L1, CASP8, AR, IL1B, CCL2, CDKN1A, IL10, PPARG, RELA, SPP1, ICAM1, PGR and SERPINE1. The significantly enriched GO targets were presented (adjusted *p*-value <0.001) in Figure 3. The top five GO enrichment targets included (1) transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding (GO:0000982); (2) transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding (GO:0001228); (3) ubiquitin-like protein

ligase binding (GO:0044389); (4) cytokine receptor binding (GO:0005126); and (5) transcriptional activator activity, RNA polymerase II proximal promoter sequence-specific DNA binding (GO:0001077). Detailed GO enrichment information was shown in Table 4. Thus, we speculated that FAA probably executed its pharmacological effects on breast cancer by simultaneously involving these molecular functions.

3.5. KEGG Enrichment

Meanwhile, we further carried on KEGG [32] enrichment analysis on the 75 common targets in order to clarify the integral regulation of FAA for the treatment of breast cancer. We obtained 74 pathways in total which belonged to several categories, including human diseases, cellular processes, and drug resistance, among others, of which the top 30 significantly enriched KEGG targets were presented (adjusted p -value <0.001) in Figure 4. In the cancer-related disease, prostate cancer (hsa05215), bladder cancer (hsa05219), pancreatic cancer (hsa05212), breast cancer (hsa05224), colorectal cancer (hsa05210), non-small cell lung cancer (hsa05223), small cell lung cancer (hsa05222), gastric cancer (hsa05226), endometrial cancer (hsa05226), renal cell carcinoma (hsa05211), thyroid cancer (hsa05216), and small cell lung cancer (hsa05222) data were processed using KEGG enrichment analysis. Detailed KEGG information was shown in Table 5. This result showed that FAA had the highly potential to treat a wide range of cancers, such as breast cancer [10], prostate cancer [49], bladder cancer [50], colorectal cancer [51], and gastric cancer [52], which were confirmed by other researchers. Further, the results in Figure 5 also verified that these signaling pathways remarkably enriched by the potential targets of FAA in breast cancer were strongly associated with signal transduction, endocrine system, replication and repair, cell growth and death, most of which played a essential role in the development and progression of cancers, such as pathways in PI3K/AKT signaling pathway (hsa04151), Estrogen signaling pathway (hsa04915), MAPK signaling pathway (hsa04010), mammalian target of rapamycin (mTOR) signaling pathway (hsa04150), apoptosis (hsa04210) and cell cycle (hsa04110). Therefore, we speculated that the underlying mechanism of FAA against breast cancer might be attributed to coordinated regulation of several cancer-related pathways.

Conclusions

So far, though many researches verified that FAA exhibited arresting antitumor activities, the underlying mechanisms of its antitumor activities had not yet been totally understood. The network pharmacology emphasized the integration of bioinformatics, systems biology and pharmacology, which not only explained the complex interactions between diseases and Chinese herbs at a systematic level, but also demonstrated to the systematic and holistic perspective of the traditional Chinese medicine theory [12]. To better explore the pharmacological mechanisms of FAA as a treatment for breast cancer, we applied the network pharmacology approach to dig out the potential mechanisms of FAA as a treatment against breast cancer by compound-target network construction, PPI network, GO and KEGG enrichment analysis. We took advantage of OB and DL to explore the potential active ingredients of FAA. Until now, the studies on the pharmacokinetics of FAA were few. Eunjeong et al. [17] found that FAA had the

anticancer activities through the inhibition of cell growth and induction of apoptosis in breast cancer cells. In this study, we acquired 9 active ingredients and 236 potential targets from FAA in total, and validated a synergistic herb strategy featuring multi-component, multi-target and multi-pathway characteristics. The compound-target network confirmed that quercetin served as the major ingredient in FAA. Moreover, the PPI network provided information concerning the source of the interactions. The PPI analysis indicated that FAA had a significant effect on breast cancer by influencing the whole biological network, including targets such as AKT1, MYC, CASP3, EGFR, JUN, CCND1, VEGFA, ESR1, MAPK1, and EGF. The PPI cluster demonstrated that AKT1 was the seed, suggesting that AKT1 played a crucial role in connecting other nodes in the PPI network. Next, the enrichment analysis presented that FAA was strongly related to signal transduction, endocrine system, replication and repair, cell growth and death. The enrichment results also showed that the underlying mechanism of FAA against breast cancer might be attributed to coordinated regulation of several cancer-related pathways, such as MAPK signaling pathway, mammalian target of rapamycin (mTOR) signaling pathway, among of others.

In conclusion, this study applied a network approach demonstrating how FAA compounds alter different pathways against breast cancer, which was supplementary to other researches on drugs against breast cancer. Furthermore, we confirmed that FAA substantially influenced lots of breast cancer related targets, a finding which was consistent with present cancer study trends showing that the occurrence and development of breast cancer was the gradual accumulation of distinct genome modifications in cancer cells [53, 54]. We fully expect that our research can help to promote the employment of network pharmacology in uncovering the potential mechanisms of anticancer Chinese herbs, and provide clues to assess the synergy of herbs in the treatment of other complex diseases, especially cancer.

Abbreviations

ADME	Absorption, distribution, metabolism, and excretion
DL	Drug-likeness
FAA	Folium Artemisiae Argyi
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
OB	Oral bio-availability
OMIM	Online Mendelian Inheritance in Man
PPI	Protein–protein interaction
STRING	The Search Tool for the Retrieval of Interacting Genes
TCMSP	Traditional Chinese Medicine Systems Pharmacology Database

Declarations

Authors' Contributions

Conceptualization, J.W., H.J. and Q.Z.; Methodology, J.W. and X.W.; Software, J.W.; Validation, J.W., H.J. and Q.Z.; Formal Analysis, J.W. and X.W.; Writing-Original Draft Preparation, J.W.; Writing-Review & Editing, J.W.; Visualization, H.Z., M.L. and X.N.; Supervision, L.Y. and Y.W.; Project Administration, Y.G. and D.L.; Funding Acquisition, Q.Z..

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Competing interests

The authors have declared that no competing interests exist.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Authors' information

Jinlu Wang, e-mail: 78257999@qq.com.

Xiuli Wang, e-mail: 952309910@qq.com.

Han Zhang, e-mail: 1063149956@qq.com.

Minghui Lu, e-mail: 107380392@qq.com.

Xingjian Niu, e-mail: 596609177@qq.com.

Lei Yin, email: 719084058@qq.com.

Yiran Wang, e-mail: yiranwang@zju.edu.cn.

Yucui Gu, e-mail: 82072809@qq.com.

Dongbo Li, e-mail:444171044@qq.com.

Hongfei Ji, e-mail: ji-allen@hotmail.com.

Qingyuan Zhang, e-mail: zqyHMU1965@163.com.

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Tables

Due to technical limitations, table 1-5 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Seventy-five target genes for FAA against breast cancer. (a) Venn diagram revealing the overlapping target genes for FAA against breast cancer. (b) Network of target genes for FAA against breast cancer. Pink diamond represents active ingredients of FAA compounds; green ellipse represents target genes of both FAA and breast cancer.

Figure 2

PPI network of FAA compound targets against breast cancer. (a) The PPI network constructed using Cytoscape. Different colors represent the degree, as the scale indicates. The size of the circle also indicates the degree. (b) The top 30 target genes of FAA against breast cancer screened by PPI network. (c) The cluster generated from (a).

Figure 3

GO enrichment analysis for the 75 common targets shared by the FAA compound targets and breast cancer related targets. The color represents the different adjusted p-values, while the size of the circle represents the count.

Figure 4

KEGG enrichment analysis for the 75 common targets shared by the FAA compound targets and breast cancer related targets. The color represents the different adjusted p-value, while the size of circle represents the count.