

ORIGINAL RESEARCH

Study on the mechanism of Zhimu in the treatment of ovarian cancer based on network pharmacology

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Abstract

We aimed to investigate the mechanism of action of Zhimu in the treatment of ovarian cancer (OC) using network pharmacology. OC targets were screened using the DisGeNET and Online Mendelian Inheritance in Man databases. Common OC and Zhimu targets were identified using the Traditional Chinese Medicine System Pharmacology, UniProt databases, and Venny 2.1.0. The protein-protein interaction (PPI) network in the Search Tool for the Retrieval of Interacting Genes/Proteins database was created using Zhimu/OC targets and a Zhimu active ingredient-target-pathway network in the Cytoscape 3.9.1 software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted using the Metascape database. And overall, 15 active ingredients in addition to 93 related targets were identified. The PPI network had 52 targets that overlapped with it, with the 10 most relevant targets being the tumour protein p53, tumour necrosis factor, serine/threonine kinase 1, vascular endothelial growth factor A, caspase-3, prostaglandin G/H synthase-2, hypoxia-inducible factor-1 alpha, interleukin-1 beta, heat-shock protein 90-alpha, and progesterone receptor. According to GO and KEGG analyses, Zhimu and OC had the nuclear factor NF-kappaB signalling pathway, oxidative stress, and the advanced glycation end product (AGE)/the receptor for the advanced glycation end product (RAGE) signalling pathway as common targets. This study highlighted the active ingredients in Zhimu and identified potential molecular therapeutic mechanisms for the treatment of OC. It also provided suggestions and directions for future research into molecular mechanisms.

Keywords

Cancer; Network pharmacology; Molecular mechanism; Zhimu

1. Introduction

Ovarian cancer (OC) is the leading cause of death among women. Patients with early-stage OC have no clinical symptoms due to the intricate anatomical structure and endocrine function of ovarian tissues. According to a report, over 70% of women diagnosed with OC have advanced disease (stages III and IV). OC has a high mortality rate, accounting for 5% of all cancer-related deaths.

Various forms of alternative and complementary medicine have been employed in recent decades to combat cancer worldwide [1]. Traditional Chinese medicine (TCM) has been practised in China for thousands of years, and an increasing number of Chinese cancer patients accept it as standard supplementary therapy and alternative treatment [2, 3]. Zhimu is a Chinese medicinal herb that is also known in Latin as *Rhizoma Anemarrhenae*. This plant belongs to the genus *Anemarrhenae* in the Liliaceae family, and the dried rhizome of this plant is used to make medicine. It has a bitter taste, which reduces fire and heat while nourishing yin and strengthening yang

[4]. The primary components of Zhimu, ginsenosides and saponins, have anticoagulant, antioxidant, antibacterial, and anticancer properties. It has been used to treat diseases in Asian countries for thousands of years [5]. Zhimu has been widely used in the treatment of ovarian tumours, but its therapeutic mechanism remains unknown. Network pharmacology is an interdisciplinary approach that connects drug action networks with biological networks, analyses how drugs relate to nodes or network modules in the network, and shifts from single target identification to integrated network analysis, allowing for a more detailed understanding of cells and organs at the molecular level, significantly improving the identification of therapeutic targets and the discovery of new biomarkers.

This study employs network pharmacology to investigate the mechanism of action of Zhimu in the treatment of OC, utilising numerous components, multiple targets, and multiple pathways to create a theoretical foundation for the research and clinical application of Zhimu.

2. Materials and methods

2.1 Identification of Zhimu's active ingredients and screening of potential targets

The constituents of Zhimu were acquired using a platform of herbal medicine systems pharmacology for drug discovery (<http://tcmspw.com/tcmsp.php>) [6], and the ingredients were subjected to an ADME (absorption, distribution, metabolism, and excretion) screening test. Two criteria were used to filter ingredients: drug-likeness (DL, the similarity of compounds to known drugs) greater than or equal to 0.18 and oral bioavailability (OB, the rate and extent of drug absorption into the human circulation) greater than or equal to 30%. The targets associated with the active compounds in Zhimu were extracted, the repeating targets were removed, and the remaining targets were converted into gene identities in the UniProt database (<https://www.uniprot.org/uploadlists/>).

2.2 OC target screening

The potential OC targets were identified using the Online Mendelian Inheritance in Man (OMIM) (<https://omim.org/>) and DisGeNET (<https://www.disgenet.org/home/>) databases.

2.3 Protein-protein interaction (PPI) network construction and core target screening

The prospective pharmacological targets for treating OC were identified by intersecting the active components in Zhimu with the OC-associated protein targets using Venny 2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/>). The active components were subjected to additional scrutiny. Following the integration of the crossover targets into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, <https://www.string-db.org>) database with a minimum interaction score requirement of 0.4 and a restriction to humans ("*Homo sapiens*"), PPI networks were constructed using PPI network-related functional enrichment analysis. The results were then imported into Cytoscape 3.9.1 (Cytoscape is available as a platform-independent open-source Java application and funding for continued development and maintenance of Cytoscape is provided by the U.S. National Institute of General Medical Sciences (NIGMS) under award number R01 GM070743. Cytoscape user support, education and new initiatives are supported by the National Resource for Network Biology (NRNB) under award number P41 GM103504.) to investigate the known Zhimu targets in OC-related proteins. The network analysis component of the software was used to examine their functions and count the nodes.

2.4 Enrichment analysis using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG)

Metascape (<https://metascape.org/gp/index.html#/main/step1>), a 2015 online database, uses an automated analytic process called CAME to obtain KEGG/GO enrichment analysis

results [7]. This process includes identifier conversion, gene annotation, membership search, and enrichment analysis. The prospective targets for OC therapy were imported into the Metascape database with a p value < 0.01 and a species restriction of "*H. sapiens*". The results of GO (Biological Process, Molecular Function, Cellular Component) and KEGG pathway enrichment analysis were visualized using online biology tools.

2.5 Building the ingredient-target-pathway network

The network integrating drug-active components, targets, and pathways was created using Cytoscape 3.9.1 (Fig. 1).

3. Results

A total of 81 active compounds of Zhimu were acquired from the TCMSp database, and 15 of them were evaluated according to the ADME principles (Table 1). A total of 407 Zhimu-related targets were identified, of which 93 were retained after the removal of duplicates. The resultant targets were translated into gene names using the UniProt database.

After removing duplicate targets using the DisGeNET (1224 targets) and OMIM (237 targets) databases, 1341 OC-related disease targets remained. Following that, Venny 2.1.0 was used to intersect active components in Zhimu with predicted target genes, yielding 52 common target genes (Fig. 2).

The STRING database was used to import the 52 common Zhimu and OC potential target genes, and the resulting network (Fig. 3) contained 52 nodes and 374 edges, with an average node degree of 14.4 and a PPI-enriched p value < 0.01 . The STRING analysis results were imported into the Cytoscape software. The network analysis plug-in was used to count the nodes and examine their connectivity in the network. As the node's degree increases, so will the number of biological activities in the network (Fig. 4). According to the core target with more than three parameters (betweenness centrality, closeness centrality, and degree), the 10 most relevant targets were identified based on the mean and median of AKT serine/threonine kinase 1 (AKT1), tumour protein p53 (TP53), tumour necrosis factor (TNF), vascular endothelial growth factor A (VEGFA), prostaglandin G/H synthase-2 (PTGS2), interleukin-1 beta (IL1B), hypoxia-inducible factor-1 alpha (HIF1A), caspase-3 (CASP3), heat-shock protein 90-alpha (HSP90AA1), and progesterone receptor (PGR).

Using the Metascape data platform, an enrichment analysis of 52 OC-related Zhimu targets was conducted, and the results were visualised using online biology tools. The results of the Zhimu enrichment study of prospective targets for the treatment of OC are depicted in Figs. 5 and 6 (A–C). The BPs of GO enrichment must include positive regulation of cell migration, response to oxidative stress, initiation of reproductive structures, response to xenobiotic stimulus, regulation of reactive oxygen species metabolic activities, response to decreasing oxygen levels, positive regulation of protein phosphorylation, response to wounds, radiation, and toxic materials, and negative regulation of catabolic processes. In MF, protein homodimerization activity, oxidoreductase ac-

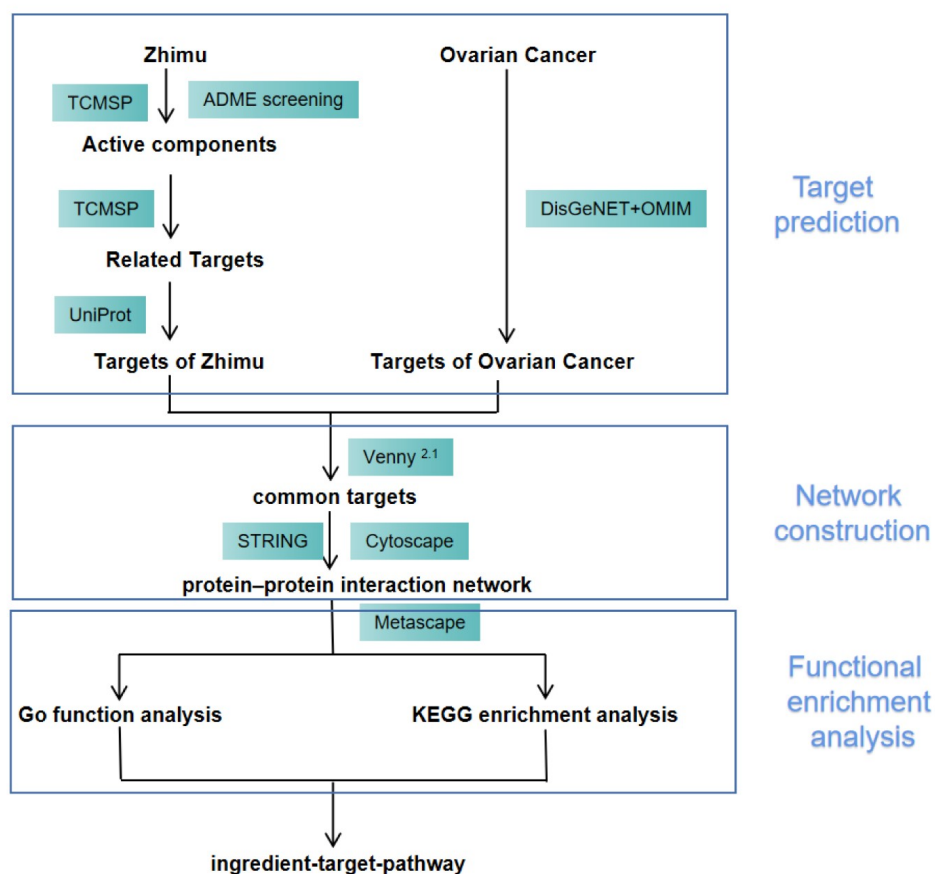


FIGURE 1. Flowchart of the network pharmacology study. ADME: absorption, distribution, metabolism, and excretion; OMIM: Online Mendelian Inheritance in Man; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; TCMSP: Traditional Chinese Medicine System Pharmacology.

TABLE 1. Basic information on the main active ingredients of Zhimu.

Mol ID	Molecule Name	Oral bioavailability (%)	Drug-likeness
MOL004542	Anemarsaponin E qt	30.67	0.86
MOL004514	Timosaponin B III qt	35.26	0.87
MOL004540	Anemarsaponin C qt	35.50	0.87
MOL003773	Mangiferolic Acid	36.16	0.84
MOL004492	Chrysanthemaxanthin	38.72	0.58
MOL004528	Icariin I	41.58	0.61
MOL000422	Kaempferol	41.88	0.24
MOL000449	Stigmasterol	43.83	0.76
MOL004373	Anhydroicaritin	45.41	0.44
MOL004497	Hippeastrine	51.65	0.62
MOL001677	Asperglaucide	58.02	0.52
MOL004489	Anemarsaponin F qt	60.06	0.79
MOL000546	Diosgenin	80.88	0.81
MOL000631	Coumaroyltyramine	112.90	0.20
MOL000483	(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-(2-(4-hydroxyphenyl)ethyl) acrylamide	118.35	0.26

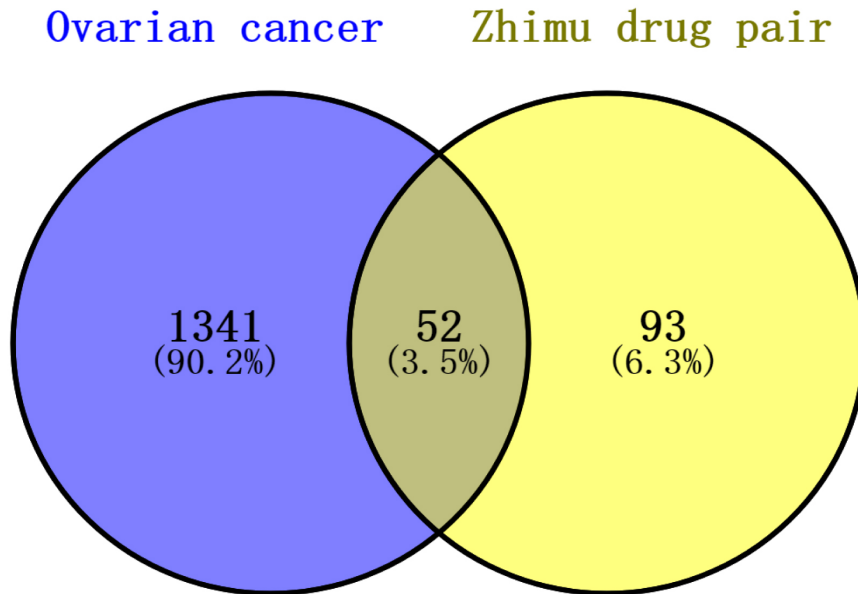


FIGURE 2. A Venn diagram showing the intersection of the active ingredients in Zhimu with the predicted targets of ovarian cancer.

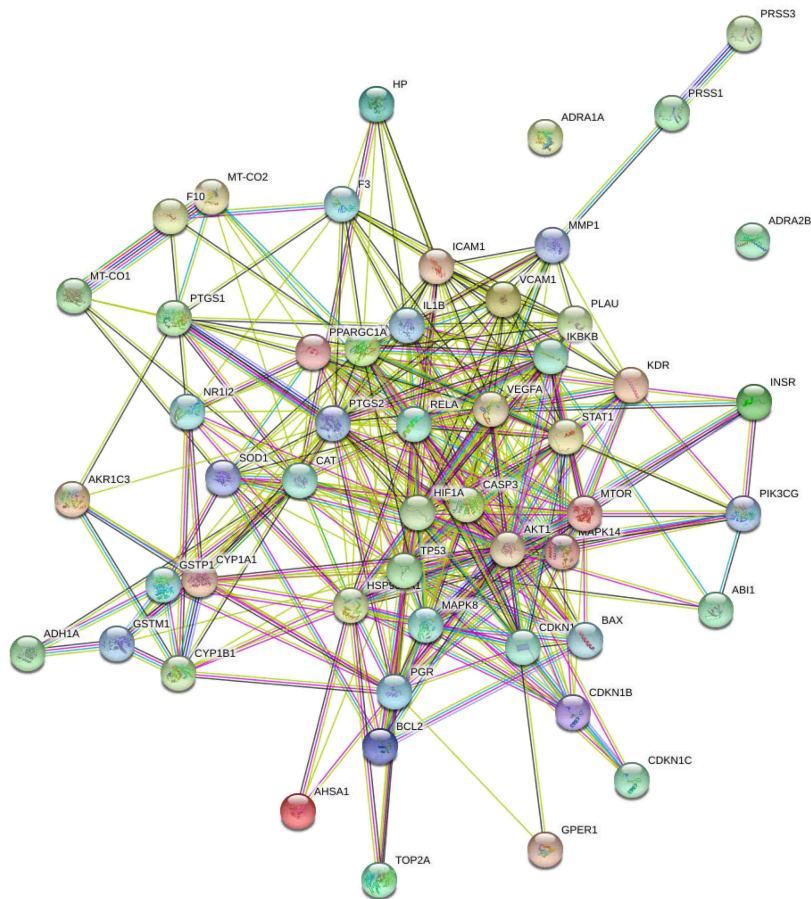


FIGURE 3. PPI network. Network nodes—query proteins and the first shell of interactors, coloured; the second shell of interactors, white; proteins with an unknown 3D structure, unfilled; and proteins with known or anticipated 3D structure, filled. Protein-protein connections are indicated by edges—co-occurring genes, dark blue; text mining, light green; co-expression, black; protein homology, light purple; curated databases, light blue; empirically determined, fuchsia; localised genes, green edges; and gene fusions, red. In the illustration, the thickness of the line represents the magnitude of the force.

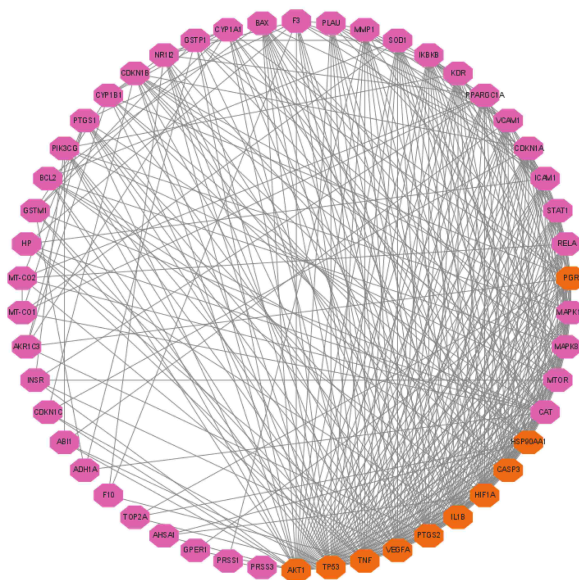


FIGURE 4. Potential targets are arranged counter-clockwise by their degree values, from largest to smallest.

tivity, protein kinase binding, transcription factor binding, cyclin-dependent protein serine/threonine kinase inhibitor activity, and heat shock protein binding were all found to be significantly elevated. Membrane rafts, mitochondrial envelopes, secretory granule lumens, protein kinase complexes, and transcription regulator complexes were the most enriched CCs. The results of the KEGG pathway enrichment analysis focused primarily on the AGE-RAGE signalling pathway in diabetic complications, chemical carcinogenesis-reactive oxygen species, prostate cancer, the NF- κ B signalling pathway, and proteoglycans in cancer, chemical carcinogenesis-receptor activation, ovarian steroidogenesis, and the oxytocin signalling pathway (Fig. 5D).

We built a network of the active ingredients in Zhimu, potential targets, and enrichment pathways using the Cytoscape 3.9.1 software (Fig. 6). The blue rectangle represents the active ingredients in Zhimu, the pink oval represents the targets, and the green diamond represents the relevant pathways.

4. Discussion

OC is the third most common malignancy in women, following uterine and cervical cancers. OC has the highest recurrence and mortality rates of all gynaecologic tumours because it lacks early and atypical late symptoms [8]. Less than 45% of OC patients have a five-year survival rate. In the majority of high-income countries, the age-standardized incidence rate of OC has remained stable or decreased, whereas it has increased in many low- and middle-income countries. Moreover, as life expectancy increases, an increasing number of OC cases are diagnosed [9]. TCM injections are an available adjuvant therapy for OC. TCM practitioners in China have been using compound fuling granules to treat OC for over 20 years. Despite its unidentified therapeutic mechanism, Zhimu has been used to treat OC in China for thousands of years.

Using network pharmacology, we elucidated the novel molecular biological mechanisms by which Zhimu acts to treat

OC. The first step was to identify Zhimu's active ingredients using the criteria of OB \geq 30% and DL \geq 0.18. This process yielded 15 active ingredients and 93 potential targets for a complex-disease target modulation network. The primary active ingredients included kaempferol, asperglauclide, anhydroicaritin, diosgenin, coumaroyltyramine, icariin I, and others. Kaempferol inhibits the G0/G1 cell cycle, induces apoptosis, and modulates the Mitogen-activated protein kinase (MEK)/extracellular-signal-regulated kinase (ERK), Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathways, halting the OC progression [10]. Icariin inhibits cell cycle transition and migration of the OC cells [11]. Diosgenin regulates the vascular endothelial growth factor receptor 2 (VEGFR2) and phosphoinositide 3-kinase (PI3K)/AKT/Mitogen-activated protein kinase (MAPK) signalling pathways as well as the viability of OC cells [12]. Coumaroyltyramine and anhydroicaritin interact with prostaglandin G/H synthase-1, influencing tumorigenesis and progression. Therefore, these active ingredients of Zhimu are thought to be effective in the treatment of OC.

PPI network topology analysis revealed that the primary targets are AKT1, TP53, TNF, VEGFA, PTGS2, IL1B, HIF1A, CASP3, HSPA90AA1 and PGR. These key proteins may be targeted by Zhimu in the treatment of OC. PTGS2 activates the PI3K/AKT pathway and promotes the proliferation and migration of Caov-3 human ovarian carcinoma cells [13]. HSPA90AA1 RNAi inhibits both proliferation and apoptosis in human ovarian cancer SKOV3 cells [14]. AKT1 protein kinase targets serine and threonine residues. PI3K/AKT targets activate approximately 70% of OC cells and stimulate several signalling pathways involved in angiogenesis, cell growth, proliferation, survival, and metabolism [15]. This implies that Zhimu aids in the treatment of OC through these targets.

GO analysis revealed that Zhimu's BP prioritises response to oxidative stress and other exogenous agents, cell migration regulation, and reproductive structure development. These functions promote tumour proliferation and migration. The

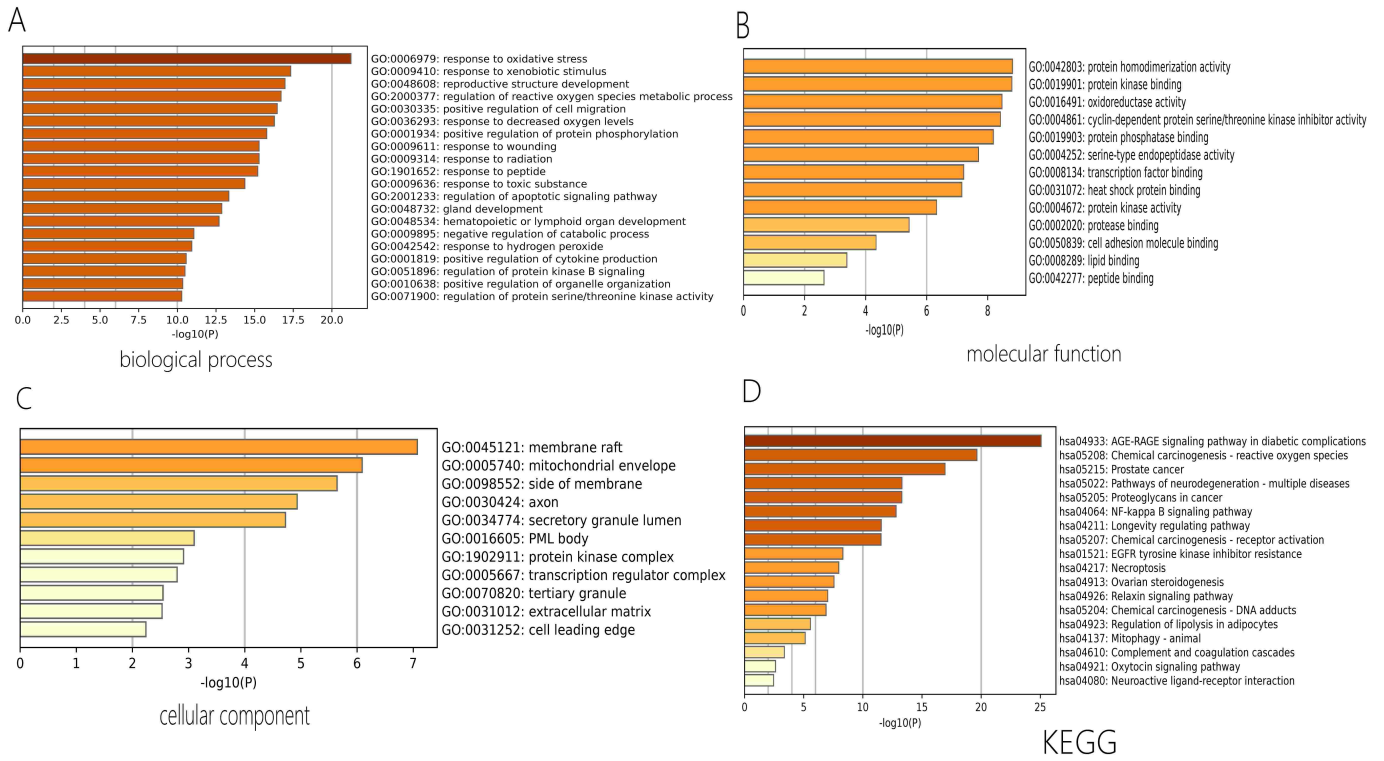


FIGURE 5. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. (A) biological process (BP); (B) molecular function (MF); (C) cellular component (CC); and (D) KEGG. (A–D) sorted by the importance of $\log_{10}(P)$ of each lane.

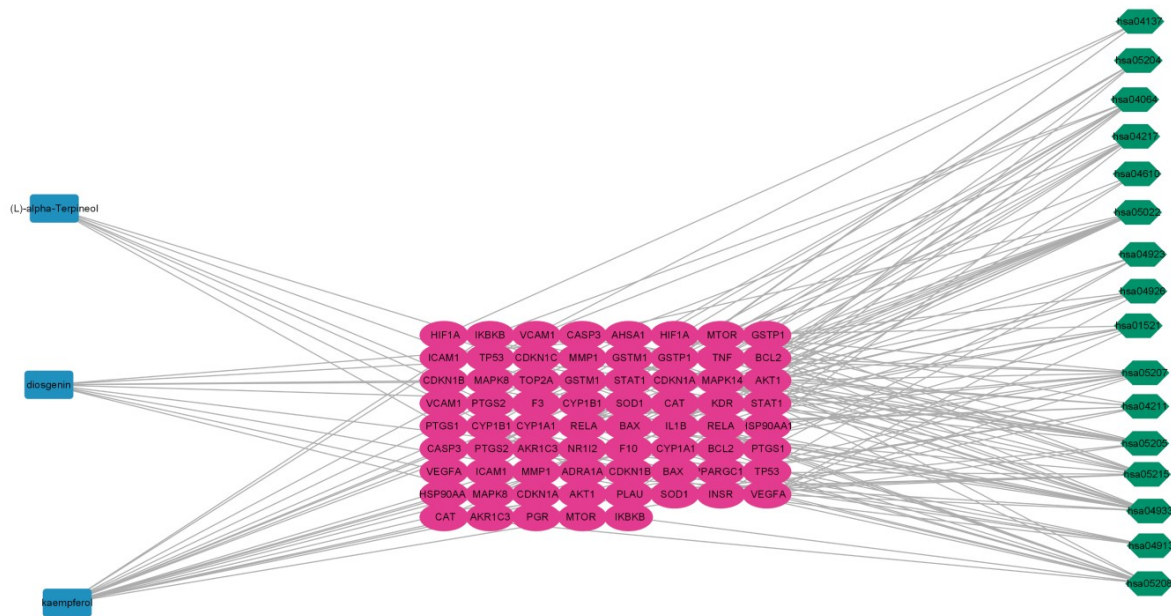


FIGURE 6. Network diagram of “component-target-pathway”.

NF- κ B and AGE/RAGE signalling pathways were the key KEGG pathways. AGE accumulation and elevated RAGE levels are associated with various pathological conditions. The AGE/RAGE signalling pathway perturbs cellular redox homeostasis and cell death pathways, contributing to various diseases, including cancer [16]. The NF- κ B signalling pathway

is also linked to OC progression *via* multiple mechanisms [17–19].

This study lays the groundwork for Zhimu-based treatment of OC. Here, targets associated with TP53, TNF, AKT1, VEGFA, CASP3, PTGS2, HIF1A, IL1B, HSP90AA1, PGR, as well as the oxidative stress, NF- κ B and AGE/RAGE

signalling pathways, were identified. These serve as references for the clinical application of Zhimu. This study has some limitations as well. In network pharmacology, we focused exclusively on the effect of Zhimu in OC. To comprehend intricate multi-target, multi-pathway, and synergistic interactions, pharmacodynamic and mechanistic studies must be conducted, and their results should be validated.

5. Conclusions

Based on the results of network pharmacology, Zhimu interacts with multiple active components, targets, and processes to regulate the progression of OC.

AUTHOR CONTRIBUTIONS

WM—designed and conceived the study. SG—analyzed data and drafted the manuscript. CP—completed the manuscript. ZY—revised the manuscript. QS and XL—provided advice and technical assistance. All authors have contributed to and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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