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Original Research Article

Mechanisms of Salidroside in Treating Premature Ovarian Insufficiency: Network Pharmacology and Molecular Docking

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Abstract

Background: Salidroside has multiple functions such as antiinflammatory, neuroprotection, immunomodulation, antioxidative stress, and antiapoptosis. However, its application in the treatment of ovarian insufficiency is not yet clear.
Objectives: By means of network pharmacology, we analyzed the mechanism of salidroside in premature ovarian insufficiency (POI) and verified it with molecular docking technology.
Materials and Methods: We collected the target of salidroside using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, screened the target genes of POI from GeneCards, and constructed protein–protein interactions (PPI) of salidroside for treating POI by Cytoscape software. We performed PPI, gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of key genes using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) and performed molecular docking verification of salidroside and key proteins by AutoDock software.
Results: From 335 salidroside and 5,177 POI target genes, 223 key genes were identified at their intersection. The top 20 targets, including insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2), and estrogen receptor 1 (ESR1), were selected based on maximal clique centrality (MCC), Degree, and maximum neighborhood component (MNC) rankings. These genes play roles in signal transduction, phosphorylation, and apoptosis regulation, and are located in the cytosol, cytoplasm, and nucleus. They are involved in protein binding and adenosine triphosphate (ATP) binding, with pathways related to cancer, phosphatidylinositol (PI), and lipid metabolism. Molecular docking revealed salidroside's strong binding to these proteins, indicating its potential in POI treatment through multi-target action.
Conclusion: Salidroside exerts a multi-target effect on pathways such as signal transduction, demonstrating translational potential to be developed into an effective treatment for POI patients. Its multi-target characteristics may regulate the physiological processes of POI patients, further highlighting its promise in translating research findings into clinical practice for POI therapy.

Keywords

Salidroside, premature ovarian insufficiency, network pharmacology, molecular docking

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Introduction

Premature ovarian insufficiency (POI) refers to a clinical syndrome of ovarian activity decline occurring in women before the age of 40, characterized by menstrual disorders (such as amenorrhea or oligomenorrhea) accompanied by high gonadotropins and low estrogen levels (Guo et al., 2023). In China, the incidence of POI is 2.8%, showing an increasing trend year by year, and the disease also tends to occur at a younger age (European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI et al., 2016). Hormone replacement therapy (HRT) can improve the low-estrogen symptoms of POI patients and prevent long-term complications. With definite clinical efficacy, it is the main method for Western medicine in treating POI. However, the long-term application of HRT has raised many concerns in

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clinical practice. Studies have shown that long-term use of HRT significantly increases the risks of breast cancer, stroke, and cardiovascular diseases (Cold et al., 2022). This not only imposes an additional health burden on patients but also places clinicians in a dilemma when choosing treatment options. Traditional Chinese medicine has demonstrated obvious efficacy in improving menstrual abnormalities and female reproductive capacity, with relatively high safety (Choi & Kim, 2023; Gan et al., 2023).

Rhodiola rosea (family name: Crassulaceae), a herbal medicine, has the effects of replenishing qi, promoting blood circulation, unblocking the channels (meridians in the traditional Chinese medicine concept), and relieving asthma (Huang et al., 2020). Salidroside, one of the main active ingredients in *R. rosea*, has multiple functions such as anti-inflammatory, neuroprotection, immunomodulation, anti-oxidative stress, and anti-apoptosis (He et al., 2023; Liang et al., 2023). Currently, for the treatment of POI, Western medicine mainly relies on HRT. However, the side effects of HRT limit its long-term application. Although traditional Chinese medicine has certain advantages, it lacks in-depth modern scientific interpretations. Due to its multi-faceted pharmacological activities, salidroside theoretically has the potential to be a new-type drug that can counteract menstrual disorders, correct ovarian function, and act on multiple targets. Nevertheless, its action targets and mechanisms remain unclear. Network pharmacology integrates multiple disciplines such as polypharmacology, systems biology, and network analysis, and can reveal the network-based regulation of the body by traditional Chinese medicine (Chen et al., 2022; Liao et al., 2024; Yuan et al., 2023). By constructing a "compound–gene–disease" interaction network system, this method can interpret the relationship between drugs and diseases and further reveal the mechanism of action of drugs on diseases (Han et al., 2022).

Based on the above-mentioned situation, there is an urgent need for a safe and effective treatment plan for POI. The side effects of HRT limit its long-term use, and although traditional Chinese medicine has potential, it lacks in-depth exploration of modern scientific mechanisms. As a key component of *R. rosea*, salidroside has various beneficial pharmacological activities, but its specific targets and mechanisms in the treatment of POI have not been clarified. Network pharmacology and molecular docking techniques provide powerful tools to analyze the synergistic mechanism of salidroside in treating POI from the perspectives of multiple components, targets, and pathways. Therefore, this study uses these techniques to analyze the mechanism of salidroside in treating POI deeply. This study anticipates filling the gap in the understanding of the mechanism of salidroside in treating POI. This will provide a scientific basis for the rational clinical application of *R. rosea* in treating POI. Moreover, it may open up new avenues for the treatment of POI, provide patients with safer and more effective treatment options, and thus be of great practical significance in improving patients' quality of life and long-term health.

Materials and Methods

Database and Software

A computational pipeline was constructed using the following resources: PubChem database, SwissTargetPrediction (http://swisstargetprediction.ch/), PharmMapper (http://lilab-ecust.cn/pharmmapper/), Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) website (http://string-db.org/), Similarity Ensemble Approach (SEA) prediction website, Draw Venn Diagram website (http://bioinformatics.psb.ugent.be/webtools/Venn/), Genome Annotation Database Platform (GeneCard) database (https://www.genecards.org/), AutoDockTools 1.5.7 software, and Cytoscape 3.8.0 software.

Screening of Salidroside Targets

The structure data file (SDF) of salidroside was obtained from the PubChem database and imported into the SwissTargetPrediction and PharmMapper websites for prediction analysis. In addition, simplified molecular input line entry system (SMILES) molecular expressions obtained from SDF were imported into the Similarity ensemble approach prediction website to screen out human-related target genes for drugs (Jiang et al., 2022).

POI Disease Target Screening

With "premature ovarian insufficiency" as the keyword, POI-related target genes were retrieved on the GeneCard database, and the disease target was obtained by deleting duplicates after all target genes were merged.

Screening and Protein Interaction of Salidroside and POI Intersection Targets

We constructed a protein–protein interaction (PPI) network. The targets of salidroside and POI were uploaded to the Draw Venn Diagram website to draw a diagram showing the intersection genes. We uploaded the intersection genes to STRING. After obtaining and transferring the PPI network to Cytoscape 3.9.1 software, we carried out visualization mapping according to the protein degree value, from high to low.

Core Target Gene Mining

Cytoscape 3.8.0 used molecular complex detection (MCODE) and cytoHubba plug-ins to discover the core modules and genes of the POI PPI network acted by salidroside, with the plug-in parameters set by default. After the cytoHubba plug-in performed maximum neighborhood component (MNC), maximal clique centrality (MCC), and maximal mutual Degree topological algorithm to analyze PPI, we further intersected the top 20 target interaction networks.

Intersection Criteria

The top 20 target interaction networks obtained by analyzing the PPI network using the MNC, MCC, and Degree topological algorithms of the cytoHubba plug-in are regarded as gene sets, and only the target genes that exist simultaneously in these 20 sets are retained as the final intersection result. This criterion aims to screen out target genes that show importance and high consistency under different topological analysis algorithms, and these genes are more likely to play a key and stable role in the regulatory mechanism of salidroside on POI.

Functional Enrichment Analysis of Genes at the Intersection of Salidroside and POI

Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Zhang et al., 2024) enrichment analysis were carried out for key targets using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). We imported the biology information of the bioinformatics platform (https://www.bioinformatics.com.cn/) for online mapping.

Salidroside and Core Target Gene Molecules Docking

We applied AutoDockTools 1.5.7 software for molecular docking and selected the core target protein for docking with salidroside. The 2D structural formula SDF file of salidroside compounds was obtained using the PubChem database. The target protein structure was obtained from the protein data bank (PDB) (https://www.rcsb.org/). After removing water molecules from the obtained structure, it was simulated with the structure file of salidroside, and the molecular binding energy and three-dimensional structure diagram of the docking were obtained.

For the validation part of the docking protocol, we must honestly point out that due to experimental conditions and time constraints, a systematic validation has not been carried out, which is a limitation of this study.

Statistical Analysis

Target Screening

The structural information of salidroside was imported into SwissTargetPrediction, PharmMapper, and SEA websites. The number of predicted target genes from each platform was counted, and the total count was obtained by merging and removing duplicates. Consistency was evaluated by calculating the overlap ratio.

POI Target Screening

POI target genes were retrieved from GeneCards using "premature ovarian insufficiency." The retrieved gene count was noted, and the intersection gene count with salidroside target genes was determined.

Core Target Gene Mining

A PPI network of intersection genes was constructed using STRING. Node and edge counts were taken. Module analysis with MCODE plugin included noting the highest module score and gene count. Topological analysis via cytoHubba's MNC, MCC, and Degree algorithms involved counting the top 30 targets, and core target genes were identified by intersecting the top 20.

Functional Enrichment Analysis

Enrichment analysis was performed on the intersection genes of salidroside and POI. Enrichment information for biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways was statistically described.

Results

Salidroside Target Gene

The chemical structure of *R. rosea* and *R. rosea* glycoside is shown in Figure 1. The SMILES molecular expression "C1=CC(=CC=C1CCOC2C(C(C(C(C(O2)CO)O)O)O)O))" of salidroside was imported into the SEA website, and 40 human-related target genes were screened. After merging all salidroside-related target genes obtained above, the duplicate values were deleted, and 335 targets were finally obtained.

POI Target Gene

We retrieved 5,177 POI target genes from the GeneCards database. After intersecting this set of genes with salidroside target genes, 223 crossover genes (key genes) were obtained (Figure 2).

Core Target Gene Network Map and Analysis

The PPI network map of intersected genes was acquired through the STRING network. It had 221 nodes and 2627 interacting edges (Figure 3). The module with the highest score of MCODE analysis is 30.054 and contains 38 genes (Table 1). The topological analysis algorithm of cytoHubba was used, and the top 30 targets of MNC, MCC, and Degree were shown in Table 2 and Figure 4A–C. The intersection of the top 20 targets in MCC, Degree and MNC was performed, and 12 common targets were obtained, including insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2), kinase insert domain receptor (KDR), matrix metalloproteinase 9 (MMP9), matrix metalloproteinase 2 (MMP2), peroxisome proliferator-activated receptor gamma (PPARG), Harvey rat sarcoma viral oncogene homolog (HRAS), cell division cycle 42 (CDC42), SRC proto-oncogene,

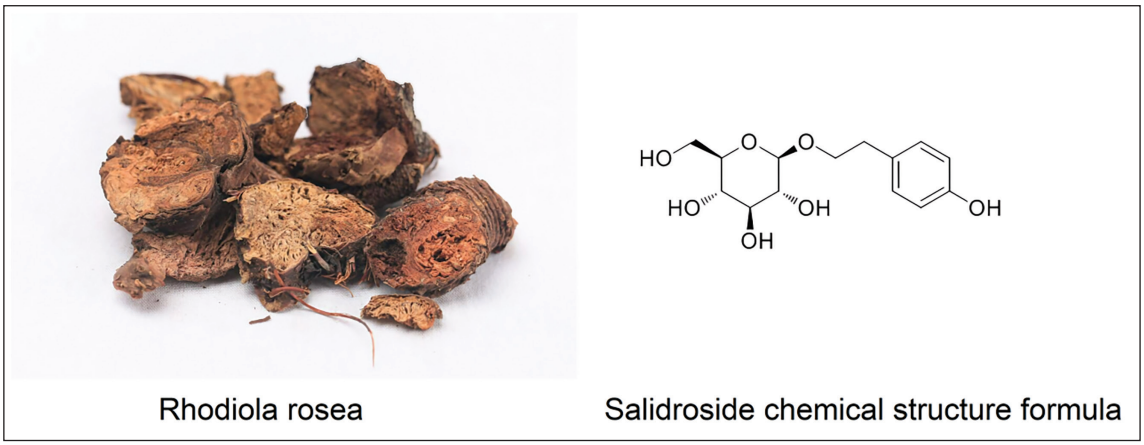


Figure 1. *Rhodiola rosea* and Chemical Structure Formula of *Rhodiola rosea* Glycoside.

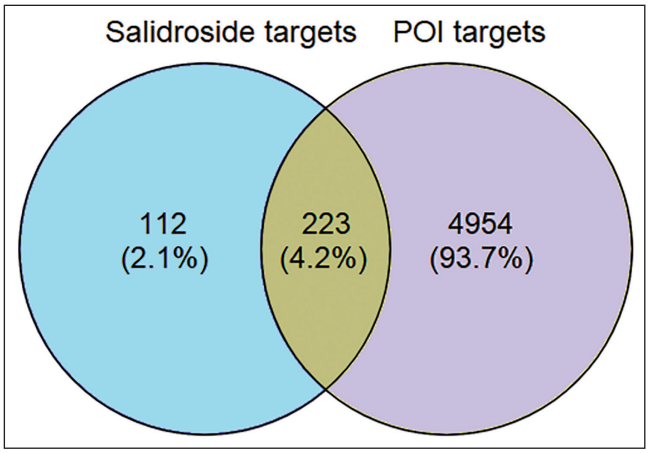


Figure 2. Wayne Map of Intersection Target Genes of *Rhodiola rosea* and Premature Ovarian Insufficiency (POI).

non-receptor tyrosine kinase (SRC), estrogen receptor 1 (ESR1), interleukin 6 (IL6) and ras-homologous gene family member A (RHOA) (Figure 4D). These genes were incorporated into the modules with the highest scores in the MCODE analysis. This indicates that they play a significant role in the treatment of POI with salidroside and are core target genes.

Pathway Enrichment of Intersection Targets

In terms of BP enrichment of biological processes, there are signal transduction, phosphorylation, negative regulation of apoptotic process, and others (Zhang et al., 2023). For CC enrichment of cell components, there are cytosol, cytoplasm, nucleus, extracellular exosome, plasma membrane, and so on. In terms of MF enrichment of cell functions, there are protein binding, identical protein binding, adenosine triphosphate (ATP) binding, and the like. The signaling pathways

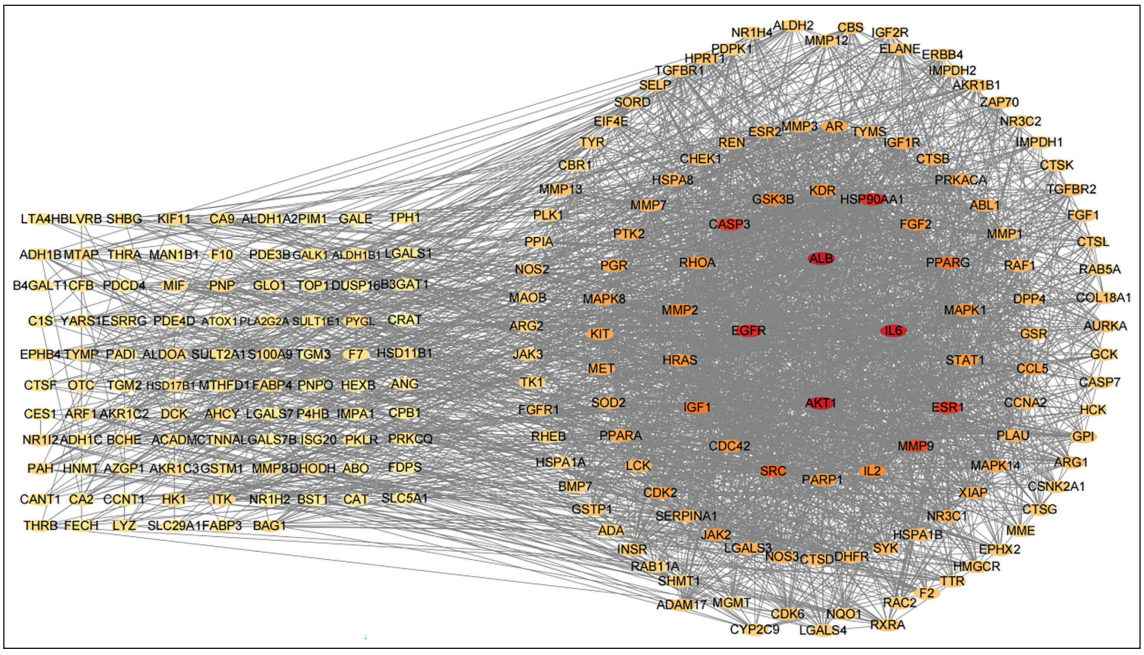


Figure 3. Protein–Protein Interactions (PPI) of Intersecting Genes.

Table 1. Genes of the Highest Scoring Modules Analyzed by MCODE.

node_name	MCODE Scores	node_name	MCODE Scores
MAPK1	20.0	HSP90AA1	17.5
JAK2	19.8	GSK3B	17.3
PTK2	19.8	IL6	17.2
IGF1	19.4	EGFR	17.2
MAPK8	19.2	ALB	17.2
MMP2	18.8	CASP3	17.2
MMP9	18.6	AR	17.2
STAT1	18.6	PPARG	17.1
HRAS	18.4	MET	17.0
IGF1R	18.4	PLAU	17.0
ESR1	18.3	IL2	16.8
TGFBR2	18.3	PGR	16.6
KDR	18.2	PRKACA	16.6
PARP1	17.9	FGFR1	16.5
SRC	17.8	CCL5	16.5
AKT1	17.7	CDC42	16.1
RHOA	17.7	ESR2	16.1
IGF2	17.6	XIAP	16.1
MAPK14	17.5	ABL1	16.1

Note: CDC42: Cell division cycle 42; ESR1: Estrogen receptor 1; IGF2: Insulin-like growth factor 2; HRAS: Harvey rat sarcoma viral oncogene homolog; IGF1: Insulin-like growth factor 1; IL6: Interleukin 6; KDR: Kinase insert domain receptor; MMP2: Matrix metalloproteinase 2; MMP9: Matrix metalloproteinase 9; PPARG: Peroxisome proliferator-activated receptor gamma; RHOA: Ras-homologous gene family member A; SRC: SRC proto-oncogene, non-receptor tyrosine kinase.

enriched by KEGG include pathways in cancer, phosphatidylinositol (PI), lipid and atherosclerosis, the mitogen-activated protein kinase (MAPK) signaling pathway, Proteoglycans in cancer, and so on (Figure 5).

Molecular Docking of Salidroside with Key Genes of POI

In the molecular docking of salidroside with 12 key genes, such as IGF1 (binding energy: −5.93 kcal/mol), IGF2 (binding energy: −3.26 kcal/mol), KDR (binding energy: −2.94 kcal/mol), MMP9 (binding energy: −6.09 kcal/mol), MMP2 (binding energy: −6.61 kcal/mol), PPARG (binding energy: −3.98 kcal/mol), HRAS (binding energy: −5.99 kcal/mol), CDC42 (binding energy: −5.65 kcal/mol), SRC (binding energy: −4.33 kcal/mol), ESR1 (binding energy: −2.78 kcal/mol), IL6 (binding energy: −5.81 kcal/mol), and RHOA (binding energy: −5.46 kcal/mol), this molecular docking indicates a high degree of docking affinity. The smaller the value of molecular binding energy, the greater the binding degree between the two molecules, resulting in a stronger interaction. The docking mode between salidroside and the above key targets is shown in Figure 6.

Discussion

POI is characterized by a progressive decline in ovarian function. Early treatment plays a crucial role in halting the progression of the disease in a timely manner (Su et al., 2023). The etiology of POI is complex and diverse, and its pathogenesis remains unclear to date. As of now, medical researchers have not developed a drug with proven efficacy for the treatment of this disease. Theoretically, salidroside has the potential to be a promising candidate for the treatment of POI. However, its action targets and mechanisms still need to be explored in depth. Therefore, this study aims to investigate the mechanism of action of salidroside in the treatment of POI by combining network pharmacology analysis and molecular docking. The approach combining modern network pharmacology and molecular docking can comprehensively explore the action mechanisms of traditional Chinese medicines and preliminarily elucidate the pharmacological principles of traditional Chinese medicines (Ju et al., 2023).

In this study, the PPI network diagram of the intersection genes was obtained through the STRING network. This diagram showcases the complex interaction relationships among genes, consisting of 221 nodes and 2,627 edges. From the diagram, we can visually observe that dense and intricate network connections are formed among the genes. For instance, some genes are surrounded by a relatively large number of edges, indicating that they may interact with multiple other genes in the network and are in relatively crucial positions. This complex connection pattern strongly implies that in the process of salidroside treating POI, multiple genes do not function independently but collaborate synergistically. Furthermore, through MCODE analysis, the module with the highest score is 30.054, and this module contains 38 genes. In the PPI network diagram, this module exhibits a highly tight connection state, suggesting that the genes within the module have a stronger functional relevance and synergy. Since this module is derived from the analysis of the network's tight connectivity and modularity, it is highly likely that these genes jointly participate in the key biological processes of salidroside in treating POI. For example, these genes may jointly regulate an important cellular signaling pathway, playing a crucial role in maintaining or restoring ovarian function. Meanwhile, by applying the topological analysis algorithms of cytoHubba (MNC, MCC, and Degree), the importance of genes in the network is evaluated from different perspectives. Degree mainly considers the connectivity of nodes, that is, the number of other genes connected to this gene. The higher the connectivity, the greater the direct influence of this gene in the network. MCC and MNC, on the other hand, comprehensively consider factors such as the topological structure of the network, enabling a more comprehensive reflection of the importance of genes in the entire network. Through these

Table 2. Topological Analysis Results of MCC, Degree, and MNC Algorithms (Top 30).

Node	MCC	Node	Degree	Node	MNC
FGFR1	9.22E+13	ALB	121	ALB	121
IGF1	9.22E+13	AKT1	116	AKT1	116
JAK2	9.22E+13	IL6	109	IL6	109
PLAU	9.22E+13	EGFR	99	EGFR	99
TGFBR2	9.22E+13	HSP90AA1	96	HSP90AA1	96
MET	9.22E+13	ESR1	92	ESR1	92
IGF2	9.22E+13	CASP3	91	CASP3	91
KDR	9.22E+13	MMP9	85	MMP9	85
MMP9	9.22E+13	SRC	76	SRC	76
MMP2	9.22E+13	PPARG	69	PPARG	69
PPARG	9.22E+13	IGF1	68	IGF1	68
PTK2	9.22E+13	GSK3B	61	GSK3B	60
HRAS	9.22E+13	IL2	60	IL2	60
CDC42	9.22E+13	MMP2	59	MMP2	59
SRC	9.22E+13	IGF2	56	IGF2	56
ESR1	9.22E+13	KDR	54	KDR	54
IL6	9.22E+13	CDC42	54	CDC42	54
IGF1R	9.22E+13	MAPK1	54	MAPK1	54
AR	9.22E+13	HRAS	53	HRAS	53
RHOA	9.22E+13	RHOA	53	RHOA	53
MAPK8	9.22E+13	PARP1	50	PARP1	50
AKT1	9.22E+13	STAT1	50	STAT1	50
XIAP	9.22E+13	JAK2	47	JAK2	47
PARP1	9.22E+13	MAPK8	46	MAPK8	46
STAT1	9.22E+13	CDK2	46	CDK2	46
HSP90AA1	9.22E+13	CCL5	44	CCL5	44
GSK3B	9.22E+13	PGR	44	PGR	44
CASP3	9.22E+13	KIT	43	KIT	43
ALB	9.22E+13	MAPK14	43	MAPK14	43
EGFR	9.22E+13	PPARA	42	PPARA	42

Note: AKT: Protein kinase B; CDC42: Cell division cycle 42; ESR1: Estrogen receptor 1; IGF2: Insulin-like growth factor 2; HRAS: Harvey rat sarcoma viral oncogene homolog; IGF1: Insulin-like growth factor 1; IL6: Interleukin 6; KDR: Kinase insert domain receptor; MAPK: Mitogen-activated protein kinase; MMP2: Matrix metalloproteinase 2; MMP9: Matrix metalloproteinase 9; MCC: Maximal clique centrality; MNC: Maximum neighborhood component; PPI: Protein–protein interaction; PPARG: Peroxisome proliferator-activated receptor gamma; RHOA: Ras-homologous gene family member A; SRC: SRC proto-oncogene, non-receptor tyrosine kinase.

analysis methods, we can screen out the genes in key positions in the network. For example, in the Degree analysis, genes with high connectivity may play important roles in information transmission or function regulation, while genes with high scores in the MCC and MNC analyses may be crucial for maintaining the stability of the network structure and the integrity of its functions. By taking the intersection of the top 20 targets ranked by MCC, Degree, and MNC, 12 common targets were obtained, including IGF1, IGF2, KDR, MMP9, MMP2, PPARG, HRAS, CDC42, SRC, ESR1, IL6, and RHOA. These common targets not only stand out in their respective topological analyses but are also selected in multiple analysis methods, further demonstrating their significant positions in the process of salidroside treating POI. At the same time, these genes are included in the module with the highest score in the MCODE analysis, and this result strongly supports the conclusion that these genes are core target genes. Because they are both in key positions in the network and within the important tightly-connected module, they are likely to play important functions in the treatment of POI with salidroside. For example, they may participate in the regulation of key signaling pathways, cell function

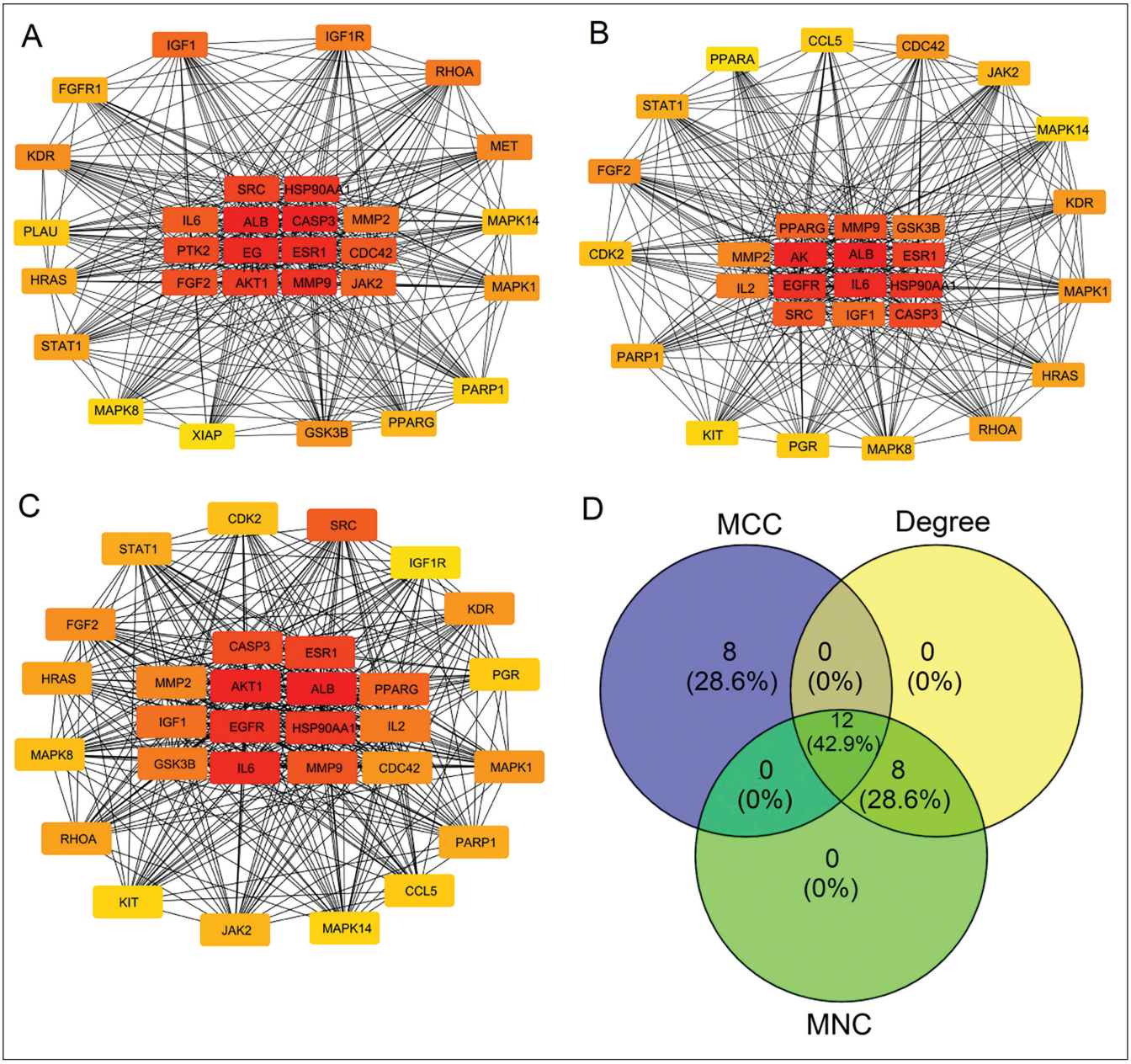


Figure 4. Targets of Maximum Neighborhood Component (MNC), Maximal Clique Centrality (MCC), Degree Calculation (Top 30), and Their 12 Common Targets (Top 20).

regulation, and other processes, thereby affecting ovarian function and providing potential action targets and mechanism clues for salidroside in treating POI.

GO and KEGG enrichment analyses showed that these key genes are mainly enriched in BP such as signal transduction, phosphorylation, and negative regulation of the apoptotic process; CC such as cytosol, cytoplasm, and nucleus; MF such as protein binding, identical protein binding, and ATP binding; and signaling pathways in the KEGG such as pathways in cancer, PI-related pathway, and pathways in lipid and atherosclerosis. From a clinical perspective, the decline of ovarian function in patients with POI may be accompanied by abnormal intracellular signal transduction, increased apoptosis, and metabolic disorders. The pathways enriched by these key genes may be closely related to these clinical features of POI. For example, abnormalities in the signal transduction pathway may lead to abnormal responses of ovarian cells to hormonal signals, thus affecting the normal function of the ovaries. Abnormalities in the negative regulation of apoptosis may result in increased apoptosis of ovarian cells, accelerating the decline of ovarian function. Salidroside may play a therapeutic role in POI by regulating these pathways.

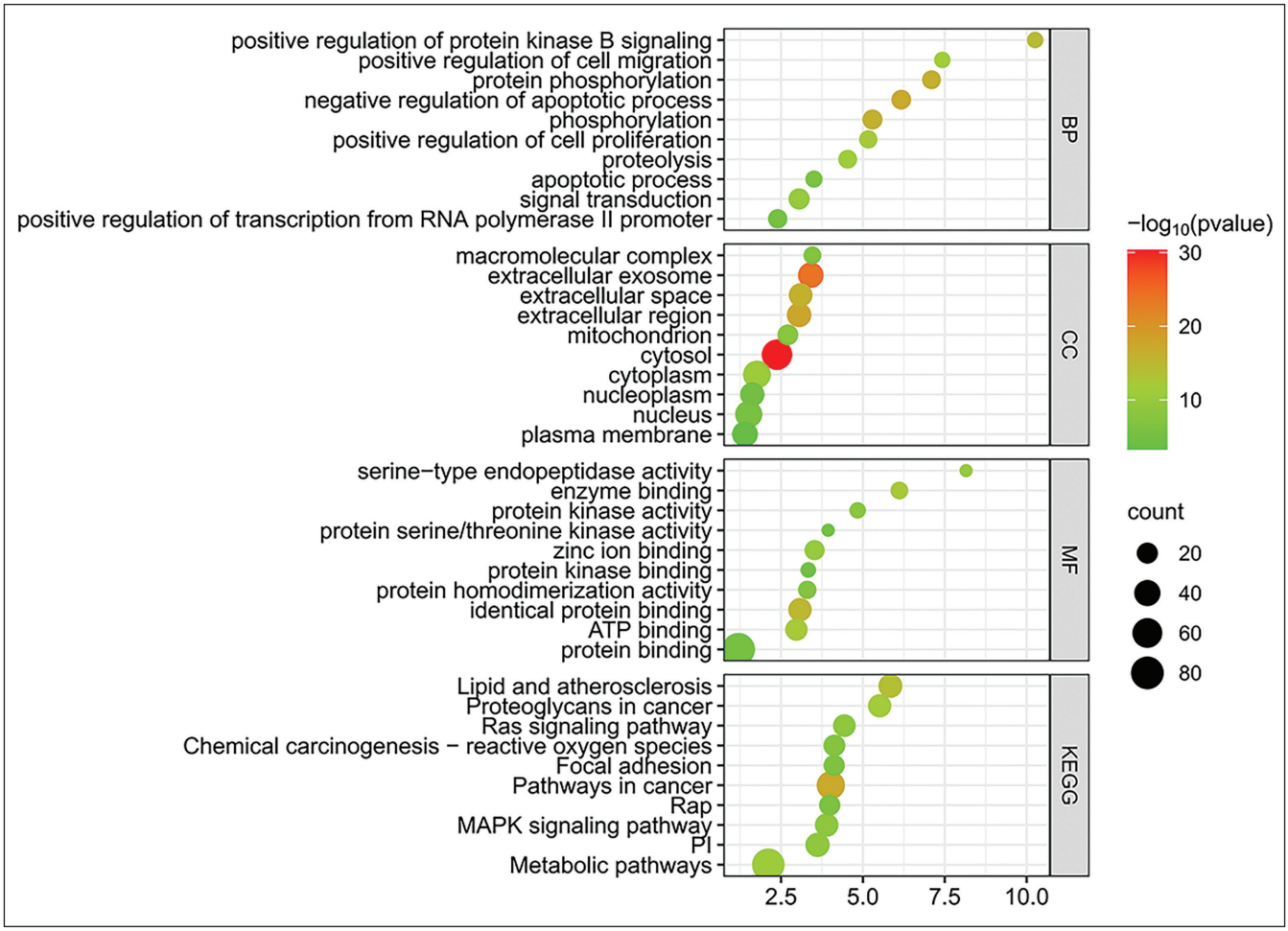


Figure 5. Pathway Enrichment of Intersection Targets.

To further explore the interactions between salidroside and these key genes, we conducted molecular docking experiments. In the molecular docking models of salidroside with 12 key genes, namely, IGF1, IGF2, KDR, MMP9, MMP2, PPARG, HRAS, CDC42, SRC, ESR1, IL6, and RHOA, the binding energy values reflect the tightness of the binding between salidroside and these genes as well as the strength of their interactions. Generally, the smaller the molecular binding energy value, the closer the binding between the two, enabling a stronger interaction. Notably, the binding energies of salidroside docking with IGF1, MMP9, MMP2, HRAS, CDC42, IL6, and RHOA are all lower than −5 kcal/mol. This result is of great significance. From a biological perspective, these relatively low binding energies imply that there may be a strong affinity and specific binding between salidroside and these key genes, suggesting that salidroside may exert its therapeutic effect on POI through direct interactions with these genes.

R. rosea has the effects of invigorating the spleen and stomach and replenishing qi, as well as nourishing yin and promoting fluid production. Salidroside, the main active ingredient of *R. rosea*, may play a role in regulating the hormone levels in the ovaries of patients with POI and resisting ovarian cell apoptosis. As Chen et al. (2024) demonstrated, salidroside can improve the condition of cyclophosphamide-induced premature ovarian failure (POF) in rats (Chen et al., 2024). After treatment with salidroside, the secretion levels of follicle-stimulating hormone (FSH), estradiol (E2), and anti-Müllerian hormone (AMH) in POF rats were restored, follicular atresia decreased, and the number of antral follicles increased. In addition, the fertility of POF rats was significantly enhanced, and the level of oxidative stress decreased. However, it still needs to be further clarified whether salidroside has other action targets and mechanisms. From the perspective of specific gene functions, IGF1, as an insulin-like growth factor, is related to insulin in structure and function. However, it has higher growth-promoting activity and plays a role in glycogen and deoxyribonucleic acid (DNA) synthesis, as well as synaptic maturation. Promoting DNA synthesis may be one of the pathways through which it participates in the correction of POI (Cao et al., 2024). In POI patients, ovarian function decline may be accompanied by abnormal cell

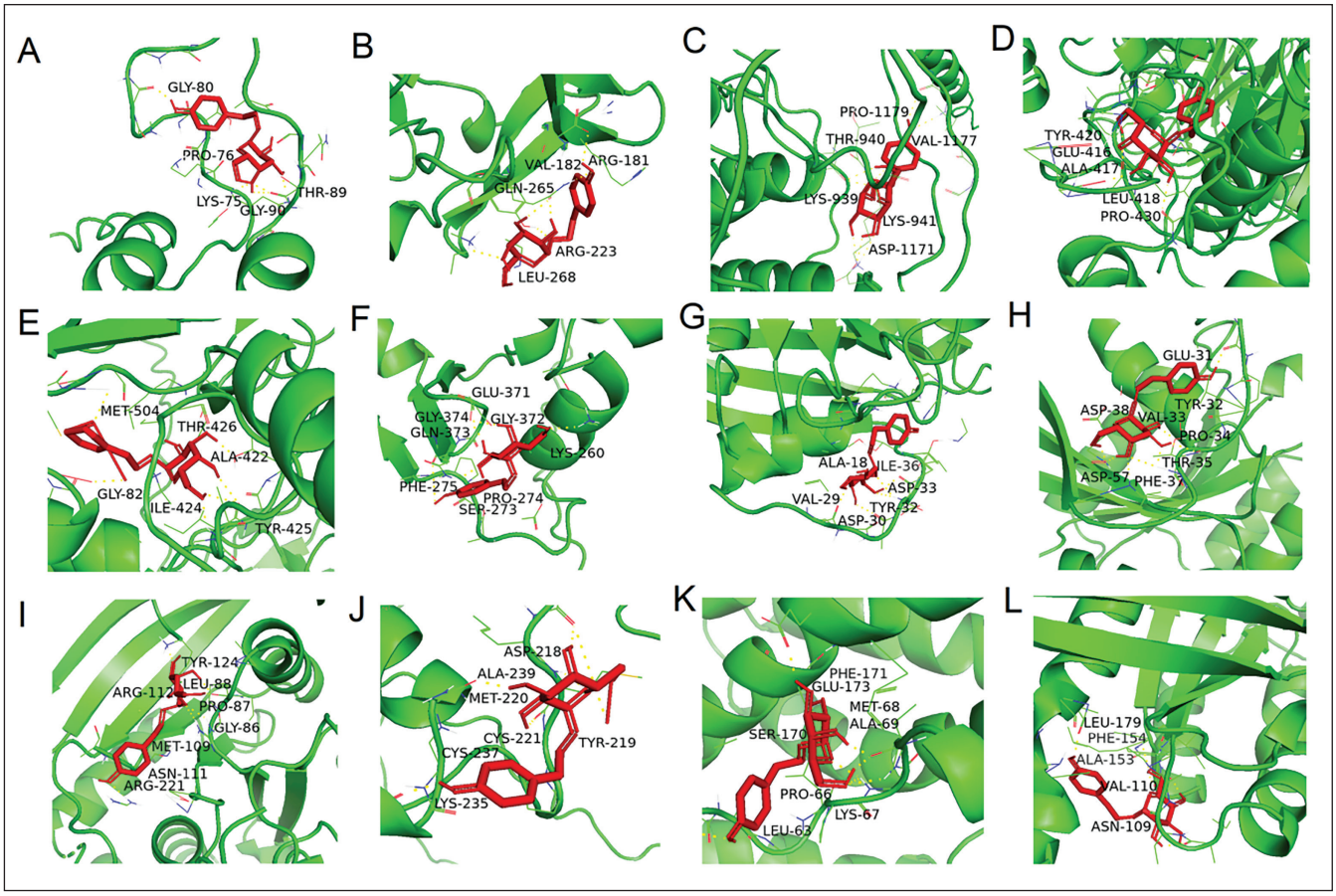


Figure 6. Molecular Docking Patterns of Salidroside and Key Targets of Premature Ovarian Insufficiency (POI). (A), (B), (C), (D), (E), (F), (G), (H), (I), (J), (K), and (L) are the Modes of Salidroside With Insulin-like Growth Factor 1 (IGF1), Insulin-like Growth Factor 2 (IGF2), Kinase Insert Domain Receptor (KDR), Matrix Metalloproteinase 9 (MMP9), Matrix Metalloproteinase 2 (MMP2), Peroxisome proliferator-activated Receptor Gamma (PPARG), Harvey Rat Sarcoma Viral Oncogene Homolog (HRAS), Cell Division Cycle 42 (CDC42), SRC Proto-oncogene, Non-receptor Tyrosine Kinase (SRC), Estrogen Receptor 1 (ESR1), Interleukin 6 (IL6), and Ras-homologous Gene Family Member A (RHOA), Respectively.

proliferation and differentiation. IGF1 may regulate the proliferation and differentiation of ovarian cells by promoting DNA synthesis, thereby improving ovarian function. MMP9 is a matrix metalloproteinase that plays a crucial role in local proteolysis of the extracellular matrix and leukocyte migration. In the ovaries of POI patients, the remodeling of the extracellular matrix may be abnormal, and the abnormal expression of MMP9 may affect the normal structure and function of the ovaries (Pawar et al., 2023). MMP2, as a ubiquitin-related metalloproteinase, is widely involved in various functions such as vascular remodeling, angiogenesis, tissue repair, tumor invasion, and inflammation (Sadat Eshaghi et al., 2022). In addition to degrading extracellular matrix proteins, its C-terminal non-catalytic fragment, PEXtin domain (PEX), also has the property of inhibiting cell apoptosis. During the occurrence of POI, ovarian angiogenesis and tissue repair may be impaired. MMP2 and its fragment PEX may play a protective role in ovarian function by regulating these processes. HRAS is involved in the activation of Ras protein signal transduction (Tang et al., 2023). Ras proteins bind to guanosine diphosphate (GDP)/guanosine triphosphate (GTP) and possess intrinsic guanosine triphosphatase (GTPase) activity. By regulating cell signaling, they interact with a series of downstream proteins, initiate specific signal transduction pathways, and influence biological processes such as cell proliferation, differentiation, and survival. This series of processes may play a crucial role in the abnormal changes of ovarian cells in POI patients, and the regulation of HRAS may affect the fate of ovarian cells. CDC42, a small GTPase associated with the plasma membrane, cycles between the active GTP-bound state and the inactive GDP-bound state. In the active state, it binds to various effector proteins to regulate the biochemical or physical responses of cells to external stimuli (Kang et al., 2019). In the ovaries of POI patients, the response of cells to external stimuli may be altered, and CDC42 may be involved in regulating these abnormal responses, thus affecting ovarian function. Cytokines have extensive biological functions in aspects such

as immunity, tissue regeneration, and metabolism. IL6 is an effective inducer of the acute-phase response. The research by He et al. (2022) has confirmed that the inhibition of IL6 has a protective effect on ovarian dysfunction. In the bodies of POI patients, the inflammatory response may be involved in the damage of ovarian function. The abnormal expression of IL6 may exacerbate inflammation, thereby affecting ovarian function. The research by Hu et al. (2023) indicates that Jinfeng Pills improve the condition of POI in rats by regulating the IL-17A/IL6 axis and the MEK/ERK signaling pathway, further demonstrating the importance of IL6 in the pathogenesis of POI. RHOA, a small GTPase, binds to various effector proteins in its active state and regulates cellular responses such as cytoskeleton dynamics, cell migration, and the cell cycle. The study by Chen et al. (2023) revealed that RHOA/Rho-associated coiled-coil forming protein kinase 1 (ROCK1)-mediated actin cytoskeleton rearrangement regulates the production of ovarian steroids such as estrogen. In patients with POI, abnormal estrogen production may be one of the important factors leading to ovarian function decline. RHOA may influence the occurrence and development of POI by regulating estrogen production. In addition, the RhoA/Rho-associated coiled-coil forming protein kinase (ROCK) pathway also mediates estrogen-regulated epithelial-mesenchymal transition and proliferation in endometriosis, suggesting the importance of this pathway in reproductive system diseases (Huang et al. 2020). Overall, the mechanism of salidroside in treating POI may involve multiple aspects such as cellular responses, cell proliferation, and signal transduction. However, these still need to be confirmed by more research data.

However, the use of network pharmacology to study drug molecular targets for disease treatment is subject to certain limitations. On the one hand, network pharmacology is predictive in nature. It predicts action targets and pathways through databases and algorithms. Although it can provide a comprehensive theoretical basis, the prediction results may deviate from the actual situation. On the other hand, the mechanism of salidroside in treating POI proposed in this study lacks experimental verification. It is only based on network analysis and molecular docking and has not been verified in an actual biological system. In response to these limitations, future research can be carried out in the following aspects. First, conduct *in vitro* experiments. Use cell models such as ovarian granulosa cells and theca cells to verify the effects of salidroside on the screened key genes and related pathways, and observe changes in biological behaviors such as cell proliferation, apoptosis, and hormone secretion. Second, carry out *in vivo* experiments. Establish an animal model of POI, intervene with salidroside, and further verify its therapeutic effect and mechanism on POI at the whole animal level. Ultimately, based on the verification of *in vitro* and *in vivo* experiments, clinical trials can be considered to evaluate the safety and effectiveness of salidroside in POI patients, providing a more reliable basis for its clinical application. It should be noted that in the current study, we have not performed a validation of the docking protocol. This is a potential limitation of our work, as the lack of validation may affect the reliability and interpretation of the docking results. Future studies could incorporate validation methods such as cross-docking validation, redocking experiments, or comparison with experimental data to enhance the robustness of the docking protocol. Cross-docking validation, for instance, involves selecting known ligand-protein complexes from the PDB, separating the ligands and proteins, and re-docking the ligands onto their original proteins to assess the accuracy of the protocol based on the comparison of predicted and experimental binding poses and energies.

Conclusion

Based on network pharmacology and molecular docking techniques, this study identified that salidroside may treat POI through multi-target effects, influencing signal transduction, cytoplasm-related functions, protein binding, and cancer-related pathways. The research findings provide a theoretical basis for the mechanism of action of salidroside in treating POI in terms of multi-components, multi-targets, and multi-pathways, and also point out a new direction for subsequent experimental research and clinical applications. However, it must be acknowledged that this study has limitations. The action targets and pathways predicted by network pharmacology need further verification, and the proposed mechanism of salidroside in treating POI lacks *in vitro* and *in vivo* experimental verification. Future research should focus on thorough verification of the mechanism of action and effectiveness of salidroside in treating POI through *in vitro* cell experiments, *in vivo* animal experiments, and clinical trials, thereby promoting the practical application of salidroside in the treatment of POI.

Abbreviations

AKT: Protein kinase B; AMPK: AMP-activated protein kinase; AMH: Anti-Müllerian hormone; ATP: Adenosine triphosphate; CDC42: Cell division cycle 42; DAVID: Database for Annotation, Visualization, and Integrated Discovery; DNA: Deoxyribonucleic acid; E2: Estradiol; ESHRE: European Society for Human Reproduction and Embryology; ERK: Extracellular signal-regulated kinase; ESR1: Estrogen receptor 1; FANCI: Fanconi anemia complementation group I; FOXO3a: Forkhead box O3a; FSH: Follicle-stimulating hormone; GDP: Guanosine diphosphate; GO: Gene Ontology; GTP: Guanosine triphosphate; GTPase: Guanosine triphosphatase; HMGB1: High mobility group box 1; HRAS: Harvey rat sarcoma viral oncogene homolog; HRT: Hormone replacement therapy; IGF1: Insulin-like growth factor 1; IGF2: Insulin-like growth factor 2; IL6: Interleukin 6; KEGG: Kyoto Encyclopedia of Genes and Genomes; KDR: Kinase insert domain receptor; MAPK: Mitogen-activated protein kinase; MCC: Maximal clique centrality; MEK: Mitogen-activated protein kinase kinase; MMP2: Matrix metalloproteinase 2; MMP9: Matrix metalloproteinase 9; MCODE: Molecular complex detection; MNC: Maximum neighborhood component; NF-κB: Nuclear factor kappa B; PAR: Protease-activated

receptor; PDB: Protein data bank; PEX: PEXtin domain; PI: Phosphatidylinositol; PI3K: Phosphoinositide 3-kinase; POI: Premature ovarian insufficiency; POF: Premature ovarian failure; PPI: Protein–protein interaction; PPARG: Peroxisome proliferator-activated receptor gamma; PTEN: Phosphatase and tensin homolog; RHOA: Ras-homologous gene family member A; ROCK: Rho-associated coiled-coil forming protein kinase; ROCK1: Rho-associated coiled-coil forming protein kinase 1; ROS: Reactive oxygen species; SDF: Structure data file; SEA: Similarity ensemble approach; Sirt1: Sirtuin 1; SMILES: Simplified molecular input line entry system; SRC: SRC proto-oncogene, non-receptor tyrosine kinase; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; TCMSP: Traditional Chinese Medicine Systems Pharmacology; TLR4: Toll-like receptor 4.

Author Contributions

Lili Wei and Baoman Ma: Conceptualization, data curation, software, formal analysis, validation, investigation, visualization, methodology, writing—original draft, and writing—review & editing. Dan Wu: Resources, data curation, software, supervision, visualization, methodology, and writing—review & editing. Le Wang: Data curation, software, visualization, methodology, and writing—review & editing. Xueting He: Software, supervision, visualization, methodology, and writing—review & editing. Ying Gong: Visualization, methodology, and writing – review & editing. Chujuan Lin: Conceptualization, resources, supervision, funding acquisition, project administration, and writing—review & editing.

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Informed Consent

The participant has provided informed consent for the submission of the article to the journal.

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