Exploring the mechanism of *Astragalus membranaceus* against uterine fibroids based on network pharmacology

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Abstract

Background: Uterine fibroids (ULs) are the most common benign tumors of the reproductive tract in gynecology and their clinical presentations include menorrhagia, pelvic pressure, dysmenorrhea, and anemia. Surgical resection and the hormonal drug administration are the primary treatment. The plant *Astragalus membranaceus* (astragalus) has a long history of use in traditional Chinese medicine and studies have shown that it has antitumor effects. However, the role and mechanism of astragalus in ULs are not completely clear. The present study aimed to investigate the astragalus mechanism of action against ULs based on network pharmacology approach, in order to provide insights for the development of a safe and effective drug for the ULs treatment.

Methods: The astragalus active ingredients and the potential drug targets were screened by the Traditional Chinese Medicine System Pharmacology Database and Analytical Platform (TCMSP). The gene expression profiles of ULs were obtained from Gene Expression Omnibus (GEO). The intersection of astragalus components target genes and differentially expressed genes between UL and normal patients were obtained using Perl software to provide the astragalus-ULs drug regulatory network. The protein–protein interaction (PPI) network was established using the STRING online database and Cytoscape software, followed by the topological properties analysis of the PPI networks. GO (Gene ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analyses were conducted by R software. The KEGG relational network was constructed using Cytoscape software.

Results: A total of 21 astragalus active ingredients and 406 drug targets were obtained from the TCMSP. Seventeen of these targets overlap with ULs disease targets and were considered potential targets for the ULs treatment by astragalus. The analysis of the regulatory network showed that the astragalus active components with the most targets are quercetin, kaempferol, mangiferin, tetrodotoxin and isorhamnetin. Target genes with the highest Dgree values obtained from the PPI network analysis are estrogen receptor 1 (ESR1), tumor suppressor factor p53 (TP53), neurotrophic tyrosine kinase receptor 1 (NTRK1) and E3 ubiquitin ligase protein (CUL3). GO and KEGG enrichment analyses indicate that these targets are mainly involved in biological processes related to cellular response to reactive oxygen species, oxidative stress and response to lipopolysaccharides. The main signal transduction pathways involved include the IL-17 and TNF signaling pathways, the AGE-RAGE signaling pathway in diabetic complications and proteoglycans in cancer.

Conclusions: The present study demonstrates that the astragalus therapeutic use against ULs have multicomponent and multi-target properties, providing a novel approach to further investigate the astragalus mechanism of action in the treatment of ULs.

1. Introduction

Uterine fibroids (ULs) are benign monoclonal tumors of the smooth muscle layer of the uterus, whose growth is dependent on estrogen and progesterone^[1]. Epidemiological studies show that the ULs

incidence increases with age, with women over 45 years of age having more than 60% risk of developing ULs^[2]. The ULs causes are unknown, but it is currently believed that people of African descent, over 40, with the age of first menarche below 10 years, family history of ULs and obesity are associated with an increased fibroid risk. ULs are the most common reproductive tract tumor in childbearing age women and their most common symptom is abnormal bleeding from the uterus, usually with heavy menstrual flow. Other symptoms include pelvic discomfort, menstrual pain, urinary incontinence, anemia and painful intercourse, which can seriously affect the patient's quality of life.

Approximately 3% to 7% of untreated fibroids in pre-menopausal women disappear within six months to three years and decrease in size after menopause. Treatment for patients with clinically severe symptoms includes medication (hormonal contraceptives, tranexamic acid, NSAIDs, hormones) and surgery. However, the current medications have many side effects and large fibroids increase the hysterectomy risk. Therefore, there is a need for research on more effective and safer drugs to improve the prognosis and survival of ULs patients.

Traditional Chinese Medicine (TCM) has played an increasingly important role in treating disease and improving the quality of life in recent years. *Astragalus membranaceus* (astragalus) is a plant with a long history of medicinal use in China and is widely used clinically to treat various ailments. astragalus is rich mainly in polysaccharides, saponins and flavonoids. Studies have shown that astragalus active ingredients have antitumor effects, and their main mechanisms include immunity regulation, inhibition of tumor cell proliferation and metastasis, tumor cell apoptosis induction and resistance to oxidative stress^[3-4]. However, the astragalus mechanism of action in the ULs treatment is still unclear. As the diseases treatment by TCM has multicomponent, multi-target and multi-channel characteristics, the mechanism of action of many TCM procedures is still uncertain and a new approach is necessary to explain their molecular mechanisms and pharmacological effects.

Network pharmacology has become a systematic approach to analyzing drug targets with the development of big data biomedical research. Network pharmacology targets biological networks and analyzes the molecular links and mechanisms between drugs, targets and diseases within these networks to facilitate the shift from a "one-target, one-drug" model to a "network-target, multicomponent therapy" model^[5-6]. This study aimed to explore the astragalus potential mechanisms on ULs disease through network pharmacology, targeted drug interaction databases and biological analysis methods. The workflow based on a network pharmacology integration strategy used in this work is shown in Fig. 1.

2. Materials And Methods

2.1 Astragalus active ingredients screening and target prediction

The Traditional Chinese Medicine System Pharmacology Database and Analytical Platform (TCMSP; <http://sm.nwsuaf.edu.cn/lsp/tcmsp.php>) was used to screen the astragalus chemical composition. The TCMSP database contains 499 herbs, 29,384 ingredients, 3,311 targets and 837 diseases registered in

the Chinese Pharmacopoeia. The process by which drugs are absorbed, distributed, metabolized, and excreted after entering the body is called the ADME process. TCMSP provides twelve important parameters related to ADME, such as oral bioavailability (OB), drug-like properties (DL), half-life (HL), Caco-2 permeability (Caco-2), and blood-brain barrier (BBB)^[7]. The astragalus active components were screened based on the criteria $OB \geq 30\%$ and $DL \geq 0.18$ and the corresponding values of all components of astragalus target point were obtained. In addition, the terms "*Astragalus membranaceus*", "*Astragalus propinquus*" and "*Astragalus mongholicus*" were used as subject words (mesh word) to manually search for published articles in the PubMed database (<https://www.ncbi.nlm.nih.gov/pubmed>). Articles related to the research of astragalus active ingredients research and their targets were incorporated into this study. The drug active ingredients and targets obtained were collated and the targets corresponding to drug ingredients that did not meet the above criteria are excluded. The ID conversion of drug targets was performed using Perl software to obtain the final gene symbol of the drug targets.

2.2 Screening of ULs-related targets

GSE31699 microarray data was downloaded from the Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo/>) database using the microarray platform GPL6947 (Affymetrix Human Gene Expression Array). The aim of GSE31699 was to compare the changes in the gene expression changes between ULs and normal uterine myometrium patients, in line with the requirements of this study. The ID conversion of downloaded data was performed using Perl software to obtain disease-associated Gene Names (Gene Names). The screening for differentially expressed genes (DEGs) was carried out using the "limma" and "pheatmap" packages of the R software. $P < 0.05$ and $|\log FC| > 1$ were used as the screening criteria to obtain 703 disease-related DEGs, of which 298 were up- and 406 were down-regulated. The selection of the top 20 most significantly up- and down-regulated genes was performed based on heatmaps and volcano plots.

2.3 Construction of drug control networks

The intersection of astragalus components target genes and the identified DEGs was performed using Perl software to obtain the final astragalus-ULs regulatory network. The Cytoscape software (v.3.6.2; <http://www.cytoscape.org/>) was used to build the regulatory network between astragalus active ingredients and ULs-disease related target genes.

2.4 PPI network topology analysis

Two methods were used to construct the protein-protein interaction (PPI) network for the screened genes. In the first method, the disease and drug intersection genes obtained were imported into the STRING database (<https://string-db.org/>), the specie was defined as "*Homo sapiens*" and the astragalus-ULs common target protein interaction network was generated. In the second method, the PPI interaction network was built using the "Bisogenet" package of the Cytoscape software (v.3.6.2), in which the "organism" chosen was "*Homo sapiens*". The PPI protein interaction network was derived from DIP (<https://dip.doe-mbi.ucla.edu/>), BIOGRID (<https://thebiogrid.org/>), HPRD (<http://www.hprd.org/>), INTACT

(<https://www.ebi.ac.uk/intact/>), MINT (<https://mint.bio.uniroma2.it/>) and BIND (<http://www.bindingdb.org/>) databases. The network topology analysis of the PPI networks was performed using the “CytoNCA” package of the Cytoscape software. The $DC > 61$ condition was defined for the first screening of the PPI networks, and then the Betweenness (BC) > 600 condition was defined for the second screening of the networks, thus resulting in 254 proteins.

2.5 GO and KEGG enrichment analyses

The “colorspace”, “stringi”, and “ggplot2s” packages of the R software and the “DOSE”, “clusterProfiler” and “enrichplot” packages of the Bioconductor software were used for Gene Ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analyses ($p < 0.05$), respectively. Cytoscape software (v.3.6.2) was used to construct the KEGG relational network.

3. Results

3.1 Analysis of astragalus active ingredients

Of the 87 astragalus components found in the TCMSP database, 20 were screened using the criteria $OB \geq 30\%$ and $DL \geq 0.18$. In addition, a literature search using the PubMed database revealed that, in addition to the 20 active ingredients selected, astragaloside appeared in 175 studies. Astragaloside is a saponin that previous studies have demonstrated to have excellent antitumor effects^[8]. Therefore, 21 astragalus active ingredients were finally selected for further studies (Table 1). The active ingredients and drug targets obtained were collated, duplicates and invalid targets were removed and, finally, 406 drug targets were identified.

Table 1: Twenty-one active components from the plant *Astragalus membranaceus* (astragalus) studied in the present work.

Mol ID	Molecule name	OB%	DL
MOL000211	Mairin	55.38	0.78
MOL000239	Jaranol	50.83	0.29
MOL000296	hederagenin	36.91	0.75
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
MOL000354	isorhamnetin	49.6	0.31
MOL000371	3,9-di-O-methylnissolin	53.74	0.48
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69
MOL000378	7-O-methylisomucronulatol	74.69	0.3
MOL000379	9,10-dimethoxypterocarpan-3-O- β -D-glucoside	36.74	0.92
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
MOL000387	Bifendate	31.1	0.67
MOL000392	formononetin	69.67	0.21
MOL000398	isoflavanone	109.99	0.3
MOL000417	Calycosin	47.75	0.24
MOL000422	kaempferol	41.88	0.24
MOL000433	FA	68.96	0.71
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26
MOL000439	isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48
MOL000098	quercetin	46.43	0.28
MOL000407	astragaloside	22.5	0.15

Twenty components were selected from the Traditional Chinese Medicine System Pharmacology Database and Analytical Platform (TCMSP) using the criteria oral bioavailability (OB) $\geq$ 30% and drug-like properties (DL) $\geq$ 0.18. In addition to these 20 components, astragaloside was also included in this study due to its excellent antitumor effects previously demonstrated.

3.2 ULs disease target prediction

The disease targets obtained from the GEO database were screened and 703 UL-associated DEGs were obtained, being 298 up- and 406 down-regulated genes ($P < 0.05$, $|\log FC| > 1$). Heat maps and volcano plots were drawn for the top 20 most significantly up- and down-regulated genes (Fig. 2). The black dots represent genes that were not differentially expressed between the UL and normal groups, in green are represented the genes that were up-regulated in the normal group compared to the UL group, and in red are represented the genes that were up-regulated in the UL group compared to the normal group.

The black dots represent the genes that were not differentially expressed between both groups of patients. The green dots represent the up-regulated genes in the normal group compared to the UL group and the red dots represent the up-regulated genes in the UL group compared to the normal group.

3.3 Construction of the astragalus-ULs regulatory network

The 406 astragalus active ingredients and 703 ULs disease-related targets obtained were intersected and the results were used for further analysis by the Cytoscape software, as well as for constructing drug regulatory networks (Fig. 3) and for performing Wayne's plot (Fig. 4). The drug control network obtained was composed of 30 nodes and 38 edges. The results showed that the astragalus active ingredients with the most targets were quercetin, isorhamnetin, hederagenin, kaempferol and formononetin. The data suggest that these five compounds may be the main astragalus components for the ULs treatment.

3.4 PPI Network Topology Analysis

The intersectional target genes obtained from ULs-related genes and astragalus active components were inserted into the STRING database to construct the PPI network (Fig. 5). The node size in the PPI network represents the Dgree value size. The larger and brighter the node, the higher the Dgree value. The edges between the nodes represent the strength of the interaction between the nodes. According to the strength of the interaction node, the edges vary from thin to thick and their distribution from dark to light. The PPI network includes 17 nodes, 98 edges, and an average node count of 11.5. After screening using $DC > 61$ and Betweenness (BC) > 600 , 254 proteins were obtained and used to plot the PPI network topological analysis (Fig. 6). The results showed that the proteins with the highest Dgree values were ESR1, TP53, NTRK1, and CUL3.

In the graph, the node size represents the Dgree value and the edge between nodes represents the intensity of interaction between nodes. The graph includes 17 nodes, 98 edges and the average number of nodes is 11.5.

3.5 GO and KEGG enrichment analyses

GO and KEGG enrichment analyses of the astragalus action targets in the ULs treatment were performed and screened using $P < 0.5$ as criteria. GO enrichment results showed (Fig. 7) that these targets are involved in a total of 13 molecular functions (MFs). The top ten MFs are R-SMAD binding, RNA polymerase II transcription factor binding, serine type endopeptidase activity, peptidase activator activity, serine type peptidase activity, serine hydrolase activity, peptidase regulatory activity, RNA polymerase II

activated transcription factor binding, collagen binding and SMAD binding. Twenty biological processes (BP) were identified in which the targets are involved, the top ten being cellular response to reactive oxygen species, oxidative stress, response to lipopolysaccharides, coagulation, hemostasis, response to molecules of bacterial origin, neuroinflammatory response and coagulation regulation. A total of six cellular components are involved in the targets, including the collagen-containing extracellular matrix and the platelet alpha particle. The KEGG enrichment analysis revealed a total of 52 signaling pathways in which the identified targets are involved and the top 20 most significantly enriched entries were selected to be plotted as bubble plots (Fig. 8). These pathways include the interleukin (IL)-17 and tumor necrosis factor (TNF) signaling pathways, the AGE-RAGE signaling pathway in diabetic complications and proteoglycans in cancer. The Cytoscape (v.3.6.2) software was used to construct the KEGG relational network (Fig. 9).

4. Discussion

Uterine fibroids (ULs) are the most common benign tumors of the reproductive tract in gynecology. The incidence of ULs has been increasing in recent years and hormonal drugs have serious side effects. Therefore, it is increasingly important to find a safe and effective antitumor medication for the ULs treatment. The *Astragalus membranaceus* (astragalus) plant has a long history of use in Chinese medicine. Recent studies have proven that astragalus has a higher safety over traditional anti-cancer drugs and less side effects.

We found that the astragalus active ingredients with the most targets in the ULs are quercetin, isorhamnetin, hederagenin, kaempferol and formononetin based on the drug regulatory network. They can be the main astragalus components for the ULs treatment. Quercetin belongs to the group of flavonoid compounds that have a variety of effects, such as anti-inflammatory and anti-tumor properties^[9]. Cancer cells may be more sensitive to reactive oxygen species (ROS) accumulation compared to normal cells and the gradual increase in ROS production is essential to the occurrence and development of tumors. Quercetin induces apoptosis in tumor cells by scavenging ROS to exert antioxidant effects^[10]. Studies have shown that quercetin can effectively attenuate the expression of TNF- α -induced inflammatory genes (such as IL-6, IL-1 β , IL-8 and monocyte chemoattractant protein 1 (MCP-1)), which are closely related to the tumor cell cycle regulation and neovascularization^[11]. Kaempferol is a common flavonoid compound that has been reported to treat a variety of gynecological cancers, including breast, cervical, and ovarian cancer^[12]. Kaempferol and its glycosylated derivatives have been shown to have anti-inflammatory, antioxidant and antitumor activities^[13]. The kaempferol antitumor mechanisms includes apoptosis induction, G2/M-phase cell cycle blockade promotion, phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT) down-regulation, inhibition of epithelial-mesenchymal transition (EMT)-related markers (N-cadherin, E-cadherin) and matrix metalloproteinase 2 (MMP-2) expression^[14]. Formononetin has been demonstrated to have antitumor properties in vivo and in vitro. Its main antitumor mechanisms include multiple signals via the regulation of the endogenous pathways of Bax, Bcl-2 and caspase-3 proteins, which induce apoptosis by regulating

mediators, such as cyclin A, cyclin B1 and cyclin D1, and causing the cell cycle arrest through signal transduction and transcriptional activator (STAT) activation. Formononetin can also involve the phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT) and mitogen-activated protein kinase (MAPK) signaling pathways to inhibit cell proliferation by regulating the vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) and to inhibit cell invasion by recruiting matrix metalloproteinases (MMP)-2 and MMP-9 proteins^[15]. Although astragaloside was not considered as one of the compounds with the most targets within the drug-disease regulatory network here provided and the TCMSP did not provide corresponding targets for this compound, a literature search using the PubMed database revealed that astragaloside has potent antitumor effects, and therefore it can be also considered a key astragalus compound. The astragaloside antitumor effects may be related to the PI3K/Akt signaling pathway regulation, which inhibits the proliferation and differentiation of tumor cells^[31]. In addition, astragaloside can induce apoptosis by up-regulating caspase-3 expression and inhibiting the Bcl-2 expression. Astragaloside has been shown to exert antitumor effects by down-regulating regulatory T cells (Tregs) and up-regulating cytotoxic T lymphocytes (CTLs)^[32]. Thus, astragaloside interferes with indoleamine 2,3-dioxygenase (IDO) expression and blocks the immune tolerance.

From the regulatory network, we know that many disease targets can be modulated by various compounds, suggesting that ULs treatment by astragalus is multi-targeted and multi-component. From the PPI network topological analysis, we obtained target genes with high Degree scores, namely ESR1 (Degree = 762), NTRK1 (Degree = 460), TP53 (Degree = 335) and CUL3 (Degree = 329), suggesting that these may be core targets in the astragalus treatment network for ULs. ESR1 has long been considered a *de novo* marker in hormone receptor-positive breast cancer, and ESR1 mutations have been shown to be a key mechanism for resistance to endocrine therapy. Many mutations in the ESR1 ligand-binding domain are associated with hormonal resistance and anti-estrogens^[16]. In our study, the growth of ULs was estrogen-dependent, which can be a new mechanism for the ULs treatment by astragalus. NTRK1 was first identified as an oncogene in 1982 by Mariano Barbacid and colleagues in a gene transfer assay. Somatic NTRK mutations have been reported in a variety of tumors, including colon, rectal, lung (large cell neuroendocrine and non-small cell lung cancers) and melanoma^[17]. Although NTRK1 oncogenes can be highly enriched in some tumor types and rarely found in others, they have been used clinically as diagnostic markers in common tumors. Tumor Suppressor Factor P53 (TP53) mutations are prevalent in a variety of tumors. Its primary role is to inhibit tumorigenesis by controlling cell cycle progression, senescence, DNA repair, cell death and metabolism^[18]. TP53 mutations are the most frequent compared to other genes and are also potential tumor and target predictive markers for drug interventions^[19]. Under normal conditions, Kelch-like ECH-associated protein 1 (KEAP1) down-regulates NRF2 expression through CUL3-mediated ubiquitination and proteasomal degradation^[20]. NRF2 is a master transcription factor that regulates antioxidant-related genes, and in the occurrence of genetic changes (e.g., amplification of NRF2, loss of KEAP1 and CUL3), they can lead to different types of cancers^[21-24].

To predict the astragalus mechanism in the ULs, we performed GO and KEGG enrichment analyses. GO analysis showed that the targets identified are involved in biological processes related to cellular

responses to reactive oxygen species, oxidative stress and responses to lipopolysaccharides. Studies have demonstrated that hypoxia is a universal phenomenon in tumors and that hypoxia inducible factor-1 α (HIF-1 α) is a transcription factor that plays an important role in hypoxia. HIF-1 α promotes tumor growth by activating several genes related to tumor biology, including VEGF, PI3K, AKT, NF- κ B and MMP-2^[25]. These data are consistent with the antitumor mechanism of action previously investigated of the major astragalus components. KEGG analysis revealed significant enrichment in the IL-17 and TNF signaling pathways, involving molecular targets, including PTGS2, JUN, FOS, MMP9, IL-6 and CCL2. The ULs development is associated with many cytokines and the tumor necrosis factor (TNF)- α is one of the most important myometrium-associated cytokines^[26]. TNF- α has multiple biological functions, including fever induction, promotion of adhesion molecule expression, phagocytic stimulation, appetite suppression and insulin resistance modulation^[27]. TNF- α can induce multiple intracellular signaling pathways, including apoptosis, cell survival and inflammation pathways, and increased TNF- α serum levels have been identified in ULs patients^[28]. IL-17 is a founding member of a novel inflammatory cytokine family and activates NF- κ B signal transduction and the induction of NF- κ B-dependent cytokines. IL-17 also activates mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK), p38 and JUN N-terminal kinase (JNK) pathways, thereby playing a role in the progression of several tumor types^[29]. Recent studies have shown that changes in Th1/Th2 equilibrium can lead to tumor initiation and development. When the equilibrium drifts towards Th2, the immune escape occurs which leads to tumor development. Th1/Th2 homeostasis is regulated by the cytokines secreted by Th cells, and Th1 cells mainly secrete IFN- γ and TNF- α ^[30]. Our previous studies have demonstrated that ULs are associated with cytokine-mediated drift of the Th1/Th2 balance towards the Th2 state and consequent immune escape. The results of the present study indicate that the targets for the ULs treatment by astragalus are mainly involved in TNF- α and IL-17 signaling pathways, providing evidence of the astragalus mechanism of action involved in the ULs treatment.

5. Conclusion

The biological functions of astragalus active components and their ULs disease-related targets were analyzed by network pharmacology. The data revealed the molecular biological mechanism underlying the treatment of ULs by astragalus and provided a theoretical basis for the development of a safe and effective new drug for the ULs clinical treatment.

Abbreviations

ULs
Uterine fibroids
Astragalus
Astragalus membranaceus
TCMSP
Traditional Chinese Medicine System Pharmacology Database and Analytical Platform

GEO
Gene Expression Omnibus
PPI
The protein–protein interaction
GO
Gene ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
ESR1
Estrogen receptor 1
TP53
Tumor suppressor factor p53
NTRK1
Neurotrophic tyrosine kinase receptor 1
CUL3
E3 ubiquitin ligase protein
TCM
Traditional Chinese Medicine
OB
Oral bioavailability
DL
Drug-like properties
HL
Half-life
Caco-2
Caco-2 permeability
BBB
Blood-brain barrier
MFs
Molecular functions
BP
Biological processes
TNF
Tumor necrosis factor
ROS
Reactive oxygen species
MCP-1
Monocyte chemotactic protein 1
EMT
Epithelial-mesenchymal transition

MMP-2

Matrix metalloproteinase 2

PI3K/AKT

Phosphatidylinositol 3 kinase/protein kinase B

STAT

Signal transduction and transcriptional activator

MAPK

Mitogen-activated protein kinase

VEGF

Vascular endothelial growth factor

FGF2

Fibroblast growth factor 2

Tregs

Regulatory T cells

CTLs

Cytotoxic T lymphocytes

IDO

Indoleamine 2,3-dioxygenase

KEAP1

Kelch-like ECH-associated protein 1

HIF-1 α

Hypoxia inducible factor-1 α

TNF- α

Tumor necrosis factor- α

ERK

Extracellular signal-regulated kinase

JNK

JUN N-terminal kinase

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and material

All available data and material can be accessed.

Competing interests

The authors declare that they have no conflicts of interest

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Authors' Contributions

QTT and LDH conceived and designed the study. QTT wrote the paper. LY and GLF provided technical support. RH and YX reviewed the manuscript. All authors read and approved the final manuscript.

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References

- [1] McWilliams MM, Chennathukuzhi VM. Recent Advances in Uterine Fibroid Etiology. *Semin Reprod Med.* 2017;35(2):181-189.
- [2] De La Cruz MS, Buchanan EM. Uterine Fibroids: Diagnosis and Treatment. *Am Fam Physician.* 2017;95(2):100-107.
- [3] Wu Y, Zhang X, Li Z, Yan H, Qin J, Li T. Formononetin inhibits human bladder cancer cell proliferation and invasiveness via regulation of miR-21 and PTEN. *Food Funct.* 2017;8(3):1061-1066.
- [4] Zajkowska M, Lubowicka E, Malinowski P, Szmitkowski M, Ławicki S. Plasma levels of VEGF-A, VEGF B, and VEGFR-1 and applicability of these parameters as tumor markers in diagnosis of breast cancer. *Acta Biochim Pol.* 2018;65(4):621-628.
- [5] Yuan H, Ma Q, Cui H, et al. How Can Synergism of Traditional Medicines Benefit from Network Pharmacology?. *Molecules.* 2017;22(7):1135. Published 2017 Jul 7.
- [6] Zhang RZ, Yu SJ, Bai H, Ning K. TCM-Mesh: The database and analytical system for network pharmacology analysis for TCM preparations. *Scientific Reports.* 2017 Jun;7(1):2821.
- [7] Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform.* 2014;6:13. Published 2014 Apr 16.

- [8] Li L, Hou X, Xu R, Liu C, Tu M. Research review on the pharmacological effects of astragaloside IV. *Fundam Clin Pharmacol*. 2017;31(1):17-36.
- [9] Khan F, Niaz K, Maqbool F, et al. Molecular Targets Underlying the Anticancer Effects of Quercetin: An Update. *Nutrients*. 2016;8(9):529. Published 2016 Aug 29.
- [10] Galadari S, Rahman A, Pallichankandy S, Thayyullathil F. Reactive oxygen species and cancer paradox: To promote or to suppress?. *Free Radic Biol Med*. 2017;104:144-164.
- [11] Kashyap D, Mittal S, Sak K, Singhal P, Tuli HS. Molecular mechanisms of action of quercetin in cancer: recent advances. *Tumour Biol*. 2016;37(10):12927-12939.
- [12] Wang X, Yang Y, An Y, Fang G. The mechanism of anticancer action and potential clinical use of kaempferol in the treatment of breast cancer. *Biomed Pharmacother*. 2019;117:109086.
- [13] Imran M, Salehi B, Sharifi-Rad J, et al. Kaempferol: A Key Emphasis to Its Anticancer Potential. *Molecules*. 2019;24(12):2277. Published 2019 Jun 19.
- [14] Imran M., Rauf A., Shah Z.A., Saeed F., Imran A., Arshad M.U., Ahmad B., Bawazeer S., Atif M., Peters D.G., et al. Chemo-preventive and therapeutic effect of the dietary flavonoid kaempferol: A comprehensive review. *Phytother. Res*. 2019;33:263–275.
- [15] Tay KC, Tan LT, Chan CK, et al. Formononetin: A Review of Its Anticancer Potentials and Mechanisms. *Front Pharmacol*. 2019;10:820. Published 2019 Jul 26.
- [16] Carausu M, Bidard FC, Callens C, et al. ESR1 mutations: a new biomarker in breast cancer. *Expert Rev Mol Diagn*. 2019;19(7):599-611.
- [17] Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol*. 2018;15(12):731-747.
- [18] Kruiswijk F, Labuschagne C.F., Vousden K.H. p53 in survival, death and metabolic health: A lifeguard with a licence to kill. *Nat. Rev. Mol. Cell Biol*. 2015;16:393–405.
- [19] Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol*. 2010;2(1):a001008.
- [20] Namani A, Matiur Rahaman M, Chen M, Tang X. Gene-expression signature regulated by the KEAP1-NRF2-CUL3 axis is associated with a poor prognosis in head and neck squamous cell cancer. *BMC Cancer*. 2018;18(1):46. Published 2018 Jan 6.
- [21] Cescon DW, She D, Sakashita S, Zhu CQ, Pintilie M, Shepherd FA, Tsao MS. NRF2 pathway activation and adjuvant chemotherapy benefit in lung Squamous cell carcinoma. *Clin Cancer Res*. 2015;21(11):2499–2505.

- [22] Qian Z, Zhou T, Gurguis CI, Xu X, Wen Q, Lv J, Fang F, Hecker L, Cress AE, Natarajan V, et al. Nuclear factor, erythroid 2-like 2-associated molecular signature predicts lung cancer survival. *Sci Rep*. 2015;5:16889.
- [23] MacLeod AK, Acosta-Jimenez L, Coates PJ, McMahon M, Carey FA, Honda T, Henderson CJ, Wolf CR. Aldo-keto reductases are biomarkers of NRF2 activity and are co-ordinately overexpressed in non-small cell lung cancer. *Br J Cancer*. 2016;115(12):1530–1539.
- [24] Namani A, Cui QQ, Wu Y, Wang H, Wang XJ, Tang X. NRF2-regulated metabolic gene signature as a prognostic biomarker in non-small cell lung cancer. *Oncotarget*. 2017;8(41):69847–69862.
- [25] Chen J, Bai M, Ning C, et al. Gankyrin facilitates follicle-stimulating hormone-driven ovarian cancer cell proliferation through the PI3K/AKT/HIF-1 α /cyclin D1 pathway[J]. *Oncogene*. 2016;35(19):2506-17.
- [26] Islam MS, Protic O, Stortoni P, et al. (2013) Complex networks of multiple factors in the pathogenesis of uterine leiomyoma. *Fertility and Sterility* 100(1): 178–193.
- [27] Nair S, Al-Hendy A. (2011) Adipocytes enhance the proliferation of human leiomyoma cells via TNF- α proinflammatory cytokine. *Reproductive Sciences* 18(12): 1186–1192.
- [28] Ciebia M, Włodarczyk M, Wrzosek M, et al. TNF- α serum levels are elevated in women with clinically symptomatic uterine fibroids [published correction appears in *Int J Immunopathol Pharmacol*. 2018 Jan-Dec;32:2058738418789805]. *Int J Immunopathol Pharmacol*. 2018;32:2058738418779461.
- [29] Amatya N, Garg AV, Gaffen SL. IL-17 Signaling: The Yin and the Yang. *Trends Immunol*. 2017;38(5):310-322. doi:10.1016/j.it.2017.01.006
- [30] Wang Y, Zhou J, Fu S, Wang C, Zhou B. A Study of Association Between Oral Lichen Planus and Immune Balance of Th1/Th2 Cells. *Inflammation*. 2015;38(5):1874-1879.

Figures

Figure 1

The entire workflow based on a network pharmacology integration strategy.

Volcano

Figure 2

Volcano diagram of differentially expressed genes between uterine fibroids (ULs) and normal uterine myometrium patients.

Figure 3

Astragalus active ingredients-uterine fibroids (ULs) disease-related targets regulatory network. The red node indicates the potential active component from astragalus and the yellow node indicates its corresponding target.

Figure 4

Target gene matching ULs-related gens and astragalus (Huang Qi) active components, with a total of 17 targets.

Figure 5

Diagram of protein-protein interaction (PPI) network of the astragalus pharmacological targets in the uterine fibroids (ULs) treatment.

Figure 6

Topological analysis of protein-protein interaction (PPI) network of the astragalus pharmacological targets in the uterine fibroids (ULs) treatment.

Figure 7

Gene Ontology (GO) enrichment analysis of the astragalus pharmacological targets in the uterine fibroids (ULs) treatment.

Figure 8

KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis of the astragalus pharmacological targets in the uterine fibroids (ULs) treatment.

Figure 9

KEGG (Kyoto Encyclopedia of Genes and Genomes) relationship network of the astragalus pharmacological targets in the uterine fibroids (ULs) treatment.

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