Original Article

Mechanisms of the Jian Pi Tiao Gan Yin in the treatment of simple obesity revealed by network pharmacology

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Background: This study sought to examine the mechanism of the Jian Pi Tiao Gan Yin in the treatment of obesity by network pharmacology.

Methods: The active components and corresponding targets of the Jian Pi Tiao Gan Yin were identified using the traditional Chinese medicine systems pharmacology database and analysis platform, and the obesity-related targets were acquired from the Online Mendelian Inheritance in Man database. The drug and disease targets were also identified. Cytoscape software was used to construct the "active component target" network diagram. The protein-protein interaction network was drawn using the Search Tool for the Retrieval of Interacting Genes/Proteins platform, and the Cytoscape MCODE plugin was used to find clusters for the protein cluster analysis. The gene annotation and analysis were performed with the Metascape database via functional databases, such as the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), and Autodock and PyMOL were used for the molecular docking.

Results: The GO analysis identified 244 target genes of the Jian Pi Tiao Gan Yin, 1,378 targets of obesity, and 123 targets of drug and disease. Additionally, 208 biological process items, 38 molecular function items, and 33 cell component items were also identified. The KEGG pathway analysis identified the hypoxia-inducible factor, forkhead box O, cyclic adenosine monophosphate, and vascular endothelial growth factor signaling pathways. The results of the molecular docking showed that the main active components of the Jian Pi Tiao Gan Yin in the treatment of obesity were quercetin, kaempferol, stigmasterol, luteolin, isorhamnetin, β-sitosterol, sapogenin, tanshinone, and formononetin, all of which have been proven to bind to core obesity-related proteins, such as AKT1, interleukin-6 (*IL-6*), vascular endothelial growth factor A (*VEGFA*), tumor necrosis factor (*TNF*), tumor protein 53 (*TP53*), prostaglandin-endoperoxide synthase 2 (*PTGS2*), caspase-3 (*CASP3*), mitogen-activated protein kinase 1 (*MAPK1*), JUN, and epidermal growth factor (*EGF*). Thus, our study revealed the potential mechanism of the Jian Pi Tiao Gan Yin as a multi-component, multi-target, and multi-channel treatment for obesity. These findings lay the foundation for further studies on the mechanism of the Jian Pi Tiao Gan Yin in obesity treatment.

Conclusions: The Jian Pi Tiao Gan Yin can be used as a multi-component, multi-target, and multi-channel treatment for obesity.

Keywords: Jian Pi Tiao Gan Yin; obesity; network pharmacology; molecular docking;

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Introduction

A variety of mis-regulated factors and processes in the body are involved in obesity, and lead to fat accumulation, and abnormal weight gain (1). Obesity may also be accompanied by dizziness, physical fatigue, mental fatigue, lazy speech, difficult movement, shortness of breath, and other clinical symptoms. According to the World Health Organization (WHO), in 2016, 39% of the world's adult population was overweight, while 13% was obese (2). Obesity can cause a variety of diseases, such as dyslipidemia, hypertension, diabetes, and coronary heart disease, which seriously endanger human health. Additionally, obesity is related to adipose tissue, which is the long-term energy storage reservoir and a key regulator of energy balance, satiety, glucose metabolism, and many other physiological processes. White adipose tissue stores and releases fatty acids and glycerol in response to the body's energy needs, while brown adipose tissue generates heat to maintain a constant temperature during cold exposure.

Traditional Chinese medicine (TCM) believes that obesity is closely related to the liver and spleen. The spleen is the hub for the rise and fall of Qi. After the food and water valley is decomposed through the stomach, its fine components are transmitted through the transformation of the spleen, up to the heart and lung, down to the bladder, nourish the five internal organs and spread the limbs of the whole body, so as to maintain the normal metabolic activities of the human body. If the function of the spleen and stomach is strong, eat more than the normal needs of the human body. The essence of water and grain is more than enough and turns into phlegm turbidity, ointment and fat, which stagnates in the body and becomes fat. If the key movement of spleen deficiency is lost, it can be either spleen deficiency of vegetarian body, so it is easy to produce phlegm and dampness, and can "eat less and be fat": it can also be that the spleen and stomach will be damaged by improper diet after the day, which will also cause spleen deficiency constitution over time, leading to spleen dyskinesia, cycle and phlegm turbidity. The liver governs catharsis. If the liver Qi is depressed, it will lead to emotional disorder, injury by seven emotions, involving the five internal organs, qi stagnation and Tianjin stagnation. The accumulation of phlegm dampness and water cannot disperse, the blood stasis stops, and the viscera are distributed on the skin, causing obesity. It can be seen from the above that spleen deficiency and liver depression are the fundamental pathological basis of simple obesity, and the treatment needs to strengthen the spleen and regulate the liver.

Jian Pi Tiao Gan Yin is composed of raw Astragalus, bupleurum, Poria cocos, white peony, Yimi, Salvia miltiorrhiza, Perrin, cassia seed, Alisma orientalis, raw rhubarb and hawthorn. All drugs are used together to make the spleen healthy, the liver Qi sparse, the water wet, the phlegm and blood stasis open, so as to treat obesity. Network pharmacology is a comprehensive and holistic discipline. It organically combines drugs with the body, so that we can find the therapeutic targets of drugs more accurately.

Research has been conducted on a number of TCM compounds in the development of anti-obesity drugs in recent years because of their minimal side effects, and multi-component and multi-target characteristics. Previous studies have shown that the Jian Pi Tiao Gan Yin has an ideal curative effect in the treatment of obesity (3,4). To further understand the action mechanism of the Jian Pi Tiao Gan Yin, we used the popular research approach of network pharmacology to predict the active components, therapeutic targets, and signal pathways of the Jian Pi Tiao Gan Yin. We also used molecular docking technology to verify the docking of the key effective components and targets to provide the scientific basis to further elucidate its active components, mechanism of action, and clinical efficacy.

Methods

Collection of potentially active components from the Jian Pi Tiao Gan Yin and target prediction

The TCM system pharmacology database and analysis platform (TCMSP; https://old.tcmsp-e.com/tcmsp.php) was used (5). The main objective was to identify the effective components of the Jian Pi Tiao Gan Yin, which consists of 30 g of raw Astragalus, 15 g of Chaihu, 15 g of Fuling, 15 g of Baishao, 15 g of Yimi, 15 g of Danshen, 15 g of Peilan, 15 g of cassia seeds, 12 g of Alisma orientalis, 6 g of rhubarb, and 12 g of hawthorn. An oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were used as the screening conditions (6,7). The potential target information of the active ingredients selected from Jian Pi Tiao Gan Yin were obtained from the UniPort (http://www.Uniport.org) database. The UniPort KB protein sequence database was further used to search for and obtain the official gene symbols for all of the protein targets.

Construction of a network of targets of the TCM active ingredients

The main aim was to upload the TCM, active ingredients and related targets of the Jian Pi Tiao Gan Yin using the network image visualization software Cytoscape (v3.7.2) (http://www.cytoscape.org/), and build the TCM active ingredient target network. The important targets of the Jian Pi Tiao Gan Yin *in vivo* were identified as those that intersected with the active components of the TCM.

Annotating the obesity-related genes

The GeneCard (https://genealacart.genecards.org/) and the Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/) databases were searched using the keyword "obesity" for obesity-related genes, and after summarizing the results of the 2 database targets, the obesity targets were detected. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Intersection set of the Jian Pi Tiao Gan Yin and the obesity targets

The composition targets of the studied Jian Pi Tiao Gan Yin (see section "Collection of potentially active components from the Jian Pi Tiao Gan Yin and target prediction") and the obesity targets (see section "Annotating the obesity-related genes") were used to make the intersection map of the composition and disease targets using a Venn map production platform. This allowed us to identify the intersection targets and the potential therapeutic targets of the Jian Pi Tiao Gan Yin in the treatment of obesity.

Construction of the protein-protein interaction (PPI) network to screen core genes

The Venn diagram (see section "Annotating the obesity-related genes") was obtained using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/). The PPI network model was constructed by setting the conditions for homo sapiens, and the data were imported into Cytoscape 3.7.2 software. The score of each node was calculated using the CytoNCA, a Cytoscape plugin, and the nodes with higher scores were screened to construct the network to determine the core genes in the PPI network.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses

Using the Metascape platform (https://metascape.org/gp/index.html), the GO enrichment analysis, and KEGG pathway enrichment analysis, the intersection among the targets of obesity and certain drugs were discovered. The GO analysis included the biological process (BP), cellular component (CC), and molecular function (MF) modules for the genes' function annotation. We also conducted a WeChat data analysis, and used an online visualization platform (http://www.bioinformatics.com.cn). The data were visualized to determine the biological functions and the related signaling pathways of the Jian Pi Tiao Gan Yin in the treatment of obesity.

Potential active ingredient—core target molecular docking test and verification

Ten active components with the highest degree in the TCM active component target network diagram were linked to 10 core proteins with the highest degree in the PPI network. The structures of the compounds were identified by the ZINK database (http://zinc.docking.org). The protein structure was obtained from the PDB (Protein Data Bank) database (http://www.rcsb.org). AutoDock software was used for molecular docking, and PyMOL software was used for output optimization.

Table 1 TCM active ingredients and disease targets

No.	TCM	Active ingredient	Disease targets
1	Raw Astragalus	16	426
2	Bupleurum chinense	13	319
3	Poria cocos	6	27
4	Radix Paeoniae Alba	8	115
5	Yimi	6	45
6	Salvia miltiorrhiza	59	855
7	Perrin	7	111
8	Cassia seed	12	107
9	Alisma orientalis	7	9
10	Rhubarb	7	99
11	Hawthorn	6	274

TCM, traditional Chinese medicine.

Results

Screening of the effective components and target prediction of the Jian Pi Tiao Gan Yin

By searching the TCMSP database with an OB ≥30% and DL ≥0.18 as screening conditions, a total of 147 active ingredients were identified, including 16 raw Astragalus, 13 Bupleurum, 6 Poria cocos, 8 Paeonia lactiflora, 6 Yimi, 59 Salvia miltiorrhiza, 7 Pelagic, 12 Cassia seed, 7 Alisma orientalis, 7 raw Rhubarb, and 6 hawthorn active ingredients. After sorting, 2,387 predicted targets were identified by the UniPort database (https://www.uniprot.org/). After standardized annotation and the deletion of repeated targets, 244 targets remained. The results are summarized in *Table 1*.

Network diagram of the active components and the corresponding targets of the Jian Pi Tiao Gan Yin

A network diagram of the active components and corresponding targets of the Jian Pi Tiao Gan Yin was constructed using Cytoscape 3.7.2 software (see *Figure 1*, in which the hexagon represents the active components, the circle represents the drugs, the diamond represents the drug targets, and each edge represents the interaction between the active components and targets).

Obesity-related genes

In the OMIM and GeneCards databases, the applied keyword was "obesity". After all the target genes were combined, the duplicate values were eliminated, and 1,378 disease targets were identified.

An intersection of the Jian Pi Tiao Gan Yin drugs and the disease targets

The analysis identified 244 potential active component targets and 1,378 corresponding disease targets. The drug targets and disease targets were simultaneously introduced into an online mapping tool platform to construct a Venn diagram, and 123 intersection genes were identified (see *Figure 2*).

PPI network for screening core obesity-related genes

A PPI network (see *Figure 3*) was built by importing the common obesity targets into the STRING database, which contains 123 target proteins and 2,489 target interconnection proteins with an average degree of 39.8. The PPI protein interaction network was further visualized using Cytoscape (v3.7.2) software (see *Figure 4*). In the network, the darker the node color and the more lines, the higher the degree of association between the target protein corresponding to the node and the other proteins, and the more important the target protein. Among them, the genes of *AKT1*, interleukin-6 (*IL-6*), vascular endothelial growth factor A (*VEGFA*), tumor necrosis factor (*TNF*), tumor protein 53 (*TP53*), prostaglandin-endoperoxide synthase 2 (*PTGS2*), caspase-3 (*CASP3*), mitogen-activated protein kinase 1 (*MAPK1*), *JUN*, epidermal growth factor (*EGF*), *CXCL8*, mitogen-activated protein kinase 8 (*MAPK8*), matrix metalloprotein 9 (*MMP9*), signal transducer and activator of transcription 3 (*STAT3*), interleukin 1 beta (*IL1B*), Fos proto-oncogene (*FOS*), epidermal growth factor receptor (*EGFR*), *MYC*, and chemokine (C-C motif) ligand 2 (*CCL2*) ranked in the top 20 degrees of freedom, with degree values of 94, 92, 88, 86, 85, 79, 79, 78, 77, 76, 75, 74, 72, 72, 71, 71, 71, 71, 67, and 66, respectively, and 123 targets were identified from the intersection of drugs and

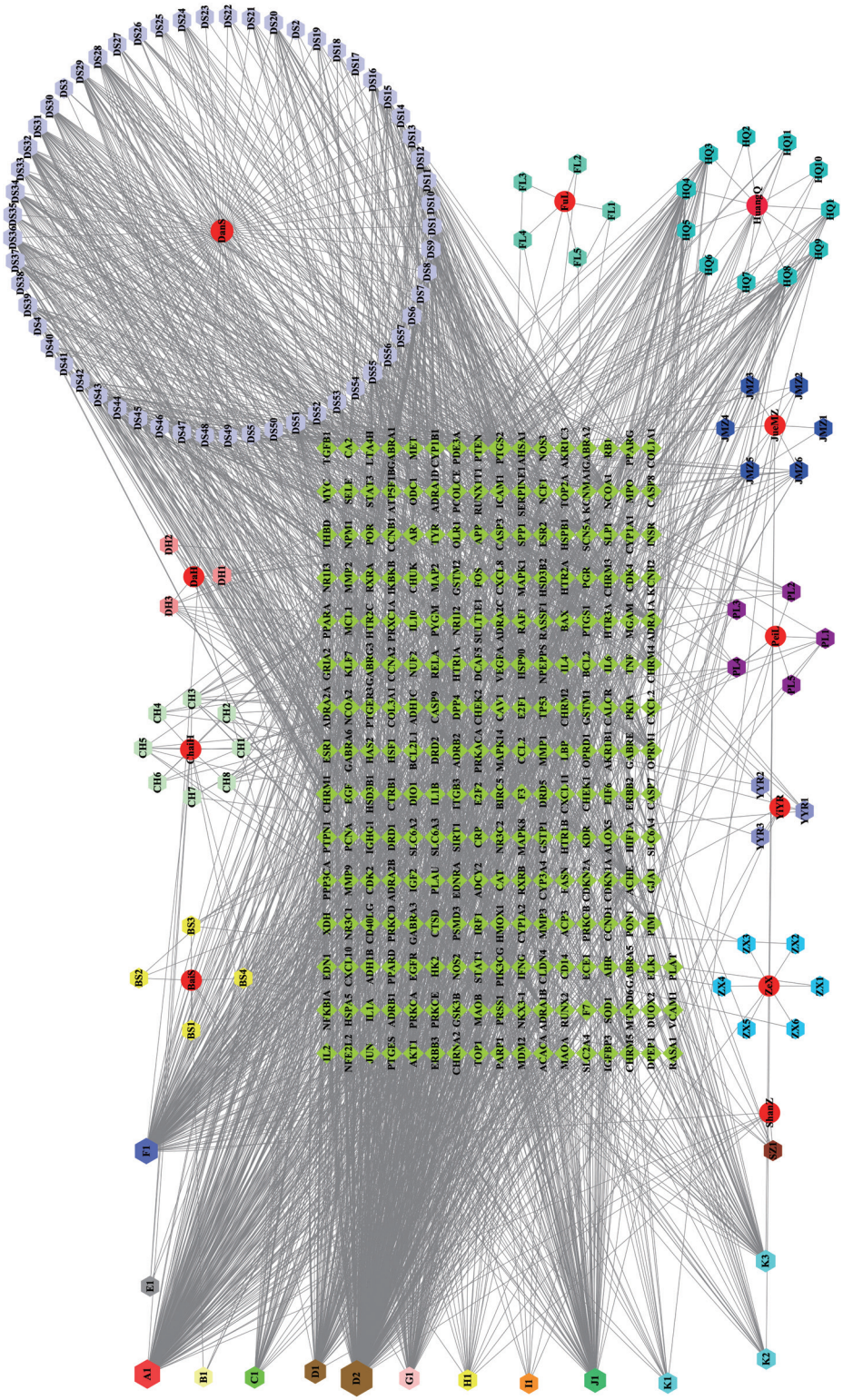

Figure 1 Network of traditional Chinese medicine compound targets of the Jian Pi Tiao Gan Yin.

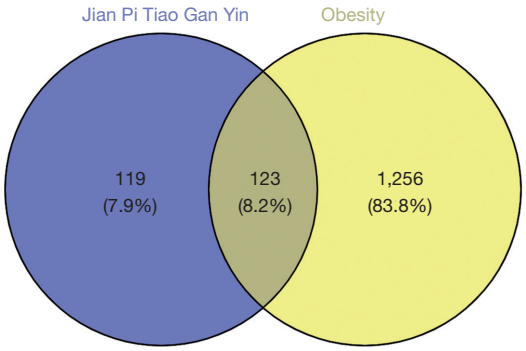

Figure 2 Intersection diagram of the active ingredients of the drugs and disease targets.

diseases. We found that the higher the degree of ganyin, the greater its importance. Ganyin may be the core target of the Jian Pi Tiao Gan Yin in the treatment of obesity.

GO and KEGG pathway enrichment analyses

In total, 2,333 BPs, 96 CCs, and 172 MFs in the 123 potential targets were obtained using the Metascape platform (P<0.01). The results showed that the biological mechanisms of action of the Jian Pi Tiao Gan Yin in the treatment of obesity were mainly related to the reaction of cells to inorganic and organic cyclic compounds, and

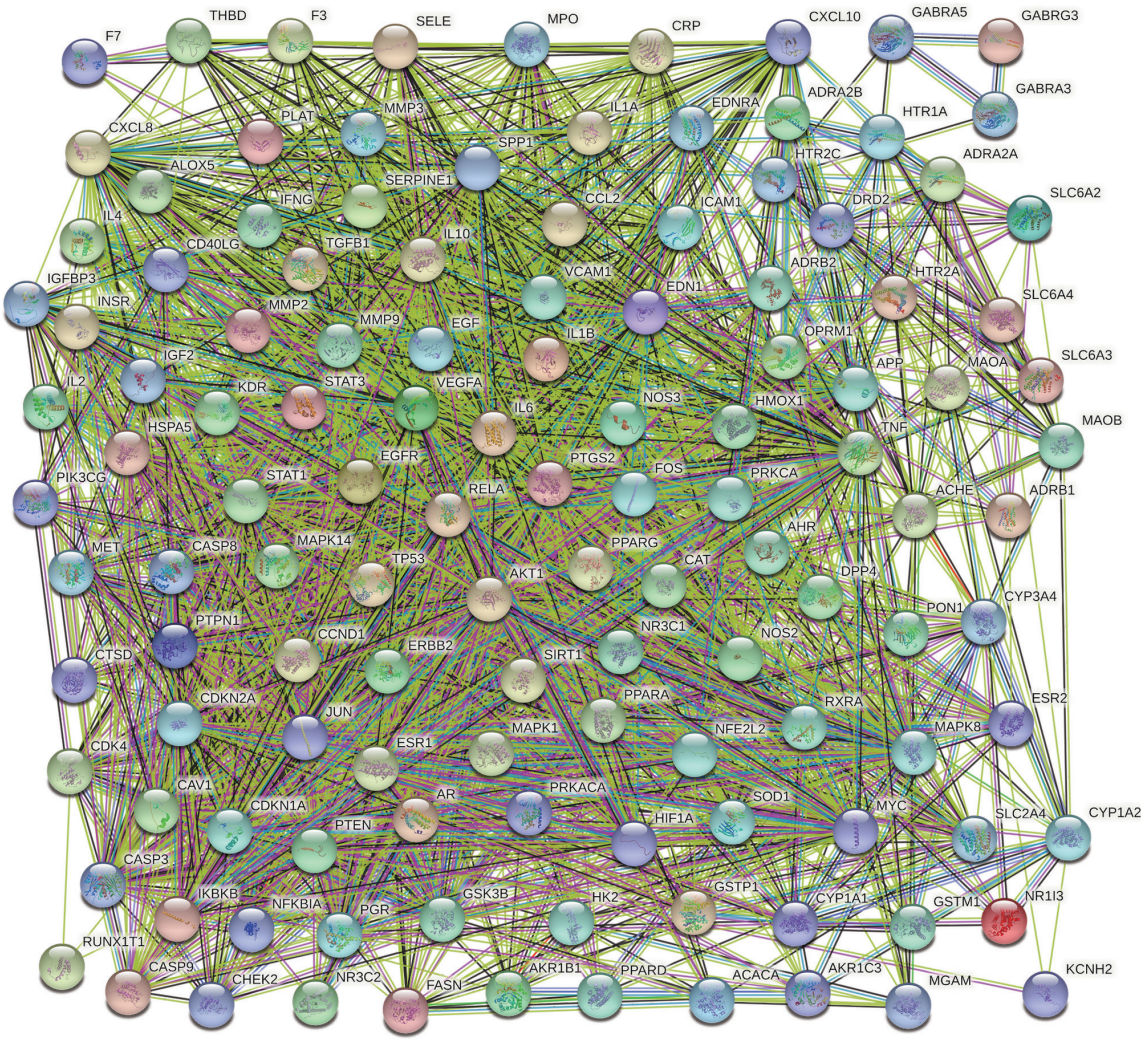

Figure 3 The protein-protein interaction network of the Jian Pi Tiao Gan Yin in obesity.

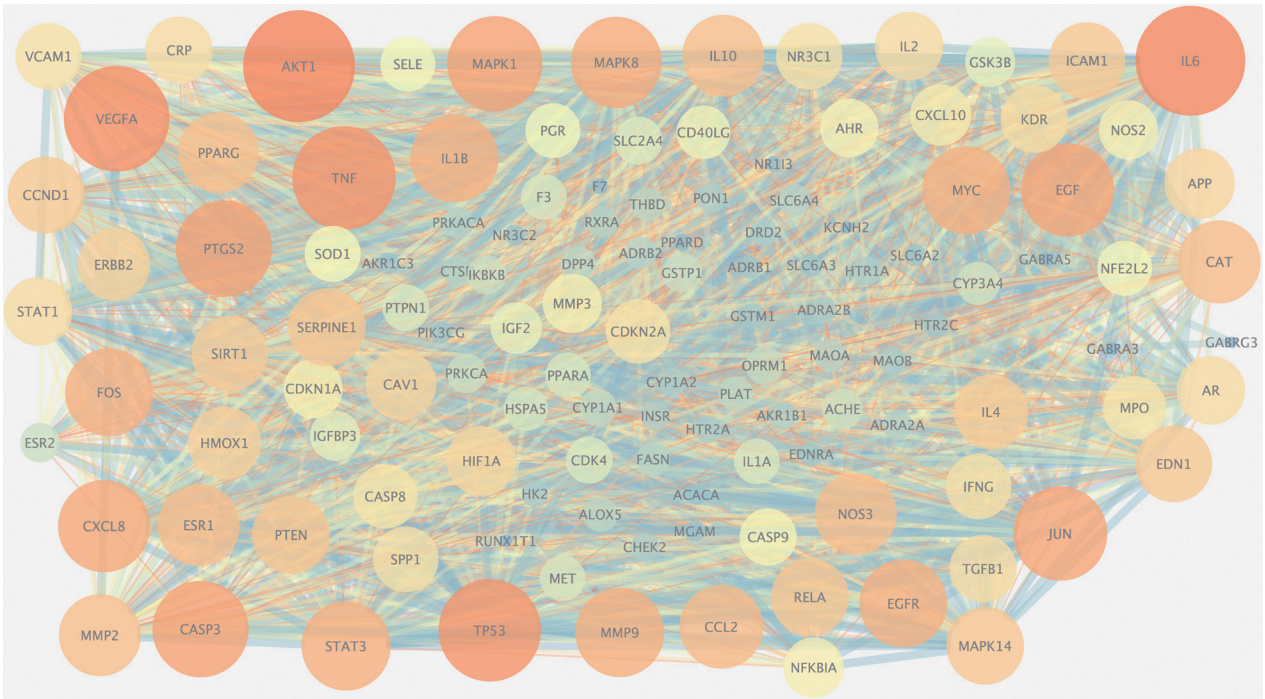

Figure 4 The protein-protein interaction network of the common targets of the Jian Pi Tiao Gan Yin and obesity.

lipopolysaccharides. The metabolic processes of reactive oxygen species and cellular responses to oxidative stress were also involved. Cellular migration and responses to steroid hormones were also found to be involved in the mechanism of action of the Jian Pi Tiao Gan Yin. Cell components, such as membrane rafts, vesicle cavities, cytoplasmic perinuclear regions, and receptor complexes, were also involved. The MFs mainly involved in the action of the Jian Pi Tiao Gan Yin were related to the nuclear and cytokine receptors. Further, transcription factors, protein kinases, phosphatases, and protein domain specific binding were proven to be involved in the molecular mechanism of the Jian Pi Tiao Gan Yin activity. The KEGG analysis (P<0.01) revealed a total of 345 signaling pathways, which were closely related to the hypoxia-inducible factor (HIF)-1, forkhead box O (*FOXO*), cyclic adenosine monophosphate (*cAMP*), and vascular endothelial growth factor (*VEGF*) signaling pathways. The results are shown in *Figures 5*,*6*.

Docking results of the potential active ingredients and the core target molecules of the Jian Pi Tiao Gan Yin mechanism of action

Quercetin, kaempferol, stigmasterol, luteolin, isorhamnetin, beta-sitosterol, aloe-emodin, hederagenin, and tanshinone IIA and formononetin. were identified as the top 10 active compounds in degree. These major active components, were linked with the following genes: *AKT1*, *IL-6*, *VEGFA*, *TNF*, *TP53*, *PTGS2*, *CASP3*, *MAPK1*, *JUN*, and *EGF*. It is generally believed that a docking score <–4.25 kcal/mol indicates some binding activity, whereas a value <–5.0 kcal/mol indicates good binding activity, and a value <–7.0 kcal/mol indicates strong binding acclivity (8). The docking results obtained during the present study are set out in *Table 2*. The top 3 compounds were selected for the demonstration of the molecular docking analysis, and the results are shown in *Figure 7*.

Discussion

The Jian Pi Tiao Gan Yin was prepared by Professor Yunshen Xu according to the characteristics of obesity. It was intended to strengthen the spleen and replenish the qi, soothe the liver, relieve depression, remove turbid pathogens, and have a significant effect in the treatment of obesity. However, to date, there has been no systematic study on its material basis, targets, and mechanisms of action. In this study, we used different databases, such as

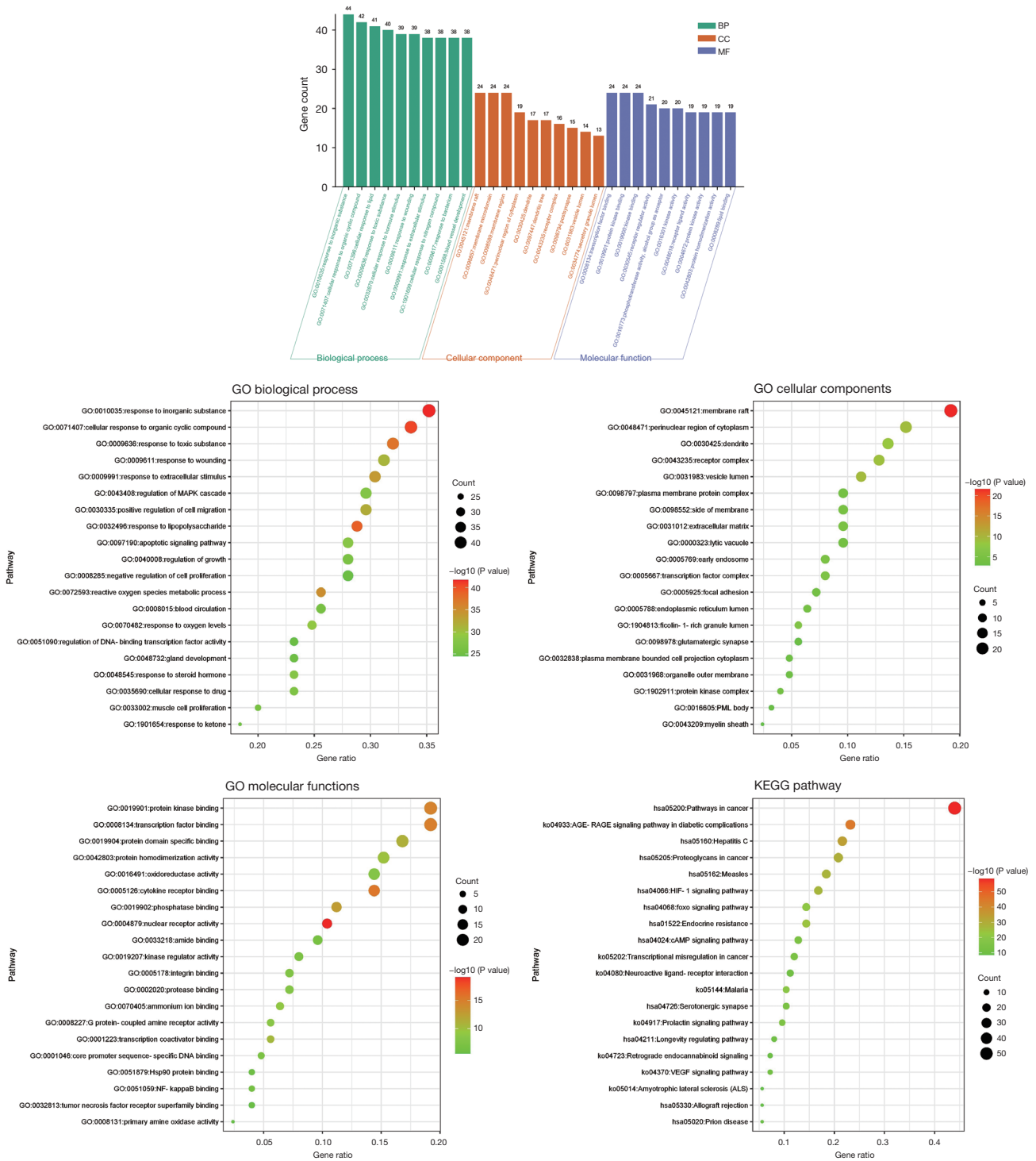

Figure 5 Enrichment analyses of the potential targets of the Jian Pi Tiao Gan Yin in the treatment of obesity. BP, biological process; CC, cellular component; MF, molecular function; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

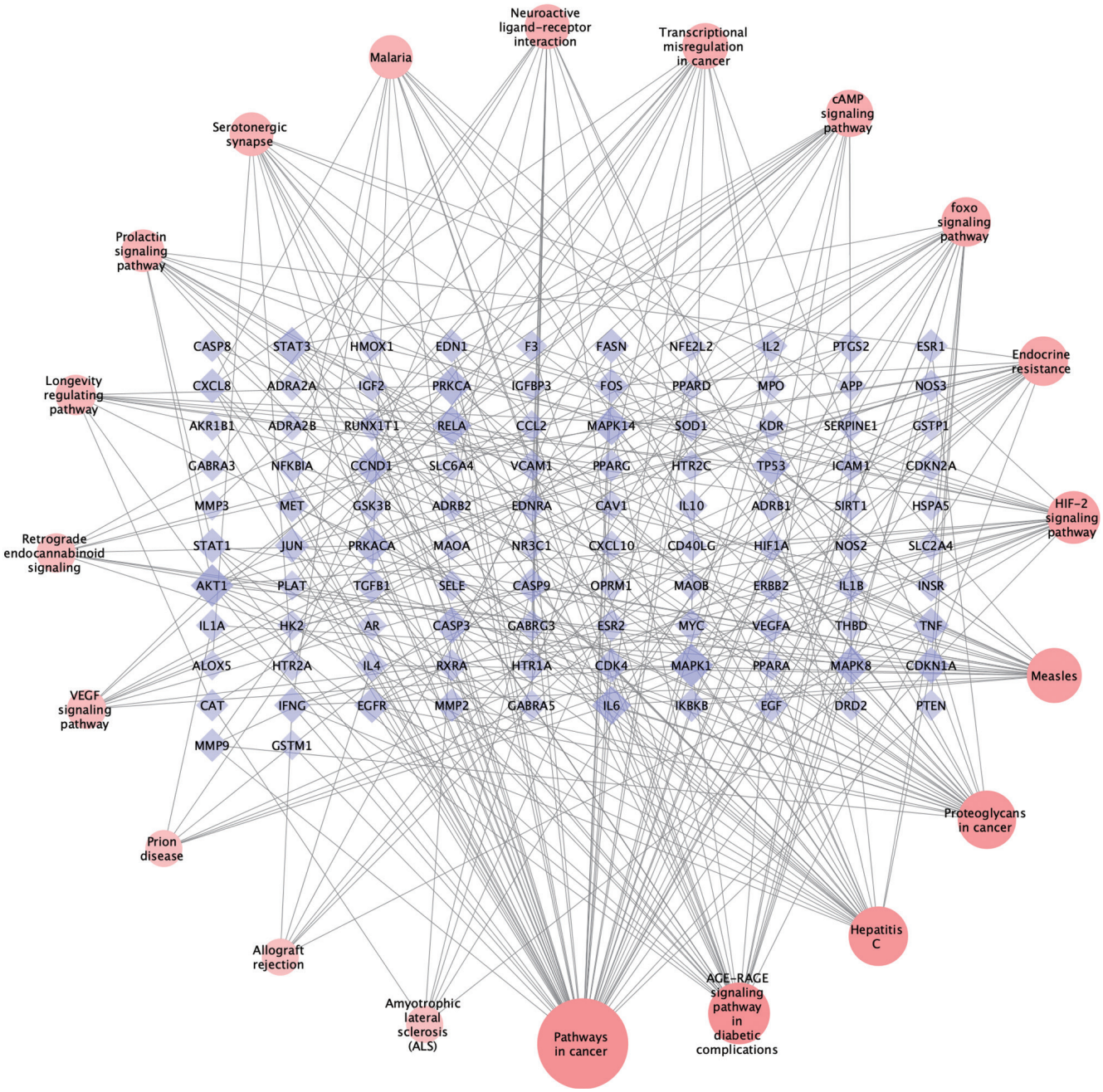

Figure 6 Network of target-pathways.

TCMSP, GeneCards, OMIM, STRING, and Metascape, to analyze the active ingredients and mechanisms of action of the Jian Pi Tiao Gan Yin in the treatment of obesity.

According to TCM, the active ingredient target network diagram showed that quercetin, kaempferol, stigmasterol, luteolin, isorhamnetin, β-sitosterol, aloe-emodin, sapogenin, tanshinone, and formononetin were the most potent active components of the Jian Pi Tiao Gan Yin. Quercetin has a variety of physiological activities (9); for example, it has antioxidant (10), lipid-lowering, weight-reducing, anti-tumor (11), and anti-inflammatory effects. Study (12) has shown that hypertrophic and proliferative adipocytes can

Table 2 Molecular docking results of the active drug ingredients and core targets

Compound	Binding energy (kcal/mol)									
	AKT1	IL-6	VEGFA	TNF	TP53	CASP3	PTGS2	MAPK1	JUN	EGF
Quercetin	–7.34	–5.60	–5.88	–7.82	–5.87	–6.21	–6.14	–5.27	–7.16	5.48
Kaempferol	–7.63	–5.61	–5.88	–7.23	–6.14	–5.99	–7.00	–4.45	–6.89	5.71
Stigmasterol	–8.76	–6.61	–8.02	–9.37	–8.39	–8.59	–10.35	–8.43	–7.92	–8.62
Luteolin	–7.70	–5.70	–6.11	–7.70	–7.06	–6.20	–7.74	–5.83	–7.62	–5.67
Isorhamnetin	–7.66	–4.90	–6.25	–6.87	–6.13	–6.33	–6.68	–5.05	–5.06	–5.54
Beta-sitosterol	–8.88	–6.23	–7.63	–9.60	–8.48	–8.76	–10.63	–8.46	–8.29	–7.91
Aloe-emodin	–6.02	–3.76	–4.70	–6.78	–4.79	–4.71	–5.89	–4.51	–5.34	–5.00
Hederagenin	–8.82	–6.78	–7.37	–10.80	–8.79	–9.32	–8.95	–8.10	–7.97	–7.87
Tanshinone IIA	–7.42	–7.21	–7.38	–7.39	–6.72	–6.53	–8.61	–6.64	–7.70	–7.11
Formononetin	–7.27	–7.21	–7.39	–7.57	–8.30	–6.63	8.46	–6.64	–7.55	–7.11

IL-6, interleukin-6; VEGFA, vascular endothelial growth factor A; TNF, tumor necrosis factor; CASP3, caspase-3; PTGS2, prostaglandin-endoperoxide synthase 2; MAPK1, mitogen-activated protein kinase 1; EGF, epidermal growth factor; TP53, tumor protein 53.

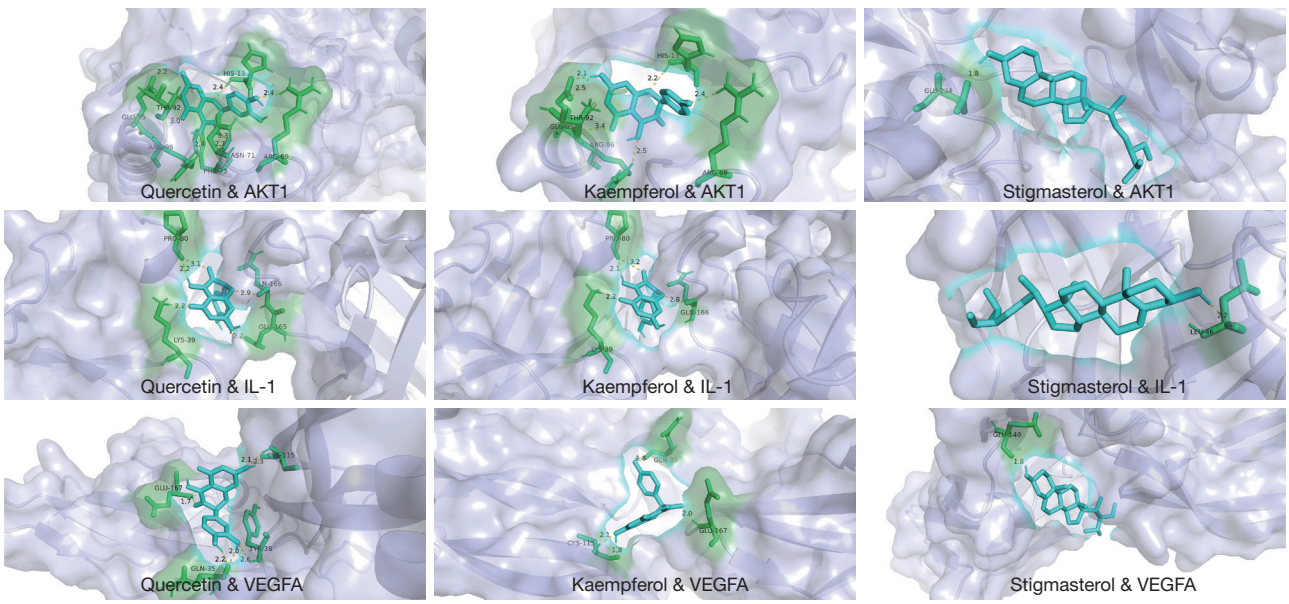

Figure 7 Molecular docking model diagram of the key pharmacodynamic substances-core targets. IL-1, interleukin-1; VEGFA, vascular endothelial growth factor A.

secrete pro-inflammatory factors, such as IL-6 and TNF-α, which can cause a variety of diseases. Forney *et al.* (13) found that the levels of IL-6 in the white fat of mice after the oral administration of quercetin was significantly lower than those of high-fat mice. Dandona *et al.* (14) found that quercetin significantly reduced the secretion of TNF-α in rats, and quercetin significantly upregulated the expression of acetyl coenzyme A oxidase and other genes related to fatty acid β-oxidation and ketogenesis, reduced the content of free fatty acid in serum, and promoted fat catabolism (15). Quercetin also inhibits adipogenesis by reducing the gene expression levels of key adipogenic factors, such as

peroxisome proliferator-activated receptor gamma (PPAR-γ) and CCAAT/enhancer-binding protein α (C/EBPα) (16).

Lin *et al.* (17) showed that luteolin reduced the fat storage of Caenorhabditis elegans by promoting the central serotonin signal. Lee *et al.* (18) found that kaempferol induced cell cycle arrest in the S phase by inactivating the mammalian target of rapamycin (mTOR)/P70S6K/Akt axis, thereby inhibiting adipogenesis accompanied by the downregulation of C/EBPα and PPAR-γ expression. Isorhamnetin has been shown to improve the symptoms of metabolic syndrome through a variety of mechanisms; for example, it has been shown to have an anti-inflammatory effect on obesity, and inhibit the adipogenesis of adipocytes by inhibiting the Wnt/catenin signaling and directly binding to and inducing the transactivation of lxr-s (19). Rodríguez-Rodríguez *et al.* (20) found that isorhamnetin reduced weight gain, and increased the insulin secretion and energy consumption of mice fed a high-fat diet, reduced the accumulation of fat in adipose tissue, and prevented fat cell hypertrophy. Tanshinone is a major diterpene that can be isolated from Salvia miltiorrhiza. Jung *et al.* (21) found that tanshinone prevented obesity induced by a high-fat diet by inhibiting early fat production, and improved glucose metabolism and insulin sensitivity.

The PPI protein interaction analysis showed that the main targets were the proteins coded by *AKT1*, *IL-6*, *VEGFA*, *TNF*, *TP53*, *PTGS2*, *CASP3*, *MAPK1*, *JUN*, *EGF*. Akt, also known as protein kinase B (PKB), is an important downstream effector of phosphatidylinositol 3-kinase (PI3K), which phosphorylates many downstream factors, such as enzymes, kinases, and transcription factors, to regulate cell function (22). AKT1 is involved in glucose metabolism, cell growth, and angiogenesis (23,24). It is a positive regulator of the mTOR pathway, and mediates its activation by insulin. AKT1 is also a key inhibitor of the energy metabolism of 5'-adenosine monophosphate-activated protein kinase (AMPK) (25). The activation of the AKT1/mTOR pathway is related to the inhibition of the liver kinase B1 (LKB1)/AMPK signal. The relationship between AMPK and the mTOR pathway, as regulators of energy balance and targets for the treatment of metabolic disorders, may have important clinical significance.

Shearin *et al.* (26) showed that Akt signaling acts downstream of insulin and insulin-like growth factor 1 (IGF-1) receptors, and is crucial for proper fat expansion and/or maintenance. IL-6 is a cytokine with both pro-inflammatory and anti-inflammatory properties. It was first considered a predictor or pathogenic mediator of insulin resistance and cardiovascular disease (27). It is related to excessive abdominal adipose tissue and can regulate the secretion of other inflammatory factors, such as TNF-α. One of the predictors of disease or pathogenic mediators is related to excessive abdominal adipose tissue. IL-6 and TNF-α are well-known pro-inflammatory cytokines produced in adipose tissue. The serum levels of these 2 cytokines increase proportionally with bodyweight (28). VEGFA is an angiogenic factor, which stimulates the proliferation and migration of endothelial cells. TP53 plays an important role in the regulation of glycolysis, lipolysis, and glycogen synthesis. Molchadsky *et al.* (29) reported that TP53 protects diet-induced obesity by enhancing the oxidation of brown fat. It is speculated that the Jian Pi Tiao Gan Yin treats obesity by affecting the above-related targets.

According to the KEGG pathway analysis, the HIF-1, FOXO, endocrine resistance, cAMP, and VEGF signaling pathways are the main therapeutic pathways involved in the Jian Pi Tiao Gan Yin way of action. The HIF-1 signaling pathway is closely related to fat homeostasis (30), and potentiates the hypomethylation of adipocytes and inflammatory adipocytokines in response to hypoxia (31). Ali *et al.* (32) found that the HIF-1 signaling pathway plays a negative role in the thermogenic regulation of adipocytes. Fibroblast growth factor 9 (*FGF9*) induces the upregulation of HIF-1 expression, and the corresponding changes of its downstream molecules at the early stage of adipocyte differentiation. FGF9 inhibits the browning of white adipocytes by activating the HIF-1 signaling pathway. FOXO is involved in many cellular physiological events, such as apoptosis, cell cycle control, glucose metabolism, and antioxidant stress. The cAMP signaling pathway is a kind of cyclic nucleotide system. In this system, the extracellular signals bind to the corresponding receptors and induce responses by regulating the level of the intracellular second messenger, cAMP. cAMP regulates key physiological processes, including metabolism, secretion, calcium homeostasis, muscle contraction, cell fate, and gene transcription. cAMP levels increase in the absence of gnai2. As a result, lipolysis is enhanced and triglycerides stored in adipocytes are reduced, thereby reducing fat accumulation. Additionally, the cAMP signaling pathway is correlated with the regulation of lipid metabolism. VEGF is a major regulator of vascular development and blood and lymphatic function in healthy and diseased adults. Zhao *et al.* (33) found that the local overexpression of VEGFA in adipose tissue induced by doxycycline promoted fat decomposition

and browning. These results indicate that the HIF-1, FOXO, cAMP and VEGF signaling pathways are closely related to the occurrence and development of obesity.

We conducted a molecular docking analysis, and found out that the binding energy of ligands and receptors was good, and the conformations were stable. The results of the molecular docking showed that the active components of the Jian Pi Tiao Gan Yin bind with receptor proteins stably, thus potentially playing a role in obesity treatment.

Our study investigated the mechanisms of action of the Jian Pi Tiao Gan Yin in the treatment of obesity, and further confirmed the process of the multi-target, multi-channel, and collaborative treatment of diseases by Chinese herbal compounds. The Jian Pi Tiao Gan Yin may regulate the HIF-1, FOXO, camp, and VEGF signaling pathways by combining active components (e.g., quercetin, kaempferol, stigmasterol, luteolin, isorhamnetin, β-sitosterol, aloe-emodin, hederagenin, tanshinone, and formononetin) with proteins (e.g., AKT1, IL-6, VEGFA, TNF, TP53, PTGS2, CASP3, MAPK1, JUN, and EGF). This could be a multi-targeted goal for obesity treatment. Jian Pi Tiao Gan Yin has little toxic and side effects and is convenient to take. It has obvious advantages in the treatment of obesity. Additionally, our results pave the road for detailed future research.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-553/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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