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Original Research

Computational systems pharmacology analysis of Tong-Jing-Yi formula in the treatment of dysmenorrhea

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Abstract

Background: Tong-Jing-Yi (TJY) formula consists of Leonurus, fried Toosendan and processed Cyperus, etc. The therapeutic effect of TJY on dysmenorrhea has been clinically validated, but the underlying mechanism remains unclear. The present study aimed to explore the possible molecular targets of TJY and the potential mechanisms. **Methods**: The components of TJY formula were identified by ultra performance liquid chromatography–quadrupole-time of flight/mass spectrometry. SwissTargetPrediction database was used to predict the targets of TJY formula, and targets associated with primary dysmenorrhea were also collected through other databases. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted. **Results**: A total of 91 compounds with identified structures were screened, including 3 groups of isomers. The results predicted 854 TJY formula-related targets and 363 disease-related targets. GO and KEGG analysis showed that the top 5 target genes were *PIK3CA*, *AKT1*, *EGFR*, *AKT2* and *CYP19A1*. PI3K-Akt signaling, chemokine signaling, focal adhesion, and Rap1 signaling were ranked in the top 15 pathways. **Conclusion**: TJY formula might play roles in the treatment of dysmenorrhea underlying mechanisms relating to the involvement of TNF-α, interleukin and PI3K-Akt signaling pathway. Potential pathways have been identified that need mechanistic confirmation in a laboratory setting in the future.

Keywords: Tong-Jing-Yi formula; Dysmenorrhea; Network pharmacology; PI3K-Akt signaling pathway; Component identification; Bioinformatic analysis

1. Introduction

Dysmenorrhea refers to abdominal pain during menstruation that leads to lumbosacral pain, nausea, loss of appetite, headache, restlessness, insomnia, fatigue, diarrhea, and even syncope [1,2]. Dysmenorrhea can be classified into two main categories: primary dysmenorrhea (PD) and acquired dysmenorrhea (AD) [3]. PD is defined as pain during the menstrual cycle in the absence of an identifiable cause [3]. AD refers to dysmenorrhea caused by pelvic organic diseases such as endometriosis, adenomyosis, chronic pelvic inflammation, uterine malformation and so on [4]. The incidence of PD in adolescent women ranges from 16% to 93% [5]. PD is related to an increase of endometrial prostaglandin (PG) and interleukin (IL) during menstruation. Prostaglandins (PGF2-α) stimulates the contraction of uterus and reduces the amount of blood perfusion through myometrial compression of the blood vessels. Then the uterus cramps by lack of oxygen and the body feels abdominal pain [2]. Both PG synthase inhibitors such as nonsteroidal anti-inflammatory drugs [6] and oral contraceptives are prescribed for dysmenorrhea. PG synthetase inhibitors suppress PG production by inhibiting the activity of PG synthetase, which prevents excessive uterine contraction and spasm and consequently alleviates or eliminates dysmenorrhea [3]. Oral contraceptives reduce the PG content in menstrual blood by inhibiting ovulation [7]. Vitamins (V_D, V_E, V_K), calcium, magnesium, olive oil, fennel, dietary fiber, zinc, Omega-3 and Omega-6 Fatty Acids are also used as adjuvant therapy for dysmenorrhea [2,8]. However, these treatments show limited clinical efficacy as well as various side effects, and thus more effective treatments for dysmenorrhea are required.

Traditional Chinese medicine has been widely used for the treatment of dysmenorrhea in China [9,10]. Tong-Jing-Yi (TJY) formula, consisting of Leonurus, fried Toosendan, processed Cyperus, Bergamot, raw Hawthorn, vinegar Corydalis, honey bran roasted green peel, *Artemisia argyi* leaf, raw Radix Paeoniae Alba, *Achyranthes bidentata*, fried mustard seed and processed *Evodia rutaecarpa*, has been applied for the treatment of dysmenorrhea in the Obstetrics and Gynecology Hospital of Fudan University. The prescription uses Artemisia argyi leaf and Cyperus to warm the menstruation, regulating Qi and dispersing cold as the monarch medicine. Evodia rutaecarpa, Leonurus, corydalis, toosendan and fried mustard seed are used to warm the menstruation, disperse the knot, and relieve pain as the minister medicine. Radix Paeoniae Alba is as the adjuvant, entering the liver meridian, nourishing the blood, softening the liver and relieving pain. It can also assist other hot drugs in the prescription to prevent excessive heat and nour-

ish Yin. Hawthorn, bergamot and green peel are as the adjuvant, and assist the minister medicine to soothe the liver, promote Qi and relieve pain. Achyranthes bidentata is used to induce all kinds of drugs to go down. The whole prescription is used together to warm the uterus and disperse cold. It regulates Qi, nourishes blood, and relieves pain. Although the efficacy of TJY formula on dysmenorrhea and menstrual disorders has been clinically verified, both clinical studies and exploration of the pharmacological mechanisms of TJY in dysmenorrhea are limited.

In the present study, a network pharmacology-based analysis of TJY formula was performed to explore the underlying mechanism of TJY formula in the treatment of dysmenorrhea.

2. Methods

2.1 Preparation of TJY formula solution

To prepare TJY, 15 g Leonurus, 12 g fried toosendan, 12 g processed cyperus, 6 g bergamot, 24 g raw hawthorn, 12 g vinegar corydalis, 12 g honey bran roasted green peel, 6 g *Artemisia argyi* leaf, 12 g raw radix paeoniae alba, 12 g *Achyranthes bidentata*, 12 g fried mustard seed, and 6 g processed *Evodia rutaecarpa* were weighed and placed in a dish with 1000 mL deionized water (3 cm above the medicinal material). After soaking for 1 h, medicinal materials were decocted on low fire for 30 min and then filtered with a 1000 mesh gauze while hot. The filtrate was collected and 700 mL of water was added to the residue. Medicinal materials were decocted on low fire for 30 min after the water was boiled and then the samples were filtered with 1000 mesh gauze while hot. The filtrate was collected and mixed with previously collected samples. The filtrate was cooled to room temperature and centrifuged (12,000 rpm, 5 min). The supernatant was filtered using a 0.22 μm membrane. The filtrate was concentrated and lyophilized.

2.2 Component identification

The components of TJY formula were analyzed by ultra performance liquid chromatography-quadrupole-time of flight/mass spectrometry (UPLC-Q-TOF/MS) [11–14]. Briefly, Chromatographic column: Waters ACQUITY UPLC HSS T3 (2.1 $\times$ 100 mm, 1.8 μm); Batch No.: 0200372571; Column temperature: 25 °C ; Injection volume: 1 μL; Detection wavelength: 280 nm. Mobile phase ratio and flow rate: 0.1% formic acid aqueous solution for phase A, 0.1% formic acid acetonitrile for phase B. Mass spectrometric detection was performed in Negative/Positive ion mode.

2.3 Bioinformatic analysis

The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to confirm the molecular structures. The SwissTargetPrediction database (http://www.swisstargetprediction.ch/) was used to predict the target of selected compounds from TJY formula. Targets related to PD were collected through a series of databases by performing keyword search of "dysmenorrhea" and the species was set as "human", including Genecards (https://www.genecards.org/), TTD (http://db.idrblab.net/ttd/), DiGSeE (http://www.disgenet.org/), Drugbank (https://www.drugbank.ca/) and OMIM (https://omim.org/). The PPI network diagram was depicted based on the targets of candidate components of the TJY formula and PD listed in the STRING database (http://string-db.org/). GO and KEGG pathway enrichment analysis of core targets were performed via the DAVID database (FDR $<$0.05).

3. Results

3.1 Identification of the components of the TJY formula

A total of 96 compounds were identified from TJY formula according to the multi-level mass spectrometry information of samples and the database of high-resolution mass spectrometry of natural products. Among the 96 compounds, 5 compounds with unidentified structures were removed. A total of 91 compounds were selected (**Supplementary Table 1**), including 3 group isomers. We used PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to confirm the molecular structures. The results showed that 12 compounds were from *Artemisia argyi* leaf (Monarch drug) and 1 compound was from processed Cyperus (Monarch drug). A total of 13 compounds were detected from Corydalis vinegar (Minister drug), 7 compounds were detected from processed *Evodia rutaecarpa* (Minister drug), 6 compounds were detected from fried Toosendan (Minister drug), 4 compounds were detected from fried Mustard Seed (Minister drug), and 3 compounds were detected from Leonurus (Minister drug). In addition, 9 compounds were detected from raw Radix Paeoniae Alba (Assistant drug), 8 compounds were detected from green peel (Assistant drug), 7 compounds were detected from raw Hawthorn (Assistant drug), and 6 compounds were detected from Bergamot (Assistant drug). Eight compounds were detected from *Achyranthes bidentata* (Guide drug). The sources of the remaining compounds were unclear. The corresponding structures of Canonical SMILES are shown in **Supplementary Table 1**. The result in UPLC-HRMS basic peak ion diagram-negative ion mode is shown in Fig. 1A, in UPLC-HRMS basic peak ion current diagram-positive ion mode is shown in Fig. 1B, and in UPLC-UV chromatogram-UV 280 nm is shown in Fig. 1C.

3.2 Target prediction of the components of TJY formula

We used the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) to predict the targets of selected compounds from TJY formula. A total of 4701 targets were collected, and 854 targets were obtained after deduplication and imported into STRING database (http://string-db.org/cgi/input.pl). Protein-protein interaction (PPI) network analysis was conducted (Fig. 2).

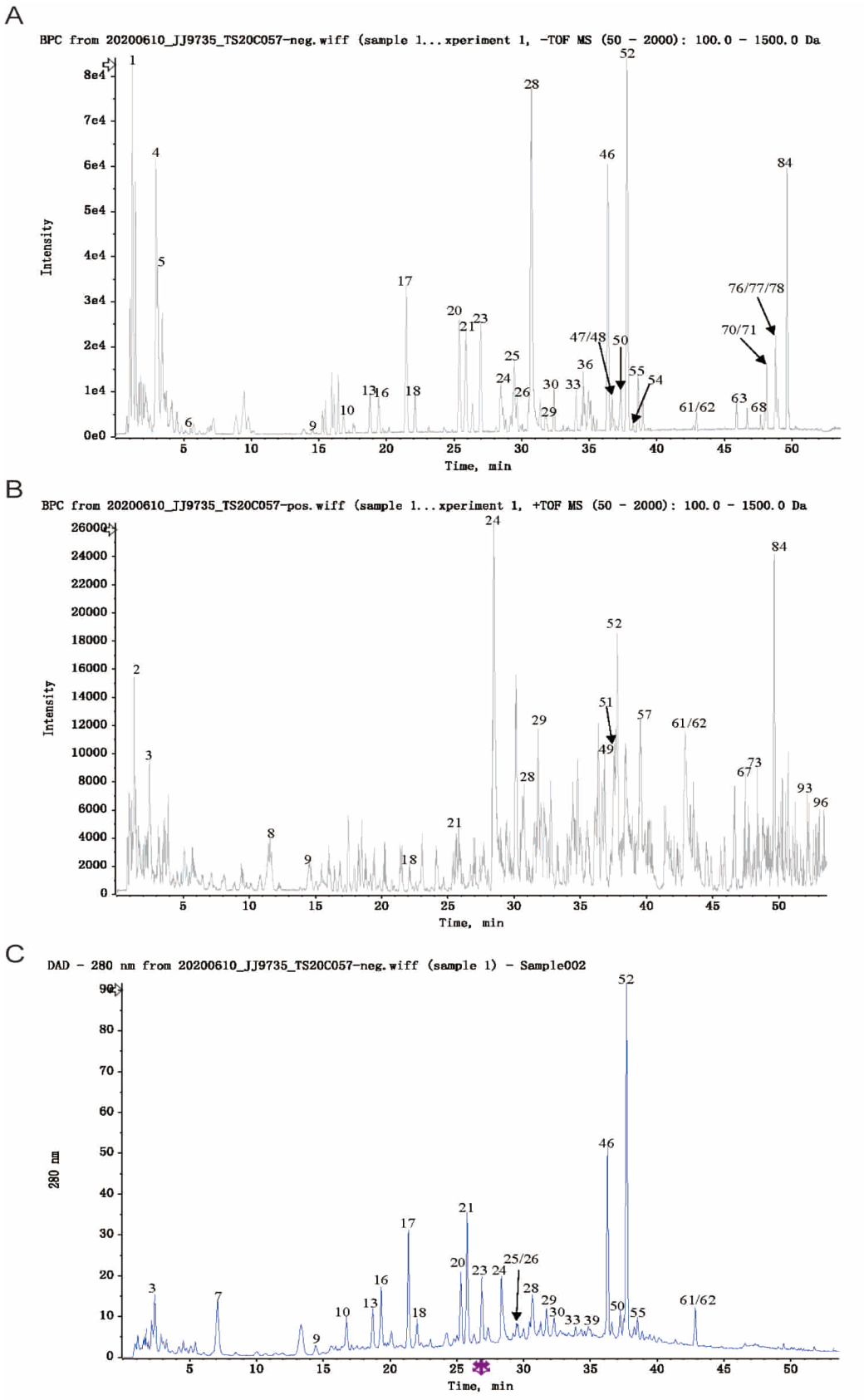

Fig. 1. Identification of the components of TJY formula. (A) UPLC-HRMS basic peak ion diagram-negative ion mode. (B) UPLC-HRMS basic peak ion current diagram-positive ion mode. (C) UPLC-UV chromatogram-UV 280 nm.

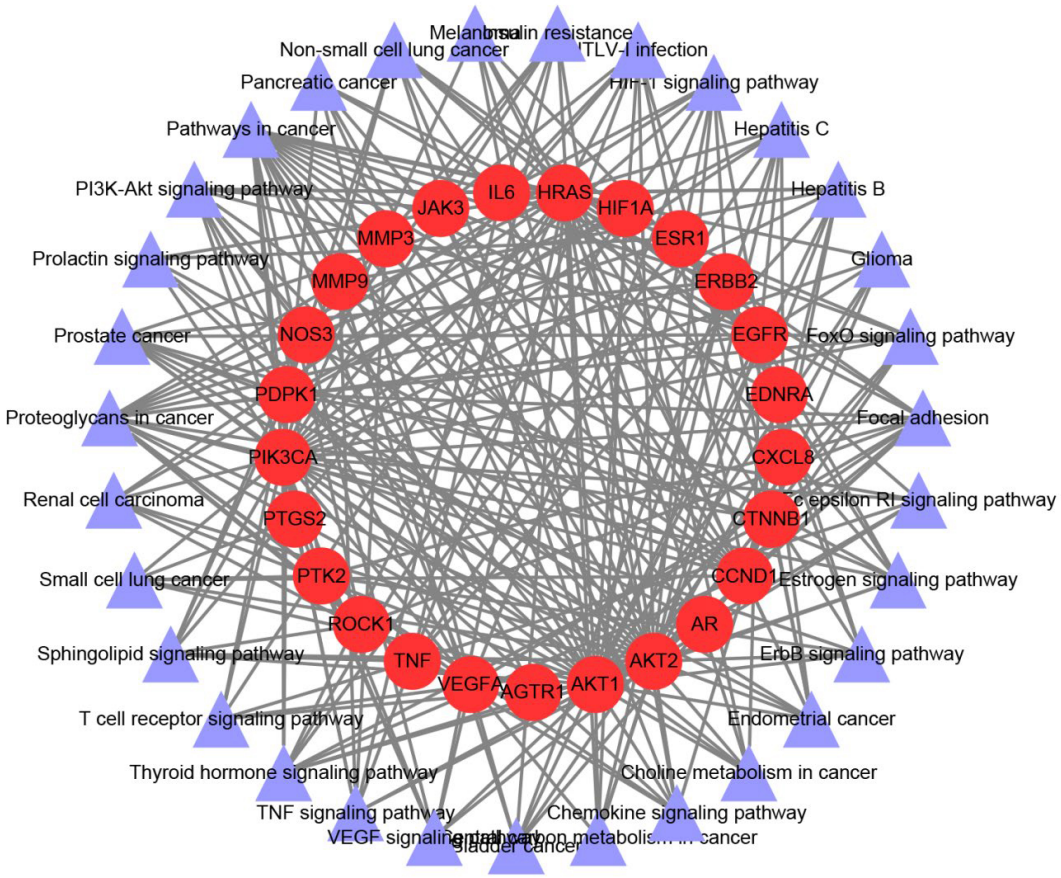

Fig. 2. PPI network analysis of TJY formula.

3.3 Disease-related targets query

Targets related to PD were collected through a series of databases by performing keyword searches of "dysmenorrhea" and the species set as "human", including in Genecards (https://www.genecards.org/), TTD (http://db.idrblab.net/ttd/), DiGSeE (http://www.disgenet.org/), Drugbank (https://www.drugbank.ca/) and OMIM (https://omim.org/). We collected 545 targets from Genecards (n = 287), TTD (n = 12), DiGSeE (n = 9) and Drugbank (n = 237). All targets were normalized to genesymbol by BioDBnet database, and 363 disease-related targets were obtained after de-duplication. Based on the PD-related targets, the data were retrieved in the STRING database (https://string-db.org/). The PPI network was established as the background network of gene mapping (Fig. 3). The PPI relationship with a confidence score $\geq$0.9 was selected. The PPI of component-related targets were "merged" with those of disease-related targets via Cytoscape 3.6.1 software (https://cytoscape.org/) and the overlapping targets were obtained. Three topological properties were calculated, including "degree", "closeness centrality" and "betweenness centrality". The predicted target was selected only when three parameters were greater than the median.

3.4 Core targets screening

To further explore the function and mechanism of candidate targets related to components and disease, the corresponding PPI network diagram was depicted based on the targets of candidate components of TJY formula and of PD listed in the STRING database (http://string-db.org/). The diagrams show a disease-target PPI (Fig. 2) and component-target PPI (Fig. 3). The PPI network of the core target consists of 56 nodes and 211 edges. The core target information is shown in Fig. 4. The top five targets are PIK3CA, AKT1, EGFR, AKT2 and CYP19A1, followed by HRAS, PTGS2, and MMP9. The top 15 targets are shown in Table 1.

3.5 GO and KEGG analysis of core targets

The GO and KEGG pathway enrichment analysis of 56 core targets were performed using the DAVID database (FDR $<$0.05). GO analysis revealed 36 items related to biological process, 13 items related to molecular function and 3 items related to cellular component. A total of 27 signaling pathways were involved according to KEGG pathway enrichment analysis. GO (Fig. 5) and KEGG analysis (Fig. 5B) results are shown in Fig. 5. Based on the results of enrichment analysis, the "target pathway" network diagram of the TJY formula in the treatment of PD was estab-

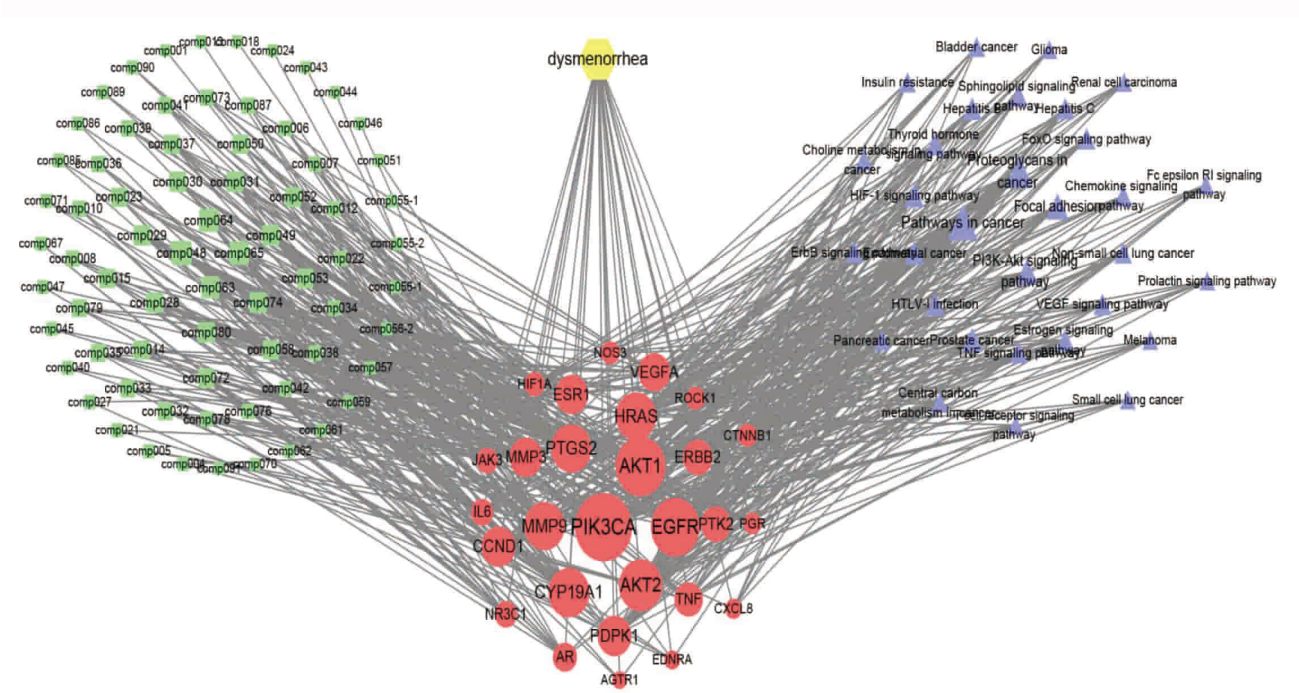

Fig. 3. PPI network analysis of dysmenorrhea.

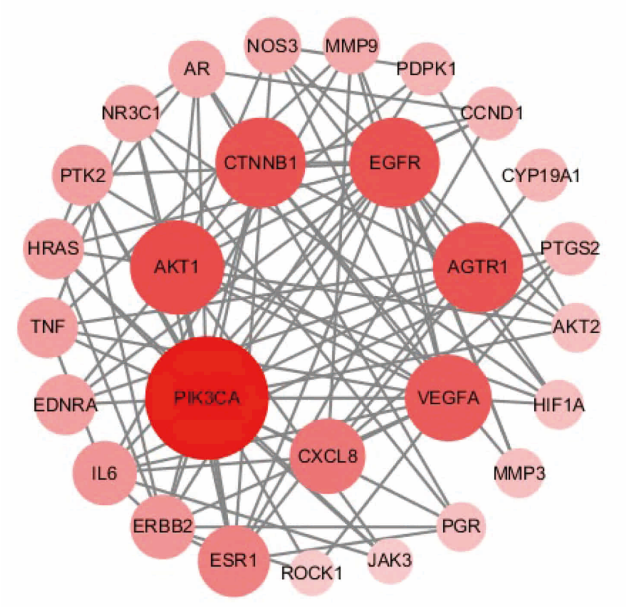

Fig. 4. PPI network of the core targets. The size and color of the nodes in the graph positively correlated with the degree value.

Table 1. The top 15 central targets in the PPI network.

Top 15 targets	Nodes
PIK3CA	45
AKT1	38
EGFR	37
AKT2	32
CYP19A1	30
HRAS	29
PTGS2	29
MMP9	29
CCND1	23
PDPK1	23
ESR1	22
MMP3	22
VEGFA	21
PTK2	19
ERBB2	19

lished. The top 15 signaling pathways and targets are shown in Table 2. The PI3K-Akt signaling pathway also ranked in the top 15 signaling pathways, which was consistent with the predicted core target genes. GO and KEGG analysis of core targets suggested that the TJY formula might play an anti-inflammatory role in the treatment of dysmenorrhea by affecting the expressions of core target genes.

4. Discussion

The etiology of dysmenorrhea is not precisely understood, but is associated with altered prostaglandin synthesis by the uterus (PGF2α) [15]. Excessive release of PG from disintegrating cells during endometrial sloughing causes myometrial hypercontractility, resulting in ischemia and hypoxia of the uterine muscle as well as pain [16,17]. PGs are ubiquitously distributed intracellular substances that are derived from long-chain polyunsaturated fatty acids, such as arachidonic acid, a common component of cell membrane phospholipids [18]. PGs have a range of biological

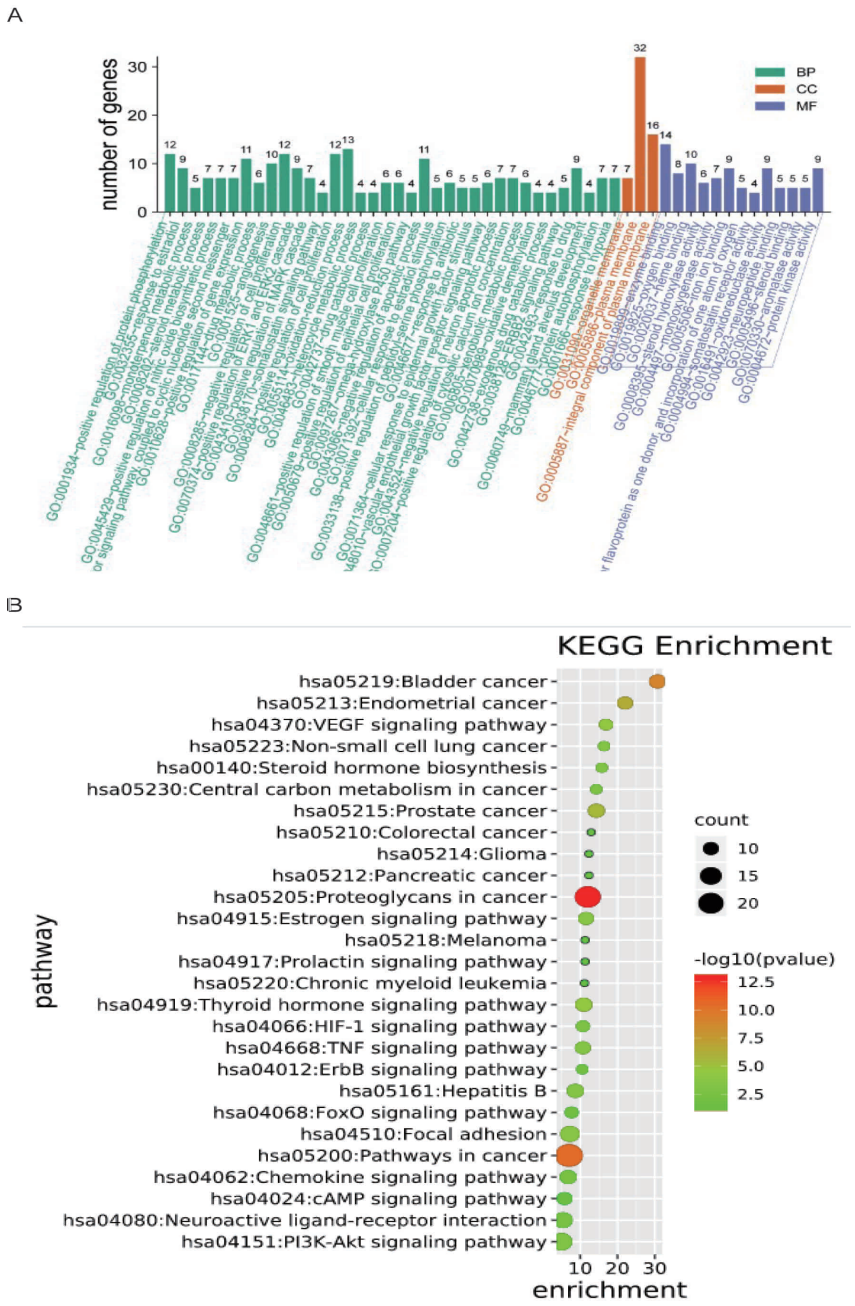

Fig. 5. GO and KEGG analysis of core targets. (A) GO analysis. (B) KEGG analysis.

effects on a wide variety of physiological and pathological activities including pain, inflammation, body temperature, and sleep regulation [16]. In the theory of traditional Chinese Medicine, dysmenorrhea is caused by blocking of Qi and blood. For the treatment of dysmenorrhea, there are multiple effective and clinically validated formulas of traditional Chinese medicine, such as *Wenjing Tang*, *Shaofu Zhuyu Tang*, and *Danggui Sini Tang* [19–21]. TJY formula also has been demonstrated to possess good clinical efficacy against dysmenorrhea in our hospital, which is worth further study.

In the present study, the TJY formula was analyzed and 96 compounds were detected. Among them, 91 compounds were identified while 5 compounds with unidentified structure were not identified. Among the 91 compounds, 13 compounds were from monarch drugs, 33 compounds were from minister drugs, 30 compounds were from assistant drugs, and 8 were compounds from guide drugs. The monarch drug *Artemisia argyi* leaf can be used as a therapeutic agent for abdominal pain and dysmenorrhea as well as eczema and itching. The anti-inflammatory effect of *Artemisia argyi* leaf is closely related to the inhibition of inflammatory mediators released from macrophages and inflammatory cytokine production in inflamed tissues [22]. As the most commonly used single herbs, Corydalis and Cyperus were found in 33.1% and 29.2% of all prescrip-

Table 2. The top 15 enriched KEGG pathways of core targets.

NO.	KEGG_ID	Name	Pathway class	Degree
1	hsa05200	Pathways in cancer	Cancer: overview	36
2	hsa05205	Proteoglycans in cancer	Cancer: overview	32
3	hsa04151	PI3K-Akt signaling pathway	Signal transduction	29
4	hsa05161	Hepatitis B	Infectious disease: viral	27
5	hsa04062	Chemokine signaling pathway	Immune system	24
6	hsa04510	Focal adhesion	Cellular community - eukaryotes	24
7	hsa04015	Rap1 signaling pathway	Signal transduction	24
8	hsa05212	Pancreatic cancer	Cancer: specific types	22
9	hsa05142	Chagas disease (American trypanosomiasis)	Infectious disease: parasitic	22
10	hsa05203	Viral carcinogenesis	Cancer: overview	22
11	hsa05215	Prostate cancer	Cancer: specific types	21
12	hsa04066	HIF-1 signaling pathway	Signal transduction	21
13	hsa05166	HTLV-I infection	Infectious disease: viral	21
14	hsa04668	TNF signaling pathway	Signal transduction	20
15	hsa04014	Ras signaling pathway	Signal transduction	20

tions in a study of women aged from 13 to 25 years with a single diagnosis of primary dysmenorrhea in Taiwan [23]. The composition of TJY formula is consistent with that previously described [22,23], which indicates that its composition is rational and effective.

The PPI network, GO and KEGG analyses showed that the top target and signaling pathways were the PIK3CA and PI3K-Akt signaling pathways, respectively. PIK3CA, AKT1 and AKT2 are members of the PI3K-Akt pathway. PIK3CA is a family of lipid kinases consisting of a regulatory subunit (p85) and a catalytic subunit (P110). The binding of ligand to the membrane receptor activates p85 and recruits P110, which catalyzes the formation of PI3P from PIP2 on the intracellular surface. As a second messenger, PI3P further activates Akt and PDK1 [24]. AKT, also known as PKB, is an important downstream molecule of PI3K. There are three main isoforms of AKT, namely AKT1, AKT2 and AKT3. They are essential in the regulation of cell growth, proliferation, survival and glucose metabolism [25]. EGFR (epidermal growth factor receptor, also known as ErbB-1 or HER1) is a member of epidermal growth factor receptor family. The EGFR signaling pathway plays an important role in cell growth, proliferation and differentiation [26]. The CYP19A1 gene encodes an aromatase that catalyzes the conversion of testosterone and androstenedione to estrone and estradiol. Aromatase, also known as estrogen synthase, is expressed in endometrial cancer, breast cancer, endometriosis and uterine leiomyoma, and its overexpression is related to the occurrence and development of these diseases [27]. Among the top 15 targets, PIK3CA, AKT1, EGFR and AKT2 were reported to be related to inflammation. A study showed that IL-8 promotes integrin β3 upregulation and cell invasion through the PI3K/Akt pathway in hepatocellular carcinoma [28]. PIK3CA, AKT1 and AKT2 are also related to inflammatory factors, such as IL-33 [29], IL-1β [30], and TNF-α [31], among others. Another study demonstrated an involvement of the IL-1β/EHD1/TUBB3 axis in EGFR-tyrosine kinase inhibitor resistance [32]. There are two major mechanisms responsible for the activation of PI3K-Akt signaling pathway: one is the interaction with growth factor receptor or connexin with phosphorylated tyrosine residues, resulting in the change of dimer conformation; the other is the direct binding of Ras and P110. PI3K activation results in the production of the second messenger PIP3 on the plasma membrane; PIP3 binds to signal proteins namely Akt and PDK1. Akt can be also activated by PDK-mediated phosphorylation of Thr473 (such as integrin linked kinase, ILK). Activated Akt regulates downstream target proteins (e.g., Caspase9, NF-κB, GSK-3) and thus plays important a role in many biological processes, including cell cycle progression, cell growth, survival, actin rearrangement, migration, and intracellular vesicular transport. In addition, the chemokine signaling pathway, focal adhesion [33], Rap1 signaling pathway [34], HIF-1 signaling pathway [35], TNF signaling pathway and Ras signaling pathway [36] have been reported to be associated with inflammation. The PI3K-Akt signaling pathway can be activated by a variety of cell stimuli or toxic damage, and it mainly regulates basic cell functions such as transcription, translation, proliferation and growth [37]. Previous studies have shown that PI3K-Akt signaling activation promotes the proliferation of endometrial cells and also increases the contractility of human endometriosis stromal cells [38], which might be associated to dysmenorrhea in endometriosis [39].

There are some limitations of this study. Although the components of TJY formula were identified followed by network pharmacology analysis, the specific relationships among compounds, targets and signaling pathways remain unclear. Experiments in cells and animal models need to

be supplemented to explore the mechanisms and verify our speculation.

5. Conclusions

TJY formula might play roles in the treatment of dysmenorrhea underlying mechanisms relating to the involvement of IL-8, L-33, IL-1β and TNF-α as well as the PI3K-Akt signaling pathway. Potential pathways have been identified that need mechanistic confirmation in a laboratory setting in the future.

Abbreviations

TJY, Tong-Jing-Yi; PD, primary dysmenorrhea; AD, acquired dysmenorrhea; PG, prostaglandin; IL, interleukin; UPLC-Q-TOF/MS, ultra performance liquid chromatography-quadrupole-time of flight/mass spectrometry; PI3K, phosphoinositide 3-kinase; PI3P, phosphatidylinositol 3-phosphate; PIP2, phosphatidylinositol 4,5-bisphosphate; PDK1, phosphoinoside dependent kinase 1; PKB, protein kinase B; EGFR, epidermal growth factor receptor; HIF-1α, hypoxia inducible factor-1α.

Author contributions

YYL—analysis of data, manuscript revision; JLZ—extraction and drafting of the manuscript; JT—design and revision and provide funding support. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Obstetrics and Gynecology Hospital of Fudan University (approval number: 2021-84).

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.ceog4904099.

References

[1] Libarle M, Simon P, Bogne V, Pintiaux A, Furet E. Management of dysmenorrhea. Revue Medicale de Bruxelles. 2018; 39: 264–272.

[2] Ciebiera M, Esfandyari S, Siblini H, Prince L, Elkafas H, Wojtyla C, *et al.* Nutrition in Gynecological Diseases: Current Perspectives. Nutrients. 2021; 13: 1178.

[3] Ferries-Rowe E, Corey E, Archer JS. Primary Dysmenorrhea. Obstetrics & Gynecology. 2020; 136: 1047–1058.

[4] Piessens S, Edwards A. Sonographic Evaluation for Endometriosis in Routine Pelvic Ultrasound. Journal of Minimally Invasive Gynecology. 2020; 27: 265–266.

[5] De Sanctis V, Soliman A, Bernasconi S, Bianchin L, Bona G, Bozzola M, *et al.* Primary Dysmenorrhea in Adolescents: Prevalence, Impact and Recent Knowledge. Pediatric Endocrinology Reviews. 2015; 13: 512–520.

[6] Oladosu FA, Tu FF, Hellman KM. Nonsteroidal antiinflammatory drug resistance in dysmenorrhea: epidemiology, causes, and treatment. American Journal of Obstetrics and Gynecology. 2018; 218: 390–400.

[7] Kho KA, Shields JK. Diagnosis and Management of Primary Dysmenorrhea. Journal of the American Medical Association. 2020; 323: 268.

[8] Kaplan Ö, Nazıroğlu M, Güney M, Aykur M. Non-steroidal anti-inflammatory drug modulates oxidative stress and calcium ion levels in the neutrophils of patients with primary dysmenorrhea. Journal of Reproductive Immunology. 2013; 100: 87–92.

[9] Fu M, Meng X. Assessment of clinical efficacy of traditional Chinese medicine for the management of primary dysmenorrhea in the UK. Medicine. 2020; 99: e23246.

[10] Lin J, Liao W, Mo Q, Yang P, Chen X, Wang X, *et al.* A systematic review of the efficacy comparison of acupuncture and traditional Chinese medicine in the treatment of primary dysmenorrhea. Annals of Palliative Medicine. 2020; 9: 3288–3292.

[11] Ren D, Ran L, Yang C, Xu M, Yi L. Integrated strategy for identifying minor components in complex samples combining mass defect, diagnostic ions and neutral loss information based on ultra-performance liquid chromatography-high resolution mass spectrometry platform: Folium Artemisiae Argyi as a case study. Journal of Chromatography. a. 2018; 1550: 35–44.

[12] Kandikattu HK, Rachitha P, Krupashree K, Jayashree GV, Abhishek V, Khanum F. LC-ESI-MS/MS analysis of total oligomeric flavonoid fraction of Cyperus rotundus and its antioxidant, macromolecule damage protective and antihemolytic effects. Pathophysiology. 2015; 22: 165–173.

[13] Li C, Hou X, Xu Y, Gao W, Li P, Yang H. Manual annotation combined with untargeted metabolomics for chemical characterization and discrimination of two major crataegus species based on liquid chromatography quadrupole time-of-flight mass spectrometry. Journal of Chromatography A. 2020; 1612: 460628.

[14] Li W, Sun X, Liu B, Zhang L, Fan Z, Ji Y. Screening and identification of hepatotoxic component in Evodia rutaecarpa based on spectrum-effect relationship and UPLC-Q-TOFMS. Biomedical Chromatography. 2016; 30: 1975–1983.

[15] Tang B, Liu D, Chen L, Liu Y. NLRP3 inflammasome inhibitor MCC950 attenuates primary dysmenorrhea in mice via the NF-kappaB/COX-2/PG pathway. Journal of Inflammation. 2020; 17: 22.

[16] Crofford LJ. Prostaglandin biology. Gastroenterology Clinics of North America. 2001; 30: 863–876.

[17] Marjoribanks J, Ayeleke RO, Farquhar C, Proctor M. Nonsteroidal anti-inflammatory drugs for dysmenorrhoea. The Cochrane Database of Systematic Reviews. 2015; 2015: CD001751.

[18] Li X, Guo S, Chen Z, Ren K, Zhang H, Yu S, *et al.* Regula-

tion of Mild Moxibustion on Uterine Vascular and Prostaglandin Contents in Primary Dysmenorrhea Rat Model. Evidence-Based Complementary and Alternative Medicine. 2021; 2021: 1–12.

[19] Ushiroyama T, Sakuma K, Nosaka S. Comparison of Effects of Vitamin E and Wen-jing-tang (Unkei-to), an Herbal Medicine, on Peripheral Blood Flow in Post-menopausal Women with Chilly Sensation in the Lower Extremities: a Randomized Prospective Study. The American Journal of Chinese Medicine. 2006; 34: 969–979.

[20] Wen S, Chang W, Shen H, Wu H. Prescription patterns and factors influencing the use of Chinese herbal medicine among pregnant women in Taiwan: a population-based retrospective study. BMC Complementary Medicine and Therapies. 2020; 20: 240.

[21] Yao J, Yu L, Chen Q, Chen Z, Wang D, Qiu X, *et al*. Comparison of ferulic acid content in Radix Angelicae Sinensis, Danggui-Buxue-Tang and Danggui-Sini-Tang. Experimental and Therapeutic Medicine. 2014; 7: 1364–1368.

[22] Yun C, Jung Y, Chun W, Yang B, Ryu J, Lim C, *et al*. Anti-Inflammatory Effects ofArtemisiaLeaf Extract in Mice with Contact DermatitisIn VitroandIn Vivo. Mediators of Inflammation. 2016; 2016: 1–8.

[23] Chen H, Lin Y, Su IH, Chen Y, Yang S, Chen J. Investigation on Chinese herbal medicine for primary dysmenorrhea: implication from a nationwide prescription database in Taiwan. Complementary Therapies in Medicine. 2014; 22: 116–125.

[24] Arafeh R, Samuels Y. PIK3CA in cancer: the past 30 years. Seminars in Cancer Biology. 2019; 59: 36–49.

[25] Revathidevi S, Munirajan AK. Akt in cancer: Mediator and more. Seminars in Cancer Biology. 2019; 59: 80–91.

[26] Talukdar S, Emdad L, Das SK, Fisher PB. EGFR: an essential receptor tyrosine kinase-regulator of cancer stem cells. Advances in Cancer Research. 2020; 467: 161–188.

[27] Parween S, DiNardo G, Baj F, Zhang C, Gilardi G, Pandey AV. Differential effects of variations in human P450 oxidoreductase on the aromatase activity of CYP19A1 polymorphisms R264C and R264H. The Journal of Steroid Biochemistry and Molecular Biology. 2020; 196: 105507.

[28] Sun F, Wang J, Sun Q, Li F, Gao H, Xu L, *et al.* Interleukin-8 promotes integrin beta3 upregulation and cell invasion through PI3K/Akt pathway in hepatocellular carcinoma. Journal of Experimental & Clinical Cancer Research. 2019; 38: 449.

[29] Nakajima S, Ishimaru K, Kobayashi A, Yu G, Nakamura Y, Ohoka K, *et al*. Resveratrol inhibits IL-33–mediated mast cell activation by targeting the MK2/3–PI3K/Akt axis. Scientific Reports. 2019; 9: 18423.

[30] Yan S, Jiang C, Li H, Li D, Dong W. FAM3A protects chondrocytes against interleukin-1beta-induced apoptosis through regulating PI3K/Akt/mTOR pathway. Biochemical and Biophysical Research Communications. 2019; 516: 209–214.

[31] Liu S, Cao C, Zhang Y, Liu G, Ren W, Ye Y, *et al.* PI3K/Akt inhibitor partly decreases TNF-alpha-induced activation of fibroblast-like synoviocytes in osteoarthritis. Journal of Orthopaedic Surgery and Research. 2019; 14: 425.

[32] Huang J, Lan X, Wang T, Lu H, Cao M, Yan S, *et al.* Targeting the IL-1beta/EHD1/TUBB3 axis overcomes resistance to EGFR-TKI in NSCLC. Oncogene. 2020; 39: 1739–1755.

[33] Wong VW, Rustad KC, Akaishi S, Sorkin M, Glotzbach JP, Januszyk M, *et al*. Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. Nature Medicine. 2011; 18: 148–152.

[34] Ghosh AS, Tergaonkar V. Telomeres and inflammation: Rap1 joins the ends? Cell Cycle. 2010; 9: 3834–3835.

[35] Balamurugan K. HIF-1 at the crossroads of hypoxia, inflammation, and cancer. International Journal of Cancer. 2016; 138: 1058–1066.

[36] Kortlever RM, Sodir NM, Wilson CH, Burkhart DL, Pellegrinet L, Brown Swigart L, *et al*. Myc Cooperates with Ras by Programming Inflammation and Immune Suppression. Cell. 2017; 171: 1301–1315.e14.

[37] Xie Y, Shi X, Sheng K, Han G, Li W, Zhao Q, *et al.* PI3K/Akt signaling transduction pathway, erythropoiesis and glycolysis in hypoxia (Review). Molecular Medicine Reports. 2019; 19: 783–791.

[38] Yin X, Pavone ME, Lu Z, Wei J, Kim JJ. Increased activation of the PI3K/AKT pathway compromises decidualization of stromal cells from endometriosis. The Journal of Clinical Endocrinology and Metabolism. 2012; 97: E35–E43.

[39] Xu XY, Zhang J, Qi YH, Kong M, Liu SA, Hu JJ. Linc-ROR promotes endometrial cell proliferation by activating the PI3K-Akt pathway. European Review for Medical and Pharmacological Sciences. 2018; 22: 2218–2225.