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Study on Molecular Mechanism of Tongqi No.1 Prescription for Tubal Obstructive Infertility Based on Network Pharmacology and Molecular Docking

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Abstract: In order to investigate the mechanism of action of Tongqi No. 1 Formula in the treatment of tubal obstructive infertility, a network pharmacology method was used to analyze the active ingredients and drug targets of Tongqi No. 1 Formula, and the disease targets of tubal obstructive infertility, respectively. The drug targets, disease targets, and active ingredient were then analyzed, and the results were finally validated by using molecular docking methods. It was found that 61 drug active ingredients and 215 drug targets were obtained in Tongqi No. 1 Formula. The GeneCards database, the OMIM database combined with the DRUGBANK database, was merged and duplicate values were removed, resulting in 1382 disease targets and 119 drug-disease intersecting genes. GO enrichment revealed that Tongqi No. 1 Formula has biological functions such as response to steroid hormones, regulation of apoptotic signaling pathway, positive regulation of cell death, response to growth factors, and response to estradiol. After KEGG pathway enrichment analysis, the main pathways involved are NF-KB, JAK-STAT, p13k-AKT, MAPK, etc. Molecular docking results showed that tanshinone IIA stably docked into the active pocket of AKT1 protein structure 4EJN, and the two were hydrogen bonded through amino acid residues ASN53, ASN51, VAL-271, VAL-270, SER-205, and GLN-203. The present study initially revealed that the treatment of tubal obstruction infertility with "Tongqi No. 1 Formula" is carried out through multiple components, multiple targets and multiple pathways, which provides a basis for further research.

1. Introduction

At present, the number of infertility patients in the world reaches 186 million [1], and the infertility rate is about 15.5%, showing an increasing trend year by year.By 2020, about 25 percent of couples of child-bearing age in China were suffering from infertility, an increase from 2018.Factors that cause infertility include tubal factors, ovulation disorders and immune factors,

among which tubal factors, tubal obstruction accounts for 14%-45% of all infertility causes [2] , and is the main factor that causes infertility.Modern medicine mainly uses endoscopic therapy, interventional therapy and assisted reproductive technology therapy, but there are some problems such as postoperative adhesion and easy recurrence.At present, most of the Chinese medical scientists recognized that the main pathogenesis of tubal obstructive infertility is "blood stasis", the treatment principle is to promote blood stasis and dredging collagals, clinical treatment effect is significant, shortcomings for the relevant mechanism of action still need further research.

Tongqi No.1 prescription is the experience prescription of Liu Maolin, director of Yulin Hospital of Traditional Chinese Medicine. The prescription has Chuanxiong, which has the effect of promoting blood circulation and removing blood stasis, qi and dispelling wind.Fang Zhong Sanling, Zedoary turmeric broken blood to eliminate accumulation and relieve pain, good stasis.Take pangolin to promote blood and eliminate disease, through the effect of activating collaterals.Soap thorn soft and firm scattered knot, promote mass, effusion disappear.Danshen Qi promoting blood circulation and removing blood stasis, Pueraria Gan insipid regulation, can prevent the problem of ligusticum Chuanxiong too much, fructus aurantii dissipates blood by removing blood stasis, asarum, cassia twig, Tongcao, road through the meridian activating collaterals, Wang does not leave the line of blood through the meridian, all drugs cooperate, play blood activating qi, dredge the blocking work, make the fallopian tube smooth.Since the establishment of gynecology department of Yulin Hospital of Traditional Chinese Medicine in 1989, more than 10,000 patients with tubal obstructive infertility have been cured by Director Liu Maolin.In long-term clinical work, this research team found that Tongqi No.1 formula has a significant effect on tubal obstructive infertility [3,4], which can improve the symptoms of tubal obstruction and improve the pregnancy rate.Therefore, this study used the method of network pharmacology to analyze the mechanism of action of Tongqi No.1 formula with definite clinical efficacy in the treatment of tubal obstructive infertility, which can provide a new idea for clinical application and scientific basis for the next experimental research.

2. Data and methods

2.1. Collection of components and targets of the No.I prescription

I will pass disambiguation party (rhizoma ligustici wallichii, pangolin scales, asarum, soap, ricepaperplant pith, cowherb seed, passepartout, rhizoma sparganii, rhizoma zedoariae, salvia miltiorrhiza, puerarin, acid-insoluble ash, cassia twig) in each taste traditional Chinese medicine name input TCMSP [5] (Traditional Chinese Medicine Systems Pharmacology, http://temspw.com/tcmsp.php)database, combined OB (oral bioavailability) and DL (drug-like) as the effective active ingredient and protein target screening of each drug [5], set OB>=30%, DL>=0.18, and refer to related literature for supplement.When the screening was completed, the target proteins corresponding to each drug were standardized using the Uniprot (http://www.www.uniprot.org/) database, and the corresponding genes of the target proteins were retrieved using the UniprotKB database. The search condition was set as Organism: Homosapiens (Human) for protein and gene transformation and construction of a composition-target protein database.

2.2. Target collection for tubal obstructive infertility

Through GeneCards (https://www.genecards.org), OMIM (http://www.omim.or) and TDD (http://bidd.nus.edu.sg/group/cjttd)),With "fallopian tube complication infertility" as the key word,The DRUGBANK database (https://www.drugbank.ca) was used to find clinical first-line

targets of Western drug action for the treatment of tubal obstructive infertility as a supplement[6] .Duplicate values were merged and deleted from the disease database to obtain the final disease target protein, and its final results were collated through the Uniprot database.

2.3. Composition of Tongqi No.1 Formula -- PPI network construction of tubal obstructive infertility related targets

In order to study the correlation between the drug target of Tongqi No.1 prescription and the target gene of tubal obstructive infertility disease, Tongqi No.1 prescription was compared with the target gene of tubal obstructive infertility. The intersection gene of Tongqi No.1 prescription was selected and STRING11.0 [7] (http: the rest of the data were set as Homosapiens and highestconfidence (>0.9), and PPI was constructed.CytoScape3.7.1 software is used to visualize the results, and MCODE plug-in is used to describe the biological processes and corresponding functions of the core target.

2.4. Enrichment analysis of core target function and pathway of Tongqi No.1 formula component - tubal obstructive infertility.

The Metascape platform [8,9] (http://metascape.org/gp/index.html) has two features, first, a comprehensive explanation of its annotation function, and second, the timely update, once a month, is achieved. If the core targets are entered into the Metascape platform with a setting of $P<0.01$, the main biological processes and metabolic pathways of the core targets could be enriched and analyzed. The results were visualized using the online website platform of Microbiology Letter (www.bioinformatics.com.cn) to further elucidate the mechanism of action of Tongqi No. 1 Formula for the treatment of tubal obstructive infertility.

2.5. Tongqi No.1 formula component - tubal obstructive infertility target - pathway network map construction

The composition of Tongqi No.1 prescription -- target of tubal obstructive infertility -- pathway network diagram was constructed using Cytoscape3.7.1 to analyze the effective components and target parameters of Tongqi No.1 prescription. According to the obtained results, the main core targets and main active components of Tongqi No.1 prescription were determined.

2.6. Molecular docking verification of active ingredients and key targets

Based on the previous research content, PyRx software was used to verify the molecular docking of key active ingredients and action targets of Tongqi Formula I, and the best docking results were visualized through pymol, and the other molecular docking results were drawn heat map.

3. Results

3.1. Acquisition of active ingredient targets for Tongqi Recipe I.

Initial extraction of pharmaceutical chemical components: There were 7 species of ligusticum ligusticum, 8 species of asarum asarum, 11 species of saponaria saponata, 4 species of Fructus vermicelli, 4 species of luputong, 5 species of tricolor, 3 species of zedoary rhizome, 65 species of salvia miltiorrhiza, 4 species of pueraria rhizoma, 15 species of Fructus aurantii, 7 species of cassia twig and 7 species of pangolin. A total of 61 active compounds were obtained by deleting many of the same compounds, only one of which was kept and no corresponding target compound was

removed.See Table 1.

Table 1: Main components of Tongqi NO.1ingredient

Drug	MOLID	Lable	Main active ingredient	OB (%)	DL
Chuanxiong	MOL001494	CX1	Mandenol	42	0.19
	MOL002135	CX2	Myricanone	40.6	0.51
	MOL002157	CX3	wallichilide	42.31	0.71
	MOL000359	A1	sitosterol	36.91	0.75
Danshen	MOL001601	DS1	1,2,5,6-tetrahydrotanshinone	38.75	0.36
	MOL001659	DS2	Poriferasterol	43.83	0.76
	MOL002222	DS3	sugiol	36.11	0.28
	MOL002651	DS4	Dehydrotanshinone II A	43.76	0.4
	MOL000006	DS5	luteolin	36.16	0.25
	MOL007041	DS6	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23
	MOL007049	DS7	4-methylenemiltirone	34.35	0.23
	MOL007050	DS8	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde2	62.78	0.4
	MOL007063	DS9	przewalskin a	37.11	0.65
	MOL007082	DS10	Danshenol A	56.97	0.52
	MOL007088	DS11	cryptotanshinone	52.34	0.4
	MOL007093	DS12	dan-shexinkum d	38.88	0.55
	MOL007094	DS13	danshenspiroketallactone	50.43	0.31
	MOL007142	DS14	salvianolic acid j	43.38	0.72
	MOL007145	DS15	salviolone	31.72	0.24
	MOL007154	DS16	tanshinone iia	49.89	0.4
Ezhu	MOL000296	B1	hederagenin	36.91	0.75
Gegen	MOL000392	GG1	formononetin	69.67	0.21
	MOL000358	C1	beta-sitosterol β	36.91	0.75
	MOL002959	GG2	3'-Methoxydaidzein 3'	48.57	0.24
Guizhi	MOL001736	GZ1	(-)-taxifolin (-)	60.51	0.27
	MOL000358	C1	beta-sitosterol β	36.91	0.75
	MOL000359	A1	sitosterol	36.91	0.75
	MOL000492	GZ2	(+)-catechin(+)	54.83	0.24
	MOL004576	GZ3	taxifolin	57.84	0.27
Lulutong	MOL000358	C1	beta-sitosterol	36.91	0.75
	MOL000359	A1	sitosterol	36.91	0.75
	MOL000517	LLT1	isostyracin epoxide	92.53	0.22
	MOL000519	LLT2	coniferin	31.11	0.32
Sanleng	MOL001297	SL1	trans-gondoic acid	30.7	0.2
	MOL000296	B1	hederagenin	36.91	0.75
	MOL000358	C1	beta-sitosterol	36.91	0.75
Tongcao	MOL000359	A1	sitosterol	36.91	0.75
Wangbuliuxing	MOL000449	D1	Stigmasterol	43.83	0.76
	MOL002322	WBLX1	isovitexin	31.29	0.72
	MOL000098	D2	quercetin	46.43	0.28
Xixin	MOL012140	XX1	4,9-dimethoxy-1-vinyl-$b-carboline	65.3	0.19
	MOL012141	XX2	Caribine	37.06	0.83
	MOL001460	XX3	Cryptopin	78.74	0.72
	MOL001558	XX4	sesamin	56.55	0.83
	MOL002501	XX5	[(1S)-3-[(E)-but-2-enyl]-2-methyl-4-oxo-1-cyclopent-2-enyl] (1R,3R)-3-[(E)-3-methoxy-2-methyl-3-oxoprop-1-enyl]-2,2-dimethylcyclopropane-1-	62.52	0.31

			carboxylate[(1S)		
	MOL002962	XX6	5-isopropyl-2-methylbicyclo[3.1.0]hex-2-ene 5	48.23	0.33
	MOL000422	E1	kaempferol	41.88	0.24
Zaojiaoci	MOL000422	E1	kaempferol	41.88	0.24
	MOL013296	ZJC1	Fustin	50.91	0.24
	MOL000073	ZJC2	ent-Epicatechin	48.96	0.24
	MOL013179	ZJC3	fisetin	52.6	0.24
	MOL002914	F1	Eriodyctiol (flavanone)	41.35	0.24
	MOL000098	D2	quercetin	46.43	0.28
	MOL000359	A1	sitosterol	36.91	0.75
	MOL000358	C1	beta-sitosterol	36.91	0.75
	MOL000449	D1	Stigmasterol	43.83	0.76
Zhishi	MOL013277	ZS1	Isosinensetin	51.15	0.44
	MOL013279	ZS2	5,7,4'-Trimethylapigenin	39.83	0.3
	MOL013437	ZS3	6-Methoxy aurapten	31.24	0.3
	MOL001941	ZS4	Ammidin	34.55	0.22
	MOL002914	F1	Eriodyctiol (flavanone)	41.35	0.24

3.2. Analysis of active ingredients and drug target network of Tongqi Prescription I

The 61 active ingredients of Tongqi I formula correspond to 215 target genes. Using Cytoscape3.7.1 software, the relationship between compounds and targets is made into a network and the network diagram is drawn, as shown in Figure 1.The result is analyzed and the degree value is calculated by using the plug-in NetworkAnalyzer in the software. The larger the degree value is, the more important the node is in the network.The statistical results showed that there were 275 nodes in the network. According to the degree value, the top 5 active ingredients in the network nodes were quercetin, β-sitosterol, kaempferol, luteolin and stigmasterol, respectively, and the corresponding drug targets were KCNH2, PGR, AR, RELA and PGR.Through the analysis of the active ingredients and drug targets of Tongqi No.1 prescription, it can be concluded that the therapeutic effect of Tongqi No.1 prescription may play a role through multiple active ingredients corresponding to multiple targets.

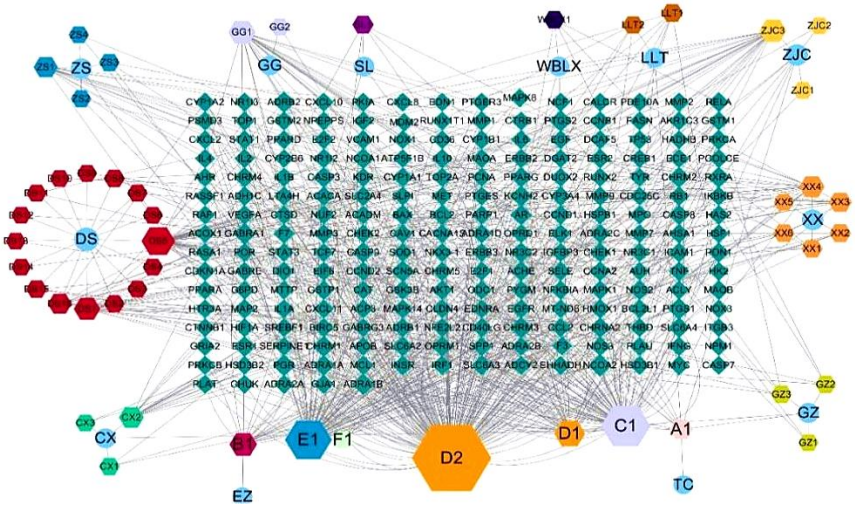


Figure 1: Network of "compound-target" of Tongqi Yihao Formula

3.3. Disease target collection for tubal obstructive infertility

The disease database was merged and duplicates were removed, and the final results were

collated through the Uniprot database, resulting in 1382 disease targets, as shown in Figure 2 (1).

3.4. Composition of Tongqi No.1 Formula - PPI network construction related to tubal obstructive infertility

Venny2.1.0 website was used for online analysis to obtain the intersection target genes of disease genes and drug targets, as shown in Figure 2 (2).

By using STRING database, the intersection genes were included to construct the drug-disease PPI network. The PPI network had 119 nodes and 2358 edges, with an average interval of 0.701 and an average node degree of 39.6.Topological analysis results showed that target proteins with high degree values included luteolin, quercetin, isovitexin, etc., which were visualized on Cytoscape3.7.1, as shown in Figure 3.

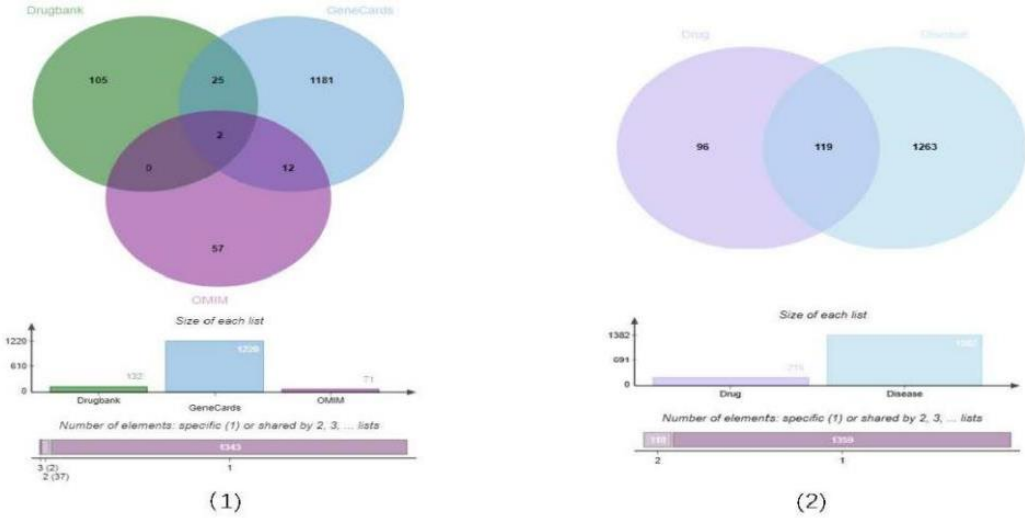


Figure 2: Venn diagram of common targets of disease and drug diaease

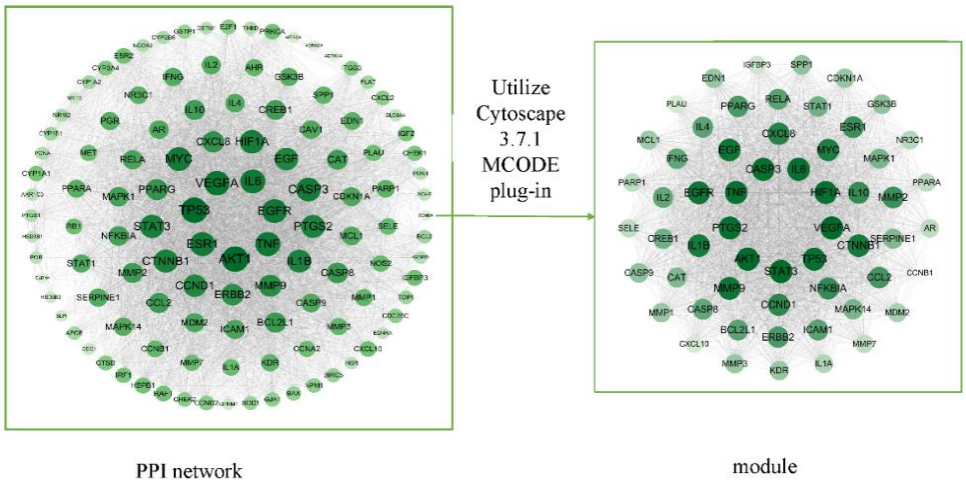


Figure 3: Intersection gene PPI network and potential subnet module

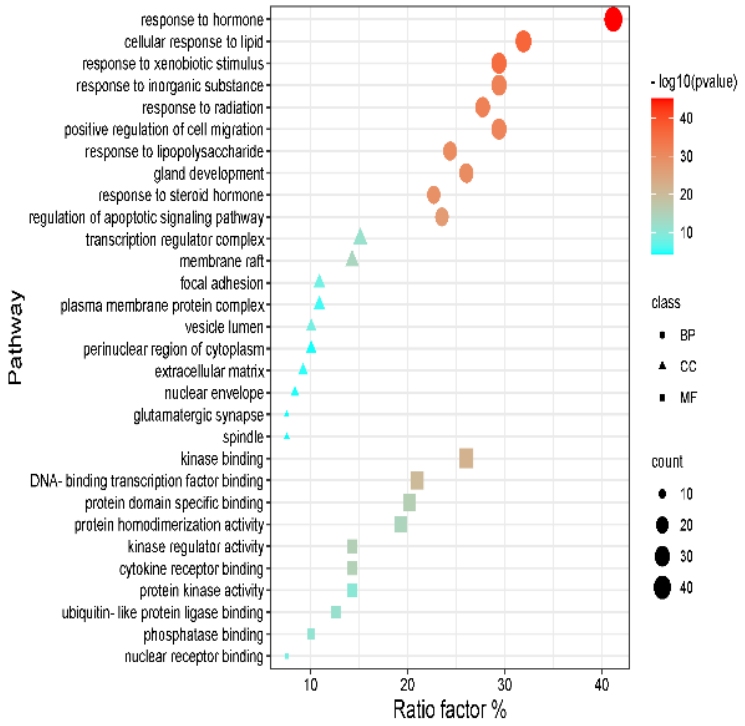
After PPI network is obtained, module is obtained by using MCODE plug-in in CytoScape3.7.1, as shown in Figure 3. According to the P value, descriptions of the functions of the top 3 biological processes in the PPI network and Module are obtained, as shown in Table 2.

Table 2: Biological process table for best score

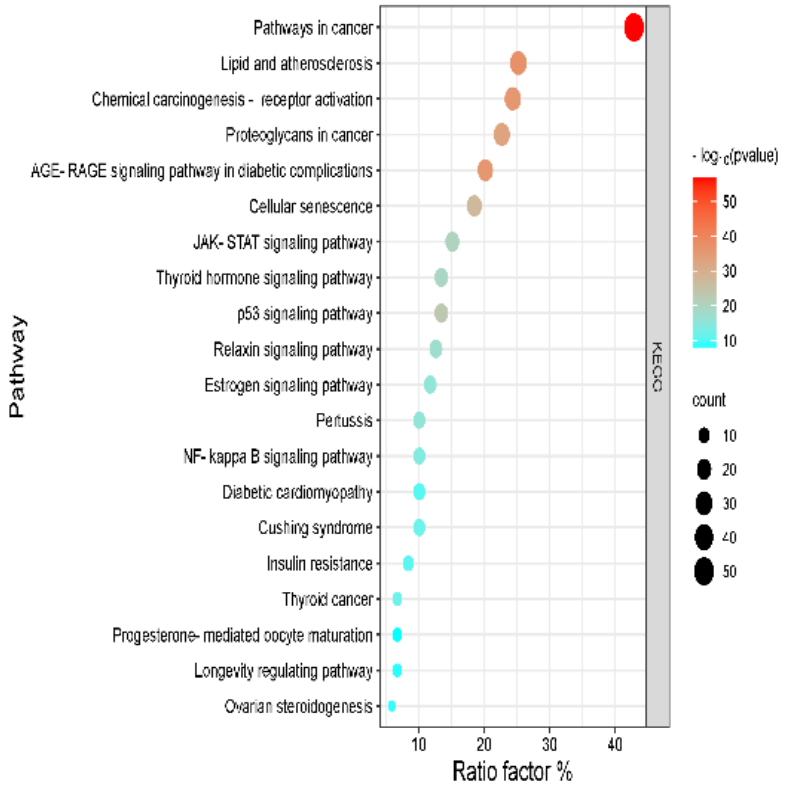
GO	Description	Log10(P)
GO:0009725	response to hormone	-45.9
GO:0071396	cellular response to lipid	-37.2
GO:0071407	cellular response to organic cyclic compound	-36.2

3.5. Enrichment analysis of target function and pathway

Through retrieval on Metascape data platform, signal pathway analysis was carried out on targets related to tubal obstructive infertility treated by Tongqi No.1 formula, and the results were visualized using Weisheng platform.The results showed that the occurrence of tubal obstructive infertility was closely related to the function of multiple targets.GO enrichment analysis was performed on the top 10 items and bubble map was drawn. Tongqi Formula 1 mainly participated in biological processes including regulation of apoptotic signaling pathway, positive regulation of cell death, response to growth factors, response to estradiol and other biological functional targets for the treatment of tubal obstructive infertility mainly concentrated in protein kinase activity and nuclear receptor binding.It mainly involves adhesion, platelet a granules, Bcl-2 family protein complex and so on.GO enrichment results were shown in Figure 4 (1), and it was found that AGE-RAGE signaling pathway, p53 signaling pathway, JAK-STAT signaling pathway, NFκB signaling pathway, etc., were the main pathways for Tongqi Formula 1 treatment of tubal obstructive infertility. KEGG analysis results were shown in Figure 4 (2), and target pathway enrichment results were shown in Table 3.



(1) GO enrichment



(2) KEGG pathway

Figure 4: Target function and pathway enrichment map

Table 3: Enrichment results of tubal obstructive infertility pathway treated by Tongqi No.1formula

GO	Description	Count	Log10(P)	Hits
hsa05200	Pathways in cancer	51	-56.85	AKT1,BIRC5,AR,BAX,CCND1,BCL2,BCL2L1,CASP3,CASP8,CASP9,CCNA2,CCND2,CDKN1A,CTNNB1,E2F1,EDN1,EDNRA,EGF,EGFR,ERBB2,ESR1,ESR2,GSK3B,GSTM1,GSTP1,HIF1A,IFNG,IGF2,IL2,IL4,IL6,CXCL8,MDM2,MET,MMP1,MMP2,MMP9,MYC,NFKBIA,NOS2,PPARG,PRKCA,MAPK1,PTGS2,RAF1,RB1,RELA,STAT1,STAT3,TP53,VEGFA
hsa05417	Lipid and atherosclerosis	30	-37.46	AKT1,APOB,BAX,BCL2,BCL2L1,CASP3,CASP8,CASP9,MAPK14,CYP1A1,CYP2B6,CXCL2,GSK3B,ICAM1,IL1B,IL6,CXCL8,MMP1,MMP3,MMP9,NFKBIA,PPARG,PRKCA,MAPK1,RELA,CCL2,SELE,STAT3,TNF,TP53
hsa04933	AGE-RAGE signaling pathway in diabetic complications	24	-35.98	AKT1,BAX,CCND1,BCL2,CASP3,MAPK14,EDN1,ICAM1,IL1A,IL1B,IL6,CXCL8,MMP2,SERPINE1,PRKCA,MAPK1,RELA,CCL2,SELE,STAT1,STAT3,THBD,TNF,VEGFA
hsa05207	Chemical carcinogenesis - receptor activation	29	-35.92	AHR,AKT1,BIRC5,AR,CCND1,BCL2,CREB1,CYP1A1,CYP1A2,CYP1B1,CYP2B6,CYP3A4,E2F1,EGF,EGFR,ESR1,ESR2,GSTM1,MYC,PGR,PPARA,PRKCA,MAPK1,RAF1,RB1,RELA,STAT3,VEGFA,NR1I3
hsa05205	Proteoglycans in cancer	27	-32.92	AKT1,CCND1,CASP3,CAV1,CDKN1A,MAPK14,CTNNB1,EGFR,ERBB2,ESR1,HIF1A,IGF2,ITGB3,KDR,MDM2,MET,MMP2,MMP9,MYC,PLAU,PRKCA,MAPK1,RAF1,STAT3,TNF,TP53,VEGFA
hsa04218	Cellular senescence	22	-27.44	AKT1,CCND1,CCNA2,CCNB1,CCND2,CDKN1A,CHEK1,MAPK14,E2F1,IGFBP3,IL1A,IL6,CXCL8,MDM2,MYC,SERPINE1,MAPK1,R

				AF1,RB1,RELA,TP53,CHEK2
hsa04115	p53 signaling pathway	16	-23.28	BAX,CCND1,BCL2,BCL2L1,CASP3,CASP8,CASP9,CCNB1,CCND2,CDKN1A,CHEK1,IGFBP3,MDM2,SERPINE1,TP53,CHEK2
hsa04630	JAK-STAT signaling pathway	18	-20.33	AKT1,CCND1,BCL2L1,CCND2,CDKN1A,EGF,EGFR,IFNG,IL2,IL4,IL6,IL10,MCL1,MYC,RAF1,STAT1,STAT3
hsa04919	Thyroid hormone signaling pathway	16	-19.52	AKT1,CCND1,CASP9,CTNNB1,ESR1,GSK3B,HIF1A,ITGB3,MDM2,MYC,PRKCA,MAPK1,RAF1,STAT1,TP53,NCOA2
hsa04926	Relaxin signaling pathway	15	-17.45	AKT1,CREB1,MAPK14,EDN1,EGFR,MMP1,MMP2,MMP9,NFKBIA,NOS2,PRKCA,MAPK1,RAF1,RELA,VEGFA
hsa05133	Pertussis	12	-15.7	CASP3,MAPK14,IL1A,IL1B,IL6,CXCL8,IL10,IRF1,NOS2,MAPK1,RELA,TNF
hsa04915	Estrogen signaling pathway	14	-15.46	AKT1,BCL2,CREB1,CTSD,EGFR,ESR1,ESR2,MMP2,MMP9,OPRM1,PGR,MAPK1,RAF1,NCOA2
hsa04064	NF-kappa B signaling pathway	12	-13.99	PARP1,BCL2,BCL2L1,CXCL2,ICAM1,IL1B,CXCL8,NFKBIA,PLAU,PTGS2,RELA,TNF
hsa04934	Cushing syndrome	12	-11.89	AHR,CCND1,CDKN1A,CREB1,CTNNB1,E2F1,EGFR,GSK3B,HSD3B1,HSD3B2,MAPK1,RB1
hsa05216	Thyroid cancer	8	-11.79	BAX,CCND1,CDKN1A,CTNNB1,MYC,PPARG,MAPK1,TP53
hsa04931	Insulin resistance	10	-10.76	AKT1,CREB1,GSK3B,IL6,INSR,NFKBIA,PPARA,RELA,STAT3,TNF
hsa05415	Diabetic cardiomyopathy	12	-10.51	PARP1,AKT1,MAPK14,CTSD,G6PD,GSK3B,INSR,MMP2,MMP9,PPARA,PRKCA,RELA
hsa04913	Ovarian steroidogenesis	7	-8.91	CYP1A1,CYP1B1,HSD3B1,HSD3B2,INSR,PTGS2,AKR1C3
hsa04211	Longevity regulating pathway	8	-8.6	AKT1,BAX,CAT,CREB1,INSR,PPARG,RELA,TP53
hsa04914	Progesterone-mediated oocyte maturation	8	-8.13	AKT1,CCNA2,CCNB1,CDC25C,MAPK14,PGR,MAPK1,RAF1

3.6. Construction of the pathway network diagram of Tongqi No.1 formula component - tubal obstructive infertility

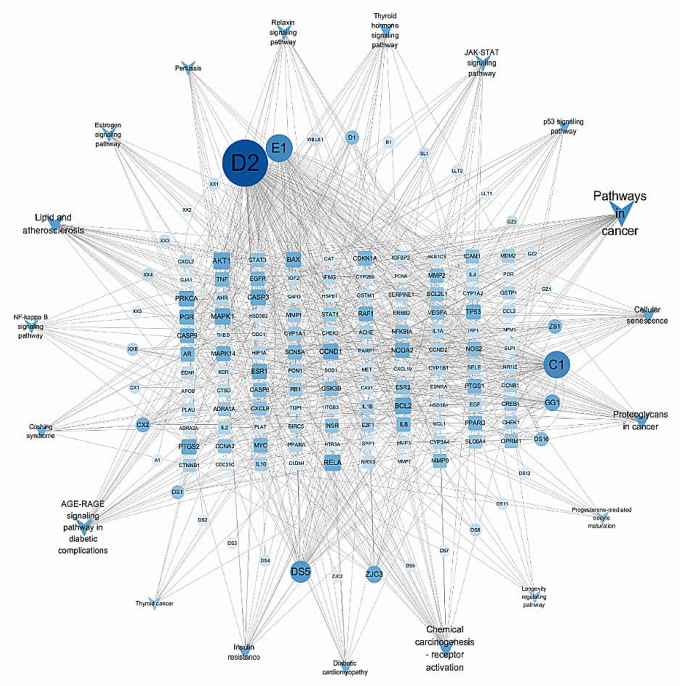


Figure 5: Tongqi No.1-tubal obstructive infertility-access network diagram

CytoScape3.7.1 software is required to construct the pathway network diagram of Tongqi Square No.1 - Tubal obstructive infertility, and the result is shown in Figure 5.The built-in NetworkAnalyzer subfunction of the software was used to analyze the network topology parameters of "Tongqi No.1 prescription" for tubal obstructive infertility, and the core components and core action targets were obtained.

Table 4: Node characteristic parameters of main active component network of Tongqi No.1 formula

MOLID	name	Degree	BetweennessCentrality	ClosenessCentrality
MOL000098	quercetin	117	0.31549765	0.49142857
MOL000422	kaempferol	54	0.08431593	0.40375587
MOL000358	beta-sitosterol	52	0.03717659	0.36909871
MOL000006	luteolin	34	0.04869032	0.37885463
MOL013179	fisetin	18	0.02863796	0.34677419
MOL000392	formononetin	17	0.03923164	0.38222222
MOL002135	Myricanone	11	0.01335066	0.36134454
MOL013277	Isosinensetin	10	0.01178996	0.34677419
MOL007154	tanshinone iia	9	0.01478842	0.32089552
MOL000449	Stigmasterol	9	0.02780135	0.33076923

The analysis of Cytoscape network showed that, Degree of quercetin was 117, betweenness 0.315, ClosenessCentrality 0.491.According to the analysis of the results, it is predicted that the main components of the treatment of tubal obstructive infertility are quercetin, followed by kaempferol, β-sitosterol, etc. The main active components are shown in Table 4.

The connection degree of AKT1 in the network was 18, the mediality was 0.03, and the tightness was 0.45. It was predicted that AkT1 was the most important target of Tongqi No.1 formula in the treatment of tubal obstructive infertility.The next are BCL2, MAPK1, RELA, CCND1, CASP3, PPKCA, BAX, ESR1 and PTGS2, as shown in Table 3.

3.7. Molecular Docking

The binding energy of ligand and receptor is inversely proportional to the required energy. Binding energy <0kcal/mol indicates that the receptor and ligand can spontaneously bind, while binding energy <-5kcal/mol indicates that the receptor and ligand have good binding energy activity.The main active ingredients of Tongqi No.1 formula and its important targets were respectively docked. Tongqi No.1 formula had 12 main active ingredients and 10 important targets, a total of 120 docked times.The results are shown in the form of heat map, as shown in Figure 6. The binding energy of AKT1 and tanshinoneiia (-11.4kcal/mol) was the best. The molecular docking results showed that tanshinone IIA could stably bind to the active pocket of AKT1 protein structure 4EJN, and the two could bind through amino acid residues ASN53, ASN51, VAL-271, VAL-270,SER-205 and GLN-203 were hydrogen-bonded, and their binding results were visualized by pymol, as shown in Figure 7. Except HIFIA protein had poor docking with its active components, other active components had good docking with proteins.

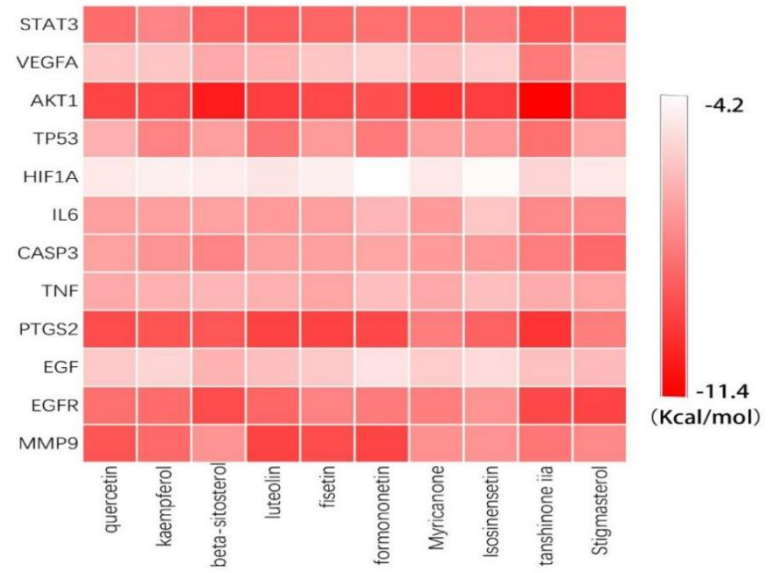


Figure 6: Heat map

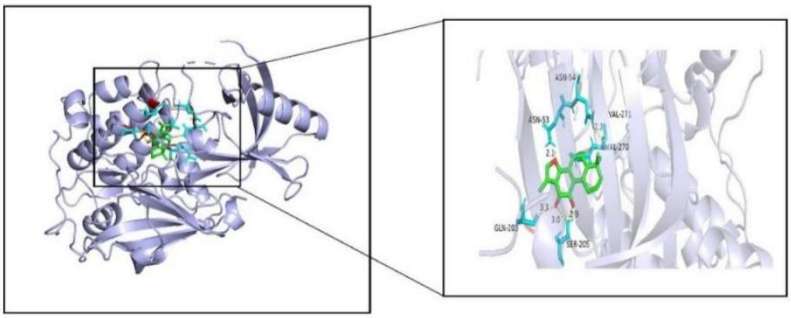


Figure 7: Results of tanshinone IIA and AKT1

4. Discuss current research

Currently, it is believed that tubal obstruction caused by inflammation is the most common pathogenic factor in tubal obstructive infertility.Inflammation or scar formation in the fallopian tube can cause fallopian tube obstruction, resulting in abnormal peristaltic function of the fallopian tube, and eventually infertility [10]. At present, most traditional Chinese medicine experts believe that the disease that causes tubal obstructive infertility is caused by "blood stasis", and its pathogenesis is that blood stasis obstructs cell collaterals and eventually leads to infertility. Therefore, the method of promoting blood stasis and smoothing collaterals and dispersing nodes should be used throughout the treatment, with the same treatment of specimens [11]. Based on more than 50 years of clinical and basic research, Tongqi I prescription was established to treat tubal obstructive infertility, and the clinical effect is remarkable.

Through this study on the molecular mechanism of "Tongqi No. I prescription" in the treatment of tubal obstructive infertility, the results showed that Tongqi No.1 prescription contained 61 active ingredients corresponding to 215 target genes, and the main active ingredients included quercetin, urushiflavin, kaempferol, etc. Topological analysis of 119 predicted therapeutic targets in PPI showed that STAT3, VEGFA, AKT1, TP53, HIFIA, IL-6, CASP3, TNF, PTGS2, EGF, EGFR and MMP9 were important targets for Tongqi No.1 prescription in the treatment of tubal obstructive infertility. According to KEGG cluster analysis and target pathway analysis, Tongqi No.1

prescription has multi-target and multi-pathway effects in the treatment of tubal infertility.Among them, the most involved pathways were JAK-STAT signaling pathway (hsa04630), NF-κB signaling pathway (hsa04064), estrogen signaling pathway (hsa04915) and P53 signaling pathway (hsa04115).

Modern medicine believes that inflammation can lead to local inflammatory exudation, edema, hyperplasia, and eventually obstruction of the fallopian tube.In the process of inflammation and infection, plasma proteins and immune cells will appear at the site of infection and injury, which play the role of mediating inflammatory response to eliminate invading pathogens and promote tissue repair [12]. At the same time, the intracellular signaling pathway will be activated to further promote inflammatory response. In this process, inflammatory cells will produce many inflammatory mediators, the most important pro-inflammatory factors are IL-6 and TNF-a [13]. IL-6 is a kind of glycoprotein with various biological activities [14], which is mainly secreted by macrophages or epithelial cells. It phosphorylates AKT in macrophages, keeps macrophages viable, enhances the contraction activity of fibroblasts, and eventually causes severe scar. Various chlamydia can enter host cells using different but related cell surface receptors that may share a common downstream signaling process for scarring via G proteins, particularly through the P13K-AKT pathway [15]. Bai Xue et al. [16] found that the mrna expression of pro-inflammatory factor IL-6 in patients with tubal inflammatory obstructive infertility was higher than that of normal people, suggesting that IL-6 could participate in chronic inflammatory response of the body to further promote fibrosis of the inflammatory injury machine, and the final outcome would be tubal obstruction. Schols [17], a foreign scholar, confirmed that IL-6 is closely related to systemic inflammatory response, and Ulich [18] found that IL-6 may participate in the process of transforming acute inflammation into chronic inflammation, making the acute episode of tubal inflammation turn into chronic episode, and further lead to local tissue adhesion of the tubal. Further leads to granulation tissue, fibrous tissue growth, resulting in tubal hyperplasia, fibrosis, and eventually the formation of tubal obstructive infertility. Tumor necrosis factor a (TNF-a) is a protein in essence, and fibrinous adhesion is closely connected with it, and it is also the crossing center of different signal transduction pathways. It activates fibroblasts and has strong adhesion to white blood cells while participating in the inflammatory and immune responses of the body, thus aggravating the damage of fallopian tubes.TNF-a can also mediate the release of IL-6, IL-8 and other inflammatory factors [14,19]. JendroMC et al. [20] have shown that macrophages infected with chlamydia can escape the killing effect of Tcell, which is beneficial to the survival of chlamydia in cells and the establishment of continuous infection. The amount of TNF-A produced is proportional to the damage to the fallopian tubes. When IL-6 and TNF-a act synergistically, inflammatory mediators network can be formed. When the inflammatory mediators network is formed, vascular endothelial cells can be indirectly or directly damaged and a large number of inflammatory substances can be exudated, thus aggravating the damage to the fallopian tube [21]. Relevant studies [22,23] have shown that: The main causes of tubal obstructive infertility are related to TNF-a and IL-6. TNF-a can synthesize IL-6 through mediated immune response, and IL-6 can damage the epithelium and mucosa of the tubal by participating in the chronic inflammatory response of the body, thus causing tubal obstructive infertility.As epidermal growth factors and receptors, EGF, EGFR and ERBB2 can participate in cell proliferation, apoptosis and local adhesion formation.Epidermal growth factor (EGF) can promote MAPK phosphorylation, but this study was conducted in bovine oviduct epithelial cells and was not found in human oviduct epithelial cells.The EGFR pathway can also regulate Notch signaling, generate a variety of cilia in the fallopian tube, regulate the fallopian tube microenvironment, and facilitate fertilization and embryo survival.Tubal epithelial cells are activated with the participation of TP53 proteins and bal-2 family apoptosis proteins. Severe inflammation can lead to aggregation of glycoproteins and

mucus, accelerate the apoptosis of tubal epithelial cells, and cause the loss of tightly connected tubal epithelium, which is not conducive to fertilization [15].

In addition, cytokines interact with immune cell surface receptors to trigger inflammatory signals, which are mainly NF-KB, JAK/STST and MAPK signaling pathways. For NF-κB signaling pathway, studies have shown that in tubal obstructive infertility patients reproductive tract infection produced a variety of inflammatory factors, can activate TLR2 and TLR4 in the body, when TLRs activated, the signal will be transmitted along the cell to NF-κB, activate downstream NFκB molecules, and at the same time TLRs and MyD88 activation, Inflammatory cytokines such as TNF-A accumulate and inhibit the expression of factors such as IL-10. The expression of inflammatory cytokines leads to the continuous activation of NF-κB, which again releases a large number of inflammatory cytokines, forming a cascade of worsening reactions. The persistence of the above reactions will lead to the damage of the tubal tissues, the loss of cilia in large numbers in the tubal group, the atresia of the tubal umbrellas, the adhesion to the surrounding tissues, and even the formation of tubal scar due to the sustained inflammatory damage, and eventually the loss of its function leading to infertility [24].

The main active ingredient in formula I can play a role. Quercetin can delay inflammatory factors and reduce neutrophils. At the same time, it can also lower neutrophils, thus playing an anti-inflammatory role [25]. Lurushetin can inhibit the activation of NF-κB and phosphorylation of p38MAPK, which can downregulate the activity of mast cells and inhibit the interaction between mast cells and activated T cells, thus playing an anti-inflammatory role. Studies have reported that urushetin can play an anti-inflammatory role by using NF-KB, JAK-STAT, p13k-AKT, MAPK and other pathways to restrict the expression of toll-like receptor 4, TNF-a, interleukin, chemokines and other inflammatory mediators [26,27]. The anti-inflammatory effects of luteolin [28] are mainly directed at transcription factors, such as signal transduction and the regulation of transcription activator 3 (STAT3), and can alter various signaling pathways involved in inflammation, or its anti-inflammatory effects can inhibit the release of PGE2 and the expression of NF-κB in the nucleus and bind to DNA.Further down-regulation of COX-2 expression was associated with [28,29]. Studies have shown that the imbalance between the coagulation system and the fibrinolytic system is related to repeated infection and chronic inflammation, which increases the blood viscosity and leads to the occurrence of tissue adhesion. Tanshinone IIA can directly block the abnormal activation of NF-KB and regulate the expression of VCAM-1 and ICAM-1 induced by inflammatory factors to achieve clinical anti-inflammatory purposes [30]. Kaempferol prevents NF-κB from entering the nucleus, thereby reducing the release of inflammatory mediators [31]. JNC et al. [32] found that β-sitosterol plays an anti-inflammatory role by reducing the synthesis of NO, inhibiting the activity of IL-6 in macrophages, and reducing the secretion of IL-1, TNF-a and other inflammatory factors.

In summary, this study found that the possible mechanism of Tongqi No.1 prescription in treating tubal obstructive infertility is as follows: 1.Through NF-κB, JAK-STAT, p13k-AKT, MAPK and other pathways, the activation of NF-κB can be inhibited, the expression of toll-like receptor 4, TNF-a and other inflammatory mediators can be inhibited, and the activity of mast cells can be downregulated, and the interaction between mast cells and activated T cells can be weakened to play the anti-inflammatory role.2. The abnormal activation of NF-κB may play a role in blocking, and then regulate the expression of VCAM-1 and ICAM-1 induced by inflammatory factors to achieve clinical anti-inflammatory purposes [32]. By reducing the synthesis of NO, it can also act on IL-6 in macrophages to decrease its IL-6 activity and reduce the secretion of IL-1, TNF-a and other inflammatory factors.It can be seen that Tongqi No.1 prescription treats tubal obstructive infertility through multiple components, multiple targets and multiple approaches, which provides new clinical guidance for the treatment of tubal obstructive infertility and also provides theoretical

support for the treatment of tubal obstructive infertility. However, the dosage of Tongqi No.1 prescription is not taken into account in this study.And the changes of TCM after decocting, its metabolites in vivo were not included in the analysis.Further experimental verification will be carried out in the future to further study the main molecular mechanism of Tongqi I formula.

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