Research Paper

Network Pharmacological Study of Yiyi-Fuzi-Baijiang powder in Treating Colorectal Cancer

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This investigation dealt with the analysis of the active ingredients of *Yiyi-Fuzi-Baijiang* powder in the treatment of colorectal cancer using computer network pharmacology technology to predict the mechanism of action. The chemical constituents and targets of *Yiyi-Fuzi-Baijiang* powder were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform and the common targets in the treatment of colorectal cancer were obtained from Online Mendelian Inheritance in Man, Therapeutic Target Database and PharmGkb database. Excel was used to screen the molecular target and Cytoscape software was used to establish the network of Chinese medicine components target of *Yiyi-Fuzi-Baijiang* powder. The gene function and metabolic pathway were analyzed using the biological information annotation database. Nineteen components of *Yiyi-Fuzi-Baijiang* powder were found to interact with 121 target proteins of colorectal cancer, including progesterone receptor, prostaglandin G/H synthase 2, gamma-aminobutyric acid receptor subunit alpha-1, prostaglandin G/H synthase 1, nuclear receptor co-activator 2, beta-2 adrenergic receptor, sodium channel protein type 5 subunit alpha, alpha-1B adrenergic receptor. It is the target of *Chaihushugansan* in the treatment of post-stroke depression, mainly involving in neuroactive ligand-receptor interaction, endocrine regulated calcium reabsorption, TGF-β signaling pathway, Hedgehog signaling pathway, inflammatory mediator regulation transient receptor potential channels, cholinergic synapse, through the regulation of the nervous system, cell cycle, apoptosis, inflammatory regulation, cell communication and other biological processes to treat colorectal cancer. The possible mechanism of *Yiyi-Fuzi-Baijiang* powder in treating colorectal cancer was revealed by network pharmacological study, which laid a foundation for further elucidation of its action target.

Key words: Coix seed, monkshood, Patrinia villosa, colorectal cancer, network pharmacology

Colorectal cancer, including colorectal and rectal cancer, is the third most common cause of cancer death in the world. Its 5 y survival rate is only 30-65 %[1]. In recent years, the incidence of colorectal cancer has increased gradually in developing countries. The incidence and mortality of colorectal cancer in China ranks fifth and sixth, respectively among all the malignant tumours in the urban population, which has seriously threatened the health of people in China[2]. Its prevention and treatment has become a hot research topic[3-5]. Colorectal cancer in traditional Chinese clinical medicine clinical was grouped under intestinal *Qin*, accumulation, locking anal hemorrhoids, visceral toxin and scattered in enteropathy, intestinal wind, diarrhoea, colon *Yin*, intestinal wind blood and other diseases. The treatment outcomes of traditional Chinese medicine is significant[6-8]. The early clinical research and animal experiment of the research group confirmed that *Yiyi-Fuzi-Baijiang* powder showed good curative effect in the treatment of colorectal cancer[9]. However, due

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to the regulation of multi-component and multi-target of traditional Chinese medicine, the mechanism of *Yiyi-Fuzi-Baijiang* powder in the treatment of colorectal cancer is not clear.

Network pharmacology is a new method to study the mechanism of traditional Chinese medicinal compounds[10]. The purpose of this study is to explore the potential effective components and possible mechanism of *Yiyi-Fuzi-Baijiang* powder in the treatment of colorectal cancer and to systematically explore the overall regulatory effect of the multi-component and multi-target drug on colorectal cancer at the molecular level, so as to provide a basis for the further research and development of compounds in traditional Chinese medicine.

MATERIALS AND METHODS

Traditional Chinese medicine systems pharmacology (TCMSP) is a platform integrating pharmacokinetics, pharmaceutical chemistry and drug target protein network disease network[11]. Based on the TCMSP database, the chemical constituents of Coix, aconite and *Patrinia villosa* were searched. All the chemical components were screened by Excel and the component data were obtained.

Research tools:

TCMSP; UniProt Database to obtain gene information of components, targets, interrelations and targets of traditional Chinese Medicine; Online Frontal Analysis Mendelian Inheritance in Man (OMIM)[12,13], Drug target database, Databases/TTD/TTD.asp and PharmGkb database acquisition of target proteins for stroke and depression; Cytoscape 3.2.1 software building a network of compound target interaction[12-16].

Screening of active compounds of *Yiyi-Fuzi-Baijiang* powder:

The target organs and tissues for traditional Chinese medicine should be achieved through the process of absorption, distribution, metabolism and excretion (ADME). Oral bioavailability (OB), drug likeness (DL), blood-brain barrier (BBB) and half-life (HL) are the key parameters of ADME. In this study, TCMSP data platform was used to evaluate OB, DL, BBB and HL of each active component of *Yiyi-Fuzi-Baijiang* powder and selected chemical components that simultaneously meet the requirements of OB ≥30 %, DL ≥0.18, BBB ≥0.3 and HL ≥4 h as the candidate active components.

Target selection of *Yiyi-Fuzi-Baijiang* powder in the treatment of colorectal cancer:

GADCC, therapeutic target database (TTD) and PharmGKB databases were used to search the coding genes of colorectal cancer to determine the disease target. Based on the molecular docking technology, the effective components and disease targets of *Yiyi-Fuzi-Baijiang* powder were screened and the target of *Yiyi-Fuzi-Baijiang* powder in the treatment of colorectal cancer was obtained.

Biological process and metabolic pathway analysis of target:

Database for annotation, visualization and integrated discovery (DAVID) can provide annotated information of biological functions and find the most significant biological annotation. By inputting the list of target gene names and defining the species as human, and correcting all the target gene names to their official names, the enrichment analysis of go biological process and the metabolism of Kyoto Encyclopaedia of genes and genomes (KEGG) pathway analysis were carried out pathway enrichment analysis.

RESULTS AND DISCUSSION

The results showed that there were 19 active molecules in *baizao* powder that included 6, sitosterol α1, mandenol, (2R)-2,3-dihydroxypropyl(z)-octadec-9-enoate, sitosterol, stigmasterol and CLR; 6, 11,14-eicosadienoic acid, deltoin, karakoline, karanjin, neonadsuranic acid B, sitosterol and 7 of *Patrinia villosa* L, morrianine C, asperglauccide, acacetin, sinoacutine, β-sitosterol, sitosterol, and stigmasterol (Table 1). Through UniProt database, the target points of the above ingredients are transformed into gene names, and the composition target map of traditional Chinese medicine is constructed (fig. 1).

Using GADCC, TTD and PharmGKB database to search the coding genes of colorectal cancer, there are 121 targets as disease targets (Table 2). Based on the molecular docking technology, 19 active ingredients and 121 disease targets of *Coix Fuzi baizao* powder were screened, and 8 well docking targets were obtained as the targets of *Coix Fuzi baizao* powder in the treatment of colorectal cancer (Table 3), respectively progesterone receptor, prostaglandin G/H synthase 2, γ-aminobutyric acid receptor subunit -1, prostaglandin G/H synthase 1, nuclear receptor coactivator 2, β-2 adrenergic receptor, sodium channel protein type 5 subunit α, α-1B adrenergic receptor.

TABLE 1: CANDIDATE ACTIVE COMPONENTS AND PHARMACOKINETIC PARAMETERS OF *YIYI-FUZI-BAIJIANG* POWDER

Number	Component	OB/%	DL	BBB	HL	Medicinal Materials
MOL001323	Sitosterol α1	43.28	0.78	0.97	5.64	Coix seed
MOL001494	Mandenol	42	0.19	1.14	5.39	Coix seed
MOL002882	(2R)-2,3-dihydroxypropyl (Z)-octadec-9-enoate	34.13	0.3	-0.22	5.19	Coix seed
MOL000359	Sitosterol	36.91	0.75	0.87	5.37	Coix seed
MOL000449	Stigmasterol	43.83	0.76	1	5.57	Coix seed
MOL000953	CLR	37.87	0.68	1.13	4.52	Coix seed
MOL002211	11,14-eicosadienoic acid	39.99	0.2	0.76	5.6	monkshood
MOL002392	Deltoin	46.69	0.37	-0.12	7.7	monkshood
MOL002397	Karakoline	51.73	0.73	-0.03	11.1	monkshood
MOL002398	Karanjin	69.56	0.34	0.62	13.15	monkshood
MOL002401	Neokadsuranic acid B	43.1	0.85	-0.01	12.05	monkshood
MOL000359	Sitosterol	36.91	0.75	0.87	5.37	monkshood
MOL001676	Vilmorrianine C	33.96	0.22	0.14	21.71	*Patrinia villosa*
MOL001677	Asperglaucide	58.02	0.52	-0.22	6.88	*Patrinia villosa*
MOL001689	Acacetin	34.97	0.24	-0.05	17.25	*Patrinia villosa*
MOL001697	Sinoacutine	63.39	0.53	0.36	4.9	*Patrinia villosa*
MOL000358	β-sitosterol	36.91	0.75	0.99	5.36	*Patrinia villosa*
MOL000359	Sitosterol	36.91	0.75	0.87	5.37	*Patrinia villosa*
MOL000449	Stigmasterol	43.83	0.76	1	5.57	*Patrinia villosa*

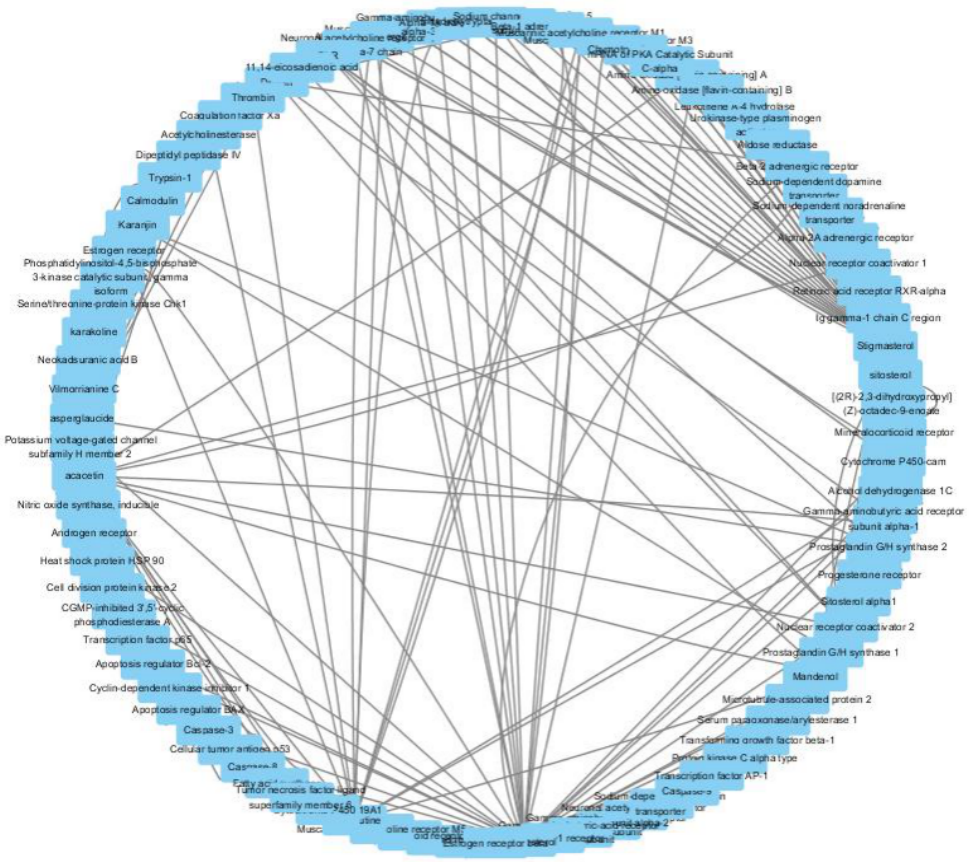

Fig. 1: Composition target map of *Yiyi-Fuzi-Baijiang* powder

The results showed that the 8 predicted targets were related to biological processes such as neural regulation, cell cycle, apoptosis, inflammatory regulation, cell communication and so on (Table 4). It is suggested that *Yiyi-Fuzi-Baijiang* powder may play an anticancer role by improving these biological processes. The results of

TABLE 2: DISEASE TARGETS

1	Vascular endothelial growth factor receptor 1	65	PTGS1
2	Vascular endothelial growth factor receptor 2	66	PTGS2
3	Potassium-transporting ATPase α chain 1	67	ADRB2
4	Retinoic acid receptor α	68	ADH1B
5	DNA topoisomerase I	69	FH
6	DNA topoisomerase II	70	ZFHX3
7	FL cytokine receptor	71	MXI1
8	Histone deacetylase 1	72	ADRA1B
9	Tumor necrosis factor	73	EIF4G1
10	Calcium channel	74	OPCML
11	MLH3	75	NKX2-1
12	AXIN2	76	GABRA-1
13	MCC	77	PTPRJ
14	PIK3CA	78	HMMR
15	AKT1	79	SMAD4
16	FGFR3	80	ATR
17	MSH6	81	ACVR1B
18	CHEK2	82	BARD1
19	TP53	83	NCOA-2
20	MUTYH	84	STK11
21	DCC	85	PRKN
22	BRAF	86	FOXE1
23	PMS2	87	MAD1L1
24	DLC1	88	PHB
25	PDGFRL	89	RAD51C
26	CTNNB1	90	FGFR2
27	MSH2	91	RAD51D
28	APC	92	AURKA
29	MLH1	93	PPP2R1B
30	TGFBR2	94	RAD54L
31	NRAS	95	HABP2
32	CCND1	96	RAD54B
33	PLA2G2A	97	SCN5A
34	BAX	98	ELAC2
35	POLE	99	RAD51
36	POLD1	100	BRIP1
37	BUB1	101	RNASEL
38	EP300	102	IL1RN
39	BUB1B	103	IL1B
40	SMAD7	104	SRGAP1
41	TLR2	105	RB1CC1
42	FLCN	106	MPO
43	GALNT12	107	ATM
44	EPCAM	108	SASH1
45	BRCA2	109	PALLD
46	KRAS	110	ERCC6
47	CDH1	111	EHBP1
48	BRCA1	112	CHRNA3
49	EGFR	113	CHRNA5
50	ERBB2	114	RNF43
51	CDKN2A	115	SDCCAG8
52	EPHB2	116	CYP2A6
53	PTEN	117	RHBDF2
54	CASP8	118	MSMB
55	KLF6	119	HRAS
56	SLC22A18	120	SRC
57	SLC22A18	121	NQO1
58	PPM1D		
59	IRF1		
60	AR		
61	PALB2		
62	RB1		
63	MAP3K8		
64	PGR		

TABLE 3: POTENTIAL TARGET OF *YIYI-FUZI-BAIJIANG* POWDER IN THE TREATMENT OF COLORECTAL CANCER

number	Target protein	Gene name
MOL000359	Progesterone receptor	PGR
MOL000449	Prostaglandin G/H synthase 2	PTGS2
MOL000449	Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1
MOL000449	Prostaglandin G/H synthase 1	PTGS1
MOL000359	Nuclear receptor coactivator 2	NCoA-2
MOL000449	Beta-2 adrenergic receptor	ADRB2
MOL000449	Sodium channel protein type 5 subunit alpha	SCN5A
MOL000449	Alpha-1B adrenergic receptor	ADRA1B

TABLE 4: GO BIOLOGICAL PROCESS ENRICHMENT ANALYSIS OF TARGET

Biological Process	Related Targets	P	Benjamini
Neural regulation	PTGS2, ADRB2	0.00575	0.21
Cell cycle	PGR, SCN5A, ADRA1B	0.01532	0.43
Apoptosis	PTGS1, GABRA1, SCN5A	0.00784	0.20
Inflammation regulation	PTGS2, ADRB2, NCoA-2	0.01074	0.38
Cell communication	PTGS1, GABRA, SCN5A	0.00058	0.04

enrichment analysis of KEGG signal pathway of *Yiyi-Fuzi-Baijiang* powder 's anticolorectal cancer target (Table 5) showed that 8 therapeutic targets participate in 4 categories and 6 signal pathways, including neural regulation, apoptosis, cell cycle and inflammation regulation, as shown in Table 5, mainly including neuroactive ligand-receptor interaction, endocrine regulated calcium reabsorption, TGF-β signaling pathway, Hedgehog signaling pathway, inflammatory

TABLE 5: ENRICHMENT ANALYSIS OF TARGET KEGG METABOLIC PATHWAY

Metabolic pathway	Related targets	P	Benjamini
Neuroactive ligand-receptor interaction	PGR, NCoA-2, SCN5A	0.032	0.45
Endocrine regulated calcium reabsorption	NCoA-2, ADRA1B, SCN5A	0.045	0.65
TGF-β signaling pathway	PTGS2, ADRB2	0.061	0.72
Hedgehog signaling pathway	SCN5A, ADRA1B	0.015	0.33
Inflammatory mediator regulation TRP channels	PTGS1, GABRA1, SCN5A	0.050	0.24
Cholinergic synapse	PTGS2, ADRB2	0.012	0.46

mediator regulation TRP channels, cholinergic synapse. The results showed that the main active components of *Yiyi-Fuzi-Baijiang* powder were distributed in different metabolic pathways, and the possible mechanism of action was to coordinate and regulate each other.

Yiyi-Fuzi-Baijiang powder comes from one of the 4 classics of traditional Chinese medicine, synopsis of the Golden Chamber ulceration, carbuncle, intestinal abscess, soakage, pulse syndrome and treatment of the 18th: the intestine carbuncle is a disease where the body is wrong, the abdomen is urgent, according to it, if it is swollen, the abdomen is not accumulated, the body is not hot, the pulse number, this is the intestine has carbuncle pus, *Yiyi-Fuzi-Baijiang* powder main. This prescription is composed of Coix seed, aconite and *Patrinia villosa*. It was first recorded in the treatment of intestinal carbuncle. It is mainly used for the treatment of intestinal carbuncle and pus[17]. It has the function of eliminating carbuncle and removing pus, and strengthening *yang qi*. In modern times, it is used in intestinal, skin, gynecology, andrology and other diseases, as well as dry skin, abnormal keratosis and other diseases[18-20]. It is most commonly used in the treatment of abscess and purulent inflammation[18]. In the prescription, Coix seed is used to eliminate carbuncle and swelling, *Patrinia villosa* is used to clear heat, detoxify and discharge pus, while *Fuzi Wenyang* powder is used to relieve cold and pain. Modern pharmacology research showed that many monomers in *Yiyi-Fuzi-Baijiang* powder have anticancer effect.

In this investigation it was found that sitosterol α-1, mandenol, (2R)-2,3-dihydroxypropyl (Z)-octadec-9-enoate, sitosterol, stigmasterol, CLR, 11,14-eicosadienoic acid, deltoin, karakoline, karanjin, neokadsuranic acid B, sitosterol, vilmorrianine C, asperglaucide, acacetin, sinoacutine, β-sitosterol, sitosterol, stigmasterol as 19 main active ingredients in Coix, aconite and *Patrinia*, acting on progesterone receptor, prostaglandin G/H synthase 2, γ-aminobutyric acid receptor subunit α-1, prostaglandin G/H synthase 1, nuclear receptor coactivator 2, β-2 adrenergic receptor, sodium channel protein type 5 subunit α, α-1B adrenergic receptor several potential targets. These targets may play an important role in the antitumor network. The enrichment analysis of go biological process showed that the target genes of *Yiyi-Fuzi-Baijiang* powder were involved in the biological processes of neural regulation, cell cycle, apoptosis, inflammation regulation, cell communication and so on. These biological processes are closely related to the development of nervous system and cancer cells and may be involved in the pathophysiological process of colorectal cancer. Some targets are related to cell cycle, apoptosis and inflammation control, which proved that the alkaloid extract of *Yiyi-Fuzi-Baijiang* powder has good analgesic and antiinflammatory effect, could regulate the central nervous system and has a certain central nervous inhibitory effect[21-23]. Therefore, *Yiyi-Fuzi-Baijiang* powder can produce antitumor activity by inhibiting tumor growth and migration, regulating energy, regulating neural system multi phenotype intervention network mode, and has a certain relieving effect on cancer pain.

The results of KEGG metabolism pathway analysis showed that the related targets of the active components in the powder were, progesterone receptor, prostaglandin G/H synthase 2, γ-aminobutyric acid receptor subunit α-1, prostaglandin G/H synthase 1, nuclear receptor coactivator 2, β-2 adrenergic receptor, sodium channel protein type 5 subunit alpha, α-1B adrenergic receptor participating in neuroactive ligand-receptor interaction, endocrine regulated calcium reabsorption, TGF-β signalling pathway, Hedgehog signaling pathway, inflammatory mediator regulation TRP channels, cholinergic synapse signal pathway. Neuroactive ligand-receptor interaction[24], endocrine regulated calcium reabsorption[25], cholinergic synapse[26], Hedgehog signaling pathway[27] are mainly related to the inflammatory reaction and the occurrence and development of cancer cells and participates in the process of cell cycle change and apoptosis. TGF-β signaling pathway[28] can affect PTGS2 –and ADRB2 gene to mediate brain activity, thus affecting the transmission of neural information. Inflammatory mediator regulation TRP channels[29] mainly involved

in cell communication and related to the growth and development of cancer cells. In this study, the target of *Coix Fuzi-Baijiang* powder in the treatment of colorectal cancer was involved as neuroactive ligand-receptor interaction (3 targets), endocrine regulated calcium reabsorption (3 targets), TGF-β signalling pathway (2 targets), Hedgehog signalling pathway (2 targets), inflammatory mediator regulation TRP channels (3 targets) and cholinergic synapse (2 targets). It is suggested that the active components of *Yiyi-Fuzi-Baijiang* powder may play an antiinflammatory and analgesic role by participating in the above pathway, thereby reducing the inflammatory response and regulating the nervous system.

In conclusion, this study described the reticular relationship among the main active components, targets and pathways of *Yiyi-Fuzi-Baijiang* powder. It was found that *Yiyi-Fuzi-Baijiang* powder not only has neuromodulatory effect, but also has multi-dimensional pharmacological effects such as anticancer and antiinflammatory. However, network pharmacology research is inseparable from the integrity and practicability of related databases and there are some differences between the technology of molecular simulation docking and the environment *in vivo*. Therefore, the relevant targets and molecular mechanisms of the research still need further experimental verification.

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Conflict of interest:

All authors report no conflicts of interest in this work.

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