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Network-Based Pharmacology for the Treatment of Chronic Atrophic Gastritis with Danqi Quyu Zhitong Granules and its Mechanism of Action in Regulating Ferroptosis

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Abstract

Objective: Based on bioinformatics and network pharmacology, we investigated the active ingredients, targets and pathways of Danqi Quyu Zhitong Granules in the treatment of chronic atrophic gastritis（CAG) and the regulation of Ferroptosis.

Methods: The active ingredients and CAG-associated targets of DQQYZT were screened by TCMSP, PubChem, STRING, OMIM, Gene Cards and DisGeNet platforms; The active ingredient-CAG target network of DQQYZT was constructed by Cytoscape software; The interaction network between the compound and CAG target protein was constructed by combining with STRING database. The DAVID database was used for enrichment analysis; The FerrDb database was used to obtain iron death regulatory genes, and finally the common target genes were obtained to construct the DQQYZT active ingredient-CAG-iron death network; The PPI network was combined with the STRING database; The DAVID database was used for KEGG analysis.

Results: Screening of 192 drug active ingredients and 86 CAG-associated target genes by GO

and KEGG analysis, the biological process of DQQYZT for the treatment of CAG is related with ofextrinsic apoptotic signaling pathway in absence of ligand, response to antibiotic, protein phosphorylation, striated muscle cell differentiation, peptidyl-serine phosphorylation, RNA polymerase II sequence-specific DNA binding transcription factor binding, protein kinase activity and so on. And it plays a role in regulating Apoptosis, Apoptotic Signaling in Response to DNA Damage, Role of Mitochondria in Apoptotic Signaling, p53 signaling pathway and Cellular senescence pathways. 27 genes common to the regulation of Ferroptosis may be involved in Signaling pathways such as Signaling by Interleukins, Th17 cell differentiation pathway, Interleukin-4 and Interleukin-13 signaling, IL-17 signaling pathway and Cytokine Signaling in Immune system.

Conclusion: This study predicts the main compounds, targets and pathways of DQQYZT for the treatment of CAG and its regulation of iron death, providing theoretical reference.

Keywords: Danqi Quyu Zhitong Granules; Chronic Atrophic Gastritis; Network Pharmacology; Bioinformatics; Ferroptosis; Mechanism of Action

Introduction

Chronic atrophic gastritis (CAG) is a chronic digestive disease characterised by atrophy, reduction or thinning of the intrinsic gastric glands, pyloric glandular hyperplasia and intestinal glandular hyperplasia as a result of repeated long-term damage to the gastric mucosa epithelium. The progression of the disease is characterised by intestinal epithelial metaplasia and heterogeneous hyperplasia, and the incidence of gastric cancer in CAG with intestinal epithelial metaplasia and heterogeneous hyperplasia is as high as 6%. Therefore, treatment and reversal of CAG is an important strategy to control the development of gastric cancer [1]. Recent studies have found that Chinese medicine treatment can effectively improve clinical symptoms and reverse the progression of CAG.

"Danqi Quyu Zhitong Granules" is an in-hospital preparation of our old experts in the treatment of CAG. It consists of Huang-Qi, Dan-Shen, Bai-Shao, Wu-Yao, Bei-Shashen, Di-Yu, E-Zhu, Ku-Shen, Gou-Qi-Zi, Hou-Pu, San-Qi, Chai-Hu, Huang-Lian, Gui-Zhi, Ding-Xiang and Da-Huang. Huang-Qi nourishes the vital energy of the lung and spleen, while Dan-Shen invigorates blood stasis and nourishes the blood. The combination of the two herbs benefits the Qi and invigorates the blood, so that the Qi can flow through the blood and the blood can flow through the Qi. E-Zhu, which is warm in nature, belongs to the liver and spleen meridians, and is effective in breaking up blood and moving Qi, eliminating stagnation and relieving pain. The combination of Huang Qi and E-Zhu is the first remedy used by Mr Zhu Liangchun, a master of Chinese medicine, to treat chronic atrophic gastritis, in the sense of Mr Zhang Xichun's "Shiquan Yuzhen Decoction", which treats consumptive disease by both attacking and tonifying. Bupleurum detoxifies the liver and relieves depression, moves Qi and relieves pain; Hou-Pu regulates Qi and harmonizes the stomach, relieves lumpiness and fullness; Wu-Yao smooths Qi and opens up depression, disperses cold and relieves pain; The three herbs together detoxify the liver, move Qi and open up depression to help Qi move and disperse stasis. Da-Huang, Huang-Lian, Ku-Shen and Di-Yu all have the function of clearing heat and removing toxins and turbidity. The above ten herbs work together as subjects to move Qi and disperse stasis, detoxifying and resolving turbidity. As this disease is prolonged, it is easy to consume Yin and injure Blood, so it is supplemented with Sha-Shen and Gou-Qi-Zi to nourish Yin and nourish Blood. Ding-Xiang warms the middle and subdues the rebellious, disperses cold and relieves pain. Gui-Zhi warms the middle and disperses cold to relieve pain, aromatically strengthens the stomach, and also

serves as an ambidextrous medicine. These two herbs are warm in nature and can control the cold nature of Dan-Shen. The whole formula is based on tonifying the deficient and dipping the actual, treating both the symptoms and the root cause, taking the main and secondary aspects into account, mainly tonifying Qi and resolving blood stasis, supplemented by detoxifying and resolving turbidity, as well as nourishing Yin and regulating Qi to relieve pain, which together can invigorate the blood and create muscle, dispel blood stasis and relieve pain. Previous clinical studies have found that DQQYZT can improve the symptoms and clinical efficacy of CAG patients, which may be related to the regulation of intestinal flora, reduce the oxidative stress damage of gastric mucosa, and promote the recovery of gastric mucosa [2,3].

Ferroptosis is a novel form of programmed cell death in which iron-dependent reactive oxygen species (ROS) are upregulated, distinguishing it from other programmed cell death such as apoptosis, cell necrosis and cell autophagy [4]. There is growing evidence that Ferroptosis is associated with the development of a variety of diseases, including cancer [5], cardiovascular disease [6] and kidney injury [7]. Recent studies have found that Ferroptosis is involved in the development of a variety of gastrointestinal diseases, including intestinal ischemia-reperfusion injury [8], inflammatory bowel disease [9], gastric cancer [10] and colorectal cancer [11].

Studies on Ferroptosis and CAG are not yet available. However, it has been reported that Astragalus can stimulation inhibits the iron death process by regulating key iron death factors [12], which may be the reason for the association between Ferroptosis and CAG. Network pharmacology is a new subject based on the theory of systems biology, the analysis of biological systems network and the selection of specific signal nodes for multi-target drug molecular design. TCM network pharmacology methods include network-based disease gene prediction, drug target prediction, disease-specific drug function prediction, Chinese herbal medicine network construction, and drug-gene-disease network construction and analysis [13]. In recent years, a large number of studies have shown that TCM network pharmacology is a promising method to reveal the pharmacological mechanism of traditional Chinese medicine prescriptions [14], such as, a combination of computational strategies and experiments has been used to reveal the role of WFC in regulating immune response and inhibiting inflammation in CAG treatment [15]; There is also a study based on network pharmacology to elucidate the mechanism of action of Moluodan in the treatment of CAG [16]. Therefore, this study systematically investigated the active ingredients, targets and pathways of DQQYZT using network pharmacology to further reveal its mechanism of action against CAG. Secondly, the possible mechanism of action of DQQYZT was elaborated around the Ferroptosis regulatory network, and its pharmacological mechanism of action was explored to provide reference for subsequent clinical and basic application studies.

Data and Methods

Collection and screening of active ingredients of DQQYZT

The active ingredients and the corresponding target genes of all drugs of DQQYZT were screened by searching the TCMSP database (https://tcmsp.com/index.php) separately, using oral bioavailability (OB) ≥ 30% and drug similarity (DL) ≥ 0.18 as screening conditions. All the compounds included in the screening were again searched through TCMSP and PubChem databases (https://pubchem.ncbi.nlm.nih.gov) to find the corresponding potential targets.

Acquisition of CAG-related target genes

The GeneCards database (https://www.genecards.org/), OMIM database (https://omim.org/), DisGeNET database (https://www.disgenet.org/search) were searched for the keyword “Chronic Atrophic Gastritis” to obtain CAG-related target genes.

Acquisition of DQQYZT active ingredients and corresponding common target genes of CAG

The STRING database (https://cn.string-db.org/) was used to convert the potential targets of DQQYZT drug into gene IDs to obtain the corresponding gene targets. The keyword “chronic atrophic gastritis” was used to search for all CAG-related target genes in Disgenet and OMIM, and the duplicates

were removed and compared with the DQQYZT drug target genes to obtain the common target genes.

Construction of DQQYZT-CAG-related target protein interaction network

Using the STRING database (species set to "Human", threshold set to 0.4), the potential targets of DQQYZT and CAG were obtained and imported into Cytoscape 3.6.0 software to map the interaction network between the drug and CAG target proteins.

Construction of common target by gene GO analysis and KEGG pathway enrichment analysis

The targets of action of DQQYZT drugs were imported into the DAVID database (https://david.ncifcrf.gov/), and the threshold value was selected as $P < 0.001$, and the top 20 GO analysis and KEGG pathways were finally selected in combination with literature search, and bar graphs of GO analysis were plotted using Excel. OmicShare platform (https://www.omicshare.com) ploted pathway bubble maps.

Acquisition of Ferroptosis regulatory genes and comprehensive analysis of their common targets with DQQYZT and CAG

The FerrDb database (http://www.zhounan.org/ferrdb) was used to retrieve genes related to the Ferroptosis process, compile and remove duplicate genes. Finally, iron death regulatory genes were obtained and further collated with DQQYZT and CAG common target genes, and then the common genes were obtained using OmicShare analysis. The DQQYZT active ingredient-RCAG and iron death common target network were constructed, and the KEGG pathway bubble map was drawn, and the PPI network map of common target genes was constructed.

Results

Pharmacological study of DQQYZT and CAG network

Analysis of the active ingredients of DQQYZT formulae

The TCMSP analysis platform was used to search for "Huang-Qi", "Dan-Shen", "Bai-Shao", "Wu-Yao", "Bei-Sha-Shen", "Di-Yu", "E-Zhu", "Ku-Zhen", "Gou-Qi-Zi", "Hou-Pu" "San-Qi", "Chai-Hu", "Huang-Lian", "Gui-Zhi", "Ding-Xiang", "Da-Huang" pharmaceutical ingredients, etc. A total of 291 ctive ingredients were collected, 20 from Huang-Qi, 65 from Dan-Shen, 13 from Bai-Shao. 20 from Huang-Qi, 65 from Dan-Shen, 13 from Bai-Shao, 9 from Wu-Yao, 8 from Bei-Sha-Shen, 13 from Di-Yu, 3 from E-zhu, 45 from Gou-Qi-Zi, 2 from Hou-Pu, 8 from San-Qi, 17 from Chai-Hu, 14 from Huang-Lian, 7 from Gui-Zhi, 6 from Ding-Xiang, 16 from Da-Huang, among which 39 common components were identified through the TCMSP platform and PubChem database. Sixty active ingredients with no potential targets found were removed, resulting in a final collection of 192 active ingredients in Table1.

Table 1: Table of active ingredients of DQQYZT

Mol ID	Active ingredient name	OB(%)	DL	Name source of traditional Chinese medicine
MOL000211	Mairin	55.37707338	0.7761	Huangqi, Baishao, Diyu
MOL000239	Jaranol	50.82881677	0.29148	Huangqi
MOL000296	hederagenin	36.91390583	0.75072	Huangqi, Ezhu

MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.22847056	0.78288	Huangqi
MOL000354	isorhamnetin	49.60437705	0.306	Huangqi, Chaihu
MOL000371	3,9-di-O-methylnissolin	53.74152673	0.47573	Huangqi
MOL000378	7-O-methylisomucronulatol	74.68613752	0.29792	Huangqi
MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.73668801	0.9243	Huangqi
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.25545452	0.42486	Huangqi
MOL000387	Bifendate	31.09782391	0.66553	Huangqi
MOL000392	formononetin	69.67388061	0.21202	Huangqi,Kushen
MOL000417	Calycosin	47.75182783	0.24278	Huangqi
MOL000422	kaempferol	41.88224954	0.24066	Huangqi, Diyu, Chaihu, Dingxiang, Baishao
MOL000433	FA	68.96043622	0.7057	Huangqi
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.04541112	0.47943	Huangqi
MOL000098	quercetin	46.43334812	0.27525	Huangqi, Beishashen, Wuyao, Diyu, Kushen, Gouqizi, Sanqi, Chaihu, Huanglian, Dingxiang
MOL001601	1,2,5,6-tetrahydrotanshinone	38.75	0.36	Danshen
MOL001659	Poriferasterol	43.83	0.76	Danshen
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	Danshen
MOL001942	isoimperatorin	45.46	0.23	Danshen, Beishashen
MOL002222	sugiol	36.11	0.28	Danshen
MOL002651	Dehydrotanshinone II A	43.76	0.4	Danshen
MOL002776	Baicalin	40.12	0.75	Danshen, Chaihu
MOL000569	Digallate	61.85	0.26	Danshen
MOL000006	Luteolin	36.16	0.25	Danshen, Kushen
MOL007036	5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	33.77	0.29	Danshen
MOL007041	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23	Danshen
MOL007045	3α-hydroxytanshinone II a	44.93	0.44	Danshen

MOL007048	(E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic acid	48.24	0.31	Danshen
MOL007049	4-methylenemiltirone	34.35	0.23	Danshen
MOL007050	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.4	Danshen
MOL007058	formyltanshinone	73.44	0.42	Danshen
MOL007059	3-beta-Hydroxymethyllenetanshiquinone	32.16	0.41	Danshen
MOL007061	Methylenetanshinquinone	37.07	0.36	Danshen
MOL007063	przewalskin a	37.11	0.65	Danshen
MOL007064	przewalskin b	110.32	0.44	Danshen
MOL007068	Przewaquinone B	62.24	0.41	Danshen
MOL007069	przewaquinone c	55.74	0.4	Danshen
MOL007070	(6S,7R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g] benzofuran-10,11-dione	41.31	0.45	Danshen
MOL007071	przewaquinone f	40.31	0.46	Danshen
MOL007077	sclareol	43.67	0.21	Danshen
MOL007079	tanshinaldehyde	52.47	0.45	Danshen
MOL007081	Danshenol B	57.95	0.56	Danshen
MOL007082	Danshenol A	56.97	0.52	Danshen
MOL007085	Salvilenone	30.38	0.38	Danshen
MOL007088	cryptotanshinone	52.34	0.4	Danshen
MOL007093	dan-shexinkum d	38.88	0.55	Danshen
MOL007094	danshenspiroketallactone	50.43	0.31	Danshen
MOL007098	deoxyneocryptotanshinone	49.4	0.29	Danshen
MOL007100	dihydrotanshinlactone	38.68	0.32	Danshen
MOL007101	Dihydrotanshinone I	45.04	0.36	Danshen
MOL007105	epidanshenspiroketallactone	68.27	0.31	Danshen
MOL007107	C09092	36.07	0.25	Danshen
MOL007108	isocryptotanshi-none	54.98	0.39	Danshen
MOL007111	Isotanshinone II	49.92	0.4	Danshen
MOL007115	manool	45.04	0.2	Danshen
MOL007119	miltionone I	49.68	0.32	Danshen
MOL007120	miltionone II	71.03	0.44	Danshen
MOL007121	miltipolone	36.56	0.37	Danshen

MOL007122	Miltirone	38.76	0.25	Danshen
MOL007124	neocryptotanshinone ii	39.46	0.23	Danshen
MOL007125	neocryptotanshinone	52.49	0.32	Danshen
MOL007127	1-methyl-8,9-dihydro-7H-naphtho [5,6-g]benzofuran-6,10,11-trione	34.72	0.37	Danshen
MOL007130	prolithospermic acid	64.37	0.31	Danshen
MOL007132	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid	109.38	0.35	Danshen
MOL007141	salvianolic acid g	45.56	0.61	Danshen
MOL007142	salvianolic acid j	43.38	0.72	Danshen
MOL007143	salvilenone I	32.43	0.23	Danshen
MOL007145	salviolone	31.72	0.24	Danshen
MOL007150	(6S)-6-hydroxy-1-methyl-6-methylol-8,9 -dihydro-7H-naphtho[8,7-g] benzofuran-10,11-quinone	75.39	0.46	Danshen
MOL007151	Tanshindiol B	42.67	0.45	Danshen
MOL007152	Przewaquinone E	42.85	0.45	Danshen
MOL007154	tanshinone iia	49.89	0.4	Danshen
MOL007155	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g] benzofuran-10,11-dione	65.26	0.45	Danshen
MOL007156	tanshinone Ⅵ	45.64	0.3	Danshen
MOL001924	paeoniflorin	53.87	0.79	Baishao
MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl -2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.56	0.53	Baishao
MOL001918	paeoniflorgenone	87.59	0.37	Baishao
MOL000492	(+)-catechin	54.83	0.24	Baishao、Guizhi
MOL001939	Alloisoimperatorin	34.8	0.22	Beishashen
MOL001941	Ammidin	34.55	0.22	Beishashen
MOL001951	Bergaptin	41.73	0.42	Beishashen
MOL001956	Cnidilin	32.69	0.28	Beishashen
MOL000449	Stigmasterol	43.83	0.76	Beishashen, Gouqizi, Sanqi, Chaihu, Dingxiang
MOL010495	6,7-dimethoxy-2-(2-phenylethyl)chromone	31.93	0.3	Wuyao
MOL010496	DMPEC	32.38	0.39	Wuyao
MOL010907	Norboldine	40.92	0.46	Wuyao

MOL010913	C09495	77.09	0.25	Wuyao
MOL010916	nubigenol	42.55	0.19	Wuyao
MOL010917	Boldine	31.18	0.51	Wuyao
MOL000358	beta-sitosterol	36.91	0.75	Baishao, Beishashen, Wuyao, Gouqizi, Sanqi, Guizhi, Dingxiang, Dahuang
MOL000359	sitosterol	36.91	0.75	Baishao, Wuyao, Guizhi
MOL005399	alexandrin_qt	36.91	0.75	Diyu
MOL005853	methyl-2,3,6-tri-O-galloyl-β-D-glucopyranoside	44.95	0.67	Diyu
MOL005858	3,7,8-Tri-O-methylellagic acid	37.54	0.57	Diyu
MOL005860	3-O-galloylprocyanidin B-3	30.06	0.33	Diyu
MOL005862	Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside	48.07	0.68	Diyu
MOL005864	methyl-6-O-galloyl-β-D-glucopyranoside	44.85	0.29	Diyu
MOL005869	daucostero_qt	36.91	0.75	Diyu
MOL005880	sauvissimoside R1	37.39	0.31	Diyu
MOL005883	gambiriin B-3	34.99	0.75	Diyu
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one	42.36	0.21	Kushen
MOL001484	Inermine	75.18	0.54	Kushen
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39	Kushen
MOL003627	sophocarpine	64.26	0.25	Kushen
MOL003648	Inermin	65.83	0.54	Kushen
MOL003673	Wighteone	42.8	0.36	Kushen
MOL003680	sophoridine	60.07	0.25	Kushen
MOL004580	cis-Dihydroquercetin	66.44	0.27	Kushen
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl) chroman-4-one	71.12	0.18	Kushen
MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27	Kushen
MOL005944	matrine	63.77	0.25	Kushen
MOL006596	Glyceollin	97.27	0.76	Kushen
MOL003347	hyperforin	44.03	0.6	Kushen

MOL006604	(2S)-7-hydroxy-2-(4-hydroxyphenyl)-5 -methoxy-8-(3-methylbut-2-enyl) chroman-4-one	48.09	0.39	Kushen
MOL006613	kushenin	47.62	0.38	Kushen
MOL006620	kushenol J_qt	50.86	0.24	Kushen
MOL006623	kushenol,t	51.28	0.64	Kushen
MOL006626	leachianone,g	60.97	0.4	Kushen
MOL006630	Norartocarpetin	54.93	0.24	Kushen
MOL000456	Phaseolin	78.2	0.73	Kushen
MOL006650	(-)-Maackiain-3-O-glucosyl-6'-O-malonate	48.69	0.52	Kushen
MOL001323	Sitosterol alpha1	43.28	0.78	Gouqizi
MOL003578	Cycloartenol	38.69	0.78	Gouqizi
MOL001495	Ethyl linolenate	46.1	0.2	Gouqizi
MOL001979	LAN	42.12	0.75	Gouqizi
MOL005406	atropine	45.97	0.19	Gouqizi
MOL005438	campesterol	37.58	0.71	Gouqizi
MOL006209	cyanin	47.42	0.76	Gouqizi
MOL007449	24-methylidenelophenol	44.19	0.75	Gouqizi
MOL008173	daucosterol_qt	36.91	0.75	Gouqizi、Dahuang
MOL008400	glycitein	50.48	0.24	Gouqizi
MOL000953	CLR	37.87	0.68	Gouqizi
MOL009604	14b-pregnane	34.78	0.34	Gouqizi
MOL009612	(24R)-4alpha-Methyl-24-ethylcholesta -7,25-dien-3beta-ylacetate	46.36	0.84	Gouqizi
MOL009617	24-ethylcholest-22-enol	37.09	0.75	Gouqizi
MOL009618	24-ethylcholesta-5,22-dienol	43.83	0.76	Gouqizi
MOL009620	24-methyl-31-norlanost-9(11)-enol	38	0.75	Gouqizi
MOL009621	24-methylenelanost-8-enol	42.37	0.77	Gouqizi
MOL009622	Fucosterol	43.78	0.76	Gouqizi
MOL009633	31-norlanost-9(11)-enol	38.35	0.72	Gouqizi
MOL009634	31-norlanosterol	42.2	0.73	Gouqizi
MOL009635	4,24-methyllophenol	37.83	0.75	Gouqizi
MOL009639	Lophenol	38.13	0.71	Gouqizi
MOL009640	4alpha,14alpha,24-trimethylcholesta -8,24-dienol	38.91	0.76	Gouqizi
MOL009641	4alpha,24-dimethylcholesta-7,24-dienol	42.65	0.75	Gouqizi

MOL009642	4alpha-methyl-24-ethylcholesta-7,24-dienol	42.3	0.78	Gouqizi
MOL009644	6-Fluoroindole-7-Dehydrocholesterol	43.73	0.72	Gouqizi
MOL009646	7-O-Methylluteolin-6-C-beta-glucoside_qt	40.77	0.3	Gouqizi
MOL009656	(E,E)-1-ethyl octadeca-3,13-dienoate	42	0.19	Gouqizi
MOL009660	methyl (1R,4aS,7R,7aS)-4a,7-dihydroxy-7-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-1,5,6,7a-tetrahydrocyclopenta[d]pyran-4-carboxylate	39.43	0.47	Gouqizi
MOL009665	Physcion-8-O-beta-D-gentiobioside	43.9	0.62	Gouqizi
MOL009677	lanost-8-en-3beta-ol	34.23	0.74	Gouqizi
MOL009678	lanost-8-enol	34.23	0.74	Gouqizi
MOL009681	Obtusifoliol	42.55	0.76	Gouqizi
MOL005970	Eucalyptol	60.62	0.32	Houpi
MOL005980	Neohesperidin	57.44	0.27	Houpi
MOL001494	Mandenol	42	0.19	Sanqi
MOL001792	DFV	32.76	0.18	Sanqi
MOL002879	Diop	43.59	0.39	Sanqi
MOL005344	ginsenoside rh2	36.32	0.56	Sanqi
MOL001645	Linoleyl acetate	42.1	0.2	Chaihu
MOL004598	3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone	31.97	0.59	Chaihu
MOL004609	Areapillin	48.96	0.41	Chaihu
MOL013187	Cubebin	57.13	0.64	Chaihu
MOL004624	Longikaurin A	47.72	0.53	Chaihu
MOL004653	(+)-Anomalin	46.06	0.66	Chaihu
MOL004718	α-spinasterol	42.98	0.76	Chaihu
MOL000490	petunidin	30.05	0.31	Chaihu
MOL001454	berberine	36.86	0.78	Huanglian
MOL002894	berberrubine	35.74	0.73	Huanglian
MOL002897	epiberberine	43.09	0.78	Huanglian
MOL002903	(R)-Canadine	55.37	0.77	Huanglian
MOL002904	Berlambine	36.68	0.82	Huanglian
MOL002907	Corchoroside A_qt	104.95	0.78	Huanglian
MOL000622	Magnograndiolide	63.71	0.19	Huanglian
MOL000785	palmatine	64.6	0.65	Huanglian

MOL001458	coptisine	30.67	0.86	Huanglian
MOL002668	Worenine	45.83	0.87	Huanglian
MOL001736	(-)-taxifolin	60.51	0.27	Guizhi
MOL000073	ent-Epicatechin	48.96	0.24	Guizhi
MOL004576	taxifolin	57.84	0.27	Guizhi
MOL013219	Strictosamide_qt	76.3	0.76	Dingxiang
MOL001749	ZINC03860434	43.59	0.35	Dingxiang
MOL002235	EUPATIN	50.8	0.41	Dahuang
MOL002268	rhein	47.07	0.28	Dahuang
MOL002280	Torachrysone-8-O-beta-D-(6'-oxayl) -glucoside	43.02	0.74	Dahuang
MOL002281	Toralactone	46.46	0.24	Dahuang
MOL002288	Emodin-1-O-beta-D-glucopyranoside	44.81	0.8	Dahuang
MOL000471	aloe-emodin	83.38	0.24	Dahuang
MOL000554	gallic acid-3-O-(6'-O-galloyl)- glucoside	30.25	0.67	Dahuang
MOL000096	(-)-catechin	49.68	0.24	Dahuang

DQQYZT drug-Cag common target prediction

The ID transformation of DQQYZT compound corresponding to potential targets was performed using STRING database, and 237 target genes were obtained. As shown in Figure 1, disgenet, OMIM and GeneCards databases were used to search for CAG related targets, and 3192 targets were obtained by taking union and removing duplicates. Using the OmicShare website, 86 common targets were obtained.

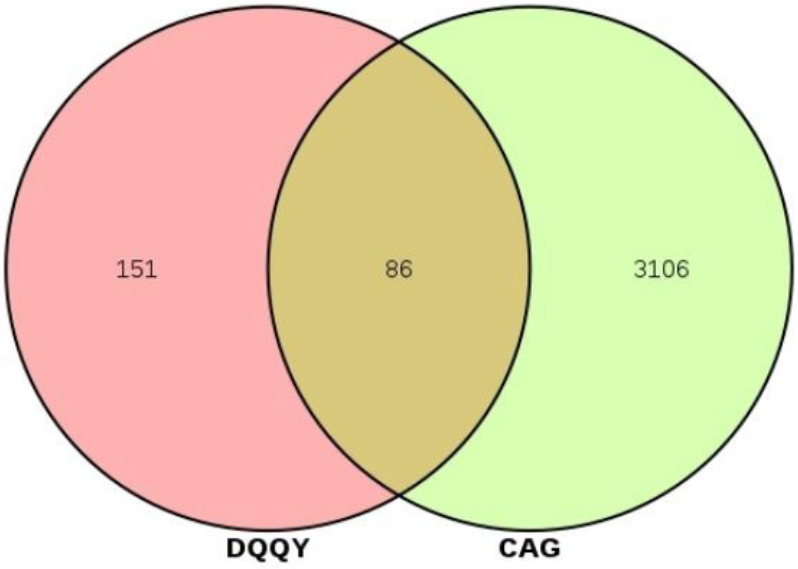

Figure 1: Venn diagram. DQQY: Danqi Quyu Zhitong granule drug target; CAG: Disease target gene

Construction of DQQYZT active ingredient-CAG common target network

The DQQYZT active ingredient and the CAG target were imported into Cytoscape software to construct the formula active ingredient-CAG target network, Figure 2 [hexagons represent the DQQYZT active ingredient; squares represent the CAG target]. It includes 252 nodes and 681 edges. The average degree value of the drug active ingredients was 4.1, and 40 active ingredients greater than the average were screened according to the degree value, Table 2. Multiple disease targets can be seen in the figure corresponding to the same prescription active ingredient, or different prescription active ingredients.

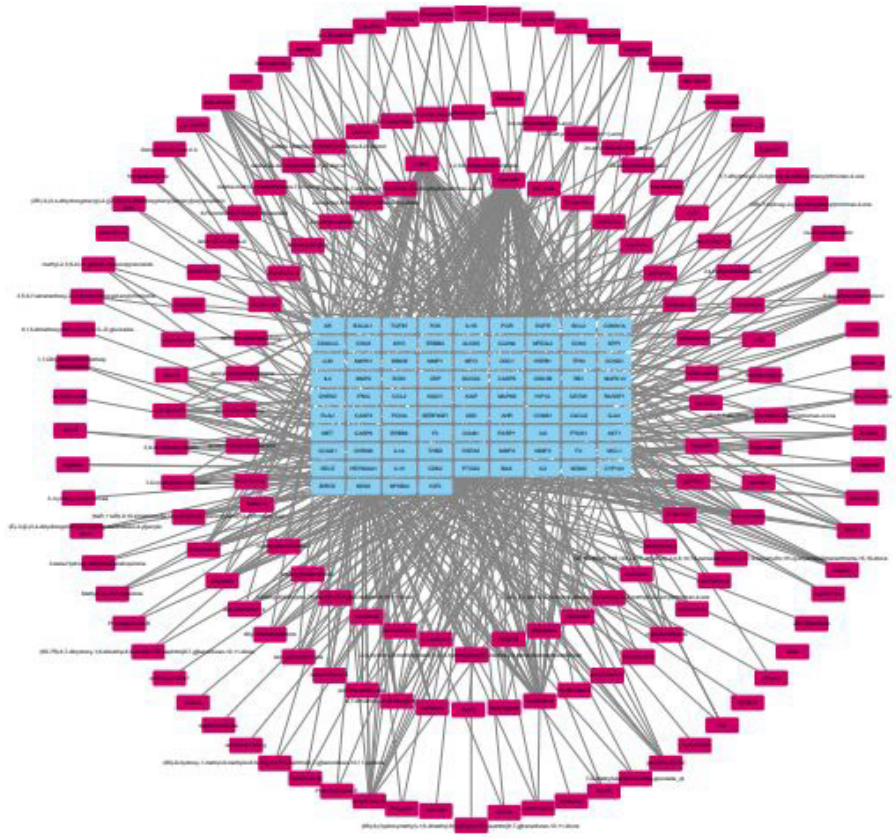

Figure 2: Construction diagram of active ingredient-CAG target network

Table 2: DQQYZT degree values ranked the top 40 active ingredients

Compound	Subordinate Chinese medicine	Degree
quercetin	Huangqi, Beishashen, Wuyao, Diyu, Kushen, Gouqizi, Sanqi, Chaihu, Huanglian, Dingxiang	68
luteolin	Danshen, Kushen	34
kaempferol	Huangqi, Diyu, Chaihu, Dingxiang, Baishao	24
beta-sitosterol	Baishao, Beishashen, Wuyao, Gouqizi, Sanqi, Guizhi, Dingxiang, Dahuang	12
aloe-emodin	Dahuang	12

tanshinone iia	Danshen	12
formononetin	Huangqi, Kushen	11
isorhamnetin	Huangqi, Chaihu	10
7-O-methylisomucronulatol	Huangqi	10
glycitein	Gouqizi	10
Calycosin	Huangqi	9
6,7-dimethoxy-2-(2-phenylethyl) chromone	Wuyao	9
Phaseolin	Kushen	9
cryptotanshinone	Danshen	8
DMPEC	Wuyao	8
8-Isopentenyl-kaempferol	Kushen	8
2-(4-hydroxy-3-methoxyphenyl) -5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	Danshen	7
Wighteone	Kushen	7
petunidin	Chaihu	7
neocryptotanshinone ii	Danshen	7
ginsenoside rh2	Sanqi	7
Stigmasterol	Beishashen, Gouqizi, Sanqi, Chaihu, Dingxiang	6
Jaranol	Huangqi	6
dan-shexinkum d	Danshen	6
dihydrotanshinlactone	Danshen	6
isocryptotanshi-none	Danshen	6
Isotanshinone II	Danshen	6
Berlambine	Huanglian	6
palmatine	Huanglian	6
deoxyneocryptotanshinone	Danshen	6
miltionone I	Danshen	6
matrine	Kushen	6
3,9-di-O-methylnissolin	Huangqi	5
(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c] chromen-3-ol	Huangqi	5
prolithospermic acid	Danshen	5
Glyceollin	Kushen	5

berberine	Huanglian	5
2-isopropyl-8-methylphenanthrene -3,4-dione	Dnshen	5
epidanshenspiroketallactone	Danshen	5
Boldine	Wuyao	5

Analysis of the interaction network between DQQYZT and CAG disease target proteins

The 86 targets were used to obtain interaction relationships using the STRING database and imported into Cytoscape software to draw a network diagram, Figure 3. 86 nodes (i.e. common targets) with 1596 edges are visible. The average Degree value of the Degree network in the figure is 37.1. The targets with Degree values higher than the average are AKT1, TP53, IL6, JUN, CASP3, HIF1A, PTGS2, IL1B, MYC, MMP9, CCND1, EGFR, etc., which may be key targets, Table 3.

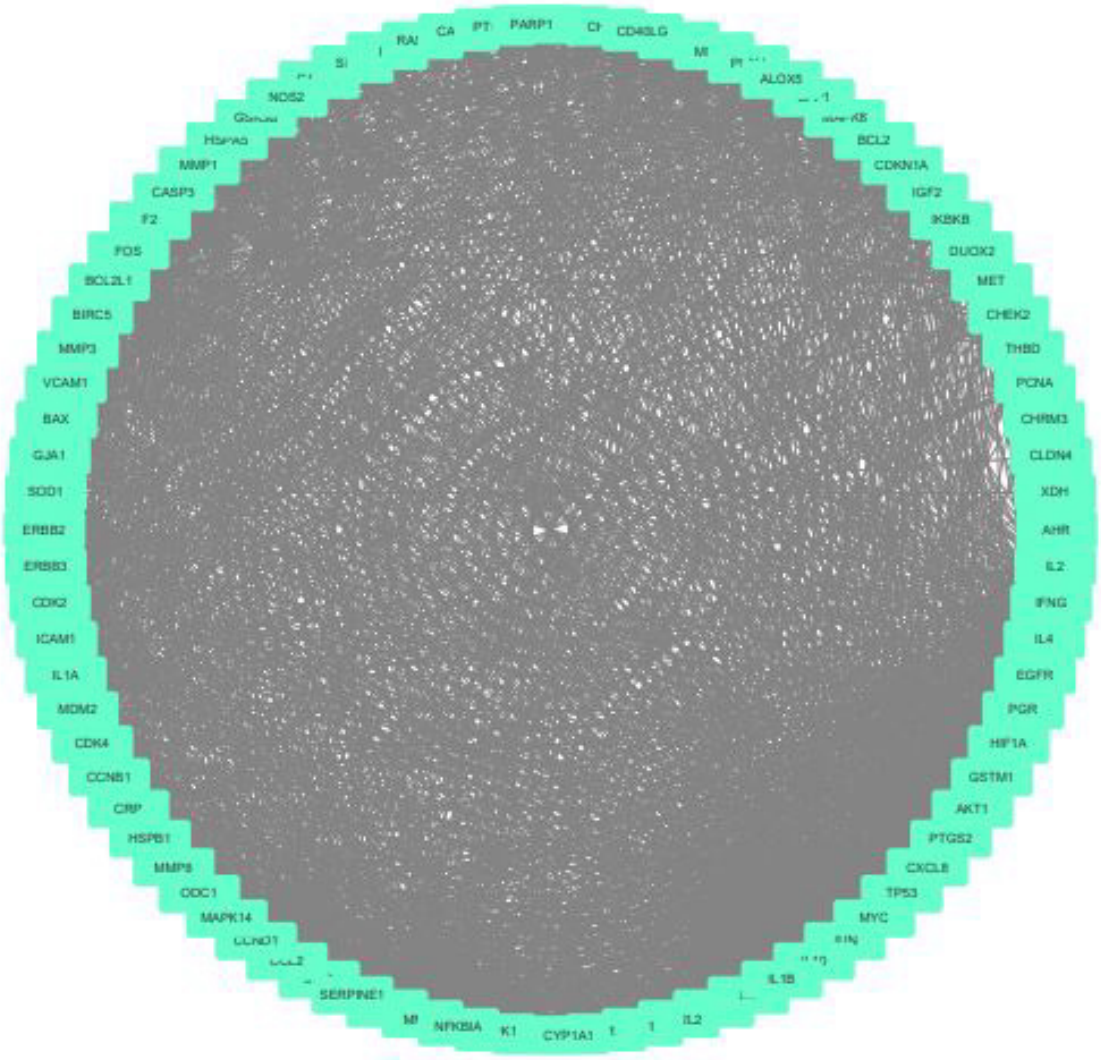

Figure 3: Interaction network of DQQYZT and CAG associated target proteins

Table 3: DQQYZT drugs treat CAG-related targets

Degree	Target	Degree	Target	Degree	Target
76	AKT1	56	FOS	44	MDM2
75	TP53	55	HSP90AA1	44	MCL1
74	IL6	54	IL10	42	IFNG
71	JUN	54	NFKBIA	42	IL4
69	CASP3	53	MMP2	42	CDKN1A
67	HIF1A	53	ERBB2	41	MAPK1
67	PTGS2	53	CASP8	41	MAPK14
67	IL1B	52	CCL2	40	TGFB1
65	MYC	52	BCL2L1	40	VCAM1
65	MMP9	46	ICAM1	39	PARP1
63	CCND1	46	MAPK8	38	AR
62	EGFR	45	CASP9	38	SERPINE1
58	CXCL8	44	IL2	38	IKBKB

GO and KEGG pathway enrichment analysis

Using the GO biological processes and KEGG metabolic pathways obtained from the DAVID database, the top 20 pathways associated with CAG were selected, Figure 4 and Figure 5. From Figure 4, it can be seen that the GO biological processes involved in DQQYZT treatment of CAG are mainly likely to be associated with extrinsic apoptotic signaling pathway in absence of ligand, response to antibiotic, protein phosphorylation, striated muscle cell differentiation, peptidyl-serine phosphorylation, RNA polymerase II sequence-specific DNA binding transcription factor binding, protein kinase activity, lipopolysaccharide-mediated signaling pathway, liver regeneration, transcription factor binding, ovarian follicle development positive regulation of pri-miRNA transcription from RNA polymerase II promoter, cytosol, cellular response to organic cyclic compound, response to gamma radiation, cytokine activity, cellular response to UV, cellular response to gamma radiation, nucleoplasm, positive regulation of reactive oxygen species metabolic process, etc; From Figure 5, it can be seen that the treatment of CAG with Danqi Quyu Zhitong Granules involves the KEGG pathway which may be associated with Apoptosis, Apoptotic Signaling in Response to DNA Damage, Role of Mitochondria in Apoptotic Signaling, p53 signaling pathway, Cellular senescence, DNA damage response, TGF-beta signaling pathway, ErbB signaling pathway, IL-17 signaling pathway, Gastrin signaling pathway, TNF signaling pathway, Th17 cell differentiation pathway, T cell receptor signaling pathway, C-type lectin receptor signaling pathway, Toll-like receptor signaling pathway, Signal transduction through IL1R, MAPK signaling pathway, NOD-like receptor signaling pathway, etc.

Acquisition of Ferroptosis regulatory genes and comprehensive analysis of their common targets with DQQYZT and CAG

Acquisition of Ferroptosis regulatory genes

The Ferferrdb database was used to obtain Ferroptosis driver gene (264), Ferroptosis suppressor gene (238), Ferroptosis marker gene (9), Ferroptosis unclassified gene (110), and the duplicate genes were removed and finally, we obtain 564 genes. More detailed genetic information are shown in Supplementary Table S1.

Acquisition of common target genes

The pre-collated DQQYZT and CAG common target genes with Ferroptosis genes were plotted using the OmicShare website to obtain a total of 27 common genes. Details of the 27 genes are shown in Figure 6, Supplementary Table S2.

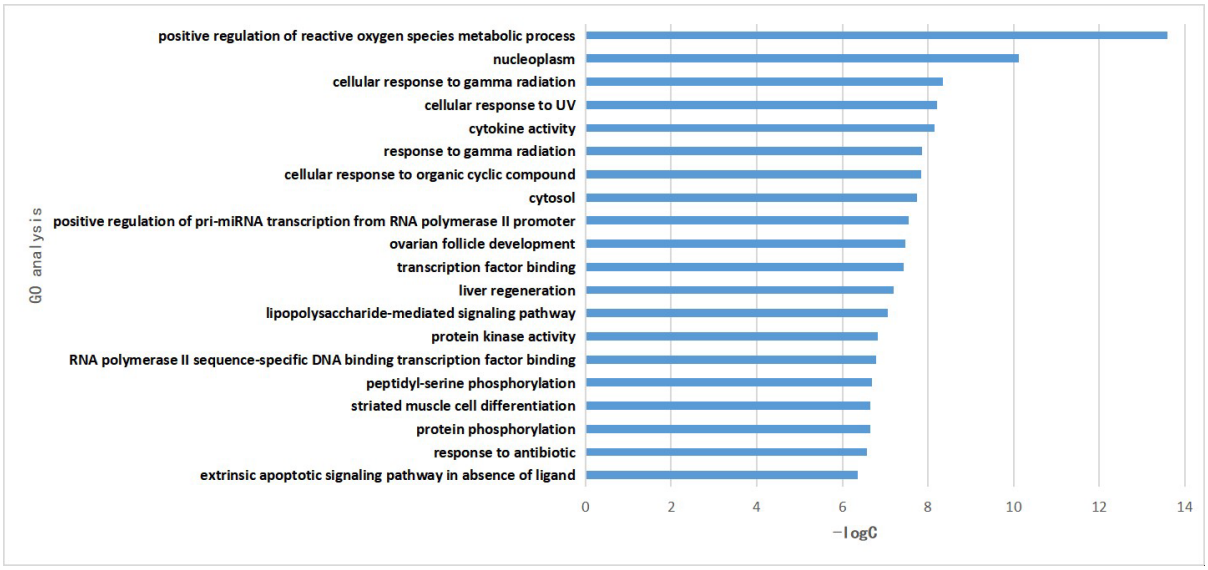

Figure 4: GO biological process for DQQYZT treatment of CAG

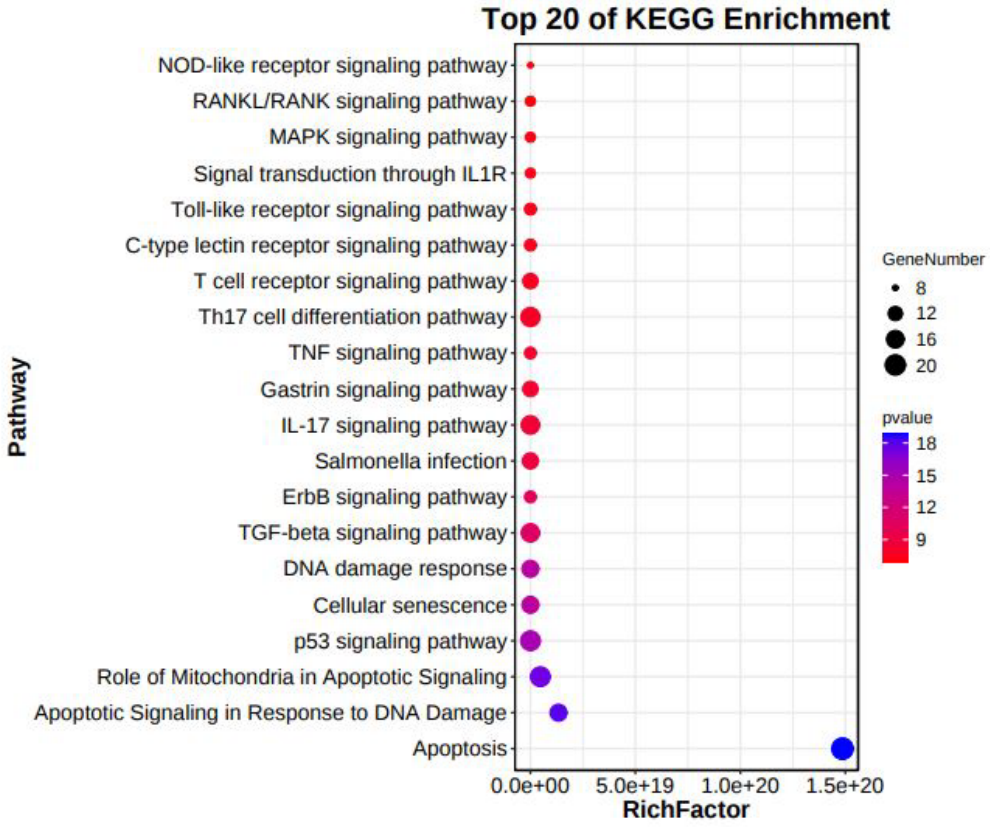

Figure 5: KEGG pathways for DQQYZT treatment of CAG

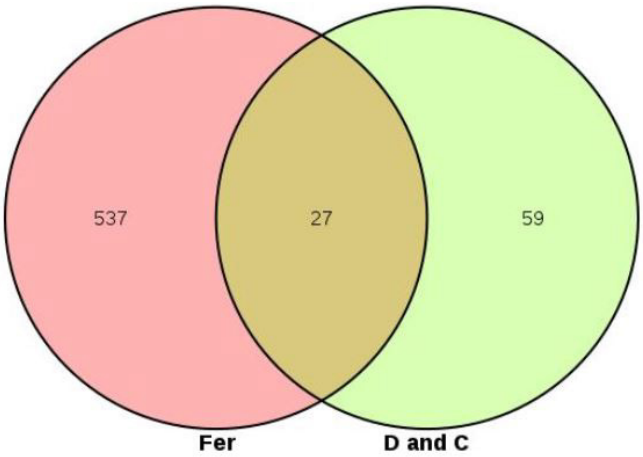

Figure 6: The Venn diagram of DQQYZT and CAG shared target genes and ferroptosis genes. Fer:Ferroptosis D and C:DQQYZT and CAG

The common target genes and Ferroptosis genes of DQQYZT and CAG sorted earlier were drawn by using OmicShare website. A total of 27 common genes were obtained.

DQQYZT active constituents-CAG, Ferroptosis common target network

The corresponding active ingredient of DQQYZT and CAG target were imported into Cytoscape software to construct the active ingredient-target network in prescription, as shown in Figure 7 [circles represent DQQYZT active ingredients; triangles represent CAG targets]. It includes 160 nodes and 298 edges. As shown in Table 4, the average degree value of the active ingredients of the drug was 2.2, and 38 active ingredients larger than the average were screened according to the degree value.

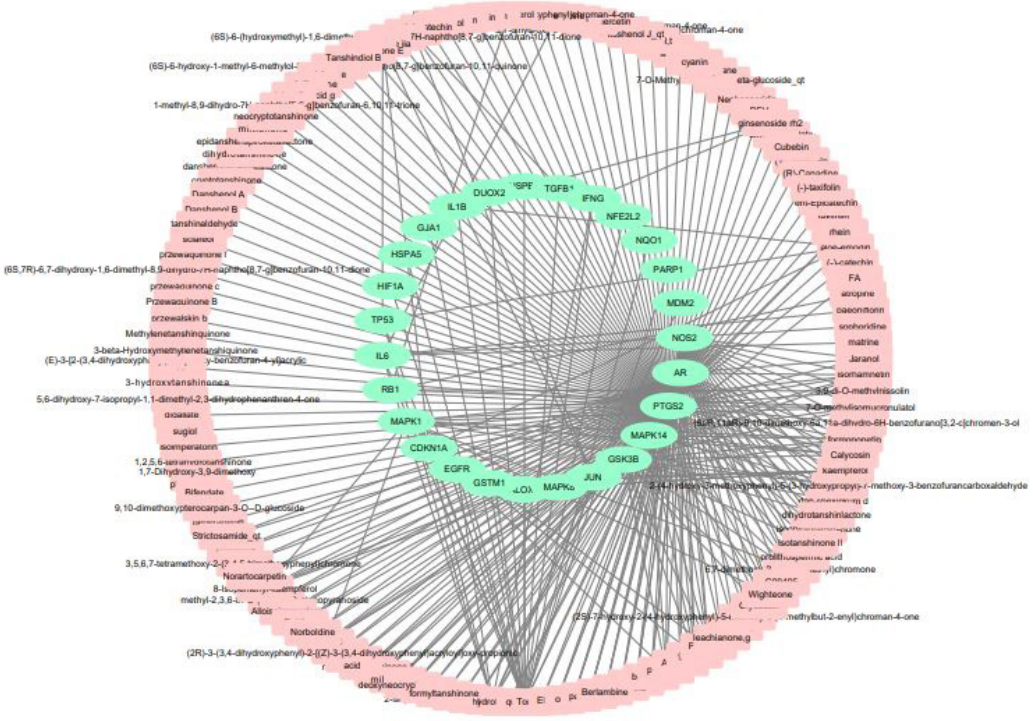

Figure 7: Network diagram of the active ingredients-ferroptosis and CAG targets of DQQYZT

Table 4: DQQYZT ranked among the top 38 active components in the degree value of regulating ferroptosis against CAG

Compound	Degree	Chinese materia medica
quercetin	20	Huangqi, Beishashen, Wuyao, Diyu, Kushen, Gouqizi, Sanqi, Chaihu, Huanglian, Dingxiang
luteolin	9	Danshen, Kushen
formononetin	7	Huangqi, Kushen
kaempferol	7	Huangqi, Diyu, Chaihu, Dingxiang, Baishao
isorhamnetin	6	Huangqi, Chaihu
7-O-methylisomucronulatol	6	Huangqi
Calycosin	6	Huangqi
6,7-dimethoxy-2-(2-phenylethyl) chromone	6	Wuyao
Wighteone	6	Kushen
Phaseolin	6	Kushen
glycitein	6	Gouqizi
2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	5	Danshen
petunidin	5	Chaihu
DMPEC	5	Wuyao
8-Isopentenyl-kaempferol	5	Kushen
dan-shexinkum d	4	Danshen
dihydrotanshinlactone	4	Danshen
Isotanshinone II	4	Danshen
tanshinone iia	4	Danshen
aloe-emodin	4	Dahuang
Jaranol	3	Huangqi
3,9-di-O-methylnissolin	3	Huangqi
isocryptotanshi-none	3	Danshen
prolithospermic acid	3	Danshen
Glyceollin	3	Kushen
Areapillin	3	Chaihu
berberine	3	Huanglian
berberrubine	3	Huanglian
epiberberine	3	Huanglian
Berlambine	3	Huanglian

palmatine	3	Huanglian
coptisine	3	Huanglian
EUPATIN	3	Dahuang
deoxyneocryptotanshinone	3	Danshen
miltionone I	3	Danshen
neocryptotanshinone ii	3	Danshen
beta-sitosterol	3	Baishao, Beishashen, Wuyao, Gouqizi, Sanqi, Guizhi, Dingxiang, Dahuang
ginsenoside rh2	3	Sanqi

KEGG pathway analysis

KEGG signaling pathway analysis was performed using the DAVID database on the common genes obtained between the three. The results were exported to an Excel sheet with a screening condition of $P < 0.05$, while the top 20 figures after non-signaling pathways were artificially removed and the KEGG pathway bubble map was drawn using the OmicShare platform (as shown in Figure 8). The bubble map shows the possible association with Signaling by Interleukins, Th17 cell differentiation pathway, Interleukin-4 and Interleukin-13 signaling, IL-17 signaling pathway, Cytokine Signaling in Immune system, Aryl hydrocarbon receptor pathway, Colorectal cancer, IL-18 signaling pathway, Cellular Senescence, ErbB signaling pathway, AGE/RAGE pathway, Gastrin signaling pathway, FoxO signaling pathway, TNF-related weak inducer of apoptosis (TWEAK) signaling pathway, Chemical carcinogenesis-reactive oxygen species, MAPK signaling pathway, NAD metabolism in oncogene-induced senescence and mitochondrial dysfunction-associated senescence, TNF signaling pathway, Activation of the AP-1 family of transcription factors and RAC1/PAK1/p38/MMP2 pathway.

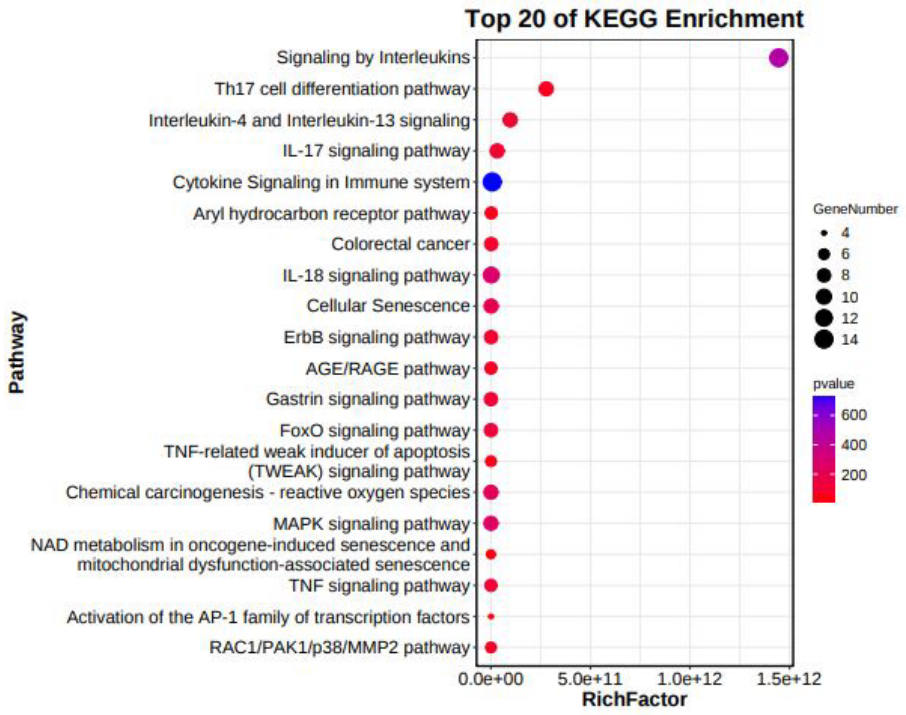

Figure 8: DQQYZT regulates the KEGG pathway of ferroptosis against CAG

Construct PPI network of DQQYZT regulating Ferroptosis against CAG

The STRING database was used to obtain interaction graph for the 27 targets, see Figure 9. There are 27 visible nodes (i.e. co-acting targets) and 201 edges. The average Degree value of the degree network in the graph is 14.9, and the targets of Degree higher than the average Degree values are (13) tumour protein P53 (TP53), interleukin-6 (IL6), hypoxia-inducing factor 1a (HIF1A), JUN proto-oncogene protein, interleukin-1β (IL1B), prostaglandin endoperoxide synthase 2 (PTGS2), epidermal growth factor receptor (EGFR), MAPK14, MAPK1, CDKN1A, MAPK8, AR, PARP1, etc. as possible key targets, as shown in Table 5.

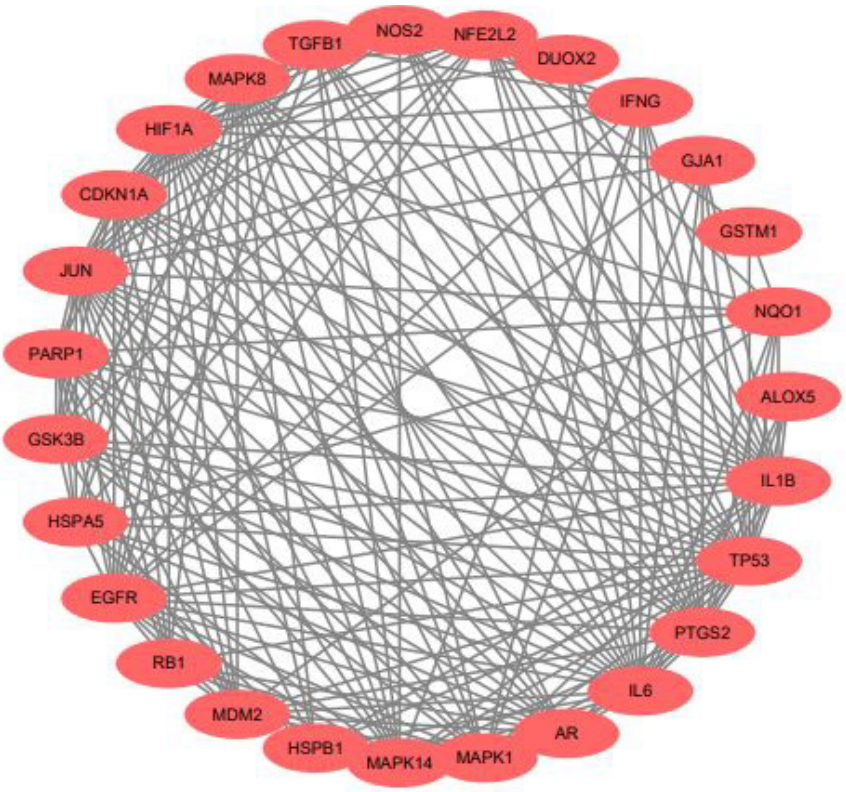

Figure 9: DQQYZT regulates the PPI network of ferroptosis resistance to CAG

Table 5: DQQYZT regulates ferroptosis against CAG related targets

Degree	Target	Degree	Target	Degree	Target
25	TP53	24	IL6	24	HIF1A
23	JUN	22	IL1B	21	PTGS2
20	EGFR	18	MAPK14	17	MAPK1
17	CDKN1A	17	MAPK8	16	AR
15	PARP1				

Discussion

Chronic atrophic gastritis (CAG) is a refractory gastrointestinal disease and is classified as a high risk factor for gastric cancer. At present, there is no definite therapeutic drug for the treatment of chronic atrophic gastritis in clinical practice. Danqi Quyu Zhitong Granules is an in-hospital preparation that has shown remarkable efficacy in the treatment of chronic atrophic gastritis. A previous study found that Danqi Quyu Zhitong Granules could improve gastric precancerous lesions and block their transformation to gastric cancer by inhibiting cell apoptosis and abnormal proliferation and reducing symptoms of intestinal epithelial metaplasia or abnormal hyperplasia.

Based on the method of network pharmacology, the mechanism of action of Danqi Quyu Zhitong Granules in the treatment of chronic atrophic gastritis was investigated by using relevant databases. There were 86 overlapping genes between Danqi Quyu Zhitong Granules and chronic atrophic gastritis. In conclusion, Danqi Quyu Zhitong Granules in the treatment of CAG have the characteristics of multi-components and multi-targets. Network analysis suggested that the average degree value was 37.1, indicating high association and complex mechanism of action. The biological process of its treatment of CAG was found to involve multiple signaling pathways.

It has been found that Danqi Quyu Zhitong Granules include quercetin, luteolin, kaempferol, β-sitosterol, aloe emodin, tanshinone IIA, methoxy isoflavone, isorhamnetin, etc. Among them, quercetin, luteolin, kaempferol, methoxy isoflavone and isorhamnetin are all flavonoids. Some studies have shown that flavonoids can produce superoxide ions and increase the inhibitory DNA damage, thus regulating apoptosis. Among them, quercetin and kaempferol are probably the most important components in the treatment of CAG [17]. Quercetin, a major constituent of Huang Qi, Bei Sha Shen, Wu Yao, Di Yu, Ku Shen, Gou Qi Zi, San Qi, Chai Hu, Huang Lian, Ding Xiang, Dan Shen, is a free radical scavenger that inhibits inflammation and proteoglycan degradation through down-regulating TNF-α and MMP-9 [18]. Quercetin has good binding activity with potential targets of CAG, and the molecular conformation is stable, and its binding energy is superior to that of omeprazole [17]. Kaempferol can play a role in the treatment of CAG by reducing IL-6 and IL-1β levels through modulation of the hedgehog signaling pathway [19]. Kaempferol can also resist inflammatory responses and reduce inflammatory mediators by blocking the activation of p38, ERK and NF-κB signaling pathways [20,21]. Luteolin can cause a significant increase in IL-10 mRNA expression. And IL-10 has recently been recognized as the most potent anti-inflammatory cytokine [22]. β-sitosterol has been found to affect gastrointestinal disorders directly or indirectly [23]. Quercetin, luteolin and kaempferol are the main compounds in the Danqi Quyu Zhitong Granules, which have strong binding affinity to the target proteins JUN, TP53, AKT1, MAPK and IL6 [24]. The related targets of Danqi Quyu Zhitong Granules in the treatment of CAG include AKT1, TP53, IL6, JUN, CASP3, HIF1A, PTGS2, IL1B, MYC, MMP9, CCND1 and EGFR, etc. The expression of AKT1 in CAG rats is significantly increased [25]. The mutations of the tumour protein p53 (TP53) gene occurs in the early stage of gastric cancer, and is involved in gastric cancer [26]. IL-6 and IL-1β are key targets for the treatment of CAG [19]. Dysregulation of IL-6 sequential synthesis is decisive for chronic inflammation and autoimmunity [27]. IL1B strongly inhibits gastric acid secretion. This inhibition is 100-fold stronger than that of proton pump inhibitors (Beales and Calam) and 6000 times stronger than that of H_2 receptor antagonists. In this case, nitro compounds which play an important role in exacerbating inflammatory are prone to develope atrophic inflammation and increase the incidence of gastric cancer [28]. CAG can be treated by reversing down-regulation of cleaved caspase-3 protein expression in the pyloric stomach of rats [29]. The expression of EGFR and MMP-2 is higher in the gastric tissues of CAG rats [30]. The high positive rate of EGFR in gastric precancerous lesions suggests that this protein is involved in the progression of gastric precancerous lesions and is an early event of gastric cancer. The expression of EGFR can be used as a marker to detect early carcinogenesis [31]. Helicobacter pylori gastritis induces the expression and secretion of several matrix metalloproteinases, including MMP-2 and MMP-9 [32].

Analysis of the KEGG pathway enrichment results showed

that the key pathways involved in the treatment of CAG with Danqi Quyu Zhitong Granules were apoptosis, apoptotic signaling pathway in response to DNA damage, the role of mitochondria in apoptotic signaling, P53 signaling pathway, cellular senescence pathway, DNA damage response, TGF-beta signaling pathway, ErBb signaling pathway, IL-17 signaling pathway, gastrin signaling pathway, TNF signaling pathway, T--cell receptor signaling pathway, C-type lectin receptor signaling pathway, toll-like receptor signaling pathway, signal translation via IL1R, MAPK signaling pathway, Nod-like receptor signaling pathway, etc.

Studies have shown that AKT1, TP53, IL6, JUN, CASP3, PTGS2, MMP9, EGFR, CXCL8, HSP90AA1, MAPK8, MAPK1, these proteins are mainly involved in the regulation of cell proliferation and apoptosis processes. Numerous studies have shown that cell proliferation and apoptosis play a key role in the pathogenesis of CAG [33]. CAG is associated with the gastric mucosal barrier damage, cell proliferation and apoptosis imbalance in rats. One of its mechanisms may be the reduced expression of the epidermal growth factor receptors EGFR and NF-Kb in the gastric mucosa [31]. Mitochondrial damage, P53 signaling pathway and DNA damage response can all cause early gastric carcinogenesis by inducing apoptosis. In the early stages of gastric cancer, mitosis is associated with tumour suppression and mitosis removes mitochondrial and mitochondrial DNA damage, which protects healthy cells from malignant transformation and tumour cells from [34]. p53 targets are involved in apoptotic pathway and the occurrence of gastric carcinogenesis [35]. DNA damage response-1 (REDD1) is a ubiquitous conserved protein that can be induced by multiple stimuli. However, the regulation, function and clinical relevance of REDD1 in H. pylori-associated gastritis is currently unknown [36]. Cellular senescence is a predisposing factor for carcinogenesis and is a distinct phenomenon in the gastric mucosal tissue of H. pylori positive CAG patients [37]. Gastric mucosal atrophy is highly correlated with cellular senescence [38].

TGF-β1 can prevent the benign growth of gastric glands and activate the conversion of myofibroblasts into smooth muscle cells, which plays a key role in the occurrence and development of CAG. The expression of TGF-β is positively correlated with H. pylori-induced chronic gastritis and is significantly expressed in precancerous gastric cells [39]. Knockdown of ErbB2 promotes apoptosis in DNA-damaged (high) apoptotic (low) cells. pEGFR, pEGFR-ERBB2 and pERBB2 are mainly increased in gastritis or atrophic gastritis tissues [40]. Recent studies have shown that gastrin plays an important role in the occurrence and development of gastric cancer, and has certain impact on the growth and deterioration of cancer cells [41]. About 95% of gastrin in the human body is biologically active and exists in the form of α-amidated gastrin, of which the highest proportion of G-17 is synthesized and secreted by gastric sinus G cells. When AG occurs, the gastric sinus gland is reduced, the number of G cells in the sinus decreases, and serum G-17 levels decrease, which can be used as a marker to determine G cell function. The results of the G-17 assay in this paper show that when the gastric mucosa is atrophic, gastrin secretion is affected, the intrinsic glands disappear or are reduced, and both G-17 secreted by the gastric sinus and PGI secreted by the gastric body are significantly reduced [42]. Inflammation is an important factor affecting gastric mucosal proliferation and carcinogenesis, among which the most characteristic cell is TNF-α [43]. HP infection leads to chronic atrophic gastritis through activation of TLR2, TLR4/MAPK/NF-κB/iNOS/NO signaling pathways [44]. Anti-inflammatory effects of rhein in chronic atrophic gastritis through Nrf2 and MAPK signaling pathways [45].

Quercetin [46], luteolin [47], kaempferol [48], and aloe emodin [49] can regulate the process of cell Ferroptosis. The field of cell death is rapidly evolving and a variety of cell death pathways have been identified, including apoptosis, necrosis, pyroptosis, ferroptosis, and autophagy-dependent cell death. [50] EGFR-related pathways of Ferroptosis and apoptosis in ovarian cancer [51]. P53 can enhance ferroptosis by inhibiting the expression of SLC7A11 (solute carrier family 7 member 11) or by enhancing the expression of SAT1 (spermidine/spermidine N1-acetyltransferase 1) and GLS2 (glutaminase 2). On the other hand, p53 inhibited ferroptosis by directly inhibiting DPP4 (dipeptidyl peptidase 4) activity or inducing CDKN1A/p21 (cell cycle protein-dependent kinase inhibitor 1A) expression [52]. The ferroptosis associated gene HIF1A is an effective biomarker for gastric adenocarcinoma [53].

High circulating Th17 and Th22 cells are associated with poor progression and survival of gastric cancer [54]. T cell of CD8+ that produce interleukin (IL)-17 (Tc17 cells) promote inflammatory progression and may be associated with poor prognosis. [55] AP-1 is a key transcription factor regulated by the mitogen activated protein kinase (MAPK) signaling pathway and plays a critical role in inflammation and cancer [56]. IL-13 is closely associated with the repair of gastric mucosal damage [57]. GPX4 is an important negative factor of ferroptosis, which has recently been shown to inhibit Caspase-11-dependent apoptosis and the release of IL-1β [50].

In summary, I speculated that DQQYZT's anti-CAG and its regulation of ferroptosis is a process involving multi-components, multi-targets and multi-pathways. However, the present study did not experimentally validate the tested active components, targets and pathways, but was only a predictive exploration relying on the existing relevant databases. Based on network pharmacology, the mechanism of action of drug molecules can be calculated, analyzed and predicted and the therapeutic effect of drugs can be discovered, which provides more powerful theoretical evidence for accelerating drug development and determining the direction of clinical trials, and provides scientific ideas and directions for subsequent basic research. However, network pharmacology still faces some challenges in practical application, such as the database is not perfect, and few people in network pharmacology research use animal tests or cell tests to verify the efficacy. Therefore, network pharmacology needs to be continuously refined and improved to make the screening of active ingredients and mechanisms of action more convincing [58].

Data Availability

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declared that they have no conflicts of interest regarding this work.

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Author Contributions

Yingying Fang,Jingyu Zhan and Ming Chen designed the study and was responsible for the feasibility analysis and supervision of the study. Bing Wang and Bingbing He drafted the manuscript. Jiajia Chen and Yue Shi collected data. Wenyu Fan,Caihong Song and Ying Li analyzed data. Hongliang Geng, Xiaoming Yan and Yuhua Xiang revised manuscript drafts. All authors have made substantive intellectual contributions to the conceptualization and development of this study. All authors have read and agreed to the final version of the manuscript.

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Supplementary Tables

Table S1: Fig4 GO analysis FDR

Term	FDR
extrinsic apoptotic signaling pathway in absence of ligand	4.1803957848819E-12
response to antibiotic	1.36076958082222E-08
protein phosphorylation	2.59975271311843E-07
striated muscle cell differentiation	5.30324755898448E-07
peptidyl-serine phosphorylation	9.16106063250039E-06
RNA polymerase II sequence-specific DNA binding transcription factor binding	1.81187651381221E-08
protein kinase activity	2.19944159145798E-06
lipopolysaccharide-mediated signaling pathway	0.00545771718076575
liver regeneration	2.15553028349695E-06
transcription factor binding	3.13805172438134E-08
ovarian follicle development	0.000579682752662651
positive regulation of pri-miRNA transcription from RNA polymerase II promoter	6.58446678044713E-09
cytosol	9.43851015168387E-08
cellular response to organic cyclic compound	2.96147729064398E-09
response to gamma radiation	0.0000116452081377176
cytokine activity	8.50887337122904E-06
cellular response to UV	0.0000112337292162234
cellular response to gamma radiation	0.0000156104485456097
nucleoplasm	0.0000140305127285327
positive regulation of reactive oxygen species metabolic process	0.0000158943050893103

Table S2: Fig5 KEGG analysis FDR

Term	FDR
Apoptosis	1.35230949185594E-18
Apoptotic Signaling in Response to DNA Damage	7.47748161687235E-18
Role of Mitochondria in Apoptotic Signaling	9.76790724901033E-17
p53 signaling pathway	3.49603972789562E-15
Cellular senescence	3.09540308646683E-14
DNA damage response	3.09540308646683E-14

TGF-beta signaling pathway	2.50989568614349E-11
ErbB signaling pathway	2.0645524118022E-10
Salmonella infection	3.38160647626141E-09
IL-17 signaling pathway	3.85345825256248E-09
Gastrin signaling pathway	7.17078258530604E-09
TNF signaling pathway	8.02061426782535E-07
Th17 cell differentiation pathway	1.3272200271744E-08
T cell receptor signaling pathway	2.20011845464403E-08
C-type lectin receptor signaling pathway	6.31554185157756E-08
Toll-like receptor signaling pathway	0.0000001034563507122
Signal transduction through IL1R	1.11810920727782E-07
MAPK signaling pathway	1.25514112135826E-07
RANKL/RANK signaling pathway	2.21394313447953E-07
NOD-like receptor signaling pathway	2.95650334462283E-07

Table S3: Fig8 KEGG analysis FDR

Term	FDR
Signaling by Interleukins	4.03355565240682E-09
Th17 cell differentiation pathway	0.0000000007433349616
Interleukin-4 and Interleukin-13 signaling	0.0000000206203642618
IL-17 signaling pathway	5.39158318428213E-09
Cytokine Signaling in Immune system	4.43844045210427E-07
Aryl hydrocarbon receptor pathway	9.21189751961778E-08
Colorectal cancer	3.5201926001644E-08
IL-18 signaling pathway	0.000125487012385075
Cellular Senescence	0.0000007710848214061
ErbB signaling pathway	9.98348916602591E-06
AGE/RAGE pathway	5.76340203988136E-07
Gastrin signaling pathway	5.76340203988136E-07
FoxO signaling pathway	3.9659049676454E-07
TNF-related weak inducer of apoptosis (TWEAK) signaling pathway	0.0000376333275919208
Chemical carcinogenesis - reactive oxygen species	7.7907689190084E-07
MAPK signaling pathway	4.50480570414064E-06

NAD metabolism in oncogene-induced senescence and mitochondrial dysfunction-associated senescence	0.000188397935373128
TNF signaling pathway	3.30103265022945E-06
Activation of the AP-1 family of transcription factors	0.0000512762889988551
RAC1/PAK1/p38/MMP2 pathway	0.00001548534330439

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