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ARTICLE

Analysis of the Effective Mechanism of Fructus Aurantii Immaturus in the Treatment of Chronic Atrophic Gastritis Based on Network Pharmacology and Molecular Docking Technology

Jing-Jie Wu[1*], Ying-Bing Mei[1,2*], Nie-Rui Huang[1], Si-Si Li[1]

[1]Hubei University of Chinese Medicine, Wuhan, China,430000;
[2]Hubei Provincial Hospital of Traditional Chinese medicine, Wuhan, China,430000.

***Corresponding to**: Ying-Bing Mei, Hubei University of Chinese Medicine, Wuhan, China,430000, email: meiyingbing@hbhtcm.com; Jing-Jie Wu, Hubei Provincial Hospital of TCM, Wuhan, China,430000, email: 296798091@qq.com.

Abstract: Background: Chronic atrophic gastritis (CAG) is a common precancerous disease, which has attracted extensive attention. Fructus Aurantii Immaturus (FAI) is a Chinese herbal medicine widely used to treat gastritis for thousands of years, but its pharmacological mechanism is still unclear. In this study, the potential pharmacological mechanism of FAI in treating CAG was investigated through network pharmacology and molecular docking.
Methods: The corresponding targets of FAI were collected from TCMSP database, and the targets of CAG were obtained from GeneCards database and DisGeNET database. Then, the disease-components-action targets network and protein-protein interaction (PPI) network were constructed. By running the packages of Bioconductor platform with R software, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were carried out for the relevant targets. After that, we verified our prediction of candidate targets by docking with the important components in FAI.
Results: There were 22 active ingredients in FAI, and we obtained 40 action targets, including AKT1, CASP3, VEGFA, JUN, etc. The signaling pathways regulated by these targets are closely related to signal transduction, apoptosis and proliferation, including PI3K-Akt signaling pathway, NF-kappa B signaling pathway, etc. Molecular docking showed that the hydrogen bond, π-π stacking and cation –π were the main forms of interaction.
Conclusion: The treatment of CAG by FAI is the result of multi-molecule and multi-target interaction. The mechanism of action involves multiple biological functions and multiple signal transduction pathways. This study can provide more ideas for the development of new drugs for CAG in the future.
Key words: chronic atrophic gastritis, gene network, ethnopharmacology, traditional Chinese medicine, protein interaction maps, molecular docking

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***Abbreviations*:** CAG, Chronic atrophic gastritis; FAI, Fructus Aurantii Immaturus; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GC, gastric cancer; TCM, Traditional Chinese medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB, bioavailability; DL, drug-likeness; BP, Biological Process; MF, Molecular Function; CC, Cellular Component; VEGF, vascular endothelial growth factor; RKTs, receptor tyrosine kinase.

***Authors' Contributions*:** The manuscript has been read and approved by all of the authors.

***Competing interests*:** The authors declare that they have no conflict of interest.

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1. Introduction

Chronic atrophic gastritis (CAG) is a common gastrointestinal disease, which is defined as inflammation and atrophy of the gastric mucosa and reduction of gastric glands [1]. As a commonly precancerous lesions, the existence of CAG in the background of gastric cancer (GC) has been widely recognized, which has attracted extensive attention [2, 3]. According to a global epidemiological survey in 2018, GC is one of the most common types of cancer with the top five morbidity and mortality rates among all malignant tumor. The situation seems to be worse in China, where the mortality rate of GC ranks second among all cancers [4, 5]. Therefore, early intervention should be used to delay or even reverse the progress of CAG. The treatment options for CAG includes *Helicobacter pylori* eradication, acid suppressive therapy (PPI, H2-antagonists), prokinetic agents, neuromodulators and regular endoscopic detection [6, 7]. However, these options can hardly delay the pathological progress of CAG.

Nowadays, Traditional Chinese medicine (TCM) is increasingly recognized by the people at home and abroad because of the high therapeutic performance and few side effects. TCM has the advantages of 'accurate syndrome differentiation and treatment' and 'individualized prescription medicine', which can not only reduce the clinical symptoms, but also delay or even block the process of CAG to a certain extent [8]. FAI is the dried immature fruit of Citrus aurantium L and Citrus sinensis Osbeck [9], widely applied in treating gastrointestinal diseases. In TCM theory, FAI has the effects of promoting digestion, removing food retention, reducing phlegm and resolving masses [10, 11]. Some clinical data showed [12, 13] that FAI was a common herb for CAG and had wide application in compatibility with herbs such as Baizhu and Houpo. Studies have shown that compounds like luteolin and naringenin in FAI had various beneficial effects, including anti-oxidative, anticancer and anti-inflammatory effects, which can improve the gastrointestinal function and inhibit tumor growth [14, 15]. However, most studies of FAI are limited to a single component or a single pathway, the underlying mechanism of FAI has yet to be fully elucidated.

Due to the complexity of ingredients and diversity of targets, it is difficult to determine the effective mechanism of TCM by standard pharmacological methods. Network pharmacology is an emerging discipline, it can elucidate the relationship among diseases, drugs, molecules, targets and pathways, and explain the mechanism of TCM prescriptions from a more comprehensive perspective based on bioinformatics and computer technology [16, 17]. In this study, the active components, targets and signaling pathways of FAI in treating CAG were obtained through the network pharmacological method, and some biomolecular networks were constructed to analyze the potential mechanism. We also studied the interactions between active components and action targets by molecular docking. Our results may clarify the therapeutic effect of FAI on CAG, and provide some ideas for the follow-up drug development.

2. Materials and Methods

2.1. Materials

Data used in this study were collected from the Traditional Chinese Medicine Systems Pharmacology Database and AnalysisPlatform (TCMSP, http://www.tcmspw.com/tcmsp.php, Version 2.3), the GeneCards database (https://www.genecards.org/, Version4.12), the DisGeNET database (https://www.disgenet.org/home/), the UniProt,(https://www.uniprot.org/), R software (R3.6.1 for Windows), the Venn Diagram, the STRING database (https://string-db.org/, Version11.0), Cytoscape software (version 3.6.1), the Bioconductor (https://bioconductor.org/bioLite.R) and its packages: org.Hs.eg.db, clusterProfiler (version 3.10), the KEGG PATHWAY database (https://www.kegg.jp/kegg/pathway.html, updated on October 24, 2018), the RCSB Protein Data Bank (http://www.pdb.org/), and Schroedinger Software Maestro 11.1.

2.2. Methods

2.2.1. Collection of Potential Active Ingredients and Prediction of Their Targets

Active ingredients were mainly obtained from TCMSP database, the largest pharmacological platform of Chinese herbal medicine, through which we could find out the relationship among drugs, targets and diseases. To analyze the druggability of herbs at the molecular level, ADME evaluations are important for drug discovery and development, including bioavailability (OB), Caco-2 permeability (Caco-2), drug-likeness (DL) of drugs, blood-brain barrier (BBB), etc. OB is an important indicator for evaluating the internal quality of drugs, which can evaluate the absorption efficiency of oral drug. DL refers to the similarity between a compound and a known drug, reflecting the possibility of a compound becoming a new drug. Since FAI was absorbed orally, OB and DL are the common criteria for screening active ingredients [18, 19]. Under the conditions of OB >30% and DL >0.18, 22 active ingredients and their corresponding targets were obtained. The "Protein names" of these targets were converted to "Gene names" by the UniProt Knowledgebase. Then those targets were uploaded into the STRING database and analyzed with Cytoscape software.

2.2.2. Collection of Target Genes Associated with CAG

The GeneCards database is an integrative database that

provides comprehensive information on all annotated and predicted human genes [20]. The DisGeNET database contains a wealth of information about genes and variants associated with human disease [21]. Genes related to CAG were obtained from these databases through searching the term “chronic atrophic gastritis” or “gastritis, atrophic”.

2.2.3. Construction of the “Disease-components-action targets” Network and PPI Network

Action targets of FAI for treating CAG were identified by Venn Diagram packages in R software. Then, we used Cytoscape 3.6.1 software to create a “disease-components-action targets” network, and analyzed it with emphasis on “degree”. Afterward, the identified action targets were input into the STRING database for multiple searches with the term “Homo sapiens”, and the obtained PPI network was saved as PNG image and TSV file. In order to reveal the importance of each gene, we used R software to calculate the number of adjacent genes in the downloaded TSV file, and output a histogram of the core gene.

2.2.4. GO Enrichment Analysis and KEGG Pathway Analysis.

GO enrichment analysis and KEGG pathway analysis are used to characterize action targets [22]. The "org.hs.eg.db" packages of the Bioconductor platform were run through R software to convert the action targets into Entrez IDs. The clusterProfiler package was used for functional clustering and visual analysis of those genes. After selecting "org.hs.eg.db" as the OrgDb type and setting the cutoff value of p as 0.05, clusterProfiler was used to enrich GO functions (including Biological Process (BP), Molecular Function (MF) and Cellular Component (CC)) and KEGG pathways.

2.2.5. Molecular Docking

Molecular docking is a method to identify and predict the structure of a receptor-ligand complex by simulating molecular geometry and intermolecular force [23]. Molecular docking of the core gene with the ingredients acting on it could help us further understand the effective mechanism of FAI. The three-dimensional structure of target protein with crystal structure was downloaded from PDB database. Then, the protein preparation, hydrogenation, charging and deletion of water molecules was carried out by using the Protein Preparation Wizard module of Schrodinger molecular docking software. Next, the central protein receptor lattice was created with the original ligand by the receptor glide generation module, with the box size set at 1 nm (10 A) and other parameters as the system default values. Subsequently, ligands were treated with the LigPrep module, all conformations of pH 7.0 ± 2.0 were generated by the Epik method, and other parameters were set to be system default values. Finally, ligand molecules were docked using the Ligand Docking module.

3. Results

3.1. Potential Active Ingredients and Their Targets of FAI

The roadmap of the integrated network pharmacology approach is shown in figure 1. There were 22 active ingredients and 307 targets that met the requirements of OB>30% and DL>0.18. The information (TCMSP ID, molecular name, OB and DL) of the active ingredients is shown in table 1. After deleting overlapping genes, we got 100 targets of FAI. Then, we input these targets into the STRING database to obtain the PPI network (Fig.2A) and analyzed them with Cytoscape software. The PPI network had 100 nodes and 1209 edges. The average node degree and betweenness centrality of the network were 24.18 and 88.62, respectively. A total of 26 genes (Fig.2B) were considered as core targets because their betweenness centrality and average node degree were above the mean. Most of them were related to oxidative stress and apoptosis, indicating that FAI had the potential of resisting inflammation and tumor. This effect of FAI was also indicated by KEGG pathway analysis of these 100 genes (Figs. 2C and 2D).

3.2. Analysis of Genes Associated with CAG

A total of 506 CAG-related targets were obtained from the GeneCards database, and 126 targets from the DisGeNET database. After removing the duplicates, we got 541 targets. The GO enrichment analysis (Fig.3) suggested that growth factor activity, cytokine activity, receptor-ligand activity, cytokine receptor binding and receptor-regulator activity were the main molecular functions.

3.3 Networks of Action Targets

After the intersection of CAG-related targets and FAI-related targets, we got 40 action targets for further analysis (Fig.4). The types of targets included transcription factors, enzymes, receptors, enzyme inhibitors, etc., involving redox reactions, cell proliferation, differentiation, tumor cell migration, and most importantly, apoptosis.

The "disease-components-action targets" network constructed based on active ingredients and action targets consisted of 58 nodes, with a centrality of 0.627 and a heterogeneity of 1.415. According to figure 4B, the top 3 compounds were mol000006 (luteolin, degree = 33), mol005828 (nobiletin, degree = 14), and mol004328 (naringenin, degree = 11), suggesting that the above compounds might play an important role in the treatment of CAG.

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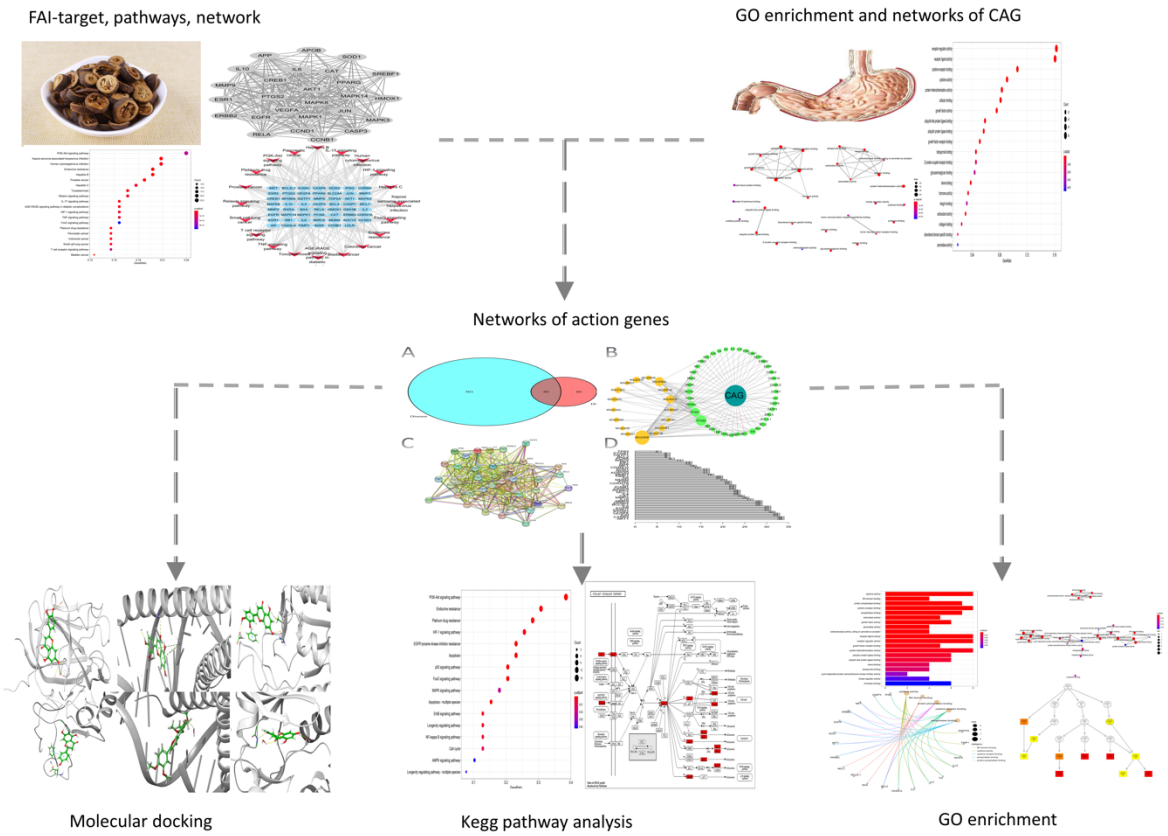

Figure 1. Roadmap of this study.

Table 1. Active ingredients in FAI

TCMSP ID	Molecule Name	OB(%)	DL
MOL000006	luteolin	36.16	0.25
MOL001798	neohesperidin_qt	71.17	0.27
MOL001803	Sinensetin	50.56	0.45
MOL001941	Ammidin	34.55	0.22
MOL002914	Eriodyctiol (flavanone)	41.35	0.24
MOL004328	naringenin	59.29	0.21
MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27
MOL005828	nobiletin	61.67	0.52
MOL005849	didymin	38.55	0.24
MOL007879	Tetramethoxyluteolin	43.68	0.37
MOL009053	4-[(2S,3R)-5-[(E)-3-hydroxyprop-1-enyl]-7-methoxy-3-methylol-2,3-dihydrobenzofuran-2-yl]-2-methoxy-phenol	50.76	0.39
MOL013276	poncirin	36.55	0.74
MOL013277	Isosinensetin	51.15	0.44
MOL013279	5,7,4'-Trimethylapigenin	39.83	0.3
MOL013352	Obacunone	43.29	0.77
MOL013428	isosakuranetin-7-rutinoside	41.24	0.72
MOL013430	Prangenin	43.6	0.29
MOL013433	prangenin hydrate	72.63	0.29
MOL013435	poncimarin	63.62	0.35
MOL013436	isoponcimarin	63.28	0.31
MOL013437	6-Methoxy aurapten	31.24	0.3
MOL013440	citrusin B	40.8	0.71

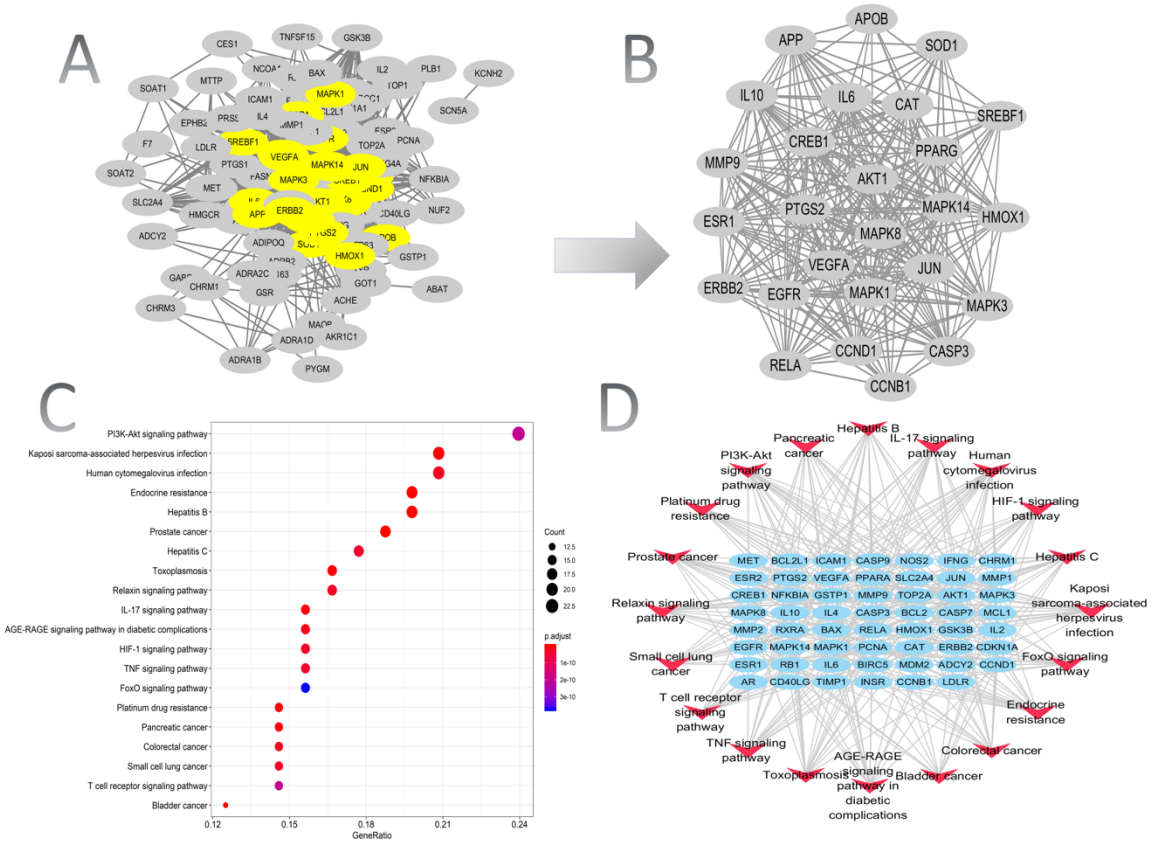

Figure 2. Analysis of genes associated with FAI. (A) PPI network of FAI. Yellow node represents the Core genes of PPI network. (B) The network of core genes. (C) A dot plot of top 20 enriched KEGG pathways, the color from red to blue represents that the adjusted P-value varies from small to large. (D) Network of top 20 KEGG pathways and enriched genes. Blue node represents the enriched genes and red node represents the KEGG pathways.

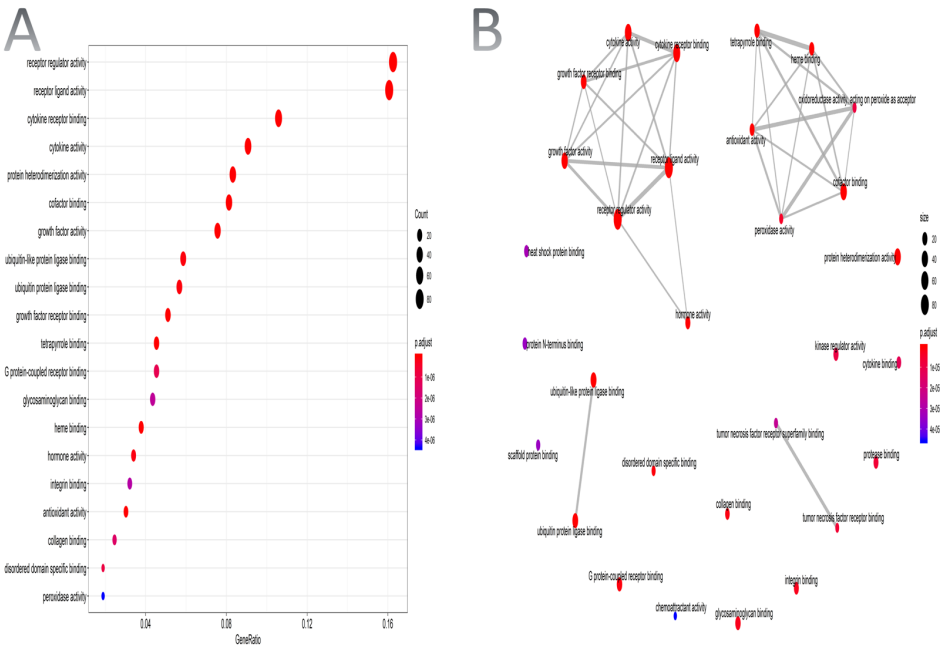

Figure 3 Analysis of genes associated with CAG. the color from red to blue represents that the adjusted P-value varies from small to large. (A) A dot plot of top 20 enriched GO terms with corresponding adjusted p-values. (B)Interaction networks between enriched GO terms analyzed by Enrichplot.

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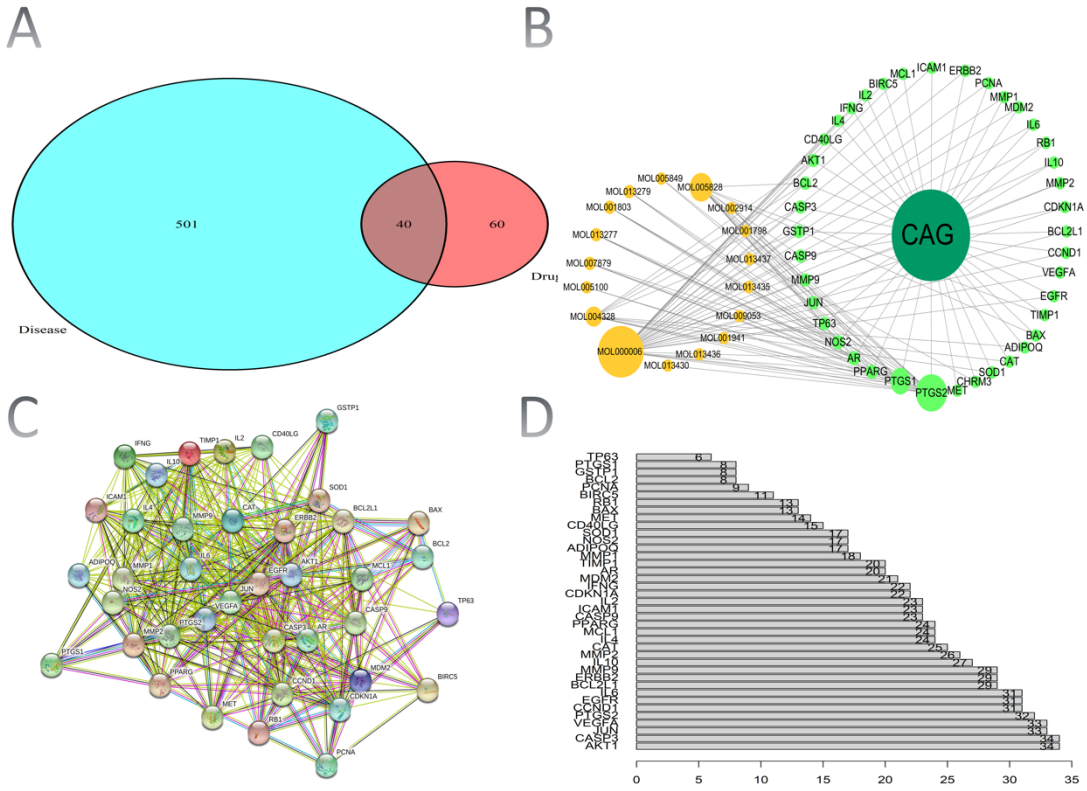

Figure 4. Networks of action genes. (A) Venn diagram of action targets of FAI for CAG; Blue represents the targets of CAG, red represents the targets of FAI, and the intersection of red and blue is the action targets of FAI for CAG. (B) The "disease-components-action targets" network; the dark green node represents the disease, the green nodes represent action targets, the yellow nodes represent the effective components of FAI for treatment of CAG, and the edges represent the interaction relationships; Each node's degree is represented by the node size. (C) PPI network of action targets. (D) Degree of action genes.

PPI network of action targets in FAI (Fig.4C) contained 39 nodes and 422 edges, with an average degree of 21.64 and an average betweenness centrality of 16.67. We used R software to calculate the degree of each target and ranked them, and then the related histogram was obtained (Fig.4D). There were 14 targets whose degree and betweenness centrality exceeded the average, including AKT1, CASP3, JUN, VEGFA, etc., (Table 2) indicating that they might be the core targets of the pharmacological effects.

3.4. GO Enrichment Analysis

The GO enrichment analysis of 40 action targets was carried out by clusterProfiler (pV<0. 05, qV<0. 05), and dot plots related to BP, CC and MF (Fig.5) were derived respectively.

GO-BP (Fig.5A) enrichment contained 1361 terms, including toxic substance response, oxidative stress response, steroid hormone response, reactive oxygen species response, cell oxidative stress response, regulation of smooth muscle cell proliferation, smooth muscle cell proliferation, endogenous wilting apoptotic signaling pathway, etc. GO-CC (Fig.5B) enrichment contained 30 terms, including extracellular matrix, capsule, nuclear chromosome, chromatin, cyclin dependent protein kinase whole enzyme complex, white kinase complex, mitochondrial outer membrane, etc. It suggested that the main action sites of FAI were matrices, nucleus and mitochondria.

Table 2. Information on 14 core targets

	Gene symbol	Betweenness centrality	Degree
1	AKT1	54.97678	34
2	CASP3	49.454	34
3	VEGFA	41.30384	33
4	JUN	38.67731	33
5	PTGS2	37.54077	32
6	CCND1	52.06555	31
7	EGFR	50.13217	31
8	IL6	28.82886	31
9	ERBB2	37.08426	29
10	BCL2L1	28.21332	29
11	MMP9	16.81658	29
12	CAT	31.92482	25
13	MCL1	20.92886	24
14	CDKN1A	20.76966	22

And there were 56 GO terms in GO-MF(Fig.6). It could be found that the molecular biological functions of FAI in treating CAG included cytokine activity, BH domain binding, protein phosphatase binding, cytokine receptor binding, phosphatase binding.

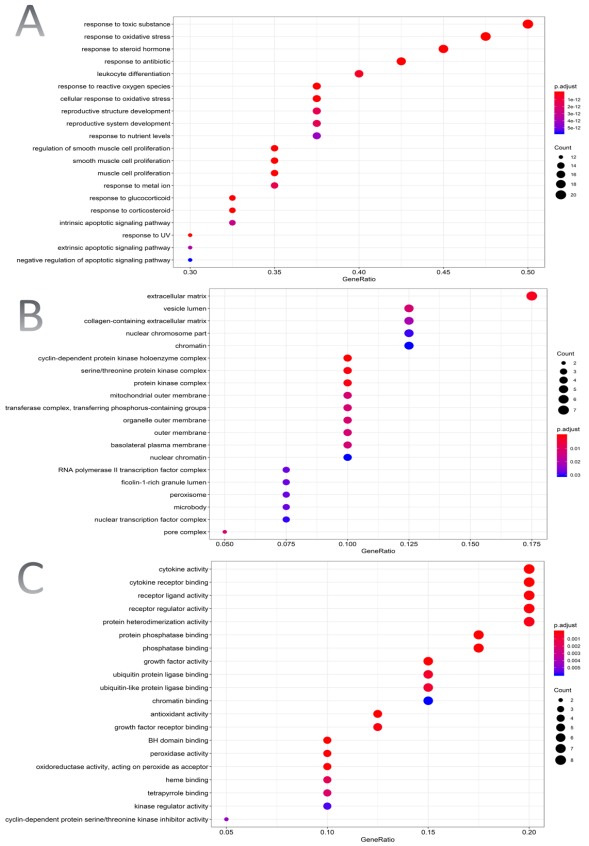

Figure 5. Dot plots of GO enrichment. The ordinate is the name of the enriched items (GO terms), and the abscissa is the proportion of targets in each term (gene ratio); the color from red to blue represents that the adjusted P-value varies from small to large, and a redder color means that the term is more significant. (A) Biological processes, (B) cellular components, (C) molecular functions.

3.5. KEGG Pathway Enrichment Analysis

Through the analysis of KEGG enrichment, we obtained 16 pathways that may be related to FAI treatment (Table 3), and made a scatter diagram according to the p value of these pathways (Fig.7A).

Among these 16 pathways, three pathways with the lowest p-value were related to drug resistance of tumor, and they were platinum resistance, endocrine resistance and EGFR tyrosine kinase inhibitor resistance. Seven pathways were related to signal transduction, including HIF-1, PI3K-Akt, FoxO, ErbB, NF-kappa B, MAPK and AMPK signaling pathways. Four pathways were related to cell growth and death, including p53 signaling pathway, cell cycle and apoptosis. The other two pathways were related to aging.

As shown in figure 7, PI3K-Akt signaling pathway have enriched the most genes (gene ratio = 15 / 39). PI3K-Akt is a classic anti-apoptosis signaling pathway, which is closely related to the occurrence and development of CAG. In order to further understand the effect of FAI in this signaling pathway, we downloaded a specific pathway map from the KEGG PATHWAY database (Fig.7B), and marked the enriched genes in the pathway, including AKT1, BCL2, CASP9, EGFR, etc.

To combine with the pathological mechanism of CAG, we searched the reference pathways of gastric cancer in KEGG PATHWAY database with the keyword "Gastritis" (Fig.8). As shown in the figure 8, PI3K-Akt, MAPK and p53 signaling pathways play an important role in the progression of GC. And these signaling pathways were also involved in the treatment of CAG by FAI, indicating FAI might delay the progress of CAG through these pathways.

3.6. Analysis of Molecular Docking

In this study, AKT1, CASP3, JUN and VEGFA were selected for molecular docking with corresponding compounds. The corresponding values are shown in table 4. The binding mode of drug ingredients and targets is shown in figures 9 to 11.

Luteolin is a natural flavonoid with four hydroxyl groups and one carbon group, which is the acceptor and donor of hydrogen bonds. As shown in table 4 and figure 9, luteolin and AKT1 combined well under the action of hydrogen bonds (glide score = -8.056). The hydroxyl groups of luteolin formed one hydrogen bond with the carbonyl group of GLU228 and the carboxyl group of GLU234, respectively. Luteolin and VEGFA interacted also mainly through hydrogen bonds (Fig. 10A). The interactions between luteolin and CASP3 included not only hydrogen bonds, but also π - π stacking (Fig.10B). And luteolin and JUN (Fig.10C)interacted mainly through hydrogen bonds and cation -π.

According to figure 11, the interactions between AKT1 and naringenin, CASP3 and naringenin, JUN and nobiletin were hydrogen bonds, hydrogen bonds and π - π stacking, hydrogen bonds and cation – π, respectively. To sum up, these three compounds had good docking results with AKT1, CASP3, JUN and VEGFA, and the interactions included π - π stacking, cation - π, and hydrogen bonds.

4. Discussion

FAI is a conventional Chinese herbal medicine that is considered effective in treating CAG. However, there are few in-depth studies on its pharmacological mechanism. The potential effect of FAI on CAG is further discussed through the network pharmacological method in this study.

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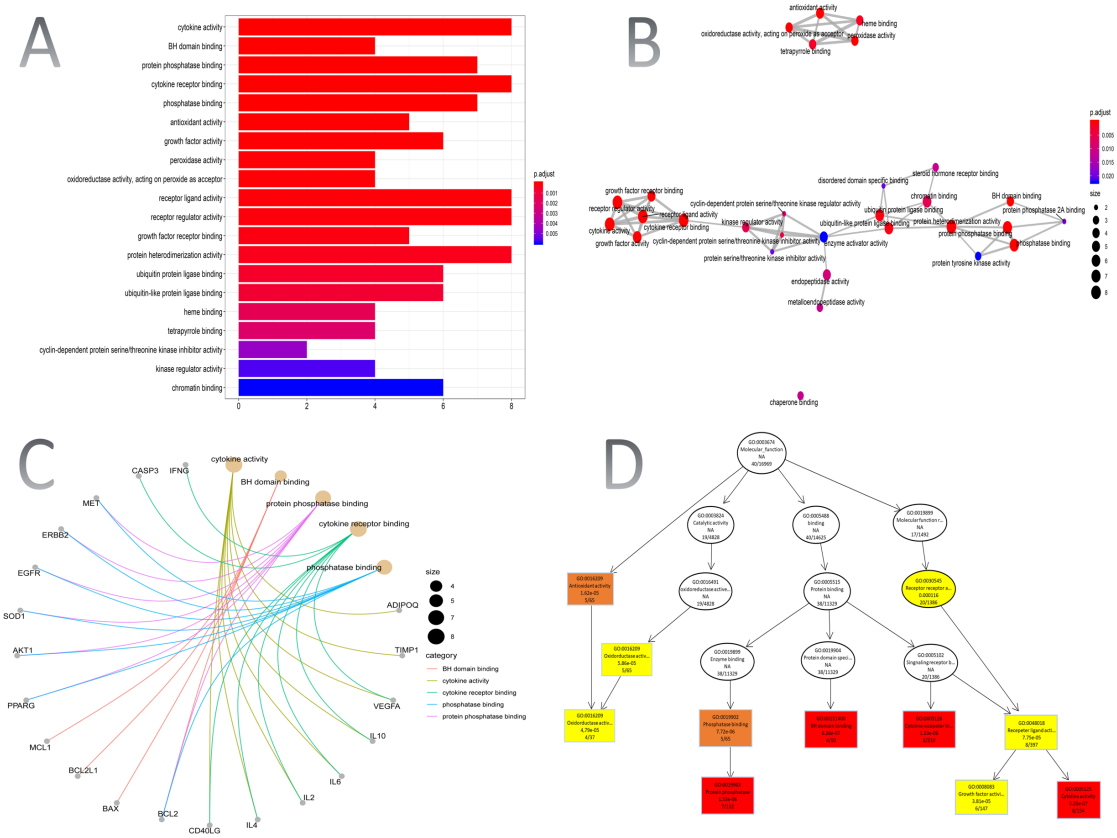

Figure 6. Further network analysis of GO-MF. (A) A bar plot of top 20 enriched GO terms with adjusted p-values. (B) Interaction networks between enriched GO terms analyzed by Enrichplot. (C) Sub-network of the top 5 GO terms and their enriched genes. (D) Directed acyclic graph of top 10 GO terms; different colors represent different enrichment significance, and the redder the color is, the higher the significance is.

Table 3. KEGG pathways of FAI in treatment of CAG

ID	Description	P value	Gene ID
hsa01524	Platinum drug resistance	2.89E-12	AKT1/BCL2/CASP3/GSTP1/BAX/CASP9/BCL2L1/CDKN1A/MDM2/ERBB2/BIRC5
hsa01522	Endocrine resistance	3.25E-12	AKT1/BCL2/BAX/MMP9/JUN/EGFR/CCND1/CDKN1A/MMP2/RB1/MDM2/ERBB2
hsa01521	EGFR tyrosine kinase inhibitor resistance	4.69E-09	AKT1/BCL2/BAX/EGFR/VEGFA/BCL2L1/IL6/ERBB2/MET
hsa04066	HIF-1 signaling pathway	4.96E-09	NOS2/AKT1/BCL2/TIMP1/EGFR/VEGFA/CDKN1A/IL6/ERBB2/IFNG
hsa04151	PI3K-Akt signaling pathway	1.69E-08	AKT1/BCL2/CASP9/EGFR/VEGFA/CCND1/BCL2L1/CDKN1A/IL6/MDM2/ERBB2/MCL1/IL2/IL4/MET
hsa04115	p53 signaling pathway	4.60E-08	BCL2/CASP3/BAX/CASP9/CCND1/BCL2L1/CDKN1A/MDM2
hsa04215	Apoptosis-multiple species	1.05E-07	BCL2/CASP3/BAX/CASP9/BCL2L1/BIRC5
hsa04210	Apoptosis	5.61E-07	AKT1/BCL2/CASP3/BAX/CASP9/JUN/BCL2L1/MCL1/BIRC5
hsa04068	FoxO signaling pathway	4.81E-06	AKT1/CAT/EGFR/CCND1/CDKN1A/IL10/IL6/MDM2
hsa04012	ErbB signaling pathway	0.000421	AKT1/JUN/EGFR/CDKN1A/ERBB2
hsa04211	Longevity regulating pathway	0.000521	PPARG/AKT1/CAT/ADIPOQ/BAX
hsa04064	NF-kappa B signaling pathway	0.000972	PTGS2/BCL2/BCL2L1/ICAM1/CD40LG
hsa04110	Cell cycle	0.002325	CCND1/CDKN1A/RB1/MDM2/PCNA
hsa04010	MAPK signaling pathway	0.006252	AKT1/CASP3/JUN/EGFR/VEGFA/ERBB2/MET
hsa04213	Longevity regulating pathway - multiple species	0.011451	AKT1/SOD1/CAT
hsa04152	AMPK signaling pathway	0.012695	PPARG/AKT1/ADIPOQ/CCND1

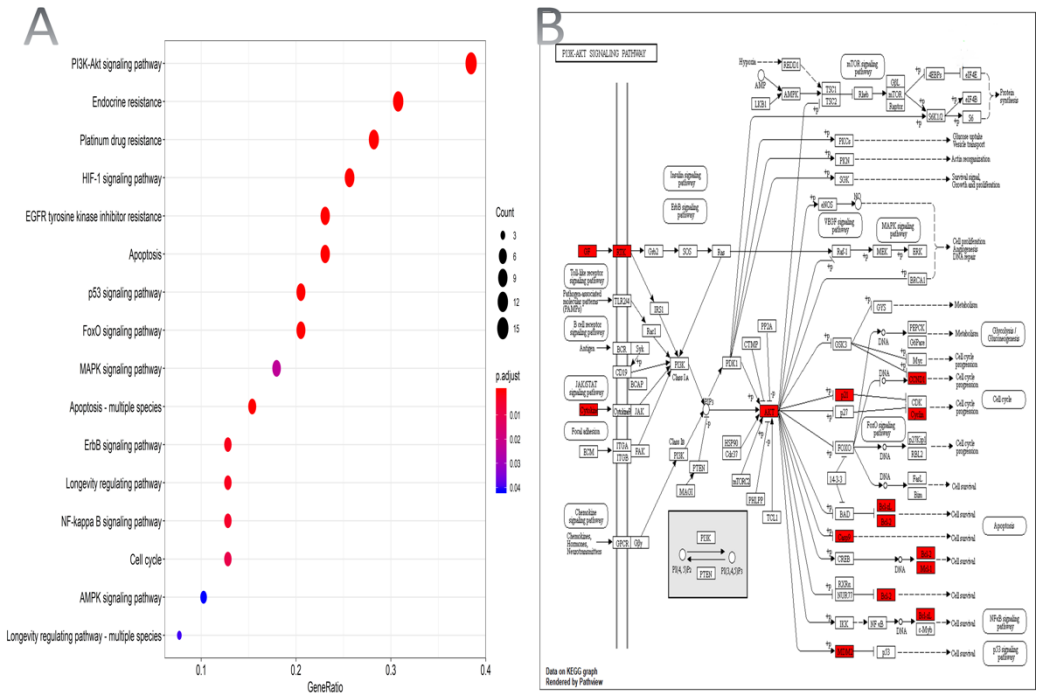

Figure 7. Analysis of KEGG pathways. (A) A dot plot of enriched KEGG pathways. (B) PI3K-Akt signaling pathway; the red boxes are the genes enriched in this pathway

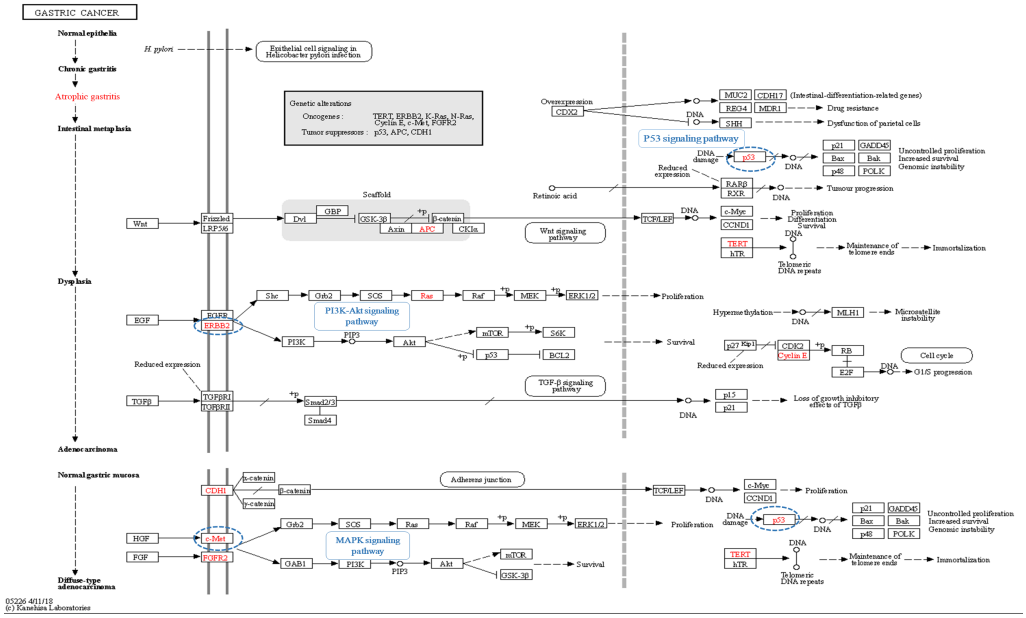

Figure 8 Gastric cancer-the reference pathway. The red nodes represent core genes in these reference pathways, the nodes circled in blue are the core genes related to FAI, the blue nodes are the signaling pathways involved in the treatment of CAG by FAI.

Table 4. Glide score values for targets with compounds

Gene ID	PDB ID	Compounds	Glide score
AKT1	4EKL	luteolin	-8.056
AKT1	4EKL	naringenin	-7.87
CASP3	6BDV	luteolin	-5.293
CASP3	6BDV	naringenin	-5.71
JUN	5T01	luteolin	-5.59
JUN	5T02	nobiletin	-5.613
VEGFA	5DN2	luteolin	-5.765

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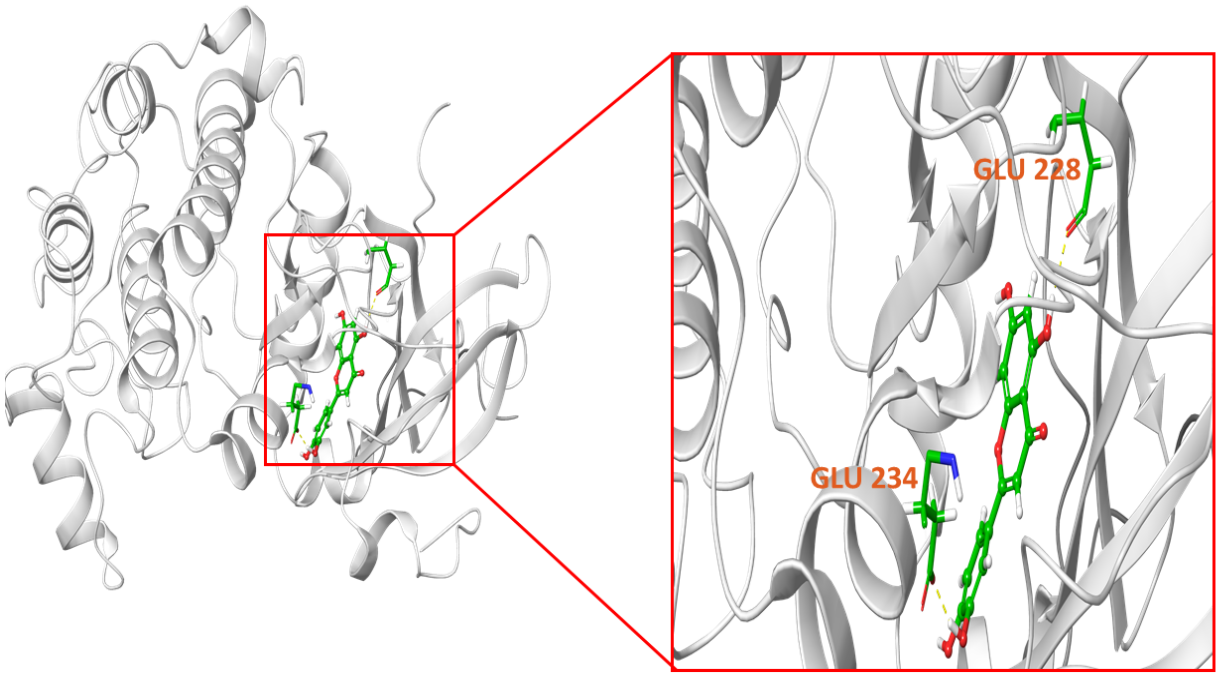

Figure 9. Molecular docking of luteolin and AKT1. The green compound is luteolin, the white compound is the protein structure of AKT1, the yellow dotted line represents the hydrogen bond.

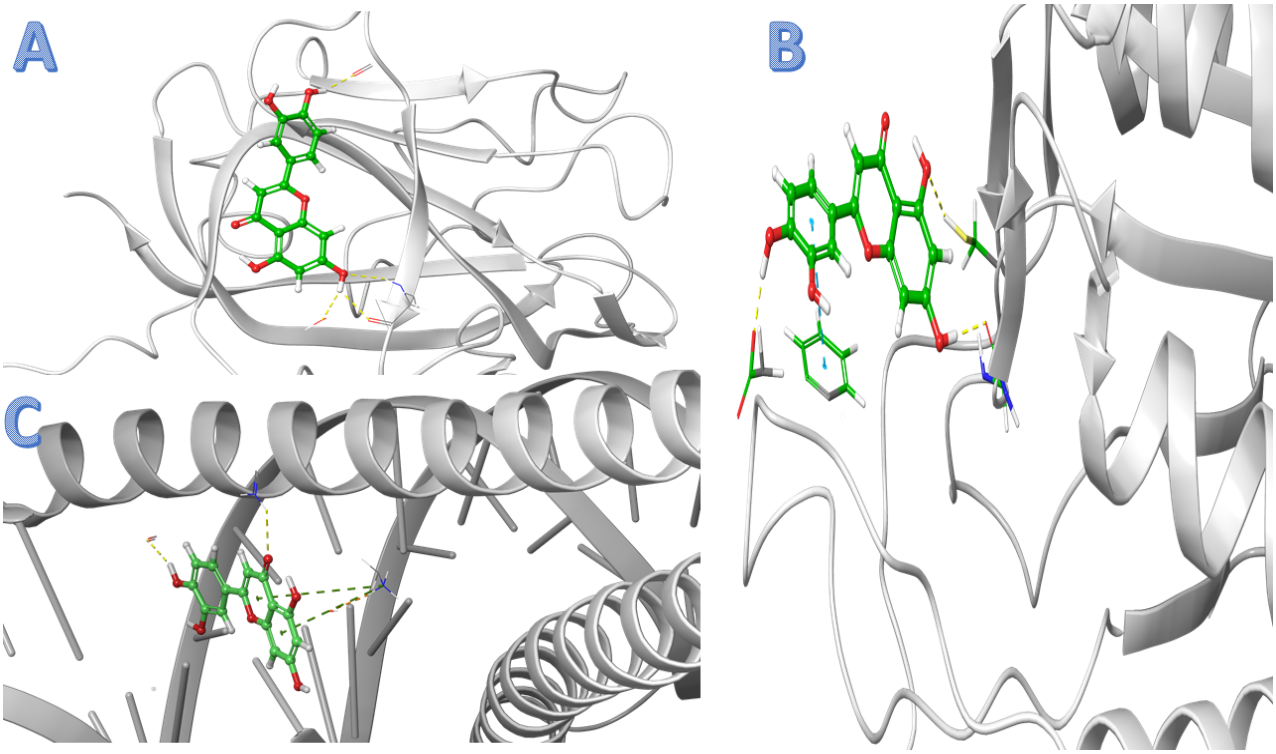

Figure 10. Binding modes of luteolin with selected targets. The green compound is luteolin, the white compounds are protein structure of the selected targets, the yellow dotted line represents the hydrogen bond, the green dotted line represents cation -π, the blue dotted line represents π - π stacking. (A) Luteolin with VEGFA. (B) Luteolin with CASP3; (C) Luteolin with JUN.

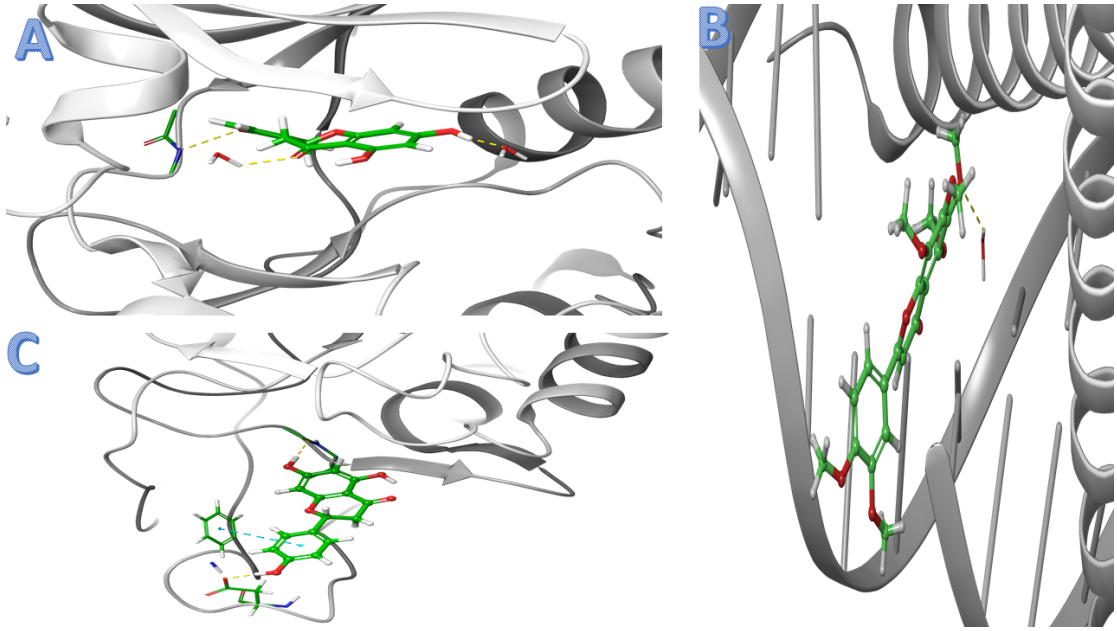

Figure 11. Binding modes of selected compounds with selected targets. The green compounds are naringenin and nobiletin, the white compounds are protein structure of the selected targets, the yellow dotted line represents the hydrogen bond, the green dotted line represents cation -π, the blue dotted line represents π - π stacking. (A) Naringenin with AKT1. (B) Nobiletin with JUN; (C)Naringenin with CASP3.

A total of 22 active ingredients were screened out, including neohesperidin_qt, naringenin, nobiletin, Sinensetin, luteolin Ammidin and so on. Most ingredients have been proven to have anti-inflammatory, antitumor and anti-apoptosis effects [24, 25]. For example, some studies have shown that luteolin (MOL000006, OB = 36.16%, DL = 0.25, degree = 33) can inhibit the release of inflammatory factors, and has anti-inflammatory and anti-tumor effects [26, 27]. In some reports, luteolin was found to inhibit the progress of chronic gastritis and regulate the apoptosis of gastric mucosal cells [28, 29]. Nobiletin (MOL005828, OB = 61.67%, DL = 0.52, degree = 14) has been proven to have anti-tumor effect. It can regulate Ras, MEK, ERK, JAK2, STAT3 and PI3K-Akt signaling pathways, reduce Akt and STAT phosphorylation levels, inhibit tumor cell proliferation and induce apoptosis [30, 31]. What's more, nobiletin also has anti-inflammatory, hypoglycemic, and adipocyte-inhibiting effects [31, 32], which has a wide application prospect. Naringenin (MOL004328, OB = 59.29%, DL = 0.21, degree = 11) is good at anti-tumor, anti-inflammatory, antioxidant and neuroprotective [33, 34]. It has an excellent inhibitory effect on malignant tumors, which can reduce the activity of Akt and caspase proteins, promote the apoptosis of damaged gastric epithelial cells, and maintain the integrity of gastric mucosa [35]. In addition, naringenin has excellent anti-inflammatory and analgesic effects by inhibiting the release of proinflammatory cytokines such as TNF-α, NF-κB, etc.[36]. In short, FAI contains various anti-tumor and anti-inflammatory compounds, which can control the apoptosis of gastrointestinal cells, reduce gastrointestinal inflammation, and regulate the gastrointestinal tract. It reflects the synergistic therapeutic effect of multiple components of FAI in treating CAG.

PPI analysis demonstrated that AKT1, CASP3, JUN and VEGFA were the core genes. Akt phosphorylation is an important anti-apoptosis regulatory factor, which can mediate the cell growth induced by insulin and various growth factors, maintain cell activity and inhibit cell apoptosis. Reducing Akt phosphorylation can promote apoptosis of tumor cells and inhibit metastasis of tumor [37]. CASP3 is a member of the caspase family. As an important cleavage enzyme for apoptosis, CASP3 plays an important role in the mechanism of apoptosis and is also a hot spot in tumor research. Caspase-3 mainly exists in the cytoplasm. When the cells are damaged, CASP3 is activated and can cut PARP polymerase, promote DNA cleavage, and regulate the immune response of tumor cells [38]. JUN is involved in coding the transcription factor AP-1, a leucine zipper protein that is a key transcription factor for regulating cell proliferation and apoptosis. AP-1 is highly expressed in many types of tumors, which can accelerate tumor growth and metastasis and promote tumor cell apoptosis [39, 40]. VEGFA belongs to the vascular endothelial growth factor (VEGF) family and can promote the vascular endothelial growth. Therefore, VEGFA is closely related to tumor angiogenesis and metastasis. Overexpression of VEGFA can be found in many tumor types, especially in liver cancer and melanoma [41].

CAG is a precancerous lesion whose key to treatment is to prevent its deterioration. As described

in the Results section, most of the targets of FAI in the treatment of CAG are related to cell proliferation and apoptosis. FAI may promote the apoptosis of damaged cells by enhancing the susceptibility of gastric epithelial cells and regulating their cycles. FAI can not only delay the development and deterioration of CAG by inhibiting the expression of anti-apoptosis genes such as AKT1 and JUN and up-regulating the expression of pro-apoptosis genes such as CASP3, but also inhibit abnormal neovascularization by reducing the overexpression of VEGFA gene, which is important for the development of CAG. Meanwhile, other core genes such as PTGS2, CCND1, EGFR, IL6, ERBB2 and CDKN1A are also considered important and worthy of further study.

In this study, 16 signaling pathways were obtained, among which PI3K-Akt, NF-kappaB, MAPK and p53 signaling pathways are closely related to cell apoptosis and proliferation. As shown in figure 7, FAI mainly regulates PI3K-Akt signaling pathway through Akt genes and its downstream targets such as CCND1, BCLS, MDMS and CASP9, and acts on growth factors and receptor tyrosine kinase (RKTs) to regulate PI3K expression. Under physiological conditions, the PI3K-Akt signaling pathway regulates various metabolic activities such as glucose metabolism, macromolecular protein synthesis and redox reactions to promote cell survival and growth. However, hyperphosphorylated Akt in tumor cells can change metabolism, enhance intracellular nutrient transport, inhibit cell apoptosis and promote tumor development and metastasis [42]. The P53 signaling pathway is a classic tumor suppressor pathway. It is usually closed under normal circumstances, but when cells are damaged, it is activated and can block cell cycles, repair or clear damaged DNA, and induce programmed cell death [43]. P53, as a key gene, is the first-line "protector" of anti-tumor [44]. We also found another signaling pathway called NF-kappaB signaling pathway that can promote the progress of CAG. NF-kappaB, as the central protein of this pathway, can inhibit the expression of p53 [45]. NF-kappaB signaling pathway is an important anti-apoptosis pathway, which inhibits cell differentiation, accelerates the proliferation of tumor cells, and promotes tumor neovascularization and tumor-related inflammatory responses [46]. According to the above analysis, we concluded that the compounds of FAI can directly or indirectly inhibit Akt phosphorylation and have different effects on its downstream targets, such as inhibiting the overexpression of CCND1, BCLS and MDMS, upregulating the expression of CASP9, thereby preventing PI3K-Akt signaling pathway is overactivated and induces apoptosis of damaged cells. Meanwhile, the genes mentioned above also affect the conduction of other signaling pathways such as NF-kappaB, p53 signaling pathways, thereby promoting cell apoptosis and inhibiting the deterioration of CAG. However, the above conclusions still need to be verified by further research.

In conclusion, the pharmacological mechanism of FAI in treating CAG is systematically summarized in this paper, aiming to provide a more comprehensive research of FAI, and give some ideas for the development and research of new drugs for treating CAG. However, it is worth noting that the key genes and pathways obtained through network pharmacology and molecular docking still need to be verified in vivo or in vitro.

Data availability statement

The data used to support the findings of this study are included within the article.

References

1. J. Watari, N. Chen, P.S. Amenta,et al. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development, World J Gastroenterol, 20 (2014):5461-73.
2. P. Correa. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention, CANCER RES, 52 (1992): 6735-40.
3. Y.H. Park, N. Kim. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer, J Cancer Prev, 20 (2015) :25-40.
4. F. Bray, J. Ferlay, I. Soerjomataram, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin, 68 (2018): 394-424.
5. W. Chen, R. Zheng, P.D. Baade, et al. A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, CA Cancer J Clin, 66 (2016): 115-32.
6. P. Pimentel-Nunes, D. Libanio, R. Marcos-Pinto, et al. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019, ENDOSCOPY, 51 (2019): 365-388.
7. E. Lahner, R.M. Zagari, A. Zullo, et al. Chronic atrophic gastritis: Natural history, diagnosis and therapeutic management. A position paper by the Italian Society of Hospital Gastroenterologists and Digestive Endoscopists [AIGO], the Italian Society of Digestive Endoscopy [SIED], the Italian Society of Gastroenterology [SIGE], and the Italian Society of Internal Medicine [SIMI],

Dig Liver Dis, 51 (2019): 1621-1632.
8. S. Zhang, G. Li, X.Tang, et al. Consensus on TCM diagnosis and treatment of chronic atrophic gastritis, Journal of Traditional Chinese Medicine, 51 (2010): 749-753.
9. Y. Liu, Z. Liu, C. Wang, et al. Study on essential oils from four species of Zhishi with gas chromatography-mass spectrometry, CHEM CENT J, 8 (2014): 22.
10. Q. Hao, Y. Li. The effect and mechanism of different varieties of Bupleurum and citrus on gastro-intestinal motility, China Medical Abstracts(Internal Medicine), 4(2001): 221-224.
11. The Pharmacopoeia Committee of China: Chinese Pharmacopoeia, 2015. Beijing: People's Medical Publishing House;1 (2015): 172
12. W. Xu, R. Zhao, D. Li, et al. TCM master LI Dian-gui's clinical experience in the diagnosis and treatment of atrophic gastritis. China Journal of Traditional Chinese Medicine and Pharmacy 34 (2019) 4350-4353.
13. C. Jiang. Clinical observation of Zhishi Xiaopi pill in the treatment of chronic atrophic gastritis. Journal of Practical Traditional Chinese Medicine, 35(2019):21-22.
14. Y. Stevens, E.V. Rymenant, C. Grootaert, et al. The Intestinal Fate of Citrus Flavanones and Their Effects on Gastrointestinal Health, NUTRIENTS, 11 (2019): 1464.
15. M. Imran, A. Rauf, T. Abu-Izneid, et al. Luteolin, a flavonoid, as an anticancer agent: A review, BIOMED PHARMACOTHER, 112 (2019): 108612.
16. T. Pei, C. Zheng, C. Huang, et al. Systematic understanding the mechanisms of vitiligo pathogenesis and its treatment by Qubaibabuqi formula, J ETHNOPHARMACOL, 190 (2016): 272-87.
17. X. Xie, X. Ma, S. Zeng, et al. Mechanisms of Berberine for the Treatment of Atherosclerosis Based on Network Pharmacology, Evid Based Complement Alternat Med, 2020 (2020) 3568756.
18. X. Xu, W. Zhang, C. Huang, et al. A novel chemometric method for the prediction of human oral bioavailability, INT J MOL SCI, 13 (2012): 6964-82.
19. Ru, J., Li, P., Wang, J., et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. Journal of cheminformatics, 6, 13 (2014): PMC4001360.
20. M. Safran, V. Chalifa-Caspi, O. Shmueli, et al. Lancet: Human Gene-Centric Databases at the Weizmann Institute of Science: GeneCards, UDB, CroW 21 and HORDE, NUCLEIC ACIDS RES, 31 (2003): 142-6.
21. J. Pinero, J.M. Ramirez-Anguita, J. Sauch-Pitarch, et al. The DisGeNET knowledge platform for disease genomics: 2019 update, NUCLEIC ACIDS RES, 48 (2020) D845-D855.
22. Chen J, Li C, Zhu Y, et al. Integrating GO and KEGG terms to characterize and predict acute myeloid leukemia-related genes. Hematology 2015, 20, (6): 336-42.
23. G.M. Morris, M. Lim-Wilby. Molecular docking, Methods Mol Biol, 443 (2008): 365-82.
24. L. Cheng, Y. Ren, D. Lin, et al. The Anti-Inflammatory Properties of Citrus wilsonii Tanaka Extract in LPS-Induced RAW 264.7 and Primary Mouse Bone Marrow-Derived Dendritic Cells, MOLECULES, 22 (2017): 1213.
25. J.A. Emim, A.B. Oliveira, A.J. Lapa. Pharmacological evaluation of the anti-inflammatory activity of a citrus bioflavonoid, hesperidin, and the isoflavonoids, duartin and claussequinone, in rats and mice, J PHARM PHARMACOL, 46 (1994): 118-22.
26. M.J. Tuorkey. Molecular targets of luteolin in cancer, EUR J CANCER PREV, 25 (2016): 65-76.
27. X. Fan, Du K, N. Li, et al. Evaluation of Anti-Nociceptive and Anti-Inflammatory Effect of Luteolin in Mice, J Environ Pathol Toxicol Oncol, 37 (2018): 351-364.
28. C.M. Park, K. Jin, C.W. Cho, et al. Luteolin inhibits inflammatory responses by downregulating the JNK, NF-κB, and AP-1 pathways in TNF-α activated HepG2 cells, FOOD SCI BIOTECHNOL, 21 (2012): 279-283.
29. M. Kimata, M. Shichijo, T. Miura, et al. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells, CLIN EXP ALLERGY, 30 (2000): 501-8.
30. K. Aoki, A. Yokosuka, Y. Mimaki, et al. Nobiletin induces inhibitions of Ras activity and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signaling to suppress cell proliferation in C6 rat glioma cells, BIOL PHARM BULL, 36 (2013) 540-7.
31. D. Wei, G. Zhang, Z. Zhu, et al. Nobiletin Inhibits Cell Viability via the SRC/AKT/STAT3/YY1AP1 Pathway in Human Renal Carcinoma Cells, FRONT PHARMACOL, 10 (2019): 690.
32. K. Kanda, K. Nishi, A. Kadota, et al. Nobiletin suppresses adipocyte differentiation of 3T3-L1 cells by an insulin and IBMX mixture induction, Biochim Biophys Acta, 1820 (2012): 461-8.
33. Z. Nouri, S. Fakhri, F.F. El-Senduny, et al. On the Neuroprotective Effects of Naringenin: Pharmacological Targets, Signaling Pathways, Molecular Mechanisms, and Clinical Perspective, Biomolecules, 9 (2019): 690.
34. R. Joshi, Y.A. Kulkarni, S. Wairkar.

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doi: 10.53388/ghr2021-03-028

Pharmacokinetic, pharmacodynamic and formulations aspects of Naringenin: An update, LIFE SCI, 215 (2018): 43-56.
35. H. Zhang, X. Zhong, X. Zhang, et al. Enhanced anticancer effect of ABT-737 in combination with naringenin on gastric cancer cells, EXP THER MED, 11 (2016): 669-673.
36. F.A. Pinho-Ribeiro, A.C. Zarpelon, V. Fattori, et al. Naringenin reduces inflammatory pain in mice, EUROPHARMACOLOGY, 105 (2016): 508-519.
37. L.L. Wang, L. Zhang, X.F. Cui. Downregulation of long noncoding RNA LINC01419 inhibits cell migration, invasion, and tumor growth and promotes autophagy via inactivation of the PI3K/Akt1/mTOR pathway in gastric cancer, THER ADV MED ONCOL, 11 (2019): 1758835919874651.
38. M.E. Rodriguez-Ruiz, A. Buque, M. Hensler, et al. Apoptotic caspases inhibit abscopal responses to radiation and identify a new prognostic biomarker for breast cancer patients, ONCOIMMUNOLOGY, 8 (2019): e1655964.
39. S. Trop-Steinberg, Y. Azar. AP-1 Expression and its Clinical Relevance in Immune Disorders and Cancer, AM J MED SCI, 353 (2017) 474-483.
40. H. Mirzaei, N. Khodadad, C. Karami, et al. The AP-1 pathway; A key regulator of cellular transformation modulated by oncogenic viruses, REV MED VIROL, 30 (2020) e2088.
41. L. Claesson-Welsh. What is normal? Apelin and VEGFA, drivers of tumor vessel abnormality, EMBO MOL MED, 11 (2019): e10892.
42. G. Hoxhaj, B.D. Manning. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism, NAT REV CANCER, (2019): 74-88 .
43. G. Pistritto, D. Trisciuoglio, C. Ceci, et al. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies, Aging (Albany NY), 8 (2016): 603-619.
44. B. Hong, A.P. van den Heuvel, V.V. Prabhu, S. Zhang, W.S. El-Deiry, Targeting tumor suppressor p53 for cancer therapy: strategies, challenges and opportunities, CURR DRUG TARGETS, 15 (2014): 80-89.
45. R.F. Johnson, N.D. Perkins. Nuclear factor-kappaB, p53, and mitochondria: regulation of cellular metabolism and the Warburg effect, TRENDS BIOCHEM SCI, 37 (2012): 317-324.
46. Y. Ben-Neriah, M. Karin. Inflammation meets cancer, with NF-kappaB as the matchmaker, NAT IMMUNOL, 12 (2011) 715-723.