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Network pharmacology and bioinformatics to identify the molecular mechanisms of Gleditsiae Spina against colorectal cancer

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ABSTRACT

Objective: In this study, network pharmacology, bioinformatics and molecular docking were used to explore the active phytochemicals, hub genes, and potential molecular mechanisms of Gleditsiae Spina in treating of colorectal cancer..

Methods: The targets of Gleditsiae Spina, and targets related to CRC were derived from databases. We identified the hub genes for Gleditsiae Spina anti-colorectal cancer following the protein–protein-interaction (PPI) network. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were used to analyze the hub genes from a macro perspective. Finally, we verified the hub genes by molecular docking, GEPIA, HPA, and starBase database.

Results: We identified nine active phytochemicals and 36 intersection targets. The GO enrichment analysis results showed that Gleditsiae Spina may be involved in gene targets affecting multiple biological processes, including response to radiation, response to ionizing radiation, cyclin-dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, cyclin-dependent protein serine/threonine kinase regulator activity and protein kinase regulator activity. KEGG enrichment analysis results indicated that the P53 signaling pathway, IL-17 signaling pathway, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, and JAK-STAT signaling pathway were mainly related to the effect of Gleditsiae Spina on colorectal cancer. Molecular docking analysis suggested that the active phytochemicals of Gleditsiae Spina could combine well with hub genes (*PTGS1*, *PIK3CG*, *CCND1*, *CXCL8* and *ADRB2*).

Conclusion: This study provides clues for further study of anti-CRC phytochemicals as well as their mechanisms of provides a basis for their development model.

Introduction

Colorectal cancer (CRC) is still a global health challenge, as it is one of the most common malignant tumors in the digestive system (Dekker et al., 2019). In the past 20 years, the age of onset of colorectal cancer has gradually become younger (Patel & Ahnen, 2018), which deserves global concern. Chemotherapy and radiotherapy are the two main types of treatment for CRC patients today. However, they frequently cause adverse side effects in patients, including liver dysfunction, gastrointestinal problems, and suppression of the bone marrow (Baidoun et al., 2021), making it difficult for patients to complete the entire treatment. Given this, there is an urgent need for colorectal cancer medicines that are more efficient and less toxic (Islam et al., 2022).

Gleditsiae Spina, belonging to the Rubiaceae family, is the thorn of *Gleditsia sinensis* Lam., which is a natural herb commonly found in eastern and tropical Asian countries such as China, Japan, and Indonesia (Gao et al., 2016). From the perspective of traditional Chinese medicine theory, Gleditsiae Spina clears heat, detoxifies, promotes blood

Abbreviations: AUC, The area under ROC curve; BP, Biological process; CC, cell composition; CRC, Colorectal cancer; DEGs, Differential genes; DL, Drug-like properties; FC, Fold change; GEO, Gene expression omnibus; GO, Gene ontology; GEPIA, Gene expression profiling interactive analysis; HPA, The human protein atlas; HRs, Hazard ratio; IL17, Interleukin-17; KEGG, Kyoto encyclopedia of genes and genomes; LASSO, Least absolute shrinkage and selection operator; MF, Molecular function; OB, Oral bioavailability; PDB, Protein data bank; PPI, Protein–protein-interaction network; RB, Retinoblastoma; RF, Random forest; ROC, Receiver operating characteristic; SVM, Support vector machine; TCM, traditional Chinese medicine; TCMSP, traditional Chinese medicine systems pharmacology database and analysis platform.

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circulation, and removes blood stasis. It has been widely used in a variety of Chinese medicine formulations for a long time and is used in treating of inflammation and infectious diseases such as pharyngitis, bronchitis, hepatitis, urethritis, and appendicitis. According to reports, Gleditsiae Spina has a potent pharmacological impact against many cancers, including colon, liver, stomach, lung, and breast cancers, with mild side effects (Cai et al., 2019; Kim et al., 2016; Lee et al., 2010; Lee et al., 2013; Yu et al., 2019). However, the fundamental mechanism of its anti-colorectal cancer activity has not been completely clarified.

Network pharmacology is the combining of drug action targets and disease targets to create an interaction network between diseases, targets, medications, and pathways to thoroughly and systematically understand the mechanism of drug action, which is consistent with the overall concept of traditional Chinese medicine (TCM) treatment of diseases and the theory of syndrome differentiation and treatment (Wang et al., 2021).

In this study, network pharmacology was used to determine the pharmacologic target network of Gleditsiae Spina, and bioinformatics was employed to assemble data on the pertinent targets of colorectal cancer. GO and KEGG enrichment analyses were performed on the intersection targets. We verified the hub genes utilizing molecular docking, GEPIA, HPA, and StarBase database to unravel the pathophysiology of colorectal cancer and provide a novel therapeutic intervention therapy perspective.

Materials and Methods

Active phytochemicals and targets of Gleditsiae Spina

The active phytochemicals and potential targets of Gleditsiae Spina were queried and screened using the traditional Chinese medicine systems pharmacology database and analysis platform (Ru et al., 2014) (TCMSP, https://tcmsp-e.com/tcmsp.php), in which oral bioavailability (OB $\geq$ 30 %) and drug-like properties (DL $\geq$ 0.18) were essential indicators to evaluate their drug development potential. The Uniprot database (UniProt, 2023) (Uniprot, http://www.uniprot.org/) was used to correct the entered protein target names to their Official Symbols and the species was set as human.

Target and differential expression analysis in colorectal cancer

"Colorectal cancer" was used as a keyword to retrieve the CRC data set from the Gene Expression Omnibus database (Edgar et al., 2002) (GEO, https://www.ncbi.nlms.nih.gov/geo). As a training dataset for discovery, the GSE23878 dataset was constructed by the GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array platform. Colon tissues were from 24 CRC patients and 35 healthy people. Since the whole data set came from the GEO database, no informed permission nor ethical approval was necessary.

The "limma" package in R software was used to screen differential genes (DEGs) for CRC. Statistically significant DEGs were selected according to *p-value* < 0.05 and $|\log2FC|>1$ (Fold change, FC refers to the multiple of difference) after calibration, and the volcano map and heat map were drawn by R software for visualization to understand the distribution of up-regulated genes and down-regulated genes.

Screening core genes and hub genes

To identify potential interactions between CRC-associated DEGs and targets related to major phytochemicals of Gleditsiae Spina, the intersection of CRC targets and Gleditsiae Spina targets were taken to identify the core genes of Gleditsiae Spina in the treatment of CRC and a Venn graph was made by using the "VennDiagram" package in R software. Subsequently, the PPI network map and drug- phytochemicals -targets-disease map were constructed using the string database and Cytoscape software. Then, hub genes were screened according to Degree, BC and CC values.

GO and KEGG enrichment analysis

To further explore the biological functions of core genes, GO gene enrichment analysis and KEGG signaling pathway analysis were performed and visualized on core genes by using the "clusterProfiler", "org.Hs.eg.db", and "ggplot2" packages in R software to explore the main molecular biological processes and signaling pathways of potential targets of Gleditsiae Spina. Finally, the compound–therapeutic targets-core pathways network diagram was constructed using Cytoscape software with nodes in the network representing compound, targets, and pathways. Nodes were connected by edges that represented different meanings depending on the ways the network was built.

Exploration of the significance of core genes in the diagnosis of CRC

In this study, three machine learning algorithms, including least absolute shrinkage selector operator (LASSO), support vector machine recursive feature elimination (SVM-RFE), and random forest (RF) (Chen et al., 2022b) were used to explore the significance of core genes for evaluating the diagnosis potential of CRC. Firstly, the LASSO and SVM algorithms were used to classify the diagnostic markers of CRC. Then, 10-fold cross-validation was performed using the "glmnet" package in R software to distinguish CRC patients from healthy people. To avoid overfitting, SVM-RFE algorithm was used to screen high-quality genes using the "e1071" and "svmRadial" packages in R software. Subsequently, RF algorithm was used to build binary trees using recursive partitions. The "randomForest" package in R software was used to construct an RF classification model and ranked the therapeutic targets according to the Gini index to screen the therapeutic targets expressed by the features. The intersetion genes of three machine learning algorithms were considered to have better diagnostic analysis of CRC. We used the "pROC" package in R software to evaluate the predictive value of the CRC diagnosis. AUC, which refers to the area under receiver operating characteristic (ROC) curves, was calculated to evaluate the prediction model's precision. The larger the AUC value, the higher the accuracy of the prediction model.

mRNA expression lever, pathological stage, overall survival, and protein expression of hub genes

The hub genes were inputted into the online tool GEPIA database (Tang et al., 2017) (GEPIA, http://gepia.cancer-pku.cn/index.html) to verify their mRNA expression levels and pathological stage. The protein expression of hub genes was then studied in the HPA database (Uhlen et al., 2015) (HPA, https://www. proteinatlas.org/). The hub genes' overall survival was analyzed using Kaplan-Meier plotter (Gyorffy, 2021) (Kaplan-Meier plotter, https://www.kmplot.com/). The hazard ratios (HRs; 95 % confidence interval) and *p-value* were calculated, and *p-value* < 0.05 served as the significance threshold.

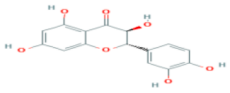
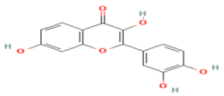
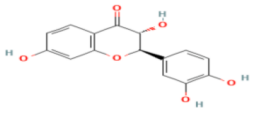
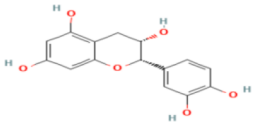
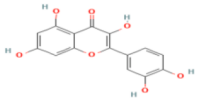
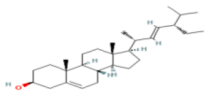
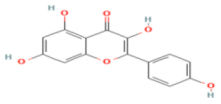
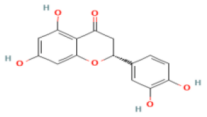
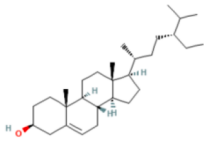
Analysis of immune cell infiltration

The CIBERSORT algorithm (Chen et al., 2018) (https://cibersortx.stanford.edu) was used to determine the relative proportions of 22 invading immune cell types in each tissue. The immunological score of each sample was calculated using the "ESTIMATE" algorithm. In addition, the associations of signature genes with the number of infiltrating immune cells were determined by using "Spearman" rank correlation analysis in R software. Finally, the graphing method of the "ggplot2" package in R software was used to visualize the resulting correlations.

ceRNA network construction

StarBase database (Li et al., 2014) (starBase, http://starbase.sysu.

Table 1
Basic information of active ingredients in Gleditsiae Spina.

Mol ID	Phytochemical Name	MW	Stucture	OB	DL
MOL001736	(-)-taxifolin	304.25		60.51	0.27
MOL013179	Fisetin	286.24		52.6	0.24
MOL013296	Fustin	288.25		50.91	0.24
MOL000073	ent-Epicatechin	290.27		48.96	0.24
MOL000098	Quercetin	302.23		46.43	0.28
MOL000449	Stigmasterol	412.7		43.83	0.76
MOL000422	Kaempferol	286.24		41.88	0.24
MOL002914	Eriodyctiol (flavanone)	288.25		41.35	0.24
MOL000358	beta-sitosterol	414.7		36.91	0.75

edu.cn) was used to predict mRNA-miRNA interaction pairs of hub genes. Then, the predicted miRNAs were searched in starBase database again and miRNA-lncRNAs were screened to obtain the ceRNA network of mRNA-miRNA-lncRNAs. Among them, Cytoscape software was used for visual analysis.

Molecular docking

The main active phytochemicals of Gleditsiae Spina were imported into Chem3D software to construct their chemical structure formula and minimize its energy, then saved as PDB format file. Hub genes were brought into the PDB database, and corresponding proteins were downloaded as PDB format files and imported into PyMol software to remove water molecules and small molecules. The protein was imported into autodocktool software for hydroprocessing and saved as a PDBQT format file. Finally, PyMol and MOE software were utilized for molecular docking and visualization analysis.

Results

Screening of active phytochemicals in Gleditsiae Spina

Through searching and screening the TCMSP database, nine active phytochemicals whose OB value was higher than 30 % and DL value was higher than 0.18 were found in Gleditsiae Spina: (-)-taxifolin, fisetin, fustin, ent-epicatechin, quercetin, stigmasterol, kaempferol, eriodyctiol (flavanone), and beta-sitosterol. Then, 204 related targets were screened after removing duplicate values, as shown in Table 1.

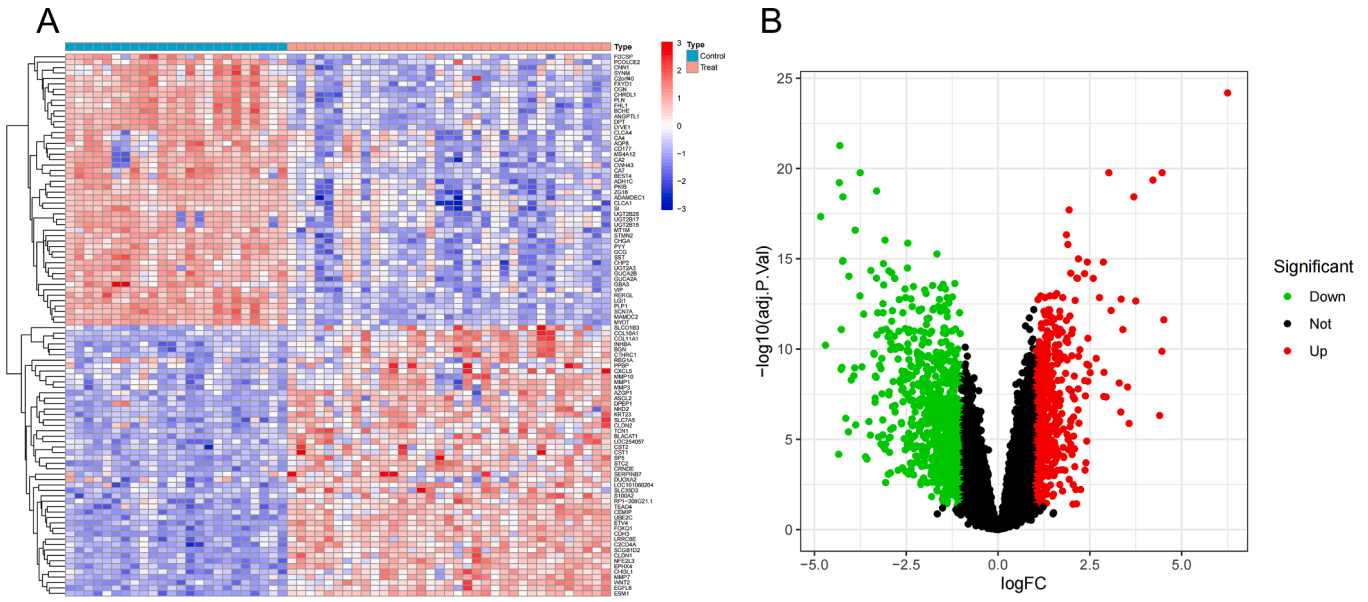


Fig. 1. Analysis of expression levels of DEGs of CRC, A is a heat map of DEGs, B is a volcano map of DEGs, and green represents down-regulated genes, while red represents up-regulated genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

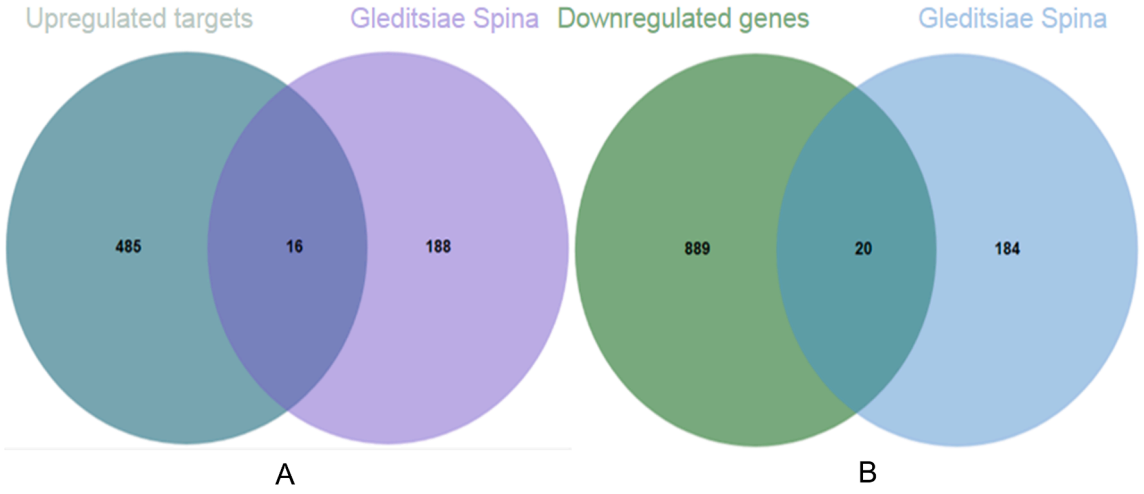


Fig. 2. Venn diagram, yellow circle represents up-regulated genes of CRC, blue circle represents down-regulated genes of CRC, and green circle represents Gleditsiae Spina relative targets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Dentification of CRC-associated DEGs, and identification of core genes and hub genes

The GSE23878 dada set included colon tissue from 24 CRC patients and 35 healthy people. Differential analysis results showed that a total of 1327 genes were found to be different between CRC colon tissue samples and healthy samples, including 501 up-regulated genes and 909 down-regulated genes, as shown in Fig. 1. A total of 36 intersection targets were acquired by the intersection of CRC related-DEGs and Gleditsiae Spina-related targets, which including 16 up-regulated gene (*CCND1, CDK4, MMP7, CXCL8, NUF2, DPEP1, TOP2A, MMP3, PLAU, MMP1, MYC, BIRC5, CCNB1, COL1A1, CXCL11,* and *E2F1*) and 20 down-regulated genes (*PTGS1 PIK3CG, CA2, CD36, DPP4, AKR1B10 ADRB2, FOS, XDH, CAV1, CCL2, MGAM, ABCG2, HAS2, NR3C2, ADH1C, ADRA2A, MAOA, CHRM2,* and *PDE3A*). These 36 intersection genes were known as core genes, as shown in Fig. 2. Then, a Drug - phytochemicals - targets - disease network was constructed via Cytoscape software, as shown in Fig. 3. We used Cytoscape software to construct a PPI network map for core genes, as illustrated in Fig. 4. The nine active phytochemicals of Gleditsiae Spina with their degree values in the hub network were presented as a bar graph in Fig. 5, and MOL000098 (quercetin), MOL013179 (fisetin), MOL000449 (stigmasterol), MOL000422 (kaempferol) and MOL000358 (beta-sitosterol) were, respectively, the top five active phytochemicals that interact with CRC related targets. Finally, according to DC, BC and CC values as shown in Table 2, we considered *PTGS1, PIK3CG, CCND1, CXCL8* and *ADRB2* as the hub genes.

GO and KEGG enrichment analysis of core genes

We conducted GO and KEGG enrichment analysis of core genes to understand the biological function of core genes in CRC and the related regulating pathways. A total of 335 GO entries were obtained (p-*value* $<$ 0.05), and the main category was the biological process (BP), cell composition (CC), and molecular function (MF). The results of GO enrichment analysis showed that, among BP entries, core genes were correlated with “response to radiation”, “response to ionizing radiation”, “cyclin-dependent protein kinase holoenzyme complex”, “serine/

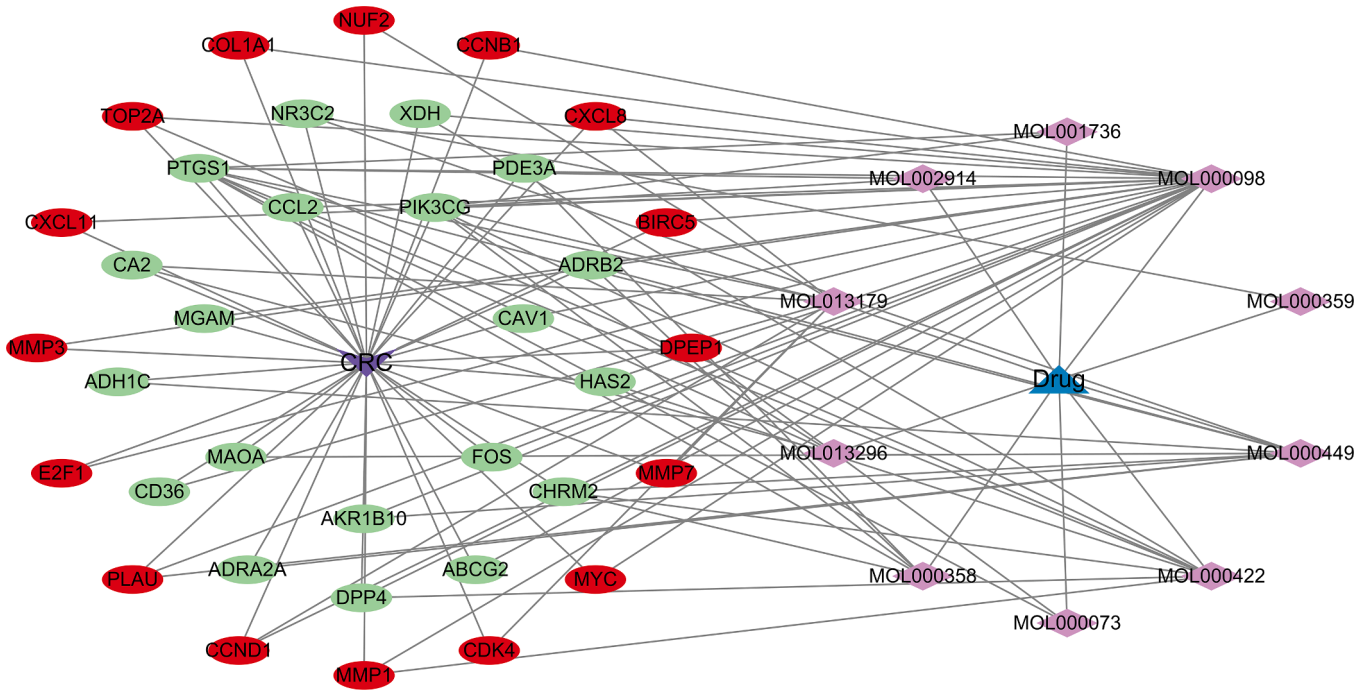


Fig. 3. Drug - phytochemical - targets - disease diagram, the red oval represents the up-regulated genes of CRC, the green oval represents the down-regulated genes of CRC, and the purple diamond represents the active phytochemicals of Gleditsiae Spina. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

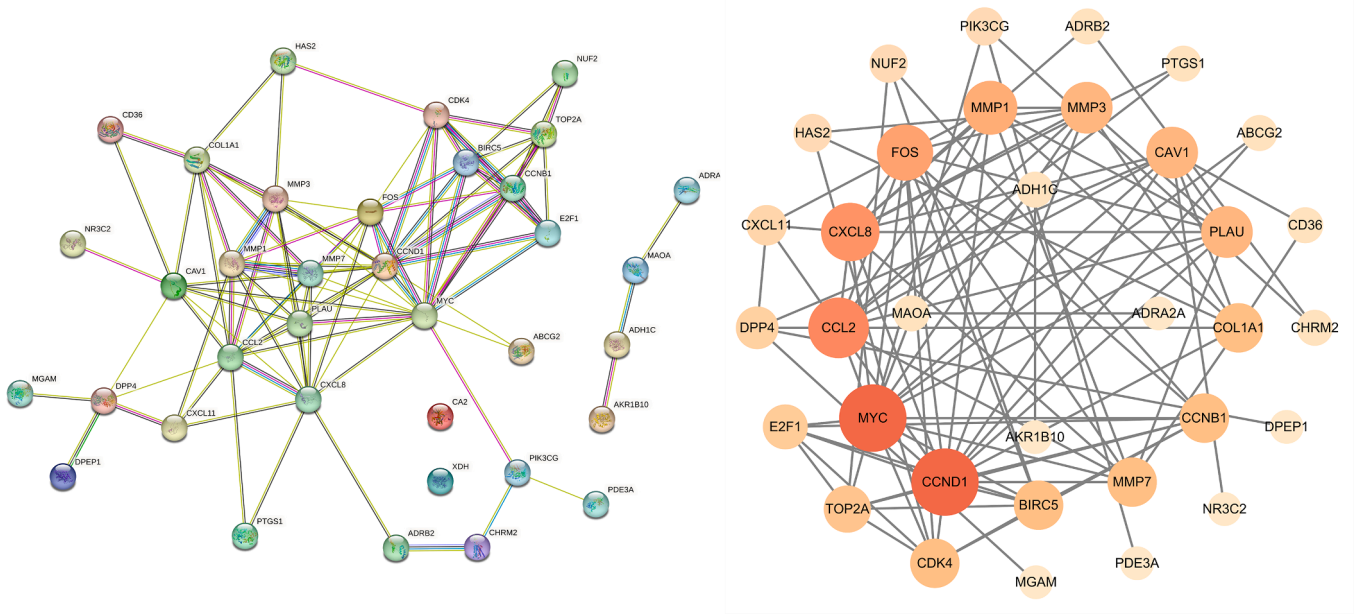


Fig. 4. PPI network of core genes, the darker the color, the more important it is in against CRC by Gleditsiae Spina.

threonine protein kinase complex", "cyclin-dependent protein serine/threonine kinase regulator activity", and "protein kinase regulator activity", as shown in Fig. 6A. There were 11 CC entries, mainly related to serine/threonine protein kinase complex, protein kinase complex, apical part of cell, and so on. Also, 19 MF entries were mainly related to cyclin-dependent protein serine/threonine kinase regulator activity, protein kinase regulator activity, kinase regulator activity, and so on. The above analysis showed that Gleditsiae Spina is likely to act on these cell structures, affect these biological processes, and exert these molecular functions to achieve the purpose of anti-colorectal cancer, as indicated in Fig. 6.

By utilizing the "cluster profiler" package in R language software, the KEGG pathway enrichment analysis of the 36 core genes of Gleditsiae Spina on CRC was carried out. The results showed 50 signal pathways (p-*value* < 0.05) in which Gleditsiae Spina interfered with CRC. IL-17 signaling pathway, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway as well as JAK-STAT signaling pathway were significantly enriched (Fig. 6B). According to the results of the enrichment analysis, core genes may significantly influence the onset and progression of CRC by controlling PI3K-AKT signaling pathway, IL-17 signaling

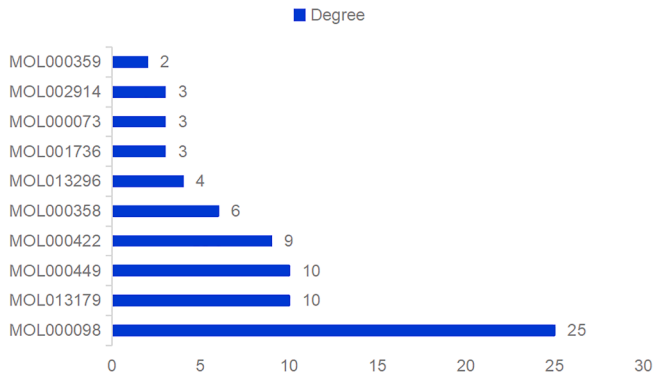


Fig. 5. Active phytochemical of Gleditsiae Spina in relation to hub network, MOL000098 is quercetin, MOL013179 is fisetin, MOL000449 is Stigmasterol, MOL000422 is kaempferol, and MOL000358 is beta-sitosterol.

Table 2
Using three algorithms of cytoHubba to discover the hub genes.

Gene	Degree	Betweenness Centrality	Closeness Centrality
PTGS1	10	0.0859	0.5529
PIK3CG	8	0.0528	0.5280
CCND1	3	0.0038	0.4747
CXCL8	3	0.0038	0.4747
ADRB2	4	0.0100	0.4845

pathway, and Toll-like receptor signaling pathway. Then, the core signaling pathways were visualized through the KEGG data set, as shown in Fig. 7. Finally, the targets-pathways network and phytochemicals-targets-pathways network were constructed using Cytoscape software, as shown in Figs. 8-9.

Considering the differences between CRC patients and healthy people, we aimed to evaluate the diagnostic potential of core genes in CRC. We screened the meaningful genes through three machine learning algorithms (LASSO, SVM-RFE, and RF) in the training dataset to achieve the role of distinguishing CRC patients from healthy people. In the LASSO algorithm, 13 genes were selected, including *CCND1, CDK4, MMP7, DPEP1, MMP1, MYC, CCNB1, COL1A1, PTGS1, CD36, CCL2, ADH1C*, and *PDE3A* (Fig. 10A-B). Meanwhile, in the SVM-RFE algorithm, the first five targets could be determined as the best targets according to the maximum accuracy and minimum error value and five targets (*CD36*, *CCNB1*, *PTGS1*, *CCND1*, and *CDK4*) were identified, as illustrated in Fig. 10C-D. In addition, we used the RF algorithm to determine the gene importance of core genes. Random forest results indicated that there were 19 related genes whose gene importance was greater than 0, including *E2F1, MAOA, CDK4, CHRM2, DPEP1, ADRB2, CCND1, MMP1, NUF2, HAS2, PTGS1, COL1A1, TOP2A, ADH1C, MMP7, CD36, CAV1, ABCG2,* and *MYC* (Fig. 10E-F). Finally, we combined the results of this three machine learning algorithms, and four genes (*CCND1 CDK4 PTGS1*, and *CD36*) of core genes had better diagnostic potential (Fig. 10H). To clarify the diagnostic potential of these four genes in distinguishing CRC samples from healthy people samples, ROC curves were drawn with diagnostic significance, as shown in Fig. 10G, and their AUC values were all greater than 0.9. The above evidence suggested that *CCND1, CDK4, PTGS1*, and *CD36* of core genes had high accuracy and specificity in differentiating CRC patients from healthy people.

Immune infiltration analysis of hub genes

We utilized the CYBERSORT method to analyze whether there were differences in the immune microenvironment between the normal group and the CRC group, and the difference in immune cell content between the two groups could be visually seen in Fig. 11A -B. Then, we used the statistical Wilcox. Test method to compare the difference between the two groups, as shown in Fig. 11C, and found that B cell naive, Dendritic cells resting, Macrophages M1, Macrophages M2, Mast cells activated, Mast cells resting, NK cells activated, T cells CD4 memory activated, and T cells gamma delta were statistically significant between the two groups with $p\text{-value} < 0.05$. Besides, in the CRC group, the infiltration of immune cells including Dendritic cells resting, Macrophages M1, Mast cells activated, and NK cells activated was higher. It could be seen in Fig. 11D that there were strong correlation lines among these five hub genes, among which *ADRB2*, *PIK3CG*, and *PTGS1* were strongly associated with Macrophages M2, *PIK3CG* was negatively correlated with CD8 T cells, *CXCL8* was negatively correlated with Plasma cells and

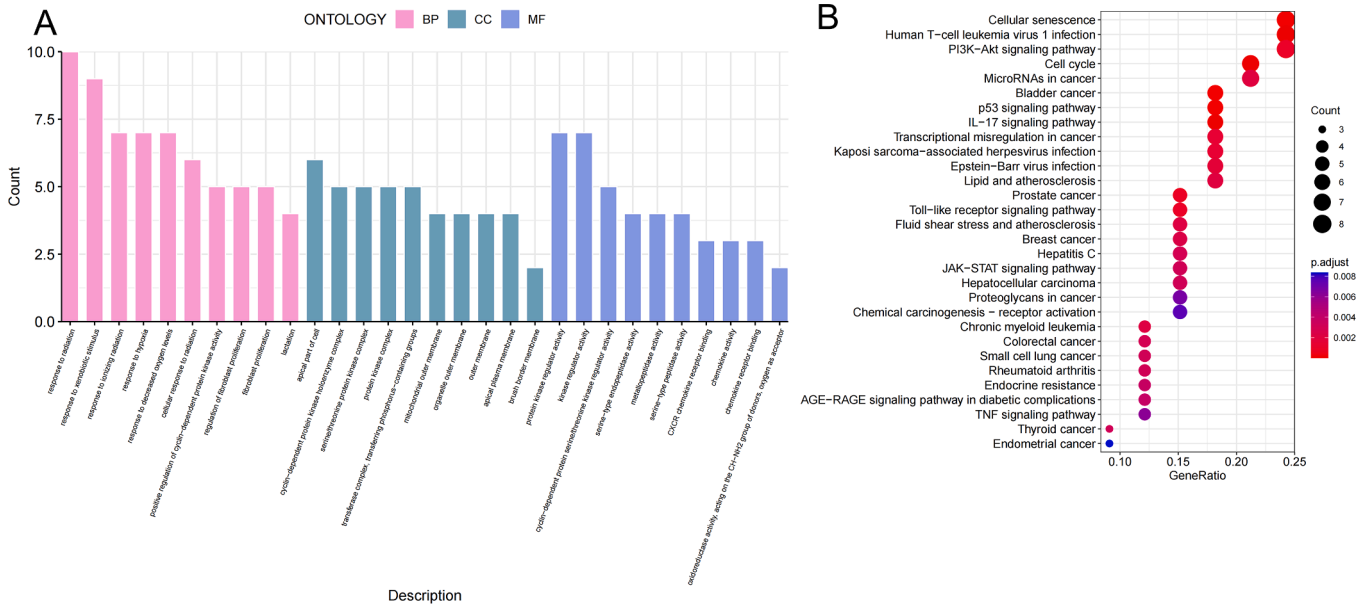


Fig. 6. GO and KEGG enrichment analysis, (A) GO enrichment analysis: the top 10 biological processes (BP), cell components (CC), and molecular functions (MF). The Y-axis represents the target number of enrichment, $p\text{-value} < 0.05$. (B) Bubble chart of the top 20 KEGG pathways, the bubble size represents the number of targets enriched in terms, and the color indicates the *p-value*, $p\text{-value} < 0.05$.

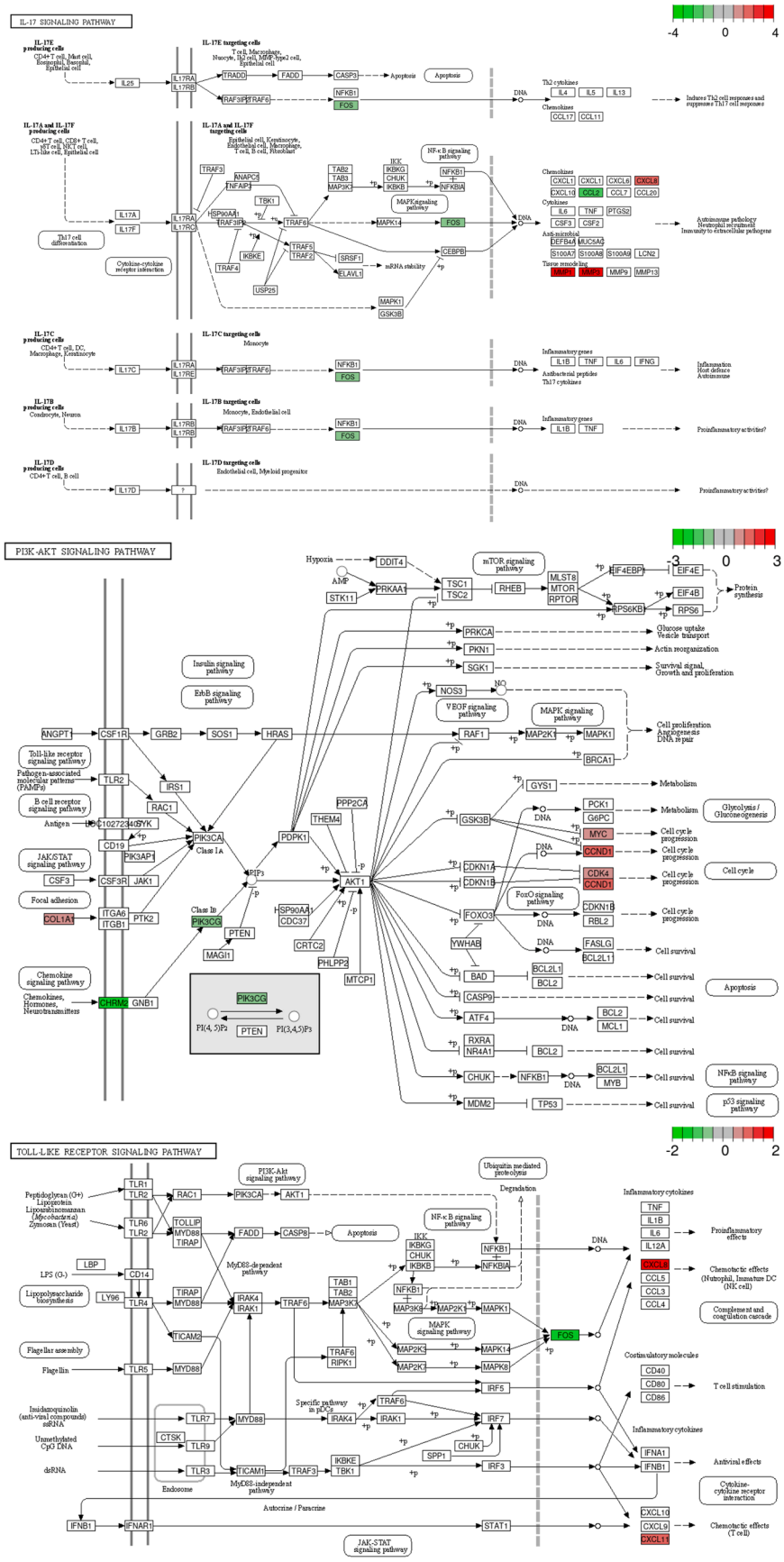


Fig. 7. Core pathways diagram, red represents up-regulation, while green represents down-regulation. The darker the color, the higher expression of this gene. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

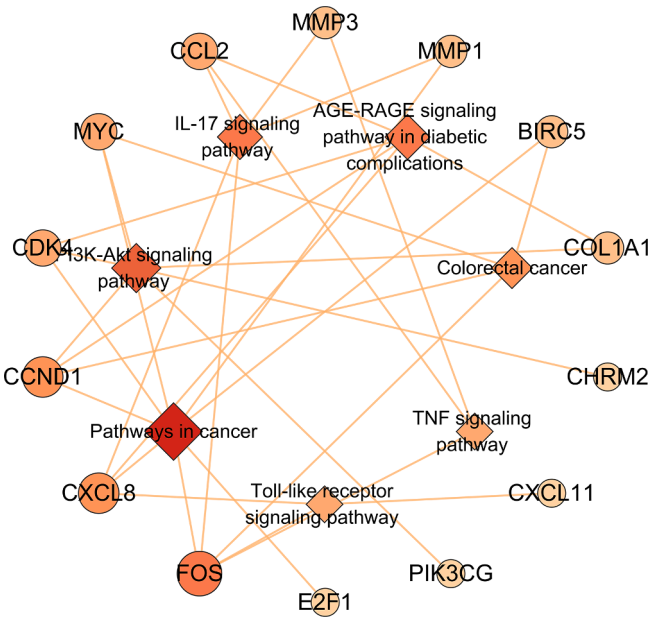


Fig. 8. Targets – pathways diagram, the circle represents genes, and the diamond represents pathway. The darker the color, the more important it is.

positively correlated with neutrophils.

Construction of ceRNA network of hub genes

To further study the intrinsic mechanism of lncRNA-based miRNA-mediated mRNA, we established a ceRNA network. We predicted 5 hub genes-activated miRNAs using the starBase online database. A total of 768 mRNA-miRNA pairs were identified, including 5 mRNAs and 317 miRNAs. We further searched miRNA-targeted lncRNAs from the starBase database and identified a total of 348 miRNA-lncRNA pairs, including 8 miRNAs and 125 lncRNAs. According to the ceRNA theory, we use shared miRNAs as nodes. Finally, a total of 388 interacting ceRNA networks were constructed, including 8 miRNAs, 125 lncRNAs and 5 mRNAs, as illustrated in Fig. 12.

Differential mRNA expression, pathological stage, overall survival (OS) analysis and immunohistochemical analysis of hub genes

The results of the GEPIA database showed that the mRNA levels of *CXCL8* and *CCND1* were significantly highly expressed in CRC (Fig. 13A). We analyzed the relations between the mRNA levels of hub genes and the pathological stages of CRC. The results suggested that as the disease progressed, the mRNA expression of all hub genes gradually increased (Fig. 13B). We inputted hub genes into Kaplan-Meier plotter for overall survival analysis. The results were shown in Fig. 13C: when the expression levels of *CCND1* and *PIK3CG* were low, the overall survival of the patient was worse; when the expression of *ADRB2* was high, the overall survival of the patient was worse, and the expression levels of *CXCL8* and *PTGS1* had less impact on patients' overall survival statistics. HPA database showed that, hub genes were expressed in normal colon tissues to varying degrees, except for *ADRB2*, which had no corresponding data in the database. Compared with normal colon tissues, *CXCL8* and *PIK3CG* were increased in CRC tissues, while the expression of *PTGS1* and *CCND1* decreased in CRC tissues as shown in Fig. 13D.

Molecular docking

The results of molecular docking were plotted as a heatmap as illustrated in Fig. 14. It can be seen from the figure that *ADRB2* with beta-sitosterol, *CXCL8* with kaempferol, *PIK3CG* with stigmasterol, *CCND1* with fustin, *PTGS1* with fustin had good binding activities, respectively. We selected the molecular docking results of *ADRB2*, *CCND1*, *CXCL8* and *PTGS1* with the main active phytochemicals, and visual analysis was carried out by PyMol software as shown in Fig. 15. This finding indicates that the active phytochemicals in the Gleditsiae Spina had good binding with the core targets.

Discussion

Colorectal cancer has risen to the position of one of the most prevalent malignant tumors in the world due to changes in lifestyle, the pressure to survive, and other causes. There is still a need to develop drugs with high efficacy and low toxicity. Chinese medicine has been utilized for thousands of years to treat various types of illnesses in people, making it a potential gold mine for new drug development. In this study, the key active phytochemicals, and their molecular mechanisms of Gleditsiae Spina in the treatment of colorectal cancer were investigated, and the hub genes were verified by bioinformatics and molecular docking. The results suggested that nine phytochemicals of Gleditsiae Spina have therapeutic effects on colorectal cancer. The majority of active phytochemicals in Gleditsiae Spina possess multi-target capabilities and can modulate a wide range of targets associated with colorectal cancers. In view of the above results, we can conclude that the main active phytochemicals of Gleditsiae Spina are likely to have a synergistic effect in the treatment of colorectal cancer. Besides, we found some core genes have the diagnostic potential in distinguishing CRC samples from healthy people samples. In addition, bioinformatics analysis of hub genes was performed, and the results demonstrated that hub genes were critical for prognosis of colorectal cancer. Therefore, we speculate that hub genes may be the essential targets for regulating the occurrence and development of colorectal cancer.

In our research, nine active phytochemicals of Gleditsiae Spina were

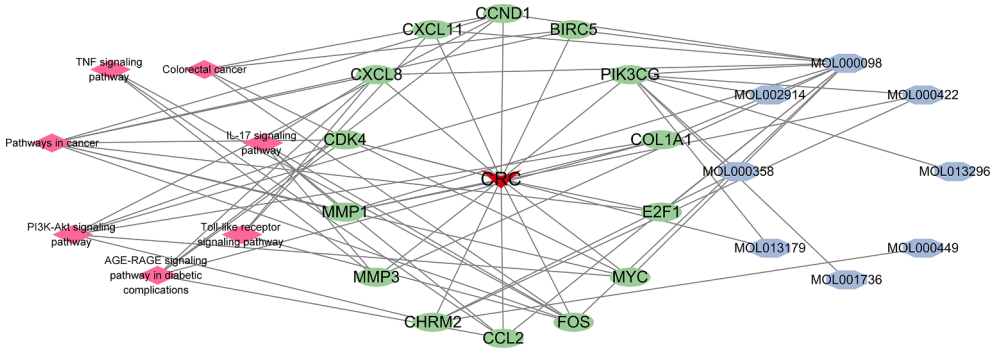


Fig. 9. Active phytochemical - targets - pathways diagram, blue oval represents active phytochemical of Gleditsiae Spina, and pink diamond represents pathways. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

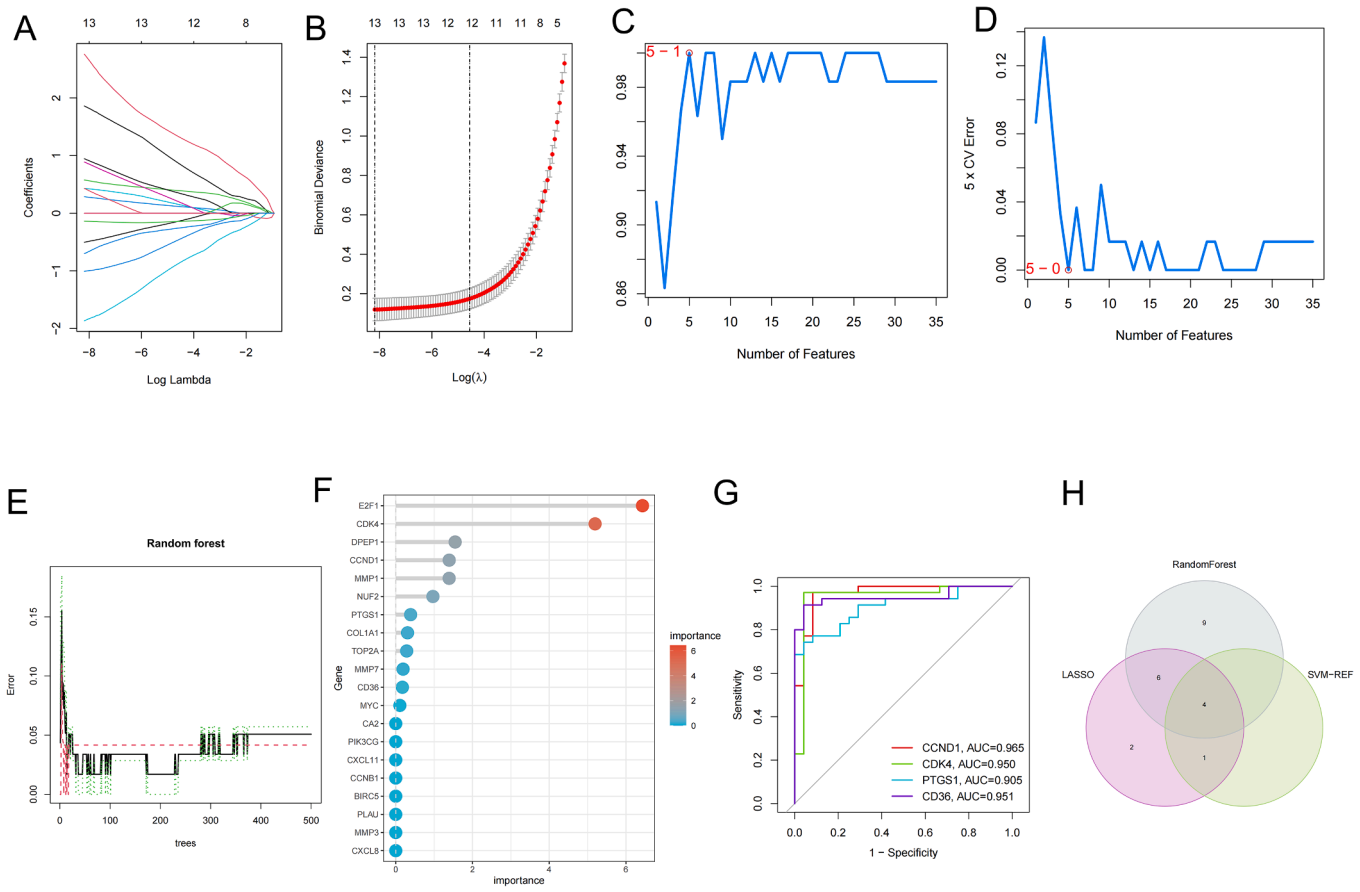


Fig. 10. Significance of core genes in the diagnosis of CRC, (A-B) means that in the LASSO algorithm, 13 genes from 36 therapeutic targets were selected, (C-D) indicates that in the SVM-RFE algorithm, the first five genes could be determined as the best targets according to the maximum accuracy and minimum error value, (E-F) show that Random forest results identified 19 related genes whose gene importance was greater than 0, (G) is the ROC curves, and AUC of *CCND1*, *CDK4*, *PTGS1*, and *CD36* are greater than 0.9, (H) indicates the intersection of the three machine learning algorithms, and four genes (*CCND1 CDK4 PTGS1* and *CD36*) had the better diagnostic potential.

examined, including (-)-taxifolin, fisetin, fustin, ent-epicatechin, quercetin, stigmasterol, kaempferol, eriodyctiol (flavanone), and beta-sitosterol. Quercetin is a dietary flavonoid with antioxidant, anti-inflammatory and anticancer properties. According to related studies, quercetin inhibits the tumorigenesis of CRC by inhibiting the polarization of M2 macrophages and downregulating hsa_circ_0006990 (Chen et al., 2022a). As a drug, quercetin has also been studied for use in combination with drug delivery systems for the treatment of colorectal cancer. However, some studies have pointed out that quercetin may have some adverse effects. For example, increasing the intracellular concentration of quercetin may promote aerobic reactions not only in cancer cells but also in normal tissues, resulting in side effects that require careful monitoring. But specific data on the toxicity and therapeutic dose of quercetin is currently unclear (Russo et al., 2014). Fisetin, a dietary flavanol, is emerging as a potent anticancer agent, however, its tumor-specific pharmacological targets remain largely unexplored. There is a correlation between fisetin-mediated inhibition of autophagy and induction of apoptosis in colorectal cell lines. It is also associated with inflammation (Pandey & Trigun, 2023), improving the inflammatory state of colorectal cancer patients, which is likely to be a new supplementary anti-tumor agent (Farsad-Naeimi et al., 2018). Nanopreparations of fisetin and quercetin secondary plant metabolites have profound effects on a variety of colorectal cancer preclinical models (Biganeh et al., 2023). To investigate fisetin's anticancer potential and determine the ideal therapeutic dosage, more clinical trials are necessary (Rahmani et al., 2022). Stigmasterol can prevent and treat cardiovascular diseases such as hypertension and coronary heart disease. Studies suggested that quercetin and stigmasterol, the core compounds in the treatment of rectal cancer, have the function of hindering the formation of cancer cells (Shi et al., 2021). Toxicological research is required to assess stigmasterol's safety, nonetheless, as certain investigations have suggested that it can occasionally result in cardiac damage (Tao et al., 2019) . Although kaempferol is a representative element of traditional Chinese medicine and has pharmacological properties against tumor, it is unclear how kaempferol affects the onset of CRC. According to a recent study, kaempferol enhances patients' immune systems by modulating key genes, helping with the therapy of CRC (Gu et al., 2023a). Besides, kaempferol has the ability to reverse the drug resistance of colorectal cancer cells to 5-Fluorouracil (5-Fu), suggesting that kaempferol, either alone or in combination with 5-Fu, holds promise for the treatment of colorectal cancer. Furthermore, this flavonoid compound exhibits toxic activity exclusively towards cancer cells while it shows limited toxicity to healthy cells (Qattan et al., 2022). Beta-sitosterol may hinder the growth of human colon cancer cells by obstructing the Wnt/β-catenin pathway (Gu et al., 2023b). But as of right now, no information has been published on the potential toxicity and suggested dosages of beta-sitosterol for colorectal cancer, so future study needs to pay close attention to these issues. Eriodictyol can suppress colorectal cancer by downregulating tissue specific transplantation antigen P35B (TSTA3) expression (Huang et al., 2022). Fisetin is a nontoxic as well as dietary agent which possesses antiproliferative properties against several cancers (Khan et al., 2013) . Also, it has previously been proposed that fisetin selectively targets cancer cells, showing little to no effect on normal cells (Khan et al.,2012). According

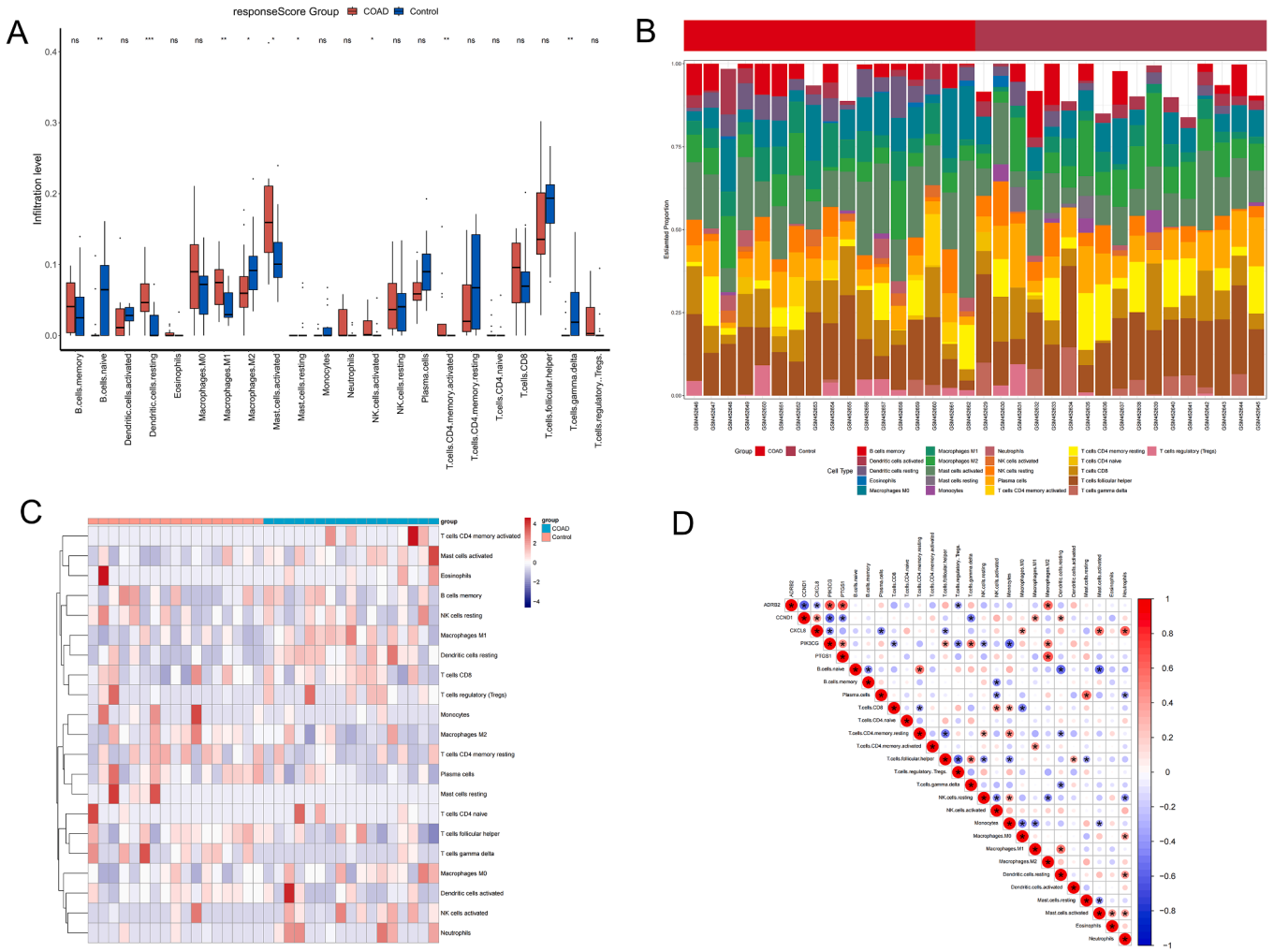


Fig. 11. Immune Infiltration analysis of hub genes, (A) reflects the difference of immune cell infiltration between normal group and cancer group, (B) shows the difference in immune cell infiltration between each sample, (C) represents that the difference of immune cell infiltration between the normal group and the tumor group is statistically analyzed, and "ns" indicates that the difference is statistically insignificant, (D) indicates that five genes are strongly correlated with each other, with red representing positive correlation, blue representing negative correlation, and "*" indicating statistically correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to certain research, taxifolin can cause CRC regression by inducing cell cycle arrest (Razak et al.,2018) . Yet, no research has been undertaken to evaluate its toxic doses, which remains further comprehensive study. Chiaino etc. reported that Acacia Catechu Willd Extract, which contains Epicatechin and catechin, can induce colon cancer cells apoptosis (Chiaino et al., 2020). However, further research is needed to determine how epicatechin affects colon cancer. Phytochemicals are the constituents of Gleditsiae Spina, and numerous literatures back up the claim that these compounds are crucial in the development of anti-CRC cancers. Therefore, it can highlight the importance of Gleditsiae Spina in the treatment of CRC and improve the prognosis of patients.

In order to explore the significance of core genes in the diagnosis of CRC, we conducted three kinds of machine learning algorithms on the core genes, and we found that *CCND1*, *CDK4*, *PTGS1* and *CD36* of the core genes had significant significance in the diagnosis of colorectal cancer. Besides, according to the values of DC, BC and CC value, we considered *CCND1*, *ADRB2*, *PIK3CG*, *CXCL8* and *PTGS1* as the hub genes, and the bioinformatics analysis was carried out of them. *CCND1* is a regulatory component of the cyclin D1-CDK4 (DC) complex that phosphorylates and inhibits members of the retinoblastoma (RB) protein family, including RB1, and regulates the cell cycle during the G1/S transition (Feng et al., 2019). Feng, Y., et al found that *CCND1* promotes CRC cell proliferation and tumorigenesis by activating the PI3K/AKT pathway. RAP1A promotes the occurrence of CRC through PTEN/FOXO3/CCND1 signaling pathway. As a cell cycle protein, *CCND1* plays an important role in regulating cell proliferation and division. As a result, *CCND1* might be a crucial molecule in the diagnosis of CRC (Liu et al., 2018). *PTGS1* is a molecule on the inflammatory pathway and is involved in the development of inflammation-related diseases (Frank et al., 2010). Gene variants of *PTGS1* inflammatory pathway were significantly associated with increased risk of CRC, and *PTGS1* gene polymorphisms were identified as a risk factor for CRC. The beta2 adrenergic receptor (*ADRB2*) is positively associated with T-cell exhaustion features in various tumors (Lin et al., 2023). T cells are crucial anti-tumor lymphocytes, and the exhaustion of these cells is frequently linked to the malignant progression of malignancies. Down-regulation of *ADRB2* expression inhibits the migration, invasion, and epithelial-mesenchymal transition of colorectal cancer cells (Lu et al., 2022). We found the corresponding results in the database, *ADRB2* low expression is possibly a protective factor which indicated a longer survival time. Phosphoinositide 3-kinase (*PIK3CG*) phosphorylates inositol lipid and participates in immune response (Zhang et al., 2019). The molecular switch in the phosphatidylinositol 3 kinase (PI3K)-AKT signaling pathway can be used as a potential target for the treatment of CRC, and the expression of *PIK3CG* in the PI3K-AKT signaling pathway down-regulated. *CXCL8* is involved in tumor proliferation, migration,

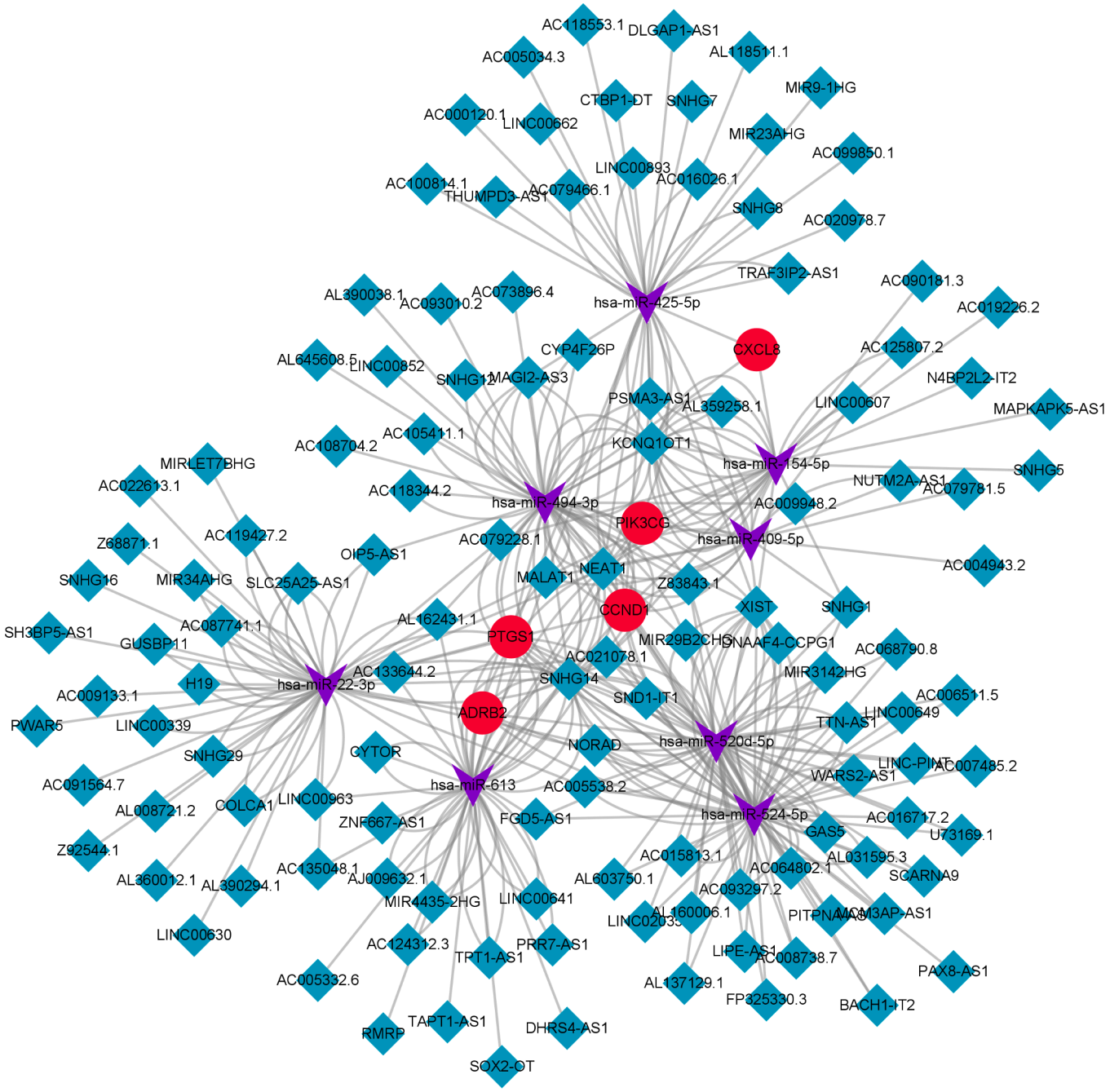


Fig. 12. ceRNA network of hub genes, blue square represents IncRNA, purple triangle represents miRNA, and red circle represents mRNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and invasion. High expression of *CXCL8* is positively correlated with cAMP response element binding protein 1 (CREB1) /ribosomal protein S6 kinase B1 (RPS6KB1) expression, thereby promoting cell proliferation and differentiation (Li et al., 2019). As shown in Fig. 13A, CRC patients had higher expression of *CXCL8*.

Tumor-infiltrating immune cells play an important role in the process of tumorigenesis and malignant progression. Once recruited into the tumor microenvironment, immune cells can promote the development of cancer cell phenotypes toward malignancy. Through the correlation analysis of 5 hub genes with tumor microenvironment immune cells, we believe that the hub genes are likely to play a certain role in the immune invasion process of tumor cells. Macrophages represent a heterogeneous cell population that plays an important role in defense mechanisms and homeostasis. M2 macrophages have been shown to promote tumor growth, while M1 macrophages have pro-tumor and anti-tumor properties (Strizova et al., 2023). A strong correlation line was found among the five hub genes, including *ADRB2*, *PIK3CG*, and *PTGS1*, which were strongly correlated with macrophages M2, and M2 is mainly induced by Th2 cytokines, such as IL-4, IL-13, glucocorticoids, and MCSF (Rudd et al., 2009). CD8 T cell, Plasma cells are often associated with immune killer of tumor, macrophages M0 and others are immunosuppressive. CD8 T cells and Plasma cells tend to imply immune killing of a tumor, while macrophages M0 has immunosuppressive properties. Immune infiltration analysis of hub genes indicated that *PIK3CG* was negatively correlated with CD8 T cells, *CXCL8* was negatively correlated with Plasma cells, and *CXCL8* was positively correlated with neutrophils. *CXCL8* is an inflammatory chemokine that is elevated in the colorectal cancer microenvironment and plays an important role

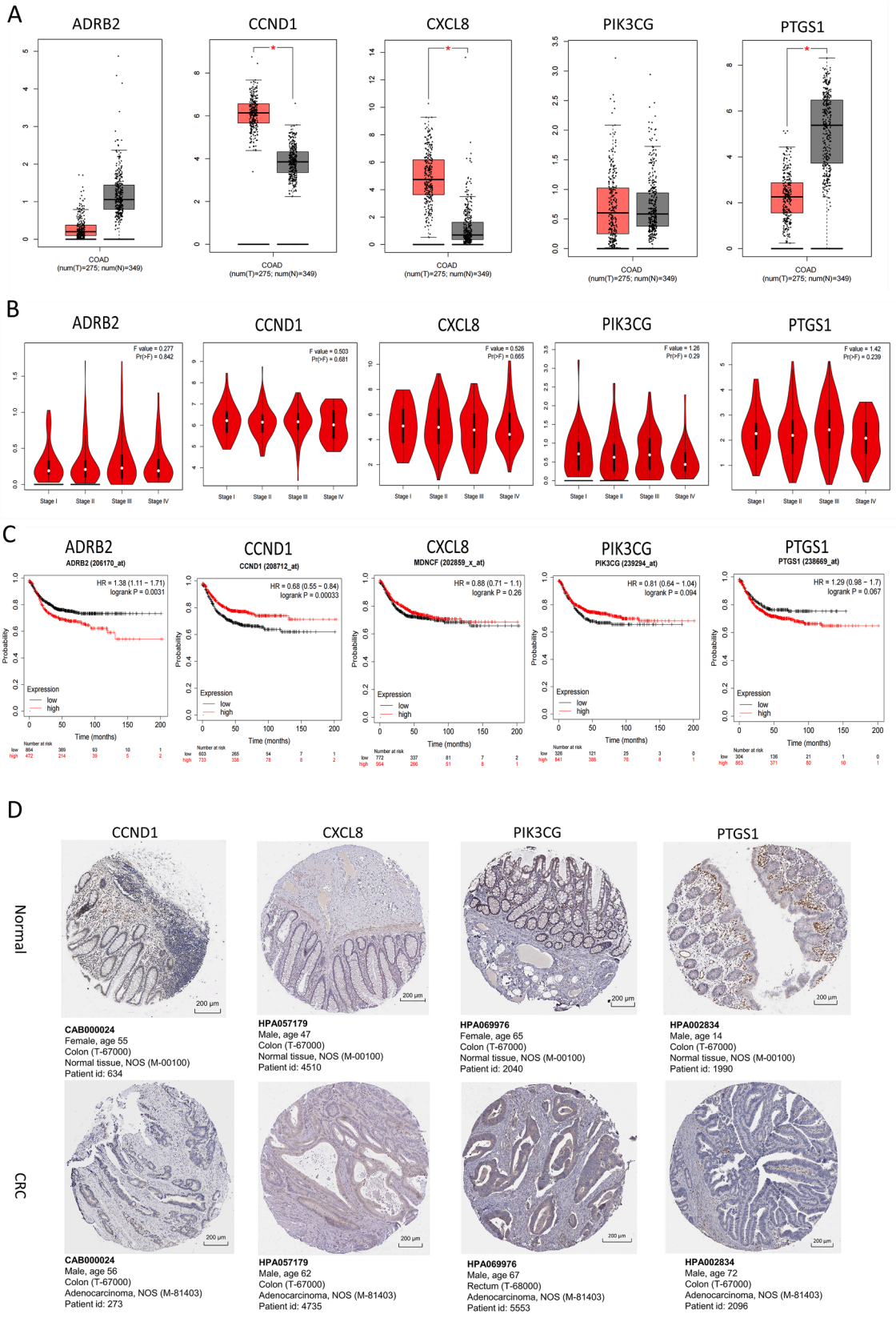


Fig. 13. Hub genes bioinformatics analysis. (A) Box plots showing the mRNA expression levels of *ADRB2, CCND1, CXCL8*, *PIK3CG* and *PTGS1*. Red represented tumor group, gray represents normal group. (B) The violin diagram indicated the stage plot of mRNA expression level and pathological stage in the GEPIA database. (C) The line charts showed the Overall survival (OS) of hub genes. The survival curve comparing the patients with high (red) and low (black) expression in CRC. (D) Protein expression in normal tissue and CRC tissue in HPA database. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

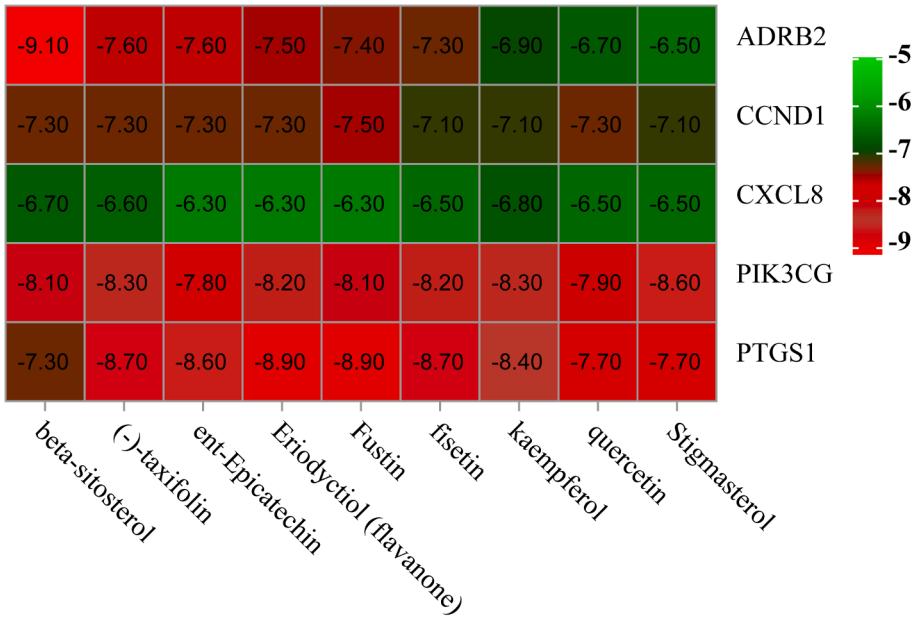


Fig. 14. Heatmap of binding between main active phytochemicals and hub genes. The darker the color, the better the binding activity.

in neutrophil chemotaxis, promoting angiogenesis, invasion, and metastasis in the cancer environment (Pennel et al., 2022).

GO enrichment analysis results suggested that the BP mainly related to metabolically related protein kinases and their complex derivatives, which are involved in signal transduction and the co-regulation of complex functions such as the cell cycle. Cyclin-dependent kinases (CDKs) and Cyclins are the core molecules in the whole cell cycle regulation mechanism. Cyclin-dependent kinases, a member of the serine/threonine kinase family, are dimeric complexes composed of cell cycle catalytic kinase subunits and regulatory subunits. Cell cycle dysregulation is a common feature of human cancers. It has been found that activated YES-associated protein (YAP) can reprogram tumor stem cells into a low-Wnt signaling pathway, thereby inhibiting the growth of primary and metastatic cancer cells of CRC. Serine/threonine kinases 3 and 4 of Hippo signaling pathway and large tumor suppressor factor 1/2 can inhibit YAP and activate Wnt signaling pathway of tumor stem cells, leading to the growth of colorectal cancer cells (Cheung et al., 2020). Cell components mainly associated with serine/threonine protein kinase complex, protein kinase complex, apical part of cell, etc. Molecular functions mainly included related to cyclin-dependent protein serine/threonine kinase regulator activity, protein kinase regulator activity, kinase regulator activity, etc.

KEGG enrichment analysis results showed that P53 signaling pathway, IL-17 signaling pathway, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway and JAK-STAT signaling pathway were likely to be the main pathways that regulating CRC. In normal human cell activities, P53 gene plays a role in a variety of biological functions, such as cell cycle arrest, promoting cell apoptosis and DNA repair, regulating cell differentiation, aging, and maintaining genome stability. It has been reported that treating colon cancer with 5-fluorouracil in vitro could gradually increase the expression of P53 in cells, and then activate bcl2-associated X protein (Bax), leading to cell apoptosis (Lu et al., 2000). PI3K-AKT pathway is an intracellular signal transduction pathway that promotes metabolism, proliferation, cell survival, growth, and angiogenesis in response to extracellular signals. After the activation of PI3K-AKT signal, it is transmitted to the downstream pathway, resulting in the activation of a series of pathways, including protein translation, cell cycle, apoptosis, P53 pathway, etc. Activation of PI3K/Akt signaling pathway is one of the classical signaling pathways that regulate the proliferation and apoptosis of tumor cells, which is closely related to the development of CRC (Yang et al., 2018). DUAN et al. found that activation of PI3K-AKT-mTOR signaling pathway can promote the invasion, migration and epithelial-mesenchymal transformation of CRC cells (Duan et al., 2018). Therefore, the treatment of cancer by inhibiting the level of PI3K/Akt signaling pathway may become the future direction of CRC treatment. The IL-17 family signals through its corresponding receptors to activate downstream pathways, including NF-κB, MAPKs, and C/ EBPs, inducing the expression of antimicrobial peptides, cytokines, and chemokines. IL-17A is a new player in the colorectal cancer cytokine environment and has been implicated in tumorigenesis, angiogenesis, and metastasis of colorectal cancer (Razi et al., 2019). Some evidence suggests that IL-17A can increase the expression of PD-L1 and promote tumor progression in HCC or ovarian cancer (Gaffen et al., 2014; Monin & Gaffen, 2018; Wei et al., 2019). Other studies have observed that IL-17A can stimulate increased levels of PD-L1 protein in colorectal cancer cells and tissues (Liu et al., 2021). TLR-related signaling pathways, involving in epithelial cell proliferation and IgA production, play an important role in maintaining tight epithelial cell connections, recognizing PAMPs, and inducing antimicrobial peptide expression (Aboushousha et al., 2020). TLRs transmit signals by recruiting specific adaptor molecules, leading to activation of transcription factors NF-κB and IRFs, which determine the outcome of innate immune responses (Kawasaki & Kawai, 2014). Chronic inflammation in the tumor microenvironment is known to have an impact on tumor progression. Damaged or damage-associated molecular patterns (DAMP) expressed by cancer cells stimulate TLRS in immune cells, leading to chronic inflammation. Studies have shown that in patients with ulcerative colitis, the expression of TLR2 and NF-κB is often significantly increased, and the TLR2 signaling pathway is activated (Zhang et al., 2020). TLR-induced NF-κB activation in colorectal cancer has been shown to promote tumor cell survival (Fukata et al., 2006). The JAK/STAT pathway constitutes a rapid membrane-to-nuclear signaling module and induces the expression of several key mediators of cancer and inflammation (Darnell, 1997). Some studies have shown that abnormal activation of JAK-STAT signaling pathway plays an important role in the proliferation and growth of colorectal cancer cells, and inhibiting the activation of JAK-STAT signaling can inhibit tumor proliferation and growth (Corvinus et al., 2005; Lin et al., 2005).

Molecular docking results showed that the main active compounds of Gleditsiae Spina had good binding activity with the hub targets, and then we identified the hub targets in different databases. While molecular docking suggests that active phytochemicals in Gleditsiae Spina

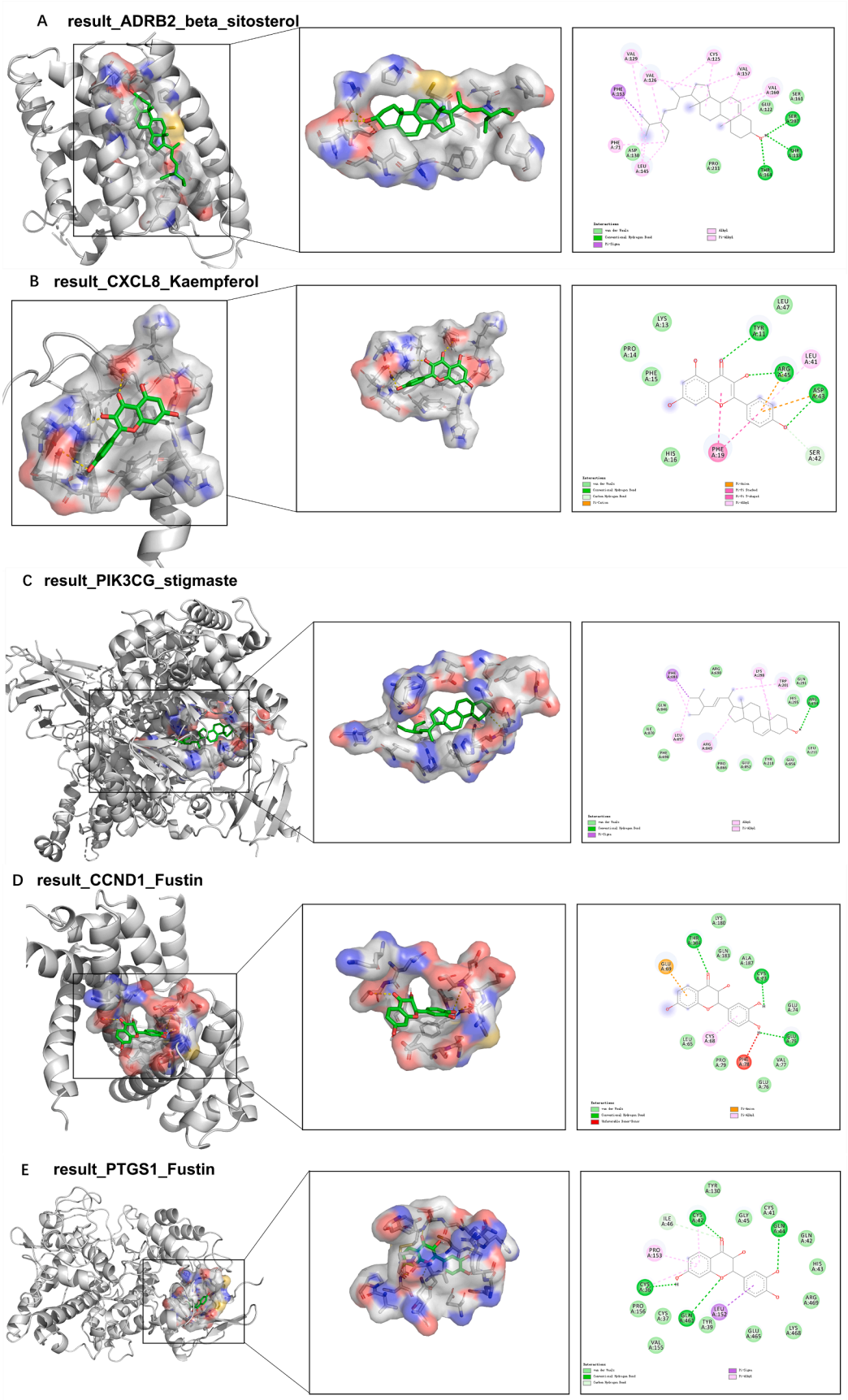


Fig. 15. Molecular docking between active phytochemicals and hub genes. (A) Represents the molecular binding of *ADRB2* with beta-sitosterol, (B) represents the molecular binding of *CXCL8* with kaempferol, (C) represents the molecular binding of *PIK3CG* with stigmasterol, (D) represent the molecular binding of *CCND1* with fustin, and (E) represent the molecular binding of *PTGS1* with fustin, respectively.

bind with core targets, it's important to note that further studies are required to determine the potential toxicity and therapeutic efficacy of using these nine ingredients together. Prior to clinical trials, animal studies are recommended to evaluate the safety and efficacy of these components. Additionally, dose–response studies are needed to establish the optimum dosage for treatment. And we will conduct these cellular and animal experiments in our future studies.

Conclusions

In this study, we successfully identified the active phytochemicals and molecular pathways of Gleditsiae Spina for the treatment of colorectal cancer. Currently, five anti-colorectal cancer hub genes and 9 key active phytochemicals have been identified. This study also showed that among the 36 intersection targets, the hub genes (*ADRB2*, *CCND1*, *CXCL8*, *PIK3CG*, and *PTGS1*) may be related to the anti-colorectal cancer effect of Gleditsiae Spina active components. The underlying mechanism of Gleditsiae Spina active phytochemicals showing anti-CRC effects is the inhibition/regulation of multiple BP (response to radiation, response to ionizing radiation, cyclin-dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, cyclin-dependent protein serine/threonine kinase regulator activity and protein kinase regulator activity. We identified five key pathways (P53 signaling pathway, IL-17 signaling pathway, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway and JAK-STAT signaling pathway) involved in the treatment of CRC by active phytochemicals of Gleditsiae Spina. Thus, our findings suggest a synergy among multiple anti-CRC core targets, multi-molecular pathways, and key active components. In addition, molecular docking, bioinformatics analysis, and network pharmacology screening results were consistent, indicating the effectiveness of network pharmacology and bioinformatics in this study. Therefore, these findings provide a basis for the further development of anti-CRC drugs based on the active components of Gleditsiae Spina in the future.

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CRediT authorship contribution statement

Yingzi Wu: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Jinhai Luo:** Conceptualization, Formal analysis, Investigation. **Baojun Xu:** Funding acquisition, Methodology, Project administration, Software, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Aboushousha, T., Hammam, O., Aref, A., Kamel, A., Badawy, M., Abdel Hamid, A., 2020. Tissue profile of CDK4 and STAT3 as possible innovative therapeutic targets in urinary bladder cancer. Asian Pac. J. Cancer Prev. 21 (2), 547–554. https://doi.org/10.31557/APJCP.2020.21.2.547.

Baidoun, F., Elshiwy, K., Elkeraie, Y., Merjaneh, Z., Khoudari, G., Sarmini, M.T., Gad, M., Al-Husseini, M., Saad, A., 2021. Colorectal cancer epidemiology: Recent trends and impact on outcomes. Curr. Drug Targets 22 (9), 998–1009. https://doi.org/10.2174/1389450121999201117115717.

Biganeh, H., Dizaji, S.M., Taghipour, Y.D., Murtaza, G., Rahimi, R., 2023. Nanoformulations of plant-derived compounds as emerging therapeutic approach for colorectal cancer. Curr. Drug Deliv. 20 (8), 1067–1094. https://doi.org/10.2174/1567201819666220823155526.

Cai, Y., Zhang, C., Zhan, L., Cheng, L., Lu, D., Wang, X., Xu, H., Wang, S., Wu, D., Ruan, L., 2019. Anticancer effects of *Gleditsia sinensis* extract in rats transplanted with hepatocellular carcinoma cells. Oncol. Res. 27 (8), 889–899. https://doi.org/10.3727/096504018X15482423944678.

Chen, B., Khodadoust, M.S., Liu, C.L., Newman, A.M., Alizadeh, A.A., 2018. Profiling tumor infiltrating immune cells with CIBERSORT. Methods Mol. Biol. 1711, 243–259. https://doi.org/10.1007/978-1-4939-7493-1_12.

Chen, D., Liu, J., Zang, L., Xiao, T., Zhang, X., Li, Z., Zhu, H., Gao, W., Yu, X., 2022b. Integrated machine learning and bioinformatic analyses constructed a novel stemness-related classifier to predict prognosis and immunotherapy responses for hepatocellular carcinoma patients. Int. J. Biol. Sci. 18 (1), 360–373. https://doi.org/10.7150/ijbs.66913.

Chen, B., Wu, L., Tang, X., Wang, T., Wang, S., Yu, H., Wan, G., Xie, M., Zhang, R., Xiao, H., Deng, W., 2022a. Quercetin inhibits tumorigenesis of colorectal cancer through downregulation of hsa_circ_0006990. Front. Pharmacol. 13, 874696 https://doi.org/10.3389/fphar.2022.874696.

Cheung, P., Xiol, J., Dill, M.T., Yuan, W.C., Panero, R., Roper, J., Osorio, F.G., Maglic, D., Li, Q., Gurung, B., Calogero, R.A., Yilmaz, O.H., Mao, J., Camargo, F.D., 2020. Regenerative reprogramming of the intestinal stem cell state via hippo signaling suppresses metastatic colorectal cancer. Cell Stem Cell 27 (4), 590–604 e599. https://doi.org/10.1016/j.stem.2020.07.003.

Chiaino, E., Micucci, M., Durante, M., Budriesi, R., Gotti, R., Marzetti, C., Frosini, M., 2020. Apoptotic-induced effects of Acacia catechu Willd. Extract in human colon cancer cells. Int. J. Mol. Sci. 21 (6), 2102. https://doi.org/10.3390/ijms21062102.

Corvinus, F.M., Orth, C., Moriggl, R., Tsareva, S.A., Wagner, S., Pfitzner, E.B., Baus, D., Kaufmann, R., Huber, L.A., Zatloukal, K., Beug, H., Ohlschlager, P., Schutz, A., Halbhuber, K.J., Friedrich, K., 2005. Persistent STAT3 activation in colon cancer is associated with enhanced cell proliferation and tumor growth. Neoplasia 7 (6), 545–555. https://doi.org/10.1593/neo.04571.

Darnell Jr., J.E., 1997. Stats and Gene Regulation. *Science* 277 (5332), 1630–1635. https://doi.org/10.1126/science.277.5332.1630.

Dekker, E., Tanis, P.J., Vleugels, J.L.A., Kasi, P.M., Wallace, M.B., 2019. Colorectal cancer. Lancet 394 (10207), 1467–1480. https://doi.org/10.1016/S0140-6736(19)32319-0.

Duan, S., Huang, W., Liu, X., Liu, X., Chen, N., Xu, Q., Hu, Y., Song, W., Zhou, J., 2018. IMPDH2 promotes colorectal cancer progression through activation of the PI3K/AKT/mTOR and PI3K/AKT/FOXO1 signaling pathways. J. Exp. Clin. Cancer Res. 37 (1), 304. https://doi.org/10.1186/s13046-018-0980-3.

Edgar, R., Domrachev, M., Lash, A.E., 2002. Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30 (1), 207–210. https://doi.org/10.1093/nar/30.1.207.

Farsad-Naeimi, A., Alizadeh, M., Esfahani, A., Darvish Aminabad, E., 2018. Effect of fisetin supplementation on inflammatory factors and matrix metalloproteinase enzymes in colorectal cancer patients. Food Funct. 9 (4), 2025–2031. https://doi.org/10.1039/c7fo01898c.

Feng, Y., Qian, W., Zhang, Y., Peng, W., Li, J., Gu, Q., Ji, D., Zhang, Z., Wang, Q., Zhang, D., Sun, Y., 2019. CDCA2 promotes the proliferation of colorectal cancer cells by activating the AKT/CCND1 pathway in vitro and in vivo. BMC Cancer 19 (1), 576. https://doi.org/10.1186/s12885-019-5793-z.

Frank, B., Hoffmeister, M., Klopp, N., Illig, T., Chang-Claude, J., Brenner, H., 2010. Polymorphisms in inflammatory pathway genes and their association with colorectal cancer risk. Int. J. Cancer 127 (12), 2822–2830. https://doi.org/10.1002/ijc.25299.

Fukata, M., Chen, A., Klepper, A., Krishnareddy, S., Vamadevan, A.S., Thomas, L.S., Xu, R., Inoue, H., Arditi, M., Dannenberg, A.J., Abreu, M.T., 2006. Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: Role in proliferation and apoptosis in the intestine. Gastroenterology 131 (3), 862–877. https://doi.org/10.1053/j.gastro.2006.06.017.

Gaffen, S.L., Jain, R., Garg, A.V., Cua, D.J., 2014. The IL-23-IL-17 immune axis: From mechanisms to therapeutic testing. Nat. Rev. Immunol. 14 (9), 585–600. https://doi.org/10.1038/nri3707.

Gao, J., Yang, X., Yin, W., 2016. From traditional usage to pharmacological evidence: A systematic mini-review of Spina Gleditsiae. Evid. Based Complementary Altern. Med. 2016, 3898957. https://doi.org/10.1155/2016/3898957.

Gu, S., Liu, F., Xie, X., Ding, M., Wang, Z., Xing, X., Sun, X., 2023b. β-Sitosterol blocks the LEF-1-mediated Wnt/β-catenin pathway to inhibit proliferation of human colon cancer cells. Cell Signal. 104, 110585 https://doi.org/10.1016/j.cellsig.2022.110585.

Gu, C., Tang, L., Hao, Y., Dong, S., Shen, J., Xie, F., Han, Z., Luo, W., He, J., Yu, L., 2023a. Network pharmacology and bioinformatics were used to construct a prognostic model and immunoassay of core target genes in the combination of quercetin and kaempferol in the treatment of colorectal cancer. J. Cancer 14 (11), 1956–1980. https://doi.org/10.7150/jca.85517.

Gyorffy, B., 2021. Survival analysis across the entire transcriptome identifies biomarkers with the highest prognostic power in breast cancer. Comput. Struct. Biotechnol. J. 19, 4101–4109. https://doi.org/10.1016/j.csbj.2021.07.014.

Huang, H., He, Y., Li, Y., Gu, M., Wu, M., & Ji, L. (2022). Eriodictyol suppresses the malignant progression of colorectal cancer by downregulating tissue specific

transplantation antigen P35B (TSTA3) expression to restrain fucosylation. *Bioengineered*, 13(3), 5551-5563. http:// 10.1080/21655979.2022.2039485.
Islam, M.R., Akash, S., Rahman, M.M., Nowrin, F.T., Akter, T., Shohag, S., Rauf, A., Aljohani, A.S.M., Simal-Gandara, J., 2022. Colon cancer and colorectal cancer: Prevention and treatment by potential natural products. Chem. Biol. Interact. 368, 110170 https://doi.org/10.1016/j.cbi.2022.110170.
Kawasaki, T., Kawai, T., 2014. Toll-like Receptor Signaling Pathways. *Front. Immunol.* 5, 461. https://doi.org/10.3389/fimmu.2014.00461.
Khan, N., Afaq, F., Khusro, F.H., Mustafa Adhami, V., Suh, Y., Mukhtar, H., 2012. Dual inhibition of phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin signaling in human nonsmall cell lung cancer cells by a dietary flavonoid fisetin. Int. J. Cancer 130 (7), 1695–1705. https://doi.org/10.1002/ijc.26178.
Khan, N., Syed, D.N., Ahmad, N., Mukhtar, H., 2013. Fisetin: a dietary antioxidant for health promotion. Antioxid Redox Signal. 19 (2), 151–162. https://doi.org/10.1089/ars.2012.4901.
Kim, K.H., Han, C.W., Yoon, S.H., Kim, Y.S., Kim, J.I., Joo, M., Choi, J.Y., 2016. The fruit hull of Gleditsia sinensis enhances the anti-tumor effect of cis-Diammine Dichloridoplatinum II (Cisplatin). Evid. Based Complementary Altern. Med. 2016, 7480971. https://doi.org/10.1155/2016/7480971.
Lee, S.J., Park, K., Ha, S.D., Kim, W.J., Moon, S.K., 2010. *Gleditsia sinensis* thorn extract inhibits human colon cancer cells: The role of ERK1/2, G2/M-phase cell cycle arrest and p53 expression. Phytother Res. 24 (12), 1870–1876. https://doi.org/10.1002/ptr.3214.
Lee, S.J., Ryu, D.H., Jang, L.C., Cho, S.C., Kim, W.J., Moon, S.K., 2013. Suppressive effects of an ethanol extract of *Gleditsia sinensis* thorns on human SNU-5 gastric cancer cells. Oncol. Rep. 29 (4), 1609–1616. https://doi.org/10.3892/or.2013.2271.
Li, J.H., Liu, S., Zhou, H., Qu, L.H., Yang, J.H., 2014. starBase v2.0: Decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. 42 (Database issue), D92–D97. https://doi.org/10.1093/nar/gkt1248.
Li, J., Liu, Q., Huang, X., Cai, Y., Song, L., Xie, Q., Liu, F., Chen, X., Xu, P., Zeng, F., Chu, Y., Zeng, F., 2019. Transcriptional profiling reveals the regulatory role of CXCL8 in promoting colorectal cancer. Front. Genet. 10, 1360. https://doi.org/10.3389/fgene.2019.01360.
Lin, Q., Lai, R., Chirieac, L.R., Li, C., Thomazy, V.A., Grammatikakis, I., Rassidakis, G.Z., Zhang, W., Fujio, Y., Kunisada, K., Hamilton, S.R., Amin, H.M., 2005. Constitutive activation of JAK3/STAT3 in colon carcinoma tumors and cell lines: Inhibition of JAK3/STAT3 signaling induces apoptosis and cell cycle arrest of colon carcinoma cells. Am. J. Pathol. 167 (4), 969–980. https://doi.org/10.1016/S0002-9440(10)61187-X.
Lin, Y., Liu, Y., Gao, Z., Jing, D., Bi, R., Cui, X., Cao, Q., Zhao, Q., Gao, R., Su, Y., Liu, S., Zhao, M., Yang, Y., Chen, A., Dai, B., Gao, X., 2023. Beta-adrenergic receptor blocker propranolol triggers anti-tumor immunity and enhances irinotecan therapy in mice colorectal cancer. Eur. J. Pharmacol. 949, 175718 https://doi.org/10.1016/j.ejphar.2023.175718.
Liu, C., Liu, R., Wang, B., Lian, J., Yao, Y., Sun, H., Zhang, C., Fang, L., Guan, X., Shi, J., Han, S., Zhan, F., Luo, S., Yao, Y., Zheng, T., Zhang, Y., 2021. Blocking IL-17A enhances tumor response to anti-PD-1 immunotherapy in microsatellite stable colorectal cancer. J. Immunother Cancer 9 (1). https://doi.org/10.1136/jitc-2020-001895.
Liu, L., Yan, X., Wu, D., Yang, Y., Li, M., Su, Y., Yang, W., Shan, Z., Gao, Y., Jin, Z., 2018. High expression of Ras-related protein 1A promotes an aggressive phenotype in colorectal cancer via PTEN/FOXO3/CCND1 pathway. J. Exp. Clin. Cancer Res. 37 (1), 178. https://doi.org/10.1186/s13046-018-0827-y.
Lu, J., Moochhala, S., Kaur, C., Ling, E., 2000. Changes in apoptosis-related protein (p53, Bax, Bcl-2 and Fos) expression with DNA fragmentation in the central nervous system in rats after closed head injury. Neurosci. Lett. 290 (2), 89–92. https://doi.org/10.1016/s0304-3940(00)01307-0.
Lu, T., Zheng, C., Fan, Z., 2022. Cardamonin suppressed the migration, invasion, epithelial mesenchymal transition (EMT) and lung metastasis of colorectal cancer cells by down-regulating ADRB2 expression. Pharm. Biol. 60 (1), 1011–1021. https://doi.org/10.1080/13880209.2022.2069823.
Monin, L., Gaffen, S.L., 2018. Interleukin 17 Family Cytokines: Signaling mechanisms, biological activities, and therapeutic implications. Cold Spring Harb. Perspect. Biol. 10 (4) https://doi.org/10.1101/cshperspect.a028522.
Pandey, A., Trigun, S.K., 2023. Fisetin induces apoptosis in colorectal cancer cells by suppressing autophagy and down-regulating nuclear factor erythroid 2-related factor 2 (Nrf2). J. Cell. Biochem. 124 (9), 1289–1308. https://doi.org/10.1002/jcb.30447.
Patel, S.G., Ahnen, D.J., 2018. Colorectal cancer in the young. Curr. Gastroenterol. Rep. 20, 1–12. https://doi.org/10.1007/s11894-018-0618-9.
Pennel, K.A., Quinn, J.A., Nixon, C., Inthagard, J., van Wyk, H.C., Chang, D., Rebus, S., Group, G., Hay, J., Maka, N.N., Roxburgh, C.S., Horgan, P.G., McMillan, D.C., Park, J.H., Roseweir, A.K., Steele, C.W., Edwards, J., 2022. CXCL8 expression is associated with advanced stage, right sidedness, and distinct histological features of colorectal cancer. J. Pathol. Clin. Res. 8 (6), 509–520. https://doi.org/10.1002/cjp2.290.
Qattan, M.Y., Khan, M.I., Alharbi, S.H., Verma, A.K., Al-Saeed, F.A., Abduallah, A.M., Al Areefy, A.A., 2022. Therapeutic importance of kaempferol in the treatment of cancer through the modulation of cell signalling pathways. Molecules 27 (24), 8864. https://doi.org/10.3390/molecules27248864.
Rahmani, A.H., Almatroudi, A., Allemailem, K.S., Khan, A.A., Almatroodi, S.A., 2022. The potential role of fisetin, a flavonoid in cancer prevention and treatment. Molecules 27 (24), 9009. https://doi.org/10.3390/molecules27249009.
Razak, S., Afsar, T., Ullah, A., Almajwal, A., Alkholief, M., Alshamsan, A., Jahan, S., 2018. Taxifolin, a natural flavonoid interacts with cell cycle regulators causes cell cycle arrest and causes tumor regression by activating Wnt/β-catenin signaling pathway. BMC Cancer 18, 1–18. https://doi.org/10.1186/s12885-018-4959-4.
Razi, S., Baradaran Noveiry, B., Keshavarz-Fathi, M., Rezaei, N., 2019. IL-17 and colorectal cancer: From carcinogenesis to treatment. Cytokine 116, 7–12. https://doi.org/10.1016/j.cyto.2018.12.021.
Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., Xu, X., Li, Y., Wang, Y., Yang, L., 2014. TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. J. Cheminformatics 6, 1–6. https://doi.org/10.1186/1758-2946-6-13.
Rudd, C.E., Taylor, A., Schneider, H., 2009. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunol. Rev. 229 (1), 12–26. https://doi.org/10.1111/j.1600-065X.2009.00770.x.
Russo, G.L., Russo, M., Spagnuolo, C., Tedesco, I., Bilotto, S., Iannitti, R., Palumbo, R., 2014. Quercetin: A pleiotropic kinase inhibitor against cancer. In: Advances in Nutrition and Cancer, pp. 185–205. https://doi.org/10.1007/978-3-642-38007-5_11.
Shi, H., Tian, S., Tian, H., 2021. Network pharmacology interpretation of Fuzheng-Jiedu Decoction against colorectal cancer. Evid. Based Complementary Altern. Med. 2021, 1–16. https://doi.org/10.1155/2021/4652492.
Strizova, Z., Benesova, I., Bartolini, R., Novysedlak, R., Cecrdlova, E., Foley, L. K., & Striz, I.2023. M1/M2 macrophages and their overlaps - myth or reality? *Clin. Sci. (1979), 137*(15), 1067-1093. 10.1042/CS20220531.
Tang, Z., Li, C., Kang, B., Gao, G., Li, C., Zhang, Z., 2017. GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 45 (W1), W98–W102. https://doi.org/10.1093/nar/gkx247.
Tao, C., Shkumatov, A.A., Alexander, S.T., Ason, B.L., Zhou, M., 2019. Stigmasterol accumulation causes cardiac injury and promotes mortality. Commun, Biol. 2 (1), 20. https://doi.org/10.1038/s42003-018-0245-x.
Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C.A., Odeberg, J., Djureinovic, D., Takanen, J.O., Hober, S., Alm, T., Edqvist, P.H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., Ponten, F., 2015. Proteomics. Tissue-based map of the human proteome. Science 347 (6220), 1260419. https://doi.org/10.1126/science.1260419.
UniProt, C., 2023. UniProt: The universal protein knowledgebase in 2023. Nucleic Acids Res. 51 (D1), D523–D531. https://doi.org/10.1093/nar/gkac1052.
Wang, X., Wang, Z.Y., Zheng, J.H., Li, S., 2021. TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. Chin. J. Nat. Med. 19 (1), 1–11. https://doi.org/10.1016/S1875-5364(21)60001-8.
Wei, Y., Shi, D., Liang, Z., Liu, Y., Li, Y., Xing, Y., Liu, W., Ai, Z., Zhuang, J., Chen, X., Gao, Q., Jiang, J., 2019. IL-17A secreted from lymphatic endothelial cells promotes tumorigenesis by upregulation of PD-L1 in hepatoma stem cells. J. Hepatol. 71 (6), 1206–1215. https://doi.org/10.1016/j.jhep.2019.08.034.
Yang, J., Pi, C., Wang, G., 2018. Inhibition of PI3K/Akt/mTOR pathway by apigenin induces apoptosis and autophagy in hepatocellular carcinoma cells. Biomed. Pharmacother. 103, 699–707. https://doi.org/10.1016/j.biopha.2018.04.072.
Yu, J., Li, G., Mu, Y., Zhou, H., Wang, X., Yang, P., 2019. Anti-breast cancer triterpenoid saponins from the thorns of *Gleditsia sinensis*. Nat. Prod. Res. 33 (16), 2308–2313. https://doi.org/10.1080/14786419.2018.1443092.
Zhang, Y., Han, D., Yu, S., An, C., Liu, X., Zhong, H., Xu, Y., Jiang, L., & Wang, Z. 2020. Protective effect of iridoid glycosides of the leaves of *Syringa oblata Lindl.* on dextran sulfate sodium-induced ulcerative colitis by inhibition of the TLR2/4/MyD88/NF-kappaB signaling pathway. *Biomed Res. Int., 2020*, 7650123. 10.1155/2020/7650123.
Zhang, T., Ma, Y., Fang, J., Liu, C., Chen, L., 2019. A deregulated PI3K-AKT signaling pathway in patients with colorectal cancer. J. Gastrointest. Cancer 50 (1), 35–41. https://doi.org/10.1007/s12029-017-0024-9.