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Exploring the potential mechanisms of luteolin against ulcerative colitis and colorectal cancer via network pharmacology and molecular docking

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Author contributions

ZL designed the research. QZ, SL and ZL analyzed the data and wrote the paper. YS and LK selected the materials. All authors read and approved the submitted version.

Competing interests

The authors declare no conflicts of interest.

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Peer review information

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Abbreviations

UC, ulcerative colitis; CRC, colorectal cancer; CTD, Comparative Toxicogenomics Database; DEGs, differentially expressed genes; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Citation

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Abstract

Background: Patients diagnosed with ulcerative colitis (UC) are known to have an increased susceptibility to colorectal cancer (CRC). However, the shared underlying mechanisms between UC and CRC remain unclear. Given the therapeutic potential of luteolin in both UC and CRC, this study aims to elucidate the molecular targets and mechanisms through which luteolin exerts its effects against these diseases. **Methods**: The GeneCards database, DisGENet database, and Gene Expression Omnibus database were utilized to analyze the targets associated with UC and CRC. Subsequently, the Traditional Chinese Medicine Systems Pharmacology and SwissTargetPrediction databases were employed to identify luteolin-related targets. The identified luteolin-related targets were then mapped to official gene symbols using the UniProt database. The Cytoscape 3.9.0 software was utilized to construct a network of luteolin-associated targets. Venn diagram analysis was performed to identify common targets among UC, CRC, and luteolin. The common targets were further analyzed using the STRING database to construct a protein-protein interaction network. The "cytoHubba" plugin in Cytoscape 3.9.0 was employed to identify hub targets within the PPI network. Gene Ontology functional analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were conducted on the hub targets. Finally, molecular docking using AutoDock and PyMOL software was performed to assess the binding affinity between luteolin and the hub targets. **Results**: Luteolin was found to interact with a total of 149 pharmacological targets, while UC and CRC were associated with 1232 and 3278 targets, respectively. Forty-six common targets were identified among luteolin, UC, and CRC. Through the application of seven different algorithms, seven hub targets were identified, TP53, AKT1, TNF, SRC, EGFR, and MMP9. Bioinformatics enrichment analysis revealed 49 enriched pathways through Kyoto Encyclopedia of Genes and Genomes analysis, while Gene Ontology analysis yielded a total of 245 biological processes, 4 cellular components, and 7 molecular functions. Molecular docking simulations demonstrated a good binding affinity between luteolin and the hub targets. **Conclusion**: This study identified multiple potential pharmacological targets and elucidated various biological pathways through which luteolin may exert its therapeutic effects in the treatment of UC and CRC. These findings provide a solid theoretical foundation for further experimental investigations in the treatment of UC and CRC.

Keywords: ulcerative colitis; colorectal cancer; luteolin; network pharmacology; gene enrichment

Introduction

Ulcerative colitis (UC) is a chronic, non-specific inflammation that primarily affects the mucosa and submucosa of the colon and rectum [1]. Its major clinical symptoms include diarrhea, abdominal discomfort, and the presence of mucopurulent and bloody stools [2]. Current clinical treatments for UC involve the use of aminosalicylic acids, glucocorticoids, and immunosuppressants to reduce inflammation and alleviate symptoms. However, these medications often come with side effects such as glucocorticoid-induced Cushing's syndrome, bone marrow suppression, opportunistic infections caused by immunosuppressants, and nausea and vomiting associated with salicylic acid [3]. Furthermore, UC increases the risk of developing colorectal cancer (CRC), which ranks as the third most common cancer worldwide. Chronic inflammatory stress leads to genetic damage in the intestinal mucosa and excessive tissue repair, resulting in abnormal hyperplasia of colonic epithelial tissue and ultimately leading to the development of CRC [4]. Patients with CRC may exhibit a range of symptoms, including rectal bleeding, changes in bowel habits, anemia, and abdominal discomfort [5]. Clinical treatment options for CRC typically involve the use of capecitabine, tegafur, and oxaliplatin [6]. However, these treatments can cause side effects such as damage to the hematopoietic system, reduced white blood cell and platelet counts, alopecia, and peripheral neuropathy [7]. Therefore, there is a need to explore potential drugs for the treatment of UC and CRC.

Luteolin, a naturally occurring tetrahydroxyflavonoid, is abundantly present in various fruits, vegetables, and herbs [8], including celery, carrots, broccoli, lemons, marjoram, juniper berry, mint, and honeysuckle. Luteolin has been found to possess multiple pharmacological effects, including anti-tumor [9], anti-oxidative [10], anti-inflammatory [11], anti-allergic [12], anti-bacterial [13], neuroprotective [14], and cardioprotective effects [15]. Studies have also shown that luteolin can inhibit CRC cell metastasis by regulating the miR384/pleiotrophin axis [16] and prevent epithelial-to-mesenchymal transition in CRC cells by maintaining the expression of CREB1 [17]. Furthermore, luteolin may reduce UC through the SHP-1/STAT3 pathway [18]. However, the specific pharmacological targets and underlying mechanisms of luteolin's anti-UC and anti-CRC activities are still not fully understood.

To address this knowledge gap, we employed network pharmacology, a holistic and systematic approach that analyzes the relationships between various components, multiple targets, and diseases through network analysis [19]. In this study, we used network pharmacology to identify hub targets and elucidate potential mechanisms of luteolin's anti-UC and anti-CRC effects. The identified hub targets were further analyzed using bioinformatics tools for enrichment analysis. Additionally, molecular docking was performed to investigate the binding affinity between luteolin and the hub targets. This study provides valuable insights and potential reference strategy for the treatment of UC and CRC, enhancing our understanding of the pharmacological applications of luteolin.

Methods

Identification of targets associated with UC and CRC

To identify targets associated with UC and CRC, we conducted comprehensive searches in the Genecards [20], DisGent [21], and Comparative Toxicogenomics Database (CTD) [22] databases. Additionally, we utilized relevant datasets from the Gene Expression Omnibus database to validate these targets [23]. Specifically, we analyzed the GSE134025 dataset, consisting of total RNA samples from UC patients and a control group, as well as the GSE44076 dataset, which included total RNA samples from CRC tissues and healthy colon mucosa. By applying appropriate scoring criteria, we obtained target information from Genecards, DisGent, and CTD. The data were analyzed using R programming language along with the DESeq2 and limma packages. Differentially expressed genes (DEGs) were identified from the Gene Expression Omnibus database using cutoff criteria of $P_{adj} < 0.05$ and $\log_2$ fold change > 1. Furthermore, we employed the Venny online tool (Venny 2.1.0) to identify common targets shared between UC and CRC.

Screening for potential targets of luteolin

In order to identify potential targets of luteolin, we queried the TCMSP database, a comprehensive Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, using the keyword "luteolin". We selected targets with an oral bioavailability greater than or equal to 30% and drug-likeness greater than or equal to 0.18 [24]. After data refinement, we cross-referenced the obtained targets with the Uniprot database, utilizing the Swiss-Prot and Human settings [25]. Additionally, we utilized the SMILES structural formula of luteolin obtained from the PubChem database to predict potential targets using the SwissTargetPrediction database [26]. Following the removal of duplicates, we confirmed the final potential targets of luteolin by integrating information from both online databases.

Construction of protein-protein interaction (PPI) network

The identified common targets were incorporated into the STRING database [27] with a medium confidence score threshold of 0.4 to construct a PPI network. To further visualize and determine the hub targets within the PPI network, we employed the "cytoHubba" plug-in in Cytoscape 3.9.0, which calculated the weight of each target and identified the hub targets.

Functional mechanism of common genes in UC and CRC

We conducted a series of enrichment analyses using the Metascape database [28] to elucidate the functional mechanisms underlying the common genes in UC and CRC. These analyses included Gene Ontology (GO) analysis, which provides insights into biological processes, molecular functions, and cellular components, as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, which identifies relevant biological pathways. We considered changes significant based on a threshold of $P_{adj} < 0.05$ and $\log_2$ fold change > 1. To gain a comprehensive understanding of the relevant signaling pathways, we integrated the results with information from Reactome [29] and GeneMANIA [30], in addition to employing KEGG pathway analysis. Finally, the results were visualized using bioinformatics tools available at bioinformatics.com.cn.

Molecular docking

To investigate the molecular interactions between luteolin and specific target proteins, we searched the Protein Data Bank database [31] and identified seven hub targets, including VEGFA, TP53, AKT1, TNF, SRC, EGFR, and MMP9, which act as protein receptors. The 2D structure of luteolin, a small molecular ligand, was obtained from the PubChem database [32] and converted into MOL2 format using chemical office software. Molecular docking simulations were performed using AutoDock Vina software, and we selected the top four receptor proteins with the lowest energy values. The ligand-receptor interactions were visualized using Pymol software.

Results

Identification of DEGs and shared targets between UC and CRC

Figure 1 illustrates the overall procedure of this study. In the GSE134025 dataset for UC, a total of 2577 DEGs were identified, with 1298 genes showing upregulation and 1279 genes showing downregulation (Figure 2A). In the case of CRC, the GSE44076 dataset revealed 159 DEGs, including 122 upregulated genes and 37 downregulated genes (Figure 2B). Supplementary Table S1 and S2 provide detailed information on the DEGs from GSE134025 and GSE44076, respectively. Subsequently, we searched the GeneCards, CTD, and DisGeNET databases to identify targets associated with UC and CRC. We obtained the top 500 targets from each database, following the scoring guidelines provided by the respective websites (Table 1) to ensure the reliability of the data. If the raw data contained

fewer than 500 targets, all available targets were included. This process yielded 500 UC-related targets from CTD, DisGeNET, and GeneCards, respectively. Similarly, for CRC-related targets, we obtained 26,500,500 from CTD, DisGeNET, and GeneCards. We integrated the target information from the three databases and the GSE134025 dataset to obtain UC-related targets (Figure 2C). Likewise, for CRC-related targets, we combined the targets from the GSE44076 dataset and the three databases (Figure 2D). After removing duplicates, we obtained 1232 UC-related targets and 3278 CRC-related targets. By taking the intersection of these sets, we identified 500 genes that were common to both UC and CRC (Figure 2E).

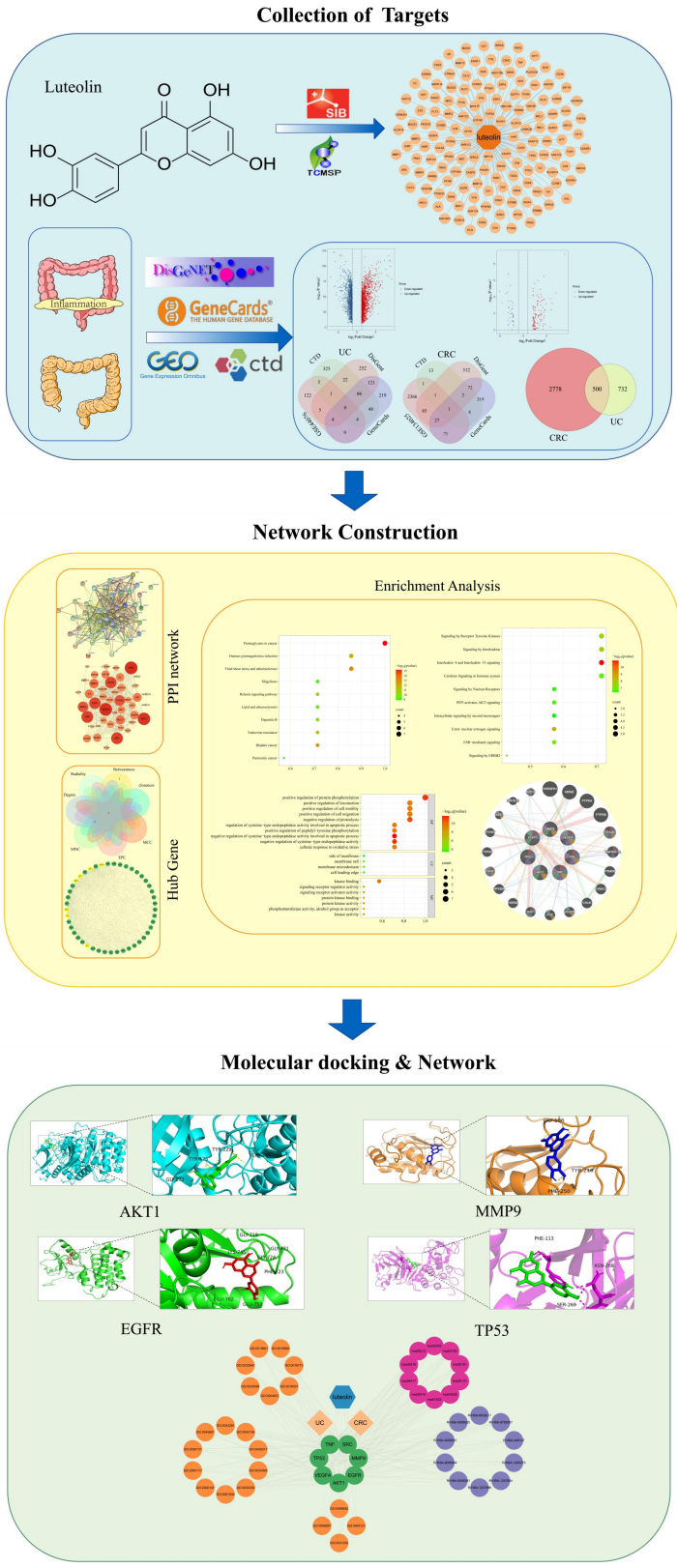

Figure 1 The workflow of this study

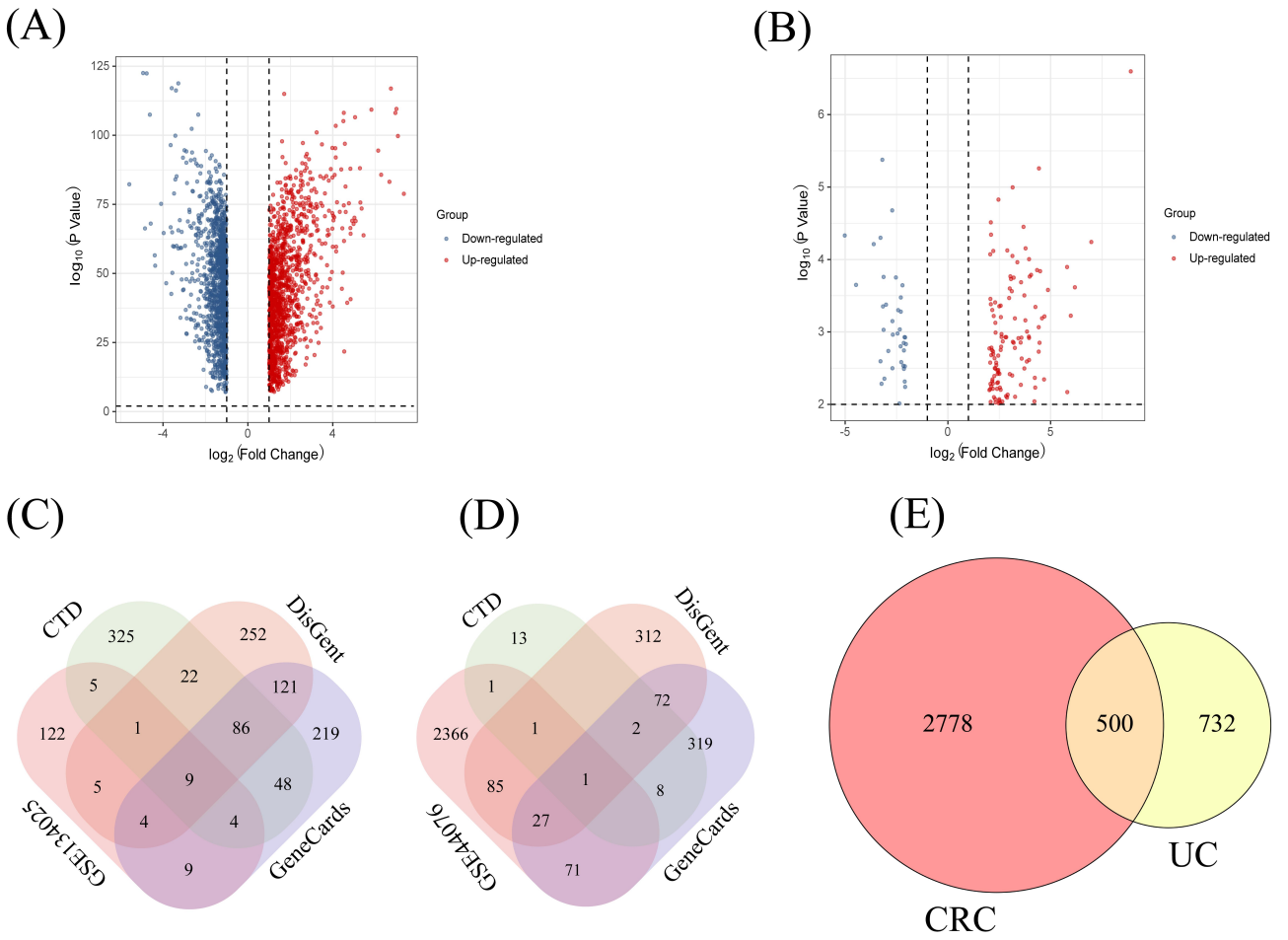

Figure 2 Analysis of common targets between UC and CRC. (A) The volcano map of GSE44076. (B) The volcano map of GSE134025. (C) UC-related targets from union set of CTD, DisGent, GeneCards, and GSE44076. (D) CRC-related targets from union set of CTD, DisGent, GeneCards, and GSE134025. (E) Venn diagram showed 500 common targets between CRC and UC. UC, ulcerative colitis; CRC, colorectal cancer; CTD, Comparative Toxicogenomics Database.

Table 1 Identification of UC and CRC related targets

Disease	Data sources	Amount of raw data	Filter criteria	Amount of selected data
UC	CTD	18099	TOP 500 in inference Score	500
	GeneCards	1582	TOP 500 in relevance score	500
	DisGeNET	1458	TOP 500 in score	500
	GSE134025	60298	$P_{adj} < 0.05$ and $\log_2$fold change > 1	159
CRC	CTD	26	ALL	26
	GeneCards	11208	TOP 500 in relevance score	500
	DisGeNET	5473	TOP 500 in score	500
	GSE44076	19831	$P_{adj} < 0.05$ and $\log_2$fold change > 1	2577

UC, ulcerative colitis; CRC, colorectal cancer; CTD, Comparative Toxicogenomics Database.

Identification of luteolin-related targets and intersection with UC and CRC

We utilized the TCMSP database and the SwissTargetPrediction database to explore the pharmacological targets of luteolin and their intersection with UC and CRC. After removing duplicates, we found a total of 149 targets associated with luteolin (Figure 3A) (Supplementary Table S3 provides detailed information). By comparing these luteolin-related targets with the UC-related targets and CRC-related targets, we identified 46 common targets shared between luteolin, UC, and CRC (Figure 3B).

Construction of PPI network and identification of hub targets

The STRING database was utilized to import the 46 common targets to construct the PPI network. The resulting PPI network consisted of 45 nodes and 376 edges, which were visualized using Cytoscape software (Figure 4A). One target, "AKR1B10," was removed from the network as it did not have any connections to other targets (Figure 4B). To identify hub targets within the network, topological analysis was performed using the CytoHubba plug-in in Cytoscape, which includes

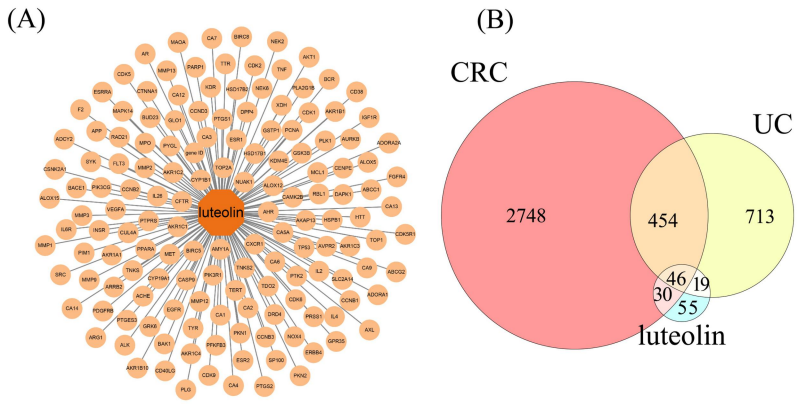

Figure 3 Common targets among luteolin, UC and CRC. (A) luteolin-related targets. (B) Venn diagram showed 46 common targets among luteolin, UC and CRC. UC, ulcerative colitis; CRC, colorectal cancer.

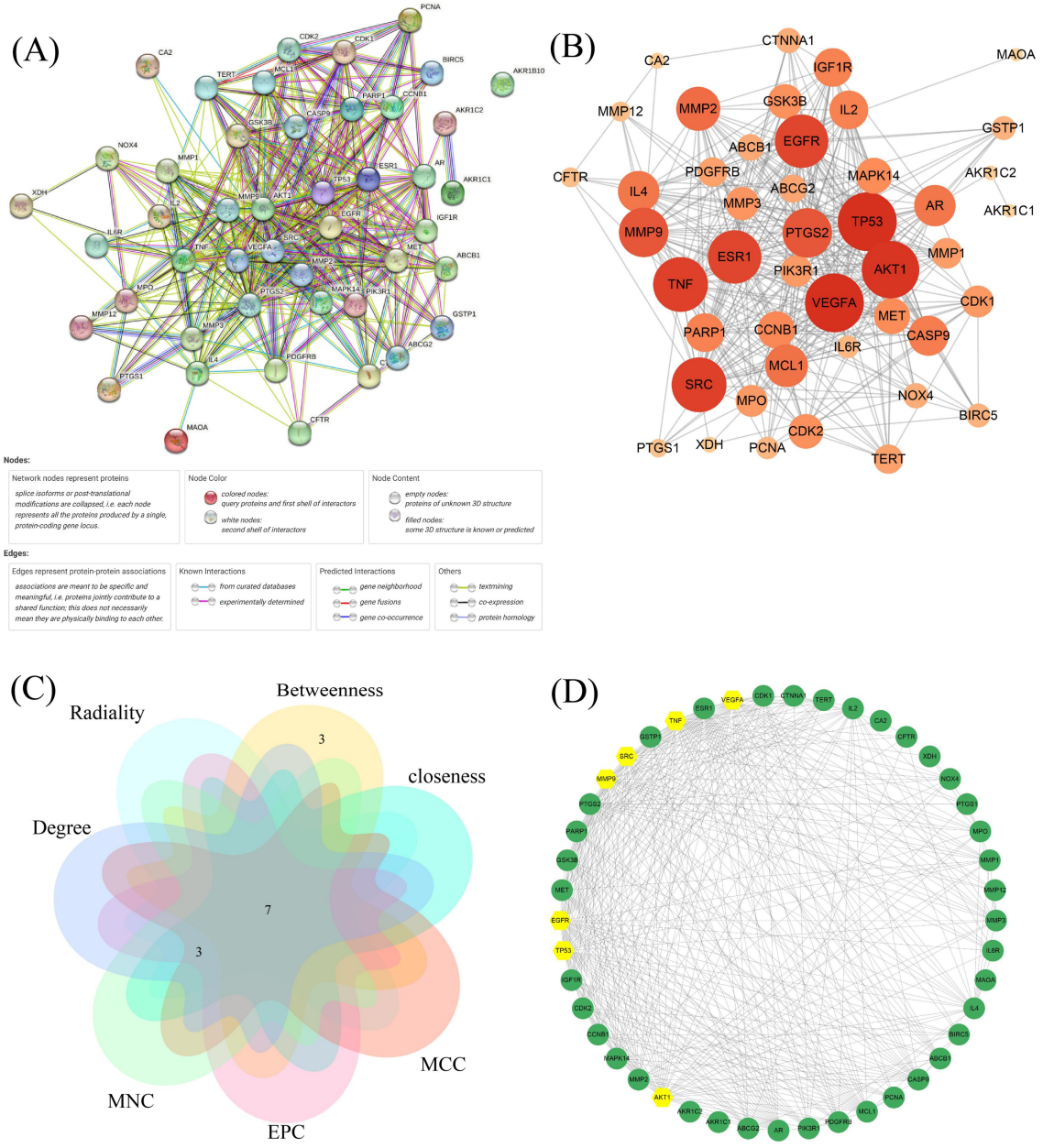

Figure 4 PPI network of common targets among luteolin, UC and CRC and identification of hub targets. (A) PPI network of common targets among luteolin, UC and CRC. (B) Visualization of PPI network using cytoscape. Nodes from small to large represent the degree value from low to high. The deeper color represents the higher degree of targets. (C) Intersection of 7 algorithm to identify 7 hub targets among luteolin, UC and CRC. (D) The highlighted yellow 7 hub targets are AKT1, TP53, EGFR, MMP9, SRC, TNF and VEGFA. UC, ulcerative colitis; CRC, colorectal cancer; PPI, protein-protein interaction.

seven algorithms: "Radiality," "Degree," "MNC," "EPC," "MCC," "Closeness," and "Betweenness." The top 10 targets identified by each algorithm were scored and the results are presented in Supplementary Table S4. The outputs from the seven algorithms were merged to identify the hub targets (Figure 4C). Ultimately, seven hub targets were identified, and their connections with other targets in the PPI network are illustrated in Figure 4D.

Analysis of pathway enrichment and GO

We utilized two global databases of KEGG and Reactome to analyze the pathway enrichment of the seven hub targets in relation to UC, CRC, and luteolin. In the KEGG pathway database, we found a total of 49 signal pathways associated with the hub targets (Supplementary Table S5). Similarly, in the Reactome database, we identified 29 related pathways (Supplementary Table S6). To visualize the results, we selected the top 10 pathways and created a KEGG pathway and Reactome dot plot based on the respective *P*-values (Figures 5A and 5B). The pathways are summarized in Table 2. For the GO analysis, we examined the biological processes, cellular components, and molecular functions related to the hub targets. In terms of biological processes, we identified 245 processes, primarily associated with positive regulation of protein phosphorylation, regulation of proteolysis, and positive regulation of locomotion. Regarding cellular components, we found four components, including membrane raft, membrane microdomain, cell leading edge, and side of the membrane. In the category of molecular functions, seven functions were identified, mainly related to kinase binding, signaling receptor activator activity, and signaling receptor regulator activity. The GO terms are summarized in Table 3. To visualize the results, we selected the top 20 biological processes based on their *P*-values, along with the four cellular components and seven molecular functions, and constructed a dot plot (Figure 5C). Additionally, using the GeneMANIA tool, we created a co-expression network to explore the genetic relationships between the seven hub targets and their co-expressed targets. A total of 20 predicted targets were included in the co-expression study, considering various connection qualities, such as 61.12% physical interactions, 17.49% genetic expression, 8.61% predicted, 6.58% co-expression, 3.34% co-localization, and 2.85% pathway. These connections are represented in Figure 5D.

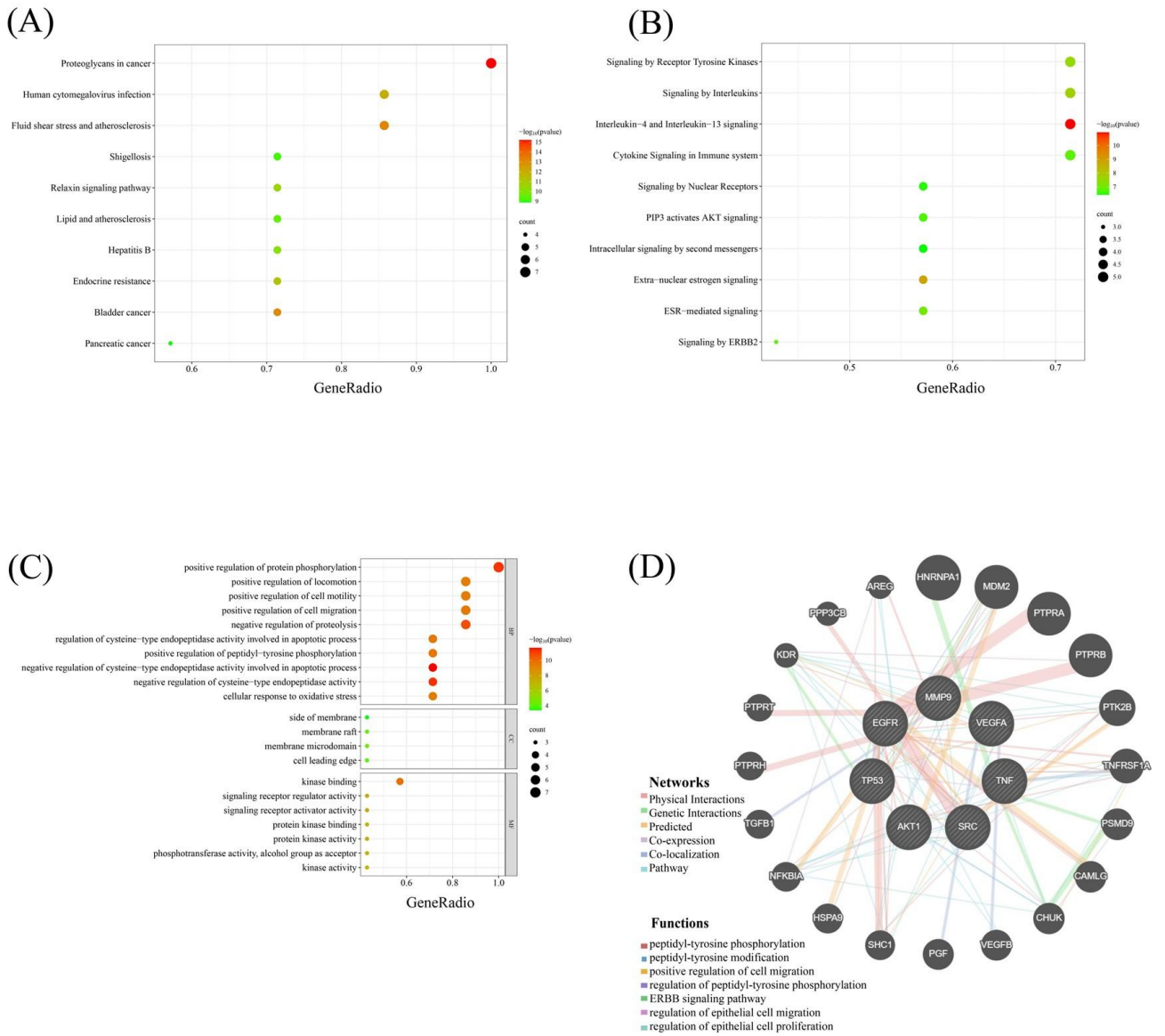

Figure 5 GO terms and pathway enrichment analysis of hub genes. (A) KEGG pathway dot plot. (B) Reactome enrichment analysis. (C) The dot plot of GO showing Top 20 BP terms, CC terms and MF terms based on the *P* value. (D) The functional network of hub targets generated by GeneMANIA. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; MF, molecular function; CC, cellular component.

Table 2 Pathway enrichment analysis of 7 hub targets among UC, CRC and luteolin

Category	Pathways	*P*-values	Genes
KEGG pathway	Proteoglycans in cancer	6.04E-16	AKT1, MMP9, SRC, TNF, TP53, VEGFA
	Fluid shear stress and atherosclerosis	5.98E-14	EGFR, MMP9, SRC, TP53, VEGFA
	Bladder cancer	7.54E-14	AKT1, EGFR, SRC, TNF, TP53, VEGFA
	Human cytomegalovirus infection	1.12E-12	AKT1, EGFR, MMP9, SRC, TP53
	Endocrine resistance	6.81E-12	AKT1, EGFR, MMP9, SRC, VEGFA
	Relaxin signaling pathway	2.76E-11	AKT1, MMP9, SRC, TNF, TP53
	Hepatitis B	8.74E-11	AKT1, MMP9, SRC, TNF, TP53
	Lipid and atherosclerosis	3.64E-10	AKT1, EGFR, SRC, TNF, TP53
	Shigellosis	7.32E-10	AKT1, EGFR, TP53, VEGFA
	Pancreatic cancer	1.29E-09	AKT1, EGFR, TP53, VEGFA
Reactome	Interleukin-4 and Interleukin-13 signaling	1.12E-11	AKT1, MMP9, TNF, TP53, VEGFA
	Extra-nuclear estrogen signaling	1.36E-09	AKT1, EGFR, MMP9, SRC
	Signaling by Interleukins	1.9E-08	AKT1, MMP9, TNF, TP53, VEGFA
	Signaling by Receptor Tyrosine Kinases	3.08E-08	AKT1, EGFR, MMP9, SRC, VEGFA
	ESR-mediated signaling	1E-07	AKT1, EGFR, MMP9, SRC
	Signaling by ERBB2	1.49E-07	AKT1, EGFR, SRC
	Cytokine Signaling in Immune system	1.6E-07	AKT1, MMP9, TNF, TP53, VEGFA
	PIP3 activates AKT signaling	2.06E-07	AKT1, EGFR, SRC, TP53
	Signaling by Nuclear Receptors	3.23E-07	AKT1, EGFR, MMP9, SRC
	Intracellular signaling by second messengers	3.69E-07	AKT1, EGFR, SRC, TP53

UC, ulcerative colitis; CRC, colorectal cancer; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 3 GO analysis of 7 hub targets among UC, CRC and luteolin

Category	GO ID	Term	*P*-values	Genes
GO-biological process	GO:0043154	Negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	2.12E-12	AKT1, MMP9, SRC, TNF, VEGFA
	GO:2000117	Negative regulation of cysteine-type endopeptidase activity	4.67E-12	AKT1, MMP9, SRC, TNF, VEGFA
	GO:0001934	Positive regulation of protein phosphorylation	5.66E-12	AKT1, EGFR, MMP9, SRC, TNF, TP53, VEGFA
	GO:0045861	Negative regulation of proteolysis	1.68E-11	AKT1, MMP9, SRC, TNF, TP53, VEGFA
	GO:0050731	Positive regulation of peptidyl-tyrosine phosphorylation	2.11E-10	EGFR, SRC, TNF, TP53, VEGFA
	GO:0043281	Regulation of cysteine-type endopeptidase activity involved in apoptotic process	2.87E-10	AKT1, MMP9, SRC, TNF, VEGFA
	GO:0030335	Positive regulation of cell migration	2.93E-10	AKT1, EGFR, MMP9, SRC, TNF, VEGFA
	GO:2000147	Positive regulation of cell motility	3.83E-10	AKT1, EGFR, MMP9, SRC, TNF, VEGFA
	GO:0034599	Cellular response to oxidative stress	4.28E-10	AKT1, EGFR, MMP9, SRC, TP53
	GO:0040017	Positive regulation of locomotion	4.45E-10	AKT1, EGFR, MMP9, SRC, TNF, VEGFA
GO-cellular components	GO:0045121	Membrane raft	4.24E-05	EGFR, SRC, TNF
	GO:0098857	Membrane microdomain	4.28E-05	EGFR, SRC, TNF
	GO:0031252	Cell leading edge	9.06E-05	AKT1, EGFR, SRC
	GO:0098552	Side of membrane	0.00038101	AKT1, SRC, TNF
GO-cellular components	GO:0019900	Kinase binding	4.83393179	AKT1, EGFR, SRC, TP53
	GO:0004672	Protein kinase activity	3.66514579	AKT1, EGFR, SRC
	GO:0016773	Phosphotransferase activity, alcohol group as acceptor	3.44349367	AKT1, EGFR, SRC
	GO:0019901	Protein kinase binding	3.39692799	AKT1, SRC, TP53
	GO:0016301	Kinase activity	3.34151040	AKT1, EGFR, SRC
	GO:0030546	Signaling receptor activator activity	3.82698986	EGFR, TNF, VEGFA
	GO:0030545	Signaling receptor regulator activity	3.70896397	EGFR, TNF, VEGFA

GO, Gene Ontology; UC, ulcerative colitis; CRC, colorectal cancer.

Molecular docking

For molecular docking analysis, we selected four hub targets. The results of the matching analysis provided the binding affinity scores between luteolin and these targets, which are displayed in Table 4. All four hub targets, namely AKT1, EGFR, MMP9, and EGFR (PDB IDs: 3OCB, 1AO7, 2ITY, and 6ESM, respectively), showed strong binding affinity with luteolin, with binding affinity scores below 5. This indicates a favorable interaction between luteolin and the selected hub targets. Figure 6 illustrates the docking results for these proteins.

Discussion

UC is a chronic inflammatory bowel disease that affects the colon, rectum, and submucosa. It is characterized by recurrent episodes and a gradual onset [33]. Clinical manifestations of UC include constipation, mucous stools, diarrhea, abdominal discomfort, and tenesmus [34]. Prolonged inflammatory stress can lead to genetic damage in the intestinal mucosa, resulting in excessive tissue repair and abnormal proliferation of colonic epithelial cells, eventually leading to CRC [35]. However, there is limited research exploring the targets and pathways of luteolin for the treatment of UC and CRC from a network pharmacology perspective, although previous studies have shown the beneficial effects of luteolin in both UC [36] and CRC [37]. Therefore, our study aimed to identify the pharmacological targets and pathways of luteolin for UC and CRC treatment. By analyzing the GES134025 dataset, we identified 1298 upregulated and 1279 downregulated genes. Similarly, the analysis of the GSE44076 dataset revealed 122 upregulated and 37 downregulated genes. After merging these datasets with disease-related genes from GeneCards, DisGeNET, CTD, and the Gene Expression Omnibus database, we obtained 1232 targets for UC and 3278 targets for CRC. Filtering out 149 luteolin targets from the TCMSP database and SwissTargetPrediction database, we identified 46 common targets among luteolin, UC, and CRC. From these common targets, we further identified seven hub targets (VEGFA, TP53, AKT1, TNF, SRC, EGFR, MMP9) using the CytoHubba plug-in in Cytoscape software.

Table 4 Binding affinity of representative four hub targets with luteolin

	Target proteins	E-value (Kcal/mol)	No of H bonds	Binding site
Luteolin	AKT1 (PDB ID:3OCB)	–6.28	5	GLY:173 TYR:175 TYR:229 LYS:284
	TP53 (PDB:6RZ3)	–7.16	3	PHE:113 ASN:268 SER:269
	EGFR (PDB ID:2ITY)	–6.94	2	THR:102 ASN:131
	MMP9 (PDB ID:6ESM).	–5.10	4	GLN:37 GLU:38 TYR:39 ASN:75

PDB, Protein Data Bank.

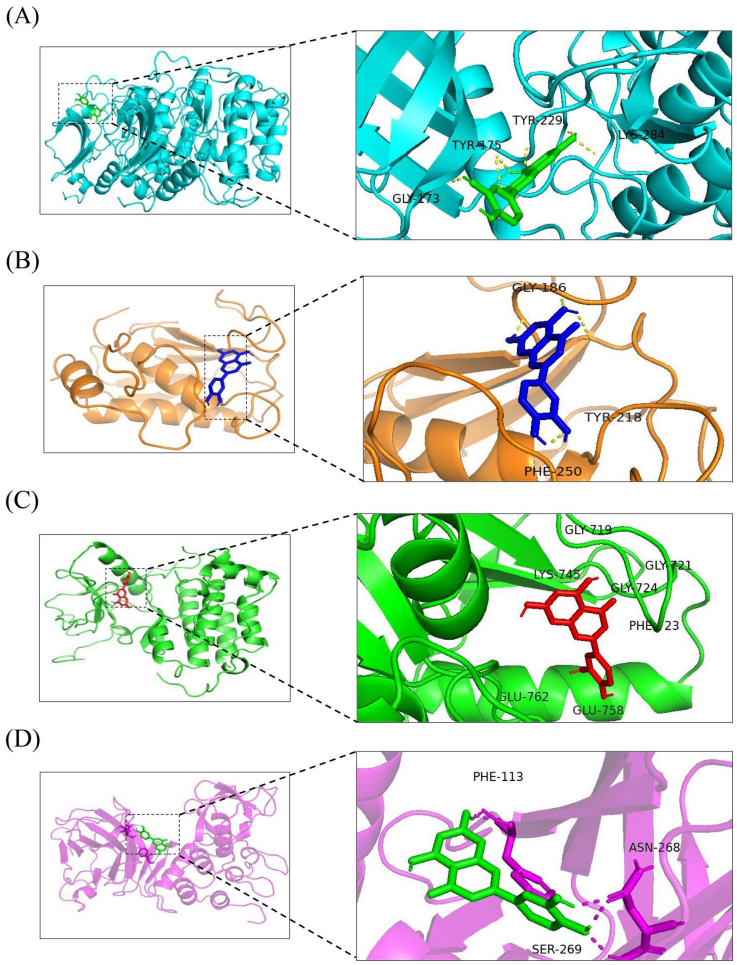

Figure 6 The representative molecular docking results. (A) Luteolin bound to the pocket of AKT1. (B) Luteolin bound to the pocket of MMP9. (C) Luteolin bound to the pocket of EGFR. (D) Luteolin bound to the pocket of TP53.

Next, we explored the pathways influenced by luteolin in the treatment of UC and CRC using KEGG pathway and Reactome enrichment analyses. The top 10 enriched KEGG pathways included proteoglycans in cancer, fluid shear stress and atherosclerosis, hepatitis B, lipids and atherosclerosis, bladder cancer, cancer pathways, shigellosis, endocrine resistance, and the MAPK signaling pathway. The involvement of proteoglycans in the cancer pathway has been linked to functional gene modules and microRNAs associated with the pathological stage of colon cancer [38]. Additionally, human cytomegalovirus infection has been found to enhance epithelial-to-mesenchymal transition markers in CRC, promoting cell migration and proliferation [39]. Regarding the GO analysis, we observed that the most significant GO biological processes related to luteolin treatment included positive regulation of protein phosphorylation, cell migration, cell motility, and locomotion. Negative regulation of proteolysis, catalytic activity, and positive regulation of the enzyme-linked receptor protein signaling pathway were also prominent. Protein phosphorylation plays a crucial role in controlling protein and cellular functions. For example, in CRC, IL-6 regulates autophagy and chemotherapy resistance by promoting BECN1 phosphorylation, suggesting that luteolin may exert an anti-CRC/UC effect by modulating key protein phosphorylation processes [40]. The top molecular function identified was kinase binding. Previous studies have indicated that the PI3K, AKT, mTOR, and MAPK signaling pathways are activated by growth factors and play crucial roles in CRC development, suggesting that kinase activity might mediate CRC development [41]. For cellular components, the identified terms were membrane raft, membrane microdomain, cell leading edge, and side of the membrane. These components are relevant to the localization and organization of cellular structures. Furthermore, we performed molecular docking between luteolin and four hub targets (AKT1, MMP9, EGFR, TP53), and the results indicated a good binding affinity between luteolin and these targets. Akt1, also known as protein kinase B, is a serine/threonine kinase that plays a crucial role in various cellular processes, including cell survival, proliferation, and inflammation [42]. Akt1 signaling has been implicated in the modulation of inflammatory pathways in the colon, which are relevant to the pathogenesis of UC. Activation of Akt1 has been observed in colonic epithelial cells of UC patients, particularly during active disease phases [43]. In addition, AKT1 is a key component of the PI3K/AKT signaling pathway, which is frequently dysregulated in CRC [44]. The PI3K/AKT pathway can be activated through various mechanisms in CRC, including genetic alterations [45]. Yang [46] found that luteolin, as a component from Hedyotisdiffusa- Scutellariabarbata herb, could treat CRC thorough AKT1 targets based on network pharmacology and molecular docking. MMP9 is an enzyme that belongs to the matrix metalloproteinase family, which plays a crucial role in extracellular matrix remodeling and degradation [47]. Lakatos [48] showed that elevated levels of MMP9 in biopsy samples from UC patients compared to healthy individuals. This suggests that MMP9 may play a role in the pathogenesis of UC by contributing to the tissue damage and remodeling observed in the inflamed colon. For CRC, Pandurangan [49] showed that luteolin inhibits MMP9 expression to exert treatment in CRC. EGFR, a cell surface receptor, which belongs to the family of receptor tyrosine kinases. It plays a crucial role in regulating cell growth, survival, and proliferation [50]. EGFR activation in CRC has been linked to the activation of some signaling pathways, such as EGF or TGF-α, EGFR triggers intracellular signaling cascades, including the RAF/MEK/ERK and PI3K/Akt pathways, all of which are critical for CRC growth and progression [51, 52]. For UC, EGFR expression is increased in the colonic epithelium of UC patients compared to healthy individuals [53]. This upregulation may be a response to the chronic inflammation and tissue damage observed in UC, as EGFR signaling is involved in tissue repair and regeneration [54]. Luteolin, as a main component from Yifei Jianpi Tongfu formula, binds with EGFR to potentially interfere with the survival and invasion of CRC [55]. TP53, also known as the tumor protein 53 or p53, is a well-known tumor suppressor gene that plays a critical role in maintaining genomic stability and regulating cell cycle progression [56]. Patients with TP53-mutated CRC often have a higher risk of disease recurrence, lymph node involvement, distant metastasis, and reduced overall survival compared to those with wild-type TP53 [57]. TP53 alterations in UC have been linked to a more severe disease phenotype [58, 59]. In conclusion, the identification of these four hub targets (AKT1, MMP9, EGFR, TP53) suggests their potential as therapeutic targets for luteolin in the treatment of UC and CRC. Furthermore, these findings provide valuable theoretical support for the development of drugs targeting UC and CRC.

However, it is important to acknowledge the limitations of our study. The results are based on bioinformatics analysis and require experimental validation to confirm the predicted targets and pathways. Additionally, while our findings contribute to the understanding of luteolin's pharmacological function, further preclinical and clinical studies are necessary to evaluate its efficacy and safety in the context of UC and CRC.

Conclusion

Overall, our study identified common targets and pathways between UC, CRC, and luteolin, a natural compound with potential therapeutic effects. Through bioinformatics and computational analysis, we identified seven hub targets (VEGFA, TP53, AKT1, TNF, SRC, EGFR, MMP9) and explored their associated pathways and biological processes (Figure 7). These findings provide insights into the potential mechanisms of luteolin in the treatment of UC and CRC. It offers

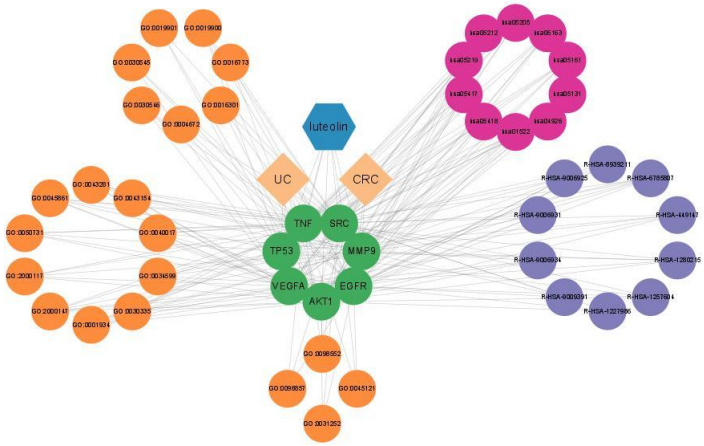

Figure 7 Interaction network showing hub targets and main signal pathways of luteolin against UC and CRC. Hexagon represents luteolin. Diamond represents UC and CRC respectively. Green ellipse represents hub targets. Yellow ellipse represents GO enrichment. Pink ellipse represents KEGG pathway. Purple ellipse represents Reactome pathway. GO, Gene Ontology; UC, ulcerative colitis; CRC, colorectal cancer; KEGG, Kyoto Encyclopedia of Genes and Genomes.

guidance for the development of medications targeting these diseases and underscores the need for further research to translate these findings into clinical applications.

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