

Research on the Treatment of Colorectal Cancer Based on Network Pharmacology and Molecular Docking

Xinyao Shu

Chengdu University of Traditional Chinese Medicine

Yunwu Zhang (✉ zhangyunwu@stu.scu.edu.cn)

Sichuan University

Yujia Wang

Chengdu University of Traditional Chinese Medicine

Jing Wu

Chengdu University of Traditional Chinese Medicine

Xingyu Chen

Chengdu University of Traditional Chinese Medicine

Ruitong Xu

Chengdu University of Traditional Chinese Medicine

Xingting Liu

Chengdu University of Traditional Chinese Medicine

Jingsong Luo

Chengdu University of Traditional Chinese Medicine

Tingting Deng

Chengdu University of Traditional Chinese Medicine

Research Article

Keywords: Sophora flavescens, decoction, colorectal cancer, network pharmacology, molecular docking

Posted Date: March 25th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-263797/v2>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: To predict the mechanism of Huaijiao Diyu Decoction in the treatment of colorectal cancer.

Methods: The active components and related targets of 7 kinds of Chinese medicinal materials were collected from the database of Chinese medicinal materials. CRC related targets are obtained from the Genes card database. cross targets of disease and drug targets were input into the STRING database to construct protein-protein interaction networks. GO enrichment analysis and KEGG enrichment analysis using clusterProfiler packets in the language, Finally, the interaction between core components and core targets is discussed by molecular docking.

Result: TCMSP database prompts, There are 34 active compounds mapping 114 targets in Sophora japonica decoction; G encards database prompts, A total of 858 targets are closely related to CRC, The two data sets map each other to obtain 114 intersection targets. Core components of Sophora flavescens decoction in treating CRC may be quercetin, kaempferol, luteolin, The core therapeutic targets may be PTGS2, PTGS1, HSP90AA1 and AR, CRC related pathways involve multiple molecular functions such as cell proliferation, apoptosis, cell signal transduction, metabolism, endocrine, tumor immunity, transcription and cell metabolism. Molecular docking results show that the binding ability of PTGS1, PTGS2, AR to core components is strong, The Vina value (binding energy) of the interaction between PTGS1 protein and core components is the best, -7.9kcal/mol .

Conclusion: This study demonstrates the mechanism of multi-component, multi-target and multi-pathway action in the treatment of CRC, Can provide the idea for clarifying the specific mechanism of Huaijiao Diyu decoction in the treatment of CRC in the future.

1. Background

Colorectal cancer is one of the most common malignancies in gastrointestinal tract[1], typical symptoms are hematochezia, abdominal pain, abdominal mass, weight loss, anemia, intestinal obstruction [2]. According to the latest report in 2018, globally, the incidence of colorectal cancer ranks fourth among malignant tumors, death rate ranked second [3]. January 2019, The National Cancer Center released the latest issue of national cancer statistics, the incidence and mortality of colorectal cancer in China ranked third and fifth respectively, The incidence of colorectal cancer in China is on the rise. The western medicine treatment of colorectal cancer is still mainly surgical treatment. And the only cure for colorectal cancer, Clinical use of surgery, radiotherapy, chemotherapy, targeted therapy and other treatment methods combined, fluorouracil (5 FU) based regimens and oxaliplatin were generally accepted as a standard adjuvant regimen [4]. But since most patients are diagnosed as advanced, Difficult treatment, Poor prognosis or long-term radiotherapy and chemotherapy lead to a serious decline in the quality of life of patients, Produce serious adverse reactions.

Traditional Chinese medicine is an important tumor-assisted treatment [5]. A number of studies have shown that Chinese medicine has played a positive role in blocking tumor energy metabolism, inducing

cancer cell apoptosis, reducing tumor cell recurrence and metastasis rate, and inhibiting tumor cell growth and value-added, surgery, chemotherapy and taking Chinese herbal decoction were protective factors for CRC recurrence and metastasis has a positive effect on the survival time of CRC patients [6,7]. TCM believes that colorectal cancer has spleen and kidney yang deficiency, qi and blood deficiency, spleen deficiency dampness Sheng, spleen deficiency deficiency and other syndromes. Clinical practice has proved that Huaijiao Diyu decoction has a significant effect in the treatment of colorectal cancer, but its treatment mechanism is not clear and needs further exploration.

Sophora flavescens decoction from the "political criteria", for the main prescription for clearing heat and cooling blood, this prescription by Sophora flavescens, Radix Paeoniae Alba, Gardenia, Fructus Aurantii, Scutellariae, Schizonepeta. A result suggest that pretreatment with noncytotoxic concentrations of fructus sophorae extract (FSE) xhibitsanti-inflammatory activity by inhibiting inflammatory media and cytokines through the inactivation of NF- κ B, ERK and JNK, and it may offer therapeutic potential for creating inflammatory diseases associated with macroactivity [8]. The present results suggest that FSE may protect against inflammation and bone damage, and would be a valuable candidate for further investigation as a novel anti-arthritic agent [9]. Data suggested that the addition of SOWPa (from *Sanguisorba officinalis* L. Rosaceae) to PRP increased the reproductive capacity of ACL fibers by blocking the TLR-4/NF- κ B pathway. The addition of sowpa to prp can improve the regeneration ability of acl fibroblasts by blocking the tlr-4/nfb pathway. components from *Sanguisorba officinalis* L. on *Vibrio vulnificus* have anti-bacterial effects and their soluble epoxide hydrolase inhibitory activity [11]. However, due to the characteristics of multi-component, multi-pathway and multi-target synergistic effect, this complexity makes the substance basis of traditional Chinese medicine is not clear, the mechanism of action is not clear, the quality of traditional Chinese medicine and traditional Chinese medicine is difficult to control, and the evaluation system of efficacy and safety is lacking. It is difficult to carry out comprehensive and systematic research from the whole to the level of tissues, organs, cells and molecules. Network pharmacology through the construction of "disease-gene-target-drug" interaction network, docking drugs and multiple disease targets and network analysis, systematic and comprehensive observation of drug intervention and impact on the disease network [12], It is helpful to reveal the material basis and mechanism [13] of traditional Chinese medicine or compound.

2. Materials And Methods

Chinese medicine believes that the pathogenesis of early colorectal cancer (colorectal cancer) is dampness and heat toxin accumulation of large intestine, qi and blood stasis is not smooth, its treatment principles should be to clear heat and dampness, blood stasis and detoxification, commonly used prescriptions such as Huaijiao Diyu decoction, Baeweng decoction, Xuefu Zhuyu decoction, etc. As shown in Fig.1.

2.1 Collection of active compounds and targets

Through the TCMSP database (<http://tcmsp.com/tcmsp.php>), Search "Sophora japonica "," Ulmus pumila "," Fructus Aurantii "," Scutellaria baicalensis "," Schizonepeta "," Gardenia "," Radix Paeoniae Alba" seven herbs. Oral bioavailability (oral bioavailability,) in pharmacokinetic (ADME) parameters was used in this study OB and drug-like properties (drug-likeness,); and DL) as a standard, $30\% \text{ OB} \geq \text{set}$, $\text{DL} \geq 0.18$ to screen active compounds and predict target [14] of active compounds in Sophora flavescens decoction. The Uniprot database (<https://www.uniprot.org/>) is used to standardize the target name.

2.2 Collection of CRC targets of active compounds in Sophora japonica L. Decoction

Through the GeneCards database (<https://www.genecards.org/>) to "Colorectal cancer" as the keyword retrieval CRC action target [16]. The related targets of active compounds and CRC targets in Huaijiao Diyu decoction were mapped to obtain the intersection targets, and the R package VennDiagram was used to draw the Venn map [17] of Huaijiao Diyu decoction and CRC related targets.

2.3 Traditional Chinese Medicine - Active Compounds - Construction of CRC- Target Network

The Chinese medicine, active compound, target and disease of Huaijiao Diyu decoction were introduced into the CRC Cytoscape 3.8.0 software to construct the "Chinese medicine-active compound- CRC- target" network. Analysis of topology parameters of network nodes using Network Analysis plug-ins, The core active compounds and core target [18] of Huaijiao Diyu decoction were screened by Degree value.

2.4 Building protein interaction networks

Using the string database (<https://string-db.org/>) to obtain target interaction [19], Homo sapiens", by selecting species Check "Hidden Network Interrupt Node hide disconnected nodes in the network", And set the "minimum required interaction score minimum required interaction score">0.9 to get the protein interaction network diagram and the core genes of the network, According to the number of connections with adjacent genes, the top 30 core genes histogram was obtained by R software. Import data cytoscape database to draw protein interaction network. Analysis of topology parameters of network nodes using Network Analysis plug-ins, According to the Degree value, the Sophora japonica decoction was screened.

2.5 Analysis of biological functions and pathways

ClusterProfiler is a software package for enrichment clustering and visualization of gene sets in R languages. Using the R language (version 4.0.3) to call the clusterProfiler package to analyze the GO molecular function and KEGG pathway enrichment of the target, Among them, the screening condition is that the P value is less than 0.05. The histogram and bubble chart [20] of GO function and KEGG path are drawn by using R package.

2.6 Molecular docking analysis of core active compounds with core protein receptors

The core active compounds of the first 4 and the core protein receptors of the first 4 were selected from the interaction network of the CRC targets treated Degree Huaijiao Diyu decoction. The structure of target

protein and core components are downloaded from PDB website (<http://www.rcsb.org/>) and pubchem website (<https://pubchem.ncbi.nlm.nih.gov/>) respectively, Using Pymol software to remove water and raw PDB, Autodock Tolls software for targeting protein receptor molecules with polar hydrogen, molecular docking through Autodock Vina and Python scripts. The lower the binding energy, the better the affinity between the receptor and the ligand, This study selected the binding energy ≤ -5.0 kJ/mol as the screening basis for the effective binding of compounds and targets.

3. Results

3.1 Screening results of active compounds and targets in *Sophora japonica*

Enter the drug name in the TCMSP database, And according to $OB \geq 30$, $DL \geq 0.18$, the active components of the drug were obtained. There were 13 active compounds of *E. officinalis*, Six active compounds, *Fructus Aurantii* active compounds 5, There are 36 active compounds in *Scutellaria baicalensis*, There are 11 active compounds, *Gardenia* 5 active compounds, *Radix Paeoniae Alba* active compounds 13, A total of 64 active compounds were obtained after mapping, A total of 247 corresponding targets.

3.2 Results of intersection of active compounds in the treatment CRC action targets of *Sophora japonica*

The 858 CRC related potential targets were retrieved in the GeneCards database, and 114 intersection targets were obtained by mapping them with 247 targets corresponding to *Huaijiao Diyu* decoction, as shown in Fig.2.

3.3 Screening results of targets for the treatment of CRC by active compounds in *Sophora flavescens* decoction

To construct the network map of Chinese medicine - active compound - CRC- target by introducing CRC Chinese medicine, active compound, target and disease into the software, The network diagram consists of 172 nodes and 491 edges, Common goals and diseases containing 58 ingredients and 114 drugs. 114 common targets are considered as potential targets for the treatment of CRC by *huajiao diyu* decoction (Fig.3). degree represents the total number of routes that other nodes in the network connect to that node. The higher the value, The more important the corresponding component or target is. After analysis using the circuit network analyzer plug-in in the Cytoscape software, Tables 1 and 2 list 10 major active components and 10 major targets, respectively. Table 1 shows, The highest degree component is PTGS2(degree 48).

3.4 Building protein interaction networks

Use the string database (<https://string-db.org/>) to obtain target interaction relationships, *Homo sapiens*", by selecting species Check "Hidden Network Interrupt Node hide disconnected nodes in the network", And set the "minimum required interaction score minimum required interaction score" >0.9 to get the protein interaction network diagram and the core genes of the network (Fig.4), According to the number of

connections with adjacent genes, the top 30 core genes were obtained by R software (Fig.5). Import data cytoscape database to draw protein interaction network. Network Analysis plug-in analysis of the network diagram found, sorted by Degree values from high to low, AKT1, is the top 10 MAPK1, MAPK3, HSP90AA1, JUN, MAPK14, ESR1, CCND1, RB1, IL6(Fig.6).

3.5 Analysis of biological functions and pathways

GO function analysis shows, 114 intersection targets predicted 145 enrichment results (pvalueCutoff <0.05, qvalueCutoff <0.05). DNA-binding transcription factor binding, included RNA polymerase II-specific DNA-binding transcription factor binding, ubiquitin-like protein ligase binding and transcription coactivator binding, Based on the results of screening the top 20 of the P values, See Fig.7, Fig.8.

KEGG enrichment analysis of *Sophora flavescens* decoction (p <0.05), The results show that 164 pathways were obtained, PI3K-Akt signaling pathway, MAPK signaling pathway and TNF signaling pathway were the representatives.. The top 20 pathways in KEGG enrichment analysis was demonstrated according to P value (Fig.9, Fig.10) Furthermore, they were mainly involved in antiviral, immunomodulatory and anti-inflammatory effects according to the function of these top 20 pathways (Table 3). Hepatitis B ID hsa05161) is the clearest pathway, see Fig.11.

3.6 Molecular docking

Molecular docking results show, There were 12 pairs of quercetin, luteolin, kaempferol, baicalin and other four core components < the Vina score of 5.0, indicating that the binding ability of PTGS1-PTGS2-AR and core components is strong, The Vina value (binding energy) of the interaction between PTGS1 protein and core components is the best, kcal/mol,-7.9 The specific docking results are shown in Table 4. Combined with Fig.12, quercetin form hydrogen bonds ARG376-GLN374 the amino acid residues of the PTGS2 protein, luteolin small molecules form hydrogen bonds THR206 the amino acid residues of the PTGS1 protein, wogonin small molecules form hydrogen bonds LYS93-TYR92 the amino acid residues of the AR protein.

4. Discussion

CRC is a common malignant tumor of digestive system, which can seriously affect the quality of life of patients. Huaijiao Diyu decoction has the function of reducing poison and increasing efficiency in the treatment of CRC, but its specific molecular mechanism and pharmacodynamic substance have not been fully elucidated. At present, network pharmacology and molecular docking technology have been applied to the screening of active compounds of traditional Chinese medicine and the docking of key targets on a large scale, which provides a possibility for the preliminary exploration of the action law of Huaijiao Diyu decoction in the treatment of CRC.

Analysis of active ingredients active ingredient analysis

Among the active ingredients, key ingredients with higher degree includes quercetin, luteolin, kaempferol and wogonin. these four components belong to flavonoids, flavonoid phytoestrogens have been suggested to be associated with reduced risk of colorectal cancer [21]. A recent research showed that the combination of quercetin and exercise training exerts potent anti-tumour and anti-depressive effects through suppression of inflammation and upregulation of the BDNF/Trk β / β -catenin axis in the prefrontal cortex in CRC treatment [22]. The combination of quercetin (Q) and alantolactone (A) is capable of reactive activity of immunity by education ICD, causing cell toxicity and modulating the immune-suppressive tumor microenvironment [23]. Jiamei Qi [24] found that alternating the consumption of β -glucan and quercetin alleviated colon damage and reduced the mortality rate in CRC mice. During colon cancer genesis, luteolin known to reduce oxidative stress thereby protects the cell to undergo damage in vivo. Wnt/ β -catenin signaling, deregulated during neoplastic development, is modified by luteolin [25]. Thermosensitive in situ gel containing luteolin micelles has shown strong ability to promote tumor apoptosis, suppress tumor proliferation and block tumor angiogenesis [26]. luteolin uppresses colorectal cancer cell metastasis via regulation of the miR 384/pleiotrophin axis [27]. Kaempferol has a synergistic effect with 5FU by inhibiting cell proliferation and inducing apoptosis in colorectal cancer cells via suppression of TS or attenuation of p Akt activation. The combination of kaempferol and 5FU may be used as an effective therapeutic strategy for colorectal cancer [28]. Reactive Oxygen Species and p53 Mediated Activation of p38 and Caspases is Critically Involved in Kaempferol Induced Apoptosis in Colorectal Cancer Cells [29]. Wogonin could inhibit the proliferation of SW480 cells through Wnt/ β -catenin pathway [30]. Wogonin exerts growth inhibitory effects on the SW48 colorectal cancer cells by autophagic and apoptotic cell death, wogonin could also inhibit the PI3K/AKT and STAT3 signal transduction pathways [31]. Wogonin not only reduced tumor multiplicity, preserved colon length to normal but also didn't induce side effects on various organs [32].

Analysis of potential targets Potential Target Analysis

The target with the highest degree was PTGS2. The following targets were PTGS1, HSP90AA1, AR in the network of ingredient-target-disease. prostaglandin endoperoxide synthase (PTGS), also known as cyclooxygenase (COX), which include inducible PTGS2 (cox-2) and constitutive PTGS1 (cox-1). HCT116 and HT29 could induce depolarization of granulocyte membrane potential after silencing by COX-1 gene, inhibiting adenosine triphosphate (ATP) production, increasing the content of reactive oxygen species, leading to apoptotic [33] in cancer cells. The association of tumour PTGS2 (COX-2) expression with colour cancer quality is strong in BRAF-mutated tumours than in BRAF-wild-type tumours [34]. Liu J and other [35] found Hsp90aa1 and dab2ip involved in the occurrence and metastasis mechanism of colorectal cancer. Heat shock protein 90 (HSP90) AA1 functions as an onco-protein to regulate the assembly, manipulation, folding and degradation of its client proteins, including c-MYC and Weidong Shi's data revealed an unknown FBXL6-HSP90AA1-c-MYC axis which might contribute to the oncogenesis of HCC, and we propose that inhibition of FBXL6 might represent an effective therapeutic strategy for HCC treatment [36]. Repression of β 2-AR but not β 1-AR signaling selectively suppressed cell viability, induced G1-phase cell cycle arrest, caused both intrinsic and extrinsic pathways-mediated apoptosis of specific CRC cells and inhibited CRC-xenograft growth in vivo [37].

Analysis of PPI network

PPI network analysis

The target protein levels of AKT1,MAPK1,MAPK3,HSP90AA1 and JUN were excessively expressed in PPI network. This result indicated the series of targets of Huaijiao Diyu decoction treating CRC. AKT, also known as protein kinase B (PKB), is a downstream effector of the PI3K and is directly activated by it (PI3K Sator). Copanlisib promotes growth inhibition and apoptosis by modulating the AKT/FoxO3a/PUMA axis in colorectal cancer [38]. A novel coordination complex of platinum (PT) leads cell death in colony cancer by after reducing balance and modelling MAPK pathway [39]. ROS/JNK/c-Jun axis is involved in oridonin-induced caspase-dependent apoptosis in human colorectal cancer cells [40].

Analysis of signaling pathways Signal Pathway Analysis

The results of GO molecular functional clustering of 114 potential target genes in clusterProfiler package showed that the top 30 enrichment results involve many molecular functions, such as cell signal transduction, cell metabolism, transcription and redox, as well as antiviral, immunomodulatory and anti-inflammatory effects. KEGG pathway enrichment suggests, Possible CRC and PI3K-Akt signaling pathway, of Tongxie prescription MAPK signaling pathway and TNF signaling pathway closely related. Among them, Most CRC patients with abnormal PI3K/AKT signaling pathway and PI3K/Akt signal inhibitors are considered to be effective CRC therapeutic agents. PI3K/AKT signaling pathways can regulate the transcriptional expression of key miRNA, To enhance the [42] of resistance to proliferation and migration of CRC cells. Huijie Zhang and other [43] studies have found that regulating the activity of mapk signaling pathway can improve the depression behavior and survival state of the rat depression model. The RAS/MAPK axis regulates cancer cell proliferation, apoptosis, inflammation, migration, and metastasis [44]. Jobin and colleagues [45] found that targeting inflammation with TNF therapy has a preventative effect on carcinogenic activity of the microbiota in mouse models of colitis-associated colorectal cancer. molecular docking indicates that the core components of Huaijiao Diyu decoction interact strongly with PTGS1-PTGS2 A R, suggesting that PTGS1-PTGS2 A R is probably an important target for the treatment of Huaijiao Diyu decoction.

Conclusion

This study used network pharmacology, molecular docking and other means to obtain 58 active compounds and 114 targets of CRC treated by Huaijiao Diyu decoction, and demonstrated the molecular relationship and the signal pathway covered by the GO function enrichment analysis, the KEGG pathway enrichment analysis, the construction of the CRC- target network and the key target verification. The results showed that there was a synergistic effect between traditional Chinese medicine, and showed that Huaijiao Diyu decoction could play a pharmacological role at the molecular level through multi-component, multi-target and multi-way, mainly involving many molecular functions such as cell signal transduction, cell metabolism, transcription and redox, as well as antiviral, immunomodulatory and anti-inflammatory effects. The overall understanding of the treatment CRC of Huaijiao Diyu decoction from an

intuitive point of view provides an important reference for the further study of the intervention CRC of Huaijiao Diyu decoction, which presents some advantages compared with the traditional pharmacological research. Limited by this study, only considering the quantitative relationship between traditional Chinese medicine, active compounds and targets, but not taking into account the effective intensity factors of active compounds and targets, the mechanism of action of drugs in vivo needs to be verified by further experiments. Therefore, more research methods should be carried out in the future to further reveal the mechanism of common prescriptions and their derived compounds in the treatment of colorectal cancer.

Abbreviation

CRC: Colorectal Cancer; TCM: Traditional Chinese medicine; TCMSP: Traditional Chinese Medicine Systems Pharmacology; OB: Oral bioavailability; DL: Drug likeness; PPI: Protein–protein interaction; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins database; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PDB: Protein Data Bank.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

Funding

Not applicable

Authors' contributions

Xinyao Shu- SXY

Yunwu Zhang- ZYW

Yujia Wang- WYJ

Jing Wu- WJ

Xingyu Chen- CXY

Ruitong Xu- XRT

Xingting Liu- LXT

Jingsong Luo- LJS

Tingting Deng- DTT

ZYW, DTT contributed to the conception of the study;

SXY performed the experiment;

SXY, WYJ contributed significantly to analysis and manuscript preparation;

ZYW, SXY, WJ performed the data analyses and wrote the manuscript;

CXY, XRT, LXT, LJS helped perform the analysis with constructive discussions.

Acknowledgements

Not applicable

References

1. Yao, Y., et al., *Luteolin suppresses colorectal cancer cell metastasis via regulation of the miR-384/pleiotrophin axis*. *Oncology Reports*, 2019. 42(1).
2. Di Leo M, Z.R., Puzzono M, Ditunno I, Mannucci A, Antoci G, Russo Raucci A, Patricelli MG, Elmore U, Tamburini AM, Albarello L, Azzolini F, Bonura GF, Esposito D, Fanti L, Notaristefano C, Viale E, Perea J, Testoni PA, Rosati R, Cavestro GM, Risk factors and clinical characteristics of early-onset colorectal cancer vs. late-onset colorectal cancer—a case-case study. *European journal of gastroenterology & hepatology*, 2020.
3. Bray, F., et al., *Global cancer statistics 2018—GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*. *CA: A Cancer Journal for Clinicians*, 2018. 68(6) p. 394-424.
4. Yuji, M., et al., *Precision medicine for adjuvant chemotherapy of resected colorectal cancer*. *Annals of Gastroenterological Surgery*, 2020. 4(6).
5. Shi-Yun, S. and L. Qi, *Research progress in the treatment of colorectal cancer in classical prescriptions*. *TMR Cancer*, 2019. 2(3).
6. Yuli, W., et al., *The Effect of Long-Term Traditional Chinese Medicine Treatment on Survival Time of Colorectal Cancer Based on propensity Score Matching—A Retrospective Cohort Study*. *Evidence-based complementary and alternative medicine —eCAM*, 2020. 2020.

7. Shao,C.,et al.,*Effect of Chinese Herbal Medicine on the Survival of Colorectal Cancer Patients With Liver-Limited Metastases—A Retrospective Cohort Study,2008to 2017.Integrative Cancer Therapies,2019.18.*
8. Hyun,C.Y.and K.Hye-Joo,*Fructus sophorae attenuates secretion of proinflammatory mediators and cytokines through the modulation of NF- κ B and MAPK signaling pathways in LPS-stimulated RAW 264.7macrophages.General physiology and biophysics,2016.35(3).*
9. Han,H.M.,et al.,*Protective effects of Fructus sophorae extract on collagen-induced arthritis in BALB/c mice.Experimental and Therapeutic Medicine,2017.13(1).*
10. Hong,Z.,et al.,*Positive effects of platelet-rich plasma (PRP) and a Sanguisorba officinalis polysaccharide on the protection and differentiation of anteriorcruciate ligament (ACL) fibroblasts in vitro.Pharmacological biology ,2020.58(1).*
11. Dong,S.X.,et al.,*Anti-bacterial effects of components from Sanguisorba officinalis L.on Vibrio vulnificus and their soluble epoxide hydrolase inhibitory activity.Natural product research,2019.33(23).*
12. Bo,Z.,et al.,*Matrine Is Identified as a Novel Macropinocytosis Inducer by a Network Target Approach.Frontiers in pharmacology,2018.9.*
13. Runzhi,Z.,et al.,*Network Pharmacology Databases for Traditional Chinese Medicine—Review and Assessment.Frontiers in pharmacology,2019.10.*
14. Ru,J.,et al.,*TCMSP—a database of systems pharmacology for drug discovery from herbal medicines.Journal of Cheminformatics,2014.6(1).*
15. Wenjuan,Z.,et al.,*Systems Pharmacology for Investigation of the Mechanisms of Action of Traditional Chinese Medicine in Drug Discovery.Frontiers in pharmacology,2019.10.*
16. Yunxia,W.,et al.,*Therapeutic target database 2020—enriched resource for facilitating research and early development of targeted therapeutics.Nucleic acids research,2020.48(D1).*
17. Chen,H.and P.C.Boutros, *VennDiagram—a package for the generation of highly-customizable Venn and Euler diagrams in R.BMC Bioinformatics,2011.12(1).*
18. T,D.N.,et al.,*Cytoscape StringApp—Network Analysis and Visualization of Proteomics Data.Journal of proteome research,2019.18(2).*
19. Szklarczyk,D.,et al.,*The STRING database in 2017—quality-controlled protein–protein association networks,made broadly accessible.Nucleic Acids Research,2017.45(D1).*
20. Walter,W.,F.t.Saanchez - Cabo,and M.Ricote,*GOplot—an R package for visually combining expression data with functional analysis.Bioinformatics,2015.31(17).*
21. Ruijingfang,J.,et al.,*Serum Concentration of Genistein,Luteolin and Colorectal Cancer Prognosis.Nutrients,2019.11(3).*
22. Sadighparvar,S.,et al.,*Combination of quercetin and exercise training attenuates depression in rats with 1,2-dimethylhydrazine-induced colorectal cancer—Possible involvement of inflammation and BDNF signalling.Experimental Physiology,2020.105(9).*

23. Jing,Z.,et al.,*Nanoformulated Codelivery of Quercetin and Alantolactone Promotes an Antitumor Response through Synergistic Immunogenic Cell Death for Microsatellite-Stable Colorectal Cancer*.*ACS nano*,2019.13(11).
24. Qi,J.,et al.,*Alternating consumption of β -glucan and quercetin reduces mortality in mice with colorectal cancer*.*Food Science & Nutrition*,2019.7(10).
25. Kumar,P.A.and E.N.Mohd, *Luteolin, a bioflavonoid inhibits colorectal cancer through modulation of multiple signaling pathways—a review*.*Asian Pacific journal of cancer prevention —APJCP*,2014.15(14).
26. Yuzhu,H.,et al.,*Thermosensitive In Situ Gel Containing Luteolin Micelles is a Promising Efficient Agent for Colorectal Cancer Peritoneal Metastasis Treatment*.*Journal of biomedical nanotechnology*,2020.16(1).
27. Yuanyuan,Y.,et al.,*Luteolin suppresses colorectal cancer cell metastasis via regulation of the miR-384/pleiotrophin axis*.*Oncology reports*,2019.42(1).
28. Qiongyu,L.,et al.,*Synergistic effect of kaempferol and 5-fluorouracil on the growth of colorectal cancer cells by regulating the PI3K/Akt signaling pathway*.*Molecular medicine reports*,2019.20(1).
29. Jhin-Baek,C.,et al.,*Reactive Oxygen Species and p53 Mediated Activation of p38 and Caspases is Critically Involved in Kaempferol Induced Apoptosis in Colorectal Cancer Cells*.*Journal of agricultural and food chemistry*,2018.
30. Yan-Ping,L.,et al.,*[Inhibitory effect of wogonin on human colorectal cancer cell SW480 based on network pharmacology]*. *Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica*,2020.45(8).
31. Hao,T.,et al.,*A flavone, Wogonin from Scutellaria baicalensis inhibits the proliferation of human colorectal cancer cells by inducing of autophagy, apoptosis and G2/M cell cycle arrest via modulating the PI3K/AKT and STAT3 signalling pathways*.*Journal of B.U.ON: official journal of the Balkan Union of Oncology*,2019.24(3).
32. Qian,F.,et al.,*Prevention of Wogonin on Colorectal Cancer Tumorigenesis by Regulating p53 Nuclear Translocation*.*Frontiers in pharmacology*,2018.9.
33. Lei,D.,et al.,*Downregulation of cyclooxygenase-1 stimulates mitochondrial apoptosis through the NF- κ B signaling pathway in colorectal cancer cells*.*Oncology reports*,2019.41(1).
34. Kosumi,K.,et al.,*Prognostic association of PTGS2(COX-2) over-expression ordering to BRAF mutation status in color cancer: Results from two constructive cases and CALGB 89803(Alliance) priority*.*European Journal of Cancer*,2019.111.
35. J., L.*Bioinformatic Analysis and Mechanism Study of Expression Relationship between HSP90AA1 and DAB2IP in Colorectal Cancer*.in *17th International Immunology Conference*.2019. Beijing, China.
36. Shi,W.,et al.,*FBXL6 governs c-MYC to promote hepatocellular carcinoma through ubiquitination and stabilization of HSP90AA1*.*Cell Communication and Signaling*,2020.18(1).
37. Chin,C.C.,et al.,*Selective β 2-AR Blockage Suppresses Colorectal Cancer Growth Through Regulation of EGFR-Akt/ERK1/2 Signaling, G1-Phase Arrest, and Apoptosis*.*Journal of Cellular*

Physiology,2016.231(2).

38. Ji, Y., et al., *Copanlisib promotes growth inhibition and apoptosis by modulating the AKT/FoxO3a/PUMA axis in colorectal cancer. Cell Death & Disease, 2020. 11(11).*
39. Al-Khayal, K., et al., *A novel coordination complex of platinum (PT) leads cell death in coloral cancer by after reducing balance and modelling MAPK pathway. BMC Cancer, 2020. 20(1).*
40. Di, Z., et al., *ROS/JNK/c-Jun axis is involved in oridonin-induced caspase-dependent apoptosis in human colorectal cancer cells. Biochemical and Biophysical Research Communications, 2019. 513(3).*
41. Kumar, P.A., *Potential targets for prevention of colorectal cancer—a focus on PI3K/Akt/mTOR and Wnt pathways. Asian Pacific journal of cancer prevention —APJCP, 2013. 14(4).*
42. Soleimani, A., et al., *Role of Regulatory Oncogenic or Tumor Suppressor miRNAs of PI3K/AKT Signaling Axis in the Pathogenesis of Colorectal Cancer. Current Pharmaceutical Design, 2018. 24(39).*
43. Zhang H, C.M., Liu Y, Dong X, Zhang C, Jiang H, Chen X, *Paroxetine combined with fluorouracil plays a therapeutic role in mouse models of colorectal cancer with depression through inhibiting IL-22 expression to regulate the MAPK signaling pathway. Experimental and therapeutic medicine, 2020. 20(6).*
44. Soleimani, A., et al., *The potential role of regulatory microRNAs of RAS/MAPK signaling pathway in the pathogenesis of colorectal cancer. Journal of Cellular Biochemistry, 2019. 120(12).*
45. Yang, Y., et al., *Amending microbiota by targeting intestinal inflammation with TNF blockade attenuates development of colorectal cancer. Nature Cancer, 2020. 1(7).*

Tables

Table 1 Core targets of the top 10 in the network of Degree CRC- targets

Gene	Degree	TopologicalCoefficient	NeighborhoodConnectivity	Radiality
PTGS2	48	0.078683036	9.8125	0.989747095
PTGS1	38	0.094976077	11.44736842	0.987924356
HSP90AA1	38	0.097054563	11.57894737	0.987772461
AR	24	0.139823718	15.54166667	0.984886459
PRSS1	23	0.142140468	15.7826087	0.984734564
NOS2	21	0.200746965	11.23809524	0.976380345
ESR1	13	0.211538462	10.30769231	0.974101921
PPARG	11	0.21991342	24.09090909	0.98306372
PGR	10	0.136363636	5.5	0.971671603
CASP3	10	0.236792453	26.1	0.98306372

(Notes: PTGS2 prostaglandin-endoperoxide synthase 2, Annex PTGS1 prostaglandin-endoperoxide synthase 1, HSP90AA1 heat shock protein 90alpha family class A member 1, AR androgen receptor, PRSS1 serine protease 1, NOS2 nitric oxide synthase 2, ESR1 Estrogen Receptor 1, PPARG peroxisome proliferator activated receptor gamma, PGR progesterone receptor, CASP3 caspase 3)

Table 2 Core Compounds with Top 10 Values in the Network of Degree CRC- Targets

MOL ID	Molecule Name	Degree	Source
MOL000098	quercetin	77	diyu,huaijiao,jingjie,zhizi
MOL000006	luteolin	40	jingjie
MOL000422	kaempferol	28	baishao,diyu,huaijiao,zhizi
MOL000173	wogonin	27	huangqin
MOL002714	baicalein	23	huaijiao,huangqin
MOL005828	nobiletin	21	zhike
MOL001689	acacetin	18	huangqin
MOL004328	naringenin	18	zhike
MOL002928	oroxilin	12	huangqin
MOL002933	5,7,4'- Trihydroxy-8-methoxyflavone	12	huangqin

Table 3 Information of potential targets and signaling pathways

ID	Description	pvalue	Count
hsa04151	PI3K-Akt signaling pathway	3.73E-20	34
hsa05161	Hepatitis B	2.23E-30	33
hsa05167	Kaposi sarcoma-associated herpesvirus infection	3.71E-25	31
hsa05163	Human cytomegalovirus infection	9.96E-21	29
hsa05166	Human T-cell leukemia virus 1infection	6.46E-20	28
hsa05215	Prostate cancer	8.43E-29	27
hsa05169	Epstein-Barr virus infection	1.01E-19	27
hsa04210	Apoptosis	3.99E-23	26
hsa05205	Proteoglycans in cancer	2.05E-18	26
hsa05206	MicroRNAs in cancer	5.60E-14	26
hsa05165	Human papillomavirus infection	2.64E-13	26
hsa01522	Endocrine resistance	1.28E-25	25
hsa05162	Measles	1.49E-21	25
hsa05160	Hepatitis C	3.43E-20	25
hsa05170	Human immunodeficiency virus 1infection	7.11E-16	24
hsa04010	MAPK signaling pathway	1.11E-12	24
hsa05022	Pathways of neurodegeneration -multiple diseases	2.22E-08	24
hsa04933	AGE-RAGE signaling pathway in diabetic complications	1.84E-22	23
hsa04218	Cellular senescence	7.86E-18	23
hsa05225	Hepatocellular carcinoma	4.31E-17	23

Table 4 Binding energies of key active compounds with core targets

Mol ID	Molecule Name	PTGS1	PTGS2	HSP90AA1	AR
MOL000098	quercetin	-5.4	-7.9	-4.2	-7.5
MOL000006	luteolin	-6.3	-7.9	-4.2	-7.3
MOL000422	kaempfero	-5.4	-7.9	-4.2	-7.1
MOL000173	wogonin	-6.2	-7.9	-4.2	-7.5

Figures

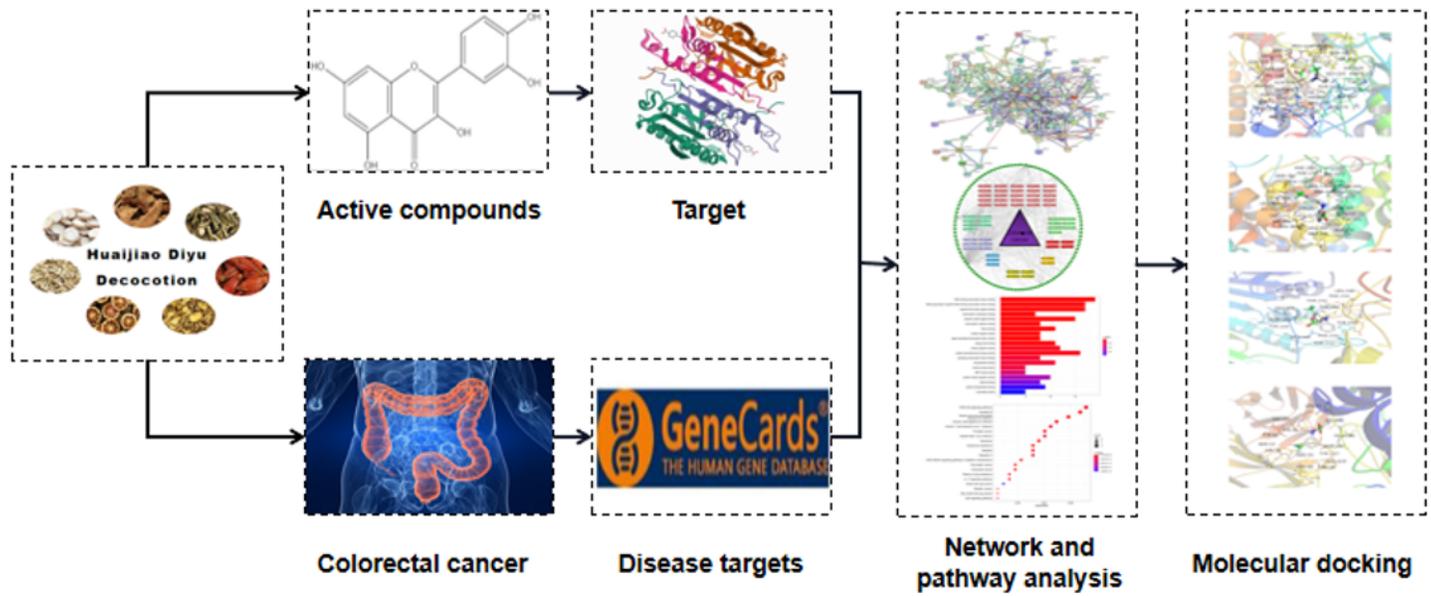


Figure 1

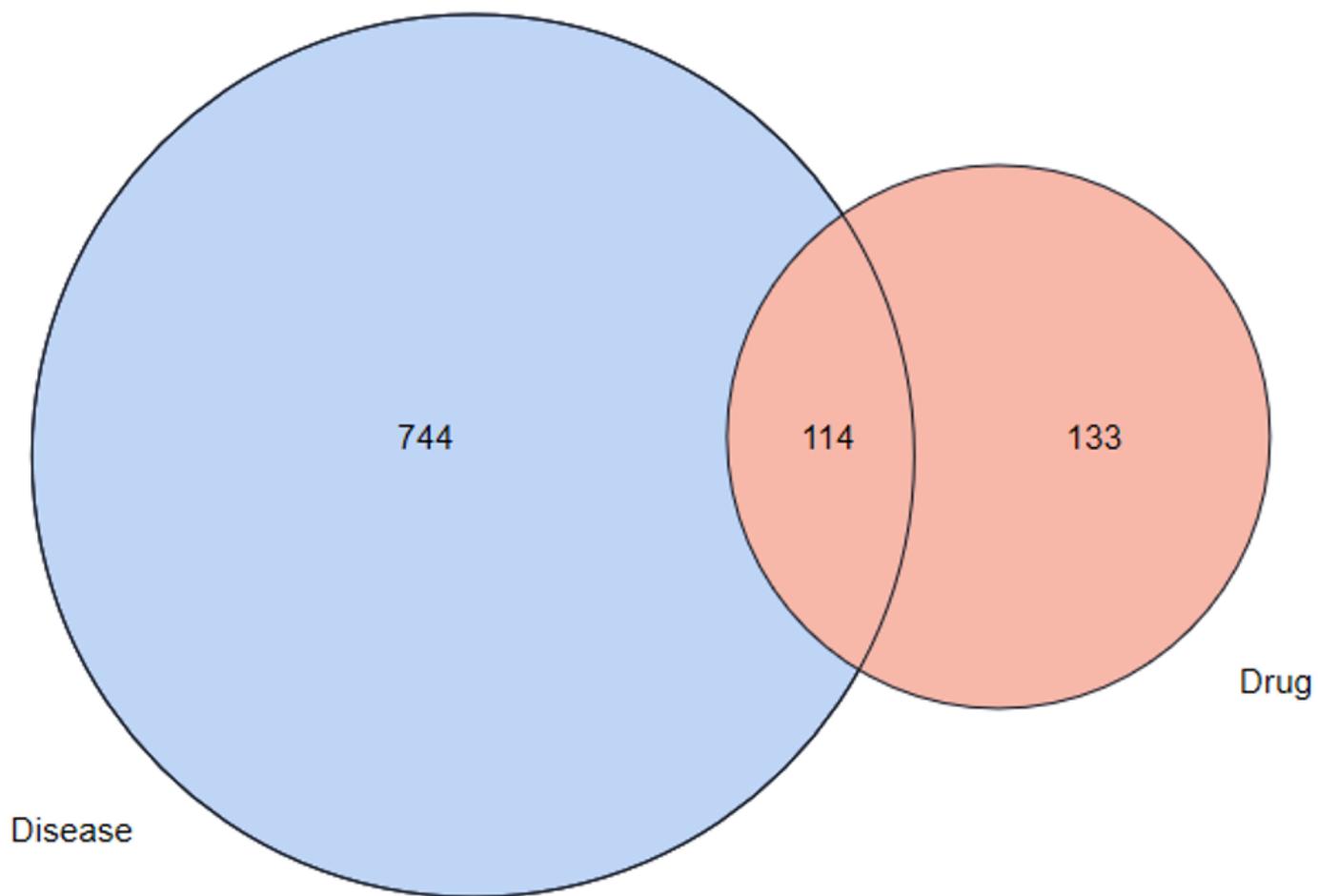


Figure 2

Venn diagram of Huaijiao Diyu decoction-CRC related targets (the large circle on the left represents the CRC related potential target, the small circle on the right represents the related potentiVenn diagram of Huaijiao Diyu decoction-CRC related targets (the large circle on the left represents the CRC related potential target, the small circle on the right represents the related potential target of Huaijiao Diyu decoction, and the middle represents the intersection target.)al target of Huaijiao Diyu decoction, and the middle represents the intersection target.)

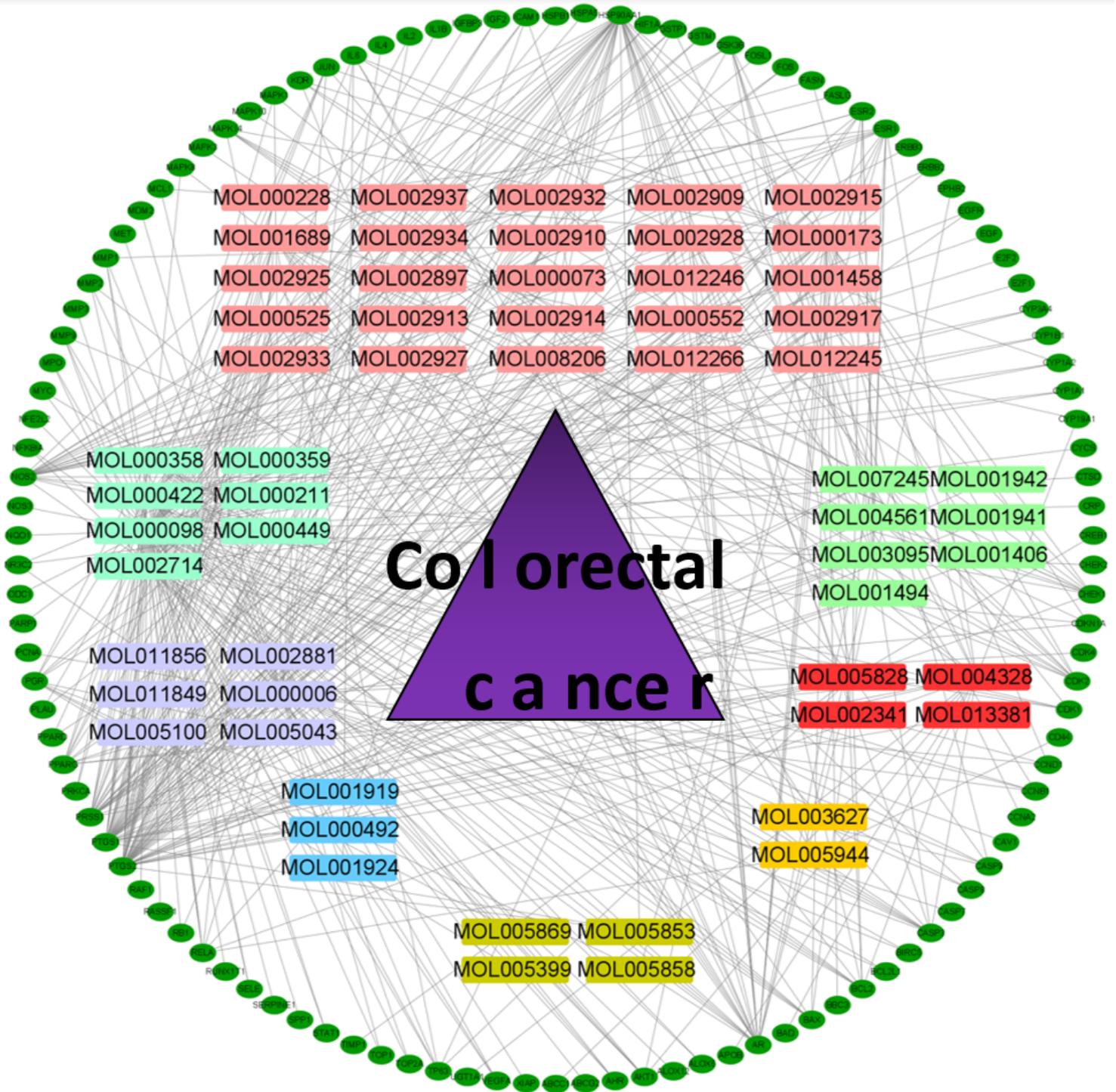


Figure 3

The network of diagram of herbs-active compounds-targets (triangle represents CRC, rectangle represents the corresponding active compounds of traditional Chinese medicine, oval represents the target of active compounds, light pink represents *Scutellaria baicalensis* and its corresponding active compounds, light blue indicates *Schizonepeta mutiDrug*, gray indicates *Schizonepeta* and its corresponding active compounds, dark yellow indicates *Ulmus pumila* and its corresponding active compounds, orange indicates *Sophora japonica* and its corresponding active compounds, red indicates *Fructus Aurantii* and its corresponding active compounds, light green indicates *gardenia* and its corresponding active compounds.)

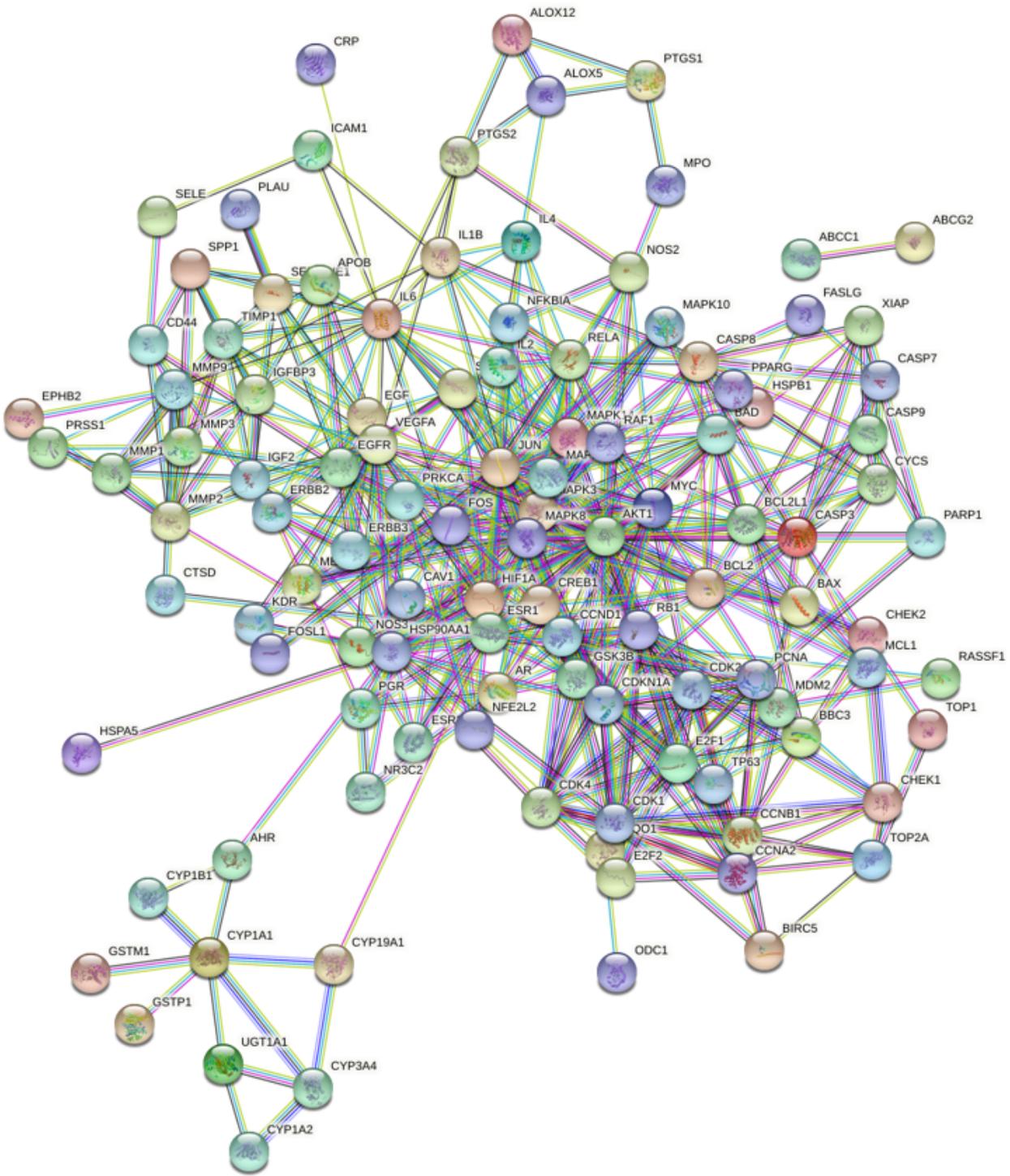


Figure 4

The network of protein to protein interaction (PPI)

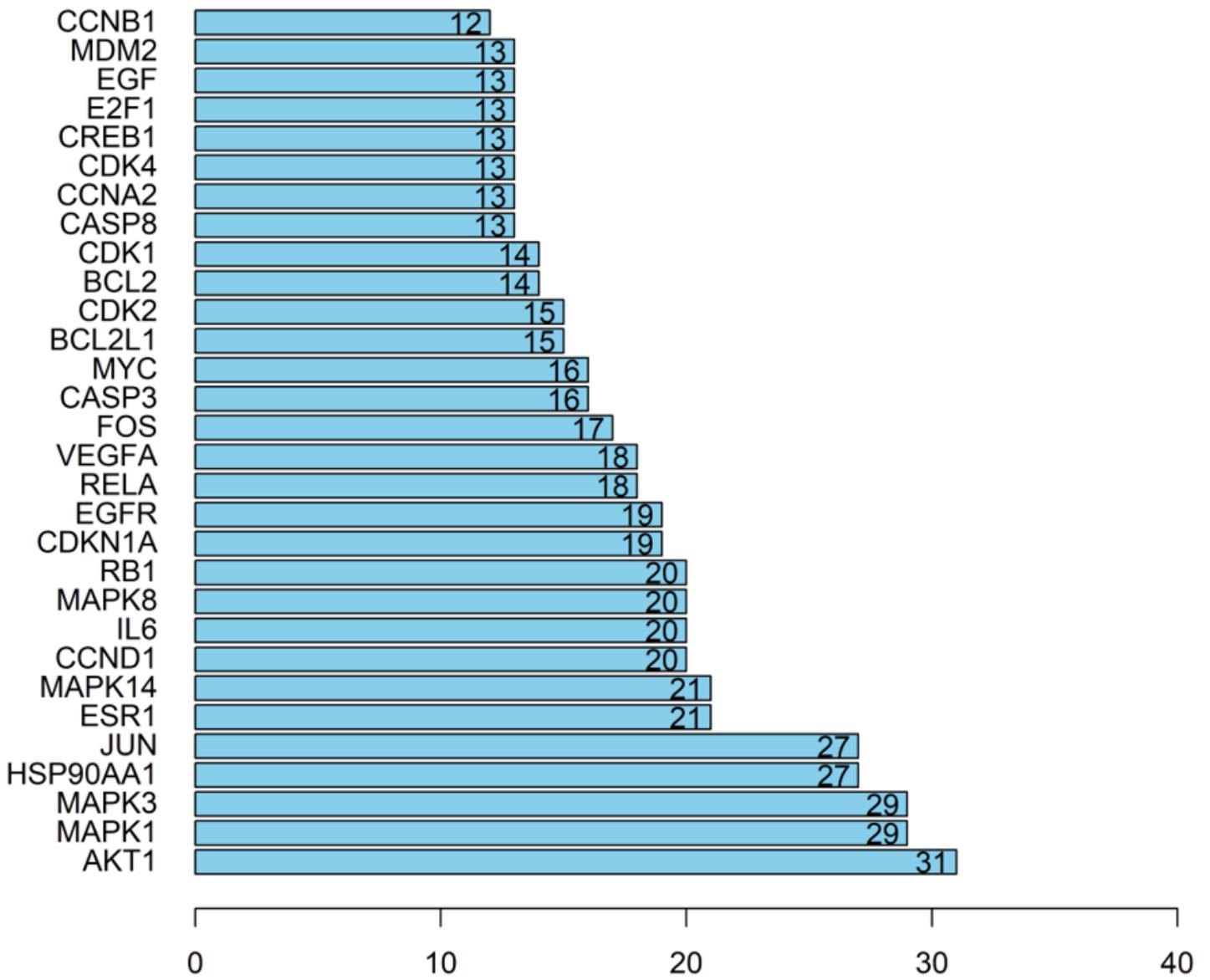
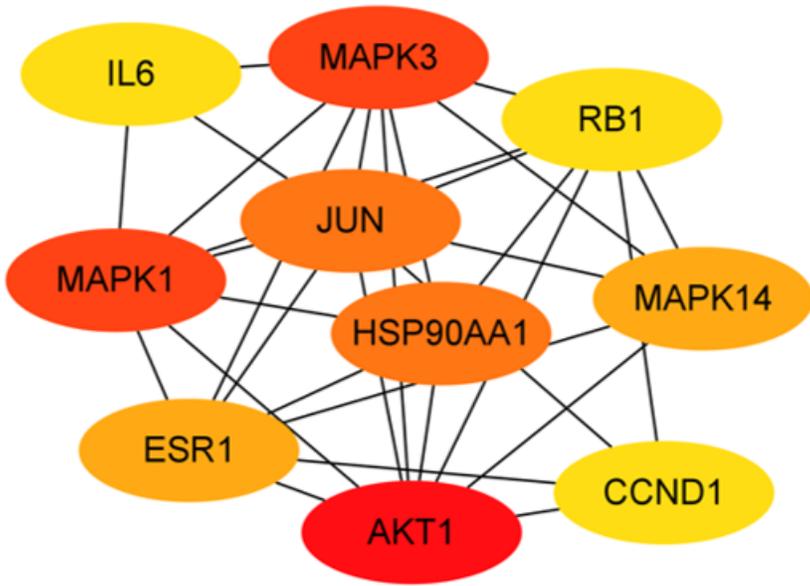


Figure 5

The top 30 core genes



Rank	Node
1	AKT1
2	MAPK1
2	MAPK3
4	HSP90AA1
4	JUN
6	MAPK14
6	ESR1
8	CCND1
8	RB1
8	IL6

Figure 6

The core target of Degree top 10 in the network of protein interactions (AKT1,MAPK1, MAPK3, HSP90AA1, JUN, MAPK14, ESR1, CCND1, RB1, IL6)

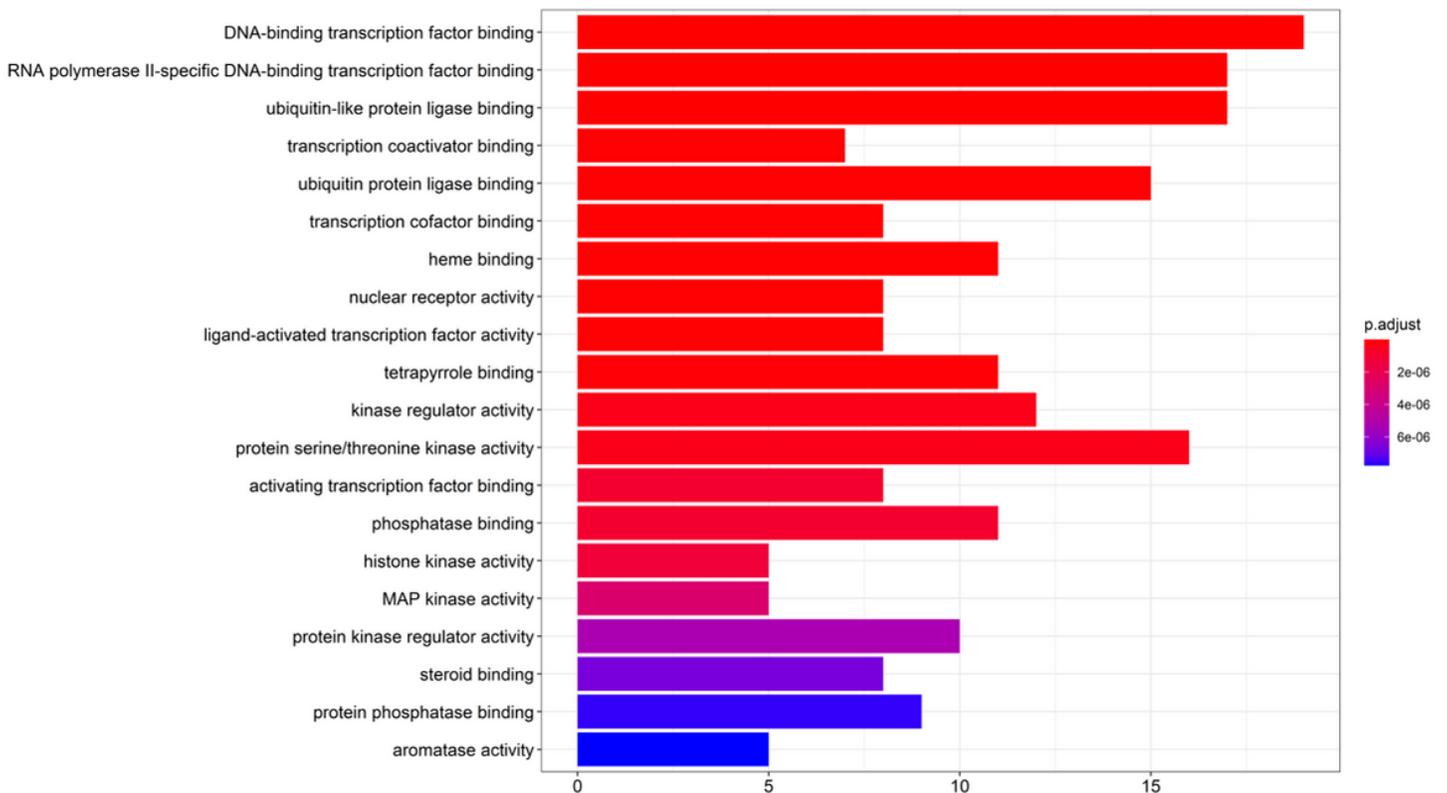


Figure 7

GO function histogram of the key targets of Huaijiao Diyu decoction in treatment of CRC

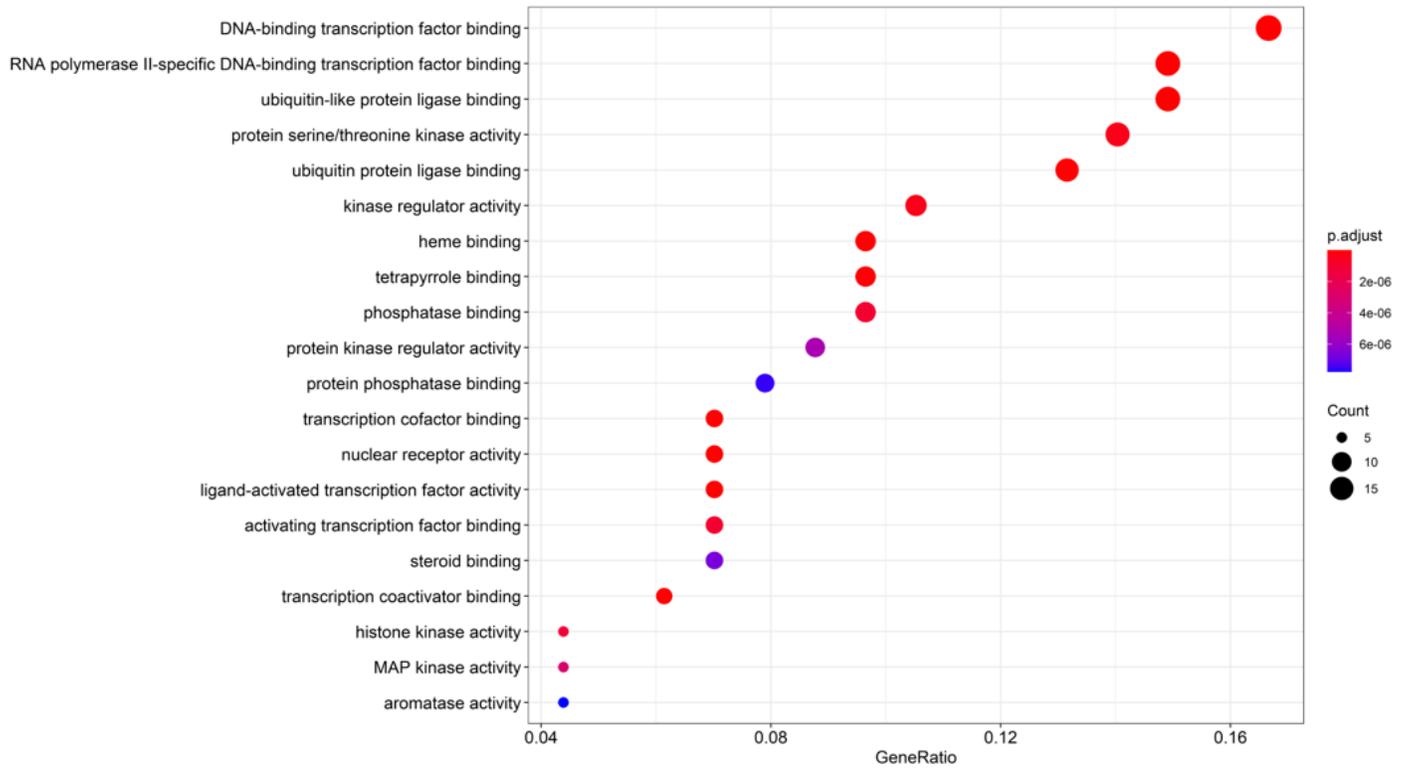


Figure 8

GO pathway enrichment bubble chart of the key targets of Huaijiao Diyu decoction in treatment of CRC

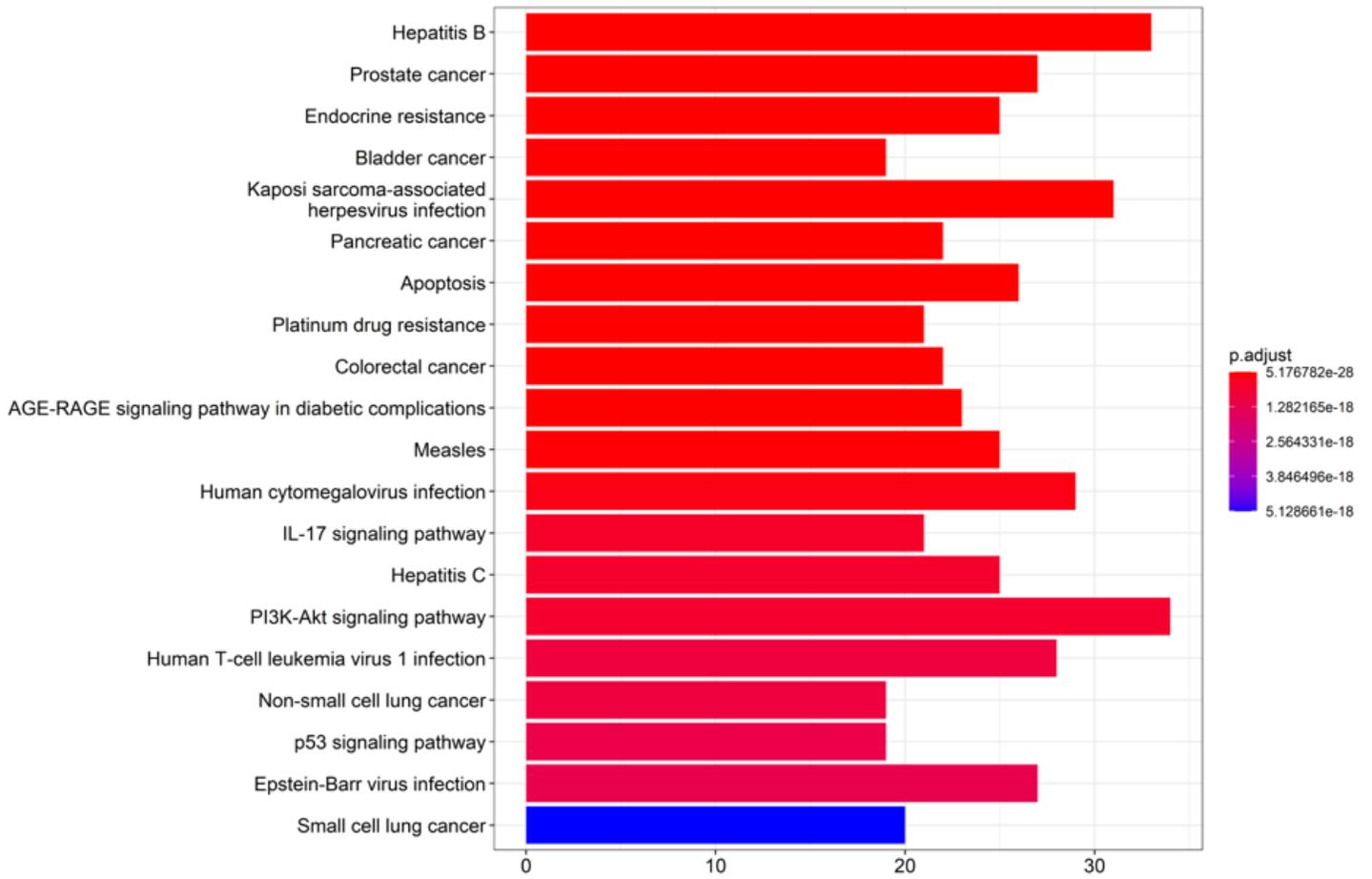


Figure 9

KEGG function histogram of the key targets of Huaijiao Diyu decoction in treatment of CRC

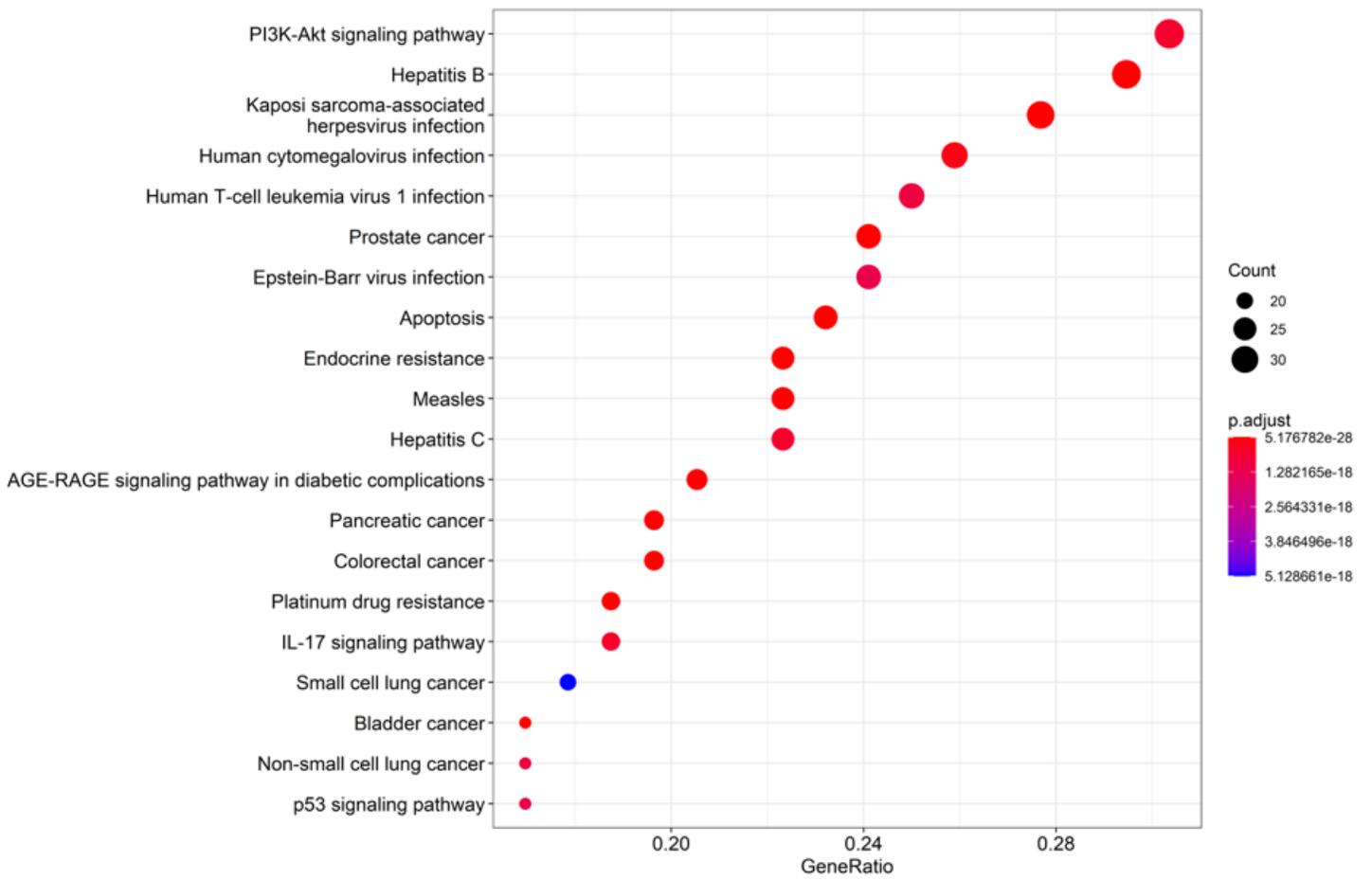


Figure 10

KEGG pathway enrichment bubble chart of the key targets of Huaijiao Diyu decoction in treatment of CRC

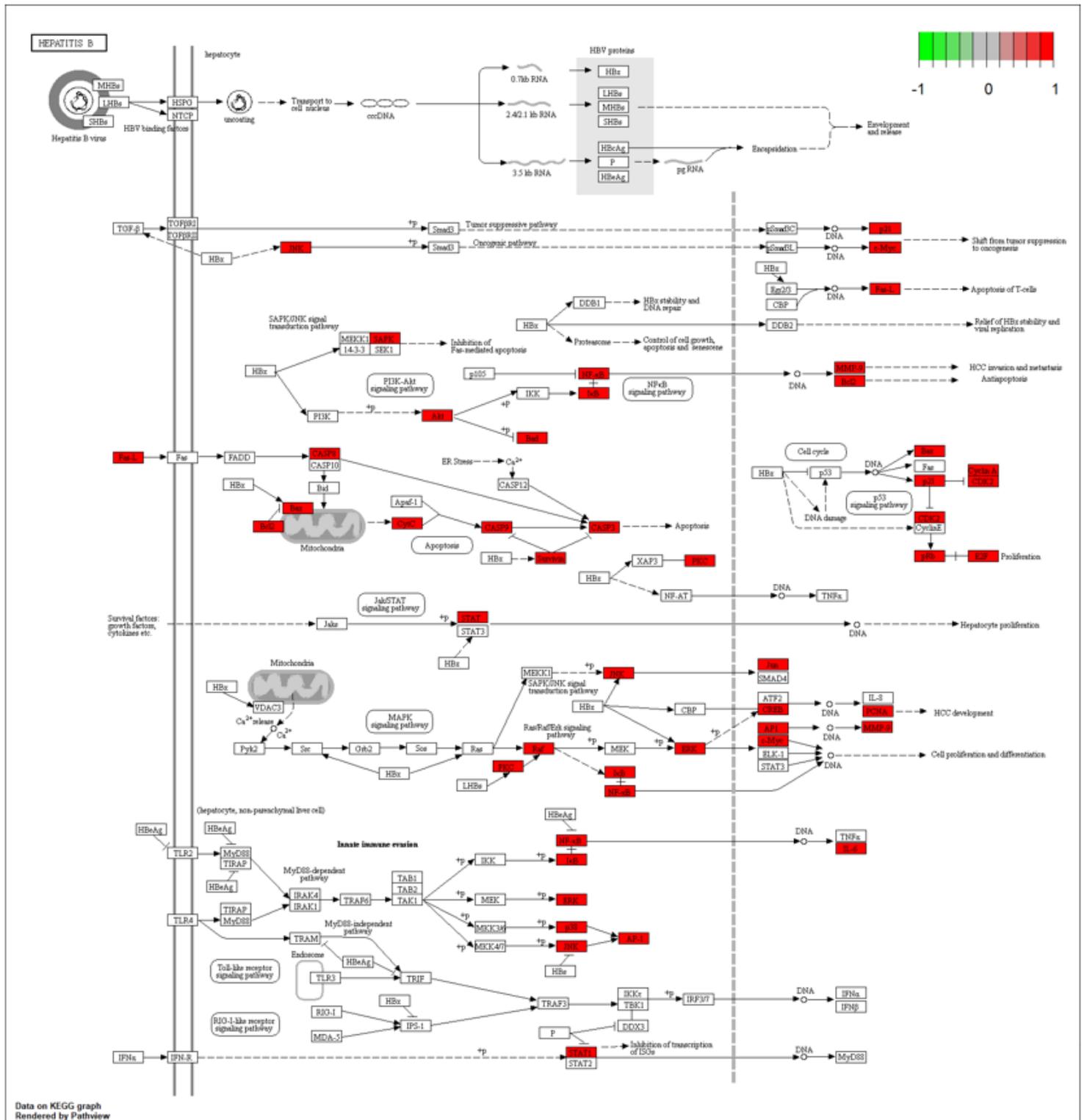
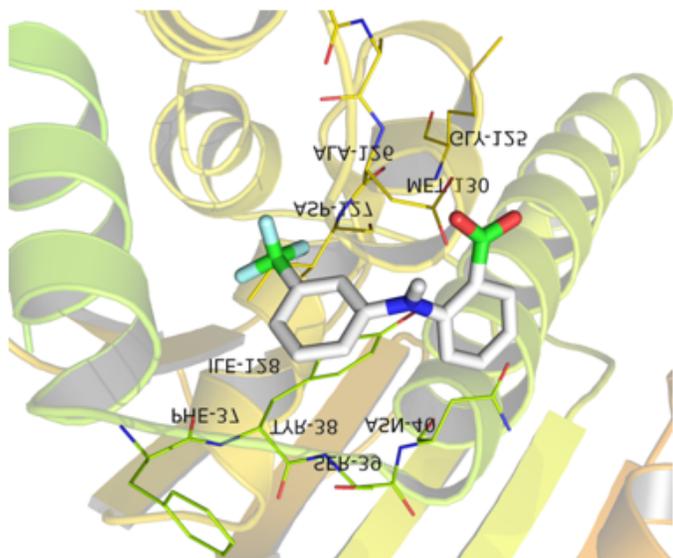
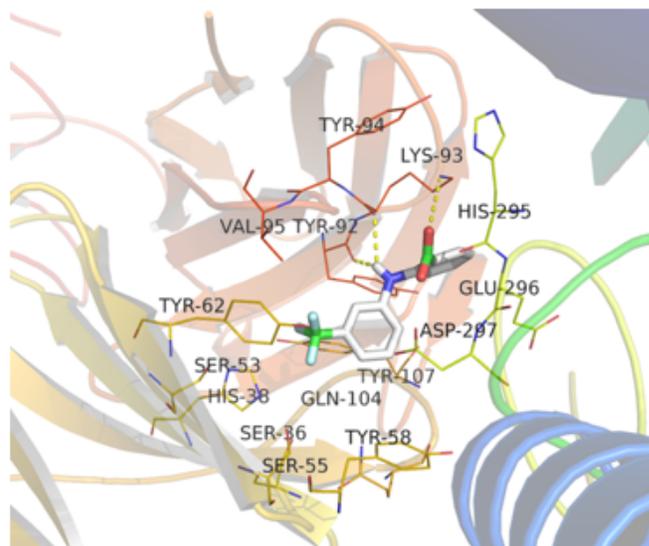


Figure 11

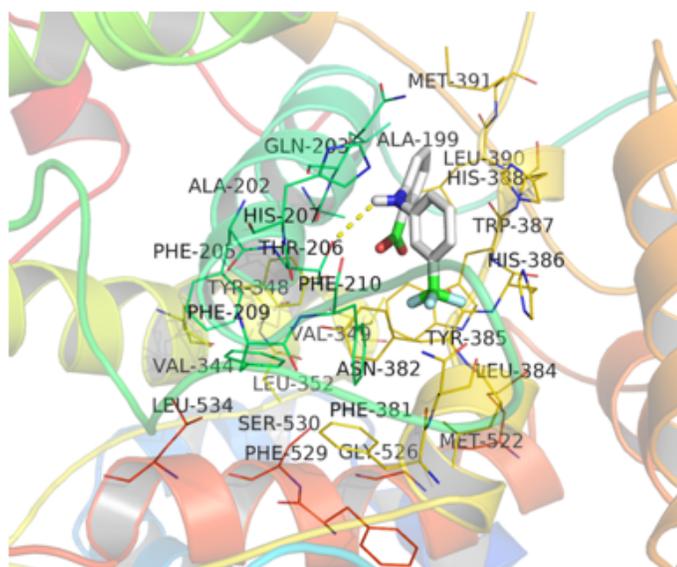
Distribution of target protein in the prediction pathway of *Sophora japonica*



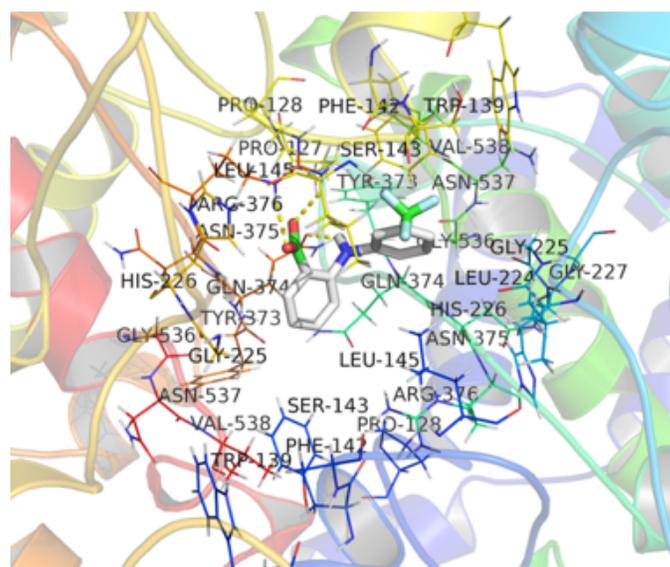
PTGS1



PTGS2



HSP90AA1



AR

Figure 12

The molecular docking diagram of quercetin and PTGS2, Luteolin and PTGS1, Kaempferol and HSP90AA1, Wogonin and AR

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryinformation.docx](#)