A study reveals anti-colorectal cancer effects of *Cassia tora* L. based on network pharmacology and bioinformatics

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ABSTRACT

Recent studies suggest that Cassia tora L. may have beneficial effects on colorectal cancer. However, the mechanisms and targets through which Cassia tora exerts its effects on colorectal cancer remain inadequately understood. This study aims to elucidate the active components, targets, and mechanisms of Cassia tora in colorectal cancer using network pharmacology and bioinformatics approaches. The results reveal that Cassia tora contains 18 active components associated with 266 targets relevant to colorectal cancer. The top 15 core targets identified include TP53, SRC, PTGS2, ESR1, CYP19A1, HSP90AA1, PIK3CA, TNF, EGFR, HRAS, MMP9, CASP3, UGT2B7, HSP90AB1, and CYP2C19. Differential expression analysis, survival analysis, single-cell transcriptome analysis, and drug sensitivity analysis indicate that PTGS2, TP53, and CASP3 are promising targets for pharmacological treatment of colorectal cancer. Additionally, this study reveals that the mechanisms by which Cassia tora exerts its effects on colorectal cancer involve pathways associated with prostate cancer, lipid metabolism and atherosclerosis, and endocrine resistance. In conclusion, this study is the first to employ network pharmacology and bioinformatics to elucidate the active components, targets, and mechanisms of Cassia tora in the context of colorectal cancer, offering new insights for the development of innovative therapies for this disease.

Keywords: Bioinformatics, *Cassia tora* L, colorectal cancer, network pharmacology, single-cell transcriptome analysis

1. INTRODUCTION

Colorectal cancer (CRC), which is also known as bowel cancer, colon cancer, or rectal cancer, comprises cancer developed from the colon or rectum [1]. CRC is the third most commonly diagnosed cancer in men and the fourth most commonly diagnosed cancer in women worldwide [2]. Treatment options for CRC currently include surgical resection, targeted therapy, adjuvant chemotherapy, and radiotherapy [3]. However, these treatments vary significantly in their effectiveness and side effects. Additionally, emerging therapies such as targeted therapy and immunotherapy have shown promising results in some patients but require further research to validate their efficacy and applicability. Thus, exploring new treatment strategies and mechanisms is essential for improving CRC treatment outcomes.

In recent years, traditional Chinese medicine (TCM) has attracted increasing attention from researchers worldwide for its potential in treating colorectal cancer. Several studies have employed network pharmacology to investigate the mechanisms through which TCM may impact CRC. In 2020, Ruirong He and colleagues [4] examined the anticancer mechanisms of compound Kushen injection (CKI), focusing on the interactions among multiple components, targets, and pathways. That same year, Tian Xu et al. [5] used network pharmacology to identify the active ingredients and anticancer mechanisms of the Huangqin-Baishao combination. In 2021, Wancai Que et al. [6] explored the bioactive components and mechanisms of Huanglian for CRC treatment, while Jingyun Jin et al. [7] predicted the targets and signaling pathways associated with Xiaochaihu decoction (XCHT) in CRC therapy. Additionally, Ying Zhu et al. [8] assessed the effectiveness of Chinese medicine in treating metastatic CRC and investigated the effective components and potential targets. Jin-Hua Fan et al. [9] elucidated the mechanism of "Zuo Jin Capsule" in inhibiting colorectal cancer using network pharmacology. In 2022, Jie Sun et al. [10] emphasized that "Compound Sophora flavescens injection"

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plays a key role in preventing colorectal cancer through cell cycle arrest, and Luorui Shang et al. [11] explored the mechanisms of "Si Junzi Tang" in treating. These studies collectively contribute to understanding the role of Chinese medicine in colorectal cancer treatment through network pharmacology. It is worth emphasizing that all of the above studies have promoted drug development for colorectal cancer.

Research in the field of using network pharmacology to study TCM for treating colorectal cancer is well-established. From the perspective of network pharmacology, these studies have preliminarily explored and validated the holistic regulatory characteristics of various TCM treatments for colorectal cancer, involving multiple components, targets, and pathways. They have predicted the potential mechanisms of TCM in treating colorectal cancer, providing scientific basis for further research on active ingredients and experimental studies. This paves the way for the clinical development of new anti- drugs, offering more choices and theoretical support.

2. METHODS

2.1 Data acquisition

The active ingredients of *Cassia tora* were retrieved from the TCMSP database, using criteria of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. The identified active ingredients of *Cassia tora* were then used as inputs to obtain their corresponding target proteins through the PubChem and SwissTargetPrediction databases. For colorectal cancer (CRC) targets, databases such as GeneCards, TTD, OMIM, DisGeNET, and DrugBank were searched using the keywords “colorectal cancer” and “CRC.”

2.2 Network pharmacology analysis

The intersection targets of *Cassia tora* and colorectal cancer were input into the STRING database to obtain target interaction data, which was then imported into Cytoscape 3.9.1 to construct the PPI network. The top 15 core targets were determined based on the Degree value. All data on the drug, compounds, and targets were imported into Cytoscape 3.9.1 to create the Traditional Chinese Medicine-Compound-Target network diagram.

The KEGG pathway enrichment analysis of the common targets of *Cassia tora* and colorectal cancer was performed using the Microbiota Perception platform, with the gene species and background limited to Homo sapiens. The results were visualized as a bubble chart.

2.3 Survival analysis and differential expression analysis

Access the GEPIA database, click on "Expression DIY" and select "Boxplot." Set the disease type to COAD, adjust |log2FC| to 1.0, and the *p*-value to 0.01. Sequentially input the 15 core targets to obtain differential expression images.

Access the GEPIA database, click on "Survival" and select "Survival Plots." Set the disease type to COAD, with the group cut-off set to median, and both cut off-High (%) and cut off-Low (%) set to 50%. Check the "Yes" option to display "Hazards Ratio" and "95% Confidence Interval," and set the x-axis units to "Months." Sequentially input the 15 core targets to obtain survival analysis curves.

2.4 Single-Cell transcriptome analysis

Single-cell transcriptomics is a high-resolution gene expression analysis method that sequences RNA from individual cells. This technique reveals cellular heterogeneity and uncovers differences in gene expression between various cell types and states. By using the SingleCell database, selecting the species Homo sapiens (human), and querying for colorectal cancer, one can obtain a clustering analysis of colorectal cancer cells. Within the clustering analysis, further investigation of core genes can reveal their expression levels in colorectal cancer. Expression levels are indicated by color intensity, with deeper colors representing higher expression levels.

2.5 Drug sensitivity analysis of genes

Using the GSCA database, select "Drug" and input the genes identified from differential expression and survival analysis. Observe the drug sensitivity of each gene in the GDSC and CTRP databases. Drug sensitivity analysis helps in understanding how gene expression levels affect drug treatment efficacy, thereby guiding the development of personalized treatment strategies. By identifying the relationship between these genes and drug sensitivity, drug selection can be optimized to improve therapeutic outcomes.

3. RESULTS

3.1 Data on active pharmaceutical ingredients, drug targets, and disease targets

In the TCMSP database, 13 active chemical components of Cassia tora were identified. Additionally, using CNKI with 'quality control of Cassia tora' as keywords, 4 main chemical components were further selected, bringing the total to 18 chemical components. SMILES and 2D structures of these 18 components were obtained from the PubChem database and inputted into the SwissTargetPrediction database to acquire corresponding targets. After merging and removing duplicates, a total of 266 drug targets were obtained.

Using “colorectal cancer” and “CRC” as keywords, disease targets for colorectal cancer were retrieved from GeneCards, TTD, OMIM, DisGeNET, and DrugBank databases, resulting in 4,775 targets after merging and removing duplicates. Intersection processing of *Cassia tora* and colorectal cancer targets yielded 190 intersecting targets.

3.2 Network pharmacology analysis

Intersection analysis of *Cassia tora* and colorectal cancer targets yielded a total of 190 intersecting targets, as shown in Figure 1A. The data of these intersecting targets obtained from the String website were uploaded to Cytoscape software for analysis. The top 15 targets ranked by Degree were selected as core targets, namely: TP53, SRC, PTGS2, ESR1, CYP19A1, HSP90AA1, PIK3CA, TNF, EGFR, HRAS, MMP9, CASP3, UGT2B7, HSP90AB1, and CYP2C19.

KEGG enrichment analysis revealed 271 signaling pathways, with the top 10 pathways selected based on P-values, as depicted in Figure 1D. These genes primarily participate in pathways related to prostate cancer, lipid metabolism and atherosclerosis, endocrine resistance, EGFR tyrosine kinase inhibitor resistance, and central carbon metabolism in cancer.

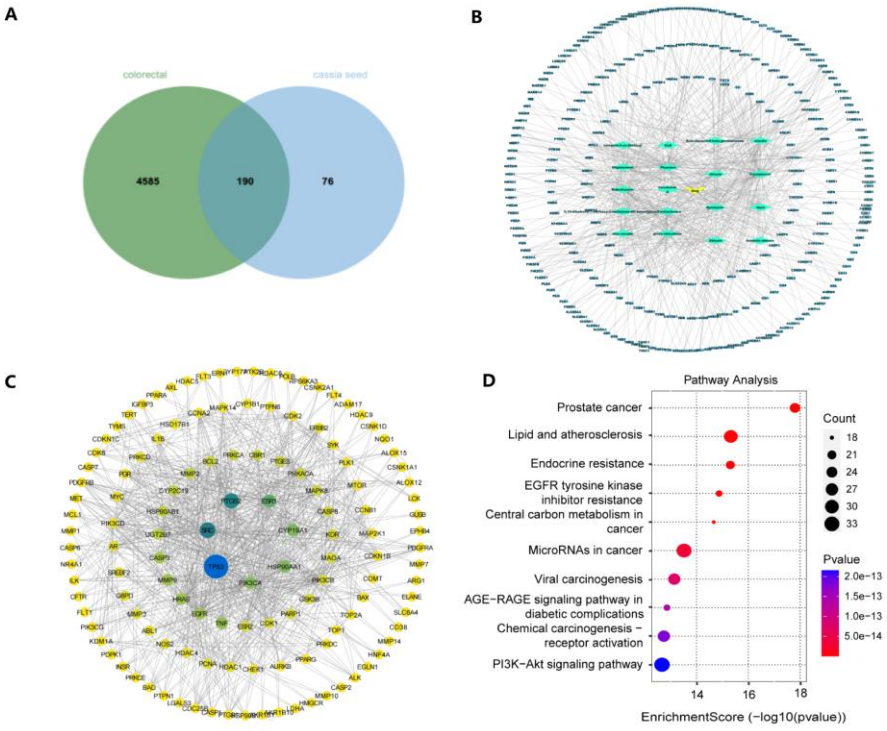

Figure 1. (A) Venn Diagram of Drug Components-Disease Targets. (B) Herb-Component-Target Network Diagram. (C) PPI Network Diagram. (D) KEGG Enrichment Analysis Bubble Chart

3.3 Differential expression analysis and survival analysis

In the GEPIA database, differential expression analysis of core targets is shown in Figure 2. Red indicates tissue, while gray represents normal tissue. The results indicate elevated expression levels of TP53, HSP90AA1, MMP9, HSP90AB1, and CASP3 in colorectal cancer tissue.

Survival prognosis analysis of colorectal cancer patients is depicted in Figure 3. "n (high)" denotes the number of patients with high gene expression, while "n (low)" denotes those with low gene expression. The results reveal significant differences in overall survival rates between high and low expression groups of TP53 (Logrank *p*=0.012), PTGS2 (Logrank *p*=0.0078), CYP19A1 (Logrank *p*=7.4e-05), and UGT2B7 (Logrank *p*=0.041).

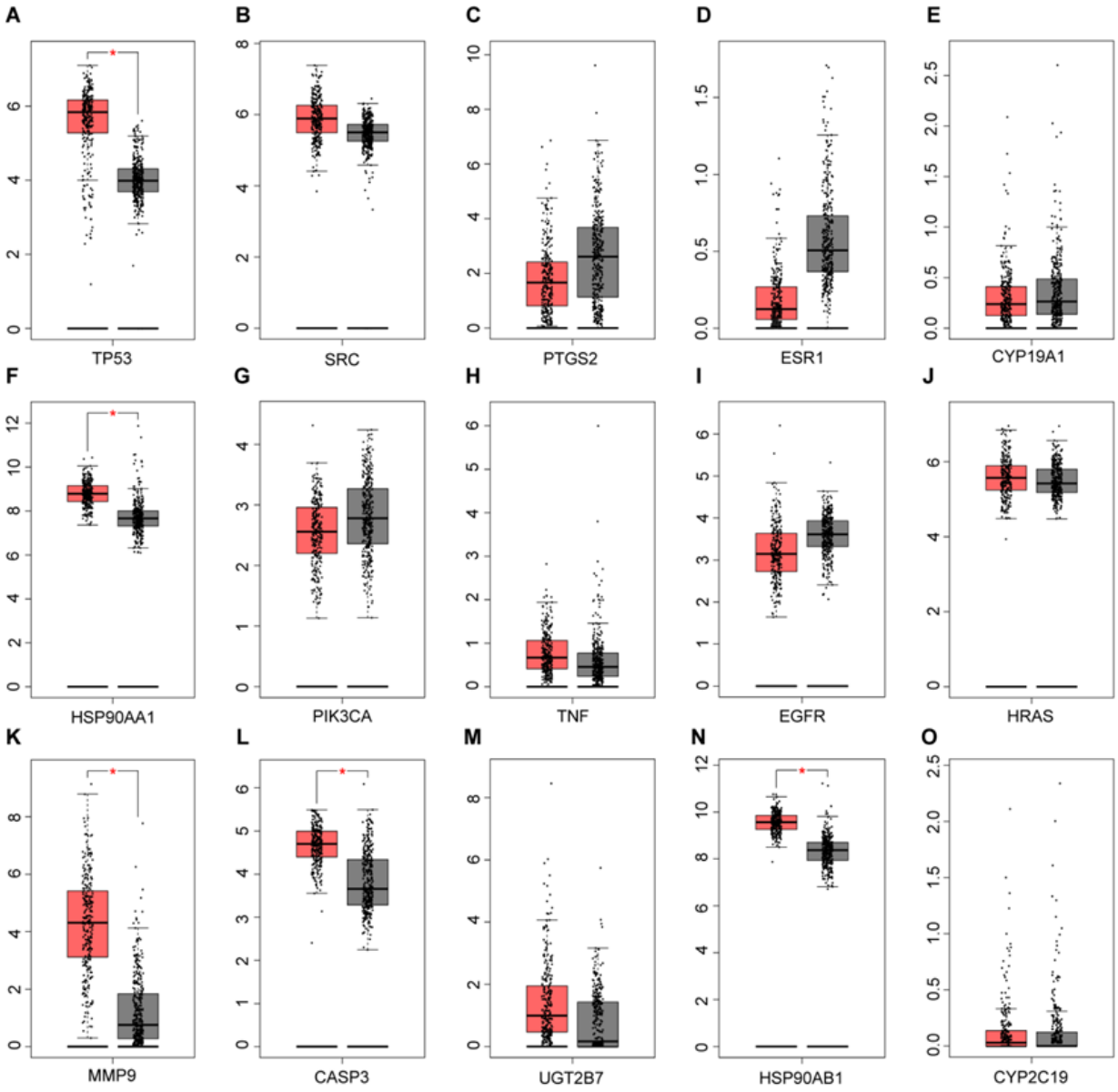

Figure 2. Differential Expression Analysis of Core Targets

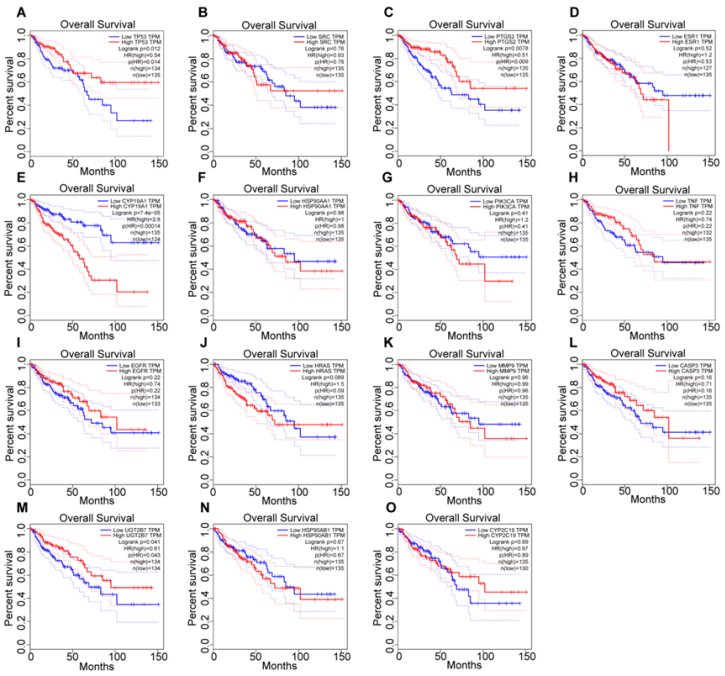

Figure 3. Survival analysis of core targets

3.4 Single-cell transcriptome analysis

Continuing with the cell subtype analysis, the expression of previously identified genes in colorectal cancer was observed. The results are depicted in the figure, where darker colors indicate higher expression levels. From Figures 4B, C, F, G, H, and I, it is evident that TP53, PTGS2, HSP90AA1, MMP9, CASP3, and HSP90AB1 exhibit elevated expression in colorectal cancer.

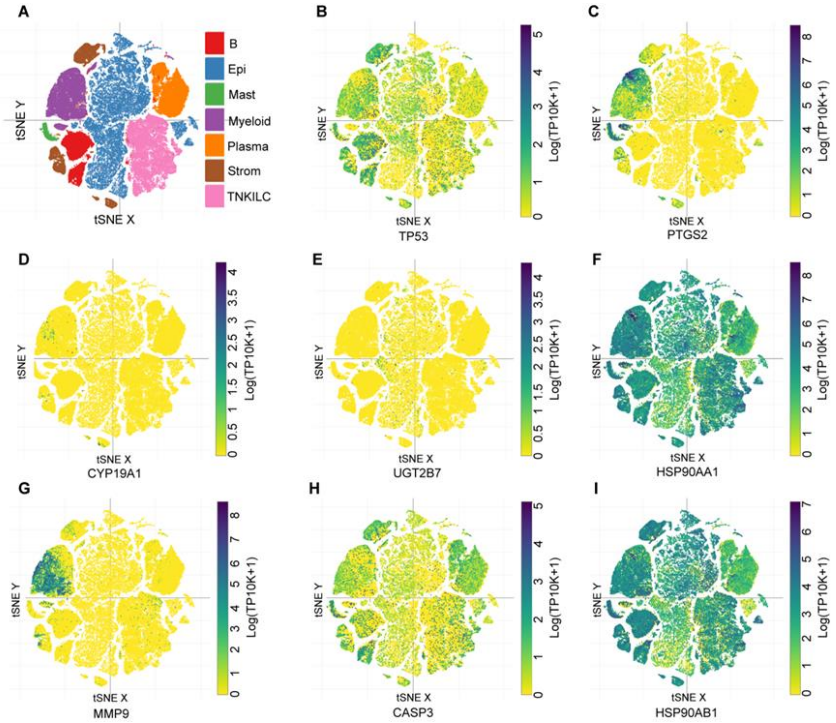

Figure 4. Single-cell transcriptomic analysis

3.5 Single-cell transcriptome analysis

Using the GSCA database's Drug section, we retrieved genes significantly associated with differential expression and prognosis in colorectal cancer. Subsequently, we obtained bubble plots from the GDSC and CTRP drug databases, as depicted in Figure 5, illustrating the sensitivity analysis. The bubble plots depict the correlation between gene expression and drug sensitivity. The x-axis represents a variety of anti-cancer drugs, including both commonly used chemotherapeutic agents and experimental compounds found in the GDSC and CTRP databases. Bubble color indicates the correlation between mRNA expression and IC50 values. From the figure, it is evident that PTGS2 mRNA expression correlates positively with drug sensitivity, whereas TP53 and CASP3 mRNA expressions correlate negatively with drug sensitivity.

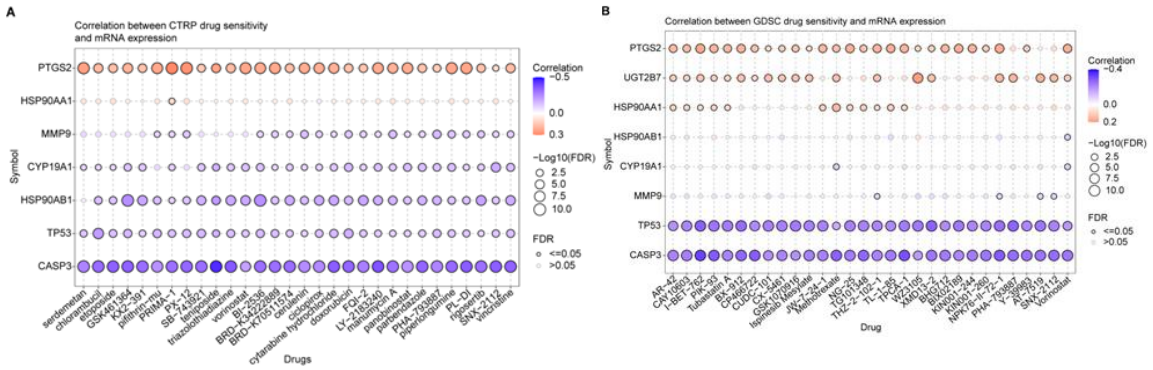

Figure 5. Drug sensitivity analysis of core targets

4. CONCLUSIONS

This study investigates the mechanisms by which *Cassia tora* treats colorectal cancer using bioinformatics and network pharmacology techniques. The research identified that Cassia tora contains various active compounds, including, but not limited to, aloe-emodin, rhein, cassioside, and rubrofusarin, among a total of eighteen. These compounds have oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18, indicating that they are not only efficiently absorbed when administered orally but also possess favorable chemical and physical properties for drug development, demonstrating significant potential in pharmaceutical applications. These compounds share 190 common targets with colorectal cancer targets. Using the STRING database and Cytoscape, a "herb-compound-target" network and a protein-protein interaction (PPI) network were constructed, identifying the top 15 core targets, including TP53, SRC, PTGS2, ESR1, and CYP19A1, which may be closely related to colorectal cancer. KEGG pathway enrichment analysis suggests that *Cassia tora* may exert its effects through pathways related to prostate cancer, lipid and atherosclerosis, endocrine resistance, EGFR tyrosine kinase inhibitor resistance, and central carbon metabolism in cancer.

Further analysis of core targets included differential expression, survival analysis, single-cell transcriptome analysis, and drug sensitivity analysis. Differential expression analysis showed elevated levels of TP53, HSP90AA1, MMP9, HSP90AB1, and CASP3 in colorectal cancer tissues compared to normal tissues. Survival analysis indicated significant differences in overall survival rates between high and low expression groups for TP53, PTGS2, CYP19A1, and UGT2B7, suggesting these genes may effectively inhibit the occurrence of colorectal cancer. Single-cell transcriptome analysis revealed high expression of TP53, PTGS2, HSP90AA1, MMP9, CASP3, and HSP90AB1 in colorectal cancer. Drug sensitivity analysis indicates that TP53, CASP3, and PTGS2 are associated with drug sensitivity, suggesting that they are common drug targets involved in apoptosis, inflammation, and tumor progression. These genes are interrelated: TP53 can influence apoptosis and inflammatory responses by regulating CASP3 and PTGS2. Additionally, the inflammatory effects of PTGS2 may in turn affect CASP3-mediated apoptosis, thereby forming a complex regulatory network.

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