Anti-Colorectal Cancer Mechanisms of Formononetin Identified by Network Pharmacological Approach

Authors' Contribution:
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Background: The network pharmacological approach was used to identity the anti-colorectal cancer (CRC) targets of formononetin (FN) and the molecular mechanisms of FN against CRC.

Material/Methods: A tool of the DisGeNET database was used for collection of CRC-based targets. Other tools of SuperPred, herbal ingredients target (HIT), and SwissTargetPrediction databases were applied in prediction of pharmacological targets of FN against cancer. A protein-protein interaction (PPI) network of FN against CRC was obtained by using a STRING database. All top biological functional processes and signaling pathways of FN against CRC were identified by using Database for Annotation, Visualization and Integrated Discovery (DAVID) software and Omicshare cloud platform.

Results: The most key anti-CRC targets of FN were identified as tumor protein p53 (TP53), cytochrome P450 3A4 (CYP3A4), ATP binding cassette subfamily G member 2 (ABCG2), tumor necrosis factor (TNF), epidermal growth factor receptor (EGFR), Erb-B2 receptor tyrosine kinase 2 (ERBB2), and cytochrome P450 1A1 (CYP1A1). In further assays, the treatment of CRC by FN was mainly involved in biological functional processes of reactive oxygen species metabolic process, positive regulation of transcription, DNA-templated, positive regulation of nucleic acid-templated transcription, and positive regulation of RNA metabolic process. anti-CRC by FN of signaling pathways were associated with amyotrophic lateral sclerosis (ALS), allograft rejection, cytokine-cytokine receptor interaction, asthma, mitogen-activated protein kinase (MAPK) signaling pathways, and others.

Conclusions: The anti-CRC molecular mechanisms of FN are implicated in suppression of cellular proliferation and regulation of cancer-related metabolic pathways. Interestingly, 8 optimal biological targets may be used as potential molecular markers for predicting and treating CRC.

MeSH Keywords: Colorectal Neoplasms • Molecular Mechanisms of Pharmacological Action • Pharmacology

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Background

Globally, epidemiological data indicate that CRC is one of the most common cancer-related causes of death [1]. In clinical practice, most of CRC cases are found with advanced stage, limiting opportunities for medical chemotherapy [2]. Further development of an alternative chemotherapy with high efficiency and low toxicity is a promising anti-CRC research topic. Formononetin (FN), a pharmacologically active compound isolated form *Astragalus* root, reportedly has potent anti-tumor activities [3]. Notably, growing evidence indicates that FN has pharmacological effects against CRC cells, and the underlying mechanism is involved in induction of apoptosis-related pathways [4,5]. However, more detailed anti-CRC mechanisms by FN remain unrevealed, and further study is needed. Interestingly, use of a tool of network pharmacology is a newly emerging strategy for revealing biological targets, functional processes, and molecular pathways of existing treatments for against clinical disease [6]. In addition, the predictive findings may function as possible pharmacological biotargets of chemotherapy against disease prior to in-depth experimental validation [7,8]. At present, there are still limited studies focusing on the biological target and molecular mechanism of FN against CRC. In the present study, a network pharmacological approach was used for identification and visualization of biological targets, functional processes, and signaling pathways of FN against CRC before clinical and experimental validations.

Material and Methods

Experimental design

Figure 1 presents a flow chart showing the experimental design.

Collection and identification of anti-CRC targets of FN

The SuperPred database was used to obtain known targets of FN, and the SwissTargetPrediction database was employed in identification of predictive targets of FN. The CRC-diseased targets were identified by using the DisGeNET database. Subsequently, the combined targets of FN and CRC were further used to produce the anti-CRC targets by FN through the UniProt database.

Construction of PPI network of FN against CRC and topological analysis

The mapped anti-CRC targets of FN were used to obtain the function-related proteins by using a STING database, and protein interaction with confidence score greater than 0.9 was set to exclude targets. The collective targets were imported into Cytoscape software to construct a PPI network of FN against CRC. The Network-Analyzer in Cytoscape was used to further analyze the average degree of freedom and degree of freedom, and thus the core targets were screened and identified according to degree value. The upper limit of the screening range was the large degree value in the topological data, and the lower limit was twice the average degrees of freedom [9].

Cluster analysis

A multivariate conditional outlier detection (MCODE) algorithm was used to cluster the PPI network of FN-target-CRC, as reported previously [10].

Biological function and pathway enrichment analyses of core targets

The Database for Annotation, Visualization and Integrated Discovery (DAVID) database was used to obtain biological functions and molecular pathways of core targets in FN against CRC before being visualized through the Omicshare cloud platform. Advanced bubble diagrams of signaling pathways and biological processes were plotted according to P values. The Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analyses of all identifiable targets were performed to further reveal detailed pharmacological mechanisms of FN in the treatment of CRC.

Results

Target information

All 6229 genes found using the DisGeNET database were used for pathogenetic targets. The top 200 targets with P values greater than 0.05 were selected as the optimal targets of CRC. Ten anti-cancer targets were obtained through the HIT database, 23 known targets and 20 predictive targets were screened through the SuperPred database, and 15 identifiable targets of FN were identified through the SwissTargetPrediction database. After mapping, 19 intersecting targets of FN against CRC were obtained, followed by 86 interacted and correlated edges (Figure 2). In addition, the most key anti-CRC targets of FN were identified as TP53, CYP3A4, ABCG2, TNF, EGFR, ERBB2, and CYP1A1 (Figure 3).

Cluster analysis and enrichment assay

The subnetworks clustered by the MCODE algorithm using Cytoscape are shown in Figure 4. The predictive targets of CYP2C19, CYP2D6, CYP3A4, and CYP2C9 were clustered as a group. IL4, PPARG, TNF, TP53, ESR1, ESR2, EGFR, BRCA1, ERBB2, ABCG2 targets were clustered and grouped. The Cluego plugin and GraphPad Prism software were used to achieve visualization
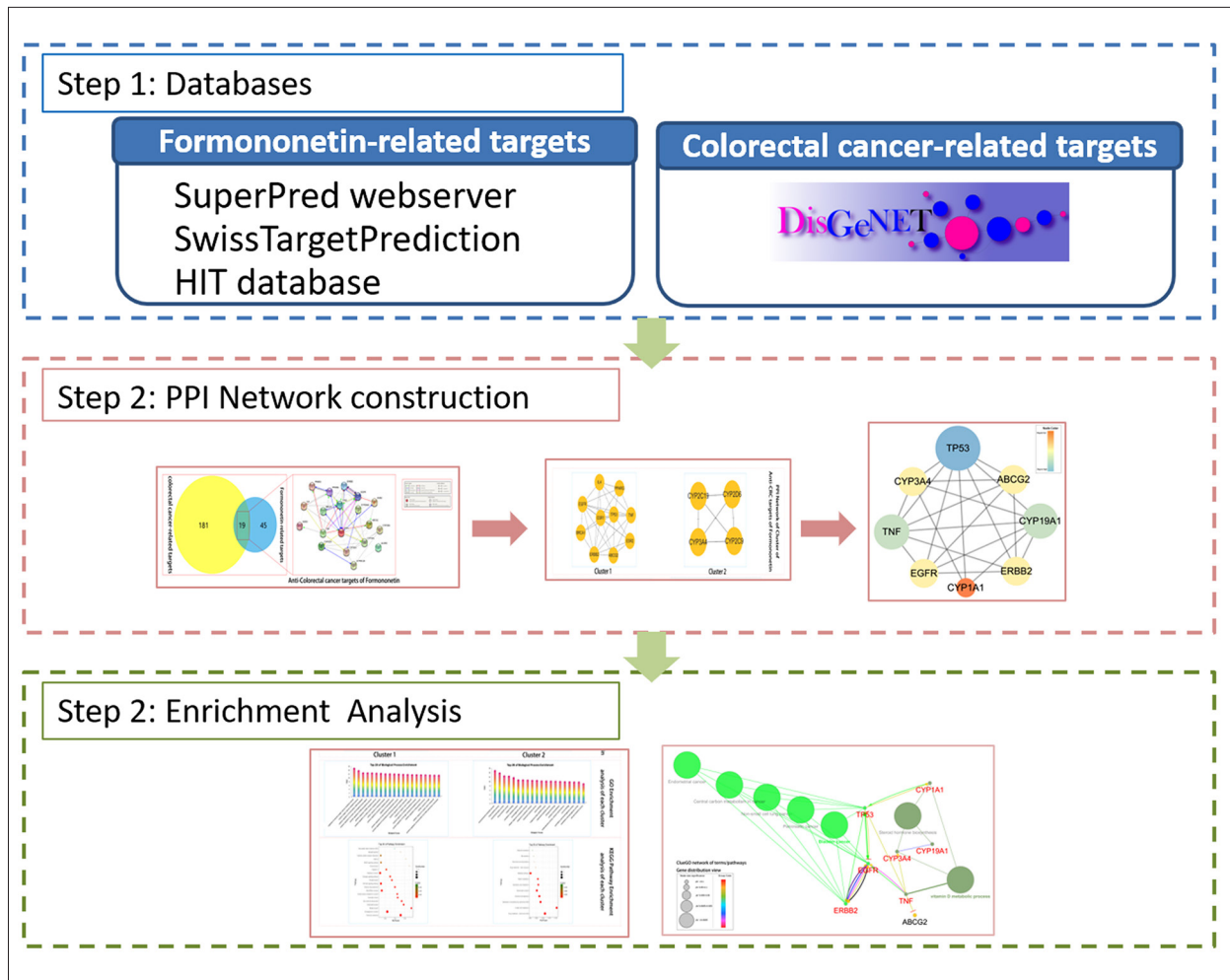
Figure 1. Flow chart of FN against CRC by using a strategy of network pharmacological approach.
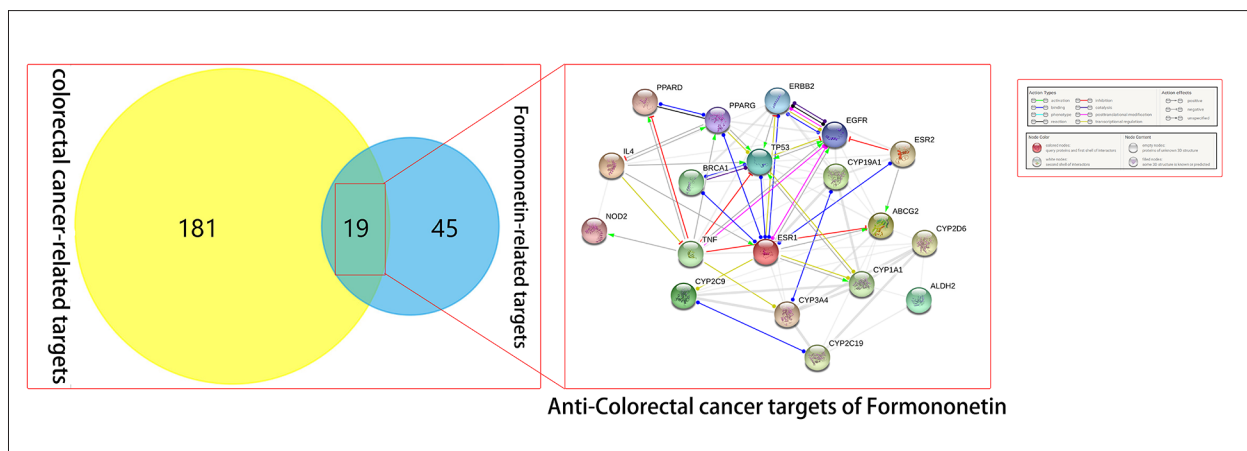

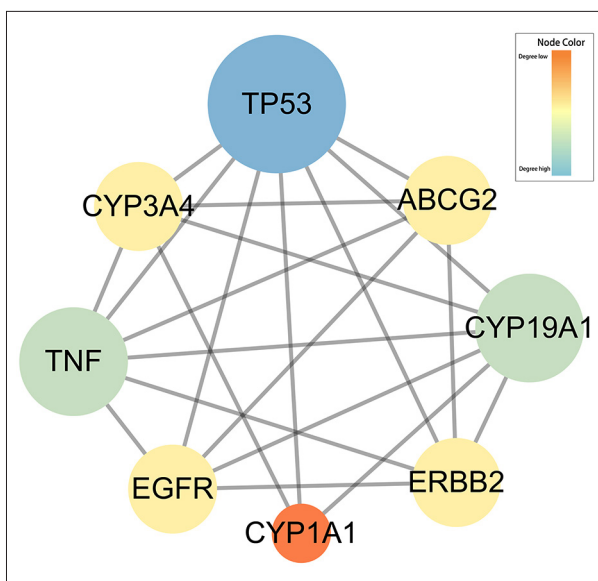**Figure 2.** Mapper targets of FN and CRC for construction of a PPI network.

Figure 3. Hub targets of the most important molecules of FN and CRC.

of molecular functions and biological pathways of the most key anti-CRC targets of FN, as revealed in Figure 5.

Discussion

CRC is the third most life-threatening cancer in the world, with a high mortality rate. The lethality rate of CRC ranks the second of fatal malignancies, just after lung carcinoma and breast cancer [11]. Moreover, most CRC patients are diagnosed with advanced stage because of inapparent early symptoms and insufficient clinical inspection [12]. In recent medical regimens, therapeutic bioactive compounds against CRC have been developed as a substitute for traditional cancer treatment methods such as surgery, radiotherapy and chemotherapy. Growing evidence indicates that marked TP53 mutations are found in CRC cells and human samples. Abnormally mutated changes of the TP53 gene play a key role in advanced CRC [13]. Compared with non-cancer tissues, the methylation levels of microRNA-34a in TP53 PIN A1A1 and TP53 MSP GG genotypes in CRC tumors
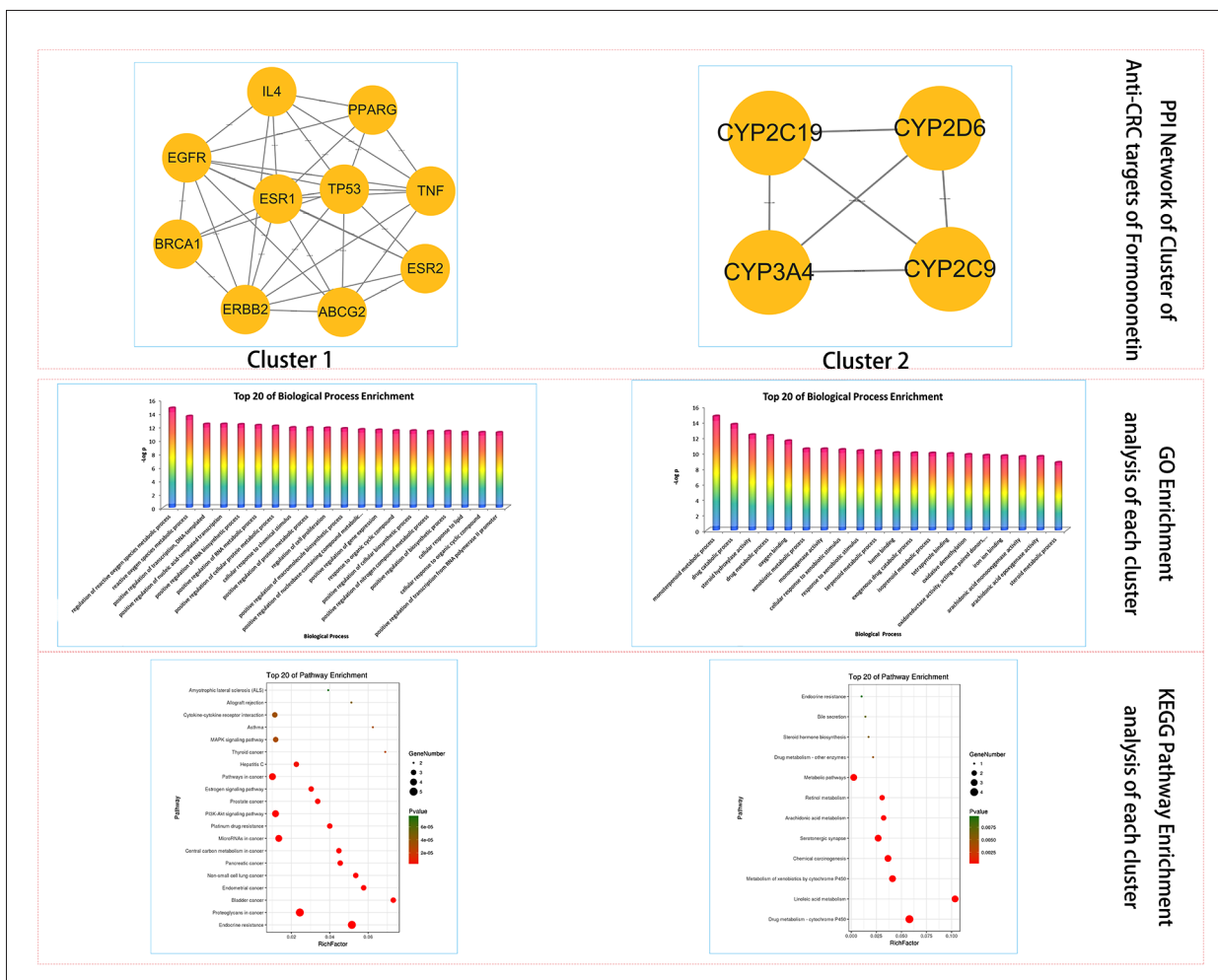

Figure 4. Cluster analysis for correlative association of core biotargets of FN and CRC.
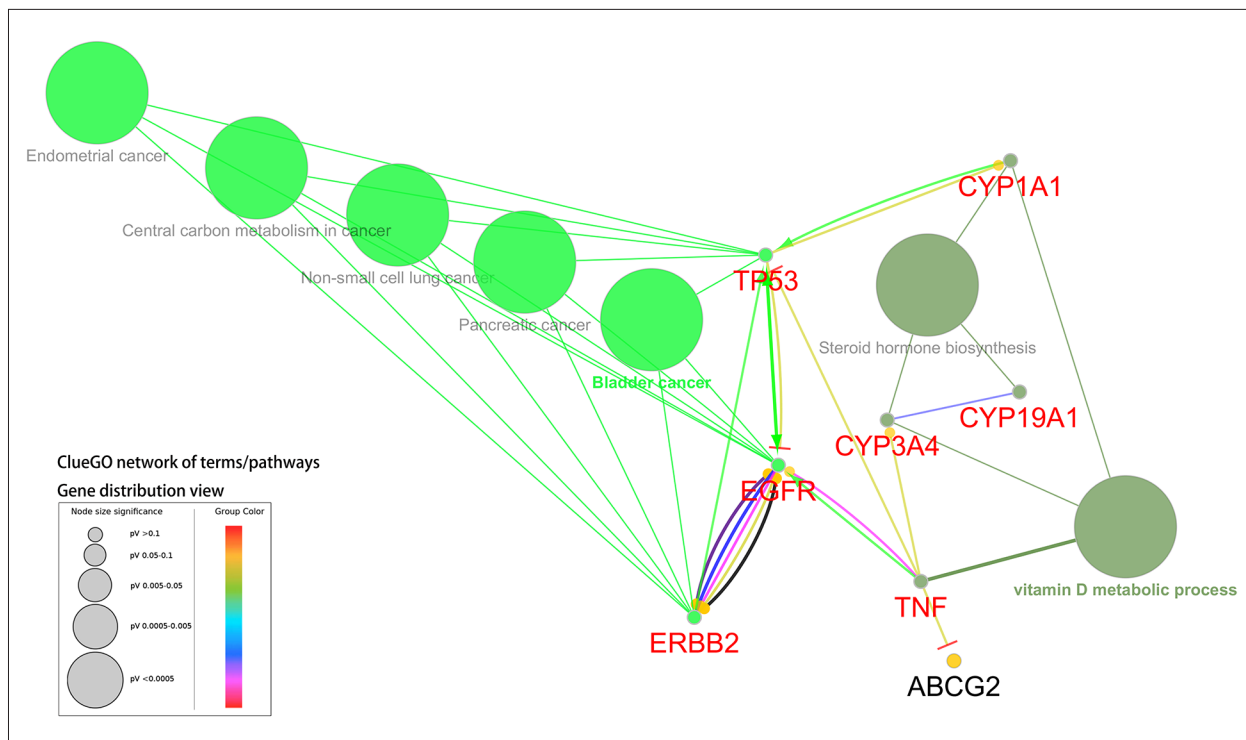
Figure 5. Targets-pathways for anti-CRC exerted by FN, associated with hub target.

are clearly higher [14]. Thus, overexpression of TP53 mutation is a pathological target in CRC progression. Oxidative stress and inflammation play important roles in malignant CRC, and the high expression of ABCG2 in CRC tissue may be a feedback of overoxidation reaction, associated with poor prognosis. A cell culture study showed that downregulation of ABCG2 can inhibit the production of antioxidants, inducing tumor cell growth [15], suggesting that ABCG2 is a potential therapeutic target of CRC. Excessive expression of CYP3A4 is detected in colon tissue [16]. Intestinal CYP3A and P-glycoprotein (P-gp), some important components in the host defense barrier, exert an important effect in the pathogenesis of disease, drug exposure, and environmental stimulus. Clinical and experimental data suggest that the expressions of CYP3A and P-gp in the intestinal tract of patients with inflammatory bowel disease is clearly lower, accompanied with imbalance of the gut microbiome [17]. The steroid-related gene CYP19A1 can affect estrogen metabolic processes, and it can increase cancer risk through inflammatory stress [18]. Cytochrome P450 1A1 (CYP1A1) is a crucial metabolic enzyme involved in the metabolism of many xenobiotic organisms. One of the pathogenetic mechanisms of CYP1A1-mediated carcinogenesis is reported be that CYP1A1 metabolizes polycyclic aromatic hydrocarbons (PAHs) to active epoxy intermediates, and then covalently binds into DNA for inducing tumors [19]. Collectively, cytochrome P450 enzymes (CYP3A4, CYP19A1, and CYP1A1) may function as potent biological and therapeutic targets against CRC. In addition, endogenous TNF/TNF receptor (TNFR) pathways affect survival rates

of patients with cancer [20], suggesting that TNF exerts a key role in CRC development. High expression of EGFR is observed in cancer cells and tissues [21]. EGFR is functionally involved in proliferation, survival, invasion, and immunodeficiency of metastatic CRC [22]. ERBB2 (HER2) amplification is an emerging biomarker in CRC, increasing sensitivity to combination therapy against HER2 [23]. According to the literature, it can be speculated that FN plays an effective anti-cancer role by regulating the expression of predictive targets in CRC cells. Molecular classification and identification of new and emerging biomarker indicates a key role in cancer progression, and it may provide insight into clinical information for prolonging the survival of patients with CRC. In addition, GO annotation analysis and KEGG pathway assay of biological targets of FN against CRC showed most of the enriched top signaling pathways.

Conclusions

The prediction of biotargets and molecular mechanisms by using a tool of network pharmacology is accurate and practical, and this approach sheds light on an attractive scientific foundation for further exploring the pharmacological targets and signaling mechanisms of FN against CRC.

Conflict of interests

None.

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