Discovery of the Anti-Tumor Mechanism of Calycosin Against Colorectal Cancer by Using System Pharmacology Approach

Background: The aim of our study was to elucidate the biological targets and pharmacological mechanisms for calycosin (CC) against colorectal cancer (CRC) through an approach of system pharmacology.

Material/Methods: Using a web-based platform, all CRC-causing genes were identified using a database of gene-disease associations (DisGeNET), and all well-known genes of CC identified using the databases of prediction of protein targets of small molecules (Swiss Target Prediction), drug classification, and target prediction (SuperPred). The carefully selected genes of CRC and CC were concurrently constructed by using a database of functional protein association networks (STRING), and use of software for visualizing complex networks (Cytoscape), characterized with production of protein-protein interaction (PPI) network of CC against CRC. The important biological targets of CC against CRC were identified through topological analysis, then the biological processes and molecular pathways of CC against CRC were further revealed for testing these important biotargets by enrichment assays.

Results: We found that the key predictive targets of CC against CRC were estrogen receptor 2 (ESR2), ATP-binding cassette sub-family G member 2 (ABCG2), breast cancer type 1 susceptibility protein (BRCA1), estrogen receptor 1 (ESR1), cytochrome p450 19A1 (CYP19A1), and epidermal growth factor receptor (EGFR). Visual analysis revealed that the biological processes of CC against CRC were positively linked to hormonal metabolism, regulation of genes, transport, cell communication, and signal transduction. Further, the interrelated molecular pathways were chiefly related to endogenous nuclear estrogen receptor alpha network, forkhead box protein A1 (FOXA1) transcription factor network, activating transcription factor 2 (ATF2) transcription factor network, regulation of telomerase, plasma membrane estrogen receptor signaling, estrogen biosynthesis, androgen receptor, FOXA transcription factor networks, estrogen biosynthesis, and phosphorylation of repair proteins.

Conclusions: Use of system pharmacology revealed the biotargets, biological processes, and pharmacological pathways of CC against CRC. Intriguingly, the identifiable predictive biomolecules are likely potential targets for effectively treating CRC.

MeSH Keywords: Colorectal Neoplasms • Defense Mechanisms • Pharmacology, Clinical • Phytoestrogens

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/918250
Background

Colorectal cancer (CRC) is a highly lethal gastroenteric tumor with high invasiveness [1]. CRC has the third highest morbidity and mortality rates of all cancers worldwide. Diagnostically, the early symptoms of CRC are difficult to detect, and the terminal stage of CRC is hard to treat due to lack of effective biomarkers for clinical screening. If left untreated, advanced CRC will develop to cancer-induced remote metastasis, and the fatality rate increases sharply [2,3]. Pathologically, the exact pathogenesis of CRC is unclear. There are few currently available chemotherapeutic options for advanced CRC, especially in metastatic stage. The clinical effectiveness of surgical resection, chemotherapy, and radiotherapy for CRC is insufficient [4,5]. Therefore, more effective and less cytotoxic bioactive treatments for CRC need to be screened and developed, and there is an urgent need to define the pharmacological mechanisms and biotargets of potential CRC treatments.

Traditional Chinese medicine uses Astragalus root to enhance immunity, protect cardiovascular function, and treat hypoglycemia. Astragaloside A, formononetin, and calycosin are among the bioactive ingredients extracted from Astragalus root that have promising pharmacological benefits [6,7]. Calycosin (CC), a functional phytoestrogen, is pharmacologically beneficial due to its neuroprotection, cytoprotection, antioxidative, hypolipidemic, and hypoglycemic effects [8]. Moreover, CC has potent anti-neoplastic activities, including action against breast cancer and colorectal cancer. In some biological mechanisms, the signaling pathways exerted by CC are related to regulation of WDR7-7-GPR30, HOTAIR/p-Akt, ERβ/miR-17, ERβ/MiR-95, and IGF-1R [9,10]. However, the network molecular mechanisms of CC against CRC are not entirely defined. System pharmacology, also known as network pharmacology, is an emerging methodology to uncover the bioinformatic findings of predictive targets, signaling pathways, and protein-to-protein interaction networks in “drug treating disease” [11]. Interestingly, mounting evidence shows herb-isolated components to treat disorders using the system pharmacological approach [12]. In the present study, we used the system pharmacological approach to screen and reveal the biological targets, bio-processes, and signaling pathways of CC in treating CRC. As a result, the whole schematic diagram was illuminated, as shown in Figure 1.

Material and Methods

Data collection and assay of CC in treating CRC

The well-known pharmacological genes of CC were obtained from the web-available databases of Swiss Target Prediction and SuperPred, and then the disease-conditioning genes of CRC were collected from the DisGeNET database. Subsequently, identified genes were pooled prior to obtaining the therapeutic targets of CC in treating against CRC [13].

Network target construction and analysis of CC treating CRC

Further, the web-available STRING database was accessed for analysis of pooled therapeutic targets of CC in treating CRC,
followed by identification of interrelated network proteins. Then, the system PPI networks with all core targets were created, resulting from the reference data greater than 0.9 using Cytoscape software. In addition, topological indexes following Network Analyzer were screened and determined for identification of core targets. Briefly, clustering assay of system PPIs of CC in treating CRC was performed with the MCODE algorithm, which is a method for automatically finding molecular complexes in large protein interaction networks [14].

Biological processes and molecular pathways by enrichment tests

The web-available functional enrichment analysis tool (Funrich software) was used to find the functional processes and biological pathways of CC in treating CRC. Then, the graphical visualization of these biological functions and signaling pathways was performed in accordance with referencing -LogP value in computational setting [15].

Results

Data collection and identification of biotargets

We isolated and collected 2753 well-established pathogenic genes from the DisGeNET database. According to scores, the optimal 200 genes were identified for further data analysis, including 1 microRNA. We also screened and identified 40 pharmacological targets of CC, and 11 intersection targets of CC in treating CRC were obtained using FunRich software. In further analysis, a PPI network of CC in treating CRC was constructed using these 11 pooled biotargets (Figure 2).

Topology parameters of PPI network and core targets

Network Analyzer was used to analyze the topological indexes and PPI network of CC in treating CRC. The median of the calculated degrees of freedom was set as 4, and the maximum degrees of freedom was set as 6. Thus, the core target screening conditions were set as 4 to 6, and 6 core target proteins were identified as ESR2, ABCG2, BRCA1, ESR1, CYP19A1, and EGFR (Figure 3).
Excessive expression of neoplastic ESR2 is associated with low getting tissues or cells [16]. Increasing evidence indicates that closely related to the development of CRC. ESR2 (estrogen receptor) is a nuclear receptor that is highly expressed in malignant tissues, and it promotes cell proliferation and growth of cancer cells in a spatiotemporal manner [22]. In estrogenic signaling, ESR1 is a potential target for treating colorectal cancer [23]. CYP19A1 is a functional enzyme that is involved in gonadal development, sex differentiation, and androgen biosynthesis [24]. CYP19A1 has a functional role in the development of colon and rectal cancer through regulating inflammation-associated pathways [25]. EGFR is a transmembrane tyrosine kinase that regulates cellular proliferation, differentiation, neoplastic growth [26]. Thus, anti-EGFR inhibitor may be a promising treatment of colorectal cancer, including metastatic stage [27]. The literature and our present results indicate that these genes/proteins may be potential pharmacological targets of CC in treating CRC, but this needs to be verified by further research. In addition, these biotargets are consistent with signaling pathways induced by FN, such as endogenous nuclear estrogen receptor alpha network, transcription factor network, estrogen receptor signaling, estrogen biosynthesis, androgen receptor, estrogen biosynthesis, and phosphorylation of repair proteins.

**Biological processes and molecular pathways of core biotargets**

To reveal the detailed mechanisms of CC in treating CRC, enrichment analyses were conducted. Visualization data from FunRich assay showed that the biological processes of explicit core targets were hormone metabolism, regulation of gene expression, epigenetic, regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolism, transport, cell communication, and signal transduction (Figure 4). Furthermore, the bioinformatic findings of signaling pathways of explicit core targets in CC in treating CRC were predominantly associated with validated nuclear estrogen receptor alpha network, FOXA1 transcription factor network, ATF2 transcription factor network, regulation of telomerase, plasma membrane estrogen receptor signaling, estrogen biosynthesis, androgen receptor, FOXA transcription factor networks, estrogen biosynthesis, and ATM-mediated phosphorylation of repair proteins (Figure 5).

**Discussion**

By using the bioinformatic assays of system pharmacology, we obtained detailed information on functional processes and signaling pathways of CC in treating CRC. More importantly, the core biotargets of CC in treating CRC were obtained, and the following optimal biotargets were screened: ESR2, ABCG2, BRCA1, ESR1, CYP19A1, and EGFR. Based on our literature review, we reasoned that these important genes/proteins were closely related to the development of CRC. ESR2 (estrogen receptor α) is a nuclear hormone receptor that is functionally linked to modulation of cellular proliferation and differentiation in targeting tissues or cells [16]. Increasing evidence indicates that excessive expression of neoplastic ESR2 is associated with low survival and poor prognosis of colorectal cancer patients [17]. ABCG2 (ATP-binding cassette transporter G2) is a glycosylated transmembrane protein that has a key role in the multidrug resistance of tumor cells [18]. Increasing evidence shows that overexpression of ABCG2 is associated with the progression of stem cell-derived cancer cells, and ABCG2 may be a potential therapeutic target for treating colorectal cancer [19]. BRCA1 is a susceptibility protein that mutates in cancer cells, and it has roles in regulating cell cycle, ubiquitination, and apoptosis [20]. Based on the literature, elevated BRCA2 mutation is related to the risk of developing colorectal cancer [21]. ESR1 (estrogen receptor β) is a nuclear receptor that is highly expressed in malignant tissues, and it promotes cell proliferation and growth of cancer cells in a spatiotemporal manner [22]. In estrogenic signaling, ESR1 is a potential target for treating colorectal cancer [23]. CYP19A1 is a functional enzyme that is involved in gonadal development, sex differentiation, and ontogenesis [24]. CYP19A1 has a functional role in the development of colon and rectal cancer through regulating inflammation-associated pathways [25]. EGFR is a transmembrane tyrosine kinase that regulates cellular proliferation, differentiation, neoplastic growth [26]. Thus, anti-EGFR inhibitor may be a promising treatment of colorectal cancer, including metastatic stage [27]. The literature and our present results indicate that these genes/proteins may be potential pharmacological targets of CC in treating CRC, but this needs to be verified by further research. In addition, these biotargets are consistent with signaling pathways induced by FN, such as endogenous nuclear estrogen receptor alpha network, transcription factor network, estrogen receptor signaling, estrogen biosynthesis, androgen receptor, estrogen biosynthesis, and phosphorylation of repair proteins.
Conclusions

These bioinformatic data shed light on the clinical and pharmacological significance of CC in treating CRC. Intriguingly, the core biotargets, biological functions, and molecular pathways of CC in treating CRC are identified through system pharmacological methods. Further, these optimal biotargets of ESR2, ABCG2, BRCA1, ESR1, CYP19A1, and EGFR may be used for screening and treating colorectal cancer.

Conflict interest

None.

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