Prediction of triptolide targets in colorectal cancer using network pharmacology and molecular docking

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Abstract

Network pharmacology is an approach that uses bioinformatics to predict and identify multiple drug targets and interactions in disease. Here, we applied network pharmacology to investigate the potential mechanisms of action of triptolide, an active component in the traditional Chinese medicine *Tripterygium wilfordii* Hook F, in colorectal cancer (CRC). We first searched public databases for genes and proteins known to be associated with CRC, as well as those predicted to be targets of triptolide, and then used Ingenuity Pathway Analysis (IPA) to identify enriched gene pathways and networks. Networks and pathways that overlapped between CRC-associated proteins and triptolide target proteins were then used to predict candidate protein targets of triptolide in CRC. The following proteins were found to be expressed in both CRC-associated networks and triptolide target networks: JUN, FOS, CASP3, BCL2, IFNG, and VEGFA. Docking studies suggested that triptolide can fit in the binding pocket of the four top candidate triptolide target proteins (CASP3, BCL2, VEGFA and IFNG). The overlapping pathways were activation of neuroinflammation signaling, glucocorticoid receptor signaling, T helper (Th) cell differentiation, Th1/Th2 activation, and colorectal cancer metastasis signaling. These results show that network pharmacology can be used to generate hypotheses about how triptolide exerts therapeutic effects in CRC. Network pharmacology may be a useful method for characterizing multi-target drugs in complex diseases.

1. Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the world [1]. Many Asian countries, including China, South Korea, Singapore, and Japan, are experiencing an escalating incidence of CRC [2-9]. A lot of effort has been conducted to enhance the treatment of CRC. Despite improved surgical techniques and advancements in radio- and chemotherapy over the past few decades, the overall survival rate of patients with CRC has not improved substantially [10]. It is therefore imperative to devise novel strategies for safe and effective treatment of CRC.

Triptolide is a diterpene triepoxide purified from *Tripterygium wilfordii* Hook F, commonly known as '*lei gong teng*' or 'thunder god vine', a medicinal plant whose extracts have been used in traditional Chinese medicine for treating rheumatoid arthritis and other inflammatory diseases [11-16]. Recent studies have shown that triptolide kills CRC and other cancer cells *in vitro* with high potency [17, 18]. Animal studies have shown that triptolide inhibits the growth of CRC cells in a mouse xenograft model [19, 20]. However, the mechanisms underlying the therapeutic effects of triptolide in CRC are unclear.

Network-based drug discovery is a promising, cost-effective drug development approach based on bioinformatics, systems biology and pharmacology. Instead of the current "one target, one drug" approach, network pharmacology utilizes a "network target, multicomponent" strategy. Because network pharmacology can provide a good understanding of the principles of network theory and systems biology, it has been considered the next paradigm in drug discovery [21-24]. Herbal medicines such as

triptolide are particularly promising drugs for developing new multicomponent and multitarget synergistic cancer therapeutics [25-27].

In this study, we investigated the potential mechanisms by which triptolide acts in CRC using network pharmacology. Our results revealed potential mechanisms that may underlie the therapeutic effects of triptolide in CRC and showed that network pharmacology is a useful tool to facilitate the development of novel CRC drugs.

2. Materials And Methods

2.1. Identification of genes and proteins associated with CRC and potential triptolide target proteins

We searched the National Center for Biotechnology Information (NCBI) GenBank Database (<https://www.ncbi.nlm.nih.gov/>) for genes related to the search term “colorectal cancer”. Search hits were filtered to retain only studies performed in *Homo sapiens*. Potential human protein targets of triptolide were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (<http://lsp.nwu.edu.cn/tcmsp.php>) [32] and PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) [33].

2.2. Prediction of pathways and networks associated with CRC and triptolide targets

The protein symbols of candidate triptolide targets and CRC-associated genes were uploaded into Ingenuity Pathway Analysis (IPA) software version 2019 (<https://www.qiagenbioinformatics.com>, Redwood City, CA, US) for network and pathway analysis. IPA was used to construct pathways and networks based on known interactions between genes and proteins. Enrichment analysis of CRC target Gene Ontology (GO) enrichment and network was performed using R (version 3.6.0 for Windows) and Cytoscape version 3.6.1 (<http://www.cytoscape.org>). Pathways and networks were ranked according to the number of molecules in pathways and networks, and cut-off p value < 0.05 was used to identify significantly enriched pathways/networks. Pathways and networks involving CRC-associated genes and candidate triptolide targets were identified using the “Compare” module in IPA.

2.3. Construction of common networks and core target screening

Proteins that were both previously associated with CRC and predicted to be targets of triptolide were collected using Venny 2.1.0 software (<http://bioinfogp.cnb.csic.es/tools/venny/>), and potential protein-protein interactions were analyzed in STRING 10.5 (<https://string-db.org/>) after filtering by *Homo sapiens*.

Target proteins whose values for the topological attributes of node degree distribution and betweenness centrality were above the means were defined as "core targets". The degree of a node is the number of nodes to which it is linked, while betweenness reflects the extent to which nodes lie between one another. Finally, core targets were analyzed using IPA.

2.4. Prediction of binding between triptolide and candidate target proteins

The crystal structures of candidate proteins bound by triptolide were downloaded from the RCSB Protein Data Bank (<http://www.pdb.org/>) and modified using YASARA software (<http://www.modekeji.cn>) to remove ligands, add hydrogen, remove water, and optimize and patch amino acids. Before docking, ChemBioDraw 3D was used to generate 3D chemical structures and minimize binding energies for all candidate triptolide targets. We used YASARA software to test the precision of docking between triptolide and candidate target proteins because it had the highest accuracy and consistency (data not shown). The best docking poses were identified as those showing the smallest root mean square deviation (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation. Models with an $\text{RMSD} \leq 4 \text{ \AA}$ were considered reliable and those with an $\text{RMSD} \leq 2 \text{ \AA}$ were considered accurate [34]. The network pharmacology approach in this study is summarized in Fig. 1.

3. Results

3.1. Networks and enriched functions in CRC-associated genes

A total of 3,298 CRC-associated genes were identified in GenBank. IPA identified a total of 596 pathways and 25 networks associated with these genes. The top/majority of pathways were involved in molecular mechanisms of cancer, CRC metastasis signaling and Wnt/ β -catenin signaling. The majority of networks were involved in cancer, cellular movement, organismal injury and abnormalities, cellular development, embryonic development, organismal development, cell-to-cell signaling and interaction, protein synthesis, and RNA damage and repair (Fig. 2).

Gene Ontology (GO) enrichment and network analysis of CRC-associated proteins showed that the top three functions were epithelial cell proliferation, ameboidal-type cell migration and regulation of vasculature development (Fig. 3).

3.2. Networks and enriched functions in triptolide target genes and proteins

A total of 33 proteins were identified as candidate triptolide targets, and IPA revealed a total of 294 enriched pathways and 10 networks. The most significantly enriched pathways were neuroinflammation signaling, glucocorticoid receptor signaling, T helper (Th) cell differentiation, Th1 and Th2 activation, and CRC metastasis signaling. The top networks identified were involved in gene expression, cellular function and maintenance, cell cycle, inflammatory response, organismal injury and abnormalities, cell-to-cell signaling and interaction, cell death and survival, dermatological diseases and conditions, and infectious diseases (Fig. 4).

3.3. Networks of shared proteins and special proteins targeted by triptolide

The intersections between the set of potential triptolide targets and CRC-related proteins were analyzed using Venny software, which identified 29 shared proteins (Fig. 5A). STRING suggested that the 29 proteins can interact with one another via 269 interactions (edges) (Fig. 5B).

Proteins linked to CRC and potentially targeted by triptolide participate in several canonical pathways involved in a range of biological activities. To demonstrate the ability of our integrative bioinformatic approach to propose specific protein targets for further mechanistic studies, we selected the top pathway in the IPA categories “molecular mechanisms of cancer” and “colorectal cancer metastasis signaling” that were linked to CRC and targeted by triptolide. Several nodes in this pathway emerged as potential direct targets of triptolide in CRC: JUN, FOS, CASP3, BCL2, IFNG, and VEGFA (Fig. 5C and D). Combining these results with STRING analysis, we identified JUN, FOS, CASP3, BCL2, IFNG, and VEGFA as particularly likely targets of triptolide in CRC.

3.4. Predicted binding of triptolide to target proteins in CRC

To further validate candidate triptolide targets in CRC, we tested the precision of docking between triptolide and the following potential target proteins by YASARA software (Fig. 6): (A) CASP3 (PDB: 5IAN), (B) BCL2 (PDB: 6GL8), (C) VEGFA (PDB: 4KZN) and (D) IFNG (PDB: 1EKU). As shown in Fig. 6, triptolide binds to the active sites of these target proteins and interacts with several amino acid residues, with most interactions being hydrophobic.

For instance, in the combination of triptolide with VEGFA, there are different hydrophobic interactions between triptolide and residues of VEGFA such as Gln-22, Tyr-25, His-27 and Pro-28. In addition, triptolide can form a hydrogen bond with a length of 1.8 Å and a bond energy of 8.40 kJ/mol with the Arg-207 residue of CASP3, and its aromatic ring forms a π - π interaction with the aromatic ring in Phe-256 of CASP3. Overall, these results provide further evidence that these four proteins may act as triptolide targets in CRC.

4. Discussion

The network pharmacology approach is relatively new and was first proposed by Li et al. in 2014 [28]. Because it provides a more complete understanding of network theory and systems biology, it has been considered the next paradigm in drug discovery [29, 30]. Network pharmacology has been used to study pathways of interaction between drugs and proteins or genes and diseases, and it is capable of describing complexities among biological systems, drugs and diseases from a network perspective [31-34]. Therefore, the development of network pharmacology techniques that can predict multiple drug-target interactions may hold the key to future drug discoveries in complex diseases such as CRC. In this work, we integrated information from publicly available databases to predict interactions between triptolide and its potential targets in CRC, as well as the signaling pathways and networks involved. Pathway analysis suggested that triptolide regulates the activation of neuroinflammation signaling, glucocorticoid receptor signaling, Th cell differentiation, Th1 and Th2 activation, and metastasis signaling in CRC.

These results are consistent with several *in vitro* and *in vivo* studies. Previous work [35] suggested that triptolide is able to induce G1 cell cycle arrest by inhibiting transcriptional activation of E2F1. Triptolide was found to reduce both tumor number and tumor size in mice carrying mutations to promote growth of adenomatous polyposis coli as well as in mice treated with azoxymethane/dextran sodium sulfate to induce cancer[36]. Triptolide effectively inhibits CRC cell proliferation, colony formation, and organoid growth *in vitro*, and these effects are associated with down-regulation of target genes transcribed by RNA polymerase III. Moreover, triptolide inhibits cyclooxygenase-2 and inducible nitric oxide synthase expression in human colon cancer [37].

Our study presents several limitations. Although we predicted and verified potential triptolide target proteins by molecular docking, our results are based on bioinformatic predictions and require experimental confirmation. As next steps, we intend to use triptolide to treat CRC cells, identify its targets using transcriptomics and proteomics, and thereby elucidate its mechanisms of action.

In summary, our results predict that the therapeutic effects of triptolide in CRC are mediated, at least in part, via JUN, FOS, CASP3, BCL2, IFNG, and VEGFA. These results may be useful in guiding further research to determine the molecular targets of triptolide in CRC, and they may inspire further application of network pharmacology to drug discovery.

Declarations

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Authors' contributions

Xinqiang SONG conceived and designed the study, and wrote the manuscript.

Yu Zhang, Erqin Dai, Qiyue Zhang, Nongyi Zheng, Lei Wang, Hongtao Du analyzed the data.

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

Triptolide-targets,
pathways and networks

Proteins, pathways and networks
associated with colorectal cancer

Molecular docking of triptolide on
potential target proteins

Shared pathways, networks and targets
of triptolide on colorectal cancer

Figure 1

Flow diagram of network-based analysis of possible molecular mechanisms by which triptolide acts in colorectal cancer.

Figure 2

Pathways and networks of genes and proteins associated with colorectal cancer (CRC). (A) Top ten pathways in proteins associated with CRC. The yellow threshold line indicates $p=0.05$. (B-C) Representative networks of proteins associated with CRC. For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this article.

Figure 3

Enriched functional categories in colorectal cancer (CRC)-associated genes. (A) Dot plot showing the top 15 enriched biological processes with adjusted p-values analyzed by clusterProfiler. The color scale indicates the adjusted p-value, and the dot size represents the gene count in each term. (B) Interaction networks between enriched biological processes analyzed by enrichMap in the clusterProfiler package. The color scale indicates the adjusted p-value, and the dot size represents the gene count in each term.

(C) Sub-network showing important genes in the top three Gene Ontology (GO) terms. The subnetwork depicts the relationships among the three GO terms and CRC-associated genes. For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this article.

Figure 4

Pathways and networks of predicted triptolide target proteins. (A) Top ten pathways in predicted triptolide target proteins. The yellow threshold line indicates $p=0.05$. (B-C) Representative networks of predicted triptolide target proteins. For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this article.

cancer” and (D) “colorectal cancer metastasis signaling” that have been linked to CRC and are predicted to be targeted by triptolide. Proteins likely to be targeted by triptolide are marked with purple boxes. Triangles mean proteins with enzymatic activity and circles mean proteins without enzymatic activity.

Figure 6

Molecular models of triptolide binding to the predicted target proteins: (A) CASP3 (PDB: 5IAN), (B) BCL2 (PDB: 6GL8), (C) VEGFA (PDB: 4KZN) and (D) IFNG (PDB: 1EKU). The blue dashed lines indicate H-bonds and the red dashed lines indicate π - π interactions, with interaction distances indicated above the lines. The thick sticks represent the triptolide molecule, and the thin lines represent residues in the protein binding site. For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this article.