Mechanism Analysis of Coix Seed in Gastric Cancer Treatment Based on Biological Network Modules

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Abstract
Coix seed, the mature seed of *Coix lacryma-jobi* L., is a traditional herb widely used in various cancer adjuvant treatments; however, its mechanism is unknown. The aim of this study was to reveal the multitarget mechanisms of Coix seed in the treatment of gastric cancer (GC) by biological network and modular analysis methods. Forty-one ingredients and 482 targets of Coix seed and 165 GC-related genes were obtained from databases. Twelve on-target genes (*AICDA*, *CASP3*, *EP300*, *ERBB2*, *FGFR2*, *IL12A*, *IL12B*, *IL1B*, *LOX*, *TJP1*, *TP53*, and *TRIB3*) of Coix seed overlapped with GC-related genes. Using compound-target and protein–protein interaction network analyses, we discovered the core targets of Coix seed. Markov cluster algorithm-based modular analysis identified 5 potential module targets of Coix seed for GC. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated the vast actions of Coix seed, which involve pathways in cancer, the cell cycle, receptor signal transduction, deoxyribonucleic acid damage response, transcriptional regulation, apoptosis, and cell connections. This study elucidated the potential mechanisms of Coix seed on GC, which may lead to the development of an effective drug. Additionally, this study showed the feasibility of network and modular analysis methods to investigate traditional Chinese medicinal herbal mechanisms and may provide a new angle for further research in the field of anticancer mechanisms and multitarget drugs.

Keywords
biological network, coix seed, gastric cancer, targeted module

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Introduction
Gastric cancer (GC) is the fourth most common disease worldwide, with high global incidence and mortality rates.¹² Surgical treatment is limited by the low early diagnosis rate, and chemotherapy is limited by its side effects and drug resistance.³⁴ Although novel biochemical targeting agents have been used to improve the outcomes of patients with GC, the efficacy of these agents for advanced and recurrent GC remains unsatisfactory.⁵ Therefore, traditional Chinese medicine (TCM) is an important alternative treatment for patients with GC, especially for those in the advanced stage.⁶ Many TCM herbs and their extracts have antitumor effects; however, understanding the multitarget molecular mechanisms of the herbs is challenging.⁷

Coix seed (Semen Coicis), the mature seed of *Coix lacryma-jobi* L., is used as both a food and drug in TCM. As a drug, Coix seed has been traditionally used to treat cancers.⁸ The active components of Coix seed include sterols, triterpenoids, polysaccharides, oils, and starches.⁹¹⁰ These components have anticancer, anti-inflammatory, antiproliferative, and antiallergic activities.⁸ Extracts of Coix seed significantly suppress the growth of GC cells.¹¹ Kanglaite Injection, mainly composed of Coix seed oil, enhances the efficacy and reduces the side effects of chemotherapy in patients with GC.¹²¹³ Kanglaite Injection is efficacious in the treatment of various cancers and...
improves the quality of life; however, its mechanisms are unclear.

The holistic philosophy of TCM shares basic ideas with network biology and network pharmacology. As a rational strategy, network-based methods are widely applied in TCM research, including studies on formula compatibility, target prediction, and pharmacology. In this study, biological network and modular analysis methods were used to explore the potential targets and action mechanisms of Coix seed for the treatment of GC to explain the potential mechanisms behind the antitumor activity of Coix seed from the system and modular biology network levels.

Materials and Methods

Datasets

The composite compounds of Coix seed were obtained from the Chinese Medicine and Chemical Composition Database (http://www.sioc.ac.cn/) and Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://lsp.nwsuaf.edu.cn/tcmsp.php), which are specifically designed to store information on the active components of Chinese herbs. In total, 41 compounds of Coix seed were collected after the removal of duplicated records. For each compound, we collected related gene targets from the TCMSP and Comparative Toxicogenomics Database (CTD, http://ctdbase.org). The targets obtained from TCMSP were either recorded in DrugBank (https://www.drugbank.ca/) or experimentally validated, and targets from CTD were bound to or regulated by Coix seed compounds, with at least 1 reference to show this. Then, GC-related genes were collected from Online Mendelian Inheritance in Man (www.omim.org/) and Human Phenotype Ontology (http://human-phenotype-ontology.github.io) databases. The Medical Subject Heading term “Stomach Neoplasms” was used as the keyword. Detailed information on Coix seed compounds and GC-related genes is listed in Supplemental Table S1.

Gene Set Comparison Analysis

The Coix seed targets and GC-related gene sets were compared and overlapping genes were detected, which were defined as the on-target genes. The similarity of the 2 gene sets was analyzed by Jaccard’s similarity coefficient, which is a pair-wise similarity tool defined as the intersection size divided by the union size of 2 sample sets. All targets were converted to the canonical gene symbol. A higher Jaccard’s similarity coefficient means a higher proportion of on-target genes.

Network Construction

Known protein–protein interactions (PPIs) were downloaded from the Human Protein Reference Database (http://www.hprd.org) and BioGRID (http://thebiogrid.org). The species was restricted to Homo sapiens and 216,938 existing PPIs were obtained. We obtained gene target networks by mapping Coix seed target genes to the PPI dataset with official gene symbols. Cytoscape 3.0.0 was used to visualize and analyze the Coix seed compound-gene network and gene target network and topological properties were analyzed by the NetworkAnalyzer plug-in.

Network Module Identification

The disease mechanism and drug action have a modular basis in network biology, as genes and proteins interact in a network to execute certain functions. To understand better the underlying gene interactions and cellular functional mechanisms, we identified the densely connected multitarget modules of Coix seed. A Markov cluster algorithm (MCL) was used to identify the target modules of Coix seed. The minimum module size was set as 3, all edges were assumed to be unidirectional, and the inflation value was set as 2.

Module Functional Enrichment Analysis

To characterize the function of the Coix seed target network and modules, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID 6.8) to elucidate the pharmacological mechanisms of Coix seed. For the network and each module, an overrepresentation of a functionally relevant annotation was defined by a modified Fisher's exact P-value with a Benjamini–Hochberg adjustment. The enrichment background was selected as “Homo sapiens”, and GO terms and pathways with a P < 0.05 were considered significant.

Results

Similarity of Coix Seed Target Genes and GC Gene Sets

Using the databases, 26 compounds and 482 gene targets of Coix seed and 165 GC-related genes were obtained. Among the target and GC-related genes, 12 overlapping genes were identified (ALCDA4, CASP3, EP300, ERBB2, FGFR2, IL12A, IL12B, IL1B, LOX, TJP1, TP53, and TRIB3), that is, the on-target genes. The Jaccard’s similarity coefficient of the 2 datasets was 0.019. This showed the multitargeted regulating effect of Coix seed in the treatment of GC.

Coix Seed Compound-Targets Network

Based on the compounds and their targets, we constructed the compound-target network of Coix seed, as shown in Figure 1. This network consisted of 523 nodes (26 components and 481 genes) and 858 edges. Among the gene nodes, GC-related CASP3 had the highest degree, as 7 compounds of Coix seed acted on it. The other high-degree genes included NCOA2
(14), PTGS2 (14), PTGS1 (12), TNF (10), ADH1C (8), and PPARA (8). These genes may have important roles in the pharmacological action of Coix seed.

**PPI Network of Coix Seed Gene Targets**

By mapping the Coix seed target genes to known PPI interactions, we obtained the Coix seed PPI network, which consisted of 120 nodes and 137 edges (Figure 2). The network density was 0.02, clustering coefficient was 0.01, connected components value was 13, network heterogeneity was 1.09, characteristic path length was 3.93, and average number of neighbors was 2.02. The hub nodes included RXRA, MAPK3, BCL2, and CDK1.

**Target Module of Coix Seed in GC Treatment**

Based on the Coix seed PPI network, the MCL algorithm was used to identify the target modules of Coix seed. Sixteen modules (nodes ≥3) were obtained, the largest consisting of 11 nodes. We labeled these 16 modules as Mod-1 to Mod-16. The detailed information is shown in Figure 3. Five modules included on-target genes related to GC. The 5 modules were 3, 4, 7, 12, and 14 and the on-target genes were CASP3, EP300, IL1B, TJPI, and TP53, respectively. Through a literature analysis, we found that 41 of 75 genes in the modules were reported to be risk factors of Helicobacter-induced GC.

**Biological Functions of Targeted Modules**

To characterize the biological functions of the Coix seed target network and modules, we performed GO and KEGG pathway enrichment analysis for the 5 on-target modules that had overlapping genes. All significant 237 GO terms and 106 KEGG pathways of the Coix seed target network are listed in Supplemental Table S2. The most significant GO term and KEGG pathway were positive regulation of transcription from ribonucleic acid (RNA) polymerase II promoter and pathways.
in cancer, respectively. In Table 1, we show the 5 most significant GO functions and KEGG pathways of each target module. The compounds of Coix seed were mainly involved in the deoxyribonucleic acid (DNA) damage response, transcription factor binding, and the apoptotic process according to GO and pathways in cancer and the cell cycle, according to KEGG. In the 5 most significant pathways in Mod-4, Mod-14 genes were directly enriched in cancer-related pathways.

Figure 2. Protein–protein interaction network of Coix seed gene targets. Node degree from high to low is labeled by deep to light red.
Discussion

In this study, biological network and modular methods were used in the analysis of the multitarget pharmacological mechanisms of Coix seed in the treatment of GC. Twelve overlapping genes were identified between Coix seed compound targets and GC-related genes; thus, these may have important roles in GC development and therapy. Based on the Coix seed PPI network, 5 modules with GC-related genes were identified that may be Coix seed target modules for GC therapy. The functional analysis of the target modules revealed that the pharmacological actions of Coix seed in GC treatment may involve pathways in cancer, the DNA damage response, transcription factor binding, the apoptotic process, and the cell–cell junction. Our results may provide new clues for further exploration of the antitumor mechanism of Coix seed.

Among the module target genes, CASP3 was the highest degree node in the Coix seed compound-target network. CASP3 mutations play an important role in the development of several human tumors, including GC.25 Another overlapping module gene, EP300, functions as histone acetyltransferase and is an important modulator of chromatin remodeling. Frameshift mutations of EP300 and its expressional loss may be a feature of gastric and colorectal cancers with high microsatellite instability.26 Gene polymorphisms of IL1B, IL12A, and IL12B affect gastric mucosal carcinogenesis in patients with Helicobacter pylori infection.27,28 The overlapping TJP1, TP53, and TRIB3 genes are also closely related to the treatment of GC.29-31 This suggests that Coix seed can regulate the development of GC through these targets.

Using the PPI network and functional enrichment analysis, several hub genes and important pathways of Coix seed were identified. Fifty-five percent of the identified module genes had important roles in the development and progression of H. pylori-associated GC (Figure 3). The target gene IL1B upregulates the hub gene RXRA via the activation of nuclear factor-kappa-light-chain-enhancer of activated B cells signaling,32 which suggests the possible clinical significance of hub genes in the diagnosis and treatment of GC. The hub gene MAPK3 is an independent prognostic marker for patients with resected GC.33 Several enriched pathways are involved in the development or treatment of GC, such as pathways in cancer, cell adhesion, p53, and Wnt/beta-catenin.34-36 These indicate the efficacy of the identified key genes and pathways for GC treatment.

Figure 3. Modules of the Coix seed protein–protein interaction network. Blue nodes represent target genes related to gastric cancer (GC). Red font represents genes associated with the development and progression of Helicobacter pylori-associated GC.
Systems biology and network research are holistic and systemic. TCM is guided by systems science to regulate disease via multiple components and targets. The study of systems biology, network research, and TCM has resulted in new concepts and strategies in the research of Chinese herbal compound action. Modular network analysis may also provide further insight into the multitarget pharmacological mechanisms of drugs at the network and systematic levels.

In conclusion, this study identified the gene targets of Coix seed in GC and potential pathways behind the therapeutic effect of Coix seed in GC. This showed the feasibility of network and modular analysis methods to investigate TCM herbal mechanisms and may provide a new angle for further research in the field of anticancer mechanisms and multitarget drugs.

Table 1. Top 5 Significant Enriched GO and KEGG Biological Functions of Coix Seed On-Target Modules.

| Module ID | GO functions | P-value | KEGG pathways          | P-value |
|-----------|--------------|---------|------------------------|---------|
| Mod-3     | GO:0006977—DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest | 2.52E-09 | hsa04110: Cell cycle | 2.96E-11 |
|           | GO:0034644—cellular response to ultraviolet | 1.09E-04 | hsa04115: p53 signaling pathway | 1.19E-07 |
|           | GO:0005654—nucleoplasm | 4.38E-04 | hsa04010: mitogen-activated protein kinase signaling pathway | 9.15E-04 |
|           | GO:0006461—protein complex assembly | 6.97E-04 | hsa04068: FoxO signaling pathway | 5.32E-03 |
|           | GO:0005634—nucleus | 7.03E-04 | hsa05161: Hepatitis B | 6.21E-03 |
| Mod-4     | GO:0037113—transcription coactivator activity | 2.58E-07 | hsa05211: Renal cell carcinoma | 2.60E-04 |
|           | GO:008134—transcription factor binding | 4.44E-07 | hsa04919: Thyroid hormone signaling pathway | 8.01E-04 |
|           | GO:0045944—positive regulation of transcription from ribonucleic acid polymerase II promoter | 6.99E-07 | hsa04310: Wnt signaling pathway | 1.17E-03 |
|           | GO:0036382—chromatin binding | 1.64E-06 | hsa05161: Hepatitis B | 1.29E-03 |
|           | GO:005667—transcription factor complex | 1.27E-05 | hsa05164: Influenza A | 1.86E-03 |
| Mod-7     | GO:0001666—response to hypoxia | 1.34E-04 | hsa05134: Legionellosis | 1.59E-02 |
|           | GO:0032611—interleukin-1 beta production | 7.79E-03 | hsa04621: NOD-like receptor signaling pathway | 1.84E-02 |
|           | GO:0071310—cellular response to organic substance | 5.66E-02 | hsa04623: Cytosolic deoxyribonucleic acid-sensing pathway | 2.39E-02 |
|           | GO:0033198—response to adenosine triphosphate | 6.65E-02 | hsa05133: Pertussis | 2.16E-02 |
|           | GO:0007566—embryo implantation | 5.24E-03 | hsa05132: Salmonella infection | 1.56E-02 |
| Mod-12    | GO:001327—apical-lateral plasma membrane | 3.75E-05 | hsa04530: Tight junction | 3.90E-04 |
|           | GO:0005923—bicellular tight junction | 2.14E-03 | hsa04670: Leukocyte transendothelial migration | 3.39E-02 |
|           | GO:0007043—cell junction assembly | 3.28E-02 | hsa05160: Hepatitis C | 3.81E-02 |
|           | GO:0005911—cell junction | 1.87E-02 | hsa04514: Cell adhesion molecules | 4.07E-02 |
|           | GO:0019904—protein domain specific binding | 5.06E-02 | | |
| Mod-14    | GO:0034349—glial cell apoptotic process | 7.15E-04 | hsa05200: Pathways in cancer | 3.23E-03 |
|           | GO:0032025—response to cobalt ion | 9.53E-04 | hsa05014: Amyotrophic lateral sclerosis | 1.44E-02 |
|           | GO:0008635—activation of cysteine-type endopeptidase activity involved in apoptotic process by cytochrome c | 1.07E-03 | hsa05134: Legionellosis | 1.56E-02 |
|           | GO:0097153—cysteine type endopeptidase activity involved in apoptotic process | 1.59E-03 | hsa05416: Viral myocarditis | 1.64E-02 |
|           | GO:0030220—platelet formation | 2.14E-03 | hsa05210: Colorectal cancer | 1.79E-02 |

GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Declaration of Conflicting Interests
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