Systematic elucidation of the mechanism of geraniol via network pharmacology

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Background: Geraniol is an acyclic monoterpene alcohol, which is extracted from the ethereal oils of aromatic plants. A systematic analysis of its mechanism of action has not yet been carried out.

Methods: In this study, the druggability of geraniol was assessed via Traditional Chinese Medicine Systems Pharmacology Database (TCMSP), and the potential targets of geraniol were identified using the Comparative Toxicogenomics Database (CTD). Additionally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using WebGestalt. Drug-target-pathway networks were constructed using Cytoscape to give a visual view.

Results: Our findings showed that geraniol has superb druggability with 38 putative identified target genes. GO, KEGG, and network analyses revealed that these targets were associated with cancer, inflammatory immunoreactions, and other physiological processes.

Conclusion: Geraniol is predicted to target multiple proteins and pathways that shape a network which can exert systematic pharmacological effects.

Keywords: geraniol, druggability, target prediction, enrichment analysis, network pharmacology

Introduction

Natural products and traditional Chinese medicine (TCM) are the most abundant resources of active compounds for drug discovery. Monoterpenes, for example, are dietary compounds extracted from the ethereal oils of many vegetables, fruits, and especially TCM. Geraniol (Figure 1A) is an acyclic monoterpene alcohol, which is found in the ethereal oils of aromatic plants.¹ Geraniol has been shown to exert a wide spectrum of pharmacological activities, for example anti-inflammatory, antimicrobial, antitumor, and so on.²⁻⁵ Close attention has been paid to geraniol due to its potential role in the treatment of a variety of diseases, such as chronic or allergic rhinitis, lung cancer, etc.⁶⁻⁸ These results suggest that geraniol can be utilized as a valuable chemical probe or a chemical moiety for the dissection of complex biological processes, discovery of hidden molecular relationships, and identification of therapeutic target molecules and pathways. Accordingly, the molecular mechanisms which geraniol induces and the resulting changes in cellular phenotypes are rarely studied. Meanwhile, employment of computational methodologies for the identification of drug targets and the underlying mechanisms is becoming mainstream in order to save money, time, and effort.^{9,10} In particular, computational target identification and following the molecular mechanisms can accelerate the drug discovery and drug design processes.

Therefore, we elucidated the pharmacological actions of geraniol systematically using computational methodologies. First, the druggability of geraniol was assessed

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Figure 1 (A) Chemical structure of geraniol downloaded from the PubChem database (CID: 637566); (B) Workflow for the identification of potential geraniol target genes that integrates ADME evaluation, chemical-gene interaction, GO and KEGG pathway analyses and network construction.

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CTD, Comparative Toxicogenomics Database.

using the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) server.¹¹ Next the potential candidate target genes were predicted by chemical-gene interaction analysis.¹² Furthermore, gene ontology and pathway analyses were investigated using the identified target genes. Finally, drug-target network was constructed to provide a systematic overview of the potential target genes and the mechanism of action for geraniol. A schematic diagram of the analysis procedures for geraniol target gene prediction is shown in Figure 1B.

Materials and methods

Assessment of pharmacokinetics properties using TCMSP database (<http://lsp.nwu.edu.cn/tcmsp.php>) is a resource of systems pharmacology for TCMs or related compounds.¹¹ It can provide information on the absorption, distribution, metabolism, and excretion (ADME) properties of a drug with potential biological effects at a systematic level, for example, oral bioavailability (OB), drug likeness (DL), Caco-2 permeability (Caco-2), blood-brain barrier (BBB), and so on.^{13,14}

Of all pharmacokinetics properties, OB is the foremost feature of orally administered drugs, since it acts as a vital role in evaluating the efficacy of the drug distribution to the systemic circulation. In TCMSP database, OB was calculated on OBioavail1.1 based on an in-house model.^{11,13} For orally administered drugs, the movement across the intestinal epithelial barrier is one of the biggest obstacles of human absorption and its bioavailability.^{11,13} In present study, the chemical name “geraniol” was entered to the search box

Table 1 Putative targets of geraniol

Num.	Gene ID	Gene symbol	Gene name
1	1260	CNGA2	Cyclic nucleotide-gated channel alpha 2
2	1350	COX7C	Cytochrome c oxidase subunit 7C
3	1537	CYC1	Cytochrome c1
4	1555	CYP2B6	Cytochrome P450 family 2 subfamily B member 6
5	2099	ESR1	Estrogen receptor 1
6	2100	ESR2	Estrogen receptor 2
7	2554	GABRA1	Gamma-aminobutyric acid type A receptor alpha1 subunit
8	2560	GABRB1	Gamma-aminobutyric acid type A receptor beta1 subunit
9	2641	GCG	Glucagon
10	26762	HAVCR1	Hepatitis A virus cellular receptor 1
11	2769	GNA15	G protein subunit alpha 15
12	2774	GNAL	G protein subunit alpha L
13	2936	GSR	Glutathione-disulfide reductase
14	3162	HMOX1	Heme oxygenase 1
15	3265	HRAS	HRas proto-oncogene, GTPase
16	3575	IL7R	Interleukin 7 receptor
17	3576	CXCL8	C-X-C motif chemokine ligand 8
18	3949	LDLR	Low density lipoprotein receptor
19	43	ACHE	Acetylcholinesterase
20	4790	NFKB1	Nuclear factor kappa B subunit 1
21	4953	ODC1	Ornithine decarboxylase 1
22	5111	PCNA	Proliferating cell nuclear antigen
23	5594	MAPK1	Mitogen-activated protein kinase 1
24	5595	MAPK2	Mitogen-activated protein kinase 2
25	5743	PTGS2	Prostaglandin-endoperoxide synthase 2
26	581	BAX	BCL2 associated X, apoptosis regulator
27	5894	RAF1	Raf-1 proto-oncogene, serine/threonine kinase
28	596	BCL2	BCL2, apoptosis regulator
29	7083	TK1	Thymidine kinase 1
30	7157	TP53	Tumor protein p53
31	7298	TYMS	Thymidylate synthetase
32	7442	TRPV1	Transient receptor potential cation channel subfamily V member 1
33	836	CASP3	Caspase 3
34	8383	OR1A1	Olfactory receptor family 1 subfamily A member 1
35	8390	OR1G1	Olfactory receptor family 1 subfamily G member 1
36	841	CASP8	Caspase 8
37	842	CASP9	Caspase 9
38	847	CAT	Catalase

and its pharmacokinetic properties were investigated at the molecular level.

Target identification by the Comparative Toxicogenomics Database

The Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>), is a robust, publicly available database for toxicogenomic information. It provides manually curated key information about chemical-gene/protein interactions,

chemical-disease and gene-disease relationships, from peer-reviewed scientific literature. Currently, CTD includes more than 30.5 million toxicogenomic relationships related to chemicals, proteins, and so on.¹² Given a compound, CTD can provide the corresponding target genes sorted by the interactions between them by descending order. Candidate targets of geraniol were predicted using CTD with default parameters.

Analysis by GeneMANIA

GeneMANIA (<http://www.genemania.org>), is an user-friendly and flexible web server, for generating hypotheses in regard to gene function, analyzing gene lists, and prioritizing genes for functional assays.¹⁵

Given a query list, GeneMANIA can list the genes that have shared properties, or function similarly with the original query. It also shows a functional relationship network, expounding the association among the list as well as curated genomics and proteomics data. The potential candidate target

genes were entered into the search bar after selecting *Homo sapiens* from the organism option, and the results were further collated.

Gene function and pathway enrichment analysis

Web-based gene set analysis toolkit (WebGestalt, <http://www.webgestalt.org/option.php>) can be utilized to thoroughly understand the functional and pathway enrichment information on the gene of interest.¹⁶ Potential candidate targets were input to the WebGestalt server using over-representation enrichment analysis method with the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

GO analysis is a commonly used approach for annotating genes and gene products with functions including molecular function, biological pathways, and cellular components.¹⁷ KEGG is a useful resource for systematic analysis of gene functions and related high-level genome functional information.^{18,19}

Figure 2 Protein network of geraniol. Black nodes represent target proteins, and connecting colors indicate different correlations. Functional associations between targets were investigated using GeneMANIA. Genes in black circles were query terms while these in gray circle indicate genes associated with query genes.

Figure 3 GO map of putative target genes. (A) Biological process categories. (B) Cellular component categories. (C) Molecular function categories.

Network construction

In order to understand the complex relationships among compound, targets, and diseases, we used Cytoscape (v 3.6.1; <https://www.nigms.nih.gov/>) to construct and analyze the three-layer networks.

Results

Pharmacokinetics properties of geraniol

ADME describes the disposition of a pharmaceutical compound and TCMCP provides information on 12 very important characteristics on ADME-related properties like

Figure 4 KEGG pathway analysis of putative target genes.

Figure 5 Geraniol-target-pathway network.

Caco-2, human OB, BBB, and Lipinski's rule of five for drug screening and evaluation.¹¹ AMDE-related properties of geraniol were thoroughly investigated using TCMSP. Notably, the OB of geraniol was calculated to be 23.93%.

Targets identification of geraniol

Potential targets of geraniol were predicted using the CTD as described in the "Materials and methods" section.¹² In total, 41 candidate target genes were identified by CTD. Afterwards, we filtered these genes by using the threshold

chemical-gene interaction ≥ 1 and removed the non-human genes. Finally, 38 unique target genes for geraniol remained (Table 1). These 38 identified interacting genes were used for further investigation.

GeneMANIA analysis

Among the 38 targets and their interacting proteins, it was found that 37.05% had physical interactions, 20.16% exerted co-localization, and 19.43% displayed similar co-expression characteristics. Other results, including pathway,

Table 2 Pharmacological and molecular properties of geraniol

Name	MW	AlogP	Hdom	Hacc	OB (%)	Caco-2	BBB	DL	FSAF	TPSA	RBN
Geraniol	154.28	2.93	1	1	23.93	1.19	1.14	0.02	0.27	20.23	4

Abbreviations: Caco-2, Caco-2 permeability; OB, oral bioavailability; DL, drug likeness; BBB, blood–brain barrier.

shared protein domains and genetic interactions, are shown in Figure 2.

GO and pathway analysis

In order to study further the 38 identified target genes, GO and KEGG enrichment analyses were carried out using WebGestalt. As shown in Figure 3, the top seven functions were used as response to stimulus (35/38), biological regulation (33/38), metabolic process (31/38), membrane (30/38), protein binding (30/38), multicellular organismal process (29/38), and cell communication (28/38). These functional terms are highly relevant to anti-inflammatory activities, especially for chronic or allergic rhinitis.

As for pathway analysis, the 38 targets participate in 10 KEGG pathways with significant false discovery rate (FDR)-adjusted *P*-value including apoptosis, pathways in cancer, and so on, which were shown in Figure 4.

Network analysis

Based on target and pathway analyses, an entire compound, targets and diseases network was constructed using Cytoscape (v 3.6.1). As shown in Figure 5, this compound, targets, and diseases interaction network has 80 nodes and 129 edges. The red oblong, green inverted triangles, and blue circles correspond to geraniol, target genes, and pathways, respectively.

Discussion

Poor pharmacokinetics and toxicity are the most important causes of costly delays in drug discovery and development. There is, therefore, growing belief that certain features in the drug discovery process should be prioritized.²⁰ In silico analysis can improve predictions and pharmacokinetic modeling, as well as metabolic and toxicity endpoints; all of which accelerate and streamline the drug discovery process.^{9,10}

Lipinski's rule of five can identify some very important drug properties, which should be taken into account for compounds developed with the aim of oral delivery.²¹ The rule of five takes into consideration molecular weights (MWs) <500 Da, a LogP <5, as well as numbers of hydrogen-bond donors and acceptors less than 5 and 10,

respectively. Today, the rule of five is generally referred to as a guideline for drug optimization.²² As shown in Table 2, the pharmacokinetic properties of geraniol meet these requirements, meaning geraniol is a superior candidate for drug development.

In drug discovery, target gene identification is the first step. More and more active compounds or drugs are being shown to interact with multiple genes or proteins.^{23–26} A variety of in silico target identification approaches have been developed and are broadly applied toward this aim. As listed in Table 1, 38 potential targets of geraniol were identified using computational methods. The results of GeneMANIA provided information on physical interactions, co-localization, co-expression as well as shared protein domains, and implied that the targets and their interacting proteins may have identical or similar functions.

We identified an inflammatory role for geraniol in allergic rhinitis. Similarly, Madankumar et al reported geraniol exerts antimicrobial, antioxidant, antitumor, and anti-inflammatory activities via activation of apoptotic pathways.^{6,27} These results closely coincide with our findings from GO and KEGG analyses.

The drug-target network shown in Figure 5 also revealed that geraniol has multiple targets and further indicated that it possesses multiple pharmacological activities. Cho et al have also revealed that geraniol exerts systematic pharmacological effects by targeting multiple proteins and pathways.^{28,29} Multiple target therapeutic medicaments are more effective for the treatment of complex diseases, for instance allergic rhinitis, cancers, and are less vulnerable to adaptive resistance. Hence, geraniol could be a promising resource that may be utilized as chemical moiety, lead compound, or an active ingredient for future drug discovery.

In summary, we would like to underline that geraniol is an active ingredient or a promising compound for the development of a safe and effective multi-targeted anticancer medicament. This study provides novel insight into the perspectives and challenges for geraniol research and its application in future clinical investigation.

Disclosure

The authors report no conflicts of interest in this work.

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