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Research

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Exploration in the mechanism of fucosterol for the treatment of non-small cell lung cancer Based on network pharmacology and molecular docking

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Abstract

Background

It has been demonstrated that fucosterol induces a therapeutic effect on cancer. However, the molecular mechanisms underlying the effects of fucosterol in the treatment of non-small cell lung cancer are still unclear.

Methods

In this study, pharmMapper and GeneCards databases were utilized to gather the prediction of fucosterol targets and NSCLC-related targets. The mechanisms of fucosterol against NSCLC were identified in DAVID6.8 by enrichment analysis of GO and KEGG, and protein-protein interaction data was obtained from Sting

Database. Molecular docking was used to predict the docking of GRB2. Moreover, the relationship of GRB2 expression and immune infiltrates was analyzed by TIMER database.

Results

The results suggest that fucosterol acts against by candidate targets, such as MAPK1, EGFR, GRB2, IGF2, MAPK8 and SRC, which regulate biological processes including negative regulation of apoptotic process, peptidyl-tyrosine phosphorylation, positive regulation of cell proliferation. The Raf / MEK / ERK signaling pathway initiated by GRB2 maybe the most significant pathway for fucosterol to treat NSCLC.

Conclusions

These results show that GRB2 is the key target for fucosterol in the treatment of NSCLC, which laying a theoretical foundation for further research and providing scientific support for the development of new drugs.

Key words: Fucosterol; Non-small cell lung cancer; Network pharmacology; Molecular docking; Ingenuity pathway analysis

Background

Lung cancer is the leading causes of cancer death worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for about 85% of lung cancer[2]. At present, the main treatment methods of NSCLC are chemotherapy, surgery, radiotherapy and targeted therapy [3], but the five-year survival rate as low as 18% and may lead to serious side effects and drug resistance [4, 5]. Therefore, there is an urgent need to develop effective drugs for the treatment of non-small cell lung cancer.

25% of the Earth's total species are consist of marine species. Many of these compounds have special biological activities and chemical structures that can be used as potential medicines for many diseases[6, 7]. Most of these marine plants extracts have been confirmed to be anti-cancer [8, 9], anti-inflammatory[10, 11], anti-viral [12, 13], etc. Marine medicines derived from marine plant extracts have received more and more attention. Brown algae is a kind of macroalgae in the ocean. Fucosterol is a sort of algal phytosterol in ethanol extract of brown algae, which has been proved to have multiple biological activities, including antioxidant[14-16], anti-inflammatory[17-19], anticancer[20], antimicrobial[21], anti-depression[22], etc. Previous research have reported fucosterol on anti-cervical cancer[20], anti-leukemia[23], anti-colorectal cancer[24], etc., but there are few studies on the mechanisms of fucosterol in treating non-small cell lung cancer, and its potential therapeutic targets and related pathways have not been reported in detail.

In the past few years, network pharmacology, as a new discipline based on system biology and multi-direction pharmacology, can effectively implement the predictive analysis of drug action mechanisms[25], identify new drug targets[26], and better explain the mechanism of interactions between bioactive molecules and cellular pathways. Molecular docking technology is an advantageous tool for modernization research and can achieve virtual screening of drugs[27]. Reverse molecular docking refers to the docking of a small molecule drug in a group of potential binding cavities of clinically relevant large molecule targets. Through detailed analysis of its binding characteristics, the potential small molecule targets are identified and ranked according to the binding tightness[28, 29].

In this study, network pharmacology and molecular docking were utilized to elucidate the mechanisms of fucosterol treatment of NSCLC, providing new ideas for the treatment of NSCLC and promoting the development of new drugs.

Materials and Methods

Data preparation

Fucosterol chemical structure

The PubChem database(<https://pubchem.ncbi.nlm.nih.gov/>) was used to retrieve the 3D chemical structure of fucosterol and saved it as “sdf” format which imported into Pymol for minimizing the MM2 energy, and finally reserved as mol2 format.

Prediction of fucosterol targets

Fucosterol's mol2 format was uploaded to the PharmMapper database (<http://www.lilab-ecust.cn/pharmmapper/>) to determine the fucosterol-related predictive targets. Set parameters: Generate Conformers: Yes; Maximum Generated Conformations: 300; Select Target Set: Human Protein Target Only; Number of Reserved Matched Targets: 300. The results of predictive targets with z-score>0 as screening criteria were retained for further study.

Protein name correction

Fucosterol-related predictive targets were input into UniProtKB database (<http://www.uniprot.org/>) which was utilized to collect the official symbols with the species limited to “*Homo sapiens*” by eliminating duplicate and nonstandard targets.

Prediction of targets of fucosterol for NSCLC treatment

NSCLC-related targets were gathered from GeneCards database(<https://www.genecards.org/>) using the phrase “non-small cell lung cancer” as a keyword and the top 500 targets were retained according to the relevance score. The predictive targets of fucosterol were compared with the NSCLC-related targets in BioVenn(<http://www.biovenn.nl/index.php>) to determine the candidate targets of fucosterol for NSCLC treatment.

Protein-Protein interaction data

The data on protein-protein interaction (PPI) were obtained from String database (<https://string-db.org/>) with uploading the candidate targets of fucosterol relevant to the treatment of NSCLC while the species limited to “*Homo sapiens*” and the confidence score > 0.9 (low confidence: score <0.400; medium confidence: 0.400 < score <0.700; high confidence: 0.700 < score <0.900; highest confidence: score > 0.900).

Enrichment analysis

DAVID 6.8 (<https://david.ncifcrf.gov/home.jsp>) was applied to carry out GO enrichment analysis and KEGG pathway enrichment analysis of the candidate targets. First, the species option was selected as “*Homo sapiens*”. Second, four analysis items were selected: KEGG pathway, cellular components, molecular functions, and biological processes. Third, Enriched GO terms and pathways were screened for $P\text{-value} \leq 0.05$ using the Bonferroni correction. Finally, KEGG pathway and the top 20 items of GO with ranking by their P-value based on the third step were chosen for further analysis.

Network construction

Network construction method

The Cytoscape3.7.2 software was utilized for network topology analysis and network visualization. Construct the following networks:(1) Cluster 1 for non-small cell lung cancer network; (2) Fucosterol-NSCLC candidate targets PPI network; (3) important biological processes and candidate targets crosstalk network; (4) KEGG pathway and candidate targets crosstalk network

Cluster for NSCLC network

The disease module is a set of network components that collectively disrupts cellular function and lead to a specific disease phenotype. Owing to the disease module, the functional module and the topology module have the same meaning in the network, the function module is equal to the topology module, and the disease can be considered as interference and destruction of functional module[30, 31].The plug-in MCODE of Cytoscape3.7.2 software was used to analyze the protein interaction network of non-small cell lung cancer to find the dense regions, and the first cluster of results was retained.

Screening of important biological processes

The biological processes of candidate targets enrichment analysis retained based on the above-mention steps were compared and screened with the results obtained by NSCLC the first cluster network enrichment analysis. The relationship between the important biological processes and the candidate targets was visualized through a network.

Screening of KEGG pathways

Results of KEGG enrichment analysis were removed disease and generalized pathways after filtering by the above steps, and the relationship between pathways and the candidate targets was presented by a network.

An Integrated pathway map construction

For exploring the comprehensive mechanism of fucosterol treatment of NSCLC, an integrated pathway map related to NSCLC was established, including the non-small cell lung cancer pathway and the key signal transduction pathways.

Prediction of binding between fucosterol and GRB2

In this work, the initial structure of the ligand-free structure of the GRB2(PDB code: 6SDF), was obtained from X-ray diffraction which in Protein Data Bank and the 3D structure of fucosterol was obtained from Pubchem (compound CID: 5281328). Then, we using AutoDock 4 which based on the standard docking procedure for a rigid protein and a flexible ligand. At first we used pymol2.3 to remove the water molecules and the original ligands from the GRB2, and then used AutoDock4 to add hydrogen and use the vina to dock the GRB2 with fucosterol, and then select the compound with the best binding effect. Finally, we use pymol2.3 to observe and analyze the interaction and binding mode between ligand and receptor.

GRB2 expression and immune infiltrates

In order to explore the correlation between GRB2 expression and immune infiltrates in LUAD, the TIMER database (<https://cistrome.shinyapps.io/timer/>) , a friendly comprehensive tool set for integrated analysis of immune infiltrates across diverse types of cancer, was carried out. There were six immune cells involving in the analysis, including CD4+T cells, CD8+T cells, B cells, neutrophils, macrophages and dendritic cells in the TIMER database. Besides, we performed the association between gene GRB2 and EGFR after adjusting tumor purity.

Prognostic values of GRB2

The correlation between GRB2 expression and survival in lung adenocarcinoma was analyzed by Kaplan-Meier plotter (<http://kmplot.com/analysis/>), a tool for assessing the functions of 54,675 genes and 10,188 tumor tissue samples, including breast cancer, ovarian cancer, lung cancer, and gastric cancer.

Results

Prediction of fucosterol related targets

A diagrammatic overview displaying the different steps of network pharmacology and molecule was illustrated in Figure 1. We identified 210 predictive targets from the Pharmmapper database, and ultimately, we obtained 135 official symbols of fucosterol related targets for limiting to “*Homo sapiens*” in the UniProtKB database after filtering by z-score>0 (Table 1).

Cluster analysis of NSCLC network

Although we selected high-relevance score of NSCLC targets from GeneCards, the NSCLC bio-network is still huge. In order to further analyze biological processes of the function module in NSCLC network, we obtained protein-protein interaction network (PPI) data for NSCLC from the String database, and Cytoscape3.7.2 plug-in MCODE was used for clustering to find out the topology module of NSCLC's PPI network. The cluster 1 was retained since it is the most significant for PPI network of NSCLC (Figure 2). We imported the data of cluster 1 into the DAVID6.8 to discover its GO biological processes which were shown in a bubble chart (Figure 3). Cluster 1 is mainly involved in cell proliferation, apoptosis, cell cycle, angiogenesis, NSCLC gene expression, invasion and migration, signal transduction, and NSCLC related signaling pathways.

Analysis of fucosterol-NSCLC PPI network

In order to construct the interaction network between proteins and dig out the core regulatory genes, fucosterol-NSCLC PPI network of candidate targets was constructed by Cytoscape3.7.2 based on the String database (Figure 4). Fucosterol shares 37 targets with NSCLC, and the network consists of 36 nodes (one of which does not interact with other target proteins) and 177 edges. The color of each node is related to its degree; the darker nodes have the larger value of Degree. The size of the node is linked to its Edge; the bigger nodes have the larger value of Edge Between. Based on the network topology analysis, which business centrality is 0.0261, average node degree is 9.83, average closeness centrality is 0.535, which suggests the presence of a central hub between candidate targets. Cytoscape3.7.2 plug-in cytoHubba was utilized to obtain six hub genes, which between centrality, node degree and closeness centrality as screening conditions. We speculate that these hub genes play a significant role in NSCLC treated fucosterol: EGFR, MAPK8, MAPK1, GRB2, SRC, IGF1 (Table 2).

Gene ontology analysis of candidate targets

GO enrichment allows a better understand of the gene function and biological significance of the candidate targets of fucosterol for NSCLC treatment on a systematic level. To obtain the biological processes, molecular functions, and cellular components of the candidate targets, we performed a GO enrichment analysis which was displayed the top 20 significantly terms($p\text{-value}\leq 0.05$) of each module in Figure 5. It is suggested that the candidate targets could act through protein tyrosine kinase activity, protein phosphatase binding, negative regulation of apoptotic process, peptidyl-tyrosine phosphorylation, positive regulation of cell proliferation in the nucleus, cytosol, extracellular space, nucleoplasm, extracellular region. Among them, 14 vital biological processes directly affecting the most significant cluster 1 of

NSCLC disease module were presented with candidate targets independently (Figure 6). This network diagram reveals candidate targets were mainly involved in cell proliferation and apoptosis, angiogenesis, cell migration and signal transduction, meanwhile it illustrates that hub genes were strongly associated with various biological processes.

Evaluation of target-pathway network

As showed in Table 3, 37 candidate targets were further mapped to 73 pathways for more intuitive explain the mechanism of fucosterol in treating NSCLC at the pathway level. Cytoscape3.7.2 was utilized to construct the T-P network which showed the relationship between 37 candidate targets and the pathways that getting rid of generalized and disease terms (Figure 7). It is found that some targets are mapped to multiple pathways and multiple targets also regulated various pathways, which suggest that candidate targets may mediate interaction and crosstalk of different pathways. These pathways may be the major factor for fucosterol's resistance to non-small cell lung cancer, such as PI3K-Akt signaling pathway, VEGF signaling pathway, ErbB signaling pathway. The PI3K-Akt signaling pathway is widely recognized as a prominent cancer signaling pathway, which is closely related to affect the proliferation, survival and apoptosis of NSCLC cells[32-34]. Also, VEGF signaling pathway involves in tumor cell-dependent continuous vascular supply, which has a profound effect on tumor cell growth and metastasis[35]. In addition, for interaction of ErbB receptors with many signal transduction molecules which can activate multiple intracellular pathways, the ErbB signaling pathway plays a significant role in the development of cancer[36]. According to T-P network analysis, we discover fucosterol maybe to regulate NSCLC therapy through PI3K-Akt signaling pathway, VEGF signaling pathway, ErbB signaling pathway by apoptosis, tumor angiogenesis, and cell cycle arrest.

Analysis of an Integrated pathway map

To further explicate the complex mechanism of fucosterol in the treatment of NSCLC, an integrated pathway map (Figure 8) was constructed by integrating the non-small cell lung cancer disease pathway map and the key signal transduction pathways map that obtained from evaluating of T-P network. The key signal transduction pathways map comprises of three signaling pathways: hsa04151: PI3K-Akt signaling pathway, hsa04012: ErbB signaling pathway, hsa04370: VEGF signaling pathway. As shown in Figure 8, the signaling transduction pathways and NSCLC disease pathway reflect multiple modules such as cell proliferation, apoptosis, migration and angiogenesis. From the perspective of non-small cell lung cancer disease pathway (Figure 8A), it was found that the fucosterol treatment of NSCLC can crosstalk the inflammatory module and the tumor module, involving the joint action of multiple pathways.

Fucoesterol can directly activate the Ras signaling pathway, PI3K-Akt signaling pathway, and ErbB signaling pathway, and indirectly activate the MAPK signaling pathway to regulate cell proliferation and apoptosis to achieve the therapeutic effect on NSCLC. From the perspective of the key signal transduction pathways(Figure 8B), it was found that both VEGF signaling pathway and ErbB signaling pathway can activate downstream signals to control angiogenesis, affect cell proliferation and migration, and play a crucial role in the development and metastasis of tumors[37-39]. Meanwhile, the PI3K-Akt signaling pathway participates in the regulation of cell cycle progression[40-42]. According to this map, we found the three signaling pathways can jointly activate the Raf / MEK / ERK signaling pathway which was reported playing an anti-proliferation role in curing cancer[43]. More importantly, the downstream pathway-Raf / MEK / ERK signaling pathway mainly activated by GRB2 to regulate cell proliferation and migration. We will further discuss GRB2 which is a significant target in fucoesterol for treating NSCLC.

The expression of GRB2 and immune infiltrates in NSCLC

The docking mode and hydrogen bonding residues of fucoesterol with GRB2 after docking are shown in Figure 9. Then,the binding sites of ligands and GRB2 and the surrounding residues are shown by pymol in figure 9 A. In the docking model of fucoesterol and GRB2, the action site are ASP-16, LYS-11,LYS-21,Ser-33,ASN-30, which forms hydrogen bonds with Ser-33, while the interaction between ligands and surrounding residues is analyzed in figure 9 B. It was able to be seen that the connection between ligands and GRB2 mainly depends on hydrophobic interaction, only Ser-33 forms hydrogen bonds, and the binding energy is-9.9kcal/mol, indicating that the binding effect between them is still decent. Besides, we conducted the TIMER database to analyze the relationship GRB2 expression and immune infiltrates. As shown in Figure 10A-B, the expression of GRB2 was significantly negatively associated with tumor purity while significantly positively correlated with infiltrating levels of immune cells, CD4⁺T cells ($r = 0.385729$ $P = 1.28e-18$), CD8⁺T cells($r = 0.307506$, $P = 3.99e-12$), B cells($r = -0.196239$, $P = 1.37e-05$), neutrophils($r = 0.541071$, $P = 4.43e-38$), macrophages($r = 0.404903$, $P = 1.47e-20$) and dendritic cells($r = 0.544328$, $P = 5.45e-39$), suggesting that the GRB2 expression was mainly related to the immune infiltration of CD4⁺T cells, CD8⁺T cells, B cells, neutrophils, macrophages and dendritic cells. Besides, the result in KM plotter showed that the lower expression level of GRB2 has a better overall survival rate and gene GRB2 was an independent prognosis indicator for OS of patients with LUAD(Figure 10C-D). Hence, we speculated that GRB2 exerted a more significant effect on the prognosis of LUAD, for it was highly associated with various immune cells in LUAD.

Discussion

Non-small cell lung cancer is characterized by high malignancy, low 5-year survival rate and poor prognosis. However, for patients with advanced lung cancer, having specific predictive biomarkers and receiving targeted therapy or immunotherapy significantly improves quality of life and progression-free survival (PFS) compared to chemotherapy[44-49]. Hence, finding effective targeted drugs to anti-lung cancer has become an urgent problem to be solved. Network pharmacology can analyze and explain the complexity between biological systems, diseases, and drugs from a network perspective, becoming a frontier method of drug discovery[50, 51]. Therefore, the research on the mechanism of fucosterol in the treatment of NSCLC based on network pharmacology may serve as the foundation for the development of targeted drugs for NSCLC in the future.

Based on the reverse of molecular docking technology for predicting fucosterol targets, and compare with NSCLC disease targets, we obtained 37 candidate targets. Fucosterol-NSCLC PPI network analysis uncovered that fucosterol probably exerted pharmacological effects on NSCLC via 37 candidate targets which including 6 hub genes: GRB2, EGFR, MAPK1, SRC, IGF2, MAPK8. According to GO biological process analysis, 37 candidate targets were found to mainly responsible for cell proliferation, apoptosis, migration, signal transduction and angiogenesis. Meanwhile, KEGG analysis of candidate targets disclosed that fucosterol has an effective effect on the treatment of NSCLC mainly via multiple modules such as cell proliferation, apoptosis, migration and angiogenesis. Through the integrated pathway map, we discover that the Raf / MEK / ERK pathway that GRB2 can participate as a trigger or initiating signal is critical to the treatment of NSCLC.

Growth factor receptor-bound protein 2 (Grb2) is a universally expressed adaptor protein that can play a pivotal downstream mediator role in a variety of oncogenes signaling pathways and has a significant effect on signal transduction in normal and cancer cells[52, 53]. Grb2 is a downstream protein of epidermal growth factor receptor (EGFR) that is known to be closely related to NSCLC, and may serve as an adaptor protein to bind to phosphorylated tyrosine in the EGFR known to be closely associated with NSCLC, thereby linking receptor activation to intracellular signaling cascade[54-56]. Recently, research has shown that the balance of Grb2 monomer-dimer is a determinant of its normal and carcinogenic functions[57]. Grb2, which regulates angiogenesis and cell movement, is highly overexpressed in tumors and may be useful as a target for anticancer agents. In the present study, we found Grb2 can be targeted by fucosterol, and activate the Raf / MEK / ERK pathway to achieve the purpose for treating NSCLC[43, 58-60].

Conclusion

In summary, our study indicated the molecular and pharmacological mechanism of fucosterol against NSCLC from a systematic perspective. We unveiled GRB2, as an anticancer target in fucosterol, to initiate the Raf / MEK / ERK pathway for treating non-small cell lung cancer. And, this strategy provides a new idea of anti-NSCLC and lays a foundation for the development of new medicines. Nonetheless, network pharmacology has certain limitations, and more experiments are needed to verify the validity of our findings. Moreover, we hope that our study will be useful for fostering innovative research of marine drugs against cancers.

Abbreviations

NSCLC	Non-small cell lung cancer
GRB2	Growth factor receptor-binding protein 2
KEGG	Kyoto Encyclopedia of Gene and Genome
GO	Gene Ontology
PPI	Protein-protein interaction
TIMER	Tumor IMmune Estimation Resource

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author contributions

LX Luo conceived the idea; XL Li, YS Zheng, ZP Lin, BX Lin, Q Wang contributed to the acquisition, analysis, and interpretation of data. LX Luo and BX Lin wrote the

manuscript; H Luo, L Cui, and LX Luo reviewed the paper and provided comments, and all authors reviewed the manuscript.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1. Diagram of the study design.

Figure 2. Cluster 1 for NSCLC network

Figure 3. Biological process bubble diagram of cluster 1. The color scales indicate different thresholds of adjusted p-values, and the sizes of the dots represent the gene count of each term.

Figure 4. The related targets of NSCLC and predicted targets of fucosterol were screen and combined for constructing a PPI network.

Figure 5. GO analysis of candidate targets. GO enrichment analysis identified genes involved in (A) indicate different thresholds of adjusted p-values, and the sizes of the dots represent the gene count of each term.

Figure 6. Important biological processes and candidate targets crosstalk in the treatment of NSCLC with fucosterol. Node in red is the hub gene, node in blue is targets in fucosterol, and yellow is the specific biological process.

Figure 7. Target-pathway network. The T-P network is built by the candidate targets and a pathway if the pathway is lighted at the target. Yellow nodes represent signaling pathway from enrichment analysis. Blue nodes represent the candidate targets in fucosterol. Red nodes represent the hub genes screened by PPI network.

Figure 8. The integrated pathways map of fucosterol in the treatment of NSCLC include (A) the non-small cell lung cancer pathway, marked in yellow as the functional targets of fucosterol, and (B) the key signal transduction pathways.

Figure 9. The expression of GRB2 and immune infiltrates in NSCLC. (A-B) Correlation of GRB2 expression with immune infiltration level in LUAD. GRB2 expression had significant positive correlations with infiltrating levels of CD4+T cells, CD8+T cells, B cells, neutrophils, macrophages and dendritic cells (C) Relationship between GRB2 and EGFR in LUAD. GRB2 expression showed a strong correlation with EGFR expression after adjusting tumor purity in LUAD. (D) Prognostic values of GRB2 (n=2437) in lung adenocarcinoma (OS in Kaplan–Meier plotter). Line in red with high expression while in black with low expression. Low expression of GRB2 was connected with better over survival.

Figure 10. Simulation results of GRB2, with fucosterol. (A) The docking site of GRB2 and fucosterol. (B) Display of the interaction between GRB2 with fucosterol.

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