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.

1. 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.

2. 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-mentioned 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 used 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.

3. Results

Prediction of fucosterol related targets

A diagrammatic overview displaying the different steps of network pharmacology and molecule was illustrated in Fig. 1. We identified 210 predictive targets from the Phrammapper 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).

Table 1
Prediction targets of fucosterol

Pharma Model	Gene symbol	z-score
3f0r_v	HDAC8	2.82743
1qyx_v	HSD17B1	2.70536
1o6u_v	SEC14L2	2.68742
1n83_v	RORA	2.39228
1a28_v	PGR	2.2175
1ya3_v	NR3C2	2.13319
1s9j_v	MAP2K1	2.12046
1q22_v	SULT2B1	1.98504
1pic_v	PIK3R1	1.88125
1lv2_v	HNF4G	1.83771
1rhr_v	CASP3	1.80882
1osh_v	NR1H4	1.79715
830c_v	MMP13	1.78365
1rbp_v	RBP4	1.71609
1lho_v	SHBG	1.70322
1mrq_v	AKR1C1	1.70301
1sz7_v	TRAPPC3	1.6669
3czt_v	HSD11B1	1.65102
2piw_v	AR	1.6069
1fd0_v	RARG	1.59522
1d4p_v	F2	1.55496
1gse_v	GSTA1	1.54788
1uhl_v	NR1H3	1.52908
1hrk_v	FECH	1.52472
2iiv_v	DPP4	1.50916
1s0z_v	VDR	1.49636
1dkf_v	RARA	1.49513

Pharma Model	Gene symbol	z-score
2i6b_v	ADK	1.38413
1o1v_v	FABP6	1.35079
2shp_v	PRKACA	1.28799
2fjm_v	PTPN11	1.27676
2j14_v	PTPN1	1.27187
1 × 0n_v	PPARD	1.2585
3bbt_v	GRB2	1.25573
1l8j_v	ERBB4	1.23987
2vx0_v	PROCR	1.23974
1he3_v	EPHB4	1.19363
1pq2_v	BLVRB	1.19205
1j99_v	CYP2C8	1.08311
1cbs_v	SULT2A1	1.06101
1sm2_v	CRABP2	1.04514
1upw_v	ITK	1.03078
1qpd_v	NR1H2	1.00159
1uym_v	LCK	0.997933
1hov_v	HSP90AB1	0.978531
1h9u_v	MMP2	0.976261
1u59_v	RXRB	0.97084
1fe3_v	ZAP70	0.963876
19gs_v	FABP7	0.95545
2hzi_v	GSTP1	0.954306
1w6k_v	ABL1	0.954084
1yvj_v	LSS	0.922657
1 m48_v	JAK3	0.917557
1t84_v	IL2	0.909365
1dxo_v	WAS	0.886825

Pharma Model	Gene symbol	z-score
1j78_v	NQO1	0.867991
2p4i_v	GC	0.803103
1iz2_v	TEK	0.79593
1oiz_v	SERPINA1	0.795317
1ln3_v	TTPA	0.794336
1t4e_v	PCTP	0.78729
1xap_v	MDM2	0.775562
1qkt_v	RARB	0.767258
1r9o_v	ESR1	0.742597
1qip_v	CYP2C9	0.733894
1hmt_v	GLO1	0.731703
1bl6_v	FABP3	0.730803
2rfn_v	MAPK14	0.721237
1i7g_v	MET	0.707586
1pmn_v	PPARA	0.678619
1e7a_v	MAPK10	0.644744
2oi0_v	ALB	0.634049
1cg6_v	ADAM17	0.623444
1ma0_v	MTAP	0.608206
1g3m_v	ADH5	0.594411
1xjd_v	SULT1E1	0.557908
2uwd_v	PRKCQ	0.555214
1oj9_v	HSP90AA1	0.540688
1njs_v	MAOB	0.529254
1xvp_v	GART	0.502645
2pjl_v	NR1I3	0.488399
1nhz_v	ESRRA	0.486782
1j96_v	NR3C1	0.454473

Pharma Model	Gene symbol	z-score
2ipw_v	AKR1C2	0.434142
1p49_v	AKR1B1	0.426598
1xbb_v	STS	0.421996
1okl_v	SYK	0.418201
2qu2_v	CA2	0.398063
1ih0_v	BACE1	0.394868
1sa4_v	TNNC1	0.387846
1l6l_v	FNTA	0.385162
2fgi_v	APOA2	0.36325
2pe0_v	FGFR1	0.355588
3hvc_v	PDPK1	0.353361
2h8h_v	SRC	0.34725
2itp_v	EGFR	0.342717
1xor_v	PDE4D	0.333997
1jqe_v	HNMT	0.312737
1shj_v	CASP7	0.309471
2iku_v	REN	0.285831
2pg2_v	KIF11	0.27567
3bgp_v	PIM1	0.259029
2ywp_v	CHEK1	0.245709
1t46_v	KIT	0.241973
1s95_v	PPP5C	0.227167
1itu_v	DPEP1	0.226319
2b53_v	CDK2	0.222033
2jbp_v	MAPKAPK2	0.216487
3cjb_v	KDR	0.212231
1n7i_v	PNMT	0.205326
1egc_v	ACADM	0.198463

Pharma Model	Gene symbol	z-score
1so2_v	PDE3B	0.191704
1l9n_v	TGM3	0.19101
2bxx_v	MAOA	0.187567
1hw8_v	HMGCR	0.158181
1tt6_v	TTR	0.155605
2o9i_v	NR1H2	0.148658
1 × 89_v	LCN2	0.147746
1j4i_v	FKBP1A	0.145396
1xlz_v	PDE4B	0.141599
1s8c_v	HMOX1	0.137498
1tjj_v	GM2A	0.121157
1 mx1_v	CES1	0.098769
2g01_v	MAPK8	0.092468
1reu_v	BMP2	0.090768
1fzv_v	PGF	0.088164
1s1p_v	AKR1C3	0.08785
1r7y_v	ABO	0.071475
1pme_v	MAPK1	0.068987
1g4k_v	MMP3	0.067658
1h6g_v	CTNNA1	0.067305
1vj5_v	EPHX2	0.059538
2nn7_v	CA1	0.049744
2pin_v	THRB	0.011683
1gxr_v	IGF1	0.0063

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 (Fig. 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 (Fig. 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 (Fig. 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).

Table 2
Hub genes of fucosterol against NSCLC

Name	Betweenness Centrality	Closeness Centrality	Degree
GRB2	0.03441407	0.614035	16
EGFR	0.06936033	0.648148	19
MAPK1	0.12911792	0.729167	22
SRC	0.03183796	0.660377	18
IGF1	0.09323304	0.660377	18
MAPK8	0.18473443	0.7	20

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 Fig. 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 (Fig. 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 (Fig. 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.

Table 3
KEGG analysis of candidate targets of fucosterol against NSCLC

Term	Pathways	Count	PValue
hsa05200	Pathways in cancer	21	1.21E-16
hsa05205	Proteoglycans in cancer	17	3.47E-16
hsa04151	PI3K-Akt signaling pathway	15	4.36E-10
hsa04014	Ras signaling pathway	13	5.06E-10
hsa05215	Prostate cancer	11	9.84E-12
hsa04015	Rap1 signaling pathway	11	5.35E-08
hsa04068	FoxO signaling pathway	10	1.42E-08
hsa04510	Focal adhesion	10	5.84E-07
hsa04914	Progesterone-mediated oocyte maturation	9	8.41E-09
hsa04012	ErbB signaling pathway	9	8.41E-09
hsa04915	Estrogen signaling pathway	9	2.36E-08
hsa04550	Signaling pathways regulating pluripotency of stem cells	9	3.57E-07
hsa05203	Viral carcinogenesis	9	6.39E-06
hsa05218	Melanoma	8	4.93E-08
hsa04917	Prolactin signaling pathway	8	4.93E-08
hsa04912	GnRH signaling pathway	8	2.79E-07
hsa04722	Neurotrophin signaling pathway	8	1.85E-06
hsa05161	Hepatitis B	8	6.56E-06
hsa04010	MAPK signaling pathway	8	2.33E-04
hsa05206	MicroRNAs in cancer	8	4.92E-04
hsa05230	Central carbon metabolism in cancer	7	6.71E-07
hsa05214	Glioma	7	7.37E-07
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	7	8.84E-07
hsa05220	Chronic myeloid leukemia	7	1.36E-06
hsa04668	TNF signaling pathway	7	1.38E-05
hsa05219	Bladder cancer	6	1.66E-06
hsa05223	Non-small cell lung cancer	6	8.03E-06

Term	Pathways	Count	PValue
hsa05221	Acute myeloid leukemia	6	8.03E-06
hsa04370	VEGF signaling pathway	6	1.23E-05
hsa05211	Renal cell carcinoma	6	1.81E-05
hsa04664	Fc epsilon RI signaling pathway	6	2.10E-05
hsa04066	HIF-1 signaling pathway	6	1.11E-04
hsa04660	T cell receptor signaling pathway	6	1.35E-04
hsa05231	Choline metabolism in cancer	6	1.41E-04
hsa04114	Oocyte meiosis	6	2.21E-04
hsa04919	Thyroid hormone signaling pathway	6	2.60E-04
hsa04650	Natural killer cell mediated cytotoxicity	6	3.43E-04
hsa05169	Epstein-Barr virus infection	6	3.43E-04
hsa04380	Osteoclast differentiation	6	4.76E-04
hsa05160	Hepatitis C	6	5.10E-04
hsa04062	Chemokine signaling pathway	6	2.30E-03
hsa04810	Regulation of actin cytoskeleton	6	3.89E-03
hsa05213	Endometrial cancer	5	1.28E-04
hsa05210	Colorectal cancer	5	2.54E-04
hsa05131	Shigellosis	5	2.87E-04
hsa05212	Pancreatic cancer	5	3.05E-04
hsa04115	p53 signaling pathway	5	3.43E-04
hsa04520	Adherens junction	5	4.29E-04
hsa04540	Gap junction	5	9.67E-04
hsa04750	Inflammatory mediator regulation of TRP channels	5	1.45E-03
hsa05142	Chagas disease (American trypanosomiasis)	5	1.80E-03
hsa04620	Toll-like receptor signaling pathway	5	1.93E-03
hsa04071	Sphingolipid signaling pathway	5	3.04E-03
hsa04910	Insulin signaling pathway	5	5.01E-03
hsa04630	Jak-STAT signaling pathway	5	5.97E-03

Term	Pathways	Count	PValue
hsa05202	Transcriptional misregulation in cancer	5	9.76E-03
hsa05164	Influenza A	5	1.12E-02
hsa05152	Tuberculosis	5	1.19E-02
hsa04320	Dorso-ventral axis formation	4	3.25E-04
hsa04621	NOD-like receptor signaling pathway	4	2.78E-03
hsa04662	B cell receptor signaling pathway	4	5.03E-03
hsa05133	Pertussis	4	6.34E-03
hsa05145	Toxoplasmosis	4	1.80E-02
hsa04670	Leukocyte transendothelial migration	4	2.02E-02
hsa04110	Cell cycle	4	2.46E-02
hsa04611	Platelet activation	4	2.78E-02
hsa05162	Measles	4	2.95E-02
hsa04921	Oxytocin signaling pathway	4	4.00E-02
hsa04960	Aldosterone-regulated sodium reabsorption	3	1.66E-02
hsa04930	Type II diabetes mellitus	3	2.45E-02
hsa04150	mTOR signaling pathway	3	3.48E-02
hsa04730	Long-term depression	3	3.70E-02

Analysis of an Integrated pathway map

To further explicate the complex mechanism of fucosterol in the treatment of NSCLC, an integrated pathway map (Fig. 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 Fig. 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 (Fig. 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. Fucosterol 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(Fig. 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 fucosterol for treating NSCLC.

The expression of GRB2 and immune infiltrates in NSCLC

The docking mode and hydrogen bonding residues of fucosterol with GRB2 after docking are shown in Fig. 9. Then, the binding sites of ligands and GRB2 and the surrounding residues are shown by pymol in Fig. 9A. In the docking model of fucosterol 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 Fig. 9B. 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 -9.9 kcal/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 Fig. 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 (Fig. 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.

4. 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].

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

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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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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.

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Figures

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 screened 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.