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The Mechanism of Celastrol in the Treatment of Metastatic Lung Adenocarcinoma Revealed by Network Pharmacology and Molecular Docking

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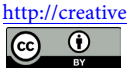

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Abstract

Background: Celastrol is an active ingredient extracted from Traditional Chinese Medicine (TCM), which can restrain the progression of lung cancer, whereas its underlying mechanism is unclear. In our study, the underlying mechanism of celastrol in the treatment of lung adenocarcinoma (LUAD) with metastasis was investigated by network pharmacology and molecular docking. Method: Potential targets of celastrol were collected from TCMSP, Batman-TCM and GeneCard database, and its potential targets were predicted using the STP platform and the TargetNet server. Metastasis marker genes (MGs) were obtained from the HCMDB. The genes correlated with LUAD were gathered from the GeneCard and OMIM database. And the common targets among celastrol potential targets, MGs and LUAD were analyzed. The protein-protein interaction (PPI) networks were obtained from the STRING database. SangerBox and the Xiantao bioinformatics tool were applied to visualize GO and KEGG analysis. Molecular docking tested the binding affinity between celastrol and core genes. Result: A total of 107 targets of celastrol against metastasis LUAD were obtained. The core targets were obtained from the PPI network, namely AKT1, JUN, MYC, STAT3, IL6, TNF, NFKB1, BCL2, IL1B, and HIF1A. GO and KEGG enrichment analysis indicated celastrol for the treatment of metastasis LUAD most refers to cellular response to chemical stress, DNA-binding transcription factor binding, transcription regulator complex and pathways in cancer. And some of these targets are associated with differential expressions and survival rates in LUAD. Moreover, Molecular docking shows celastrol can bind with BCL2 well by hydrogen bond and hydrophobic interaction. Conclusion: This finding roundly expounded the core genes and potential mechanisms of celastrol for the treatment of metastasis LUAD, offering the theoretical basis and antitumor mechanism of TCM in the treatment of lung cancer.

Keywords

Celastrol, Lung Adenocarcinoma, Metastasis, Network Pharmacology, Molecular Docking

1. Introduction

Lung cancer is one of the most highly common cancers and its mortality is the dominating position in the world [1]. It is primarily classified into two types, including small-cell lung cancer (approximately accounts for 15%) and non-small cell lung cancer (NSCLC, approximately accounts for 85%) [2]. NSCLC is to be divided into three classes: large cell carcinoma, squamous cell carcinoma and adenocarcinoma (AD) [3]. AD constitutes about 40% of all lung cancer patients, and is the most frequently histologic type in both smokers and nonsmokers [4]. For the past few years, a mass of new therapeutic methods for AD patients have emerged, including immunotherapy and targeted therapies as monotherapy or in combination with chemotherapy [5]. In spite of the progress of therapy methods, lung carcinoma is still the top cause of cancer-related deaths around the world, representing approximately 10% of all cancer-related deaths [6]. More than 50% NSCLC patients are diagnosed with metastasis, which is the main reason for the high mortality rate [7]. Accordingly, it is urgent to explore the molecular markers to identify metastatic lung cancer early and seek efficient therapeutics to prolong the survival duration. In future cancer treatment, applying new approaches and utilizing the superiority of phytochemicals may be effective in the treatment of lung cancer patients [8].

Celastrol is a pentacyclic triterpenoid compound derived from a classical clinical used Traditional Chinese Medicine (TCM) named *Tripterygium wilfordii* Hook F [9]. Celastrol has various pharmacological effects and exerts therapeutic properties against many diseases, including obesity, systemic lupus erythematosus, infection, and hepatic fibrosis [10] [11] [12] [13]. Furthermore, numerous studies have revealed that celastrol exerts an anti-tumor effect on many cancers by its pro-apoptotic, anti-angiogenic, anti-metastatic, and anti-inflammatory activities [14]. Celastrol has shown enormous therapeutic potential in colorectal cancer, leukemia, and gastric cancer [15] [16] [17]. Celastrol could suppress the proliferation of LUAD cells by regulating non-coding RNAs and cell death pathways [18] [19] [20]. Equally, celastrol increases the targeted drug susceptibility and radiation sensitivity in LUAD [21] [22]. However, researchers have not provided the specific mechanisms of the effect of celastrol on advanced LUAD.

In this study, the correlations among celastrol potential targets, metastasis marker genes (MGs) and LUAD were conducted by network pharmacology. Furthermore, molecular docking technology was used to explore the binding mode and affinity between celastrol and core action targets to get a clearer mechanism. Ultimately, our study can provide potential proof for the clinical use of celastrol in the treatment of metastasis LUAD.

2. Materials and Methods

2.1. Screening and Target Prediction of Celastrol

To clarify the effective targets of celastrol, we gathered all targets of celastrol from the following five databases: including TCMSP [23], BATMAN-TCM [24], GeneCards database [25], the STP database [26], and TargetNet web server [27]. Then removing the duplicated genes, intersecting genes were obtained.

2.2. Determination of Metastasis Marker Genes (MGs) and LUAD Related Targets

MGs were obtained from the Human Cancer Metastasis Database (HCMDB) [28], which contains 1938 genes obtained by collecting metastasis-related expression profiles and analyzing them. The keyword "lung adenocarcinoma" was used to get the LUAD related target in GeneCards [29] and OMIM [30] databases. Finally, cross-targets of celastrol, LUAD and MGs were deemed as pharmacological targets.

2.3. Construction of Protein-Protein Interaction (PPI) Networks

The intersection targets were imported into the STRING database to build PPI network [31]. And using Cytoscape 3.9.0 software to process the PPI network to visualize and screen the core targets [32].

2.4. Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) Enrichment Analysis

GO functional annotations were employed to analyze the top 30 targets ranked by degree value, including the biological processes (BP), cellular component (CC) and molecular function (MF) [33]. KEGG and SangerBox platform was used to gather the target-related pathways [34]. The threshold of significance was set to $p < 0.05$ and FDR < 0.1.

2.5. Core Targets Expressions in LUAD

Xiantao bioinformatics tool (https://www.xiantao.love/products) is an online bioinformatics analysis tool that can analyze RNA expression differences between tumor and normal samples obtained from TCGA database. We performed an unpaired analysis of core targets mRNA expressions in LUAD.

2.6. Survival Analysis

Overall survival was analyzed in the GEPIA database [35]. The samples were divided into high and low expression groups by minimum p-value. Ultimately, survival plots of the core targets in LUAD were obtained.

2.7. Docking of Celastrol with Target Molecules

The 2D structure of celastrol was downloaded from the Pubchem database, and Chem3D software was used to transform it into 3D structure. The 3D structure

of the docking genes was obtained from the PDB database [36]. Then, import the structures of receptor and ligand to AutoDockTools [37] to add hydrogen and other pretreatments. And docking of the celastrol and core genes was conducted to investigate its binding action. PyMol software was used to visualize the docking results [38].

3. Results

3.1. Determination Targets of Celastrol Against Metastasis LUAD

Searching from the following databases: TCMSP, BATMAN-TCM, GeneCards, STP and TargetNet web server, with duplicate targets among databases removed, 227 genes related with celastrol were obtained. 10732 genes of LUAD were obtained from the GeneCards and OMIM databases. 1938 MGs were obtained from the HCMDB. 107 intersection genes were regarded as potential targets and conducted further study (**Figure 1**).

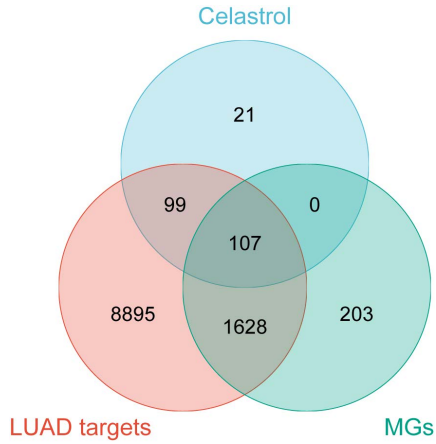


Figure 1. The venn diagram of celastrol MGs and LUAD.

3.2. Screening Core Gene and Analyzing Topological Network

The PPI network of 107 genes was obtained from the STRING platform. Then 107 nodes and 4534 edges were acquired. 10 nodes and 45 edges were obtained under the qualification of degree > 154, namely AKT1, JUN, MYC, STAT3, IL6, TNF, NFKB1, BCL2, IL1B, HIF1A. Further, the PPI network information was imputed into Cytoscape software for visualization, the darker color of the targets meant more significance in the regulatory network (**Figure 2**).

3.3. GO and KEGG Enrichment Analysis

To further investigate the molecular mechanisms of celastrol for the therapy of metastasis LUAD, GO and KEGG enrichment analysis of the top 30 genes ranked by betweenness value were performed. A total of 2432 GO items were obtained, including 2281 BP, 51 CC, and 100 MF. Based on the count, results indicated that celastrol in the treatment of metastasis LUAD mostly involves BP such as gland development, cellular response to chemical stress, response to oxidative stress, ep-

ithelial cell proliferation, response to lipopolysaccharide, cellular response to oxidative stress, response to peptide, response to metal ion, peptidyl-serine modification. These targets pass through DNA-binding transcription factor binding, DNA-binding transcription activator activity, RNA polymerase II-specific DNA-binding transcription factor binding, protein serine/threonine/tyrosine kinase activity, ubiquitin-like protein ligase binding, protein serine kinase activity, protein serine/threonine kinase activity, ubiquitin protein ligase binding, histone deacetylase binding. And they play a role in the transcription regulator complex, membrane raft, membrane microdomain, RNA polymerase II transcription regulator complex, secretory granule lumen, vesicle lumen, cytoplasmic vesicle lumen, nuclear envelope, caveola, plasma membrane raft (Figure 3).

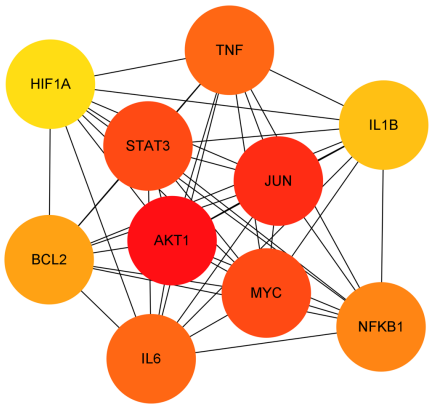
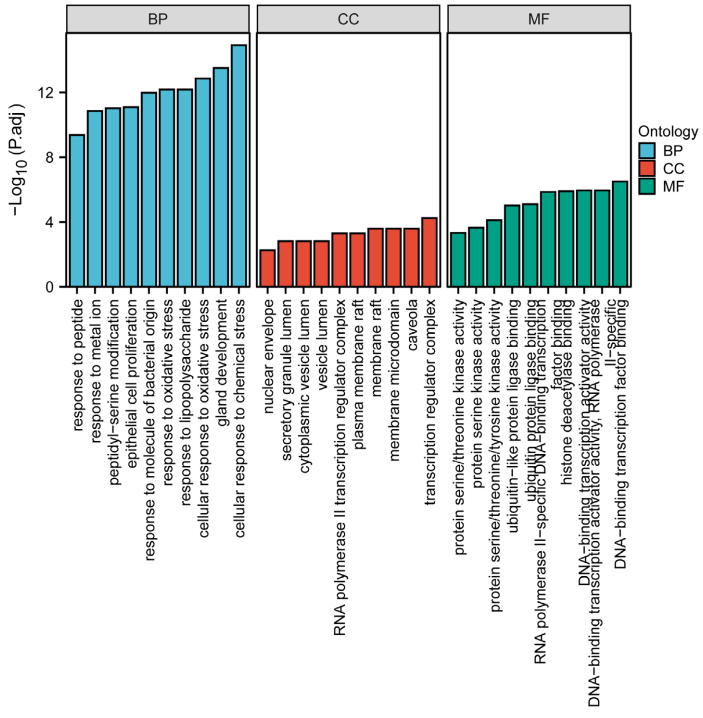
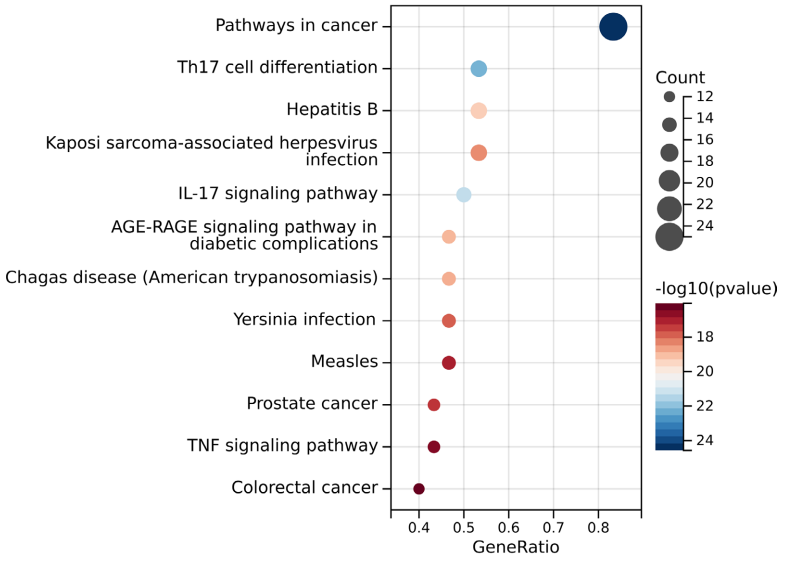


Figure 2. The PPI network of core targets. The darker color of the targets meant more significance.



(A)



(B)

Figure 3. GO functional enrichment and KEGG pathway enrichment analysis of core genes of celastrol against metastasis LUAD. (A) GO functional enrichment analysis, (B) KEGG pathway enrichment analysis.

In addition, the KEGG enrichment analysis for the targets in response to celastrol in the treatment of metastasis LUAD was involved in below pathways, including pathways in cancer, Th17 cell differentiation, hepatitis B, Kaposi sarcoma-associated herpesvirus infection, IL-17 signaling pathway, prostate cancer, AGE-RAGE signaling pathway in diabetic complications, Chagas disease, yersinia infection, measles (**Figure 3**).

3.4. Core Targets Expressions and Survival Analysis in LUAD

Core target expressions and survival analysis of the 8 targets in LUAD were acquired. The gene differential expression p values of seven targets were less than 0.05, namely AKT1, JUN, STAT3, IL6, TNF, NFKB1, BCL2, IL1B, HIF1A. And the survival analysis p values of three targets were less than 0.05, namely MYC, BCL2, and HIF1A. Therefore, these targets may play a critical role in celastrol reaction on metastasis LUAD and can prolong the overall survival of LUAD patients. Above all, BCL2 and HIF1A may be the promising targets of celastrol reaction on metastasis LUAD.

3.5. Molecular Docking Results and Analysis

Molecular docking proceeded between celastrol and the two targets (BCL2 and HIF1A). The binding energies of BCL2 and HIF1A were both less than -7kcal·mol-1. It is shown that celastrol may influence the function of core targets by restraining the binding activity and play a vital role in the treatment of metastasis LUAD. As shown, celastrol was docked in the binding pocket of BCL2 through one hydrogen bond with ARG-146, but had no hydrogen bond in the binding pocket of HIF1A (**Figure 4**). The docking results revealed that celastrol

could bind into the docking pocket well between BCL2, suggesting BCL2 plays a significant role in the response to celastrol in metastasis LUAD.

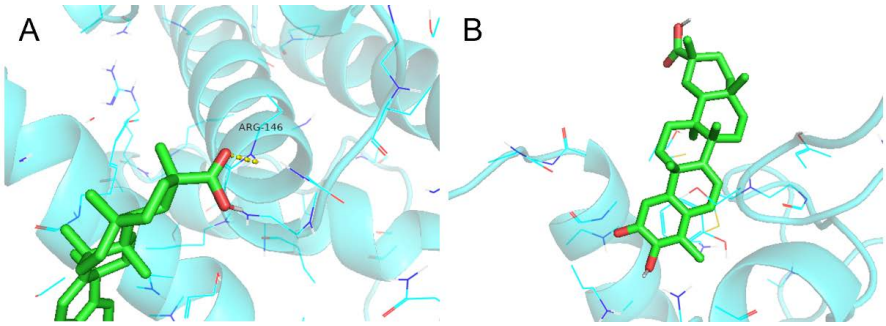


Figure 4. Molecular docking of celastrol and the two targets. Proteins (A) BCL2, (B) HIF1A are shown interacting with celastrol molecule.

4. Discussion

Lung cancer is one of the malignant carcinomas with a rapid increase in morbidity and the leading cause of worldwide cancer deaths, which is the most serious problem to human health. The etiology of lung cancer is still not completely clear. TCM has been widely used for treating lung cancer and has shown significant advantages in prolonging survival time and improving living quality in the last several years [39] [40]. TCM has the characteristics of multiple compounds, targets, pathways, and BPs. So far, there are few researches on the therapeutic action of celastrol on metastasis LUAD. Therefore, effective methods are needed to explore its targets and its antitumor mechanism in advanced lung cancer.

In the present study, we got the gene targets from public databases of celastrol in the treatment of metastatic LUAD and used network pharmacology to uncover the network characteristics of celastrol and explore the drug targets. Through our research, 107 genes were deemed as potential targets for celastrol in the treatment of metastatic LUAD. Next, 30 genes were used to conduct GO and KEGG pathways enrichment analysis to explain the underlying mechanism of celastrol reaction on metastatic LUAD. Further expression difference and survival analysis screened BCL2 and HIF1A may be promising targets. And molecular docking results showed BCL2 plays an important role in the reaction of celastrol in metastasis LUAD.

5. Conclusion

The present research studies the underlying mechanisms of celastrol in the treatment of metastatic LUAD using network pharmacology. This work showed that celastrol exerts pharmacological effects in metastatic LUAD in a multitarget and multi-pathway manner, mainly including pathways in cancer, response to oxidative stress, IL-17 signaling pathway and so on. Our research provides a possibility for further investigation of the underlying mechanism of the thera-

peutic action of celastrol in metastatic LUAD.

Fund

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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