The mechanism of Icariside II on NSCLC/COVID-19 based on network pharmacology and molecular docking

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Research Article

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Abstract

Patients with non-small cell lung cancer (NSCLC) are susceptible to coronavirus disease-2019 (COVID-19), but related treatments are limited. Icariside II (IS), a metabolite of plant Epimedin, showed anti-inflammation and immunoregulation effects in various diseases. This study aimed to evaluate the effect and mechanisms of IS on NSCLC/COVID-19. Targets of NSCLC/COVID-19 were defined as the common targets of NSCLC and COVID-19. The clinical characteristics of NSCLC patients were collected from The Cancer Genome Atlas Program (TCGA) database and analyzed by the R package of “survival”, univariate and multivariate Cox proportional hazards regression model. Further, the targets in IS treatment of NSCLC/COVID-19 were defined as the overlapping targets of IS and NSCLC/COVID-19 targets. Gene Ontology (Go) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of the treatment targets found IS might affect the leucocyte migration, inflammation response, and active oxygen species metabolic process, and regulate the IL-17, TNF, and HIF-1 signaling pathway in NSCLC/COVID-19. Protein-protein interaction (PPI) network identified six hub targets of IS in the treatment of NSCLC/COVID-19 including F2, SELE, MMP1, MMP2, AGTR1, and AGTR2. Molecular docking showed above target proteins had a great binding degree to IS. Our finding indicated that IS exerts therapeutic effects in NSCLC patients infected with COVID-19, supporting a further pre-clinical study to validate the related effect and underlying mechanism.

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a devastating pandemic—Coronavirus disease 2019 (COVID-19)(1). However, no specific therapy has been developed to overcome this life-threatening epidemic disease yet. Previous study reported an increased incidence of COVID-19 in patients with cancer compared with the general population(2). Cancer patients need to visit the hospital for long-term treatment, which increases the risk of COVID-19 infection. As any external infection can deteriorate their condition, among patients with cancer and COVID-19, 30-day all-cause mortality was higher than patients with the individual disease(3). Lung cancer is the first leading cause of cancer death(4). More than 350 people will die each day from lung cancer, which is more than breast, prostate, and pancreatic cancers combined and 2.5 times more than CRC, the second leading cause of cancer death(4). Therefore, finding the hub targets and effective therapy in the treatment of NSCLC patients with COVID-19 is of great significance in clinical.

Icariside II (IS), also knowns as Baohuoside I, is a metabolite of Icariin from Herba epimedium. IS has been extensively studied for its anti-inflammation and immunoregulation. Recent studies found that IS exhibits a broad spectrum of cytotoxicity against multiple cancer types both in vitro and in vivo(5). Moreover, studies demonstrated that IS could enhance the anti-tumor effect of the mainstream medication including paclitaxel(6), TRAIL(7), and cisplatin(8). In addition, COVID-19 was considered with cytokine storm syndromes and immunosuppression(9). Thus, we hypothesize that IS might exert potent pharmacological activity in patients with NSCLC combined with COVID-19.
Public databases provide giant resources for data mining. Network Pharmacology is a convenient tool for exploring the potential targets and mechanisms of the therapeutic effect of bioactive components. As non-small cell lung cancer (NSCLC) accounts for about 85% of lung cancer cases, in this study, we collected the NSCLC-related targets from the TCGA database for interaction analysis with COVID-19-related targets and performed Clinicopathological analysis of common targets. Then, we explored the mechanism underlying the therapeutic effect of IS on patients of NSCLC combined with COVID-19, based on network pharmacology and molecular docking.

**Methods**

**Identification of common targets of NSCLC and COVID-19**

Transcriptome profiles of NSCLC patients (LUAD) were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) on 05 May 2022. NSCLC-related targets were obtained by comparing the differential gene expression (DGE) between tumor and normal. And DGEs were analyzed by using the R package of "limma" with a false discovery rate<0.05 and \(|\log \text{fold change (FC)}|>1\). Furthermore, COVID-19 related targets were selected from the database of Genecard(10) (https://www.genecards.org/), OMIM(11) (https://www.omim.org/) and NCBI (https://www.ncbi.nlm.nih.gov/gene). Finally, NSCLC-related targets and COVID-19-related targets were intersected to obtain the common targets in NSCLC and COVID-19.

**Clinicopathological analysis of NSCLC/COVID-19-related targets**

The correlation between NSCLC/COVID-19 targets and survival rates in patients with NSCLC/COVID-19 was analyzed by the R package of "survival". Prognostic analyses were performed using univariate Cox proportional hazards regression. Moreover, different characteristics of diseased targets and patients with NSCLC/COVID-19 were analyzed using a multivariate Cox proportional hazards regression model. Finally, patients were categorized into low-risk and high-risk groups based on the risk score[30]. The optimal cutoff value of risk score was calculated by the R package of maxstat (Maximally selected rank statistics with several P-value approximations version: 0.7-25), setting the minimum number of grouping samples to be greater than 25%, and the maximum number of samples to be grouped less than 75%. The prognostic difference between the two groups was further analyzed by the R package of "survival" and "survfit". The log-rank test method was used to evaluate the significance of the prognostic difference between groups.

**Identification of hub targets of IS in treatment of NSCLC/COVID-19**

IS-related targets were predicted by database including HERBs(12) (http://herb.ac.cn), Targetnet(13) (http://targetnet.scbdd.com/), SwissTargetPrediction(14) (http://swisstargetprediction.ch), GeneCards(10) (https://www.genecards.org), and STITCH(15) (http://stitch.embl.de). The overlapping targets of IS and NSCLC/COVID-19 were analyzed by the Venn program on SangerBox (http://vip.sangerbox.com) and imported into the STRING database(16) (version 11.5, https://cn.string-
db.org) to construct protein-protein interactions (PPI) network. Topological parameters of the PPI network (.tsv) were analyzed by the Network-Analyzer setting in Cytoscape software (version 3.9.4). Further, hub targets were collected according to the Degree and MNC value of the algorithm.

**Enrichment analyses and network visualization of targets that IS in the treatment of NSCLC/COVID-19**

The c5.go.bp (cc and mf).v7.4.symbols.gmt and KEGG rest API(https://www.kegg.jp/kegg/rest/keggapi.html) were downloaded from the molecular signatures Database (DOI:10.1093/bioinformatics/btr260, http://www.gsea-msigdb.org/gsea/downloads.jsp) as a background. For gene set functional enrichment analysis, genes were mapped into the background set and enriched using the R package clisterProfiler (version 3.14.3) to obtain the results of gene set enrichment. The minimum gene was set to 5, the maximum gene set to 5000, P-value <0.05, and a FDR<0.1 were considered statistically significant. R packages of “GOplot” were used for visualization of gene ontology (GO) biological process (BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of the overlapping gene targets of IS against NSCLC/COVID-19. Finally, a graphical network of drug-target-GO/KEGG pathway-disease of IS in the treatment of NSCLC/COVID-19 was visualized by Cytoscape (3.9.1).

**Molecular docking of hub targets and IS**

The molecular structure of IS was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The protein structure of hub targets was obtained from the PDB database (https://www.rcsb.org/). The Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) structure files necessary for virtual screening were created by the AutoDock software (AutoDockTools-1.5.6). Hydrogenation and Gasteiger charge for merging nonpolar hydrogen atoms were performed by the AutoDock software. The visualization was conducted by Pymol software.

**Results**

**Common targets of NSCLC and COVID-19**

A total of 2799 NSCLC-related targets were identified by using the TCGA database and 488 COVID-19-related targets were collected from the database including Genecard (178), OMIM (22), and NCBI (436) (Figure 2A). Further, sixty-one intersected targets were identified between NSCLC and COVID-19 and regarded as the NSCLC/COVID-19 targets (Figure 2A Supplementary Table 1). Among 61 targets, 22 were up-regulated and 39 were down-regulated in comparing the differential gene expression in tumor and normal (Figure 2B).

**Clinicopathological analysis of CRC/COVID-19-related targets**

A total of 61 differential targets were subjected to Cox analyses, to understand the correlation between NSCLC/COVID-19-related targets and pathology of NSCLC/COVID-19. First, the univariate Cox analysis identified 17 targets to be significantly associated with NSCLC/COVID-19, including five factors of
reduction in hazard (HR>1): TRPA1, LDHA, KPNA2, and HAVCR1, and 13 factors of increase in hazard (HR<1): AGTR1, ERVFRD-1, CPA3, SFTPC, ERG, C5AR1, CAT, IL33, C4BPA, TNFRSF13C, TEK, AGER and SELP (P<0.01, Figure 3A; P<0.05, Table 1). Then, multivariate Cox analysis identified six targets out of 17, including three factors of reduction in hazard (HR>1): TRPA1, LDHA, and HAVCR1, and two factors of reduction in hazard (HR<1): C5AR1, CAT, and ERG (P<0.05, Table 2). Then, patients were divided into two groups of high-risk and low-risk based on the optimal cut-off value of 0.035. Finally, a significant prognostic difference was observed between the high-risk and low-risk groups in the overall survival analysis (P=2.4e-11, Figure 3B).

Further, the relationship between risk score, survival status, and expression distribution of the six individual targets in each patient was analyzed. The results demonstrated that greater risk value in patients correlated with higher risk score (Figure 3C), decreased survival rate of patients (Figure 3D), and increased expression levels of the three targets—TRPA1, LDHA and HAVCR1 (Figure 3E).

Table 1 Univariate Cox proportional hazards regression analysis of NSCLC/COVID-19 targets

<table>
<thead>
<tr>
<th>Target</th>
<th>HR</th>
<th>HR.95 Lower</th>
<th>HR.95 Upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPA1</td>
<td>1.00177661</td>
<td>1.00123572</td>
<td>1.002317786</td>
<td>8.82E-14</td>
</tr>
<tr>
<td>LDHA</td>
<td>1.00000818</td>
<td>1.00000476</td>
<td>1.000011608</td>
<td>2.89E-06</td>
</tr>
<tr>
<td>KPNA2</td>
<td>1.00003858</td>
<td>1.00001365</td>
<td>1.000063501</td>
<td>0.00233233</td>
</tr>
<tr>
<td>HAVCR1</td>
<td>1.00061885</td>
<td>1.00022202</td>
<td>1.001015839</td>
<td>0.00244873</td>
</tr>
<tr>
<td>AGTR1</td>
<td>0.99936415</td>
<td>0.99890783</td>
<td>0.999820671</td>
<td>0.00610345</td>
</tr>
<tr>
<td>ERVFRD-1</td>
<td>0.99982342</td>
<td>0.99969459</td>
<td>0.99995226</td>
<td>0.00886555</td>
</tr>
<tr>
<td>CPA3</td>
<td>0.99983949</td>
<td>0.99971883</td>
<td>0.999960169</td>
<td>0.00910464</td>
</tr>
<tr>
<td>SFTPC</td>
<td>0.99999794</td>
<td>0.99999623</td>
<td>0.999999652</td>
<td>0.01540084</td>
</tr>
<tr>
<td>ERG</td>
<td>0.99967663</td>
<td>0.99940843</td>
<td>0.99994905</td>
<td>0.01841015</td>
</tr>
<tr>
<td>C5AR1</td>
<td>0.99974811</td>
<td>0.999535</td>
<td>0.999961255</td>
<td>0.0208624</td>
</tr>
<tr>
<td>CAT</td>
<td>0.99994987</td>
<td>0.99990712</td>
<td>0.999992625</td>
<td>0.02120623</td>
</tr>
<tr>
<td>IL33</td>
<td>0.99999615</td>
<td>0.99999286</td>
<td>0.999999449</td>
<td>0.02300936</td>
</tr>
<tr>
<td>C4BPA</td>
<td>0.9999906</td>
<td>0.99998197</td>
<td>0.99999223</td>
<td>0.0306276</td>
</tr>
<tr>
<td>TNFRSF13C</td>
<td>0.99945886</td>
<td>0.99896013</td>
<td>0.99995783</td>
<td>0.0329582</td>
</tr>
<tr>
<td>TEK</td>
<td>0.99967141</td>
<td>0.9993648</td>
<td>0.99978119</td>
<td>0.03539589</td>
</tr>
<tr>
<td>AGER</td>
<td>0.99997238</td>
<td>0.99994508</td>
<td>0.99999684</td>
<td>0.04531652</td>
</tr>
<tr>
<td>SELP</td>
<td>0.98937643</td>
<td>0.97900712</td>
<td>0.999855559</td>
<td>0.04955128</td>
</tr>
</tbody>
</table>
### Table 2 Multivariate Cox proportional hazards regression analysis of NSCLC/ COVID-19 targets

<table>
<thead>
<tr>
<th>Target</th>
<th>HR</th>
<th>HR.95 Lower</th>
<th>HR.95 Upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPA1</td>
<td>1.00164135</td>
<td>1.00107823</td>
<td>1.00220477</td>
<td>1.09E-08</td>
</tr>
<tr>
<td>LDHA</td>
<td>1.00000854</td>
<td>1.0000044</td>
<td>1.00001269</td>
<td>5.37E-05</td>
</tr>
<tr>
<td>C5AR1</td>
<td>0.99968439</td>
<td>0.99946258</td>
<td>0.99990625</td>
<td>0.00530242</td>
</tr>
<tr>
<td>CAT</td>
<td>0.99993784</td>
<td>0.99988325</td>
<td>0.99999243</td>
<td>0.02563203</td>
</tr>
<tr>
<td>HAVCR1</td>
<td>1.00047229</td>
<td>1.00001187</td>
<td>1.00093293</td>
<td>0.04437616</td>
</tr>
<tr>
<td>ERG</td>
<td>0.99964026</td>
<td>0.99922616</td>
<td>1.00005453</td>
<td>0.08874963</td>
</tr>
</tbody>
</table>

Furthermore, an association of six genes with clinical characteristic in NSCLC were explored (Table 3). The expression of LDHA, ERG, and risk score were higher in older (>65 years old) than that in younger (≤65 years old) (Figure 4A C and E). The expression of LDHA, CAT, and risk score were higher in patients with lymph node metastasis (N1-2) than that in patients without lymph node metastasis (N0), indicating that LDHA, CAT, and risk score were related to the number of lymph node metastasis (Figure 4B D and F). While no significant associations were found between six genes and gender, tumor scope and size (T1-2 and T3-4), and advanced stage (Stage I-II and Stage III-IV).

### Table 3 Clinical characteristic correlation analysis of NSCLC/ COVID-19 targets

<table>
<thead>
<tr>
<th>Gene</th>
<th>&lt;=65 versus &gt;65</th>
<th>Female versus Male</th>
<th>T1-2 versus T3-4</th>
<th>N0 versus N1–2</th>
<th>Stage I-II versus Stage III-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPA1</td>
<td>-0.007(0.994)</td>
<td>-0.161(0.872)</td>
<td>-0.727(0.467)</td>
<td>-1.177(0.239)</td>
<td>-1.033(0.302)</td>
</tr>
<tr>
<td>LDHA</td>
<td><strong>-2.306(0.021)</strong></td>
<td>-0.172(0.863)</td>
<td>-0.672(0.501)</td>
<td><strong>-2.562(0.01)</strong></td>
<td>-0.378(0.706)</td>
</tr>
<tr>
<td>C5AR1</td>
<td>-0.552(0.581)</td>
<td>-0.317(0.751)</td>
<td>-0.0866(0.386)</td>
<td>-0.590(0.555)</td>
<td>-0.191(0.849)</td>
</tr>
<tr>
<td>CAT</td>
<td>-1.031(0.303)</td>
<td>-0.253(0.800)</td>
<td>-0.733(0.463)</td>
<td><strong>-2.183(0.029)</strong></td>
<td>-0.506(0.613)</td>
</tr>
<tr>
<td>HAVCR1</td>
<td>-0.848(0.397)</td>
<td>-0.587(0.557)</td>
<td>-0.532(0.595)</td>
<td>-1.595(0.111)</td>
<td>-0.312(0.755)</td>
</tr>
<tr>
<td>ERG</td>
<td><strong>-2.331(0.02)</strong></td>
<td>-1.020(0.308)</td>
<td>-1.245(0.213)</td>
<td>-1.401(0.161)</td>
<td>-0.569(0.569)</td>
</tr>
<tr>
<td>Risk score</td>
<td><strong>-2.34(0.019)</strong></td>
<td>-1.385(0.166)</td>
<td>-0.458(0.647)</td>
<td><strong>-3.769(0.000)</strong></td>
<td>-0.684(0.494)</td>
</tr>
</tbody>
</table>

**Hub targets of IS in treatment of NSCLC/COVID-19**
A total of 565 targets related to IS were predicted from five drug databases. Eleven intersection targets of IS and NSCLC/COVID-19 were constructed into the PPI network by the STRING database (Figure 5A, Supplementary Table 2). Further calculation of the topological parameters of the PPI network suggested that the median value of Degree and MNC was two, and the maximum was four. Thus, the hub target screening criteria was set to two-four. Finally, six hub targets were identified, including F2, SELE, MMP1, MMP2, AGTR1, and AGTR2 (Figure 5B, Supplementary Table 3).

**Enriched GO and KEGG pathway of targets that IS in the treatment of NSCLC/COVID-19**

The 11 targets were submitted for GO and KEGG enrichment analyses, to explore the possible mechanism that IS against NSCLC/COVID-19. Go analysis indicated that IS might affect a series of BPs related to cell (leucocyte) migration, regulation of inflammation response, and regulation of active oxygen species metabolic process (Figure 6A); IS might affect a series of CCs related to an intrinsic component of plasma member, membrane microdomain, external encapsulating signature, and perinuclear region of cytoplasm (Figure 6B); IS might affect a series of MFs related to molecular transducer activity, serine hydrolase activity (Figure 6C). KEGG analysis suggested that IS could regulate the inflammation-related signaling pathway of IL-17 and TNF, HIF-1 signaling pathway, and others including rheumatoid arthritis, renin-angiotensin system, complement, and coagulation cascades, AGE-RAGE signaling pathway in diabetic complications, neuroactive ligand-receptor interaction, serotonergic synapse, phospholipase D signaling pathway and adrenergic signaling in cardiomyocytes (Figure 6D and E). An interaction network showing hub targets, pharmacological functions, and signaling pathways of IS against NSCLC/COVID-19 was constructed (Figure 8, Supplementary Table 4).

**Results of molecular docking of hub targets and IS**

Docking simulation studies were carried out to investigate the binding sites of six hub targets with IS. Docking results showed that the main interaction interface that binding with the IS was SER-156 and ILE-128 in F2 (Figure 7A), LYS-32 and LYS-74 in SELE (Figure 7B), GLU-219, ALA-182, and LEU-181 in MMP1 (Figure 7C), MET-395 in MMP2 (Figure 7D), GLU-46, ASP-61 and ASN-58 in ARTG1 (Figure 7E), ASP-297 in ARTG2 (Figure 7F). According to the binding energy, IS has a high affinity with F2 and MMP2 (Supplementary Table 5).

**Discussion**

COVID-19 pandemic, leading to a gigantic increase in incidence and mortality rates worldwide. It has been reported that patients with lung cancer are more likely to be infected with SARS-COV-2 and have a higher death risk because the poor condition and immunosuppression(17). IS was reported as a potential natural component in anti-inflammation and immunoregulation. To our knowledge, this is the first study to explore the hub targets and possible signaling pathways underlying the therapeutic effect of IS on NSCLC/COVID-19. We found the hub targets that IS in the treatment of NSCLC/COVID-19 include F2, SELE, MMP1, MMP2, AGTR1, and AGTR2. IS might affect the leucocyte migration, inflammation response, and active oxygen species metabolic process, and regulate the IL-17, TNF, and HIF-1 signaling.
pathway in NSCLC/COVID-19. Taken together, our work showed that a natural small compound IS might exert therapeutic effects in NSCLC patients infected with COVID-19. Considering the advantages of low cytotoxicity compared to chemotherapeutic drugs, we believe that IS might be a potential agent in the combination strategy for cancer and COVID-19 treatment. At least our work provides evidence for the further preclinical evaluation of IS as a potential natural agent to improve therapies for cancer patients with COVID-19.

In the current study, we first screened out and identified hub genes and 61 intersecting genes of NSCLC combined with COVID-19. The DGE analysis showed 22 upregulated and 39 downregulated genes in patients with NSCLC and/or COVID-19. The DGE-based analysis might be used for determining clinical characteristics in NSCLC patients with COVID-19. Further independent prognostic and survival analyses found some important DGE, including TRPA1, LDHA, KPNA2 HAVCR1, AGTR1, ERVFRD1, CPA3, SFTPC, ERG, C5AR1, CAT, IL33, C4BPA, TNFRSF13C, TEK, AGER, and SELP. These DGE may function as potent biomarkers for screening and characterizing different stages of NSCLC patients with COVID-19. Taken together, these 61 intersection targets could be potential treatment targets in NSCLC combing with COVID-19.

In the network pharmacology analyses, we identified 11 overlapping targets with IS treatment against NSCLC and COVID-19. The DGE analysis identified significant differences in the expression of AGTR1, LDHA, and TEK. Moreover, NSCLC and COVID-19 showed increased expression of LDHA, and decreased expression of AGTR1 and TEK, along with a lower survival rate. LDHA encodes Lactate dehydrogenase A, which is one of five isoforms of the lactate dehydrogenase family and plays a crucial role in aerobic glycolysis that is a feature of cancer cells (the Warburg effect)(18). A previous study found phosphorylation-mediated activation of LDHA promotes cancer cell invasion and tumor metastasis(19). Therefore, LDHA is widely regarded as a desirable target for cancer therapeutics. AGTR1 encodes the type 1 receptor which is thought to mediate the major cardiovascular effects of angiotensin II. During SARS coronavirus-2/SARS-CoV-2 infection, it can recognize and internalize the complex formed by secreted ACE2 and SARS-CoV-2 spike protein through DNM2/dynamin 2-dependent endocytosis(20). TEK, encoding receptor tyrosine kinase, belongs to the protein tyrosine kinase Tie2 family. It acts as a cell-surface receptor for ANGPT1, ANGPT2, and ANGPT4 and regulates angiogenesis, endothelial cell survival, proliferation, migration, adhesion, cell spreading, and reorganization of the actin cytoskeleton, but also maintenance of vascular quiescence(21). Receptor tyrosine kinase has anti-inflammatory effects by preventing the leakage of proinflammatory plasma proteins and leukocytes from blood vessels(22). These findings indicated that these 11 intersection targets might be potent pharmacological targets of IS in the treatment of NSCLC and COVID-19.

Our GO and KEGG analysis suggested that the mechanism underlying the anti-NSCLC and anti-COVID-19 effects of IS were mediated by regulating leukocyte migration, inflammation response, and active oxygen species metabolic process, and regulate the IL-17, TNF, and HIF-1 signaling pathway in NSCLC/COVID-19. IS, a metabolite of Herba Epimedii has been used for impotency, osteoporosis, and amnestic treatment for thousands of years. IS was reported as a potential anti-inflammatory drug for a series of
inflammatory diseases such as atherosclerosis, Alzheimer's disease, depression, osteoarthritis, and asthma(23). Moreover, a recent study demonstrated that IS could potentiate cisplatin-induced apoptosis in non-small cell lung cancer cells(8), overcome TRAIL resistance of melanoma cells(7), and enhance paclitaxel-induced apoptosis in human melanoma A375 cells. Thus, we infer that IS might be a promising agent for patients with NSCLC and COVID-19. Our findings support the further preclinical study of IS as an anti-tumor and anti-COVID-19 drug.

Moreover, the anti-NSCLC/COVID-19 action of IS could be modulated by hub genes, including F2, SELE, MMP1, MMP2, ARTG1, and ARTG2. Using molecular docking analysis, we identified the best binding activities of IS with SER-156 and ILE-128 structures in the hub target F2, indicating that IS can effectively bind to specific proteins in the novel coronavirus. These findings suggested that IS may be able to bring the F2 to target the COVID-19. Additionally, we believe that adjuvant supplementation of IS may enhance the therapeutic efficacy of current clinical antiviral agents and immunotherapy to treat the fatal COVID-19, or NSCLC combined with COVID-19.

**Conclusion**

Network pharmacology and molecular docking indicated IS exerts therapeutic effects in NSCLC patients infected with COVID-19, supporting further pre-clinical studies to validate the related effect and underlying mechanism.

**Declarations**

**Availability of data and materials**

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

**Competing interests**

The authors declare that there are no conflicts of interest.

**Funding**

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**Author’s contributions**

Qing Kong designed the study and conducted the network pharmacology analysis. Huahe Zhu conducted the molecular docking analysis. Qing Kong wrote the manuscript. Jingcheng Dong and Baojun Liu revised the manuscript. All authors read and approved the final manuscript.
Acknowledgments

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References


20. AGTR1 Gene - Angiotensin II Receptor Type 1 [ 


Figures

Figure 1
Flowchart of the study.

**Figure 2**


**Figure 3**

Prognostic value of NSCLC/COVID-19 related targets. (A) Univariate Cox analysis identifying 10 targets (P<0.01). (B) Survival analysis indicating a significant difference in overall survival between high-risk and low-risk groups (P=2.4e-11). (C) Risk score using Cox proportional hazards regression in patients of the high-risk and low-risk groups. (D) Survival time in the high-risk and low-risk groups. (E) Expression levels of targets in the high-risk and low-risk groups.

**Figure 4**

Clinical characteristic association of the six targets in NSCLC. (A-B) Association of gene expression of LDHA with age and the number of lymph node metastasis in NSCLC. (C) Association of gene expression of ERG and age in NSCLC. (D) Association of gene expression of CAT and the number of lymph node metastasis in NSCLC. (E-F) Association of risk score with age and the number of lymph node metastasis of NSCLC.

**Figure 5**

Targets network of Icariside II (IS) against NSCLC/COVID-19. (A) Protein-protein interaction (PPI) network representing 11 intersecting targets of Icariside II and NSCLC/COVID-19. (B) Topological parameters analysis of PPI network indicating six hub targets.

**Figure 6**

Enrichment analysis of targets of Icariside II in treatment of NSCLC/COVID-19. (A) Biological process. (B) Cellar component. (C) Molecular function. (D) Kyoto Encyclopedia of Genes and Genomes (KEGG)
pathway.

**Figure 7**

Molecular docking results of (A) F2, (B) SELE, (C) MMP1, (D) MMP2, (E) ARTG1, and (F) ARTG2 with IS.

**Figure 8**

Drug-targets-GO/KEGG pathway-disease network.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1VenndataofNSCLCCOV.xlsx
- SupplementaryTable2VennDataofIS.xlsx
- SupplementaryTable3Hubtargets.xlsx
- SupplementaryTable4Enrichment.xlsx
- SupplementaryTable5Dock.xlsx