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Ginsenoside Rg3, a promising agent for NSCLC patients in the pandemic: a large-scale data mining and systemic biological analysis

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ABSTRACT

Introduction: Non-small cell lung cancer (NSCLC) patients are particularly vulnerable to the Coronavirus Disease-2019 (COVID-19). Currently, no anti-NSCLC/COVID-19 treatment options are available. As ginsenoside Rg3 is beneficial to NSCLC patients and has been identified as an entry inhibitor of the virus, this study aims to explore underlying pharmacological mechanisms of ginsenoside Rg3 for the treatment of NSCLC patients with COVID-19.

Methods: Based on a large-scale data mining and systemic biological analysis, this study investigated target genes, biological processes, pharmacological mechanisms, and underlying immune implications of ginsenoside Rg3 for NSCLC patients with COVID-19.

Results: An important gene set containing 26 target genes was built. Target genes with significant prognostic value were identified, including baculoviral IAP repeat containing 5 (BIRC5), carbonic anhydrase 9 (CA9), endothelin receptor type B (EDNRB), glucagon receptor (GCGR), interleukin 2 (IL2), peptidyl arginine deiminase 4 (PADI4), and solute carrier organic anion transporter family member 1B1 (SLCO1B1). The expression of target genes was significantly correlated with the infiltration level of macrophages, eosinophils, natural killer cells, and T lymphocytes. Ginsenoside Rg3 may benefit NSCLC patients with COVID-19 by regulating signaling pathways primarily involved in anti-inflammation, immunomodulation, cell cycle, cell fate, carcinogenesis, and hemodynamics.

Conclusions: This study provided a comprehensive strategy for drug discovery in NSCLC and COVID-19 based on systemic biology approaches. Ginsenoside Rg3 may be a prospective drug for NSCLC patients with COVID-19. Future studies are needed to determine the value of ginsenoside Rg3 for NSCLC patients with COVID-19.

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1. Introduction

In December 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was first detected , which was accountable for the outbreak of Coronavirus Disease-2019 (COVID-19) across the whole world. As of August 18, 2022, there had been 598,311,686 confirmed cases with 6,464,291 reported deaths [1]. COVID-19 has disproportionately affected certain subgroups of patients, including patients with cancer [2]. Lung cancer patients are one of the highest risk of deaths from COVID-19, in particular [3]. All results, despite the heterogeneity of available data, consistently identify lung cancer patients as being a remarkably vulnerable population for a rapid clinical deterioration and a higher rate of hospitalization and mortality; among these are patients with NSCLC [2,4]. Chronic inflammation in cancer can cause a surge of pro-inflammatory immune responses, leading to increased secretions of cytokines from T cells and phagocytes [5]. In lung cancer patients, the underlying tumor microenvironment (TME) supports SARS-CoV-2 proteins by activating cytokine storms and cellular metabolic variations-related pathways, further accelerates infection and weakens immune system [6,7]. As the ongoing pandemic affects an increasing number of NSCLC patients, there is an unmet need of efficacious drugs in NSCLC patients with COVID-19.

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Panax ginseng, a widely used herbal medicine, has gained in popularity due to its glycosylated triterpenoid saponins known as ginsenosides [8]. A monomer preparation of ginsenoside Rg3, Shenyi capsule, has been approved by the National Medical Products Administration (approval number Z20030044) and is frequently used for various cancers, particularly in NSCLC [9]. In NSCLC patients, ginsenoside Rg3 improved the survival rate [10] and objective response rate [11] in combination with chemotherapy. Previous studies suggested that ginsenoside Rg3 inhibited cell proliferation, migration, invasion, apoptosis, autophagy, and angiogenesis in cancers [11,12]. In addition, ginsenoside Rg3 hindered the differentiation of immune cells, such as Th1 and Th17 cells, presenting potential for resolving immune-mediated inflammatory diseases [13,14]. A recent study has reported that ginsenoside Rg3 effectively inhibited the interaction of receptor-binding domain and angiotensin-converting enzyme 2 (ACE2), further blocked the entry of SARS-CoV-2 into cells [15]. Therefore, ginsenoside Rg3 may be a prospective drug for NSCLC patients with COVID-19.

Based on high-throughput technologies, systemic biological analysis is an efficient tool for identifying active ingredients, target genes, molecular interactions, and signaling pathways linked to certain diseases [16]. Herein, we performed a large-scale data mining and systemic biological analysis to explore the underlying mechanisms of ginsenoside Rg3 for NSCLC and COVID-19.

2. Materials and methods

2.1. Identification of NSCLC/COVID-19-related target genes

To identify COVID-19-related genes, we searched six online platforms, including Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/), Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), DisGeNET (https://www.disgenet.org/covid/diseases/summary/), GeneCards (https://www.genecards.org/), and Comparative Toxicogenomics Database (CTD, http://ctdbase.org/). We downloaded three COVID-19 transcriptomic RNA-sequencing (RNA-seq) datasets from Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) on 21 October 2021, including GSE147507 [17], GSE157103 [18], and GSE166190 [19]. Then, based on the clinical data of each dataset, differentially expressed genes (DEGs) were filtered by R package *Deseq2* with threshold |log2FoldChange|>1 and adjusted $P < 0.05$. Genes collected from public databases and GEO datasets were merged for subsequent analyses.

To identify NSCLC-related genes, we searched six public databases, including OMIM, Drugbank (https://go.drugbank.com/), CTD, TTD, DisGeNET, and GeneCards. RNA-seq data of NSCLC were downloaded from The Cancer Genome Atlas (TCGA) data portal on UCSC Xena database (https://xenabrowser.net/datapages/). In the TCGA-NSCLC cohort, a total of 1,135 tissues (1,027 cancer tissues and 108 para-cancerous tissues) from 1,016 NSCLC patients were obtained and 995 of them had complete clinical data. Raw counts data and fragments per kilo base per million mapped reads (FPKM) were downloaded. Furthermore, NSCLC-related DEGs of the TCGA-NSCLC cohort were identified by R package *Deseq2* with threshold |log2FoldChange|>1 and adjusted $P < 0.05$. NSCLC-related genes were obtained by intersecting genes from the TCGA-NSCLC cohort and public databases. The main sources of public data and the acquisition of COVID19/NSCLC related genes had been reported in our previous study, but processed by a new analysis strategy [20]. Finally, we built a NSCLC/COVID-19-related gene set by intersecting target genes of NSCLC and COVID-19.

2.2. Obtaining a ginsenoside Rg3 target and NSCLC/COVID-19-related gene set

To build a ginsenoside Rg3-related gene set, we collected pharmacological targets of ginsenoside Rg3 from six online databases, including Similarity ensemble approach (SEA, http://sea.bkslab.org/), Parm Mapper (http://www.lilab-ecust.cn/pharmmapper/), CTD (http://ctdbase.org/), Target Net (http://targetnet.scbdd.com/), Swiss Target Prediction (http://www.swisstargetprediction.ch/), and Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org.cn/batman-tcm/). A ginsenoside Rg3 target and NSCLC/COVID-19-related gene set was obtained by intersecting the ginsenoside Rg3-related gene set and the NSCLC/COVID-19-related target genes.

2.3. Identification of target genes of ginsenoside Rg3 in NSCLC/COVID-19

Based on the ginsenoside Rg3 target and NSCLC/COVID-19-related gene set, GeneMANIA (http://genemania.org/) was used to construct the Protein-Protein Interaction (PPI) network and detect interactions between targets. To analyze topological parameters, we applied the network-analyzer setting of Cytoscape 3.9 (https://cytoscape.org/). In the PPI network, the significance of target genes was estimated by three topological parameters, including Betweenness-Centrality, Degree, and Closeness-Centrality.

2.4. Gene ontology (GO) enrichment analyses and network construction

To explore shared mechanisms of NSCLC and COVID-19, we conducted GO analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis based on identified NSCLC/COVID-19-related target genes. $P < 0.05$ was set to filter the enriched terms. The GO analysis comprised three categories, including biological processes (BP), cellular components (CC), and molecular functions (MF). In addition, to reveal underlying mechanisms of ginsenoside Rg3 for treating NSCLC patients with COVID-19, we performed another GO and KEGG pathway analysis based on the target genes of ginsenoside Rg3 in NSCLC/COVID-19. The above GO and KEGG analyses were performed by R package *clusterProfiler*.

2.5. Exploring the clinical significance of target genes

The product-limit method (KM method), log-rank test, and Cox regression analysis were conducted to determine the impact of target genes on the overall survival (OS) of NSCLC patients. The complete RNA-seq data of 995 NSCLC patients were obtained from TCGA in a log2 (FPKM+1) format. R package *survminer* [21] was used to calculate the cutoff value of each gene for classifying patients into the high- or the low-expression group. Then, target genes significantly associated with the OS ($P < 0.05$) were identified as hub target genes of ginsenoside Rg3 in NSCLC/COVID-19. A Cox proportional-hazards model was built to calculate risk scores and divide patients into the high- or the low-risk group. Variables included age, gender, smoking status and exposure, clinical stage, Tumor Node Metastasis (TNM) stage, radiation therapy, and treatment outcome were adjusted in the multivariate Cox analysis to estimate the significance of hub target genes.

2.6. Discovering expression patterns and correlations of hub target genes of ginsenoside Rg3 in NSCLC/COVID-19

To discover expression patterns of hub target genes of ginsenoside Rg3 in NSCLC/COVID-19, the RNA-seq data of the TCGA-NSCLC cohort from 1,135 tissues were applied. The RNA-seq data of 288 normal lung tissue on the Genotype-Tissue Expression (GTEx) database [22] were downloaded from UCSC Xena website [23]. To guarantee the statistical comparability, all data were downloaded in a log2 (FPKM+1) format. The Wilcoxon test was implemented to compare statistical differences in gene expressions among the cancer group, the para-cancerous group, and the GTEx normal group. The Spearman correlation test was conducted to determine associations between hub target genes. $P < 0.05$ indicated a statistical significance.

2.7. Analysis for underlying immune implications of hub target genes

To evaluate underlying immune implications of hub target genes of ginsenoside Rg3 in NSCLC/COVID-19, single-sample gene set enrichment analysis (ssGSEA) was used to explore associations between target genes and infiltration status of 28 types of immune cells by R package *GSVA*. The "Estimation of Stromal and immune cells in Malignant tumors using expression data" (ESTIMATE) method [24] was utilized to evaluate the tumor purity of NSCLC samples by R package *estimate*. Spearman's correlation analysis was performed based on gene expression, immune infiltration score, and tumor purity score. $P < 0.05$ and $r \geq 0.3$ were considered as statistical significances. The Wilcoxon test was also conducted to detect the difference of immune cell infiltrations between the high- and the low-risk group with $P < 0.05$ as threshold.

2.8. Molecular docking

To predict predominant binding modes of ginsenoside Rg3 with NSCLC/COVID-19-related proteins, a molecular docking analysis was performed. First, structures of related proteins and ginsenoside Rg3 were obtained from the RCSB PDB database (https://www.rcsb.org/) and PubChem (PubChem CID: 9918693, https://pubchem.ncbi.nlm.nih.gov/compound/9918693), respectively. Then, Pymol software was used to remove redundant ligands and the hydrone of hub genes [25]. Next, to explore molecular root and chemical bond, the ginsenoside Rg3 molecule was loaded into the Autodock tools 1.5.6 and the molecular docking was performed by the Autodock Vina [26]. The exhaustiveness value was set as 10 to increase the probability of detecting global minimum of scoring function inbuilt in AutoDock Vina. Docking affinity score < -5.0 kcal/mol indicated a strong binding interaction.

3. Results

3.1. Identification of the NSCLC/COVID-19-related genes and the ginsenoside Rg3 target genes

he number of COVID-19-related genes derived from OMIM, TTD, PubChem, DisGeNET, GeneCards, and CTD was 2, 78, 628, 1,832, 2,572, and 9,860, respectively (Supplementary file 1). The number of COVID-19-related genes collected from GSE147507, GSE157103, and GSE166190 was 4,825, 3,089, and 494, respectively (Supplementary file 2). These results were accorded with our previous study [20]. After removing duplication, a total of 14,666 COVID-19-related genes were identified by merging obtained genes.

The number of NSCLC-related genes gathered from OMIM, Drugbank, CTD, TTD, DisGeNET, and GeneCards was 15, 30, 144, 167, 447, and 4,536, respectively (Supplementary file 3). In total, 8,229 up-regulated DEGs and 2,139 down-regulated DEGs were obtained from the TCGA-NSCLC cohort (Supplementary figure 1, Supplementary file 4). After removing duplication, a total of 618 NSCLC-related DEGs were identified by intersecting 10,368 DEGs and the NSCLC-related genes.

A NSCLC/COVID-19-related gene set containing 362 genes was acquired by combining the identified genes (Fig. 1A). A total of 431 target genes of ginsenoside Rg3 were obtained from SEA (14 genes), Pharm Mapper (28), CTD (42), Target NET (100), Swiss Target Prediction (106), and BATMAN-TCM (141) (Supplementary file 5). After deleting duplication, 377 target genes were identified (Fig. 1B). Finally, a gene set with 26 target genes was built by intersecting the ginsenoside Rg3-related genes and the NSCLC/COVID-19-related genes (Supplementary file 6).

3.2. Exploration of potential mechanisms by enrichment analyses and network constructions

The GO and KEGG pathway analysis of 362 NSCLC/COVID-19-related genes identified 603 GO terms (BP, 507; CC, 38; MF, 38) and 251 KEGG pathways. Based on NSCLC/COVID-19-related genes, the functional enrichment analysis highlighted biological processes that mainly related to the regulation of ERK1/2 cascade, mitosis, cell cycle, and anti-microbial immune response (Fig. 1C), while the KEGG analysis identified 15 pathways that mainly related to anti-inflammation processes, cell cycle, chemical carcinogenesis, microRNAs in cancer, and complement and coagulation (Fig. 1D). Meanwhile, a total of 138 GO terms (BP, 119; MF, 19; CC, 0) were identified based on ginsenoside Rg3 target and NSCLC/COVID-19-related gene set. The identified BPs were mainly associated with hemodynamics and calcium homeostasis (Fig. 1E), while the KEGG analysis of the same gene set highlighted 15 pathways that primarily related to cellular signal transduction, cell fate, chemical carcinogenesis, anti-microbial process, and blood coagulation (Fig. 1F). A PPI network containing 46 nodes and 284 edges was built based on target genes (Fig. 1G and Supplementary file 7). The median Betweenness-Centrality, mean Degree, and mean Closeness-Centrality was 0.030, 12.35, and 0.440, respectively. Plasminogen (PLG), fibrinogen beta chain (FGB), GCGR, EDNRB, and BIRC5 had the most superior topological values in the network (Fig. 1H).

3.3. Clinical significance of hub target genes

The univariate Cox analysis and log-rank test suggested that BIRC5, CA9, EDNRB, GCGR, IL2, PADI4, and SLCO1B1 were significantly associated with the OS of NSCLC patients ($P < 0.05$). High level expressions of BIRC5 (HR, 1.30; 95% CI, 1.03-1.64; $P = 0.026$), CA9 (HR, 1.25; 95% CI, 1.03-1.53; $P = 0.027$), EDNRB (HR, 1.26; 95% CI, 1.01-1.58; $P = 0.044$), PADI4 (HR, 1.32; 95% CI, 1.06-1.65; $P = 0.012$), and SLCO1B1 (HR, 1.36; 95% CI, 1.11-1.67; $P = 0.003$) were associated with a poor OS. Whereas high level expressions of GCGR (HR, 0.68; 95% CI, 0.54-0.87; $P = 0.002$) and IL2 (HR, 0.78; 95% CI, 0.62-0.98; $P = 0.032$) were associated with a better OS (Fig. 2A-H).

Subsequently, a prognostic model was built by the multivariate Cox regression analysis and the risk score was calculated for every patient in the TCGA-NSCLC cohort based on expression levels of target genes and their relative coefficients. With the median cutoff value = 1.89, patients were classified into the high- (n = 472) or the low-risk group (n = 523) (Fig. 2I). Patients in the high-risk group had a higher mortality than those in the low-risk group (46.6% *vs.*

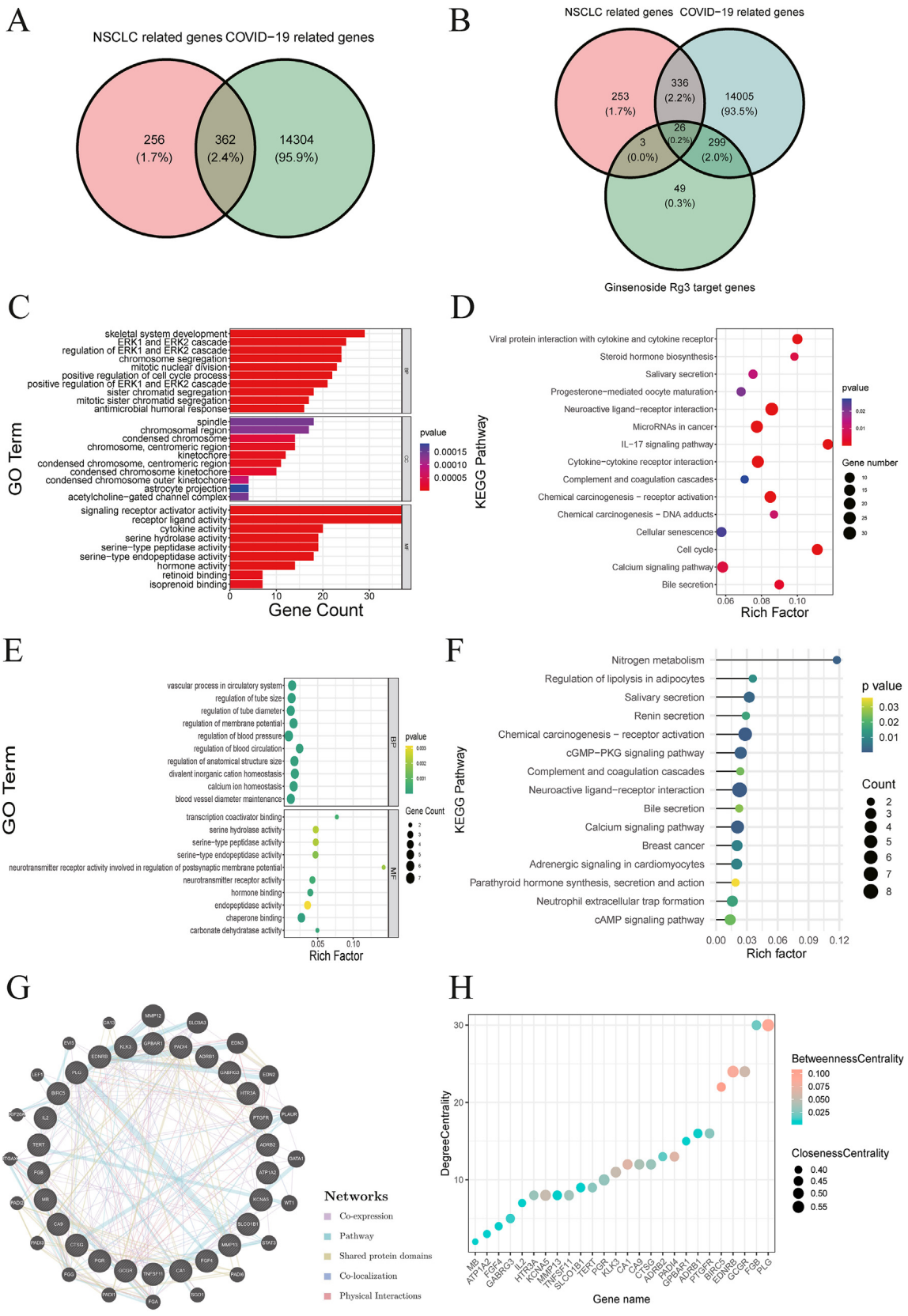
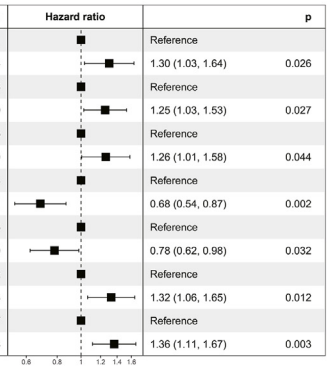
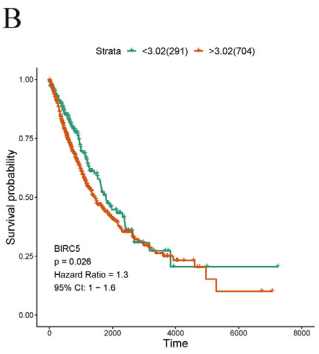


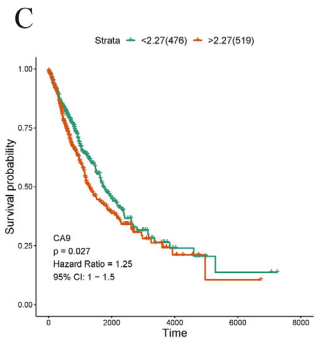
Fig. 1. Generalized analysis of the importance of NSCLC/COVID-19-related target genes. A, Identification of NSCLC/COVID-19-related genes. B, Obtaining a ginsenoside Rg3 target and NSCLC/COVID-19-related gene set by intersecting ginsenoside Rg3 targets and NSCLC/COVID-19-related genes. C, GO analysis of NSCLC/COVID-19-related genes. D, KEGG pathway analysis of NSCLC/COVID-19-related genes. E, GO analysis of target genes of ginsenoside Rg3 in NSCLC/COVID-19. F, KEGG pathway analysis of target genes of ginsenoside Rg3 in NSCLC/COVID-19. G, The PPI network based on the target genes of ginsenoside Rg3 in NSCLC/COVID-19. H, A bubble chart of topological value of target genes.

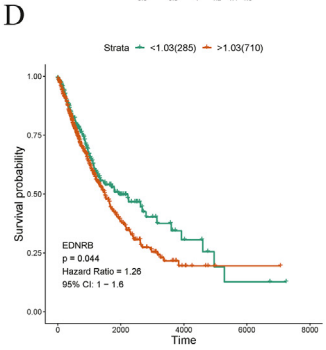
A

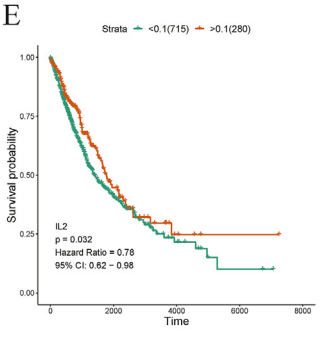
Variable		N	Hazard ratio	p
BIRC5	low	291	Reference	
	high	704	1.30 (1.03, 1.64)	0.026
CA9	low	476	Reference	
	high	519	1.25 (1.03, 1.53)	0.027
EDNRB	low	285	Reference	
	high	710	1.26 (1.01, 1.58)	0.044
GCGR	low	724	Reference	
	high	271	0.68 (0.54, 0.87)	0.002
IL2	low	715	Reference	
	high	280	0.78 (0.62, 0.98)	0.032
PADI4	low	742	Reference	
	high	253	1.32 (1.06, 1.65)	0.012
SLCO1B1	low	637	Reference	
	high	358	1.36 (1.11, 1.67)	0.003

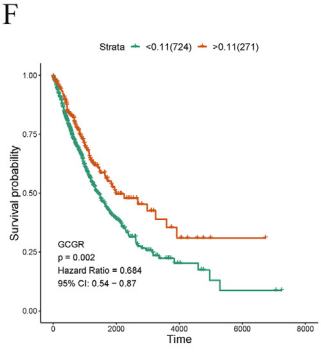


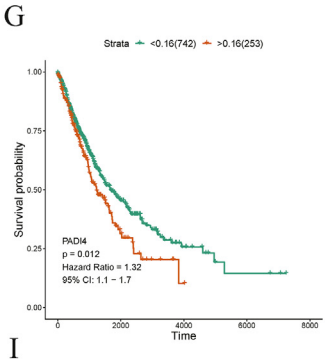


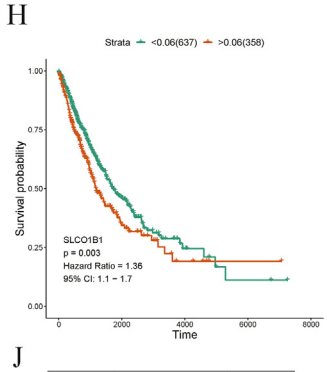


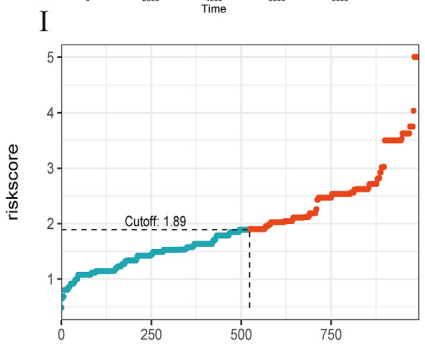


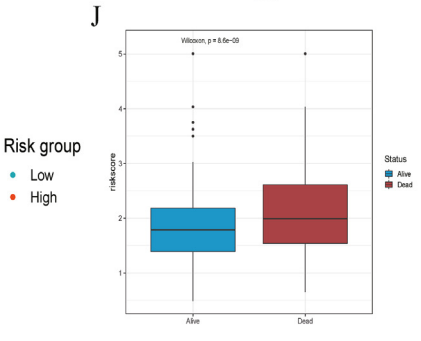


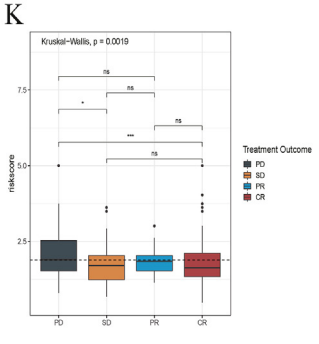


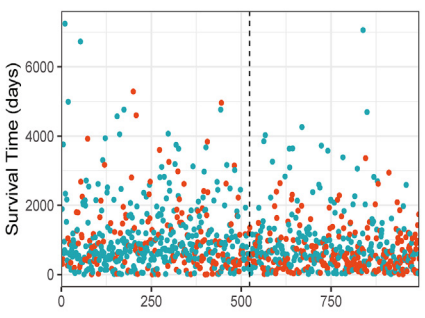


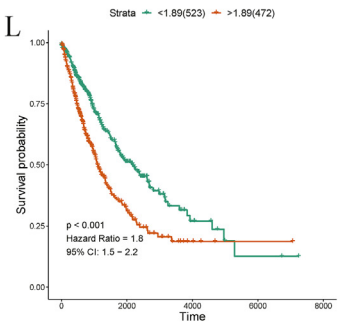












33.1%) (Fig. 2J and K), while patients with progressive disease (PD) had a significantly higher risk than those with stable disease (SD) or complete remission (CR) ($P < 0.05$) (Fig. 2K and L). Moreover, the higher risk score indicated a worse OS (HR, 1.8; 95% CI, 1.5-2.2; $P < 0.001$) (Fig. 2L).

After adjusting potential confounding factors, the multivariate Cox analysis still demonstrated that expression levels of target genes were significantly associated with the prognosis (HR, 1.8; 95% CI, 1.45-3.2; $P < 0.001$) (Table 1). The Cox risk proportional model suggested that BIRC5, CA9, GCGR, and SLCO1B1 had a significantly higher expression in cancer tissues than in non-cancer tissues, while they also had a significantly higher expression in para-cancerous tissues than in normal tissues ($P < 0.05$) (Fig. 3A, B, D, H, and J). By contrast, EDNRB and PADI4 had a significantly lower expression in cancer tissues than in non-cancer tissues ($P < 0.05$) (Fig. 3C and G). In addition, IL2 had a significantly higher expression in cancer tissues and para-cancerous tissues than in normal tissues, whereas it had a lower expression in cancer tissues than in para-cancerous tissues ($P < 0.05$) (Fig. 3E and I).

Furthermore, based on the Spearman correlation test, expression levels of BIRC5, CA9, GCGR, IL2, and SLCO1B1 were positively correlated with each other (Fig. 3K) (Supplementary file 9). The expression levels of EDNRB and PADI4 were positively correlated, but negatively correlated with expression levels of BIRC5, CA9, GCGR, IL2, and SLCO1B1. In the treatment of NSCLC and COVID-19, ginsenoside Rg3 may target at hub genes and induce a chain reaction based on potential interactions of these genes.

3.4. Immune implications of target genes for NSCLC/COVID-19

BIRC5 expression was positively correlated with infiltration levels of activated CD4 T-cell ($r = 0.42$, $P < 0.001$) and CD56 bright natural killer cell ($r = 0.38$, $P < 0.001$) but negatively correlated with infiltration levels of 11 types of immune cell, such as eosinophil ($r = -0.5$, $P < 0.001$), mast cell ($r = -0.49$, $P < 0.001$), and monocyte ($r = -0.46$, $P < 0.001$) (Fig. 4A); expressions of both CA9 and GCGR were positively correlated with the infiltration level of CD56 bright natural killer cell (both $r = 0.33$, $P < 0.001$) (Fig. 4B and D). What's more, expression levels of EDNRB, IL2, and PADI4 were all significantly and positively correlated with infiltration levels of immune cells. The count of correlated immune cells was 20, 16, and 15, respectively. EDNRB expression was mainly correlated with infiltration levels of mast cell ($r = 0.62$, $P < 0.001$), eosinophil ($r = 0.61$, $P < 0.001$), and natural killer cell ($r = 0.51$, $P < 0.001$) (Fig. 4C). IL2 expression was mainly correlated with infiltration levels of immature B cell ($r = 0.51$, $P < 0.001$), effector memory CD8 T-cell ($r = 0.47$, $P < 0.001$), and type 1 T helper cell ($r = 0.46$, $P < 0.001$) (Fig. 4E). PADI4 expression was mainly correlated with infiltration levels of neutrophil ($r = 0.46$, $P < 0.001$), plasmacytoid dendritic cell ($r = 0.47$, $P < 0.001$), and central memory CD8 T-cell ($r = 0.39$, $P < 0.001$) (Fig. 4F). In addition, NSCLC patients with higher expression levels of these genes had significantly higher infiltration levels of six types of T-cell (*e.g.* CD4+ type, CD8+ type, and helper T-cell) and five others (*e.g.* macrophage and neutrophil) (Fig. 4G). Moreover, a significantly positive correlation was found between BIRC5 expression and tumor purity score, whereas expressions of IL2, EDNRB, and PADI4 had a significantly negative correlation with tumor purity score (Fig. 4H ~ K). These findings suggested that target genes might involve in the pathophysiologic processes of NSCLC and COVID-19 through immunity regulation.

3.5. Binding ginsenoside Rg3 to hub target proteins of NSCLC/COVID-19

A molecular docking analysis was performed to discover the possible binding mode of ginsenoside Rg3 with hub target proteins encoded by the hub target genes. The result showed that ginsenoside Rg3 successfully bound to all hub target proteins. The rank of docking affinity score was CA9 (−14.4) < GCGR (−14.1) < BIRC5 (−13.0) < IL2 (−12.5) < SLCO1B (−10.3) < PADI4 (−8.7) < EDNRB (−8.5) (Table 2). Docking affinity scores were all less than −5 kcal/mol (Supplementary figure 2).

4. Discussion

Among 150 different types of ginsenoside extracted from *Panax ginseng*, Rg3 is the most frequently investigated ingredient in studies for cancers [27] (Supplementary Table 1). One of noteworthy features of ginsenoside Rg3 is its structure—steroid glycoside—which reasonably explains its immune regulation capacity and steroid-like anti-inflammatory activity [27]. In lipopolysaccharide-induced acute lung injury, ginsenoside Rg3 reduced pro-inflammatory mediators, increased anti-inflammatory cytokines, and boosted anti-inflammation ability through the PI3K/Akt/mTOR signaling pathway [28]. In H460 tumor-bearing mice, ginsenoside Rg3 preparation inhibited tumor growth by improving host immunity [29]. In addition, ginsenoside Rg3 attenuated allergic inflammation as it inhibited Th1 cell differentiation *in vitro*, reduced Th1 cell responses *in vivo*, and decreased proinflammatory cytokines by suppressing mast cell-mediated inflammation via MAPK signaling pathway [30]. In COVID-19, although the current evidence is controversial and limited, steroids decreased the duration of oxygen dependence in the acute phase [31] and reduced mortality when the dosage was appropriate [32]. Recently, ginsenoside Rg3 has been found to hinder SARS-CoV-2 from entering cells by inhibiting ACE2 [15]. Given the above information, one could hypothesize ginsenoside Rg3 may be a drug that is beneficial to patients with NSCLC and COVID-19. Thus, we conducted the first comprehensive bioinformatic analysis focusing on mechanisms of ginsenoside Rg3 for the treatment of NSCLC patients with COVID-19.

The result of GO and KEGG enrichment analysis offers a clue for further study on shared mechanisms of NSCLC and COVID-19. In GO BP analysis based on NSCLC/COVID-19-related genes, potential therapeutic targets of both NSCLC and COVID-19 involved inflammation-related BPs, such as ERK1 and ERK2 cascade. Activated by mitogens, ERK1/2 signaling pathway is essential for cell proliferation, tumor development, and gene expression by regulating cell cycle, migration, invasion, and autophagy [33]. Upstream signals of the ERK1/2 signaling pathway are frequently altered in cancer, such as Akt and Ras signaling molecule, which closely related to cell proliferation and radiation-/chemo-resistance [33]. Clinically, high basal levels of PI3K-Akt and Ras-ERK1/2 activation indicated an aggressive form of NSCLC [34,35]. Notably, rennin angiotensin system (RAS) is a fundamental regulator of blood pressure, blood circulation, and blood vessel diameter [36] and an important player in anti-inflammatory processes. Mediated the production of angiotensin (Ang) II [37], ACE2 is a crucial component of the RAS and an entry receptor for SARS-CoV-2, as well. One of the determinants of COVID-19 infection is the binding capacity of SARS-CoV-2 spike protein to ACE2 [38]. A cohort study included 12

Fig. 2. Clinical significance of hub target genes of ginsenoside Rg3 in NSCLC/COVID-19. A, Forest plot of the univariate Cox proportional analysis of the hub target genes. B-H, Kaplan-Meier curve analysis reveals the impact of hub target genes on overall survival. I, Risk score grouping and survival distribution of patients in the TCGA-NSCLC cohort. J, Risk scores distribution of NSCLC patients with different survivals. K, Risk scores distribution of NSCLC patients with different treatment outcomes. L, Kaplan-Meier analysis between the high- and the low- risk group.

Table 1
Univariate and multivariate Cox regression analysis based on hub target genes of ginsenoside Rg3 in NSCLC and COVID-19

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*p*
riskscore (High vs.Low)	1.8 (1.47,2.2)	**<0.001**	2.15 (1.45,3.2)	**<0.001**
Age (≥65 vs.<65)	1.26 (1.02,1.56)	**0.029**	0.98 (0.65,1.49)	0.934
Treatment Outcome (SD vs.PD)	0.27 (0.15,0.47)	**<0.001**	0.34 (0.17,0.67)	**0.002**
Treatment Outcome (PR vs.PD)	0 (0,Inf)	0.993	0 (0,Inf)	0.995
Treatment Outcome (CR vs.PD)	0.11 (0.08,0.16)	**<0.001**	0.15 (0.09,0.23)	**<0.001**
Cigeret packs somked (≥40/year vs. <40/year)	1.07 (0.84,1.35)	0.597	-	-
M Stage (M1 vs. M0)	2.27 (1.44,3.58)	**<0.001**	0.76 (0.26,2.25)	0.617
N Stage (N2–N3 vs. N0–N1)	1.76 (1.34,2.31)	**<0.001**	1.04 (0.41,2.68)	0.931
T Stage (T3-T4 vs. T1-T2)	1.88 (1.47,2.41)	**<0.001**	1.24 (0.52,2.93)	0.624
Accepted Radiation Therapy (Yes vs.No)	1.61 (1.2,2.17)	**0.002**	1.09 (0.67,1.78)	0.74
Gender (Female vs. Male)	0.88 (0.72,1.08)	0.226	-	-
Clinical Stage (III-IV vs. I-II)	2.09 (1.66,2.65)	**<0.001**	1.75 (0.61,4.99)	0.297
Smoking exposure (≥20 years vs < 20 years)	0.91 (0.53,1.55)	0.73	-	

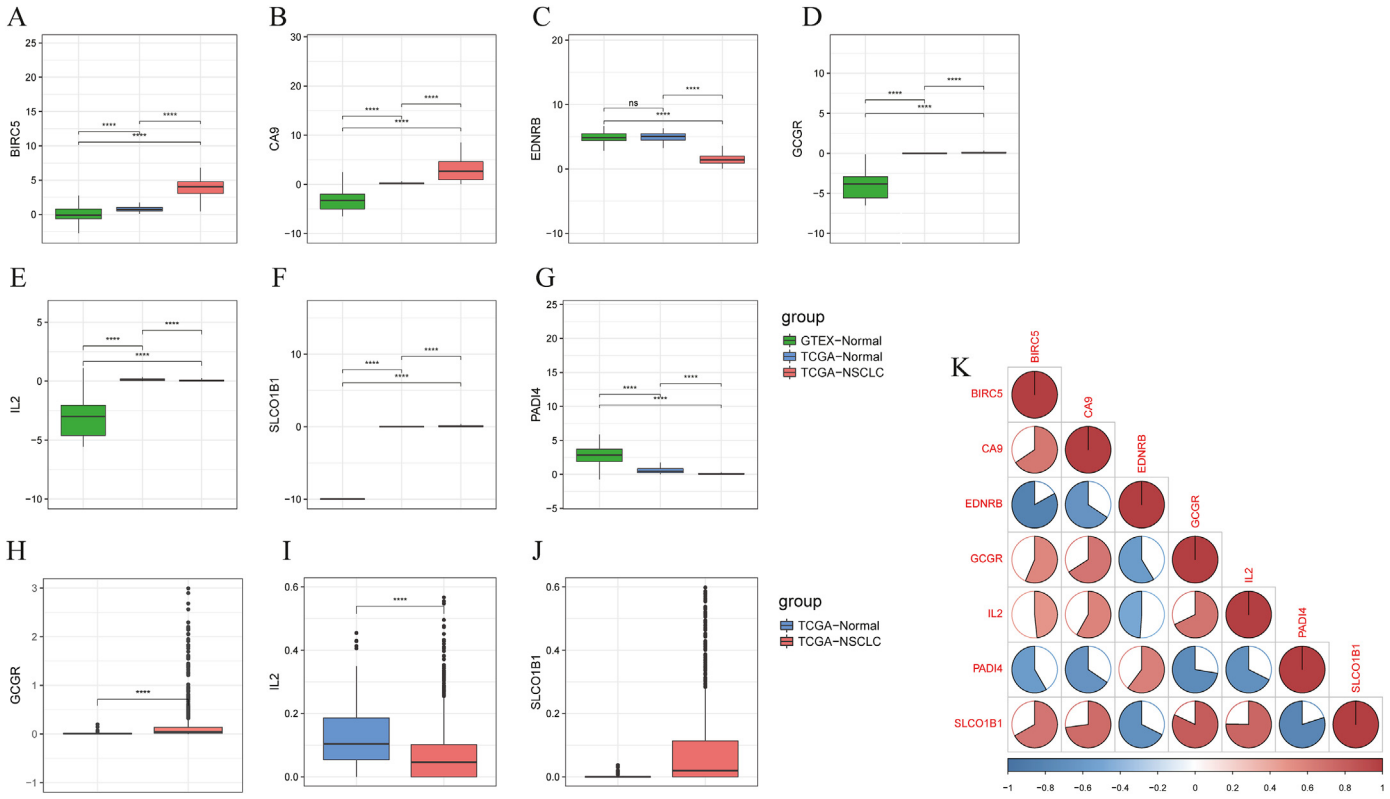


Fig. 3. Expression patterns and correlations of hub target genes of ginsenoside Rg3 in NSCLC/COVID-19. A-J, Expression levels of hub target genes in different tissues. K, Expression correlations between hub target genes.

COVID-19 patients reported that the level of Ang II in plasma samples from SARS-CoV-2 infected patients was higher than that from uninfected individuals. Moreover, the level of Ang II significantly correlated with viral titer and lung injury [39].

One of the identified target genes, FGB, is involved in complement and coagulation cascades which is a series of enzymatic activations that trigger the conversion of soluble fibrinogen to insoluble fibrin clots in the presence of thrombin [40]. It is found that the expression of FGB significantly increased both in COVID-19 [41] and lung adenocarcinoma patients [40]. The interaction between FGB and ACE2 could aggravate thrombosis, one of the main pathologies of COVID-19 [42]. FGB could intensify systemic inflammation and promote a procoagulant and prothrombotic status by triggering inflammatory signal via NF-κB signaling pathway [43]. As shown in the present study, FGB participated in multiple identified pathways, including ERK1 and ERK2 cascade, regulation of tube diameter, tube size, anatomical structure size, blood circulation, blood vessel diameter maintenance, and vascular process in circulatory system. Thus, FGB could be a potential target of ginsenoside Rg3 for the treatment of NSCLC patients with COVID-19.

In the multivariate Cox analysis, BIRC5, CA9, EDNRB, GCGR, IL2, PADI4, and SLCO1B1 were identified to be significantly associated with the prognosis. According to molecular docking, ginsenoside Rg3 could effectively bind to these target genes. Among these genes, the high expression of BIRC5, CA9, and SLO1B1 predicted a worse OS of NSCLC patients. As a mitotic spindle checkpoint gene, BIRC5 (also named survivin) is a well-known cancer therapeutic

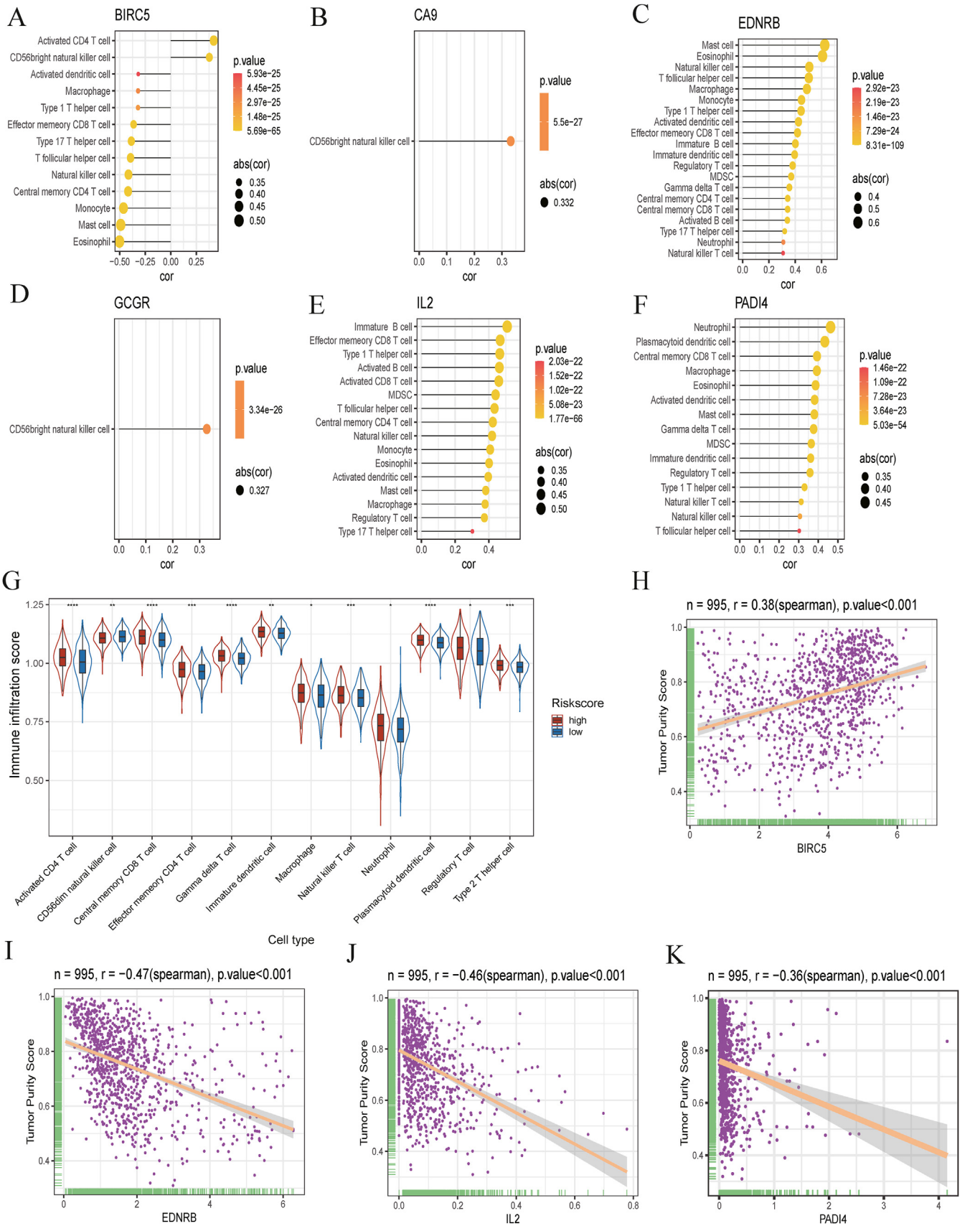


Fig. 4. Immune implications of hub target genes of ginsenoside Rg3 in NSCLC/COVID-19. A-G, Associations between hub target genes and different types of immune cell. H-K, Correlations between hub target genes and tumor purity score.

Table 2
Molecular docking affinity score of ginsenoside Rg3 and hub target proteins

Drug Hub target proteins	ginsenoside Rg3
	Docking Affinity (kcal/mol)
BIRC5	−13.0
CA9	−14.4
EDNRB	−8.5
GCGR	−14.1
IL2	−12.5
PADI4	−8.7
SLCO1B	−10.3

target which plays an important role in tumorigenesis, metastasis, and therapy resistance [44]. Over-expressed BIRC5 was related to worse outcomes in NSCLC [45]. BIRC5 not only inhibited apoptosis and regulated mitosis through suppressing activities of caspase-3 and caspase-7, but also stalled cell cycle through activating *P53* gene *P21* gene [44]. In addition, BIRC5 promoted cell proliferation, migration, and invasion via the β-catenin pathway, transforming growth factor-β (TGF-β), and the PI3K/Akt pathway, respectively [46–48]. It should be noted that ginsenoside Rg3 could induce the apoptosis of SK-MES-1 cells in a dose-dependent manner and significantly down-regulated the expression of BIRC5 [49]. In consistence with a previous study, we found that the BIRC5 expression was positively correlated with the activation of CD56 natural killer cell and CD4 T-cell, but negatively correlated with the dormancy status of mast cells [30]. These findings indicated that BIRC5 might be a biomarker for cancer vaccines.

Participated in nitrogen metabolic pathways, CA9 is one of the most strongly hypoxia-induced genes, as well as the hallmark feature of many solid tumors, such as NSCLC [50]. In a hypoxic condition, CA9 involved in tumor growth, proliferation, invasion, and metastasis by encoding CA9 protein, an effective catalyst for the reversible hydration of carbon dioxide to bicarbonate and proton [50]. In consistence with the result, previous studies have also found that high levels of CA9 and other cytokines including IL-1ra, IL-8, IL-10, monobasic calcium phosphate-1 (MCP-1), and supercritical fluid (SCF) were significantly correlated with the higher mortality rate in patients with COVID-19 [51,52]. Moreover, hypoxia increased the intracellular Ca^{2+} concentration in pulmonary arterial myocytes and hijacked calcium channels and pumps through triggering mitochondrial reactive oxygen species (ROS), then increased airway responsiveness and aggravated COVID-19 [53]. Thus, it could be worthwhile to investigate CA9 as a regulator or biomarker in NSCLC patients with COVID-19.

Expressed on the basolateral domain of hepatocytes, organic anion transporter polypeptides (OATPs) facilitated the uptake of anionic endogenous compounds and drugs from blood before eliminating into bile [54]. OATP1B1 variants (encoded by SLCO1B1), a type of isoforms of OATPs, were found to involve in the hepatic transport of SN-38 disposition which resulted in the variability in toxicity and efficacy of irinotecan-based chemotherapy in NSCLC patients [55]. Additionally, as SLCO1B1 variants affected the pharmacokinetics of hydroxychloroquine/chloroquine and lopinavir/ritonavir, it may also theoretically affect clinical response and toxicity in COVID-19 [56].

The analysis of immune implications provided available reference for future research on the effect of ginsenoside Rg3 on TME in the context of viral infection. In lung cancer, TME is remarkably active and highly complicated. The understanding of the relationship between the tumor and immune system is still at an early stage. According to prior studies, components of TME, such as lymphocytes, neutrophils, dendritic cells and natural killer cells, are modulated by signals from infiltrating tumor cells and then regulate related signaling pathways which increase tumor survival and growth [57]. On the one hand, lung infections like COVID-19 induce inflammation and expose tumor cells in a microenvironment filled with pro-inflammatory cytokines through various signals such as TNF-α-mediated NF-κB pathway [58] and IL-6-mediated STAT3 pathway [59], which may further lead to metastasis; on the other hand, the chronic pulmonary inflammation caused by tumor microenvironment makes lung cancer patients more susceptible to virus infections that can result in increased tumor growth and survival chances mainly due to immunosuppression, apoptosis reduction, cellular communication transformation, and increased angiogenesis [60]. Although the exploration of immune implications provided limited evidence, it indicated that future experimental research on immunomodulatory effects of ginsenoside Rg3 and its interactions with tumor infiltrating-lymphocytes could be promising.

In summary, we developed an in *silico* framework to identify ginsenoside Rg3 as a promising drug for NSCLC patients with COVID-19, through regulating biological processes and signaling pathways mainly related to anti-inflammation, immunomodulation, cell cycle, cell fate, carcinogenesis, and hemodynamics. These findings may provide available reference for further research on ginsenoside Rg3 and give insights into the drug discovery for NSCLC patients with COVID-19. Nevertheless, this study may be subject to several limitations. The first is the probable partial retrieval of common DEGs due to the insufficient sample size of studies on NSCLC and COVID-19. The second would be limited nature of computational methods. Last but not least, experiments and translational research should be performed in the future to further explore the effect of ginsenoside Rg3 on NSCLC and COVID-19.

5. Conclusions

Based on a large-scale data mining and systemic biological analysis, this study has identified 1) potential therapeutic targets, including BIRC5, CA9, EDNRB, GCGR, IL2, PADI4, and SLCO1B1; 2) signaling pathways primarily related to anti-inflammation, immunomodulation, cell cycle, cell fate, carcinogenesis, and hemodynamics of ginsenoside Rg3 for the treatment of NSCLC and COVID-19. This study increased the understanding of the application value of ginsenoside Rg3 for NSCLC patients with COVID-19. In addition to the findings, further experiments and clinical studies should be done with ginsenoside Rg3 to explore its precise pharmacological mechanisms and evaluate its efficacy in NSCLC patients with COVID-19.

Authors' contributions

YT, ZZ conceived and designed the study. ZZ, QC performed data analysis and data interpretation. ZZ, QC, XZ conducted the bioinformatics and statistical analyses. ZZ, QC plotted the figures. QC, XZ, HC, RY prepared the manuscript. All authors read and approved the final manuscript.

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Declaration of competing interest

All authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data of this article can be found online at https://doi.org/10.1016/j.jgr.2022.09.006.

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