Potential Mechanism of Tibetan Medicine Dali in the Treatment of Respiratory Diseases via Network Pharmacology

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Research

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Abstract

Background: As a source of Tibetan medicine “Dali”, *Rhododendron anthopogonoides* Maxim (RAM) has good medically helpful effect on respiratory diseases. However, due to its complex components, there is still a lack of pharmacological and pharmacodynamic research on its active components.

Methods: The research method of the network pharmacology was used in this study, to obtain the mechanism of RAM in the treatment of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). The active components of RAM were obtained by searching the relevant database, and the protein targets of each chemical component in RAM were screened by Traditional Chinese Medicine Database and Analysis Platform (TCMSP) and PubChem. Then, the protein targets were normalized to the corresponding gene name through UniProt. Meanwhile, genes related to ALI were searched by Online Mendelian Inheritance in Man (OMIM) database, GeneCards database, DrugBank database and DisGeNET database. The “Component-Target-Protein” network was established by Cytoscape software, and the core targets were screened by combining with STRING software. Finally, GO and KEGG enrichment analysis were carried out by David database.

Results: Combined with all above results, 92 active components of RAM and 176 gene targets of ALI/ARDS were screened, including 46 common targets, which were related to biological processes such as inflammation, immune and inflammatory response, cell growth and differentiation and apoptosis.

Conclusions: It can be concluded through the investigation of network pharmacology that RAM regulates a variety of physiological processes such as cell growth, differentiation, apoptosis and death by activating the Toll-like receptor signaling pathway, TNF signaling pathway, cAMP signaling pathway, MAPK signaling pathway, as well as regulating the inflammatory response of cells, the immune response of the body and promoting the inflammatory cytokines NF-κB dependent expression and other processes to treat ALI/ARDS. This study revealed the mechanism of RAM multi-component, multi-target and multi-pathway, and provided scientific basis and research ideas for its clinical application.

1. Introduction

Acute lung injury (ALI), a lung disease caused by some direct or indirect causes such as trauma, infection and inhalation of harmful gases(1). Its main pathological manifestations are pulmonary edema, respiratory distress and hypoxemia, and might damage various processes of lung function in different ways(2). Without proper intervention, it might develop into acute respiratory distress syndrome (ARDS), a common critical disease in clinic, which will lead to multiple organ failure and pose a serious threat to human health(3).

Although great progress had been made in the treatment of ALI/ARDS, the incidence rate and mortality rate of ALI/ARDS were still high. A study showed that in the United States, the incidence rate of ALI and ARDS in patients over 15 years old was 78.9 cases and 58.7 cases in 100 thousand cases respectively, with a mortality rate of 38.5% and 41.1% respectively(4). A retrospective cohort study reported that from

2007 to 2016, incidence rate and mortality rate of 146,058 ICU patients under 18 years old with ARDS were 1.8% and 20% respectively(5). Moreover, 56% of patients still needed continuous nursing because of different degrees of severe lung function injury after rehabilitation(5), which also brought a great economic burden to patients and their families.

At present, according to the pathological characteristics of ALI, its treatment methods mainly include respiratory support treatment and pharmacological treatment. Respiratory supportive therapy mainly includes mechanical ventilation(6) and extracorporeal membrane oxygenation (ECMO), which aims to deliver sufficient oxygen and nutrition to various organs of the whole body to support the normal operation of the human body. Clinically, although respiratory support therapy can alleviate the symptoms of patients with ALI to a certain extent, it can only appropriately prolong the survival time of patients, which can't curb the deterioration of ALI, nor reduce the mortality of patients, and the existing respiratory support treatment model is not suitable for the daily care of patients with ALI(7–9).

The pharmacological treatments of ALI mainly include corticosteroid (CS), antioxidant stress drugs and so on. CS has strong effects of anti-inflammatory and dilatation on pulmonary vessels. Nevertheless, the treatment of CS has no remarkable impact on mortality. At the same time, the use of CS also has some side effects, especially pediatric patients are prone to drug withdrawal reaction, infection and other risks after stopping the use of CS(10). Antioxidant stress drugs such as N-acetylcysteine can only shorten the stay time of patients in ICU to a certain extent, which also can't reduce the mortality of patients(11). Up to now, there is still no specific drug that can effectively prevent ALI. Although the treatment of ALI has improved with the development of medicine, and gene therapy and cell transplantation have been proved effective in ALI treatment, but there are still different degrees of limitations(12). Therefore, the incidence rate and mortality rate of ALI is high as before(13) .

As an important part of traditional Chinese medicine (TCM), Tibetan medicine has a history of more than 3800 years(14), with a medical system perfected by the Tibetan people who living in the harsh cold and hypoxia environment of the snow covered plateau through long-term life practice(15). Due to their hypoxic living environment, the Tibetan people have a great demand for respiratory drugs, and statistics showed that 37.55% of Tibetan medicine preparations can be used for the treatment of respiratory diseases(16). In recent years, Tibetan medicine has played an important role in the prevention and treatment of severe acute respiratory syndrome (SARS). At the same time, by analyzing the pharmacological active components of disease-related Tibetan drugs and their compounds, it can be seen that they have significant therapeutic effects on respiratory diseases such as COVID-19, and can effectively alleviate the symptoms of inflammation, cough and so on(17).

Rhododendron anthopogonoides Maxim(RAM) is a *Rhododendron* L. plant of Ericaceae(18), a plant source of Tibetan medicine "Dali" (□□□□□□ in Tibetan language)(19). As a shrub, it grows on the wet hillside of high-altitude areas such as Gansu and Qinghai(20). The Rhododendron has effects of relieving cough and asthma, eliminating phlegm, clearing away heat and toxic material, promoting digestion and detumescence with a pungent and bitter taste and a mild property(21). Research showed that

Rhododendron contained volatile oils, flavonoids, coumarins, triterpenoids and steroids, with pharmacological effects such as antitussive and antiasthmatic, expectorant, bacteriostatic and anti-inflammatory, and played a certain role in the treatment of respiratory diseases(22). It is mainly used to prevent and treat senile chronic bronchitis and has a wide application prospect in medicine. However, due to its complex components, there is still a lack of pharmacological and pharmacodynamic research on its active components(18).

2. Materials And Methods

2.1 Active ingredients and potential targets of RAM

Taking “RAM (*Rhododendron anthopogonoides* Maxim)” and “chemical constituents” as the key words, the relevant literatures were searched in The National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/), CNKI (https://www.cnki.net/), VIP (http://www.cqvip.com/), WANFANG DATA (https://www.wanfangdata.com.cn/), and the search scope was from the establishment of the database to April 29, 2021. Then synthesized all the searched active components of RAM and queried CAS number through the chemical source network (https://www.chemsrc.com/) of each one. After screened out the active components with CAS number, the related targets of each component were predicted in Traditional Chinese Medicine Database and Analysis Platform (TCMSP, https://tcmsp-e.com/) through CAS number. The compounds not recorded in TCMSP were searched in PubChem (https://pubchem.ncbi.nlm.nih.gov/) continuously, exported the target proteins under the directory of “Chemical-Gene Co-Occurrences in Literature”, and screened out compounds without target proteins after integrating the information obtained in TCMSP and PubChem. Input the obtained target information into Uniprot (https://www.uniprot.org/), set the category as “human”, then eliminated the genes without “Homo sapiens” and “Reviewed” information, and standardize the protein name into the gene entries.

2.2 Collecting therapeutic targets of ALI/ARDS

The known therapeutic targets of ALI/ARDS were acquired from four sources: Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/), GeneCards database (https://www.genecards.org/), DrugBank database (https://go.drugbank.com/) and DisGeNET database (https://www.disgenet.org/), with combination of all data and elimination of the coincident gene entries, the ALI/ARDS related genes were obtained.

2.3 Screening of common targets of RAM and ALI/ARDS

The Wayne diagram of RAM and ALI/ARDS targets was drawn on OmicShare website (https://www.omicshare.com/), and the intersection targets might be the key targets of treatment of ALI/ARDS with ALI.

2.4 Construction of Components-Target-Protein network

The interaction network diagram of Components-Target-Protein was constructed by Cytoscape3.8.0 software. In order to make the network relationship clear, the active components and targets were represented by “Nodes”, while the interactions between nodes were represented by “Edge”. After analyzing the network, the core targets were screened by taking “Degree, Betweenness Centrality (BC) and Closeness Centrality (CC)” greater than their corresponding median respectively.

2.5 Construction of Protein–Protein Interaction (PPI) network

The core targets of RAM and ALI/ARDS were uploaded to the STRING11.5 (https://string-db.org/) in the form of gene symbol to construct the PPI network, then set organism to “Homo sapiens”, minimum required interaction score to “highest confidence” (> 0.9), then hide disconnected nodes in the network, and other parameters remain the default settings for export after operation.

2.6 Gene Ontology function (GO)and Kyoto Encyclopedia of Genes and Genomes database (KEGG) pathway enrichment analysis

Database for Annotation, Visualization and Integrated Discovery (David) database (https://david.ncifcrf.gov/) was used to analyze the GO function and KEGG pathway enrichment of the core targets of RAM and ALI/ARDS. After screening the items with $P < 0.05$, all data were exported, and visualized with Omicshare to explain the biological function and related signal pathways of treatment of ALI/ARDS with ALI. The results were presented in the form of column chart and bubble chart.

The workflow of the study on the treatment of ALI/ARDS with ALI based on network pharmacology was shown in Figure 1.

3. Results

3.1 Screening of RAM active components

Through TCMSP and PubChem database, the components without corresponding target proteins were eliminated, and 92 active compounds of RAM were obtained.

3.2 Screening of ALI/ARDS targets

By searching the disease action targets of ALI/ARDS in the disease target database (OMIM and GeneCards), mining and deleting duplicate targets, 172 potential disease targets were collected and standardized by UniProt ID.

3.3 Screening of common targets of drugs and diseases

As shown in the Venn chart (Fig. 2), 46 common targets of RAM and ALI/ARDS were obtained. It also shown that RAM played a therapeutic role by acting on multiple related targets of ALI/ARDS through a variety of components.

3.4 Construction and analysis of Components-Target-Protein network

RAM active components and action targets were imported into Cytoscape3.8.0 software to construct the “Components-Target-Protein” network diagram, including 982 nodes and 1665 edges (Fig. 3). Through the network topology analysis, the core targets of RAM in the treatment ALI/ADRS were filtrated by condition that the Degree, BC and CC were greater than their respective median. As shown in Table 1, there were 13 core targets screen out, including CXCL8, CASP8, IL6, CCND1, NOS2, etc. The core targets might be closely related to RAM in the treatment of ALI/ARDS, and adopted in further GO and KEGG analysis.

Table 1
The core targets of RAM in the treatment of ALI/ARDS

Uniprot ID	Gene names	Betweenness Centrality (BC)	Closeness Centrality (CC)	Degree
P10145	CXCL8	0.0186	0.2290	5
Q14790	CASP8	0.0169	0.2251	8
P05231	IL6	0.0319	0.2456	11
P24385	CCND1	0.0314	0.2460	11
P35228	NOS2	0.0545	0.2598	11
P01375	TNF	0.0609	0.2515	13
P05412	JUN	0.0314	0.2145	14
P37231	PPARG	0.0124	0.2174	15
P31749	AKT1	0.0659	0.2448	16
P25963	NFKBIA	0.0263	0.2496	19
P19838	NFKB1	0.0325	0.2474	22
Q04206	RELA	0.0491	0.2558	26
P35354	PTGS2	0.1645	0.2672	58

3.5 Construction and analysis of PPI network

To explore the relationship between the 13 core targets for treatment of ALI/ARDS, the PPI network was constructed through STRING database. As shown in the Fig. 4, strong interactions were observed between the core targets in the PPI network, which contained a total of 13 nodes, 36 edges, and the average node degree was 5.54, the avg. local clustering coefficient was 0.682.

3.6 GO function enrichment analysis

As shown in Fig. 5, by using David database, GO function enrichment analysis was performed on the core targets of treatment of ALI/ARDS, a total of 38 GO-related ($P < 0.05$) information were obtained, including 26 biological processes, 4 cellular components and 8 molecular functions. The enrichment results (Fig. 5A) showed that the biological processes of RAM in the treatment of ALI/ARDS were mainly related to cellular response to lipopolysaccharide, inflammatory response, positive regulation of transcription from RNA polymerase II promoter, positive regulation of nitric oxide biosynthetic process, cellular response to nicotine and so on. Cellular components (Fig. 5B) were mainly involved I-κB/NF-κB complex, cytosol, nucleoplasm and nucleus. Molecular functions (Fig. 5C) were mainly focused on identical protein binding, transcription factor binding, transcription regulatory region DNA binding, RNA polymerase II distal enhancer sequence-specific DNA binding, protein homodimerization activity, chromatin binding, transcription factor activity, sequence-specific DNA binding, protein heterodimerization activity. The results suggested that ALI/ARDS involved multiple biological processes in *vivo*, and RAM might play a role in the treatment of ALI/ARDS by regulating these biological processes. The top 20 biological processes and all cellular components and molecular functions were concluded for visual analysis.

3.7 KEGG pathway enrichment analysis

The David platform was used for conducting KEGG pathway enrichment analysis for the core targets of RAM and ALI/ARDS with the screening condition $P \leq 0.05$. Excluding the pathways unrelated to ALI/ARDS diseases, and a total of 18 pathways were obtained, and output all KEGG pathways in the form of bubble diagram (Fig. 6). The size of the nodes indicated the number of enriched targets, and the color of the nodes from red to blue indicated the *p*-value from large to small. Therefore, the larger the red node, the higher the significance of the signal path. After analysis, the signal pathways were mainly distributed in Toll-like receptor signaling pathway, TNF signaling pathway, NOD-like receptor signaling pathway, small cell lung cancer, apoptosis, RIG-I-like receptor signaling pathway and the details were listed in Table 2..

Table 2
Main KEGG pathways significantly relating to core targets

Term	Pathway	Count	*P*-Value	Pop Hits
hsa04620	Toll-like receptor signaling pathway	9	1.15E-12	106
hsa04668	TNF signaling pathway	9	1.24E-12	107
hsa04621	NOD-like receptor signaling pathway	7	1.97E-10	56
hsa05222	Small cell lung cancer	7	2.59E-09	85
hsa04210	Apoptosis	6	3.81E-08	62
hsa04622	RIG-I-like receptor signaling pathway	6	7.08E-08	70
hsa04064	NF-kappa B signaling pathway	6	2.13E-07	87
hsa04660	T cell receptor signaling pathway	6	4.29E-07	100
hsa04662	B cell receptor signaling pathway	5	4.32E-06	69
hsa04920	Adipocytokine signaling pathway	5	4.58E-06	70
hsa04623	Cytosolic DNA-sensing pathway	4	1.59E-04	64
hsa04062	Chemokine signaling pathway	5	2.16E-04	186
hsa04024	cAMP signaling pathway	5	2.75E-04	198
hsa04010	MAPK signaling pathway	5	7.00E-04	253
hsa04071	Sphingolipid signaling pathway	4	1.02E-03	120
hsa04151	PI3K-Akt signaling pathway	5	2.23E-03	345
hsa04068	FoxO signaling pathway	3	2.19E-02	134
hsa04630	Jak-STAT signaling pathway	3	2.54E-02	145

4. Discussion

As one of the sources of Tibetan medicine “Dali”, Rhododendron has the effects of relieving cough and asthma, clearing heat and detumescence and tonifying the kidney(23). It is clinically used to treat respiratory diseases such as cough, asthma, phlegm and so on(24). Its chemical constituents mainly include flavonoids, sesquiterpenoids, triterpenoids, chromogen ketones, phenolic acids and etc.(22). In this study, the active components of RAM were obtained to the greatest extent through multiple literature search databases, after screening the components in TCMSP and PubChem database, the related targets of each component were integrated, and the active targets and involved pathways of ALI/ARDS were studied by using those databases. The mechanism of RAM in the treatment of ALI/ARDS was explained from the perspective of targets and signal pathways, the “Component-Target-Protein” interaction network

diagram of RAM in the treatment of ALI/ARDS was constructed, 92 active components and 46 common action targets of RAM were screened, and 13 significant and effective action targets were screened by STRING and Cytoscape software. At the same time, GO and KEGG enrichment showed that they involved 26 biological processes, 4 cellular components, 8 molecular functions and 18 signal pathways. Through the network pharmacological analysis of the components and targets of RAM, the targets and pathways of RAM in the treatment of ALI/ARDS had a scientific basis.

By searching literatures and online pharmacological research methods, most of the screening criteria for drug active ingredients included integrated model ADME information screening to identify the potential bioactive, including the evaluation of Caco2 permeability, oral bioavailability (OB), and drug-likeness (DL), and half-life (HL) were used to apply ADME-related models(25–27). In this study, because most Tibetan drugs were natural drugs, with diverse sources of drugs, complex chemical components, and the active components that exert the efficacy were not clear. Meanwhile, there were few studies on the mechanism of drug action, and many factors such as cultivation, harvesting, processing and storage might have a certain impact on the components of drugs(28). Combined with various literature databases, it was found that there were few studies on RAM with messy data information, and its active components and action targets were less recorded in TCMSP and PubChem databases. Based on the above reasons, in the current study, we did not screen the active components of RAM at the ADME level. Therefore, the chemical components of RAM that might play a role but were not retrieved in TCMSP and PubChem need to be studied.

Through the screening of active components and target prediction of RAM, it could be seen that RAM mainly contains 92 chemical components such as naringenin, isorhamnetin, kaempferol and avicularin. A Study had reported that naringenin can regulate T cell subsets and reduce the serum concentration of proinflammatory cytokines such as TNF-α, INF-γ, IL-6, and the globular inflammatory reaction, so as to achieve the effect of treating inflammation(29). Isorhamnetin inhibited the activity of myeloperoxidase (MPO), the levels of TNF-α and IL-6, to abolish inflammation and support the mRNA expression of pro-inflammatory mediators(30). Kaempferol reduced the infiltration of $CD3^+$ T cell and gene expression of major pro-inflammatory cytokines including IL-6, IL-17A and TNF-α, at the same time, it also reduced the signal of regulated pro-inflammatory NF-κB(31). Avicularin prevented the activation of MEK/NF-κB pathway, inhibited inflammatory response, prevented cell viability, and induced TNF-α treatment of apoptosis of human RA synovial cells(32). It can be seen that the active ingredients represented by naringenin, isorhamnetin, kaempferol and avicularin play an important role RAM in the treatment of ALI/ARDS, especially in terms of anti-inflammatory.

Based on the“components-target-protein” network and PPI network, 13 core targets were screened for RAM treatment of ALI/ARDS, including IL-6, TNF, CXCL8, NOS2, RELA, NF-κB1, NF-κBIA, etc, which were closely related to inflammation, immune and inflammatory response, cell growth, differentiation and apoptosis. As an inflammatory factor, IL-6 is an indicator of inflammatory response, and TNF can promote the expression of IL-6 and mediate inflammatory response(33). The abnormal regulation of CXCL8 and its receptors was related to many inflammatory mediated diseases. In response to external

stimuli, lung macrophages secrete CXCL8, which coordinated the migration of PMN to the lungs and led to airway inflammatory diseases(34). Through enhancing macrophage migration and survival, circulating inflammatory signals, and impairing lipid metabolism, the lung SCC was promoted by NOS2. Also, inflammatory signals between macrophages and epithelial cells could be circulated by NOS2(35). As macrophages, RELA was significant in inflammatory response, excessive RELA activation in macrophages during inflammation could lead to serious tissue damage and endanger health(36). A group of transcription factors composed of NF-κB family contained NF-κB1, NF-κBIA were crucial mediators of inflammatory response. In addition to the inflammatory response, there were various signal transduction processes gathered on the NF-κB pathway, including cell proliferation, apoptosis, angiogenesis and many other processes(37). From the above description, it was not difficult to see that the above targets were closely related to the subsequent pathways

Go and KEGG enrichment analysis showed that the biological processes of RAM in the treatment of ALI/ARDS were mainly related to the response of cellular response to lipopolysaccharide, inflammatory response, positive regulation of nitric oxide biosynthetic process, etc. Molecular functions were mainly focused on identical protein binding, transcription factor binding, etc. Cellular components were mainly involved in I-κB/NF-κB complex, cytosol and so on. At the same time, combined with KEGG pathway enrichment analysis, RAM treatment of ALI/ARDS involved Toll-like receptor signaling pathway, TNF signaling pathway, etc. By querying the role and mechanism of each pathway in PubChem database, it could be concluded that cAMP signaling pathway, MAPK signaling pathway, Sphingolipid signaling pathway,PI3K-Akt signaling pathway, FoxO signaling pathway can regulate many physiological processes such as cell growth, differentiation, apoptosis and death, while other 8 pathways such as Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, RIG-I-like receptor signaling pathway, NF-κB signaling pathway were related to cellular inflammatory response, immune response and the NF-κB dependent expression of inflammatory cytokines. And Jak-STAT signaling pathway, TNF signaling pathway, Adipocytokine signaling pathway can participate in many important biological processes such as cell proliferation, differentiation, apoptosis and immune regulation at the same time. In addition, when screening all KEGG pathways provided by David website, we retained small cell lung cancer, and as the only lung related disease in the results, we believe that it might be related to ALI/ARDS to some extent.

In conclusion, this study shown that RAM might act on 46 targets such as TNF, TP53, IL6, PTGS2, CCL2 and VEGFA through 92 active components to regulate Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, RIG-I-like receptor signaling pathway and NF-κB signaling pathway, to involve in anti-inflammatory, immune response and promoting inflammatory cytokines NF-κB dependent expression and other biological processes, which play a therapeutic role in the treatment of ALI/ARDS. This study made an exploratory study on the action pathway and mechanism of RAM on ALI/ARDS. It was found that RAM played an effective role in the treatment of ALI/ARDS through the synergistic action of multi-component, multi-target and multi-pathway, which provided a theoretical basis for the follow-up in-depth research, in order to provide new ideas and references for the development and utilization of RAM. However, further experimental research and demonstration are still needed to lay a foundation for further exploring the mechanism of RAM’s drug-target interaction

Abbreviations

RAM: *Rhododendron anthopogonoides* Maxim

ALI: acute lung injury

ARDS: acute respiratory distress syndrome

TCMSP: Traditional Chinese Medicine Database and Analysis Platform

OMIM: Online Mendelian Inheritance in Man

ECMO: extracorporeal membrane oxygenation

CS: corticosteroid

TCM: traditional Chinese medicine

SARS: severe acute respiratory syndrome

NCBI: The National Center for Biotechnology Information

BC: Betweenness Centrality

CC: Closeness Centrality

PPI: Protein–Protein Interaction

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

David: Database for Annotation, Visualization and Integrated Discovery

OB: oral bioavailability

DL: drug-likeness

HL: half-life

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to publish.

Data Availability

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

Competing Interests

The authors declare that they have no conflicts of interest.

Authors' contributions

YF is the main contributor to this manuscript. YF analyzed and summarized the literature and wrote the manuscript. YY, YW, JW, LH collected data. YY, YW analyzed and interpreted the data. XZ and SG (corresponding authors) conceived and coordinated the study and critically evaluated the data. All authors read and approved the final manuscript.

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References

1. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012;307(23):2526-33.
2. Standiford TJ, Ward PA. Therapeutic targeting of acute lung injury and acute respiratory distress syndrome. Transl Res. 2016;167(1):183-91.
3. Butt Y, Kurdowska A, Allen TC. Acute Lung Injury A Clinical and Molecular Review. Arch Pathol Lab Med. 2016;140(4):345-50.
4. Suresh R, Kupfer Y, Tessler S. Acute respiratory distress syndrome. N Engl J Med. 2000;343(9):660-1.
5. Killien EY, Mills B, Watson RS, V Avilala MS, Rivara FP. Morbidity and Mortality Among Critically Injured Children With Acute Respiratory Distress Syndrome. Critical Care Medicine. 2018.
6. Nieman GF, Gatto LA, Andrews P, Satalin J, Habashi NM. Prevention and treatment of acute lung injury with time-controlled adaptive ventilation: physiologically informed modification of airway pressure release ventilation. Annals of Intensive Care. 2020;10(1).

7. Luh SP, Chiang CH. Acute lung injury/acute respiratory distress syndrome (ALI/ARDS): the mechanism, present strategies and future perspectives of therapies. Journal of Zhejiang University SCIENCE B. 2007;8(1):60-9.
8. Zhang Z, Chen L, Ni H. The effectiveness of Corticosteroids on mortality in patients with acute respiratory distress syndrome or acute lung injury: a secondary analysis. Scientific Reports. 2015;5:17654.
9. Shen TZ, Guo JH, Wang XJ, Jiang SP. Research Progress in the Treatment of Acute Lung Injury. Chinese Journal of Modern Applied Pharmacy. 2021;38(3):5.
10. Mokra, Mikolka, Kosutova, Mokry. Corticosteroids in Acute Lung Injury: The Dilemma Continues. International Journal of Molecular Sciences. 2019;20(19):4765.
11. Zhang L, Ding SX, Li CF, Wang YF, Chen Z, Wang ZQ. Effects of N-acetylcysteine treatment in acute respiratory distress syndrome: A meta-analysis. Experimental Therapeutic Medicine. 2017;14(4):2863-8.
12. Zhou Y, Li P, Goodwin AJ, Cook JA, Halushka PV, Chang E, et al. Exosomes from endothelial progenitor cells improve outcomes of the lipopolysaccharide-induced acute lung injury. Critical Care Medicine. 2019;23(1).
13. He YQ, Zhou CC, Yu LY, Wang L, Chen WS. Natural product derived phytochemicals in managing acute lung injury by multiple mechanisms. Pharmacological Research. 2020;163(1):105224.
14. Zhan D, Duojiciren, Mei CN. A Brief History of Tibetan Medicine. Herald of Medicine. 2019;38(04):456-60.
15. Cang KC, Xie JP, Shao R. On Current Situation and Development of Tibetan Medicine Industry in the Tibet Autonomous Region. Journal of Tibet University: Social Science Edition. 2021;36(2):8.
16. Kuang TT, Sun M, Wang Z, Zhu XQ, Tang TH, Cao MD, et al. Study on Varieties, Indications and Usage Features of Tibetan Medicine Preparations Based on Drug Standards. China Pharmacy. 2017;28(16):2231-4.
17. Liu DH, Zhong WL, Zhu YY, Wu HC, Du SY. Pharmacological Effects of Tibetan Medicine on New Coronavirus Pneumonia. Chinese Traditional Patent Medicine. 2021:1-7.
18. Hou KS, Wu DL. A Dictionary of the Families and Genera of Chinese Seed Plants. Beijing, China: Science Press; 1982.
19. Li XF, Jin HZ, Chen G, Yan SK, Shen YH, Yang M, et al. Study Advances on Chemical Constituents and Pharmacological Activities of the Tibetan Medicine Dali. Natural Product Research And Development. 2008(6):1125-8.
20. Jing L, Ma H, Fan P, Gao R, Jia Z. Antioxidant potential, total phenolic and total flavonoid contents of Rhododendron anthopogonoides and its protective effect on hypoxia-induced injury in PC12 cells. BMC complementary and alternative medicine. 2015;15:287.
21. Dai SJ, Chen RY, Yu DQ. Studies on the flavonoid compounds of Rhododendron anthopogonoides. China Journal of Chinese Materia Medica. 2004(01):48-51.

22. Fan MX, Zhao JQ, Yuan Q, Tao YD, Shao Y, Mei LJ. Chemical constituents from Rhododendron anthopogonoides. Chinese Herbal Medicine. 2016;47(21):3769-72.
23. Peng Y, Zeng R, Wu SJ, Lei S, Sun ZN, He XQ, et al. Content determination of five flavonoids in Tibetan medicine Rhododendron anthopogonoides by quantitative analysis of multi-components by single marker (QAMS). China Journal of Chinese Materia Medica. 2021;46(9):8.
24. Pharmacopoeia Committee of Ministry of Health P. Drug standard of the Ministry of health of the people's Republic of China: Tibetan Medicine. Beijing, China.1995.
25. Hu W, Fu W, Wei X, Yang Y, Lu C, Liu Z. A Network Pharmacology Study on the Active Ingredients and Potential Targets of Tripterygium wilfordii Hook for Treatment of Rheumatoid Arthritis. Evidence-based complementary alternative medicine : eCAM. 2019;2019:5276865.
26. Lu C, Fu W, Zhou R, Hu W. Network pharmacology-based study on the mechanism of Yiganling capsule in hepatitis B treatment. BMC Complementary Medicine Therapies. 2020;20(1):1-17.
27. Qu SY, Li XY, Xia H, Qi YY, Zhu HX. Analysis of Antidepressant Activity of Huang-Lian Jie-Du Decoction Through Network Pharmacology and Metabolomics. Frontiers in pharmacology. 2021;12:619288.
28. Wang J, Zhang B. Clinical Chinese Pharmacy. China: People's Medical Publishing House(PMPH); 2012.
29. Abrego-Peredo A, Romero-Ramírez H, Espinosa E, López-Herrera G, Rodríguez-Alba J. Naringenin mitigates autoimmune features in lupus-prone mice by modulation of T-cell subsets and cytokines profile. PLoS ONE. 2020;15(5):e0233138.
30. Dou W, Zhang J, Li H, Kortagere S, Sun K, Ding L, et al. Plant flavonol isorhamnetin attenuates chemically induced inflammatory bowel disease via a PXR-dependent pathway. Journal of Nutritional Biochemistry. 2014;25(9):923-33.
31. Liu C, Liu H, Lu C, Deng J, Yan Y, Chen H, et al. Kaempferol attenuates imiquimod-induced psoriatic skin inflammation in a mouse model. Clinical and experimental immunology. 2019;198(3):403-15.
32. Wang W, Zheng H, Zheng M, Liu X, Yu J. Protective effect of avicularin on rheumatoid arthritis and its associated mechanisms. Experimental and therapeutic medicine. 2018;16(6):5343-9.
33. Cao LH, Yang X, Zhao YY, Li XM, Miao MS. Effect of Rhizoma Phragmitis on Chronic Bronchitis Based on Network Pharmacology and Its Mechanism. Pharmacology and Clinics of Chinese Materia Medica. 2021;37(02):96-103.
34. Ha H, Debnath B, Neamati N. Role of the CXCL8-CXCR1/2 Axis in Cancer and Inflammatory Diseases. Theranostics. 2017;7(6):1543-88.
35. Wang X, Gray Z, Willette-Brown J, Zhu F, Shi G, Jiang Q, et al. Macrophage inducible nitric oxide synthase circulates inflammation and promotes lung carcinogenesis. Cell death discovery. 2018;4:46.
36. Chatterjee B, Banoth B, Mukherjee T, Taye N, Vijayaragavan B, Chattopadhyay S, et al. Late-phase synthesis of IκBα insulates the TLR4-activated canonical NF-κB pathway from noncanonical NF-κB signaling in macrophages. Science signaling. 2016;9(457):ra120.

37. Zhang JW, Chen QS, Zhai JX, Lv PJ, Sun XY. Polymorphisms in NF-κB pathway genes & their association with risk of lung cancer in the Chinese population. Pakistan journal of medical sciences. 2015;31(6):1411-6.

Figures

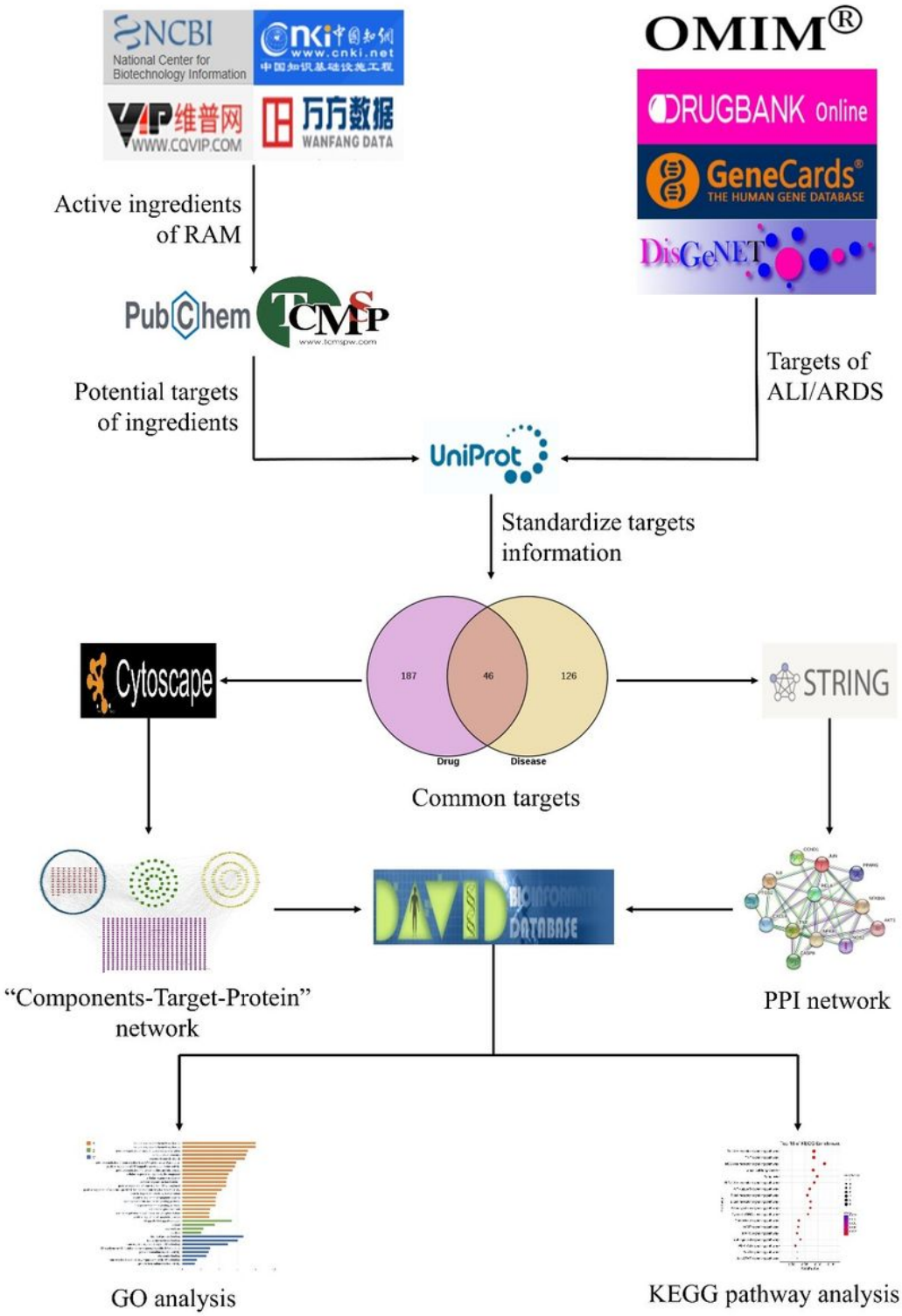

Figure 1

The workflow of the study on the treatment of ALI/ARDS.

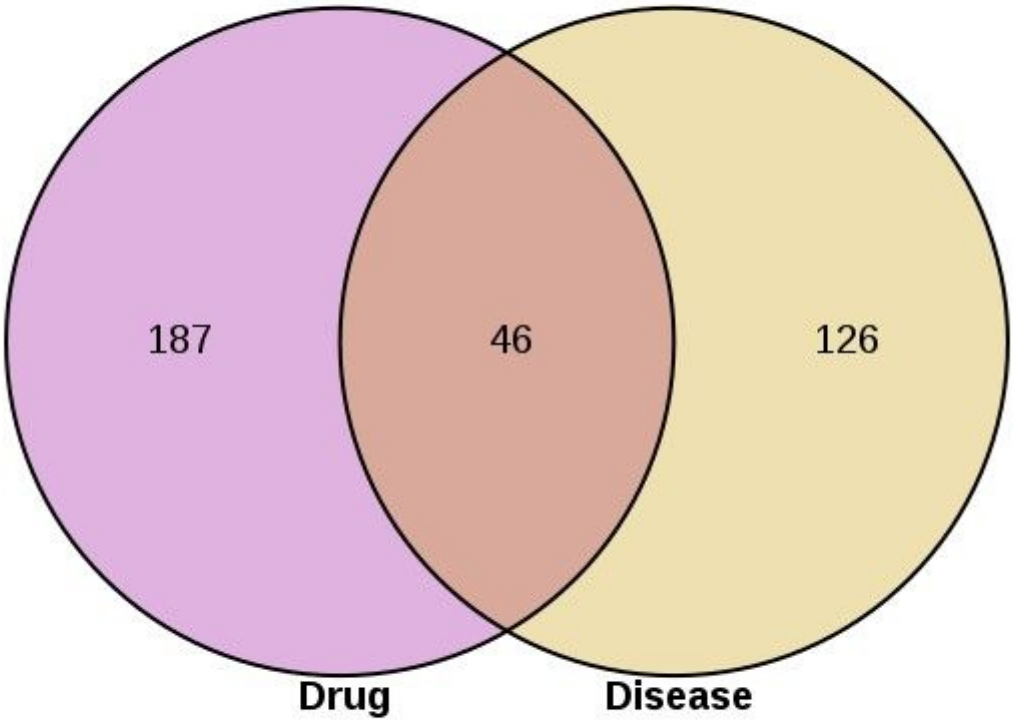

Figure 2

Venn diagram of “drug-disease” intersection targets of RAM in the treatment of ALI/ARDS.

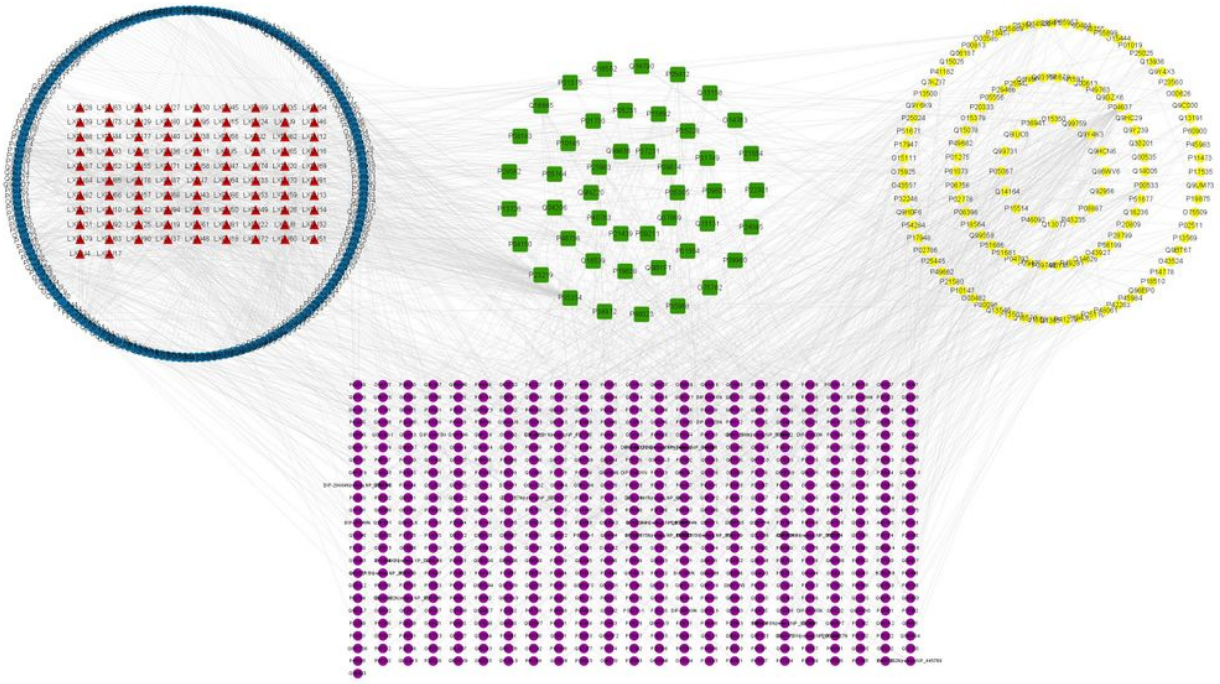

Figure 3

“Components-Target-Protein” network diagram of RAM in the treatment of ALI/ARDS. Note: the red triangle represented drug active components, the blue circle represented the drug/component targets, the yellow circle represented disease targets, the green rectangular represented the disease targets directly affected by the drug/component, and the rose red circle represented proteins interacting with the targets.

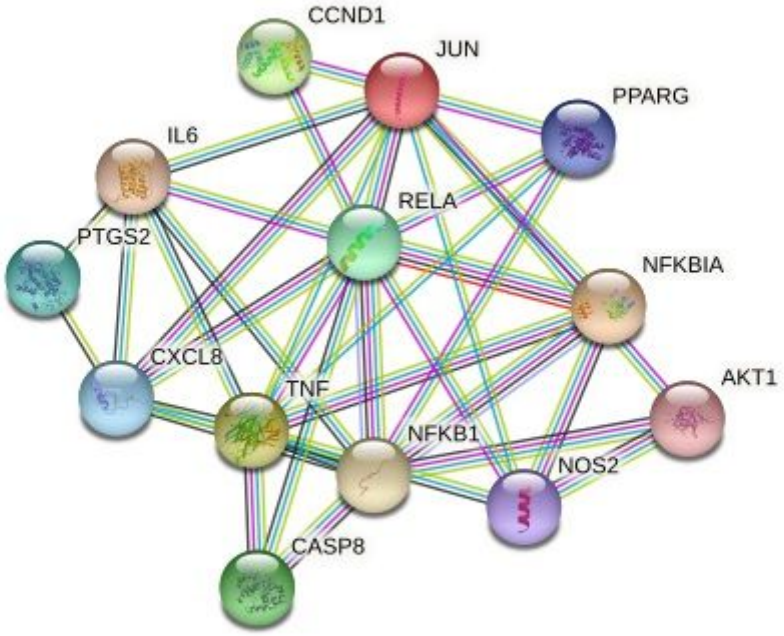

Figure 4

PPI network of core targets on the treatment of ALI/ARDS.

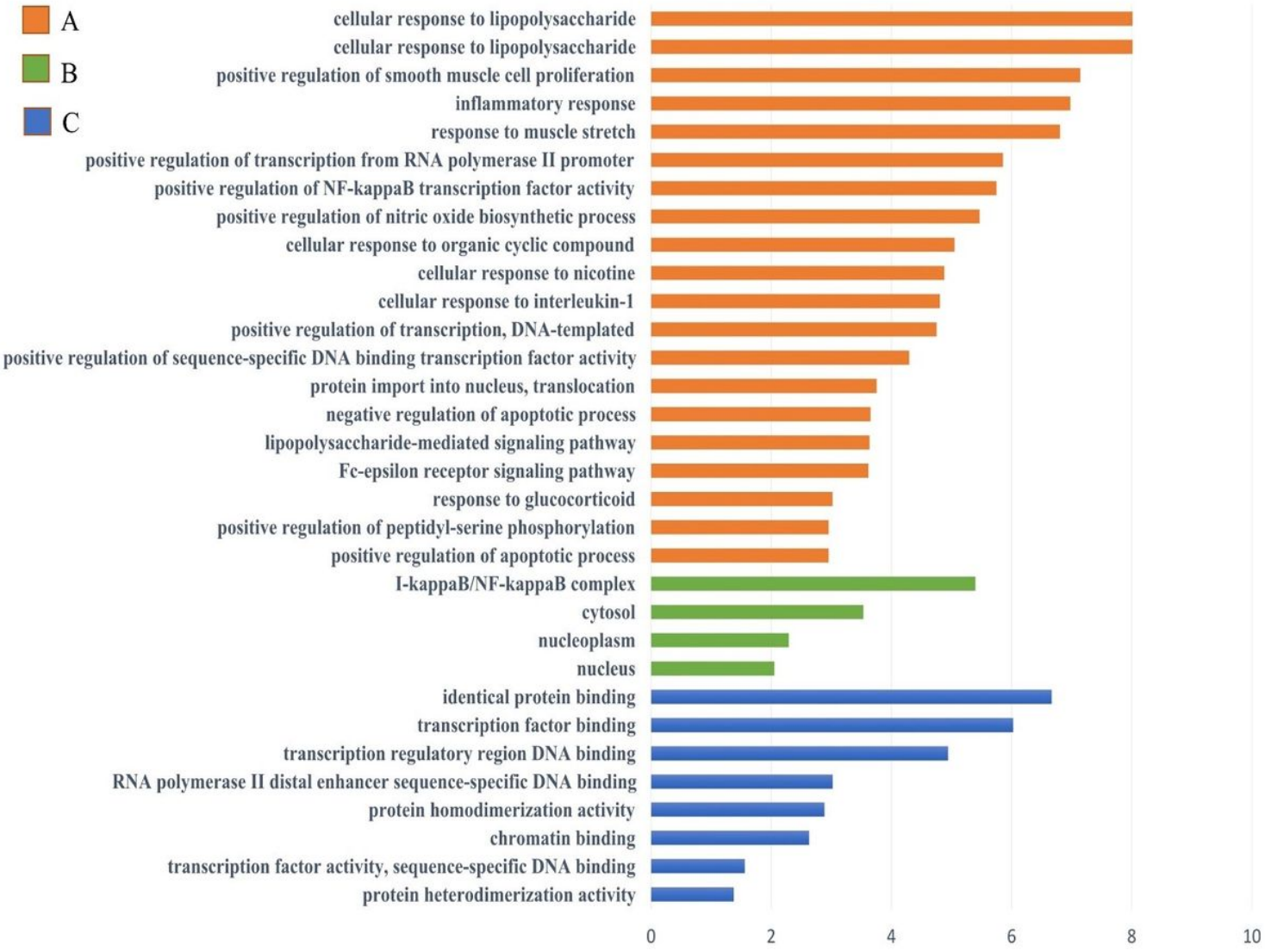

Figure 5

GO enrichment results of RAM in the treatment of ALI/ARDS. Note: (A) biological processes, (B) cellular components, (C) molecular functions

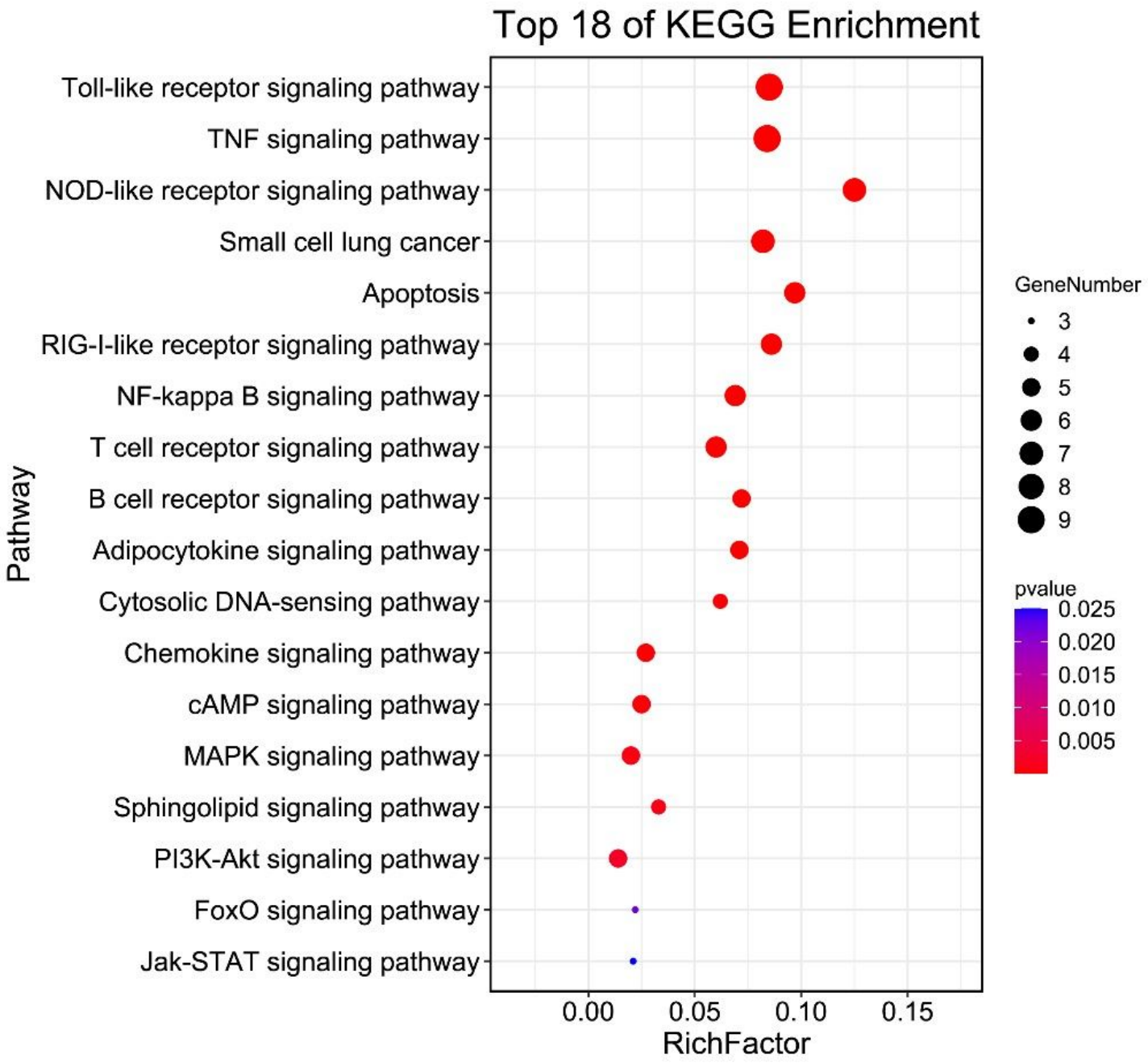

Figure 6

KEGG pathway enrichment analysis results