Hindawi
Journal of Food Biochemistry
Volume 2024, Article ID 1552878, 13 pages
https://doi.org/10.1155/2024/1552878

Research Article

Analysis and Validation of the Network Pharmacology of the Mechanism of Glycyrrhetinic Acid for the Treatment of High-Altitude Pulmonary Hypertension

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Received 3 May 2023; Revised 25 March 2024; Accepted 22 April 2024; Published 10 May 2024

Academic Editor: Ali Akbar

Glycyrrhetinic acid (GA) is a pentacyclic triterpene component in *Glycyrrhize glabra* L, it has demonstrated an inhibitive effect on high-altitude pulmonary hypertension (HAPH), but the molecular action is still not known. We aimed to explore the mechanism of GA for the treatment of HAPH based on network pharmacology and molecular docking method. Cell experiment validation was also conducted. The targets for GA were screened using the Swiss Target Prediction and Batman databases. The HAPH-related targets were obtained using the GeneCards and OMIM databases. The common targets for diseases and drugs were obtained using a Venn diagram. The core targets were screened using the String database. Then, a component-target-disease diagram and protein–protein interaction (PPI) network mutual assistance diagram were developed using Cytoscape3.9.1 software. GO functional and KEGG pathway enrichment analyses were conducted using the Metascape database. Finally, molecular docking of the target and its corresponding active components were performed using Autodock software. A total of 68 common targets for glycyrrhetinic acid high-altitude pulmonary hypertension were screened out. The core targets include PTGS1, MMP1, SERPINA6, and nitric oxide synthase (NOS2), involving PPAR signal pathway, human cytomegalovirus infection, IL-17 signal pathway, proteoglycans in cancer, and other pathways. The molecular docking affinity was $-8.4\,\text{kcal}\cdot\text{mol}^{-1}$ in average, indicating that GA has a good binding stability with key target proteins. In the PDGF-BB-induced PASMC proliferation model, PASMC proliferation and the p-p38, p38, p-ERK1/2, and ERK1/2 protein expression were inhibited. The pharmacological mechanism of GA for the treatment of HAPH was characterized by multi-target and multi pathway. GA may serve as a promising therapeutic candidate for HAPH but still needs further *in vivo/in vitro* experiment.

1. Introduction

Glycyrrhetinic acid (GA), which has an oleanolane-type skeleton, is a pentacyclic triterpene component in *Glycyrrhize glabra* L extract, a traditional Chinese medicine, and is widely distributed in nature. With multiple biological activities such as anti-inflammatory [1], anti-tumor [2, 3], antioxidant [4], liver protection [5, 6], and enhanced cellular immune regulation [7], GA has significant curative effects on ventricular arrhythmias induced by various reasons, can protect myocardium, and can reduce myocardial ischemic damage for acute myocardial infarction [8]. The biological

role of GA in improving lung function has been confirmed. For example, 18β-GA can reduce radiation-induced lung injury and fibrosis in mice by inhibiting inflammation [9]. 18β-GA can effectively inhibit the proliferation of non-small cell lung cancer [10]. 18β-GA regulates the activity of RhoA-ROCK signaling pathway, inhibits the proliferation of HPASMCs, and has potential value in the treatment of PAH [11]. Previous studies have shown that 18β-GA not only reduces pulmonary arterial pressure in HAPH model rats but also can alleviate the metabolic disorder of HAPH rats through anti-oxidation and anti-inflammatory effects, improve their bodies' ability to resist hypoxia, and restore various metabolic pathways (energy metabolism, amino acid metabolism, and lipid metabolism) [12], as well as have a certain protective effect against cardiac injury in HAPH rats [13]. However, 18β-GA has potential therapeutic effects on HAPH rats, but its target needs to be further studied.

High-altitude pulmonary hypertension (HAPH) is a complication of altitude diseases, and it is mainly caused by maladaptation or inadaptation to altitude acclimatization. It is characterized by severe symptomatic excessive erythrocytosis and hypoxemia. Approximately 0.14 billion people live at a high-altitude (above 2,500 m), and the number of people who go to the high-altitude areas for working or travelling is also increasing annually [14]. The cause of HAPH is mainly related to environment, gene, and heredity. Its pathogenesis is that long-term hypoxia and low pressure cause a decrease in alveolar oxygen partial pressure, which leads to the contraction of small and medium-sized pulmonary arteries, pulmonary vascular remodeling, and increased pulmonary blood flow resistance, resulting in hypoxic pulmonary hypertension [15].

Based on network pharmacology, drug molecular targets and disease-related targets were screened out by using computer simulations and various databases. It can reveal complex network connections among drugs, targets, and diseases by using high-throughput screening, network visualization, and network analysis, to analyze and predict the drug mechanism [16]. In this paper, network pharmacology was combined with molecular docking to explore the possible mechanism of glycyrrhetinic acid in treating HAPH, which can provide a new theoretical support for the clinic treatment of HAPH.

2. Materials

2.1. Drugs and Reagents. The reagents used include glycyrrhetinic acid (Shanghai Yuanye Biotechnology Co., Ltd.) Phospho-p38 MAPK (p-p38 MAPK) monoclonal antibody, Phospho-ERK1/2 (p-ERK1/2) monoclonal antibody, p38 MAPK monoclonal antibody, ERK1/2 monoclonal antibody, GAPDH monoclonal antibody (US CST Company, Batch No. 9377S, 4511T, 8460T, 9145T, and 4967S).

2.2. Database and Analysis Platform

Compound target prediction database: Swiss Target Prediction (https://swisstargetprediction.ch/) [17] and Batman (https://bionet.ncpsb.org/batman-tcm/) databases [18].

Disease target database: GeneCards (https://www.genecards.org/) [19] and OMIM (https://omim.org/) databases [20].

Protein sequence database: UniProt (https://www.uniprot.org/) database.

Protein interaction database: String (https://string-db.org) [21].

Protein structure database: Protein Data Bank (PDB) database (https://www.rcsb.org/).

Chemistry Database: PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Enrichment analysis database: DAVID (https://david.ncifcrf.gov/).

Bibliographic databases: China national knowledge Internet (https://www.cnki.net) and Pubmed (https://pubmed.ncbi.nlm.nih.gov/).

Molecular docking software: DockThor Version 2.0software.

Image-based rendering software: InteractiVenn, Bioinformatics (https://www.bioinformatics.com.cn), Cytoscape 3.9.0, PyMol 2.4.0, and GraphPad Prism 7.

Statistical software: SPSS 21.0.

3. Methods

3.1. Information Query on Glycyrrhetinic Acid-High Altitude Pulmonary Hypertension Common Targets and Collection of Key Targets. The targets of glycyrrhetinic acid were retrieved from the BATMAN and Swiss Target Prediction databases. Among them, the targets with probability >0 were included in the Swiss Target Prediction database, and the targets with score >20 were included in Batman Database. After removing duplicate data from the retrieved targets by using the Uniprot database, the potential targets for GA were obtained. High-altitude pulmonary hypertension and hypoxic pulmonary hypertension were used as the keywords to retrieve the GeneCards and OMIM databases, the obtained disease targets were combined, and duplicate data were removed. The screened GA and HAPH targets were used as input into Venny 2.1, a Vann diagram software, for the analysis, and the intersection target was obtained.

3.2. PPI Network Construction and Core Target Screening. By using the Interacti Venn software, the potential targets of GA were mapped to the disease targets of HAPH. Then, GA high-altitude pulmonary hypertension common targets were obtained. By taking GA and HAPH common targets as "nodes" and the interactions between targets as "edges," String database was used to construct GA high-altitude pulmonary hypertension common targets and PPI. The PPI network was introduced into Cystoscape 3.8.0 to perform topology analysis by using the network analyzer. Four parameters such as degree, betweenness centrality, average shortest path length, and closeness centrality were used as the reference standard. Based on degree ranking, genes with scores greater than the average were selected as key targets. At the same time, the MCODE module was used to analyze gene clusters and screen core targets [22].

3.3. Enrichment Analysis on Glycyrrhetinic Acid-High-Altitude Pulmonary Hypertension Common Targets. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for glycyrrhetinic acid high-altitude pulmonary hypertension common targets. The top 20 biological process (BP), molecular function (MF), cellular component (CC), and KEGG pathways with FDR (Q value) < 0.05 were screened out. a small FDR value indicates that biological process or pathway is closely related to GA for the treatment of HAPH. Finally, the results were visualized using the Bioinformatics platform, and the results are displayed in bar and bubble charts.

3.4. GA–Key Targets–Main Pathways–High-Altitude Pulmonary Hypertension Network. By taking GA, key targets, main pathways, and HAPH as "nodes" and the interactions between nodes as "edges," Cytoscape 3.8.0 software was used to construct a "GA–key targets–main pathways–high-altitude pulmonary hypertension" network pharmacology map.

3.5. Molecular Docking. The molecular structure of GA was extracted from PubChem database, and the protein molecular structure of key targets was extracted from PDB database. DockThor software was used to perform molecular docking. The evaluation index of intermolecular binding ability was the docking affinity. The binding mode was visualized using PyMol software.

3.6. Cell Experiment Validation

3.6.1. Detecting the Effect of GA on the Proliferation of PASMCs Induced by PDGF-BB by Using the CCK-8 Method. The effect of GA on the activity of PASMCs was determined by performing CCK-8 cell proliferation experiment to study the effect of different doses of GA (20–80 μM) on 24 h of proliferation. PASMCs grow to 70%–80% fusion and then serum starvation was performed 24 h before the experiment. Cells were treated with PDGF-BB (20 ng/mL) for 24 h, treated with GA for another 24 h, and finally incubated with CCK-8 for 4 h. Cell proliferation was determined by measuring the absorbance at 450 nm.

3.6.2. Detecting the Effect of GA on the Cell Cycle of PASMCs Induced by PDGF-BB by Using Flow Cytometry. Cells in the six-well plate reached 70%–80% fusion after 24 h of starvation. Cells were pretreated with PDGF-BB (20 ng/mL) for 24 h and treated with 18β-GA (80 μM) for another 24 h for subsequent analysis. Cells were harvested using the trypsin method and fixed at 4°C with 70% ethanol. The fixed cells were centrifuged at 800 rpm for 5 min and resuspended in 500 μL of staining buffer before testing. A total of 10 μL of RNaseA was added to the mixture and then 25 μL propidium iodide was added. The suspension was incubated in the water bath at 37°C for 30 min. Finally, the cells were filtered through a 400-mesh filter and detected by flow cytometry.

3.6.3. Detecting the Effect of Glycyrrhetinic Acid on Protein Expression in PASMCs Induced by PDGF-BB by Using Western Blot. Proteins were extracted from HPASMC in lysis buffer by using a protein extraction reagent (BCA protein quantitative kit). The concentration of proteins was measured using a BCA protein assay kit. Lysates were centrifuged (2,000 rpm) at 4°C for 10 min. The protein expression (40 μg/swimlane) was detected using SDS PAGE (8% and 15% gel), and the protein was electroblotted onto the nitrocellulose membrane. Next, the membrane was sealed with PBST containing 5% skimmed milk at room temperature for 2 h and then anti-p38 antibody (diluted in 1 : 500, Abcam), anti-ERK1/2 antibody (diluted in 1 : 5,000, Abcam), anti-AKT antibody (diluted in 1 : 50, Abcam), and anti-β-Actin (1 : 1000, Proteintech Group) were used respectively overnight at 4°C. Then, the membrane was washed with PBST and incubated with horseradish peroxidase-conjugated goat anti rabbit IgG antibody (diluted in 1 : 2,000, Proteintech Group) for 2 h at room temperature. The protein strips were visualized using the ECL protein blot kit (Beyotime Biotechnology). The gray value of each strip on the blot was analyzed using Quantitative One software and standardized by using anti-β-actin antibody.

4. Results

4.1. Information on GA High-Altitude Pulmonary Hypertension Common Targets. Compound component targets were retrieved from the Swiss Target Prediction and Batman databases. The targets with probability >0 were included in the Swiss Target Prediction database, while the targets with score >20 were included in the Batman database. A total of 111 targets were obtained after the retrieved targets were corrected and de-duplicated based on the Uniprot database. After retrieving the disease target database, 2,232 hypoxic pulmonary hypertension-related targets and 319 high-altitude pulmonary hypertension-related targets were retrieved in GeneCards Database. A total of 94 genes related to hypoxic pulmonary hypertension and 149 targets related to high-altitude pulmonary hypertension were obtained from the OMIM database. A total of 2,385 disease-related genes were obtained after combining and de-duplicating genes in the two databases. The screened drug targets and disease targets were used as input into Venny 2.1, a Venn diagram software. Subsequently, 68 common targets were obtained and then used as predictive targets for drug acting on diseases for the following pathway enrichment analysis (Figure 1).

4.2. PPI Network Construction. The drug and disease common targets were used as input in the String database [5] () for PPI network construction. The biological species was set as "Homo sapiens" to obtain the PPI network. A total of 68 nodes and 323 edges were included in this network. The average value was 9.5 (Figure 2).

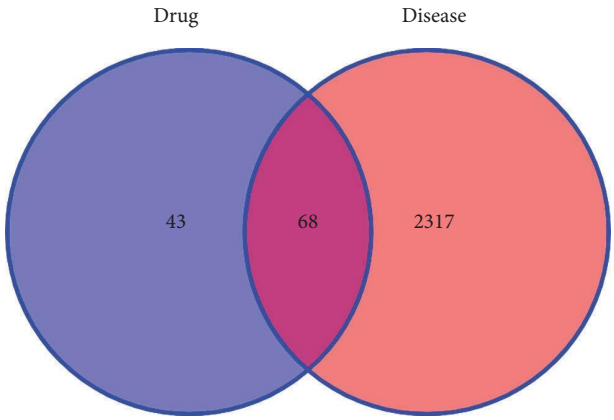


Figure 1: Venn diagram of GA high-altitude pulmonary hypertension common targets.

4.3. Topology Analysis. PPI network was introduced into Cystoscape 3.8.0 to perform topology analysis by using a network analyzer. Four parameters such as degree, betweenness centrality, average shortest path length, and closeness centrality were used as the reference standard. Based on degree ranking, genes with scores greater than the average were selected as key targets. A total of 25 key targets were screened out. These 25 targets were drawn into pictures by using R 3.6.3, with the abscissa being the degree value of each target (Figure 3).

4.4. MCODE Clustering Analysis. As show in Table 1, In this paper, key genes were screened out by using MCODE analysis [23]. The established PPI network was introduced into Cytoscape3.8.0. The MCODE module was opened to analyze gene clusters and screen core targets. A total of four gene clusters and four core genes are obtained. The core genes include PTGS1, MMP1, SERPINA6, and NOS2.

4.5. GO Biological Function Enrichment Analysis on Glycyrrhetinic Acid-High Altitude Pulmonary Hypertension Common Targets. GO enrichment was performed on the drug-disease common targets and disease targets. Based on the String database, the items with corrected P value < 0.05 were filtered. A total of 994 BP s were enriched in intersection targets, involving the regulation of inflammatory response, steroid metabolic process, regulation of lipid metabolic process, regulation of small molecule metabolic process, cellular ketone metabolic process, fatty acid metabolic process, lipid localization, fatty acid transport, positive regulation of lipid metabolic process, response to steroid hormone; 65 MFs were enriched, involving and steroid binding, nuclear receptor activity, ligand-activated transcription factor activity, drug binding, prostaglandin receptor activity, prostanoid receptor activity, icosanoid receptor activity, steroid hormone receptor activity, transcription coactivator binding, and fatty acid binding; 7 CCs were enriched, involving lipid droplet, nuclear envelope lumen, membrane raft, membrane microdomain, membrane region, peroxisome, and microbody. Based on the R 3.6.3 software, the clusterProfiler, enrichplot, and ggplot2 packages were installed to draw the histogram and bubble charts (Figure 4).

4.6. KEGG Pathway Enrichment Analysis on GA High-Altitude Pulmonary Hypertension Common Targets. KEGG pathway enrichment analysis was performed on the drug and disease common targets. Based on the String database, items with corrected P value < 0.05 were filtered, and a total of 30 signal pathways were enriched. Based on R 3.6.3 software, clusterProfiler package was installed to draw the histogram and bubble charts. The figure shows that it mainly involves the PPAR signaling pathway, IL-17 signaling pathway, Leishmaniasis, human cytomegalovirus infection, C-type lectin receptor signaling pathway, bladder cancer, ovarian steroidogenesis, regulation of lipolysis in adipocytes, endocrine resistance, proteoglycans in cancer, chemical carcinogenesis-receptor activation, steroid hormone biosynthesis, arachidonic acid metabolism, insulin resistance, inflammatory bowel disease, TNF signaling pathway, Fc epsilon RI signaling pathway, serotonergic synapse, adipocytokine signaling pathway, and prolactin signaling pathway. The above signal pathways may be closely related to the effect of GA for the treatment of HAPH (Figure 5).

4.7. Construction of 18β-GA High-Altitude Pulmonary Hypertension–Pathway–Target Network. The component–disease–pathway–target network document was introduced into Cytoscape3.8.0 to draw a pathway network diagram to effectively display the component, multi-target functional characteristics of the active ingredients of traditional Chinese medicine for disease treatment (Figure 6).

4.8. Molecular Docking. As shown in Table 2, the molecular docking affinity was $-8.4\,\text{kcal}\cdot\text{mol}^{-1}$ in average, indicating that 18β-GA has a strong affinity with key target proteins, and a good binding stability is present. Especially, the molecular docking between 18β-GA and the protein corresponded by NOS2 was the best. Figure 7 shows the molecular docking pattern between 18β-GA and key target protein, in which the gray molecule is 18β-GA.

4.9. Effect of GA on the Activity of PDGF-BB-Induced ASMC Proliferation Model. The PASMC proliferation model in rats induced by platelet-derived growth factor-BB (PDGF-BB) was constructed. The experimental result shows that after being intervened by 20 ng/mL PDGF-BB, the proliferation level of pulmonary artery smooth muscle cells remarkably increased. After being intervened by GA (20, 40, and 80 μM) combined with PDGF-BB, the proliferation level decreased and recovered to the normal level. After being intervened by PDGF-BB (20 ng/mL) alone, G1-phase cells became more than that of the normal group, and S-phase cells decreased. In comparison with the PDGF-BB group, G2-phase and S-phase cells intervened by 80 μM 18β-GA increased, and the G1-phase cells decreased.

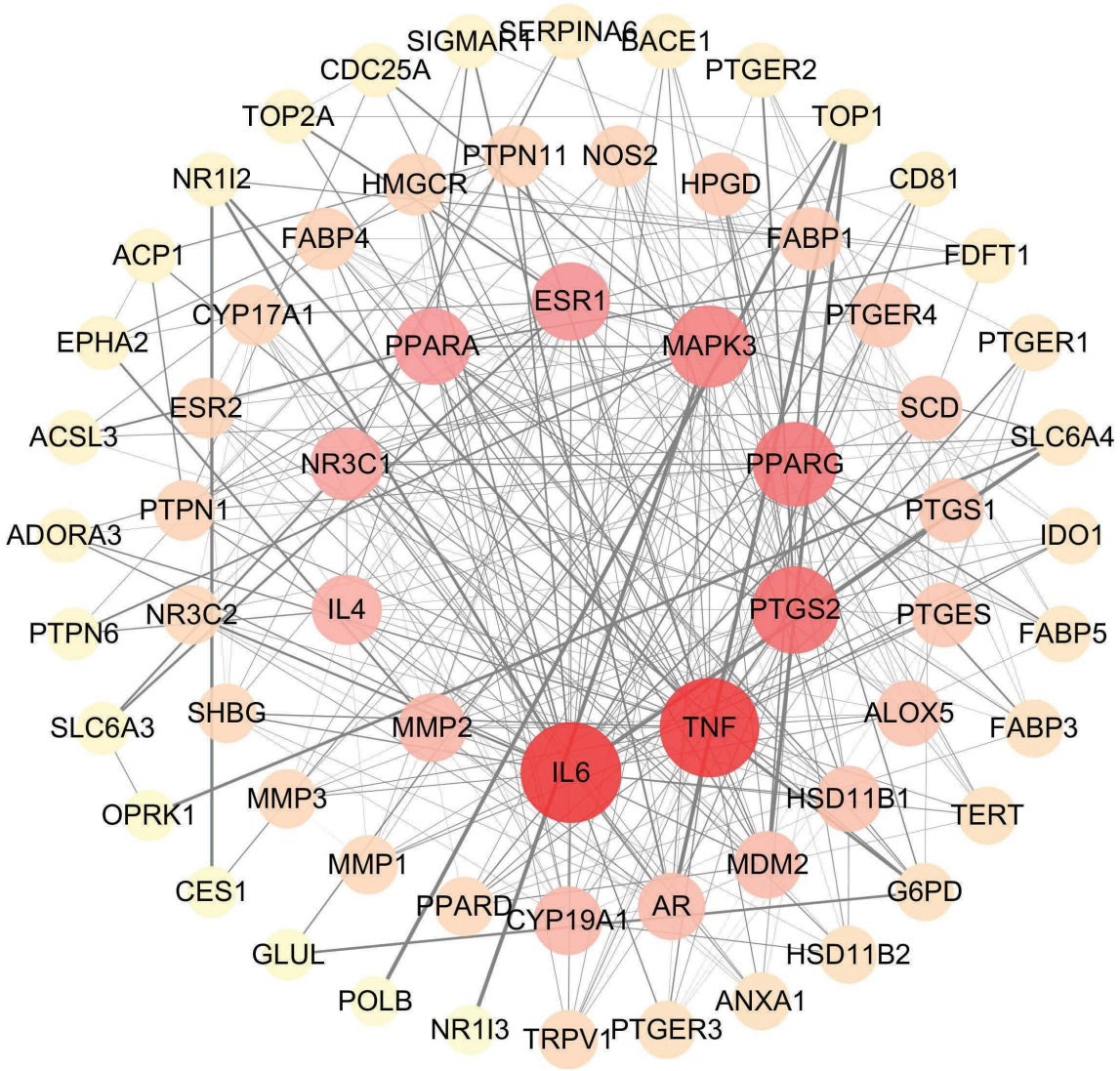


Figure 2: PPI network diagram of GA and HAPH intersection targets.

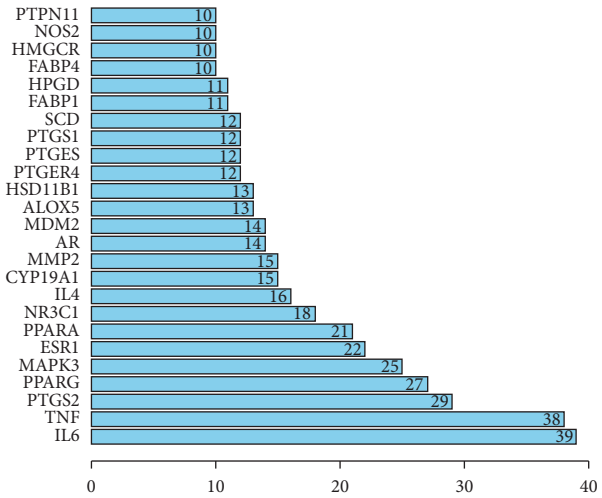
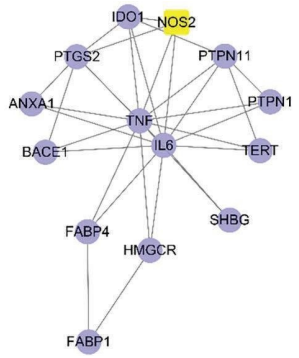
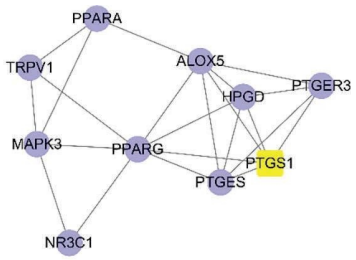
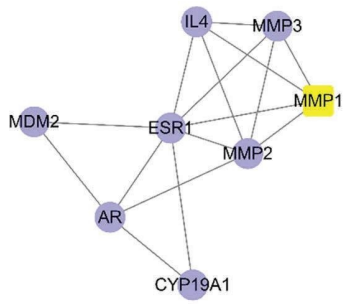
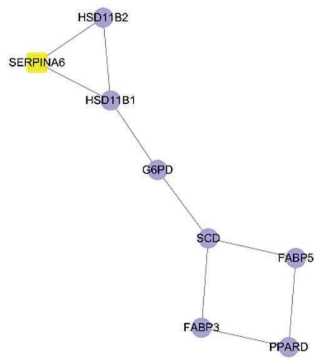


Figure 3: Topology analysis.

Table 1: MCODE clustering analysis.

Cluster	Network	Nodes	Edges	Node IDs
1		14	33	IL6, BACE1, ANXA1, SHBG, PTGS2, TERT, NOS2, HMGCR, IDO1, PTPN1, PTPN11, FABP4, FABP1, TNF
2		10	22	NR3C1, MAPK3, TRPV1, HPGD, PPARG, PTGS1, PPARA, ALOX5, PTGER3, PTGES
3		8	16	CYP19A1, MDM2, MMP1, IL4, AR, ESR1, MMP2, MMP3
4		8	9	PPARD, G6PD, SCD, SERPINA6, FABP3, HSD11B1, FABP5, HSD11B2

This finding indicates that 18β-GA may inhibit the proliferation of cells induced by PDGF-BB in the S phase by blocking the cell cycle (Figure 8).

4.10. Effect of 18β-GA on the MAPK Pathway of PDGF-BB Induced ASMC Proliferation Model. Western blot analysis was used to detect the expression levels of p-p38, p38, p-ERK1/2, and ERK1/2 proteins in various groups of cells. 18β-GA can remarkably decrease the expression levels of p-p38, p38, p-ERK1/2, and ERK1/2 proteins in the PASMC proliferation model, indicating that 18β-GA can inhibit the proliferation of pulmonary artery smooth muscle by inhibiting the MAPK pathway (Figure 9).

5. Discussions

At present, HAPH is a common disease in clinical medicine. It is a complication caused by long-term hypoxia in people at high-altitude areas. Its disability rate in patients is very high.

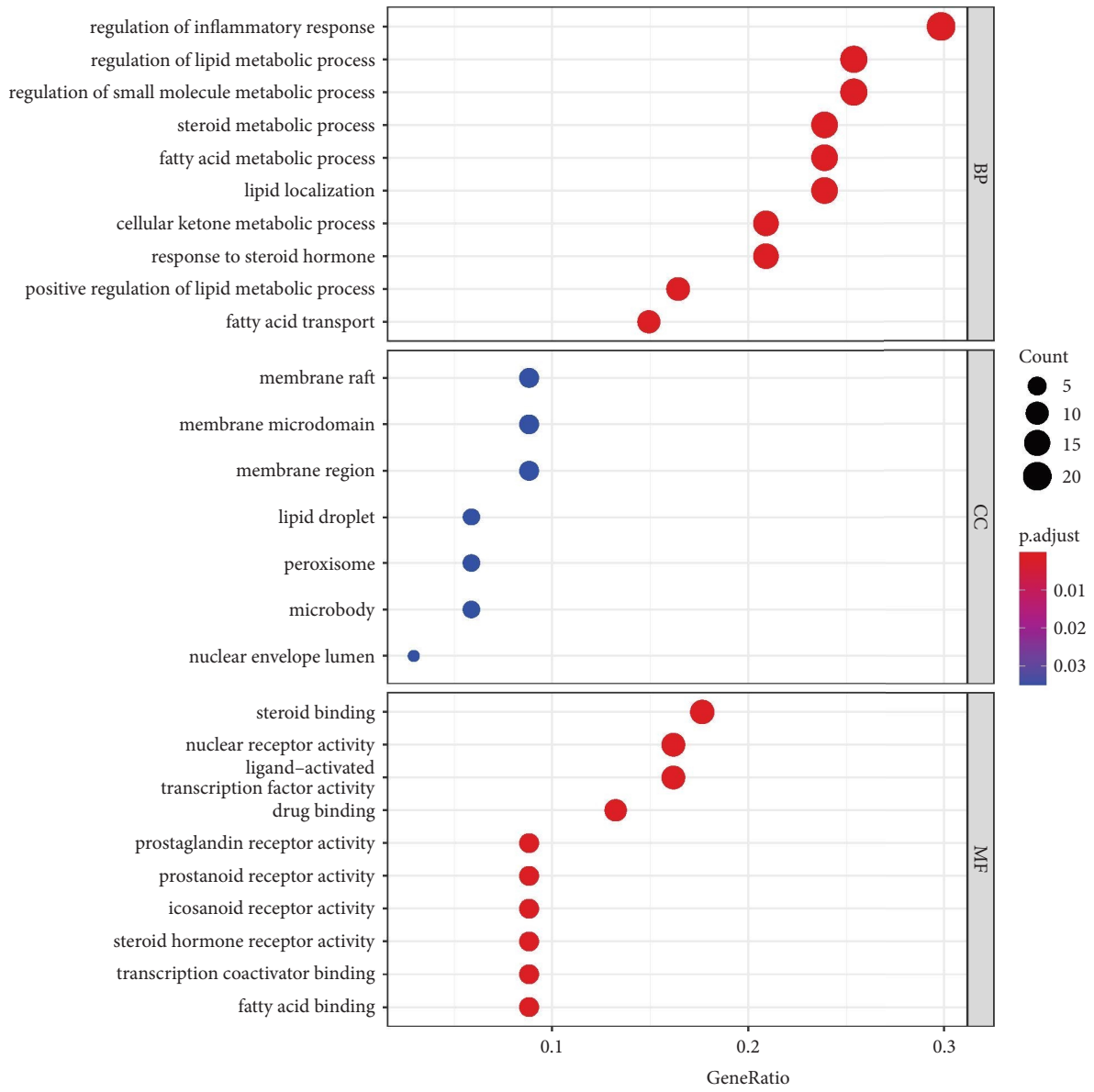


Figure 4: GO functional enrichment analysis results.

This condition may cause severe heart failure and endanger life in the late stage of the disease [24]. The pathogenesis of HAPH is complex. Long-term hypoxia will cause pulmonary vasoconstriction and pulmonary vascular remodeling in patients, thus increasing pulmonary vascular resistance and elevated pulmonary arterial pressure. Consequently, hypoxic pulmonary hypertension occurs. This pathological change is largely irreversible [25]. At present, the clinical treatment methods for HAPH mainly include leaving high-altitude environment, long-term oxygen therapy, and the use of calcium channel blockers, prostacyclins, endothelin receptor antagonists, and phosphodiesterase inhibitors. However, the efficacy of existing drugs is poor. Therefore, safer and more effective drugs need to be developed for clinical use. The study on the mechanism of 18β-GA for the treatment of HAPH can provide a new therapeutic idea for the treatment of pulmonary hypertension.

18β-GA, as the main active ingredient of liquorice, has various pharmacological activities, including antibacterial, anti-inflammatory, antiviral, anticancer, and treatment of lung diseases. In recent years, studies on the effect of18β-GA in treating lung diseases have greatly increased. The study results show that 18β-GA can effectively prevent and treat non-small cell lung cancer, and precautions should be taken against early radiation lung injury [9, 26]. Based on network pharmacology and molecular docking technology, this paper provides a certain research basis and theoretical basis for deeply exploring the mechanism of 18β-GA for the treatment of HAPH.

Mitogen-activated protein kinases (MAPKs) are a family of central signaling molecules, which are mainly composed of several subgroups such as p38 mitogen-activated protein kinases (MAPKs), extracellular signal-regulated protein kinases (ERKs), and c-Jun N-terminal kinases (JNKs).

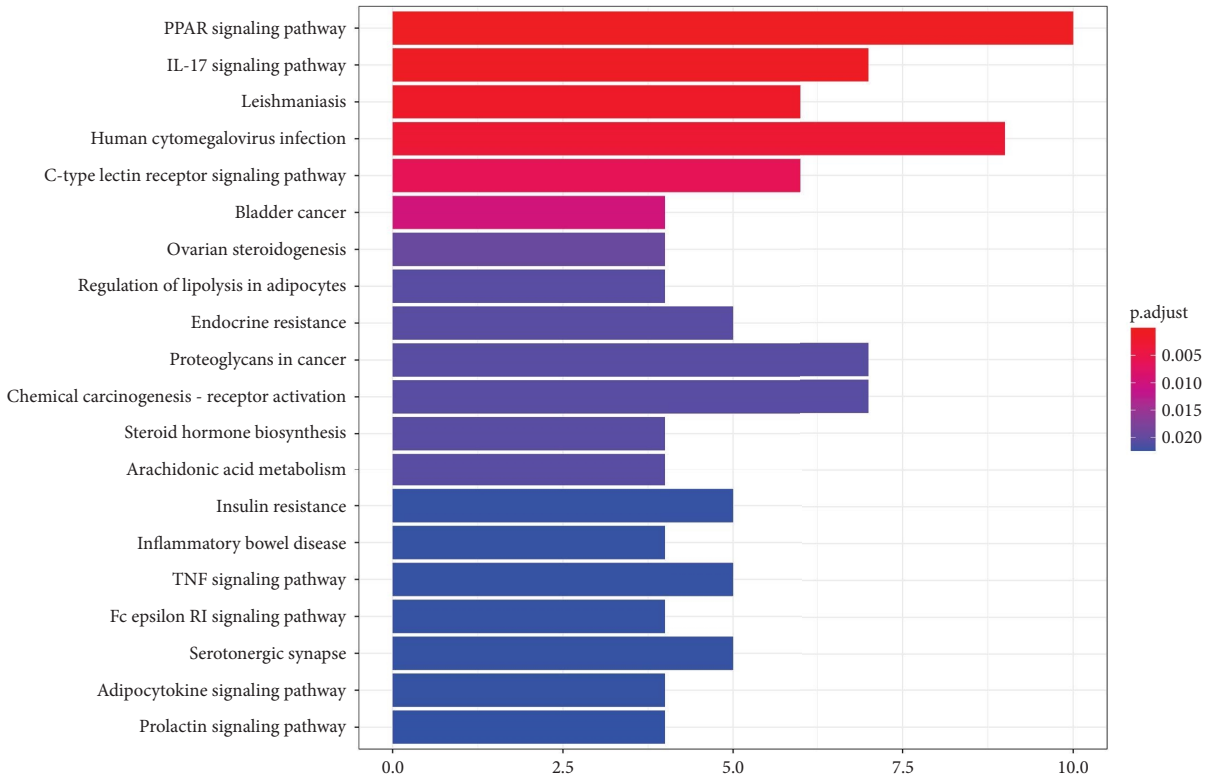


Figure 5: Top 20 pathways closely related to GA for the treatment of HAPH obtained from KEGG enrichment analysis.

The study shows that chronic hypoxia inhibits the expression of Kv1.5 protein by activating ERK1/2 and p-38 MAPK pathways, resulting in the depolarization of PASMCs, elevated intracellular free Ca^{2+} concentration, and pulmonary vasoconstriction [27]; moreover, chronic hypoxia stimulation can upregulate the expression and phosphorylation levels of ERK1/2 and p38 MAKP proteins in pulmonary artery vessels, causing excessive proliferation of pulmonary artery smooth muscle cells, triggering pulmonary artery remodeling, and thereby promoting the occurrence and development of HAPH; finally, inhibiting the ERK1/2 signal pathway can prevent pulmonary vascular remodeling, increase right ventricular pressure, and improve right ventricular hypertrophy in chronic hypoxic-induced pulmonary hypertension models [28, 29]. Zhu et al. [30] found that Baicalin inhibits the remodeling of pulmonary arteries and improves pulmonary hypertension by inhibiting the activation of MAPK signaling pathways, including p38, ERK, and JNK.

Network pharmacology analysis showed that the potential targets of 18β-GA for the treatment of HAPH include PTGS1, MMP1, SERPINA6, and NOS2. PTGS1 is a prostaglandin synthase with important physiological significance, which plays a key role in the pathophysiological progression of inflammation, arthritis, and cancer [31]. Matrix metalloproteinase 1 (MMP1), an activated factor of collagenase and G protein coupled protease activated receptor 1 (PAR1), is a target related to tumor formation and migration, which has been discovered in recent years [32]. SERPINA6 is a protein that binds cortisol in the blood and is the main transport protein of glucocorticoids and progesterone in the blood of most vertebrates [33]. Inducible NOS2 assists macrophages in fighting pathogens in the immune system by using the oxidative stress of nitric oxide. It also exists in the cardiovascular system, and it functions only after cells are stimulated and activated, producing a large amount of nitric oxide [34]. KEGG enrichment analysis shows that 18β-GA anti-HAPH mainly involves the PPAR signal pathway, human cytomegalovirus infection, IL-17 signal pathway, and proteoglycans in cancer.

To sum up, based on network pharmacology and molecular docking technology, this paper predicts the molecular mechanism of 18β-GA in the treatment of HAPH. 18β-GA may function on PTGS1, MMP1, SERPINA6, and NOS2 and other targets to regulate PPAR signal pathway, human cytomegalovirus infection, IL-17 signal pathway, and proteoglycans in cancer to exert anti-HAPH effects. GA may play an anti-HAPH role through a "multi target-multi pathway" approach.

In the past, drugs and treatments for pulmonary hypertension were primarily developed for primary pulmonary hypertension. Currently, it is more imperative to study drugs specifically targeting the pathogenesis of HAPH after verifying their effectiveness in its treatment. Existing researchers have conducted drug research with a focus on HIF as a target. For instance, digoxin, a classic heart failure treatment, can inhibit the synthesis of HIF-1α protein, thereby reducing hypoxia-induced pulmonary vascular remodeling in mice. Additionally, a small molecule compound called C76 acts as a targeted inhibitor of

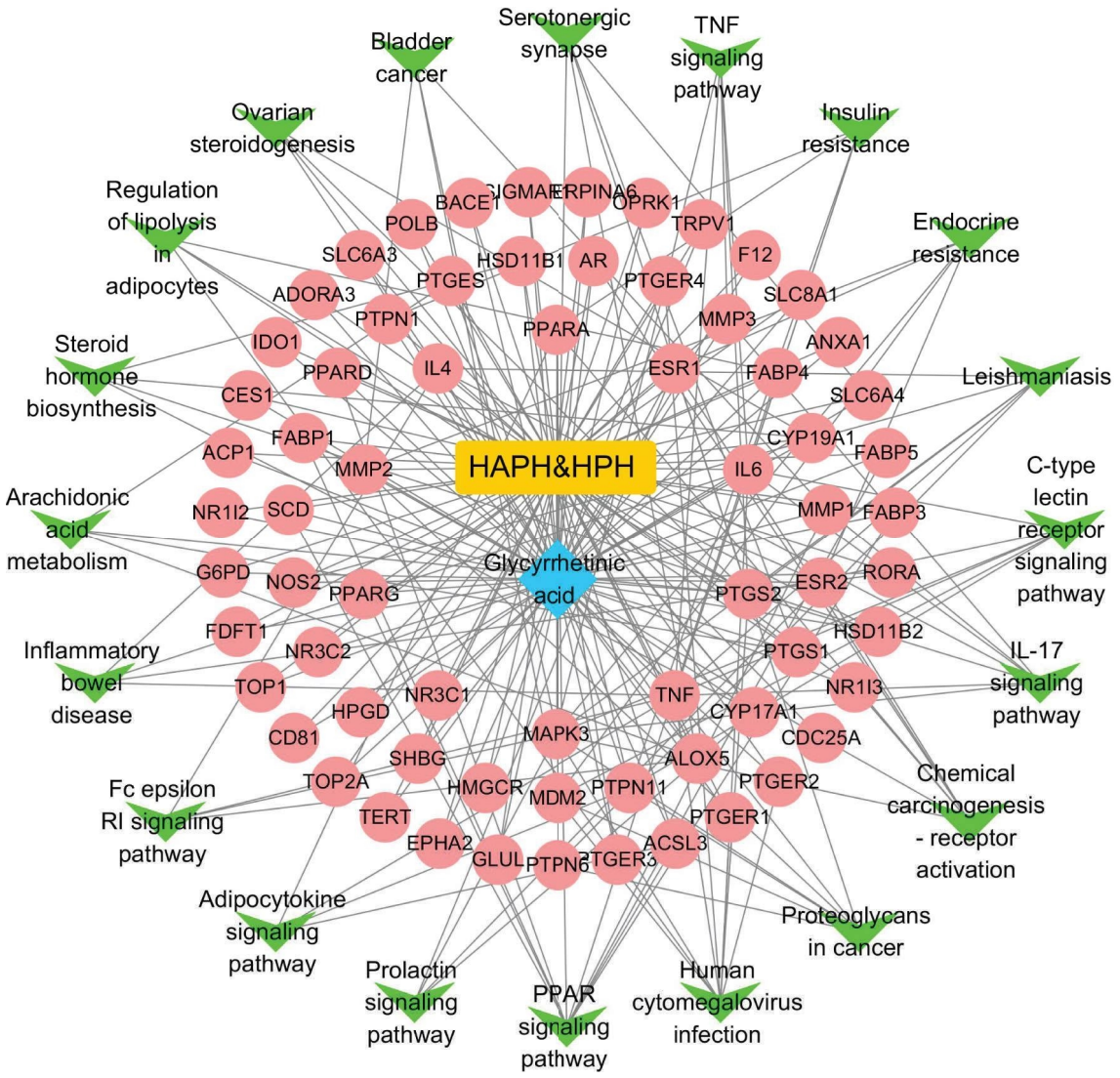


Figure 6: Construction of 18β-GA high-altitude pulmonary hypertension–pathway–target network. Remark: as shown in the diagram, the compound is in blue; the Chinese medicine target in disease is in pink; the top 20 most significant pathways are in green; and the disease is in yellow.

Table 2: Molecular docking between 18β-GA and the key target protein.

Protein	Identifier	Score
PTGS1	6Y3C	−9.4
MMP1	1CGE	−7.4
SERPINA6	2VDX	−7.9
NOS2	1NSI	−9.7

HIF-2 and significantly reduces experimental animal's pulmonary artery pressure, right heart failure, and mortality. The study of circRNA and miRNA also provides novel insights into the diagnosis and treatment of pulmonary hypertension. Detecting changes in circRNA and miRNA levels during the pathogenesis of HAPH can serve as potential diagnostic markers; however, targeted treatment methods still require further exploration. Despite significant improvements in survival rates and quality of life for patients with HAPH due to advancements in new

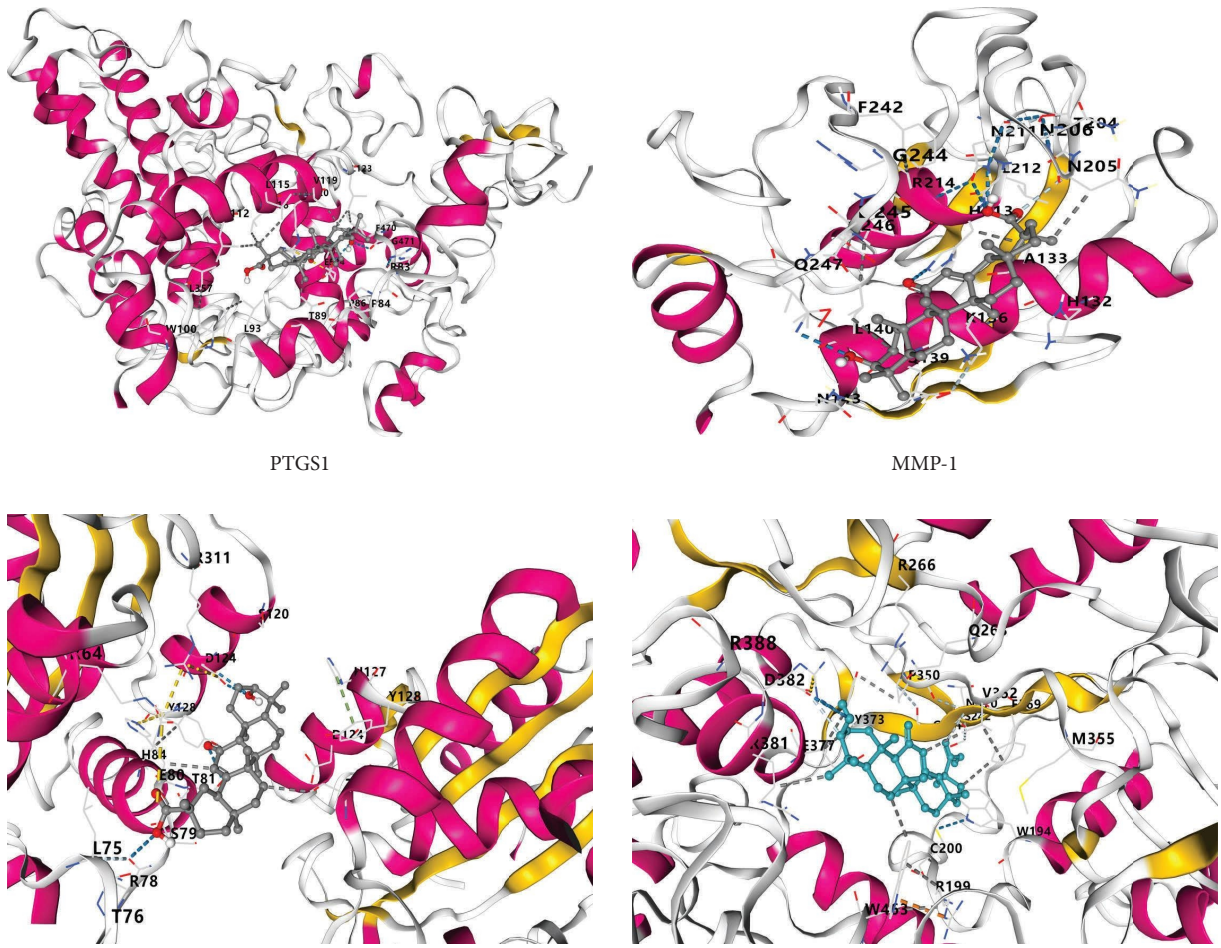


Figure 7: The result of molecular docking.

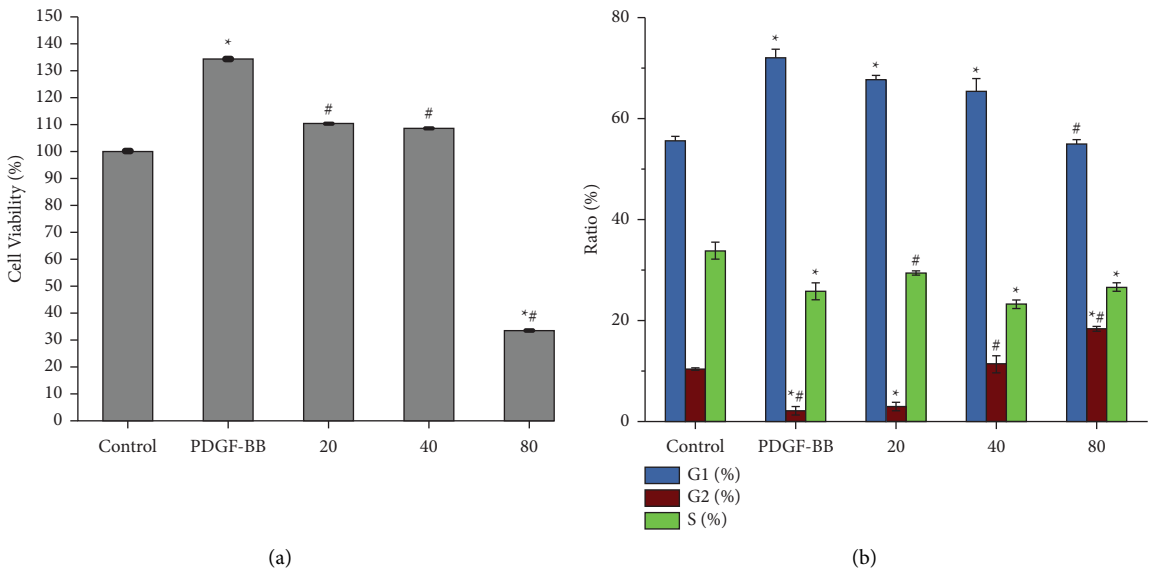


Figure 8: Continued.

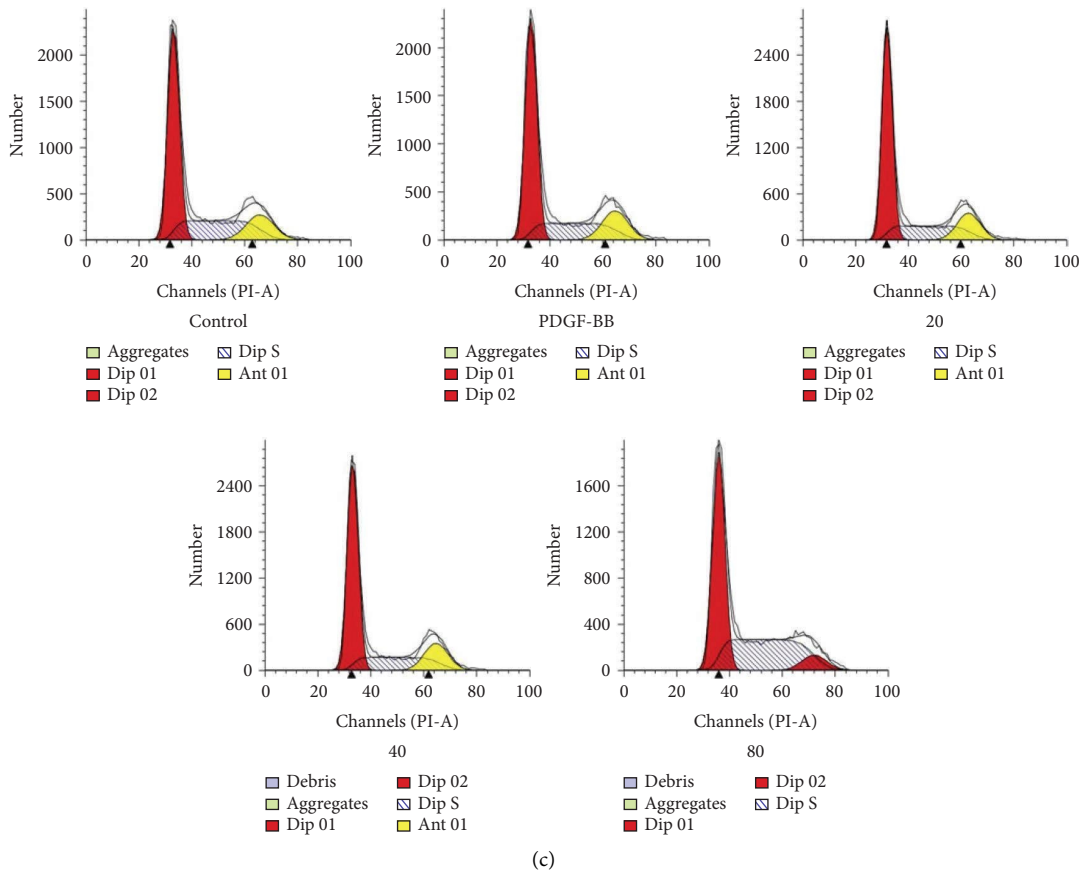


Figure 8: Effect of GA on the activity of PDGF-BB-induced PASMC proliferation model.

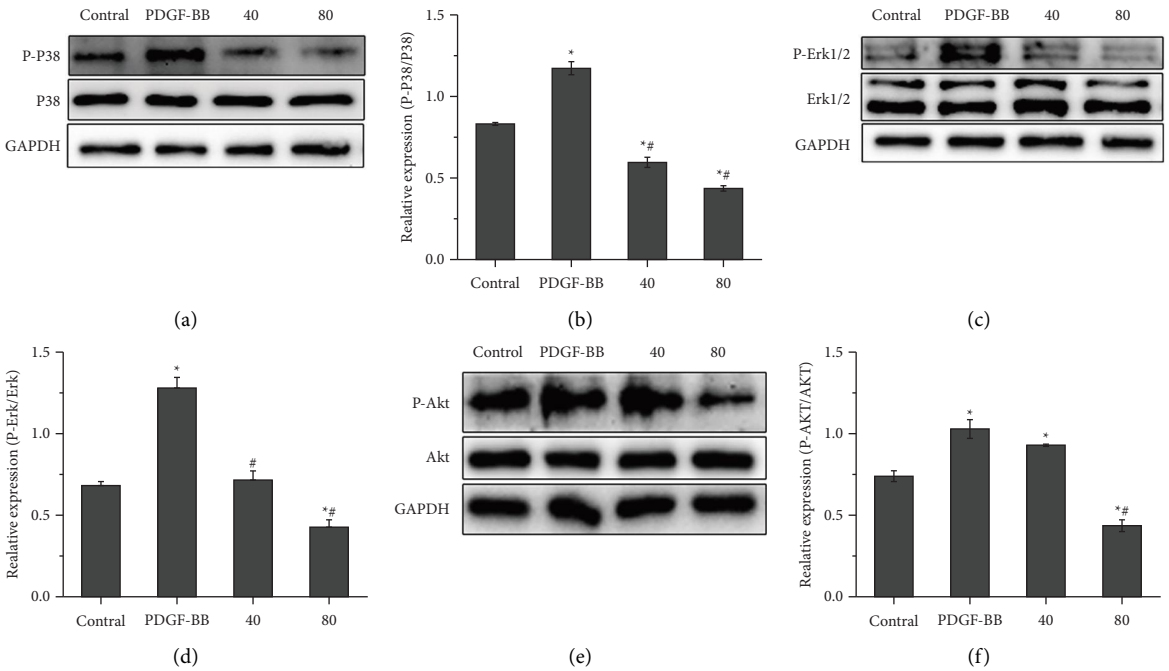


Figure 9: Effect of 18β-GA on the MAPK pathway of PDGF-BB induced ASMC proliferation model.

drugs and treatment approaches, long-term prognosis remains unfavorable. Therefore, continuous comprehensive research on its pathogenesis and treatment methods is necessary.

This study also has some limitations. although a large number of targets and pathways can be screened through network pharmacology technology, the findings need to be confirmed through *in vivo/in vitro* experiment, which is the focus of our next study.

6. Conclusion

In this study, the therapeutic effects of GA on HAPH were preliminarily analyzed by means of network pharmacology and molecular docking. The pharmacological mechanism of GA for the treatment of HAPH was characterized by multi-target and multi pathway. GA may serve as a promising therapeutic candidate for HAPH but still needs further *in vivo/in vitro* experiment.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Zulipiye Ainaisi and Yang Tao designed the experiment and drafted the manuscript. Dilinuer Maimaitiyiming and Ainiwaer Aikemu contributed to the study design and gave the theoretical guidance. Yiliyaer Nijiati, Fang Lei and Liao Xijiang contributed to statistical analyses. Yang Tao was responsible for the revision of the final version. All authors read and approved the final manuscript. Zulipiye Ainaisi and Yang Tao contributed equally to this work.

Acknowledgments

This work was supported by Natural Science Foundation of Xinjiang Uygur Autonomous Region (2023D01D09).

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