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Original Article

Anti-Inflammatory and Antioxidant Effects of *Ganoderma Lucidum* in Preventing a Mouse Model of High-Altitude Pulmonary Edema

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Abstract:

Objective: High-altitude pulmonary edema (HAPE) is a life-threatening illness from acute hypobaric hypoxia. *Ganoderma lucidum*, with anti-inflammatory/antioxidant effects, is a potential HAPE therapy. This study explored key biological pathways of its protective effect on HAPE mice.

Methods: First, bioinformatics methods were used to predict the key target genes and main biological pathways of *Ganoderma lucidum* in regulating HAPE. Then, *Ganoderma lucidum* was administered to HAPE mice as an intervention. Lung histopathological changes were assessed via hematoxylin-eosin (HE) and Masson staining; inflammation-related factor levels were detected by enzyme-linked immunosorbent assay (ELISA); finally, key target gene expression was measured by Western blot (WB) analysis.

Results: Bioinformatics analysis revealed a strong functional correlation between *Ganoderma lucidum* and HAPE pathological regulation. Compared with the HAPE model group, the *Ganoderma lucidum* group had alleviated lung tissue damage—characterized by reduced alveolar septal edema, inflammatory cell infiltration, and collagen deposition. ELISA confirmed it downregulated pro-inflammatory proteins, while WB showed it significantly decreased expression of key HAPE targets (Adora2b, Ccr2, Ttk, Ace, Cma1, Hspa1a).

Conclusion: *Ganoderma lucidum* may involve in HAPE-related pathways, inhibiting HAPE progression via anti-inflammatory/antioxidant effects, providing a new basis for HAPE treatment.

Keywords: High-altitude pulmonary edema (HAPE), *Ganoderma lucidum*, anti-inflammatory, antioxidant, network pharmacology, transcriptomics

1. Introduction

High-altitude pulmonary edema (HAPE) is a clinical syndrome caused by acute exposure to a hypobaric hypoxic environment above 2,500 meters (m), characterized by initial symptoms of stamina loss, dyspnea, and dry cough during exertion, which are followed by dyspnea at rest, rales, cyanosis, cough, and pink frothy sputum(Sacks, Baxter et al. 2018, Sydykov, Mamazhakypov et al. 2021, Richalet, Jeny et al. 2023). The risk of HAPE increases with higher altitudes and faster ascent rates. Without treatment, the estimated mortality rate of HAPE is 50%(Bärtsch and Swenson 2013, Woods and Alcock 2021). As a noncardiogenic pulmonary edema, HAPE is caused by exaggerated hypoxic pulmonary vasoconstriction (HPV), which leads to abnormally elevated pulmonary artery pressure and capillary pressure(Sharma Kandel, Mishra et

al. 2020, Swenson 2020). Concurrently, acute hypoxia induces excessive reactive oxygen species (ROS) production, which exacerbates oxidative stress and impairs the function of pulmonary microvascular endothelial cells(Desireddi, Farrow et al. 2010, Sommer, Strielkov et al. 2016, Sharma Kandel, Mishra et al. 2020). Together, these elevated pressures and endothelial damage lead to noninflammatory, hemorrhagic leakage of alveolar capillaries; this leakage then secondarily triggers an inflammatory response, and the resulting inflammation further reduces the permeability of the alveolar capillary membrane, ultimately worsening fluid accumulation in the alveoli(Paul, Gangwar et al. 2019, Ge, Li et al. 2024). Given that HAPE's pathology involves such interconnected complexity, it is urgent to advance research into its pathogenesis and identify more effective intervention targets(Wang, Huang et al. 2022, Si, Wang et al. 2023).

Ganoderma lucidum, also known as Reishi, is a valued medicinal fungus used in traditional medicine for over 2,000 years. Extensive preclinical and clinical studies have confirmed the anti-inflammatory and antioxidant properties of *Ganoderma lucidum*(Cör, Knez et al. 2018, Cör Andrejč, Knez et al. 2022). Animal experiments demonstrated that, in a natural aging mouse model, *Ganoderma lucidum* polysaccharide (GLSP) plays a role in protecting the endothelial barrier. The findings indicate that GLSP can alleviate reactive oxygen species (ROS)-induced oxidative stress and the subsequent endothelial damage, thereby further supporting the integrity of the endothelial barrier(Jin, Zhu et al. 2017, Wen, Sheng et al. 2022). In addition, studies have demonstrated that GLP treats atherosclerosis (AS) by exerting antioxidant effects and improving endothelial cell function—primarily via increasing the number of endothelial progenitor cells (EPCs) to repair endothelial dysfunction and reducing the number of circulating endothelial cells (CECs, a marker of endothelial damage)(Ma, Han et al. 2024). However, the role of *Ganoderma lucidum* in the pathogenesis of HAPE has not been investigated so far. Therefore, it is imperative to explore whether *Ganoderma lucidum* can affect HAPE by improving the endothelial cell barrier and regulating vascular permeability, thereby providing more insights for HAPE prevention(Cör Andrejč, Knez et al. 2022).

Network pharmacology combined with RNA-seq-based transcriptomics was used to explore the potential mechanism of *Ganoderma lucidum* against high-altitude pulmonary edema (HAPE)(Swallah, Bondzie-Quaye et al. 2023, Ding, Shangguan et al. 2025). First, bioinformatics was employed to screen for potentially effective active components in *Ganoderma lucidum*, and the GeneCards database was utilized to identify core cross-gene targets between *Ganoderma lucidum* and HAPE. Subsequently, a mouse HAPE model was established, and RNA-seq technology was applied to obtain the global gene expression profile of lung tissues; core targets were further cross-screened via bioinformatics methods, and their importance was ranked using a random forest algorithm(Ge, Son et al. 2018, Kong and Yu 2018). Meanwhile, protein–protein interaction (PPI), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to explore potential associations among these core targets, as well as the mechanism of action and key pathways underlying *Ganoderma lucidum*'s therapeutic effects on HAPE. Finally, pathological and molecular biology experiments were performed to validate these core targets in vivo, aiming to systematically clarify the molecular mechanism of *Ganoderma lucidum* in preventing and treating HAPE and provide a theoretical basis for its clinical development and application(Ahmad 2020).

Materials and Methods

Main Instruments and Reagents

Preparation of *Ganoderma lucidum* Extract

The laboratory-cultured *Ganoderma lucidum* was collected and the culture medium was subjected to freeze-drying to obtain solid sheet-like substances. These substances were then dissolved in water, and a stock solution of 3 mg/mL was prepared based on the measured polysaccharide content, resulting in the *Ganoderma lucidum* extract.

Collection of Target Points for *Ganoderma lucidum*- High Altitude Pulmonary Edema

The active components of *Ganoderma lucidum* and their corresponding target points were collected from the TCMSP database (https://www.tcmsp-e.com/). The corresponding target points of the active components of *Ganoderma lucidum* were collected from the Genecards database (https://www.genecards.org/) and duplicates were removed. With "High Altitude Pulmonary Edema (HAPE)" as the keyword, disease target points related to HAPE were collected from the Genecards database to obtain the defined target points for HAPE.

Establishment of Animal Models and Grouping

This study used 6-week-old, specific pathogen-free (SPF) grade male C57BL/6J mice, weighing 18-22 g, provided by Beijing Huafukang Biotechnology Co., Ltd. (Production License No.: SCXK(京)2024-0003). The mice were housed in a standardized animal laboratory and randomly divided into 3 groups (n=9). They were acclimatized for 7 days, with free access to food and water. Their behavior and health status were observed daily to ensure the scientific nature of the experiment and the stability of the results. Experimental groups: ① Blank control group (Control), raised under normal pressure and oxygen conditions, and given normal saline by gavage for 7 consecutive days before modeling; ② High Altitude Pulmonary Edema (HAPE) model group, placed in a low-pressure oxygen chamber simulating an altitude of 5000 meters and an oxygen concentration of 10% for 72 hours to replicate the HAPE model, and given normal saline by gavage for 7 consecutive days before modeling; ③ *Ganoderma lucidum* treatment group (GL), given *Ganoderma lucidum* extract (100 mg/kg) by gavage for 7 consecutive days before modeling. This study was approved by the Medical Ethics Committee of The Third People's Hospital of Chengdu (Approval No: 成都三院伦字2025-S-116).

Collection of Lung Tissue Samples and RNA-seq

After the modeling was completed, the mice were anesthetized with isoflurane inhalation. The whiskers on both sides of the mice were cut with dry scissors, and blood samples were collected from the orbital artery. The blood was collected in a 1.5 mL sterile and enzyme-free centrifuge tube and left at 4°C for 2 hours. The samples were then centrifuged at 4°C and 3000 rpm for 15 minutes. The separated serum was quickly transferred to a clearly labeled sterile and enzyme-free centrifuge tube and frozen at -80°C for subsequent determination. The mice were sacrificed by cervical dislocation and the lung tissues were immediately removed. Some of the tissues were fixed in 4% paraformaldehyde for pathological sections, and some were frozen at -80°C for RNA and protein extraction. The lung tissue samples from the three groups of mice were sent to a company for RNA-seq. The raw data were processed through quality control, alignment, and gene expression quantification normalization.

Transcriptome Data Analysis and Key Gene Screening

DEGs were screened for the Control group vs. HAPE group (dataset A), and HAPE group vs. GL group (dataset B). Gene set integration: ① The union of dataset A and the HAPE disease target points obtained from network

pharmacology was taken to obtain the "disease-related gene set (Set 1)"; ② The union of dataset B and the *Ganoderma lucidum* action target points obtained from network pharmacology was taken to obtain the "treatment-related gene set (Set 2)".

Bioinformatics Analysis

The 149 intersection targets were imported into the DAVID database for Gene Ontology (GO) enrichment analysis in three parts: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper was used for target gene pathway annotation analysis (<0.05), and visual bubble charts and bar charts were created. Random forest algorithm was used for feature importance analysis to screen out the top 10 core differential genes. The expression patterns of the top 10 genes were analyzed using heat maps through the Microbiomics tool to visually present their expression differences in the three groups of samples.

Histopathological and Molecular Biological Verification

The mouse lung tissues were fixed in 4% paraformaldehyde at 4 ℃ for 48 hours to maintain the integrity and stability of the tissue structure. The fixed tissues were then subjected to dehydration treatment through a concentration gradient to ensure the removal of water from the tissues. Subsequently, the tissues were cleared with xylene and then embedded in melted paraffin. The paraffin blocks were sectioned into continuous 5 μm thick slices, and HE and Masson staining were performed on every 5th slice to observe the pathological morphology changes, inflammatory cell infiltration, and collagen fiber deposition in the lung tissues.

Western Blot Analysis

The blood and impurities in the lung tissues were removed using pre-cooled phosphate buffered saline. The tissues were homogenized on ice, and the supernatant was collected as the protein extract. The protein concentration was determined using a BCA protein quantification kit, and the absorbance of each well was measured at 562 nm using a microplate reader. The protein concentration was calculated based on the standard curve. The proteins were separated by SDS-PAGE (80 V for 20 min for the stacking gel, 120 V for 65 min for the separating gel) and transferred to a polyvinylidene fluoride (PVDF) membrane at 200 mA for 120 min. The membrane was washed with TBST, blocked with skim milk, and incubated overnight at 4 ℃ with Adora2b (1:1000), Ccr2 (1:1000), Ttk (1:1000), Ace (1:1000), Cma1 (1:1000), and Hspa1a (1:1000). GAPDH (1:10000) and β-Actin were used as internal controls. The membrane was then incubated with secondary antibodies (1:5000) at room temperature for 2 hours. ECL was used for color development, and the gray values were analyzed using ImageJ software.

Detection of Serum Inflammatory Factor Levels

To evaluate the regulatory effect of *Ganoderma lucidum* on the inflammatory response of acute high-altitude pulmonary injury, the levels of inflammation-related cytokines in the mouse serum were detected. The experiment was conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Take the mouse serum samples stored at -80°C and slowly thaw them at 4°C. Before testing, centrifuge them at 4°C and 3000 rpm for 10 minutes to thoroughly remove the precipitates. Use the supernatant for the assay. The experiment employs commercial mouse-specific ELISA kits to detect the concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α). All operations strictly follow the instructions of each kit. Use an enzyme-linked immunosorbent assay (ELISA) reader to measure the OD value of each well at a wavelength of 450 nm. Generate a standard curve

based on the concentrations of the standards and their corresponding OD values. Substitute the average OD value of the samples into the standard curve equation to calculate the actual concentrations of each cytokine in the serum.

Statistical Analysis

All data are presented as mean ± standard deviation (Mean ± SD) and analyzed using GraphPad Prism 10.0 software. One-way analysis of variance (ANOVA) was used for comparisons among multiple groups, and Tukey's test was used for pairwise comparisons between groups. A P value < 0.05 was considered statistically significant.

Results

Drug-Disease Target Screening

Through screening in the TCMSP database (OB≥30%, DL≥0.18), a total of 61 active components of *Ganoderma lucidum* were obtained. The corresponding targets were annotated and de-duplicated in the Genecards database, resulting in 640 potential targets of *Ganoderma lucidum*. Using "High Altitude Pulmonary Edema" as the keyword, 639 HAPE-related disease targets were retrieved and de-duplicated in the same database. To verify the intervention effect of *Ganoderma lucidum* on HAPE, we successfully established a mouse model. Compared with the Control group, the HAPE group mice showed obvious disease states after 72 hours of low-pressure hypoxia treatment, including rapid breathing, fluffed fur, reduced activity, and significant congestion and edema in the lung tissue. However, in the GL group, these symptoms were significantly alleviated.

RNA-seq analysis was performed on the lung tissues of the three groups of mice. The results of differential expression analysis showed that 438 DEGs were identified when comparing the HAPE group with the Control group, defined as dataset A; 244 DEGs were identified when comparing the GL group with the HAPE group, defined as dataset B. To systematically screen the key targets of *Ganoderma lucidum* in the treatment of HAPE, this study adopted an integrated analysis strategy of network pharmacology and transcriptomics: the union of dataset A and 639 HAPE disease targets was taken to obtain a set of 1037 genes (Set 1), aiming to comprehensively cover the pathologically related genes of HAPE; the union of dataset B and 640 *Ganoderma lucidum* action targets was taken to obtain a set of 872 genes (Set 2), to comprehensively capture the genes that *Ganoderma lucidum* may regulate. The intersection of Set 1 and Set 2 ultimately yielded 149 key genes (Figure 1). These genes are involved in the pathological process of HAPE and may be regulated by *Ganoderma lucidum*. This strategy effectively overcomes the limitation of a limited number of direct intersection targets in traditional network pharmacology, providing a reliable basis for subsequent mechanism exploration.

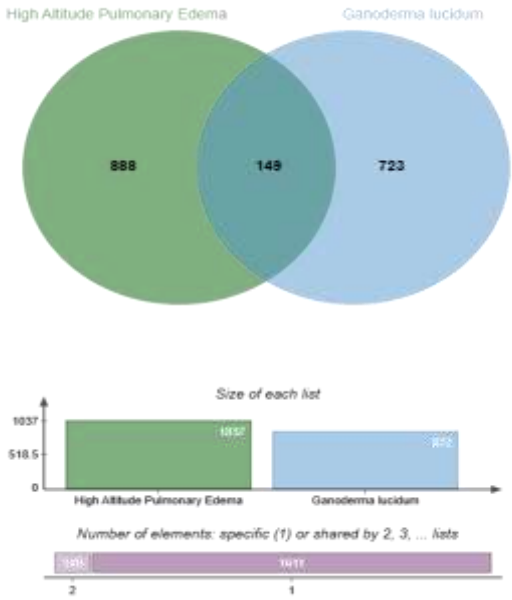

Figure 1 Venny diagram of the intersection genes of high-altitude acute lung injury (HAPE) and *Ganoderma lucidum* (GL). The overlapping area represents 149 key genes involved in HAPE pathogenesis and regulated by GL.

Screening and Functional Analysis of Keaniy Genes

Through the above integrated analysis strategy, 149 key genes were finally screened out. GO enrichment analysis showed that these genes were mainly involved in biological processes such as regulation of heart rate, positive regulation of tyrosine phosphorylation of STAT protein, DNA damage response, and regulation of DNA - templated transcription (Fig. 2A). KEGG pathway analysis indicated that these genes were significantly enriched in pathways such as TNF signaling pathway, Human T - cell leukemia virus 1 infection pathway, and Neurotrophin signaling pathway.

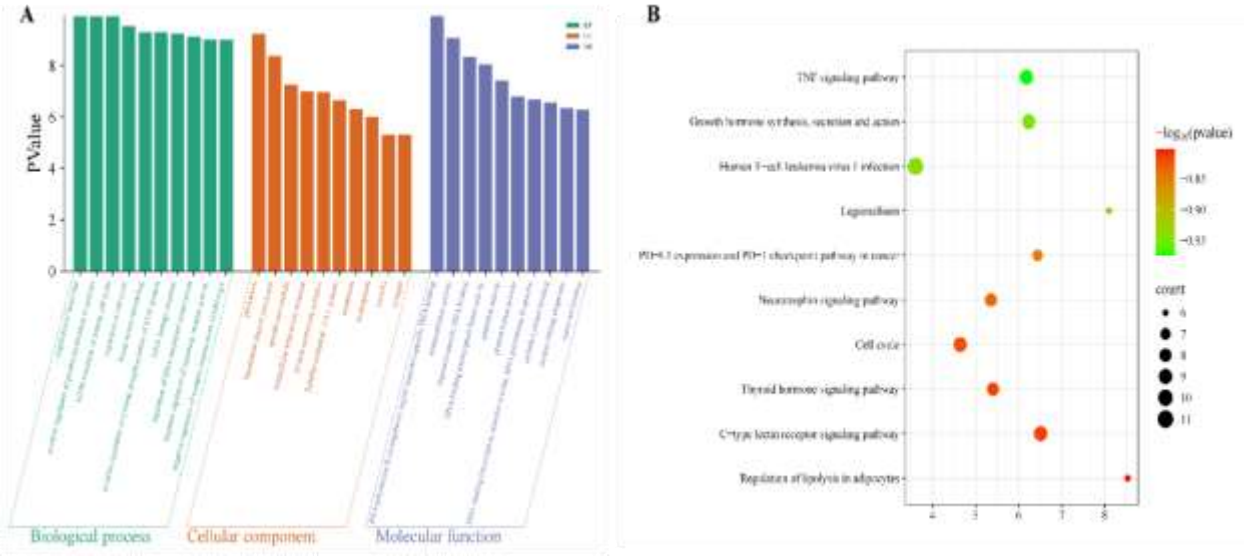

Figure 2 Enrichment analysis of HAPE-GL target genes (A) Visualization of the top 10 results of GO clustering analysis; (B) Visualization of the top 10 results of KEGG pathway analysis

Random Forest Screening of Core Targets

Based on the random forest algorithm, the importance scores of 149 key genes were calculated, and the top 10 core genes were screened out, namely Adora2b, Ccr2, Ttk, Ace, Cma1, Hspa1a, Flt1, Hspa1b, Gm15542, and Gm43595 (Figure 3 A). The heatmap shows that these 10 genes can clearly distinguish the three groups of samples, and their expression trends are reversed in the HAPE group and the GL group (Figure 3 B).

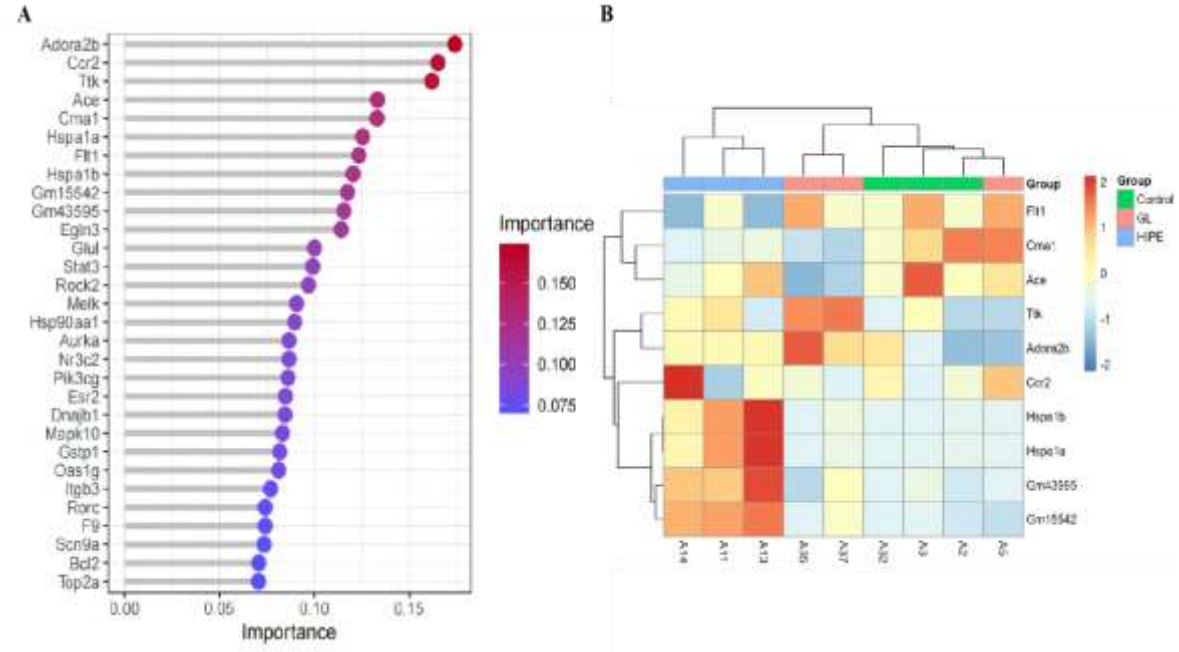

Figure 3 Target integration learning of HAPE-GL
(A) Random forest number analysis; (B) Heatmap analysis of the top 10 results of random forest number

Ganoderma Lucidum Alleviates the Pathological Damage of HAPE

HE staining showed that the alveolar structure in the Control group was intact, with uniform intervals, and no obvious hemorrhage or inflammatory cell infiltration was observed. The lung tissue in the HAPE group presented typical pathological features of acute lung injury. The alveolar structure was severely damaged, with a large number of alveolar walls ruptured and fused, forming emphysema-like changes. The alveolar septum was significantly widened and congested, and a large number of red blood cells (hemorrhage) and infiltrating inflammatory cells were visible in the alveolar cavity. The pathological damage of the lung tissue in the GL group was significantly improved. Most alveolar structures were maintained intact, and the thickening and congestion of the alveolar septum were significantly reduced (Figure 4 A). Semi-quantitative pathological scoring (Total Score) analysis of HE-stained sections indicated that the pathological score of the HAPE group was significantly higher than that of the Control group (p<0.05). After treatment with *Ganoderma lucidum*, the pathological score of the GL group was significantly lower than that of the HAPE group (p<0.05) (Figure 4 C), statistically confirming that pretreatment with *Ganoderma lucidum* can effectively alleviate the destruction of lung tissue architecture and acute inflammatory response caused by low-pressure hypoxia exposure. Additionally, Masson staining showed

that only a very small amount of blue collagen fibers were deposited in the interstitial tissue of the lung in the Control group, with a normal structure. In the HAPE group, a large number of blue collagen fibers were observed in the thickened alveolar septum, around blood vessels, and around the bronchi. After treatment with *Ganoderma lucidum*, the deposition of collagen fibers in the interstitial tissue of the lung was significantly reduced compared to the HAPE group, with the blue staining area becoming lighter and smaller (Figure 4 B). Further quantitative analysis of the thickness of collagen in the alveolar septum (Collagen Thickness of Alveolar Septum) (Figure 4 D) showed that the collagen deposition thickness in the HAPE group was significantly greater than that in the Control group (p<0.05). Importantly, the collagen thickness in the GL group was significantly reduced compared to the HAPE group (p<0.05). These data objectively indicate that *Ganoderma lucidum* intervention not only alleviates acute inflammation and edema but also significantly inhibits the early collagen fiber deposition secondary to HAPE, thereby potentially delaying the progression of pulmonary fibrosis.

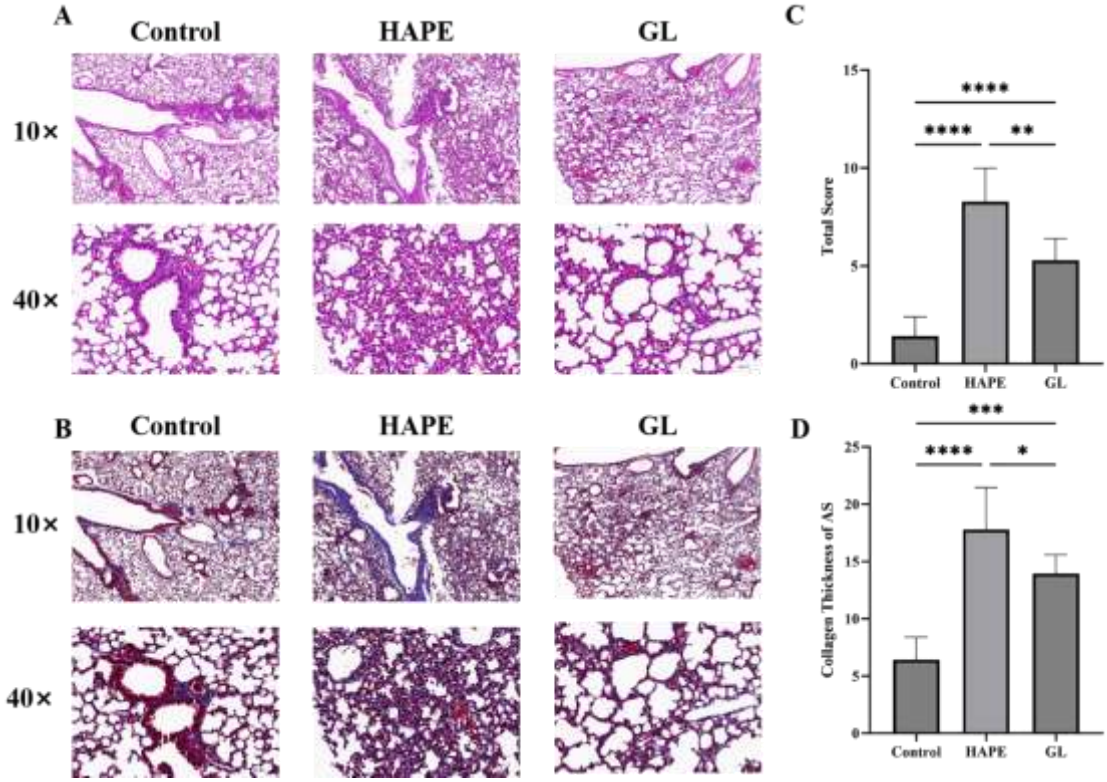

Figure 4 Pathological staining results

(A) Hematoxylin-eosin (HE) staining results (magnification: 10×-40×); (B) Masson staining results (magnification: 10×-40×); (C) Semi-quantitative pathological score of HE staining; (D) Quantitative analysis of alveolar septal collagen thickness, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Validation of Core Target Protein Levels

To validate the predictions from transcriptomics and bioinformatics at the protein level and further explore the mechanism of action of *Ganoderma lucidum*, we detected the expression levels of six key proteins in lung tissue by Western Blot (Figure 5A). The results showed that compared with the Control group, the expression levels of multiple proteins in the lung tissue of HAPE group mice were significantly changed. Among them, the expressions of pro-inflammatory

mediators Ccr2 and Cma1 were significantly upregulated (Figure 5C, 5F). The expressions of hypoxia-sensitive receptor Adora2b and cell cycle-related kinase Ttk were also significantly increased (Figure 5B, 5D). The expression of Ace, which is involved in angiotensin generation, showed an upward trend (Figure 5E). Meanwhile, the expression of Hspa1a, a cellular stress protection protein, was significantly increased (Figure 5G), which might be a compensatory protective response of the body against hypobaric hypoxia injury. At the same time, the protein expression profile in the GL group was reversed, showing the characteristics of multi-target regulation. Compared with the HAPE group, the protein expression levels of pro-inflammatory targets Ccr2 and Cma1 in the GL group were significantly inhibited (Figure 5C, 5F). The overexpression of Adora2b and Ttk was also significantly downregulated (Figure 5B, 5D). Notably, *Ganoderma lucidum* treatment reduced the protein level of Ace (Figure 5E), suggesting that it may improve pulmonary vascular function by regulating the renin-angiotensin system. In contrast, *Ganoderma lucidum* treatment further significantly upregulated the expression of protective protein Hspa1a (Figure 5G), indicating that *Ganoderma lucidum* can not only inhibit inflammatory and injury-related proteins but also actively enhance the body's endogenous stress protection mechanism.

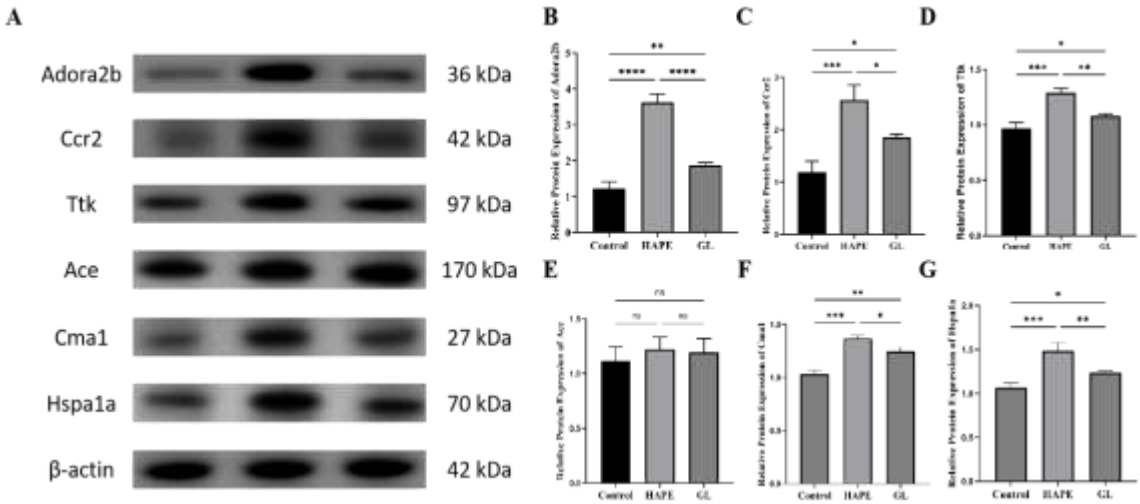

Figure 5 Western blot verification results of the top 6 of random forest

(A) Western blot detection of the expression levels of the top 6 proteins; (B) Quantitative analysis of Adora2b protein expression; (C) Quantitative analysis of Ccr2 protein expression; (D) Quantitative analysis of Ttk protein expression; (E) Quantitative analysis of Ace protein expression; (F) Quantitative analysis of Cma1 protein expression; (G) Quantitative analysis of Hspa1a protein expression, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Ganoderma Lucidum Regulates the Levels of Serum Inflammatory Factors in HAPE Mice

To evaluate the systemic inflammatory response level in the HAPE model and the anti-inflammatory effect of *Ganoderma lucidum*, we detected the concentrations of key pro-inflammatory factors IL-1β, IL-6, TNF-α and anti-inflammatory factor IL-10 in the serum of mice by ELISA. The results showed that compared with the Control group, the levels of pro-inflammatory factors in the serum of HAPE group mice were significantly increased (Figure 6 A-C), while the concentration of anti-inflammatory factor IL-10 was significantly decreased in the HAPE group (Figure 6 D). After

pretreatment with *Ganoderma lucidum* extract, the serum inflammatory factors in the GL group mice were significantly reversed in a favorable direction. Compared with the HAPE group, the levels of IL-1β and TNF-α in the serum of the GL group were significantly decreased (p<0.05), but IL-6 did not significantly decrease (Figure 6 B). Additionally, the concentration of anti-inflammatory factor IL-10 did not show a significant increase in the GL group (Figure 6 D). This result indicates that low-pressure hypoxia exposure successfully induced a systemic inflammatory response in mice, with a severe tilt towards a pro-inflammatory state. *Ganoderma lucidum* treatment has a clear inhibitory effect on the over-activated inflammatory response in the HAPE model, but its regulatory effect is selective, significantly reversing the levels of IL-1β and TNF-α, while the regulatory trend for IL-6 and IL-10 was not obvious under the conditions of this study. This suggests that *Ganoderma lucidum* may mainly exert its core anti-inflammatory effect by inhibiting the expression of IL-1β and TNF-α, thereby systematically reversing the inflammatory imbalance state, providing key systemic evidence for the alleviation of HAPE.

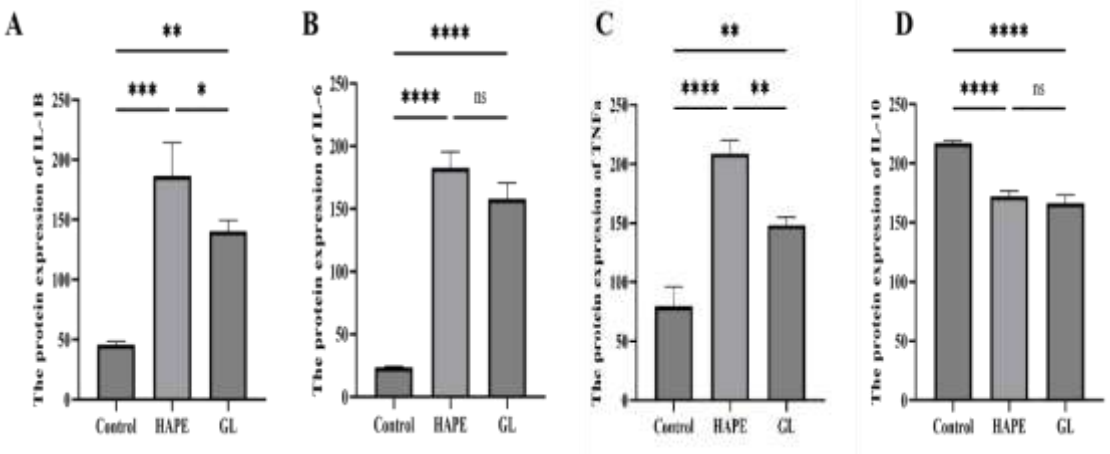

Figure 6 Detection of serum inflammatory factor levels
(A) Quantitative analysis of serum IL-1β concentration; (B) Quantitative analysis of serum IL-6 concentration; (C) Quantitative analysis of serum TNF-α concentration; (D) Quantitative analysis of serum IL-10 concentration, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Discussion

HAPE is a life-threatening hypoxic lung disease, and the current lack of effective preventive. *Ganoderma lucidum*, a traditional medicinal fungus, has been widely reported for its anti-inflammatory and antioxidant properties in various inflammatory diseases; however, its role and underlying mechanisms in HAPE remain largely unaddressed, leaving a critical gap in the field. The present study systematically investigated the protective effects of *Ganoderma lucidum* against HAPE and clarified its molecular targets, providing new insights into HAPE prevention and treatment.

First, in our work, morphological and pathological observations have confirmed the protective effect of *Ganoderma lucidum* against HAPE. Hematoxylin-eosin (HE) staining and Masson staining revealed that compared with the HAPE group, the GL group exhibited significantly alleviated pathological damage—specifically reduced alveolar septal edema, decreased inflammatory cell infiltration, and diminished collagen deposition. These findings are consistent with previous studies demonstrating the anti-inflammatory and anti-

fibrotic effects of *Ganoderma lucidum* in lung diseases(Li, Liu et al. 2022, Ding, Shangguan et al. 2025). ELISA results further supported this conclusion: pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) that were markedly upregulated in the HAPE group were significantly downregulated after *Ganoderma lucidum* pretreatment (p<0.01). This indicates that suppressing excessive inflammatory responses is a key contributor to *Ganoderma lucidum*'s protective effects against HAPE, aligning with its well-recognized immunomodulatory properties(Kou, Gao et al. 2021, Xu, Xiao et al. 2021) .

Second, on this basis, we further explored the specific pathways and targets through which *Ganoderma lucidum* improves HAPE. By intersecting the potential targets of *Ganoderma lucidum* and HAPE (obtained via network pharmacology) with differentially expressed genes from transcriptomics, we identified 149 key genes(Luo, Tan et al. 2022). Subsequent GO and KEGG pathway enrichment analyses of these genes revealed the following distribution across functional categories: 659 pathways in the Biological Process (BP) category, 82 pathways in the Cellular Component (CC) category, and 184 pathways in the Molecular Function (MF) category. Among these enriched pathways, the "positive regulation of tyrosine phosphorylation of STAT protein" pathway and the "regulation of DNA damage response" pathway were significantly enriched—findings that suggest Ganoderma lucidum may modulate HAPE progression by targeting inflammation- and oxidative stress-related pathways(Luo, Tan et al. 2022, Lian, Yang et al. 2024). To prioritize critical targets, we applied the random forest algorithm to calculate the importance scores of the 149 key genes, ultimately identifying the top 20 core genes (including Adora2b, Ccr2, Ttk, Ace, Cma1, Hspa1a, Flt1, Hspa1b, Gm15542, Gm43595, Egln3, Glul, Stat3, Rock2, Melk, Hsp90aa1, Aurka, Nr3c2, Nr3c3g, and Pik3cg). Among these 20 core genes, Adora2b, Ccr2, Ttk, Ace, Cma1, and Hspa1a ranked in the top six and were selected for subsequent experimental validation, given their well-documented associations with oxidative Stress and inflammation(Nie, Deng et al. 2024). Relevant studies have indicated that during hypoxia, extracellular adenosine levels increase, and Adora2b (as an adenosine receptor) participates in the endogenous feedback loop regulating hypoxia-associated inflammation. Ccr2 can mediate the recruitment of inflammatory cells to damaged or inflamed sites to exacerbate local inflammatory responses, and its abnormal activation may also contribute to oxidative stress by regulating reactive oxygen species production in inflammatory cells(Boniakowski, Kimball et al. 2018, El Sayed, Patik et al. 2022). Ttk is involved in DNA damage repair and cell cycle regulation, while Ace not only promotes vasoconstriction and local inflammation but also induces inflammatory cells to release reactive oxygen species(Poetsch 2020). Cma1 can directly hydrolyze inflammation-related proteins to amplify inflammatory responses, and Hspa1a—acting as a stress-responsive protein—plays a role in balancing inflammation and oxidative stress(He, Deng et al. 2025). Western blot (WB) analysis verified the protein expression changes of the top six core targets (Adora2b, Ccr2, Ttk, Ace, Cma1, Hspa1a) and clarified their roles in the protective mechanism of *Ganoderma lucidum*. Compared with the control group, the protein expression levels of the above six targets in the lung tissue of the HAPE group were significantly upregulated (p<0.01)—which is consistent with the pathological roles of these targets in HAPE: excessive activation of Adora2b exacerbates hypoxic pulmonary vascular leakage, Ccr2 mediates the recruitment of inflammatory cells to lung injury sites, abnormal expression of Ttk is associated with disorders of alveolar epithelial cell proliferation and apoptosis, high expression

of Ace aggravates lung tissue inflammatory damage by promoting vasoconstriction and reactive oxygen species release, excessive secretion of Cma1 hydrolyzes inflammation-related proteins to amplify pulmonary inflammatory responses, and abnormal upregulation of Hspa1a may disrupt the balance between inflammation and oxidative stress and indirectly affect lung tissue homeostasis. Importantly, *Ganoderma lucidum* pretreatment significantly downregulated the protein expression of these six targets ($p<0.05$), directly demonstrating that *Ganoderma lucidum* exerts a protective effect against HAPE by regulating these key molecules(Cör Andrejč, Knez et al. 2022).

In conclusion, the present study demonstrates that *Ganoderma lucidum* exerts a protective effect against HAPE. Its mechanisms involve two key aspects: 1) mitigating lung pathological damage and suppressing pro-inflammatory cytokine release; 2) regulating HAPE-related pathways through multi-target synergism (i.e., targeting Adora2b, Ccr2, Ttk, Ace, Cma1, and Hspa1a) This finding is of great significance. It not only offers novel insights into how *Ganoderma lucidum* ameliorates HAPE but also lays a solid foundation for further exploring the specific molecular mechanisms underlying its protective effect(Ahmad 2020).

Conclusion

In summary, this study demonstrates that *Ganoderma lucidum* (GL) exerts a significant protective effect against high-altitude pulmonary edema (HAPE) in a mouse model. The therapeutic mechanism is primarily mediated through its potent anti-inflammatory and antioxidant properties. By integrating network pharmacology with transcriptomic analysis, we identified key target genes, including Adora2b, Ccr2, and Ttk, and validated that GL intervention effectively modulates their expression. These findings not only elucidate the multi-target mechanism underlying GL's action against HAPE but also provide a compelling theoretical basis for its potential application as a novel preventive and therapeutic strategy for HAPE in clinical settings.

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Author Contributions

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acquisition.

Conflict of Interest Statement

The authors declare no conflicts of interest related to this work.

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References

1. Ahmad, M. F. (2020). "Ganoderma lucidum: A rational pharmacological approach to surmount cancer." J Ethnopharmacol 260: 113047.
2. Bärtsch, P. and E. R. Swenson (2013). "Clinical practice: Acute high-altitude illnesses." N Engl J Med 368(24): 2294-2302.
3. Boniakowski, A. E., A. S. Kimball, A. Joshi, M. Schaller, F. M. Davis, A. denDekker, A. T. Obi, B. B. Moore, S. L. Kunkel and K. A. Gallagher (2018). "Murine macrophage chemokine receptor CCR2 plays a crucial role in macrophage recruitment and regulated inflammation in wound healing." Eur J Immunol 48(9): 1445-1455.
4. Cör Andrejč, D., Ž. Knez and M. Knez Marevci (2022). "Antioxidant, antibacterial, antitumor, antifungal, antiviral, anti-inflammatory, and nevro-protective activity of Ganoderma lucidum: An overview." Front Pharmacol 13: 934982.
5. Cör, D., Ž. Knez and M. Knez Hrnčič (2018). "Antitumour, Antimicrobial, Antioxidant and Antiacetylcholinesterase Effect of Ganoderma Lucidum Terpenoids and Polysaccharides: A Review." Molecules 23(3).
6. Desireddi, J. R., K. N. Farrow, J. D. Marks, G. B. Waypa and P. T. Schumacker (2010). "Hypoxia increases ROS signaling and cytosolic Ca(2+) in pulmonary artery smooth muscle cells of mouse lungs slices." Antioxid Redox Signal 12(5): 595-602.
7. Ding, L., H. Shangguan, X. Wang, J. Liu, Y. Shi, X. Xu and Y. Xie (2025). "Extraction, purification, structural characterization, biological activity, mechanism of action and application of polysaccharides from Ganoderma lucidum: A review." Int J Biol Macromol 288: 138575.
8. El Sayed, S., I. Patik, N. S. Redhu, J. N. Glickman, K. Karagiannis, E. S. Y. El Naenaeey, G. A. Elmowalid, A. M. Abd El Wahab, S. B. Snapper and B. H. Horwitz (2022). "CCR2 promotes monocyte recruitment and intestinal inflammation in mice lacking the interleukin-10 receptor." Sci Rep 12(1): 452.
9. Ge, S. X., E. W. Son and R. Yao (2018). "iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data." BMC Bioinformatics 19 (1): 534.
10. Ge, Y. L., P. J. Li, Y. R. Bu, B. Zhang, J. Xu, S. Y. He, Q. L. Cao, Y. G. Bai, J. Ma, L. Zhang, J. Zhou and M. J. Xie (2024). "TNF-α and RPLP0 drive the apoptosis of endothelial cells and increase susceptibility to high-altitude pulmonary edema." Apoptosis 29(9-10): 1600-1618.
11. He, X., B. Deng, C. Zhang, G. Zhang, F. Yang, D. Zhu, Y. Yang, B. Ma, X. Hu, Y. Wang and X. Kang (2025). "HSPA1A inhibits pyroptosis and neuroinflammation after spinal cord injury via DUSP1 inhibition of the MAPK signaling pathway." Mol Med 31(1): 53.
12. Jin, M., Y. Zhu, D. Shao, K. Zhao, C. Xu, Q. Li, H. Yang, Q. Huang and J. Shi (2017).

"Effects of polysaccharide from mycelia of Ganoderma lucidum on intestinal barrier functions of rats." Int J Biol Macromol 94(Pt A): 1-9.

13. Kong, Y. and T. Yu (2018). "A Deep Neural Network Model using Random Forest to Extract Feature Representation for Gene Expression Data Classification." Sci Rep 8(1): 16477.
14. Kou, R. W., Y. Q. Gao, B. Xia, J. Y. Wang, X. N. Liu, J. J. Tang, X. Yin and J. M. Gao (2021). "Ganoderterpene A, a New Triterpenoid from Ganoderma lucidum, Attenuates LPS-Induced Inflammation and Apoptosis via Suppressing MAPK and TLR-4/NF-κB Pathways in BV-2 Cells." J Agric Food Chem 69(43): 12730-12740.
15. Li, D. W., M. Liu, Y. Q. Leng, J. F. Hu, S. Deng, A. J. Leng, X. C. Ma, R. Y. Wang, J. Zhou and C. Wang (2022). "Lanostane triterpenoids from Ganoderma lucidum and their inhibitory effects against FAAH." Phytochemistry 203: 113339.
16. Lian, W., X. Yang, Q. Duan, J. Li, Y. Zhao, C. Yu, T. He, T. Sun, Y. Zhao and W. Wang (2024). "The Biological Activity of Ganoderma lucidum on Neurodegenerative Diseases: The Interplay between Different Active Compounds and the Pathological Hallmarks." Molecules 29(11).
17. Luo, H., D. Tan, B. Peng, S. Zhang, C. T. Vong, Z. Yang, Y. Wang and Z. Lin (2022). "The Pharmacological Rationales and Molecular Mechanisms of Ganoderma lucidum Polysaccharides for the Therapeutic Applications of Multiple Diseases." Am J Chin Med 50(1): 53-90.
18. Ma, Y., J. Han, K. Wang, H. Han, Y. Hu, H. Li, S. Wu and L. Zhang (2024). "Research progress of Ganoderma lucidum polysaccharide in prevention and treatment of Atherosclerosis." Heliyon 10(12): e33307.
19. Nie, X., W. Deng, H. Zhou and Z. Wang (2024). "Long noncoding RNA MCM3AP-AS1 attenuates sepsis-induced cardiomyopathy by improving inflammation, oxidative stress, and mitochondrial function through mediating the miR-501-3p/CADM1/STAT3 axis." Int Immunopharmacol 128: 111500.
20. Paul, S., A. Gangwar, H. Patir, K. Bhargava and Y. Ahmad (2019). "Reverse translating SULT1A1, a potential biomarker in roentgenographically tested rat model of rapid HAPE induction." Life Sci 229: 132-138.
21. Poetsch, A. R. (2020). "The genomics of oxidative DNA damage, repair, and resulting mutagenesis." Comput Struct Biotechnol J 18: 207-219.
22. Richalet, J. P., F. Jeny, P. Callard and J. F. Bernaudin (2023). "High-altitude pulmonary edema: the intercellular network hypothesis." Am J Physiol Lung Cell Mol Physiol 325(2): L155-l173.
23. Sacks, D., B. Baxter, B. C. V. Campbell, J. S. Carpenter, C. Cognard, D. Dippel, M. Eesa, U. Fischer, K. Hausegger, J. A. Hirsch, M. Shazam Hussain, O. Jansen, M. V. Jayaraman, A. A. Khalessi, B. W. Kluck, S. Lavine, P. M. Meyers, S. Ramee, D. A. Rüfenacht, C. M. Schirmer and D. Vorwerk (2018). "Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke." Int J Stroke 13(6): 612-632.
24. Sharma Kandel, R., R. Mishra, J. Gautam, A. Alaref, A. Hassan and N. Jahan (2020). "Patchy Vasoconstriction Versus Inflammation: A Debate in the Pathogenesis of High Altitude Pulmonary Edema." Cureus 12(9): e10371.
25. Si, L., H. Wang, R. Wang, L. Tsering, Q. Long, Y. Jiang, Y. Yi and Y. Zhao (2023). "Suggestive evidence of CYP4F2 gene polymorphisms with HAPE susceptibility in the Chinese Han population." PLoS One 18 (1): e0280136.
26. Sommer, N., I. Strielkov, O. Pak and N.

Weissmann (2016). "Oxygen sensing and signal transduction in hypoxic pulmonary vasoconstriction." Eur Respir J 47(1): 288-303.

27. Swallah, M. S., P. Bondzie-Quaye, Y. Wu, A. Acheampong, F. L. Sossah, S. M. Elsherbiny and Q. Huang (2023). "Therapeutic potential and nutritional significance of Ganoderma lucidum - a comprehensive review from 2010 to 2022." Food Funct 14(4): 1812-1838.
28. Swenson, E. R. (2020). "Early hours in the development of high-altitude pulmonary edema: time course and mechanisms." J Appl Physiol (1985) 128(6): 1539-1546.
29. Sydykov, A., A. Mamazhakypov, A. Maripov, D. Kosanovic, N. Weissmann, H. A. Ghofrani, A. S. Sarybaev and R. T. Schermuly (2021). "Pulmonary Hypertension in Acute and Chronic High Altitude Maladaptation Disorders." Int J Environ Res Public Health 18(4).
30. Wang, Y., X. Huang, F. Peng, H. Han, Y. Gu, X. Liu and Z. Feng (2022). "Association of variants m.T16172C and m.T16519C in whole mtDNA sequences with high altitude pulmonary edema in Han Chinese lowlanders." BMC Pulm Med 22(1): 72.
31. Wen, L., Z. Sheng, J. Wang, Y. Jiang and B. Yang (2022). "Structure of water-soluble polysaccharides in spore of Ganoderma lucidum and their anti-inflammatory activity." Food Chem 373(Pt A): 131374.
32. Woods, P. and J. Alcock (2021). "High-altitude pulmonary edema." Evol Med Public Health 9(1): 118-119.
33. Xu, J., C. Xiao, H. Xu, S. Yang, Z. Chen, H. Wang, B. Zheng, B. Mao and X. Wu (2021). "Anti-inflammatory effects of Ganoderma lucidum sterols via attenuation of the p38 MAPK and NF-κB pathways in LPS-induced RAW 264.7 macrophages." Food Chem Toxicol 150: 112073.