Quantitative proteomics reveals the effects of resveratrol on high-altitude polycythemia treatment

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Abstract

High-altitude polycythemia (HAPC) is a common plateau chronic disease in which red blood cells (RBCs) are compensatory hyperproliferative due to high altitude hypoxic environment. HAPC severely affects the physical and mental health of populations on the plateau. However, the pathogenesis and treatment of HAPC has been rarely investigated. Here, the hypoxia-induced HAPC model of rat was established, in which hemoglobin concentration significantly increased and platelets clearly decreased. The effect of resveratrol upon hypoxia enabled HAPC remission and maked hemoglobin and platelet tend to a normal level. Furthermore, we applied quantitative proteomics to investigate the plasma proteome variation and the underlying molecular regulation during HAPC occurrence and treatment with resveratrol. Hypoxia promoted erythrocyte developing and differentiating and disrupted cytoskeleton organization. Notably, the resveratrol administration reversed the proteome change pattern due to hypoxia and contributed to plateau adaption. Quantitative verification of differentially expressed proteins confirmed the roles of resveratrol in HAPC. Resveratrol is expected to be useful for HAPC treatment.

Statement of significance of the study

High-altitude polycythemia (HAPC) is a common plateau chronic disease, which severely affects the physical and mental health of populations on the plateau. However, the pathogenesis and treatment of HAPC has been rarely investigated. In our study, we established a rat model of the hypobaric hypoxia-induced HAPC. The detection of biochemical indicators, especially hemoglobin contents, confirmed the occurrence of HAPC. Meanwhile, the use of resveratrol upon hypoxia enabled HAPC remission and maked hemoglobin and platelet tend to a normal level. Furthermore, we applied label-free quantitative proteomics to investigate the plasma proteome changes during HAPC development and remission. Hypoxia promoted erythrocyte development and differentiation and disrupted cytoskeleton organization. Notably, the resveratrol administration reversed the proteome change pattern due to hypoxia and contributed to plateau adaption. These results will increase understanding of the role and potential molecular mechanism of resveratrol in HAPC.

1. Introduction

The high-altitude environment is characterized by low oxygen, low air pressure, cold and strong radiation, among which hypoxia is the main factor affecting human life activities.[1] High-altitude polycythemia (HAPC) is a common plateau chronic disease in which red blood cells (RBCs) are compensatory hyperproliferative due to high altitude hypoxic environment in the plateau at an altitude of more than 3 km. It has been reported that the incidence of

HAPC was about 13% above 4500 m above the sea level.[2] HAPC severely affects the physical and mental health of populations on the plateau, which could lead to pulmonary heart disease, congestive heart failure and neurological disorders in the late stage of the disease. HAPC is also a risk factor for cerebral thrombosis and cerebral hemorrhage.[3] At present, it is recognized in the world that hypoxia is the most important research clue of HAPC,[4] but its pathogenesis is still unclear.

Plasma proteome profiling demonstrated Jersey cattle exposed to high altitude hypoxia could adapt to that condition through regulation of inflammatory homeostasis by inhibiting the acute phase response, coagulation system, and complement system[5]. Also, differential plasma proteomes of high altitude natives and lowland normal controls by a TMT-based proteomic approach revealed the protective mechanisms on adaptation to high-altitude hypobaric hypoxia. Recently, the plasma proteome profiling of HAPC was studied using the quantitative proteomics method.[6] The disordered proteins between HAPCs and healthy controls were enriched in complement and coagulation cascades, inflammation and immune response, playing essential roles in the HAPC progress.[7] Unfortunately, there is no safe and effective treatment for HAPC.

Resveratrol is a natural non-flavonoid polyphenolic compound widely found in natural plants and fruits such as grapes, pine, peanuts, Polygonum cuspidatum, and Cassia seed.[8] It has been studied that resveratrol has multiple roles of anti-tumor, anti-cardiovascular diseases, anti-oxidation, blood vessel dredge, anti-platelet aggregation, immune regulation, liver protection, antibacterial and anti-inflammatory.[9] However, the application of resveratrol in

HAPC treatment has rarely been studied. In view of the various physiological and pharmacological effects of resveratrol, it was anticipated to have the effects of HAPC intervention and treatment.

In our study, we established a rat model of the hypobaric hypoxia-induced HAPC. The detection of biochemical indicators, especially hemoglobin contents, confirmed the occurrence of HAPC. Meanwhile, we also examined the effects of resveratrol on HAPC. Furthermore, we applied label-free quantitative proteomics to investigate the plasma proteome changes during HAPC development and remission, in order to explore the potential molecular mechanism of HAPC.

2. Materials and methods

2.1. HAPC model of rat

The male Wistar rats (180 - 200 g) were used in our study according to the ethics committee guidelines of the Tianjin Institute of Environmental and Operational Medicine. The intermittent hypoxia exposure was used to develop HAPC, referring to a previous report.[10] Rats were randomly divided into five experimental groups (Figure 1A), which were the normoxia control, hypoxia exposure, hypoxia exposure with low -, middle- and high -dose of resveratrol. The rats in hypoxia exposure with low -, middle- and high -dose resveratrol group were respectively administrated resveratrol of 50, 100, 200 mg/kg for 16 weeks after 4 weeks of hypoxia exposure.

2.2. Hematological and hemorheological measurement

After hypoxia exposure at the 4th and 20th week, venous blood was collected from rat tails and hemoglobin concentration was determined by the cyanmethemoglobin (HiCN) method.[11] The rats were sacrificed under anaesthesia at the 20th week after hypoxia exposure. One part of blood samples was collected into vacuum blood collection tubes with a suitable amount of K2-EDTA for whole blood cell analysis using the Automatic hematology analyzer (Pulang, China). Another part of blood samples was collected into vacuum blood collection tubes with a suitable amount of lithium heparin for blood viscosity determination using an Automatic Blood Rheometer SA-6900 (Beijing Success Technology Development Co. Ltd., Beijing, China).

2.3. SDS-PAGE and protein digestion

Control, hypoxia and high-dose resveratrol groups were followed into label-free proteomics research. Blood samples were collected into anticoagulation tubes. The plasma was separated RBCs from whole blood by centrifugation (3000 *g* for 10 min at 4 °C). Each group has nine samples. To eliminate the individual differences, every three samples were pooled into one sample for the following proteomics identification. High abundance proteins were removed using Albumin & IgG Depletion SpinTrap (GE Healthcare) according to the instruction manual. The remaining proteins were solubilized in the lysis buffer and heated at 100 °C for eight minutes. Then, the proteins were separated by SDS-PAGE and visualized by Coomassie Brilliant Blue staining (Supplemental Figure 1). Each gel line was cut into three slices for trypsin digestion performed in a microcentrifuge tube. After discoloration and lyophilization, samples were digested by 50 μL trypsin (0.01 μg/μL) in 25 mM ammonium at 37 °C for 15

hours. The digested peptides were extracted and acidified with 5% TFA at 40 °C for one hour and 2.5% TFA, 50% acetonitrile at 30 °C for one hour. The peptides were pooled with the supernatants and then dried by centrifugal lyophilization.

2.4. LC-MS/MS analysis

Thereafter, the collected peptides in each fraction were dissolved in 2% methyl alcohol and 0.1% formic acid, loaded into an Easy-nLC nanoflow HPLC system coupled to an Orbitrap Lumos mass spectrometer (Thermo Fisher Scientific). Spectrals were obtained from 350 to 1,800 m/z. Resolving powers of MS and MS/MS scan at 200 m/z were set at 70,000 and 17,5000. The raw MS data has been uploaded into iProx database with an accession number IPX0001940001 (link for reviewers: https://www.iprox.org/page/SSV024.html;url=1577005240184SXx6; password: gL7b).

2.5. Data analysis

The MS raw data was searched against rat protein sequences in UniProt database using the ProteomeDiscovery software (Thermo Fisher Scientific). A maximum of two missed cleavage sites was allowed using tryptic digestion. Precursor mass tolerance was set at 20 ppm, fragment mass tolerance was set at 0.5 Da. Carbamidomethyl (C) was selected as a static modification, Oxidation (M) and Acetyl (Protein N-term) were selected as dynamic modifications. Decoy database searching was performed to control the false discovery rate (FDR) < 0.05. A total of 1,297 proteins were identified. Missing values of protein intensity were imputed using Perseus.[12] Differential expression was analyzed by Student's t-test based on protein intensity values in triplicate of each group, and the proteins with p value <

0.05 and fold change > 1.2 were treated as significantly differential proteins. Gene ontology (GO) and KEGG pathways enrichment were performed by the online Metascape tool.[13] Clustering analysis was accomplished by the pheatmap R package. The volcano plots were generated by the ggplot2 R package.

2.6. Western blotting

Immunoblotting analysis was performed as previously reported.[14] Briefly, Equal amounts of proteins underwent SDS-polyacrylamide gel electrophoresis and were transferred to polyvinylidene difluoride membranes. Membranes were incubated with primary antibodies (anti-Hbb, anti-Ywhae, anti-Vwf and anti-Serpinc1, Abcam), and then probed with horse-radish peroxidase-conjugated secondary antibodies. The blots were visualized using the SuperSignal® West Femto Maxi-mum Sensitivity Substrate (Thermo Scientific, Rockford, IL), and the images were captured with an Amersham Imager 680 (GE, USA).

2.7 Statistical Analysis

All statistical analyses were performed using SPSS 17.0 software (IBM Corp., Armonk, NY, USA). For the three or more groups of data, statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test. Student's t-test was used to determine the significance of the differences between two groups. A two-sided value of p value < 0.05 or p value <0.01 was considered statistically significant.

3. Results

3.1. HAPC rat model

A rat model of intermittent hypobaric hypoxia-induced polycythemia was established (Figure 1A). The rats' body weight gradually decreased after exposure to hypoxia for 4 weeks (Figure 1B). Importantly, we found that the hemoglobin content increased to 242.8 g/L and was 45.1% higher than that of the control group (Figure 1C). From the 5th week to the 20th week upon hypobaric hypoxia exposure, rats in drug groups were treated with resveratrol. The rats were sacrificed at the 20th week after the hypobaric hypoxia exposure under anaesthesia (Figure 1A). Rats that received resveratrol for 16 weeks exhibited no significant changes in body weight compared with those in the hypoxia control group (Figure 1D). The hemoglobin concentration in the hypoxia group always maintained a high level (>210 g/L). Compared to the hypoxia group, the hemoglobin concentration in the resveratrol group significantly reduced in a dose dependent manner. In the subsequent proteomics analysis, we only used the high-dose group to investigate the roles of the resveratrol in high-altitude polycythemia.

In addition to the detection of hemoglobin concentration, we also observed other indicators such as red blood cell (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), red blood cell volume distribution width - CV (RDW-CV), red blood cell volume distribution width - SD (RDW-SD), platelet count (PLT) and viscosity (Table 1 and 2). In our study, the platelet counts in intermittent hypobaric hypoxia for 20 weeks were 45.0% lower than those in the control group, while those in the low-, medium- and high-dose resveratrol groups decreased by 30.2%, 28.1% and 22.8% respectively. The number of platelets in the high-dose resveratrol group was significantly higher than that in the hypoxic group ($p < 0.05$). The increase of platelet level after hypoxia may contribute to the improvement of hemodynamics. Furthermore, we found

hypoxia significantly increased the whole blood viscosity. Compared with the hypoxia group, the blood viscosity of the high-dose resveratrol group was significantly reduced, indicating that long-term use of resveratrol at a dose of 200 mg/kg could alleviate the alternations of blood viscosity induced by hypoxia exposure. Additionally, there were no changes both hematological and hemorheological parameters after using resveratrol alone under the normoxic condition (Supplementary Table 1 and 2), hence we excluded the potential effects of resveratrol on normal controls under the normoxic exposure. Next, the normoxic control, hypoxia exposure and high-dose resveratrol upon hypoxia exposure groups were analyzed by the proteomics method, in order to investigate the plasma proteome variation and the underlying molecular regulation during HAPC occurrence and treatment using resveratrol.

3.2. Proteome alternation upon the hypoxia exposure.

Label-free proteomics was firstly used to investigate the plasma proteome alternation between the hypoxia and control groups. Compared with the control group, 25 up-regulated proteins and 29 down-regulated proteins were identified in the hypoxia group (Figure 2A and Supplementary Table 3). Up-regulated proteins concentrated on erythrocyte development, coenzyme biosynthetic process, myeloid cell development and so on (Figure 2B), from which we could conclude that the erythrocyte production was significantly promoted in the hypoxia group. Regulation of T cell proliferation indicated the disrupted immune response process due to the hypoxia environment, as previously reported.[6] While down-regulated proteins were mainly enriched in import into cell, endocytosis and cytoskeleton organization. KEGG pathway enrichment analysis also revealed that the hypoxia environment changed

trans-endothelial migration and cytoskeleton organization (Figure 2C). Meanwhile, there was a significant change in platelet activation, consistent with the above findings of decreased platelet counts during the HAPC progress (Table 1). Overall, the rat HAPC model was confirmed by the plasma proteome diversification and erythrocyte development and cytoskeleton organization were the primary responses to hypoxia during the HAPC progress.

3.3. HPAC remission induced by the resveratrol

To explore the effects of resveratrol on the rat HAPC model, we further analyzed the plasma proteome changes between the resveratrol and hypoxia groups. Quantitative label-free proteomics identified 31 up-regulated proteins and 46 down-regulated proteins when comparing the resveratrol group with the hypoxia group (Figure 3A and Supplementary Table 4). Highly-expressed proteins in the resveratrol group were enriched in signaling by Rho GTPases and actin filament-based process (Figure 3B). When resveratrol was used to treat the HAPC model, previously decreased proteins involving the actin filament-based process began to show up-regulation. Inversely, cofactor metabolic process-related proteins were down-regulated after resveratrol intervention. Importantly, the blood coagulation associated with HAPC previously reported was alleviated in the resveratrol group. We also observed that both up- and down-regulated proteins participated in the progress of response to toxic substance, potentially causing by the need for the resveratrol drug metabolism.

The unsupervised hierarchical clustering for differently expressed proteins revealed that the resveratrol group and the control group were more similar among all the three groups (Figure 3C), which demonstrated that the use of resveratrol was beneficial for the remission of HAPC

to a healthy state. Differential proteins were mainly grouped into 3 protein clusters. Cluster 1 was composed of hypoxia-specific high-expressing proteins and was linked to small molecule biosynthetic process, cofactor metabolic process and erythrocyte development. In resveratrol group, increased erythrocyte development under hypoxia appeared to return to near the normal level, consistent with the red blood cell count results (Table 1). In contrast, Cluster 3 included the hypoxia-specific low-expressing proteins and focused on signaling by Rho GTPases, actin cytoskeleton organization and endocytosis, demonstrating that resveratrol could assist actin cytoskeleton remodeling of HAPC. However, Cluster 2 did not show that the resveratrol and the control were closer. Proteins in Cluster 2 were enriched in response to L-ascorbic acid, acute inflammatory response and blood coagulation. These function adjustments contributed to better plateau adaptation.[7] Collectively, the resveratrol helped HAPC remission by regulating erythrocyte development and actin cytoskeleton on the one hand, and contributed to plateau adaptation on the other.

3.4. Verification of key proteins

To confirm the changes in plasma due to hypoxia exposure and resveratrol treatment, we intended to verify expressions of several important proteins using the Western blotting (WB) method. Hbb was a significantly highly expressed protein in the hypoxia group compared to the control and resveratrol groups. Hbb, as a hemoglobin subunit beta-1, was involved in oxygen transport from the lung to the various peripheral tissues.[15] The average MS intensity of the hypoxia group was 2.0 times that of the control group ($p < 0.019$) and 3.5 times that of the resveratrol group ($p < 0.004$) (Figure 4A). Accordingly, the WB results of Hbb showed

the same protein expression trend. Ywhae was a low expressed protein in the hypoxia group. The MS intensity in control group increased to 1.6 times that of the hypoxia group and the resveratrol group increased to 2.0 times (Figure 4B). Ywhae was also highly expressed in control and resveratrol groups using WB. Another two proteins Vwf and Serpinc1 belonged to Cluster 2 in Figure 3C and both of them participated in the blood coagulation. There were no significant differences between the control and hypoxia groups. But the resveratrol decreased their expression (Figure 4C and D). Vwf is important to the hemostasis maintenance, which promotes adhesion of platelets to the sites of vascular injury by forming a molecular bridge between sub-endothelial collagen matrix and platelet-surface receptor complex.[16] The reduced Vwf under the resveratrol treatment aids to platelet increase. Serpinc1 (antithrombin III) could interact with coagulation factor F9, thrombin, FXII, FXI, FIX and FX, directly participating in the intrinsic pathway of blood coagulation.[17] Overall, changes of protein expression were experimentally confirmed by WB and these important proteins were positively regulating the response to hypoxia (Supplemental file 1).

4. Discussion

In our study, we established the rat model of high-altitude polycythemia by simulating the plateau hypobaric hypoxia environment. Although there are no exact diagnostic criteria for rats, the hemoglobin content of our rat model reached 242.8 g/L and was significantly higher than that of the control group, which is higher than the diagnostic level 210 g/L of human HAPC. Meanwhile, the decreased platelets due to hypobaric hypoxia in our rat model also illustrated the HAPC development.[18] Label-free proteomics provided a more basic and direct

evidence of HAPC. We observed the up regulation of erythrocyte development and differentiation and disruption of platelet activation. Hence, the HAPC definitely happened in our rat model.

Compared to the hypoxia group, the hemoglobin concentration and the number of RBCs in the resveratrol group significantly reduced in a dose dependent manner, indicating the positive roles of resveratrol in relieving HAPC were unneglectable. Also, Platelets are small pieces of cytoplasm that are shed from megakaryocytes and have a hemostatic effect. It has been reported that hypoxia can reduce platelet counts.[18] Compared with the plain people, platelet counts decreased by 12% under plateau hypoxia for 3 months and by 31% for 13 months. We found that resveratrol could significantly increase the number of platelets. Furthermore, long-term use of resveratrol could alleviate the alternations of blood viscosity induced by hypoxia exposure, indicating that resveratrol intervention upon hypoxia enabled HAPC remission.

Metabolomic and proteomic profiling were widely used in the study of the responses to high-altitude hypoxia. It had provided supportive evidence of RBC metabolic adaptations that ensue within hours from exposure to high altitude hypoxia.[19] Nonbiased high-throughput metabolomic profiling revealed S1P as an intracellular hypoxia-responsive biolipid promoting erythrocyte glycolysis, O_2 delivery for adaptation to high-altitude hypoxia.[20] Also, using high throughput unbiased metabolomic profiling found that plasma adenosine concentrations and soluble CD73 (sCD73) activity rapidly increased at high altitude and were associated with elevated erythrocyte 2,3-BPG levels and O_2 releasing capacity.[21]

Metabolomic and proteomic profiling comprehensively defined the physiological responses of skeletal muscle energy metabolism to 16 days of high-altitude hypoxia (5260 m) in healthy volunteers.[22] In our studies, plasma proteomics not only confirmed the increase in red blood cells upon hypoxia exposure, but also revealed other important function changes, such as regulation of T cell proliferation and actin cytoskeleton organization. The molecular mechanism of cytoskeleton organization on HAPC has not been unclear, but a recent study has shown that declining partial pressure of oxygen (pO_2) gradient could reduce essential cytoskeletal elements and housekeeping proteins, such as actin, tubulin and GAPDH.[23] Inflammation and immune response has been reported to be associated with HAPC in humans.[6] High-altitude hypobaric hypoxia could promote the selective differentiation of hematopoietic stem cells into red blood cells by up-regulating some inflammatory cytokines, and then induce erythropoietin-independent erythrocyte proliferation.[24] Hence, immune response to high-altitude hypoxia may be an important non-erythropoietin mechanism of erythrocytosis.

Recently, the red blood cell proteome has gradually developed. It has been used multiplex tandem mass tag labeling combined with nano-LC-MS/MS to compare the proteome of adult and cord RBCs and reticulocytes.[25] Bryk et al provides the first “in-depth” quantitative analysis of the RBC proteome, which promotes future studies of erythrocyte structure, functions, and disease.[26] Also, further extensions of RBC protein complexity and biochemistry are anticipated, stemming from the application of advancing bioanalytical technologies.[27] The application of RBC proteomic profiling in HPAC will also be the focus of the next study.

In view of decreased hemoglobin concentration and increased platelets after resveratrol administration, the positive roles of resveratrol in relieving HAPC were unneglectable. The increase of platelet levels upon the hypoxia environment would help improve hemodynamics. Differentially expressed proteins revealed by label-free proteomics also demonstrated the resveratrol could reverse the disrupted proteome pattern caused by hypoxia. Cofactor metabolic process, erythrocyte development, cytoskeleton organization and so on in resveratrol group were close to the normal control. Additionally, proteins involved in acute inflammatory response and blood coagulation further reduced in resveratrol group, such as Vwf and Serpinc1. Their decreases would contribute to plateau adaptation without inducing and aggravating HAPC. It is expected to open up the prospects of the resveratrol application in the field of high-altitude medicine.

Supporting Information

Supplementary Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure legends

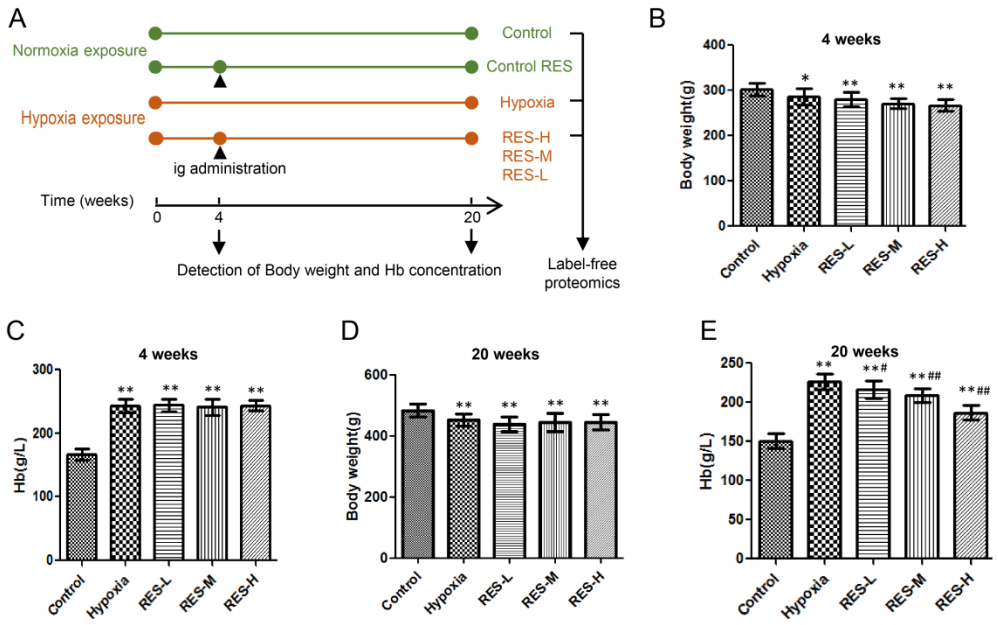

Figure 1. Scheme of the experimental design and the rat HAPC model. A. Workflow of the entire study. Detection of biochemical indicators is used to measure the establishment of rat models. Label-free proteome identification is used to reveal the underlying molecular alternation. **B.** Body weight changes in different groups at 4 weeks under hypoxia exposure. **C.** Hb abundance changes in different groups at 4 weeks under hypoxia exposure. **D.** Body weight changes in different groups at 20 weeks under hypoxia exposure and resveratrol administration. **E.** Hb abundance changes in different groups at 20 weeks under hypoxia exposure and resveratrol administration. (**, p value <0.01 vs Control; *, p value < 0.05 vs Control; $^{\#\#}$, p value <0.01 vs Hypoxia; $^{\#}$, p value < 0.05 vs Hypoxia)

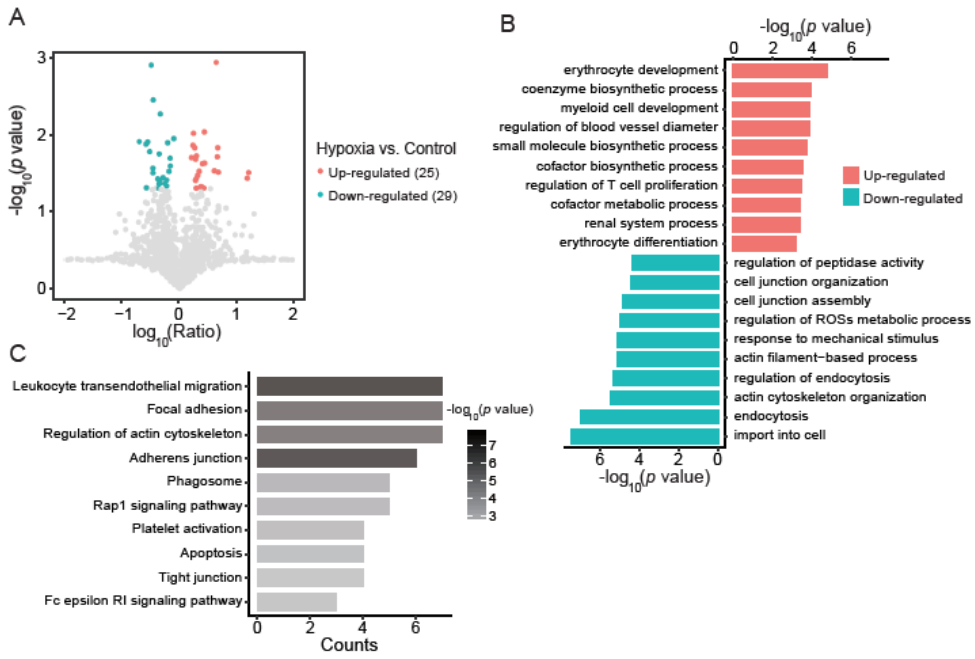

Figure 2. Plasma proteome alternation upon the hypoxia exposure. A. Volcano plot illustrates the differentially expressed proteins between hypoxia and control groups. **B.** Enriched gene ontology terms of up- and down-regulated proteins. **C.** Enriched KEGG pathways of differentially expressed proteins.

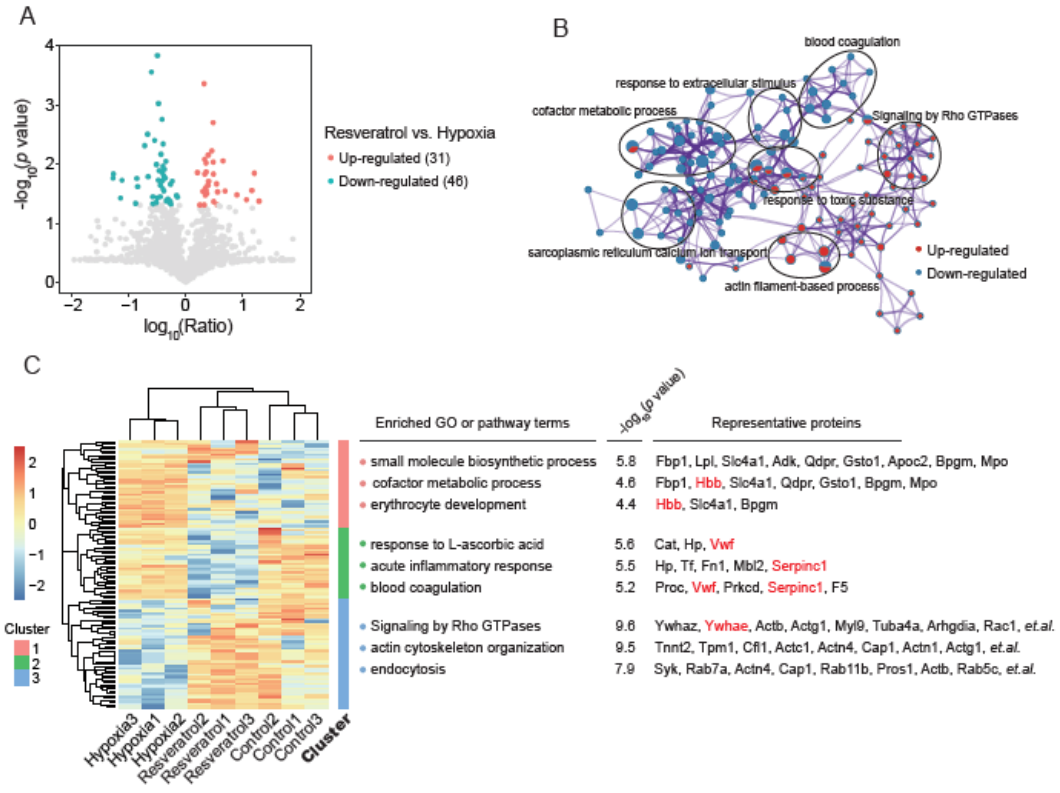

Figure 3. HAPC remission induced by the resveratrol. A. Differential expression analysis between resveratrol and hypoxia groups. **B.** Enriched ontology clusters revealed by Metascape. Representative terms are converted into a network layout. Each term is represented by a circle pie. Each pie sector is proportional to the number of hits originated from up- (red color) and down-regulated (blue color) protein list. The thickness of the edge represents the similarity score between terms. **C.** Unsupervised hierarchical clustering between all the groups based on protein intensities provided by label-free proteomics. Differentially expressed proteins form three protein clusters. Their enriched GO and pathway terms and some representative proteins are illustrated. Proteins in red are verified by the Western Blotting in the following.

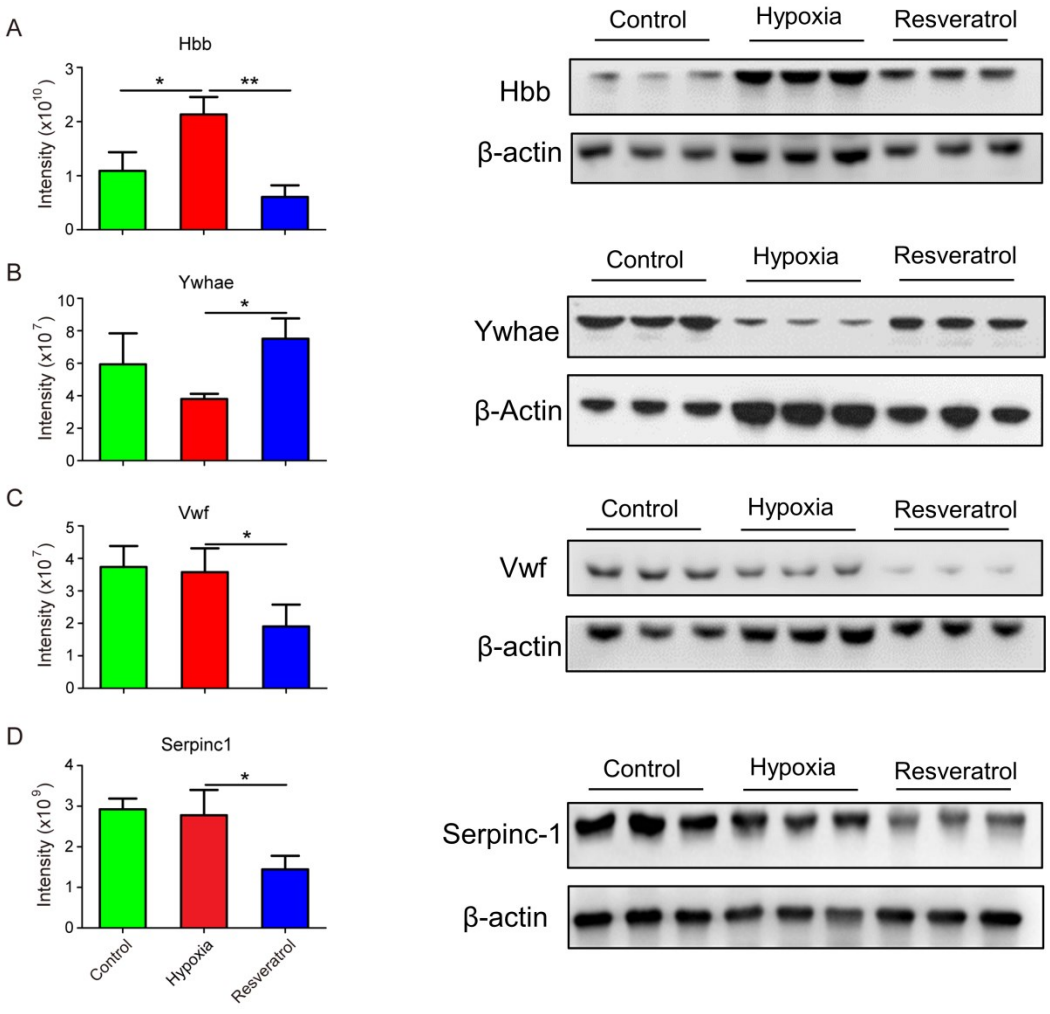

Figure 4. Quantification confirmation of key proteins. MS intensities (left) and Western Blotting (right) of Hbb (**A**), Ywhae (**B**), Vwf (**C**) and Serpinc1 (**D**) in control, hypoxia and resveratrol groups. The column height in the left presents the average intensity of triplicates and the error bar means standard deviation. Differential analysis used Student's t-test (**, p value <0.01; *, p value < 0.05).

Table 1. Changes in hematological parameters after hypoxia exposure in the female rats.

	Control	Hypoxia	RES-L	RES-M	RES-H
RBC(10^{12}/L)	9.01±0.46	11.73±0.36**	11.57±0.61**	10.93±0.81**	9.82±0.53*$^{\#}$
MCH	28.16±0.37	30.68±1.07	29.75±0.66*	29.22±0.64*$^{\#}$	28.13±0.57$^{\#\#}$
MCHC g.dL^{-1}	33.69±0.48	33.62±0.83	34.05±0.25	33.27±0.55	33.68±0.48
HCT %	44.43±1.45	65.47±4.47**	61.59±4.24**$^{\#}$	54.49±3.41$^{**\#\#}$	45.69±2.34$^{\#\#}$
RDW-CV(%)	15.2±1.4	22.4±2.5*	19.2±2.1*	19.1±1.3*	18.2±1.5*$^{\#}$
RDW-SD(fl)	30.6±1.3	39.5±3.1*	36.9±3.9*	37.1±1.7*	36.3±2.7 *
PLT(10^{9}/L)	1537±149	1014±173**	1088±186*	1108±150*	1182±112* $^{\#}$

*p<0.05 vs Control, **p<0.01 vs Control, $^{\#}p$<0.05 vs Hypoxia, $^{\#\#}p$<0.01 vs Hypoxia.

Table 2. Changes in hemorheological parameters after hypoxia exposure in the female rats.

	Control	Hypoxia	RES-L	RES-M	RES-H
Blood viscosity mPa.s^{-1} (1s-1)	27.26±3.35	75.33±11.20*	74.18±10.93*	65.848±8.88*	43.61±7.65*$^{\#\#}$
Blood viscosity mPa.s^{-1} (5s^{-1})	11.56±1.13	31.54±3.20*	33.66±4.90*	29.09±3.40*	20.17±2.10**$^{\#\#}$
Blood viscosity	6.39±0.47	17.26±1.52	20.26±5.08*	16.75±1.87*	12.12±1.37**$^{\#\#}$

mPa.s^{-1} (30s^{-1})		*			
Blood viscosity mPa.s^{-1} (200s^{-1})	4.67±0.27	12.52±1.31 *	15.76±1.27*	12.55±1.45*	9.33±1.16**$^{\#}$
Casson viscosity mPa.s-1	3.72±0.17	9.92±1.28* *	11.36±1.36* *	10.21±1.25**	7.75±1.04**$^{\#}$
Blood viscosity mPa.s^{-1} (1s-1)	27.26±3.3 5	75.33±11.2 0*	74.18±10.93 *	65.848±8.88*	43.61±7.65*$^{\#\#}$
RCI	6.08±1.13	5.82±1.42	5.19±1.65	5.26±1.18	4.71±0.93*
ERI	5.22±0.72	13.66±1.28 **	11.87±1.04* *	11.74±0.92**	8.61±1.24*$^{\#\#}$

*p<0.05 vs Control, **p<0.01 vs Control, $^{\#}p$<0.05 vs Hypoxia, $^{\#\#}p$<0.01 vs Hypoxia.