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ARTICLE Network pharmacology prediction, molecular docking and in vitro experiment explored the potential mechanism of Gaoyuan'an capsule in improving hypoxia tolerance

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BACKGROUND: Tibetan medicine Gaoyuan'an capsule (GYAC) is widely used to prevent pulmonary edema at high altitude, but the specific mechanism has not been explored. In this study, we analyzed the mechanism of GYAC in hypoxia tolerance, and provided a new idea for the prevention and treatment of altitude disease.

METHODS: The effective components and corresponding targets of GYAC were screened out by the Chinese herbal medicine network database, and the key targets of hypoxia tolerance were retrieved by Genecards, OMIM and PubMed database. Cytoscape 3.7.2 was used to construct GYAC ingredient-target-hypoxia tolerance-related target network. GO function annotation and KEGG enrichment analysis were performed to predict the pathways in which target genes may be involved, and molecular docking was used to verify the binding ability of the compound to target genes. In vitro, the above results were further verified by molecular experiment.

RESULTS: We found that GYAC can improve hypoxia tolerance by regulating various target genes, including IL6, IFNG, etc. The main regulatory pathways were HIF-1 signaling pathway. Molecular docking showed that the affinity between luteolin and target genes (IL6, IFNG) were better. In vitro, we observed that hypoxia can inhibit cell viability and promote apoptosis of H9C2 cell. And hypoxia can promote the expression of LDH. After the addition of luteolin, the decrease of cell viability, the increase of cell apoptosis, LDH release and the decrease of mitochondrial membrane potential were inhibited. Besides, inflammatory related factors (IL-6, IL-10, IL-2, IFNG and VEGFA) expression were also inhibited hypoxic cell models.

CONCLUSIONS: The results of network pharmacology and molecular docking showed that luteolin, a monomeric component of GYAC, played a role in hypoxia tolerance through a variety of target genes, such as IL6, IFNG. What's more, we have discovered that luteolin can reduce the inflammatory response in cardiac myocytes, thereby alleviating mitochondrial damage, and ultimately enhancing the hypoxia tolerance of H9C2 cardiomyocytes.

The Pharmacogenomics Journal (2024) 24:8; 1-10; https://doi.org/10.1038/s41397-024-00327-0

INTRODUCTION

Continuous hypoxia under low-pressure is a defining characteristic of high-altitude environments, significantly impacting the metabolic functions of skeletal muscles and heart depending on the duration of exposure [1]. Hypoxic environments contribute to various diseases, acute alpine disease, high-altitude cerebral edema and high-altitude pulmonary edema [2]. As global development continues, an increasing number of people are visiting high-altitude areas for tourism and work, making the prevention of altitude sickness a critical concern.

Current studies have shown that traditional Chinese medicine (TCM) has certain potential in hypoxia tolerance [3, 4]. GYAC is a kind of Tibetan medicine, its main ingredients include rhodiola, astragalus, salvia miltiorrhiza and so on. It is widely used to prevent high-altitude reactions. Its primary function is to enhance hypoxia tolerance and boost immunity, but the specific mechanism remains unclear. The ingredients of TCM have attracted the

attention of researchers. Astragalus extract-Astragaloside IV (AST-IV) exhibits anti-inflammatory, anti-fibrotic, antioxidant, anti-asthmatic, anti-diabetic and immunomodulatory properties [5, 6]. It has been proved that AST-IV can inhibit hypoxiainduced apoptosis of PC-12 cells by down-regulating miR-124 [7]. Hypoxia inducible Factor-1a (HIF-1a) has become a key regulator of hypoxia responses, and is also a major regulator of homeostasis in hypoxic cells and systems by activating transcription of numerous genes [8], and AST-IV can upregulate HIF-1 [9]. Rhodiola rosea is a common medicinal plant in Asian countries. It is reported that rhodiola rosea has significant anti-hypoxic, neuroprotective, anti-fatigue, and radiation-protective activities [10, 11]. Rhodiola rosea can alleviate apoptosis and mitochondrial energy metabolism within rat models of hypoxia-induced brain injury by regulating HIF-1/microRNA 210/ISCU1/2(COX10) signaling pathway [12]. Saponins, a key bioactive component of Panax quinquefolium, exhibit a range of pharmacological effects, such

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as blood glucose regulation, blood lipid reduction, anti-tumor, anti-oxidation, anti-arrhythmic properties and enhancement of myocardial ischemia recovery. Danhong injection (DHI) is extracted from Salvia miltiorrhiza and safflower, has been shown to protect myocardial cells from hypoxia/reoxygenation and H₂O₂-induced injury by inhibiting the opening of mitochondrial permeability transition pore [13].

TCM is frequently utilized in treating various diseases. Nonetheless, the intricacies of TCM's mechanisms of action remain elusive, primarily due to the absence of robust methodologies for analyzing its complex chemical constituents [14]. The integration of network biology and multi-pharmacology is anticipated to broaden the scope for identifying drug-actionable targets, thereby establishing a solid foundation for efficacious drug discovery strategies [15]. Molecular docking, a theoretical simulation technique, predicts the binding mode and affinity of a drug based on the interactions between the drug molecule and its receptor. It can reduce the time and consumables we spend in drug development, at the same time, there is great practical value in drug design, making it a highly attractive tool [16].

In the present study, we used network pharmacology and molecular docking to predict the potential mechanism of GYAC in improving hypoxia tolerance. In vitro experiments, we constructed a cell hypoxia model for drug administration to verify the above results. This study may reveal new therapeutic pathways or drug targets for high-altitude capsules, and provide new ideas for their application in hypoxia related diseases. The flow chart of this research is shown in Supplementary Fig. 1.

MATERIALS AND METHODS

Composition of GYAC and the potential targets of GYAC

In order to collect the chemical constituents of five herbs in GYAC, we used the traditional Chinese medicine system (TCMSP, http://lsp.nwu.edu.cn/TCMSP.php; HERB, http://herb.ac.cn/; ETCM, http://www.tcmip.cn/ETCM/; Pubchem, https://pubchem.ncbi.nlm.nih.gov/; swisstargetprediction, http://www.swisstargetprediction.ch/] [17], and the criteria of drug screening was: OB (oral bioavailability) \geq 30%, DL (similarity of patent medicine) \geq 0.18. Finally, the effective compound information and corresponding targets of GYAC were obtained. In addition, comprehensive database, such as the traditional Chinese medicine integrative database (TCMID, http://183.129.215.33/TCMID/search/) [18], PubMed (https://pubmed.ncbi.nlm.nih.gov/) and OMIM (https:// www.omim.org/) were used to retrieve the compounds.

Targets associated with hypoxia tolerance

Target genes related to hypoxia were obtained from Genecards (https:// www.genecards.org/), OMIM (https://omim.org/) and PubMed database. The common targets were obtained by intersecting the targets of active ingredients and hypoxia related targets. Taking "Homo sapiens" as research species, the predicted targets were selected and standardized by UniProt database.

Construction of the GYAC ingredient-target-hypoxia tolerance-related target network

Based on the target genes of active ingredients of GYAC and the target genes of hypoxia tolerance, the common targets were introduced into STRING database (https://string-db.org/cgi/input.pl) to establish PPI network [19]. We set the minimum required interaction score at 0.4. Only when the interaction score between two proteins reaches or exceeds 0.4 will they be included in our network analysis. Based on Cytoscape 3.7.2, we visualize complex networks and integrate different types of attribute data [20]. In the network, nodes represent the targets, edges represent the interactions between these nodes. According to the degree value, the top ranked compounds were selected as ligands for molecular docking. The higher the degree value, the more likely it is that the molecule will be as a key component of GYAC.

Enrichment analysis of GO function and KEGG pathway

The functional annotation of Gene Ontology (GO) was analyzed by DAVID database (https://david.ncifcrf.gov/summary.jsp) and the pathway

enrichment analysis database-Kyoto Encyclopedia of genes and genomes (KEGG) was used to predict the mechanism of target genes. EHBIO (https://www.ehbio.com) was used to visualize the enrichment results of GO functional annotation analysis, including biological process (BP), molecular function (MF) and cell composition (CC) and KEGG enrichment analysis.

Molecular docking

The core targets (IL-6 and IFNG) were obtained from PPI network as protein receptors, and theirs PDB files were download through RCSB PDB database (http://www.rcsb.org/). The SDF format files of 2D structure of core active compounds were obtained in PubChem database, and the 3D molecular structure of luteolin from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). PyMOL software was used to remove water and original ligands from the original PDB file. Autodock charging software was used to add polar hydrogen to target protein receptor molecules. Molecular docking was accomplished by Autodock Vina and Python scripts. The lower the binding energy, the better the affinity between receptor and ligand. In this study, the binding energy ≤ -5.0 kcal/mol was selected as the screening basis for effective binding of luteolin to target proteins.

Cell culture

Rat cardiomyocytes H9C2 was obtained from the iCell. The cells were cultured in DMEM medium mixed with 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were cultured in an incubator at 37 °C, 5% CO_2 .

Cell viability

Cell viability assay was performed by Cell Counting Kit-8 (MCE, HY-K0301). Firstly, H9C2 cells were inoculated into 96-well plates at 2×10^3 cells/well and treated with luteolin (Solarbio, SL8300) at a series of concentration (0 µm/mL, 1 µm/mL, 10 µm/mL, 50 µm/mL, 100 µm/mL, 150 µm/mL) for 6 h, 12 h and 24 h, respectively. At 450 nm wavelength, we detected the absorbance value of the above groups.

Cell apoptosis

Cell apoptosis and Cell cycle were performed by cell apoptosis kit (Muse, MCH100105) based on the flow cytometer instrument. After cells were collected, we added we added the staining agents-Annexin V-fluorescein isothiocyanate (FITC) and Propidium Iodide (PI) to the above groups. Besides, the cell apoptosis of each group was determined by calculating the sum of early and late apoptosis in each group.

Quantitative real-time polymerase chain reaction (RT-qPCR)

After cells were collected, total RNA was extracted with Trizol reagent according to the instructions. Nanodrop 2000 was used to detect RNA concentration and purity. RNA integrity was detected by 0.8% denaturing gel electrophoresis. Then, reverse transcribe 500 ng RNA into cDNA with the TAKARA reverse transcription kit. Finally, we completed the PCR procedure by the fluorescence quantitative instrument (ABI 7500).

Western blotting

Western blot experiment was done with sodium dodecyl sulfatepolyacrylamide gel (SDS-PAGE). Herein, polyvinylidene fluoride (PVDF) membranes were blocked with blocking solution containing 5% nonfat milk and then incubated with primary antibodies at 4 °C overnight. Clean the PVDF membrane with TBST and the membrane was incubated with a secondary antibody for 1 h at room temperature. Finally, the electrochemiluminescence (ECL) solutions were prepared in a dark chamber to visualize the target protein bands. The antibodies used in this study were as follows: BAX (proteintech, 50599-2-lg, 1:9000), BCL2 (proteintech, 26593-1-AP, 1:3000), IgG (ZEN BIO, 511103, 1:20000).

Statistical analysis

Data was expressed as mean \pm standard deviation (SD). SPSS software 20.0 and GraphPad Prism 8.3.1 software were used to complete data collation and analysis. One-way analysis of variance was used for multiple group comparisons. p < 0.05 indicates significant.



Fig. 1 Network pharmacology identified ingredients and targets of GYAC and hypoxia tolerance-related targets and Protein-protein interaction (PPI) network. A 40 overlapping targets were obtained. B–D The overlapping targets were imported into STRING database for PPI network analysis, and the result was visualized by Cytoscape 3.7.2. E We constructed GYAC ingredient-target-hypoxia tolerance related target network.

RESULTS

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Network pharmacology identified ingredients and targets of GYAC and hypoxia tolerance-related targets and Proteinprotein interaction (PPI) network

A total of 121 active compounds were retrieved in the TCMSP, HERB, ETCM, Pubchem and swisstargetprediction database, and then 707 corresponding target genes were acquired. We searched out 121 hypoxia-related targets in Genecards and OMIM database. Finally, 40 overlapping targets were obtained through the intersection of ingredient targets and diseaserelated targets (Fig. 1A) and the top 9 targets were used as core targets (Table 1). Subsequently, in order to further explore the mechanism of action of GYAC in hypoxia tolerance, 40 overlapping targets were imported into STRING database for PPI network analysis, and the result was visualized by Cytoscape 3.7.2, as shown in Fig. 1B-D. In PPI network diagram, there were 40 nodes, 605 edges. The top targets were CCL2, IFNG, IL1B, EGFR, CXCL8, IGF1, INS, IL6, HIF1A which indicated that these proteins have strong interactions with other proteins. The above showed that GYAC has a multi-components and multi-targets synergistic effect in improving hypoxia tolerance, especially in inflammatory response.

GO and KEGG enrichment analyses

In order to further understand the potential mechanism of 40 common targets of improving hypoxia tolerance, GO enrichment analysis was performed, as shown in Supplementary Fig. 2 and Table 2. The results showed that 40 common target genes were enriched in 536 GO terms (448 BP, 29 CC and 59 MF). The top 20 GO terms of BP mainly included positive regulation of gene expression, negative regulation of gene expression, etc. The top 20 GO terms of CC mainly included extracellular region, extracellular space, etc. The top 20 GO terms f MF mainly included identical protein binding, enzyme binding, etc. In addition, the KEGG pathway enrichment analysis showed that the common target genes were enriched in 202 pathways, as shown in Supplementary Fig. 1 and Table 3. It mainly involved HIF-1 signaling pathway, IL-17 signaling pathway, etc. Again, the above results showed that GYAC may improve hypoxic tolerance by inhibiting inflammation.

Construction of the GYAC ingredient-target-hypoxia tolerance-related target network

Based on the Cytoscape software 3.7.2, we constructed GYAC ingredient-target-hypoxia tolerance related target network, as shown in Fig. 1E. The results displayed that salidroside (HBIN042854), kaempferol (HBIN031741), luteolin (MOL000006) and ginsenoside Rb1 (C0810) were considered to be the most important effective components of GYAC against hypoxia tolerance. After reviewing the relevant studies on hypoxia tolerance and traditional Chinese medicine, we chose quercetin for further study.

Molecular docking

To verify the network pharmacology results, we performed molecular docking experiments between luteolin and common targets, as shown in Fig. 2A, B. We observed that luteolin was observed to have a strong interaction with the target gene-IL-6 and IFNG, the binding energy was -8.2 kcal/mol and -9.7 kcal/ mol. It is generally believed that the lower the binding energy between ligand and receptor, the more stable the binding conformation, In Fig. 2A, the results showed that luteolin formed three hydrogen bonds (ARG-756, ASN-543, GLU-646), one Pi-Sigma (ILE-735) and one Pi-Alkyl (PRO-750) with IL-6. Luteolin and IFNG were mainly bound by four hydrogen bonds (TYR-521, LYS-185, GTP-706 and VAL-117), two bump (LYS-116 and MG-704), one donor-donor (DZ-4701), one Pi-Sigma (ILE-735) and one Pi-Alkyl (MET-115), as shown in Fig. 2B.

Construction of hypoxic tolerance model of H9C2 cardiomyocytes

In vitro, we constructed a hypoxic tolerance model of H9C2 cardiomyocytes. The cell viability and apoptosis of the model were observed under 12 h, 24 h and 36 h hypoxia conditions. In Fig. 3A–C, we observed that hypoxia can inhibit cell viability and promote apoptosis of H9C2 cell. In Fig. 3D–F, we found that hypoxia can promote the mRNA of pro-apoptotic factor Bax and inhibit the expression of anti-apoptotic factor Bcl2. This result was confirmed by the expression of Bax and Bcl2 proteins, which again confirmed that hypoxia can promote the apoptosis of H9C2 cells. According to the above results, we chose hypoxia for 24 h to construct the model and carried out the following experiments. Subsequently, we found that hypoxia can promote the expression of LDH, indicating that hypoxia can cause mitochondrial damage in cardiomyocytes (Fig. 3G).

Luteolin can improve hypoxia tolerance by reducing the inflammatory response and lowering mitochondrial damage of H9C2 cardiomyocytes

Combining with the results of network pharmacology, we selected the hypoxic tolerance cell model treated by luteolin. First, we examined the cell viability of luteolin at different concentrations (0 μm/mL, 1 μm/mL, 10 μm/mL, 50 μm/mL, 100 μm/mL, 150 μm/ mL) under hypoxia conditions for 6 h, 24 h, and 36 h (Fig. 4A–C). Finally, we chose 6 h of hypoxia for the experiment, and the concentrations of luteolin treated models were 50 µm/mL, 100 µm/mL, 150 µm/mL for the following experiments. In Fig. 4D, we observed that luteolin can inhibit the decrease of cell viability in hypoxic cell model. Besides, luteolin can inhibit increase of cell apoptosis in hypoxic cell model (Fig. 4E–J). Besides, luteolin can inhibit LDH release and the decrease of mitochondrial membrane potential in hypoxic cell models (Fig. 4K–L). Moreover, luteolin can inhibit the expression of inflammatory related factors (IL-6, IL-10, IL-2, IFNG and VEGFA) in hypoxic cell models (Fig. 4M–Q). Taken together, we suggest that luteolin can reduce the inflammatory

Serial number	ClosenessCentrality	Degree	BetweennessCentrality	Abbreviation		
1	0.872	29	0.081	CCL2		
2	0.960	23	0.072	IFNG		
3	0.955	20	0.045	IL1B		
4	0.875	24	0.061	EGFR		
5	0.886	27	0.070	CXCL8		
6	0.920	21	0.179	IGF1		
7	1.000	18	0.234	INS		
8	0.950	18	0.079	IL6		
9	0.647	5	0.024	HIF1A		

Table 1. The core targets of Gaoyuan'an capsule and hypoxia tolerance

Table 2. Top 20 enriched GO pathways of target gene.

GO-BP For Solike regulation of gene expression 19 1.03F-18 18.0114 1.60F-15 1.36F-15 GO.2010629 negative regulation of gene expression 14 1.84F-14 21.98997 2.84F-11 1.24F-11 GO.2010629 positive regulation of gene expression 17 2.01F-13 8.241792 1.37E-10 6.75E-11 GO.2010628 positive regulation of transcription, DNA-template 17 2.01E-13 3.136E-06 9.31E-10 6.75E-11 GO.2004984 positive regulation of transcription from RNA 12 1.86E-12 2.320255 2.27E-09 6.36E-10 GO.2004984 positive regulation of postidy-iserine phosphorylation 9 1.10E-11 4.747989 1.58E-08 6.30E-10 GO.20049840 positive regulation of apoptotic process 13 2.34E-11 1.741101 4.40E-68 3.35E-09 GO.20049840 positive regulation of apoptotic process 13 2.34E-10 1.228748 3.35E-07 2.24E-68 GO.20049242 positive regulation of apoptotic process 13 2.34E-10 1.238751 6.62E-67 <th>Term_ID</th> <th>Term_description</th> <th>Count</th> <th>P value</th> <th>Fold enrichment</th> <th>Bonferroni</th> <th>FDR</th>	Term_ID	Term_description	Count	P value	Fold enrichment	Bonferroni	FDR
GO201022 positive regulation of gene copression 19 1.026-16 1.801104 1.060-15 1.326-11 GO2010022 positive regulation of gene copression 14 1.446-14 2.18997 2.846-11 1.265-13 GO2001062 positive regulation of transcription, DNA templated 17 2.016-13 3.16056 9.3126-10 6.762-11 GO2000106 response to to Npoxia 11 6.006-12 3.316056 9.3162-10 6.762-11 GO2000106 response to to Npoxia 12 1.862-12 2.3265 2.976-90 3.68E-10 GO2003138 positive regulation of transcription from RNA 19 3.746-12 7.546358 5.876-90 6.30E-10 GO20030456 positive regulation of appotityl-serine phospharylation 9 1.02E-11 4.741101 4.40F-68 3.35E-09 GO2003022 regulative regulation of appotityl process 12 2.86E-10 1.26274 1.74E-68 3.53E-09 GO2004026 positive regulation of appotityl process 7 3.88E-10 1.84E-04 1.446-47 3.27E-68 <t< td=""><td>GO-BP</td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	GO-BP						
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CO0031663 lipopolysaccharide-mediated signaling pathway 8 1.91E-12 92.44762 2.97E-09 3.68E-10 GO.0045944 positive regulation of transcription from RNA 19 3.74E-12 7.546358 5.80E-09 6.30E-10 GO.0035318 positive regulation of transcription from RNA 19 1.02E-11 47.47989 1.58E-08 1.52E-09 GO.0035318 positive regulation of apoptotic process 13 2.58E-11 1.741101 4.47E-08 3.33E-07 GO.00071456 cellular response to hypoxia 9 2.14E-10 32.59813 3.33E-07 2.22E-08 GO.0003264 positive regulation of apoptotic process 13 3.88E-10 1.66275 5.94E-07 3.27E-08 GO.0004324 positive regulation of diptocybity process 6 1.04E-09 121.3375 1.61E-66 8.21E-08 GO.0004529 positive regulation of smooth muscle cell proliferation 7 1.65E-09 58.57672 2.56E-06 1.17E-07 GO.0002576 extracellular region 19 1.83E-08 4.485531 2.89E-06 1.40E-05 <td>GO:0009410</td> <td>response to xenobiotic stimulus</td> <td>12</td> <td>1.80E-12</td> <td>23.02055</td> <td>2.79E-09</td> <td>3.68E-10</td>	GO:0009410	response to xenobiotic stimulus	12	1.80E-12	23.02055	2.79E-09	3.68E-10
COOd4944 positive regulation of transcription from RNA 19 3.74E-12 7.546338 \$.80E-00 6.30E-10 GOOd033138 positive regulation of peptidyl serine phosphorylation 9 1.02E-11 47.47989 1.58E-08 1.52E-09 GOOd03035 positive regulation of apoptiduc process 12 2.28E-11 14.74194 4.00E-08 3.48E-09 GOOd071455 cellular response to hypoxia 9 2.14E-10 3.258E-11 3.33E-07 2.22E-08 GOOd071456 cellular response to hypoxia 9 2.14E-10 3.258E-10 7.546338 3.33E-07 2.22E-08 GOOd071456 cellular response to hypoxia 13 3.38E-10 11.66275 5.94E-07 3.27E-08 GOOd04529 positive regulation of glycotytic process 6 1.04E-09 121.3375 1.61E-06 8.21E-08 GOOd04529 positive regulation of smooth muscle cell proliferation 7 1.65E-09 5.827672 2.52E-06 2.40E-07 GOOd04529 glucose homeostasis 8 3.56E-09 3.268E-06 1.17E-07 GOOd04591 <td>GO:0031663</td> <td>lipopolysaccharide-mediated signaling pathway</td> <td>8</td> <td>1.91E-12</td> <td>92.44762</td> <td>2.97E-09</td> <td>3.68E-10</td>	GO:0031663	lipopolysaccharide-mediated signaling pathway	8	1.91E-12	92.44762	2.97E-09	3.68E-10
CO003138 positive regulation of peptidyl-serine phosphorylation 9 1.02E-11 47.4799 1.58E-08 1.52E-08 GO000954 inflammatory response 13 2.58E-11 14.74194 4.00E-08 3.48E-09 GO0001365 positive regulation of apoptic process 12 2.88E-11 17.81101 4.47E-08 3.33E-07 GO001365 negative regulation of apottic process 13 3.83E-10 11.62E-07 3.22E-08 GO0001365 negative regulation of entric oxide biosynthetic process 7 3.88E-10 7.355761 6.02E-07 3.27E-08 GO0001325 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.17E-07 GO0001525 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.17E-07 GO0001525 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.17E-07 GO0001525 angiogenesis 10 1.52E-09 19.0333 2.36E-05 1.40E-05 GO0005561 extracellular space 11 2.01E-07 <t< td=""><td>GO:0045944</td><td>positive regulation of transcription from RNA polymerase II promoter</td><td>19</td><td>3.74E-12</td><td>7.546358</td><td>5.80E-09</td><td>6.30E-10</td></t<>	GO:0045944	positive regulation of transcription from RNA polymerase II promoter	19	3.74E-12	7.546358	5.80E-09	6.30E-10
CO0006954 inflamatory response 13 2.58E-11 14.74194 4.00E-08 3.48E-09 GO.00401202 cellular response to lipopolyaccharide 10 1.12E-10 2.554474 1.74E-07 2.22E-08 GO.00401202 cellular response to lipopolyaccharide 13 2.34E-10 12.08726 3.35E-07 2.24E-08 GO.00401306 negative regulation of apoptotic process 13 2.84E-10 12.08726 3.95E-07 2.44E-08 GO.0045429 positive regulation of nulric oxide biosynthetic process 7 3.88E-10 7.32E-08 6.02E-07 3.27E-08 GO.0045429 positive regulation of smooth muscle cell proliferation 7 1.05E-09 9.03333 2.36E-06 1.17E-07 GO.004553 anglogenesis 0 0.5ZE-09 9.03333 2.36E-06 1.47E-07 GO.0005576 extracellular region 19 1.88E-08 4.485531 2.49E-05 1.40E-05 GO.0005576 extracellular space 11 4.07E-07 8.27E-04 1.40E-05 1.40E-05 GO.0000564	GO:0033138	positive regulation of peptidyl-serine phosphorylation	9	1.02E-11	47.47989	1.58E-08	1.52E-09
CO0043065 positive regulation of apoptotic process 12 2.88E-11 17.1101 4.47E-08 3.33E-09 GO007122 cellular response to hypoxia 9 2.14E-10 3.259813 3.33E-07 2.22E-08 GO00071450 cellular response to hypoxia 13 2.54E-10 1.208726 3.93E-07 2.22E-08 GO0004521 positive regulation of apoptotic process 6 1.04E-09 121.3375 1.61E-06 8.21E-08 GO0004522 positive regulation of glycolytic process 6 1.04E-09 121.3375 1.61E-06 8.21E-08 GO0004523 angiogenesis 10 1.52E-09 120.333 2.36E-06 1.17E-07 GO0004561 extracellular region 7 1.65E-09 32.62857 5.52E-06 2.40E-07 GO000551 extracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO0005501 cavacellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO0005501 cavacellular space 1.42 2.75E-04<	GO:0006954	inflammatory response	13	2.58E-11	14.74194	4.00E-08	3.48E-09
COO071222 cellular response to lipopolysaccharide 10 1.12E-10 25.5474 1.74E-07 1.26E-08 GO.0014366 cellular response to hipopolis 9 2.14E-10 32.59813 3.33E-07 2.22E-08 GO.0003066 negative regulation of cell proliferation 13 3.38E-10 11.66275 5.94E-07 3.27E-08 GO.00045429 positive regulation of intric oxide biosynthetic process 6 1.04E-09 121.3375 1.01E-06 8.21E-08 GO.00045251 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.17E-07 GO.00045251 positive regulation of smooth muscle cell proliferation 7 1.58E-09 58.57672 2.56E-06 1.70E-07 GO.0005576 extracellular region 19 1.83E-08 4.485531 2.89E-06 2.40E-07 GO.0005676 extracellular space 17 2.01E-07 4.1248 4.59E-05 1.40E-05 GO.0005667 transcription factor complex 11 4.07E-07 8.278E-04 1.000738 0.001598 0.001598 0.001598	GO:0043065	positive regulation of apoptotic process	12	2.88E-11	17.81101	4.47E-08	3.53E-09
GO0011456 cellular response to hypoxia 9 2.14E-10 32.59813 3.33E-07 2.22E-08 GO0043066 negative regulation of apoptotic process 13 2.34E-10 11.05275 5.94E-07 3.32F-07 3.27E-08 GO0045429 positive regulation of ally colytic process 6 1.04E-09 121375 1.01E-06 8.21E-08 GO0045421 positive regulation of smooth muscle cell proliferation 7 1.65E-09 58.57672 2.56E-06 1.17E-07 GO.0005293 glucose homeostasis 8 3.56E-09 32.6857 5.22E-06 2.40E-07 GO.0005576 extracellular region 19 1.83E-08 4.485531 2.89E-06 2.65E-06 GO.0005576 extracellular space 17 2.01E-07 4.49434 3.18E-05 1.40E-05 GO.0005051 extracellular space 11 4.07E-07 8.279708 6.43E-05 1.40E-05 GO.0005054 nucleoplasm 18 5.23E-04 1.10E017 0.136339 0.015968 GO.0005634 nucleoplasm <	GO:0071222	cellular response to lipopolysaccharide	10	1.12E-10	25.54474	1.74E-07	1.26E-08
GO.0043066 negative regulation of apoptotic process 13 2.54E-10 12.08726 3.95E-07 2.45E-08 GO.008284 positive regulation of cell proliferation 13 3.88E-10 7.385761 6.02C-07 3.27E-08 GO.0045421 positive regulation of glycolytic process 6 1.04E-09 121.3375 1.61E-06 8.21E-08 GO.004523 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.14E-07 GO.004293 glucose homostasis 8 3.56E-09 32.02E57 5.2E-06 2.040E-07 GO.0005576 extracellular space 17 2.01E-07 4.4495511 2.89E-06 2.65E-06 GO.0005501 carcacellar space 17 2.01E-07 4.44951 3.18E-05 1.40E-05 GO.000501 caveola 6 2.90E-07 4.1248 4.59E-05 1.40E-05 GO.00050291 macromolecular complex 11 4.07E-07 3.272F-04 3.08E-05 1.40E-05 GO.0005054 nucleoplasm 18 5.23E-04 2.302357<	GO:0071456	cellular response to hypoxia	9	2.14E-10	32.59813	3.33E-07	2.22E-08
GO.0008284 positive regulation of cell proliferation 13 3.83E-10 71.66275 5.94E-07 3.27E-08 GO.0045429 positive regulation of nitric oxide biosynthetic process 6 1.04E-09 121.3375 1.61E-06 8.21E-08 GO.0001525 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.17E-07 GO.00048661 positive regulation of smooth muscle cell proliferation 7 1.65E-09 58.57672 2.56E-06 2.40E-07 GO.0005576 extracellular region 19 1.83E-08 4.485531 2.89E-06 2.65E-06 GO.0005051 extracellular space 17 2.01E-07 4.49934 3.18E-05 1.40E-05 GO.0005051 extracellular space 11 4.07E-07 8.279708 6.43E-05 1.40E-05 GO.0005654 nucleoplasm 18 5.23E-04 3.08209 0.042591 0.007988 GO.0005654 nucleoplasm 18 5.23E-04 3.08209 0.01239 0.015968 GO.0005654 nucleoplasm 5 9.27E-0	GO:0043066	negative regulation of apoptotic process	13	2.54E-10	12.08726	3.95E-07	2.45E-08
GO:0045429 positive regulation of nitric oxide biosynthetic process 7 3.88E-10 73.85761 6.02E-07 3.27E-08 GO:0045821 positive regulation of glycolytic process 6 1.04E-09 121.3375 1.61E-06 8.21E-08 GO:0045923 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.17E-07 GO:004593 glucose homeostasis 8 3.56E-09 32.62857 5.52E-06 2.40E-07 GO:0005576 extracellular region 19 1.83E-08 4.485531 2.89E-06 2.65E-06 GO:0005501 cxtracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO:0005051 cxtracellular space 11 4.07E-07 8.279708 6.43E-05 1.40E-05 GO:0005054 nucleuplasm 4 2.75E-04 30.78209 0.001788 GO:0005654 nucleuplasm 22 0.001168 1.880192 0.16688 0.015968 GO:000567 transcription factor complex 5 9.27E-04 11.6017 0.13	GO:0008284	positive regulation of cell proliferation	13	3.83E-10	11.66275	5.94E-07	3.27E-08
GO.0045821 positive regulation of glycolytic process 6 1.04E-09 121.3375 1.61E-06 8.21E-08 GO.001525 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.14E-07 GO.0042639 glucose homeostasis 8 3.56E-09 32.62857 5.52E-06 2.06E-07 GO.0005576 extracellular region 19 1.83E-08 4.485531 2.89E-05 1.40E-05 GO.0005576 extracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO.0005010 caveola 6 2.90E-07 4.1248 4.59E-05 1.40E-05 GO.0005011 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO.0005067 transcription factor complex 5 9.27E-04 1.10617 0.13639 0.012639 GO.0005634 nucleoplasm 2 0.001183 1.880192 0.166321 0.015968 GO.0005739 mitochondrion 10 0.001133 3.53651 0.137453 <t< td=""><td>GO:0045429</td><td>positive regulation of nitric oxide biosynthetic process</td><td>7</td><td>3.88E-10</td><td>73.85761</td><td>6.02E-07</td><td>3.27E-08</td></t<>	GO:0045429	positive regulation of nitric oxide biosynthetic process	7	3.88E-10	73.85761	6.02E-07	3.27E-08
GO.0001525 angiogenesis 10 1.52E-09 19.03333 2.36E-06 1.14E-07 GO.0048661 positive regulation of smooth muscle cell proliferation 7 1.65E-09 58.57672 2.56E-06 2.17E-07 GO.0005076 extracellular region 19 1.83E-08 4.485531 2.89E-06 2.65E-06 GO.0005515 extracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO.0005501 caveola 6 2.90E-07 4.1248 4.59E-05 1.40E-05 GO.000501 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO.0005647 nucleoplasm 18 5.23E-04 2.302357 0.079329 0.012639 GO.0005657 transcription factor complex 5 9.27E-04 1.16017 0.13339 0.015968 GO.0005729 cytosol cytosol 21 0.001143 1.93592 0.166387 0.015968 GO.0005739 mitochondrino 10 0.00133 3.53631 <	GO:0045821	positive regulation of glycolytic process	6	1.04E-09	121.3375	1.61E-06	8.21E-08
GO.0048661 positive regulation of smooth muscle cell proliferation 7 1.65E-09 58.57672 2.56E-06 1.17E-07 GO.0042593 glucose homeostasis 8 3.56E-09 32.62857 5.52E-06 2.40E-07 GO.005756 extracellular region 19 1.83E-08 4.485531 2.89E-05 1.40E-05 GO.0005501 extracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO.0005501 caveola 6 2.90E-07 4.248 4.59E-05 1.40E-05 GO.0005631 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO.0005654 nucleoplasm 18 5.23E-04 2.302357 0.079329 0.012639 GO.0005667 transcription factor complex 5 9.27E-04 11.16017 0.136339 0.015968 GO.0005739 tratech and anter 5 0.001133 1.52245 0.166637 0.015968 GO.0005739 mitochondrion 10 0.001131 3.536351 0.18	GO:0001525	angiogenesis	10	1.52E-09	19.03333	2.36E-06	1.14E-07
GO.0042593 glucose homeostasis 8 3.56E-09 32.62857 5.52E-06 2.40E-07 GO.005576 extracellular region 19 1.83E-08 4.485531 2.89E-06 2.65E-06 GO.0005576 extracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO.0032991 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO.0005654 nucleoplasm 4 2.75E-04 30.78209 0.042591 0.007988 GO.0005654 nucleoplasm 18 5.22E-04 1.16017 0.136339 0.015968 GO.0005654 nucleus 22 0.001108 1.880192 0.16688 0.015968 GO.0005674 mambrane raft 5 0.001133 1.052245 0.166837 0.001596 GO.0005739 mitochondrion 10 0.001313 3.536351 0.18743 0.015968 GO.0005737 cytoplasm 21 0.00147 16.76748 0.225668 0.015752 <t< td=""><td>GO:0048661</td><td>positive regulation of smooth muscle cell proliferation</td><td>7</td><td>1.65E-09</td><td>58.57672</td><td>2.56E-06</td><td>1.17E-07</td></t<>	GO:0048661	positive regulation of smooth muscle cell proliferation	7	1.65E-09	58.57672	2.56E-06	1.17E-07
GO-CC Sector Sector </td <td>GO:0042593</td> <td>glucose homeostasis</td> <td>8</td> <td>3.56E-09</td> <td>32.62857</td> <td>5.52E-06</td> <td>2.40E-07</td>	GO:0042593	glucose homeostasis	8	3.56E-09	32.62857	5.52E-06	2.40E-07
GO.0005576 extracellular region 19 1.83E-08 4.485531 2.89E-06 2.65E-06 GO.0005615 extracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO.0005901 caveola 6 2.90E-07 41.248 4.59E-05 1.40E-05 GO.00032991 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO.000564 nucleoplasm 18 5.23E-04 2.302357 0.079329 0.012639 GO.0005647 transcription factor complex 5 9.27E-04 11.16017 0.136339 0.015968 GO.0005643 nucleus 22 0.001183 1.88192 0.1666321 0.015968 GO.0005737 mitochondrion 10 0.001131 3.536351 0.187453 0.015968 GO.0005737 ctyplasm 21 0.001422 1.903921 0.02572 0.015968 GO.0005737 ctyplasm 21 0.001422 1.903921 0.02572 0.015968 <	GO-CC						
GO.0005615 extracellular space 17 2.01E-07 4.44934 3.18E-05 1.40E-05 GO.0005901 caveola 6 2.90E-07 41.248 4.59E-05 1.40E-05 GO.0032991 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO.003564 nucleoplasm 4 2.75E-04 30.78209 0.042591 0.007988 GO.0005654 nucleoplasm 18 5.23E-04 30.78209 0.012639 GO.0005667 transcription factor complex 5 9.27E-04 11.16017 0.136339 0.015968 GO.0005634 nucleus 22 0.001183 1.880192 0.166637 0.015968 GO.0005739 mitochondrion 10 0.00131 3.536351 0.015968 GO.0005737 cytosl statistription factor complex 4 0.001617 16.7648 0.225668 0.015968 GO.0005737 cytoplasm 21 0.01432 1.903921 0.22572 0.015968 GO.000791	GO:0005576	extracellular region	19	1.83E-08	4.485531	2.89E-06	2.65E-06
GO:0005901 caveola 6 2.90E-07 41.248 4.59E-05 1.40E-05 GO:0032991 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO:0031093 platelet alpha granule lumen 4 2.75E-04 30.78209 0.042591 0.007988 GO:0005654 nucleoplasm 18 5.23E-04 2.302357 0.073039 0.015968 GO:0005634 nucleoplasm 22 0.001108 1.880192 0.160688 0.015968 GO:0005634 nucleus 21 0.001133 1.52245 0.166637 0.015968 GO:0005739 mitochondrion 10 0.00131 3.536351 0.187453 0.015968 GO:0005737 cytoslasm 21 0.001312 1.933921 0.022572 0.015968 GO:0005737 cytoplasm 21 0.00137 53.3793 0.194721 0.015968 GO:000791 euchromatin 3 0.005943 2.535738 0.610076 0.057449 GO:0000705	GO:0005615	extracellular space	17	2.01E-07	4.44934	3.18E-05	1.40E-05
GO:0032991 macromolecular complex 11 4.07E-07 8.279708 6.43E-05 1.47E-05 GO:0031093 platelet alpha granule lumen 4 2.75E-04 30.78209 0.042591 0.007988 GO:0005664 nucleoplasm 18 5.23E-04 2.302357 0.079329 0.012639 GO:0005667 transcription factor complex 5 9.27E-04 11.16017 0.136339 0.015968 GO:0005632 nucleus 22 0.001108 1.880192 0.16682 0.015968 GO:0005121 membrane raft 5 0.001133 1.052245 0.166637 0.015968 GO:0005739 mitochondrion 10 0.001313 3.536351 0.187453 0.015968 GO:0005737 cytoplasm 21 0.001432 1.903921 0.202572 0.015968 GO:0005737 cytoplasm 3 0.00137 53.33793 0.194721 0.015968 GO:000791 euchromatin 3 0.001432 1.903921 0.202572 0.015968	GO:0005901	caveola	6	2.90E-07	41.248	4.59E-05	1.40E-05
GO:0031093 platelet alpha granule lumen 4 2.75E-04 30.78209 0.042591 0.007988 GO:0005654 nucleoplasm 18 5.23E-04 2.302357 0.079329 0.012639 GO:0005667 transcription factor complex 5 9.27E-04 11.16017 0.136339 0.015968 GO:0005629 cytosol 22 0.001108 1.880192 0.166387 0.015968 GO:0005739 mitochondrion 5 0.001133 1.93592 0.165321 0.015968 GO:0005739 mitochondrion 10 0.001133 3.536351 0.187453 0.015968 GO:0005737 cytoplasm 21 0.001432 1.903921 0.202572 0.015968 GO:000737 cytoplasm 21 0.01432 1.903921 0.202572 0.015968 GO:000791 euchromatin 3 0.00543 25.35738 0.610076 0.057449 GO:000785 chromatin 7 0.01346 3.42754 0.88222 0.114689 GO:000785	GO:0032991	macromolecular complex	11	4.07E-07	8.279708	6.43E-05	1.47E-05
GO:0005654 nucleoplasm 18 5.23E-04 2.302357 0.079329 0.012639 GO:0005667 transcription factor complex 5 9.27E-04 11.16017 0.136339 0.015968 GO:0005634 nucleus 22 0.001108 1.880192 0.16688 0.015968 GO:0005829 cytosol 21 0.001143 1.93592 0.165321 0.015968 GO:0005739 membrane raft 5 0.001133 10.52245 0.166637 0.015968 GO:0005737 membrane raft 5 0.001313 3.53651 0.187453 0.015968 GO:0005737 cytoplasm 21 0.001432 1.903921 0.020572 0.015968 GO:000575 RNA polymerase II transcription factor complex 4 0.001617 16.76748 0.22568 0.016752 GO:000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 GO:0003031 caspase complex 2 0.013144 147.3143 0.876774 0.114689 <t< td=""><td>GO:0031093</td><td>platelet alpha granule lumen</td><td>4</td><td>2.75E-04</td><td>30.78209</td><td>0.042591</td><td>0.007988</td></t<>	GO:0031093	platelet alpha granule lumen	4	2.75E-04	30.78209	0.042591	0.007988
GO:0005667 transcription factor complex 5 9.27E-04 11.16017 0.136339 0.015968 GO:0005634 nucleus 22 0.001108 1.880192 0.166688 0.015968 GO:0005829 cytosol 21 0.001143 1.93592 0.165321 0.015968 GO:0005739 mitochondrion 10 0.001313 3.536351 0.187453 0.015968 GO:0005737 cytoplasm 21 0.001432 1.903921 0.202572 0.015968 GO:0005757 RNA polymerase II transcription factor complex 4 0.001617 16.76748 0.225668 0.01672 GO:000791 euchromatin 3 0.00543 25.35738 0.610076 0.057449 GO:000785 chromatin 7 0.013464 147.3143 0.876774 0.114689 GO:000785 chromatin 7 0.013468 15.94639 0.900334 0.116713 GO:000785 chromatin 7 0.013468 1.42544 0.88222 0.114689	GO:0005654	nucleoplasm	18	5.23E-04	2.302357	0.079329	0.012639
GO:0005634 nucleus 22 0.001108 1.880192 0.160688 0.015968 GO:0005829 cytosol 21 0.001143 1.93592 0.165321 0.015968 GO:0005739 mitochondrion 10 0.001313 3.536351 0.187453 0.015968 GO:0005739 mitochondrion 10 0.001313 3.536351 0.187453 0.015968 GO:0005737 cytoplasm 21 0.001422 1.903921 0.202572 0.015968 GO:0000775 RNA polymerase II transcription factor complex 4 0.001617 16.76748 0.225668 0.016722 GO:0000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 GO:0000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:000785 chromatin 7 0.013446 3.42754 0.88222 0.11489 GO:000785 chromatin 3 0.019968 13.45043 0.958701 0.144834 GO:000785 <td>GO:0005667</td> <td>transcription factor complex</td> <td>5</td> <td>9.27E-04</td> <td>11.16017</td> <td>0.136339</td> <td>0.015968</td>	GO:0005667	transcription factor complex	5	9.27E-04	11.16017	0.136339	0.015968
GO:0005829 cytosol 21 0.001143 1.93592 0.165321 0.015968 GO:0045121 membrane raft 5 0.001153 10.52245 0.166637 0.015968 GO:0005739 mitochondrion 10 0.001313 3.536351 0.187453 0.015968 GO:0012506 vesicle membrane 3 0.00137 53.33793 0.194721 0.015968 GO:0005737 cytoplasm 21 0.001432 1.903921 0.202572 0.015968 GO:000575 RNA polymerase II transcription factor complex 4 0.001617 16.76748 0.225668 0.01672 GO:0000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 GO:0000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:0003141 secretory granule 3 0.01489 15.94639 0.900334 0.116713 GO:0005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834	GO:0005634	nucleus	22	0.001108	1.880192	0.160688	0.015968
GO:0045121membrane raft50.00115310.522450.1666370.015968GO:0005739mitochondrion100.0013133.5363510.1874530.015968GO:0012506vesicle membrane30.0013753.337930.1947210.015968GO:0005737cytoplasm210.0014321.9039210.2025720.015968GO:00090575RNA polymerase II transcription factor complex40.00161716.767480.2256680.016752GO:0000791euchromatin30.00594325.357380.6100760.057449GO:000303caspase complex20.013164147.31430.8767740.114689GO:000785chromatin70.0134463.427540.882220.114689GO:00030141secretory granule30.01996813.450430.9587010.144834GO:0034774secretory granule lumen40.020116.7179150.9596310.144834GO:0042802identical protein binding221.22E-126.1148772.88E-102.55E-10GO:002020protease binding79.84E-082.9868242.32E-055.88E-06GO:002020protease binding79.84E-082.9868242.32E-055.88E-06GO:005515protein binding401.41E-071.4978653.32E-055.88E-06	GO:0005829	cytosol	21	0.001143	1.93592	0.165321	0.015968
GO:0005739 mitochondrion 10 0.001313 3.536351 0.187453 0.015968 GO:0012506 vesicle membrane 3 0.00137 53.33793 0.194721 0.015968 GO:0005737 cytoplasm 21 0.001432 1.903921 0.202572 0.015968 GO:000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 GO:000785 chromatin 7 0.01346 3.42754 0.88222 0.114689 GO:000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:000785 chromatin 3 0.01489 15.94639 0.900334 0.116713 GO:000786 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0005788 endoplasmic reticulum lumen 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:0005789 enzyme binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 GO:00198	GO:0045121	membrane raft	5	0.001153	10.52245	0.166637	0.015968
GO:0012506 vesicle membrane 3 0.00137 53.33793 0.194721 0.01598 GO:0005737 cytoplasm 21 0.001432 1.903921 0.202572 0.015968 GO:000575 RNA polymerase II transcription factor complex 4 0.001617 16.76748 0.225668 0.015752 GO:000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 GO:000785 chromatin 7 0.013164 147.3143 0.876774 0.114689 GO:000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:000785 chromatin 3 0.01489 15.94639 0.900334 0.11671 GO:0030141 secretory granule lumen 3 0.019968 13.45043 0.958701 0.144834 GO:0005786 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0005786 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10	GO:0005739	mitochondrion	10	0.001313	3.536351	0.187453	0.015968
G0:0005737 cytoplasm 21 0.001432 1.903921 0.202572 0.015968 G0:0090575 RNA polymerase II transcription factor complex 4 0.001617 16.76748 0.225668 0.016752 G0:000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 G0:0008303 caspase complex 2 0.013164 147.3143 0.876774 0.114689 G0:000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 G0:000785 chromatin 3 0.01489 15.94639 0.900334 0.116713 G0:0030141 secretory granule lumen 3 0.01968 13.45043 0.958701 0.144834 G0:0005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 G0:0019899 enzyme binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 G0:0002020 protease binding 7 9.84E-08	GO:0012506	vesicle membrane	3	0.00137	53.33793	0.194721	0.015968
GO:0090575 RNA polymerase II transcription factor complex 4 0.001617 16.76748 0.225668 0.01672 GO:0000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 GO:0008303 caspase complex 2 0.013164 147.3143 0.876774 0.114689 GO:000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:0030141 secretory granule 3 0.014489 15.94639 0.900334 0.116713 GO:0034774 secretory granule lumen 3 0.019968 13.45043 0.958701 0.144834 GO:0005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:0002020 protease binding 7 9.84E-08 29.86824 2.32E-05 5.88E-06 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 <td>GO:0005737</td> <td>cytoplasm</td> <td>21</td> <td>0.001432</td> <td>1.903921</td> <td>0.202572</td> <td>0.015968</td>	GO:0005737	cytoplasm	21	0.001432	1.903921	0.202572	0.015968
GO:0000791 euchromatin 3 0.005943 25.35738 0.610076 0.057449 GO:0008303 caspase complex 2 0.013164 147.3143 0.876774 0.114689 GO:000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:0030141 secretory granule 3 0.014489 15.94639 0.900334 0.116713 GO:003785 endoplasmic reticulum lumen 3 0.019968 13.45043 0.958701 0.144834 GO:0005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:000200 protease binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497655 5.88E-06 5.88E-06	GO:0090575	RNA polymerase II transcription factor complex	4	0.001617	16.76748	0.225668	0.016752
GO:0008303 caspase complex 2 0.013164 147.3143 0.876774 0.114689 GO:0000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:0030141 secretory granule 3 0.014489 15.94639 0.900334 0.116713 GO:0034774 secretory granule lumen 3 0.019968 13.45043 0.958701 0.144834 GO:0005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:0002020 protease binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO:0000791	euchromatin	3	0.005943	25.35738	0.610076	0.057449
GO:0000785 chromatin 7 0.013446 3.42754 0.88222 0.114689 GO:0030141 secretory granule 3 0.014489 15.94639 0.900334 0.116713 GO:0034774 secretory granule lumen 3 0.019968 13.45043 0.958701 0.144834 GO:0057788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:0019899 enzyme binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 GO:0002020 protease binding 7 9.84E-08 2.986824 2.32E-05 5.88E-06 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO:0008303	caspase complex	2	0.013164	147.3143	0.876774	0.114689
GO:0030141 secretory granule 3 0.014489 15.94639 0.900334 0.116713 GO:0034774 secretory granule lumen 3 0.01968 13.45043 0.958701 0.144834 GO:005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:0019899 enzyme binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 GO:002020 protease binding 7 9.84E-08 2.92.66824 2.32E-05 5.88E-06 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO:0000785	chromatin	7	0.013446	3.42754	0.88222	0.114689
GO:0034774 secretory granule lumen 3 0.019968 13.45043 0.958701 0.144834 GO:0005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO-MF	GO:0030141	secretory granule	3	0.014489	15.94639	0.900334	0.116713
GO:0005788 endoplasmic reticulum lumen 4 0.02011 6.717915 0.959631 0.144834 GO-MF	GO:0034774	secretory granule lumen	3	0.019968	13.45043	0.958701	0.144834
GO-MF GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:0019899 enzyme binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 GO:0002020 protease binding 7 9.84E-08 2.986824 2.32E-05 5.88E-06 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO:0005788	endoplasmic reticulum lumen	4	0.02011	6.717915	0.959631	0.144834
GO:0042802 identical protein binding 22 1.22E-12 6.114877 2.88E-10 2.55E-10 GO:0019899 enzyme binding 12 1.85E-10 14.95658 4.38E-08 1.94E-08 GO:0002020 protease binding 7 9.84E-08 29.86824 2.32E-05 5.88E-06 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO-MF						
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GO:0002020 protease binding 7 9.84E-08 29.86824 2.32E-05 5.88E-06 GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO:0019899	enzyme binding	12	1.85E-10	14.95658	4.38E-08	1.94E-08
GO:0005125 cytokine activity 8 1.15E-07 19.73438 2.72E-05 5.88E-06 GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO:0002020	protease binding	7	9.84E-08	29.86824	2.32E-05	5.88E-06
GO:0005515 protein binding 40 1.41E-07 1.497865 3.32E-05 5.88E-06	GO:0005125	cytokine activity	8	1.15E-07	19.73438	2.72E-05	5.88E-06
	GO:0005515	protein binding	40	1.41E-07	1.497865	3.32E-05	5.88E-06
GO:0004517 nitric-oxide synthase activity 3 1.24E-05 473.625 0.002916 4.31E-04	GO:0004517	nitric-oxide synthase activity	3	1.24E-05	473.625	0.002916	4.31E-04

Table 🛛	2. co	ontinue	ed
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Term_ID	Term_description	Count	P value	Fold enrichment	Bonferroni	FDR
GO:0008083	growth factor activity	6	2.26E-05	17.01647	0.00533	6.46E-04
GO:0034617	tetrahydrobiopterin binding	3	2.47E-05	355.2188	0.005815	6.46E-04
GO:0019903	protein phosphatase binding	5	3.45E-05	26.3125	0.008107	8.01E-04
GO:0000976	transcription regulatory region sequence-specific DNA binding	6	1.13E-04	12.14423	0.026222	0.00218
GO:0030235	nitric-oxide synthase regulator activity	3	1.15E-04	177.6094	0.026713	0.00218
GO:0034618	arginine binding	3	2.24E-04	129.1705	0.051601	0.003909
GO:0020037	heme binding	5	2.70E-04	15.47794	0.061733	0.00434
GO:0010181	FMN binding	3	4.87E-04	88.80469	0.108508	0.00678
GO:0005159	insulin-like growth factor receptor binding	3	4.87E-04	88.80469	0.108508	0.00678
GO:0042803	protein homodimerization activity	8	6.74E-04	5.148098	0.147094	0.008803
GO:0005516	calmodulin binding	5	8.44E-04	11.44022	0.180676	0.010377
GO:0003700	transcription factor activity, sequence-specific DNA binding	7	9.50E-04	5.888766	0.200863	0.011026
GO:0004712	protein serine/threonine/tyrosine kinase activity	6	0.001984	6.458523	0.374189	0.021825
GO:0071889	14-3-3 protein binding	3	0.002354	40.59643	0.426583	0.024597

GO gene ontology, BP biological process, MF molecular function, CC cell composition.

Table 3. Top 20 enriched KEGG pathways of target genes.

ID	Description	p value	p.adjust	Count
hsa04066	HIF-1 signaling pathway	4.69E-26	9.47E-24	19
hsa04933	AGE-RAGE signaling pathway in diabetic complications	5.26E-23	5.31E-21	17
hsa05142	Chagas disease (American trypanosomiasis)	5.06E-21	3.41E–19	16
hsa05200	Pathways in cancer	1.61E-20	8.11E-19	25
hsa05163	Human cytomegalovirus infection	8.31E-17	3.36E-15	17
hsa05145	Toxoplasmosis	2.68E-15	9.02E-14	13
hsa05167	Kaposi sarcoma-associated herpesvirus infection	3.22E-15	9.28E-14	15
hsa04657	IL-17 signaling pathway	1.01E-14	2.55E-13	12
hsa05161	Hepatitis B	1.25E-14	2.80E-13	14
hsa05133	Pertussis	4.09E-14	7.51E–13	11
hsa05140	Leishmaniasis	4.09E-14	7.51E–13	11
hsa05418	Fluid shear stress and atherosclerosis	4.72E-14	7.89E–13	13
hsa05152	Tuberculosis	5.08E-14	7.89E–13	14
hsa04659	Th17 cell differentiation	5.74E-14	8.28E-13	12
hsa05135	Yersinia infection	2.34E-13	3.15E-12	12
hsa05205	Proteoglycans in cancer	3.13E-13	3.96E-12	14
hsa05321	Inflammatory bowel disease (IBD)	3.58E-13	4.26E-12	10
hsa04625	C-type lectin receptor signaling pathway	1.45E-12	1.63E-11	11
hsa05212	Pancreatic cancer	1.61E-12	1.71E-11	10
hsa04010	MAPK signaling pathway	2.94E-12	2.97E-11	15

KEGG Kyoto Encyclopedia of genes and genomes.

response of cardiomyocytes, thereby reducing mitochondrial damage, and thus increase the hypoxia tolerance of H9C2 cardiomyocytes.

DISCUSSION

Hypoxia is a fundamental consequence of high-altitude exposure and severe illness. In high altitude areas, the partial pressure of oxygen decreases, leading to reduced oxygen utilization in the body, which may cause various pathophysiological symptoms, such as ischemia, high altitude reaction, high altitude pulmonary edema and high altitude brain edema [21], mental disorders and memory loss, insomnia, dizziness, nausea, irritation, dyskinesia [22], as well as biochemical changes of blood-brain barrier and left ventricular dysfunction [23]. In the results of network pharmacology, we found that luteolin has potential capabilities. And we observed that luteolin was observed to have a strong interaction with the target gene-IL-6 and IFNG. The results indicated that Luteolin had an effect on the inflammatory response induced by hypoxia.

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Fig. 2 Molecular docking experiments between luteolin and common targets. A, B We observed that luteolin was observed to have a strong interaction with the target gene-IL-6 and IFNG, the binding energy was -8.2 kcal/mol and -9.7 kcal/mol, respectively.



Fig. 3 Construction of hypoxic tolerance model of H9C2 cardiomyocytes. A–C Hypoxia can inhibit cell viability and promote apoptosis of H9C2 cells. D–F Hypoxia can promote the mRNA of pro-apoptotic factor Bax and inhibit the expression of anti-apoptotic factor Bcl2 of H9C2 cells. G Hypoxia can promote the expression of LDH of H9C2 cells.

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Fig. 4 Luteolin can improve hypoxia tolerance by reducing the inflammatory response and lowering mitochondrial damage of H9C2 cardiomyocytes. A–C We chose 6 h of hypoxia for the experiment, and the concentrations of luteolin treated models were 50 μm/mL, 100 μm/ mL, 150 μm/mL for the following experiments. D Luteolin can inhibit the decrease of cell viability in hypoxic cell model. E–J Luteolin can inhibit increase of cell apoptosis in hypoxic cell model. K Luteolin can inhibit LDH release in hypoxic cell models. L Luteolin can inhibit the decrease of mitochondrial membrane potential in hypoxic cell models. M–Q Luteolin can inhibit the expression of inflammatory related factors (IL-6, IL-10, IL-2, IFNG and VEGFA) in hypoxic cell models.

Luteolin is a natural compound that has significant antiinflammatory and antioxidant properties [24]. Under hypoxia conditions, inflammation is usually activated in response to the damage caused by hypoxia [25]. However, an excessive inflammatory response can lead to tissue damage and organ dysfunction [26]. Therefore, controlling the inflammatory response is essential to improve the body's tolerance to hypoxia. In the study, we found that hypoxia can promote the expression of LDH, indicating that hypoxia can cause mitochondrial damage in cardiomyocytes. After the addition of luteolin, the decrease of cell viability in hypoxic cell model and the increase of cell apoptosis in hypoxic cell model were inhibited. Besides, luteolin can inhibit LDH release and the decrease of mitochondrial membrane potential in hypoxic cell models. Moreover, luteolin can inhibit the expression of inflammatory related factors in hypoxic cell models. Thus, we suggest that luteolin can reduce the inflammatory response of cardiomyocytes, thereby reducing mitochondrial damage, and thus increase the hypoxia tolerance of H9C2 cardiomyocytes.

The results of KEGG signaling pathway indicate that HIF-1 signaling pathway is involved in the hypoxia tolerance of luteolin. HIF-1 α is a transcription complex that, by activating the transcription of multiple genes, has become a key regulator of hypoxia responses and a major regulator of homeostasis in hypoxic cells and systems. The role of HIF-1 pathway in hypoxia tolerance involves several aspects. First, HIF-1 can induce cellular adaptation to hypoxic environments [27]. Under hypoxia conditions, HIF-1 can up-regulate the expression of a variety of enzymes that are involved in the process of glycolysis and help cells gain energy in the absence of oxygen. In addition, HIF-1 can also promote angiogenesis, providing more oxygen and nutrients to hypoxic tissues [28]. However, it is regrettable that this study was unable to complete the research on the HIF-1 pathway in the enhancement of hypoxia tolerance by luteolin. We will continue to explore it in the future, providing a theoretical basis for the mechanism of luteolin in improving hypoxia tolerance.

CONCLUSION

In conclusion, our study revealed that luteolin, a monomeric component of GYAC, played a role in hypoxia tolerance through a variety of target genes, such as IL6, IFNG. What's more, we have discovered that luteolin can reduce the inflammatory response in cardiac myocytes, thereby alleviating mitochondrial damage, and ultimately enhancing the hypoxia tolerance of H9C2 cardiomyocytes.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author.

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ACKNOWLEDGEMENTS

We thank all authors for their contributions and supports.

AUTHOR CONTRIBUTIONS

Tianbo Jin wrote the manuscript; Xiaoli Liu and Yuhe Wang analyzed the data; Yijin Qi and Xuemei Li made tables and figures; Li Wang and Xue He provided research ideas.

FUNDING

Natural Science Foundation of Tibet Autonomous Region (XZ202201ZR0048G), Science and Technology Major Project of Tibetan Autonomous Region of China (XZ202201ZD0001G) and Key R&D Program of Xizang (Tibet) Autonomous Region (XZ202101ZY0018G).

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by Research Ethics Committee of Xizang Minzu University. Written informed consent was obtained from individual or guardian participants.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41397-024-00327-0.

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