Microarray Analysis Reveals the Potential Molecular Mechanism of Lp299v in Stable Coronary Atherosclerotic Disease

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Original article

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Abstract

A growing body of evidence has confirmed that inflammatory mechanisms are involved in the formation and treatment of coronary atherosclerotic disease (CAD). An increase in circulatory levels of inflammatory cytokines has been found in patients with CAD, while the molecular mechanisms of inflammation still remain elusive. This study was designed to identify differentially expressed genes (DEGs), and to explore the molecular mechanism and core genes that are involved in the effects of *Lactobacillus plantarum* 299v (Lp299v) supplementation. Microarray dataset (GSE156357) was downloaded from the Gene Expression Omnibus (GEO) database. The DEGs were identified by the R software. Then, the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses and construction of protein-protein interaction (PPI) network were performed by DAVID, STRING, and Cytoscape. In daily alcohol user (DAU) group, 7,541 DEGs were identified, including 206 up-regulated and 7,335 down-regulated DEGs. In non-daily alcohol user (non-DAU) group, 2,799 DEGs were identified (2,491 up-regulated and 308 down-regulated DEGs). The GO enrichment analysis revealed that DEGs were mainly enriched in cell division, mitosis, and chemotactic cytokines. The KEGG enrichment analysis showed that Lp299v supplementation reduced the levels of chemotactic cytokines, and weakened immune response. Proteins of G protein-coupled receptor, inflammatory response, regulation of cell proliferation and apoptosis-related proteins were found in the PPI network. The core genes were associated with G protein-coupled receptor, inflammatory response, and cell proliferation and apoptosis. The weighted gene co-expression network analysis (WGCNA) enriched the DEGs in 4 modules. This study indicated the expressions of chemokine receptors and regulation of immune response in the Lp299v supplementation. Meanwhile, it was supposed that chemokine receptors may have a cellular effect.

Introduction

Traditionally, the pathophysiology of coronary atherosclerotic disease (CAD) has been identified in the formation of coronary atherosclerotic plaques, which can cause chronic narrowing of coronary lumen, and may even cause rupture of coronary atherosclerotic plaques (CAPs) and thrombosis, eventually leading to acute myocardial infarction. An increasing number of studies have confirmed that inflammatory mechanisms are not only involved in the formation of CAD, but also affect the treatment and prognosis of patients with CAD (Garofallo et al. 2019; Libby et al. 2005; Pugliese et al. 2020). The levels of C-reactive protein (CRP) and interleukin-6 (IL-6) were reduced in patients with stable CAD who took statins compared with those who underwent elective coronary stent implantation and did not take statins, suggesting an association between CAP and inflammatory response (Moratalla et al. 2016) .The increase in the concentrations of fibrinogen and CRP causes an elevation in acute phase proteins and cytokines, such as tumor necrosis factor (TNF) and IL-6, which further activates monocytes/macrophages and T cells, and leads to atherosclerosis(Schaberg et al. 1992; Tappia et al.1995). Macrophages and T lymphocytes are thought to play an important role in the formation of CAPs (Hedrick 2015; Moore et al. 2018).

Several studies have shown that gut microbiota play a significant role in CAD, heart failure, and metabolic disorders (Brial et al.2018; Kitai et al.2018; Tang et al. 2017). The influences of gut microbiota on these diseases may be associated with regulation of intestinal or systemic inflammation. *Lactobacillus plantarum* 299v (Lp299v), a member of the Lactobacillus family, is widely found in dairy products, meat, and fermented vegetables. Lp299v has a significant antioxidant activity, and it can inhibit intestinal bacteria and intestinal inflammations (Bested et al. 2013; Bixquert Jimenez 2009). A prospective study demonstrated that Lp299v improved vascular endothelial function and reduced systemic inflammation in patients with stable CAD (Malik et al. 2018). In a controlled, randomized, double-blinded study, the experimental group who drank beverages containing Lp299v had significantly greater systolic blood pressure, leptin, and fibrinogen than those in the control group, in which a 37% reduction in F2-isoprostaglandin levels and a 42% reduction in IL-6 levels were observed as well (Naruszewicz et al. 2002). Subsequent studies confirmed that Lp299v possessed a systemic anti-inflammatory effect and reduced the activity of circulatory inflammatory markers, and it was found that the levels of Janus kinase 2 (JAK2), Guanylate-binding protein 1 (GBP1), and TNF superfamily member 10 (TNFSF10) were reduced in blood after oral Lp299v supplementation.(Hofeld et al. 2021)

Hence, the present study aimed to identify differentially expressed genes (DEGs) and the core genes, so as to explore the molecular mechanism. In this study, microarray datasets from the Gene Expression Omnibus (GEO) database were downloaded and analyzed to obtain DEGs, and serum levels were measured before and after oral Lp299v in daily alcohol user (DAU) and non-DAU patients with stable CAD (Hofeld et al. 2021). Subsequently, the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses and construction of a protein-protein interaction (PPI) network were performed to investigate the molecular mechanisms of Lp299v supplementation in patients with stable CAD.

Methods

Microarray datasets

The microarray dataset (GSE156357) was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/gds). The probes were converted into the corresponding gene symbols according to the annotation information in the platform. The GSE156357 dataset included 19 pre-supply samples and 19 post-supply samples. There were 4 and 15 patients in the DAU and non-DAU groups, respectively. A total of 38 samples were obtained from 19 patients before and after oral Lp299v supplementation (Hofeld et al. 2021).

Identification of DEGs

The DEGs of pre-supply and post-supply samples were screened using R software in the two groups. Benjamini-Hochberg adjusted P was used to control false discovery rate (FDR). Probe sets without corresponding gene symbols or genes with more than one probe set were removed or averaged. Gene expression levels of |logFC| >0.3 and $P < 0.05$ were chosen as thresholds.

GO and KEGG pathway enrichment analyses of DEGs

The GO and KEGG pathway enrichment analyses were performed by the Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.ncifcrf.gov) in two groups. $P < 0.05$ was considered statistically significant.

PPI network construction and module analysis

The PPI network was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING: http://string-db.org) to identify direct or indirect associations between proteins. The STRING does not support networks with higher than 2,000 nodes. The number of proteins was reduced by chosen thresholds of the gene expression levels of |logFC| >0.7 and $P < 0.05$ in the DAU group, and |logFC| >0.4 and $P < 0.05$ in the non-DAU group. A combined score > 0.9 was used to construct the PPI networks, which were visualized by Cytoscape software. The Molecular Complex Detection (MCODE) plug-in was used to select important functional modules of protein interaction networks.

Selection of the core genes in the network

The maximal clique centrality (MCC) of each node was calculated by CytoHubba, a plugin in Cytoscape, and the top 10 genes with the highest degree were regarded as core genes in the PPI networks. Genes with the deepest color were considered as the core genes in the network.

Weighted Gene Co-Expression Network analysis (WGCNA)

The R software was used to remove outliers and samples, and WGCNA was additionally utilized to construct a scale-free co-expression network. First, Pearson correlation matrix and average linkage method were used for all paired genes, and then, the power function was utilized to construct the weighted adjacency matrix. The adjacency was transformed into a topological overlap matrix (TOM), and the corresponding dissimilarity (1-TOM) was calculated. To classify genes with similar expression profiles into gene modules, average linkage hierarchical clustering was conducted according to the TOM-based dissimilarity measure.

Results

Identification of DEGs

After normalization, 7,541 DEGs were identified in the DAU group (206 up-regulated and 7,335 down-regulated DEGs). In the non-DAU group, 2,799 DEGs were identified, including 2,491 up-regulated and 308 down-regulated DEGs. A heatmap was plotted for each group.

Using the two heatmaps, it was found that after Lp299v treatment, the differential gene expression levels in the treatment group were significantly changed compared with the control group.

(Fig. 1here)

The GO and KEGG pathway enrichment analyses of DEGs

(Fig. 2 here)

As shown in Table 1 and Fig. 2a, changes in the cellular component (CC) of DEGs were mainly enriched in the **miosis component**.

Table 1
The top enriched CC of DEGs in the DAU group sorted by adjusted P-values in a descending order

Category	GOID	GO name	Count	Adjusted *P*-value
CC	GO:0005819	Spindle	25	8.01E-23
CC	GO:0072686	Mitotic spindle	16	2.03E-19
CC	GO:0000775	Chromosome, centromeric region	18	4.99E-19
CC	GO:0000779	Condensed chromosome, centromeric region	15	3.06E-18
CC	GO:0005876	Spindle microtubule	12	1.79E-17
CC	GO:0000776	Kinetochore	15	5.72E-16
CC	GO:0098687	Chromosomal region	22	9.42E-16
CC	GO:0000793	Condensed chromosome	18	3.30E-14
CC	GO:0000777	Condensed chromosome Kinetochore	12	9.75E-14
CC	GO:0030496	Midbody	13	2.10E-12

(Table 1here)

As presented in Table 2 and Fig. 2b, changes in the biological process (BP) of DEGs were significantly enriched in the **mitosis process**.

Table 2
The top enriched BP of DEGs in the DAU group sorted by adjusted P-values in a descending order

Category	GOID	GO name	Count	Adjust *P*-value
BP	GO:0140014	Mitotic nuclear division	34	8.01E-23
BP	GO:0000280	Nuclear division	36	2.03E-19
BP	GO:0048285	Organelle fission	37	4.99E-19
BP	GO:0000070	Mitotic sister chromatid segregation	24	3.06E-18
BP	GO:0000819	Sister chromatid segregation	25	1.79E-17
BP	GO:0007059	Chromosome segregation	29	5.72E-16
BP	GO:1902850	Microtubule cytoskeleton organization involved in mitosis	21	9.42E-16
BP	GO:0034765	Nuclear chromosome segregation	25	3.30E-14
BP	GO:0031225	Mitotic spindle organization	18	9.75E-14
BP	GO:0005179	Regulation of mitotic nuclear di-vision	19	2.10E-12

(Table 2 here)

As shown in Table 3 and Fig. 2c, changes in molecular function (MF) were mainly enriched in **microtubule binding and tubulin binding**.

Table 3
The top enriched MF of DEGs in the DAU group sorted by adjusted P-values in a descending order

Category	GOID	GO name	Count	Adjusted *P*-value
MF	GO:0008017	Microtubule binding	18	1.86E-07
MF	GO:0015631	Tubulin binding	18	1.06E-05
MF	GO:0048248	CXCR3chemokine receptor binding	4	1.06E-05
MF	GO:0035173	Histone kinase activity	5	7.82E-05
MF	GO:0008009	Chemokine activity	7	7.82E-05
MF	GO:0003774	Motor activity	10	0.000107135
MF	GO:0003777	Microtubule motor activity	8	0.000107135
MF	GO:0050786	RAGE receptor binding	4	0.000249883
MF	GO:0042379	Chemokine receptor binding	7	0.000504897
MF	GO:0045236	CXCR chemokine receptor binding	4	0.001751271

(Table 3 here)

Although the number of DEGs in the DAU group was higher than that in the non-DAU group, according to the results of the GO enrichment analysis, the number of pathways after enrichment of these genes was the same. The enrichment of these DEGs mainly targeted **microtubule binding and tubulin binding**.

In KEGG analysis, the results of the KEGG pathway enrichment analysis revealed that the DEGs in DAU group were mainly enriched in herpes simplex virus 1 (HSV-1) infection, Gonadotropin-releasing hormone (GnRH) secretion, ubiquitin-mediated proteolysis, apoptosis, endocrine resistance, and cellular senescence. These pathways showed varying levels of down-regulation. Figure S1 shows the inherent immune escape in HSV-1 pathway, as well as down-regulation of various related genes and proteins in PI3K-Akt and mitochondrial pathways, which reduced cellular senescence and apoptosis.

(Fig. 3 here)

(Table S1 here)

In addition, the results of the KEGG pathway enrichment analysis indicated that DEGs in the non-DAU group were mainly enriched in HSV-1 infection, Yersinia infection, neuroactive ligand-receptor interaction, human immunodeficiency virus-1 (HIV-1) infection, and human cytomegalovirus infection (HCMV). These pathways all caused up-regulation of DEGs to varying degrees. Figure S2 shows the Yersinia infection pathway, which inhibits the proliferation and recruitment of macrophages, weakens the immune response, and suppresses the interferon response, inflammatory response, and phagocytosis.

(Fig. 4 here)

(Table S2 here)

PPI network construction and module analysis

Figure S3 and Figure S4 show the PPI networks in the DAU and non-DAU groups, respectively.

(Figure S3 and Figure S4 here)

Using the PPI network, we found three main clusters and there were PPIs in these three clusters. Cluster 1 consisted of 25 proteins whose functions included chemotaxis and G protein-coupled receptor. Cluster 2 contained 15 proteins whose functions included cell cycle regulation, as well as regulation of calcium and nuclear factor-kappa B (NF-κB) signaling pathways. Cluster 3 covered 11 proteins whose functions included G protein-coupled receptor, regulation of cell proliferation and apoptosis, and metastasis suppressor genes.

(Table 4 here)

Table 4
Clusters of the PPI network of the DEGs in the DAU group

Cluster	Scores	Density	Nodes	Genes
1	24.917	25	299	GPSM1, ADRA2C, HTR1A, GALR2, BDKRB2, GNGT2, CXCR3, LPAR2, GRM3, TAS2R38, TAS1R1, PPY, TAS2R19, CXCL11, CXCL10, OXGR1, SST, SSTR4, GNGT1, GNG11, GNG13, TAS2R5, GNB4, GNB3, ADCY6
2	13.143	15	92	FBXO32, TRIM63, CCDC22, GPS1, UBE2C, TRAIP, FBXW12, DTX3L, FBXO27, SKP2, UBE2M, NEDD8, SKP1, RBX1, ASB12
3	11	11	55	GPR143, CHRM5, OPN4, KISS1, GPR132, P2RY2, F2RL2, CYSLTR2, GRP, GNRH2, PROKR2

Similarly, we found two main clusters in the non-DAU group. Cluster 1 consisted of 24 proteins whose functions included chemotaxis of inflammatory cells and G protein-coupled receptor. Cluster 2 covered 20 proteins whose functions included cell cycle regulation, mitosis, and inflammatory response.

(Table 5 here)

Table 5
Clusters of the PPI network of the DEGs in the non-DAU group.

Cluster	Scores	Density	Nodes	Genes
1	14.696	24	169	ADRA2B, GNB3, GNB4, TAS2R3, GNG13, GNG11, GNGT1, GLP2R, GNG3, RAMP2, CCR9, GPR45, CCL28, RLN2, SUCNR1, MC1R, GPSM2, F2RL1, CHRM4, GNRH1, GAST, TRHR, LTB4R, TRH
2	9.579	20	91	B9D2, DSN1, CENPH, CLASP1, NUP85, CENPO, CENPT, NUP43, NUP107, BUB1B, ASB1, SMURF2, KLHL11, KBTBD7, FBXL4, FBXO32, CDC23, WWP1, UBE2D2, TRIM41

Using the MCODE, the most significant module, called sub-unit in the PPI networks, was identified. The core genes and their corresponding lines in the two groups are shown in Fig. 5. The deeper color, the higher degree was.

(Fig. 5 here)

As shown in Figure S5, the WGCNA clustered the DEGs into 4 modules, and 4 different colors were used to represent 4 different modules.

(Figure S5 here)

Discussions

The results of the present study further indicated the molecular mechanism of Lp299v supplementation. Meanwhile, the core genes and the results of enrichment analyses expanded the molecular mechanism of Lp299v supplementation in patients with stable CAD. Our experimental data were categorized into the DAU group and the non-DAU group. The former was composed of 8 samples, and the latter included 30 samples. The bioinformatics analysis was performed on both the DAU and the non-DAU groups. After the results were compared between the two groups, some differences were found, which could reveal the weakening effect of alcohol on the protection of intestinal inflammation. This hypothesis will be further analyzed in the future experiments.

The composition of gut microbiota is very complex, including bacteroidetes, firmicutes, proteobacteria, fusobacteria, actinobacteria. In different populations with different diets, gut microbiota vary in terms of number and proportion (Yamashita et al. 2016). Previous studies have found that gut microbiota were associated with several diseases, such as cardio-metabolic diseases, tumors, inflammatory bowel disease, and central nervous system diseases (Cheng et al 2020; Sitkin et al. 2021; Sun et al. 2020; Zhou et al. 2020). Some scholars demonstrated that intestinal inflammation may be a risk factor for atherosclerosis, and gut microbiota and intestinal immunity could be used as therapeutic targets for the treatment of CAD (Yamashita et al. 2015). *Lactobacillus plantarum* is one of the important members of the genus Lactobacillus, and it has been identified as a probiotic, confirming its value for further research and application (Kleerebezem et al. 2010).

In both the DAU and non-DAU groups, the GO enrichment analysis indicated cell mitosis and reduced chemotactic movement, suggesting that Lp299v could promote immune regulation and attenuate immune response. Additionally, the GO enrichment analysis revealed that cell mitosis, anti-apoptosis and anti-cell senescence increased, which may be secondary to the activation of the PI3K-Akt pathway. The KEGG pathway enrichment analysis showed apoptosis and senescence of cells in the DAU group, as well as the down-regulated expression of HSV-1 infection-related signaling pathway, while in the non-DAU group, neuroactive ligand-receptor interaction and various infection-related signaling pathways were up-regulated to varying degrees. In the DAU group, activation of these pathways inhibited cellular senescence and apoptosis, attenuated innate immune escape, and reduced inflammatory response. In the non-DAU group, activation of these pathways inhibited the proliferation and recruitment of macrophages, decreased the expression of interferon-β (INF-β), and attenuated immune response and chemotactic cytokines. A previous study showed that alcohol intake could promote the growth of Gram-negative bacteria in the intestines and increase the permeability of the intestines, leading to systemic inflammations (Parlesak et al. 2000). Another study which included *in vivo* and *in vitro* experiments showed that Lp299v improved vascular endothelium. The possible mechanisms might be related to improved endothelium-dependent vasodilation and nitric oxide (NO) bioavailability, as well as reduced levels of IL-8, IL-12, and leptin (Malik et al. 2018). It may be because leptin may activate p38 and ERK signaling pathways by interacting with a leptin receptor, ObR, to induce IL-8 production in M2 macrophages (Cao et al. 2016). A previous clinical study showed that in patients with obstructive jaundice, Lp299v supplementation after biliary drainage could reduce acute inflammation of the immune system and decrease intestinal permeability (Jones et al. 2013). A number of scholars confirmed that Lp299v could attenuate the immune response in patients with coronary heart disease (Hofeld et al. 2021; Naruszewicz et al. 2002). A previous study reported that Lp299v significantly reduced cell apoptosis, which was consistent with the down-regulation of apoptosis pathway found in our study (Dykstra et al. 2011). Earlier studies showed that Lp299v-contained beverages could protect body cells against excessive production of reactive oxygen species, thereby protecting against oxidative damage (Gawlik-Dziki et al. 2021; Onning et al. 2003).

We, in the present study, found genes that were associated with G protein-coupled receptor, inflammatory response, cell cycle regulation, regulation of cell proliferation and apoptosis, and metastasis suppression in the PPI networks. A previous study reported that Lp299v could be used in the treatment of some types of cancer, possibly because Lp299v affects cell cycle, cell proliferation and apoptosis, tumor metastasis, inflammatory response, and other genes and proteins (Kazmierczak-Siedlecka et al. 2020). In our screening of core genes, it was found that the majority of the core genes were G protein-coupled receptor-related genes, followed by inflammation-activated chemotaxis-related genes. Diverse types of chemokines act through G protein-coupled receptors, which are collectively known as chemokine receptors. Interleukin receptor and histamine receptor are involved in inflammation and allergic reactions. In the current study, it was hypothesized that one or more of the components of Lp299v could modulate the G protein-coupled receptor, thereby regulating the immune system, inhibiting inflammatory response, promoting cell mitosis and proliferation, and inhibiting cellular senescence and apoptosis.

We found the expressions of the genes encoding adenylate cyclase, and β- and γ-subunit of G protein-coupled receptor. The results of enriched MF of DEGs indicated that chemokine receptors, acting as a G protein-coupled receptor, could play important roles in the immune system cell signaling pathway. Using the constructed PPI networks, it was found that the expressions of CXCL11 and CXCR3 in the DEGs of this sample had a parallel relationship. Both CXCL11 and CXCR3 are pro-inflammatory factors. In patients with irritable bowel syndrome, the mRNA levels of IL-6, CXCL-11, and CCR3 were up-regulated (Shukla et al. 2018). A recent study showed that compared with CCl4-treated mice that did not receive probiotic strains, mice that received B. pseudocatenulatum CECT7765 had a reduced inflammatory environment and bacterial translocation (Moratalla et al. 2014). In another study, the expression levels of pro-inflammatory factors (CCR6, CCR9, CXCR3, and CXCR6) were reduced in intestinal lymphocytes in mice with liver cirrhosis taking Bifidobacterium pseudocatenulatum CECT7765 compared with the control group (Moratalla et al. 2016). Hence, it was further hypothesized that Lp299v supplementation could modulate the expressions of chemokine receptors via a subsequent cellular effect.

In summary, Lp299v could treat patients with stable CAD by modulating inflammatory responses. Our study also found the role of G protein-coupled receptor, mitosis, apoptosis, and senescence of cell in the process of Lp299v supplementation. Cell mitosis, apoptosis, and senescence were associated with the PI3K-Akt pathway. The chemokine receptors could act as a G protein-coupled receptor, playing important roles in the immune system cell signaling pathway. It was supposed that chemokine receptors could have a cellular effect through the Gs-cAMP-PKA signaling pathway.

In this study, we found the following limitations. First, the small sample size might cause the bias in the experiment. Second, several core genes and molecular pathways were identified, which were correlated with the effect of Lp299v supplementation, while we did not explore the interactions among these core genes. Finally, we did not determine which type of chemokine receptors and which ligand play regulatory roles. Thus, our findings remain to be further verified by additional *in vivo* or *in vitro* experiments.

Abbreviations

CAD coronary atherosclerotic disease

Lp299v *Lactobacillus plantarum* 299v

GEO Gene Expression Omnibus

GO Gene Ontology

KEGG Kyoto Encyclopedia of Genes and Genomes

PPI protein-protein interaction

DAVID Database for Annotation, Visualization and Integrated Discovery

STRING Search Tool for the Retrieval of Interacting Genes

DAU daily alcohol user

non-DAU non-daily alcohol user

WGCNA weighted gene co-expression network analysis

CAPs coronary atherosclerotic plaques

CRP The levels of C-reactive protein

IL-6 interleukin-6

TNF tumor necrosis factor

JAK2 Janus kinase 2

GBP1 Guanylate-binding protein 1

TNFSF10 TNF superfamily member 10

FDR false discovery rate

MCODE Molecular Complex Detection

MCC maximal clique centrality

TOM topological overlap matrix

CC cellular component

BP biological process

MF molecular function

HSV-1 herpes simplex virus 1

HIV-1 human immunodeficiency virus-1

HCMV human cytomegalovirus infection

GPCR G protein-coupled receptor

NF-κB nuclear factor-kappa B

INF-β interferon-β

NO nitric oxide

IL-8 interleukin-8

IL-12 interleukin-12

CCl4 carbon tetrachloride

Declarations

Ethics approval and consent to participate

All the datasets were obtained from public database. The study was approved by the Medical Ethics Committee of The Third Affiliated Hospital of Southern Medical University to the Department of Cardiology, the Third Affiliated Hospital of Southern Medical University, China.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyses during the current study are available in the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=

GSE156357)

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Fu Z.Y: Conceptualization, Methodology, Writing - Original Draft and Review & Editing

Song X.L: Data Curation, Formal analysis

Shen A.N: Software and Visualization

Zhou T: Supervision and Project administration

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Figures

Fig.1
(a)

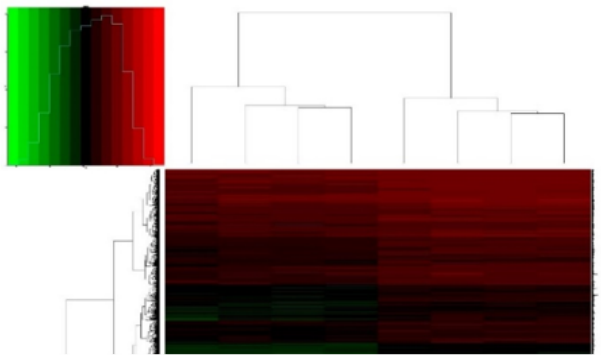

Figure 1

a In the DAU group, there are a total of 8 samples, 4 of which are the control group and 4 are the experimental group. After normalization, 206 up-regulated genes and 7,335 down-regulated DEG genes were found in the experimental group (P<0.05). b There were 38 samples in the non-DAU group, of which 19 were the control group and 19 were the experimental group. We found 2491 up-regulated genes and 308 down-regulated DEG genes in the experimental group (P<0.05).

Fig. 2

a)

Figure 2

By performing GO enrichment analysis on DEGs, we obtain the circle maps of CC, BP, and MF. a) The enriched CCs suggest changes in the composition of cell membranes, plasma membranes and biofilms. b) The enriched BP suggests activation of G protein-coupled receptor-related signaling pathways and changes in ion transport. c) The enriched BP suggested the involvement of various proteins and factors

involved in the regulation of immune response, including cytoskeletal proteins, chemokines, Toll4 receptors, etc.

Fig.3

a)

Figure 3

In the DAU group,the In KEGG analysis, the results of the KEGG pathway enrichment analysis revealed that the DEGs in DAU group were mainly enriched in herpes simplex virus 1 (HSV-1) infection,

Gonadotropin-releasing hormone(GnRH) secretion, ubiquitin-mediated proteolysis, apoptosis, endocrine resistance, and cellular senescence.

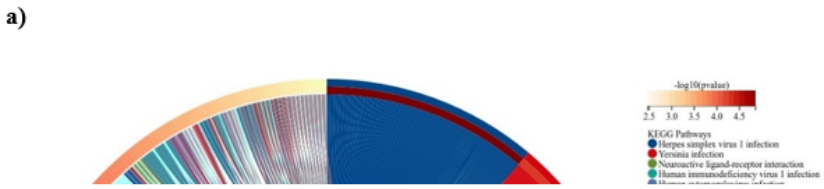

Figure 4

KEGG pathway enrichment analysis indicated that DEGs in the non-DAU group were mainly enriched in HSV-1 infection, Yersinia infection, neuroactive ligand-receptor interaction, human immunodeficiency

virus-1 (HIV-1) infection, and human cytomegalovirus infection (HCMV)

Fig.5

a)

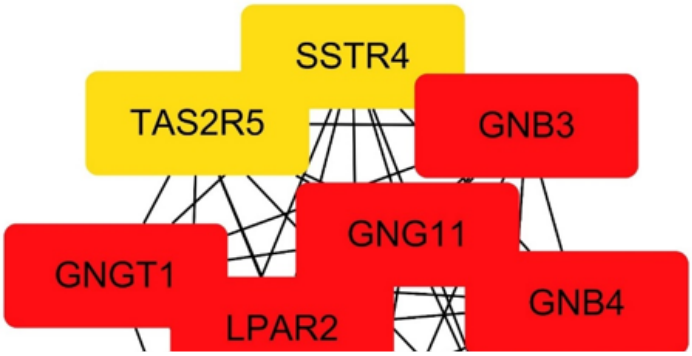

Figure 5

a) Using the CytoHubba plugin of Cytoscape to screen the top 10 genes on the PPI network of the DAU group. The darker the color, the more important the gene is. b) The top 10 genes were also screened for

the PPI network of the non-DAU group. From the two figures, it can be found that there are differences between the two groups of core genes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.pdf
- Supplementarymaterials2.docx