Two-Way Impacts Between Macrophages on Vascular Endothelium and Characteristics of TCM Syndromes in Dyslipidemic Mice with the Phlegm-Dampness Retention syndrome and the Spleen and Kidney Yang Deficiency syndrome Using RNA-Seq

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Research

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Abstract

Background: 'Treating the same disease with different methods' is a Traditional Chinese Medicine (TCM) therapeutic concept. That means although patients are diagnosed with the same disease, they may have different syndromes that require distinct drug administrations. This study aimed to identify the differentially expressed genes and related biological processes in dyslipidemia with the Phlegm-Dampness Retention (PDR) syndrome and the Spleen and Kidney Yang Deficiency (SKYD) syndrome using transcriptomic analysis.

Methods: Ten ApoE knockout (ApoE-/-) mice were used for the establishment of dyslipidemic disease-syndrome models via multifactor-hybrid modeling, with 5 in the the PDR group and 5 in the SKYD group. Five C57BL/6J mice were employed as normal controls (NC) group. Test model quality. Aortic endothelial macrophages in mice were screened using flow cytometry. Transcriptomic analysis was performed for macrophages using RNA-Seq.

Results: The quality assessment of the disease-syndrome model showed that TG, TC, and LDL-C levels significantly increased in the PDR and SKYD groups versus the NC group ($P < 0.05$). Combined with HE staining of aorta, the disease model was successfully established. The quality assessment of the syndrome models showed that mice in the PDR group presented with typical manifestations of the PDR syndrome, and mice in the SKYD group had the related manifestations of the SKYD syndrome, indicating that the syndrome models were successfully constructed. After comparing the differentially expressed gene (DEG) expressions in macrophages in dyslipidemia mice with different syndromes, 4142 genes were identified with statistical significance ($P < 0.05$). The Gene Ontology (GO) analysis for the DEGs showed that biological process of difference between PDR group and SKYD group include both adverse and protective processes were included.

Conclusion: The DEGs between the PDR syndrome and the SKYD syndrome indicate different biological mechanisms between the onset of the two syndromes. They have distinctive biological processes, including adverse and protective processes, corresponding to the invasion of pathogenic factors into the body and the fight of healthy qi against pathogenic factors, respectively, in the TCM theory. Our results have demonstrated the biological evidence behind 'treating the same disease with different treatments' in TCM.

Background

As the life quality increases and dietary pattern changes, multiple factors give rise to the growing morbidity of dyslipidemia. An epidemiological study shows that dyslipidemia closely links to cardiovascular and cerebrovascular diseases, including coronary atherosclerotic heart disease and cerebral infarction [[1]]. It is estimated that the overall morbidity of dyslipidemia in Chinese adults reaches 40.40% and continues to rise [[2]]. Dyslipidemia is characterized by abnormalities in the quantity and quality of lipids in the plasma, including a lower high-density lipoprotein cholesterol (HDL-C) level and higher triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels [[3]]. Lipid and cholesterol accumulation in the vascular wall may lead to endothelial dysfunction [[4]]. Dyslipidemia is an independent and changeable risk factor shortening the onset time of atherosclerosis [[5]]. The 2013 American College Foundation of Cardiology and American Heart Association (ACCF/AHA) guideline on the management of blood lipid in atherosclerotic cardiovascular disease (ASCVD) recommended that management for dyslipidemia is the key to control risk factors of ischemic cardiovascular events [[6]].

Numerous clinical studies and laboratory experiments have ascertained the satisfactory efficacy of TCM in dyslipidemia, enriching the therapies for the disease [[7]]. A sound effect of TCM lies in the accurate discrimination of TCM syndromes of dyslipidemia, which is the basis of TCM diagnosis and treatment. The differentiation of syndromes refers to collecting clinical information (TCM symptoms) of patients via integrated TCM diagnostic methods, including looking, listening, questioning, and feeling the pulse, and analyzing and summarizing the etiological factors and pathogenesis according to the TCM thinking mode.

As patients present with different 'TCM symptoms', dyslipidemic patients may exhibit various syndromes that can be considered different TCM subtypes of dyslipidemia. The SKYD syndrome and PDR syndrome are common in dyslipidemic patients. The vascular endothelium is accountable for delivering nutrients in a dynamic way [[8]]. Dyslipidemia acts as a risk factor of cardiovascular disease probably via promoting endothelium dysfunction, a prerequisite for the occurrence of atherosclerotic manifestations [[9]]. A critical mechanism of endothelium dysfunction is oxidative stress. Excessive nitric oxide (NO) binding with hyperoxides can form peroxynitrite anion ($ONOO^-$). $ONOO^-$ triggers oxidative stress of vascular endothelium and the endothelial injury through its oxidative effect (nitrification) on proteins, exacerbating the endothelial injury. So the severity of endothelial injury can be indicated by $ONOO^-$ levels. Our team has been studying the integration of TCM differentiation and western medicine diagnosis and investigating endothelial injury differences, as the characteristics, between the SKYD syndrome and the PDR syndrome in dyslipidemia. Our previous study suggested that dyslipidemic patients with the SKYD syndrome and the PDR syndrome exhibited different serum $ONOO^-$ concentrations, reflecting the difference in TCM syndromes in a certain sense [[10]]. It is speculated that different syndromes of dyslipidemia may correspond to different degrees of endothelial injury.

In dyslipidemia, macrophages play a pivotal role in the process of endothelial injury. Concerning the critical effect of macrophages and based on our previous study series, this work focused on aortic endothelial macrophages and explored the characteristics of the endothelial injury between the SKYD syndrome and the PDR syndrome in dyslipidemia.

Macrophages consist of two subtypes, M1 and M2 macrophages [[11]]. LDH stimulates the expressions of adhesion molecules and chemokines, thereby differentiating monocytes into macrophages through various pathways. Macrophages differentiate through different pathways. Of the two subtypes, M1 macrophages can release inflammatory factors that facilitate the progression of inflammation and further impairing vascular endothelial cells [[12]]. M2

macrophages release anti-inflammatory factors that involve in angiogenesis and tissue growth and delay the progression of inflammation [[13]]. A study showed that macrophages are indispensable in the formation of atherosclerosis [[14]]. The two subtypes exhibit antagonistic characteristics, corresponding to 'healthy *qi* (the ability to combat evils and preventing disease)' and 'evil *qi* (pathogenetic factors or factors damaging healthy *qi*)' in the TCM theory. The interactions between healthy *qi* and evil *qi* are characterized by 'healthy energy-evil struggles, mutually opposing and constraining, and the rule of waxing and waning' in the occurrence and development of diseases and syndromes.

Disease-syndrome combination is a significant mode for TCM diagnosis and treatment in the clinic. The in-depth investigation of diseases and syndromes calls for biological studies using disease-syndrome animal models in agreement with the traits of TCM theories to understand the underlying mechanisms. The characteristics of these models, such as rigorous control, high repeatability and success rate, make it easier to control the research cycle, repetitively perform experiments, collect more data, and comprehensively analyze the data. So the establishment of an appropriate disease-syndrome animal model is capturing more attention.

An important way for TCM syndrome research is investigating biological mechanisms and the characteristics of syndromes using animal models. So we developed a disease-syndrome animal model in this study for analysis. The quality of a disease-syndrome animal model can affect the accuracy and credibility of a study. We employed multifactor-hybrid modeling and established dyslipidemic mouse models with the SKYD syndrome and the PDR syndrome based on the model used in our previous study so that the quality of the animal models can be ensured as much as possible. The disease model replicated the core physiopathologic process during the occurrence and development of a disease. The syndrome models simulated the core etiology and pathology during the onset of a syndrome.

Transcriptomics is a technique used to study gene transcription and regulation related to TCM syndromes and is conducive to in-depth biological research for a better understanding of the pathogenesis of TCM syndromes, which is worthy of more prevalent application in TCM syndrome research [[15]]. Currently, studies about the characteristics of the SKYD syndrome and the PDR syndrome of dyslipidemia are rarely reported. Our study focused on the two-way impacts between the severity of the endothelial injury and macrophages and analyzed the differences in biological processes and signaling pathways between different TCM syndromes (subtypes) of dyslipidemia using transcriptomic techniques. This study provided a reference for in-depth mechanical research of TCM syndromes and studies of targets of TCM drugs.

1. Materials And Methods

1.1 Experimental animals

Ten ApoE knockout mice, male, 6 weeks old, body mass about 20±5g. Five C57BL/6J mice of the same strain, male, 6 weeks old, body mass about 20±5g. All animals were raised in Beijing Changyang Xishan Farm. Rearing environment: room temperature 21-25°C, humidity 50%-70%, 12h alternating shade. The ethics of this study was approved by the animal ethics review committee of the Institute of Basic Theories of Chinese medicine, Chinese Academy of Chinese Medical Sciences, approval no. 201908006 (Beijing, China).

1.2 Experimental main reagents and mold-making feed

Main reagents for the experiment: LDL Cholesterol Test Kit (Nanjing Jiancheng Bioengineering Institute Co., Ltd.); Total Cholesterol Lipoprotein Test Kit (Nanjing Jiancheng Bioengineering Institute Co., Ltd.); HDL Cholesterol Test Kit (Nanjing Jiancheng Bioengineering Institute Co., Ltd.); Triglyceride Test Kit (Nanjing Jiancheng Bioengineering Institute Co., Ltd.); FITC Anti-Mouse F4/80 Antigen (BM8.1) (Tonbo Biosciences Co., Ltd); PE Anti-Human/Mouse CD11b (M1/70) (Tonbo Biosciences Co., Ltd); Hematoxylin-eosin staining solution (Aijia Biotechnology Co., Ltd).

Experimental modeling feed: The dyslipidemic feed formula consisted of 63.6% basal feed + 15% lard + 20% sucrose + 1.2% cholesterol + 0.2% sodium cholate, provided by Beijing Keaoxieli Feed Co.

1.3 Modeling and evaluation methods of animal models of diseases and syndromes

Modeling methods: (1) the PDR group: 5 ApoE knockout mice were randomly selected and fed a high-fat diet for 4 weeks from weeks 1 to 4; (2) the SKYD group: 5 ApoE knockout mice were randomly selected and given 0.1% propylthiouracil by gavage at a dose of 10 mg/(kg·d) during weeks 1-4, and high-fat chow for 2 weeks during weeks 5-6; (3) the NC group: 5 C57BL/6J mice were given normal chow for modeling for a total of 4 weeks from weeks 1-4.

Model evaluation methods: (1) evaluation of model quality of dyslipidemia by means of serum lipid index testing and aortic pathology staining; (2) evaluation of model quality of the PDR group and the SKYD group by observing the characteristic manifestations of the syndromes.

1.4 Sampling and macrophage screening methods

Mice were fasted without water for 18h before sampling and given anesthesia for execution; (1) animal serum: animals in the PDR group, the SKYD group and the NC group, after taking whole blood, placed in conventional serum tubes, 3000 rpm for 10 minutes, separated serum, divided and immediately stored in -80 degrees Celsius refrigerator; (2) Animal tissues: aortic tissues were taken from animals in the PDR group and the SKYD group; (3) Macrophages: aorta of animals from the PDR group and the SKYD group were isolated and removed intact, digested by adding trypsin, sieved and ground, digestion was terminated, centrifuged, supernatant was decanted, resuspended by adding PBS, centrifuged again, supernatant was decanted,

and labeled with FITC Anti-Mouse F4/80 Antigen (BM8.1) and PE Anti-Human/ Mouse CD11b (M1/70), blown and resuspended, and stored away from light. After labeling macrophages, macrophages were screened by fluorescence-activated cell sorting (FACS) using the MoFlo XDP Ultra-Fast Flow Cell Sorting System (Beckman Coulter Co., Ltd).

1.5 Measurement of indicators

1.5.1 General observation of the model: observing and recording mental status, body hair glossiness, movement, feces, etc.

1.5.2 Lipid index testing: serum testing of triglyceride, total cholesterol lipoprotein, high density lipoprotein cholesterol and low density lipoprotein cholesterol levels in each group.

1.5.3 Aortic histopathology: Specimens were fixed in 10% neutral formalin at room temperature for 24 hours. The specimens were fixed for 48 hours at room temperature using freshly prepared 4% formaldehyde, followed by paraffin embedding and sectioning. Specimens were cut into 4-mm-thick sections and subjected to histological hematoxylin and eosin staining to observe the morphological features of the vascular endothelium.

After staining, the slices were photographed and examined using an optical microscope (AE41; Motic) equipped with a digital scanner (Pannoramic MIDI; 3DHISTECH) to record images of the stained slices.

1.5.4 Macrophage transcriptomic sequencing and data analysis.

Total RNAs of macrophages were extracted in accordance with the manual of TRIzol®(Life Technologies, Inc., Gaithersburg, MD). Preparation of library and sequencing of transcriptome were carried out using Illumina HiSeq X Ten (Novogene Bioinformatics Technology Co., Ltd., Beijing,China). The mapping of 100-bp paired-end reads to genes was undertaken using HTSeq v0.6.0 software, while fragments per kilobase of transcript per million fragments mapped (FPKM) were also analyzed. Raw reads from RNA-seq libraries were trimmed to remove the adaptor sequence and the reads with adaptor contaminants and low-quality reads (the mass value Q-score < 5 of the base number accounts for more than 50%) and reads from N (N indicates that the base information that cannot be determined) which is >10%. After filtering, reference genome and gene model annotation files were downloaded from a genome website browser (NCBI/UCSC/Ensembl). Indexes of the reference genome were built using Bowtie v2.0.6 and paired-end clean reads were aligned to the reference genome using TopHat v2.0.9. Bowtie was used for a BWT (Burrows–Wheeler Transformer) algorithm for mapping reads to the genome and Tophat can generate a database of splice junctions based on the gene model annotation file and thus achieve a better mapping result than other non-splice mapping tools. For the quantification of gene expression level, HTSeq V0.6.1 was used to count the read numbers mapped for each gene. The RPKM of each gene was calculated based on the gene read counts mapped to this gene. A differential expression analysis was performed using the DESeq R package (3.18.1). The P values were adjusted using the Benjamini & Hochberg method. Corrected P-value of 0.05 and absolute foldchange of 2 were set as the threshold for significantly differential expression. Gene Ontology (GO) enrichment analysis of differentially expressed genes, GO enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected Pvalue less than 0.05 were considered significantly enriched by differential expressed genes.

1.6 Statistical methods

Subject-related data were statistically processed by applying SPSS19.0 statistical software. The measurement data were expressed as mean plus or minus standard deviation. And one-way ANOVA with randomized group design was used for comparison between groups, and the LSD method test was used for two-way comparison if the data variance was the same, and the Tamhane's method test was used for two-way comparison if the variance was not the same, and the difference was considered statistically significant at $P<0.05$. The test level was $\alpha=0.05$, and the confidence interval for parameter estimation was 95%.

2. Results

2.1 General information and macro behavioral performance

Ten ApoE knockout mice and Five C57BL/6J mice of the same strain without deletion were used in the experiment, and 15 mice were entered into the result analysis. Before sampling, the mice in the PDR group showed loss of body hair brightness, lethargy and laziness; the mice in the SKYD group showed arching of the back and curling up, chilling, lethargy and lethargy, lying down and preferring to pile up.

2.2 Comparison of blood lipid indexes

By comparing the lipid indexes, compared with the NC group, TG, TC, LDL-C were significantly higher in the PDR group and the SKYD group ($P < 0.05$). Compared with the normal control group, no significant statistical differences were seen in the comparison of HDL-C indexes between the PDR and SKYD groups. No significant statistical differences were seen between the PDR and SKYD groups in the comparison of TG, TC, HDL-C. No statistically significant differences were found in the comparison of TG, TC, HDL-C, LDL-C and LDL-C indexes between the PDR and SKYD groups. See **Tables 1 – 4.**

2.3 Comparison of hematoxylin and eosin staining examination

The aortas in the PDR group and the SKYD group were examined by hematoxylin and eosin staining. And the endothelial surface of the vessels was smooth, and the inner, middle and outer membranes showed clear, and no obvious lipid deposition or lipid streak production was seen. See **Figure 2.**

2.4 Transcriptome sequencing analysis of macrophages in mice with dyslipidemia syndrome model

2.4.1 Analysis of differentially expressed genes in macrophages of mice with different syndromes of dyslipidemia generated by RNA sequencing

After comparing the DEG expressions in macrophages in dyslipidemia mice with different syndromes, 4142 genes were identified with statistical significance ($P < 0.05$). Among them, 1781 genes were up-regulated and 2361 genes were down-regulated. See **Figure 3.**

Combined with bioinformatics information, a total of 284 differentially expressed genes were selected in this study for in-depth analysis of content related to vascular endothelial injury. In the comparison of the 74 differentially expressed genes between the PDR group and the SKYD group, the number of reads in the PDR group was statistically higher than that in the SKYD group ($P < 0.05$). See **Table 5.**

2.4.2 GO enrichment analysis of differentially expressed genes associated with vascular endothelial injury in macrophages

On the basis of GO enrichment analysis based on differentially expressed genes, macrophage sequencing data of mice in the PDR group and the SKYD group were analyzed, focusing on the enrichment results of biological processes related to vascular endothelial injury.

The differential pathways that were upregulated in the PDR group compared to the SKYD group mainly included: arachidonic acid metabolic process epoxygenase P450 pathway response to interferon-gamma cellular response to interferon-beta. See **Figure 4.**

The differential pathways that were upregulated in the SKYD group compared to the PDR group mainly included: blood vessel morphogenesis angiogenesis response to growth factor cellular response to growth factor stimulus chemotaxis taxis. See **Figure 5.**

Discussion

Dyslipidemia may significantly increase the morbidity and mortality of cardiovascular diseases [[16]]. A study shows that effective control of blood lipid levels may reduce the possibility of relapsed coronary heart disease and its mortality [[17]]. Therefore, proactive diagnosis and treatment for dyslipidemia, the most critical risk factor of atherosclerosis, is essential for decreasing the incidence and mortality of coronary heart disease and cerebral infarction [[18]].

Treatment based on syndrome differentiation is the quintessence of TCM. Syndromes as a distinctive concept in TCM is the core content of the theory and vital evidence for treatment and prescription. TCM treatment exhibits advantages in the management of dyslipidemia [[19]]. The predominant mechanisms encompass inhibiting cholesterol absorption in the intestines and biological synthesis of endogenous lipids, regulating lipoprotein lipase activity and cholesterol transport, promoting the conversion of cholesterol into bile acids and cholesterol emission, and regulatory effects of lipid-metabolism-related transcription factors in TCM drugs [[20]]. Precise discrimination of exact TCM syndromes of dyslipidemia is the prerequisite and basis of TCM treatment. Therefore, TCM syndrome research is a critical link in TCM modernization, wherein biological syndrome research is most significant, providing evidence for the onset and evolution of syndromes and effective mechanisms behind specific interventions for syndromes.

RNA-Seq is a next-generation approach using high throughput sequencing available for low-abundance genomes. It is sensitive for gene structure analysis and gene expression and function assessments to unveil internal molecular mechanisms behind specific biological processes and the pathogenesis of diseases. The features of these genes consist of classic genetic features (nucleotide sequence changes) and epigenetic features (heritable phenotype changes without nucleotide sequence alterations). Our study investigated the syndrome-biological mechanism of dyslipidemia using RNA-Seq.

Our previous serum metabonomics studies of dyslipidemic patients with the PDR syndrome and the SKYD syndrome found that the accumulation of harmful metabolites is the predominant metabolic trait in patients with the PDR syndrome. A lack of protective metabolites is the main metabolic feature in patients with the SKYD syndrome. Further analysis showed that oxidation and inflammatory responses are essential contributors to the different metabolic characteristics between the two syndromes [[21]]. Thus, in-depth study of the SKYD syndrome and the PDR syndrome in dyslipidemia necessitate research on oxidation and inflammatory responses.

Macrophages are pivotal in the formation of atherosclerosis following oxidative stress damage to the vascular endothelium. Endothelial dysfunction and the subsequent oxidative inflammatory reaction is the core pathological mechanism of dyslipidemia. Macrophages are recruited towards endothelial cells, which is an early stimulus of atheromatous plaques formation [[22]] and the important basis of the incidence of atherosclerosis. Based on our previous research, we established the disease-syndrome animal models and explored the characteristics of the aortic endothelial macrophages between the PDR syndrome and the SKYD syndrome of dyslipidemia using RNA-Seq. Our conclusions were as follows.

1 Quality assessment of the dyslipidemia disease model using serum lipids analysis and HE staining of the aorta, revealing success in disease modeling

Compared with the NC group, TG, TC, and LDL-C levels significantly increased in mice of the PDR and SKYD groups and exceeded the upper limit of the normal range, in conformity to the diagnostic criterion of dyslipidemia. The HE staining of the aorta revealed a smooth endothelial surface and clear borders between inner, medial, and outer layers in mice of the PDR and SKYD groups, without pronounced lipid accumulation and fatty streaks. These results indicated that there were no pathological manifestations of atherosclerosis in mice of the two groups. The HE staining results implied the satisfactory quality of dyslipidemia modeling.

However, there were no significant differences in TG, TC, HDL-C, and LDL-C levels between the PDR and SKYD groups. This result suggested that differences in transcriptomic traits and biological results between the two groups using RNA-Seq reflected the characteristics of different syndromes (subtypes).

2 Quality assessment of the two syndrome models (the PDR models and the SKYD models) through behavioral tests, indicating the feasibility of syndrome modeling

The characteristics of mice in the PDR group included fat in body shape, reduced brightness of hair, lethargy, slow response, lazy to move, as well as soft, formed, and sticky feces, consistent with clinical manifestations of the PDR syndrome. The behavioral features of mice in the SKYD group incorporated matted hair, slight paw and nail colors, shrinking the body, chilly, fatigue, sleepy, sticking together, low-temperature tail, decrease in food and water intake, loose and watery stools. These were in agreement with clinical manifestations of the SKYD syndrome. These results indicated that the syndrome modeling could simulate typical symptoms of the corresponding syndrome.

3 Transcriptomic data of macrophages showed pro-inflammatory activities in the vascular endothelium in dyslipidemic mice with the PDR syndrome and the SKYD syndrome, consistent with the theory: evil *qi* leading to the incidence of disease. But this process was achieved via different biological processes in different syndromes, indicating distinct mechanisms of vascular endothelial injury in different syndromes.

3.1 IFN-γ and IFN-β expressions were upregulated in macrophages in dyslipidemic mice with the PDR syndrome, promoting endothelial inflammation

It is known that IFN-γ can facilitate the progression of inflammatory diseases, for example, inflammatory bowel disease and atherosclerosis. In-vitro and in-vivo studies have found IFN-γ may damage epithelial cells and endothelial barrier integrity [[23]]. IFN-γ exerts significant impacts on the biological properties of the vascular endothelial cells. It may initiate vascular remodeling around microvascular endothelial cells [[24]]. Monocyte-derived macrophages are induced by the pro-inflammatory factor IFN-γ, which are of vital importance during plaque formation. Both IFN-γ and macrophages are major players in oxidative stress. Just like other pro-inflammatory factors, IFN-γ acts as a significant trigger of the synthesis and release of reactive oxygen species (ROS) [[25]].

IFN-β mRNA can effectively induce endothelial chemokine expression [[26]]. It enhances endothelial cell adhesion to eosinophils mainly through upregulating vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expressions [[27]]. IFN-β fuels the formation of macrophage foam cells via SR-A-mediated cholesterol influx and ABCA1-mediated efflux of mechanisms, thus expediting the incidence of atherosclerosis [[28]].

The PDR syndrome is a type of sthenia syndrome. The phlegm evil is both the pathological product and pathogenic factor, impeding the delivery and movement of *qi*, thus resulting in body fluid stagnation and hydrops or damp evil accumulation and phlegm. Finally, the vascular endothelium is impaired.

Our results showed that IFN-γ and IFN-β expressions in macrophages were upregulated in dyslipidemic mice with the PDR syndrome, significantly higher than the levels in mice with the SKYD syndrome. This finding suggested that vascular endothelial injury induced by IFN-γ and IFN-β overexpression in vascular endothelial macrophages is a characteristic of the PDR syndrome in dyslipidemia.

3.2 Macrophage chemotaxis and taxis was enhanced in dyslipidemic mice with the SKYD syndrome, promoting endothelial inflammation

Macrophages are inflammatory cells, and their accumulation can stimulate cytokine and chemokine release, initiating immune responses and accelerating plaque formation [[29]]. The secreted chemokines infiltrate atherosclerotic plaques at the early stage [[30]]. Despite the role in lipid accumulation, macrophage foam cells also release pro-inflammatory factors and chemokines, further stimulating vascular endothelial cells, fueling vascular endothelial inflammation, and exacerbating the disease [[31]]. A study found that resveratrol could exert a protective effect on the heart via inhibiting endothelial cell migration and monocyte chemotaxis [[32]].

The TCM mechanism of the SKYD syndrome in dyslipidemia refers to spleen-kidney *yang* deficiency. Furthermore, as the body lacks warmth from *yang-qi* and protection, the vascular endothelium can be easily injured.

Our results showed that macrophage chemotaxis and taxis were significantly enhanced in dyslipidemic mice with the SKYD syndrome, compared with the PDR mice. This finding suggested that vascular endothelial injury induced by enhanced macrophage chemotaxis and taxis were the main characteristic of the SKYD syndrome in dyslipidemia.

The above results showed that different biological processes resulted in vascular endothelial injury in the PDR syndrome and the SKYD syndrome of dyslipidemia, indicating different injury mechanisms of the two syndromes. These results also imply that there exist biological bases behind the pathogenesis of TCM syndromes.

4 Transcriptomic data of macrophages revealed their protection for the vascular endothelium in dyslipidemic mice with the PDR syndrome and the SKYD syndrome. These results coincide with the TCM trait 'the waxing and waning of healthy energy-evil struggles.' But this trait consists of distinct biological processes in different syndromes, showing different vascular protective mechanisms of different syndromes.

4.1 Arachidonic acid metabolic process and epoxygenase P450 pathway levels increased in macrophages in dyslipidemic mice with the PDR syndrome, exerting protection effects on the vascular endothelium

Growing evidence has shown that AA metabolism is crucial in maintaining vascular homeostasis, closely associated with the occurrence and development of cardiovascular diseases [[33]]. AA is an amphiphilic compound affecting endothelial cell migration without the involvement of receptor-specific signaling, it affects endothelial cell metabolism and membrane viscosity [[34]]. AA metabolic pathways are pivotal in platelet activation and gastric damage [[35]]. Suppressing AA metabolism can further block endothelial cell migration, inducing cell apoptosis [[36]]. A study reported that Panax notoginseng saponins combined with aspirin inhibited platelet activity via enhancing AA metabolism [[37]].

Cytochrome P450 (CYP450) refers to a third pathway for AA metabolism [[38]]. CYP450 metabolites of AA in endothelial cells may impact endothelial function. AA is metabolized by CYP450 and cyclooxygenase (COX) into bioactive eicosanoids, exerting vascular protection effects [[39]]. AA is also metabolized by CYP450 and COX into four regioisomeric epoxyeicosatrienoic acids (EETs) used for bioprotection and cadioprotection [[40]]. EETs have multiple nutritive functions, including the anti-inflammatory effect, in their cadioprotection [[41]]. Decreases in EETs expressions may lead to the onset of cardiovascular diseases and endothelial dysfunction [[42]].

Our analysis showed significant upregulations of arachidonic acid metabolic process and epoxygenase P450 pathway levels in macrophages in dyslipidemic mice with the PDR syndrome versus the SKYD mice. These results indicated that endothelial protection from macrophages via AA and CYP450 overexpressions is another trait of the PDR syndrome in dyslipidemia.

4.2 Biological process items, including angiogenesis, blood vessel morphogenesis, response to growth factor, and cellular response to growth factor stimulus, whose activities were significantly enhanced for macrophages in dyslipidemic mice with the SKYD syndrome, which facilitated angiogenesis and vascular repair

Angiogenesis consists of multiple intricate, highly-coordinated processes, wherein endothelial cells with dynamic changes are of great importance [[43]]. Angiogenesis may occur in the pathological environment [[44]] and be initiated by endothelial cell activation. The genetic program of endothelial cells triggers the modulation of angiogenic phenotype. Macrophages are significant regulators for tissue homeostasis, growth, and repair, and morphogenesis. The growing endothelial cells can respond to extracellular signaling molecules, such as extracellular matrix molecules, chemokines, growth factors, and cell adhesion molecules [[45]]. Growth factors and cytokines secreted from macrophages [[46]] may promote the formation of new blood vessels via recruiting new blood vessels and modifying the extracellular matrix [[47]]. Vascular endothelial growth factors (VEGFs) are considered the most robust booster for angiogenesis, increasing the survival of endothelial cells and enhancing mitosis [[48]]. Numerous studies have proven that VEGFs are expressed in macrophages [[49]]. They can stimulate assorted cell functions of endothelial cells via high-affinity binding to two tyrosine kinase receptors, VEGF receptor VEGFR1 and VEGFR2 [[50]].

Our results demonstrated that the top biological process items enriched in macrophages in dyslipidemic mice with the SKYD syndrome were as follows: angiogenesis, blood vessel morphogenesis, response to growth factor, cellular response to growth factor stimulus, whose activities were significantly enhanced versus the the PDR group. This finding suggested that angiogenesis and vascular repair via enhancing macrophage chemotaxis and taxis are another critical feature of the SKYD syndrome in dyslipidemia.

The above results indicated that vascular protection mechanisms are distinct between the PDR syndrome and the SKYD syndrome in dyslipidemia. It further implies different protection mechanisms in the two syndromes. This further proves that there exist biological bases behind the pathogenesis of TCM syndromes.

According to the results in Sections 3 and 4, the vascular endothelial injury was induced by different biological processes in the PDR syndrome and the SKYD syndrome of dyslipidemia. But there also existed angiogenesis and vascular repair in the pathogenetic process. The two opposite processes, injury and repair, are consistent with the TCM rule 'healthy *qi* and evil *qi* are struggling throughout the incidence and dynamic development of diseases and syndromes.' As the injury effect outweighs the repairing effect, the state of diseases takes place.

5 Different transcriptomic characteristics of aortic endothelial macrophages between dyslipidemic mice with the PDR syndrome and the SKYD syndrome, which are manifested by distinct biological control processes during both harmful and protective biological processes, indicating different biological bases behind different syndromes of the same disease and providing biological evidence for the TCM theory 'treating the same disease with different treatments' (Figure 6)

In the TCM clinic, western medicine diagnosis is often combined with TCM syndrome diagnosis for the management of a disease. Research about the association between western diseases and TCM syndromes is believed to be one of the most important steps for modern TCM diagnostics studies. Based on the confirmation of a western disease, disease-syndrome research can not only elucidate the biological bases for TCM differentiation but also help push innovative research on 'disease-syndrome-therapy-formula' and thereby provide precise and rational treatment.

Previous TCM syndrome research emphasizes harmful factors in a syndrome scenario, without much attention to the body's self-protection in this process. Our study did both, focusing on the analysis of harmful factors and protective factors, which is an innovative dimension. The unity of opposites, protective and harmful effects, is achieved by dynamic balancing between healthy *qi* and evil *qi* through mutual conflicts, mutual restriction, and mutual repulsion. Diseases can occur when evil *qi* outstrips healthy *qi*, just as a TCM rule saying 'when there is sufficient healthy *qi* inside, pathogenic factors have no way to invade the body; where pathogenic factors accumulate, the parts of the body must be deficient in the healthy *qi*.' TCM treatment should be implemented based on accurate discrimination of diseases and syndromes and hit the mark by correcting the imbalance between healthy *qi* and evil *qi* through prescriptions and formulas.

Conclusions

Our transcriptomic analysis of aortic endothelial macrophages in dyslipidemic mice with the PDR syndrome and the SKYD syndrome showed the results as follows. First, differentially expressed genes were identified between dyslipidemic mice with the PDR syndrome and the SKYD syndrome, proving different biological mechanisms during the pathogenesis of different syndromes, from the perspective of syndrome research. Second, there existed different biological processes between the PDR syndrome and the SKYD syndrome of dyslipidemia, including harmful and protective biological processes. When evil *qi* invades the body to produce harmful effects, healthy *qi* also responds to it and thereby generates protective responses in a syndrome scenario, which agrees with the TCM rule 'healthy *qi* and evil *qi* are struggling throughout the incidence and dynamic development of diseases and syndromes.' Therefore, the occurrence of syndromes is a result of healthy energy-evil struggles. That is why TCM treatment for one disease with various therapies and formulas can achieve satisfactory efficacy, which may be attributed to different drugs targeting different biological processes. Our work offers biological mechanisms for the TCM theories 'treating different syndromes with different treatments' and 'formula corresponding to the syndrome.' Third, though patients may be diagnosed with the same disease dyslipidemia, different formulas should be selected according to their syndromes, considering distinct biological processes during the PDR syndrome and the SKYD syndrome. Our study has demonstrated the biological evidence behind 'treating the same disease with different treatments' in TCM, embodying the scientificity of 'treatment based on syndrome differentiation.'

Abbreviations

TCM	Traditional Chinese Medicine
PDR syndrome	Phlegm-Dampness Retention syndrome
SKYD syndrome	Spleen and Kidney Yang Deficiency syndrome
DEGs	DEG analysis

Declarations

Ethics approval and consent to participate

The ethics of this study was approved by the animal ethics review committee of the Institute of Basic Theories of Chinese medicine, Chinese Academy of Chinese Medical Sciences, approval no. 201908006 (Beijing, China). All the. methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

All authors read and approved the final manuscript.

Availability of data and materials

Please contact author for data requests.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.This manuscript/data, or parts thereof, has not been submitted for possible publication to another journal or that the work has previously been published elsewhere.

Author Contributions

XX and JC conceived and designed the study. JC, CY, SZ and PL performed the modeling and evaluation of animal models of diseases and syndromes. JC, TW, ZY and BX performed the sampling and macrophage screening.JC, CY, ZY, BX, LP and XX acquired the data. JC, CY, ZY and BX analyzed and interpreted data. JC and CY drafted the manuscript. PL and ZY critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Tables

Table 1 Total cholesterol index in 3 groups of mice (mean ± standard deviation)

Group	TG index
PDR group	23.32±2.33 [a]
SKYD *group*	29.32±6.32 [bc]
NC *group*	2.38±0.38
F	65.82
P	0.000

Note: a was compared with NC group, *P*<0.05; b was compared with NC group, *P*<0.05; c was compared with PDR group, *P*>0.05;

Table 2 Triglycerides index in 3 groups of mice (mean ± standard deviation)

Group	TC index
PDR group	2.96±0.11 [a]
SKYD *group*	4.71±1.15 [bc]
NC *group*	0.80±0.79
F	43.07
P	0.000

Note: a was compared with NC group, *P*<0.05; b was compared with NC group, *P*<0.05; c was compared with PDR group, *P*>0.05;

Table 3 HDL-C index in 3 groups of mice (mean ± standard deviation)

Group	HDL-C index
PDR group	5.17±2.15
SKYD *group*	3.03±1.92 [c]
NC *group*	3.55±0.68
F	2.13
P	0.162

Note: c was compared with PDR group, *P*>0.05;

Table 4 LDL-C index in 3 groups of mice (mean ± standard deviation)	
Group	LDL-C index
PDR group	2.54±0.22 [a]
SKYD *group*	3.60±1.39 [bc]
NC *group*	0.89±0.12
F	13.88
P	0.001

Note: a was compared with NC group, *P*<0.05; b was compared with NC group, *P*<0.05; c was compared with PDR group, *P*>0.05;

Table 5 Injury or protection of macrophages and vascular endothelium in mice with different syndromes of dyslipidemia related differentially expressed genes

Gene ID	PDR_ count	SKYD_ count	*p* value	Gene ID	PDR_ count	SKYD_ count	*p* value
ENSMUSG00000022445	323	47	0.00	ENSMUSG00000026104	1169	355	0.00
ENSMUSG00000032487	57	2	0.00	ENSMUSG00000035042	2000	645	0.00
ENSMUSG00000025002	73	8	0.00	ENSMUSG00000018930	875	280	0.00
ENSMUSG00000060407	456	100	0.00	ENSMUSG00000035373	2723	943	0.00
ENSMUSG00000068086	866	203	0.00	ENSMUSG00000055170	39	6	0.00
ENSMUSG00000025479	3178	776	0.00	ENSMUSG00000022504	1244	432	0.00
ENSMUSG00000060613	1120	279	0.00	ENSMUSG00000024411	16	0	0.00
ENSMUSG00000052974	706	175	0.00	ENSMUSG00000029298	686	256	0.00
ENSMUSG00000094806	514	128	0.00	ENSMUSG00000041515	5566	2360	0.00
ENSMUSG00000003053	3283	926	0.00	ENSMUSG00000074151	241	96	0.00
ENSMUSG00000025197	100	20	0.00	ENSMUSG00000000791	204	83	0.00
ENSMUSG00000054827	1406	415	0.00	ENSMUSG00000049093	19	3	0.00
ENSMUSG00000092008	30	2	0.00	ENSMUSG00000030966	800	382	0.01
ENSMUSG00000030483	61	13	0.00	ENSMUSG00000026866	485	240	0.01
ENSMUSG00000005547	252	82	0.00	ENSMUSG00000054072	5433	269	0.00
ENSMUSG00000033174	211	67	0.00	ENSMUSG00000078921	5214	719	0.00
ENSMUSG00000042248	216	71	0.00	ENSMUSG00000068606	130	9	0.00
ENSMUSG00000068083	54	13	0.00	ENSMUSG00000073555	1016	177	0.00
ENSMUSG00000025004	57	14	0.00	ENSMUSG00000090942	146	20	0.00
ENSMUSG00000074882	299	123	0.00	ENSMUSG00000048852	104	15	0.00
ENSMUSG00000062624	200	82	0.00	ENSMUSG00000046879	10788	2545	0.00
ENSMUSG00000067225	324	140	0.00	ENSMUSG00000074896	2248	623	0.00
ENSMUSG00000024292	105	40	0.00	ENSMUSG00000078920	14916	4448	0.00
ENSMUSG00000061740	96	40	0.01	ENSMUSG00000078853	6044	1992	0.00
ENSMUSG00000057666	4375	77	0.00	ENSMUSG00000058163	432	208	0.01
ENSMUSG00000040264	4138	114	0.00	ENSMUSG00000004814	326	5501	0.00
ENSMUSG00000022126	1783	66	0.00	ENSMUSG00000076431	19	455	0.00
ENSMUSG00000078922	3013	362	0.00	ENSMUSG00000040152	392	5031	0.00
ENSMUSG00000028270	7705	1058	0.00	ENSMUSG00000022309	0	90	0.00
ENSMUSG00000004296	121	13	0.00	ENSMUSG00000031785	0	88	0.00
ENSMUSG00000028268	6736	1336	0.00	ENSMUSG00000036856	0	77	0.00
ENSMUSG00000069874	1240	268	0.00	ENSMUSG00000019997	1	89	0.00
ENSMUSG00000105096	93	13	0.00	ENSMUSG00000018593	187	1817	0.00
ENSMUSG00000104713	389	83	0.00	ENSMUSG00000022018	848	7779	0.00
ENSMUSG00000035385	5307	1309	0.00	ENSMUSG00000022469	66	674	0.00
ENSMUSG00000030895	4179	1033	0.00	ENSMUSG00000061878	15	217	0.00
ENSMUSG00000060550	12164	3456	0.00	ENSMUSG00000031740	4	98	0.00
ENSMUSG00000079363	1587	447	0.00	ENSMUSG00000010660	6	114	0.00
ENSMUSG00000018899	5290	1555	0.00	ENSMUSG00000038545	1	69	0.00
ENSMUSG00000105504	237	63	0.00	ENSMUSG00000006445	37	359	0.00

Gene ID	PDR_ count	SKYD_ count	*p* value	Gene ID	PDR_ count	SKYD_ count	*p* value
ENSMUSG00000032011	149	1190	0.00	ENSMUSG00000044317	0	14	0.00
ENSMUSG00000004951	310	2300	0.00	ENSMUSG00000003032	70	229	0.00
ENSMUSG00000029373	3690	26049	0.00	ENSMUSG00000018500	418	1239	0.00
ENSMUSG00000024486	0	53	0.00	ENSMUSG00000023031	71	227	0.00
ENSMUSG00000050711	0	49	0.00	ENSMUSG00000019256	52	170	0.00
ENSMUSG00000025355	290	1978	0.00	ENSMUSG00000020063	46	153	0.00
ENSMUSG00000037095	2041	13391	0.00	ENSMUSG00000049130	1852	5261	0.00
ENSMUSG00000037362	0	44	0.00	ENSMUSG00000022836	0	13	0.00
ENSMUSG00000049791	24	201	0.00	ENSMUSG00000010175	0	13	0.00
ENSMUSG00000034353	1199	6646	0.00	ENSMUSG00000045930	0	13	0.00
ENSMUSG00000067336	1	44	0.00	ENSMUSG00000032494	0	13	0.00
ENSMUSG00000037411	0	33	0.00	ENSMUSG00000028195	12	54	0.00
ENSMUSG00000050953	0	33	0.00	ENSMUSG00000005413	1251	3480	0.00
ENSMUSG00000002603	72	381	0.00	ENSMUSG00000054364	185	535	0.00
ENSMUSG00000016494	58	313	0.00	ENSMUSG00000022475	96	284	0.00
ENSMUSG00000044337	304	1399	0.00	ENSMUSG00000026836	0	12	0.00
ENSMUSG00000001300	35	189	0.00	ENSMUSG00000073599	10	43	0.00
ENSMUSG00000019929	1375	5747	0.00	ENSMUSG00000030069	4	26	0.00
ENSMUSG00000004791	0	24	0.00	ENSMUSG00000031565	198	544	0.00
ENSMUSG00000021611	0	24	0.00	ENSMUSG00000017417	75	214	0.00
ENSMUSG00000034881	247	976	0.00	ENSMUSG00000031503	2	19	0.00
ENSMUSG00000028108	7728	28858	0.00	ENSMUSG00000022505	2	19	0.00
ENSMUSG00000038742	16	89	0.00	ENSMUSG00000042745	416	1089	0.00
ENSMUSG00000040289	0	20	0.00	ENSMUSG00000068196	0	11	0.00
ENSMUSG00000025473	497	1724	0.00	ENSMUSG00000042258	0	11	0.00
ENSMUSG00000001827	0	19	0.00	ENSMUSG00000031465	0	11	0.00
ENSMUSG00000031250	0	18	0.00	ENSMUSG00000026883	14	54	0.00
ENSMUSG00000045005	0	17	0.00	ENSMUSG00000033191	1	14	0.00
ENSMUSG00000044562	0	17	0.00	ENSMUSG00000031613	877	2203	0.00
ENSMUSG00000006235	0	17	0.00	ENSMUSG00000024241	15	55	0.00
ENSMUSG00000000392	0	17	0.00	ENSMUSG00000015468	11	41	0.00
ENSMUSG00000020676	18	84	0.00	ENSMUSG00000024087	0	10	0.00
ENSMUSG00000022893	0	16	0.00	ENSMUSG00000055254	0	10	0.00
ENSMUSG00000027996	0	16	0.00	ENSMUSG00000023885	0	10	0.00
ENSMUSG00000015957	80	277	0.00	ENSMUSG00000049001	0	10	0.00
ENSMUSG00000027276	4	32	0.00	ENSMUSG00000028868	338	835	0.00
ENSMUSG00000069763	16	73	0.00	ENSMUSG00000030123	43	121	0.01
ENSMUSG00000001761	26	101	0.00	ENSMUSG00000016933	51	140	0.01
ENSMUSG00000057789	9	46	0.00	ENSMUSG00000022528	153	382	0.01
ENSMUSG00000030605	213	650	0.00	ENSMUSG00000031902	227	558	0.01

Gene ID	PDR_ count	SKYD_ count	*p* value	Gene ID	PDR_ count	SKYD_ count	*p* value
ENSMUSG00000022425	47	129	0.01	ENSMUSG00000014932	0	18	0.00
ENSMUSG00000024290	275	672	0.01	ENSMUSG00000031451	85	308	0.00
ENSMUSG00000027665	76	194	0.01	ENSMUSG00000024526	7	44	0.00
ENSMUSG00000006311	0	9	0.01	ENSMUSG00000041120	175	585	0.00
ENSMUSG00000016458	0	9	0.01	ENSMUSG00000027500	0	16	0.00
ENSMUSG00000026586	0	9	0.01	ENSMUSG00000029096	56	200	0.00
ENSMUSG00000035458	0	9	0.01	ENSMUSG00000015619	42	156	0.00
ENSMUSG00000031963	0	9	0.01	ENSMUSG00000025351	1157	3589	0.00
ENSMUSG00000057722	0	9	0.01	ENSMUSG00000009406	35	131	0.00
ENSMUSG00000027985	279	656	0.01	ENSMUSG00000045817	241	759	0.00
ENSMUSG00000004328	4	21	0.01	ENSMUSG00000022817	296	921	0.00
ENSMUSG00000028763	4	21	0.01	ENSMUSG00000021253	5	34	0.00
ENSMUSG00000020458	156	372	0.01	ENSMUSG00000021943	0	14	0.00
ENSMUSG00000026043	122	291	0.01	ENSMUSG00000026842	27	101	0.00
ENSMUSG00000026193	2529	9983	0.00	ENSMUSG00000022521	80	252	0.00
ENSMUSG00000000094	0	9	0.01	ENSMUSG00000029050	46	153	0.00
ENSMUSG00000023078	153	2061	0.00	ENSMUSG00000020919	495	1411	0.00
ENSMUSG00000049907	0	103	0.00	ENSMUSG00000038400	78	240	0.00
ENSMUSG00000021319	0	71	0.00	ENSMUSG00000038872	7	36	0.00
ENSMUSG00000003665	0	68	0.00	ENSMUSG00000055653	39	125	0.00
ENSMUSG00000036699	0	62	0.00	ENSMUSG00000044674	16	63	0.00
ENSMUSG00000033295	12	168	0.00	ENSMUSG00000006958	0	12	0.00
ENSMUSG00000024544	73	516	0.00	ENSMUSG00000020176	0	12	0.00
ENSMUSG00000029661	21	187	0.00	ENSMUSG00000011096	189	520	0.00
ENSMUSG00000025854	317	1960	0.00	ENSMUSG00000024940	16	57	0.00
ENSMUSG00000030669	0	36	0.00	ENSMUSG00000024975	436	1087	0.00
ENSMUSG00000020108	18	133	0.00	ENSMUSG00000050071	0	10	0.00
ENSMUSG00000021388	16	120	0.00	ENSMUSG00000036333	73	189	0.01
ENSMUSG00000008999	0	29	0.00	ENSMUSG00000060216	563	1334	0.01
ENSMUSG00000006205	20	130	0.00	ENSMUSG00000062312	0	9	0.01
ENSMUSG00000038508	20	127	0.00	ENSMUSG00000026383	0	9	0.01
ENSMUSG00000040488	6	59	0.00	ENSMUSG00000031681	241	555	0.01
ENSMUSG00000024598	0	24	0.00	ENSMUSG00000033585	2	52	0.00
ENSMUSG00000046058	17	96	0.00	ENSMUSG00000021214	113	659	0.00
ENSMUSG00000022150	264	971	0.00	ENSMUSG00000006930	9	35	0.01
ENSMUSG00000042757	49	204	0.00	ENSMUSG00000059146	2	981	0.00
ENSMUSG00000031074	0	20	0.00	ENSMUSG00000029530	131	4774	0.00
ENSMUSG00000023047	0	20	0.00	ENSMUSG00000029379	0	159	0.00
ENSMUSG00000034612	0	20	0.00	ENSMUSG00000021702	0	110	0.00
ENSMUSG00000032726	0	19	0.00	ENSMUSG00000031780	217	2882	0.00

Gene ID	PDR_ count	SKYD_ count	*p* value	Gene ID	PDR_ count	SKYD_ count	*p* value
ENSMUSG00000047898	0	87	0.00	ENSMUSG00000047379	174	416	0.01
ENSMUSG00000042306	0	41	0.00	ENSMUSG00000031443	596	1380	0.01
ENSMUSG00000000869	1	49	0.00	ENSMUSG00000009281	621	1427	0.01
ENSMUSG00000027962	512	2993	0.00	ENSMUSG00000023235	145	341	0.01
ENSMUSG00000032118	0	36	0.00				
ENSMUSG00000048251	144	701	0.00				
ENSMUSG00000055994	3	47	0.00				
ENSMUSG00000052957	3	44	0.00				
ENSMUSG00000040026	27911	115085	0.00				
ENSMUSG00000056427	0	25	0.00				
ENSMUSG00000022015	0	24	0.00				
ENSMUSG00000021508	24	128	0.00				
ENSMUSG00000042190	205	820	0.00				
ENSMUSG00000026235	0	22	0.00				
ENSMUSG00000062380	74	308	0.00				
ENSMUSG00000037868	30	140	0.00				
ENSMUSG00000025959	134	517	0.00				
ENSMUSG00000028126	56	230	0.00				
ENSMUSG00000033542	0	19	0.00				
ENSMUSG00000019122	31799	107706	0.00				
ENSMUSG00000014158	16	77	0.00				
ENSMUSG00000041120	175	585	0.00				
ENSMUSG00000029371	0	16	0.00				
ENSMUSG00000038668	32	122	0.00				
ENSMUSG00000021194	0	15	0.00				
ENSMUSG00000028341	0	15	0.00				
ENSMUSG00000029071	30	115	0.00				
ENSMUSG00000026640	0	14	0.00				
ENSMUSG00000062991	32	111	0.00				
ENSMUSG00000020900	0	13	0.00				
ENSMUSG00000072596	4981	13817	0.00				
ENSMUSG00000037613	14	59	0.00				
ENSMUSG00000059714	877	2413	0.00				
ENSMUSG00000055633	6	31	0.00				
ENSMUSG00000031385	0	11	0.00				
ENSMUSG00000042265	799	2015	0.00				
ENSMUSG00000061731	134	349	0.00				
ENSMUSG00000019194	58	160	0.00				
ENSMUSG00000074657	0	10	0.00				
ENSMUSG00000039115	37	100	0.01				

Figures

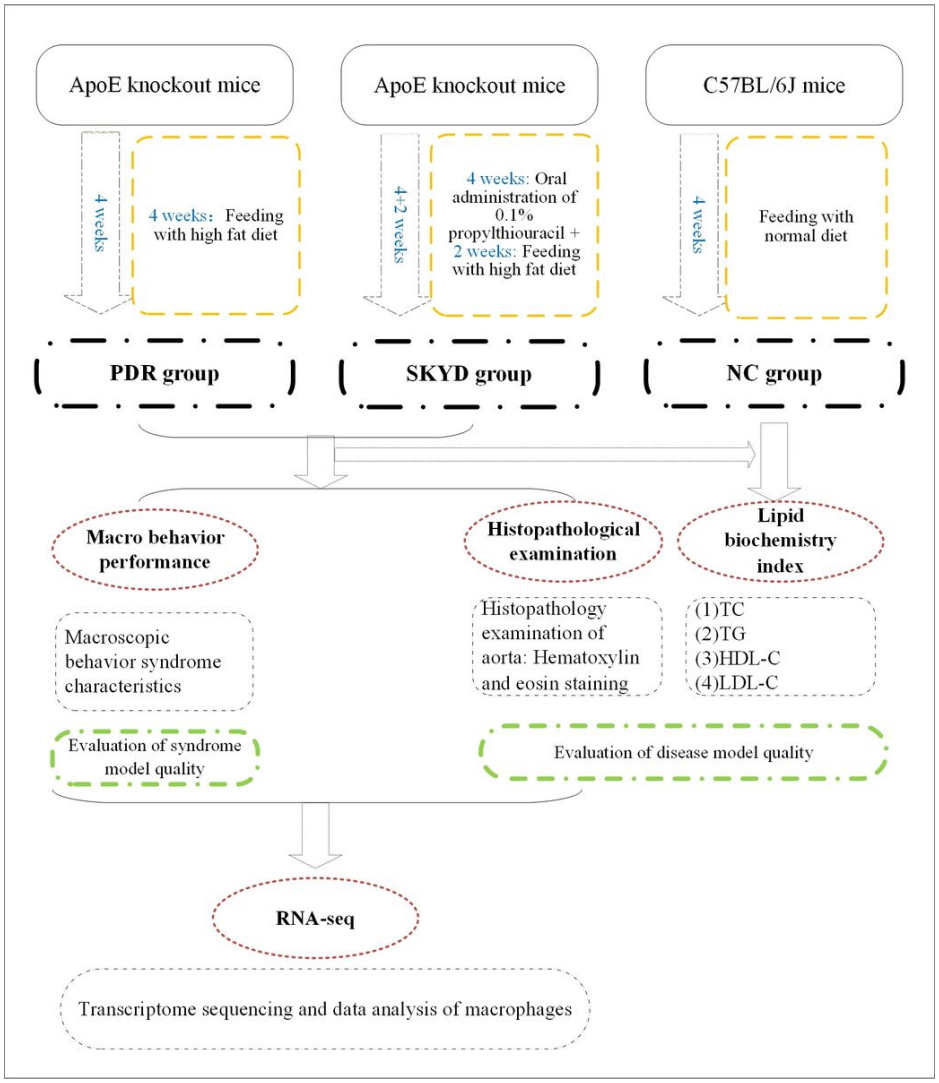

Figure 1

Flowchart of the study.

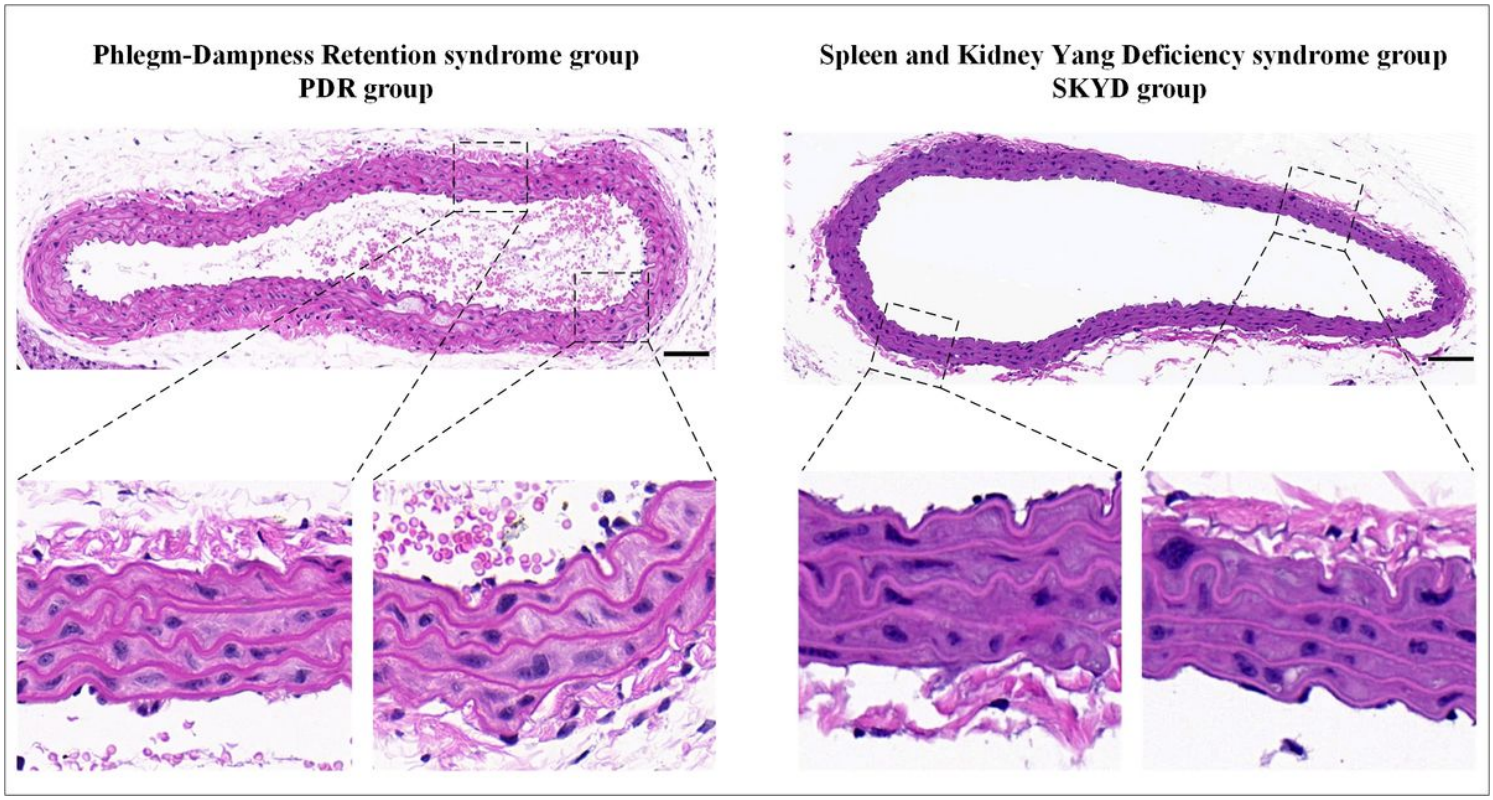

Figure 2

The aorta was stained with hematoxylin and eosin. Scale bars represent 100 μm.

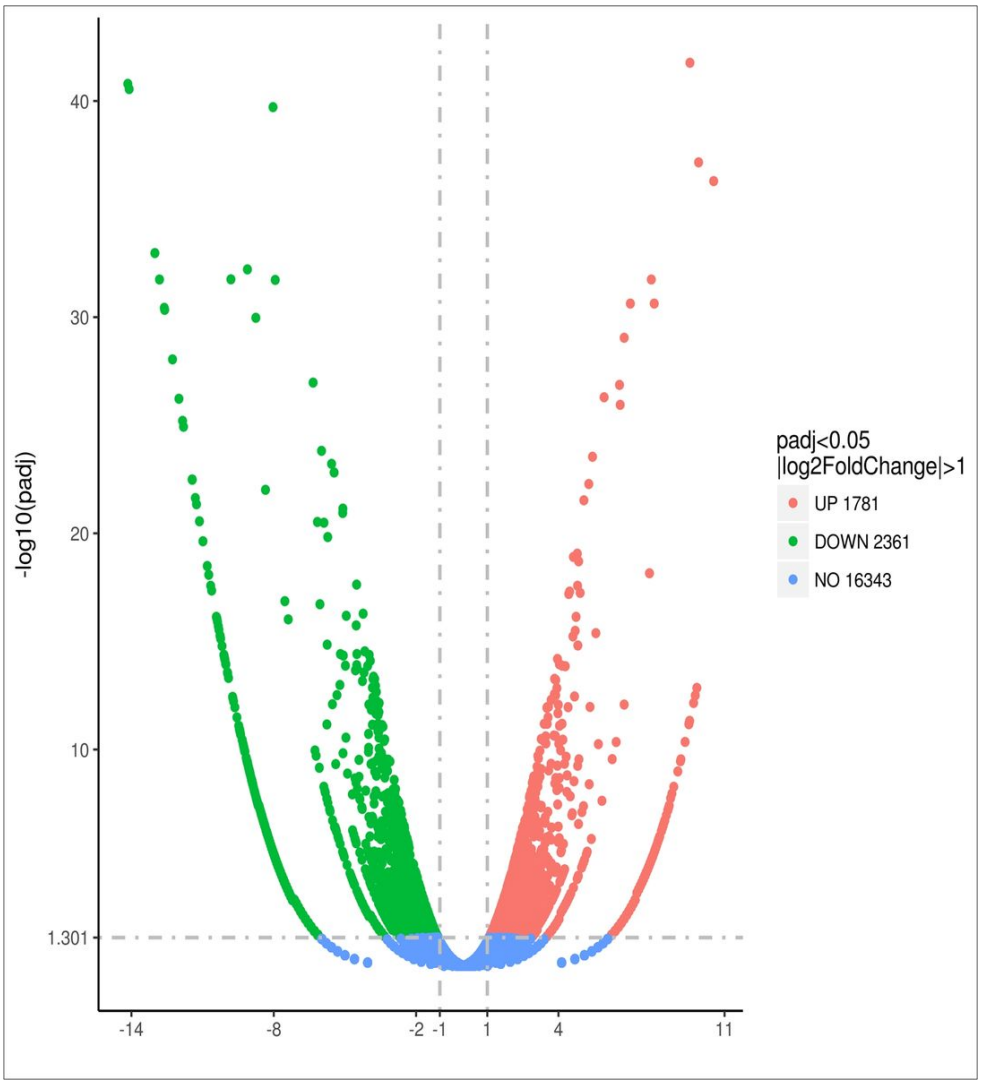

Figure 3

Quantitative comparison of gene expression levels in aortic macrophages of mice with dyslipidemia. Quantitative comparison of gene expression levels in aortic macrophages of mice with dyslipidemia: Phlegm-Dampness Retention syndrome group (PDR group) VS Kidney Yang Deficiency syndrome group (SKYD group).

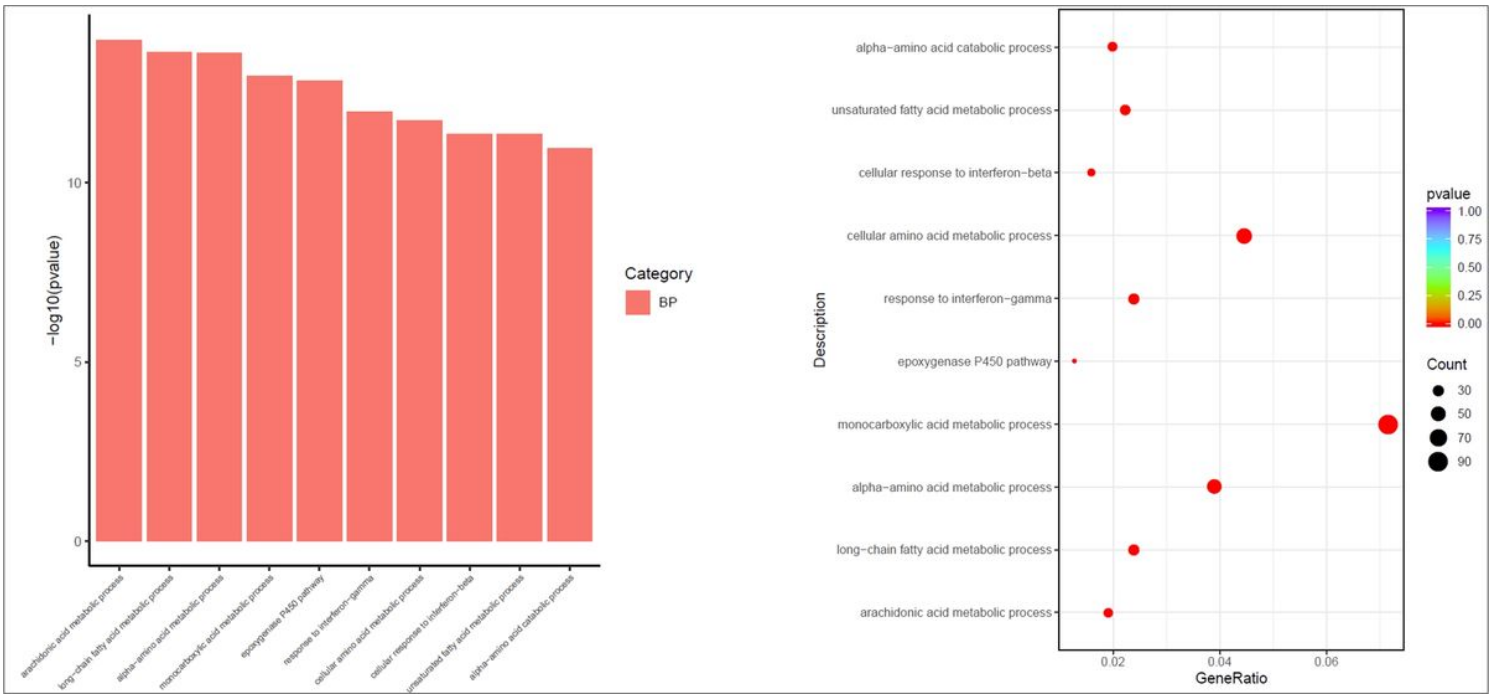

Figure 4

Different biological process of upregulation the PDR syndrome compared with the SKYD syndrome. Different biological process of upregulation in the group with Phlegm-Dampness Retention syndrome compared with the group with Kidney Yang Deficiency syndrome: Bar graph and Dot plot.

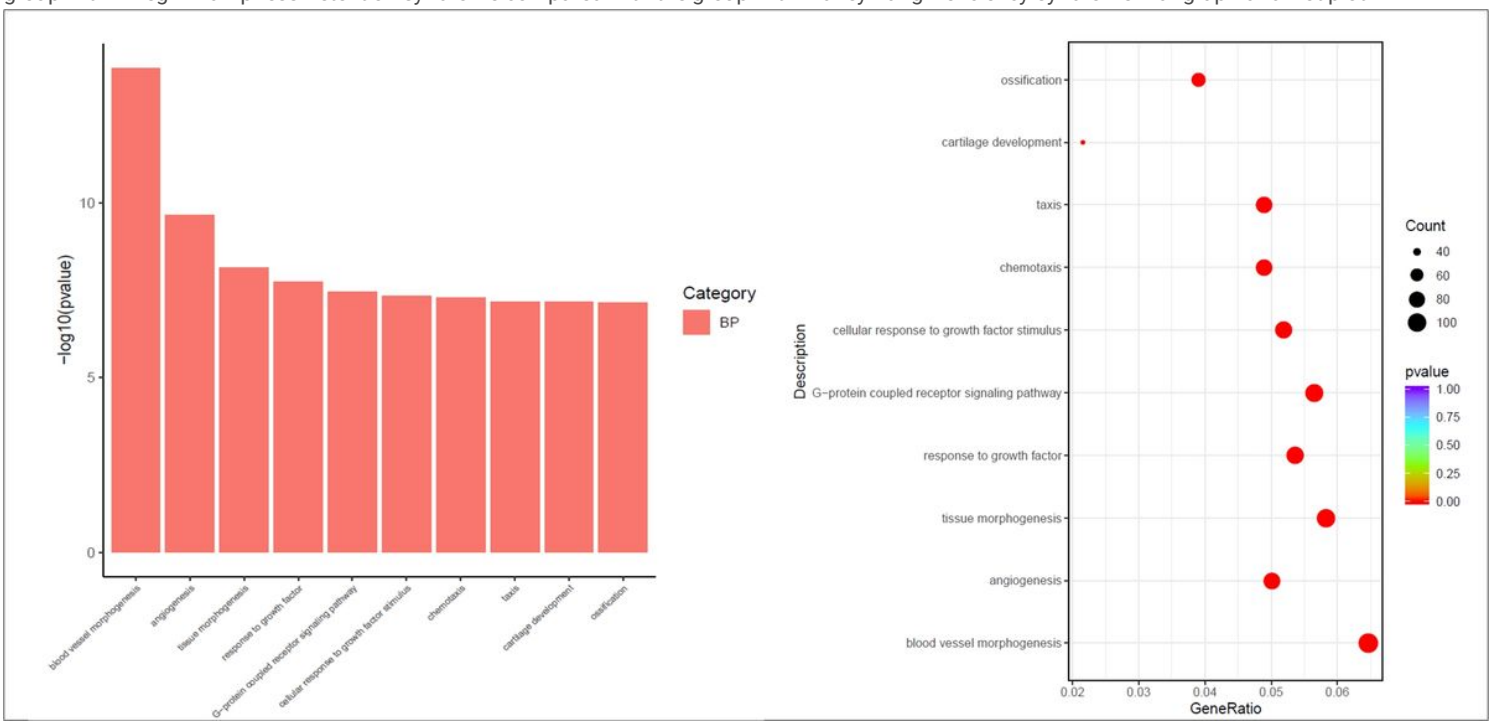

Figure 5

Different biological process of upregulation the SKYD syndrome compared with the PDR syndrome. Different biological process of upregulation in the group with Kidney Yang Deficiency syndrome compared with the group with Phlegm-Dampness Retention syndrome: Bar graph and Dot plot

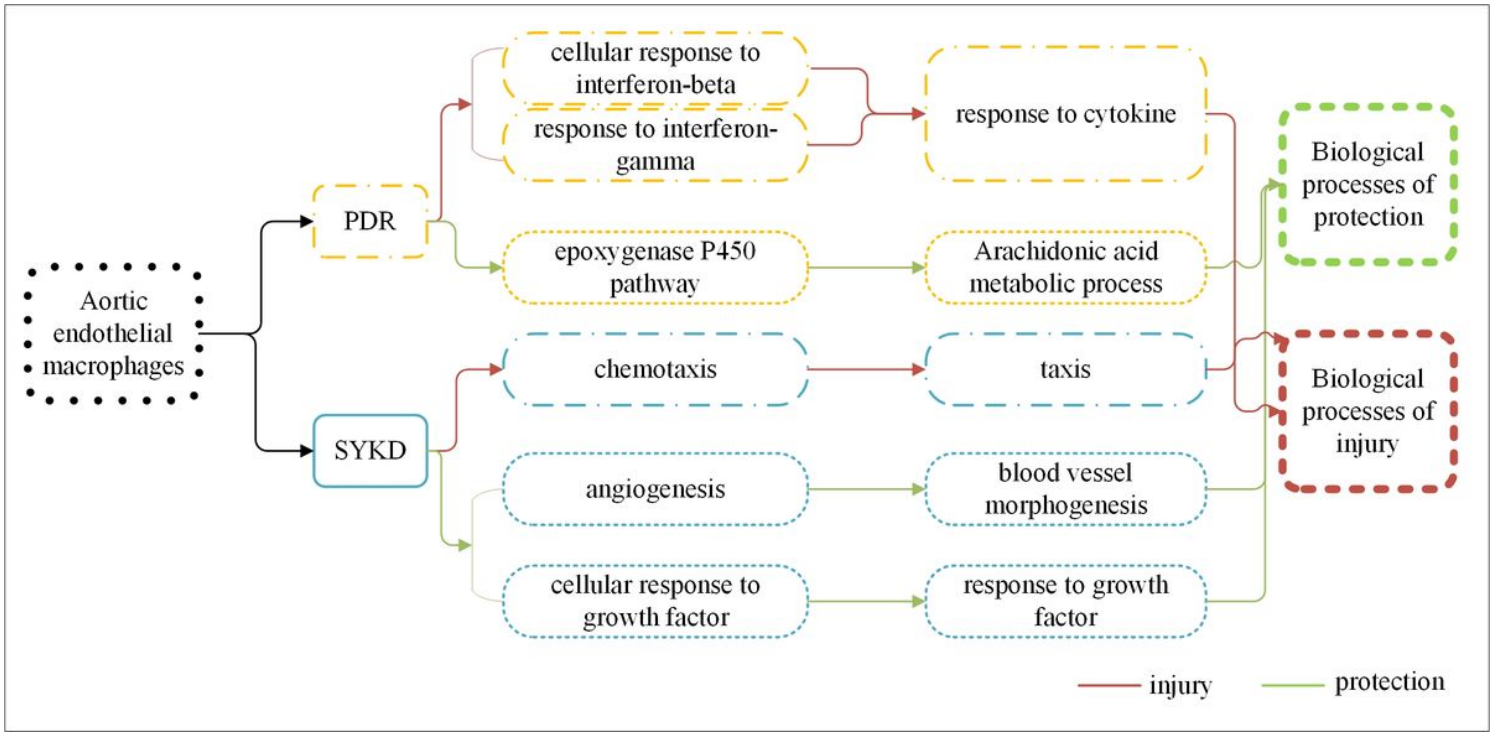

Figure 6

There are different biological processes of macrophages in dyslipidemia PDR syndrome and SKYD syndrome; There are different biological processes of macrophages in dyslipidemia Phlegm-Dampness Retention syndrome group (PDR) and Kidney Yang Deficiency syndrome group (SKYD);

Supplementary Files

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- Highlights20210219.docx
- GraphicalAbstracts.jpg