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Hypolipidemic Effect of Puerarin and Underlying Mechanism Investigation

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Abstract: Excessive intake of fat in the diet is one of the main reasons leading to hyperlipidaemia, which will result in many diseases that endanger the human health. Puerarin, which is extracted from the dry roots of the legume plant *Radix Puerariae*, has been reported to be able to improve the regeneration capability of liver cells, recover the normal liver functions, and prevent the accumulation of fat in the liver. However, research on the regulation of blood lipids has never been referred. In this paper, decrease of the blood lipids in rats by puerarin and the underlying mechanism have been thoroughly discussed. Hyperlipidaemia models were established by feeding the rats with high-fat diet, to which puerarin (10mg/kg, 20mg/kg and 40mg/kg) was then given continuously for 15 days by gavage, and blood indexes of the rats were tested and shown as follows: Puerarin could reduce the serum TC, TG and LDL-C values ($P<0.05$) and elevate the HDL-C values ($P<0.05$), which was thus demonstrated to exhibit significant hypolipidemic activity. Total RNA of the rat livers of the group treated with 400mg/kg of puerarin was extracted, and cDNA library was constructed utilizing mag-bind oligo (dT) enriched mRNA. Gene sequencing was carried out, the resulting data were assessed and their saturation was also analyzed. Besides, screening, cluster analysis of expression patterns, GO functional significant enrichment analysis and PATHWAY significant enrichment analysis for the differential gene expressions were performed. Gene expression profiling exhibited that 780 gene levels of the control and hyperlipidaemia model groups changed, in which 525 genes were up-regulated, and 255 genes were down-regulated. Meanwhile, 495 gene levels of the model group changed compared to those of the puerarin groups, in which 72 genes were up-regulated, 423 genes were down-regulated, and the number of the changed genes both involved was 163. Moreover, pathway of the fatty acid metabolism of the most significant enriched GO term was most closely related to the blood lipid metabolism in the differentially expressed genes. In this pathway, alcohol dehydrogenase 6 in the fatty alcohol cycle was down-regulated by puerarin. As a result, puerarin reduced the generation of fatty acids, regulated the entire fatty acid metabolism, and lowered the blood lipids eventually.

Keywords: Puerarin, Rat, Hypolipidemic Effect, Gene Expression Profiling, cDNA Library

1. Introduction

Excessive intake of fat in the diet is one of the main reasons leading to hyperlipidaemia, which will result in many diseases that endanger the human health [1]. Abnormal fat metabolism or circulation will bring about one or more higher lipids than normal, which is names as hyperlipidaemia. Hyperlipidaemia is a systemic disease representing the over high cholesterol (TC) and/or triglyceride (TG) in blood or over low high density lipoprotein cholesterol (HDL-C), which is called dyslipidemia in modern medicine. Lipids are insoluble or slightly soluble in water, which must be combined with proteins to form lipoproteins. Therefore, hyperlipidaemia usually exists in the form of hyperlipoproteinemia. Currently, the widely recognized hyperlipidaemia includes hypercholesterolemia, hypertriglyceridemia and composite hyperlipidaemia, and the general symptoms of hyperlipidaemia include the excessive serum total cholesterol (TC) and triglyceride (TG), and the insufficient high density lipoprotein-cholesterol (HDL-C) [2, 3].

Radix Puerariae (English: Lobed Kudzuvine Root), which

is the dry root of the leguminous plant Pueraria lobata (Willd.) Ohwi, is one of the common vegetables in some provinces of southern China. Pueraria lobata (Willd.) Ohwi tastes sweet, cool and delicious, and is usually utilized to prepare soups. Starch comprises major of *Radix Puerariae*, 12% of which is composed of flavonoids including more than ten active ingredients such as daidzein, daidzin, puerarin, puerarin-7-xyloside, etc. Besides, puerarin also contains dausterols, amino acids, coumarins, etc [4, 5]. Drug use of puerarin can improve the regeneration capability of liver cells, recover the normal liver functions, stimulate the secretion of bile, prevent the accumulation of fat in the liver, facilitate the metabolism, alleviate myocardial ischemia and cerebral arteriosclerosis, and enhance the immune functions of hepatobiliary cells [6, 7].

Puerarin is one of the saponins extracted from the dry roots of Pueraria lobata (Willd.) Ohwi with the chemical name of 7-Hydroxy-3-(4-hydroxyphenyl)-8-[(3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one. Its molecular formula is C21H20O9, and its chemical structure is shown in Figure 1.

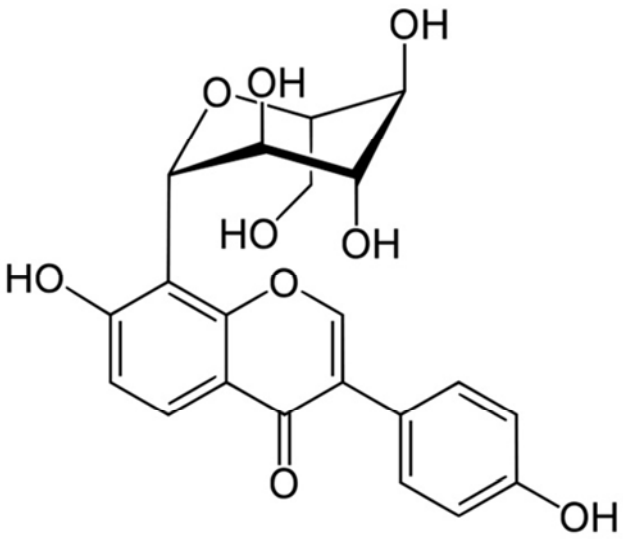

Figure 1. *Chemical structure of puerarin.*

Puerarin has been reported to be able to enhance the immunity, increase the myocardial contractility, dilate the blood vessels, reduce the blood pressure, improve the microcirculation, protect the cardiomyocytes, and resist the aggregation of platelets. Besides, puerarin can prevent the immunity of liver tissues from being damaged, which can effectively reverse the chemical induced liver fibrosis. Meanwhile, puerarin is also able to protect the nephritis and renal failure models, increase the lymphocyte transformation rates of healthy man and cancer patients, reinforce the effect of natural hormones, and apparently stimulate and induce the interference system as well [8, 9, 10].

Absorption, targeting and metabolism of drugs *in vivo* are associated with active proteins such as enzymes, and analyzing and encoding the changing gene expressions of proteins will provide theoretical basis for seeking drug targets and revealing drug metabolism pathways. Besides, analyzing the correlation between gene expression changes and phenotype characteristics of model animals after the treatment of drugs by gene expression profiling will not only provide theoretical evidences for drug research and development, but also facilitate the screening of optimal candidate drugs from series of related compounds. Moreover, principal biochemical metabolism and signal transduction pathways that differentially expressed genes involve can be determined by the new generation digital gene expression profiling [11, 12].

As is known to all, the new generation digital gene expression profiling analysis is advantageous owing to the high throughput, low cost, short sequencing time, facile sequencing process, etc, which has become an important technique among researchers to investigate the molecular biology issues [13]. Besides, the new generation high-throughput sequencing has become most influential in the field of life science [14], in which Hiseq2000 sequencing system has been replacing the traditional Sanger sequencing gradually and becoming a novel method to deal with genome sequencing and related research.

In this paper, our group revealed the hypolipidemic effect of puerarin for the first time, studied the influence of puerarin on the blood lipid metabolism and gene levels of hyperlipidaemia model rats using digital gene expression profiling based on the experimental results, and then explored the pharmaceutical mechanism of puerarin. Rats were utilized as the model animals in the experiment due to the fact that 90% of their 25000 genes matched those of human. The new genome sequencing results also showed that chromosomes of rats contained 2.75 billion base pairs, which were quite close to the 2.9 billion base pairs of human chromosomes [15].

Thereby motivated, hyperlipidaemia model rats were obtained by feeding them with high-fat diet, to which different amounts of puerarin were then fed. Then the rat livers of each group were extracted to acquire the total RNA and establish the cDNA library. Gene sequencing was then carried out employing the new generation Illumina HiSeq ™ 2000 sequencing system, the resulting data were assessed and the saturation was also analyzed. Besides, screening, cluster analysis of expression patterns, GO functional and PATHWAY significant enrichment analyses of differential gene expressions were analyzed.

2. Materials and Methods

2.1. Reagents and Apparatus

99% pure puerarin was purchased from Sobeo Biotech Co., Ltd as white needle crystal powders and classified as flavonoid. The molecular weight of puerarin was 416.38. and the melting point ranged from 187 to 189°C. The purchased puerarin was ground with 0.5% sodium carboxymethyl cellulose (CMC-Na) and prepared into the required suspensions before use. Kits for the determination of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol

(LDL-C) were bought from Biosino Bio-Techenology and Science Incorporation. RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) kits were purchased from Takara Biotechnology (Dalian) Co., Ltd. Blood components were analyzed using PRONTO-E automatic biochemistry analyzer (Pentium enhanced type, Italy). Gene analysis was carried out utilizing Illumina2000 DNA sequencer and gene analyzing system, Illumina HiSeq 2000 sequencer and ABI SOLiD system.

2.2. Animal Experiments

Experiments were approved by the Care and Use of Laboratory Animals Committee of Inner Mongolia Autonomous Region and the Animal Ethics Committee of Inner Mongolia Medical College, and were strictly performed according to the NIH guide for the Care and Use of Laboratory Animals. Clean grade male Wistar rats weighed 160-180 g were used in the experiments, which were provided by the experimental animal research center of Inner Mongolia Medical College. In the conditions of room temperature at 18-22°C, humidity at 50%-60%, lighting time at 12h/d and free drinking water, 50 rats were fed with normal diet for one week, which were then randomly divided into 5 groups with 10 rats each. The control group were fed with basic diet, and the rest were fed with high fat diet (3% cholesterol, 10% lard, 0.5% bile acid sodium and 86.5% basic diet). Then 10mg/kg, 20mg/kg, 40mg/kg of puerarin were given to three individual groups according to the optimal dosages determined by the previous experiments. Same volumes of 0.5% CMC-Na were utilized in the model and control groups. All the rats were continuously fed with 0.4 ml/100 g of drugs for 15 days. The growth of rats was observed during the experiment, and their body weights were measured every three days.

2.3. Determination of Blood Lipids

After feeding, rats that have not been fed for 12 h (water deprivation) were decollated and the blood was sampled, centrifuged to isolate the serum. Serum TC, TG, HDL-C and LDL-C values were then determined by the automatic biochemistry analyzer. Meanwhile, rat livers were rapidly exteriorized, the right lobes of which were cut (more than 100 mg), put in the freezing pipes and then stored in the liquid nitrogen.

2.4. Extraction and Determination of RNA

50 mg frozen rat livers (samples with the pharmacodynamically optimal dosages were selected, *vide infra*) were put in a mortar and quickly ground into powders in the liquid nitrogen, and the total RNA was extracted according to the requirements of the kit. 1 μL of RNA was taken, and the absorbances (A) at 260 and 280 nm were determined after the dilution with DEPC treated water. Purity was represented by the ratio of A260 nm/A280 nm, and 28S:18S>1 indicated the result was qualified. Another 3 μL of RNA was determined by 1.2% agarose gel electrophoresis to observe the integrity of RNA under UV lamp [16].

2.5. Preparation of cDNA Library and Gene Sequencing

Total RNA of the samples was extracted, and fragmentation buffer was then added to the obtained mRNA by mag-bind oligo (dT) enriched mRNA to join the fragments into short fragments. Then the resulting mRNA was used as the template, first strand cDNA was synthesized by random hexamers, to which were added the buffer, dNTPs, RNase H and DNA polymerase I to synthesize the second strand cDNA. Then the above product was purified by QiaQuick PCR kit and eluted by EB buffer, fragmented cDNAs were then end-repaired, A-tailed, and adaptor ligated. Then different lengths of fragments were purified by agarose gel electrophoresis and amplified with PCR to complete the entire library. The built library was sequenced by Illumina HiSeq2000 [17].

2.6. Data Analysis

Raw image data produced by the sequencer were transformed into sequence data by base calling and named as raw data or raw reads. The results were saved as fastq files, undesired data were removed to obtain the clean reads for further analysis. Alignment software SOAPaligner/soap2 for short reads was utilized to align the clean reads to the reference genome and reference gene sequences, respectively [18].

The number of the reads of the only matched gene and the total number of reads that have been aligned to the reference sequences were utilized to calculate the gene expression quantities by the RPKM method, and information related to the gene was then provided (e.g. coverage, Symbol, functional annotation information, etc) [19]. Then cluster software was employed to perform simultaneous hierarchical cluster analysis for the differentially expressed genes and experimental conditions utilizing Euclidean distance as the distance matrix calculation formula [20, 21].

2.7. Statistical Analysis

Results were expressed as means ± SD. All statistical analyses were carried out using one-way ANOVA and Student's t-test. A difference between mean values was considered significant if the p value obtained was less than 0.05, and the screening condition for differentially expressed genes were FDR≤0.001 and |log2Ratio|≥1.

3. Results

3.1. Significant Hypolipidemic Activity of Puerarin

All the rats during the experiment were of neat and shiny hair, normal mental states, behaviors and excrements. The weight increases of each group were comparative with no significant differences ($P > 0.05$), and no natural death occurred. Blood tests show that serum TC, TG, and LDL-C values of the model group were significantly higher ($P < 0.01$) than those of the control group, whereas HDL-C was significantly lower ($P < 0.01$), suggesting the dyslipidemia of the model group and the hyperlipidaemia rat model has been

successfully established. Meanwhile, serum LDL-C of each puerarin group apparently decreased ($P < 0.05$) compared to that of the model group, the medium high dose TC ($P < 0.01$) and the low high dose TG ($P < 0.01$) both significantly reduced, whereas the HDL-C value obviously increased ($P < 0.05$) (Figure 2). Generally speaking, puerarin was of significant hypolipidemic activity, and pharmacodynamic indexes of the group treated with 400mg/kg puerarin were best compared to those of other groups. Thus, liver tissues obtained from this group were selected for further investigations below.

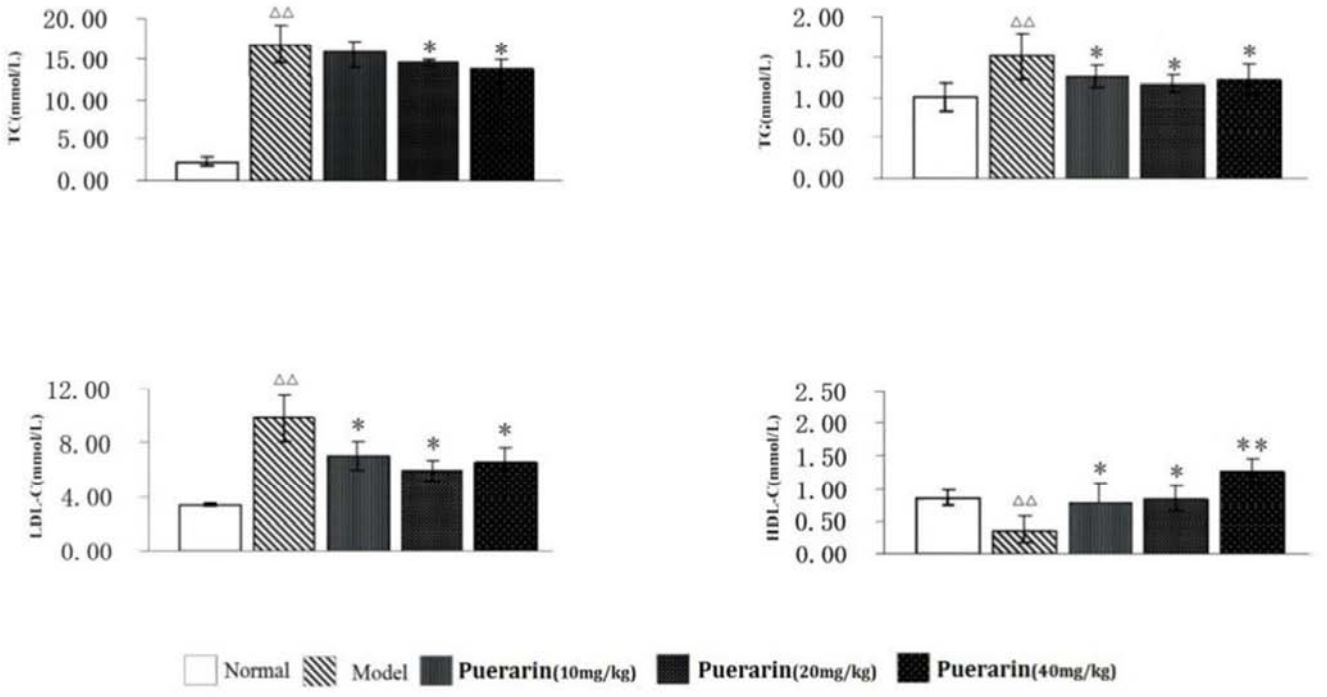

Figure 2. *Influence of piperine derivatives on serum TC, TG, LDL-C and HDL-C values of the hyperlipidemia rats.*

Compared to the control group: $\Delta\Delta P < 0.01$; Compared to the model group: $*P < 0.05$, $**P < 0.01$. Blood test show that serum TC, TG, and LDL-C of the model group all significantly increased ($P < 0.01$) compared to those of the control group, whereas the HDL-C value significantly decreased ($P < 0.01$), suggesting that the hyperlipidemia rat model has been successfully established. Serum LDL-C of each puerarin group apparently decreased ($P < 0.05$) compared to that of the model group, the medium high dose TC ($P < 0.01$) and the low high dose TG ($P < 0.01$) both significantly reduced, whereas the HDL-C value obviously increased ($P < 0.05$), in which the 400mg/kg puerarin group exhibited extremely apparent increase ($P < 0.01$).

3.2. Remarkable Changes of Gene Expression Quantities of the Liver Tissues of the Puerarin Group Compared to Those of the Hyperlipidaemia Group

Total RNA of the liver tissues of the group treated with 400mg/kg puerarin was extracted and sequenced, the data were assessed, and gene saturation was analyzed. As shown in Table 1, 28S: 18S values of the three samples were all more than 1, indicating that RNAs of the samples were integral and the tested samples were qualified. Besides, quality assessment of the sequenced reads are shown in Table 2, the overall sequencing rates of the three groups reached 88.31%, 85.66% and 87.29%, which have met the experimental requirements. Meanwhile, sequencing saturation analysis could also be used to quantify the sequences. The number of detected genes would increase with increasing sequences (reads number) and then gradually level off until the number of sequences reached certain values, suggesting that the number of the detected genes saturated and thus met the experimental requirements (Table 2).

Table 1. *Analyses of RNA integrity and sequencing saturation.*

Sample No.	Sample Name	Tube Count	Concentration (ng/μl)	Volume (μl)	Total (μg)	RIN	28S:18S
1	control	1	8258	40	319.7	7.9	1.22
2	model	1	8239	40	347.1	8.2	1.26
3	puerarin	1	11014	40	339.8	8.2	1.32

Table 2. *Quality evaluation of the reads.*

Map to Genome	blank control		model		Podophyllotoxin	
	reads number	percentage	reads number	percentage	reads number	percentage
Total Reads	114513569	100.00%	112451285	100.00%	110412583	100.00%
Total BasePairs	597548627	100.00%	594751254	100.00%	584571246	100.00%
Total Mapped Reads	101126933	88.31%	96325771	85.66%	96379144	87.29%
perfect match	60852511	53.14%	81257299	72.26%	79618514	72.11%
<=3bp mismatch	40274422	35.17%	15068472	13.40%	16760630	15.18%
unique match	92240680	80.55%	87835699	78.11%	87965705	79.67%
multi-position match	8886253	7.76%	8490072	7.55%	8413439	7.62%
Total Unmapped Reads	13386636	11.69%	16125514	14.34%	14033439	12.71%

The number of the reads of the only matched gene and the total number of reads that have been aligned to the reference sequences were utilized to calculate the gene expression quantities and provide relevant information about the specific gene. The results exhibit that 780 gene levels of the control and model groups changed, in which 525 genes were up-regulated and 255 genes were down-regulated. Meanwhile, 495 gene levels of the model group changed compared to those of the puerarin groups, in which 72 genes were up-regulated and 423 genes were down-regulated (Figure 3).

Gene expression quantities were calculated utilizing the number of the reads of the only matched gene and the total number of reads that have been aligned to the reference sequences. The results show that 780 gene levels of the control group and model group changed, in which 525 genes were up-regulated and 255 genes were down-regulated; 495 gene levels of the model group changed compared to those of the puerarin group, in which 72 genes were up-regulated and 423 genes were down-regulated.

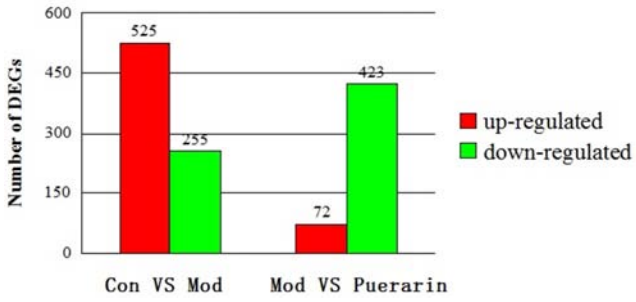

Figure 3. *Gene expression quantity changes of liver tissues.*

3.3. Functional Analysis and GO Functional Analysis of Differentially Expressed Genes

Cluster analysis of the gene expression patterns show that the expressed genes were of no more than 0.001 of FDR and no less than 2-fold of differences. Considering that genes with similar expression patterns often would exhibit similar functions, cluster results obtained with Java Treeview [22] displayed that 163 genes were qualified in total (Figure 4).

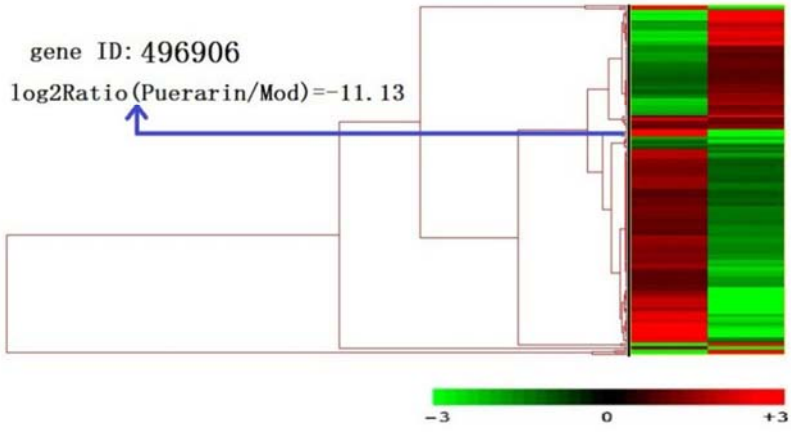

Figure 4. *Screening and cluster analysis of expression patterns of differentially expressed genes.*

Each column represents an experimental condition (The left column is control group VS model group, the right column is model group VS puerarin group). Each row represents a gene, and different multiples of expression changes are marked with different colors, in which red represents up-regulation and green represents down-regulation. The result show that 163 changed genes were involved in the two experimental conditions, control group VS model group mainly consisted of up-regulated genes, and model group VS puerarin group mainly consisted of down-regulated genes. Gene 496906 on the left of the figure was involved in the fatty acid metabolism pathway, and the interval parts were down-regulated and up-regulated, respectively.

Cluster analysis of expression patterns was also incorporated in Gene Ontology (abbreviated as GO) functional analysis, which could easily discern the expression

patterns of all the differential genes with the same function. The results show that a number of biological processes were participating in the blood lipid metabolism, and the screening of signal pathways based on the molecular functions of genes exhibit that the gene 496906 was expressed in the fatty acid metabolism signal pathway, and the value of log2 Ratio (Puerarin/Mod) was -11.12541 (Figure 4).

3.4. Pathway Significant Enrichment Analysis

Compared to the model group, the number of DEGs with pathway annotation of the puerarin group was 323, and the number of all genes with pathway annotation was 17254. GO functional analysis above has verified that the fatty acid metabolism signaling pathway was the research concern in the experiment, the expression number of differential genes annotated in this pathway was 7, which comprised 2.17% of the total differential genes. Besides, 106 genes were annotated in this pathway, which comprised 0.61% of the total genes. Qvalue was 1.88E-01, which was no more than 0.05. Therefore, this pathway has been demonstrated to be significantly enriched for the differentially expressed genes.

4. Discussion

Blood lipid metabolism disorders (such as hyperlipidaemia) were closely related to the formation and development of atherosclerosis (AS), which would lead to cardiovascular diseases and thus greatly endanger the human health in recent years. Although the traditional hypolipidemic drugs were potentially effective, their side effects were also very obvious. Besides, innovative research on these drugs in China was not competitive compared to that in the foreign countries, and seeking safe and effective hypolipidemic drugs from the natural medicines has become one of the research spotlights, which was majorly utilized to develop novel hypolipidemic drugs [23].

Previous studies have demonstrated that puerarin contains saponins which protect the liver tissues from immune damages. Besides, puerarin absorbed by stomach is of a wide range of physiological activities including protection of the liver from being damaged, induction of apoptosis of hepatic stellate cells, effective reversal of the chemical-induced liver fibrosis, prevention of the liver injury induced by CCl_4, etc [24]. Meanwhile, flavonoids of pueraria will increase the blood flow in brains and coronaries as well as moderately improve the cerebrovascular tension, flexibility and pulsation of the hypertension and coronary patients. Moreover, pueraria is not only able to stimulate the cerebral and peripheral circulations of animals and humans remarkably, but also apparently reduce the oxygen consumption of ischemic myocardium and prevent the hearts against ultrastructural damages induced by ischemia-reperfusion [25].

Regulating effect of puerarin on blood lipids was discovered by our group for the first time, which has been verified by animal experiments. The results denoted that puerarin could significantly reduce serum TC, TG and LDL-C whereas increase serum HDL-C of the hyperlipidemia rats, indicating that puerarin could obviously regulate the blood lipid metabolism disorder and reduce the risk of suffering from AS.

Blood lipids mainly consist of cholesterol (or total cholesterol TC) and triglycerides, which exist as non-free states in blood circulation and will be transported as lipoproteins after binding proteins. Closely associated lipoproteins are mainly divided into chylomicrons, very low density (pre-β) lipoproteins (VLDL), low density (β-) lipoproteins (LDL) and high density (a-) lipoproteins (HDL) according to the physical and chemical properties (such as electrophoretic mobility and density after ultracentrifugation) [26]. Major lipoproteins transported in blood are triglycerides, which are carried most frequently by chylomicrons, and exogenous triglycerides are transported to the venous system through the thoracic ducts. In the fat capillaries and muscle tissues, 90% of chylothorax triglycerides are transported by a specific group of esterase. However, the excretion of LDL is still not very clear, 70% of which are removed by livers, and the majority of circulating LDL are removed by active receptor sites, which are located on the liver cell surfaces that are bound with specific apolipoprotein B (apoB). In the LDL associated ligands, LDL bound by LDL receptors is much less than that removed by the non-LDL receptors in the circulation including the intake and clearing by the receptors of macrophages, which move to the artery wall and become foam cells of the arteriosclerosis plaque [27]. Besides, hyperlipidemia is resulted from excessive VLDL, clearing obstacle and excessive LDL transferred from VLDL. The excessive liver VLDL may be originated from obesity, diabetes, excessive alcohol, nephrotic syndrome or genetic defect. Increasing LDL and TC are also frequently associated with high triglycerides in blood, and clearing obstacle of LDL is related to the structure defect of apoB [28].

Research on drug-related genes in the entire genome will be arduous. However, the novel digital gene expression (DGE) profiling for the detection of gene expression levels is of high efficiency and throughput, which is able to examine the gene expression profiling of the liver tissues of rats and further predict the function mechanism of drugs [29]. As shown in the experimental results, effect of the puerarin group on the model group almost completely reversed the gene regulation trend of the control group by the hyperlipidemia group, which could be identified by screening the differentially expressed genes. Besides, Gene Ontology functional significant enrichment analysis showed that the reversal regulation was fairly apparent in 163 genes involved in the two conditions, whereas only a small number of genes exhibited same trends, indicating that puerarin could change the total gene levels of the hyperlipidemia models of rats by various approaches. According to the detection method of differential genes based on sequencing published in *Genome Research* by Audic S.et al [30], differentially expressed genes of the two samples were strictly screened, and the subsequent analyses including cluster analysis of expression patterns, Gene Ontology functional significant enrichment analysis and Pathway significant enrichment analysis were all based on

differentially expressed genes. Meanwhile, the differentially expressed genes were of FDR no more than 0.001 and no less than 2-fold of differences [31].

Gene Ontology (abbreviated as GO) is an international standardized gene function classification system, which provides a dynamically updated controlled vocabulary to fully describe the properties of genes and gene products in organisms. GO consists of three ontologies, which describe the molecular function of genes, the cellular component and the biological process involved, respectively. Besides, GO functional analysis also incorporates the cluster analysis of expression patterns, which could conveniently show the expression patterns of all the differential genes [32]. According to the molecular function of genes, fatty acid metabolism was of significant enrichment in the differentially expressed genes by screening GO associated with the blood lipid metabolism of animals, and some genes also participated in many biological processes related to the blood lipid metabolism. Moreover, it has been discovered that gene 496906 was involved in the fatty acid metabolism with extremely high expression quantities (Figure 5A).

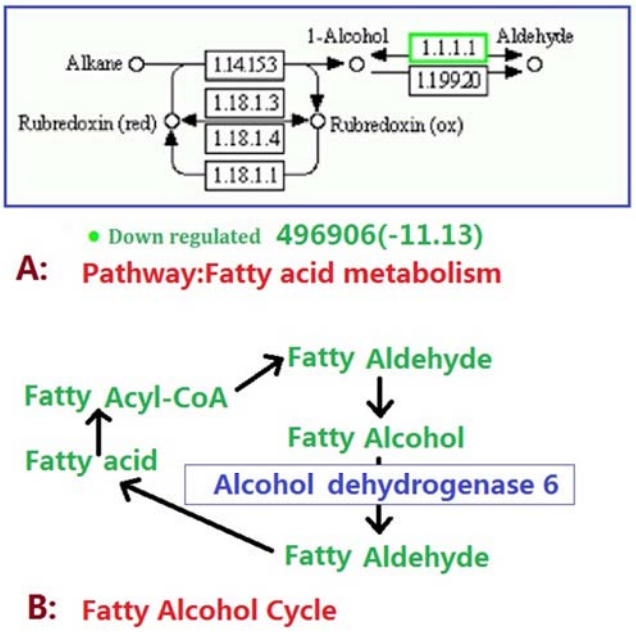

Figure 5. *A. Influence of puerarin on the fatty acid metabolism pathway of rats (KEGG database).*

The down-regulated genes are marked in green. The fatty acid metabolism of the model group was down-regulated compared to that of the puerarin group, and the corresponding log2 Ratio (Puerarin / Mod) of gene 496906 was -11.12541.

B. shows the effect of alcohol dehydrogenase on the decrease of fatty aldehyde generated by fatty alcohol and the decreasing generation of fatty acid.

Gene 496906 belongs to alcohol dehydrogenase 6 (class V), which is an enzyme in human encoded by the ADH6 gene. This gene, which is a member of the alcohol dehydrogenase family, encodes class V alcohol dehydrogenase. Gene members of the above family are able to metabolize a wide variety of substrates including retinol, ethanol, aliphatic alcohols, hydroxysteroids and lipid peroxidation products. This gene expressed both in the stomach and liver contains a glucocorticoid response element upstream of its 5' UTR that is a steroid hormone receptor binding site. Besides, the deduced amino acid sequence of the open reading frame of the gene was approximately 60% positionally identical with other alcohol dehydrogenases known before. Moreover, the gene may be of distinct physiologic functions as well. [33]

Different genes exhibit various biological functions synergically in vivo, which can be better understood by the analysis based on pathway. KEGG is the major public database related to pathway, and pathway significant enrichment analysis utilizes KEGG pathway as the unit. Hypergeometric test is applied to find out the significantly enriched pathway in the differentially expressed genes that have been aligned to the whole genome [34]. The fatty acid metabolism signal pathway in this study was significantly enriched in the differentially expressed genes. On the other hand, fat is linked by three ester bonds of one glycerol and three fatty acid molecules, which will be decomposed into glycerol and fatty acids by lipases that are of specificity. Glycerol can be phosphorylated into phosphoglycerol that will be oxidized after forming Pyr in EMP pathway, and fatty acids can be degraded by series of enzymes by α-oxidation, ω-oxidation and the most important β-oxidation. β-oxidation refers to the thiolysis of one molecule of acetyl-COA from β-carbon of the fatty acids that have undergone catalysis by series of enzymes, activation, dehydrogenation, water addition, and dehydrogenation and thiolysis, and the degradation takes place in the mitochondria [35, 36]. In the pathway of β-oxidation, the alcohol dehydrogenase gene was down-regulated by puerarin, which would increase the degradation of fatty acids. The mechanism can be more clearly expressed as follows: Fatty alcohol cycle and metabolic pathways altered consists of two components, fatty alcohol dehydrogenase (reaction 1), that catalyze the sequential oxidation of fatty alcohol to fatty acid. Alcohol dehydrogenase 6 is a member of the alcohol dehydrogenase family, the inhibition of which will eventually reduce the generation of fatty acids [37], and the mechanism resembles those of piperic acid and podophyllotoxin that are able to lower the blood lipids (the two mechanisms will be thoroughly investigated in subsequent papers) (Figure 5B).

The drawback of the experiments herein lay in the selection of male Wistar rats for the experimental animals. Although it has been proposed by Richard Gibbs that 'copies' of almost all the known genes related to human diseases could be found in rats [15], any non-human animal models are not comparative with the human environment because about 10% of the genes of rats including those of some encoded merosins are shared with mice, which do not exist in human. Therefore, genes that human do not possess may be involved in the related differential genes, which should be paid more attention in the future. In addition, rats also have more genes that can decompose the toxic substances than human, suggesting that rats will exhibit more significant toxin removing ability compared to human. Thus, animal experiments will be

replaced by tissue culture in our future investigations.

Puerarin is the single chemical compound that is of definite chemical structure extracted from *Radix Puerariae*, and puerarin is of significant hypolipidemic activity, the mechanism of which may be related to the fatty alcohol cycle in the pathway of down-regulating the fatty acid degradation and the alcohol dehydrogenase genes of β-oxidation.

In summary, gene level changes of the animals have been compared before and after the treatment of puerarin, gene changes in common under different circumstances have been studied, biological functions such as blood lipid levels have been predicted, and the target of the compound and the mechanism have been further investigated. Besides, the effect of hypolipidemic compounds based on genomics has been evaluated, the correlation between specific DNA polymorphism and different hypolipidemic environments could be obtained by comparing different samples, which further revealed the effect of the compound on the regulation of transcription and translation levels and different metabolic pathways in experimental animals, and established the relationship between signal pathways related to blood lipids and gene network. The results above will provide guidance for the hypolipidemic compounds utilized in human in the future, which will be able to be widely applied and thus of profound significance [38].

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