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Original Article

Effects of Gypenoside XLIX on fatty liver cell gene expression in vitro: a genome-wide analysis

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Abstract: Objective: To perform Genome-wide analysis of Gypenoside XLIX (Gyp-XLIX) in the treatment of fatty liver cells. Methods: The gene profiles of 3 normal liver cells, 3 fatty liver cells, and 3 fatty liver cells treated with Gyp-XLIX were detected by high-throughput sequencing to identify the differentially expressed genes (DEGs) in fatty liver treated by Gyp-XLIX. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were used to explore the biological functions of DEGs. By constructing lncRNA-mRNA co-expression network of DEGs, network node genes were mined. Possible target genes of differentially expressed lncRNA were predicted by cis regulation. Results: 782 DEGs were screened out; that is, 172 genes were highly expressed in fatty liver cells, and the expression decreased to the level of normal liver cells after Gyp-XLIX treatment; 610 genes were under expressed in fatty liver cells, and the expression increased to the level of normal liver cells after Gyp-XLIX treatment. Functional analysis of KEGG and GO showed that DEGs process DNA-binding transcription factor activity and ion transmembrane transporter activity in the plasma membrane region. This mediates glycerophospholipid metabolism, bile secretion, fatty acid degradation and other signaling pathways. lncRNA analysis showed that the expression of 16 lncRNAs was low in fatty liver cells, and the expression was increased to the level of normal liver cells after Gyp-XLIX treatment. Target gene prediction showed that 16 differentially expressed lncRNAs had cis potential to regulate target genes, among which lncRNA RPARP-AS1 had a high degree of relationship with other genes. lncRNA-mRNA co-expression network results showed that lncRNA RPARP-AS1 may acted on *NFKB2*. Conclusion: LncRNA was differentially expressed in fatty liver cells and Gyp-XLIX treated fatty liver cells, and lncRNA RPARP-AS1 may be a regulatory gene in Gyp-XLIX treated fatty liver.

Keywords: Gypenoside XLIX, fatty liver, LncRNA, mRNA, genome-wide analysis

Introduction

Fatty liver refers to the excessive accumulation of fat in liver cells caused by various reasons, which is a common pathological change of the liver. Fatty liver poses a serious threat to health, and has become the second most common liver disease after viral hepatitis, and its incidence is increasing with the onset age getting younger [1]. Fatty liver disease is generally divided into two categories: alcoholic fatty liver disease and non-alcoholic fatty liver disease (NAFLD). The prevalence of NAFLD is as high as 25% globally [2]. NAFLD is an emerging risk factor for the development of diabetes mellitus, liver fibrosis, and cardiovascular disease [3]. In addition, with the increasing incidence of obesity and related metabolic diseases, NAFLD is predicted to be a major cause of chronic liver disease. Histologically, patients with NAFLD exhibit significant hepatic steatosis, liver inflammation, and even hepatocyte necrosis. If not effectively controlled, hepatic steatosis will develop into life-threatening diseases such as cirrhosis, hepatocellular carcinoma, and liver failure [4, 5]. However, there is no evidence-based pharmacologic approach for the treatment of NAFLD. The occurrence and development of NAFLD is a complex process with multifactor effects, involving lipid peroxidation, mito-

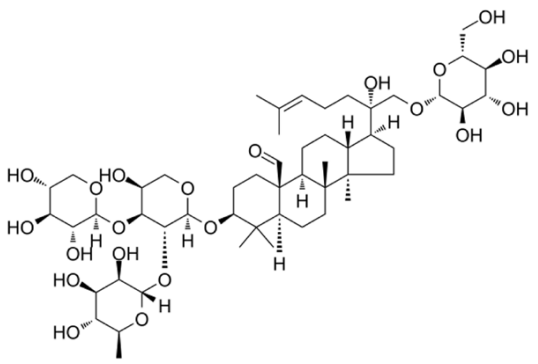

Figure 1. Gypenoside XLIX structure.

chondrial dysfunction, inflammatory reaction factor imbalance, and immune dysfunction [6-8].

Gynostemma pentaphyllum is the rhizomes or whole grass of Gynostemma pentaphyllum (Thunb.) Makino of the gourd family. Modern pharmacological studies have shown that Gynostemma pentaphyllum has the effects of regulating blood lipids, anti-tumor, lowering blood glucose, anti-aging, lowering blood pressure, enhancing immunity, calming and relieving pain [9]. Gypenoside XLIX (Gyp-XLIX) is a dammarane glycoside, which is the main component of gynostemma pentaphyllum. Gyp-XLIX is a selective peroxisome proliferator-activated receptor (PPAR)-α activator that inhibits the overexpression and hyperactivity of cytokine-induced vascular cell adhesion molecule-1 (VCAM-1) in human endothelial cells [10]. Gypenoside can effectively prevent and treat hyperlipidemia and atherosclerosis, and this regulation of blood lipids is related to inhibiting the production of free fatty acids by adipocytes and promoting the synthesis of neutral fats. Gypenoside can reduce the concentration of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) in serum of high-fat animals to varying degrees, significantly increase the blood high density lipoprotein (HDL), and reduce endothelin, so as to reduce the incidence of atherosclerosis. It is of great value in the research and development of new drugs for the prevention and treatment of cardiovascular and cerebrovascular diseases [11]. In recent years, Gypenoside isolated from Gynostemma pentaphyllum (Thunb.) Makino is considered as a lead compound with great potential for development. For example, Gypenoside has a protective effect on fatty liver induced by a high fat and high cholesterol diet and alcohol in rats [12], and can also improve non-alcoholic steatohepatitis induced by high fat diet through farnesoid X receptor activation [13]. Thus, it can be seen that Gypenoside plays an important role in the treatment of fatty liver. However, the gene expression in fatty liver treated by gypenoside has not been reported, so this study analyzed the differentially expressed genes in fatty liver treated by Gypenoside XLIX (Gyp-XLIX) through the whole genome.

Materials and methods

Materials

Human hepatocyte (LO2) cells were purchased from the Chinese Academy of Sciences Type Culture Collection (Shanghai, China) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, SH30022.01B, HyClone, UT, USA) supplemented with 10% fetal bovine serum (10082147, Gibco, MD, USA) at 37°C in a 5% CO_2 atmosphere. A lipid mixture (LM, Sigma L0288, USA) was used to model cellular steatosis. First, the cells were cultured with LM with a final concentration of 2.5% for 24 h, and the control group was cultured with ordinary medium. LM-induced hepatocytes were divided into the Lipid group and the Lipid+XLIX group. The Lipid+XLIX group was treated with Gyp-XLIX (50 μM) [14] and cultured for 24 h. Gyp-XLIX was purchased from Selleck Chemicals (S9177) and its chemical structure is shown in **Figure 1**. The research process of this study is shown in **Figure 2**.

Main reagents and instruments

TRIzol reagent was purchased from Invitrogen (Carlsbad, California, USA). Qubit RNA Assay Kit and RNA Nano 6000 Assay Kit were purchased from Life Technologies, USA. NEBNext@ UltramRNA Library Prep Kit was purchased from New England Biolabs.

The RNA-seq sequencer (Illumina Hiseq X Ten) was purchased from Illumina. The PCR instrument (Veriti) was purchased from Ap-plied Biosystems. Ultramicroscopic spectrophotometer (Nanodrop 2000) was purchased from Thermo Fisher Scientific. The Bioanalyzer

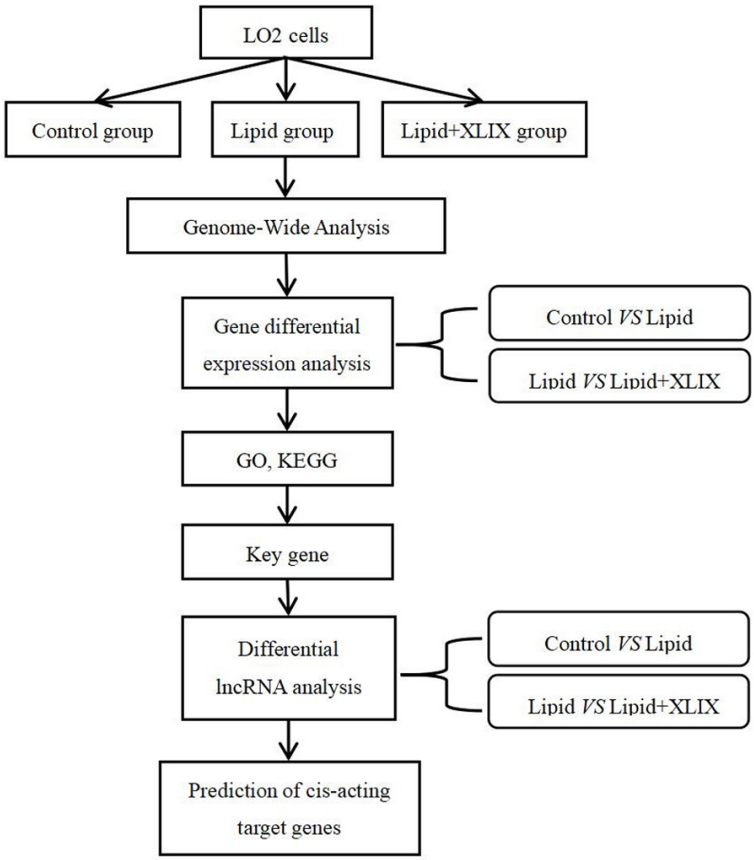

Figure 2. Diagram of the study process.

(Agilent 2100) was purchased from Agilent Technologies.

Extraction and detection of total RNA and construction of library

Total RNA was extracted by TRIzol method. The quality of total RNA was preliminarily determined by agarose gel electrophoresis and ultramicroscopic spectrophotometer. The concentration and integrity of total RNA were detected by bioanalyzer and ultramicro-spectrophotometer, respectively. The qualified total RNA samples can be used to build the database. mRNA was enriched by polyoligo (dT) method, and cDNA libraries were constructed according to the instructions for NEBNext@UltramRNA Library Prep Kit. After the completion of library construction, Wuhan Kangjian Technology Co., Ltd. was commissioned to conduct double-terminal sequencing with a read length of 150 bp on the Illumina Hiseq X Ten sequencing platform.

High throughput sequencing

Three normal liver cells, three fatty liver cells and three fatty liver cells treated with Gyp-XLIX were sequenced using an Illumina HiSeq X Ten second-generation sequencing platform. After RNA extraction, purification, and library construction, the original image data obtained by Illumina HiSeq sequencing was converted into sequence data (FASTQ format) through Base Calling, and the original sequencing data file was obtained. Quality control was conducted on the obtained sequencing data, including base mass distribution, base balance analysis and repeat sequence level analysis. The sequencing data contained some adapter and low-quality reads. In order to ensure the quality of subsequent information analysis, the sequencing data was further filtered and the raw data was cleaned using Trimmomatic software. UID deduplication data quality control: UID was parsed from reads; All the sequences under the same UID were clustered, and different clusters were generated. For multiple sequences under each cluster, consensus sequence was obtained by multi-sequence alignment. The data were straightened by merging sequences with the same consistency sequence but with high UID similarity.

Data extraction and analysis

Clean data was matched to a reference genome (GRCh38) to obtain comprehensive transcript information for transcriptome analysis and lncRNA analysis.

Gene expression level analysis mainly focused on analysis of the mRNA of protein-coding genes annotated in the genome and the evaluation of the correlation of gene expression characteristics of samples within and between groups, as well as the differentially expressed genes (DEGs). $|\log FC| > 1$ and P value < 0.05

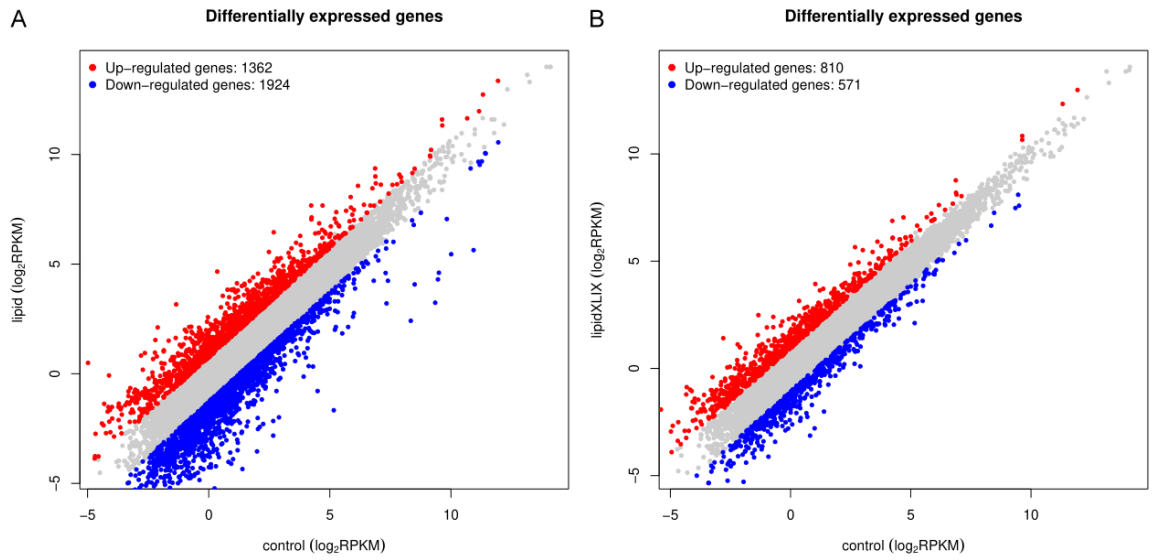

Figure 3. Screening for differentially expressed genes (DEGs). A: DEGs between the Lipid and the Control group. B: DEGs between the Lipid+XLIX group and the Control group.

were used as criteria to screen DEGs. The DEGs were shown by scatter plot and heat map. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were conducted on the screened DEGs. By using the STRING database and cytoHubba plug-in in Cytoscape software, the maximal clique centrality (MCC) was calculated, and the genes were sorted according to MCC. The top 10 genes with the highest scores were selected as the key genes.

Analysis of cis target gene for cis role of lncRNA: Biologically, it is believed that the function of lncRNA is related to the protein-coding gene adjacent to its coordinate. lncRNA located upstream and downstream of the encoding protein may have intersection with the promoter or other cis-acting elements of co-expressed genes, thus regulating gene expression at the transcriptional or post-transcriptional level. The gene encoding the protein adjacent to lncRNA gene (~100 kb upstream and downstream) was identified for functional enrichment analysis, so as to show the main function of lncRNA.

In biology, co-expression refers to the direct or indirect regulatory relationship between genes, and the regulatory process has nothing to do with the distance between genes. When the expression levels of some distant genes are positively or negatively correlated, the target genes can be predicted by correlation analysis of gene expression levels between samples or weighted gene co-expression network analysis (WGCNA). Combined with the expression data obtained by mRNA sequencing, the interaction between lncRNA and mRNA was examined from the expression level through the correlation analysis of the expression levels of lncRNA and protein-coding genes among samples. The intersection of cis and co-expression results was used to obtain the final lncRNA target gene prediction results.

Statistical methods

Statistical analysis was performed using R software (version 4.1.2). Two-tailed paired t test was used for comparison between two groups, and fold change (FC) was calculated. Hypergeometric distribution test was used for gene functional enrichment analysis. $P < 0.05$ represented a significant difference.

Results

Analysis of differentially expressed mRNA data

DEGs with $|logFC| > 1$ and $P < 0.05$ were screened through scatter plot and hierarchical cluster analysis. By comparing the Lipid group with the Control group, 3286 differentially expressed genes (1362 up-regulated genes and 1924 down-regulated genes) were screened, as shown in **Figure 3A**. By comparing the Lipid+XLIX group to the Control group, 1381 differentially expressed genes (810 up-regulat-

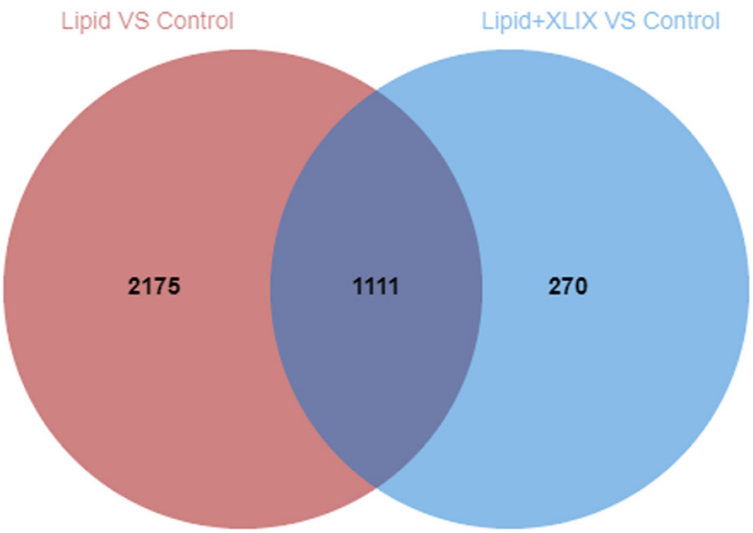

Figure 4. Venn diagram of differentially expressed genes.

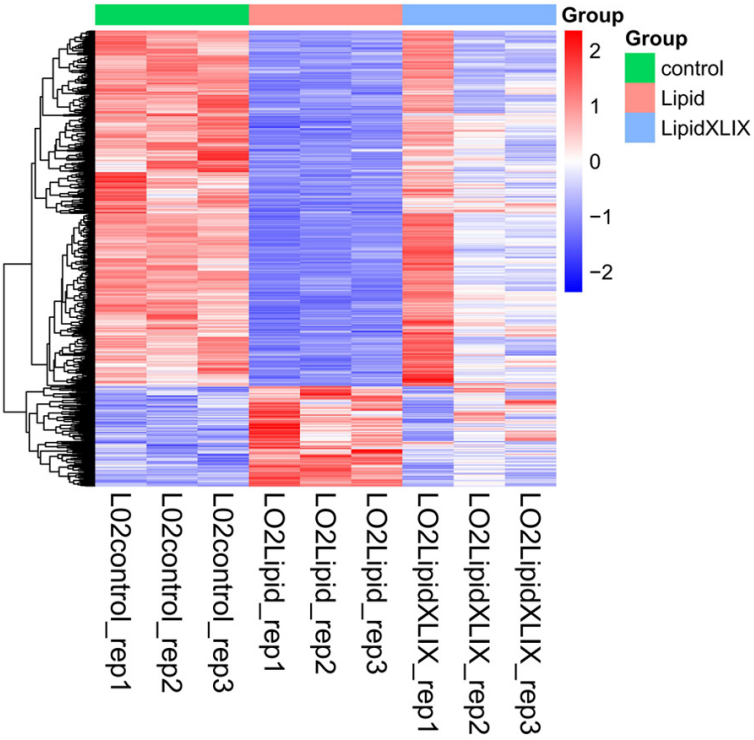

Figure 5. Heat maps of differentially expressed genes in the three groups.

ed genes and 571 down-regulated genes) were screened, as shown in **Figure 3B**. There were 1111 overlapping genes between the two sets (**Figure 4**). The 782 genes were differentially expressed in the Lipid+XLIX group and the Control group, but there was no significant difference in the Lipid+XLIX group and the Control group (see **Figure 5**; **Table 1**). In other words, 172 genes were highly expressed in fatty liver cells, and the expression decreased to the level of normal liver cells after Gyp-XLIX treatment; 610 genes were under-expressed in fatty liver cells, and the expression increased to the level of normal liver cells after Gyp-XLIX treatment.

Functional enrichment analysis of differentially expressed genes

KEGG and GO analyses of 782 DEGs were performed. KEGG results showed that the DEGs were significantly enriched in nine pathways, mainly including glycerophospholipid metabolism, bile secretion, fatty acid degradation, tight junction and other signaling pathways (**Figure 6**). GO enrichment analysis showed that the DEGs were mainly located in the plasma membrane region, and exerted their influence through the activities of DNA-bound transcription factors and ion transmembrane transport (**Figure 7**). The results showed that Gyp-XLIX may mediate phospholipid metabolism, biliary secretion, fatty acid degradation and other signaling pathways through DNA binding transcription factor activity and ion transmembrane transport activity, and thus play a role in improving fatty liver disease.

Screening of key differentially expressed genes

By using the STRING database and cytoHubba plug-in in Cytoscape software, the Maximal clique centrality (MCC) was calculated, and the genes was screened according to MCC. The top 10 genes with the highest score were selected as the key genes, as shown in **Figure 8**. The results showed that *SYT2*, *CXCR4*, *SYP*, *CACNA1A*, *WNK4*, *SCN3B*, *SYT1*, *SPI1*,

Table 1. Differentially expressed mRNA (Showing partial results)

Lipid group VS Control group					Lipid+XLIX group VS Control group				
Gene ID	logFC	F	*P* Value	FDR	Gene ID	logFC	F	*P* Value	FDR
ARID5A	1.046	59.35	4.61E-07	2.26E-06	*ARID5A*	0.337	4.642	5.52E-02	1.10E-01
ATP13A3	1.015	201.559	4.02E-11	1.12E-09	*ATP13A3*	0.243	4.783	5.22E-02	1.06E-01
BZW1	1.002	276.243	2.96E-12	1.57E-10	*BZW1*	0.22	1.738	2.21E-01	3.24E-01
CCDC126	1.072	41.521	4.95E-06	1.79E-05	*CCDC126*	0.396	3.92	7.44E-02	1.39E-01
CD274	1.153	64.428	2.60E-07	1.39E-06	*CD274*	0.311	2.323	1.57E-01	2.47E-01
COL15A1	1.064	39.128	8.72E-04	1.79E-03	*COL15A1*	0.367	5.017	5.33E-02	1.07E-01
EFNA1	1.293	115.775	8.15E-07	3.69E-06	*EFNA1*	0.432	5.14	5.22E-02	1.06E-01
HBEGF	1.217	63.831	2.77E-07	1.47E-06	*HBEGF*	0.504	2.227	1.71E-01	2.65E-01
METRNL	1.194	48.638	4.63E-04	1.02E-03	*METRNL*	0.315	1.679	2.36E-01	3.40E-01
NR4A2	1.117	91.194	2.07E-08	1.65E-07	*NR4A2*	0.21	1.339	2.73E-01	3.81E-01
ABCC2	-1.139	297.269	1.60E-12	9.78E-11	*ABCC2*	-0.372	2.562	1.53E-01	2.43E-01
ANKAR	-1.591	20.322	2.82E-04	6.51E-04	*ANKAR*	-0.746	4.233	6.52E-02	1.25E-01
APPBP2	-1.636	163.188	2.23E-10	4.09E-09	*APPBP2*	-0.81	5.043	5.96E-02	1.17E-01
BAALC-AS1	-2.42	37.009	1.01E-05	3.37E-05	*BAALC-AS1*	-0.689	4.529	5.78E-02	1.14E-01
BTBD19	-1.766	43.62	3.61E-06	1.35E-05	*BTBD19*	-0.455	2.973	1.14E-01	1.93E-01
C1QTNF1	-1.948	57.816	5.53E-07	2.64E-06	*C1QTNF1*	-0.409	3.358	9.52E-02	1.68E-01
C5orf63	-1.034	24.005	1.20E-04	3.03E-04	*C5orf63*	-0.053	0.05	8.27E-01	8.74E-01
C6orf203	-1.654	85.086	3.47E-08	2.54E-07	*C6orf203*	-0.457	2.427	1.55E-01	2.45E-01
C7orf31	-1.868	33.715	1.78E-05	5.54E-05	*C7orf31*	-0.374	2.188	1.68E-01	2.62E-01
COL16A1	-1.393	103.684	7.85E-09	7.45E-08	*COL16A1*	0.007	0.003	9.59E-01	9.71E-01

Note: FC is fold changes of RPKM value of the same gene in different samples. F value is used to test the extent to which the results of a sample are representative of the population. FDR is corrected-*P* value.

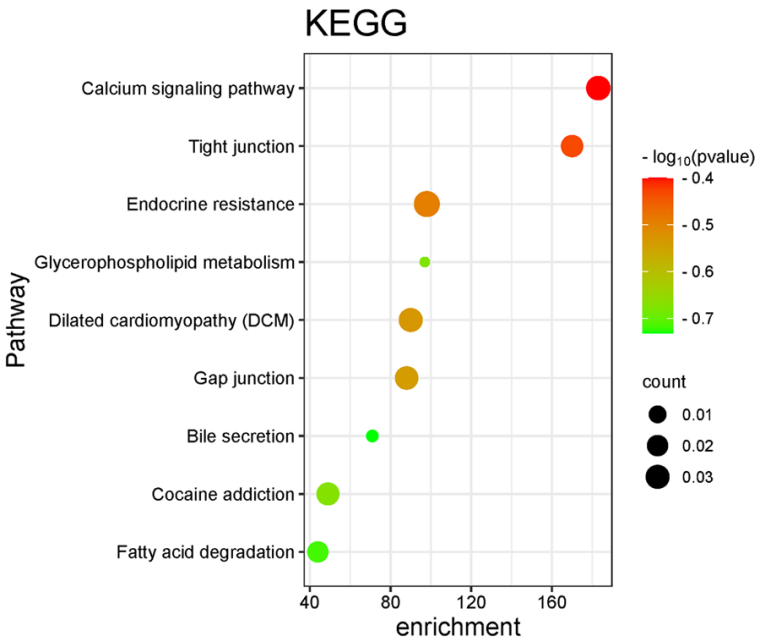

Figure 6. Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis.

GALNTL6, and *SCNN1A* played key roles in the treatment of fatty liver by Gyp-XLIX.

Analysis of differentially expressed lncRNA data

LncRNAs with significant differential expression were screened based on |logFC| > 1 and P < 0.05. Sixteen lncRNAs were differentially expressed between the Lipid group and Control group, but these 16 lncRNA were not differentially expressed in the Lipid+XLIX group and the Control group (see **Table 2**). In other words, the expression of 16 lncRNAs was low in fatty liver cells and increased to the level in normal liver cells after Gyp-XLIX treatment.

Cis-acting target gene prediction of lncRNA

Cis refers to the regulation mode of lncRNA on neighboring mRNA (such as on the same chromosome). Therefore, target genes of lncRNA can be pre-

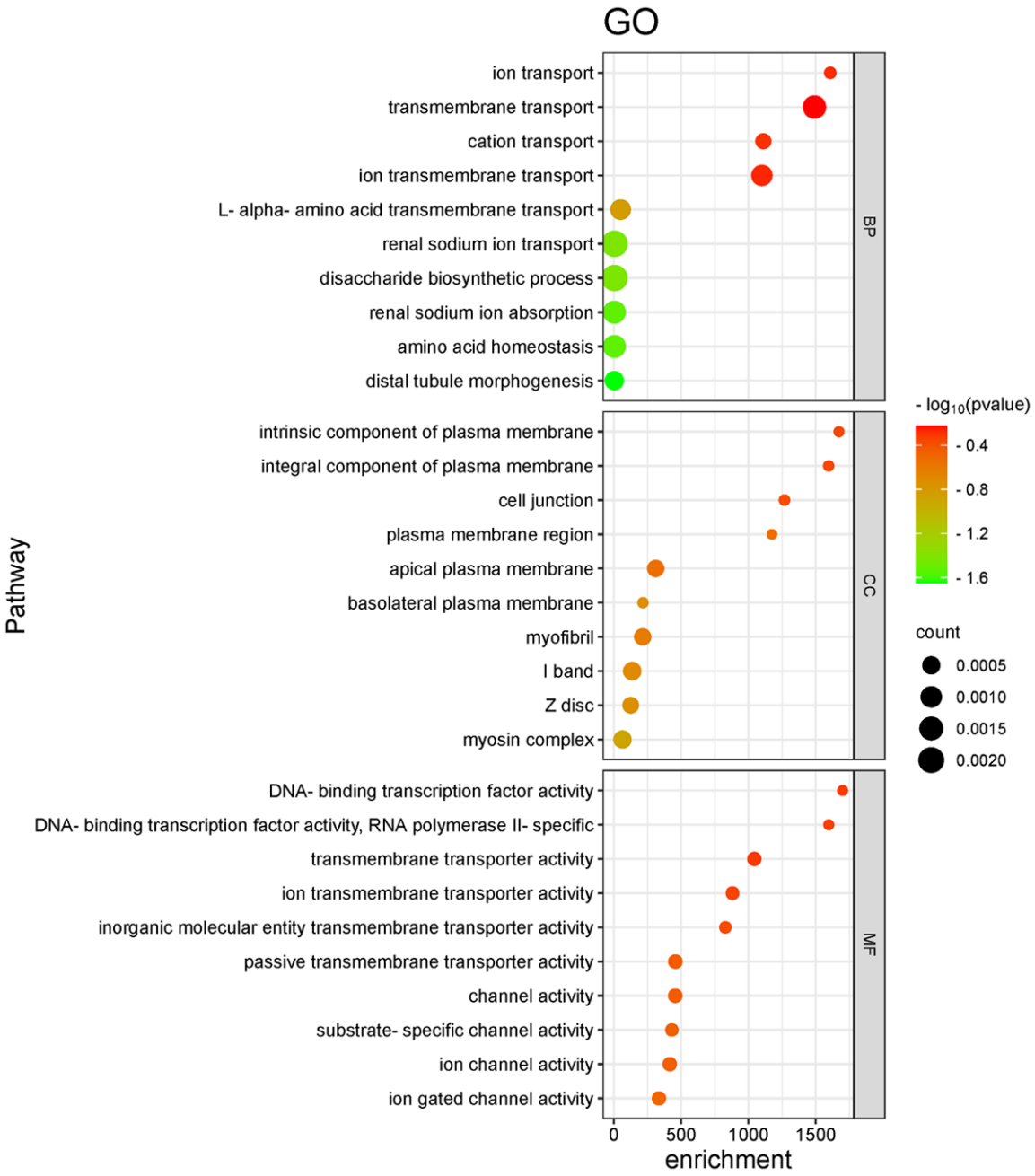

Figure 7. Gene ontology (GO) functional enrichment analysis.

dicted by looking for mRNA around lncRNA. In this study, genes encoding proteins adjacent to lncRNA genes (~100 kb upstream and downstream) were identified and their functions were analyzed to speculate the main functions of lncRNA. The prediction results of target genes by cis are shown in **Figure 9**. The results showed that 16 differentially expressed lncRNAs contained possible cis target genes, which may interact with 51 cis target genes, and lncRNA RPARP-AS1 had a high degree of relationship with other genes.

Co-expression analysis: Based on the expression file obtained from mRNA sequencing, the interaction between lncRNA and mRNA was examined from the expression level through the correlation analysis of the expression levels

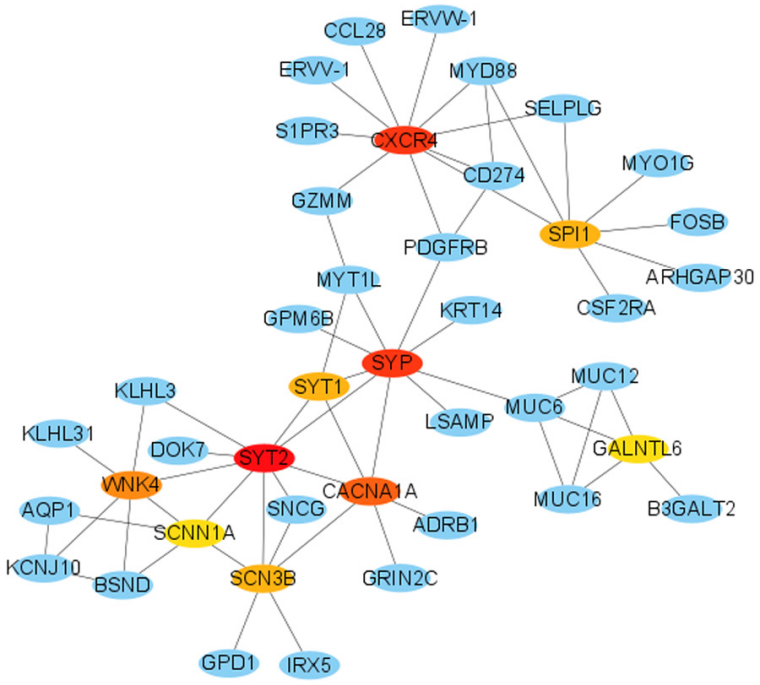

Figure 8. Key differentially expressed genes and their associated genes.

of lncRNA and protein-coding genes among samples. After the intersection of cis and co-expression results, the final target gene prediction results were obtained, as shown in **Table 3**. The results showed that lncRNA RPARP-AS1 acted on *NFKB2* gene and may be a key gene for the treatment of fatty liver.

Discussion

The treatment of fatty liver mainly includes lifestyle changes (diet, exercise, temperance and abstinence), removal of etiology and inducement (use of hepatoprotective drugs), and control of primary underlying diseases or accompanying diseases, but these treatment methods still lack definitive efficacy and have adverse reactions of varying degrees [15]. In the treatment of fatty liver, traditional Chinese medicine mainly focuses on removing dampness and phlegm, invigorating blood circulation, soothing the liver and relieving depression, which can also be supplemented by clearing heat, detoxification, cholagogue, removing food accumulation, tonifying kidney, nourishing liver and other treatment upon the personal condition [16]. The main active component of *Gynostemma pentaphyllum* is Gypenoside, which has an obvious inhibitory effect on the malignant biologic behavior of tumors cells and can cause platelet aggregation and prevent atherosclerosis. It can also effectively improve obesity and prevent liver disease, gastric, and duodenal ulcers [17-19]. In the treatment of fatty liver, it has not been reported which genes can be regulated by Gyp-XLIX drugs. Therefore, in this study, whole genome analysis was performed to screen GEGs in the fatty liver cells after Gyp-XLIX treatment to investigate the effect of Gyp-XLIX on NAFLD gene expression.

By comparing the gene expression profiles of fatty liver cells and normal liver cells, 3286 differentially expressed genes (1362 up-regulated genes, 1924 down-regulated genes) were screened. By comparing the gene expression profiles of Gyp-XLIX treated fatty liver cells and normal liver cells, 1381 differentially expressed genes (810 up-regulated genes and 571 down-regulated genes) were screened. In order to search for genes significantly regulated by the drug, we screened for genes with significant differential expression between fatty liver cells and normal liver cells, but not between fatty liver cells and normal liver cells treated with Gyp-XLIX. A total of 782 genes significantly regulated by drugs were screened. In other words, 172 genes were highly expressed in fatty liver cells, and their expression decreased to the normal level after Gyp-XLIX treatment. 610 genes were underexpressed in fatty liver cells, and their expression increased to the normal level after Gyp-XLIX treatment. Further functional enrichment analysis of these 782 DEGs showed that these genes mainly enriched in the pathways such as plasma membrane region mediated glycerophospholipid metabolism, bile secretion, fatty acid degradation and other signaling pathways, by which Gyp-XLIX plays a role in improving fatty liver through regulating DNA-binding transcription factor activity and ion transmembrane transport activity.

Glycerophospholipids are the most abundant phospholipids in the body. Besides constituting

Table 2. Differentially expressed lncRNAs

Lipid group VS Control group					Lipid+XLIX group VS Control group				
Gene ID	logFC	F	*P* Value	FDR	Gene ID	logFC	F	*P* Value	FDR
C1orf220	-2.409	44.910	2.99E-06	1.15E-05	C1orf220	-0.639	3.995	7.20E-02	1.36E-01
C8orf31	-1.876	37.850	8.83E-06	2.98E-05	C8orf31	-0.775	4.668	5.47E-02	1.10E-01
FAM225B	-3.300	106.705	6.30E-09	6.16E-08	FAM225B	-0.345	1.202	2.97E-01	4.07E-01
LINC00337	-1.121	11.936	2.88E-03	5.28E-03	LINC00337	0.004	0.000	9.90E-01	9.93E-01
LINC00570	-1.180	19.049	4.71E-04	1.03E-03	LINC00570	-0.266	0.886	3.68E-01	4.78E-01
LINC00667	-1.055	69.864	1.46E-07	8.52E-07	LINC00667	-0.101	0.463	5.11E-01	6.15E-01
LINC00863	-1.025	14.781	1.22E-03	2.42E-03	LINC00863	-0.617	3.969	7.28E-02	1.37E-01
LINC00909	-2.167	41.731	8.27E-06	2.82E-05	LINC00909	-0.644	4.761	5.27E-02	1.06E-01
LINC01021	-1.271	230.960	8.16E-11	1.89E-09	LINC01021	-0.470	3.530	1.04E-01	1.80E-01
LINC01293	-1.307	22.245	1.79E-04	4.32E-04	LINC01293	-0.120	0.195	6.67E-01	7.50E-01
LINC01547	-1.758	31.713	2.56E-05	7.64E-05	LINC01547	-0.325	1.395	2.64E-01	3.71E-01
MIR100HG	-1.134	97.475	1.41E-08	1.20E-07	MIR100HG	-0.437	4.876	5.57E-02	1.11E-01
RAB30-AS1	-1.094	105.469	6.89E-09	6.68E-08	RAB30-AS1	-0.130	1.210	2.96E-01	4.06E-01
RPARP-AS1	-1.277	83.806	4.22E-08	2.98E-07	RPARP-AS1	-0.141	0.872	3.71E-01	4.81E-01
SCAMP1-AS1	-1.106	35.660	1.27E-05	4.13E-05	SCAMP1-AS1	-0.088	0.227	6.44E-01	7.30E-01
SERPINB9P1	-1.130	36.617	1.08E-05	3.57E-05	SERPINB9P1	-0.353	1.052	3.34E-01	4.44E-01

Note: FC is fold changes of RPKM value of the same gene in different samples. F value is used to test the extent to which the results of a sample are representative of the population. FDR is corrected-*P* value.

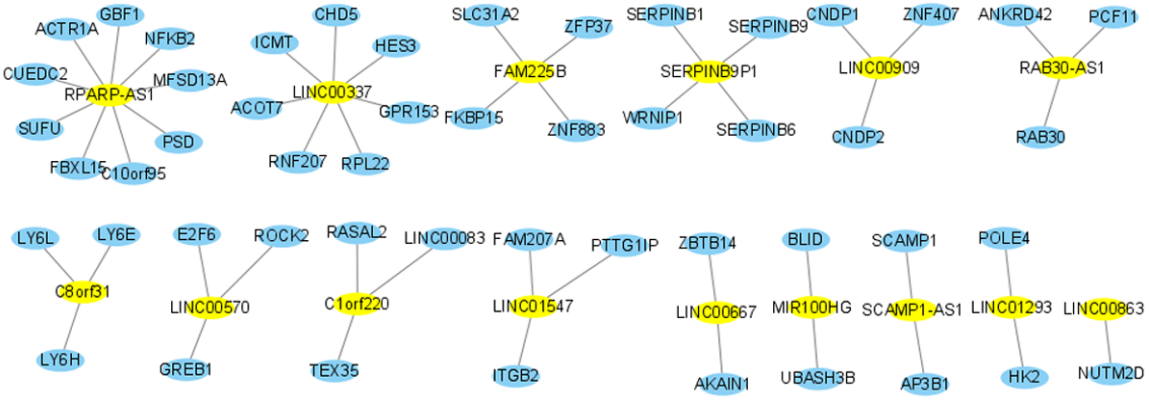

Figure 9. Candidate cis target genes of lncRNA, with yellow representing lncRNA and blue representing target genes.

Table 3. lncRNA target gene list

lncRNA	Target gene	Weighted value	Distance
RPARP-AS1	NFKB2	0.865181784	57142
LINC00863	NUTM2D	0.911910247	13872

Note: weight indicates the weight of the action. The larger the weight, the stronger the correlation.

biofilms, glycerophospholipids are also one of the components of bile and membrane surface active substances, and participate in the recognition and signal transduction of proteins in cell membranes. Previous studies have shown that gynostaphyllum pentaphyllum extract protects hepatocytes from cell death when cultured in the presence of high concentrations of insulin, glucose and linoleic acid. Also, gynostaphyllum pentaphyllum extract can prevent the accumulation of triglycerides (TGs) and cholesterol as well as oxidative stress [20]. Li et al. [21] showed that Gypenoside could regulate the key transcription factors and lipogenesis enzymes involved in fatty acid oxidation during liver lipogenesis to treat non-alcoholic steatohepatitis. Studies have found that the nuclear structure

of gypenosides is very similar to that of endogenous bile acids, which can activate farnesoid X receptors in liver, thus up-regulating the key enzymes CYP8B1 and CYP7A1 in bile acid synthesis, promoting bile acid synthesis and secretion, promoting lipid metabolism, and reducing blood lipid [22]. Lee et al. [23] found that when gypenosides were given, the increase in body weight, fat mass, white adipose tissue, and fat cell hypertrophy was inhibited, and serum triglyceride, total cholesterol and LDL cholesterol levels were lower than those of the high-fat diet group. Its lipid-lowering effect is related to the activation of AMP-activated protein kinase (AMPK), which leads to increased expression of SIRT1 [23]. Gypenosides are delivered to skeletal muscle and liver to prevent or ameliorate obesity in ob/ob mice by stimulating fatty acid oxidation in these organs and activating AMPK [24]. In addition, gypenosides can prevent obesity caused by high fat diets by promoting energy expenditure. Recent studies have shown that an isolated novel saponin JS (100 μM) can inhibit adipocyte differentiation and adipogenesis, which may be related to Wnt/β-catenin signal activation [25]. Recently, a double-blind randomized clinical trial conducted with treatment duration of 16 weeks showed that gypenosides was able to alter fat mass and fat distribution in overweight and obese males and females compared to placebo. After 16 weeks of treatment, significant reductions in body weight and total fat mass were observed [26]. These results prove that Gyp-XLIX plays a role in the treatment of fatty liver by regulating gene expression in the process of glycerol phospholipid metabolism, biliary secretion, fatty acid degradation, and other processes.

In order to further explore the key genes influenced by Gyp-XLIX, the most critical 10 genes, namely SYT2 (synaptotagmin 2), CXCR4 (C-X-C motif chemokine receptor 4), SYP (synaptophysin), CACNA1A (calcium voltage-gated channel subunit alpha 1A), WNK4 (WNK lysine deficient protein kinase 4), SCN3B (sodium voltage-gated channel beta subunit 3), SYT1 (synaptotagmin 1), SPI1 (Spi-1 proto-oncogene), GALNTL6 (polypeptide N-acetylgalactosaminyltransferase like 6), and SCNN1A (sodium channel epithelial 1 subunit alpha) were selected through STRING database and Cytoscape software in this study. The abnormal expression of some of these genes has been confirmed to be closely related to fatty liver. For example, Boujedidi et al. [27] confirmed that CXCR4 dysfunction was observed in mice and patients with nonalcoholic steatohepatitis. Takahashi et al. [28] found that WNK4 may be an adipotropic factor. WNK4-siRNA transfected adipocytes and human mesenchymal stem cells showed decreased expression of PPARγ and C/EBP-α and lipid accumulation. WNK4 protein affects the DNA binding capacity of C/EBPβ, thereby reducing the expression of PPARγ. In addition, Sancho-Knapik et al. [29] demonstrated that SYT1 expression was positively correlated with liver total cholesterol content, and the expressions of genes involved in bile acid biosynthesis, fatty acid metabolism, lipoprotein kinetics and vesicle transport were changed by increasing SYT1 expression. These results suggest that it is possible to prevent steatohepatitis and improve clinical symptoms by regulating the expression of these key genes.

Through lncRNA expression profile analysis, we screened the DEGs after Gyp-XLIX treatment of fatty liver. The expression of lncRNAs was significantly different between fatty liver cells and normal liver cells, while there were 16 lncRNAs with no significant difference between fatty liver cells and normal liver cells after Gyp-XLIX treatment. In other words, the expression of 16 lncRNAs was low in fatty liver cells and increased to the level of normal liver cells after GPR-Xlix treatment. The results showed that the number of down-regulated lncRNAs was significantly dominant, suggesting that down-regulated lncRNAs may play a leading role in the occurrence and development of fatty liver. The transcription mode of lncRNA is complex and can form a variety of secondary structures. Therefore, it is limited for predicting the biologic function of lncRNA based only on its nucleic acid sequence. Since lncRNAs contain many regulatory modes, lncRNAs are also associated with more mRNA. Different lncRNA-mRNA relationships may perform different functions. There are mainly three kinds of correlation between the two omics: Antisense relationship, Cis relationship, and Trans relationship. Cis relationship refers to the correlation between the function of lncRNA and its neighboring protein-coding genes. If lncRNA is located within 100 kb upstream or downstream of a gene, these lncRNAs may interact with mRNA and participate in the pro-

cess of transcriptional regulation [30]. In the study of cis relationship, we can predict whether there is cis relationship between lncRNA and mRNA according to the distance between lncRNA and mRNA in the genome and whether the expression level is highly correlated. For the results of lncRNA-mRNA with cis relationship, the target lncRNA-mRNA can be screened according to whether there is overlap between lncRNA and mRNA, the status of lncRNA's chain and its location in the upstream and downstream of genes. lncRNA cis target gene prediction results in this study showed that lncRNA RPARP-AS1 had a close relationship with other genes, so it was speculated that this key node lncRNA mediated the pathogenesis of fatty liver disease. lncRNA RPARP-AS1 was also found to act on NFKB2 gene through lncRNA-mRNA co-expression network. In an analysis of biologic information of the mRNA-miRNA-lncRNA regulatory network in NAFLD [31], it was found that RPARP-AS1 was differentially expressed in NAFLD and was closely related to the pathogenesis of the disease. The results are consistent with those of this study, suggesting that RPARP-AS1 may be a gene regulated by Gyp-XLIX in the treatment of fatty liver disease. The NFKB2 gene encodes a subunit of the transcription factor complex nuclear factor-κ-B (NFκB). The NFκB complex is expressed in many cell types and acts as a central activator of genes involved in inflammation and immune function [32]. Lai et al. [33] showed that increased expression of *NFKB2* could promote the accumulation of liver triglycerides, indicating that the regulation of the *NFKB2* gene through lncRNA RPARP-AS1 may be the target of GPR-XLIX treatment. There are some limitations in this study. First, the sample size is small, which may lead to false positives, so it is necessary to expand the sample size. Secondly, the screened key genes were not further verified by *in vivo* experiments.

Conclusion

Multiple mRNAs and lncRNAs were differentially expressed in fatty liver cells and Gyp-XLIX treated fatty liver cells. KEGG and GO functional analysis showed that Gyp-XLIX might regulate phospholipid metabolism, bile secretion, fatty acid degradation and other signaling pathways through DNA-binding transcription factor activity and ion transmembrane transport activity, and thus play a role in improving fatty liver disease. By lncRNA analysis, lncRNA RPARP-AS1 may be a gene regulated by Gyp-XLIX for the treatment of fatty liver, but further experimental confirmation is needed.

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Disclosure of conflict of interest

None.

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