

Identification of Key Genes and Pathways Related to Nonalcoholic Fatty Liver Disease based on GEO Databases

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) represents a growing global health crisis and is closely associated with increases in obesity and type 2 diabetes. Despite its ubiquity, its underlying biochemical mechanisms and effective therapeutic strategies remain poorly defined, hampering the development of targeted interventions. **Methods:** Candidate genes were obtained from the GEO database, and Kyoto Encyclopedia of Genes and Genomes enrichment analysis and gene set enrichment analysis were used to identify pathways involved in NAFLD-related pathways. The top genes with higher degree in the protein-protein interaction network were crossed with the top genes enriched in key pathways, and then the correlation analysis between key genes and chemotherapy response was performed. **Result:** fatty acid metabolic process, lipid transport, response to stilbenoid, etc. are key pathways related to NAFLD. Btc, Apoa4, Gdf6 AND Ifi2712b were enriched in key pathways of NAFLD. **Conclusion:** Fatty acid metabolic process, lipid transport, pathway are key pathways related to NAFLD.

Keywords

NAFLD; GEO; KEGG.

1. Overview of the Pathogenesis of NASH

The first-hit theory believes that the development of NASH starts from simple steatosis, which is not enough to induce inflammation and fibrosis. The next second-hit theory believes that oxidative stress is an important factor to aggravate liver injury [1]. NASH is the result of the simultaneous action of multiple factors, including genetic variation, abnormal lipid metabolism, oxidative stress, abnormal immune response and intestinal microbiota imbalance [2]. "Multiple hits" believe that liver inflammation is the main cause of NASH progression to fibrosis, so there may be a variety of mechanisms acting in concert to promote disease progression [3]. The overloaded lipotoxic liver injury model of NASH shows that the liver is dysfunctional in processing major metabolic energy substrates, carbohydrates, and fatty acids, leading to the accumulation of toxic lipid substances [4]. These metabolites can further induce hepatocyte stress, injury, and death, leading to fibrogenesis and genomic instability, and progression of patients to cirrhosis and liver cancer. In the development of NASH, autophagy and ferroptosis are considered to be the main factors leading to liver injury and disease progression in NASH.

2. Methods

In order to explore the key pathways affecting NAFLD, samples were screened from the NAFLD-related microarray retrieved from the GEO database, NAFLD-related expression microarray GSE24031, downloaded from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24031>). GSE24031 is included in 4 subgroups: LF-low (LFL) responders showing normal liver morphology, LF-high (LFH) responders showing benign hepatic steatosis, and HF-low (HFL) responders showing liver morphology with macrovesicles Pre-NASH of lipid droplets, as well as HF-high (HFH) responders exhibiting overt NASH, characterized by

hepatocyte expansion, presence of Mallory bodies, and activated inflammatory cells, with 4 samples each, we screened for LF-low (LFL) and HF high (HFH) 4 samples each, 8 samples in total.

3. Results

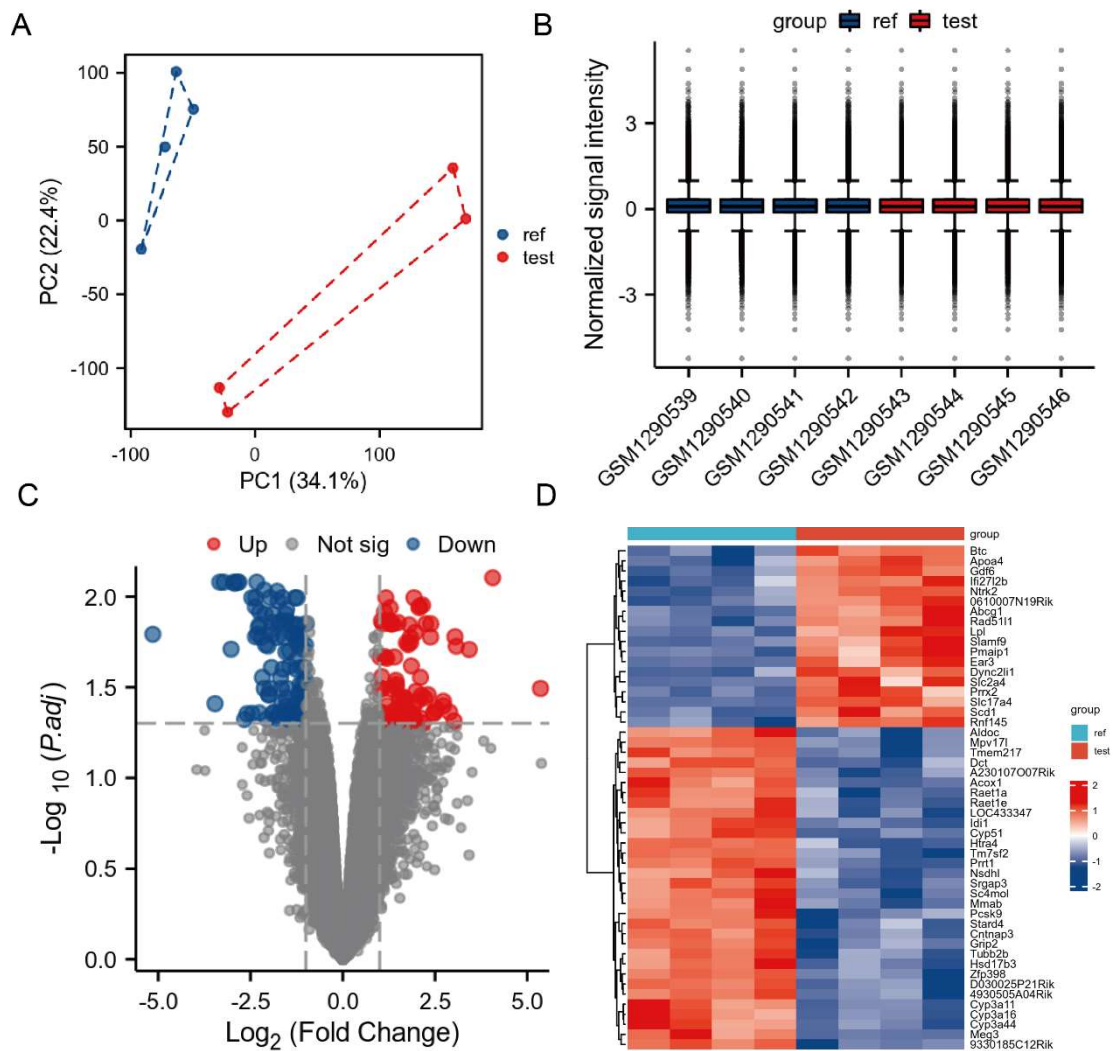


Figure 1. A, PCA plot. B,Box plot of genes in NAFLD key gene screening GSE53381 (normal group, n = 4; model group, n = 4). C, Volcano plot The red dots in Figures A and B represent significantly up-regulated genes, the green dots represent significantly down-regulated genes, and the black dots represent genes with insignificant expression differences. D cluster heat map

Retrieve NAFLD-related microarray GSE53381 through the GEO database. As shown in Figure A, the sample correction status is viewed through a box plot. The horizontal line in the middle of the box represents the median, the top of the box represents the upper quartile, and the bottom of the box represents the lower quartile. If there are black dots above and below the box, it indicates that the sample has outliers. Generally, we only need to focus on whether the median line of each sample is on the same level. We found that the samples have been corrected. The PCA plot shows the details of the differences between groups, indicating that the modeling was successful. The volcano plot displays upregulated and downregulated genes, and the heatmap shows a clear clustered distribution of differential genes by group.

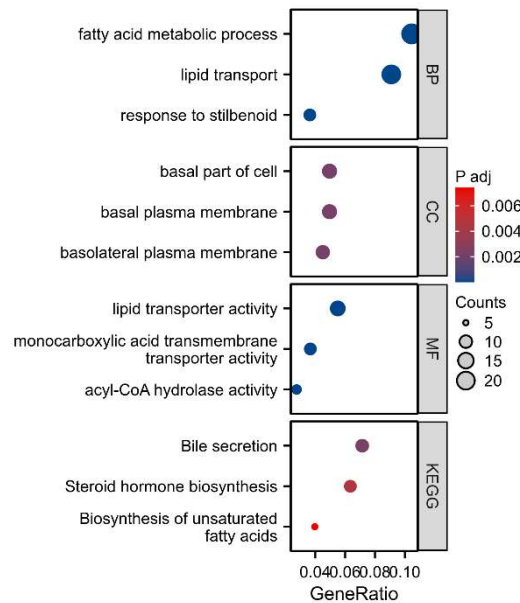


Figure 2. A, Box plot of genes in NAFLD key gene screening GSE53381 (normal group, n = 4; model group, n = 4).

The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses provided insights into the biological processes, cellular components, molecular functions, and biochemical pathways that are significantly associated with the condition under study. The bubble chart illustrates these findings across three categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF), along with a separate KEGG pathway analysis.

The GO enrichment analysis within the Biological Process category highlighted the 'fatty acid metabolic process' and 'lipid transport', which are integral to lipid homeostasis. Moreover, the response to stilbenoid was notably enriched, suggesting a potential interaction of phenolic compounds with lipid metabolism. The Cellular Component category underscored the importance of the 'basal part of the cell', 'basal plasma membrane', and 'basolateral plasma membrane', indicating a specific cellular localization of the metabolic processes identified.

In the Molecular Function category, significant enrichment was observed for 'lipid transporter activity' and 'monocarboxylic acid transmembrane transporter activity', emphasizing the role of transport proteins in the condition. Additionally, 'acyl-CoA hydrolase activity' pointed to the breakdown of acyl-CoA, a key molecule in fatty acid metabolism and energy production.

The KEGG pathway analysis further reinforced these findings, with 'bile secretion', 'steroid hormone biosynthesis', and 'biosynthesis of unsaturated fatty acids' pathways being significantly enriched. These pathways are essential for maintaining lipid balance and producing signaling molecules that regulate metabolism and inflammation, suggesting a complex interplay of biochemical pathways in the disease state.

4. Discussion

Autophagy is an intracellular lysosomal degradation pathway that regulates cellular metabolism and links catabolic and anabolic processes to maintain homeostasis in liver physiology and pathology, and autophagy has been shown to promote cellular health in the presence of lipid overload [5]. It plays an important role in cell differentiation, survival, development and homeostasis, and dysfunction of autophagy is associated with a variety of diseases, including neurodegeneration, autoimmunity, cancer, infection, inflammatory diseases and aging. During the occurrence and development of NAFLD, decreased autophagy activity is

associated with the initial development and progression of steatosis to NASH and hepatocellular carcinoma (HCC) [6]. The autophagy pathway interacts with lipid homeostasis to provide an indispensable energy and material basis for maintaining liver function. When hepatocytes are energy deficient, they can compensate by decomposing lipid droplets (LD), which are intracellular organelles storing neutral lipids, partly through selective autophagy (called lipophagy). The dysregulation of lipophagy is an important risk factor for the development of NAFLD to hepatocellular carcinoma (HCC) [7].

Autophagy degrades lipid droplets through lipophagy, and starvation and acute lipid stimulation increase the autophagy of lipid droplets and their degradation in lysosomes. Autophagy is essential for cell survival and energy homeostasis under nutrient deprivation, but the epigenetic control of autophagy gene transcription remains poorly understood. Studies have shown that liver-specific deletion of the autophagy gene *Atg7* increases liver fat content, similar to human non-alcoholic fatty liver disease [8]. *TXNIP* expression is up-regulated in NAFLD patients, methionine and choline deficient (MCD) diet fed mice, and palmitic acid (PA) treated hepatocytes, and the upregulation of *TXNIP* is positively correlated with impaired autophagy [9]. Fasting induced fibroblast growth factor 21 (FGF21) signaling has been reported to activate autophagy and lipid degradation in hepatocytes through Jumonji-D3 (JMJD3/KDM6B) histone demethylase, and JMJD3 upregulates autophagy network genes through histone H3K27-me3 demethylation. These include *Tfeb*, *Atg7*, and *Atg1*, leading to autophagy-mediated lipid degradation. Mechanologically, phosphorylation of JMJD3 Thr-1044 by PKA activated by FGF21 signaling increases its nuclear localization and interaction with nuclear receptor PPAR α , thereby activating autophagy [10].

Dysfunctional autophagy exacerbates oxidative stress and inflammation in hepatocytes and accelerates the progression of NASH. cAMP response element-binding protein H (CREBH) is a transcriptional regulator of hepatic autophagy in diet-induced NASH. Studies have shown that upregulation of CREBH in lipid-overloaded hepatocytes ameliorates cell damage, autophagy dysfunction, and the related abnormal accumulation of autophagosome marker LC3-II and autophagy substrate p62. CREBH deficiency can aggravate autophagy dysfunction and liver injury, and even cause NASH related liver fibrosis. In addition, the changes of autophagolysosome and lysosomal membrane associated protein (LAMP1) were consistent with the expression level of CREBH, suggesting that CREBH may improve the impaired autophagy function by restoring the formation of autophagolysosome to promote autophagic degradation. In addition, CREBH suppressed the expression of Coronin 1a (Coro1a), a gene involved in autophagosome-lysosome fusion, through transcriptional regulation. Overexpression of Coro1a in LO2 hepatocytes can inhibit autophagy and increase the levels of cellular inflammatory factors upon palmitic acid (PA) stimulation. Coro1a may be a new target gene of CREBH based on the autophagy pathway [11].

The lack of specific biomarkers and treatment strategies makes the management of nonalcoholic steatohepatitis (NASH) a challenging task for clinicians. Extracellular vesicles (evs) constitute a heterogeneous population of vesicles produced by budding inward or outward to the plasma membrane. The changes in the number of secreted evs and the substances they carry are also related to the disease progression and development of NASH. Autophagy constitutes a lysosomal degradation process that ensures cellular homeostasis and survival under stressful conditions, such as hypoxia and energy deprivation. It prevents cell damage by eliminating defective proteins or nonfunctional organelles. At the same time, it maintains the optimal state of cells through different mechanisms, including the removal of foreign bodies by secreting evs. Similarly, autophagy mechanism is also related to the pathogenesis of NAFLD and plays an important role in the occurrence and development of NASH [12].

The liver is a highly dynamic metabolic organ that plays a key role in plasma protein synthesis, gluconeogenesis and glycogen storage, cholesterol metabolism and bile acid synthesis, and

drug/xenobiotic metabolism and detoxification. Studies in the past decades have shown that autophagy plays an important role in maintaining liver cell function and metabolic homeostasis. Hepatic autophagy fluctuates with hormonal signals and nutrients, responding to feeding and fasting states and circadian activity. Autophagy dysfunction of liver parenchymal and non-parenchymal cells can lead to a variety of liver diseases, including non-alcoholic fatty liver disease, alcohol-related liver disease, drug-induced liver injury, cholestasis, viral hepatitis and hepatocellular carcinoma. Therefore, targeting autophagy may be a potential strategy for the treatment of these various liver diseases [13].

Liver is an important organ to regulate lipid balance and is crucial for lipid metabolism. When energy intake and consumption are unbalanced or the storage function of adipocytes is abnormal, the liver is most likely to accumulate fat, leading to hepatic steatosis and then causing systemic metabolic disorders [14]. The liver plays an important role in iron metabolism, including absorption, utilization, storage, and secretion of iron. The liver is also considered to be the most important iron depot, responsible for storing about one-third of the total iron in the human body. Dysregulation of iron metabolism in the liver may lead to massive ROS production, which significantly increases the susceptibility of hepatocytes to ferroptosis. In addition, disturbances in iron homeostasis have been demonstrated to be associated with IR and obesity [15].

Iron is absorbed by the enterocytes of the duodenum and enters the bloodstream, where it binds to transferrin and reaches the liver through the hilar circulation. Patients with biophy-proven NAFLD and iron overload have a poor long-term prognosis, which may be due to progression of liver fibrosis due to elevated insulin resistance, excessive hepatic lipid oxidation, and iron overload. GPX4 reduced ferroptosis is a key trigger for the progression of NAFLD to NASH [16], knockdown of GPX4 has been shown to increase ROS production in dietary models lacking choline and supplemented with ethoxine, and ferroptosis can be inhibited by promoting GPX4 expression [17]. Ferroptosis is iron dependent and is triggered when the balance of the REDOX system is disrupted by excess lipid peroxide accumulation. The close relationship between ferroptosis and NASH is formed by a combination of phospholipid peroxidation substrates, bioactive iron, and reactive oxygen species (ROS). The liver is essential for regulating iron balance, transfer receptor 1 (TFR1) and SLC39A14 deliver iron to hepatocytes and participate in many physiological and metabolic processes, excess iron is stored in the form of ferritin, and ferroportin (FPN) is essential for iron elimination [18].

Lipid peroxidation caused by abnormal lipid metabolism is one of the main mechanisms of ferroptosis, and lipid peroxidation of membrane phospholipids containing polyunsaturated fatty acid (PUFA) chains is the core of ferroptosis. Lipid peroxidation will oxidize PUFA into peroxides and affect the normal function of cells [19]. Free PUFAs are substrates for cell membrane phospholipid synthesis; they are esterified and oxidized into ferroptosis signals and are regulated by a variety of enzymes. Acyl-coA synthetase long-chain family (ACSL) is a key enzyme in lipid metabolism, derived from endoplasmic reticulum (ER) and mitochondrial membrane, among which ACSL4 is considered to be a key regulator of ferroptosis [20]. Ferroptosis plays a key role in the development of NAFLD. Therefore, it may be a potential therapeutic target to prevent the development of NASH, and targeting ferroptosis has good potential for the prevention and treatment of NASH.

Several studies have reported that ferroptosis is an autophagy-dependent cell death process. Inhibition of ferroptosis has been reported to rescue sepsis-induced nuclear autophagy, attenuate glutamate excitotoxicity, and reduce neuronal death in mice. Autophagy is activated under ferroptosis-inducing conditions by the conversion of LC3-I to LC3-II and GFP-LC3 fluorescent spots. Bioinformatics analysis has proved that the interaction between autophagy and ferroptosis has a synergistic effect on the prognosis, tumor microenvironment, immunity and chemotherapy resistance of squamous cell carcinoma [21]. Cellular REDOX status has a

profound effect on autophagy, lipid peroxidation can induce autophagosome formation, and excessive autophagy and lysosomes can promote ferroptosis through iron accumulation or lipid peroxidation [22].

Autophagy has been proposed as an upstream mechanism to induce ferroptosis by regulating cellular iron homeostasis and cellular ROS production. The molecular mechanism may involve a variety of pathways, such as nuclear receptor coactivator 4 (NCOA4) -dependent ferritin autophagy degradation, inhibition of SLC7A11 activity by the formation of BECN1-SLC7A11 protein complex, and RAB7A-dependent lipid autophagy degradation of lipid droplets [23]. Some researchers have found that autophagy promotes ferroptosis by regulating ferritin and transferrin receptors to increase intracellular iron levels. Inhibition of lipophagy provides a defense mechanism against ferroptosis under oxidative stress by inhibiting the breakdown of lipid droplets. Therefore, autophagy may be involved in the regulation of the ferroptosis process [24].

5. Conclusion

To sum up, autophagy and ferroptosis are considered to be the main factors leading to liver injury and disease progression in NASH.

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