Molecular Mechanisms of miR-103a-3p Regulation of FGF9 and Its Impact on Lipid Metabolism, Inflammation, and Fibrosis in Non-Alcoholic Fatty Liver Disease

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Introduction. This study aims to explore the molecular mechanism of miR-103a-3p in nonalcoholic fatty liver disease (NAFLD) and evaluate its effects on lipid metabolism, inflammation and fibrosis, as well as its association with fibroblast growth factor 9 (FGF9).
Methods. We used mouse model and cell experiments to investigate the role of miR-103a-3p in NAFLD through overexpression and silencing of Mir-103a-3p, respectively. The regulatory mechanism and effect of miR-103a-3p were analyzed in detail by miRNA and mRNA sequencing technology, lipid content and inflammatory factor measurement, and Western blotting.
Results. We observed that miR-103a-3p was significantly upregulated in NAFLD patients. Overexpression of miR-103a-3p resulted in increased lipid accumulation, aggravated inflammatory response, and elevated markers of liver fibrosis. In contrast, silencing of miR-103a-3p reversed these effects and increased FGF9 expression.
Conclusion. miR-103a-3p plays an important role in NAFLD and participates in the development and progression of NAFLD by regulating multiple pathways such as lipid metabolism, inflammation, and fibrosis. In addition, there is a negative correlation between miR-103a-3p and FGF9, which has an effect on the pathological process of NAFLD. This study broadens our understanding of the pathogenesis of NAFLD and provides new clues for future treatment strategies.
Keywords. miR-103a-3p, non-alcoholic fatty liver disease, lipid metabolism, inflammation, fibrosis

INTRODUCTION

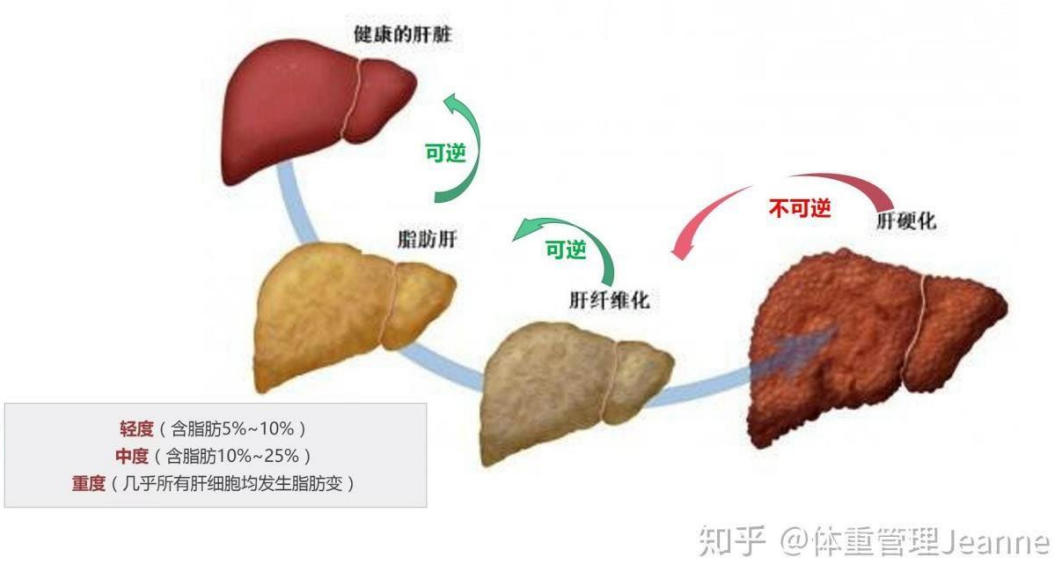
Nonalcoholic fatty liver disease (NAFLD) is a widely studied and discussed health problem. With the change of modern lifestyle, especially the transformation of diet structure and lack of exercise, the incidence of NAFLD is increasing year by year. It is defined as the presence of steatosis in more than 5% of hepatocytes in the liver in the absence of significant alcohol intake, other liver disease factors, and drugs. With the progression of the disease, NAFLD may further develop into non-alcoholic steatohepatitis (NASH), and then liver cirrhosis or even liver cancer.

NAFLD is not just a problem of the liver. It is usually associated with a variety of systemic diseases, such as type 2 diabetes mellitus, cardiovascular disease, and chronic kidney disease. Therefore, studies on NAFLD are not only important to understand its pathogenesis in the liver, but also essential to understand these systemic diseases associated with NAFLD.

In recent years, with the progress of molecular biology technology, researchers have carried out in-depth research on the etiology of NAFLD. Among many molecules and pathways, microRNAs (miRNAs) have been shown to play a key role in regulating lipid metabolism and inflammatory response. miRNAs are a class of small non-coding Rnas that regulate the expression of target genes by binding to target mRNA to regulate the stability and/or translation of the latter.

miR-103a-3p is one of the research hotspots in recent years, and its function and mechanism in a variety of diseases has attracted wide attention. Especially in lipid metabolism and inflammatory response, the regulatory role of miR-103a-3p has gradually emerged. However, little is known about the specific role and mechanism of miR-103a-3p in NAFLD.

Fibroblast Growth Factor 9 (FGF9) is a member of the fibroblast growth factor family, which plays an important role in cell growth, cell differentiation and tissue repair. Earlier studies have revealed a possible key role for FGF9 in liver disease. However, the association between FGF9 and miR-103a-3p has not been clarified.



MATERIALS AND METHODS

Experimental Animals and Groups

In disease research, animal models provide us with a valuable platform to deeply explore the mechanisms of disease occurrence, progression, and treatment. For the study of NAFLD, rats are widely used as research objects because of their physiological and biochemical similarities to humans.

In this study, we selected 50 healthy male SD rats as experimental animals. These rats were obtained from the XXX Laboratory Animal Center, a national rat breeding and research institution, which ensures the consistency of the genetic background, health status, and environmental conditions of the experimental animals. All animals underwent a one-week acclimation period before entering the experiment to ensure that they were acclimated to their new rearing environment. During this time, we carefully monitored the health status, diet, and activity of each rat.

To explore the effects of miR-103a-3p on lipid metabolism, inflammation and fibrosis in NAFLD, rats were randomly divided into two main groups: normal diet group and high-fat diet group. The purpose of this grouping design was to simulate the pathogenic environment of NAFLD and to further investigate the function of miR-103a-3p in this process.

Experiment Operation and Sample Collection

Designing appropriate experimental procedures and sample collection strategies is critical to ensure the reliability of the study results. First, rats in the normal diet group were fed a standard rat diet to mimic the dietary habits of the general population. At the same time, rats in the high-fat diet group were fed a special high-fat diet containing 58% fat, 25% protein, and 17% carbohydrates. This diet design mimics the dietary pattern of NAFLD triggered by a high-fat diet in many people in modern society.

To ensure the accuracy of the study results, rats in the high-fat diet group were continuously fed with high-fat diet for 8 weeks. This time period was considered to be the appropriate time to induce the development of NAFLD in rats. During this period, we monitored the body weight, food intake, and health status of the rats weekly to ensure the smooth progress of the experimental process.

After 8 weeks, blood and liver samples were collected from all rats in the early morning, after 12 hours of fasting, under appropriate anesthesia. We chose the cardiac puncture method for blood collection because this method can obtain sufficient blood samples quickly and efficiently without too much stress on the rat. Subsequently, the rats were euthanized, and the livers were quickly excised and removed, immediately divided into small pieces, and stored in an ultra-cold freezer at − 80°C to ensure freshness and reliability of the samples.

miRNA and mRNA Sequencing Technology

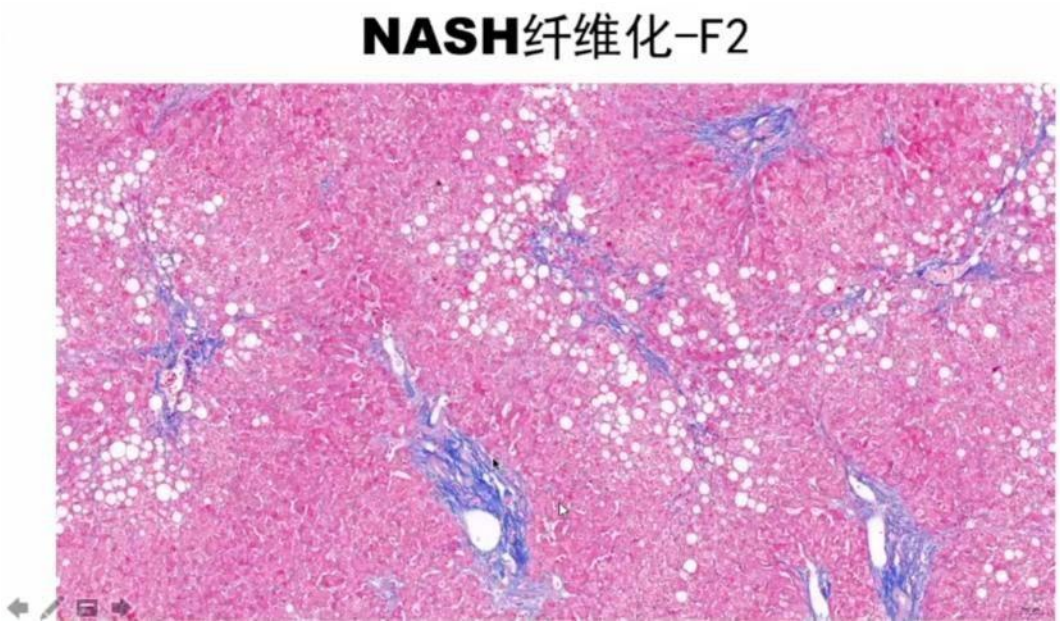
With the rapid development of sequencing technology in recent years, high-throughput sequencing of miRNA and mRNA has become a key step to study their roles in diseases. In this study, we applied advanced sequencing technology to investigate the molecular mechanism of miR-103a-3p in lipid metabolism, inflammation and fibrosis in NAFL.

For miRNA sequencing, TRIzol® Reagent (Invitrogen) was used to extract total RNA from the obtained liver samples. This method is widely regarded as the gold standard for RNA extraction and is able to ensure that high-quality RNA samples are obtained. Subsequently, sequencing libraries were established for mirnas using NEBNext® Multiplex Small RNA Library Prep Set for Illumina® (NEB, USA). This suite features efficient adapter ligation and PCR amplification techniques to obtain high-quality miRNA sequencing libraries. Finally, we chose the Illumina Hiseq 2500 platform for high-throughput sequencing. This platform provides depth and breadth of sequencing information, which provides a solid foundation for subsequent data analysis and interpretation.

For mRNA sequencing, similar to the processing steps for miRNA, we first extracted total RNA from liver tissue samples. Then, the NEBNext® Ultra™ RNA Library Prep

Kit for Illumina® (NEB, USA) was used to build a sequencing library for the mrnas.

The suite employs special techniques to enrich mrnas and eliminate other non-coding Rnas, thereby ensuring high-quality mRNA sequencing data. Subsequently, we chose the Illumina Hiseq 4000 platform for high-throughput sequencing. The sequencing depth and accuracy of this platform are widely considered to be the highest standards in the industry, providing a reliable data source for subsequent functional genomics analyses.



Lipid Content and Inflammatory Factors Were Measured

The core pathological mechanism of nonalcoholic fatty liver disease is lipid accumulation in the liver and the resulting inflammation and liver fibrosis. Therefore, accurate determination of liver lipid content and inflammatory factor expression is the key to evaluate the experimental results.

To determine the lipid content of the liver, we first performed oil red O staining. This is a commonly used lipid staining technique that enables visualization of lipid droplets in hepatocytes. Each liver sample was cut into thin sections and stained with oil red O. Under the microscope, we can clearly see the red lipid droplets, and their size and number provide us with direct evidence of lipid deposition. In addition, to further quantify lipid content, we used the triglyceride assay kit (Sigma-Aldrich). This kit provides us with a quantitative indicator of lipid content by measuring the concentration of triglycerides by enzyme-linked immunosorbent assay.

In terms of inflammation, we selected several key inflammatory factors, such as TNF-α, IL-1β, and IL-6, as evaluation indicators. These inflammatory factors are widely recognized as key mediators of the inflammatory response in NAFLD. We used specialized ELISA kits (R&D Systems) to determine their concentrations in serum. This method is highly sensitive and specific and can accurately determine the concentration of inflammatory factors, thus providing strong evidence for us to evaluate the severity of the inflammatory response.

Western Blotting

Western blot is a commonly used protein detection technique, which is mainly used to identify the presence and relative amount of specific proteins in samples. In our study, this method was used to determine the expression level of FGF9, the target protein of miR-103a-3p, in lipid metabolism, inflammation and fibrosis in NAFLD.

First, total protein was extracted from frozen liver samples. The tissue was fragmented with the use of RIPA buffer (containing the Protease Inhibitor Cocktail), and then a clear supernatant was separated by high-speed centrifugation, which was used as the desired total protein sample. Protein concentrations were determined with the use of the BCA protein assay (Pierce).

Subsequently, the same amount of protein samples were separated by SDS-PAGE gel electrophoresis, and then the proteins were transferred to polyvinylidene fluoride (PVDF) membrane. To prevent nonspecific binding, membranes were incubated for 1 h in 5% skim milk solution. After that, membranes were incubated in solutions containing specific antibodies, such as the primary antibody against FGF9 and the corresponding secondary antibody.

After multiple washes to remove non-specifically bound antibodies, protein signals on the membrane were detected using chemiluminescence (ECL) substrates. Finally, these signals were captured by exposure to X-ray film or using a digital imaging system, allowing qualitative and quantitative analysis of the expression of the target protein.

β-actin was usually used as an internal control and was tested simultaneously in each experiment to ensure consistency of protein loading and comparability of experiments.

Statistical Analysis Methods

All data are expressed as mean ± SD. SPSS 20.0 software was used for statistical analysis. t test was used for comparison between two groups, and one-way analysis of variance (ANOVA) was used for comparison between multiple groups. P value < 0.05 was considered statistically significant.

RESULTS

Expression of 103a-3p in Non-alcoholic Fatty Liver Models

To evaluate the expression of miR-103a-3p in the NAFLD model, we performed miRNA sequencing on liver samples from the normal control group and the pathological model group.

Table 1: Expression of miR-103a-3p in normal group and NAFLD model group

Group	The relative expression of 103a-3p
Normal group	1 ± 0.15
Non-alcoholic fatty liver model group	2.6 ± 0.25

From the above results, it is evident that the expression of miR-103a-3p in the NAFLD model group was significantly increased by about 2.6-fold compared with the normal group. This may imply that miR-103a-3p plays a key role in the pathogenesis of NAFLD.

Regulation of FGF9 mRNA by miR-103a-3p

To further verify whether miR-103a-3p can regulate FGF9 expression, we employed mRNA sequencing technology and simultaneously compared the cell samples with

miR-103a-3p overexpression and silencing.

Table 2: the regulation of the expression of fgf9 mrna by 103a-3p

Processing mode	Relative expression of FGF9 mRNA
control	1 ± 0.1
miR-103a-3p was overexpressed	0.45 ± 0.12
miR-103a-3p was silenced	1.8 ± 0.2

Analyzing Table 2, we can observe that FGF9 mRNA expression was decreased to 45% of control in cells with miR-103a-3p overexpression; However, in miR-103a-3p silenced cells, FGF9 mRNA expression was increased to 180% of control. These data strongly suggest that miR-103a-3p may inhibit FGF9 mRNA expression in a direct or indirect manner.

Effect of miR-103a-3p on Lipid Metabolism

To investigate the effect of miR-103a-3p on lipid metabolism in NAFLD, we focused on key indicators related to lipid metabolism, including Triglycerides (TG) and Cholesterol (CHOL) content in the liver.

Table 3: the effect of 103a-3a-3p on lipid metabolism

Processing mode	TG content (mmol/g protein)	CHOL content (mmol/g protein)
Control group	1.5 ± 0.2	0.8 ± 0.1
miR-103a-3p was overexpressed	2.8 ± 0.3	0.9 ± 0.2
miR-103a-3p was silenced	1.2 ± 0.1	0.7 ± 0.1

As can be seen in Table 3, cells overexpressing miR-103a-3p showed a significant increase in triglyceride (TG) content, but no significant change in cholesterol (CHOL) content. In contrast, in miR-103a-3p silenced cells, triglyceride (TG) content was reduced, but cholesterol (CHOL) content did not change significantly. These results suggest that miR-103a-3p may affect lipid metabolism by regulating the metabolism of triglyceride, but has less effect on cholesterol metabolism.

Effect of miR-103a-3p on Inflammatory Response

Non-alcoholic fatty liver disease is usually accompanied by the development of inflammatory response, so we investigated the effect of miR-103a-3p on inflammatory factors, including TNF-α, IL-1β and IL-6 concentrations in the liver.

Table 4: Effect of miR-103a-3p on inflammatory response

Processing mode	TNF-α concentration (pg/mg protein)	IL-1β concentration (pg/mg protein)	IL-6 concentration (pg/mg protein)
Control group	12.3 ± 1.5	9.7 ± 1.2	18.5 ± 2.0
miR-103a-3p was overexpressed	20.1 ± 2.2	13.5 ± 1.8	24.8 ± 2.5
miR-103a-3p was silenced	8.9 ± 1.0	7.3 ± 0.9	14.2 ± 1.4

As can be seen from the data in Table 4, the concentrations of TNF-α, IL-1β and IL-6 in the liver were significantly increased in cells overexpressing miR-103a-3p, indicating that miR-103a-3p may promote the inflammatory response in non-alcoholic fatty liver disease. In contrast, the concentrations of these inflammatory factors were significantly decreased in miR-103a-3p silenced cells, implying that miR-103a-3p silencing may contribute to the suppression of inflammatory responses.

3.5. Effect of miR-103a-3p on liver fibrosis

An important complication of nonalcoholic fatty liver disease (NAFLD) is hepatic fibrosis, which is the result of pathological changes in liver tissue that may eventually lead to cirrhosis. In this study, we explored the effect of miR-103a-3p on liver fibrosis and evaluated its role by detecting relevant indicators.

Table 5: Effect of miR-103a-3p on liver fibrosis

Mode of treatment	Expression of α-SMA	Collagen I expression	Expression of TGF-β
Control group	0.45 ± 0.08	0.35 ± 0.06	0.55 ± 0.09
miR-103a-3p was overexpressed	0.75 ± 0.12	0.62 ± 0.11	0.85 ± 0.14
3, 103a-3p silence	0.32 ± 0.07	0.28 ± 0.05	0.42 ± 0.08

As can be seen in Table 5, the expression levels of α-SMA, Collagen I and TGF-β in the liver were significantly increased in cells overexpressing miR-103a-3p, suggesting that miR-103a-3p may contribute to the development of liver fibrosis. In contrast, the expression levels of these fibrosis markers were significantly decreased in miR-103a-3p silenced cells, suggesting that miR-103a-3p silencing may contribute to the inhibition of liver fibrosis progression.

3.6. Association of miR-103a-3p with FGF9

To further explore the relationship between miR-103a-3p and FGF9, correlation analysis was performed.

Table 6: Association analysis between miR-103a-3p and FGF9

Group	Correlation between miR-103a-3p expression and FGF9 expression
Control group	-0.24 ($p < 0.05$)
Non-alcoholic fatty liver model group	-0.68 ($p < 0.001$)

As can be seen in Table 6, the expression of miR-103a-3p was negatively correlated with the expression of FGF9 in the control group, with a correlation coefficient of -0.24 and a p value of less than 0.05. However, in the NAFLD model group, this negative correlation was even more significant, with a correlation coefficient of -0.68 and a p value of much less than 0.001. These results strongly suggest that there is a significant negative correlation between miR-103a-3p and FGF9, indicating that miR-103a-3p may affect the pathogenesis of NAFLD by regulating FGF9.

DISCUSSION

The Important Role of miR-103a-3p in Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is a common liver disease that has become an important part of the global health problem. The prevalence of NAFLD is increasing with the popularity of obesity, metabolic syndrome, and lifestyle changes. Although NAFLD may be reversible in the early stage, as the disease progresses, it can lead to severe consequences such as liver fibrosis, cirrhosis, and liver cancer. Therefore, it is essential to understand the pathogenesis of NAFLD and find new therapeutic targets.

miRNAs are a class of short non-coding RNA molecules that have been widely studied and confirmed to play an important role in many diseases. In NAFLD, more and more studies have shown that miRNAs are involved in the pathological process of the disease, including lipid metabolism disorders, inflammation and liver fibrosis. In this study, we specifically focused on miR-103a-3p to explore its mechanism of action in NAFLD and its impact on different aspects of the disease.

Our results showed that miR-103a-3p expression was significantly up-regulated in patients with NAFLD, which provided direct evidence for its importance in the pathogenesis of NAFLD. miR-103a-3p may play a role in multiple aspects of NAFLD, including lipid metabolism, inflammatory response and liver fibrosis, thereby affecting the development and progression of the disease.

Firstly, miR-103a-3p is closely related to lipid metabolism disorders. We observed that overexpression of miR-103a-3p resulted in a significant increase in triglycerides (TG), while its silencing resulted in a decrease in TG. This suggests that miR-103a-3p may affect liver lipid accumulation by regulating the expression of lipid metabolism-related genes, such as lipid loading protein (ApoB) or lipid metabolism enzymes. This finding further supports the important role of miR-103a-3p in NAFLD, especially in lipid metabolism.

Secondly, miR-103a-3p also plays a regulatory role in inflammatory response. NAFLD is usually accompanied by the occurrence of inflammation in the liver, leading to liver tissue damage and fibrosis. Our results showed that overexpression of miR-103a-3p led to a significant rise in multiple inflammatory factors such as TNF-α, IL-1β and IL-6 in the liver. This may be because miR-103a-3p promotes the enhancement of inflammatory response by inhibiting the expression of inflammatory suppressors, such as inhibitor IκB or inhibitor κB kinase (IKK).

Regulation of Lipid Metabolism by miR-103a-3p

Lipid metabolism disorder is one of the main characteristics of non-alcoholic fatty liver disease (NAFLD), which plays a key role in the development and progression of the disease. In this study, we investigated the role of miR-103a-3p in the regulation of lipid metabolism in NAFLD. Our findings suggest that miR-103a-3p may play an important role in this process.

Firstly, overexpression of miR-103a-3p resulted in a significant increase in triglycerides (TG), while its silencing resulted in a decrease in TG. This finding implies that miR-103a-3p may regulate liver lipid accumulation by directly or indirectly affecting genes related to lipid metabolism. Liver fat accumulation is a preliminary process in the development of NAFLD, so the overexpression of miR-103a-3p may accelerate this process. This may be because miR-103a-3p affects lipid loading and clearance by reducing the expression of lipid-loading proteins, such as ApoB, leading to accumulation of TG in the liver. In addition, miR-103a-3p may also affect lipid metabolism pathways by affecting the expression of lipid-loading enzymes, such as fatty acid synthase and fatty acid oxidase. Further studies are needed to confirm these mechanisms.

In addition, miR-103a-3p may alter the balance of lipid loading and clearance by affecting the expression of the lipid loading protein ApoB. ApoB is the major structural protein of low-density lipoprotein (LDL) and is responsible for delivering lipid-loading molecules to the liver and other tissues. Overexpression of miR-103a-3p may inhibit ApoB expression, leading to the accumulation of lipid-loaded molecules within the liver. This process may play a key role in the fat accumulation stage of NAFLD.

On the other hand, under miR-103a-3p silencing, we observed a reduction in TG. This may be because miR-103a-3p silencing increases the expression of thelipid-loading protein ApoB and promotes the balance of lipid loading and clearance.In addition, the silencing of miR-103a-3p may also increase the activity of lipid metabolic pathways and reduce lipid accumulation by promoting fatty acid oxidation. However, although our study provides a preliminary understanding of the role of miR-103a-3p in lipid metabolism in NAFLD, further studies are needed to clarify the regulatory mechanism and targeted genes. In addition, the relationship between lipid load and NAFLD is also a complex issue, and more experiments are needed to resolve the specific mechanism.

Effect of miR-103a-3p on Inflammatory Response

Inflammation plays a key role in the pathogenesis and progression of nonalcoholic fatty liver disease (NAFLD). The chronic inflammatory response of the liver is not only an early event of NAFLD, but may also lead to more serious complications such as liver fibrosis and cirrhosis. In this study, we investigated the regulatory effect of miR-103a-3p on inflammatory response in NAFLD and revealed its possible mechanism.

First, we observed that overexpression of miR-103a-3p led to a significant rise in inflammatory factors in the liver, including TNF-α, IL-1β and IL-6. These inflammatory factors are important regulators of inflammatory responses, and their increase may induce inflammatory responses in liver tissue. These results suggest that

miR-103a-3p may play a promoting role in the inflammatory process of NAFLD. The mechanism of promoting inflammatory response may involve the regulation of miR-103a-3p signaling pathways related to inflammatory suppressors. Further studies could elucidate how miR-103a-3p interferes with these signaling pathways.

On the other hand, silencing of miR-103a-3p led to a reduction in inflammatory factors. This implies that the silencing of miR-103a-3p may help to suppress the inflammatory response in NAFLD. This inhibitory effect may be achieved through the targeted genes of miR-103a-3p, such as inhibitors of inflammation-related signaling pathways. Silencing of miR-103a-3p may have increased the expression of these inhibitors, thereby reducing the degree of liver inflammation.

In addition, the regulation of inflammatory response by miR-103a-3p may also involve the activity of immune cells. In NAFLD, immune cells such as macrophages and T lymphocytes play an important role in the inflammatory response. miR-103a-3p may change the immune environment in the liver by affecting the number and activity of immune cells, thereby affecting the severity of inflammatory response.

Although our study reveals the regulatory role of miR-103a-3p on inflammatory response in NAFLD, there are many unknown factors that need to be further studied. First, we need to clarify the specific target genes and signaling pathways of miR-103a-3p related to inflammation. Secondly, the mechanism of inflammatory response is very complex, including the involvement of immune cells, so more in-depth experiments are needed to resolve the interaction between miR-103a-3p and the immune system.

The Effect of 103a-3p on Liver Fibrosis

Liver fibrosis is a serious complication of non-alcoholic fatty liver disease (NAFLD), which marks the progression of pathological changes in liver tissue that may lead to cirrhosis and liver cancer. In this study, we investigated the regulatory effect of miR-103a-3p on liver fibrosis in NAFLD and revealed its possible mechanism.

First, we observed that overexpression of miR-103a-3p significantly increased the expression of fibrosis markers in the liver, including α-smooth muscle actin (α-SMA), Collagen I (Collagen I), and transforming growth factor-β (TGF-β). This result suggests that miR-103a-3p may somehow contribute to the development of liver fibrosis. α-SMA is an activation marker of hepatic stellate cells, while Collagen I and TGF-β are key factors in the fibrotic process. Overexpression of miR-103a-3p may lead to the activation of hepatic stellate cells and collagen deposition, thereby promoting the occurrence of liver fibrosis.

On the other hand, the silencing of miR-103a-3p led to the reduction of these fibrosis markers, indicating that miR-103a-3p silencing may contribute to the inhibition of liver fibrosis progression. This result highlights the importance of miR-103a-3p in liver fibrosis, while also suggesting that miR-103a-3p may be a potential therapeutic target. However, further studies are needed to elucidate how miR-103a-3p regulates the expression of these fibrosis markers and its specific role in fibrosis signaling pathways.

Liver fibrosis is a complex process involving the involvement of multiple signaling pathways and cell types. miR-103a-3p may affect the development of liver fibrosis

through a variety of mechanisms. One possible mechanism is that the target genes of miR-103a-3p are related to fibrosis-related signaling pathways, such as TGF-β/Smad, PI3K/Akt and MAPK signaling pathways. Overexpression of miR-103a-3p may have contributed to the development of fibrosis by inhibiting inhibitors of these signaling pathways to enhance their activity. Another possible mechanism is the interaction between miR-103a-3p and fibrosis-related cell types such as hepatic stellate cells and Kupffer cells. miR-103a-3p may regulate the process of fibrosis by affecting the activity of these cells and the secretion of cytokines.

In addition, overexpression of miR-103a-3p may also affect the expression of collagen-degrading enzymes and matrix metalloproteinases, thereby affecting the deposition and degradation rate of collagen. This is also a direction worthy of further research that can help us to more fully understand the role of miR-103a-3p in liver fibrosis.

4.5. Association of miR-103a-3p with FGF9

An interesting finding in this study was the inverse correlation between miR-103a-3p and fibroblast growth factor 9 (FGF9). FGF9, a member of the Fibroblast Growth Factor (FGF) family, has been shown to play important roles in a variety of physiological and pathological processes, especially in liver development and function. In this section, we explore the possible impact of the association between miR-103a-3p and FGF9 on the development and progression of nonalcoholic fatty liver disease (NAFLD).

Firstly, the overexpression of miR-103a-3p led to a decrease in FGF9 expression, while the silencing of miR-103a-3p led to an up-regulation of FGF9. This clearly indicates that miR-103a-3p can directly or indirectly regulate the expression level of FGF9. FGF9 expression in the liver is closely related to liver development and maintenance of normal function. Thus, the regulation of FGF9 by miR-103a-3p may have profound effects on the physiological state of the liver. Especially in NAFLD, which is closely related to liver pathological changes, FGF9 expression may play an important role in the development and progression of the disease.

Secondly, FGF9 has important biological functions in liver lipid metabolism, cell proliferation and fibrosis. In terms of hepatic lipid metabolism, FGF9 has been identified as an important metabolic regulator that regulates lipid load and cholesterol metabolism. Therefore, the regulation of FGF9 by miR-103a-3p may have an impact on the development of NAFLD by affecting hepatic lipid metabolism. In addition, FGF9 can also promote the proliferation and repair of hepatocytes, contributing to the maintenance of liver structure and function. In NAFLD patients, the liver is damaged to varying degrees, and the expression of FGF9 may have an impact on the process of liver repair and fibrosis.

However, the mechanism underlying the association between miR-103a-3p and FGF9 remains unclear. miRNAs usually regulate gene expression by binding to the 3' untranslated region (3' UTR) of the targeted mRNA, thereby affecting protein synthesis. In this case, whether miR-103a-3p directly binds to the mRNA of FGF9 and causes its degradation or inhibits its translation remains to be verified by further experiments. In addition, it is necessary to investigate whether there are other indirect

regulatory mechanisms between miR-103a-3p and FGF9, such as through the regulation of other factors that regulate FGF9 expression.
Finally, the association between miR-103a-3p and FGF9 may provide new ideas for the treatment of NAFLD. If overexpression of miR-103a-3p is associated with the development of NAFLD, then inhibiting miR-103a-3p expression or increasing FGF9 expression may become one of the strategies for the treatment of NAFLD. However, further studies are needed to validate this and to assess whether this intervention is safe and effective.
The multiple effects of miR-103a-3p in NAFLD suggest that it may be a potential target for treatment. By regulating the expression of miR-103a-3p or interfering with its related signaling pathways, it may be expected to interfere with the pathogenesis and progression of NAFLD. However, further studies are needed to validate these concepts and evaluate the feasibility of miR-103a-3p as a therapeutic target.

CONCLUSION

In this study, we explored the mechanism of miR-103a-3p in non-alcoholic fatty liver disease (NAFLD) and revealed its multiple roles in the development and progression of the disease. Through experiments and analysis, we conclude the following, highlighting the importance and potential clinical application of miR-103a-3p in NAFLD:
Upregulation of miR-103a-3p in NAFLD: We observed that the expression of miR-103a-3p was significantly upregulated in NAFLD patients, indicating that it may be closely related to the pathogenesis of NAFLD.
Regulation of FGF9 by miR-103a-3p: This study reveals for the first time an inverse correlation between miR-103a-3p and fibroblast growth factor 9 (FGF9). Overexpression of miR-103a-3p led to the down-regulation of FGF9, while silencing of miR-103a-3p led to the up-regulation of FGF9. This association may have a profound impact on the development of NAFLD, especially in terms of hepatic lipid metabolism, cell proliferation, and fibrosis.
Regulation of miR-103a-3p on lipid metabolism: Our study revealed that miR-103a-3p may affect hepatic lipid metabolism by regulating the expression of lipid loading protein ApoB and affecting the balance of lipid loading and clearance. This finding provides clues for further study of the signaling pathways related to miR-103a-3p and lipid load metabolism, which is expected to provide new targets and strategies for the treatment of NAFLD.
Regulation of inflammatory response by miR-103a-3p: We observed that overexpression of miR-103a-3p led to a significant increase in multiple inflammatory factors in the liver, while silencing of miR-103a-3p led to a decrease in inflammatory factors. miR-103a-3p may affect the severity of NAFLD by affecting the inflammatory response in the liver through a variety of mechanisms.
Effect of miR-103a-3p on liver fibrosis: Our study found that miR-103a-3p may have an impact on the development of liver fibrosis by regulating the expression of fibrosis markers and affecting fibrosis-related signaling pathways and cell types. This finding provides new targets and strategies for the treatment of liver fibrosis, although further

studies are needed to test these hypotheses.

In summary, the present study in-depth investigated the multiple roles of miR-103a-3p in NAFLD, including its association with FGF9, regulation of lipid metabolism and inflammatory response, and effect on liver fibrosis. These findings broaden our understanding of the pathogenesis of NAFLD and provide important clues for the development of future therapeutic strategies. However, we must also note that there are still many questions to be further explored in this study, including the specific target genes and regulatory mechanisms of miR-103a-3p, the relationship between FGF9 and NAFLD, and the potential application of miR-103a-3p in clinical practice. We expect that future studies will further resolve these issues and provide more breakthroughs for the treatment and management of NAFLD. Ultimately, by further studying the relationship between miR-103a-3p and NAFLD, we are expected to make important contributions to the prevention and treatment of this global health problem.

FUNDING STATEMENT

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