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Regulatory effects of miR-30c knockout on hepatic lipid metabolism and progression of liver fibrosis

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Abstract

Background Liver fibrosis is a chronic progressive liver disease influenced by environmental and genetic factors. Early stages of liver fibrosis are marked by abnormal fat deposition in the liver. MicroRNA-30c (miR-30c) plays key roles in various pathological processes, including lipid metabolism, metabolic disorders, and cancer. However, the precise role of miR-30c in the progression of liver fibrosis remains unclear. This study aimed to investigate the role of miR-30c in liver fibrosis using miR-30c knockout (KO) mice.

Methods KO mice were compared to wild-type (WT) mice to evaluate growth and development, with a focus on lipid metabolism markers. Liver transcriptomic analysis was performed to explore gene expression alterations due to miR-30c knockout. To induce liver fibrosis, thioacetamide (TAA) was administered to the mice, allowing the assessment of the impact of miR-30c on fibrosis initiation and progression.

Results The results showed that miR-30c knockout led to body weight loss and disrupted lipid metabolism in mice. A total of 212 upregulated genes and 267 downregulated genes were identified in the livers of KO mice by RNA-seq. Differential enrichment of lipid metabolism pathways was observed KO mice, including PPAR signaling pathway, fatty acid degradation, biosynthesis of unsaturated fatty acids, and so on. The deletion of miR-30c aggravated the liver injury induced by TAA and increased the structural disorder of hepatocytes, and miR-30c knockout mice showed more severe liver fibrosis. This study establishes miR-30c as protective against liver fibrosis progression.

Conclusion The deficiency of miR-30c might exacerbate fibrosis and inflammation, while also disrupting lipid metabolism, suggesting that miR-30c modulation shows therapeutic potential based on mechanistic evidence. Further exploration of miR-30c-mediated crosstalk between metabolic and inflammatory pathways could advance therapeutic strategies for fibrotic liver diseases.

Keywords microRNA-30c, Liver fibrosis, Lipid metabolism, Inflammation, Thioacetamide

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Introduction

Liver fibrosis is a common chronic progressive liver disease. The pathogenesis of liver fibrosis is a complex, multi-factorial process influenced by both environmental and genetic factors [1]. Current epidemiological data and laboratory research suggest that viral infections, alcohol abuse, nutritional deficiencies, industrial toxins, and metabolic disorders are all closely associated with the development of liver fibrosis. Among these, dysregulation of lipid metabolism leading to abnormal hepatic fat deposition is recognized as an early stage of liver fibrosis [2].

Investigating the potential connections between RNA and liver fibrosis can help understand how fibrogenesis occurs. MicroRNAs (miRNAs) play a crucial role by binding to specific sites on messenger RNAs (mRNAs), which can lead to the degradation of mRNAs or inhibit their translation, effectively reducing the expression of target genes after transcription [3]. As essential regulators of metabolic balance, miRNAs can influence the pathophysiological processes associated with various metabolic syndromes through the modulation of target gene expression. It suggested that miRNAs might play significant roles in the progression of liver fibrosis [4].

The microRNA-30c (miR-30c) is a member of the microRNA-30 (miR-30) family, playing significant roles in various pathological processes, including cardiovascular diseases [5], metabolic disorders [6], and cancer development [7]. Recent research has identified that miR-30c could regulate lipid synthesis by targeting Apolipoprotein B (APOB) [8]. Furthermore, it has been reported that miR-30c was downregulated in the tissues and plasma of patients with liver fibrosis, leading to abnormal activation and secretion of hepatic stellate cells [9]. The target genes of miR-30c and the signaling pathways it influences in the progression of liver fibrosis are not well understood and require further exploration. It is known that disrupted lipid metabolism can heighten the risk of developing liver fibrosis [10]; however, the specific role of miR-30c in this process is still not clear. Therefore, it is essential to investigate the role of miR-30c in the onset and progression of liver fibrosis.

In this study, miR-30c knockout (KO) mice with wild-type (WT) mice were compared to evaluate differences in growth and development, focusing on changes in biochemical markers related to lipid metabolism. Liver transcriptomic analysis was conducted to investigate the regulatory effects of miR-30c knockout on gene expression in the liver. Then, thioacetamide (TAA) intraperitoneal injection was used to induce a liver fibrosis model in these mice, to explore the impact of miR-30c on the initiation and progression of liver fibrosis. This study would help elucidate the role of miR-30c in liver fibrosis and contribute to a deeper understanding of its underlying mechanisms.

Methods

Animals

The miR-30c knockout mice C57BL/6J background) used in this study were utilized by using CRISPR/cas9-mediated genome engineering technology (Shanghai Model Organisms Center, Inc, Shanghai, China). The generation and genotyping of miR-30c KO mice were described in our previous study [11]. Mice were maintained under specific pathogen-free (SPF) conditions (Experimental Animal Use Certificate: SYXK(Wan)2023-009) in a controlled environment (22 ± 1 °C; 55 ± 5% relative humidity) with a 12 h light: dark cycle, daily health monitoring, and noise levels below 55 dB. Nesting material was replenished weekly, and animals received ad libitum access to autoclaved water and standard rodent chow. Randomization sequences were computer-generated via Research Randomizer platform (www.randomizer.org) with block randomization (block size = 4). Allocation was concealed in sealed envelopes opened after intervention assignment. Animal identifiers were recoded post-procedure by a separate technician. Mice were anesthetized by intraperitoneal injection of tribromoethanol (1.25% sterile solution, Sigma-Aldrich) at 0.2 mL/10 g body weight (250 mg/kg). Anesthesia depth was verified by loss of pedal withdrawal reflex and absence of response to tail clamp. Euthanasia was performed via cervical dislocation under sustained surgical-plane anesthesia. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Bengbu Medical University (No.2023LDK438). And methods were carried out in accordance with relevant guidelines and regulations. All procedures followed the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments), with power analysis documented before unblinding. Any post-hoc analyses are explicitly labeled.

Measurement of animal phenotypes

Mice were observed daily, and the weekly body weights of miR-30c KO mice and WT mice were recorded until 16 weeks of age. Liver weight and body weight were measured to calculate the liver index (%) as follows: Liver index (%) = liver weight/body weight. The concentrations of triglycerides (TGs) and free fatty acids (FFAs) in serum or liver samples were using reliable commercial assay kits (Cat#BC0625 for TGs and Cat#BC0595 for FFAs; both from Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacture's instructions. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by using an automatic chemistry analyzer (Celercare V5, MNCHIP, Tianjin, China).

Measurements of biochemical factors

Plasma was collected by centrifugation after blood sampling. The blood biochemistry measurements were performed according to the manufacturer's instructions. Plasma was analyzed for biochemical factors including Glucose (GLU), albumin (ALB), total protein (TP), total bilirubin (TBIL) and so on, using the fully automated biochemistry analyzer for animals Pointcare V2 (MNCHIP, China).

RNA sequencing

Total RNA of liver sample was isolated using the TRIzol reagent (Cat#15596026, Invitrogen, CA, USA). RNA concentration and purity was measured using NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The RNA samples of the WT and KO livers were sequenced with three replicates in each group at Novogene Co., Ltd. (Beijing, China). After library preparation, the samples were sequenced on the Illumina NovaSeq6000 platform. The raw data files for RNA-seq have been deposited in the Sequence Read Archive (SRA) database under PRJNA1205524.

Identification of differentially expressed genes

The differentially expressed genes (DEGs) between the WT mice and the miR-30c KO mice were identified using the package of DESeq algorithm in the R software. The resulting p-values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Criteria for differentially expressed genes (DEGs) set as $|\text{Fold change (FC)}| \geq 1.5$ and adjust p-value < 0.05. Volcano plot was used to present difference in gene expression between the WT mice and the miR-30c KO mice.

Gene function enrichment analysis

The COG (Cluster of Orthologous Groups of proteins) analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) functional pathway analysis were used to predict the function of DEGs between the WT mice and the miR-30c KO mice. The enrichment was considered to be significant when the corrected p-value was less than 0.05.

TAA-induced liver fibrosis mouse model

A toxin thioacetamide (TAA) (Cat#GC20044, GlpBio, USA) was used to induce liver fibrosis in mice. Intraperitoneal injections of TAA were administered twice per week for 6 weeks (200 mg/kg, dissolved in physiologic saline solution). The control group was administered with the same volume of normal saline (NS). The 10-week-old male WT and KO were randomly divided into four groups ($n = 6$/group): (1) NS-WT group, (2) NS-KO group, (3) TAA-WT group, and (4) TAA-KO group. All animals completed the protocol (0% attrition). Pre-defined exclusion criteria required exclusion if: (1) Technical failure in > 50% assays per sample, or (2) Evidence of non-TAA comorbidities (e.g., liver abscesses). No exclusions occurred during this study.

Pathological examinations

Liver tissues were fixed in 10% formalin. The fixed tissue was then dehydrated in a gradient ethanol series, embedded in paraffin, and stored for subsequent experiments. Tissue sections were cut into 5-μm thick slices and sequentially dewaxed by placing them in xylene, anhydrous ethanol, and alcohol. Hematoxylin eosin (H&E) staining (Cat#C0105S, Beyotime, China) was performed on the liver samples. The slides were first stained with hematoxylin for 2 min, rinsed with water, and then stained with eosin for 5 min after turning blue. The slides were subsequently dehydrated. Sirius red staining (Cat#Y026187, Beyotime, China) was also performed on the liver samples. The slices were first baked at 55 °C for 30 min for pretreatment, followed by sequential immersion in xylene, anhydrous ethanol, 95% ethanol, and 70% ethanol to complete dewaxing; subsequently, sections were rinsed in distilled water for 5 min. Sirius red staining solution was then applied for 45 min at room temperature, after which excess dye was removed by gentle rinsing under running tap water. Finally, sections were dehydrated through a graded ethanol series (70% ethanol, 95% ethanol, and three changes of anhydrous ethanol), cleared in xylene, and mounted with neutral resin under glass coverslips. Pathological changes in the liver tissue were observed under a light microscope. Pathological changes in tissue structure, inflammation, and fibrosis were observed. Tissue slides were coded by an independent technician using random identifiers. Two pathologists independently scored fibrosis stages without group information, with discordant cases arbitrated by a third expert.

Measurement of hydroxyproline levels in liver samples

The levels of hydroxyproline (Hyp) in liver samples were measured by using the Hydroxyproline assay kit (Cat#A030-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), thus assessing the deposition of collagens. Hepatic hydroxyproline content was determined using alkaline hydrolysis. Briefly, accurately weighed liver tissue samples were homogenized in alkaline solution and hydrolyzed. The mixture was subsequently neutralized following kit instructions, adjusted to a final volume of 10 mL with distilled water, and treated with activated charcoal before centrifugation to collect the supernatant for analysis. For colorimetric quantification, 1 mL of supernatant was first incubated with reagent R1 for 10 min at room temperature, followed by

the addition of reagent R2 with a further 5-minute incubation. Reagent R3 was then introduced and the solution was incubated at 60 °C in a water bath for 15 min. After centrifugation at 3,500 rpm for 10 min, the supernatant absorbance was measured at 550 nm.

Quantitative real-time polymerase chain reaction (qRT-PCR) assay

Total RNA of liver sample was isolated using the TRIzol reagent (Cat#15596026, Invotrogen, CA, USA). The cDNA was synthesized according to Vazyme reverse transcription instructions (Cat#R312-01, Vazyme, China). The amplified products were detected by RT-qPCR using SYBR Green PCR Master Mix (Cat#Q511-02, Vazyme, China). Gapdh was used as the internal reference gene. The list of tested genes and the primer sequences were shown in the Supplement Table S1. The qPCR was performed on Applied Biosystems™ 7500. Relative gene expression level was measured by $2^{-\Delta\Delta Ct}$ relative quantitative method.

Statistical analysis

Statistical analysis was carried out using the R 4.3.1 software, and the measurement data obtained from multiple independent experiments were presented as mean ± standard deviation. Differences between the two groups were analyzed using an unpaired two-tailed t-test. For multiple groups of data, overall testing (two-way ANOVA with Tukey's post hoc test) followed by multiple comparison testing were applied. Statically significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Results

Knockout of miR-30c caused body weight loss and disruption of lipid metabolism in mice

The study utilized a normal chow diet to feed wild-type mice and miR-30c knockout mice, followed by a 16-week period of weight monitoring and dissection. Results demonstrated that miR-30c knockout (KO) mice exhibited a significant reduction in body weight compared to wild-type (WT) mice (Fig. 1A). Despite no change in liver weight (Fig. 1B), knockout mice showed a notable increase in the hepatic index (Fig. 1C) alongside significantly elevated hepatic triglyceride (TG) levels in miR-30c knockout mice (Fig. 1D). These findings suggest fat accumulation in the liver. Moreover, knockout mice displayed increased levels of triglyceride and free fatty acids in serum samples (Fig. 1E and F). These findings suggested that miR-30c might play a crucial role in regulating lipid metabolism in mice. The more data of 14 kinds of biochemical factors in plasma of WT and KO mice were listed in the supplement Table S2.

Lipid metabolism pathways were differentially enriched in liver of miR-30c knockout mice

To identify the by molecular mechanisms through which miR-30c regulates that impacted lipid metabolism, RNA-seq was conducted to obtain gene expression profiles of liver tissues from wide-type and miR-30c knockout mice. According to the criteria for identifying DEGs defined as a |Fold Change (FC)| ≥ 1.5 and adjusted p-value < 0.05, 212 upregulated genes and 267 downregulated genes in the livers of KO mice compared to the WT group (Fig. 2A). Enrichment analysis of DEGs was conducted to identify functional categories significantly impacted by miR-30c knockout. The results of the Cluster of Orthologous Groups (COG) enrichment analysis indicated that genes upregulated in the liver of miR-30c knockout mice were predominantly enriched in the category of "Lipid transport and metabolism" (Fig. 2B). Conversely, genes downregulated in these mice were most significantly enriched in the category of "amino acid transport and metabolism" (Fig. 2C). This differential gene expression suggested a pronounced alteration in lipid and amino acid metabolism pathways due to the absence of miR-30c. Further KEGG enrichment analysis revealed that DEGs were prominently involved in essential pathways such as PPAR signaling pathway, Fatty acid degradation, Cysteine and methionine metabolism, Retinol metabolism, Biosynthesis of unsaturated fatty acids (Fig. 2D). KEGG analysis revealed that miR-30c knockout significantly affects lipid synthesis and metabolism. To visualize the lipid metabolism-related pathways enriched in the KEGG analysis, network diagrams were utilized to depict the relationships between DEGs and pathways (Fig. 2E). These diagrams showed that of the 40 DEGs associated with lipid metabolism pathways, 33 were upregulated in the liver of miR-30c knockout mice, whereas only 7 were downregulated. Beyond lipid metabolism pathways, we specifically interrogated fibrosis-related (e.g., TGF-β signaling, ECM-receptor interaction) and inflammatory pathways (e.g., NF-κB, TNF signaling) in KO vs. WT mice under basal conditions. While no fibrosis/inflammation pathways reached statistical significance. These data suggest that miR-30c loss primes but does not fully activate fibro-inflammatory programs without injury. Given the established crosstalk between dysregulated lipid metabolism and hepatic fibrogenesis, we next investigated whether miR-30c deficiency exacerbates liver fibrosis.

The miR-30c deletion aggravates TAA-induced liver fibrosis

Since lipid accumulation can trigger inflammation and activation of hepatic stellate cells (HSCs)—key drivers of fibrosis—we employed a thioacetamide (TAA)to induce injury in the context of miR-30c-dependent metabolic disruption. Liver histopathology was examined using

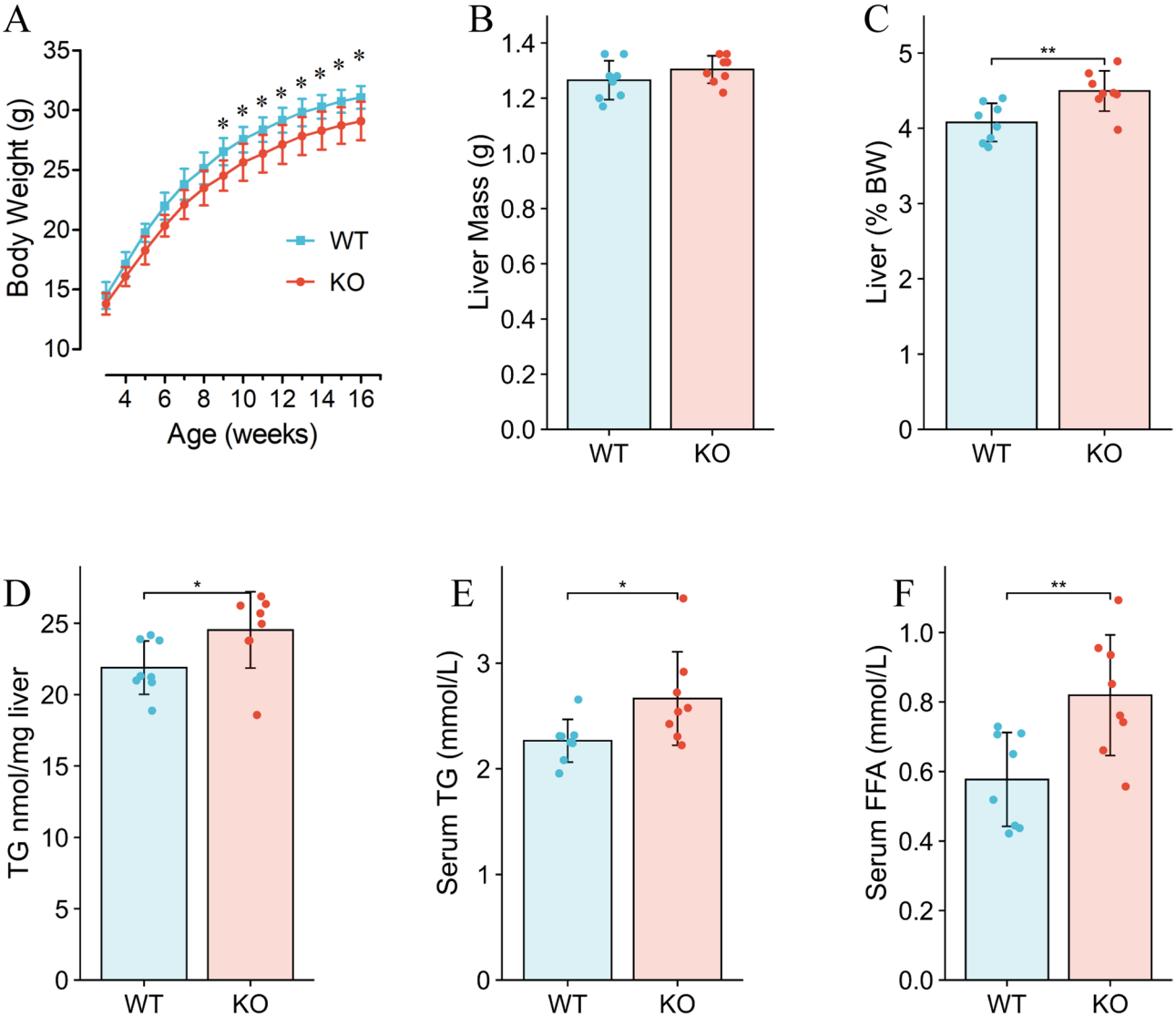

Fig. 1 Knockout of miR-30c causes body weight loss and disruption of lipid metabolism in mice
(A) The weekly body weights of WT and miR-30c KO mice were recorded from 3 week to 16 weeks of age. **(B)** The liver weights of WT and miR-30c KO mice. **(C)** The liver index of WT and miR-30c KO mice. **(D)** The hepatic triglyceride (TG) levels. **(E)** The levels of triglyceride in serum samples. **(F)** The free fatty acids in serum samples. Data expressed as mean ± SD ($n = 8$ mice/group). $^{**}P < 0.01$, $^{*}P < 0.01$ KO vs. WT by unpaired two-tailed t-test

H&E staining. Compared with the WT group, the deletion of miR-30c aggravated the liver injury induced by TAA and increased the structural disorder of hepatocytes, with notable increases in inflammatory infiltration and fibroproliferation (Fig. 3A). Sirius red staining was used to observe the extent of liver fibrosis and the condition of fibrous tissue. The staining results showed that compared with the WT mice, the miR-30c deletion increased the proliferation of liver fibrous tissue. In the mouse liver fibrosis model induced by TAA, miR-30c knockout mice showed more severe liver fibrosis (Fig. 3B). The measurement of liver hydroxyproline content also showed that the degree of liver fibrosis in miR-30c knockout mice was significantly aggravated (Fig. 3C). The serum of mice was isolated and the production levels of ALT and AST in serum were detected. The results levels of ALT and AST in serum samples showed that after TAA induction, the serum ALT and AST levels of miR-30c knockout mice were significantly higher than those of WT mice (Fig. 3D and E).

TAA-induced liver fibrosis coincides with upregulation of lipid synthesis genes in miR-30c KO mice

To further investigate the potential role of miR-30c in the progression of liver fibrosis, the expression levels of relative genes (including fibrotic genes, inflammatory genes, lipid synthesis genes) were measured by qRT-PCR. The results revealed that although miR-30c deletion did not

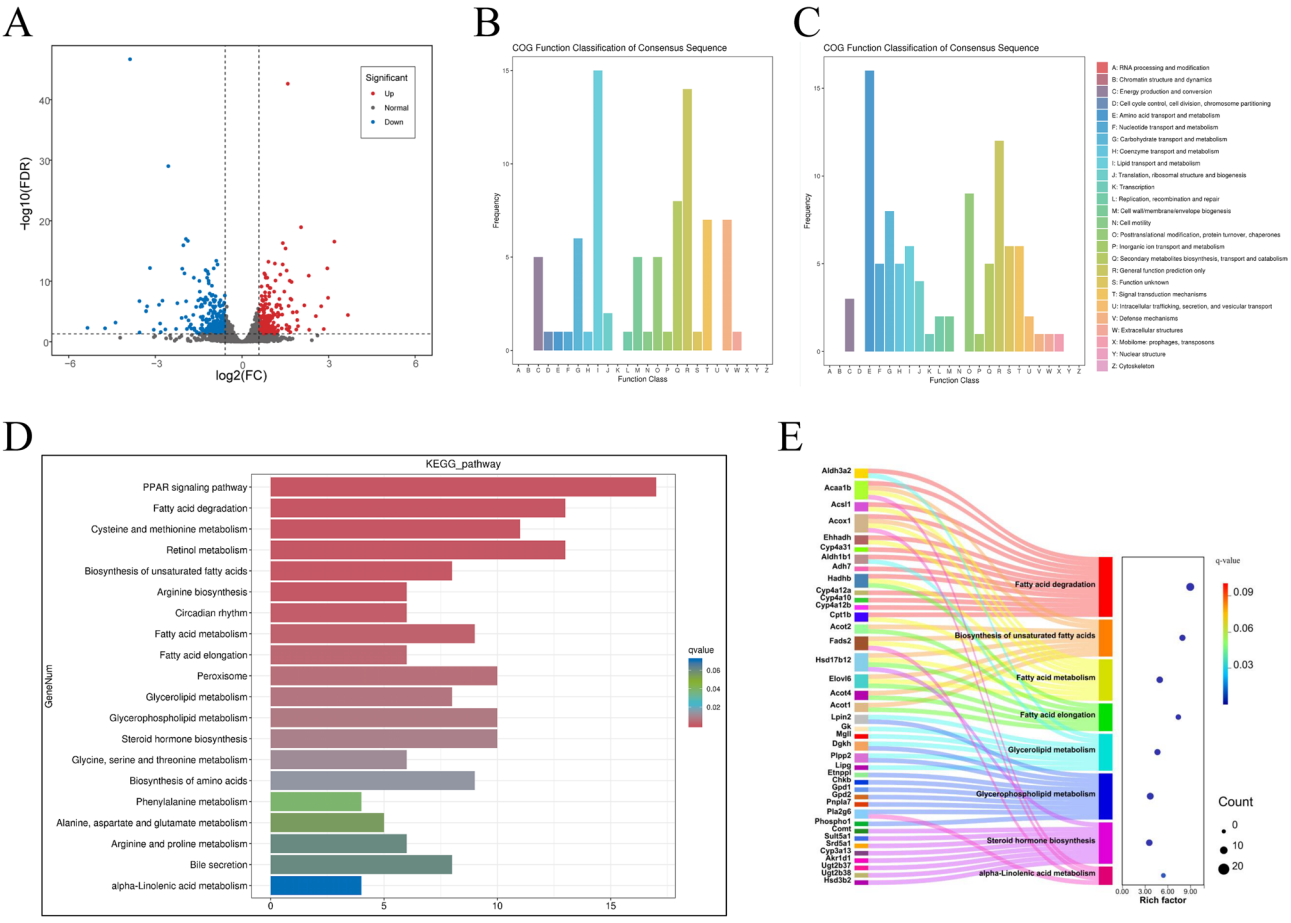

Fig. 2 Knock-out of microRNA-30c could change the gene expression profiles in liver

(A) Differential expressed genes in livers between microRNA-30c KO mice and WT mice group. **(B)** The Cluster of Orthologous Groups (COG) enrichment analysis of the up-regulated genes in the liver of miR-30c knockout mice. **(C)** The COG enrichment analysis of the down-regulated genes in the liver of miR-30c knockout mice. **(D)** The KEGG enrichment analysis of DEGs. **(E)** The network diagrams between DEGs and pathways. Functional enrichment network of DEGs in KO vs. WT livers. Nodes represent KEGG pathways; edges indicate gene-pathway associations. Node size: Rich factor; color: p-value. Differential expression analysis of two groups was performed using the DESeq2. The resulting P values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value < 0.05 found by DESeq2 were assigned as differentially expressed. ($n = 3$ mice/group)

directly induce liver fibrosis, multiple fibrosis-related genes in the livers of miR-30c knockout mice exhibited significantly increased expression with TAA treatment. In this study, without TAA, among five fibrotic genes, a significant increase in the expression of the Pdgfra was observed in the livers of the miR-30c knockout mice. However, under TAA treatment, the expression levels of several fibrosis-associated genes, such as Acta2, Col1A1, Col3A1, Trib3 and Pdgfra, were significantly upregulated in the livers of miR-30c knockout mice (Fig. 4A). In the absence of TAA, no significant differences in the expression of inflammation-related genes (Il-1b, Il-6, Tnf, Ccl2, and Adgre1) were observed between the WT and miR-30c knockout mice. With TAA stimulation, the expression levels of these inflammatory genes in both groups were all up-regulated. Moreover, the expression levels of the Il-6, Tnf, Ccl2, and Adgre1gene were significantly higher in the livers of miR-30c knockout mice compared to WT mice (Fig. 4B). Because of the important role of lipid synthesis in liver fibrosis, the expression levels of lipid synthesis genes (Acot1, Acot4, Elovl6, Fads2, and Fasn) in liver samples were also analyzed. Without TAA treatment, the expression levels of Acot1, Acot4, Elovl6, and Fasn were significantly higher in the livers of miR-30c knockout mice compared to WT mice. With TAA treatment, no significant changes in the expression of lipid synthesis-related genes were observed in the livers of WT mice. However, in miR-30c knockout mice, the expression levels of genes related to lipid synthesis, were significantly increased (Fig. 4C). This suggested that the loss of the miR-30c gene might be associated with synthesis of fatty acids and thereby altering lipid synthesis. Although no causal link is established here, the concomitant upregulation of lipid synthesis genes (Acot1,

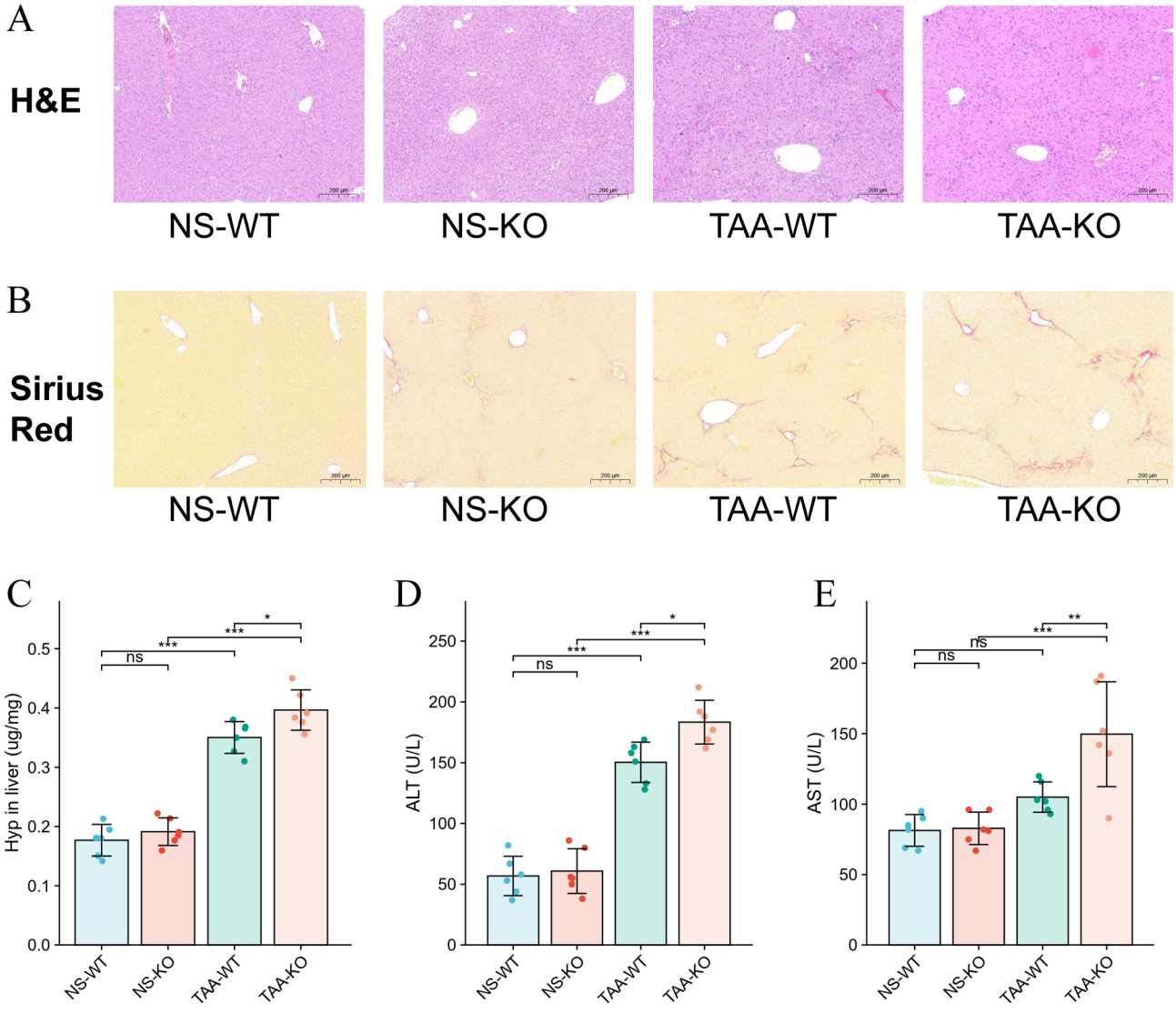

Fig. 3 Knock-out of miR-30c aggravated TAA-induced liver fibrosis
(A) H&E staining of liver tissues (Scale bar, 200 μm). **(B)** Sirius red staining of liver tissues (Scale bar, 200 μm). **(C)** Hydroxyproline contents in the liver. **(D)** Plasma levels of ALT. **(E)** Plasma levels of AST. Data expressed as mean ± SD ($n = 6$ mice/group). Overall testing (two-way ANOVA with Tukey's post hoc test) followed by multiple comparison testing were applied. Statically significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

Acot4, Fasn, etc.) and fibrosis markers in KO mice after TAA exposure suggests a potential association between metabolic dysregulation and fibrotic progression. These changes in gene expression might further contribute to liver fat accumulation, thereby playing an important role in the progression of TAA-induced liver fibrosis.

Discussion

The results of this study revealed the potential role of miR-30c in regulating lipid metabolism in mice by comparing the body weight, liver weight, and related metabolic indicators between wild-type mice and miR-30c knockout mice. Although there was no significant difference in liver weight between the two groups of mice, the liver index of miR-30c knockout mice was significantly increased. This finding suggested that there might be pathological changes such as fat accumulation in the liver of knockout mice. Further results showed that the levels of triglycerides and free fatty acids in the serum of knockout mice were increased, and it could be speculated that the deletion of miR-30c may promote lipid accumulation in the liver. Triglycerides and free fatty acids are key indicators of lipid metabolism, and their levels can reflect the balance of lipid synthesis and decomposition in the body. These results suggested that miR-30c played an important role in regulating lipid metabolism. This is consistent with previous reports on the role of microRNAs in metabolic regulation, indicating the importance of microRNAs in complex metabolic networks in organisms. Future studies should explore the specific target

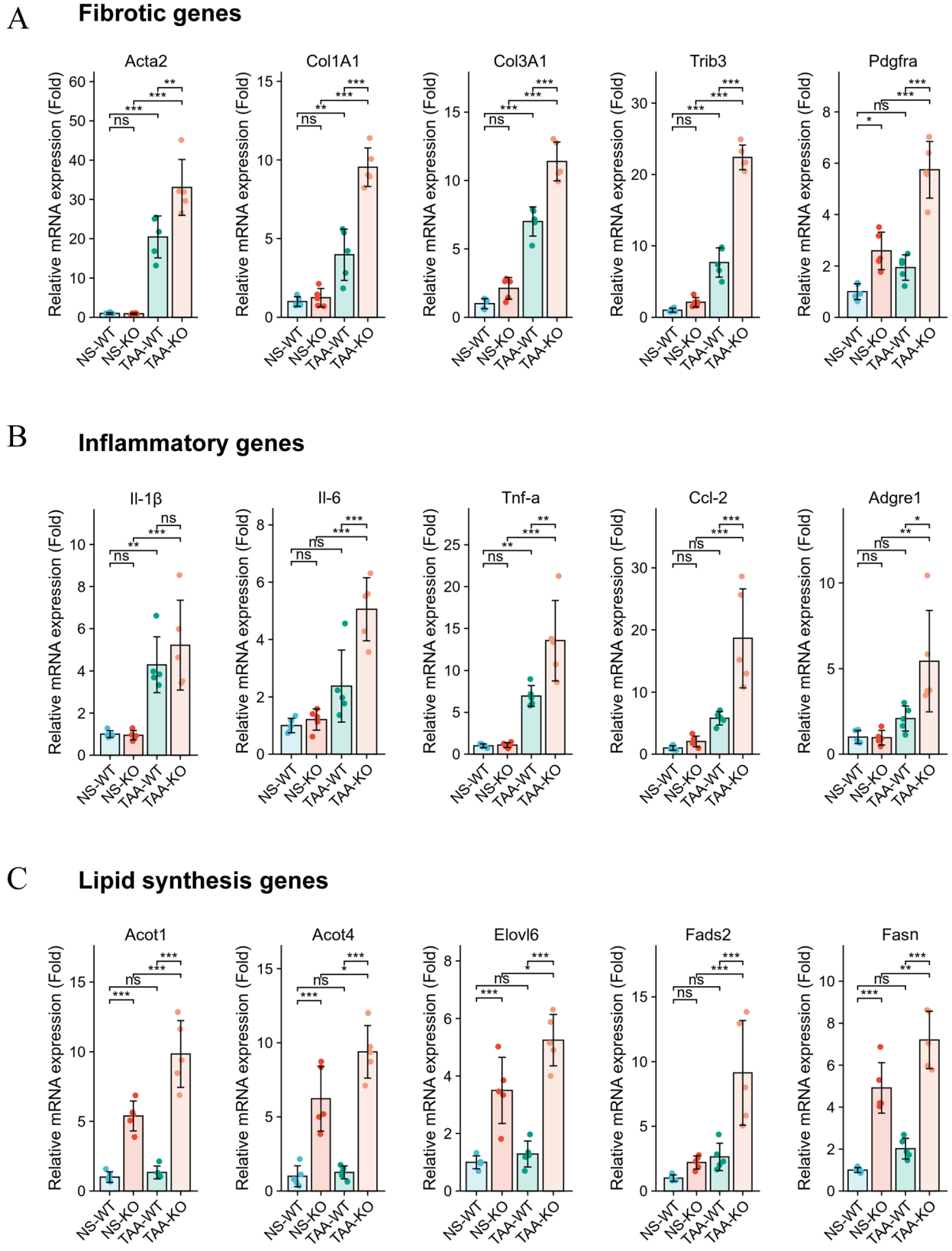

Fig. 4 Knock-out of miR-30c improved TAA-induced liver fibrosis
(A) Relative expression levels of fibrotic genes in liver samples. **(B)** Relative expression levels of inflammatory genes in liver samples. **(C)** Relative expression levels of lipid synthesis genes in liver samples. Data expressed as mean ± SD ($n = 5$ mice/group). The values of $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) by two-way ANOVA with Tukey's post hoc test

genes of miR-30c and how these target genes mediate the changes of lipid metabolism.

In this study, the results of the gene expression profiles of liver tissues of WT mice and miR-30c KO revealed the core role of miR-30c in the regulation of lipid metabolism. A total of 479 DEGs were mainly enriched in pathways related to lipid transport and metabolism. In the liver of miR-30c KO mice, some of the upregulated genes were involved in fatty acid synthesis, decomposition, transport and storage, and thus disturbed the overall homeostasis of lipid metabolism. In particular, PPAR signaling pathway and fatty acid degradation pathway were significantly affected [12]. These two processes are closely related to the oxidative decomposition of fatty acids and energy metabolism, and these changes might lead to abnormal energy metabolism in miR-30c KO mice. The results of this study were consistent with the published reports. Overexpression of miR-30c could inhibit lipid biosynthesis, thereby alleviating liver function damage caused by high-fat diet [13]. In this study, the significant changes in PPAR signaling pathway in the liver of miR-30c KO mice may be caused by the lack of negative regulation of fatty acid synthase (Fasn) by miR-30c [14]. As an important regulator of lipid metabolism, the up regulation of Fasn expression was closely related to the synthesis and storage of fatty acids, resulting in the accumulation of liver lipids and the imbalance of energy metabolism [15]. In addition, studies have pointed out that the expression of miR-30c in the liver might be closely related to the occurrence of a variety of metabolic diseases, especially playing an important role in the development of non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome [16]. By regulating genes related to lipid metabolism, miR-30c maybe provide a new target for the treatment of these diseases. Therefore, future studies should further explore the regulatory mechanism of miR-30c and its biological functions in different metabolic states, in order to provide a theoretical basis for clinical intervention.

In this study, using a TAA induced liver injury model, it was observed that miR-30c knockout mice exhibited more severe signs of liver injury and fibrosis compared to WT mice. This finding suggested a crucial role of miR-30c in maintaining liver homeostasis and alleviating fibrosis processes. With TAA treatment, the serum levels of ALT and AST in miR-30c knockout mice significantly increased, indicating an exacerbation of liver cell damage and possibly further affecting the overall function of the liver. The Sirius red staining resulted intuitively revealed the proliferation of fibrous tissue in miR-30c KO mouse liver, indicating the effect of miR-30c in regulating extracellular matrix remodeling. Meanwhile, as a key biomarker for measuring collagen deposition and fibrosis degree, the content of hydroxyproline was significantly increased in knockout mice, which is consistent with the typical characteristics of late-stage liver fibrosis. Previous studies showed that miR-30c could affect the progression of liver fibrosis by regulating the activation of hepatic stellate cells (HSCs) [17]. MiR-30c could inhibit liver fibrosis by targeting delta like ligand 4 (DLL4) in endothelial cells, demonstrating its key role in regulating the liver microenvironment and neovascularization [9]. The result about a relationship between miR-30c and the transforming growth factor-beta (TGF-β) dependent fibrosis regulatory network suggested that miR-30c might serve as a novel therapeutic target that affects the occurrence and development of liver fibrosis by regulating the behavior of HSCs (hepatic stellate cells) [18]. Another research report showed that miR-30c was downregulated during HSC activation, and its upregulation could inhibit the expression of HSC activation markers, thereby reducing the degree of liver fibrosis [17]. With a deeper understanding of the function of miR-30c, increasing evidence suggested its protective role in liver disease. These research results indicated the importance of miR-30c in the spontaneous reversal of liver fibrosis, suggesting its potential as a therapeutic target [19]. Our findings reveal that miR-30c knockout disrupts lipid homeostasis, leading to hepatic steatosis. This metabolic dysfunction primes the liver for injury by inducing lipotoxicity and oxidative stress, which we show synergizes with TAA to amplify fibrotic responses via HSC activation and collagen deposition.

While our data reveal distinct roles for miR-30c in liver fibrosis, compensatory upregulation of other miR-30 family members (e.g., miR-30a, miR-30d) may partially mitigate phenotypic severity in knockout models. Such redundancy has been observed in other miRNA families where loss of one member triggers expression changes in paralogs. However, compensatory mechanisms involving miR-30a/b/d/e remain unexplored and may mask KO phenotypes. Future studies should profile all miR-30 isoforms in KO livers to assess potential compensation.

The results of this study indicated that the absence of miR-30c could upregulate the expression of Pdgfra in the liver, suggesting its potential role in the development of liver fibrosis. The expression of Pdgfra was related to the process of liver fibrosis, especially in the activation and proliferation of hepatic stellate cells [20]. The upregulation of Pdgfra might promote the proliferation and transformation of hepatic stellate cells into myofibroblasts, further exacerbating the deposition of extracellular matrix and leading to the progression of liver fibrosis [21]. Other report indicated that Huangqi decoction could significantly downregulate the expression of PDGF related genes, thereby alleviating liver fibrosis [22]. MiR-223 restricted the progression of liver fibrosis by inhibiting the PDGF signaling pathway, further supporting the importance of miRNA in regulating liver fibrosis [23].

In TAA treated mice, the deletion of miR-30c resulted in significant upregulation of key fibrosis genes such as Acta2, Col1A1, Col3A, and Trib3, which played important roles in liver fibrosis. Research showed that upregulation of Acta2 was a key marker for the transformation of HSCs into myofibroblasts after liver injury, leading to the development of liver fibrosis [24]. In the pathological state of the liver, HSCs responded to damage by proliferating and secreting large amounts of extracellular matrix components (such as collagen), which was closely related to the expression of Acta2. A study showed that hepatic stellate cells lacking Acta2 exhibited a significant reduction in fibrosis degree in a liver fibrosis model, indicating that Acta2 plays a promoting role in the progression of liver fibrosis [25]. Additionally, in the CCl4 induced liver fibrosis model, the expression of Acta2 was closely related to the signaling pathway activity of TGF-β, which could promote the transformation of HSCs into myofibroblasts and enhance the expression of Acta2, thereby exacerbating the fibrosis process [26]. By regulating the cytoskeleton structure of HSCs, Acta2 could affect the morphology and motility of cells, and regulate their response to the external environment, thereby affecting the progression of fibrosis [27].

In the liver, the expression of collagen, especially the upregulation of Col1A1 and Col3A1, significantly promotes the process of liver fibrosis. This type of fibrosis is usually caused by chronic liver injury, accompanied by inflammation and death of liver cells [28]. In multiple studies, Col1A1 and Col3A1 have been shown to be key biomarkers of liver fibrosis, and their expression levels are positively correlated with the severity of fibrosis [29]. Another study showed that liver fibrosis could be alleviated by inhibiting the activation of HSCs and the synthesis of collagen, which involves the regulation of the p38 MAPK signaling pathway [30]. Meanwhile, the TGF-β signaling pathway also played a central role in collagen synthesis and HSC activation, promoting fibrosis progression by upregulating the expression of Col1A1 and Col3A1 [31]. In addition, Trib3 showed an increase in its expression level in different types of fibrosis models. A recent research has shown that upregulation of Trib3 expression might be associated with the activation of various cellular stress signaling pathways, such as endoplasmic reticulum stress and oxidative stress, which played a key role in the occurrence and progression of fibrosis [32]. Trib3 affected cell survival and death decisions by regulating key transcription factors and apoptosis related proteins, thereby promoting pathological changes in the fibrotic microenvironment [33]. Another research found that inhibiting the expression of Trib3 could significantly alleviate fibrosis related pathological changes, suggesting its potential therapeutic value in the fibrosis process [34].

The results of this study also suggested that miR-30c might play an important role in both liver fibrosis and inflammatory response, especially in TAA treated mouse models. The knockout of miR-30c resulted in a significant increase in inflammatory factors such as Il-1b, Il-6, Tnf, Ccl2, and Adgre1. The increase of these inflammatory factors not only reflects the exacerbation of liver inflammation, but may also further damage liver cells and promote the progression of liver fibrosis by activating pro-inflammatory signaling pathways.

In addition, analysis of lipid synthesis genes showed that even in the absence of TAA, the absence of miR-30c was associated with increased expression of several key genes involved in fatty acid metabolism. MiR-30c regulates fat synthesis and breakdown by targeting key genes such as fatty acid synthase (FASN) [35] and fatty acid transporter (FAT/CD36) [36], thereby affecting lipid accumulation and metabolic balance in liver cells. After the absence of miR-30c, abnormal accumulation of lipids in the liver might trigger a series of pathological changes, including promoting hepatocyte apoptosis and inflammatory response. In the TAA induced liver fibrosis model, the absence of miR-30c might lead to an imbalance in lipid metabolism, thereby promoting pathological changes in the liver. The abnormal accumulation of lipids could exacerbate liver cell damage, and accelerate fibrosis process through pro-inflammatory effects. Consistent with its proposed protective function, miR-30c knockout exacerbated TAA-induced fibrogenesis and heightened inflammatory responses. Thus, loss of miR-30c potentiates, rather than improves, liver fibrosis.

Beyond acute TAA-induced fibrosis, miR-30c deletion may chronically disrupt hepatic homeostasis through multifaceted pathological mechanisms. Sustained activation of HSCs could drive irreversible extracellular matrix cross-linking, promoting architectural remodeling of the liver parenchyma. Furthermore, persistent PPARa dysregulation may accelerate metabolic reprogramming, potentially exacerbating NAFLD progression. Concomitantly, altered TGF-b/TNFa ratios might impair the liver's regenerative capacity by inhibiting hepatocyte proliferation post-injury. However, the current study did not assess longitudinal adaptations such as metabolic syndrome onset or regenerative dysfunction. Future investigations should include extended longitudinal monitoring to elucidate miR-30c's role in aging-related hepatic decline, particularly regarding its impact on regenerative pathways and chronic metabolic dysregulation.

However, there are several limitations to this study. The first point was about choosing a time point. In evaluating the TAA induced liver fibrosis process, this study only selected one time point for observation. This choice might fail to fully reflect the regulatory role of miR-30c

in different stages of liver fibrosis. The second point was that there were shortcomings in the exploration of mechanisms. Although this study suggested that miR-30c deficiency might be associated with improvement in liver fibrosis, the exploration of its specific mechanism was still insufficient, and there was no in-depth analysis of how miR-30c affected the lipid synthesis pathway and the expression changes of downstream target genes. While transcriptional changes exhibit coherent biological alignment, the absence of direct immunoblotting requires consideration of potential post-transcriptional regulation. The third point was the need for further research on potential compensatory mechanisms. The data of this study show association but not causality between lipid synthesis genes and fibrosis. Genetic/pharmacologic rescue experiments are needed to test whether normalizing lipid metabolism attenuates fibrogenesis in miR-30c KO mice. The absence of miR-30c might trigger other compensatory mechanisms that could affect lipid metabolism and the progression of liver fibrosis, but this study did not fully explore these possible compensatory mechanisms. The fourth point was the lack of long-term observation. This study mainly focused on short-term effects and failed to evaluate the long-term health and liver function effects of miR-30c deficiency in mice. In future work, further research will be conducted to address these shortcomings and gain a more comprehensive understanding of the role of miR-30c in liver physiology and pathology. This study's male-specific design precludes extrapolation to female pathophysiology. The future investigations must validate findings in female models and address sex-specific therapeutic implications.

As noted in limitations, the correlative nature of lipid gene dysregulation with fibrosis severity warrants validation via functional studies. Should future work confirm lipid synthesis pathways directly modulate fibrosis (e.g., via lipotoxic HSC activation), targeting miR-30c or its effectors could represent a therapeutic strategy.

While our findings reveal miR-30c's protective role in liver fibrosis pathogenesis, therapeutic potential assertions require preclinical validation through interventional studies. Future work will deliver miR-30c mimics in TAA/NASH models to evaluate rescue effects on fibrosis endpoints.

Conclusions

In conclusion, this study found the multifaceted role of miR-30c in liver fibrosis, indicating that its deficiency may exacerbate the expression of fibrosis and inflammation related genes, and trigger changes in lipid metabolism. These findings provided important clues for further exploring the protective mechanism of miR-30c and its potential as a therapeutic target for liver fibrosis. A deeper understanding of the interaction between miR-30c, inflammation, and lipid metabolism would be expected to provide a new perspective and strategy for managing liver diseases characterized by liver fibrosis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12876-025-04237-8.

Supplementary Material 1. Table S1. The list of primer sequences for qRT-PCR. Table S2. Detection of 15 kinds of biochemical factors in WT and miR-30c KO mice plasma.

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None.

Author contributions
Conception and design: L.Z.; Data acquisition: S.M., M.Y., W.Q. and F.Z.; Statistical analysis and interpretation of data: S.M., Z.C. and Y.G.; Drafting the manuscript: S.M. and L.Z.; Figures and tables: S.M., Y.G. and L.Z. All authors reviewed the manuscript.

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Data availability
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. The raw sequence data of RNA were deposited in the NCBI SRA database with the accession number of PRJNA1205524 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1205524/).

Declarations

Ethics approval and consent to participate
All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Bengbu Medical University (No.2023LDK438). And methods were carried out in accordance with relevant guidelines and regulations. This study was carried out in compliance with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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