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ORIGINAL ARTICLE

lncRNA NEAT1 regulates fibrosis and inflammatory response induced by nonalcoholic fatty liver by regulating miR-506/GLI3

Si-Si Jin[1], Xian-Fan Lin[1], Ju-Zeng Zheng[1], Qiong Wang[2], Hua-Qin Guan[1]

[1] Department of Gastroenterology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, P.R China
[2] Department of Pharmacy, Wenzhou People's Hospital, Wenzhou 325000, Zhejiang Province, P.R China

Corresponding author: Dr. Hua-Qin Guan, Department of Gastroenterology, The First Affiliated Hospital of Wenzhou Medical University, New Campus of Nanbaixiang Wenyi First Hospital, Ouhai District, Wenzhou 325000, Zhejiang Province, P.R China: H"Q. Guan <guanhuaqin47@163.com>

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ABSTRACT. Background: As one of the most common liver disorders worldwide, nonalcoholic fatty liver disease (NAFLD) begins with the abnormal accumulation of triglyceride (TG) in the liver and can lead to inflammation and fibrosis. Long noncoding RNA (lncRNA) NEAT1 was reported to promote NAFLD progress. However, its molecular mechanism in NAFLD was not fully clear. Method: ***In vitro*** **cellular model of NAFLD was established with BRL3A cell treated by free fatty acid (FFA). Cell Counting Kit-8 (CCK-8) assay was carried out to assess cell proliferation. The expression of mRNA and protein of inflammation and fibrosis in BRL3A cell was detected by qRT-PCR and Western blot. Bioinformatics and dual-luciferase reporter assays were used to predict and validate the interaction between NEAT1 and miR-506 as well as GLI3 and miR-506. Results**: **NEAT1 was upregulated while miR-506 was downregulated in the progression of NAFLD. Meanwhile, NEAT1 and miR-506 were proved to regulate fibrosis, inflammatory response, and lipid metabolism. Knockdown of NEAT1 inhibited GLI3 expression and promoted miR-506 expression, Overexpression of miR-506 inhibited NEAT1 and GLI3 expression. Moreover, dual-luciferase reporter assays proved that miR-506 could bind to NEAT1 and GLI3, whereas NEAT1 could sponge miR-506 to regulate GLI3 expression. Conclusion**: **lncRNA NEAT1 could regulate fibrosis, inflammatory response, and lipid metabolism via the miR-506/GLI3 axis as a ceRNA, which is a novel mechanistic role in the regulation of NAFLD. These results provide a new potential treatment target for NAFLD.**

Key words: NAFLD, lncRNA-NEAT1, miR-506, GLI3

INTRODUCTION

As one of the most common liver disorders worldwide, non-alcoholic fatty liver disease (NAFLD) begins with the abnormal accumulation of triglyceride (TG) in the liver and can lead to inflammation and fibrosis [1]. HCC has recently been linked to NAFLD and the globally high prevalence of NAFLD may contribute to the rising incidence of HCC [2, 3]. Thus, it is of much significance to understand the pathogenesis of NAFLD and prevent this disease. Moreover, increasing evidence demonstrated that the hedgehog (Hh) pathway was involved in both the pathogenesis from hepatic steatosis to more serious forms of liver damage [4]. Thus, suppressing excessive Hh pathway activity is the potential approach to prevent progressive liver damage in NAFLD [4].

Long noncoding RNAs (lncRNAs) are transcripts longer than 200 nucleotides with limited coding potential, which can exhibit many functions to regulate different processes by various molecular mechanisms [5]. Emerging evidences suggested that aberrant lncRNAs participated in tumor development and progression; however, there is still a lack of in-depth understanding of its role in the regulation of chronic liver disease [6]. LncRNA nuclear enriched abundant transcript 1 (NEAT1) was involved in many diseases [7]. NEAT1 could promote colorectal cancer progression via interacting with DDX5 [8]. In ovarian cancer, it promoted cell proliferation and migration through sponging miR-506 [9]. Moreover, it enhanced the radio-resistance of cervical cancer via miR-193b-3p/CCND1 axis [10]. Recently, lncRNA NEAT1 was reported to promote NAFLD via mTOR/S6K1 signaling pathway [11]. However, there is still much to be investigated whether NEAT1 is involved in the pathogenesis of NAFLD and

Abbreviations

NAFLD	non-alcoholic fatty liver disease
HCC	hepatocellular carcinoma
NEAT1	nuclear enriched abundant transcript 1
lncRNAs	long non-coding RNAs
qRT-PCR	quantitative realtime polymerase chain reaction
FFA	free fatty acid
DMEM	dulbecco's modified eagle medium
ACC	acetyl-CoA carboxylase
CCK-8	Cell Counting Kit-8
Hh	hedgehog
ceRNA	competing endogenous RNA

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regulatory role of NEAT1 in NAFLD worth further exploration.

Recently, a new regulatory molecular mechanism of lncRNA, called competing endogenous RNA (ceRNA), was reported whereby lncRNA may function as the miRNA sponge to modulate the derepression of miRNA targets [12]. Recently, NEAT1-modualted miR-506 was reported to regulate gastric cancer development through targeting STAT3 [13]. Besides, miR-506 was reported to act as a tumor suppressor by targeting GLI3, the hedgehog pathway transcription factor in human cervical cancer [14]. On the basis of the present results, we raised the hypothesis that NEAT1 could contribute fibrosis and inflammatory response induced by NAFLD via sponging miR-506 to regulate hedgehog pathway.

In this study, we investigated the functional implication of NEAT1 in lipid metabolism using the NAFLD cell model. Our findings suggested that NEAT1 enhanced GLI3 expression to regulate fibrosis and inflammatory response induced by NAFLD via sponging miR-506. Our data provided the first clue showing the regulatory role of NEAT1 in the progression of NAFLD as a ceRNA, and it might be a potential method for the treatment of NAFLD.

MATERIALS AND METHOD

Cell culture and cell model of NAFLD

BRL3A cell was obtained from the American Type Culture Collection (ATCC, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) (ThermoFisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS, ThermoFisher Scientific, USA) at 37 °C under 5% CO_2 as described before [15].For the NAFLD cellular model, BRL3A cell was assigned into control and free fatty acid (mixture of 1 mM FFAs, containing oleic acid and palmitic acid at 2:1 volume ratio) treatment group to induce steatosis of different degrees.

Total Triglycerides (TG) Assays

The levels of TG in the BRL3A cells were determined using commercial TG assay kits from Solarbio Inc. (Shanghai, China) following the manufacturer's instructions. Briefly, the cells were lysed in ice-cold cell lysis buffer and centrifuged at 1,500 × g for 5 min. The supernant was used for the determination of TG levels.

Cell transfection

NEAT1 siRNA vector was constructed and purchased for Genechem (Shanghai, China). In brief, NEAT1 siRNA sequence was synthesized and subcloned into transfection plasmid to generate recombinant vector. Then, the recombinant vector was transfected into BRL3A cell with Lipofectamine 2000 Reagent (Invitrogen, USA). miR-506 mimic and inhibitor were designed and synthesized from GenePharma (Shanghai, China) and were transfected into BRL3A cell by Lipofectamine 3000 Reagent (Invitrogen, USA) following to the manufacturer's instruction.

RNA extraction and real-time PCR

Total RNA from BRL3A cells was isolated using Trizol regent (Invitrogen, USA). ImProm-II Reverse Transcription System (Promega, USA) was then used to generate first-strand cDNA. SYBR Green qPCR assay (Takara, China) and gene-specific primers were used for qRT-PCR following the manufacturer's protocol. The relative expression levels of genes were then calculated with the $2^{-\Delta\Delta Ct}$ method. Each sample was tested in triplicates for statistical analysis.

Western blot

Cells were collected and lysed with RIPA lysis buffer (ThermoFisher Scientific, USA). Protein concentration was then measured by the BCA Protein Assay Reagent Kit (ThermoFisher Scientific, USA). Equal amounts of protein were then separated by 10% SDS-PAGE, electrophoretically transferred to nitrocellulose membrane (Millipore, USA), and incubated with primary antibodies: ACC (Abcam, USA, 1:1000), FAS (Abcam, USA, 1:1000), SMO (Abcam, USA, 1:1000), GLI2 (Abcam, USA, 1:1000), GLI3 (Abcam, USA, 1:1000), β-actin (Abcam, USA, 1:3000), and GAPDH (Abcam, USA, 1:3000). The membrane was washed with TBST containing 0.1% Tween 20 for three times. And then the membrane was incubated with HRP-labeled goat anti-mouse/rabbit IgG (1:5000, Sigma) for 2 hours at room temperature. Signals were visualized with SuperPico chemiluminescent substrate (Pierce, USA) and film exposure.

Cell viability assay

Cell viability was measured with a CCK-8 Kit (Dojindo, Japan). Briefly, cells were seeded at a density of 4×10^3/well into 96-well plates. Then, 10 μL of CCK-8 solution was added into the culture medium, and incubated for additional 1 h at 37 °C. The OD value was read at 450 nm by using a microplate reader. The data are presented as means ± SD of multiple experiments that were performed concurrently with a single control experiment.

Dual-luciferase reporter assay

The predicted miR-506 binding site from NEAT1 or 3′ UTR of the GLI3 mRNA was amplified by PCR and cloned immediately into pGL3-control vector (Progema, USA) downstream of the stop codon of the luciferase gene. HEK-293T cells were co-transfected with the following vectors and miR-506 mimics using Lipofectamine 3000 (Invitrogen, USA). Then, Dual-luciferase Reporter Assay System (Promega, USA) was used to determine the relative dual-luciferase activity according to the manufacturer's instructions.

Immunofluorescence staining

BRL3A cells were grown on chamber slides and miR-506 or NEAT1 transfection was carried out as described before. Thirty-six hours following the transfection, the BRL3A cells were fixed in 4% paraformaldehyde/PBS for 15 min at room tempera-

ture and washed with PBS three times. Cells were blocked with 2% bovine serum albumin in PBS for 1 h followed by incubation with mouse monoclonal antibody against α-SMA and Collagen I antibody (Sigma, USA, 1:200) for 16 h at 4 °C. After being washed with PBS, FITC-labeled and Alexa Fluor 594 goat anti-mouse IgG (Invitrogen,,USA, 1:1000) were applied and incubated for 60 min. After an additional washing, cells were mounted and analyzed by fluorescence microscopy.

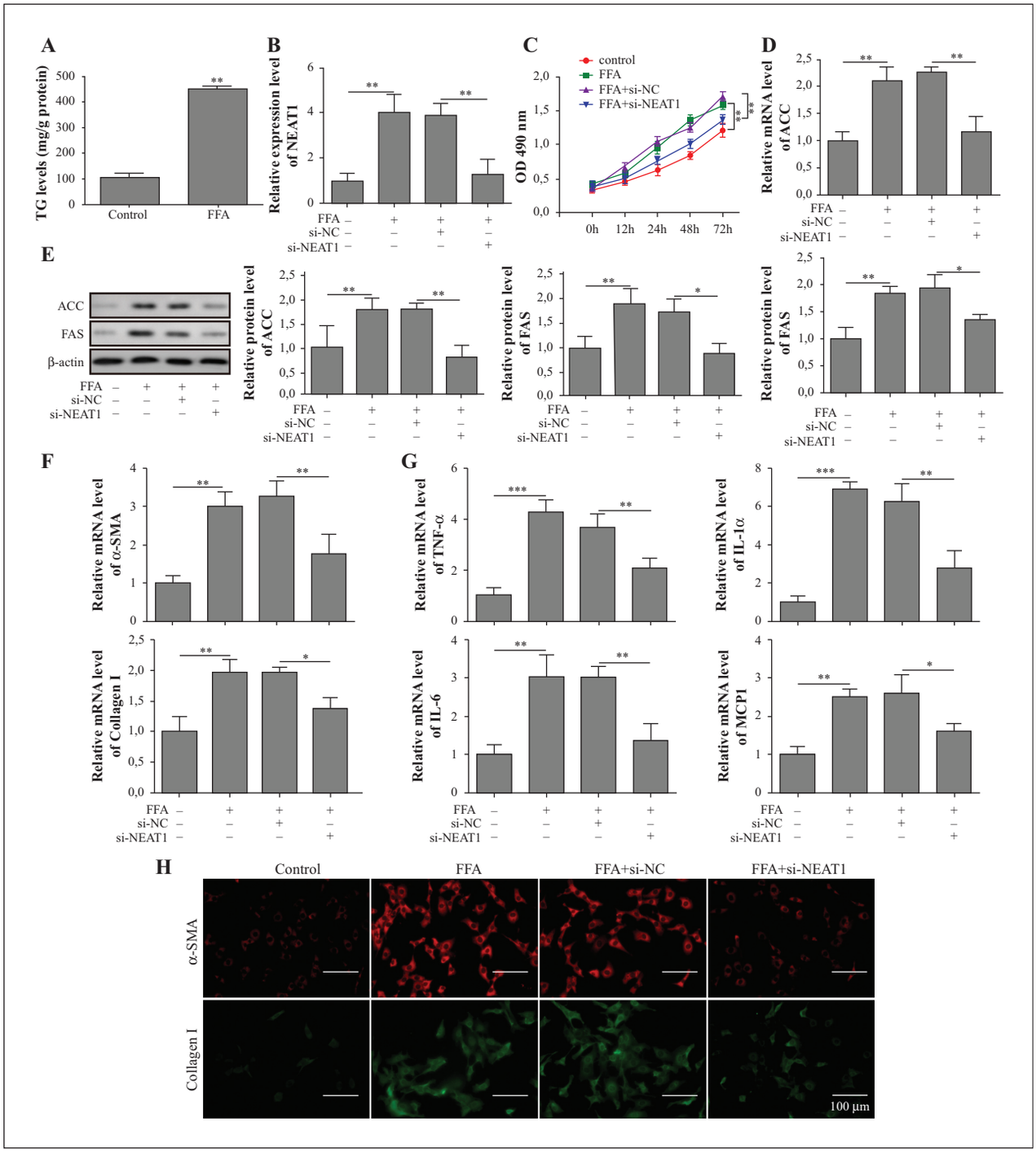

Figure 1

lncRNA NEAT1 knockdown remitted NAFLD in the FFA-induced NAFLD cellular model. Concentrations of TG were detected by ELISA in the FFA-induced NAFLD cellular model**.** B. Relative expression of NEAT1 in FFA-induced BRL3A cells transfected with NEAT1 siRNA and siRNA-NC was assessed by qRT-PCR, normalized to GAPDH. C. CCK-8 assay was performed to assess cell proliferation in FFA-induced BRL3A cell transfected with NEAT1 siRNA and siRNA-NC. D. Relative expression of molecular markers of NAFLD (FAS and ACC) in FFA-induced BRL3A cell transfected with NEAT1 siRNA and siRNA-NC was assessed by qRT-PCR, normalized to GAPDH. E. Protein levels of FAS and ACC in FFA-induced BRL3A cell transfected with NEAT1 siRNA and siRNA-NC were assessed by Western blot. F. Relative expression of two fibrosis factors α-SMA and Collagen I in FFA-induced BRL3A cells transfected with NEAT1 siRNA and siRNA-NC was assessed by qRT-PCR, normalized to GAPDH. G. Relative expression of inflammatory cytokines (TNF-α, IL-1β, IL-6, and MCP1) in FFA-induced BRL3A cells transfected with NEAT1 siRNA and siRNA-NC was assessed by qRT-PCR, normalized to GAPDH. H. Immunofluorescence staining of two fibrosis factors α-SMA and Collagen I in FFA-induced BRL3A cells transfected with NEAT1 siRNA and siRNA-NC. *P < 0.05 and **P < 0.01. All data were from three independent experiments performed in triplicate.

Statistical analysis

Graphpad prism 7 software was used to calculate and assess statistical differences between different groups. The results were presented as mean ± Standard Deviation (SD), n = 3. Comparison between two groups was performed using two-tailed student's t test and for multigroup comparison the one-way ANOVA test was used. $P < 0.05$ was considered statistically significant (*$P < 0.05$ and **$P < 0.01$).

RESULTS

LncRNA NEAT1 knockdown remitted NAFLD in FFA-induced NAFLD cellular model.

First, we used the NAFLD cellular model to evaluate the regulatory role of lncRNA NEAT1 in the pathogenesis of NAFLD. The concentration of TG in the NAFLD cellular model was detected to verify that the model was built successfully, and elevated TG secretion was observed in the NAFLD cellular model (*figure 1A*). The expression of NEAT1 was detected by qRT-PCR and was significantly upregulated in the NAFLD cellular model (*figure 1B*). In contrast NEAT1 was successfully knocked down by NEAT1 shRNA compared with shRNA-NC (*figure 1B*). Then, CCK-8 assay was carried out to assess cell proliferation. As shown in *figure 1C*, NEAT1 knockdown inhibited cell proliferation compared with the shRNA-NC group in the NAFLD cellular model. Moreover, two NAFLD-related genes (ACC and FAS) were significantly decreased by NEAT1 knockdown in the NAFLD cellular model (*figure 1D-E*). To further assess the effect of NEAT1 on fibrosis and inflammatory response in the NAFLD cellular model, two fibrosis factors α-SMA and Collagen I as well as inflammatory cytokines (TNF-α, IL-1β, IL-6, and MCP1) were measured by qRT-PCR. *Figure 1F* showed that α-SMA and Collagen I were downregulated by NEAT1 knockdown, while *figure 1G* showed that expression of those inflammatory cytokines was inhibited by NEAT1 knockdown. Moreover, α-SMA and Collagen I protein were downregulated by NEAT1 knockdown shown by immunofluorescence (*figure 1H*). Taken together, these results indicated that lncRNA NEAT1 knockdown remitted fibrosis and inflammatory response in the NAFLD cellular model.

LncRNA NEAT1 knockdown inhibited GLI3 expression and promoted miR-506 expression

To further investigate the regulatory role of NEAT1, core transcription factors SMO, GLI2, and GLI3 in Hh signaling pathway were assessed by Western blot after NEAT1 knockdown. *Figure 2A* showed that SMO, GLI2, and GLI3 were all upregulated by FFA induction, while GLI3 was downregulated by NEAT1 knockdown. miR-506 expression was also detected after NEAT1 knockdown in the NAFLD cellular model. FFA inhibited miR-506 expression, while miR-506 was upregulated by NEAT1 knockdown (*figure 2B*). Then, to determine whether NEAT1 directly regulates miR-506, dual-luciferase reporter vectors of wild-type NEAT1 (NEAT1-WT) and a mutant form (NEAT1-MUT) were constructed to carry out dual-luciferase reporter assay. Dual luciferase reporter assay confirmed that overexpression of miR-506 significantly suppressed the luciferase activity of the NEAT1-WT in HEK-293T cells, with no effect observed on the mutant form (*figure 2C*). We further

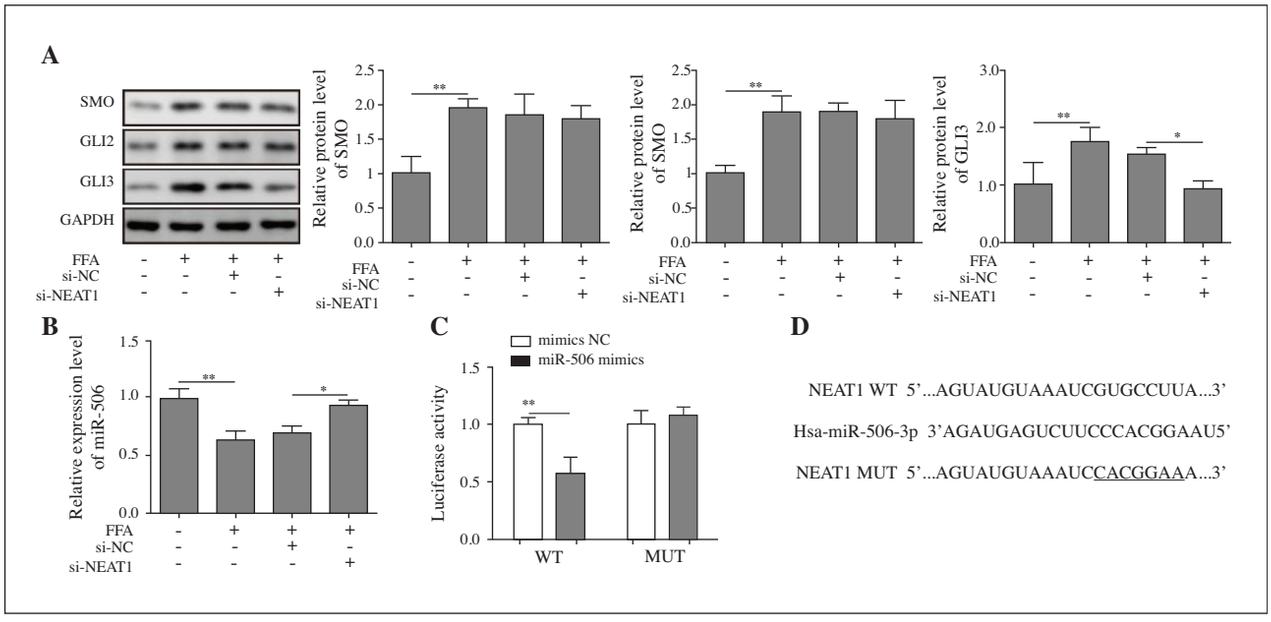

Figure 2

lncRNA NEAT1 knockdown inhibited GLI3 expression and promoted miR-506 expression. A. Protein levels of core transcription factors SMO, GLI2, and GLI3 in Hh signaling pathway in FFA-induced BRL3A cell transfected with NEAT1 shRNA and shRNA-NC were assessed by Western blot, normalized to GAPDH. B. Relative expression of miR-506 in FFA-induced BRL3A cell transfected with NEAT1 shRNA and shRNA-NC was assessed by qRT-PCR, normalized to U6. C. The dual-luciferase activity of the NEAT1-WT and NEAT1-MUT in BRL3A cells transfected with miR-506 mimics. D. Predicted binding site between miR-506 and NEAT1 by starbase software. *$P < 0.05$ and **$P < 0.01$. All data were from three independent experiments performed in triplicate.

predicted the potential binding site between miR-506 and NEAT1 by starbase software (*figure 2D*). These results indicated direct interaction of NEAT1 with miR-506 in this putative binding site, and NEAT1 could negatively regulate the expression of miR-506.

MiR-506 overexpression remitted NAFLD in the FFA-induced NAFLD cellular model

Then, we evaluated the regulatory role of miR-506 in the pathogenesis of NAFLD in the NAFLD cellular model.

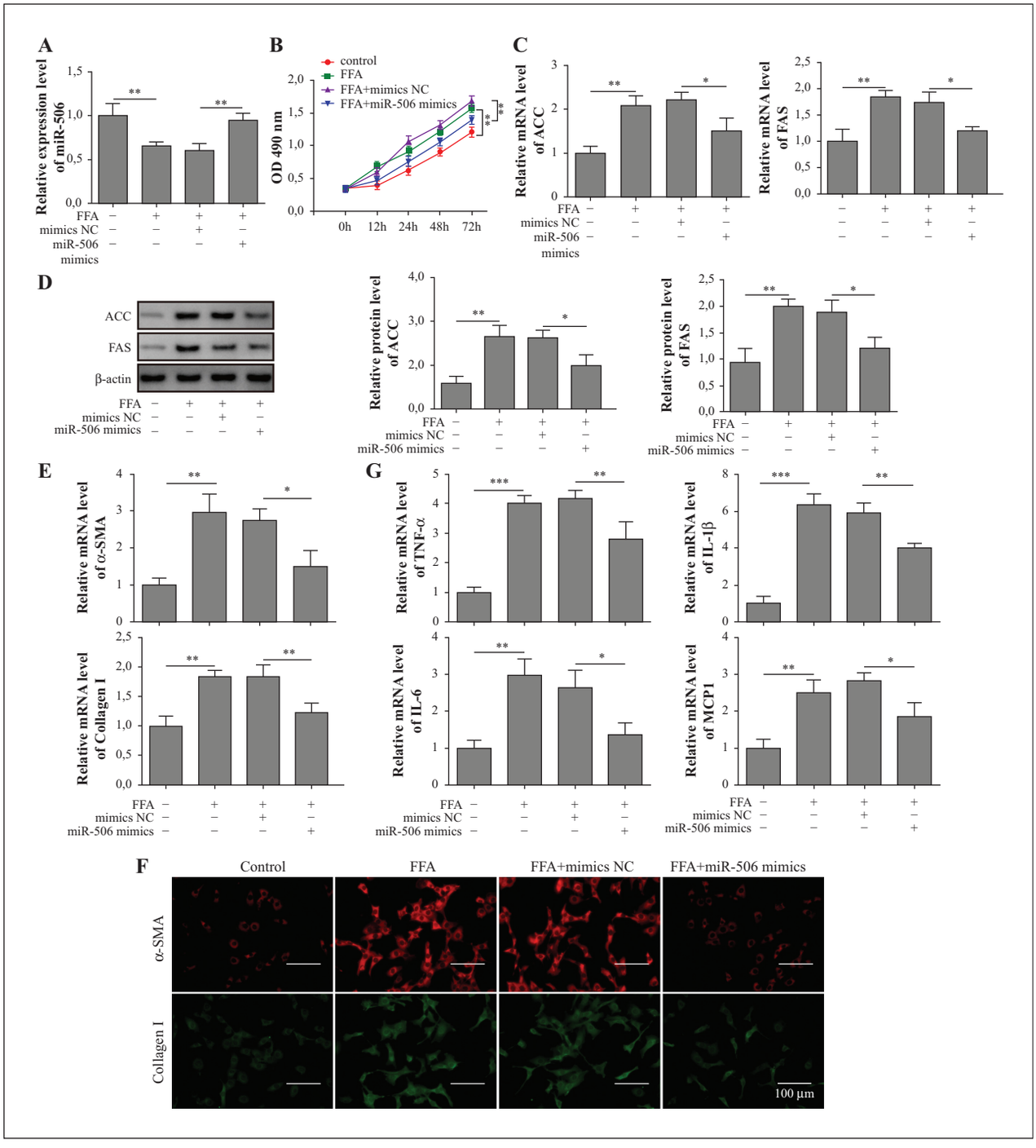

Figure 3

miR-506 overexpression remitted NAFLD in the FFA-induced NAFLD cellular model. A. Relative expression of miR-506 in FFA-induced BRL3A cells transfected with miR-506 mimics and mimics-NC was assessed by qRT-PCR, normalized to U6. B. CCK-8 assay was performed to assess cell proliferation in FFA-induced BRL3A cell transfected with miR-506 mimics and mimics-NC. C. Relative expression of molecular markers of NAFLD (FAS and ACC) in FFA-induced BRL3A cell transfected with miR-506 mimics and mimics-NC was assessed by qRT-PCR, normalized to GAPDH. D. Protein levels of FAS and ACC in FFA-induced BRL3A cell transfected with miR-506 mimics and mimics-NC were assessed by Western blot. E. Relative expression of two fibrosis factors α-SMA and Collagen I in FFA-induced BRL3A cells transfected with miR-506 mimics and mimics-NC was assessed by qRT-PCR, normalized to GAPDH. F. Immunofluorescence staining of two fibrosis factors α-SMA and Collagen I in FFA-induced BRL3A cells transfected with miR-506 mimics and NC mimics. *P < 0.05 and **P < 0.01. All data were from three independent experiments performed in triplicate. G. Relative expression of inflammatory cytokines (TNF-α, IL-1β, IL-6, and MCP1) in FFA-induced BRL3A cells transfected with miR-506 mimics and mimics-NC was assessed by qRT-PCR, normalized to GAPDH.

The expression of miR-506 was detected by qRT-PCR and was significantly downregulated in the NAFLD cellular model (*figure 3A*). In contrast miR-506 was successfully overexpressed by miR-506 mimics compared with mimics-NC (*figure 3A*). Then, the CCK-8 assay was carried out to assess cell proliferation. As shown in *figure 3B*, miR-506 overexpression inhibited cell proliferation compared with mimics-NC group in the NAFLD cellular model. Moreover, ACC and FAS were significantly decreased by miR-506 overexpression in the NAFLD cellular model (*figure 3C-D*). To further assess the effect of miR-506 on fibrosis and inflammatory response in the NAFLD cellular model, α-SMA and Collagen I, two fibrosis factors, and four inflammatory cytokines (TNF-α, IL-1β, IL-6, and MCP1) were measured by qRT-PCR. *Figure 3E* showed that α-SMA and Collagen I were downregulated by miR-506 overexpression. Moreover, α-SMA and Collagen I protein were downregulated by miR-506 overexpression shown by immunofluorescence (*figure 3F*). *Figure 3G* showed that expression of those inflammatory cytokines was inhibited by miR-506 overexpression. Taken together, these results indicated that miR-506 could remit fibrosis and inflammatory response in the NAFLD cellular model.

MiR-506 inhibited Hh signaling pathway in the FFA-induced NAFLD cell model

To further investigate the regulatory role of miR-506 in the pathogenesis of NAFLD, SMO, GLI2, and GLI3 were assessed by Western blot after miR-506 overexpression. *Figure 4A* showed that SMO, GLI2, and GLI3 were all upregulated by FFA induction, while GLI3 was downregulated by miR-506 overexpression. Then, NEAT1 was also detected after miR-506 overexpression in the NAFLD cellular model. *Figure 4B* showed that NEAT1 was downregulated by miR-506 overexpression. Then, to determine whether miR-506 could regulate GLI3 by targeting it, dual-luciferase reporter vectors of wild-type GLI3 (GLI3-WT) and a mutant form (GLI3-MUT) were constructed to carry out dual-luciferase reporter assay. Dual luciferase reporter assay confirmed that overexpression of miR-506 significantly suppressed the luciferase activity of the GLI3-WT in HEK-293T cells, with no effect observed on the mutant form (*figure 4C*). We further predicted the potential binding site between miR-506 and GLI3 via targetscan software (*figure 4D*). These results indicated direct interaction of GLI3 with miR-506 in this putative binding site, and miR-506 could negatively regulate the expression of NEAT1.

LncRNA NEAT1 regulated fibrosis, inflammatory response, and lipid metabolism through sponging miR-506

Next, we investigated whether lncRNA NEAT1 regulated fibrosis and inflammatory response through sponging miR-506. Relative expression of two fibrosis factors α-SMA and Collagen I in FFA-induced BRL3A cell transfected with miR-506 inhibitor or inhibitor-NC after lncRNA NEAT1 knockdown were assessed by qRT-PCR. As shown in *figure 5A*, NEAT1 knockdown inhibited α-SMA and Collagen I, while miR-506 inhibitor could rescue this effect. Then, inflammatory cytokines (TNF-α, IL-1β, IL-6, and MCP1) in FFA-induced BRL3A cells transfected with miR-506 inhibitor or inhibitor-NC

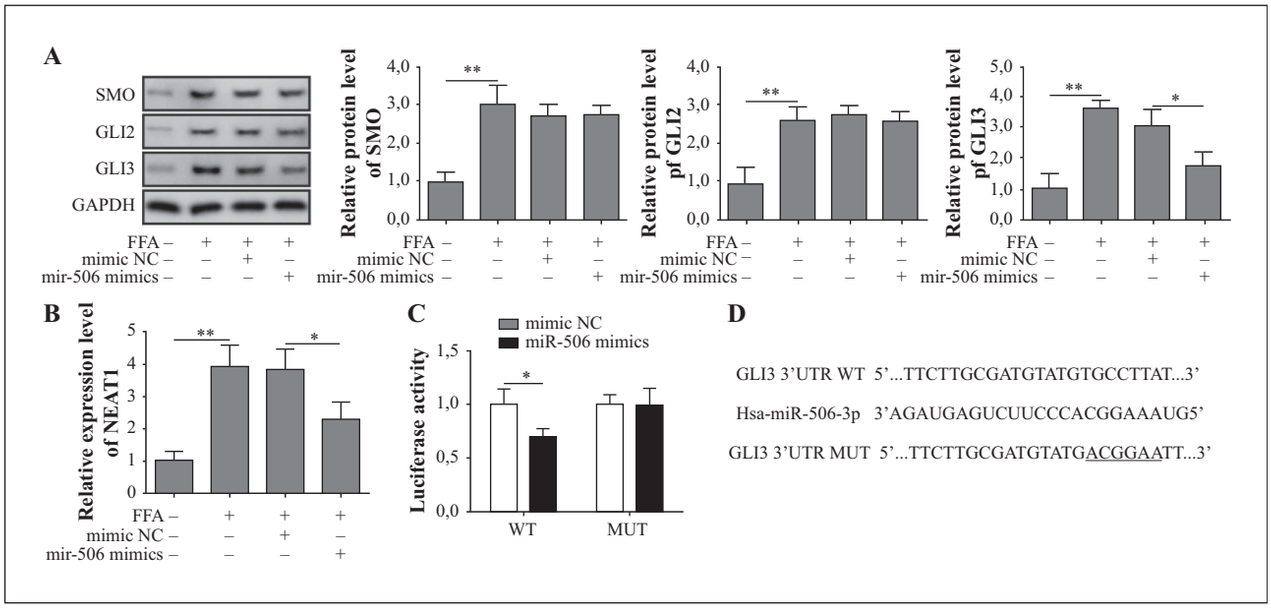

Figure 4

miR-506 overexpression inhibited Hh signaling pathway in the FFA-induced NAFLD cell model. A. Protein levels of core transcription factors SMO, GLI2, and GLI3 in Hh signaling pathway in FFA-induced BRL3A cell transfected with miR-506 mimics and mimics-NC were assessed by Western blot normalized to GAPDH. B. Relative expression of lncRNA NEAT1 in FFA-induced BRL3A cell transfected with miR-506 mimics and mimics-NC was assessed by qRT-PCR, normalized to U6. C. The dual-luciferase activity of the GLI3-WT and GLI3-MUT in BRL3A cells transfected with miR-506 mimics. D. Predicted binding site between miR-506 and GLI3 by targetscan software. *P < 0.05 and **P < 0.01. All data were from three independent experiments performed in triplicate.

after lncRNA NEAT1 knockdown were also assessed by qRT-PCR and all of those inflammatory cytokines were downregulated by NEAT1 knockdown, while miR-506 inhibitor rescued this effect (*figure 5B*). Furthermore, FAS and ACC were also downregulated by NEAT1 knockdown, while miR-506 inhibitor could compensate this effect (*figure 5C*). Moreover, NEAT1 knockdown inhibited α-SMA and Collagen I protein, while miR-506 inhibitor could rescue this effect shown by immunofluorescence (*figure 5D*). Taken together, these results indicated that lncRNA NEAT1 could regulate fibrosis, inflammatory response, and lipid metabolism by sponging miR-506 as a ceRNA.

DISCUSSION

NAFLD, one of the most common causes of liver disease, now has a prevalence about 24% worldwide [16]. According to the double-hit theory of NAFLD,

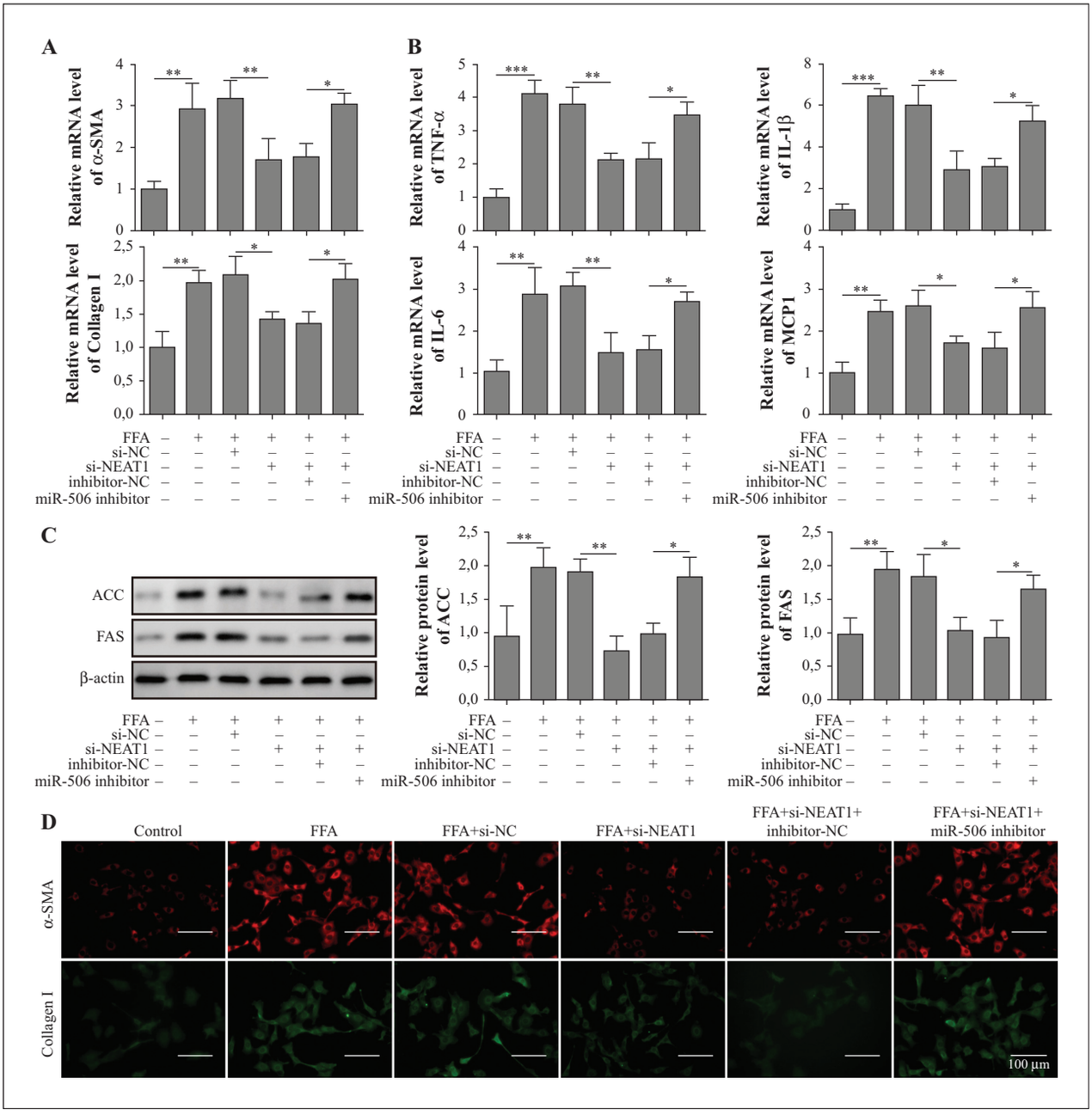

Figure 5

lncRNA NEAT1 regulated NAFLD through sponging miR-506. Relative expression of two fibrosis factors α-SMA and Collagen I in FFA-induced BRL3A cell transfected with miR-506 inhibitor or inhibitor-NC after lncRNA NEAT1 knockdown were assessed by qRT-PCR, normalized to GAPDH. B. Relative expression of inflammatory cytokines (TNF-α, IL-1β, IL-6 and MCP1) in FFA-induced BRL3A cells transfected with miR-506 inhibitor or inhibitor-NC after lncRNA NEAT1 knockdown were assessed by qRT-PCR, normalized to GAPDH. C. Protein level of FAS and ACC in FFA-induced BRL3A cell transfected with miR-506 inhibitor or inhibitor-NC after lncRNA NEAT1 knockdown were assessed by Western blot. D. Immunofluorescence staining of two fibrosis factors α-SMA and Collagen I in FFA-induced BRL3A cells transfected with miR-506 inhibitor or inhibitor-NC after NEAT1 knockdown. *P < 0.05 and **P < 0.01. All data were from three independent experiments performed in triplicate.

the first hit is the accumulation of abnormal triglycerides in hepatocytes [17, 18]. While in the second hit, inflammatory mediators further lead to liver injury, inflammation, and fibrosis, which are important indicators of NAFLD [18, 19]. Besides, accumulating evidence showed that the Hh pathway has been involved in the pathogenesis of hepatic steatosis, which indicated that suppressing excessive Hh pathway activity may be a potential approach to prevent progressive liver damage in NAFLD [4]. Recent studies showed that lncRNA has a variety of biological effects and is involved in the regulation of the development in various diseases, but there are few studies on lncRNA in NAFLD [20]. In this study, we demonstrated that lncRNA NEAT1 regulated fibrosis and inflammatory response induced by NAFLD by regulating miR-506/GLI3 axis. This study also provided a mechanistic role for lncRNAs in regulating the development of NAFLD which could be a potential treatment target for NAFLD.

A previous study showed that lncRNA NEAT1 may play important roles in NAFLD progression [11]. It was shown that downregulation of NEAT1 could alleviate the NAFLD via mTOR/S6K1 signaling pathway [14]. In this study, lncRNA NEAT1 was observed to upregulate in the NAFLD cellular model and knockdown NEAT1 could remit fibrosis and inflammatory response in the NAFLD cellular model, which is consistent with previous research, indicating that NEAT1 plays regulatory roles in NAFLD progression.

Recently, the new regulatory molecular mechanism of lncRNA as ceRNA was reported whereby lncRNA may function as a miRNA sponge to upregulate miRNA targets [21]. Recent reports also uncovered that NEAT1 could function as a ceRNA and played regulatory role in this mechanism [12]. For example, lncRNA NEAT1 promoted the growth of cervical cancer cells by sponging miR-9 [22]. In contrast NEAT1 upregulated TGF-β1 to induce hepatocellular carcinoma progression by sponging miR-139 [23]. Besides, NEAT1 enhanced the expression of the STAT3 to contribute to hepatocellular carcinoma development by sponging miR-485 [24]. In this report, we further validated that miR-506 could directly bind with NEAT1 and GLI3 through bioinformatics analysis and dual-luciferase assays. Taken together, consistent with previous reports, these data indicated that NEAT1 may interact with miR-506 to post-transcriptionally regulate the GLI3 protein.

Although we initially revealed a novel downstream molecular mechanism of NAFLD by an *in vitro* experiments, there is still much to be done to deeply understand this complex mechanism. As the situation in the animal body is more complicated and has more uncertainty than cell models, whether this mechanism could be applied to an animal model is not certain. Therefore, in the future, this novel molecular mechanism needs further verification in the mice model to prove its potential of therapeutic targets. In summary, this study demonstrated that lncRNA NEAT1 upregulation is a characteristic molecular change in NAFLD and NEAT1 promotes fibrosis, inflammatory response, and expression of NAFLD-related genes by functioning as a ceRNA to regulate the expression of GLI3 through competition with miR-506. Taken together, our study confirmed a key role of lncRNAs in regulating the development of NAFLD.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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