Annals of Hepatology 27 (2022) 100584

Contents lists available at ScienceDirect

Annals of Hepatology

journal homepage: www.elsevier.es/annalsofhepatology

Original article

Silencing lncRNA NEAT1 reduces nonalcoholic fatty liver fat deposition by regulating the miR-139-5p/c-Jun/SREBP-1c pathway

Si-Si Jin, Chun-Jing Lin, Xian-Fan Lin, Ju-Zeng Zheng, Hua-Qin Guan*

Department of Internal Medicine, the First Affiliated Hospital of Wenzhou Medical University, No. 192 Nanbaixiang Street, Wenzhou, Zhejiang 325000, China

ARTICLE INFO

Article History:
Received 25 August 2021
Accepted 25 October 2021
Available online 19 November 2021

Keywords:
lncRNA NEAT1
miR-139-5p
c-Jun
SREBP1c
Nonalcoholic fatty liver disease

ABSTRACT

Introduction and Objectives: Nonalcoholic fatty liver disease (NAFLD) starts with the abnormal accumulation of lipids in the liver. Long noncoding RNA (lncRNA) nuclear enriched abundant transcript 1 (NEAT1) was reported to modulate hepatic metabolic homeostasis in NAFLD. However, little is known about the molecular mechanisms of NAFLD.

Materials and Methods: To establish a NAFLD cellular model, HepG2 cells and LO2 cells were treated with 1 mM free fatty acids (FFAs) for 24 h. NEAT1, miRNA (miR)-139-5p, c-Jun and sterol-regulatory element binding protein-1c (SREBP-1c) were evaluated using qPCR. The protein levels of c-Jun, SREBP1c, acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS) were determined using western blotting. Moreover, Oil Red O staining was employed to assess lipid accumulation. In addition, a kit assay was performed to evaluate TG levels. Finally, the interactions among NEAT1, miR-139-5p, c-Jun and SREBP1c were identified by dual luciferase reporter gene assay.

Results: NEAT1, c-Jun and SREBP1c expression was markedly elevated, while miR-139-5p expression was reduced in the NAFLD cellular model. NEAT1 knockdown restrained lipid accumulation in the NAFLD cellular model by directly targeting miR-139-5p. Moreover, miR-139-5p overexpression suppressed lipid accumulation by directly suppressing c-Jun expression. In addition, c-Jun silencing suppressed lipid accumulation by directly targeting SREBP1c. Finally, miR-139-5p inhibition mitigated the inhibitory effect of sh-NEAT1 on lipid accumulation.

Conclusion: NEAT1 aggravated FFA-induced lipid accumulation in hepatocytes by regulating the c-Jun/SREBP1c axis by sponging miR-139-5p, indicating the potential of NEAT1 as a promising therapeutic target for NAFLD.

1. Introduction

NAFLD has become a major public health problem worldwide [1]. The prevalence of NAFLD is very high, ranging from 12.9% to 46.0% [2]. It is currently believed that the abnormal accumulation of lipids in the liver is the primary pathogenic driving factor for NAFLD [3]. The liver is an important metabolic organ of the human body. In NAFLD, the liver's ability to process major fatty acids is insufficient, leading to the accumulation of toxic lipids. These metabolites induce liver cell stress, damage and death, leading to liver fibrosis and ultimately cirrhosis and hepatocellular carcinoma. Therefore, reducing hepatic lipid accumulation is an effective treatment strategy for NAFLD. However, the molecular mechanism regulating hepatic lipid accumulation in NAFLD is largely unknown.

NAFLD progression is associated with abnormal expression of ncRNAs, including lncRNAs and miRNAs [4–6]. LncRNA NEAT1 was previously identified as an oncogene that was able to promote tumour cell proliferation and survival, thereby enhancing the progression of a variety of human malignant tumours [7,8]. Recent studies have revealed that NEAT1 is abundant in the liver and is able to promote steatosis in HepG2 cells [9]. In addition, NEAT1 was reported to be upregulated in NAFLD, and NEAT1 knockdown could alleviate lipid formation in FFA-treated HepG2 cells [10]. All this evidence suggests that NEAT1 is a risk factor affecting NAFLD. However, the specific mechanism by which NEAT1 regulates hepatic lipid accumulation in NAFLD remains unclear. miR-139-5p is enriched in the liver and has a large regulatory effect on various liver-related diseases [11,12]. In addition, miR-139-5p inhibited glycolysis in hepatocellular carcinoma [12]. More importantly, miR-139-5p was markedly reduced in NAFLD patients [13]. However, there has been no report showing that NEAT1 regulates miR-139-5p expression to participate in NAFLD progression. c-Jun is an immediate early gene that is regulated by the N-terminal kinase of c-Jun (JNK) [14]. It was proven that c-Jun plays a key role in many aspects of liver biology and diseases: c-Jun knockout mice died at approximately 13 days of

* Corresponding author.
E-mail address: guanhuaq626@163.com (H.-Q. Guan).

https://doi.org/10.1016/j.aohep.2021.100584

embryonic development and showed increased hepatoblast apoptosis, suggesting that c-Jun is essential during liver development [15]. Recently, much evidence has shown that c-Jun is involved in NAFLD progression [14],16]. Schulien et al. revealed that c-Jun expression elevated disease progression from NAFLD to NASH [16]. More importantly, JNK2 was reported to participate in regulating fatty acid synthesis in human adipocytes by regulating SREBP-1c [17]. SREBP-1 transcriptionally activates lipogenesis-related genes, such as ACC and FAS [18]. However, there has been no report showing that the c-Jun/SREBP-1c pathway, as the downstream pathway of miR-139-5p, regulates hepatic lipid accumulation in NAFLD.

Herein, we performed a systemic study to investigate the functional implication of NEAT1 in hepatic lipid accumulation using a NAFLD cellular model, which provided novel potential targets for NAFLD treatment.

2. Materials and methods

2.1. Cell culture and treatment

HepG2 and LO2 were purchased from ATCC (VA, USA). All cells were cultured in DMEM (Gibco, MD, USA) containing 10% FBS (Gibco) in a humidified atmosphere of 5% CO_2 at 37 °C. To establish the NAFLD cellular model, cells were treated with 1 mM FFA (Invitrogen, CA, USA) for 24 h, and the control cells were maintained in normal medium.

Abbreviations

Long non-coding RNA (lncRNA)
MicroRNA (miR)
Nonalcoholic fatty liver disease (NAFLD)
Triglyceride (TG)
Nuclear enriched abundant transcript 1 (NEAT1) (NEAT1)
Single stranded RNA (ssRNA)
Nuclear enriched assembly transcript 1 (NEAT1)
Free fatty acid (FFA)
N-terminal kinase of c-Jun (JNK)
Sterol-regulatory element binding protein-1c (SREBP-1c)
Acetyl-CoA carboxylase (ACC)
Fatty acid synthetase (FAS)
Stearoyl-CoA desaturase (SCD)
Quantitative real-time polymerase chain reaction (qRT-PCR)
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)
Standard deviation (SD)
Analysis of variance (ANOVA)
Western blot (WB)
Dulbecco's modified eagle media (DMEM)

2.2. Cell transfection

Short hairpin RNA against NEAT1 (sh-NEAT1), short hairpin RNA against c-Jun (sh-c-Jun), oe-Jun and mimics of miR-139-5p, as well as their negative controls, were purchased from GenePharma (Shanghai, China). Cells were transfected with sh-NEAT1, sh-c-Jun or miR-139-5p mimics and their negative controls using Lipofectamine™ 3000 (Invitrogen) for 24 h.

2.3. Measurement of triglyceride (TG) level

The intracellular TG level was examined using a TG assay kit (Jiancheng, Nanjing, China) according to the manufacturer's instructions.

2.4. Oil red O staining

Cells were fixed in 4% paraformaldehyde (10 min), rinsed in PBS once (1 min), and then rinsed in 50% isopropanol (15 s). Cells were subsequently stained with Oil Red O dye solution (Beyotime, Shanghai, China) at 37 °C (1 min). Then, the cells were detained with 60% isopropanol (15 s) and washed with PBS. Cells were viewed and photographed using an inverted microscope (Nikon, Tokyo, Japan).

2.5. Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was isolated from cells and tissues with TRIzol reagent (Thermo Fisher Scientific, CA, USA). cDNA was synthesized with a HiFi-Script cDNA synthesis kit (Life Technologies, CA, USA). Then, qPCR assays were performed on an Eppendorf MasterCycler RealPlex4 (Eppendorf, Wesseling-Berzdorf, Germany) using SYBR (Thermo Fisher Scientific). The relative expression levels of miRNA and mRNA were normalized to U6 and GAPDH and calculated by the $2^{-\Delta\Delta CT}$ method. The primers used in the study were as follows (5'-3'):

NEAT1 (F): TCCTCTACAGCCTTACCTACATC

NEAT1 (R): AGACAACCTTCAACCAACAACC miR-139-5p (F): TCTACAGTGCACGTGTCTCCAG miR-139-5p (R): GTGCAGGGTCCGAGGT

SREBP1c (F): CCACAATGCCATTGAGAAGCG

SREBP1c (R): CTGACACCAGGTCCTTCAGTG c-Jun (F): CAGGTGGCACAGCTTAAACA c-Jun (R): AACTGCTGCGTTAGCATGAG

2.6. Western blot

The proteins were isolated with RIPA, and the concentrations of protein were assessed using a BCA Kit (Beyotime). Lysate samples were separated using SDS–PAGE and then transferred to a PVDF membrane (Millipore, MA, USA). Then, membranes were incubated with primary antibodies including c-Jun (Abcam, 1:1000, ab40766), SREBP-1c (Abcam, 1:1000, ab28481), FAS (Abcam, 1:1000, ab133619), ACC (Abcam, 1:1000, ab109368) and GAPDH (Abcam, 1:5000, ab8245). After washing with PBS-T, membranes were then incubated with the corresponding secondary antibody labelled with HRP (Abcam, 1:10000, ab7090, ab6789) for 60 min. The membranes were covered with ECL reagents (Beyotime), and the images were analysed using ImageJ 1.52a.

2.7. Dual luciferase reporter gene assay

The sequence of NEAT1 containing the miR-139-5p binding site was amplified by PCR. Site-directed mutagenesis of the miR-139-5p binding site in the NEAT1 sequence was performed using a quick change site-directed mutagenesis kit (Stratagene, CA, USA). Wild-type (wt) and mutant-type (mut) reporter plasmids of NEAT1 sequences

were cloned into the PGL3 vector (GenePharma). Then, cells were cotransfected with NEAT1-wt or NEAT1-mut plasmids and miR-139-5p mimics or mimics NC by Lipofectamine™ 3000 (Invitrogen, CA, USA). Luciferase activity was examined using a dual-luciferase reporter assay system (Promega, WI, USA). The same method was used to verify the binding relationship between miR-139-5p and c-Jun.

2.8. Data analysis

The results are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS 19.0 (IBM, NY, USA). Between-group differences and multigroup comparisons were determined using Student's t test and one-way analysis of variance (ANOVA), respectively. p values less than 0.05 were considered significant.

3. Results

3.1. NEAT1, c-Jun and SREBP1c expression was significantly increased, while miR-139-5p expression was reduced in the NAFLD cellular model

To probe the roles of NEAT1, miR-139-5p, c-Jun and SREBP1c in regulating hepatic lipid accumulation in NAFLD, a NAFLD cellular model was constructed by FFA treatment. As demonstrated in **Fig. 1A**, the accumulated lipids in cells were markedly elevated after FFA treatment. Consistently, TG levels were obviously elevated in HepG2 and LO2 cells following FFA treatment (***P* < 0.05; Fig. 1B**). Then, NEAT1, miR-139-5p, c-Jun and SREBP1c expression was assessed using qPCR, and the results showed that NEAT1, c-Jun and SREBP1c expression was significantly increased in the NAFLD cellular model, while miR-139-5p was downregulated (***P* < 0.05; Fig. 1C).** Additionally, c-Jun and SREBP1c levels were increased in HepG2 and LO2 cells following FFA treatment (***P* < 0.05; Fig. 1D**). Taken together, FFA treatment promoted NEAT1, c-Jun and SREBP1c expression, suppressed miR-139-5p expression and facilitated lipogenesis in hepatocytes.

3.2. NEAT1 knockdown suppresses lipid accumulation in hepatocytes via miR-139-5p

To probe the regulatory role of NEAT1 in hepatic lipid accumulation in NAFLD, we performed NEAT1 knockdown in a NAFLD cellular model. As shown in **Fig. 2A**, NEAT1 and c-Jun expression was markedly reduced following sh-NEAT1 transfection, while miR-139-5p was significantly upregulated. A similar trend was observed in the protein level of c-Jun (***P* < 0.05; Fig. 2B**). In addition, TG levels were markedly reduced following sh-NEAT1 transfection (***P* < 0.05; Fig. 2C**). Consistently, the accumulated lipids were obviously decreased after NEAT1 knockdown (**Fig. 2D**). All these results suggested that NEAT1 knockdown inhibited lipid accumulation by regulating miR-139-5p.

3.3. miR-139-5p overexpression suppresses FFA-induced lipid accumulation in hepatocytes by regulating c-Jun

C-Jun was reported to be the target of miR-139-5p in cardiomyocytes, ovarian cancer cells and endothelial cells [6,19,20], but it is unknown whether miR-139-5p acted as a role in NAFLD by targeting c-Jun. To explore whether miR-139-5p could bind and regulate c-Jun expression in hepatocytes, miR-139-5p mimics or NC mimics were transfected into a NAFLD cellular model. As shown in **Fig. 3A (*P* < 0.05)**, c-Jun expression was markedly reduced in the NAFLD cellular model following miR-139-5p mimic transfection, while miR-139-5p was significantly upregulated. A similar trend was observed in the protein level of c-Jun (***P* < 0.05; Fig. 3B**). Additionally, TG levels were markedly reduced in the NAFLD cellular model following miR-139-5p overexpression (***P* < 0.05; Fig. 3C**). Consistently, the accumulated lipids were obviously lower in the NAFLD cellular model after miR-139-5p mimic transfection (**Fig. 3D**). Moreover, the protein levels of SREBP1c, FAS and ACC were significantly lower in the NAFLD cellular model following miR-139-5p overexpression (***P* < 0.05; Fig. 3E**). In summary, miR-139-5p overexpression reduced lipid accumulation in FFA-treated HepG2 and LO2 cells by regulating c-Jun.

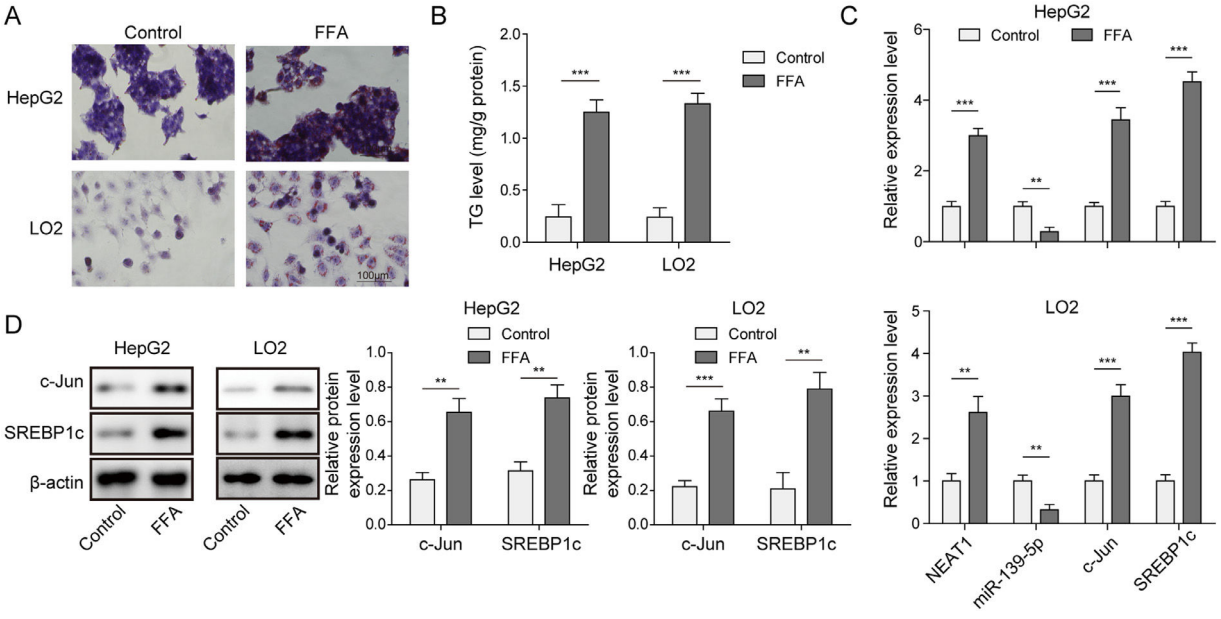

Fig. 1. NEAT1, c-Jun and SREBP1c expression was significantly higher in the NAFLD cellular model, while miR-139-5p was downregulated. HepG2 cells and LO2 cells were treated with 1 mM FFA for 24 h. (A) Lipid deposition was evaluated using Oil Red O staining. (B) TG level was assessed using kits. (C) NEAT1, miR-139-5p, c-Jun and SREBP1c expression was assessed using qPCR. (D) Western blotting was performed to evaluate the protein levels of c-Jun and SREBP1c. The data were expressed as the mean ± SD. **P* < 0.05, ** *P* < 0.01.

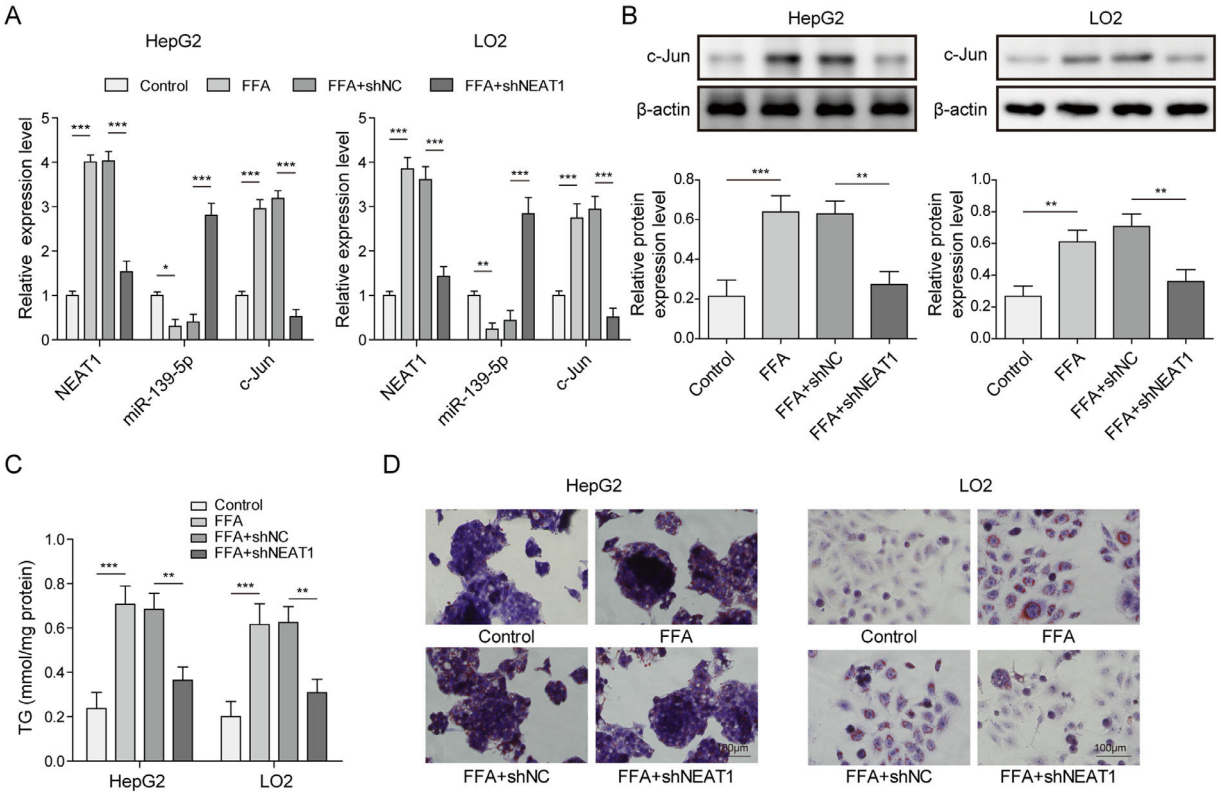

Fig. 2. NEAT1 knockdown suppresses FFA-induced lipid accumulation in hepatocytes by directly targeting miR-139-5p. NAFLD cellular models were transfected with sh-NC or sh-NEAT1. (A) NEAT1, miR-139-5p and c-Jun expression was assessed using qPCR. (B) The protein level of c-Jun was determined using western blot. (C) TG level was assessed using kits. (D) Oil Red O staining was employed to detect lipid deposition. The data were expressed as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

3.4. Silencing c-Jun inhibits FFA-induced lipid accumulation in hepatocytes by suppressing SREBP1c expression

JNK2 was reported to participate in regulating fatty acid synthesis in human adipocytes via regulation of SREBP-1c; however, it is unknown whether c-Jun plays a role in NAFLD by targeting SREBP1c. First, we found that c-Jun and SREBP1c expression was markedly reduced in the NAFLD cellular model following sh-c-Jun transfection (***P* < 0.05; Fig. 4A-B**). Additionally, TG levels were markedly reduced in FFA-treated HepG2 and LO2 cells following sh-c-Jun transfection (***P* < 0.05; Fig. 4C**). Consistently, the accumulated lipids were obviously lower in the NAFLD cellular model after sh-c-Jun transfection (**Fig. 4D**). Furthermore, reduced protein levels of ACC and FAS were observed in the FFA + sh-c-Jun group compared with the FFA + sh-NC group (***P* < 0.05; Fig. 4E**). Overexpression of c-Jun in HepG2 and LO2 cells and dual luciferase reporter assays finally revealed that c-Jun directly targeted SREBP1c (***P* < 0.05; Fig. 4F**). In total, c-Jun knockdown suppressed lipid accumulation by directly targeting SREBP1c.

3.5. lncRNA NEAT1 enhances FFA-induced lipid accumulation in hepatocytes by sponging miR-139-5p

Next, to study whether NEAT1 plays a role in regulating lipid accumulation by regulating miR-139-5p, we further predicted the potential binding site between NEAT1 and miR-139-5p through bioinformatics analysis. To further establish this binding relationship, a dual luciferase reporter gene assay was performed, and the results revealed that NEAT1 directly bound to miR-139-5p (***P* < 0.05; Fig. 5A).** Bioinformatics software predicted that miR-139-5p had a binding site for c-Jun. A dual luciferase reporter assay subsequently showed that miR-139-5p directly targeted the 3′-UTR of c-Jun (***P* < 0.05; Fig. 5B**). We knocked down NEAT1 in a NAFLD cellular model and set up a functional rescue experiment: FAA + sh-NEAT1 + inhibitor NC group and FAA + sh-NEAT1 + miR-139-5p inhibitor group. The qRT−PCR results showed that miR-139-5p expression was elevated and NEAT1 and c-Jun expression was reduced following NEAT1 knockdown; NEAT1 expression was unchanged, miR-139-5p expression was markedly reduced and c-Jun expression was obviously elevated in the FAA + sh-NEAT1 + miR-139-5p inhibitor group compared with the FAA + sh-NEAT1 + inhibitor NC group (***P* < 0.05; Fig. 5C**). In addition, sh-NEAT1 transfection resulted in reduced TG levels in the NAFLD cellular model, while this effect was eliminated by miR-139-5p inhibition (***P* < 0.05; Fig. 5D**). Consistently, the accumulated lipids were obviously lower in the NAFLD cellular model after sh-NEAT1 transfection, which was reversed by miR-139-5p inhibitor transfection (**Fig. 5E**). Western blot results subsequently showed that c-Jun, SREBP1c, ACC and FAS levels were obviously reduced in the NAFLD cellular model following NEAT1 knockdown, while this effect was eliminated by miR-139-5p knockdown (***P* < 0.05; Fig. 5F**). All these results suggested that lncRNA NEAT1 enhanced lipid accumulation in hepatocytes by downregulating miR-139-5p.

4. Discussion

NAFLD is the most common chronic liver disease worldwide, and its prevalence in the general population is 20% to 30% [21]. NAFLD has become one of the most important public health problems [22]. The main pathological feature of NAFLD is the excessive accumulation of TG in hepatocytes without consumption of alcohol [18]. As previously described, lncRNAs have many biological functions and participate in regulating the progression of various diseases; however, the roles of lncRNAs in NAFLD are largely unknown. In the current study, we found that NEAT1 could enhance lipid accumulation through the miR-139-5p/c-Jun/SREBP1c axis. Our research provides a potential target for NAFLD treatment.

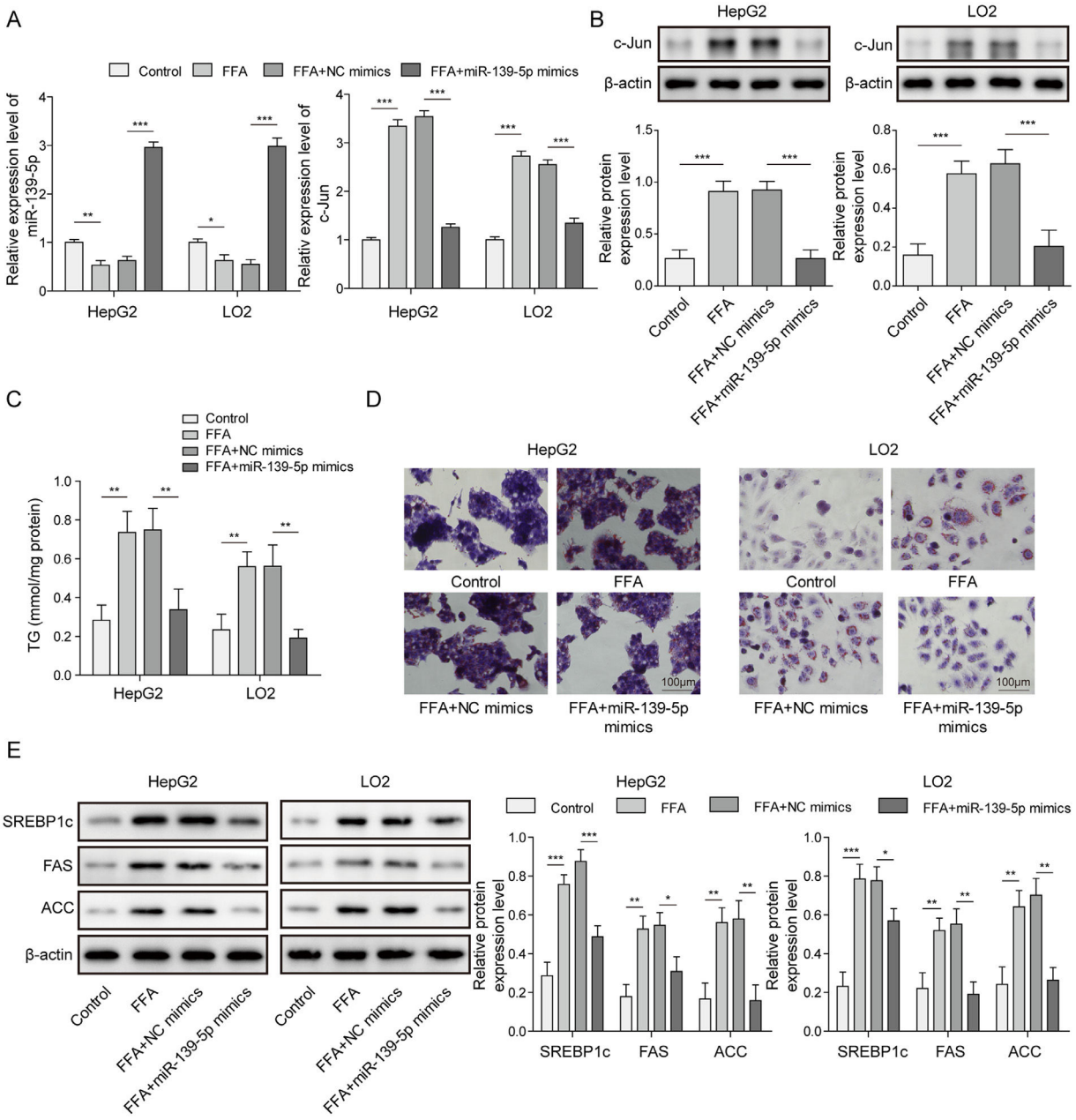

Fig. 3. miR-139-5p overexpression suppresses FFA-induced lipid accumulation in hepatocytes by directly targeting c-Jun. NAFLD cellular models were transfected with mimics NC or miR-139-5p mimics. (A) miR-139-5p and c-Jun expression was assessed using qPCR. (B) The protein level of c-Jun was detected using western blot. (C) TG level was assessed using kits. (D) Oil Red O staining was employed to detect lipid deposition. (E) Western blotting was performed to evaluate the protein levels of SREBP1c, FAS and ACC. The data were expressed as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

It was reported that NEAT1 was involved in regulating a series of biological processes, such as the inflammatory response, cell invasion and metastasis [7,23,24]. NEAT1 was reported to be markedly upregulated in NAFLD models [10,25]. In addition, NEAT1 knockdown alleviated NAFLD development by suppressing ACC and FAS expression [26]. Moreover, Fu et al. revealed that NEAT1 promoted steatosis by increasing AQP7 expression [9]. Here, NEAT1 expression was found to be elevated in the NAFLD cellular model, and NEAT1 knockdown suppressed lipid accumulation and TG secretion in FFA-treated HepG2 and LO2 cells, suggesting that NEAT1 plays a key role in NAFLD development.

The ceRNA hypothesis has been confirmed as a novel regulatory mechanism pertinent to lncRNAs [27]. By using the bioinformatics software starBase, we found that NEAT1 had a binding site for miR-139-5p. MiR-139-5p was reported to participate in regulating aerobic glycolysis in hepatocellular carcinoma [12]. Considering that NAFLD is a risk factor for hepatocellular carcinoma, the above evidence suggests that miR-139-5p may be related to NAFLD progression. More importantly, Latorre et al. showed that miR-139-5p was markedly downregulated in obese subjects with NAFLD [13]. Here, a dual luciferase reporter assay showed that NEAT1 directly bound to miR-139-5p. miR-139-5p expression in HepG2 and LO2 cells was reduced by FFA treatment. Additionally, miR-139-5p expression was increased by NEAT1 knockdown in FFA-treated HepG2 and LO2 cells. Above all, we concluded that NEAT1 acted as an endogenous sponge to suppress miR-139-5p expression. Having identified the direct relationship between NEAT1 and miR-139-5p, we assumed that NEAT1 might regulate hepatic lipid accumulation in NAFLD via its negative regulatory effects on miR-139-5p. Functional assays showed that miR-139-5p overexpression obviously inhibited lipid

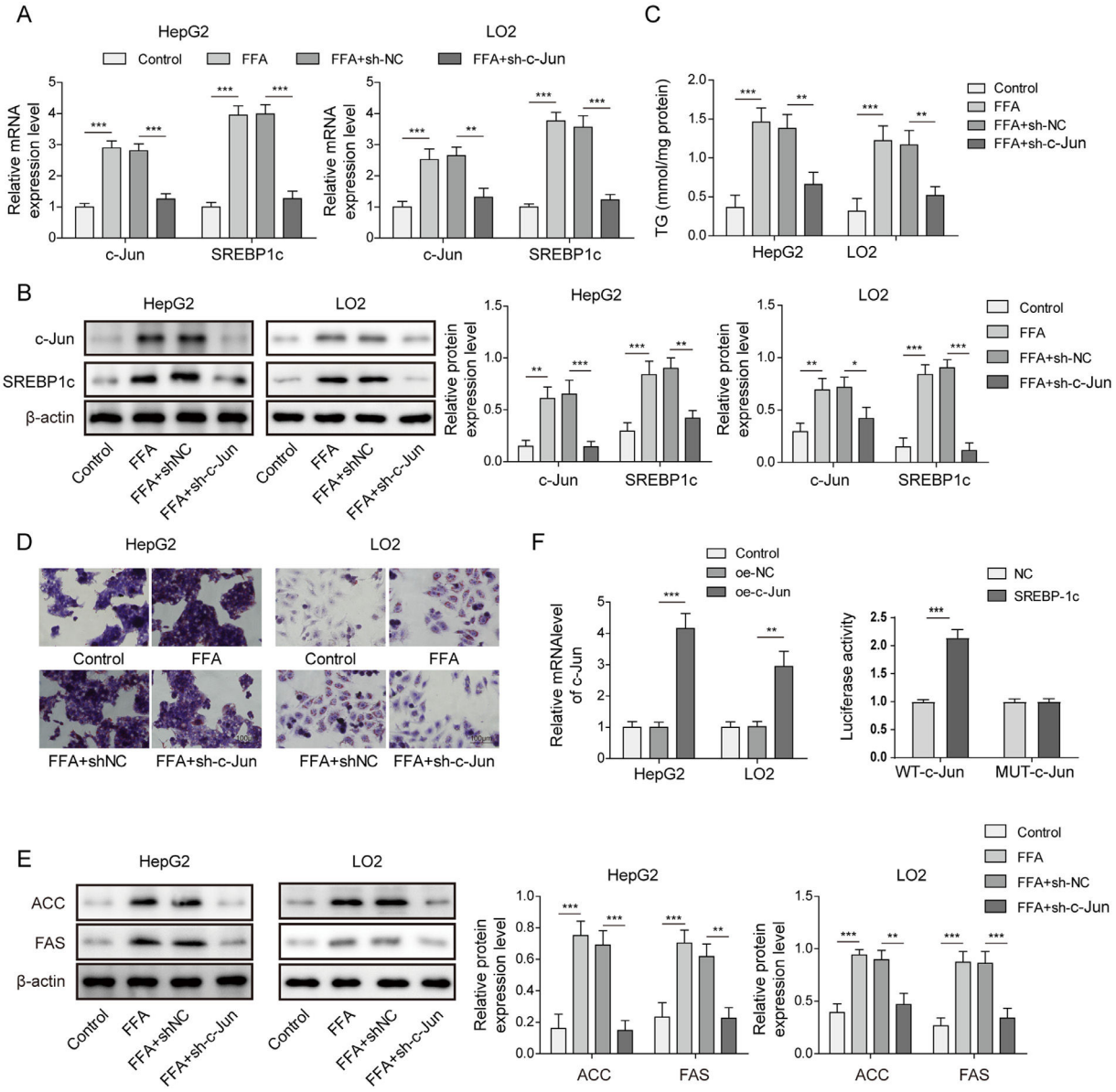

Fig. 4. Silencing c-Jun inhibits FFA-induced lipid accumulation in hepatocytes by suppressing SREBP1c expression. NAFLD cellular models were transfected with sh-NC or sh-c-Jun. (A-B) c-Jun and SREBP1c expression was determined using qPCR and western blot. (C) TG level was assessed using kits. (D) Oil Red O staining was employed to detect lipid deposition. (E) Western blotting was performed to evaluate the protein levels of FAS and ACC. (F) Overexpression of c-Jun in HepG2 and LO2 cells and the binding relationship between c-Jun and SREBP1c were verified by dual luciferase reporter gene assay. The data were expressed as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

accumulation in FFA-treated HepG2 and LO2 cells. Moreover, miR-139-5p inhibition mitigated the inhibitory effect of sh-NEAT1 on lipid accumulation. In summary, NEAT1 enhanced hepatic lipid accumulation in NAFLD by directly interacting with miR-139-5p.

We verified that c-Jun was the target of miR-139-5p. The JNK family plays a central role in hepatic physiological and pathophysiological responses [28]. The specific context and duration of activation of the JNK signalling pathway determine liver cell death to cell proliferation and canceration, as well as metabolism and survival [28]. Schulien et al. revealed that disease development from NAFLD to NASH was related to upregulated c-Jun in the liver [16]. Herein, c-Jun expression in HepG2 and LO2 cells was elevated by FFA treatment. Additionally, miR-139-5p could negatively regulate c-Jun expression. Moreover, c-Jun knockdown suppressed FFA-induced lipid accumulation in hepatocytes. SREBP1c is highly expressed in adipose tissues and plays an important role in the regulation of lipid metabolism. SREBP-1c can activate FAS and ACC through transcription to participate in fat synthesis [18]. Our results demonstrated that c-Jun could directly target SREBP1c. Therefore, we concluded that NEAT1 acted as a ceRNA to sponge miR-139-5p in HepG2 and LO2 cells to positively regulate the c-Jun/SREBP1c axis, thus enhancing hepatic lipid accumulation in NAFLD.

Overall, NEAT1 enhances hepatic lipid accumulation by activating the c-Jun/SREBP1c axis by sponging miR-139-5p. Our research confirms that NEAT1 has the potential to become a novel molecular target in NAFLD. Of course, our experiments also have shortcomings. We only performed *in vitro* experiments. In the future, we need to further verify and explore the animal level and signalling pathways, and the results will be more accurate.

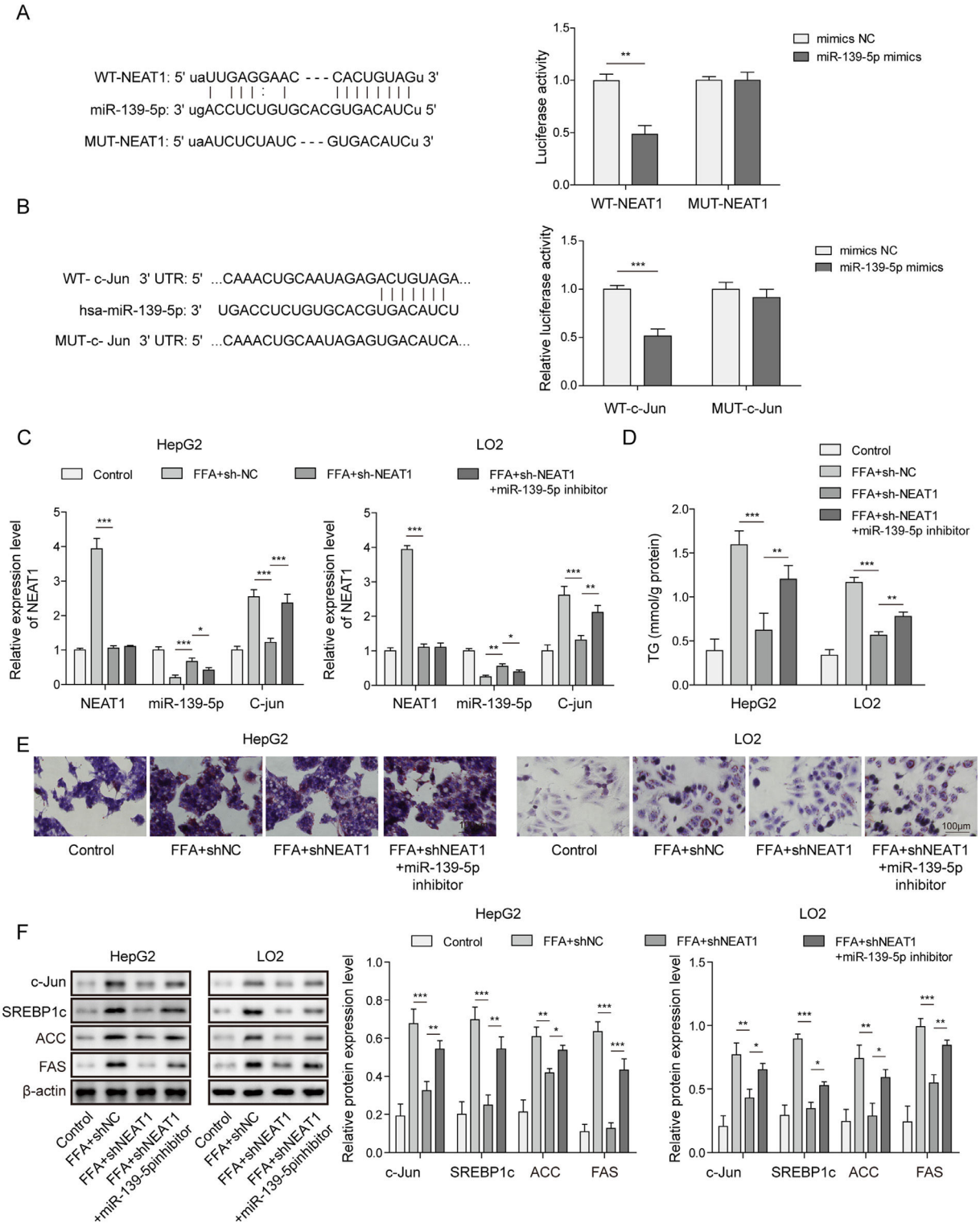

Fig. 5. lncRNA NEAT1 enhances FFA-induced lipid accumulation in hepatocytes by sponging miR-139-5p. Bioinformatics software StarBase was applied to the binding site between miR-139-5p and NEAT1. A dual luciferase reporter assay was performed to verify the binding relationship between miR-139-5p and NEAT1. (B) A dual luciferase reporter gene assay was employed to verify the binding relationship between miR-139-5p and c-Jun. HepG2 cells and LO2 cells were divided into 4 groups: control, FFA + sh-NC, FFA + sh-NEAT1 and FFA + sh-NEAT1 + miR-139-5p inhibitor. (C) NEAT1, miR-139-5p and c-Jun expression was assessed using qPCR. (D) TG level was assessed using kits. (E) Oil Red O staining was conducted to detect lipid deposition. (F) Western blotting was performed to evaluate the protein levels of c-Jun, SREBP1c, FAS and ACC. The data were expressed as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

Conflicts of interest

All authors agree with the presented findings, have contributed to the work, and declare no conflict of interest.

CRediT authorship contribution statement

Si-Si Jin: Conceptualization, Visualization, Funding acquisition, Formal analysis, Methodology, Writing – original draft, Writing –

review & editing. **Chun-Jing Lin:** Visualization, Writing – original draft, Writing – review & editing. **Xian-Fan Lin:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Ju-Zeng Zheng:** Writing – original draft, Writing – review & editing. **Hua-Qin Guan:** Writing – original draft, Writing – review & editing.

Acknowledgments

None

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