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Long noncoding RNA lncARSR promotes hepatic lipogenesis via Akt/SREBP-1c pathway and contributes to the pathogenesis of nonalcoholic steatohepatitis

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ABSTRACT

Non-alcoholic fatty liver disease and steatohepatitis (NAFLD and NASH) account for the majority of liver disease in industrialized countries. However, the pathogenesis still unclear. Long non-coding RNAs (lncRNAs) has been reported to be involved in various pathophysiological processes. Here, we reported a novel role of lncARSR in hepatic lipogenesis in NAFLD. The expression of lncARSR was induced both in NAFLD patients and mouse model, as well as in hepatocytes treated with fatty acid. Moreover, overexpression of lncARSR enhanced while knockdown of lncARSR ameliorated hepatic lipid accumulation *in vivo* and *in vitro*. Furthermore, the expression of genes related to fatty acid synthesis and oxidation increased with lncARSR overexpression *in vivo*. Mechanistically, we identified that lncARSR regulated hepatic lipogenesis via upregulating SREBP-1c, the key regulatory molecule involved in lipogenesis. Knockdown of SREBP-1c by shRNA blocked the effect of lncARSR on lipogenesis. Furthermore, we demonstrated that lncARSR regulated SREBP-1c expression by PI3K/Akt pathway. In conclusion, our data indicated that lncARSR potentially contributes to the hepatic steatosis in NAFLD, which may be a new therapeutic target against NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease, with the prevalence increases rapidly in the past a few of decades [1]. The histological spectrum of NAFLD ranged from simple steatosis to non-alcoholic steatohepatitis (NASH), which can progress to cirrhosis and even hepatocellular carcinoma (HCC) [2,3]. However, although large number of researches has been conducted, the exact pathological process of NAFLD and NASH is still unclear.

The underlying mechanism for the development and progression of NAFLD is complex and multifactorial. Ectopic accumulation of triglyceride (TG) within hepatocytes is thought to be the early stage and the hallmark of NAFLD, which is defined as hepatic TG accumulation above 5% of liver weight [4]. Evidence has shown that triglyceride (TG) de novo lipogenesis is aprominent abnormality in NAFLD and the key event that leads to massive steatosis [5].

The induction of lipogenesis is mainly controlled by sterol regulatory element binding protein 1c (SREBP-1c), which directly activates the expression of more than 30 genes, dedicated to fatty acid uptake and triglyceride synthesis [6]. Hyper-activation of SREBP-1c caused TG accumulation and lead to hepatic steatosis [7]. Increased SREBP-1c levels were found in patients with histologically diagnosed NAFLD [8].

Lon non-coding RNAs (lncRNAs) are a kind of transcripts that are more than 200 nucleotides in length without proof of protein coding potential. lncRNAs have been found to play critical roles in various pathophysiological processes including lipid metabolism, nuclear reprogramming, cell proliferation, energy expenditure, etc [9–11].

lncARSR is a recently identified lncRNA with 591 nucleotides in length [12]. It has been found to play important role in caner. lncARSR promoted renal tumor initiating cells expansion via physically interacting with YAP and facilitating YAP nuclear translocation [13]. And it also could promote doxorubicin resistance in hepatocellular carcinoma via modulating PTEN-PI3k/Akt pathway

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[14]. However, if lncASAR participate in the pathogenesis of hepatic lipid dysregulation and NAFLD is still unknown.

In this study, we demonstrated a novel role of lncARSR in the regulation of hepatic lipogenesis. We found that the expression of lncARSR was upregulated in NAFLD mice model and human patients. Knockdown of lncARSR ameliorated while overexpression of lncARSR augmented the TG accumulation induced by methionine-choline deficient (MCD) diet feeding in mice. Mechanistically, the effect of lncARSR on lipogensis was mediated by Akt/SREBP-1c pathway.

2. Methods

2.1. Human sample

The study protocol was approved by the Ethics Committee of Weifang People's Hospital. The liver tissues were previously collected from patients of benign focal hepatic lesions undergoing liver surgery. The samples were immediately shock-frozen and stored at −80 °C. Blood samples were collected from all subjects following overnight fasting. Informed written consent was obtained from every patient.

2.2. Mouse model

The protocol was approved by Research Ethics Committee of Weifang People's Hospital. C57Bl/6 male mice (6–8 weeks) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd, China. lncARSR overexpression adenovirus, lncARSR shRNA adenovirus and their empty vector adenovirus were dissolved in sterile PBS and injected through the caudal vein to mice. Four weeks later, the mice were fed with either a control chow diet (CD) or a methionine-choline deficient (MCD) diet for 2 weeks induce the NASH phenotype. After 2 weeks of the feeding, the mice were sacrificed, serum and livers were collected.

2.3. Cell culture

The human hepatocarcinoma cell line (HepG2) was cultured in DMEM medium containing glucose (4.5 g/l) and 10% FBS. All the cells were incubated at 37 °C in 5% CO2 humidity.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 19.0. All Data are presented as mean ± SEM. A Student's unpaired *t*-test was used to determine differences between two groups. $P < 0.05$ was considered significant.

3. Results

3.1. The expression of lncARSR increased in NAFLD patients and mouse model

To identify the effect of lipid accumulation on the expression of lncARSR. We recruited 12 NAFLD patients and 22 healthy controls. As shown in Fig. 1A, the mRNA levels of lncARSR both in the serum and liver were significantly increased in NAFLD patients compared with the healthy controls ($P < 0.01$).

Further, we conducted NAFLD mice model by methionine-choline deficient (MCD) diet feeding, which led to increased hepatic lipid accumulation (Fig. 1B&C). qRT-PCR revealed that lncARSR was significantly increased in the liver of MCD mice compared to chow diet fed mice (Fig. 1D). Intriguingly, the expression of lncARSR was also highly induced by FAs, including oleic acid (OA), palmitic acid (PA), and stearic acid (SA) (Fig. 1E). Taken together, our studies indicated that the expression of lncARSR is induced in NAFLD patients and mouse model.

Overexpression of lncARSR enhanced while knockdown of lncARSR ameliorated hepatic lipid accumulation *in vivo* and *in vitro*.

Transient overexpression of lncARSR using AAV8-lncARSR virus markedly induced the expression of lncARSR (Fig. 2A). And the overexpression of lncARSR caused significant increase of intracellular TG contents in HepG2 cells (Fig. 2B). The results suggest that H19 may function as a fatty acid sensor to modulate lipogenesis.

To investigate the effects of lncARSR on lipogenesis *in vivo*, we established lncARSR overexpression mice model via tail vein injection with AAV8-lncARSR, which had higher lncARSR mRNA levels (Fig. 2C). Four weeks later after AAV-lncARSR injection, the mice were followed by MCD diet feeding for 2 weeks. As predicted, compared with AAV8-con-chow diet mice, AAV8-con-MCD mice showed significant increase in hepatic lipid accumulation in AAV8-lncARSR-MCD vs AAV8-con-MCD mice, which was accompanied by the increased levels of liver TG content (Fig. 2D–F).

To further establish the endogenous effect of lncARSR in lipogenesis, we established lncARSR knockdown mice model via tail vein injection with AAV8-shlncARSR, which were challenged with a MCD diet for 2 weeks. Lipid droplets became evident in AAV8-con-MCD mice but were largely prevented from developing in AAV8-shlncARSR-MCD mice (Fig. 2G). In agreement with this observation, hepatic TG content were concurrently lower in AAV8-shlncARSR-MCD vs AAV8-control-MCD mice (Fig. 2H).

3.2. Gene expression profiles and role of SREBP-1c in lncARSR-induced lipid accumulation

Hepatic lipid metabolism is associated with lipid uptake, de novo lipogenesis (DNL), fatty acid oxidation, and lipoprotein secretion [15]. To elucidate the mechanisms underlying the influence of lncARSR, we examined the expression of genes involved in TG metabolism in lncARSR overexpression mice. As shown in Fig. 3A, the expression of key genes associated with lipogenesis (*Srebp-1c, Fasn, Acc1* and *Scd1*) were increased, while the genes related to fatty acid oxidation (Ctp1a) were decreased in the AAV8-lncARSR mouse liver.

The dysfunction of the DNL pathway has been more importantly associated with a primary disorder in NAFLD. SREBP-1c is a key regulatory molecule involved in lipogenesis. Notably, a prominent increase in the precursor and mature forms of SREBP-1c proteins was observed in the liver of both MCD and chow fed AAV-lncARSR mice (Fig. 3B and C).

By the *in vitro* study, we also confirmed that lncARSR overexpression induced the expression of genes associated with lipogenesis, such as SREBP-1c, FASN and SCD1, while when SREBP-1c was knockdown by shRNA, the effect of lncARSR on lipogenesis was blocked (Fig. 3D).

3.3. lncARSR regulated SREBP-1c via PI3K/Akt/mTOR pathway

We further elucidated the potential mechanism that lncARSR regulates SREBP-1c expression. lncARSR has been reported to active PI3K/Akt pathway [14], and SREBP-1c can be regulated by PI3K/Akt pathway [16,17]. We further checked if lncARSR regulated SREBP-1c via PI3K/Akt pathway. As shown in Fig. 4A&B, the expression of p-Akt and SREBp-1c increased significantly with lncARSR overexpression, while decreased with lncARSR knockdown. However, when the cells were treated with LY294002, the PI3K inhibitor, the effect of lncARSR on SREBP-1c disappeared (Fig. 4C). Overall, these results strongly suggest that lncARSR increased SREBP-1c levels by activating the PI3K/Akt/mTOR pathway.

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