Hepatic stellate cells-specific LOXL1 deficiency abrogates hepatic inflammation, fibrosis, and corrects lipid metabolic abnormalities in non-obese NASH mice

Aiting Yang1,3,4 · Xuzhen Yan2,4 · Xu Fan2,4 · Yiwen Shi2,4 · Tao Huang1,3,4 · Weiyu Li2,4 · Wei Chen1,3,4 · Jidong Jia2,3,4 · Hong You2,3,4

Received: 20 February 2021 / Accepted: 5 May 2021 / Published online: 20 May 2021
© Asian Pacific Association for the Study of the Liver 2021

Abstract

Background and aims Lysyl oxidase-like-1 (LOXL1), a vital cross-linking enzyme in extracellular matrix (ECM) maintenance, promotes fibrosis via enhancement of ECM stability. However, the potential role of LOXL1 in the pathogenesis of nonalcoholic steatohepatitis (NASH) has not been previously studied.

Methods We generated Loxl1flo/flo mice to selectively delete LOXL1 in hepatic stellate cells (HSCs) (Loxl1flo/floGfapcre; Loxl1flo/flo as littermate controls) and then examined liver pathology and metabolic profiles in Loxl1flo/floGfapcre fed with either a choline-deficient L-amino acid-defined (CDAA) diet or an isocaloric control diet for 16 weeks. Thereafter, the findings from the animal model were confirmed in 23 patients with biopsy-proven non-alcoholic fatty liver disease (NAFLD).

Results LOXL1 was significantly increased in CDAA induced non-obese NASH compared with the control diet, and LOXL1 deficient in HSCs ameliorated CDAA-induced inflammation and fibrosis, with reduced expression of pro-inflammation and pro-fibrogenic genes in the HSCs-specific LOXL1 knockout mice model. Interestingly, LOXL1 deficient in HSCs could attenuate hepatic steatosis and reverse the metabolic disorder by restoring adipose tissue function without altering the effect of hepatic lipogenesis gene expression in non-obese NASH model. More importantly, analyses of serum LOXL1 and leptin levels from NAFLD patients revealed that LOXL1 was positively correlated with histological fibrosis progression, whereas it was inversely correlated with leptin levels, especially in non-obese NAFLD patients.

Conclusion LOXL1 may contribute to fibrosis progression in non-obese NAFLD, and HSCs-specific knockout of LOXL1 attenuated liver steatosis, inflammation, fibrosis, and improved lipid metabolic abnormalities. Hence, LOXL1 inhibition may serve as a new therapeutic strategy for NASH.

Keywords Liver fibrosis · Hepatic stellate cell · ECM · LOXL1 · NAFLD · Crosslink · Lipid metabolism · Leptin · Non-obese NASH · CDAA

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease around the world. The demographic characteristics of NAFLD vary worldwide, usually parallel to the epidemiology of obesity, but a substantial proportion of patients who suffer from NAFLD are lean [1]. NAFLD presents a wide spectrum of pathological entities, among which nonalcoholic steatohepatitis (NASH) is highly likely to progress to end-stage liver disease and lethal cardiometabolic diseases.

The progression from NASH to cirrhosis and liver failure involves extracellular matrix (ECM) remodeling and fibrosis, which has been sequenced as the abundant production
of ECM proteins, such as collagen, elastin, and other proteins as well as incomplete fibrinolysis in activated hepatic stellate cells (HSCs) and myofibroblasts [2], and then ECM deposition through cross-linking of ECM to form an insoluble matrix. The first step of the covalent cross-linking of the ECM proteins collagen and elastin, which dictate ECM stiffness and mechanical properties is catalyzed by the LOX family members including lysyl oxidase (LOX) and lysyl oxidase-like proteins 1–4 (LOXL1-4) [3, 4].

Molecularly, the LOX family members share high homology, but the genes for the five members are located on different chromosomes associated with multifunction apart from organ fibrogenesis. Of note, among five LOX family members, expressions of LOX and LOXL1 are markedly up-regulated in fibrotic liver tissues across humans and mice [4], suggesting a possible pathogenic role of LOX and LOXL1 in liver fibrosis. Our previous study had shown that only LOXL1 upregulation paralleled with overexpression of type I procollagen and tropoelastin during later stages of liver fibrogenesis, and found Loxl1 shRNA prevented collagen and elastin from depositing in CCl4 induced-liver fibrosis [5].

Moreover, deposition of ECM in the liver not only features fibrosis, but also contributes to cell proliferation, differentiation, death, and lipid metabolism [6]. Recently, Romani et al. evidenced that cell–matrix adhesions delivered mechanical signals linked with metabolic pathways regulating lipid synthesis and accumulation by shifting the homeostatic SREBP1/2 activity [7]. In the liver, Dongiovanni et al. showed that lipotoxicity in hepatocytes enhances LOXL2 production in HSCs, which subsequently facilitating extracellular matrix stabilization [8]. Therefore, reciprocal crosstalk may exist between ECM remodeling and lipid metabolism.

Considering the critical roles of HSCs-derived LOXL1 in ECM remodeling and lipid metabolism, we hypothesized a potential link between LOXL1 and the progression of NAFLD. In this report, we generated an HSCs-specific LOXL1 knock-out mice model, through which we studied the role of LOXL1 deficiency in the progression of NASH with the mice fed with choline-deficient L-amino acid (CDAA) diet (M10530i; Moldiets) or control diet (choline supplemented amino acid, CSAA) (M10530Ci; Moldiets). CDAA dieted mice model can mimic human NASH by sequentially producing steatohepatitis, liver fibrosis, and liver cancer without any body weight loss and thus was called the “non-obese NASH” model.

Body weight and average food intake were measured weekly. At the end of the experiment, blood was collected by cardiac puncture; meanwhile, epididymal white adipose tissue, as well as the liver were rapidly harvested, weighed, and stored in liquid nitrogen at −80 °C for future analysis. A portion of each liver was fixed in 4% formalin for histology examining.

**Materials and methods**

**Detailed methods are presented in the Supplementary Materials and methods**

**Study design of animal experiment**

This study was approved by the Institutional Animal Care and Usage Committee of the Beijing Friendship Hospital, Capital Medical University (No.:20-2002). Male mice (6–8 weeks) were subjected to 16 weeks of a choline deficiency amino acid (CDAA) diet (M10530i; Moldiets) or control diet (choline supplemented amino acid, CSAA) (M10530Ci; Moldiets). CDAA dieted mice model can mimic human NASH by sequentially producing steatohepatitis, liver fibrosis, and liver cancer without any body weight loss and thus was called the “non-obese NASH” model.

The Ethics Committee of the Beijing Friendship Hospital, Capital Medical University approved the study (No.: 2018-P2-228-02). All participants provided written informed consent before they participated in the study. Eligible patients were adults (age ≥ 18 years) with histological evidence of definitive NAFLD. The histology of the participants was thereafter graded according to the NASH-CRN for Kleiner–Brunt fibrosis, steatosis, hepatocellular ballooning, lobular inflammation, and the composite NAS. NASH was diagnosed in the presence of the combination of any degree of hepatic steatosis, hepatocellular ballooning, and lobular inflammation [9]. All liver pathologists were blinded to the clinical and biochemical details of the participants. The extent of hepatic fibrosis was graded on a five-point scale as: F0 = absence of fibrosis, F1 = zone 3 perisinusoidal/perivenular fibrosis, F2 = zone 3 and periportal fibrosis, F3 = septal/bridging fibrosis, F4 = cirrhosis.

Based upon the grading, the patient participants were further grouped into no or mild fibrosis arm (F = 0–1, n = 12), and significant fibrosis arm (F ≥ 2–4, n = 11). The clinical demographics (gender, age, BMI, AST, ALT, PLT, GGT, TG, TC, HDL-C, LDL-C, LSM, and NAS) of NAFLD patients are provided in Table S2. Serum samples were
obtained from all patients within 2 weeks of liver biopsy after at least overnight fasting.

Statistical analysis

Two-group comparisons were performed using a two-tailed unpaired Student’s t test (data with normal distribution) and Mann–Whitney U test (data with non-normal distribution). Multiple groups were subjected to analysis of variance (ANOVA) with Bonferroni post-hoc test comparison. The correlations of serum LOXL1 with leptin were analyzed using Spearman rank correlation tests. \( p < 0.05 \) was considered statistically significant.

Results

Selectively depletion of LOXL1 in HSCs attenuated the CDAA induced LOXL1 up-regulation in liver

To assess the role of LOXL1 in HSCs, we first overexpressed LOXL1, which indeed promoted activation of HSCs (Figure S2), then we generated HSCs-specific LOXL1 deletion (Loxl1\(^{fl/fl}\)Gfap\(^{cre}\)) by crossing Loxl1\(^{fl/fl}\) mice to mice bearing a Gfap-Cre transgene. Loxl1\(^{fl/fl}\) mice were used as a control mice; however, the upregulated expression of LOXL1 protein levels in liver compared with those levels in CSAA-fed control mice led to LOXL1 overexpression at both mRNA and protein levels in liver compared with CDAA-fed the control mice (Fig. 1a). As expected, CDAA-fed the control mice 16 weeks (Fig. 1b, c).

To confirm the deletion of LOXL1 in activated HSCs, we performed a co-localization immunofluorescence assay on LOXL1 with \( \alpha \)-SMA in activated HSCs from CDAA-fed Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice and Loxl1\(^{fl/fl}\) mice, finding that LOXL1 expression was significantly diminished in the activated HSCs from Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice, more interestingly, LOXL1 was located in between the plates of hepatocyte (Fig. 1d).

Selectively depletion of LOXL1 in HSCs decreased steatosis, ballooning, and injury in liver

To explore the molecular mechanism of HSCs-specific LOXL1 in NAFLD development, we examined hepatic histopathological features in Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) and Loxl1\(^{fl/fl}\) mice on either CSAA or CDAA diet. CSAA-fed mice did not display any hepatic histopathological change regardless of the genetic profiles of LOXL1, whereas CDAA-fed mice developed NASH with hepatic steatosis, inflammation, and ballooning (Fig. 2a), and in further comparison, CDAA-fed Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice harbored less histologic features of NASH with a significantly lower nonalcoholic fatty liver disease activity score (NAS) with respect to the control mice, including less steatosis (2.60 ± 0.16 vs. 1.60 ± 0.22, \( p < 0.05 \)), less inflammation (2.40 ± 0.16 vs. 1.60 ± 0.22, \( p < 0.05 \)) and less hepatocyte ballooning (2.10 ± 0.23 vs. 1.40 ± 0.16, \( p < 0.05 \)) (Fig. 2b). In addition, the liver weight/body weight ratio or spleen/body weight ratio was significantly increased in CDAA-fed control mice compared with CSAA-fed control mice, but the increase was slightly decreased in CDAA-fed Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice (\( p > 0.05 \)) (Fig. 2c, d).

Also along with histological changes, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were elevated in the CDAA-fed as compared to CSAA-fed control mice; however, LOXL1 deficiency in HSCs markedly attenuated CDAA induced-elevation of ALT and AST levels by about 40–55% (\( p < 0.05 \), Fig. 2e, f), indicating a protective role of HSCs-specific LOXL1 deficiency against CDAA-induced NASH.

Selectively depletion of LOXL1 in HSCs reduced fibrosis in CDAA-induced NASH

To examine the NAFLD progression, hepatic fibrosis was stained with Sirius red (SR) staining, showing that 16-week CDAA feeding could induce typical pericellular fibrosis. Quantification of hepatic collagen deposition based upon the contrast between SR and Collagen I staining was markedly increased in the CDAA-fed control mice compared with CSAA-fed control mice, whereas this rise was abrogated in the CDAA-fed Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice (Fig. 3a–c). Further measurement of hydroxyproline level confirmed that liver fibrosis was less in CDAA-fed Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice than that in in control mice (Fig. 3d). Besides, this resolved fibrosis in the CDAA-fed Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice was associated with decreased expression of fibrosis-related genes, such as \( \alpha \)-SMA, Col1α1, Timp1, Mmp2, Mmp9, Mmp12, Mmp13, Tgfβ1, Pai1, and Pdgfrb, as assessed by qPCR analysis, compared with CDAA-fed the control mice (Fig. 3e). Beyond, we also found that the expressions of Lox were decreased significantly in CDAA-fed Loxl1\(^{fl/fl}\)Gfap\(^{cre}\) mice compared with CDAA-fed the control mice. It is worth mentioning that HSCs-specific LOXL1 in HSCs had a minor effect on Loxl2 expression (Fig. 3e).
Fig. 1 Efficient HSC-specific deletion of LOXL1 in Loxl1fl/flGfapCre mice. a Schematic representation of experiment design. Loxl1fl/flGfapCre and their matched littermate control mice were fed a CSAA diet (n = 3 mice per subgroup) or CDAA diet (n = 10 mice per subgroup) for 16 weeks. b qPCR analysis of Loxl1 expression in the livers of Loxl1fl/flGfapCre and their control mice fed with a CSAA or CDAA. c Western blot analysis (left panel) and quantification (right panel) of LOXL1 expression in the livers of Loxl1fl/flGfapCre and their control mice fed with CSAA or CDAA for 16 weeks. d Immunofluorescence detection of LOXL1 and a-SMA. Data in (b, c) are presented as the mean ± SEM. Statistical analysis was performed by unpaired two-tailed Student’s t test and one-way ANOVA followed by Tukey post hoc test (*p < 0.05)
Fig. 2 Characterization of HSCs-specific LOXL1 deletion mice fed with a CSAA or CDAA diet. a HE staining of representative liver sections in Loxl1<sup>fl/fl</sup>Gfap<sup>cre</sup> mice and their control mice after 16-week CSAA or CDAA diet. b NAFLD activity score (NAS) of the four subgroups. c Liver/body weight ratios of the four subgroups. d Spleen/body weight ratios of the four subgroups at the end of CSAA or CDAA feeding. e Serum AST and f ALT levels of the four subgroups. Data in (b–f) are presented as the mean±SEM. Statistical analysis was performed by unpaired two-tailed Student’s t test and one-way ANOVA followed by Tukey post hoc test (*p<0.05)
Selectively depletion of LOXL1 in HSCs ameliorated liver inflammation and macrophage infiltration in CDAA-induced NASH

Next, for studying whether HSCs-specific LOXL1 deletion affected hepatic inflammation, CD68 staining was used to trace macrophage infiltration, which showed much higher CD68 staining could be seen in the CDAA-fed control mice but not in the CSAA-fed control mice, and was abrogated in Loxl1<sup>fl/fl</sup>Gfap<sup>cre</sup> mice (Fig. 4a, b). Concordantly, mRNA expressions of the pro-inflammatory cytokines, such as Mcp1 and Tnf-a in CDAA-fed Loxl1<sup>fl/fl</sup>Gfap<sup>cre</sup> mice were further found to be decreased compared with CDAA-fed control mice;
Figure a: Body Weight over weeks for different genotypes.
Figure b: Epididymal fat/body weight ratio for different genotypes.
Figure c: Oil red staining images at 200X magnification for different genotypes.
Figure d: Oil red area % for different genotypes.
Figure e: Hepatic triglyceride levels for different genotypes.
Figure f: Hepatic NEFA levels for different genotypes.
Figure g: mRNA expression levels for different genes (Mixipl, Srebf1, Fasn, Acaca, Scd1) for different genotypes.
and a similar pattern was also observed for the expression of Il6, whereas no change was detected in Il10 mRNA (Fig. 4c).

**HSCs-specific LOXL1 deletion reversed the metabolic abnormalities in CDAA-induced NASH**

Afterward, the focus was turned to detect whether LOXL1 deficiency in HSCs altered the metabolic phenotype or the steatosis in CDAA-fed mice. We then extracted the metabolic profiles from each group of mice. The initial body weights for all mice were comparable, and body weights during the whole study period were measured to be similar between CSAA-fed control and CSAA-fed Lox11fl/flGfapcre mice, but lower in CDAA-fed control mice, indicating HSCs-specific LOXL1 deletion could influence the steatotic process, several key lipogenic genes (ChREBP/Mlxipl, Srebf1, Fasn, Acaca, Scd1) were then studied. It was found that the CDAA diet significantly down-regulated the expression of genes involved in fatty acid synthesis, which might result from compensatory hepatic uptake of serum lipids or by impairment in very low density lipoprotein secretion from the liver [10]. Interestingly, a tendency towards a reduced expression was observed for these genes in CDAA-fed Lox11fl/flGfapcre compared with CDAA-fed control mice, while no differences were observed in Mlxipl expression ($p>0.05$) (Fig. 6b). Interestingly, gene expression adipokines including leptin and Il6 were increased in CDAA-fed Lox11fl/flGfapcre mice compared with CDAA-fed control mice, a tendency towards an increased expression was also observed for adiponectin (Fig. 6c). Serum leptin levels confirm this finding (Fig. 6d). Following 16 weeks of CSAA or CDAA feeding, the weekly food intake per gram body weight was higher in CDAA-fed Lox11fl/flGfapcre compared with that in other groups. It is worth mentioning that after the 11th week, average food intake relative to body weight decreased progressively in CDAA-fed Lox11fl/flGfapcre mice (Fig. 6e).

**LOXL1 levels were negatively associated with leptin levels in non-obese NASH patients**

To assess the clinical relevance of our findings, we examined LOXL1 and leptin levels in NAFLD patients with no or mild or significant fibrosis (Table S2). Consistent with the mice data, we observed that serum LOXL1 level was significantly lower in patients with no or mild fibrosis than those with significant fibrosis in NAFLD patients ($F(0/1 $vs. $F > 2$), 30.0 (21.9, 38.0) vs. 66.6 (31.0, 79.7) pg/ml, $p < 0.05$) (Fig. 7a), a trend of lower serum leptin levels in patients with no or mild fibrosis than those with significant fibrosis ($F(0/1 $vs. $F > 2$), 197.5(130.0, 251.0) vs. 132(90.0, 218.0) ng/ml, $p > 0.05$) (Fig. 7b). Correlation analyses showed significant reversion in the association between serum LOXL1 and leptin in NAFLD patients (Fig. 7c). Furthermore, we noted that the prevalence of histologically detected with significant fibrosis was significantly higher among patients with non-obese NALFD (BMI <25 kg/m²) (7/9, 77.8%) compared to those obese NAFLD (BMI ≥25 kg/m²) (4/14; 8.6%; $p = 0.027$). To characterize LOXL1 expression features in human liver fibrosis disease with different etiology and pathology, we performed LOXL1...
immunohistochemistry on the samples from fatty liver, NAFLD related cirrhosis, and HBV related cirrhosis. Specifically, LOXL1 expression was weak in the fatty liver, whereas its expression within the fibrotic septa area and the portal area was associated with mass inflammatory cell infiltrating (Fig. 7d). Together, our results demonstrated LOXL1 was associated with fibrosis during NAFLD development; meanwhile, there will be a potential connection between LOXL1 and leptin in NAFLD, especially in non-obese NAFLD (Fig. 7e).

**Fig. 6** HSCs-specific LOXL1 deletion improved adipose tissue function in murine NASH. a–c qPCR analysis for Col1a1 (a), lipogenic genes (b), and adipokine-related genes (c) in adipose tissues from Loxl1fl/flGfapcre and their control mice fed with CSAA or CDAA diet for 16 weeks. d ELISA assay measurement of serum leptin levels in Loxl1fl/flGfapcre+ and their control mice fed with CSAA or CDAA diet for 16 weeks. e Average food intake (normalized per gram body weight) of the four subgroups. Data in (a–d) are presented as the mean ± SEM. Statistical analysis was performed by unpaired two-tailed Student’s t test and one-way ANOVA followed by Tukey post hoc test (*p < 0.05)
Fig. 7 Inverse correlation between the serum LOXL1 levels and the serum leptin levels in NAFLD patients. 

a Levels of serum of LOXL1 between the different stages of fibrosis ($F = 0/1$ vs. $F \geq 2$) in NAFLD patients.

b Levels of serum of leptin between the different stages of fibrosis ($F = 0/1$ vs. $F \geq 2$) in NAFLD patients.

c Correlation of serum LOXL1 with serum leptin (Spearman rank correlation test).

d Representative LOXL1 immunohistochemistry staining of liver sections in patients with chronic liver injury.

e Proposed mechanisms. Feeding the CDAA diet led to liver injury, steatosis, inflammation, and fibrogenic response with lipid metabolism abnormality. LOXL1 deficiency prevents hepatic inflammation while reducing liver fibrosis in response to CDAA feeding. In addition, depletion of LOXL1 in the liver could improve whole-body lipid metabolism abnormality in the CDAA-diet-induced NASH model, including normalized the decrease in body weight, promoted hepatic TG transport from the liver modulated by adipose tissue-derived leptin. Data in (a, b) are presented as the Median (25% Percentile, 75% Percentile). *$p<0.05$
Discussion

Non-alcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease and affects about 25% of adults in the world [12]. 13–30% of patients with NAFLD present with non-alcoholic steatohepatitis (NASH), which was characterized by hepatocyte steatosis (lipid accumulation), hepatocyte lipopapoptosis due to misdirected lipid metabolism and oxidative stress, liver inflammation, and various degrees of fibrosis, with a high risk of progression to cirrhosis and primary liver cancer. Notably, morbidity and mortality in earlier stages of NAFLD/NASH are largely due to complications of the metabolic syndrome, while the risk of liver complications increases steeply with advanced fibrosis [13, 14]. Thus, prevention of cirrhosis and reversal of advanced liver fibrosis are now considered the primary aim of NASH therapies.

The expression of most LOX family members is elevated in experimental liver fibrosis of diverse etiologies, and inhibition of the LOX and LOXLs could suppress fibrosis progression and accelerate its reversal in animal models of liver fibrosis. Among the LOX family members, LOXL2 contributes to fibrogenesis by catalyzing cross-linkage of collagen and had been taken as a therapeutic target for treating nonalcoholic steatohepatitis by catalyzing cross-linkage of collagen and had been taken as a therapeutic target for treating nonalcoholic steatohepatitis. However, simtuzumab, a humanized monoclonal antibody targeting the isoform LOXL2 failed to show an effect on the improvement of progression-free survival in two phase 2b trials of patients with bridging fibrosis or compensated cirrhosis associated with nonalcoholic steatohepatitis [20, 21], thereby highlighting the need for a deep understanding of the function of LOX family regulated ECM crosslinking.

LOXL1 from activated HSCs and myofibroblasts appears to be specifically required for cross-linking of soluble tropoelastin into insoluble elastin during ECM formation, stabilization, maintenance, and remodeling; therefore, in this study, we created HSCs-specific LOXL1 knockout mouse model to investigate the role of LOXL1 in the pathogenesis of NASH. Not surprisingly, due to LOXL1 as a key extracellular enzyme responsible for cross-linking collagen and elastin molecules to form a stable extracellular matrix, we observed that HSCs-specific LOXL1 depletion significantly reduced hepatic collagen deposition and attenuated liver fibrosis in a CDA-diet feeding NASH model. Our results are consistent with previous reports in idiopathic pulmonary fibrosis, showing LOXL1 deficiency prevented fibrosis progression through reducing lung stiffness, likely by limiting collagen cross-linking at a late stage [15]. Indeed, studies already showed that LOX/LOXL1/LOXL2 plays a crucial role in the maintenance of extracellular matrix stability and is markedly related to fibrosis.

Beyond its role in ECM maturation, our study also suggested that LOXL1 was involved in lipid metabolism. Indeed, previous studies already established the pathogenic role of LOX and LOXL2 in metabolic dysfunction by finding that Lox mRNA levels are increased in adipose tissue samples from the high-fat diet model [16], and LOXL2 expression was positively correlated with both steatosis and fibrosis in the MCD model of NASH [8]. Besides the animal models, elevated expression and activity of LOX family members are also found in sera of patients with NAFLD [8, 17]. In the molecular mechanism, ECM mechanical signaling may control lipid synthesis through direct regulation of SREBP1/2 activity [7, 18], and LOX family members are a major player in ECM maturation, which could be in part explain the LOX family involved in the regulation of lipid metabolism directly.

Our data showed that depletion LOXL1 in HSCs protects the liver against steatosis, but did not significant change lipogenesis-related genes in the liver. Furthermore, we noted that depletion of LOXL1 in the liver improved whole body lipid metabolism abnormalities in the CDA-diet-induced NASH model, including normalizing the loss in body weight, elevating serum TG levels through promoting TG transport from liver into blood, and increasing fat mass weight. More interestingly, we found that HSCs-specific LOXL1 deficiency could regulate the molecular function of adipose tissue through downregulating Col1a1 in the adipose tissue and promoting expression of leptin and IL6.

Leptin is the main adipokine predominantly made by adipose tissue that is involved in the regulation of hepatic lipid metabolism. Notably, replacement therapy with recombinant leptin potently reverses hepatic steatosis in lean rats, but not in diet-induced obesity rats [19], as well as transplantation adipose tissue treatment. Moreover, recently, Hackl et al. found CNS leptin signaling both promotes hepatic TG export and decreases de novo lipogenesis in the liver [20]. Our study also demonstrated that depletion of LOXL1 in the liver promoted TG export and reduced hepatic steatosis, we believe that leptin, which is chiefly secreted in proportion to body fat stores, regulates hepatic lipid content and improves lipid metabolic abnormalities. It is worthy of note that not only serum LOXL1 level was remarkably correlated with advanced fibrosis, but also inversely correlated with leptin production in NAFLD patients, which further confirmed the mice results. More interestingly, the proportion of BMI less than 25 in patients with progression fibrosis was significantly higher than in patients without fibrosis, suggesting non-obese NASH patients have serious fibrosis than obese NASH. A recently published meta-analysis and study also confirmed that patients with lean-NASH had higher fibrosis [21]. To our best knowledge, our current study is the very first one reporting the link between LOXL1 and leptin in NAFLD patients. Since the role of LOXL1 in the control of adipocyte function has been poorly characterized, further study should clarify how the mechanisms of LOXL1 in the liver to modulate adipose tissue function.

Based upon the findings of our and other studies, several mechanisms were proposed on the roles of LOXL1 in...
NASH. Firstly, Using single-cell RNA sequencing of fibrotic liver, Dobie et al. [22] demonstrated that LOXL1 was highly expressed in central vein-associated HSCs, one of the dominant pathogenic collagen-producing cells in cirrhotic liver fibrosis in a mouse model of NASH; then here we found that deficiency of LOXL1 in HSCs inhibits liver fibrosis in a rodent NASH model, taken together LOXL1 may play critical role in the development and progression of NASH related fibrosis; Secondly, far from being a static structure, multiple studies indicate ECM mechanics regulate cell lipid metabolism [6]. In line with that LOXL1 is well established in fibrosis via mediating ECM crosslinking and stabilization, our data showed that depletion LOXL1 in HSCs protects against steatosis in liver; however, this mechanistic link between the regulation of LOXL1 in ECM remodeling and lipid metabolism might be needed to further study; Thirdly, with the finding of selective LOXL1 deletion in the liver being able to restore adipose tissue function, a crosstalk, therefore, may exist between HSCs-specific LOXL1 and deranged metabolism in the adipose tissue in non-obese NASH.

Taken all together, in the current study, we demonstrated that selectively depletion of LOXL1 in HSCs prevented CDAA diet-induced inflammation, fibrosis, as well as improved lipid metabolic abnormalities. Therefore, LOXL1 inhibition might be an interesting therapeutic target for drug development in NASH, which may provide new insights into a clinical therapy for non-obesity-related fibrosis and metabolic diseases.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12072-021-10210-w.

Author contributions AY, and HY conceived the study. AY, XY, XF, YS, TH, WL, WC, JJ, and HY performed experiments or analyzed the data. AY drafted the manuscript, and all authors read or revised the manuscript.

Funding This study was supported by the National Natural Science Foundation of China (81970524 and 81500456).

Declarations

Conflict of interest The authors Aiting Yang, Xuzhen Yan, Xu Fan, Yiwen Shi, Tao Huang, Weiyu Li, Wei Chen, Jidong Jia and Hong You declared that they have no conflict of interest.

Animal research This study was approved by the Institutional Animal Care and Usage Committee of the Beijing Friendship Hospital, Capital Medical University (No.:20-2002).

Consent to participate The study was conducted in accordance with the principles enshrined in the Declaration of Helsinki and the Good Clinical Practices. The Ethics Committee of Beijing Friendship Hospital, Capital Medical University approved study (No.: 2018-P2-228-02).

Consent to publish All authors had access to the study data and reviewed and approved the final manuscript.

References

1. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2018;15:11–20
2. Schuppan D, Surabattula R, Wang XY. Determinants of fibrosis progression and regression in NASH. J Hepatol 2018;68:238–250
3. Schuppan D, Ashfaq-Khan M, Yang AT, Kim YO. Liver fibrosis: direct antifibrotic agents and targeted therapies. Matrix Biol 2018;68–69:345–451
4. Chen W, Yang A, Jia J, Popov YV, Schuppan D, You H. Lysyl Oxidase (LOX) Family Members: rationale and their potential as therapeutic targets for liver fibrosis. Hepatology 2020;72:729–741
5. Zhao W, Yang A, Chen W, Wang P, Liu T, Cong M, Xu A, et al. Inhibition of lysyl oxidase-like 1 (LOXL1) expression arrests liver fibrosis progression in cirrhosis by reducing elastin crosslinking. Biochim Biophys Acta Mol Basis Dis 2018;1864:1129–1137
6. Romani P, Valcarcel-Jimenez L, Frezza C, Dupont S. Crosstalk between mechano-transduction and metabolism. Nat Rev Mol Cell Biol 2021;22:22–38
7. Romani P, Brian I, Santinon G, Pocaterra A, Audano M, Pedretti S, Mathieu S, et al. Extracellular matrix mechanical cues regulate lipid metabolism through Lipin-1 and SREBP. Nat Cell Biol 2019;21:338–347
8. Dongiovanni P, Meroni M, Baselli GA, Bassani GA, Rametta R, Pietrelli A, Maggioni M, et al. Insulin resistance promotes Lysyl Oxidase Like 2 induction and fibrosis accumulation in non-alcoholic fatty liver disease. Clin Sci (Lond) 2017;131:1301–1315
9. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contois MJ, Cummings OW, Ferrell LD, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321
10. Nezvorova YA, Boyer-Diaz Z, Cubero FJ, Gracia-Sancho J. Animal models for liver disease—a practical approach for translational research. J Hepatol 2020;73:423–440
11. Wang H, Xu PF, Li JY, Liu XJ, Wu XY, Xu F, Xie BC, et al. Adipose tissue transplantation ameliorates lipodystrophy-associated metabolic disorders in seipin-deficient mice. Am J Physiol Endocrinol Metab 2019;316:E54–E62
12. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Metabolic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73–84
13. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, Colombo M, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030. J Hepatol 2018;69:896–904
14. Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic steatohepatitis: a review. JAMA Surg 2020;323:1175–1183
15. Bellaye PS, Shimbori C, Upagupta C, Sato S, Shi W, Gauldie J, Ask K, et al. Lysyl oxidase-like 1 protein deficiency protects mice from adenoaviral transforming growth factor-ß1-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 2018;58:461–470
16. Martinez-Martinez E, Rodriguez C, Galan M, Miana M, Jurado-Lopez R, Bartolome MV, Luaces M, et al. The lysyl oxidase inhibitor (ß-aminopropionitrile) reduces leptin profibrotic effects and ameliorates cardiovascular remodeling in diet-induced obesity in rats. J Mol Cell Cardiol 2016;92:96–104
17. Murawaki Y, Kusakabe Y, Hirayama C. Serum lysyl oxidase activity in chronic liver disease in comparison with serum levels of prolyl hydroxylase and laminin. Hepatology 1991;14:1167–1173
18. Bertolio R, Napoletano F, Mano M, Maurer-Stroh S, Fantuz M, Zannini A, Bicciato S, et al. Sterol regulatory element binding protein 1 couples mechanical cues and lipid metabolism. Nat Commun 2019;10:1326

19. Huang W, Dedousis N, Bandi A, Lopaschuk GD, O’Doherty RM. Liver triglyceride secretion and lipid oxidative metabolism are rapidly altered by leptin in vivo. Endocrinology 2006;147:1480–1487

20. Hackl MT, Furnsinn C, Schuh CM, Krssak M, Carli F, Guerra S, Freudenthaler A, et al. Brain leptin reduces liver lipids by increasing hepatic triglyceride secretion and lowering lipogenesis. Nat Commun 2019;10:2717

21. Shi Y, Wang Q, Sun Y, Zhao X, Kong Y, Ou X, Jia J, et al. The prevalence of lean/nonobese nonalcoholic fatty liver disease: a systematic review and meta-analysis. J Clin Gastroenterol 2020;54:378–387

22. Dobie R, Wilson-Kanamori JR, Henderson BEP, Smith JR, Matchett KP, Portman JR, Wallenborg K, et al. Single-cell transcriptomics uncovers zonation of function in the mesenchyme during liver fibrosis. Cell Rep 2019;29:1832–1847 (e1838)

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.