Research Article

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Upregulation of miR-125b by estrogen protects against non-alcoholic fatty liver in female mice

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Background & Aims: Due to the protective effect of estrogen against hepatic fat accumulation, the prevalence of non-alcoholic fatty liver disease (NAFLD) in premenopausal women is lower than that in men at the same age and in postmenopausal women. Our study was to further elucidate an underlying mechanism by which estrogen prevents NAFLD from miRNA perspective in female mice.

Methods: miRNA expression was evaluated by TaqMan miRNA assay. Luciferase and ChIP assay were done to validate regulation of miR-125b by estrogen via estrogen receptor alpha (ERα). Nile red and Oil red O staining were used to check lipid content. Overexpressing or inhibiting the physiological role of miR-125b in the liver of mice through injecting adenovirus were used to identify the function of miR-125b *in vivo*.

Results: miR-125b expression was activated by estrogen via ERα *in vitro* and *in vivo*. miR-125b inhibited lipid accumulation both in HepG2 cells and primary mouse hepatocytes. Consistently, ovariectomized or liver-specific ERα knockdown mice treated with miR-125b overexpressing adenoviruses were resistant to hepatic steatosis induced by high-fat diet, due to decreased fatty acid uptake and synthesis and decreased triglyceride synthesis. Conversely, inhibiting the physiological role of miR-125b with a sponge decoy slightly promoted liver steatosis with a high-fat diet. Notably, we provided evidence showing that fatty acid synthase was a functional target of miR-125b.

Conclusion: Our findings identify a novel mechanism by which estrogen protects against hepatic steatosis in female mice via upregulating miR-125b expression.

Keywords: Estrogen; Premenopause; NAFLD; Lipogenesis; miR-125b; FAS.
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Abbreviations: NAFLD, non-alcoholic fatty liver disease; ChIP, chromatin immunoprecipitation; HFD, high-fat diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FFA, free fatty acid; FAS, fatty acid synthase; SCD1, stearoyl coenzyme A desaturase 1; SREBP-1C, sterol regulatory element binding protein 1C; ACC, acetyl coenzyme A carboxylase; PPAR, peroxisome proliferator-activated receptor; LXRα, liver X receptor alpha; DGAT, diacylglycerol acyl coenzyme A acyltransferase; CPT1, carnitine palmitoyltransferase 1; MCD, malonyl coenzyme A decarboxylase; LCAD, long-chain acyl coenzyme A dehydrogenase; UCP2, uncoupling protein 2; NC, negative control; WAT, white adipose tissue; TG, triglyceride; ERE, estrogen response element.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic manifestation of the metabolic syndrome, which is characterized by insulin resistance, dyslipidemia, hypertension and type 2 diabetes [1,2]. It occurs mainly due to fat accumulation in the liver, and can lead to cirrhosis, which is not reversible and may ultimately progress to hepatocellular carcinoma (HCC) [3,4]. Several population-based studies have demonstrated that the prevalence of NAFLD in premenopausal women is lower than that in men between the ages of 20 and 50 years, and also lower than in postmenopausal women [5,6]. Moreover, the protective effect of estrogen against fat accumulation has been extensively validated [7–9]. Specifically, aromatase-deficient mice that lack the ability to produce estrogen developed spontaneous hepatic steatosis [8]. In addition, tamoxifen, an anti-estrogen compound used in the treatment of estrogen receptor positive breast cancer, is able to induce lipid accumulation in liver [9]. The mechanisms by which estrogen exerts these effects were identified as inhibition of fatty acid synthesis and promotion of fatty acid oxidation in liver [10–11]. However, the detailed molecular mechanisms by which estrogen protects against hepatic steatosis remain unclear.

Recent studies have shown that many miRNAs are involved in regulating glucose and lipid metabolism in liver including miR-122, miR-33, miR-24, and miR-130a-3p [12–15]. These findings indicate that the regulation of miRNAs may be a promising therapeutic strategy to ameliorate metabolic disorders.

Most reports about miR-125b were related to the field of cancer research [16–18]. Notably, a study showed that miR-125b could alter glucose and lipid metabolic homeostasis via targeting of multiple metabolic enzymes to repress cancer transformation in chronic lymphocytic leukemia [19]. However, whether miR-125b is also involved in glucose and lipid metabolism in liver remains unclear.

Interestingly, some studies showed that miR-125b can be highly expressed in ovarian tissue [20,21]. Furthermore, the relationship between estrogen and miR-125b has been reported in macrophages [22]. However, the molecular mechanisms by which estrogen regulating miR-125b expression have not been identified. Here we demonstrate that estrogen directly regulates miR-125b expression through estrogen receptor alpha (ERα) and identify a novel function of miR-125b on limiting fat accumulation in the liver of female mice.

Materials and methods

Animals and treatment

Female C57BL/6J mice were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd. (SLAC, Shanghai, China). Six-week-old female mice were maintained under 12-hour light/12-hour dark cycles and provided free access to food and water. Female mice were either bilaterally ovariectomized (OVX) or sham-operated at 6 weeks. Mice that underwent bilateral OVX were injected with adenovirus expressing miR-125b/LacZ via tail vein every 10 days for two months at 8 weeks of age. All mice were maintained on a high-fat diet (HFD) (D12492i, 60% fat, 20% protein, 20% carbohydrate, Research Diets).

Western blotting and antibodies

Cells were scraped into lysis buffer containing 2% SDS and 50 mM Tris–HCl (pH 6.8). Lysates were then quantitated and equal amounts of protein were subjected to gel for electrophoresis and immunoblotted with antibodies against ERα, HSP90 and fatty acid synthase (FAS). Antibodies against HSP90 (sc-7947), FAS (sc-55580) and ERα (sc-542) were from Santa Cruz Biotechnology.

Nile red staining

The lipid content in both primary hepatocytes and HepG2 cells was determined using Nile red. After 48 hour of free fatty acid (FFA) treatment, cell monolayers were washed twice with PBS and then fixed for 10 min with 3.7% formaldehyde, and incubated for 3 minute with Nile red and DAPI solution. Monolayers were washed thereafter with PBS and the fluorescence was determined using a fluorescence microscope. Quantification of the fluorescence was done using the Image-Pro plus software.

Histological analysis of tissues

Frozen sections of liver were stained with Oil red O. Paraformaldehyde-fixed, paraffin-embedded sections of liver were stained with hematoxylin and eosin for histology.

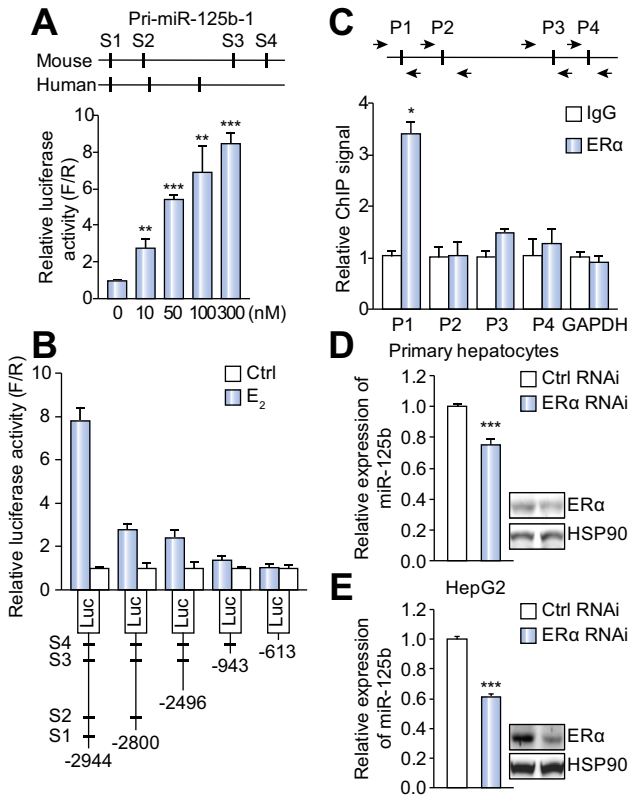

Fig. 2. Estrogen directly regulates miR-125b expression through ERα. (A) Upper panel: The ERα binding sites on the human and mouse (S1, S2, S3, and S4) promoter of miR-125b-1. Lower panel: HepG2 cells transfected with reporter constructs containing mouse miR-125b-1 promoter region were treated with different concentration of E2. After 48 h, luciferase activity was analyzed and plotted. (B) HepG2 cells transfected with reporter constructs that respectively contain truncations of the miR-125b-1 promoter were treated with E2 (100 nM). After 48 h, luciferase activity was analyzed and plotted. (C) Upper panel: The primers from P1 to P4 correspondently include ERα binding sites from S1 to S4. Lower panel: ChIP-qPCR analysis ERα occupancy on the sites using upper panel primers. (D and E) The expression of miR-125b in primary hepatocytes and HepG2 cells treated by E2 when ERα was knocked down. Knockdown of ERα was confirmed by Western blotting. Data are expressed as the mean ± SEM. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001.

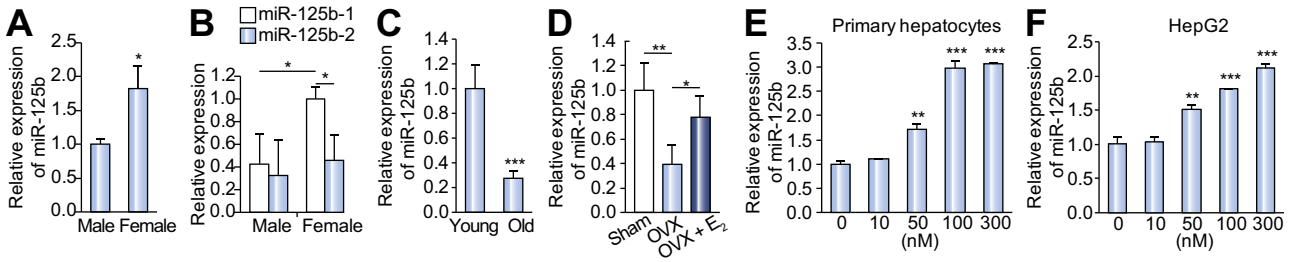

Fig. 1. Estrogen levels positively correlates miR-125b expression. (A and B) The expression of miR-125b, pri-miR-125b-1, and pri-miR-125b-2 in the livers of male and female mice at 8 week (n = 6–8 per group). (C) The expression of miR-125b in the liver of young (2 month) and old (22 month) female mice (n = 6–8 per group). (D) The expression of miR-125b in the liver of sham, OVX and OVX+E2 mice (n = 3–8 per group). (E and F) The expression of miR-125b in HepG2 cells and primary hepatocytes treated with different concentrations of E2. Data are expressed as the mean ± SEM. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001.