Liver-specific microRNA-185 knockout promotes cholesterol dysregulation in mice

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Abstract

Background: The liver plays a key role in regulating whole body cholesterol homeostasis. Hepatic cholesterol accumulation causes liver injury in fatty liver disease and hypercholesterolemia increases the risk of cardiovascular disease. MicroRNAs (miRNAs, miRs) have been shown to regulate various pathways in cholesterol metabolism. Recently, miR-185 has been shown to regulate sterol regulatory element-binding protein 2 (SREBP2) and low-density lipoprotein receptor (LDLR) to modulate cholesterol synthesis and uptake.

Materials and methods: The role of miR-185 in regulating diet-induced metabolic disorders were studied in liver-specific miRNA-185 knockout (L-miR-185 KO) mice.

Results: L-miR-185 KO mice developed worsened hepatic steatosis upon high fat high cholesterol Western diet feeding with accumulation of triglyceride and cholesterol in the liver. In addition, L-miR-185 KO mice developed hypercholesterolemia upon Western diet feeding. Gene expression analysis showed that L-miR-185 KO mice did not show increased hepatic mRNA expression of SREBP2 or its targets LDLR and HMG-CoA reductase (HMGCR). Although expression of miR-185 mimic inhibited the mRNA of SREBP2, HMGCR and LDLR in HepG2 cells, miR-185 inhibitor did not increase the mRNA of SREBP2, HMGCR or LDLR in HepG2 cells.

Conclusions: In conclusion, we reported that L-miR-185 KO mice were more sensitive to Western diet induced hepatic steatosis and hypercholesterolemia. The molecular mechanisms underlying these metabolic changes remain to be investigated in future studies.

Keywords

MicroRNA (miRNA); MicroRNA-185 (miR-185); Cholesterol; Fatty liver

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1. Introduction

Dysregulation of cholesterol homeostasis is closely associated with type-2 diabetes and non-alcoholic steatohepatitis (NASH).[1–5] Increased total and low-density lipoprotein (LDL) cholesterol is strongly associated with the risk of atherosclerosis and higher risk of cardiovascular disease, which is the leading cause of death patients with type-2 diabetes and NASH.[2] In addition, intra-hepatic accumulation of cholesterol has been reported in patients with NASH.[1,4] In experimental models, hepatic cholesterol accumulation causes lysosomal dysfunction, endoplasmic reticulum (ER) stress and mitochondrial dysfunction, which sensitize the hepatocytes to cytokine and stress signaling induced injury and cell death.[3,5] Improving overall cholesterol homeostasis is one of the key treatment goals in patients with type-2 diabetes and NASH.

The liver plays a key role in regulating whole body cholesterol homeostasis.[6] Hepatocytes acquire cholesterol through *de novo* synthesis and receptor-mediated lipoprotein uptake from the circulation, which is a critical process that affects blood cholesterol levels. The liver is quantitatively the major organ for whole body cholesterol elimination through direct biliary cholesterol secretion and conversion of cholesterol into bile acids. Sterol regulatory element-binding protein 2 (SREBP2) is a sterol sensing transcriptional factor that plays central roles in regulating cellular cholesterol homeostasis.[7,8] SREBP2 is synthesized as a protein precursor that anchors in the ER membrane by forming a complex with SREBP cleavage activating protein (SCAP) and Insulin-induced gene (Insig). When cellular cholesterol decreases, SREBP2 is translocated to Golgi where SREBP2 undergoes proteolytic cleavage to release a mature transcriptional factor that is subsequently localized to the nucleus. SREBP2 transcriptionally induces a network of genes involved in cholesterol metabolism, including numerous genes in the *de novo* cholesterol synthesis pathway and the low-density lipoprotein receptor (LDLR), leading to increased cellular cholesterol uptake and synthesis.[8] When cellular cholesterol level increases, SREBP2 proteolytic activation is inhibited and so is *de novo* cholesterol synthesis. Cholesterol accumulation in hepatocytes further activates liver X receptor (LXR) to transcriptionally induce ATP binding cassette transporter G5 (ABCG5) and G8 (ABCG8) to promote biliary cholesterol secretion and induce cytochrome P450 7A1 (CYP7A1), which encodes the rate-limiting enzyme cholesterol 7α-hydroxylase in bile acid synthesis.[9–11]

MicroRNAs (miRNAs) are short ~22 nucleotide single-stranded noncoding RNAs that regulate gene expression by binding to target messenger RNA (mRNA) 3' untranslated regions through which miRNAs promote mRNA degradation or inhibit mRNA translation. Studies have identified important roles of miRNAs in the regulation of cholesterol metabolism.[12] Previous studies found that miR-33 was located in the intron region of SREBP2 and was co-transcribed with SREBP2 gene when cholesterol levels decrease.[13,14] MiR-33 inhibits ABCA1 and ABCG1 that mediate cholesterol efflux to high-density lipoprotein (HDL) and genes in fatty acid oxidation and bile acid synthesis.[15,16] Recently, miR-33 has been shown to inhibit SREBP1c, a key lipogenic activator.[17] Inhibition of miR-33 has been shown to promote reverse cholesterol transport and attenuate atherosclerosis, while miR-33 knockout mice exhibited obesity and metabolic syndrome.[17–20] However, other studies reported lack of beneficial effects of

miR-33 inhibition on dyslipidemia and atherosclerosis progression and even worsened hyperlipidemia and hepatic steatosis.[21,22] More recently, miR-185 has been reported to inhibit SREBP2 and over-expression of miR-185 reduced SREBP2 and its target gene LDLR in liver cells.[23] Another study reported that LDLR was a direct target of miR-185.[24] Administration of miR-185 inhibitor to Apolipoprotein E (ApoE) knockout mice increased hepatic LDLR expression, lowered plasma cholesterol and attenuated atherosclerosis.[24] However, another study reported that silencing miR-185 promoted atherosclerosis progression in atherogenic diet-fed mice.[25] Furthermore, treating mice with miR-185 improved hepatic steatosis and insulin sensitivity in high-fat diet-fed mice.[26] This discrepancy needs to be further investigated. Further, these *in vivo* studies employed pharmacological approaches that did not target miR-185 in a tissue-specific manner. The liver plays a key role in regulating overall cholesterol homeostasis. In this study, the role of hepatic miR-185 in regulating hepatic and plasma lipid metabolism was further investigated in liver-specific miR-185 knockout (L-miR-185 KO) mice.

2. Materials and methods

2.1. Ethical approval

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Kansas Medical Center.

2.2. Mice and diet

MiR-185 flox mice (STOCK miR-185 ^tm1Mtm/Mmjax, Stock No. 34665) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). A targeting vector was used to delete the miR-185 stem loop in the presence of Cre recombinase. The construct was electroporated into 129P2/OlaHsd-derived E14 embryonic stem cells. Founders were crossed with C57BL/6J mice for 2 generations when we obtained them. Upon receiving these mice, we crossed these mice with C57BL/6J mice for an additional 6 generations, and then crossed them with Alb-Cre mice (Stock No. 003574, C57BL/6J background, The Jackson Laboratory). Littermates without the Cre allele were used as wild-type (WT) controls. Because females are resistant to diet-induced obesity, we only used male mice in this study. The chow diet was PicoLab Rodent Diet 20 (LabDiet, St. Louis, MO, USA) containing 13% fat calories and no added cholesterol. Western diet (WD) contains 42% fat calories and 0.2% cholesterol (TD.88,137, Envigo, Denver, CO, USA). All mice were fasted overnight from 5:00 p.m. to 9:00 a.m. and euthanized.

2.3. Measurement of cholesterol and triglycerides

About 100 mg of liver tissue was homogenized in 1 ml of PBS, and then extracted in 5 ml of chloroform:methanol (2:1; v:v) overnight. The lipid extract was then dried under nitrogen and resuspended in isopropanol containing 1% Triton X-100. Total cholesterol (TC), free cholesterol (FC) and triglyceride (TG) were measured with assay kits following the manufacturer's instructions. Cholesterol ester was calculated by subtracting FC value from TC value of the same sample. Cholesterol assay kit and TG assay kit were purchased from Pointe Scientific (Canton, MI, USA). FC assay kit was purchased from Wako Diagnostics (Richmond, VA, USA).

2.4. Measurement of bile acids

Bile acids in ~100 mg liver tissue and whole small intestine with the luminal content were extracted in 90% ethanol overnight. Whole gallbladder bile was diluted in 1 ml 90% isopropanol. Total bile acid was measured with a bile acid assay kit following the manufacturer's instruction. The bile acid pool is calculated as the sum of the amount of total bile acids in whole liver, gallbladder and small intestine. The hepatic bile acid concentration was expressed in μmol bile acids/g liver weight. Bile acid assay kit was purchased from Diazyme Laboratories (Poway, CA, USA).

2.5. Real-time polymerase chain reaction (PCR)

Total liver RNA was purified by Trizol (Sigma-Aldrich, St. Louis, MO, USA). Reverse transcription reaction was performed with Oligo dT primer and SuperScript III reverse transcriptase (Thermo Fisher Scientific, Grand Island, NY, USA). Real-time PCR was performed with iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). Amplification of 18S was used as internal reference for normalization. Relative mRNA expression was calculated by using the comparative CT (Ct) method and expressed as $2^{-\triangle Ct}$.

2.6. MiRNA mimic and inhibitor transfection

The miR-185–5p mimic, miRNA mimic negative control, miR-185–5p inhibitor, and miRNA inhibitor negative control were purchased from Dharmacon Inc. (Lafayette, CO, USA) and transfected into HepG2 cells using Lipofectamine 2000 (Thermo Scientific, Waltham, MA, USA). Forty-eight hours after transfection, cells were extracted and analyzed for relative mRNA expression by real-time PCR.

2.7. Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). Either Two-way ANOVA and Tukey post hoc test or two-sided Student's t-test was used to calculate the P-value. A P-value < 0.05 was considered statistically significant.

3. Results

3.1. L-miR-185 KO mice were more susceptible to WD-induced liver steatosis and weight gain

Liver specific miR-185 knockout was first confirmed by real-time PCR measurement which showed ~85% reduction of miR-185 in the livers of L-miR-185 KO mice (Fig. 1). MiR-185 was still detected in L-miR-185 KO mouse livers possibly due to miR-185 expression in none-parenchymal cells including macrophages.[27] To investigate the metabolic impact of liver miR-185 deficiency on lipid metabolism, we fed 6 weeks old male WT and L-miR-185 KO mice chow and WD for 12 weeks. Body weight measurement revealed that under chow-fed condition L-miR-185 KO mice and WT controls showed similar weight gain, while L-miR-185 KO mice showed modest but significantly higher weight gain than WT mice on WD (Fig. 2A). H&E staining showed that livers of L-miR-185 KO mice on chow diet appeared pale compared to that of WT mice fed chow, while after WD feeding livers

of L-miR-185 KO mice showed a higher degree of steatosis than WT mice (Fig. 2B). Biochemical measurement of hepatic lipid content showed that L-miR-185 KO mice fed a chow diet showed ~40% more TGs content although this was not apparent on H&E images (Fig. 2C). After WD feeding, L-miR-185 KO mice showed ~2-fold higher TG content than WD-fed mice (Fig. 2C), which confirmed the histological findings (Fig. 2B). Plasma TG concentration was modestly elevated in WD-fed WT mice than chow-fed WT mice, but L-miR-185 KO mice did not show significantly different plasma TG concentration than WT mice on either chow diet or WD (Fig. 2D). Plasma alanine transaminase (ALT) levels were increased in L-miR-185 KO mice on either chow or WD (Fig. 2E). These results were consistent with our observations that L-miR-185 KO mice were more prone to developing hepatic steatosis and injury. However, the ALT elevation in L-miR-185 KO mice was relatively mild and did not suggest the presence of significant liver injury.

3.2. L-miR-185 KO mice showed dysregulation of cholesterol homeostasis

Because miR-185 has been reported to regulate hepatic cholesterol synthesis and uptake, we next investigated how liver miR-185 deficiency affected cholesterol homeostasis. We found that hepatic TC content was significantly higher in L-miR-185 KO mice than WT mice on either chow diet or WD (Fig. 3A). Notably, TC elevation was largely accounted for by significantly elevated hepatic FC content (Fig. 3B) but to less extent by elevation of cholesterol ester (Fig. 3C). Plasma TC concentration was similar between WT and L-miR-185 KO mice on chow diet. WD feeding significantly increased plasma TC concentration by ~2-fold in WT mice, and WD-fed L-miR-185 KO mice showed ~20% higher plasma TC than WD-fed WT mice (Fig. 3D). WD feeding significantly increased gallbladder TC content, but L-miR-185 KO mice did not show elevated gallbladder cholesterol content compared to WT controls on either chow or WD (Fig. 3E), suggesting that liver miR-185 deficiency did not affect biliary cholesterol secretion.

3.3. L-miR-185 KO mice showed elevated bile acid pool size and hepatic bile acid concentration on WD

Bile acid synthesis is a major cholesterol elimination pathway. To determine if miR-185 KO mice show altered bile acid metabolism, we measured tissue bile acid content and hepatic bile acid concentration. We found that under chow condition, L-miR-185 KO mice and WT controls showed similar bile acid pool (Fig. 4A). WD feeding tended to decrease total bile acid pool in WT mice, which was consistent with our previous finding.[28] WD-fed L-miR-185 KO mice showed significantly larger bile acid pool than WD-fed WT mice (Fig. 4A). Further analysis revealed that L-miR-185 KO mice showed ~50% increase of hepatic bile acid concentration under both chow-fed and WD-fed condition although the increase under WD condition did not reach statistical significance (Fig. 4B). Consistent with elevated hepatic bile acid concentration, liver small heterodimer partner (SHP) mRNA was induced and liver CYP7A1 was repressed in chow-fed L-miR-185 KO mice compared to chow-fed WT mice (Fig. 4C). WD feeding also induced hepatic SHP mRNA and reduced hepatic CYP7A1 mRNA compared to chow-fed WT mice. However, under WD-fed condition, miR-185 deletion did not further increase liver SHP despite that liver bile acid concentration trended higher in WD-fed L-miR-185 KO mice than WD-fed WT mice (Fig. 4C). It is possible that WD feeding significantly increased baseline SHP mRNA expression,

which masked the effect of elevated liver bile acid concentration on hepatic SHP mRNA expression.

3.4. L-miR-185 KO mice did not show elevated hepatic SREBP or LDLR mRNA expression

To understand the mechanisms underlying the elevated hepatic lipid accumulation, we next measured hepatic gene expression critically involved in fatty acid and cholesterol metabolism. Under chow-fed condition, L-miR-185 KO mice did not show altered mRNA of SREBP1c or its target stearoyl-CoA desaturase (SCD1) (Fig. 5A). WD feeding significantly increased hepatic SREBP1c and its target SCD1, which is consistent with the key lipogenic effect of SREBP1c in hepatic steatosis.[29] L-miR-185 KO mice showed further increased SCD1 expression but not SREBP1c expression upon WD feeding, which was likely a result of higher degree of hepatic steatosis in these mice. Hepatic SREBP2 and its target genes in cholesterol metabolism including HMG-CoA reductase (HMGCR), LDLR and phosphomevalonate kinase (PMVK) were not altered in L-miR-185 KO mice compared to WT controls (Fig. 5B). WD feeding decreased the mRNA expression of SREBP2, HMGCR, LDLR and PMVK, which is consistent with increased hepatic cholesterol that is known to inhibit the SREBP2 transcriptional network.[30] L-miR-185 KO mice fed a WD showed further reduced mRNA of SREBP2, HMGCR and PMVK, but not LDLR (Fig. 5B), which is consistent with higher hepatic cholesterol content in these mice than WD-fed WT mice.

3.5. Transfection of miR-185 mimic inhibits SREBP2 and LDLR in vitro

Given that the previously reported miR-185 targets SREBP2 and LDLR were not induced in L-miR-185 KO mice, we further tested the effect of miR-185 in cultured HepG2 cells. Transfection of miR-185 mimic inhibited SREBP2 and its target HMGCR and LDLR (Fig. 6A). However, transfection of miR-185 inhibitor did not result in induction of SREBP2, HMGCR, or LDLR (Fig. 6B). Scavenger receptor-B1 (SR-B1), which was reported to be a miR-185 target, was not affected by miR-185 mimic or miR-185 inhibitor (Fig. 6A and B).

4. Discussion

The liver plays a major role in regulating cholesterol metabolism via coordinate regulation of the SREBP2 transcriptional network that controls *de novo* cholesterol synthesis, uptake and efflux.[6] Previous studies have reported that miR-185 regulates genes involved in several lipid metabolism pathways including cholesterol synthesis and uptake.[23,24] However, the beneficial effects of miR-185 inhibition remains controversial.[24,25] Furthermore, whether these effects were primarily mediated by hepatic miR-185 modulation is still not clear. In this study, we generated liver specific miR-185 knockout mice and characterized the metabolic effects of liver miR-185 deficiency under chow condition and in response to chronic WD challenge. The major finding from this study is that liver miR-185 deletion sensitized mice to diet induced weight gain, hepatic steatosis and hypercholesterolemia. Obesity is a major contributing factor to hepatic steatosis. White adipose-derived fatty acids accounts for ~70–80% of total liver fat accumulation under obesity condition.[31] Therefore, increased diet-induced weight gain was at least partially contributed to higher hepatic steatosis in WD-fed L-miR-185 KO mice. On the other hand, hepatic fat accumulation was significantly elevated in chow-fed L-miR-185 KO mice that did not show higher weight gain

than chow-fed WT controls. These results suggest that hepatic initiated mechanisms also contributed to increased hepatic fat accumulation in L-miR-185 KO mice. Our findings in L-miR-185 KO mice were consistent with a recent report that treating high-fat diet-fed mice with miR-185 attenuated hepatic steatosis and improved insulin sensitivity.[26] On the other hand, our finding was not consistent with previously reported beneficial effects of miR-185 inhibition,[24] suggesting that the beneficial effects of pharmacological miR-185 inhibition may not be mediated by liver miR-185 inhibition.

Upon examination of hepatic gene expression, we found that liver miR-185 deletion did not result in elevated SREBP2 and its target HMGCR, LDLR or PMVK. This was in contrast to previous reports that the mRNA of SREBP2 and LDLR were direct targets of miR-185.[23,24] The decreased mRNA of SREBP2, HMGCR, LDLR and PMVK upon WD feeding was likely a result of higher hepatic content of cholesterol that is known to inhibit the SREBP2 transcriptional network.[7] Similarly, lower mRNA of these genes in WD-fed L-miR-185 KO mice was likely due to higher hepatic cholesterol accumulation in these mice but not direct regulation by miR-185. Current studies identified LDLR and SR-B1 involved in plasma LDL and HDL cholesterol uptake as direct miR-185 target.[24,32] Therefore, deletion of liver miR-185 is expected to increase LDLR and SR-B1 to reduce plasma cholesterol. In contrast, L-miR-185 KO mice showed elevated plasma cholesterol than controls upon WD feeding, which was consistent with higher intrahepatic cholesterol accumulation in these mice. In cultured HepG2 cells, we showed that miR-185 mimetic indeed decreased SREBP2 and LDLR mRNA although the degree of inhibition was relatively modest. These results confirmed previous findings that miR-185 negatively inhibits these cholesterol metabolism gene mRNA.[23,24,32] On the other hand, the lack of induction of the mRNA of these miR-185 targets was consistent with our *in vivo* observations. One possible explanation is that although increasing cellular miR-185 concentration was sufficient to decrease its target genes, in HepG2 cells and in mouse livers the basal miR-185 may not play a significant role in repressing these targets. Therefore, inhibition of miR-185 did not cause a significant increase of these targets in HepG2 cells or in mouse livers. It should be noted that previous studied used human cell lines to demonstrate the miR-185 regulation of SREBP2. Species-dependent effect could exist to explain the observed discrepancy and metabolic outcome from these studies. In addition, we used a mouse model in which liver miR-185 was deleted during the embryonic stage. Since the SREBP2 transcriptional network is tightly regulated at transcriptional and posttranslational levels, it is also possible that other unknown compensatory mechanisms may act to maintain the expression of these genes independent of miR-185.

In conclusion, we report the initial characterization of the novel L-miR-185 KO mice under chow fed and WD challenged conditions, which aimed to define the roles of hepatic miR-185 in regulating lipid metabolism. The major findings from this study are that loss of hepatic miR-185 sensitized mice to diet-induced weight gain and developing hepatic steatosis and hypercholesterolemia. Despite previous reports of miR-185 inhibition of hepatic SREBP2 and LDLR,[23,24] our study did not reveal increased SREBP2 or LDLR to account for the altered lipid metabolism in L-miR-185 KO mice. Bile acid alteration could be a result but not a cause of altered cholesterol metabolism in L-miR-185 KO mice. Therefore, future studies employing unbiased liver transcriptomics analysis may

help identify novel candidate genes that are altered in L-miR-185 KO mice *in vivo*. Such information may guide further investigation aiming to elucidate the mechanisms by which liver miR-185 modulates lipid metabolism and metabolic disease pathogenesis.

Acknowledgements

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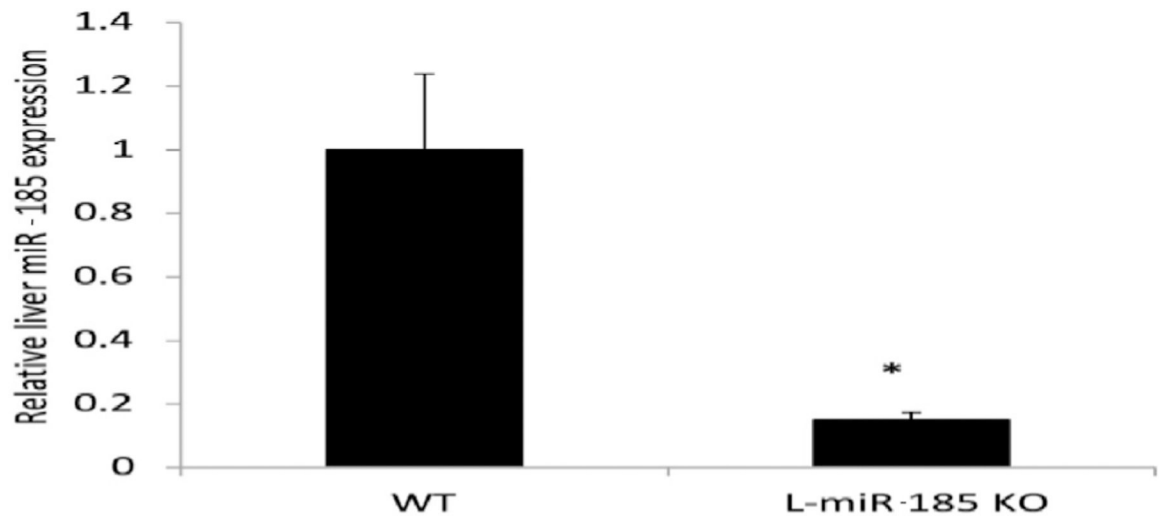
Fig. 1. Generation of L-miR-185 KO mice.

Liver miR-185 expression in WT and L-miR-185 KO mice on chow diet. Results are expressed as mean ± SEM. $N = 3$. *$P < 0.05$ (*vs.* WT). Abbreviations: L-miR-185 KO, liver-specific miR-185 knockout; WT, wild-type.
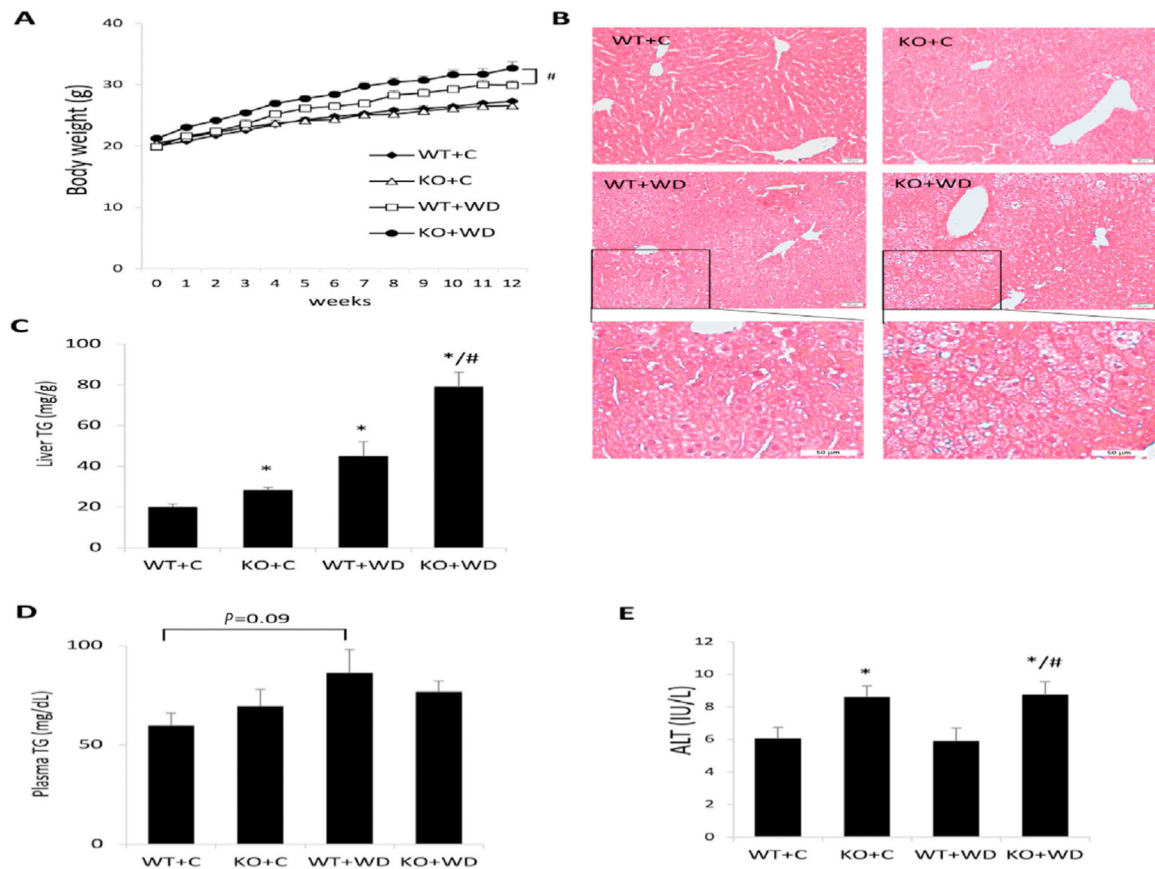
Fig. 2. L-miR-185 KO mice show higher weight gain and hepatic steatosis.
Male 6 weeks old WT and L-miR-185 KO mice were fed a chow or WD for 12 weeks. Mice were fasted for 16 h before tissue collection. **(A)** Body weight. **(B)** Liver H&E staining. Scale bar: 50 μm. **(C)** Liver total TG content. **(D)** Plasma TG concentration. **(E)** Plasma ALT. Results are expressed as mean ± SEM. N = 8–10. *P < 0.05 (*vs.* WT + C); #P < 0.05 (*vs.* WT + WD). Abbreviations: ALT, alanine transaminase; C, chow; KO, liver-specific miR-185 knockout; TG, triglyceride; WD, Western diet; WT, wild-type.
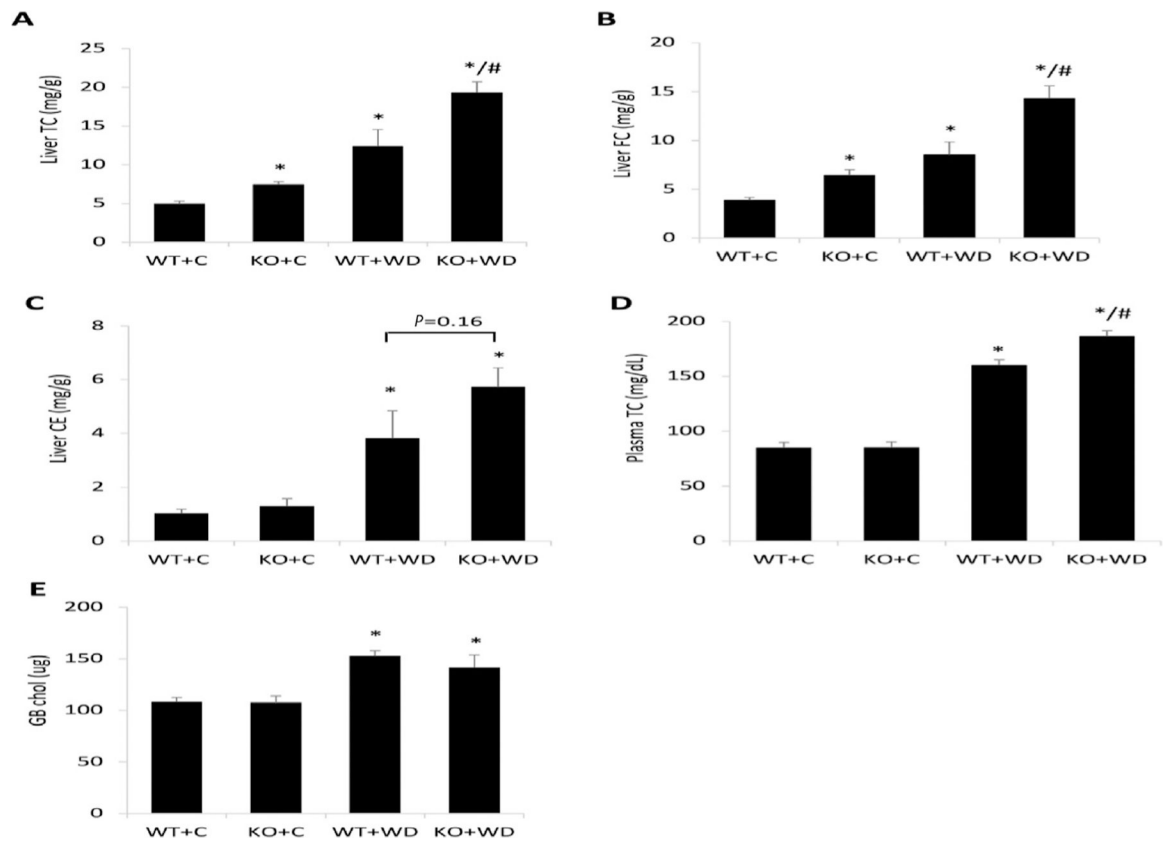
Fig. 3. L-miR-185 KO mice show cholesterol deregulation.
Male 6 weeks old WT and L-miR-185 KO mice were fed a chow or WD for 12 weeks.
Mice were fasted for 16 h before tissue collection. **(A)** Liver TC. **(B)** Liver FC. **(C)**
Liver cholesterol ester (CE). **(D)** Plasma TC. **(E)** Gallbladder (GB) cholesterol. Results are
expressed as mean ± SEM. $N = 8$–10. *$P < 0.05$ (*vs.* WT + C); #$P < 0.05$ (*vs.* WT + WD).
Abbreviations: C, chow; CE, cholesterol ester; FC, free cholesterol; GB, gallbladder; KO,
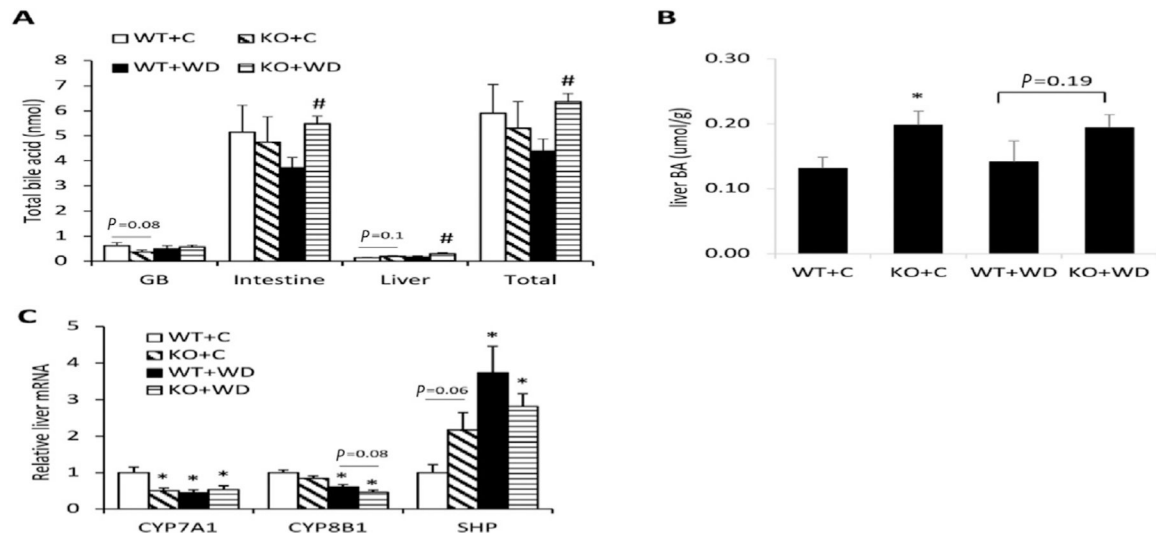liver-specific miR-185 knockout; TC, total cholesterol; WD, Western diet; WT, wild-type.

Fig. 4. Bile acid metabolism in L-miR-185 KO mice.
Male 6 weeks old WT and L-miR-185 KO mice were fed a chow or WD for 12 weeks. Mice were fasted for 16 h before tissue collection. **(A)** Tissue bile acids and total bile acid pool. **(B)** Liver bile acid concentration. **(C)** Liver mRNA expression. Results are expressed as mean ± SEM. N = 8–10. *P < 0.05 (*vs.* WT + C); [#]P < 0.05 (*vs.* WT + WD). Abbreviations: BA, bile acid; C, chow; CYP7A1, cytochrome P450 7A1; GB, gallbladder; KO, liver-specific miR-185 knockout; SHP, small heterodimer partner; WD, Western diet; WT, wild-type.
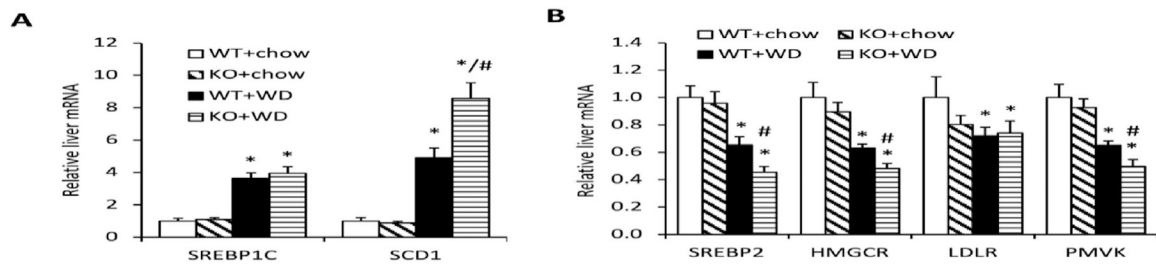
Fig. 5. Liver mRNA expression in L-miR-185 KO mice.

Male 6 weeks old WT and L-miR-185 KO mice were fed a chow or WD for 12 weeks. Mice were fasted for 16 h before tissue collection. **(A)** The mRNA expression of SREBP1c and SCD1. **(B)** The mRNA expression of SREBP2, HMGCR, LDLR and PMVK. Results are expressed as mean ± SEM. N = 8–10. *P < 0.05 (*vs.* WT + chow); #P < 0.05 (*vs.* WT + WD). Abbreviations: HMGCR, HMG-CoA reductase; KO, liver-specific miR-185 knockout; LDLR, low-density lipoprotein receptor; PMVK, phosphomevalonate kinase; SCD1, stearoyl-CoA desaturase; SREBP2, sterol regulatory element-binding protein 2; WD, Western diet; WT, wild-type.
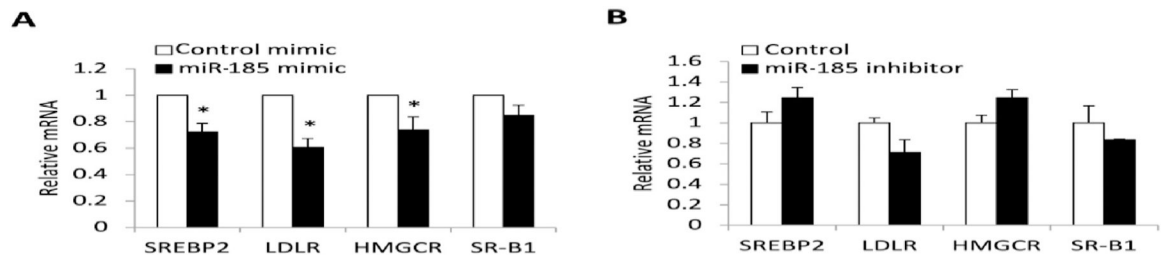
Fig. 6. MiR-185 mimic and inhibitor effects on mRNA expression in HepG2 cells.
HepG2 cells were transfected with 100 nM control or miR-185 mimic or miR-185 inhibitor for 48 h. mRNA expression was measured. **(A)** Transfection of miR-185 mimic inhibited SREBP2 and LDLR. **(B)** Transfection of miR-185 inhibitor did not show induction of SREBP2, HMGCR and LDLR. Results are expressed as mean ± SEM of triplicates. *P < 0.05 (*vs.* control). Abbreviations: HMGCR, HMG-CoA reductase; LDLR, low-density lipoprotein receptor; SR-B1, scavenger receptor-B1; SREBP2, sterol regulatory element-binding protein 2.