Abstract  High plasma cholesterol levels are found in several metabolic disorders and their reductions are advocated to reduce the risk of atherosclerosis. A way to lower plasma lipids is to curtail lipoprotein production; however, this is associated with steatosis. We previously showed that microRNA (miR)-30c lowers diet-induced hypercholesterolemia and atherosclerosis in C57BL/6J and Apoe-/- mice. Here, we tested the effect of miR-30c on plasma lipids, transaminases, and hepatic lipids in different mouse models. Hepatic delivery of miR-30c to chow-fed lepin-deficient (ob/ob) and lepin receptor-deficient (db/db) hypercholesterolemic and hyperglycemic mice reduced cholesterol in total plasma and VLDL/LDL by ~28% and ~25%, respectively, without affecting triglyceride and glucose levels. And these mice had lower plasma transaminases and creatine kinase activities than controls. Moreover, miR-30c significantly lowered plasma cholesterol and atherosclerosis in Western diet-fed Ldlr-/- mice with no effect on plasma triglyceride, glucose, and transaminases. In these studies, hepatic lipids were similar in control and miR-30c-injected mice. Mechanistic studies showed that miR-30c reduced hepatic microsomal triglyceride transfer protein activity and lipid synthesis. Thus miR-30c reduced plasma cholesterol in several diet-induced and diabetic hypercholesterolemic mice. We speculate that miR-30c may be beneficial in lowering plasma cholesterol in different metabolic disorders independent of the origin of hypercholesterolemia. — Irani, S., J. Iqbal, W. J. Antoni, L. Ijaz, and M. M. Hussain. microRNA-30c reduces plasma cholesterol in homozygous familial hypercholesterolemic and type 2 diabetic mouse models. J. Lipid Res. 2018. 59: 144–154.

Supplementary key words micro-ribonucleic acid-30c • steatosis • diabetes • lipids

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; HoFH, homozygous familial hypercholesterolemia; IVF, Invivofectamine; LDLR, LDL receptor; miR, microRNA; MTP, microsomal triglyceride transfer protein; PCSK9, proprotein convertase subtilisin/kexin type 9; STZ, streptozotocin.

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microRNAs (miRs) are ~22 nucleotide single-stranded evolutionarily conserved noncoding RNAs. They regulate gene expression post transcriptionally by interacting with the 3'-untranslated region of the target mRNA resulting in mRNA degradation, translation inhibition, or both (18).

We have previously shown that hepatic overexpression of miR-30c reduces hepatic MTP activity, plasma cholesterol, atherosclerosis, and hepatic lipoprotein production in diet-induced male and female C56BL/6j and Apoe<sup>−/−</sup> mice (19, 20). Reductions in plasma cholesterol were not associated with hepatic steatosis or increases in plasma transaminases. Here, we extended these studies to other mouse models of hypercholesterolemia and metabolic disorders to determine generality of miR-30c action in lowering plasma cholesterol. These studies suggest that miR-30c can lower hypercholesterolemia in different mouse models of metabolic disorders.

**MATERIALS AND METHODS**

**Materials**

The [3H]triolein, [3H]acetate, and [3H]glycerol were purchased from PerkinElmer Life Sciences. Chemicals and solvents were obtained from Fisher Scientific.

**Animals and diet**

WT and Ldlr<sup>−/−</sup> mice on a C57BL/6j background were bred at The State University of New York Downstate Medical Center and at New York University Winthrop Hospital. Ob/ob and db/db mice from Envigo were on chow diet (TD 8604; Harlan Teklad). Ldlr<sup>−/−</sup> mice were challenged with Western diet containing 17, 48.5, 21.2, and 0.2% (by weight) protein, carbohydrates, fat, and cholesterol, respectively (TD 88137; Harlan Teklad). All animal protocols were approved by the Institutional Animal Use and Care Committee of The State University of New York Downstate Medical Center and New York University Winthrop Hospital.

**miR-30c mimic delivery to mice**

We custom synthesized 1 g of highly purified mirVana<sup>™</sup> miR-30c mimic (Life Technologies). For its in vivo delivery, 100 µl of 1.2 mg/ml miR-30c mimic solution were prepared by mixing 50 µl of miR-30c (2.4 mg/ml) with 50 µl of complexation buffer. In a separate 1.5 ml tube, 100 µl of Invivofectamine (IVF) 3.0 (Life Technologies) were added. Afterwards, miR-30c diluted in complexation buffer was immediately added to IVF 3.0 and vortexed for 3 s to ensure miR-30c-IVF 3.0 complex formation. Next, the complex was incubated for 30 min at 50°C. The tube was centrifuged briefly to collect the sample. Finally, the complex was diluted 6-fold by adding 1 ml DEPC-treated PBS (pH 7.4) to obtain a final concentration of 0.1 mg/ml. Mice (20 g) were injected weekly with 200 µl by retro-orbital injections to obtain a 1 mg/kg/week dose. Forty-eight hours after the last injection, mice were euthanized and tissues were harvested and stored at −80°C for further analysis.

**Induction of diabetes**

Diabetes was induced in male WT chow-fed C57BL/6j mice by intraperitoneal administration of a low dose of 7.5 mg/ml streptozotocin (STZ, S0130-500MG; Sigma) dissolved in freshly prepared 0.1 M sodium citrate buffer (pH 6.3) for five consecutive days. Mice (20 g) were injected with 107 µl to obtain a 40 mg/kg body weight dose. The mice were fasted for 4 h before STZ administration (21).

**Type 2 diabetic mice**

We used two genetic models (ob/ob and db/db mice) of type 2 diabetes that are defective in leptin and leptin receptors. These mice display age-dependent increases in their plasma cholesterol levels between 1 and 4 months, with levels becoming maximal at 3–4 months of age (22, 23). Therefore, we started experiments in these mice at an age of 2 months by injecting with either PBS as a control or miR-30c on a weekly basis for 2 months. Mice were fed a chow diet ad libitum throughout the studies. This allowed us to explore whether miR-30c could avert the progression of hypercholesterolemia in these chow-fed mouse models.

**Measurements of lipids and enzymes**

Mice were fasted overnight (15 h) before blood was collected using heparinized capillary tubes from the retro-orbital venous plexus. Blood was centrifuged at 6,000 g for 5 min and then at 15,890 g for 1 min and plasma was collected to measure cholesterol and triglyceride (Thermo Scientific), ALT and AST (Biotest Diagnostics), and creatine kinase (CK) (Fisher Scientific) activities using kits according to the manufacturers’ protocols. For hepatic lipid measurements, liver pieces (~50 mg) were homogenized in 1 mM TrisCl, 1 mM EGTA, and 1 mM MgCl<sub>2</sub> (pH 7.6) and a portion was subjected to lipid extraction.

**miR and mRNA quantifications by quantitative RT-PCR**

For miR quantification, cDNA was synthesized with the TaqMan MicroRNA Reverse Transcription kit (4366597; Applied Biosystems) and used for quantitative RT-PCR. Primers specific for miR-30c and U6 were purchased from Life Technologies. miR analysis was performed using the C<sub>t</sub> method with normalization to U6 and is presented as arbitrary units.

For RNA quantification, first strand cDNA was synthesized with the Omniscript RT kit (Qiagen) and used for quantitative RT-PCR (qPCR Core kit for SYBR Green I; Eurogentec), and the C<sub>t</sub> values for each mRNA were normalized to 18S. Primers used for miRNA quantification were designed using PrimerExpress 3.0 (Applied Biosystems). These primers included: mApoB (tctattccacagaacccttc, gttataatgctcgctacctgtt), mApoAI (ggcctggtgctgtccttctcttt), mMTP (gacacctgctgtcataca, agccggtgagaccgggtct), mELOV5 (gcctcatctcgccatgtcattgtg), mLPGAT1 (tttttacggcaggaatcctccgctctcctc), mQK1 (ctttaggcagagacttgctttctc), and 18S (agtcctgctgctgcgca, gatccggctgctg).

**De novo lipogenesis, cholesterol and triglyceride synthesis**

For de novo lipogenesis, fresh liver slices were incubated with [3H]acetate (0.2 µCi) and lipids were extracted after saponification (24). Cholesterol and triglyceride synthases were studied by incubating liver slices with [14C]acetate and [3H]glycerol (0.5 µCi), respectively, extracting lipids, and separating them on a silica 60 thin-layer chromatography plate using a solvent mixture of diethyl ether, benzene, ethanol, and acetic acid at a ratio of 50:40:2:0.2. Counts were measured in a scintillation counter (Beckman LS 6000 TA).

**Aortic plaque analyses**

The aortic arches were dissected and exposed for photography. Neutral lipids in fatty streaks were visualized on the aorta with Oil Red O staining and quantified with ImageJ (25, 26).
Measurement of hepatic triglyceride production

Chow-fed C57BL/6J mice were injected weekly with PBS or miR-30c/IVF complexes. Two days after the fifth injection, mice were fasted overnight and intraperitoneally injected with 500 μl of 90 mg/ml Poloxamer 407 stock in PBS. Blood was collected before and after the injections at hourly intervals to measure triglycerides.

Statistics

Data are presented as the mean ± SD, P < 0.05. The statistical significance was determined by Student’s t-test and significant differences P < 0.05, P < 0.01, and P < 0.001 are symbolized as *, **, and ***, respectively.

RESULTS

miR-30c retards the progression of diet-induced hypercholesterolemia and atherosclerosis in Ldlr−/− mice

Previously, we have shown that miR-30c retards progression of diet-induced hypercholesterolemia and atherosclerosis in Apoer−/− mice (19, 20). Here, we hypothesized that miR-30c would impede the progression of hypercholesterolemia and atherosclerosis in Ldlr−/− mice that are deficient in the Ldlr gene and serve as a model to study homozygous familial hypercholesterolemia (HoFH) (27).

To test this hypothesis, we injected increasing doses of miR-30c for 15 weeks into 8-week-old male Ldlr−/− mice placed on a Western diet (Fig. 1). For control, mice were injected with PBS, as we had previously shown that PBS and scrambled control miR-injected mice behave similarly (19). Plasma cholesterol increased in both miR-30c- and PBS-injected groups. However, these increases were significantly lower (~27%) in the miR-30c-injected group compared with the PBS group (Fig. 1A). Doubling the miR-30c dose to 2 mg/kg/week on the tenth week further reduced plasma cholesterol levels by ~35% in the miR-30c group (Fig. 1A).

Fasting plasma triglyceride, ALT, AST, CK, and glucose (Fig. 1B–F) were not different between the two groups.

Analyses of hepatic tissue showed that miR-30c levels increased by 20-fold in mice receiving the miR (Fig. 1G). Furthermore, expression levels of miR-30c putative target genes (Mttp, Lpgat1, Qki, and Elovl5) were significantly reduced (~50%) in the miR-30c group compared with the PBS group; whereas no significant changes were observed in the nontarget genes (ApoB, ApoA1, and Gapdh) (Fig. 1H). Additionally, hepatic MTP activity was significantly lower (~60%) in the miR-30c group (Fig. 1I) with no effect on hepatic cholesterol and triglyceride levels (Fig. 1J, K). These studies suggest that the delivery of miR-30c to the liver curtailed progression of diet-induced hypercholesterolemia with no effect on hepatic lipids and plasma enzymes.

Further, we performed de novo lipogenesis and triglyceride and cholesterol biosyntheses on fresh livers at the end of the study to understand why miR-30c does not cause hepatic steatosis. miR-30c significantly reduced fatty acid, triglyceride, and cholesterol syntheses compared with the PBS group (Fig. 1L–N). These studies indicate that miR-30c suppresses hepatic lipid synthesis in Ldlr−/− mice.

To determine whether miR-30c could reduce the progression of atherosclerosis, we visualized plaques at the aortic arches and detected fewer lesions in the miR-30c group compared with the PBS group (Fig. 1O). Oil Red O staining of aortas revealed ~50% less lipid staining in the miR-30c group (Fig. 1P, Q). These studies revealed that miR-30c retards progression of atherosclerosis in Western diet-fed Ldlr−/− mice.

miR-30c dampens hypercholesterolemia in chow-fed ob/ob mice

Next, we asked whether miR-30c could reduce plasma cholesterol independent of the origin of hypercholesterolemia. For that, we used type 2 diabetic hypercholesterolemic leptin-deficient (ob/ob) mice. PBS and miR-30c were injected into male chow-fed ob/ob mice for 8 weeks (Fig. 2). We observed significant sustained reductions (~28%) in plasma cholesterol in the miR-30c group compared with the PBS group (Fig. 2A, left) and FPLC analysis of pooled plasma revealed reduced cholesterol levels in the VLDL/LDL fraction (Fig. 2A, right). Fasting triglyceride in total plasma (Fig. 2B, left) and in different lipoprotein fractions (Fig. 2B, right) as well as glucose levels (Fig. 2C) were not different between the two groups. Moreover, we did not see changes in food intake or body weight between the groups (data not shown). PBS-injected mice showed increases in their plasma ALT and AST levels and these increases were not seen in miR-30c-treated mice (Fig. 2D, E). Plasma CK levels decreased in both of the groups; however, decreases in the miR-30c group were significantly greater than in the PBS group (Fig. 2F). These studies indicate that hepatic delivery of miR-30c to chow-fed ob/ob mice reduces plasma cholesterol without affecting plasma triglyceride and glucose levels. And miR-30c prevents increases in plasma transaminases and lowers CK levels.

Hepatic tissue analysis revealed that miR-30c levels increased by 8-fold in the livers of mice injected with miR-30c (Fig. 2G). Expression levels of miR-30c putative target genes involved in lipoprotein assembly and lipid synthesis (Mttp, Lpgat1 and Elovl5) were significantly reduced in the miR-30c group compared with the PBS group; whereas no significant changes were observed for nontarget genes (ApoB, ApoA1, and Gapdh) (Fig. 2H). Additionally, MTP activity was significantly lower in the miR-30c group compared with the control group (Fig. 2I). There were no significant differences in hepatic cholesterol and triglyceride levels (Fig. 2J, K). However, miR-30c significantly reduced fatty acid, triglyceride, and cholesterol syntheses compared with PBS controls (Fig. 2L–N). Hence, these studies demonstrate that miR-30c hinders the progression of hypercholesterolemia in chow-fed type 2 diabetic hypercholesterolemic ob/ob mice, reduces plasma transaminases and hepatic lipid synthesis, and has no effect on hepatic lipid.

miR 30c curtails hypercholesterolemia in chow-fed db/db mice

Because miR-30c reduced plasma cholesterol in ob/ob mice, we asked whether it could reduce plasma cholesterol in another type 2 diabetic hypercholesterolemic mouse
miR-30c lowers plasma cholesterol

Fig. 1. miR-30c hinders the progression of diet-induced hypercholesterolemia and atherosclerosis in Ldlr−/− mice. Male Ldlr−/− mice (8 weeks old) were started on a Western diet and injected weekly with either PBS (n = 6) or increasing doses of miR-30c (n = 8) for 15 weeks, as indicated. Plasma from overnight-fasted plasma was collected on day 6 after each injection to measure cholesterol (A), triglyceride (B), ALT (C), AST (D), CK (E), and glucose (F). After 15 weeks, hepatic tissue was collected to measure miR-30c levels (G), different indicated mRNAs (H), MTP activity (I), cholesterol (J), and triglyceride (K). Fresh liver slices were also used to measure fatty acid (L), triglyceride (M), and cholesterol (N) syntheses. O: Aortic arches were exposed and photographed. Aortas were collected, fixed, and stained with Oil Red O (P), and quantified (Q). The data are a combination of two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 determined by Student’s t-test.
model. To test this, we used male lepin receptor-deficient (db/db) mice that were also fed a chow diet for 8 weeks (Fig. 3). miR-30c maximally reduced plasma cholesterol concentrations by ~25% (Fig. 3A, left), mainly by lowering cholesterol in the VLDL/LDL fraction (Fig. 3A, right). However, it did not significantly affect fasting triglyceride in total plasma (Fig. 3B, left) and in different lipoprotein fractions (Fig. 3B, right). Moreover, miR-30c had no effect on glucose levels (Fig. 3C). Plasma ALT and AST levels increased in the PBS group, but not in the miR-30c group (Fig. 3D, E). Plasma CK levels decreased in both groups and decreases in the miR-30c group were significantly lower than in the control group (Fig. 3F). Therefore, these findings reveal that miR-30c lowers plasma cholesterol, transaminases, and CK levels in another type 2 diabetic hypercholesterolemic db/db mouse model, without affecting plasma triglyceride and glucose levels.

Hepatic tissue analysis revealed that miR-30c levels increased by 6-fold in the livers of mice injected with the miR-30c (Fig. 3G). Transcript levels of Mttp increased by 6-fold in the livers of mice injected with the miR-30c group compared with the PBS group; whereas mRNA levels of ApoB, ApoA1, and Gapdh genes were not changed (Fig. 3H). Moreover, MTP activity was significantly reduced in the miR-30c group compared with the PBS group (Fig. 3I). miR-30c had no significant effect on hepatic cholesterol and triglyceride (Fig. 3J, K). We also performed de novo lipogenesis and triglyceride/cholesterol biosyntheses using fresh liver slices and observed significant reductions in fatty acid, triglyceride, and cholesterol syntheses (Fig. 3L–N). Thus, this study shows that miR-30c lowers plasma cholesterol levels in chow-fed type 2 diabetic hypercholesterolemic mice. In addition, it reduces plasma ALT, AST, and CK via unknown mechanisms.

miR-30c does not affect plasma cholesterol in STZ-induced diabetic mice

Next, we investigated to determine whether miR-30c could reduce plasma cholesterol in a mouse model of type 1 diabetes. Chow-fed male WT mice were divided into two groups. Diabetes was experimentally induced in both groups by intraperitoneal administration of 40 mg/kg STZ for five consecutive days. STZ is a chemical used for the destruction of insulin-producing cells resulting in type 1 diabetes. After STZ injections, mice were monitored for the development of hyperglycemia for 5 weeks. Fasting plasma glucose levels gradually increased in both groups, reaching a maximum of ~500 mg/dl at week 5 (Fig. 4A). Similarly, fasting plasma cholesterol and triglyceride levels increased in both groups, but these levels remained below 100 mg/dl and were close to normal levels seen in chow-fed mice (Fig. 4B, C). Next, we asked whether miR-30c could reduce plasma lipids in these mice. For this purpose, one group received PBS as a control and the other group was administered with 1 mg/kg/week of miR-30c complexed with lipid emulsions for 4 weeks. The plasma glucose levels in both groups remained elevated to similar levels over the next 4 weeks (Fig. 4A). Further, there were no significant differences in plasma cholesterol and triglyceride in these groups (Fig. 4B, C). After 4 weeks of injections, mice were euthanized and plasma was collected to measure ALT, AST, and CK levels. These enzymes were also not significantly different in the two groups (Fig. 4D–F).

Hepatic tissue analysis revealed significant increases in the miR-30c levels (7-fold) in the miR-30c-injected mice (Fig. 4G), indicating efficient delivery of miR-30c to the liver. Expression levels of miR-30c putative target genes (Mttp, Lpgat1, and Elovl5) were significantly lower in the miR-30c group compared with the PBS group; whereas no significant changes were observed for nontarget genes (ApoB, ApoA1, and Gapd) (Fig. 4H). Moreover, MTP activity was significantly lower in the miR-30c group compared with the control group (Fig. 4I). These studies indicate that hepatic delivery of miR-30c reduced MTP activity, but did not affect plasma cholesterol in STZ-induced type 1 diabetic mice.

miR-30c does not reduce plasma cholesterol in chow-fed WT mice

To test whether miR-30c affects plasma cholesterol under normal dietary conditions, we injected miR-30c or PBS in chow-fed WT mice (Fig. 5). At the end, there was an 8-fold increase in miR-30c levels in the livers of miR-30c-injected mice (Fig. 5A). Further, there were significant reductions in hepatic MTP activity (Fig. 5B). Despite these changes, there were no significant differences in fasting plasma cholesterol and triglyceride between the two groups (Fig. 5C, D). Consideration was given to the possibility that miR-30c did not reduce its target genes in these mice. However, expression analysis showed that target genes were reduced (Fig. 5E). In a different set of animals, weekly injections of miR-30c had no effect on plasma cholesterol and triglyceride (not shown). In these mice, we studied hepatic triglyceride production and observed no significant differences between the PBS and miR-30c groups (Fig. 5F). It is likely that miR-30c is unable to significantly reduce lipoprotein production in chow-fed WT mice. In short, these studies show that increasing hepatic miR-30c levels does not reduce plasma cholesterol in chow-fed mice.

**miR-30c lowers plasma cholesterol**

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**Fig. 2.** miR-30c reduces hypercholesterolemia in chow-fed ob/ob mice. Chow-fed male lepin-deficient (ob/ob) mice (8 weeks old) were retro-orbitally injected with 1 mg/kg/week miR-30c (n = 6) complexed with lipid emulsions or PBS (n = 4) for 8 weeks. Overnight fasting plasma was collected on day 6 after each injection. Plasma was used to measure total cholesterol (A, left) and triglyceride (B, left) concentrations. Plasma collected at 5 weeks was pooled and subjected to FPLC to measure cholesterol (A, right) and triglyceride (B, right) in different lipoprotein fractions. Fasting plasma was also used to measure glucose (C), ALT (D), AST (E), and CK (F) levels. After 48 h of the last injection, hepatic tissues were collected to measure miR-30c levels (G), different indicated mRNAs (H), MTP activity (I), cholesterol (J), and triglyceride (K). Fresh liver slices were used to measure fatty acid (L), triglyceride (M), and cholesterol (N) syntheses. *P < 0.05, **P < 0.01 determined by Student’s t-test. SDs are presented as error bars.
miR-30c lowers plasma cholesterol

DISCUSSION

Here, we show that miR-30c reduces plasma cholesterol in Western diet-fed hypercholesterolemic atherosclerosis-prone Ldlr<sup><del>−</del> /<del>−</del></sup> and chow-fed ob<sup>/ob</sup> and db<sup>/db</sup> hypercholesterolemic hyperglycemic type 2 diabetic mice. In these mice, miR-30c lowers plasma cholesterol without affecting plasma triglyceride and glucose. Thus, we have so far demonstrated that miR-30c reduces plasma cholesterol in five different hyperlipidemic mouse models. In agreement with previous studies, mechanistic studies performed here showed that miR-30c most likely reduces plasma cholesterol by reducing MTP activity. Novel studies in ob<sup>/ob</sup> and db<sup>/db</sup> mice indicate that miR-30c can lower plasma cholesterol in these dyslipidemic type 2 diabetic mice. These studies can be extended in the future to determine whether miR-30c can lower plasma cholesterol in diet-induced insulin-resistant diabetic models.

We observed that miR-30c lowered plasma cholesterol and atherosclerosis in Western diet-fed Ldlr<sup><del>−</del> /<del>−</del></sup> mice with no effect on plasma triglyceride, ALT, AST, and CK levels. The Ldlr<sup><del>−</del> /<del>−</del></sup> mice are models of HoFH. HoFH subjects...
lack functional LDL receptors and they do not respond to common lipid-lowering therapies, such as statins and PCSK9 inhibitors (28). Current therapies available for the treatment of HoFH are MTP inhibitors and antisense apoB (29). Both of these treatments are associated with hepatosteatosis and are administered under strict regulatory conditions (29). We speculate that miR-30c could be an alternate safer treatment modality for these HoFH subjects.

Previously we reported that miR-30c reduces hypercholesterolemia and atherosclerosis in Apoe\(^{-/-}\) mice (19, 20). The present study in the Ldlr\(^{-/-}\) mice also revealed that miR-30c can efficiently lower plasma cholesterol and atherosclerosis. Thus, it is likely that miR-30c may be a general biologic to reduce diet-induced hypercholesterolemia and atherosclerosis.

Besides Ldlr\(^{-/-}\) mice, we also studied ob/ob and db/db mice that are genetic models of type 2 diabetes (30). These mice have higher glucose levels due to the absence of leptin and leptin receptors. We observed that miR-30c reduces plasma cholesterol in these mice. However, miR-30c had no effect on plasma glucose levels. In previous studies (19, 20) and in the current Ldlr\(^{-/-}\) mice, we did not see any effect of miR-30c on plasma glucose levels and assumed that miR-30c did not affect glucose levels, as these mouse models were normoglycemic. However, with the current data in hyperglycemic ob/ob and db/db mice, it can be stated that miR-30c, in general, does not affect plasma glucose metabolism in mice.

In the Ldlr\(^{-/-}\) mice, miR-30c did not affect plasma ALT, AST, and CK levels, consistent with previous studies (19, 20). In contrast, we found that plasma ALT, AST, and CK levels were significantly reduced in miR-30c-injected ob/ob and db/db mice. At this time, we do not know the reasons for these reductions. This was unrelated to hepatic lipids, as they were not different in PBS- and miR-30c-injected mice. Further experiments are needed to explain changes in these enzymes in the plasma of miR-30c-injected mice.

It is well established that MTP inhibition reduces lipoprotein production. We have previously shown that reduced expression of MTP after miR-30c expression reduces lipoprotein production (19, 20). It is also known that when lipoprotein production is inhibited, lipids accumulate in the livers. MiR-30c suppressed MTP activity and lowered plasma cholesterol in Ldlr\(^{-/-}\), ob/ob, and db/db mice, but did not cause steatosis. Mechanistic studies suggest simultaneous reductions in de novo lipogenesis and cholesterol/triglyceride syntheses consistent with our previous studies (19, 20). Thus, it is possible that combined reductions in lipid synthesis and lipoprotein production may avoid steatosis while lowering plasma lipids.

In contrast to several animal models where miR-30c reduced plasma cholesterol, we observed that miR-30c did not lower plasma cholesterol in chow-fed mice. Chow-fed male C57BL/6J mice (8 weeks old) were retro-orbitally injected with 1 mg/kg/week miR-30c (n = 5) or PBS (n = 5) for 4 weeks. After 48 h of the last injection, hepatic tissues were collected to measure miR-30c levels (A) and MTP activity (B). Fasting plasma was collected on day 6 after each injection to measure cholesterol (C) and triglyceride (D) concentrations. E: mRNA levels of genes involved lipoprotein secretion and lipid synthesis were measured. Data are representative of two independent experiments. F: In a separate experiment, mice on chow diet were injected with miR-30c (n = 6) or PBS (n = 3) for 5 weeks. After 48 h of the last injection, mice were injected with P407 to inhibit lipoprotein clearance. Plasma was collected at indicated times to measure triglycerides. Data are representative of two independent experiments. *P < 0.05, **P < 0.01 determined by Student’s t-test. SDs are presented as error bars.
not lower plasma cholesterol despite efficient delivery of miR-30c to the liver and significant reductions in hepatic MTP activity in chow-fed STZ-induced type 1 diabetic and WT C57BL/6j mice. It is unclear why miR-30c did not reduce plasma cholesterol in these mice. Expression analyses showed that miR-30c target genes involved in lipoprotein assembly and lipid synthesis were reduced. However, lipoprotein production was not significantly reduced in chow-fed mice. Both these mouse models have lower plasma cholesterol than in other mouse models that responded to miR-30c. Therefore, it is likely that miR-30c may be effective in lowering plasma cholesterol in mice that have concentrations higher than 100 mg/dl. More mechanistic studies are needed to explain the nonresponsiveness of these mouse models to miR-30c.

miR-30c did not significantly lower fasting plasma triglyceride in all the previous (19, 20) and current studies. We do not know the reasons for the resilience of plasma triglyceride to miR-30c. Future experiments in lipoprotein lipase-deficient animals may address the effect of miR-30c on triglyceride lipolysis. Another possibility is that the Western diet feeding and leptin and leptin receptor gene deficiency have minimal effects on plasma triglyceride and that miR-30c may not regulate basal plasma triglyceride concentrations. Future studies in hypertriglyceridemic mouse models may address whether miR-30c can lower triglyceride in these mice.

These studies provide proof of concept that miR-30c can be used to lower plasma cholesterol in different metabolic diseases with no obvious unwanted side effects in mice. However, there are several issues that need to be addressed before miR-30c can be tested for human use. First, potent and long-lasting miR-30c may avoid frequent injections. Second, analogs that can be delivered via less invasive routes, such as subcutaneous or oral administration, might be desirable. Both of these issues can be addressed by developing novel chemically modified miR-30c analogs that are more stable, specific, nonimmunogenic, and easily administered. Third, a comprehensive understanding of the biological and pharmacological effects of miR-30c is needed to determine its safety.

In short, we show that miR-30c reduces plasma cholesterol in diabetic and diet-induced hypercholesterolemic mice. Thus, miR-30c may be useful in lowering plasma cholesterol in different metabolic disorders independent of the origin of hypercholesterolemia. Reductions in plasma cholesterol were not associated with hepatic steatosis. Thus, miR-30c may be a safer agent to reduce plasma cholesterol levels.

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