RESEARCH ARTICLE

Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet

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Scope: Nonalcoholic fatty liver disease (NAFLD) is a chronic disorder characterized by hepatic fat accumulation and abnormal lipid metabolism. Here, we investigated the protective effect of lycopene on high-fat diet-induced hepatic steatosis and fatty acid-induced intracellular lipid accumulation by miRNA regulation.

Methods and results: C57BL/6J mice were fed high-fat diet with or without 0.05% lycopene for 8 weeks. Hepa 1–6 cells were treated with stearic acid (SA) after 24 h pretreatment with lycopene. Treatment of lycopene improved hepatic steatosis in high-fat-fed mice and reduced intracellular lipid accumulation induced by SA in Hepa 1–6 cells. We demonstrated that miR-21 expression was decreased in livers from high-fat diet-fed mice and Hepa 1–6 cells treated with SA. Lycopene normalized the downregulation of miR-21, which led to the downregulation of fatty acid-binding protein 7 (FABP7), a direct target of miR-21, at both the transcriptional and translational levels. This specific negative regulation of miR-21 was achieved by targeting the FABP7 3′UTR. Upregulation of miR-21 markedly blocked SA-induced intracellular lipid accumulation by blocking FABP7 expression. Moreover, silencing of FABP7 reduced SA-evoked lipid accumulation in Hepa 1–6 cells.

Conclusion: The results suggest that lycopene may be a useful functional compound for treating NAFLD by regulating hepatic lipid metabolism.

Keywords:
Fatty acid-binding protein 7 / Fatty acid uptake / Lycopene / MicroRNA / Nonalcoholic steatohepatitis

1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by hepatocyte steatosis and elevated serum free fatty acids [1], which may play a central role in insulin resistance, obesity, and hepatic steatosis [2]. NAFLD has become one of the most common chronic liver diseases worldwide, affecting up to 20–40% of the general adult population [3]. However, the cellular mechanisms contributing to NAFLD development and progression remain unclear.

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Abbreviations: FABP, fatty acid-binding protein; **miRNA**, microRNA; **NAFLD**, nonalcoholic fatty liver disease; **SA**, stearic acid

MicroRNAs (miRNAs) are highly conserved small noncoding RNAs that regulate gene expression at the posttranscriptional level [4–6] by binding to complementary sites on their target transcripts, which makes miRNAs important modulators of many cellular process such as energy homeostasis, lipid metabolism, pancreatic β-cell development, adipogenesis, and high-fat induced weight gain [7–10]. Interestingly, diet diversity has been reported to induce changes in miRNA expression profiles [11], which often lead to development of diet-induced hepatic dysfunction [12].

Fatty acid-binding proteins (FABPs) are intracellular low molecular weight (14–16 kDa) polypeptides and involved in lipid metabolism, including the uptake and intracellular trafficking of long-chain fatty acids and retinoids. They also play a role in gene regulation, cell signaling, cell growth, and differentiation [13]. Although ten isoforms of FABPs have been identified in mammals [14], and hepatic functions have been attributed to many of them [15–18], the role of FABP7 in the liver remains unknown.

Lycopene is an open-chain unsaturated carotenoid antioxidant found in tomatoes, watermelons, red grapefruits, and guava [19]. Previous studies have demonstrated the protective activity of lycopene against diseases such as atherosclerosis [20], prostate cancer [21], obesity [22], and nonalcoholic steatohepatitis-promoted hepatocarcinogenesis [23]. Lycopene also reduces the development of hepatic steatosis induced by a high-fat diet [24], although the exact mechanism how lycopene ameliorates this condition has not yet been investigated.

Our previous studies demonstrated that dietary phytochemicals such as resveratrol attenuate hepatic steatosis by modulating lipid metabolism-related gene expression [25,26]. To further explore the protective mechanisms of phytochemicals against NAFLD, we examined the effects of lycopene on high-fat diet-induced hepatic steatosis.

In this study, we used a mouse model of diet-induced obesity and Hepa 1–6 cells to explore whether miRNAs contribute to the pathogenesis of NAFLD. MiRNA-21 (miR-21) downregulation followed by FABP7 upregulation was observed in steatosis. In mouse hepatocyte, miR-21 and FABP7 expression were inversely changed by FFA treatment. Normalization of miR-21 and FABP7 expression was accompanied with the protective effect of lycopene against hepatic steatosis. Overexpression of miR-21 blocked stearic acid (SA)-induced lipid accumulation in Hepa 1–6 cells. Moreover, silencing of FABP7 also reduced SA-evoked intracellular lipid storage in hepatocytes. Our results provide novel insights into the potential therapeutic mechanisms of lycopene on NAFLD and involvement of miR-21 in hepatic steatosis by controlling its downstream target, FABP7.

2 Materials and methods

2.1 Animals

Six-week-old male C57BL/6J mice (Orient Bio Inc., Seoungnam, Korea) were divided into three groups ($n = 10$): (a) control mice given a normal-fat diet (11.69% of total calories from fat), (b) mice given a high-fat diet (49.29% of total calories from fat), and (c) mice fed a high-fat diet containing 0.05% lycopene (99%, Shaanxi Scipher Biotechnology, Xi'an, China). The experimental diets were based on AIN-76A diet and the composition of the experimental diets is shown in Table 1. After 8 weeks on the diet, mice were sacrificed following a 12 h fast. All animal studies were conducted in accordance with a protocol approved by the Korea Food Research Institute's Institutional Animal Care and Use Committee.

2.2 Histological analysis and measurement of liver lipid content

For histological analysis, liver samples were fixed in 4% buffered formalin and stained with H&E. The lipid content of liver homogenates obtained from chloroform-methanol extraction was measured as described previously [27]. Tissue triglyceride and total cholesterol levels were measured with commercially available kits (Wako Chemicals, Osaka, Japan).

Table 1. Composition of the experimental diets

Ingredients \ Group	Unit: g/kg diet		
	NC	HF	HF +Ly
Casein	200	200	200
Corn oil	50	50	50
Lard	–	220	220
Cholesterol	–	5	5
Corn starch	350	125	125
Sucrose	300	300	300
Cellulose	50	50	50
Mineral mix[a)]	35	35	35
Vitamin mix[b)]	10	10	10
Methionine	3	3	3
Choline bitartrate	2	2	2
Lycopene	—	—	0.5
Calories	3850	4930	4930

a) AIN-76 mineral mixture.
b) AIN-76 vitamin mixture.
NC (normal control diet-fed mice); HF (high-fat diet-fed mice); HF + Ly (high-fat diet containing 0.05% lycopene-fed mice).

2.3 Cell culture, induction of steatosis, and lipid visualization

Hepa 1–6 cells were purchased from ATCC (Manassas, VA) and maintained in Dulbecco's Modified Eagle Medium (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. Saturated fatty acid-induced steatosis was achieved with slight modifications of previously described methods [28,29]. Cells were incubated with 50 μM SA (Sigma-Aldrich, St. Louis, MO) in 20% bovine serum albumin-PBS for 24 h. For lycopene treatment, cells were pretreated with lycopene for 24 h. After fixation with formaldehyde, neutral lipids were stained with Nile red (Sigma-Aldrich) in acetone or BODIPY 493/503 (Molecular Probes, Eugene, OR) in ethanol, and nuclei were stained with DAPI.

2.4 Quantitative PCR analysis

Total RNA from livers and Hepa 1–6 cells were isolated with a commercial kit (Macherey-Nagel, Duren, Germany). For miRNA quantification, total RNA was reverse transcribed with the TaqMan™ MicroRNA reverse transcription kit and subjected to real-time PCR with the TaqMan™ MicroRNA assay kit (Applied Biosystems, Foster City, CA). The miRNA expression was normalized to endogenous snoRNA202 (Applied Biosystems). The mRNA expression was measured as previously described [30]. The PCR primer sequence used

were as follows (5′ → 3′): FABP7, Forward: CCG AAG CTT GCA CTG GTC ACT AAT, Reverse; GGA CTA GTC CGA AGA CAA AC; peroxisome proliferator-activated receptor alpha (PPARα), Forward: AGA GCC CCA TCT GTC CTC TC, Reverse: ACT GGT AGT CTG CAA AAC CAA A; peroxisome proliferator-activated receptor gamma (PPARγ), Forward: GTC ACG GAA CAC GTG CAG C, Reverse: CAG GAG CGG GTG AAG ACT CA; carnitine palmitoyl transferase 1 alpha (CPT1α), Forward: CTC CGC CTG AGC CAT GAA G, Reverse: CAC CAG TGA TGA TGC CAT TCT; long-chain acyl-CoA dehydrogenase (LCAD), Forward: TCC AGA GGT CAG TCA ACA TGA, Reverse: CCT GGT CAA TTT TTC GAG AGT CC; fatty acid synthase (FASN), Forward: GGA GGT GGT GAT AGC CGC TAT, Reverse: TGG GTA ATC CAT AGA GCC GAG; apolipoprotein A-IV (Apoa4), Forward: CAA CAG GCT GAA GGC TAC GAT, Reverse: CGA TTT TTG CGG AGA CCT TGG; β-Actin, Forward: AATACCCCAGCCATGTGTGT, Reverse: ATG GGC ACT GTG TGT GAC C.

2.5 MiR-21 mimic and miR-21 inhibitor transfection

To specifically induce miR-21 expression, miRIDIAN™ miR-21 mimics (Thermo Scientific, Lafayette, CO) was selected. It is a double-stranded RNA oligonucleotide and effectively mimics endogenous mature miRNA function. For miR-21 inhibition, the miRIDIAN™ hairpin inhibitor (Thermo Scientific) was used. It is a single-stranded oligonucleotide and exerts effective inhibition of endogenous mature miRNA function. Mimic and inhibitor negative control oligonucleotide sequences were based on *Caenorhabditis elegans* miRNA-67. Oligonucleotides (25 pmol) were transfected using the Lipofectamine™ RNAiMAX (Invitrogen, Carlsbad, CA), and miR-21 overexpression and inhibition were verified using quantitative PCR as described above.

2.6 Western blotting

Liver tissues and harvested cells were lysed in RIPA buffer as described previously [25]. Total protein (20 μg) was separated with 12% SDS–polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Membranes were hybridized with either anti-FABP7 (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-actin (Cell Signaling, Danvers, MA) primary antibody (1:1000) and then with horseradish peroxidase-conjugated antibodies (1:2000). The immunoreactive proteins were visualized with the West-one™ Western Blot (iNtRON Biotechnology, Seoungnam, Korea).

2.7 3′UTR luciferase reporter assays

The pMIR-REPORT™ System (Ambion, Austin, TX) was used to identify the FABP7 3′UTR as the miR-21-binding site. The cDNA fragment corresponding to the 3′UTR of FABP7 was amplified with SpeI and HindIII linkers by reverse transcription PCR from Hepa 1–6 cell total RNA. The resulting amplicons were inserted at the designated multiple cloning site downstream of the firefly luciferase gene under the control of the CMV promoter of the pMIR-REPORT™ miRNA Expression Reporter Vector. Hepa 1–6 cells were cotransfected with 150 ng of either the 3′UTR luciferase reporter or the control construct and 10 pmol of the miR-21 mimic, mimic control, miR-21 inhibitor, or inhibitor control (Thermo Scientific) with the Lipofectamine™ 2000 (Invitrogen). Forty-eight hours after transfection, luciferase activity was measured using the Dual-Light® System (Applied Biosystems).

2.8 RNA interference of FABP7

Hepa 1–6 cells were transfected with 10 nM FABP7 small interfering RNA (siRNA) or control siRNA with no cellular activity (Santa Cruz Biotechnology) with Lipofectamine™ RNAiMAX. After 48 h of transfection, cells were treated with SA for 24 h and lysed. The silencing of FABP7 was confirmed by Western blotting as described above.

2.9 Statistical analysis

Data were expressed as means ± SE. Statistical analyses were performed with GraphPad Prism 5 software (San Diego, CA). One-way ANOVA was used to compare quantitative data among groups. The Bonferroni post-hoc test was used if ANOVA indicated statistical significance ($p < 0.05$).

3 Results

3.1 Lycopene protected against high-fat diet-induced hepatic steatosis

Feeding a high-fat diet is important in the development of nonalcoholic steatohepatitis of the diet-induced obesity mouse model [31]. Mice fed the high-fat diet exhibited hepatomegaly and steatosis with ballooning degeneration as well as increased levels of hepatic total cholesterol (1.4-fold), triglycerides (2.8-fold), and lipids content (2.6-fold) compared to those of NC mice (Supporting Information Table S1 and Fig. 1A). Lycopene, effectively protected high-fat diet-induced hepatic lipid accumulation and weight gain (Fig. 1A and B). Lycopene also ameliorated hepatomegaly and improved hepatic lipid profiles (Supporting Information Table S1). There were no significant differences in food intake among groups (data not shown).

To examine the effect of lycopene on hepatic lipid metabolism, we analyzed hepatic gene expressions (Fig. 1C). Interestingly, lycopene reversed the effects of high-fat diet on PPARα and PPARγ, two major transcription factors involved in hepatic lipid metabolism. Lycopene improved

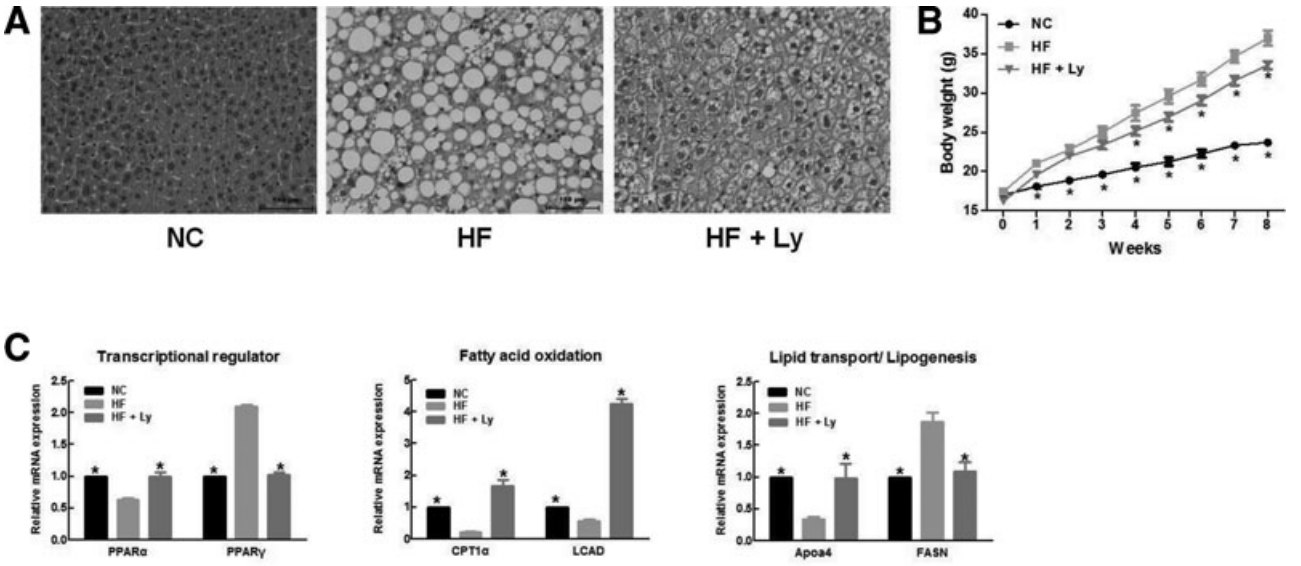

Figure 1. Protective effect of lycopene on hepatic steatosis. (A) Representative photomicrographs of liver specimens from all groups of mice (×100 magnification. NC, normal control diet; HF, high-fat diet; HF + Ly, high-fat diet containing 0.05% lycopene. (B) Body weight changes in mice fed the experimental diets for 8 weeks ($n = 10$). (C) Effect of lycopene on hepatic lipid metabolism. The mRNA expression levels are shown relative to the expression of β-actin. *$p < 0.05$ versus HF group.

the decreases of CPT1α and LCAD by high-fat diet. Moreover, lycopene increased the expression of Apoa4, a major constituent of high-density lipoprotein particles. Conversely, FASN, which regulates lipogenesis and whose expression is controlled by PPARγ, was reduced by lycopene treatment. Taken together, these results suggest that lycopene exerts its protective effect on hepatic steatosis by regulating hepatic lipid metabolism.

3.2 High-fat diet induced downregulation of miR-21 and FABP7 is the target of miR-21

To investigate whether miRNAs are involved in the protective effect of lycopene on hepatic steatosis, we compared the expression profiles of miRNAs of livers from each group using miRNA microarray analysis (data not shown). We found that high-fat diet induced downregulation of miRNA-21 and

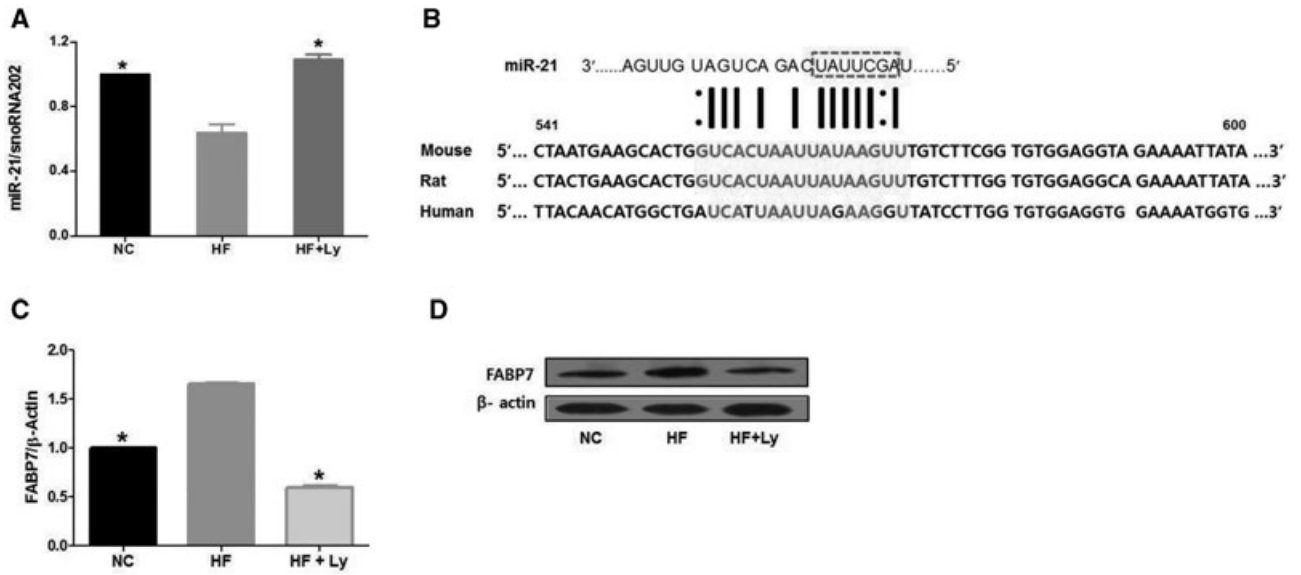

Figure 2. Effect of lycopene on HF-induced downregulation of miR-21 and FABP7 as a direct target of miR-21. (A) The miR-21 expression in the liver ($n = 5$). (B) Schematic alignment between the mature miR-21 seed sequence (dotted box) and the FABP7 mRNA target sequence (highlighted in red) in the 3′-UTR region among species. (C) qRT-PCR analysis of FABP7 mRNA expression in the liver ($n = 5$). (D) Representative Western blot of FABP7 in hepatic tissues. *$p < 0.05$ versus the HF group.

lycopene effectively prevented the decrease of miRNA-21 induced by high-fat diet (Fig. 2A). This is very interesting because miRNA-21 has emerged as a key miRNA that is dysregulated in many cancers [32]. Despite the accumulating evidences regarding the role of miR-21 in various tumors [33,34], its function in hepatic steatosis remains to be clarified. This prompted us to select miR-21 to study its role in hepatic steatosis and in the protective effects of lycopene on NAFLD.

Since miRNAs serve as post-transcriptional regulators of gene expression, we sought to identify the target gene(s) of miR-21 that may be related to the protective effect of lycopene against hepatic steatosis. Bioinformatic analysis using TargetScan, miRanda, and miRBase revealed FABP7 as one of the target genes of miR-21 and further revealed that the miRNA 5′ "seed" sequence [35] shares >85% homology with FABP7 (Fig. 2B). We observed a clear increase in mRNA and protein levels of FABP7 in fatty liver tissues (Fig. 2C and D). The upregulation of FABP7 was significantly inhibited when hepatic steatosis was improved by lycopene.

To measure the effect of miR-21 on its putative target FABP7, we modulated miR-21expression with miR-21 mimic or inhibitor in Hepa 1–6 cells. Treatment with the miR-21 mimic increased the expression of miR-21 in a concentration-dependent manner (Fig. 3A), whereas the miR-21 mimic downregulated FABP7 mRNA and protein levels (Fig. 3B and C). By contrast, treatment with the miR-21 inhibitor enhanced the expression of FABP7 mRNA and protein in the hepatocytes (Fig. 3D, E, and F).

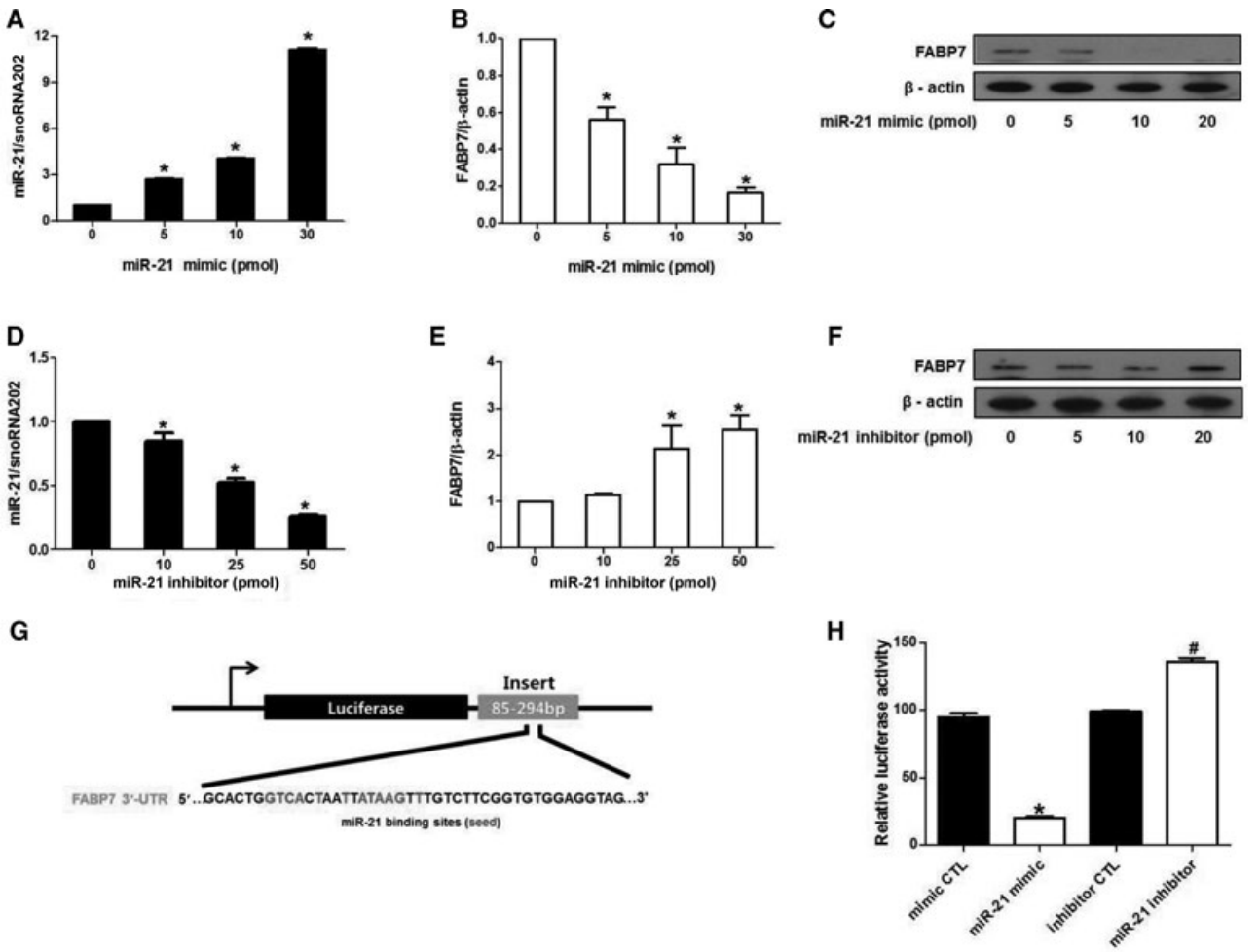

Figure 3. MiR-21 inhibits FABP7 through direct binding to 3′UTR of FABP7 (A) The expression of miR-21 in Hepa 1–6 cells treated with the miR-21 mimic. (B) The mRNA expression of FABP7 in Hepa 1–6 cells treated with the miR-21 mimic. $^{*}p < 0.05$ versus control. (C) The protein expression of FABP7 in Hepa 1–6 cells treated with the miR-21 mimic. (D) The expression of miR-21 in Hepa 1–6 cells treated with the miR-21 inhibitor. (E) The mRNA expression of FABP7 in Hepa 1–6 cells treated with the miR-21 inhibitor. $^{*}p < 0.05$ versus control. (F) The protein expression of FABP7 in Hepa 1–6 cells treated with the miR-21 inhibitor. (G) Schematic representation of the luciferase reporter vector harboring the FABP7 3′-UTR fragment (green) containing the miR-21-binding site (seed sequences in red). (H) Activity of luciferase reporter constructs fused to the 3′UTR of FABP7 in Hepa 1–6 cells transfected with control miRNAs (CTL), miR-21 mimic, or inhibitor. $^{*}p < 0.05$ versus mimic CTL. $^{\#}p < 0.05$ versus inhibitor CTL.

We performed luciferase reporter assays to determine whether miR-21 specifically targets FABP7 posttranscriptionally by binding to its 3′UTR (Fig. 3G). Introduction of the miR-21 mimic resulted in a significant decrease in luciferase activity compared with that observed in control construct-transfected cells, whereas cotransfection with the miR-21 inhibitor led to a significant increase in luciferase activity (Fig. 3H). On the contrary, there were no significant differences in the relative luciferase activity in miRNA mimic control (CTL) – or inhibitor CTL-treated hepatocytes. These data suggest that miR-21 downregulates the expression of FABP7 by targeting the FABP7 3′UTR to facilitate translational repression or mRNA degradation in hepatocytes.

3.3 Lycopene inhibited intracellular lipid accumulation through miRNA21-FABP7 pathway

To address how lycopene protects against hepatic steatosis, we induced cellular steatosis by treating Hepa 1–6 cells with saturated fatty acid, stearic acid (SA). As shown in Fig. 4A and B, SA treatment induced intracellular lipid accumulation, and pretreatment with lycopene for 24 h reduced the intracellular fat deposition in a concentration-dependent manner. We confirmed that the concentrations of lycopene that were used in this study showed no cytotoxicity in Hepa 1–6 cells (data not shown).

Incubation with SA reduced miR-21 expression, whereas lycopene pretreatment normalized miR-21 expression levels (Fig. 4C). Finally, we measured FABP7 mRNA levels and confirmed that FABP7 upregulation was blocked when lycopene inhibited SA-induced lipid accumulation (Fig. 4D). To know whether lycopene directly modulates miR-21, we treated lycopene in miR-21 downregulated cells. As shown in Fig. 4E, lycopene significantly increased the expression of miR-21. Concomitantly, mRNA expression of FABP7 was decreased by lycopene treatment (Fig. 4F). These data confirmed that lycopene directly modulates miR-21.

We treated miR-21 mimic or mimic CTL to verify the role of miR-21 in hepatic lipid accumulation (Fig. 5A). Cells transfected with the miR-21 mimic exhibited marked downregulation of FABP7 mRNA and protein levels even under SA treatment (Fig. 5B and C). Treatment with the miR-21 mimic but not the mimic CTL caused a remarkable reduction in lipid accumulation in SA-treated Hepa 1–6 cells (Fig. 5D). Based on these data, we hypothesized that miR21-mediated inhibition of FABP7 may be involved in the protection against NAFLD. To confirm this, we induced FABP7 knockdown by transfecting a specific siRNA into Hepa 1–6 cells and exposed these cells to SA (Fig. 5E). The FABP7-silenced cells exhibited decreased lipid accumulation after SA treatment, whereas si-CTL-transfected cells exhibited lipid accumulation in response to SA (Fig. 5F). These results demonstrate that miR-21 protects against hepatic steatosis by inhibiting FABP7

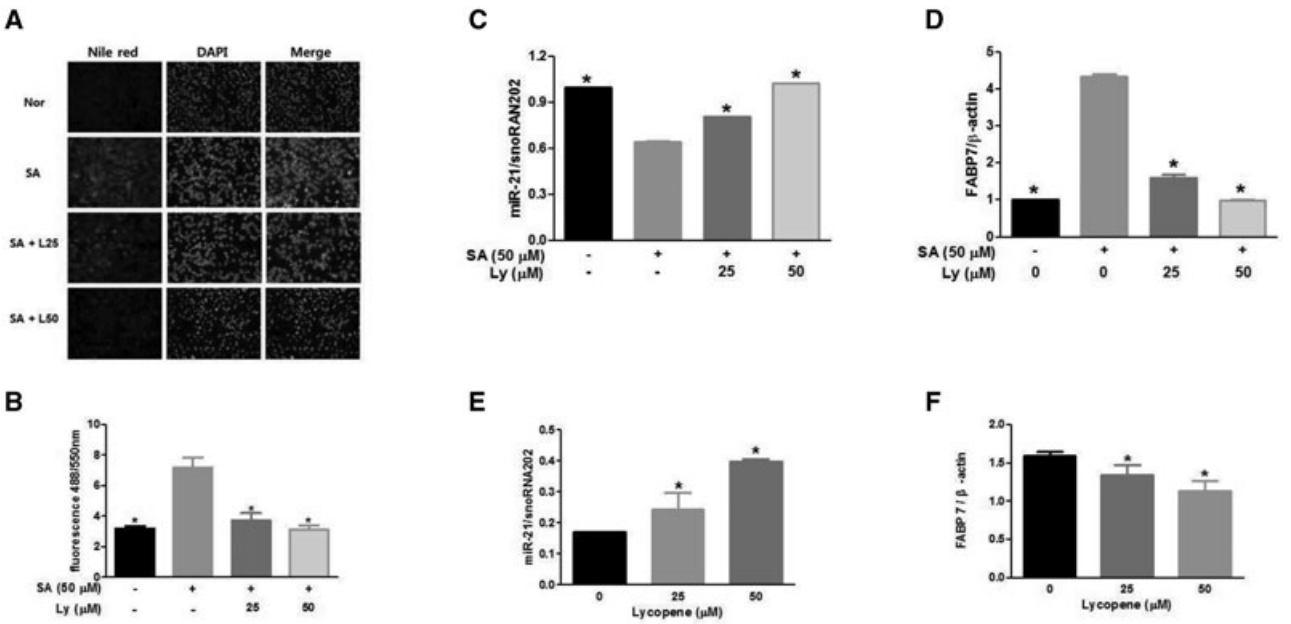

Figure 4. The inhibitory effect of lycopene on SA-induced lipid accumulation is related to miR-21 and FABP7 in Hepa 1–6 cells. (A) Representative fluorescence micrographs of hepatocytes after treatment with 50 μM SA for 24 h. Cells were pretreated with lycopene for 24 h before SA exposure. Cells were stained with Nile red and DAPI. (B) Intracellular lipid accumulation was determined by the fluorescence intensity of Nile red. (C) The expression of miR-21 in Hepa 1–6 cells. (D) The mRNA expression of FABP7 in Hepa 1–6 cells. $*p < 0.05$ versus SA-treated cells. (E) The effect of lycopene on the miR-21 expression in the miR-21 downregulated cells. (F) The effect of lycopene on the mRNA expression of FABP7 in the miR-21 downregulated cells. $*p < 0.05$ versus control.

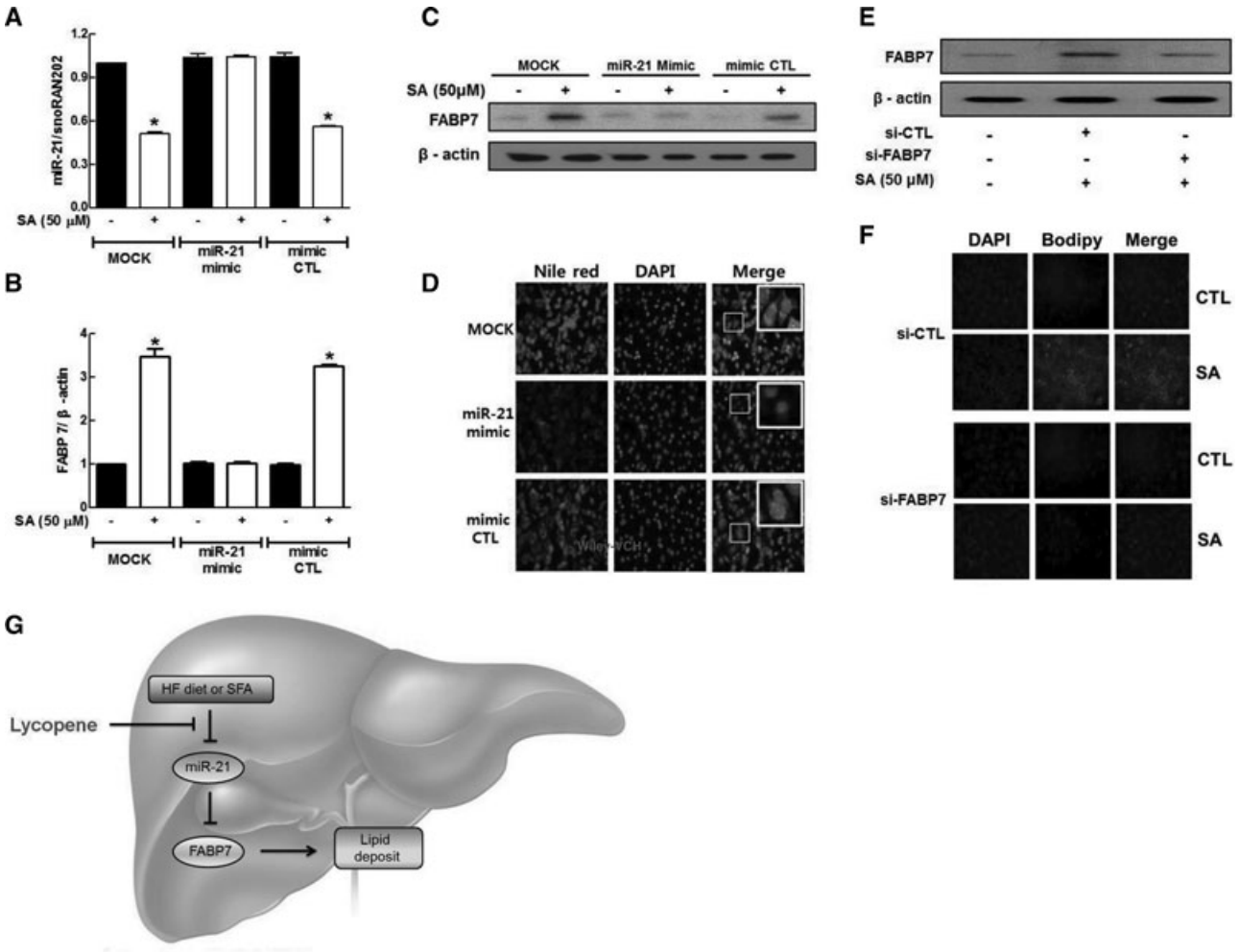

Figure 5. Effect of the miR-21 mimic and FABP7 knockdown on SA-induced lipid accumulation in Hepa 1–6 cells. (A) The expression of miR-21 in miR-21 mimic-treated Hepa 1–6 cells. Six hours after the transfection of 25 pmol of the miR-21 mimic or mimic CTL, cells were treated with 50 μM SA for 24 h. (B) The mRNA expression of FABP7 in Hepa 1–6 cells as treated above. $*p < 0.05$ versus MOCK without SA treatment. (C) The protein expression of FABP7 in Hepa 1–6 cells as treated above. (D) Representative fluorescence micrographs. Cells were stained with Nile red. The small-boxed regions were magnified and shown in large boxed regions. (E) Western blot analysis of FABP7 in siRNA-control (si-CTL) and siRNA-FABP7 (Si-FABP7) treated cells after 50 μM SA exposure for 24 h. (F) Representative fluorescence micrographs of FABP7-depleted Hepa 1–6 cells after treatment with 50 μM SA for 24 h. Cells were stained with BODIPY 493/503. (G) Schematic diagram showing the protective mechanism of lycopene on hepatic steatosis. High-fat (HF) diet or saturated fatty acid (SFA) downregulated miR-21. This, in turn, resulted in the upregulation of FABP7 and the hepatic lipid deposit was increased. Lycopene effectively ameliorated hepatic steatosis through the reverse of miR-21 downregulation evoked by high-fat diet.

and is involved in the protective effect of lycopene on NAFLD (Fig. 5G).

4 Discussion

In this study, we demonstrated that miR-21 expression was decreased in high-fat diet-induced hepatic steatosis and FFA-treated hepatocytes. Amelioration of lipid accumulation in tissues and cells by lycopene was accompanied by the normalization of miR-21 expression levels. FABP7 was one of targets of miR-21, and it was directly and inversely regulated by miR-21. Elevation of miR-21 or silencing of FABP7 notably inhibits SA-induced fatty acid uptake and lipid accumulation.

The study on hepatocyte-specific Dicer knockout mice proposed that miRNA may have an important role in hepatic function [36]. We observed miRNA expression patterns were changed by high-fat diet feeding in liver tissues. When NAFLD was ameliorated by lycopene, the expression of several miRNAs was recovered. Among them, the altered miR-21 expression was effectively improved by lycopene in the liver tissues of mice fed a high-fat diet. The miR-21 was upregulated in malignant tumor tissues including hepatocellular carcinoma tumors [37]. A close link between miR-21 and hepatic fibrosis was supported by transforming growth factor β-induced increase of miR-21 [38]. MiR-21 was also suggested as a necroinflammation marker in hepatitis C [37].

These results revealed a robust increase in miR-21 expression beyond normal levels evoked deleterious effects in the liver. Conversely, there was an interesting report that miR-21 was upregulated during liver regeneration. Moreover, miR-21 overexpression inhibited NF-κB signaling [39]. Taken together, various studies demonstrated that miR-21 is involved in several hepatic diseases. However, how miR-21 regulates hepatic lipid trafficking and metabolism is not yet known.

A target study revealed that FABP7 was a direct target of miR-21. FABPs are the single most abundant proteins in the cytosol of cells and are most active in long chain fatty acid uptake and metabolism in the liver [40]. Among FABPs, FABP1, and FABP7 are expressed in liver tissue [16]. FABP1 is the most broadly distributed mammalian FABP, and it is expressed at very high levels in the liver, intestine, and kidney [40]. FABP1 knockout mice exhibited decreased hepatic FA uptake and trafficking [41] and protection against Western diet-induced obesity and hepatic steatosis [31]. Unlike FABP1, FABP7 is predominantly expressed in the embryonic mouse brain, in which its expression gradually decreases as differentiation progresses [42] and suggested as a downstream target of Pax6 and Notch signaling pathway [43]. FABP7 knockout mice showed decrease in the cellular uptake of long chain fatty acid [44]. However, the function of FABP7 in hepatocyte has not been reported yet. Therefore, our finding that upregulation of FABP7 could be reversed by lycopene treatment is the first step in understanding the role of FABP7 role in hepatic steatosis. The downregulation of miR-21 and upregulation of FABP7 in high-fat diet-fed mice led to an increase in fatty acid uptake and their subsequent transport to the liver, which ultimately led to hepatic steatosis. We demonstrated that the miR-21 mimic decreased FABP7 mRNA and protein levels and inhibited SA-induced intracellular lipid accumulation in Hepa 1–6 cells. We obtained similar results when we treated FABP7-depleted cells with SA. Though miR-21 is an oncomir and overexpressed in cancer cells lines [45], we confirmed the downregulation of miR-21 in both Hepa 1–6 cells and liver tissues that were exposed to fatty acid and high-fat diet, respectively. Further studies will be necessary to define the role of FABP7 on lipid metabolism in the hepatocyte-specific FABP7 knockout mice

We also observed lycopene regulated two important transcription factors, PPARα and PPARγ. Recent evidence suggests that PPARα plays a central role in hepatocytes, in which it induces the transcription of genes involved in fatty acid uptake, fatty acid degradation by β-oxidation, and lipoprotein metabolism; PPARα activity is reduced by a high-fat diet [46]. In contrast, PPARγ expression is increased in high-fat diet-induced hepatic steatosis in mice [47]. A previous study reported that lycopene intake decreased the expression of PPARγ in F344 rats [48], a finding supported by our observations that lycopene induced the transcription of several fatty acid oxidation-associated genes, including CTP1α, and LCAD. Moreover, we determined that lycopene improved lipid metabolism by increasing ApoA4 and inhibiting FASN in the liver.

In conclusion, we demonstrated that dietary lycopene reduced hepatic steatosis in mice fed a high-fat diet by upregulating miR-21. We identified FABP7 as a novel target of miR-21. We also revealed that the miR-21 mimic inhibited FA-induced lipid accumulation in hepatocytes. Moreover, FABP7 depletion also reduced FA-induced fat accumulation. Given the promising preclinical findings presented in this study, we suggest that lycopene possesses great potential for preventing NAFLD and nonalcoholic steatohepatitis.

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The authors have declared no conflict of interest.

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