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MiR-126-3p and MiR-199a-3p Promote Lipid Accumulation in 3T3-L1 Adipocytes via Regulating HIF-1α and C/EBPα Expression

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Non-alcoholic fatty liver disease（NAFLD）is one major chronic liver disease emerging the primarily excessive fat accumulation in the liver. Recent studies have demonstrated that hepatic miRNAs play an important role of NAFLD pathogenesis. Previously we identified up-regulation of miR-126-3p and miR-199a-3p in the patient with NAFLD, but their roles remain unclear. In this study, we investigated the role of miR-126-3p and miR-199a-3p on lipid accumulation in 3T3-L1 adipocytes. Transfection of miR-126-3p and miR-199a-3p enhanced lipid accumulation in 3T3-L1 cells after adipocyte differentiation. Next, we evaluated lipid metabolism-related gene expression by quantitative RT-PCR. MiR-126-3p increased CCAAT/enhancer-binding protein-α（C/EBPα）mRNA expression whereas miR-199a-3p did not affect. Moreover, both miRNAs reduced the expression of hypoxia-inducible factor-1α（HIF-1α）mRNA, predicted target of miRNAs. These results suggested that miR-126-3p and miR-199a-3p promote lipid accumulation in 3T3-L1 adipocytes through regulating HIF-1α and/or C/EBPα mRNA expression.

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INTRODUCTION

Non-alcoholic fatty liver disease（NAFLD）is the most common cause of chronic liver disease characterized by hepatic steatosis in the absence of excessive alcohol consumption［1］. The emerged spectrum of NAFLD includes non-alcoholic fatty liver（NAFL）and non-alcoholic steatohepatitis（NASH）. The former generally shows a simple steatosis, while the latter shows steatosis, inflammation and fibrosis that may progress to advanced fibrosis, and cirrhosis with the potential for the development of hepatocellular carcinoma［2, 3］. NAFLD is closely associated with visceral obesity, insulin resistance, hyperlipidemia, and hypertension, all of which shared risk factors with cardiovascular disease［4, 5］. In addition, cardiovascular disease is generally recognized as the leading cause of death in patients with NAFLD［6］. NAFLD is thereby considered as a progressive disease involved with multiple organs and diverse mechanisms.

Several studies have been reported altered expression of microRNAs（miRNAs）in patients with

NAFLD stages including steatosis [7], fibrosis [8], cirrhosis, and hepatocellular carcinoma [9]. MiRNAs are small (18–25 nucleotide) non-coding RNA molecules that regulate target gene expression at the post-transcriptional level by blocking translation or promoting cleavage of specific target mRNAs or by binding to the 3′-untranslated region of their target mRNA [10, 11, 12]. Previous studies have shown that miRNAs and their regulatory gene networks are implicated in the pathogenesis of NAFLD. For example, increased miR-33a level in serum is associated with steatosis and inflammation in patients with NAFLD [13], and overexpression of miR-33a inhibits preadipocyte proliferation and adipocyte differentiation [14]. Furthermore, up-regulated miR-34a in liver tissue and serum of NAFLD patients suppresses gene expression related to lipid accumulation and steatosis [7]. Although several miRNAs have hold promise as novel candidates of therapeutic target and/or specific biomarker for NAFLD, the mechanistic role of other altered miRNAs in NAFLD development and progression remain incompletely understood.

In our previous studies, we also investigated the differential expression pattern of miRNA in biopsy samples between normal subjects and patients with NAFLD by using miRNA microarray analysis [15, 16]. Among the altered miRNAs, we demonstrated that up-regulated miR-27b promotes lipid accumulation and adipocyte differentiation through acyl-CoA thioesterase 2 (ACOT2) expression which results in NAFLD pathogenesis [17]. Our miRNA microarray data also indicated that other miRNAs are also changed their expression in NAFLD liver, but the role of them remains unclear. Therefore, we focused on miR-126-3p and miR-199a-3p that are remarkably increased in liver tissue from the early phase of NAFLD to NASH.

In this study, we have investigated the impact of miR-126-3p and miR-199a-3p on the intracellularly lipid accumulation which is an initial process of NAFLD development. We then evaluated the effect of these miRNAs on lipid metabolism-related mRNA expression. Moreover, we found that hypoxia-inducible factor-1α (HIF-1α) as a potential target of both miR-126-3p and miR-199a-3p might contribute to lipid accumulation.

MATERIALS AND METHODS

Cell culture

Mouse 3T3-L1 pre-adipocytes was obtained from the American Type Culture Collection (Manassas, VA, USA). 3T3-L1 cells were cultured in 100 mm × 20 mm polystyrene dishes (Corning, NY, USA) in Dulbecco's modified Eagle medium (Nacalai tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (Equitech-Bio, Texas, USA) containing 10 units/ml of penicillin (Nacalai tesque) and 10 μg/ml of streptomycin (Nacalai tesque). Cells were maintained at 37°C in a 5% CO_2 incubator and the fresh medium was supplemented every 3 days.

Induction of adipocyte differentiation

Briefly, 3T3-L1 cells were seeded in 24 well plates at a density of 2×10^5 cells into each well. Three days after reaching confluence the medium was replaced with a differentiation medium containing 1 μM rosiglitazone (ROSI) (Sigma-Aldrich, St. Louis, MO, USA), 150 nM insulin (INS) (Sigma Aldrich), 1 μM dexamethasone (DEX) (Sigma Aldrich), 100 μM 3-isobutyl-1-methylxanthine (IBMX) (Sigma Aldrich) or non-differentiation medium without any stimulator and grown for 3 days.

MiRNAs transfection into 3T3-L1 cells

MirVana miRNA mimic mmu-miR-126-3p mimic, mmu-miR-199a-3p mimic, and mmu-miR-33a mimic (positive control), and negative control miRNA (control-miR) were purchased from Thermo Fisher Scientific (Tokyo, Japan). Transfection of miRNAs was performed by using Lipofectamine™ RNAi MAX transfection reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. 3T3-L1 cells were grown for 3 days after plating. Cells were washed with phosphate-buffered saline (PBS) and then cultured with fresh pre-warmed Opti-MEM (Thermo Fisher Scientific). Control-miR, miR-126-3p, miR-199a-3p, or miR-33a are transfected to cells at a final concentration of 10 μM using RNAiMAX. After 4 hours of transfection cells were replaced with differentiation media.

Oil red O staining

After adipocyte differentiation, cells were washed

with pre-warmed PBS and then fixed with 200 μL of 4 % paraformaldehyde for 30 min at room temperature. Cells were stained with 500 μL of 0.2% oil red O (Sigma Aldrich) for 5 hours and observed under microscopy (CKX53, Olympus, Tokyo, Japan). For quantification of lipid accumulation, the oil red O dye staining cells were dissolved with 500 μL of 50% 2-propanol. The absorbance of oil red O dye was measured at a wavelength of 490 nm by using a Beckman DTX880 Multi Mode Microplate Reader (Beckman Coulter, Indianapolis, IN).

Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from differentiated cells by using ISOGEN II (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The first-strand cDNA was synthesized by using Super Script VILO cDNA Synthesis Kit (Thermo Fisher Scientific). The qRT-PCR was performed by a Thermal Cycler Dice Real Time System II TP900 (Takara Bio, Shiga, Japan) using Thunderbird SYBR qPCR mix (Toyobo, Osaka, Japan). The relative quantitative gene expression was determined by following the $2^{-\Delta\Delta}$Ct method and normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers used in this study were shown in Table 1.

Statistical Analysis

Data are presented as mean ± standard deviation. Statistical comparisons between non-differentiation and differentiation groups were performed by using the Student's t-test. Statistical comparisons between control-miR and miR-199a-3p, miR-126-3p, miR-33a were performed by Dunnett's test. The results were considered significantly different at $^{\dagger, *, \#}p < 0.05$ and $^{\dagger\dagger, **, \#\#}p < 0.01$.

RESULTS

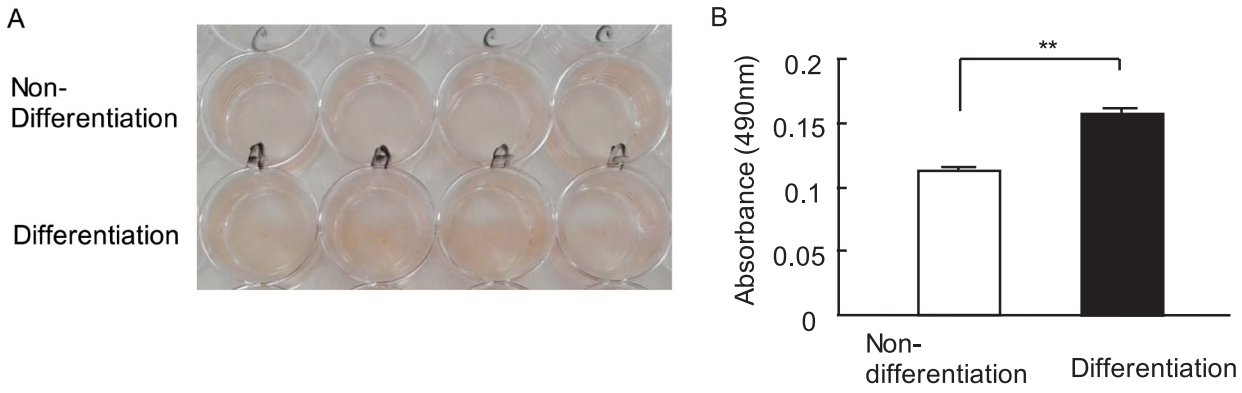
MiR-126-3p and miR-199a-3p enhance lipid deposition in 3T3-L1 adipocytes

We confirmed adipocyte differentiation from 3T3-L1 pre-adipocyte in our experimental condition. 3T3-L1 pre-adipocyte cells were stimulated with the differentiation medium including ROSI, INS, DEX, and IBMX for 3 days. Lipid accumulation, shown as the degree of oil red O staining in cells, was remarkedly increased in 3T3-L1 cells in the differentiation medium compared to cells in the non-differentiation medium (Suppl. Fig. 1). In the following experiments, we employed 3T3-L1 cells cultured in differentiation medium as the adipocyte.

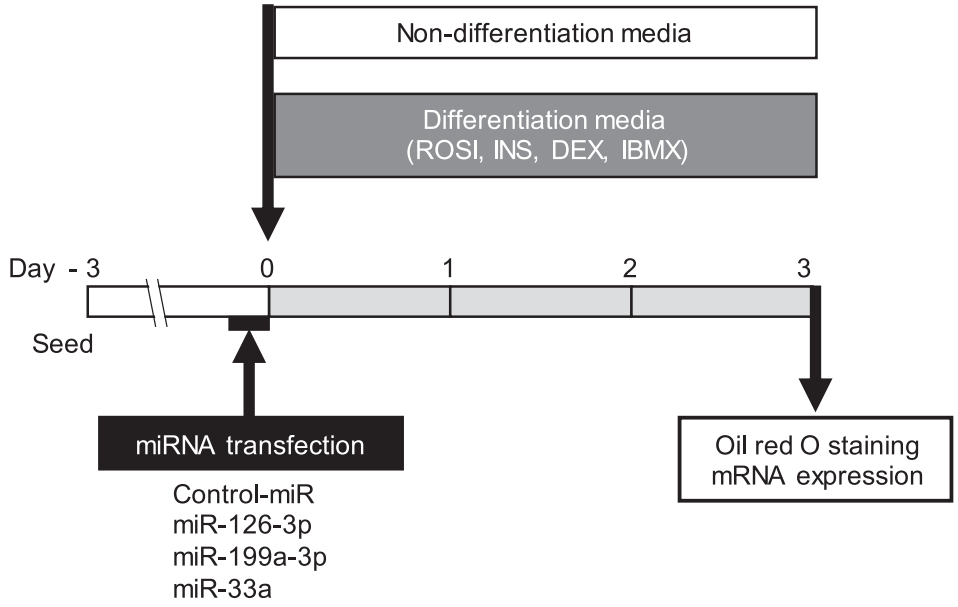
Next, we evaluated the effects of miR-126-3p and miR-199a-3p in lipid accumulation in 3T3-L1 adipocytes. We employed miR-33a as a positive control which promotes lipid deposition in hepatocytes and adipocytes [18, 19]. Before adipocyte differentiation, we transfected each miRNA mimic that intracellularly generates mature miRNA as same as endogenous miRNA in cells (Suppl. Fig. 2). The

Table 1. Primer sequences for qRT-PCR

Gene		Primer sequence
PPARγ	Forward	5'-GTACTGTCGGTTTCAGAAGTGC-3'
	Reverse	5'-ATCTCCGCCAACAGCTTCTCCT-3'
SREBP-1c	Forward	5'-ATCGCAAACAAGCTGACCTG-3'
	Reverse	5'-AGATCCAGGTTTCAGGTGGG-3'
CEBPα	Forward	5'-GTGTGCACGTCTATGCTAAACCA-3'
	Reverse	5'-GCCGTTAGTGAAGAGTCTCAGT-3'
PPARα	Forward	5'-ACCACTACGGAGTTCACGCATG-3'
	Reverse	5'-GAATCTTGCAGCTCCGATCACA-3'
HIF-1α	Forward	5'-CCTGCACTGAATCAAGAGGTTG-3'
	Reverse	5'-CCATCAGAAGGACTTGCTGGCT-3'
GAPDH	Forward	5'-AAGAAGGTGGTGAAGCAGGCATC-3'
	Reverse	5'-CGAAGGTGGAAGAGTGGGAGTTG-3'



Supplemental figure 1. Lipid deposition enhanced after adipocyte differentiation.
(A) Oil red O staining of 3T3-L1 cells after adipocyte differentiation. Lipid deposition increased in differentiated group (lower panel) compared with non-differentiated group (upper panel). Representative data from three independent experiments are shown. (B) Quantitative analysis of oil red O staining. The amount of lipid droplets in differentiation group (closed column) is increased compared with non-differentiation group (open column). Data are expressed as the mean ± SD. Significant difference **p < 0.01 was measured by student t test.



Supplemental Figure 2. Protocol of miRNAs transfection into 3T3-L1 cells.
Cells were seeded at day -3 and grown until day 0. Before differentiation, cells were transfected with control-miR, miR-126-3p, miR-199a-3p or miR-33a, by using Lipofectamine RNAi MAX reagent for 4 hours. Cells were then stimulated with differentiation media (ROSI, INS, DEX, IBMX) or non-differentiation media for 3 days. Lipid accumulation and mRNA expression in transfected cells were analyzed by oil red O staining and quantitative RT-PCR, respectively.

miRNA transfection did not alter the cell viability of 3T3-L1 cells (data not shown). After adipocyte differentiation, enhanced lipid accumulation was observed in transfection of miR-126-3p and miR-199a-3p compared to control-miR (Fig. 1A). The amount of lipid accumulation in cells transfected with miR-126-3p and miR-199a-3p were significantly increased as compared to cells transfected with control-miR (Fig. 1B). Interestingly, not only upon adipocyte differentiation, but also upon non-differentiation, miR-126-3p and miR-199a-3p promoted the lipid accumulation in 3T3-L1 cells.

MiR-126-3p and miR-199a-3p regulate lipid metabolism-related gene expression

To investigate the potential molecular mechanism of miR-126-3p or miR-199a-3p in lipid accumulation, we detected the mRNA expression of major adipogenic regulatory genes in 3T3-L1 cells. It has been well-known that increased expression of per-

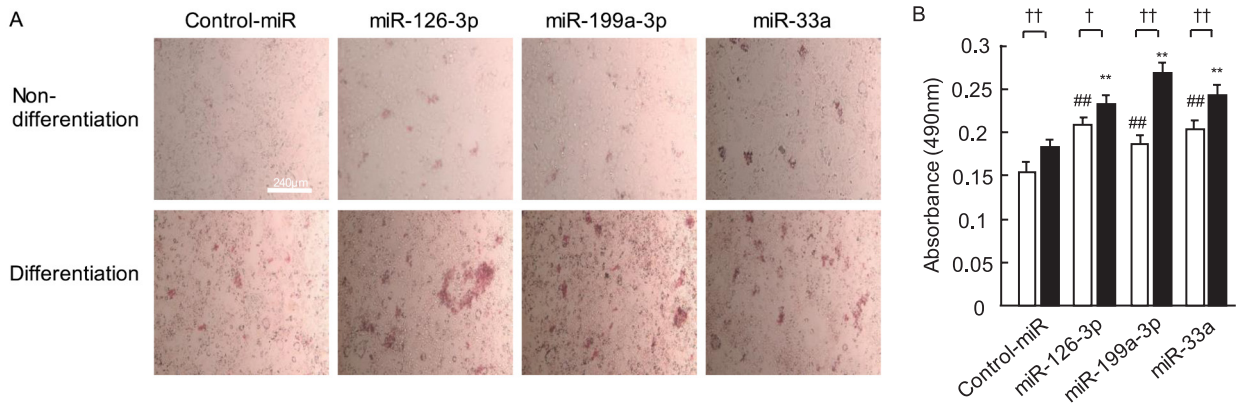


Figure 1. Effect of miR-126-3p and miR-199a-3p on lipid accumulation in 3T3-L1 adipocytes.
(A) After miRNAs transfection, 3T3-L1 pre-adipocytes were cultured in non-differentiation (upper panels) and differentiation media (lower panels). Intracellular lipid accumulation was visualized by oil red O staining. Representative data from three independent experiments are shown. Scale bars: 240 μm. (B) Quantitative analysis of oil red O staining. The amount of oil red O dye were measured by microplate reader. Data are expressed as the mean ± SD. Significant difference between non-differentiation and differentiation groups as shown as $^{\dagger}p < 0.05$ or $^{\dagger\dagger}p < 0.01$ were compared by Student's t-test. Statistical comparisons between control-miR and miR-199a-3p, miR-126-3p, miR-33a were performed by Dunnett's test. The results were considered significantly different at $^{*,\#}p < 0.05$ and $^{**,\#\#}p < 0.01$.

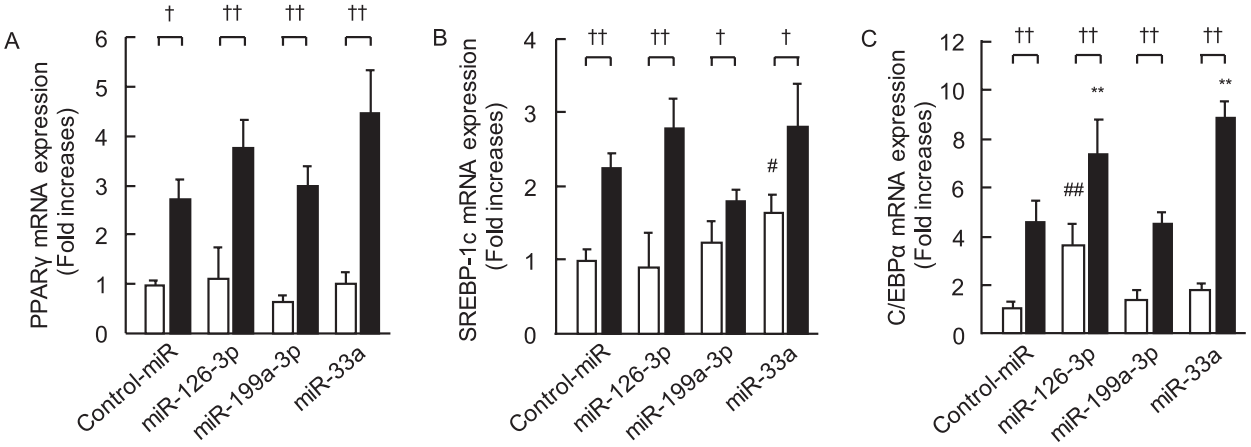


Figure 2. Effect of miR-126-3p and miR-199a-3p on lipid metabolism related gene expression.
After transfection of control-miR, miR-199a-3p, miR-126-3p, and miR-33a, 3T3-L1 pre-adipocytes were cultured in non-differentiation (open column) or differentiation media (closed column). PPARγ (A), SREBP-1c (B), and C/EBPα (C) mRNA expression were evaluated by qRT-PCR. Relative mRNA expression was normalized to GAPDH mRNA expression. Representative data from three independent experiments are shown. Data are expressed as the mean ± SD. Significant difference between non-differentiation and differentiation groups as shown as $^{\dagger}p < 0.05$ or $^{\dagger\dagger}p < 0.01$ were compared by Student's t-test. Statistical comparisons between control-miR and miR-199a-3p, miR-126-3p, miR-33a were performed by Dunnett's test. The results were considered significantly different at $^{*,\#}p < 0.05$ and $^{**,\#\#}p < 0.01$.

oxisome proliferator-activated receptor alpha and gamma (PPARα and PPARγ), sterol regulatory element binding protein 1c (SREBP-1c), and CCAAT/enhancer-binding protein-α (C/EBPα) in adipocytes contribute to lipid accumulation [20-22, 23]. We, therefore, investigated the alteration of the gene expressions after the treatment with miR-126-3p or miR-199a-3p on 3T3-L1 cells. Under the differentiation condition, PPARγ, SREBP-1c, and C/EBPα mRNA expression in each miRNA-transfected cell were increased more than them under the no-differentiation condition (Fig. 2), while PPARα mRNA expression was not detected in all groups under our experimental condition. MiR-126-3p transfection indicated the tendency of increased PPARγ and SREBP-1c mRNA expression, but these changes are not significant. Of note, miR-126-3p significantly enhanced C/EBPα mRNA expression in both no-dif-

ferentiation and differentiation conditions, compared to control-miRNA (Fig. 2C). While miR-199a-3p did not alter PPARγ, SREBP-1c, and C/EBPα mRNA expressions. Taken together, these results suggest that miR-126-3p promotes lipid accumulation via inducing C/EBPα mRNA expression in 3T3-L1 cells.

MiR-126-3p and miR-199a-3p decrease HIF-1α mRNA expression

To explore the mechanism by which miR-126-3p induces C/EBPα mRNA expression in cells, we searched target genes of miR-126-3p by using bioinformatics tools. According to Targetscan (http://www.targetscan.org), 18 genes were predicted as targets of miR-126-3p (Table 2). Among these genes, we focused on HIF-1α that implicates in not only the regulation of lipid metabolism in hepatocytes [24] but also the leukemic cell differentiation via directly interacting with C/EBPα [25]. Interestingly, HIF-1α is excluded as a candidate target of miR-199a-3p by using Targetscan, but miRSystem (http://mirsystem.cgm.ntu.edu.tw/) predicted. In addition, it has been also reported that HIF-1α is a direct target of miR-33a [26]. We, therefore, employed miR-33a as a control and tested whether miR-126-3p or miR-199a-3p down-regulate HIF-1α mRNA expression in our experimental conditions. Under the differentiation condition, HIF-1α mRNA expression in each miRNA-transfected cells were decreased than those under the no-differentiation condition (Fig. 3). Both miR-126-3p and miR-199a-3p suppressed HIF-1α mRNA expression in differentiation condition as well as miR-33a, compared to control-miRNA (Fig. 3). In addition, miR-126-3p transfection suppressed HIF-1α mRNA expression in no-differentiation condition. In contrast, miR-199a-3p did not alter the HIF-1α mRNA expression in no-differentiation condition. These results suggest that miR-126-3p or miR-199a-3p primarily reduced HIF-1α mRNA expression leading to lipid accumulation in differentiation condition.

Table 2. Predicted target genes for miR-126-3p

Gene name	Symbol
solute carrier family 7, member 5	SLC7A5
calmodulin regulated spectrin-associated protein 1	CAMSAP1
ADAM metallopeptidase domain 9	ADAM9
sprouty-related, EVH1 domain containing 1	SPRED1
v-crk avian sarcoma virus CT10 oncogene homolog	CRK
polo-like kinase 2	PLK2
insulin receptor substrate 1	IRS1
regulator of G-protein signaling 3	RGS3
DIP2 disco-interacting protein 2 homolog C (Drosophila)	DIP2C
integrin, alpha 6	ITGA6
syndecan 2	SDC2
protocadherin 7	PCDH7
EF-hand domain family, member D2	EFHD2
A kinase (PRKA) anchor protein 13	AKAP13
guanine nucleotide binding protein (G protein), alpha 13	GNA13
protein tyrosine phosphatase, non-receptor type 9	PTPN9
F-box protein 33	FBXO33
hypoxia inducible factor 1, alpha subunit	HIF1A

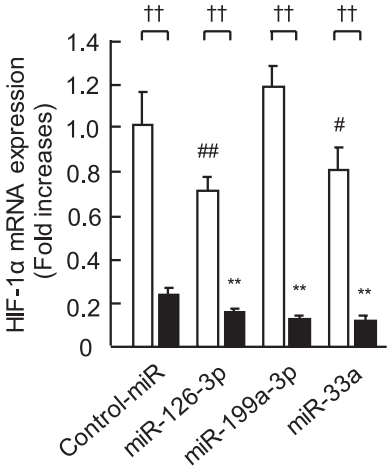


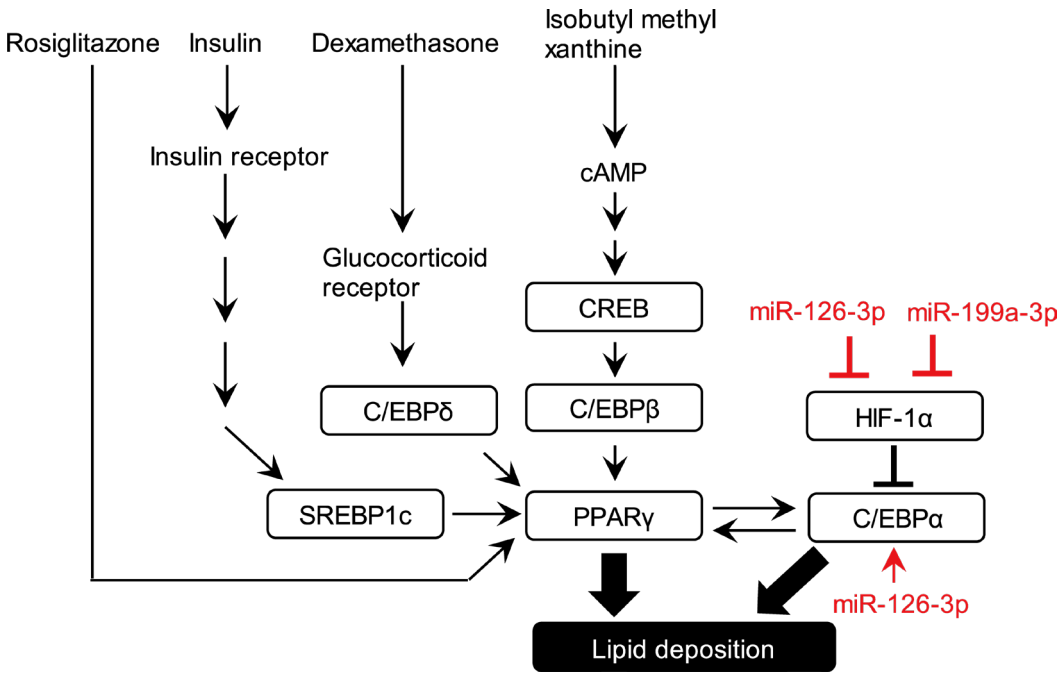
Figure 3. Effect of miR-126-3p and miR-199a-3p on HIF-1α mRNA expression. HIF-1α mRNA expression in 3T3-L1 cells cultured in non-differentiation (open column) or differentiation media (closed column) was measured by qRT-PCR. Relative mRNA expression was normalized to GAPDH mRNA expression. Representative data from three independent experiments are shown. Data are expressed as the mean ± SD. Significant difference between non-differentiation and differentiation groups as shown as $^{\dagger}p < 0.05$ or $^{\dagger\dagger}p < 0.01$ were compared by Student's t-test. Statistical comparisons between control-miR and miR-199a-3p, miR-126-3p, miR-33a were performed by Dunnett's test. The results were considered significantly different at $^{*,\#}p < 0.05$ and $^{**,\#\#}p < 0.01$.

DISCUSSION

Recently, miRNAs have been emerged as primary regulators of multiple NAFLD pathogenesis including lipogenesis, inflammation, fibrosis, and carcinogenesis. In our previous studies, we also identified the differential expression of miRNAs in the liver biopsy samples of NAFLD patients compared to healthy subjects. The result of our miRNA array analysis revealed several prominently up- or down-regulated miRNAs in NAFLD samples, however the functional targets and patho-/physiological roles of them remain unclear. Among them, we recently focused on miR-27b which is notably increased in liver and serum in patients with NAFLD and found a novel pathway that miR-27b promotes excessive lipid accumulation in adipocytes through the induction of ACOT2 expression [17]. In the present study, we, therefore, investigated the impact of other up-regulated miRNAs, miR-126-3p and miR-199a-3p, on lipid accumulation which is a primary step of NAFLD pathogenesis. Our data that miR-126-3p and miR-199a-3p promote lipid accumulation and alter lipid metabolism related gene expression suggests novel mechanisms of NAFLD pathogenesis (Suppl. Fig. 3).

MiR-126 is predominantly expressed in vascular endothelial cells and vascular smooth muscle cells and plays an important role in the physiological angiogenesis [27, 28]. MiR-126 enhances pro-angiogenic vascular endothelial growth factor (VEGF) signaling by targeting Spred1 [29] and phosphatidylinositol 3-kinase regulatory subunit beta implicated in the mitogen-activated protein kinase and phosphatidylinositol-3 kinase signaling [30]. In addition to angiogenesis, miR-126 contributes to anti-inflammatory effects in endothelial cells [31], cell proliferation [32], and remodeling in a hindlimb ischemia injury model in mice [33]. Although these studies also suggested that miR-126 might be involved in HIF-1α signaling and hypoxia-induced cellular responses in endothelial cells, however, the molecular interaction of them has not been elucidated.

In this study, we focused on up-regulated miR-126-3p in liver tissue of NAFLD patients. Exosome has been well-known as small extracellular vesicles that are available carriers of miRNAs in vivo [34]. Increases of miR-126-3p in liver is, therefore, considered due to endothelial cell-derived exosomes from blood vessels via circulating blood flow or interstitial fluid to liver tissue in patients. Our results demonstrated that miR-126-3p enhances lipid accu-



Supplemental figure 3. A schematic illustration of the predicted molecular mechanism underlying the lipid deposition promoted by miR-126-3p and miR-199-3p. MiR-126-3p and miR-199-3p mediated regulation of target gene expression might lead to adipocyte differentiation.

mulation and increased C/EBPα expression in 3T3-L1 cells. C/EBPα, is highly expressed in liver and adipose tissue, is one of the important transcription factors that involved in lipid metabolism, glucose metabolism, and adipogenesis [23, 35, 36]. It has been reported that C/EBPα-deficient mice diminish to accumulate lipids in adipocytes [37]. C/EBPα is also necessary to activate the PPARγ expression in differentiated adipocytes [38], while C/EBPβ and C/EBPδ enhance PPARγ expression [39]. Whereas PPARγ induces C/EBPα expression as well as other adipogenic genes [38]. Thus, it has been considered that these genes cooperate to accelerate lipid metabolism during adipogenesis. As shown in our data that miR-126-3p did not alter mRNA expression of PPARγ and SREBP-1c, further studies including adipogenic genes are required to elucidate the mechanism by which miR-126-3p-C/EBPα axis emerges excessive lipid accumulation in 3T3-L1 cells.

In addition, in order to explore targets mRNAs of miR-126-3p that involved in the regulation of C/EBPα expression and lipid metabolism in liver, we focused on HIF-1α predicted by bioinformatics tool Targetscan software. Numerous studies have demonstrated that HIF-1 family activation and hypoxia condition trigger lipid accumulation in NAFLD. It has been reported that hepatocyte-specific HIF-1α activation promotes alcohol-induced hepatomegaly and hepatic lipid accumulation, while hepatocyte-specific deletion of HIF-1α protects alcohol- and lipopolysaccharide-induced liver damage, hepatomegaly and lipid accumulation [40]. In contrary, the loss of the hepatic HIF-1α gene accelerated liver steatosis with enhanced lipid accumulation in choline-deficient diet-induced NAFLD [41]. These studies indicate the bi-functional role of HIF-1α on NAFLD pathology in vivo. Our in vitro data that HIF-1α mRNA expression remarkedly reduced during adipocyte differentiation and that pro-adipogenic miRNAs enhanced it suggests that HIF-1α reduction is closely associated to the promotion of lipid deposition in adipocytes. Although the molecular interaction between HIF-1α and C/EBPα are not completely understood during NAFLD pathogenesis, HIF-1-mediated down-regulation of C/EBPα in hypoxic condition is considered as an important mechanism for breast cancer cells development and leukemic cell differentiation. Thus, the miR-126-3p-HIF-1α-C/EBPα axis might be a novel molecular mechanism underlie lipid accumulation in hepatic cells and adipocytes, however, the specific mechanism needs to be further clarified.

MiR-199 has been shown to be implicated in various cancer development and progression [42], cardiomyocytes protection [43], or skeletal formation [44]. Recently, an abnormal abundance of miR-199a-3p was found in insulin-resistant and diabetic individuals [45]. In addition, it has been reported that miR-199a-3p is down-regulated in adipose tissue of the high-fat diet mice and in mouse hepatocyte cell line Hepa1-6 cell and that miR-199a-3p mimics decreased intracellular triglyceride contents both in vivo and in vitro [46]. Moreover, miR-199a-3p mimic suppressed adipocyte differentiation and triglyceride accumulation in 3T3-L1 adipocyte model [47]. These studies indicated that miR-199a-3p inhibits lipid deposition of adipocytes, with the opposite effect from our current data. In our experiment condition, PPARγ agonist ROSI was used for induction of adipocyte differentiation. It has been reported that PPARγ ligand as a molecular switch that controls its ability to modulate inflammatory responses in opposite directions [48]. Due to different experimental models with or without PPARγ activation might explain a paradoxical action of miR-199a-3p. Therefore, the correlation between PPARγ activity and miR-199a-3p action seems to be next question for understanding NAFLD pathogenesis.

CONCLUSION

Our study demonstrated the potential role of up-regulated miR-126-3p and miR-199a-3p in adipocyte lipid deposition of 3T3-L1 cells. MiR-126-3p and miR-199a-3p enhanced lipid accumulation in adipocytes by targeting HIF-1α, which facilitates adipocyte differentiation and might contribute to the pathogenesis of NAFLD.

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Conflict of Interest

All authors have nothing to disclose related to the content of this study.

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