学 位 論 文 の 要 旨

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学 位 論 文 名	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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論 文 内 容 の 要 旨

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. The emerged spectrum of NAFLD includes non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFLD is closely associated with visceral obesity, insulin resistance, hyperlipidemia, and hypertension, all of which shared risk factors with cardiovascular disease. 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, fibrosis, cirrhosis, and hepatocellular carcinoma. Therefore, 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 investigated the differential expression pattern of miRNAs between normal subjects and patients with NAFLD by using miRNA microarray analysis, however, the role of them remains unclear. Therefore, we have focused and investigated the impact of miR-126-3p and miR-199a-3p on the lipid accumulation in 3T3-L1 adipocytes. We also evaluated the effect of these miRNAs on lipid metabolism-related mRNA expression that might contribute to lipid accumulation.

MATERIALS AND METHODS

Mouse 3T3-L1 pre-adipocytes were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 10 μg/ml of streptomycin and were maintained at

37°C in a 5% CO_2. MirVana miRNA mimic mmu-miR-126-3p mimic, mmu-miR-199a-3p mimic, and mmu-miR-33a mimic, and negative control miRNA (control-miR) were transfected by using Lipofectamine™ RNAi MAX transfection reagent according to the manufacturer's instructions. After 4 hours of transfection cells were replaced with differentiation medium containing 1 μM rosiglitazone, 150 nM insulin, 1 μM dexamethasone, 100 μM 3-isobutyl-1-methylxanthine or non-differentiation medium and grown for 3 days. After adipocyte differentiation, cells were fixed with 200 μL of 4 % paraformaldehyde and stained with 500 μL of 0.2% oil red O.

Total RNA was extracted from differentiated cells and qRT-PCR was performed by a Thermal Cycler Dice Real Time System II TP900 using Thunderbird SYBR qPCR mix. The relative quantitative gene expression was determined by following the $2^{-\Delta\Delta}$Ct method and normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

RESULTS AND DISCUSSION

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. 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. 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. 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. Under the differentiation condition, peroxisome proliferator-activated receptor gamma (PPARγ), sterol regulatory element binding protein 1c (SREBP-1c), and CCAAT/enhancer-binding protein-α (C/EBPα) mRNA expressions in each miRNA-transfected cell were increased under the no-differentiation condition. Of note, miR-126-3p significantly enhanced C/EBPα mRNA expression in both no-differentiation and differentiation conditions, compared to control-miRNA. 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.

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, 18 genes were predicted as targets of miR-126-3p. Among these genes, we focused on HIF-1α that implicates in not only the regulation of lipid metabolism in hepatocytes but also the leukemic cell differentiation via directly interacting with C/EBPα. We also 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. 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. 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.

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, 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.

CONCLUSION

Our study demonstrated the potential role of up-regulated miR-126-3p and miR-199a-3p in adipocyte lipid accumulation 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.