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Original Paper

Insight into the Effects of Adipose Tissue Inflammation Factors on miR-378 Expression and the Underlying Mechanism

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Key Words
Adipose • Inflammation factor • microRNA • Mechanism

Abstract
Background/Aims: Obesity and the related metabolic syndrome have emerged as major public health issues in modern society. miRNAs have been shown to play key roles in regulating obesity-related metabolic syndrome, and some miRNAs regulated by adiponectin were identified as novel targets for controlling adipose tissue inflammation. miR-378 is a candidate target that was shown to be involved in adipose differentiation, mitochondrial metabolism and systemic energy homeostasis. However, little is known about the regulatory mechanisms of miR-378 expression. To better understand the physiological role of miR-378 in obesity and metabolic syndrome, it is crucial that we understand the regulation of miR-378 gene expression in human adipocytes. ***Methods:*** In this study, we investigated the effects of adipokines and inflammatory cytokines on miR-378 expression using Real-time PCR and the potential regulatory mechanisms using luciferase reporter assays and electrophoretic mobility shift assay (EMSA). ***Results:*** We found that adipokines and cytokines upregulated miR-378 expression primarily through SREBP and C/EBP binding sites in the miR-378 promoter region. ***Conclusion:*** Our findings showed that adipokines induced miR-378 expression and revealed the most likely mechanism of adipokine-induced miR-378 dysregulation in human adipocytes. miRNAs have been shown to function in regulating obesity-related metabolic syndrome, and miR-378 may be a novel target for controlling adipose tissue inflammation. This study offers a theoretical basis for understanding systemic adipose tissue inflammation and may provide new strategies for clinical treatment.

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Introduction

Obesity and the related metabolic syndrome have emerged as major public health issues in modern society. Traditionally, obesity has been viewed as the accumulation of adipocyte tissue due to energy imbalance and adipocyte differentiation. It is now widely recognized that chronic low-grade inflammation [1, 2] and oxidative stress [3], are involved in the initiation, propagation, and development of metabolic disorders [4]. Additionally, adipose tissue releases inflammatory cytokines and adipokines, such as leptin, resistin, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and adiponectin, which have been identified as major factors associated with obesity and metabolic syndrome [4-7].

MicroRNAs (miRNAs) are small noncoding RNAs (20~24 nt) that control gene expression by inducing target mRNA degradation or by blocking translation [8], and they are increasingly being recognized as viable therapeutic targets for a host of diseases. miRNAs have been proposed as therapeutic targets against cancer, HCV, HIV, and cardiovascular disease [9-12]. Recently, a growing body of evidence suggests that the deregulation of miRNAs is closely associated with obesity-related metabolic syndrome, including type 2 diabetes mellitus (T2DM) [13, 14]. Furthermore, some miRNAs regulated by adiponectin were identified as novel targets for controlling adipose tissue inflammation [15, 16].

miR-378 is a candidate target that was shown to be involved in adipocyte gene expression, lipogenesis, the control of mitochondrial metabolism and systemic energy homeostasis [17, 18]. miR-378 is encoded by the peroxisome proliferator-activated receptor γ coactivator 1β (*PGC-1β*) gene, which is a transcriptional coactivator that regulates metabolism and mitochondrial biogenesis and may counterbalance the metabolic actions of PGC-1β [19]. In 2010, Isabelle et al. reported that miR-378 stimulated the accumulation of triacylglycerol by specifically increasing the transcriptional activity of C/EBPα and C/EBPβ on adipocyte gene promoters [18]. In another study, Michele et al. reported that miR-378 was identified as an integral component of a regulatory circuit that functions under conditions of metabolic stress to control systemic energy homeostasis and the overall oxidative capacity of insulin target tissues [17]. However little is known about the regulatory mechanisms involved in miR-378 expression. To better understand the physiological role of miR-378 in obesity and metabolic syndrome, it is crucial that we understand the regulation of miR-378 gene expression in humans. In this study, we investigated the effects of adipokines and inflammatory cytokines on miR-378 expression and the potential regulatory mechanisms.

Materials and Methods

Cell Culture and Treatment

Human visceral preadipocytes (HPA-V; ScienCell Research Laboratories, USA) were maintained in preadipocyte medium (PAM; ScienCell Research Laboratories, USA), which contains 5 % fetal bovine serum, 1 % preadipocyte growth supplement, and 1 % penicillin/streptomycin solution, at 37 °C in a humidified atmosphere of 5 % carbon dioxide (CO_2). During the induced-differentiation phase, serum-free PAM (containing 50 nM insulin, 100 nM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 100 lM rosiglitazone) was added to confluent human preadipocytes (day 0), and the medium was replaced every 2 days for 4 days. Thereafter, the medium was replaced with serum-free PAM containing 50 nM insulin and replaced every 2 days until lipid droplets had accumulated in the cells (day 15); fat accumulation was evaluated by staining 4% paraformaldehyde-fixed cells with Oil Red O. Additionally, we collected the cells at different time points (day 0, 1, 4, 7, 10, 15). Differentiated adipocytes were used for experiments 15 days after the induction of differentiation, at which point over 80 % of the cells showed the morphological and biochemical properties of adipocytes. After an overnight incubation in serum-free PAM, human adipocytes were treated with a final concentration of 30 ng/ml IL-6 [20] (Sigma, St. Louis, MO, USA), 10 ng/ml TNF-α [21] (Sigma, St. Louis, MO, USA), 1μg/ml FFA [22] (Sigma, St. Louis, MO, USA), or 30 ng/ml leptin [23] (Sigma, St. Louis, MO, USA). Human embryonic kidney 293T (HEK293T) cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's

Table 1. The sequences of oligonucleotide primers used in promoter region PCR

Promoter region	Product size (bp)	Forward and Reverse primers (5'-3')
Pro1 (-698bp to -404bp)	295	F: GGCAGGTCCCAGCCTCAGTA
		R: TTTGCCCTTTAGCCGCCCAG
Pro2 (-403bp to -94bp)	310	F: CGAGCCTAGCTAGCAGAAAT
		R: AGTGAGCGGCTTGTATGGGA
miR-378 (-698bp to -94bp)	605	F: GGCAGGTCCCAGCCTCAGTA
		R: AGTGAGCGGCTTGTATGGGA
miR-378/SREBP (-295bp to -94bp)	202	F: ATTTAACTGAATGACAGGCC
		R: AAAAAGCAAATGTCCCAGATT

medium (DMEM) supplemented with 10% fetal bovine serum. HEK293T cells were also incubated at 37 °C under a humidified atmosphere of 5% CO_2.

RNA Isolation and Quantitative Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA from human adipocytes was extracted and purified using the TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. The integrity of the RNA was assessed by agarose gel electrophoresis and measured by spectrophotometry at 260 nm. To monitor the levels of miRNA, cDNA was synthesized from 200 ng of total RNA using a TaqMan microRNA Reverse Transcriptase Kit (ABI, Foster City, CA, USA). qRT-PCR was performed using an Applied Biosystems 7500 Sequence Detection System (ABI 7500 SDS, USA) following the manufacturer's guidelines. Briefly, samples were incubated at 95 °C for 10 min for an initial denaturation, followed by 40 PCR cycles of incubation at 95 °C for 15 s and then 60 °C for 1 min. miRNA expression was normalized to snRNA U6 and miR-103 [16]. Primer identification numbers are 000567 for hsamiR- 378, 000439 for miR-103, and 001973 for snRU6 (ABI, Foster City, CA, USA). To detect mRNA expression levels, cDNA was synthesized from 500 ng of total RNA using a High-Capacity cDNA Reverse Transcriptase Kit (ABI, USA). The qRT-PCR primer sequences are as follows: *PGC-1β* forward, 5'-TGACTCCGAGCTCTTCCAG-3'; *PGC-1β* reverse, 5'-CGAAGCTGAGGTGCATGATA-3' [24]; GAPDH forward, 5'-CGGGTCGGGAGTGGGTAAT-3'; GAPDH reverse, 5'-AGTCGCCGTGCCTACCAT-3'. Data analysis was performed using the comparative CT method [25].

Plasmids and Promoter Reporter Constructs

We examined the upstream region of miR-378 using the UCSC genome browser (http://genome.ucsc.edu/). The two predicted promoter regions (pro1 and pro2) and the mutated form of the SREBP binding site of miR-378 (miR-378/SREBP: 5'-ATTTAACCACATGAC-3' mutated into 5'-ATTTAACTGAATGAC-3') were amplified by PCR from human genomic DNA using the primer sets shown in Table 1. We reconstructed the pTA-Luc vector (Clontech, Mountain View, CA, USA) to form the vector pTB-Cherry. Then, the PCR products were subcloned into the pTB-Cherry vector using Nsi I/Xho I restriction sites (all constructs were confirmed by sequencing).

Luciferase Reporter Assays

HEK293T cells were grown to a density of 60–70 % confluence in 6-well plates and then were transiently transfected with 2 μg of reporter plasmids per well with Lipofectamine 2000 (Invitrogen, USA) (using empty pTB-Cherry plasmid as a control). The upstream fragments induced the Cherry gene to express red fluorescence protein, and the promoter activity was determined by co-transfection of the pGL3-basic promoter reporter (250 ng/well) with 25 ng/well pRL-TK (Renilla luciferase plasmid; Promega, Madison, Wisconsin) into HEK293T cells in 48-well plates. At 48 h post-transfection, the cells were harvested and cell lysates were prepared using a dual-luciferase reporter assay system according to the manufacturer's protocols (Promega) with a Luminometer (Turner Biosystems, USA). Firefly luciferase activity was normalized to Renilla luciferase activity. Furthermore, we detected the transcriptional activity of pro1, pro2, and miR-378 (PGL-3 basic as a control) with treatment of Rosiglitazone, TNF-α, IL-6, or In5 separately.

EMSA analysis

The two predicted promoter regions (pro1 and pro2) and the mutated forms of SREBP or C/EBP binding sites of miR-378 (SREBP: 5'-TTTATTTAACCA**CATG**ACAGGCCGAG-3', mutated into:

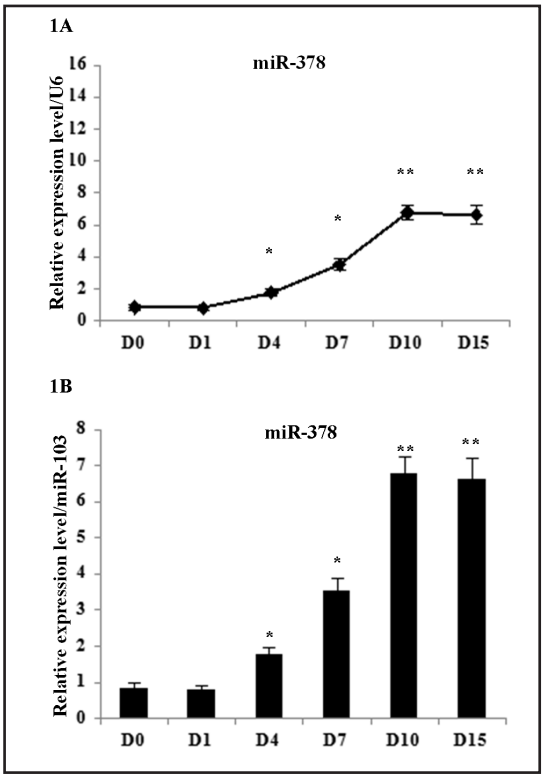

Fig. 1. We collected cells at different time points (day 0, 1, 4, 7, 10, 15) and determined the expression levels of miR-378 (snRU6 was used as the internal control). The data showed that miR-378 was upregulated over time (Fig. 1A). To verify the expression pattern of miR-378, miR-103 was used for normalization, and the results were consistent with the data using snRU6 for normalization (Fig. 1B). *p < 0.05, **p < 0.01. All experiments were performed independently in triplicate (minimum).

5'-TTTATTTAACCA**TTTT**ACAGGCCGAG-3', positive control: 5'-TTTGAAAATCACCCCATGCAAACTC-3'; C/EBP: 5'-CCCAAC**TTGGGAAAT**GTAATT-3', mutated into 5'-CCCAAC**TTTTTTTTT**GTAATT-3', positive control: 5'-CCTTACCTTTTAGTCTTTCAACAAACT-3') were amplified by PCR from human genomic DNA using the primer sets shown in Table 1. The SREBP or C/EBP binding activity was detected using an EMSA Kit (Roche, Switzerland) according to the manufacturer's protocol. The preformed probes were set as the positive control. For the supershift assay, anti-SREBP or C/EBP antibody was added during preincubation. The visualized bands were analyzed using a BioSens Gel Imaging System (BIOTOP, China). For the competition experiment, 100-fold specific oligonucleotide competitor (unlabeled probe) was added to the binding mixture, and the reaction continued 10 min before the addition of the labeled probe. For mutant detection, 100-fold mutated oligonucleotide was added to the binding mixture, and the reaction continued 10 min before the addition of the labeled probe.

Statistical Analysis

Data are presented as the mean ± SEM. Statistical analysis was performed using one-way ANOVA. Differences between groups with $p < 0.05$ were considered statistically significant. *p < 0.05; **p < 0.01. All experiments were performed independently in triplicate (at minimum).

Results

miR-378 expression pattern during adipose differentiation

To determine the expression pattern of miR-378 during adipose differentiation in human adipocytes, we collected the cells at different time points (day 0, 1, 4, 7, 10, 15) and determined the expression levels of miR-378 using Real-time PCR (snRU6 was used as the internal control). The data showed that miR-378 was upregulated over time (Fig. 1A). To verify the expression pattern of miR-378, miR-103 was used for normalization, and the results were consistent with the data using snRU6 for normalization (Fig. 1B).

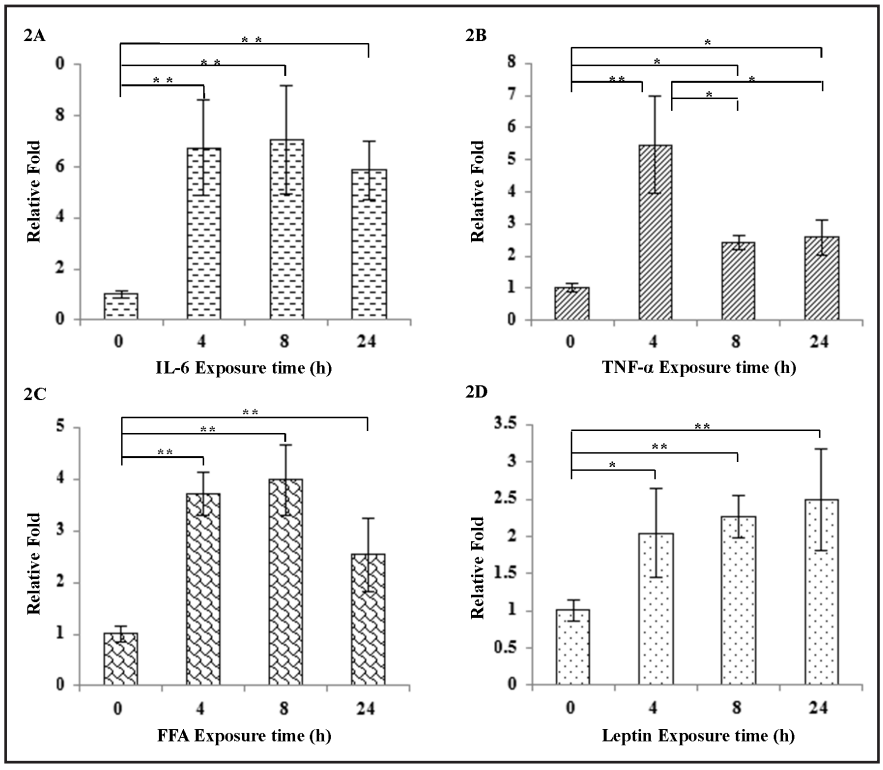

Fig. 2. Mature adipocytes were treated with 30 ng/ml IL-6, and miR-378 expression level was examined at different time points (0, 4, 8, 24 h) and normalized to snRU6 expression. We observed that miR-378 expression was significantly elevated with IL-6 (Fig. 2A), TNF-α (Fig. 2B), FFA (Fig. 2C) or leptin (Fig. 2D) treatment. *p < 0.05, **p < 0.01. All experiments were performed independently in triplicate (at minimum).

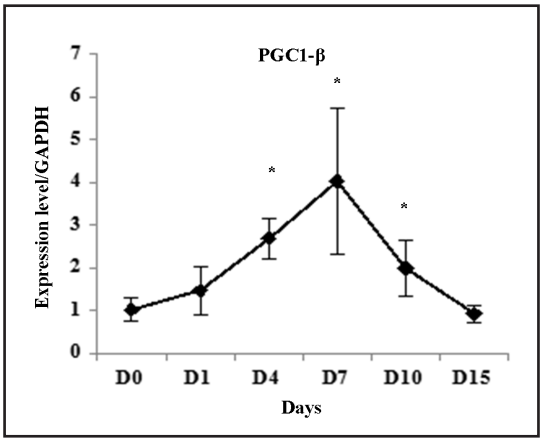

Fig. 3. We determined *PGC-1β* mRNA expression by real-time PCR. *PGC-1β* expression was evaluated from D4 to D10, and the expression peaked at D7 during adipogenesis. *p < 0.05; **p < 0.01. All experiments were performed independently in triplicate (at minimum).

The effects of IL-6, TNF-α, Leptin and FFA on miR-378 expression in Human Adipocytes

Mature adipocytes were treated with IL-6, and miR-378 expression was examined at different time points (0, 4, 8, 24 h) and normalized to snRU6 expression. We observed that miR-378 expression was significantly elevated 4 h following IL-6 exposure, with approximately 6.75-fold greater expression than the controls (Fig. 2A). miR-378 expression diminished at 24 h but, nevertheless, remained significantly upregulated compared to the control. To assess the effect of TNF-α treatment on miR-378 expression, mature adipocytes were incubated with TNF-α. We observed that TNF-α significantly upregulated miR-378 (Fig. 2B). miR-378 expression was increased 5.47-fold at 4 h, and miR-378 expression significantly diminished at 8 h and 24 h compared to 4 h but remained significantly upregulated compared to the control. Based on our results, FFA and leptin also had a significant effect on the expression of miR-378. When the cells were cultured with FFA, we detected a 3.7-fold upregulation of miR-378 expression at 4 h and a 2.5-fold increase at 24 h (Fig. 2C). In addition, the level of miR-378 was increased by approximately 2-, 2.3-, and 2.5-fold at the different time points following treatment of the adipocytes with leptin (Fig. 2D).

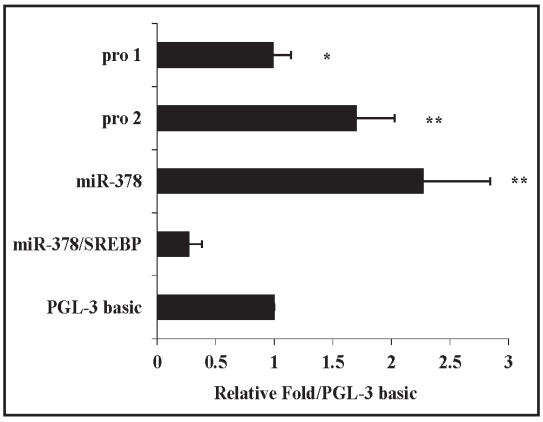

Fig. 4. We cloned two fragments from a 3-kb upstream region of hsa-miR-378 into the pTB-Cherry vector to generate pro1–pTB-Cherry and pro2–pTB-Cherry plasmids. The results indicated that compared to PGL3-basic, pro1 had no effect on inducing gene expression, while pro2 (-403bp to -94bp) induced the expression of the downstream mCherry gene because full-length pro1 and pro2 (miR-378) showed increased promoter activity (Fig. 4). Then, a mutated form of the predicted SREBP binding site (miR-378/SREBP) was constructed in the pTB-Cherry vector, and the data showed that this mutant inhibited the promoter activity (Fig. 4). *p < 0.05; **p < 0.01. All experiments were performed independently in triplicate (at minimum).

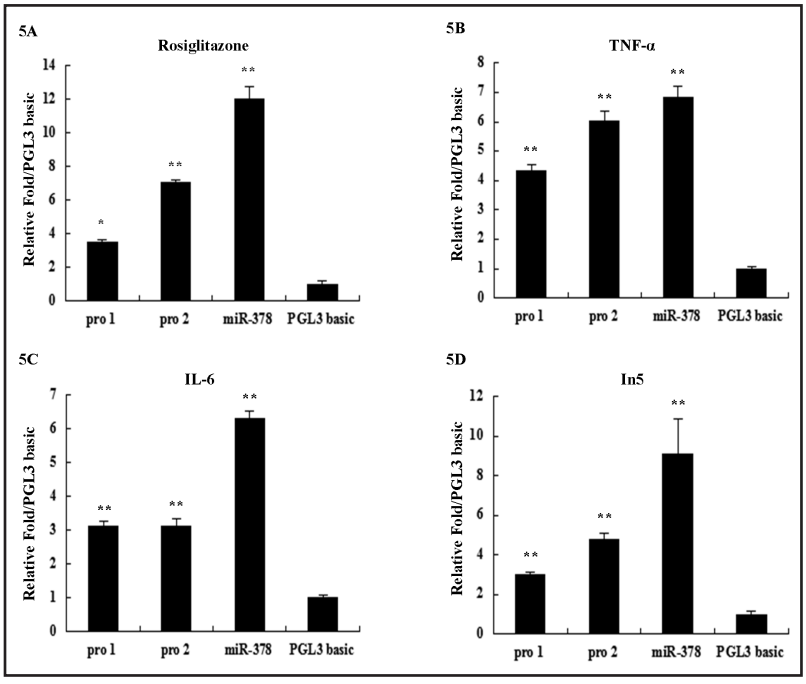

Fig. 5. We separately examined the activity of the constructs pro1, pro2, miR-378, miR-378/SREBP, and PGL3-basic following Rosiglitazone, TNF-α, IL-6, and In5 treatment in HEK293T cells and found that pro1, pro 2 and miR-378 showed increased luciferase activity compared to PGL3-basic (Fig. 5). Interestingly, pro2 exhibited higher luciferase activity than pro 1 but lower than miR-378. *p < 0.05; **p < 0.01. All experiments were performed independently in triplicate (at minimum).

The Host Gene PGC-1β Expression is not Correlated with miR-378 in Human Adipocytes

miR-378 is an intronic miRNA encoded by the *PGC-1β* gene, and some intronic miRNAs are transcribed together with the host gene. Therefore, we determined *PGC-1β* mRNA expression using real-time PCR. Interestingly, *PGC-1β* expression was not correlated with the levels of miR-378 during adipogenesis (Fig. 3).

Identification of Promoter Regions of miR-378

To explore whether miR-378 has its own transcription unit, we subcloned two fragments from a 3-kb upstream region of hsa-miR-378 into the pTB-Cherry vector to generate pro1–pTB-Cherry and pro2–pTB-Cherry plasmids. We detected promoter activity in transfected HEK293T cells. The results indicate that compared to PGL3-basic, pro1 had no effect on inducing gene expression, while pro2 (-403 bp to -94 bp) induced the expression of the downstream mCherry gene because full-length pro1 and pro2 (miR-378) showed increased promoter activity (Fig. 4). Then, a mutated form of the predicted SREBP binding site (miR-378/SREBP) was constructed in the pTB-Cherry vector, and the data showed that

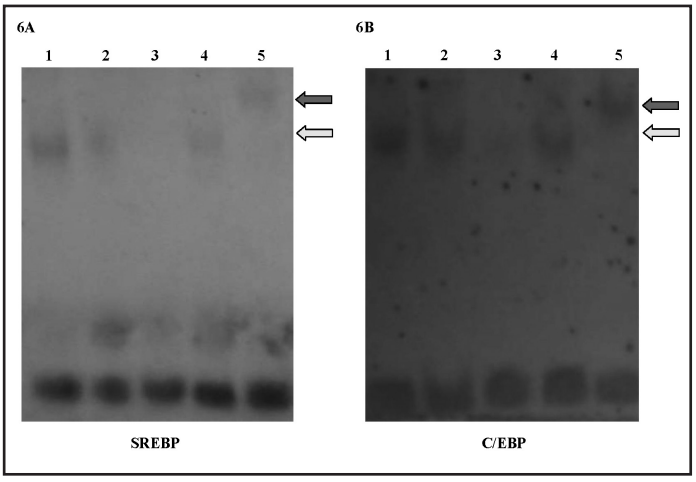

Fig. 6. To confirm the transcription factor binding sites, which are predicted to exist in the promoter region of miR-378, we performed an EMSA experiment. The data showed that Fig. 6A, Line1: positive control; line 2: sample; line 3: 100-fold unlabeled probe as a negative control; line 4: 100-fold labeled mutated-probe for the competition experiment; line 5: labeled probe and anti-SREBP antibody. Fig. 6B, Line1: positive control; line 2: sample; line 3: 100-fold unlabeled probe as a negative control; line 4: 100-fold labeled mutated-probe for the competition experiment; line 5: labeled probe and anti-C/EBP antibody. The white arrow indicates the complex of the DIG-ddUTP-labeled oligonucleotide probe and SREBP or C/EBP. The black arrow indicates the oligonucleotide probe-labeled protein-SREBP or the protein-C/EBP antibody complex.

this mutant inhibited the promoter activity compared with the wild-type miR-378 (Fig. 4). We also examined the activity of these constructs by separate treatment with Rosiglitazone, TNF-α, IL-6, and In5 in HEK293T cells and observed that pro1, pro 2 and miR-378 showed increased luciferase activity compared to PGL3-basic (Fig. 5). Interestingly, pro2 showed higher luciferase activity than pro 1 but lower than miR-378, which suggests that pro2 may be the major promoter of miR-378 and that there may be enhancers in pro1. Taken together, pro2 may be the major promoter of miR-378, and adipokines, such as Rosiglitazone, TNF-α, IL-6, and In5, may induce miR-378 promoter activity.

Confirmation of SREBP or C/EBP binding sites of miR-378

Previously, we predicted that transcription factor binding sites existed in the promoter region of miR-378 and confirmed this prediction by performing an EMSA experiment. In this study, we constructed separate wild-type and mutated probes (labeled with biotin) of SREBP or C/EBP and preincubated them with the binding mixture for 10 min before the addition of the labeled probe. The data showed that both of the wild-type oligonucleotides conjugated to the target protein, while the mutated form did not bind to the target (Fig. 6). These results indicate that there are identified SREBP and C/EBP binding sites in the promoter region of miR-378.

Discussion

A growing body of evidence suggests that the levels of adipokines, such as IL-6, TNF-α, leptin and FFA correlate with adipocyte differentiation. The dysregulation of these adipokines has been observed in obesity, type 2 diabetes, and cardiovascular diseases and an extended list of pathological changes to organs [26]. Recently, the dysregulation of miRNAs, including miR-378, was shown to closely associate with systemic energy homeostasis and the overall oxidative capacity of insulin target tissues [17]. However, little is known about the regulatory mechanisms involved in miR-378 expression. To better understand the physiological role of miR-378 in obesity and metabolic syndrome, it is crucial that we understand the regulation of miR-378 gene expression in humans. In this study, we investigated the effects of adipokines and inflammatory cytokines on miR-378 expression and the potential regulatory mechanisms. Interestingly, we observed that IL-6, TNF-α, FFA, and leptin upregulated miR-378 expression in human mature adipocytes.

We first examined the expression pattern of miR-378 during adipocyte differentiation and found that miR-378 was upregulated over time compared with the endogenous control snRU6 (Fig. 1A). In addition, miR-103 has been shown to be the most stable miRNA transcript across all biological backgrounds in human adipose tissues derived from different origins [27]. Therefore, miR-103 was also used for normalization in this study, and the results were consistent with those using snRU6 (Fig. 1B).

Increased IL-6 secretion has traditionally been thought to be induced by obesity, and IL-6 has been suggested as a novel link between obesity and metabolic syndrome [28]. Stouthard et al reported that increased glucose transport was observed in 3T3-L1 adipocytes following IL-6 treatment [29] and that IL-6 may lead to mitochondria dysfunction by inducing oxidative stress [30]. In addition, IL-6 has been shown to regulate adipocyte-related microRNA expression, such as the expression of miR-335 [16]. In this study, we explored the most likely effect of IL-6 on miR-378 during adipocyte differentiation, and we observed that IL-6 induced miR-378 expression in human mature adipocytes. While Maarten et al found that miR-378 specifically increased the transcriptional activity of C/EBP on adipocyte gene promoters [18] and multiple studies have reported that C/EBP regulated IL-6 expression [31], it is possible that IL-6-induced miR-378 expression may create a feedback loop.

TNF-α is the first identified proinflammatory cytokine that provided a link between obesity, chronic inflammation, and insulin resistance [32]. Tili et al reported that TNF-α inhibited 3T3-L1 adipocyte differentiation by inducing miR-155 expression [33], and a several reports showed that TNF-α induced mitochondrial dysfunction in adipocytes, cardiac myocytes, and muscle [34-36]. Here, we treated human mature adipocytes with TNF-α and found that TNF-α increased miR-378 expression during adipocyte differentiation. Based on the known functions of miR-378 in adipocyte gene expression, namely the control of mitochondrial metabolism and systemic energy homeostasis [17, 18], TNF-α may induce mitochondrial dysfunction in adipocytes by increasing miR-378 expression. Further studies are needed to identify the possible link between miR-378 and TNF-α-induced mitochondrial dysfunction.

Leptin is one of the most important adipocyte-derived hormones and helps regulate energy intake and metabolism [37]. It is well known that chronically elevated leptin levels are associated with obesity, inflammation-related diseases, and overeating. Recent studies have focused on the relationship between leptin and microRNAs in various diseases. Hamrick et al. demonstrated that leptin increased skeletal muscle mass by altering skeletal muscle miRNA expression profiles [38]. In another study, Zhu et al identified that leptin significantly increased miR-335 expression during adipocyte differentiation [16]. miR-378 expression levels were also elevated in human mature adipocytes after leptin exposure.

FFA is found in organisms and originates from the breakdown of triglycerides. FFA-induced insulin resistance may lead to type 2 diabetes and other cardiovascular risk factors in obesity. In 2008, Lovis et al demonstrated that the rise of miR-34a and miR-146 contributes to FFA-induced pancreatic β-cell dysfunction [39], and another study found that FFA inhibited the expression of tumor suppressor phosphatase and tensin homolog (PTEN) via miR-21 upregulation in hepatocytes [40]. In this study, we found that FFA increased miR-378 expression during adipocyte differentiation, suggesting some underlying mechanisms involving the FFA-induced miR-378 upregulation.

Proinflammatory cytokines, such as TNF-α, IL-1b, and IL-6, have been shown to play important roles in adipocyte biology [30]. Furthermore, there may be crosstalk among these factors during adipocyte differentiation. For example, elevated TNF-α levels may increase IL-6 and MCP-1 secretion in preadipocytes, and IL-6 exposure may inhibit the expression of adiponectin, resistin, glucose transporter type (GLUT)-4, and IRS-1 [41].

Our data show for the first time that these adipokines induce pronounced effects on miR-378 expression. To deduce the underlying mechanism for how adipokines regulate miR-378, we analyzed the genomic features of regions surrounding miR-378 and found that miR-378 was encoded within the gene *PGC-1β* (human chromosome 5q32). PGC-1β is a transcriptional coactivator that regulates metabolism and mitochondrial biogenesis, and

miR-378 may counterbalance the metabolic actions of PGC-1β [19]. Our results indicate that PGC-1β mRNA expression was clearly upregulated on day 7 during adipocyte differentiation, which was inconsistent with the miR-378 expression profile. Based on previous studies, some intronic microRNA transcription (such as miR-378) is initiated by promoters that are independent of their host genes [19, 42]. We speculated that adipokines may regulate miR-378 transcription through an independent mechanism. Therefore, we subcloned and identified the potential promoter of miR-378 within the intron of the *PGC-1β* gene. Our results showed that the upstream region (-698bp to -94bp) of miR-378 contained its promoter and that there may be enhancers in this region (-403bp to -94bp). Interestingly, the promoter activity was upregulated by adipokines in HEK293T cells. The region of -403 bp to -94 bp analyzed by TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) showed potential transcription factor binding sites (TFBS) for SREBP and C/EBP. To confirm this hypothesis, we performed an EMSA experiment. We constructed wild-type and mutated probes of the predicted binding sites for either SREBP or C/EBP, and we showed that both SREBP and C/EBP bound to the wild-type probes, indicating that these two transcriptional factors may regulate the expression of miR-378 by binding to sites within the miR-378 promoter. Our results show for the first time that adipokines and cytokines may induce miR-378 expression primarily through SREBP and C/EBP binding sites in the miR-378 promoter region in human adipocytes. Further studies will be needed to confirm this mechanism.

In conclusion, our findings showed that adipokines induced miR-378 expression, demonstrating the most likely mechanism of adipokine-induced miR-378 dysregulation in human adipocytes. miRNAs have been shown to function in regulating obesity-related metabolic syndrome, and miR-378 may be a novel target for controlling adipose tissue inflammation. This study offers a theoretical basis for understanding systemic adipose tissue inflammation and may provide new strategies for clinical treatment.

Acknowledgments

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