

Original Research Article

Regulation of MicroRNA-378 expression in mature human adipose tissue cells by adiponectin, free fatty acids and dexamethasone

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Abstract

Purpose: To investigate the effects of adiponectin (ADPN), free fatty acids (FFAs), growth hormone (GH), and dexamethasone (DEX) on miR-378 expression in human adipose tissue cells, and their influence on regulation of obesity and insensitivity to insulin.

Methods: Human pre-adipocytes were cultured and differentiated. Adipocytes were treated with ADPN, FFAs, GH and DEX. RNA was isolated and quantified by real-time polymerase chain reaction (RT-PCR).

Results: Stimulation with FFA led to significant up-regulation of the expression of MiR-378 (approximately 3.8-fold) relative to control at the 4th hour ($p < 0.01$) in human mature adipose tissue cells. The expression of MiR-378 was increased almost 1.5-fold by ADPN within 24 h, relative to untreated control ($p < 0.05$).

Conclusion: The results of this study demonstrate that miR-378 expression is influenced by FFAs, ADPN, and DEX, the interaction of which may be involved in the pathogenesis of obesity-induced insensitivity to insulin. Thus, miR-378 is a potential biomarker for predicting the risk of complications, especially insulin resistance in obesity

Keywords: MiR-378, Adipocytes, Adiponectin, Free fatty acids, Growth hormone, Dexamethasone, Obesity, Insulin resistance

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INTRODUCTION

The typical feature of obesity is body fat in excess of what is normal, arising from imbalance between energy intake and energy expenditure [1]. Childhood and adult obesity are two of the most critical threats to health in recent decades [2,3]. Obesity is caused by several factors, and its pathogenesis involves interplay of social,

genetic and environmental considerations [4]. A plethora of evidence point to the important roles of genetic factors in the etiology of obesity. It has been reported that ADPN delays and suppresses diabetes-associated metabolic derangements [5]. It was recently demonstrated that the adipose tissue of obese individuals contains excess levels of FFAs, a situation considered important in obesity and insulin resistance [6]. However,

the bases of the influences of ADPN, FFAs, GH and DEX on insulin sensitivity and obesity remain unclear.

MicroRNAs (miRNAs) are small (19~22 nucleotides), double-stranded RNAs that repress translation and/or destabilize mRNA [7]. One of these miRNAs, miR-378 is involved in the regulation of lipid metabolism [8,9]. In this study, the effects of ADPN, FFAs, GH and DEX on miR-378 expression in mature adipocytes were investigated with a view to unraveling the influence of miR-378 on the etiology of insulin insensitivity.

EXPERIMENTAL

Human pre-adipocytes culture and differentiation

Human pre-adipocytes (ScienCell Research Laboratories) were cultured in Pre-adipocyte Medium (PAM; ScienCell Research Laboratories) containing 5 % fetal bovine serum (FBS), 1 % pre-adipocyte growth supplement (PAGS), and 1 % streptomycin/penicillin solution (S/P) at 37 °C in a humidified atmosphere with 5 % CO₂. For induction of differentiation, confluent cells (day 0) were maintained in serum-free PAM (SF-PAM) to which 100nM DEX, 50 nM insulin, 100 µM rosiglitazone and 0.5 mM 3-isobutyl-1-methylxanthine were added. The culture medium was replaced twice within four days at 48h-intervals. Subsequently, it was replaced with SF-PAM to which 50 nM insulin was added. The medium was renewed every 48h until the appearance of fat droplets on the 15th day. Lipid production was measured by Oil Red O staining.

Treatment with ADPN, FFAs, GH and DEX

On day 15 following differentiation, the mature human adipocytes were incubated overnight in SF-PAM and thereafter treated with 3 µg/mL ADPN (Abcam), 1 mmol/L FFA mixture containing lauric, myristic, oleic, linoleic and arachidonic acids (Sigma); 10 ng/mL GH (Sigma) and 1 mM DEX (Biosharp). Three durations of exposure were used i.e. 4h, 8h and 24 h. After each period of exposure, the cells were harvested and used for further investigations [10,11].

RNA extraction and quantitation with RT-PCR

The extraction of total RNA was carried out with TRIzol reagent (Invitrogen). The amount of extracted RNA was determined spectrophotometrically using One Drop Spectrophotometer (Qite) with appropriate

standards. Relative fold-increases in expressions were computed using comparative CT procedure. Total RNA (200ng) was used to synthesize complementary DNA (cDNA) using TaqMan microRNA Reverse Transcriptase Kit (ABI) according to manufacturer's protocol. Applied Biosystems 7500 Sequence Detection System was used for Real-time RT-PCR as specified in the manufacturer's manual. Initial denaturation was effected by incubation of samples at 95 °C for 10 min, and thereafter with 40 cycles of PCR for 15 sec at 95 °C, and for 60 sec at 60 °C. All reactions were carried out in triplicate. Relative expressions were calculated by 2^{-ΔΔCt} procedure [12].

Statistical analysis

One-way analysis of variance (ANOVA) was used for data analysis with version 16 of SPSS. The results are expressed as mean ± SE. Values of $p < 0.05$ were taken as indicative of statistically significant difference.

RESULTS

Effect of ADPN on expression of miR-378

Figure 1 shows the differentiation of human pre-adipocytes into mature adipocytes. The results on Figure 2 show the effect of 3 µg/mL ADPN treatment at 4, 8 and 24 h on expression of miR-378, 15 days post-differentiation. Exposure to ADPN provoked time-determined increases in miR-378 expression, which continued up to 24 h. At 24 h, the expression of miR-378 in the ADPN-treated adipocytes was almost 0.5-fold higher than in the control ($p < 0.05$, Figure 2A).

Effect of FFAs on expression of miR-378

The effect of FFAs on miR-378 expression in cultured human adipocytes was analyzed using qPCR treatment. The adipose tissue cells were exposed in culture to 1 mM FFA cocktail. The results on Figure 3 A indicate that miR-378 expression at 4 h was up-regulated almost 3.8-fold relative to the control group by FFA ($p < 0.01$). The up-regulation continued up to 8 h and subsequently, the expression began to decrease, although it still showed statistical significance up to 24 h.

Effects of GH on expression of miR-378 in mature human adipose tissue cells

There were no significant differences in miR-378 expression between GH-treated and untreated groups (Figure 4).

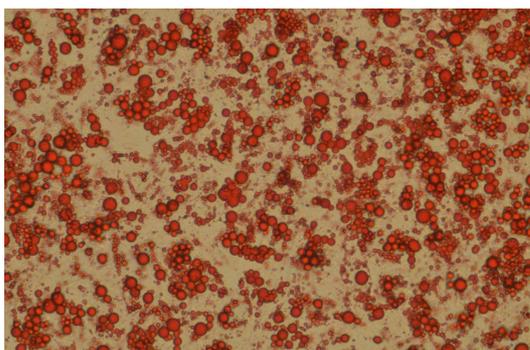


Figure 1: Micrograph of differentiated human pre-adipocytes (day 15), showing fat droplets. (Oil Red O stain)

Effect of DEX on expression of miR-378 in mature human adipose tissue cells

The expression of miR-378 was increased almost 1.5 folds after 24 h by 1mM DEX, relative to control ($p < 0.05$, Fig. 5A).

MiR-103 as an endogenous control

The stable microRNA transcript, miR-103 was employed as internal control for verifying the results obtained in miR-378 studies. There were similarities in the results obtained (Figures 2 B, Figure 3 B, Figure 4 B and Figure 5 B).

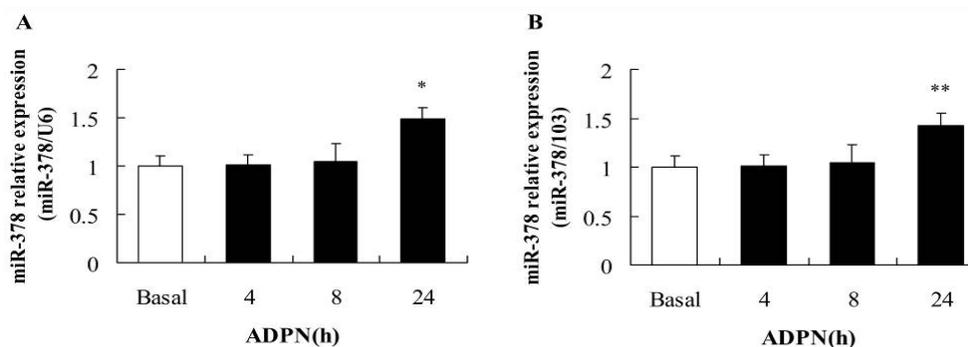


Figure 2: Effect of ADPN on miR-378 expression in mature human adipose tissue cells determined by quantitative RT-PCR relative to that of snRU6 (A) or miR-103 (B). Values are shown as mean \pm SE; * $p < 0.05$ relative to control

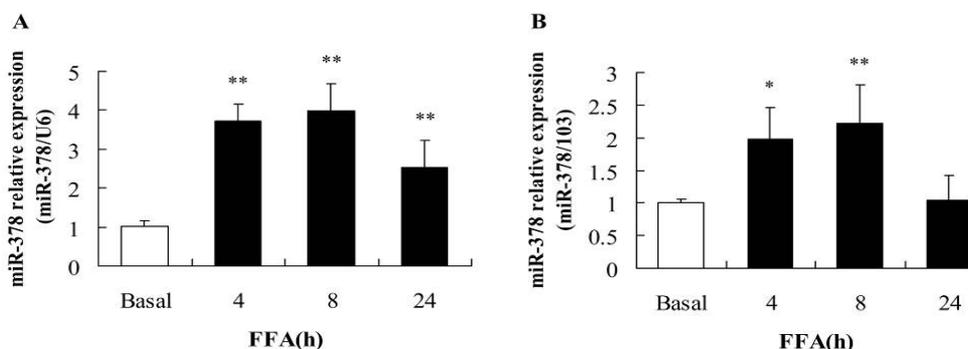


Figure 3: Effect of FFAs on miR-378 expression in mature human adipose tissue cells. The cells were exposed to 1 mmol/L FFAs for various lengths of time, and the amounts of miR-378 were determined with quantitative RT-PCR relative to the level of snRU6 (A) or miR-103 (B). Values are mean \pm SE ($n = 3$); * $p < 0.05$, ** $p < 0.01$ when compared with values for control

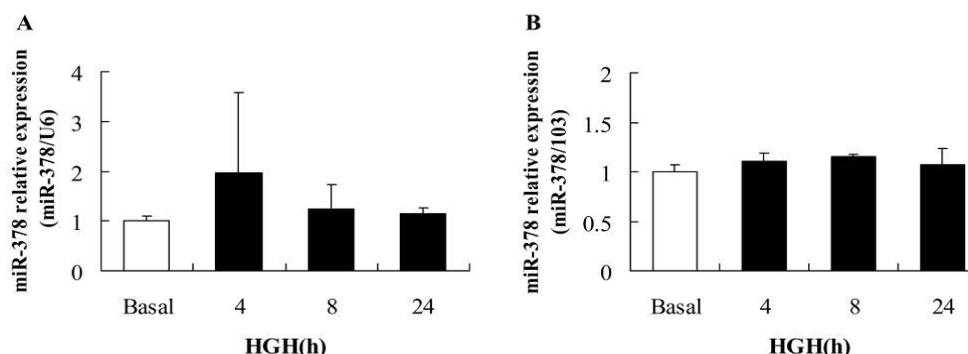


Figure 4: Effect of GH on the expression of miR-378 in mature human adipose tissue cells, determined by quantitative RT-PCR relative to snRU6 (A) or miR-103 (B). Values are expressed as mean \pm SE (n = 3)

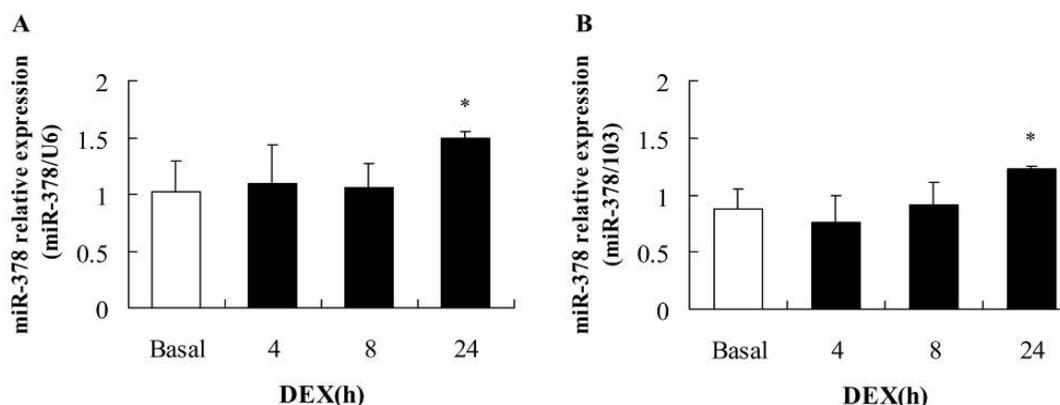


Figure 5: Effect of DEX on miR-378 expression in mature human adipose tissue cells as a function of length of exposure, determined using quantitative RT-PCR relative to snRU6 (A) or miR-103 (B). Values are expressed as mean \pm SE (n=3). * $p < 0.05$ when compared with control

DISCUSSION

Existing epidemiological studies have already shown that globally, the incidence of obesity has increased considerably. Obesity is so common in the world today that it is beginning to replace malnutrition as the most significant contributor to ill-health. Obesity is the causative factor for coronary heart disease, hypertension, sleep-breathing disorders and certain forms of cancer. However, type 2 diabetes is by far the most devastating complication of obesity. These two conditions predispose to development of insulin resistance. Obesity refers to excess accumulation of adipose tissue to an extent that negatively affects physical and psychosocial wellness [13]. Adipocytes affect metabolism through the release of FFAs, and secretion of hormones, glycerol, adiponectin, leptin and cytokines. The release of these substances is accentuated by obesity [14].

MiRNAs refer to a group of small and endogenously expressed RNAs that regulate the expression of genes post-transcriptionally. The potential of miRNAs in disease therapy has continued to receive recognition, especially in obesity and type 2 diabetes [15]. In an miRNA expression array study using total RNA extracted from human-derived pre-adipocytes and adipocytes to profile changes in miRNA expression during adipogenesis, the expressions of 70 out of 799 miRNAs assayed were significantly changed between non-induced and induced adipocytes, indicating the importance of miRNAs in adipocyte development [16]. The locus of miR-378 is located in PPARGC1B intron, and it is highly induced during adipogenesis.

In a study, it was shown that mice genetically deficient in miR-378 resisted development of obesity from high-fat diet, but exhibited increased rate of lipid metabolism in the mitochondrion [17]. This indicates the likely involvement of miR-378 in regulating energy balance and metabolic processes in the mitochondria. It has been demonstrated that in the process of adipogenesis, over-expression of miR-378 enhanced triglyceride levels due to increased rate of *de novo* lipid synthesis [9]. Moreover, Wei Liu *et al* [18] have shown that hepatic miR-378 is a key regulator of insulin signaling. On the basis of these reports, it can be hypothesized that insulin resistance and obesity may be regulated by energy sources and hormones through interaction with miR-378.

A study has demonstrated that adiponectin levels were decreased in either genetically obese ob/ob or diet-induced obese (high-fat-diet) mouse model [19]. The minor chronic inflammation seen in type 2 diabetes and obesity have also been associated with adipokine dysregulation [20]. Further studies revealed that adiponectin is an anti-inflammatory hormone which increases insulin sensitivity through enhancement of β -oxidation and suppression of hepatic gluconeogenesis [21]. Thus adiponectin regulates energy metabolism. In the present study, the concentration of adiponectin was low because its levels are decreased in obesity. However, adiponectin treatment (3 ug/ml) brought about slight increases in expression of miR-378, which reached a peak at 24 h. Based on this result, it is suggested that there exists a feedback regulation mechanism between adiponectin and miR-378. These observations suggest that a reduction in adiponectin increases

miR-378 expression and contributes to insulin resistance.

One major factor that affects insulin resistance is presence of FFAs. Type 2 diabetes and obesity are associated with high rate of FFAs release, which may be responsible for insulin resistance seen in both diseases [6]. Recently, it was shown that two key ER stress sensors, PERK and IRE-1 α were activated by FFAs, and that the use of chemical chaperons to attenuate ER stress reduced the expression of pro-inflammatory cytokines, leading to enhanced insulin sensitivity [22]. The results obtained in this study show significant up-regulation of miR-378 expression 4 h post-FFA exposure, which was sustained to the 8th hour. Thus, miR-378 may be implicated in the pathogenesis of FFAs-associated insulin insensitivity.

Growth hormone (GH) has a pronounced lipolytic effect, particularly on abdominal fat [23]. It stimulates protein synthesis, and enhances fat utilization by increasing oxidation in adipocytes and triglyceride breakdown. In addition, it exerts anti-insulin property by suppressing peripheral tissue uptake of glucose and enhancing hepatic gluconeogenesis [24]. However, the results obtained in this study suggest that GH is not associated with miR-378-linked insulin insensitivity.

In humans, excess glucocorticoids increase adipose tissue mass, and re-distribute fat between the central and peripheral depots [25]. Glucocorticoids enhance ready availability of energy substrates to cope with energy demands of stress [26]. In humans, prolonged glucocorticoid therapy is linked to central obesity and insulin resistance. The present study has demonstrated that under *in vitro* conditions, DEX enhanced the expression of miR-378 in mature human adipose tissue cells, suggesting an interaction between glucocorticoid and miR-378 in the pathogenesis of obesity-induced insulin insensitivity.

CONCLUSION

The findings of this study demonstrate that miR-378 expression is influenced by adiponectin, FFAs and dexamethasone, but not by GH. Thus, up-regulation of miR-378 expression may be implicated in the etiology of insulin resistance induced by these obesity-associated factors. Therefore, miR-378 is a potential biomarker for predicting the risk of complications of obesity, especially insulin resistance.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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