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miR-195a Inhibits Adipocyte Differentiation by Targeting the Preadipogenic Determinator *Zfp423*

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ABSTRACT

MicroRNAs (miRNAs) play essential roles in various cellular processes including proliferation and differentiation. In this study, we identified miRNA-195a (miR-195a) as a regulator of adipocyte differentiation. Differential expression of miR-195a in preadipocytes and adipocytes suggests its role in lipid accumulation and adipocyte differentiation. Forced expression of miR-195a mimics suppressed lipid accumulation and inhibited expression of adipocyte markers such as PPARγ and aP2 in 3T3-L1 and C3H10T1/2 cells. Conversely, downregulation of miR-195a by anti-miR-195a increased lipid accumulation and expression of adipocyte markers. Target prediction analysis suggested zinc finger protein 423 (Zfp423), a preadipogenic determinator, as a potential gene recognized by miR-195a. In line with this, mimicked expression of miR-195a reduced the expression of Zfp423, whereas anti-miR-195a increased its expression. Predicted targeting sequences in *Zfp423* 3′UTR, but not mutated sequences fused to luciferase, were regulated by miR-195a. Ectopic Zfp423 expression in 3T3-L1 cells increased lipid accumulation and expression of adipocyte markers, consistent with the observation that miR-195a targets *Zfp423*, resulting in suppressed adipocyte differentiation. In addition, miR-195a and *Zfp423* were inversely correlated in obese fat tissues, raising the possibility of miRNA′s role in obesity. Together, our data show that miR-195a is an anti-adipogenic regulator, which acts by targeting *Zfp423*, and further suggest the roles of miR-195a in obesity and metabolic diseases. J. Cell. Biochem. 116: 2589–2597, 2015. © 2015 Wiley Periodicals, Inc.

KEY WORDS: miR-195a; ADIPOCYTE DIFFERENTIATION; Zfp423; OBESITY

The high incidence of obesity and its related metabolic diseases worldwide has increased the focus on adipocytes over last two decades [Kopelman 2000; Yach et al., 2006]. Adipocytes, the major site of energy storage, impact energy metabolism in the entire body [Tontonoz and Spiegelman, 2008]. Adipocytes originate from precursor cells by activation of the transcriptional factors, PPARγ, and C/EBPα. Research has identified the regulators of transcriptional cascades for adipocyte differentiation [Park, Halperin et al., 2008] and signaling pathways that play roles in adipocyte differentiation have also been elucidated [Lazar 2005].

Ui Jeong Yun and No-Joon Song contributed equally to this work.

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MicroRNAs (miRNAs) are evolutionary conserved small noncoding RNAs that are known to post-transcriptionally inhibit target genes by affecting their translation and/or mRNA stability [Bartel 2004]. Numerous studies suggest that miRNAs critically regulate cell fate decisions [Ivey and Srivastava, 2010]. Recently, growing evidence indicates that regulation of miRNAs contribute to adipogenesis. Silencing of Dicer or Drosha, two crucial enzymes in the miRNA biogenesis pathway, inhibits adipogenic differentiation of human bone-marrow-derived mesenchymal stem cells [Oskowitz, Lu et al., 2008]. Several miRNAs, such as miR-24, -30c, -31, -143, and -642a-3p, have been reported to be involved in regulating adipogenesis [Esau, Kang et al., 2004; Sun et al., 2009; Zaragosi et al., 2011; Wu et al., 2012; Kang and Hata 2014].

miRNA-195a (miR-195a), a member of the miR-15 family, is aberrantly expressed in multiple diseases including cancer, heart failure, and schizophrenia [He et al., 2011]. Multiple studies have demonstrated that miR-195a suppresses tumors by targeting molecules involved in cell proliferation, migration, or invasion in various types of human cancers such as hepatocellular carcinoma, colorectal cancer, breast cancer, and glioma [Hui, Yuntao et al., 2013, Wang et al., 2014, Yang et al., 2014, Zhao et al., 2014]. Although the mechanism of miR-195a in cardiovascular diseases is not completely understood, cardiac overexpression of miR-195a results in pathological cardiac growth and heart failure in transgenic mice [van Rooij, Sutherland et al., 2006]. miR-195a is also involved in cortical development and maturation by the fine-tuning of brain-derived neurotrophic factor (BDNF) expression, specifically during late maturation and the aging of the human postmortem prefrontal cortex (PFC) [Mellios, Huang et al., 2008]. miR-195a was shown to be reduced in schizophrenia PFC [Guo et al., 2010]. Previously, miR-195a has shown to be enriched in preadipocytes [Lee et al., 2010] however, the role of miR-195a during adipogenesis still remains to be investigated.

The zinc finger protein Zfp423 was identified as a factor enriched in preadipose fibroblasts and demonstrated to be a key transcription factor committing cells to the adipogenic lineage [Gupta, Arany et al., 2010]. Overexpression of Zfp423 induces PPARγ expression, which leads to adipogenic commitment of progenitor cells. Down-regulation of Zfp423 decreases PPARγ expression and diminishes the ability of adipogenic differentiation. Thus, Zfp423 acts as a preadipocyte determinator. In this study, we describe a novel molecular mechanism controlling preadipocyte commitment. We showed that miR-195a can target a preadipogenic determinator, *Zfp423*, resulting in inhibition of adipocyte differentiation.

MATERIALS AND METHODS

CELL CULTURE

C3H10T1/2 and 3T3-L1 cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured as previously described [Park, Waki et al., 2008]. 3T3-L1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fischer Sci. Waltham, MA) media containing 10% calf serum (FCS, Hyclone, Logan, UT) and pen/strep (Thermo Fischer Sci.). C3H10T1/2 cells were grown in DMEM supplemented with 10% FBS (Hyclone) and pen/strep. Confluent cells were differentiated into adipocytes by media containing DMEM, 10% FBS, 1 μM dexamethasone (Sigma, St. Louis, MO), IBMX (0.5 mM, Sigma), and 5 μg/ml insulin (Sigma) for 2 days. Then, the medium was changed to DMEM, FBS, and insulin containing media and reloaded every two days. PPARγ agonist (1 uM, troglitazone, Sigma) was further supplemented for differentiation of C3H10T1/2 cells. Retroviral overexpression of *Zfp423* was performed using pBabe-puro and the packaging cell line Phoenix E. After 6–8 days of differentiation, the cells were fixed with 4% para-formaldehyde (Sigma) in PBS at room temperature for 1 h and stained with 0.5% Oil red O (ORO) (Sigma). The ORO stained cells were quantified by resuspending the dye in isopropanol, followed by measurement with a spectrophotometer (Colibri, Pforzheim, Germany) at 520 nm.

REAL TIME PCR ANALYSIS

Half micrograms of extracted total RNAs using TRIzol reagent (Invitrogen, Carlsbad, CA) were reverse transcribed by RTase M-MLV (2640A, Takara, Ohtsu, Japan) in a thermal cycler (Takara) as previously described [Song, Yoon et al., 2013].

The synthesized cDNAs were amplified, to measure the levels of mRNA expression, using the Power SYBR Premix Ex Taq (RP041A, Takara) with gene specific primer sets in a thermal cycler (Takara, Ohtsu, Japan). Total RNAs from mice tissue in wild type and high fat diet fed obese mice were isolated and gene expression was analyzed as described previously [Park, Waki et al., 2008]. The gene specific forward and reverse oligonucleotide primer sets were synthesized by Integrated DNA Technologies (Sandiego, CA) and their sequences were described previously [Park, Waki et al., 2008]. The relative expression levels (fold change) were obtained by using $2^{-\Delta CT}$ and normalized to the levels of 36B4. For detection of mature miRNAs, TaqMan MicroRNA assay kit (Applied Biosystems) was used according to the manufacturer's instructions and results were normalized to U6 snRNA. PCR cycling conditions were 50°C for 2 min, 95°C for 10 min and 40 cycles of (95°C for 15 sec and 60°C for 1 min).

TRANSFECTION OF miRNAs

miR-195a mimic was purchased from Genolution Pharmaceutical Inc. (Seoul, Korea). An anti-miR of miR-195a was purchased from Bioneer. Sequences were: miR-195a mimic 5′ -UAGCAGCACAGAAAUAUUGGC-3′ and anti-miR-195a 5′-GCCAATATTTCTGTGCTGCTA-3′. miRIDIAN microRNA mimic negative control (control mimic) was purchased from Thermo Scientific Dharmacon (CN-001000-01-05). Cells were seeded in a 6-well plate at 1×10^5 cells/well overnight and transfected with miRNAs using Lipofectamine RNAiMAX (Invitrogen 13778-075), according to the manufacturer's instructions. The transfected cells were in media that was exchanged with fresh media after 16 hr. Then, the cells were differentiated for 6–8 days followed by expression analysis and ORO staining. Transfections were repeated three times and showed similar results.

WESTERN BLOT

Total cell lysates were prepared and proteins were separated as previously described [Song, Yoon et al., 2013]. In brief, cells were lysed in lysis buffer (50 mM NaCl, 5 mM EDTA, 5 mM EGTA, 10 mM HEPES, and 0.1% Triton X-100), and proteins were separated in 10% SDS-PAGE and transferred to nitrocellulose paper (Invitrogen). Transferred membranes were probed for Zfp423 antibodies, followed by anti-rabbit

secondary antibodies from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and Cell Signaling Technology (Danvers, MA), respectively.

LUCIFERASE ASSAY

The predicted microRNA recognition element (MRE) sequence and MRE mutant (MRE mut) sequence were cloned into the pIS0 vector (Addgene) containing the luciferase gene. 5′-ATTTGGCCATGAGGCGCTGCTAT-3′ and 5′-ATAGCAGCGCCTCATGGCCAAAT-3′ were used for MRE and 5′-TAAACCGGTACTCCGCCTGCATA-3′ and 5′-ATACGTCCGCCTCATGGCCAAAT-3′ were used for the MRE mut. 3T3-L1 cells were cotransfected with 5 nM miR-195a or control mimic and luciferase reporter constructs using Lipofectamine 2000 (Life technologies). β-galactosidase expression plasmid was used as an internal transfection control. Twenty-four hours later, luciferase assays were performed and luciferase activities were presented after normalization to β-galactosidase activities.

STATISTICAL ANALYSIS

Differences in gene expression and lipid accumulation were analyzed with unpaired Student's *t*-test. Data are presented as means ± SEM. Statistical significance was defined as $P < 0.05$.

RESULTS

miR-195a IS REGULATED DURING ADIPOCYTE DIFFERENTIATION

Previous studies identified the preadipocyte enriched miRNAs compared to the levels in adipocytes [Lee et al., 2010]. In this study, we focused on miR-195a, one of the preadipocyte enriched miRNAs, to investigate its effects on adipocyte differentiation, and lipid accumulation. To determine the expression levels of miR-195a during adipocyte differentiation, 3T3-L1 cells were induced into adipocytes, and the expression levels of mature miR-195a were examined during adipocyte differentiation. Adipocyte

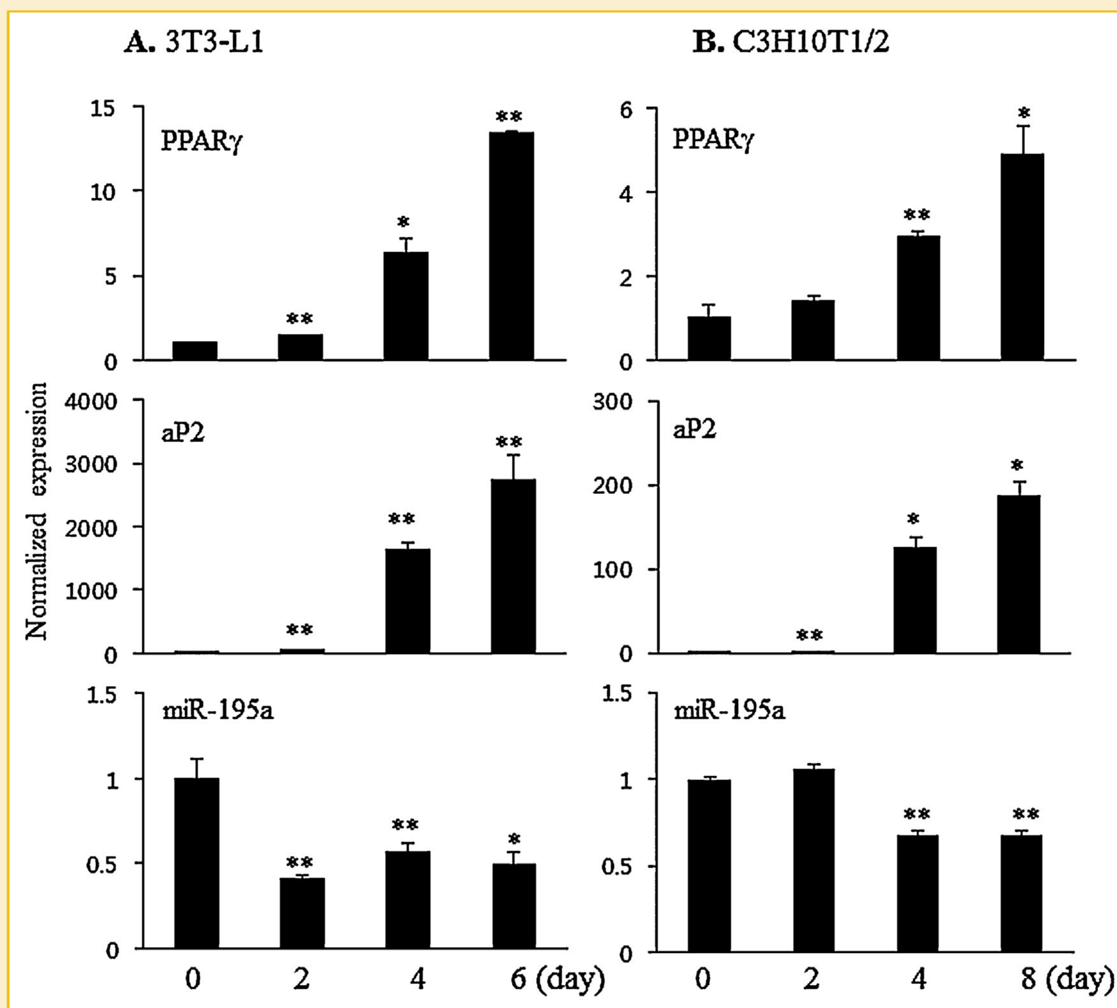

Fig. 1. Differential expression levels of miR-195a during adipocyte differentiation. (A) Expression levels of PPARγ, aP2, and miR-195a were measured during adipocyte differentiation in 3T3-L1 cells. 3T3-L1 cells were differentiated into adipocytes in the presence of an adipogenic cocktail (1 uM dexametasone, 0.5 mM IBMX, 5 ug/ml insulin) as described in the methods section. Expression of mature miR-195a normalized to U6 small nuclear RNA was examined by real time PCR. (B) C3H10T1/2 cells were induced into adipocytes and the level of expression of PPARγ, aP2, and miR-195a was determined. Data are representative of three independent experiments and given as means ± SEM. Data were analyzed using Student's *t*-test. (*$P < 0.05$, **$P < 0.001$).

differentiation can be characterized by the increased expression of adipocyte markers such as PPARγ and aP2. As expected, expression of PPARγ and aP2 was significantly increased during adipocyte differentiation in 3T3-L1 preadipocytes (Fig. 1A). By contrast, expression of miR-195a was decreased in cells post adipogenic induction compared to the levels in preadipocytes (Fig.1A). The similar expression pattern of these genes was also observed during adipocyte differentiation in mesenchymal C3H10T1/2 cells (Fig. 1B). Expression of PPARγ and aP2 was increased, whereas mature miR-195a levels began to decrease at day 4 post differentiation in

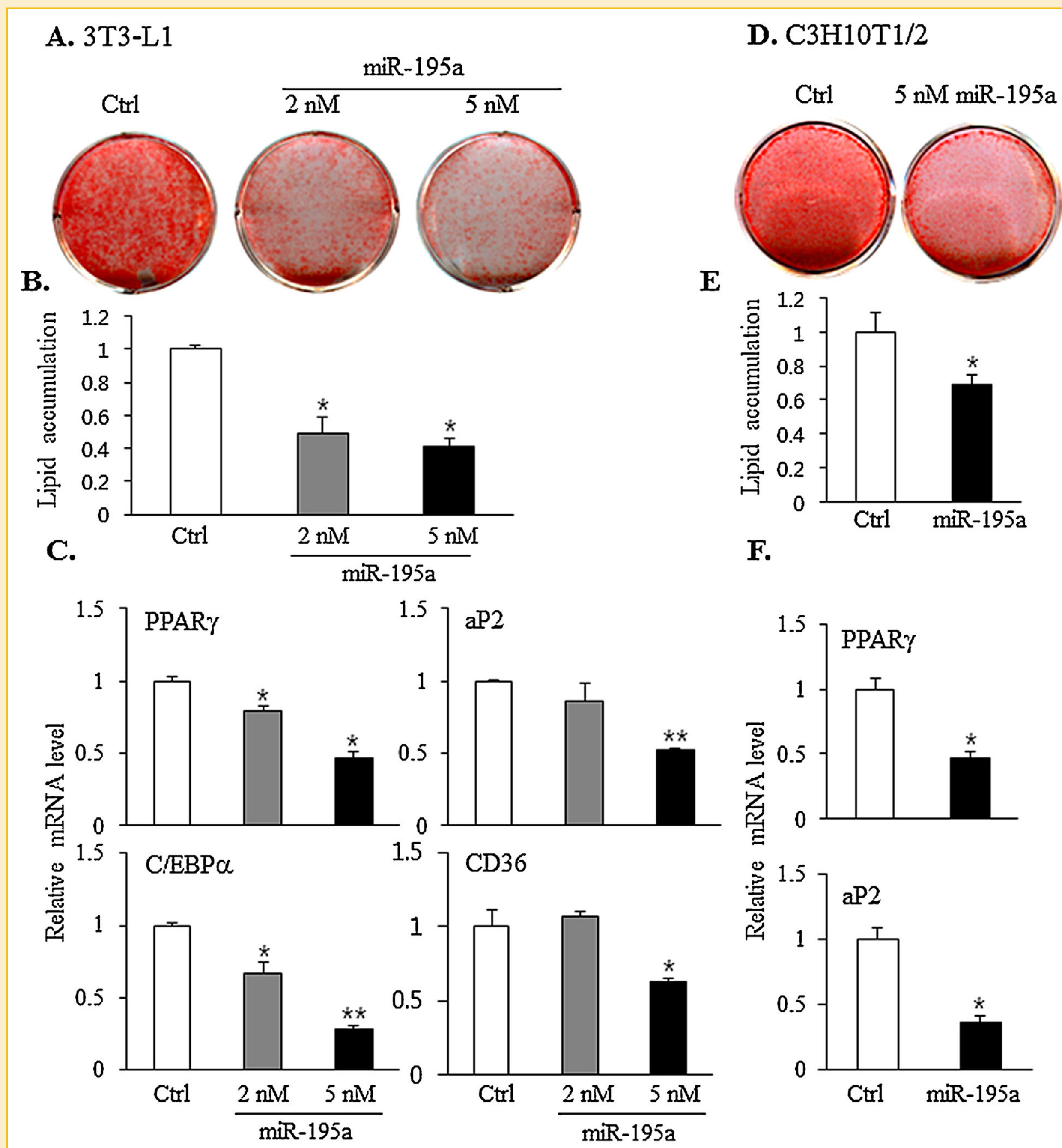

Fig. 2. miR-195a inhibits adipocyte differentiation of 3T3-L1 and C3H10T1/2 cells. (A–C) Preadipocyte 3T3-L1 cells were transiently transfected with exogenous miR-195a and differentiated into adipocytes for 6 days. (A, B) Inhibitory effects on lipid accumulation by exogenous miR-195a (2 nM and 5 nM) in 3T3-L1 cells were assessed by ORO staining (A) followed by quantification (B). (C) Reduced expression of adipocyte markers including PPARγ, aP2, C/EBPα, and CD36 mRNA was determined by real time PCR. (D–F) C3H10T1/2 cells were transiently transfected with 5 nM miR-195a and differentiated into adipocytes for 8 days. (D) miR-195a suppressed lipid accumulation in C3H10T1/2 cells. (E) Lipid accumulation was quantified. (F) Repression of adipocyte markers by miR-195a was determined by realtime PCR. Data are representative of three independent experiments and is reported as means ± SEM. Data were analyzed using Student's *t*-test. (*$P < 0.05$, **$P < 0.001$).

C3H10T1/2 cells. Thus, consistent with previous finding of the preadipocyte enriched miR-195a, the miR-195a expression was significantly decreased during adipocyte differentiation.

miR-195a INHIBITS ADIPOCYTE DIFFERENTIATION IN 3T3-L1 AND C3H10T1/2 CELLS

To investigate the roles of miR-195a in adipogenesis, preadipocytes were transfected with exogenous miR-195a mimic and stimulated to differentiate into adipocytes. 3T3-L1 cells transfected with 2 or 5 nM miR-195a inhibited lipid accumulation during adipocyte differentiation, as assessed by ORO staining, compared to negative control mimic transfected cells (Fig. 2A). Delivery of 5 nM miR-195a inhibited lipid accumulation up to 60% compared to the control cells (Fig. 2B). Similarly, expression of PPARγ, aP2, C/EBPα, and CD36 were also suppressed by the exogenous miR-195a (Fig. 2C). The anti-adipogenic effects of miR-195a were also consistently observed in mesenchymal C3H10T1/2 cells (Fig 2D–F). Similar to the effects in 3T3-L1 cells, miR-195a suppressed lipid accumulation, and expression of adipocyte markers in C3H10T1/2 cells.

DOWNREGULATION OF miR-195a IS ESSENTIAL FOR ADIPOCYTE DIFFERENTIATION

To further determine the necessity of miR-195a in adipocyte differentiation, downregulation of endogenous miR-195a by anti-miR of miR-195a (anti-miR-195a) was undertaken. 3T3-L1 cells were transfected with anti-miR-195a and differentiated into adipocytes. In

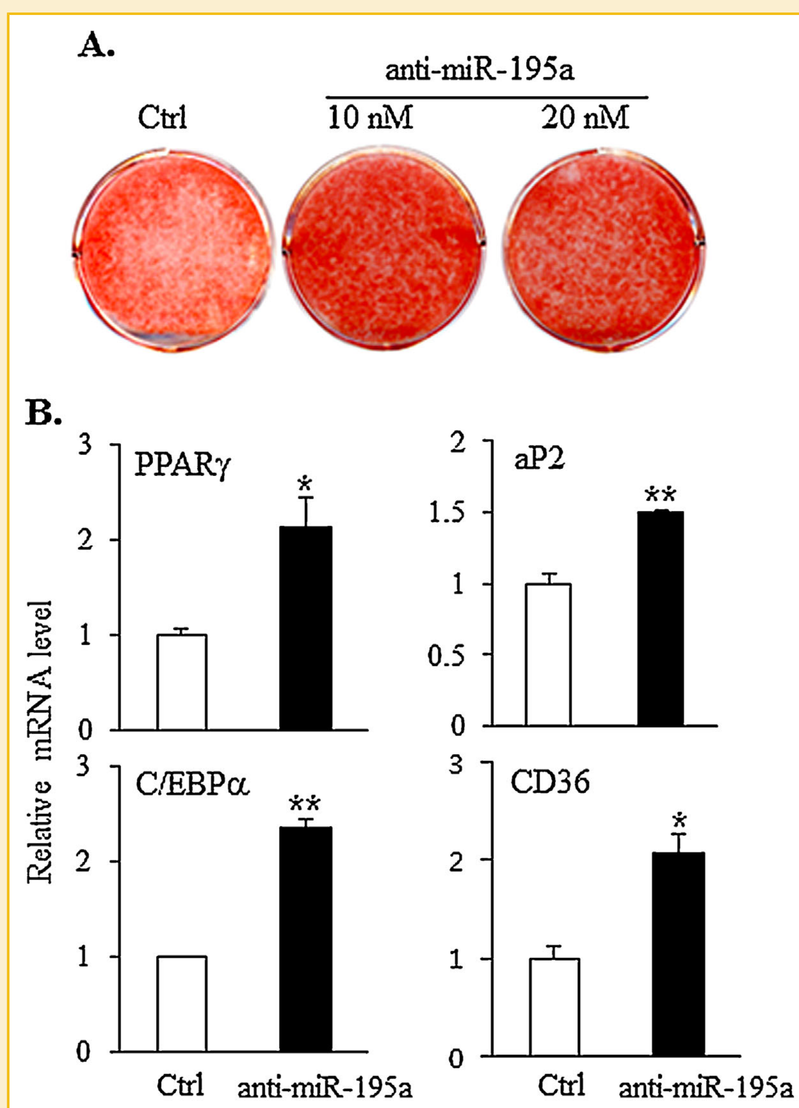

Fig. 3. Downregulation of miR-195a promotes adipocyte differentiation. 3T3-L1 cells were transiently transfected with anti-miR-195a and induced to adipocytes for 6 days. (A) Increased lipid accumulation by anti-miR-195a (10 nM and 20 nM) was assessed by ORO staining. (B) Adipocyte markers were induced by anti-miR-195a. Expression of mRNA encoding PPARγ, aP2, C/EBPα, and CD36 was measured by real time PCR. Data are representative of three independent experiments and is reported as means ± SEM. Data were analyzed using Student's *t*-test. (* $P < 0.05$, ** $P < 0.001$).

contrast to the effects by forced delivery of exogenous miR-195a, lipid accumulation was significantly increased in anti-miR-195a transfected cells compared to the control mimic transfected cells (Fig. 3A). Expression of adipocyte markers was also significantly induced by anti-miR-195a (Fig. 3B), further indicating the requirement of miR-195a downregulation in adipogenesis. Together, gain and loss of function studies indicate the roles of miR-195a in adipogenesis.

ZFP423 IS A TARGET OF miR-195a IN ADIPOCYTES

In a search for potential targets of miR-195a using miRNA target prediction altorithms such as Targetscan, Pictar, and miRanda, an evolutionarily conserved miRNA recognition element (MRE) partially complementary to miR-195a in the 3′ UTR of *Zfp423* gene was discovered. To determine whether miR-195a targets *Zfp423* and mediates destabilization of the *Zfp423* mRNA, real time PCR analysis was performed. As predicted, 2 or 5 nM miR-195a mimics transfected cells reduced *Zfp423* mRNA level to 65 and 54%, respectively (Fig. 4A). Immunoblot analysis further demonstrated that the expression of ZFP423 protein was downregulated in miR-195a transfected cells (Fig. 4B). Moreover, inhibition of endogenous miR-195a by 20 nM anti-miR-195a elevated the *Zfp423* mRNA approximately twofold (Fig. 4C).

To directly examine whether the predicted MRE is targeted by miR-195a, a luciferase reporter construct containing the putative MRE sequence at the 3′ end of the luciferase reporter gene (MRE) was generated. As a control, a reporter plasmid with mutations in the MRE sequence, which disrupted critical base pairing with miR-195a (MRE mut) was also constructed (Fig. 4D). These reporter constructs were transfected into 3T3-L1 cells together with miR-195a or negative control mimic. The luciferase activity of MRE was reduced approximately 45% upon cotransfection with miR-195a. However, the activity of the mutated construct (MRE mut) was completely unaffected, indicating that the MRE in *Zfp423* mRNA 3′ UTR is targeted by miR-195a (Fig. 4E). Taken together, these results demonstrate that *Zfp423* is a novel target of miR-195a in preadipocytes.

FORCED EXPRESSION OF ZFP423 INDUCED ADIPOCYTE DIFFERENTIATION

Zfp423 was recently identified as a preadipogenic determinator in stem cells [Gupta et al., 2010]. Thus, it is likely that the miR-195a suppresses lipid accumulation and adipocyte differentiation by targeting *Zfp423*. To directly assess the roles of Zfp423 in adipogenesis, Zfp423 was stably overexpressed in 3T3-L1 cells by using a pBabe-puro retroviral system.

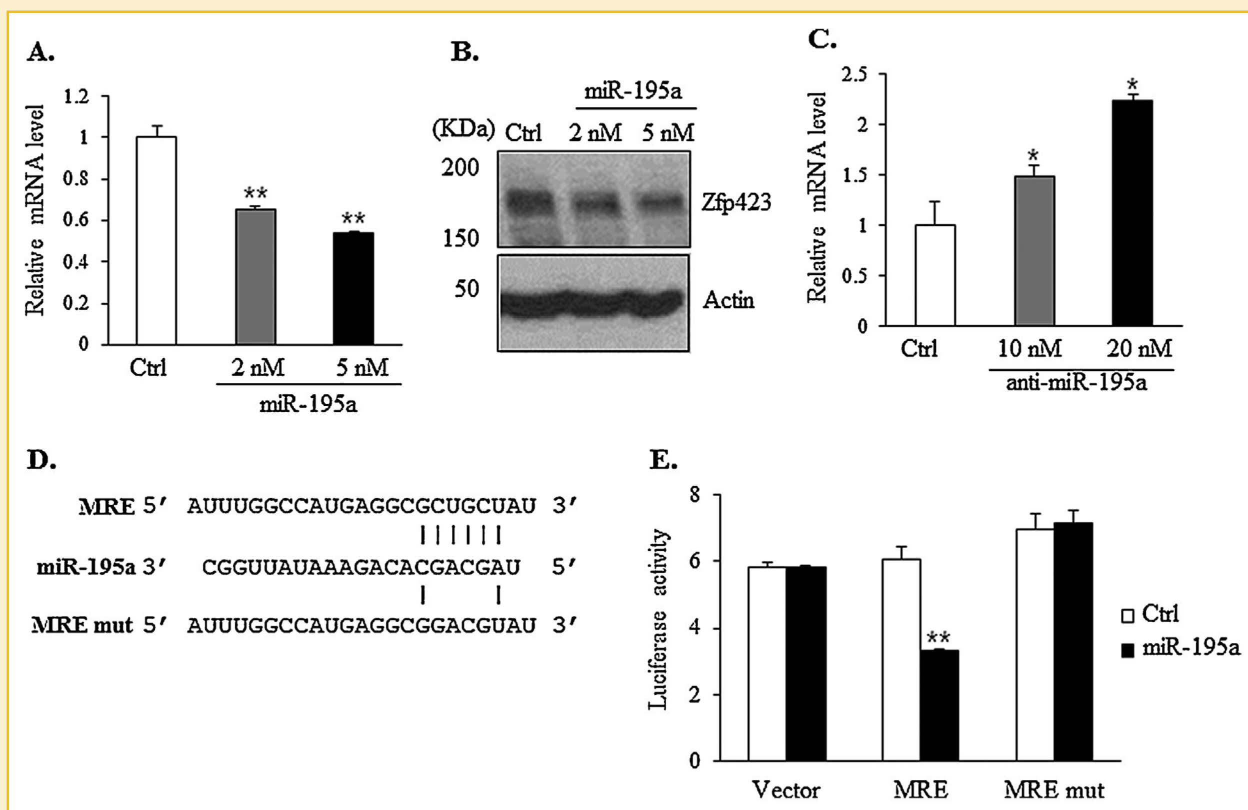

Fig. 4. Zfp423 is a novel target of miR-195a. (A–C) miR-195a decreased *Zfp423* mRNA expression, whereas anti-miR-195a dose dependently increased *Zfp423* expression in 3T3-L1 cells. (A) Exogenous miR-195a was transiently transfected into 3T3-L1 cells for 48 hr and expression of *Zfp423* mRNA was measured by realtime PCR. (B) Protein was measured by western blotting. (C) anti-miR-195a was transiently transfected into 3T3-L1 cells for 48 hrs and mRNA expression of *Zfp423* was measured. (D) Sequences of miR-195a MRE predicted by the Targetscan program in the 3′ UTR of *Zfp423*. (E) Luciferase activities of constructs with wild type (MRE) or mutated *Zfp423* MRE (MRE mut) were examined in 3T3-L1 cells by transfecting miR-195a or negative control mimic. Data are means ± SEM and were analyzed using Student's *t*-test. ($^{*}P < 0.05$, $^{**}P < 0.001$).

Enhanced lipid accumulation was consistently observed in Zfp423 stably expressing cells, compared to control empty vector (pBabe-puro) expressing cells (Fig. 5A and D). Stable overexpression of Zfp423 was confirmed by measuring the levels of *Zfp423* mRNA and protein expression (Fig. 5B and C). Stable expression of Zfp423 (pBabe-Zfp423) induced expression of adipocyte markers (Fig. 5E), verifying the pro-adipogenic roles of Zfp423 in 3T3-L1 cells. In line with this, recent studies showed that Zfp423 knockdown by shRNA in adipogenic cells prevented adipogenic differentiation [Huang et al., 2012]. Furthermore, Zfp423 deficient mouse embryos exhibited markedly impaired adipocyte differentiation [Gupta et al., 2010]. This is consistent with the idea that miR-195a targets *Zfp423*, which in turn impairs the ability of preadipocytes to undergo adipocyte differentiation.

miR-195a IS DIFFERENTIALLY REGULATED IN OBESE AND DIABETIC MICE

Our data indicate the possible roles of miR-195a in obesity and metabolic diseases. To assess this possibility, miRNA expression levels were compared in fat tissues from lean control and high fat diet induced obese mice. Expression of miR-195a is significantly increased in obese epididymal fat tissues compared to the levels in fat tissues from lean mice (Fig. 6A). Interestingly, *Zfp423* expression is lower in the obese fat tissues (Fig. 6B), further showing the inverse correlation of miR-195a, and *Zfp423* expression. Furthermore, *Zfp423* expression is higher in differentiated adipocytes compared to the levels in miR-195a enriched preadipocytes of 3T3-L1 and C3H10T1/2 cells (supplemental Fig. S1). These expression profiles suggest the role of miR-195a in obesity and further support its potential impact on *Zfp423* expression. Taken together, our data suggest regulatory roles of miR-195a in lipid accumulation and further present miR-195a as a new player in metabolic diseases.

DISCUSSION

In this study, we demonstrated that the expression of miR-195a is regulated during adipocyte differentiation and the manipulation of miR-195a regulates adipogenesis. miR-195a inhibits adipocyte differentiation and thus the expression level of miR-195a decreases during adipogenesis. We also identified *Zfp423* as a novel target of miR-195a. Both mRNA and protein expressions of Zfp423 were affected by the level of miR-195a expression. Decreased luciferase activity with miR-195a recognizing sequences, but not mutated sequences fused to luciferase by miR-195a, further suggests that miR-195a targets *Zfp423*. Finally, miR-195a is differentially expressed in obese and lean mice, suggesting the role of this miRNA in obesity, and metabolic diseases. Future studies are required to understand the regulation of miR-195a expression and to further dissect the roles of miR-195a in metabolic diseases in order to develop potent miR-195a-based therapeutics.

We showed that Zfp423 expression level is post-transcriptionally modulated by a specific miRNA, miR-195a, during adipocyte

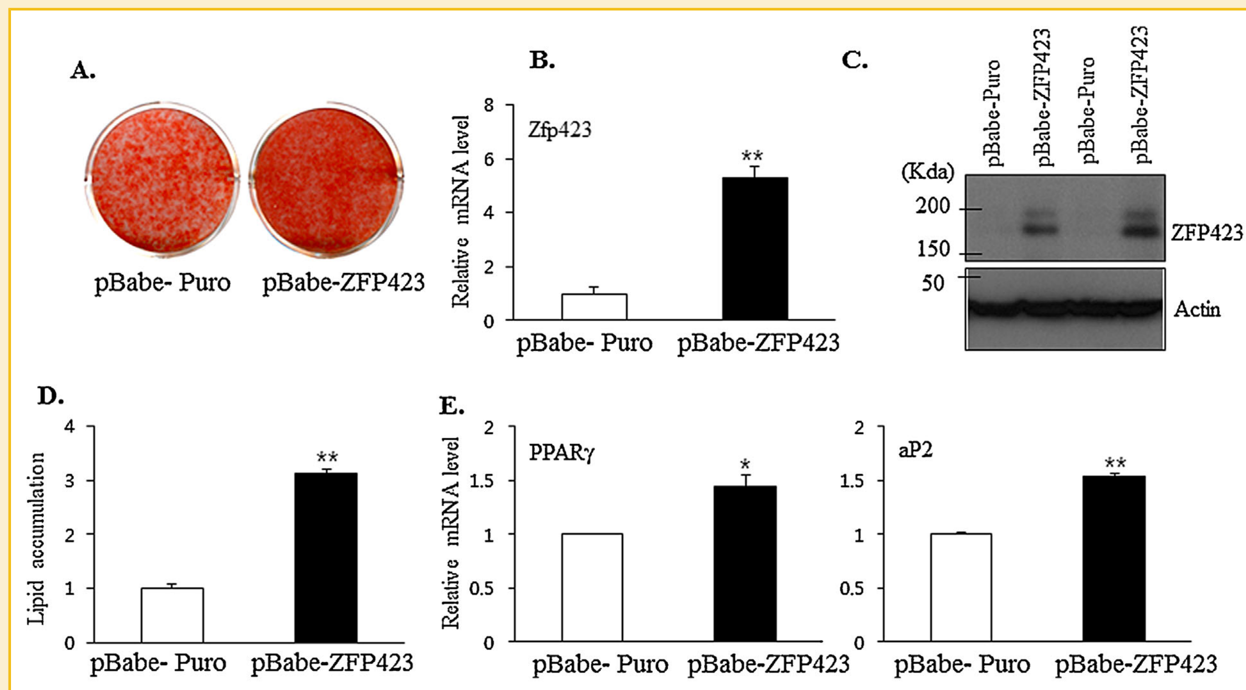

Fig. 5. Zfp423 induces adipocyte differentiation. 3T3-L1 cells were infected with pBabe retrovirus harboring control empty vector (pBabe-puro) or *Zfp423* gene (pBabe-Zfp423) and stable cells were selected with pruromycin (2 ug/ml) for 2 weeks. (A) Differentiation of cells into adipocytes was induced by DMI for 6 days and lipid accumulation was assessed by ORO staining. (B–C) Stable expression of *Zfp423* was measured by real time PCR (B) and western blotting (C). (D) Stable cells were differentiated into adipocytes for 6 days and lipid accumulation was quantified. (E) Expression of PPARγ and aP2 was measured by realtime PCR. Data are means ± SEM and were analyzed using Student's *t*-test. (*$P < 0.05$, **$P < 0.001$).

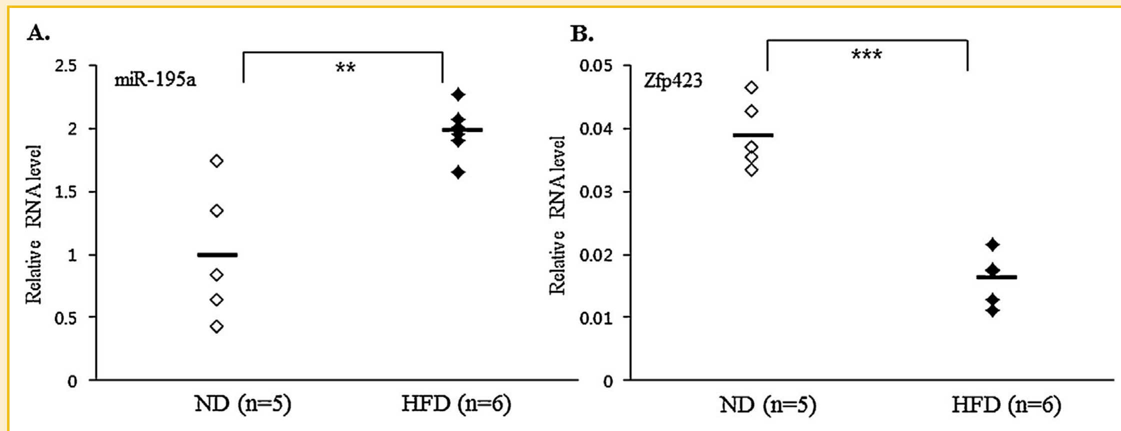

Fig. 6. miR-195a is differentially regulated in adipose tissues of obese mice. C57BL/6 mice were fed regular chow (ND) or high fat diet (HFD) for 12 weeks. Total RNA was extracted from epididymal fat tissues and expression of *Zfp423* and miR-195a was measured by real time PCR. (A) miR-195a is up regulated in high fat diet fed obese mice. (B) *Zfp423* expression in obese mice is inversely correlated with expression of miR-195a. Dots (open and closed) and bars in scatter plots represent individual mice and the average, respectively. Data were analyzed using Student's *t*-test. Differences in expression of miR-195a and *Zfp423* between ND (n = 5) and HFD (n = 6) fed groups are indicated. (**$P < 0.001$, ***$P < 0.0001$).

differentiation. Previously, Zfp423 was shown to be regulated partly through epigenetic modification during adipose tissue development [Yang, Liang et al., 2013]. Because Zfp423 is a critical transcription factor initiating adipogenic commitment, maternal obesity enhances Zfp423 expression and adipogenic differentiation during fetal development via attenuating DNA methylation and inhibitory histone modifications in the Zfp423 promoter. DNA methylation was more abundant in the Zfp423 promoter of the control than in the obesogenic tissue, consistent with lower Zfp423 expression in control fetal tissue. Furthermore, histone modification, H3K27me3, a reaction catalyzed by polycomb repressive complex 2, was lower in obesogenic fetal tissue. This provides a mechanism for the reduced DNA methylation in the Zfp423 promoter and enhanced Zfp423 expression and adipogenesis in obesogenic fetal tissue. Therefore, our study demonstrated an additional layer of the role of miRNA in post-transcriptional regulation of Zfp423 expression during adipogenesis.

Zfp423 has been shown to assemble a transcriptional complex by binding to distinct partners and DNA sequences and mediates multiple signaling pathways, which are responsible for cellular growth and differentiation. Zfp423 was first described as a transcriptional partner of Olf-1 in the development of the olfactory epithelium and pre-B lymphocytes in the rat [Tsai and Reed, 1997]. Zfp423 was then described as a DNA-binding factor that associates with Smads in response to BMP2, activating transcription of the Xenopus homeobox regulator, Xvent-2, that regulates mesoderm and neural development [Hata, Seoane et al., 2000]. Zfp423 is also required for retinoic acid (RA)-induced differentiation. Zfp423 associates with the RARα/RXRα nuclear receptor complex and is essential for transactivation in response to retinoids [Huang, Laoukili et al., 2009]. The BMP and Smad signaling pathway is implicated in the early events of adipogenesis, particularly in the induction of PPARγ [Gupta, Arany et al., 2010]. Although Zfp423 was identified as a BMP-dependent transcriptional coactivator of SMAD proteins, the SMAD-binding domain of Zfp423 is not necessary for its activity in preadipocyte commitment under basal culture conditions, but is required for Zfp423 modulation of adipogenic activity induced by BMPs. It still remains unclear which cofactors associate with Zfp423 complex to induce the transcription of PPARγ.

A mutual inhibitory relationship exists between adipogenesis and osteogenesis from mesenchymal stem cells. Accumulated evidence indicates that miRNAs act as molecular switches to control a cell fate between adipogenic and osteogenic lineages [Oskowitz, Lu et al., 2008]. As our data suggest the roles of miR-195a in adipocyte differentiation, further studies must elucidate whether the expression of miR-195a stimulates osteogenic differentiation. Of note, Zfp423 repression by another zinc finger protein mediates a BMP-induced osteoblast and adipocyte commitment [Addison, Fu et al., 2014]. Therefore, it will be interesting to test whether miR-195a, a post-transcriptional regulator of Zfp423 expression, also responds to act in the BMP induced cell fate decision. Most recently, it is reported that miR-195a alters the gene regulatory network of osteoblast differentiation and impairs the induction of BMP responsive genes [Grunhagen et al., 2014].

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