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The role and possible mechanism of lncRNA AC092159.2 in modulating adipocyte differentiation

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Abstract

Obesity is a major risk factor for metabolic diseases, while adipocyte differentiation is closely related to obesity occurrence. Long noncoding RNAs (lncRNAs) are a unique class of transcripts in regulation of various biological processes. Using lncRNA microarray, we found lncRNA AC092159.2 was highly expressed in differentiated HPA-v and located ~247 bp upstream of the TMEM18, which was associated with BMI and obesity. We aimed to explore the role of AC092159.2 in adipogenesis and the underlying mechanisms. The effects of AC092159.2 gain- and loss-of-function on HPA-v adipogenesis were determined with lentivirus and siRNA-mediated cell transduction, respectively. Lipid accumulation was evaluated by oil red O staining; the expression of AC092159.2, TMEM18 and several adipogenesis makers in HPA-v were analyzed by qPCR/Western blot. We found that the expression of AC092159.2 gradually increased during HPA-v differentiation, and its expression in omental adipose tissue was positively related with BMI among 48 human subjects. Overexpression of AC092159.2 promoted adipocytes differentiation while knockdown of it led to an adipogenic defect. Moreover, the expression of AC092159.2 and TMEM18 were positively correlated during adipogenic differentiation. AC092159.2 overexpression boosted TMEM18 expression while AC092159.2 knockdown restrained TMEM18 expression. Further rescue experiments showed that TMEM18 knockdown partially restrained adipogenic differentiation in AC092159.2 overexpressed HPA-v and adipogenic defect caused by AC092159.2 knockdown could be rescued by TMEM18 overexpression. Luciferase reporter assays revealed that AC092159.2 had a transcriptional activation effect on TMEM18. We concluded that lncRNA AC092159.2 promoted human adipocytes differentiation possibly by regulating TMEM18.

Key Words

- ▶ lncRNA
- ▶ AC092159.2
- ▶ adipocyte differentiation
- ▶ TMEM18

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Introduction

Obesity refers to excessive accumulation of adipose tissue caused by genetic and environmental factors, which can significantly increase the morbidity and mortality of cardiovascular disease, type 2 diabetes, hyperlipidemia and malignancy (Haslam & James 2005, Barth 2011, Serra-Majem & Bautista-Castano 2013). What's more, it is growing with alarming speed and is becoming a global public health crisis (Ng *et al.* 2014). Adipocyte differentiation is a vital process for adipocyte and development of obesity (Lu *et al.* 2012). Therefore, it is of great significance to study the molecular mechanism of adipocyte differentiation in the prevention and treatment of obesity and other adipocyte differentiation-related disorders. The mechanism of adipogenesis is extremely complex. At present, transcription factors and signal pathway of adipogenesis have been revealed to involve an elaborate network of transcriptional regulation that coordinates the expression of hundreds of proteins (Farmer 2006, Lefterova & Lazar 2009, Cristancho & Lazar 2011). Nevertheless, the detailed molecular mechanism of adipogenesis is not yet known.

lncRNAs are a unique class of transcripts that have regulatory roles in biological processes, such as cellular differentiation (Hu *et al.* 2012, Rinn & Chang 2012), transcriptional repression (Batista & Chang 2013), cancer metastasis (Gupta *et al.* 2010), X chromosomal inactivation (Lee 2009) and chromatin remodeling (Tsai *et al.* 2010). Recently, with the help of chip technology and high-throughput RNA sequencing, lncRNAs have been found to be widely and differentially expressed in adipose tissue (Kornfeld & Bruning 2014, Zhao & Lin 2015, Huang *et al.* 2016, Yang *et al.* 2016). Although several lncRNAs (U90926, ADINR and Uc.417) have been reported to exert important roles during adipocyte differentiation (Xiao *et al.* 2015, Cui *et al.* 2016, Chen *et al.* 2017), the exact molecular mechanisms involved in lncRNA-mediated modulation of adipogenesis still remains unclear.

Human visceral preadipocytes (HPA-v) are commonly used to study adipocyte differentiation *in vitro* (Barbosa-Desongles *et al.* 2013, Davidge-Pitts *et al.* 2014, Fujita *et al.* 2016). We used this system to identify the lncRNAs related to adipogenesis by lncRNA microarray, and found that a long noncoding RNA (lncRNA), AC092159.2, was highly expressed in differentiated HPA-v compared with undifferentiated HPA-v. Therefore, we hypothesized that lncRNA AC092159.2 may be possibly related to adipogenesis. To verify our hypothesis, we applied AC092159.2 lentivirus and siRNA mediated gain- or

loss-of-function experiments and rescue experiments in HPA-v to explore the role and possible mechanism of lncRNA AC092159.2 in modulating adipocyte differentiation.

Materials and methods

Human subjects

Omental adipose tissues were prospectively collected from patients undergoing surgery for abdominal disorders. All of the patients had no endocrine disease, malignancy or severe systemic illness. According to the Working Group on Obesity in China (WGOC) in 2003 (Wang *et al.* 2007), subjects with BMI ≥ 24 kg/m² were defined as overweight. As a result, 31 subjects with overweight/obesity and 17 controls with normal weight consented to participate in this study. Written informed consent was obtained from all participants. The study was approved by the institutional review board of Nanjing Maternity and Child Health Care Institute and the methods were carried out in accordance with the approved guidelines.

Cell culture and adipocyte differentiation

HPA-v cells (ScienCell Research Laboratories, San Diego, CA, USA) were maintained in preadipocyte medium (PAM; ScienCell Research Laboratories) supplemented with 5% fetal bovine serum (FBS; Gibco Life Technologies), 1% penicillin/streptomycin solution (P/S; ScienCell Research Laboratories) and 1% preadipocyte growth supplement (PAGS; ScienCell Research Laboratories) at 37°C in 5% CO₂. To induce differentiation, fully confluent HPA-v (day 0) were incubated in a differentiation cocktail of serum-free PAM, supplemented with 250 nM insulin, 1 mM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine and 1 μ M rosiglitazone. The culture medium was replaced 4 days later. Then, cells were incubated in serum-free DMEM containing 250 nM insulin, which were replaced every 2 days until lipid accumulated in the cells (Day 10). HPA-v cells were harvested on days 0, 1, 4, 7 and 10 for further investigations.

Lentivirus and siRNA-mediated cell transduction

Lentivirus overexpressing vectors, including the negative control (Lv-NC), AC092159.2 overexpressing lentivirus (Lv-AC092159.2) and TMEM18 overexpressing lentivirus (Lv-TMEM18) were purchased from GenePharma Inc (Shanghai, China) and transfected according to the

manufacturer's instructions. When reaching about 40% confluence, at the stage with the optimal infection efficiency, the preadipocytes were infected with 4 μ L of Lv-NC (viral titer, 1×10^8 TU/mL), Lv-AC092159.2 (viral titer, 1×10^8 TU/mL) or Lv-TMEM18 (viral titer, 1×10^8 TU/mL) in 2 mL PAM culture medium mixed with 5 μ g/mL Polybrene (Sigma-Aldrich). After 24 h of infection, fresh medium was added into the preadipocytes. The overexpression efficiency was checked by GFP expression using fluorescence microscopy and confirmed by real-time quantitative PCR (qPCR). After the infected cells reached confluency, differentiation was induced as described previously. For knockdown, cells at 60% confluence were transfected with AC092159.2 or TMEM18-specific small interfering RNA (siRNA) pool (Supplementary Table 1, see section on [supplementary data](#) given at the end of this article) using Lipofectamine 2000 (Invitrogen) at 100 nM in OptiMEM medium (Thermo Fisher Scientific) according to the manufacturer's instruction. Control cells were treated with Stealth RNAi Negative Control (Thermo Fisher Scientific). And knockdown efficiency was confirmed by qPCR.

Oil Red O staining for lipid and triacylglycerol (TG) content measurement

In vitro differentiated cells at days 7 and 10 were purged with PBS twice and afterward fixed in 4% formaldehyde for 20 min. After being rinsed by PBS twice, the fixed cells were stained with 0.2% Oil Red O (Sigma) for 60 min at 37°C. Subsequently the stained cells were purged three times with distilled water and visualized under the microscope Observer D1 (Carl Zeiss). The content of intracellular triglyceride (TG) that is relative to total protein content was measured using the triglyceride assay kit (Applygen Technologies Inc, Beijing, China) and BCA Protein Assay Kit (Pierce) according to the manufacturer's instructions. At the indicated time, the mature adipocytes were treated with lysis buffer and the cell lysates were harvested and homogenized. Subsequently, the cell suspension was retained to respectively detect the triglyceride and protein concentration.

RNA extraction and qPCR

The total RNA was isolated from HPA-v cells using TRIzol (Thermo Fisher Scientific). The high-capacity cDNA reverse transcription kit (RR047A; Takara) was used for reverse transcription of normalized RNA, and cDNA was analyzed by qPCR using the SYBR Green method. Data were normalized to PPIA and further analyzed

using the $2^{-\Delta\Delta C_t}$ method. All the primer sequences for the marker genes involved in the test were listed in Supplementary Table 2.

Protein extraction and Western blot

The protein was extracted from the differentiated adipocytes (day 7) for Western blot analysis using RIPA buffer and quantified by the BCA Protein Assay Kit (23229; Thermo Fisher Scientific). A total of 10 mg protein was separated by 10% SDS-PAGE and transferred to PVDF membranes. Membranes were immunoblotted with specific primary antibodies against rabbit polyclonal- β actin (Cell Signaling Technology, No.84575, dilution in 1:5000), rabbit polyclonal TMEM18 (Abcam, No.106597, dilution in 1:1000), rabbit polyclonal FABP4 (Abcam, No.66682, dilution in 1:1000), monoclonal rabbit PPAR γ (Cell Signaling Technology, No. 2435S, dilution in 1:1000), rabbit polyclonal C/EBP α (Cell Signaling Technology, No.2295S, dilution in 1:1000) and polyclonal rabbit C/EBP β (Cell Signaling Technology, No.3087S, dilution in 1:1000). The secondary antibody was horseradish peroxidase-conjugated goat anti-rabbit IgG (dilution in 1:5000) from Beijing Zhong Shan Biotechnology CO (Beijing, China).

Cell proliferation assay

The proliferation rates of the AC092159.2 overexpression and knockdown cells were measured using a WST-8 Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, 3000 cells were seeded into each well of the 96-well plates. The media was replaced with an equal volume of fresh media containing 10% CCK-8 after 0, 24, 48 and 72 h of growth, and the cultures were incubated at 37°C for 1 h. Cell growth was measured by microplate reader (Thermo Fisher Scientific) at an optical density (OD) of 450 nm. The results are expressed as the mean $\pm$ standard deviation (s.d.) of three separate experiments, with six determinations per experiment for each experimental condition.

Flow cytometry assay

The cell cycle and apoptosis of transfected cells were analyzed by flow cytometry. After transfection for 72 h, the cells were digested using trypsin without EDTA and washed twice with pre-cooled PBS. For cell cycle analysis, 2×10^6 cells were fixed in 70% pre-cooled ethanol (Sigma-Aldrich) and stored in fridge at -20°C overnight. Subsequently, the cells were washed and resuspended

in PBS, then stained with 500 μ L propidium iodide (PI) solution (Sigma-Aldrich) containing 100 μ g/mL RNase and 50 μ g/mL PI in 1 $\times$ PBS for 30 min. For cell apoptosis analysis, the cells were harvested according to Annexin V-FITC apoptosis detection kit instructions (BD Biosciences, San Jose, CA, USA). Subsequently, 5 μ L of PI (50 μ g/mL) and 10 μ L Annexin V-FITC were added into each tube and incubated in the dark for 15 min at room temperature. Cell cycle and apoptosis rate were analyzed on a BD FACScan (BD Biosciences).

Luciferase reporter assays

HPA-v cells or 293T cells were seeded in 96-well plates for five repeats each group, then transfected with Lv-AC092159.2 or Lv-NC. After 24 h, these cells were transfected with pGL3-Basic TMEM18 (contains TMEM18 promoter sequence) or pGL3-Basic control vector, Lipofectamine 2000, and pRL-TK vector. Cells were cultured in 37°C, 5% CO₂ for 48 h and relative luciferase activities (ratios of Renilla luciferase signal normalized to firefly luciferase) were determined.

Statistical analysis

All data were presented as mean $\pm$ s.d. Student's two-tailed *t*-test was used to analyze the differences between groups. The Pearson's correlation coefficient analysis was employed to confirm the correlations. Values were considered statistically significant at $P < 0.05$.

Results

The expression characteristics of AC092159.2

To identify the lncRNAs related to adipogenesis, HPA-v were analyzed for lncRNA expression by lncRNA microarray, and many lncRNAs changed by at least twofold ($P < 0.01$) (data have not been published). LncRNA AC092159.2 was highly expressed in differentiated HPA-v by over threefold compared with undifferentiated HPA-v. Based on GTEx Portal database, AC092159.2 has two isoforms which were expressed in multiple human normal tissues (Fig. 1A and B). However, the isoform expressed in subcutaneous and visceral adipose is mainly ENST00000445418.1, which was used for following research. Change in AC092159.2 was

confirmed by qRT-PCR. The expression of AC092159.2 gradually increased during HPA-v differentiation and peaked at day 12 of differentiation (Fig. 1C). We further evaluated the correlation between AC092159.2 expression in omental adipose tissue and BMI in 31 subjects with overweight/obesity and 17 controls with normal weight. Interestingly, remarkable positive correlation existed between AC092159.2 levels and BMI ($r=0.413$, $P=0.004$) (Fig. 1D). These findings suggested that AC092159.2 may be associated with adipocyte differentiation and obesity.

AC092159.2 promotes human adipocytes differentiation

To determine whether AC092159.2 affected adipocyte differentiation, HPA-v cells were transduced with a lentivirus expressing AC092159.2 or a mock virus for at

least 48 h before transferring to differentiation medium. Fluorescence microscopy showed that 90% of cells of the experimental group and the control group were GFP-positive and transduced cells did not emerge any morphological changes. The expression of AC092159.2 was increased 233-fold at day 0 and remained high in the whole process of differentiation (Fig. 2A). Overexpression of AC092159.2 promoted adipocytes differentiation, as indicated by the expression of adipogenesis makers FABP4, PPAR γ , C/EBP β and C/EBP α (Fig. 2B, C, D and E), triacylglycerol content (Fig. 2F) and oil red O staining (Fig. 2G). In line with qRT-PCR data, the protein levels of FABP4, PPAR γ , C/EBP β and C/EBP α were also upregulated after transduction with the AC092159.2-expressing lentivirus (Fig. 2H and I).

The effects of AC092159.2 loss-of-function on adipogenesis were also determined. We performed an

Figure 2

Overexpression of AC092159.2 in HPA-v promoted adipogenic differentiation. (A) Overexpression efficiency of AC092159.2 was verified by qPCR. (B, C, D and E) AC092159.2 overexpression in HPA-v promoted adipogenic maker gene transcription based on qPCR at days 0, 1, 4, 7 and 10 during adipogenesis. (F) Triacylglycerol content detected neutral lipid accumulation. (G) Oil red O staining indicated the effect of AC092159.2 overexpression on HPA-v adipogenic differentiation at days 7 and 10. (H) Protein levels were detected by Western blot under the same experimental conditions. (I) The quantification of Western blot. Related mRNA levels are represented as mean $\pm$ s.d. from three or more independent repeats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. A full color version of this figure is available at <https://doi.org/10.1530/JME-18-0215>.

RNAi-knockdown experiment using a pool of siRNAs to transfect them into primary preadipocyte cultures, followed by induction of differentiation. The expression of AC092159.2 was decreased by about 50% compared with the control (Fig. 3A). At the same time, knockdown of AC092159.2 inhibited adipogenesis, as indicated by reduced adipogenic transcripts FABP4, PPAR γ , C/EBP β and C/EBP α (Fig. 3B, C, D and E), triacylglycerol content (Fig. 3F) and Oil Red O staining (Fig. 3G). Likewise, the protein levels of FABP4, PPAR γ , C/EBP β and C/EBP α were also decreased (Fig. 3H and I).

AC092159.2 does not influence cell cycle, apoptosis and proliferation

To further explore other effects on adipocytes performed by AC092159.2, we detected the effect of AC092159.2

on the cell cycle, apoptosis and proliferation. First, we examined the distribution of cells at different cell cycle phases in AC092159.2 transfected HPA-v cells using flow cytometry. Overexpression of AC092159.2 did not significantly influence the cell cycle distribution (Supplementary Fig. 1A and B) while knockdown of AC092159.2 slightly decreased the percentage of preadipocytes in the G1 phase and increased the percentage in the S phase, compared with the negative control cells, but the difference was not statistically significant (Supplementary Fig. 1C and D). And we also evaluated the effect of AC092159.2 on the apoptosis of HPA-v cells. The results showed that overexpression of AC092159.2 mildly inhibited the apoptosis of HPA-v cells without any statistical difference (Supplementary Fig. 1E) and AC092159.2 siRNA had no significant effect on HPA-v cells apoptosis (Supplementary Fig. 1F). To further

Figure 3

Knockdown of AC092159.2 in HPA-v inhibits adipogenic differentiation. (A) Knockdown efficiency of AC092159.2 was verified by qPCR. (B, C, D and E) Knockdown of AC092159.2 in HPA-v inhibited adipogenic marker gene transcription based on qPCR at days 0, 1, 4, 7 and 10 during adipogenesis. (F) Triacylglycerol content detected neutral lipid accumulation. (G) Oil red O staining indicated the effect of AC092159.2 knockdown on HPA-v adipogenic differentiation at Days 7 and 10. (H) Protein levels were detected by Western blot under the same experimental conditions. (I) The quantification of Western blot. Related mRNA levels are represented as mean $\pm$ s.d. from three or more independent repeats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. A full color version of this figure is available at <https://doi.org/10.1530/JME-18-0215>.

evaluate the effect of AC092159.2 on cell proliferation, the CCK-8 assay was used to detect the growth activity of the transfected HPA-v cells in 72 h. At 24, 48 and 72 h, both overexpression and knockdown of AC092159.2 had no significant effect on HPA-v cell proliferation compared with control groups (Supplementary Fig. 1G and H). Thus, AC092159.2 is not requisite to maintain HPA-v cell self-renewal.

The correlation analysis between AC092159.2 and its potential target gene *TMEM18*

LncRNAs could modulate expression of genes that are positioned in the vicinity of their transcription sites in a *cis*-acting manner (Song *et al.* 2012). To further comprehend the mechanism underlying the regulation of adipogenesis by AC092159.2, we searched the transcription position in the UCSC genome browser and found AC092159.2 transcription sites located ~247 bp upstream of the *TMEM18* gene (Fig. 4A). Furthermore, based on GTEx Portal database, six single nucleotide polymorphisms (SNPs) in AC092159.2 were associated not only with the expression of AC092159.2, but also with *TMEM18*, and the effect sizes were similar with consistent direction (Fig. 4B). We further detected the expression of *TMEM18* by qPCR among 48 omental adipose tissues of normal and subjects with obesity. The results revealed that the expression of *TMEM18* was significantly higher in adipose tissues of subjects with obesity (Fig. 4C) and positively correlated with the expression of AC092159.2 (Fig. 4D). And the expression of *TMEM18* and AC092159.2 were also positively correlated during adipogenic differentiation (Fig. 4E). To further verify the relationship between AC092159.2 and *TMEM18*, we detected the expression of *TMEM18* with the overexpression and knockdown of AC092159.2 in HPA-v, respectively. Both qRT-PCR and Western blot revealed that overexpression of AC092159.2 boosted *TMEM18* expression while knockdown of AC092159.2 restrained *TMEM18* expression (Fig. 4F, G, H and I), suggesting that *TMEM18* may be the downstream target gene of AC092159.2.

The effect of AC092159.2 overexpression could be restrained by *TMEM18* siRNA in HPA-v during adipogenic differentiation

The above results had been preliminarily revealed that AC092159.2 participated in adipocyte differentiation possibly by regulating *TMEM18*. In order to further demonstrate this mechanism, we adopted the rescue

strategy. We tested whether the adipogenic bloom induced by overexpression of AC092159.2 could be weakened by knockdown of *TMEM18*. The results showed that knockdown of *TMEM18* partially restrained adipogenic differentiation in AC092159.2 overexpressed HPA-v, as assessed by *TMEM18*, *C/EBP β* and *C/EBP α* mRNA (Fig. 5A, B and C), as well as triacylglycerol content (Fig. 5D) and Oil Red O staining (Fig. 5E).

We also knocked down AC092159.2 in *TMEM18* overexpressed HPA-v. Adipogenic key genes *C/EBP β* and *C/EBP α* mRNA expression (Fig. 6A, B and C), triacylglycerol content (Fig. 6D) and oil red O staining (Fig. 6E) all supported that adipogenic defect caused by knockdown of AC092159.2 could be rescued by overexpression of *TMEM18*. These data revealed that, during adipogenic differentiation, AC092159.2 worked with *TMEM18* in HPA-v.

AC092159.2 has a transcriptional activation effect on *TMEM18*

The *TMEM18* promoter luciferase plasmid pGL3-Basic *TMEM18* and Lv-AC092159.2 were cotransfected to HPA-v cells or 293T cells, and the transcriptional activation was detected. The luciferase activity was significantly higher than in the control group after AC092159.2 overexpression and pGL3-Basic *TMEM18* luciferase plasmid transfection for 48 h ($P < 0.05$; Fig. 7A and B), which indicates that AC092159.2 has a transcriptional activation effect on *TMEM18*.

Discussion

In this study, we first reported lncRNA AC092159.2 and found that the expression of AC092159.2 gradually increased during HPA-v differentiation, and its expression in omental adipose tissue was positively related with BMI. Overexpression of AC092159.2 promoted adipocytes differentiation while knockdown of it led to an adipogenic defect. Moreover, the expression of AC092159.2 and *TMEM18* were positively correlated during adipogenic differentiation. AC092159.2 overexpression boosted *TMEM18* expression while AC092159.2 knockdown restrained *TMEM18* expression. Further rescue experiments showed that *TMEM18* knockdown partially restrained adipogenic differentiation in AC092159.2 overexpressed HPA-v, and adipogenic defect caused by AC092159.2 knockdown could be rescued by *TMEM18* overexpression. Luciferase reporter assays revealed that AC092159.2 had a transcriptional activation effect on *TMEM18*.

Figure 4

The correlation analysis between AC092159.2 and its potential target gene *TMEM18*. (A) The obesity susceptibility gene *TMEM18* is adjacent to AC092159.2. (B) Based on GTEx Portal database, six SNPs in AC092159.2 were associated not only with the expression of AC092159.2, but also with *TMEM18*. And the effect sizes were similar with consistent direction. (C) Relative expression of *TMEM18* by qPCR among 48 omental adipose tissues of normal and subjects with overweight/obesity. Ratios were calculated as mean $\pm$ s.d. (D) Positive correlation existed between *TMEM18* levels and BMI among the 48 omental adipose tissues of normal and subjects with obesity. (E) Relative expression of AC092159.2 and *TMEM18* during HPA-v differentiation was quantitated by qPCR. Ratios were calculated as mean $\pm$ s.d. from triplicate samples. (F, G and H) The relative expression of *TMEM18* with the overexpression and knockdown of AC092159.2 in HPA-v, respectively. (I) The quantification of Western blot. Data shown are averages of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. A full color version of this figure is available at <https://doi.org/10.1530/JME-18-0215>.

Therefore, AC092159.2 may serve as a positive regulator of adipocyte differentiation, and promoted the adipogenesis of human preadipocyte possibly by regulating *TMEM18*. It is indicated that AC092159.2 may play an essential role in obesity and related metabolic disorders. However, the precise molecular network that underlies regulation of adipocyte differentiation is still unclear, so further efforts to confirm the regulatory factors involved are of great concern. Further exploring the role and mechanism of AC092159.2 in adipocyte differentiation will ultimately open a new avenue to understand adipogenesis.

LncRNAs have vital roles in regulating cell fate during developmental and differentiation processes and are often lineage-specific in multicellular organisms (Batista & Chang 2013). Multiple lncRNAs have been identified to be involved in adipocyte differentiation (Kikuchi *et al.* 2009, Sun *et al.* 2013, Xiao *et al.* 2015) and lncRNAs were reported to regulate the expression of adjacent genes as *cis*-acting regulatory elements (Ponting *et al.* 2009). LncRNA ADINR transcribed from a position ~450bp upstream of the *C/EBP α* gene, orchestrates *C/EBP α* transcription *in vivo*. It is shown that ADINR plays an important role

Figure 5

The effect of AC092159.2 overexpression could be rescued by TMEM18 siRNA in HPA-v during adipogenic differentiation. (A, B and C) qPCR analysis of adipogenic maker genes (C/EBPβ and C/EBPα) on day 7 of adipogenic differentiation. (D) Triacylglycerol content detected neutral lipid accumulation on day 7 of adipogenic differentiation. (E) Oil red O staining on day 7 of adipogenic differentiation. Related mRNA levels are represented as mean $\pm$ s.d. from three or more independent repeats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. A full color version of this figure is available at <https://doi.org/10.1530/JME-18-0215>.

in adipogenesis through regulating C/EBPα in *cis* (Xiao *et al.* 2015). LncRNA U90926 was reported to attenuate 3T3-L1 adipocyte differentiation via inhibiting the transactivation of PPARγ2 or PPARγ (Chen *et al.* 2017). It has been confirmed that the transcription of most lncRNAs are in coordination with transcription of protein-coding genes and over 2000 lncRNAs are bidirectionally transcribed within 2|kb of the transcription start sites of protein-coding genes (Sigova *et al.* 2013). Our findings suggested that AC092159.2 exerted its effect possibly by impacting on its neighboring gene *TMEM18* which has been confirmed as an obesity susceptibility gene (Den Hoed *et al.* 2010, Jurvansuu & Goldman 2011, Berndt *et al.* 2013, Wheeler *et al.* 2013, Locke *et al.* 2015, Larder *et al.* 2017).

TMEM18 is a transmembrane protein of 140 amino acids containing four transmembrane domains which

could act as DNA-binding protein for other downstream genes (Jurvansuu & Goldman 2011) and plausible mediator of genetic variation on human adiposity (Larder *et al.* 2017). In recent years, researches have confirmed that TMEM18 was an obesity-associated gene (Den Hoed *et al.* 2010, Berndt *et al.* 2013, Wheeler *et al.* 2013, Locke *et al.* 2015, Larder *et al.* 2017) and TMEM18 was identified to be crucial for adipogenesis (Bernhard *et al.* 2013). Moreover, the methylation levels of TMEM18 promoter were possibly associated with parameters of obesity and fat distribution (Rohde *et al.* 2014). Similar to our study, mRNA expression of TMEM18 was reported to be well correlated with body weight in rats (Rask-Andersen *et al.* 2012) and knockdown of TMEM18 resulted in an obvious reduction in triacylglycerol accumulation and adipocyte differentiation in HPA-v (Bernhard *et al.* 2013). However, Rachel *et al.* reported that loss of *Tmem18* in male mice

Figure 6

The effect of AC092159.2 siRNA could be rescued by TMEM18 overexpression in HPA-v during adipogenic differentiation. (A, B and C) qPCR analysis of adipogenic maker genes (C/EBPβ and C/EBPα) on day 7 of adipogenic differentiation. (D) Triacylglycerol content detected neutral lipid accumulation on day 7 of adipogenic differentiation. (E) Oil red O staining on day 7 of adipogenic differentiation. Related mRNA levels are represented as mean $\pm$ s.d. from three or more independent repeats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. A full color version of this figure is available at <https://doi.org/10.1530/JME-18-0215>.

Figure 7

Luciferase reporter assays show that transcriptional activation of TMEM18 promoter in response to AC092159.2 overexpression. (A) In HPA-v. (B) In 293T cells. Related luciferase activities are from five repeats each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

led to an increase in body weight, which was aggravated by high-fat diet and influenced by increased food intake. Overexpression of *Tmem18* in the hypothalamic paraventricular nucleus (PVN) of wild-type mice led to decreased food intake and increased energy expenditure (Larder *et al.* 2017). But the phenotype of increased body weight in *Tmem18* null mice was sexually dimorphic (Larder *et al.* 2017). In the above research, TMEM18 exerted its effect on metabolism in PVN mainly by affecting appetite (Larder *et al.* 2017). It is shown that the

effect of TMEM18 on metabolic regulation may be tissue- and gender-specific. The tissues specificity and potential biological mechanisms relevant to this phenomenon are still controversial (Kozak 2010). So, further tissue-specific and sex-specific method may prove more productive. Although the precise molecular mechanism that underlies regulation of TMEM18 is not fully understood, we identified that TMEM18 may be the target gene of AC092159.2 and AC092159.2 may exert its function by acting on TMEM18.

Conclusion

Taken together, we identified AC092159.2 as an uncharacteristic transcriptional regulator of TMEM18 and concluded that AC092159.2 participated in the development of adipogenesis by interacting with TMEM18, which shed new light onto how lncRNAs harmonizes the regulation of downstream gene expression and adipogenic differentiation. Thus, further understanding the definite role of lncRNAs in gene regulatory networks and diseases may provide a novel potential target for the development of human therapeutics.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/JME-18-0215>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Juan Wen and Xirong Guo designed the research. Yingdi Yuan, Xinguo Cao, Jiaojiao Hu, Jingyun Li, Dan Shen, Lianghui You, Xing Wang, Yahui Zhou, Yao Gao, Lijun Zhu and Pengfei Xu performed the experiments. Lianghui You and Xing Wang analyzed the data. Yingdi Yuan and Juan Wen wrote this manuscript. Chenbo Ji supervised this work. All the authors reviewed the report and approved the final version.

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