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mRNA m^{6}A methylation downregulates adipogenesis in porcine adipocytes

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ABSTRACT

Fat Mass and Obesity-associated protein (FTO), associated with obesity, is proved to demethylate N6-methyladenosine (m^{6}A), which raises questions regarding whether m^{6}A plays vital roles in adipogenesis. To prove this, overexpression and knockdown of FTO and METTL3, as well as the chemical treatment in procine adipocytes were conducted. The results showed FTO negatively regulated m^{6}A levels and positively regulated adipogenesis, while METTL3 positively correlated with m^{6}A levels and negatively with adipogenesis. To remove the potential effect of FTO and METTL3 gene, chemical reagents of methylation inhibitor cycloleucine and methyl donor betaine were used to test the regulation effect of m^{6}A on adipogenesis. The results showed the inverse effect of m^{6}A on lipid accumulation in porcine adipocytes. These findings provide compelling evidence that m^{6}A plays a critical role in the regulation of adipogenesis.

1. Introduction

The fat mass and obesity associated gene (FTO), which was first being described in 2007, is believed to be associated with elevated body mass index and increased risk for obesity in human [1]. A large number of studies have recently proved that FTO polymorphisms are associated with pork quality, fat deposition such as intramuscular fat deposition or related traits in different pig populations [2–5], confirming the role of this gene in fatness across species.

FTO belongs to the Fe (II) − and oxoglutarate-dependent AlkB oxygenase family and was proved recently to demethylate N6-methyladenosine (m^{6}A), a wide-spread modification in mammalian messenger RNA [6]. These studies raise questions regarding whether m^{6}A plays vital roles in biological processes, such as lipid deposition. The methylation of DNA was discovered in 1925, while the methylation of RNA was not revealed until 1968. m^{6}A is the most prevalent and reversible internal messenger RNA modification in eukaryotes as well as viruses with a nuclear phase [7]. However, little was known about its precise role in biological process.

Related to FTO is methyltransferase like 3 (METTL3), a 70-kDA subunit that forms part of the N^6-adenosine methyltransferase to install m^{6}A on mRNA [8]. The m^{6}A modification is catalyzed by demethylase FTO and methylase METTL3. It prompted us to investigate whether FTO and METTL3 affect adipogenesis through regulation of m^{6}A abundance. Furthermore, in order to explore the m^{6}A methylation on adipogenesis directly, cycloleucine, a methylation inhibitor, and betaine, a methyl donor, were also studied.

Our results suggested that mRNA m^{6}A methylation of porcine adipocytes negatively regulates adipogenesis, and mRNA m^{6}A methylation could be regulated by demethylase FTO, m^{6}A methyltransferase METTL3, exogenous methylation inhibitor and methyl donors.

Abbreviations: FTO, fat mass and obesity associated gene; METTL3, methyltransferase like 3; m^{6}A, N6-methyladenosine; c/EBPβ, CCAAT/enhancer-binding protein β; PPARγ, proliferator-activated receptor γ; ACC, Acetyl-CoA carboxylase; FAS, fatty acid synthase; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; METTL14, methyltransferase-like 14.

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2. Materials and methods

2.1. Animals and cell culture

All procedures were approved by the Committee on Animal Care and Use and the Committee on the Ethics of Animal Experiments of

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Zhejiang University. The Porcine preadipocytes were isolated and cultured according to Shan et al. [9].

2.2. FT11 CMV-MCS-EF1-mcherry plasmid construction and transfection

The full-length cDNA sequence of pig FTO and METTL3 genes were amplified by reverse transcription-polymerase chain reaction (RT-PCR) using primers listed in Table 1. The recovered PCR product was ligated to the fragment collected from the CMV-MCS-EF1-mcherry (Fig. S1) after digestion with XbaI and EcoRI using CloneEZ Enzyme (Genscript, China). Recombinant vector were named as CMV-pFTO and CMV-pMETTL$_3$ which were blasted and identified (Fig. S2). The CMV-pFTO and CMV-pMETTL$_3$ plasmid were transfected into cells using Lipofectamine™ 2000 (Invitrogen Life Technologies) according to the manufacturer's instructions. The CMV empty plasmid named CMV-EF1 was served as the negative control.

2.3. RNA interference (RNAi)

The SHC203 lentiviral vectors for knockdown of FTO or METTL3 were constructed as described by Shin et al. [10]. Three potential small interference RNA (FTO-shRNA1, FTO-shRNA2, METTL3-shRNA) target sites were determined using the siRNA design program and confirmed with BLAST for specificity. The shRNA primer sequences of FTO and METTL3 were listed in Table 1. Oligonucleotides to generate the plasmid-based siRNA were cloned into SHC203 vector (supplementation Fig. 2) which was digestion with HpaI and KpnI, and all constructs were confirmed by sequencing. Lentiviruses were generated by transfecting HEK293T cells with the SHC203 vectors together with packaging vectors psPAX2, pMD2.G. Lentiviruses were collected 24 and 48 h post transfection.

2.4. Cycloleucine or betaine treatment

Porcine adipocytes were treated with 10, 20 or 40 mM cycloleucine (CL), or 0.1, 0.2 or 0.4 mM betaine (Bet) for 24 h for dose–response experiments, in which the optimal concentrations were used for further study.

2.5. mRNA m^{6}A level detection

Dot blots were performed to detect the m^{6}A level of porcine adipocytes using a previously described anti-m^{6}A antibody [11].

2.6. Triglyceride and glycerol assay

The glycerol content in culture medium was determined using glycerol kit (Applygen Technologies Inc. Beijing, China) and intracellular triglyceride was assayed using a triglyceride assay kit (Applygen Technologies Inc.) according to the manufacture's instruction.

2.7. RNA extraction, cDNA synthesis and real-time PCR

Total RNA was extracted from porcine adipocytes using Trizol Reagent according to the manufacture's instruction. Approximately 500 ng of total RNA was reverse-transcribed into cDNA using M-MuLV reverse transcriptase kit (Fermentas, EU, GlenBurnie, Maryland, USA). Gene transcript levels were determined using a SYBRR Premix ExTaq™ Kit (Takara Biotechnology Co. Ltd, Otsu, Shiga, Japan) in the ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as described by Wang et al. [12]. The PCR primers were designed using the Primer premier software v5.0 (PREMIER Biosoft International, Palo Alto, CA, USA) and synthesized by Invitrogen. Efficiency of primers was tested from two fold serial dilutions of cDNA for each primer pair. Data were analyzed by using 2-ΔΔCt and are referred to the control treatment using 18S as a reference gene. The primer sequences were listed in Table 1.

2.8. Statistical analysis

Data were presented as the mean ± SEM. A one-way analysis of variance (ANOVA) was performed to determine the significant difference in treatments using SPSS13.0. Means were considered significantly different at $P < 0.05$.

Table 1
Primers used in the study.

Gene name	Primer sequences (5′-3′)
CMV-FTO-F	acctccatagaagatAGCAGCAGCATGAAGCGAAC
CMV-FTO-R	GATCCATTTAAATTCgaattcCTTTCTCTAGGGTTTGGCTTCC
CMV-METTL3-F	acctccatagaagatAATGTCGGACACGTGGAGCTCTA
CMV-METTL3-R	GATCCATTTAAATTCgaattcTGGCCATGGATGCTTAGGTTTC
FTO-shRNA1-F	cGCACAAGCATGGCTGCTTATTtcaagagAATAAGCAGCCATGCTTGTGCTTTTT
FTO-shRNA1-R	AAAAAGCACAAGCATGGCTGCTTATTctcttgaAATAAGCAGCCATGCTTGTGCggtac
FTO-shRNA2-F	cGCGGTGGCAGTGTACAATTATtcaagagATAATTGTACACTGCCACCGCTTTTT
FTO-shRNA2-R	AAAAAGCGGTGGCAGTGTACAATTATctcttgaATAATTGTACACTGCCACCGCggtac
METTL3-shRNA-F	cGCAGTTCCTGAGCTAGCTACAtcaagagTGTAGCTAGCTCAGGAACTGCTTTTT
METTL3-shRNA-R	AAAAAGCAGTTCCTGAGCTAGCTACActcttgaTGTAGCTAGCTCAGGAACTGCggtac
PPARγ-F	AGGACTACCAAAGTGCCATCAAA
PPARγ-R	GAGGCTTTATCCCCACAGACAC
C/EBPβ-F	GCACAGCGACGAGTACAAGA
C/EBPβ-R	TATGCTGCGTCTCCAGGTTG
18S–F	CCCACGGAATCGAGAAAGAG
18S-R	TTGACGGAAGGGCACCA
FAS-F	CAGGCGAACACGATGGA
FAS-R	GAAGGGAAGCAGGGTTGATG
ACC-F	GGAGACAAACAGGGACCATTACA
ACC-R	CAGGGACTGCCGAAACATC
ATGL-F	TCACCAACACCAGCATCCA
ATGL-R	GCACATCTCTCGAAGCACCA
HSL-F	ACCCTCGGCTGTCAACTTCTT
HSL-R	ACTTTCTCCTCCTTGGTGCTAATCT

3. Results

3.1. Overexpression of FTO increased the lipid accumulation and decreased mRNA m^{6}A level in porcine adipocytes

Porcine adipocytes were transfected with CMV-pFTO, CMV-EF1 (negative control) and control, and FTO expression was determined at 48 h, 72 h and 96 h after transfection to determine the optimum transfection time. The results showed that FTO expression significantly increased 48 h after transfection and peaked at 96 h (Fig. 1A). Then transfection time of 96 h was chosen for following experiment. Overexpression of FTO in porcine adipocyte significantly increased the intracellular triglyceride content, decreased glycerol content in medium (Fig. 1B and C). The mRNA expression of adipogenesis related genes C/EBPβ ($P < 0.05$) was upregulated and that of lipolysis genes ATGL and HSL were downregulated ($P < 0.05$) by FTO overexpression (Fig. 1D). Dot blots were performed to detect the effect of FTO on m^{6}A level in porcine adipocytes (Fig. 1E). The results showed overexpression of FTO in porcine adipocytes significantly decreased the mRNA m^{6}A levels, which suggested that the FTO negatively regulated mRNA m^{6}A methylation.

3.2. Knockdown of FTO decreased the lipid accumulation and increased mRNA m^{6}A level in porcine adipocytes

To confirm the regulation of FTO on adipogenesis and mRNA m^{6}A methylation, porcine adipocytes were treated with FTO-shRNA1, FTO-shRNA2 and negative control siRNA respectively. Compared with the negative control, transfection of porcine adipocytes with FTO-shRNA1 and FTO-shRNA2 for 48 h resulted in a 68% and 58% knockdown of FTO expression (Fig. 1F). However, the interference effect decreased at 72 h and 96 h. Then we choose FTO-shRNA2 to knockdown FTO expression and 48 h for the suitable transfection time in the following experiments. Knockdown of FTO significantly decreased the intracellular triglyceride content and increased glycerol content in culture medium (Fig. 1G and H). Expression of the adipogenesis related genes c/EBPβ, PPARγ, FAS and ACC were reduced significantly ($P < 0.05$) and that of lipolysis gene ATGL was increased significantly ($P < 0.05$) after treated with FTO-shRNA2 in porcine adipocytes (Fig. 1I). The results from dot blots showed knockdown of FTO in porcine adipocytes significantly increased the mRNA m^{6}A methylation (Fig. 1J).

3.3. Overexpression of METTL3 inhibited lipid accumulation and increased mRNA m^{6}A level in porcine adipocytes

As FTO was demonstrated to modulate lipid accumulation and mRNA m^{6}A level in porcine adipocyte, we next sought to determine whether the methylase METTL3 plays a role in this process. Treating adipocytes with CMV-pMETTL3 significantly increased METTL3 expression at 48 h, 72 h and 96 h ($P < 0.01$) (Fig. 2A). The time point of 96 h was selected as the optimized transfection time for further study. The results showed overexpression of METTL3 significantly reduced the intracellular triglyceride content ($P < 0.05$) with glycerol content significant increase in the medium ($P < 0.05$) (Fig. 2B and C). The gene expression of PPARγ and FAS were significantly

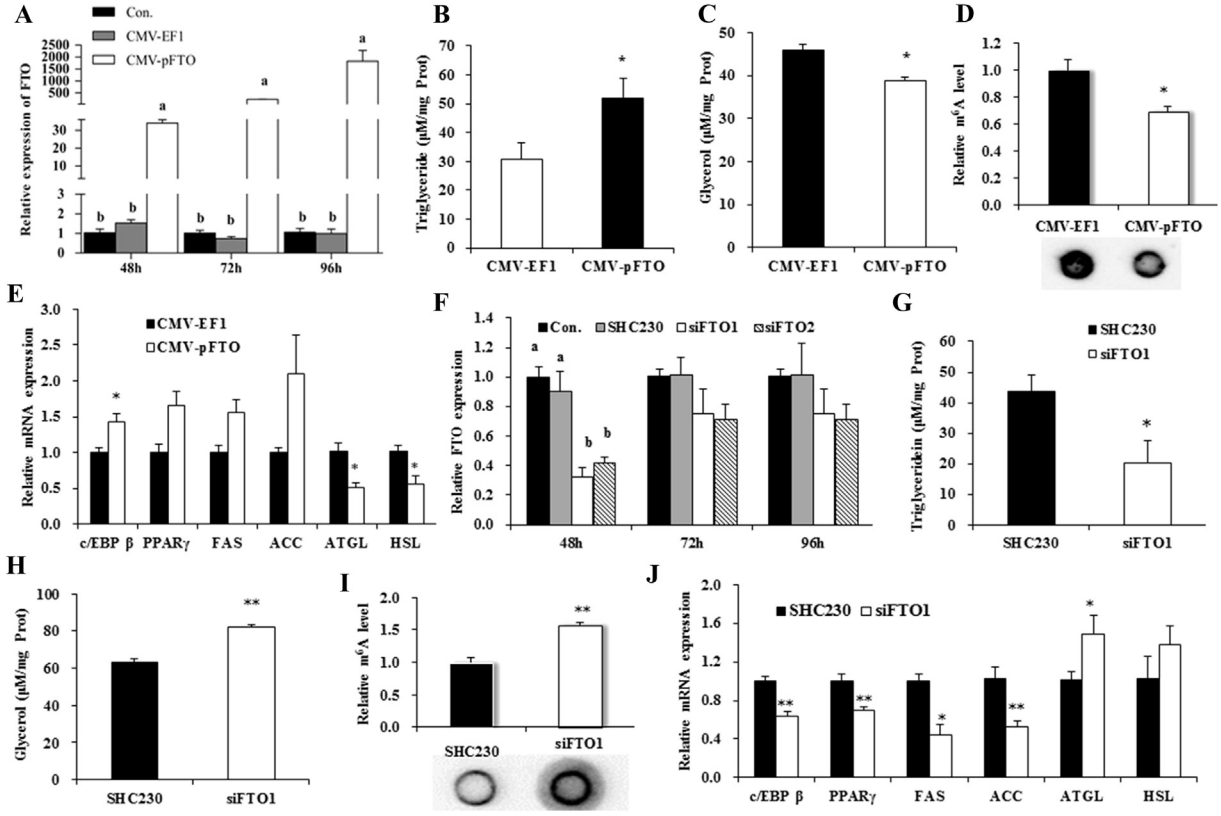

Fig. 1. Effect of FTO on lipid accumulation, mRNA m^{6}A level and gene expressions in porcine adipocytes. (A) Effect of CMV- FTO on FTO mRNA expression in porcine adipocytes. (B) Effect of FTO overexpression on triglyceride content in porcine adipocytes. (C) Effect of FTO overexpression on glycerol content in culture medium. (D) Effect of FTO overexpression on mRNA m^{6}A level in porcine adipocytes. (E) Effect of FTO overexpression on mRNA expression in porcine adipocytes. (F) Effect of FTO-shRNA1 and FTO-shRNA2 on FTO mRNA expression in porcine adipocytes. (G) Effect of FTO knockdown on triglyceride content in porcine adipocytes. (H) Effect of FTO knockdown on glycerol content in culture medium. (I) Effect of FTO knockdown on mRNA m^{6}A level in porcine adipocytes. (J) Effect of FTO knockdown on mRNA expression in porcine adipocytes. FTO, fat mass and obesity associated gene; m^{6}A, N6-methyladenosine; c/EBPβ, CCAAT/enhancer-binding protein β; PPARγ, proliferator-activated receptor γ; ACC, Acetyl-CoA carboxylase; FAS, fatty acid synthase; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase. Data are means ± SEM; n = 3. *, P < 0.05; **, P < 0.001.

downregulated ($P < 0.05$) (Fig. 2D). The mRNA m^{6}A level was significantly increased by METTL3 overexpression as determined by dot blots ($P < 0.05$) (Fig. 2E).

3.4. Knockdown of METTL3 had no effect on the lipid accumulation and mRNA m^{6}A level in porcine adipocytes

After METTL3-shRNA was transfected into porcine adipocytes for 48 h, no significant change of METTL3 expression was observed. At 72 h after transfection, METTL3 expression was significantly reduced compared to control ($P < 0.05$) (Fig. 2F). Since the inhibition effect of METTL3-shRNA was decreased at 96 h after transfection, 72 h was used in the following experiment. The results showed knowdown of METTL3 showed no significant difference in intracellular triglyceride content (Fig. 2G), glycerol content in the medium (Fig. 2H) and the adipogenesis related gene expression (Fig. 2I). The results from dot blots demonstrated mRNA m^{6}A level was not affected by the knockdown of METTL3 (Fig. 2J).

3.5. Exogenous regulation of mRNA m^{6}A methylation and lipid accumulation

To further confirm the inverse regulation effect of mRNA m^{6}A on adipogenesis, we treated porcine adipocytes with methylation inhibitor cycloleucine or methyl donor betaine to rule out the potential interference of FTO or METTL3 gene.

Treatment with different doses of CL significantly decreased mRNA m^{6}A level in a dose-dependent manner in porcine adipocytes ($P < 0.05$, Fig. 3A). Compared with the control, 10, 20 or 40 mM CL administration decreased m^{6}A levels by 23% ($P < 0.05$), 34% ($P < 0.05$) and 53% ($P < 0.05$), respectively. To determine if CL is toxic to adipocytes, cell proliferation was detected after CL treatment for 24 h using MTT method. The results showed that exposure of cultured adipocytes to 20 or 40 mM CL significantly inhibited cell growth ($P < 0.05$, Fig. 3B), and no difference were observed when cells exposed to 10 mM CL. Then the CL concentration of 10 mM (CL10) was used in the following experiment. Treatment with CL10 significantly increased intracellular triglyceride ($P < 0.05$) and decreased glycerol in culture medium ($P < 0.05$) (Fig. 3C and D). Gene expression showed c/EBPβ, PPARγ and ACC were upregulated ($P < 0.05$), and ATGL and HSL were downregulated ($P < 0.05$) by CL10 in adipocytes (Fig. 3E).

To test the effects of Betaine on mRNA m^{6}A level, porcine adipocytes were incubated with 0.1, 0.2 or 0.4 M betaine. The results showed betaine increased mRNA m^{6}A level in a dose-dependent pattern (Fig. 4A). To assess if betaine has negative effect on cell growth, adipocytes were treated with 0.1, 0.2 or 0.4 M betaine and observed by microscopy. 0.1 or 0.2 M betaine did not show any significant effects on cell growth, while higher concentration than 0.4 M inhibited cell growth as shown in Fig. 4B. Then the concentration of 0.2 M (Bet 2) was used to assay the effect of betaine on lipid accumulation. The results demonstrated Bet 2 significantly decreased intracellular triglyceride ($P < 0.05$) (Fig. 4C) and

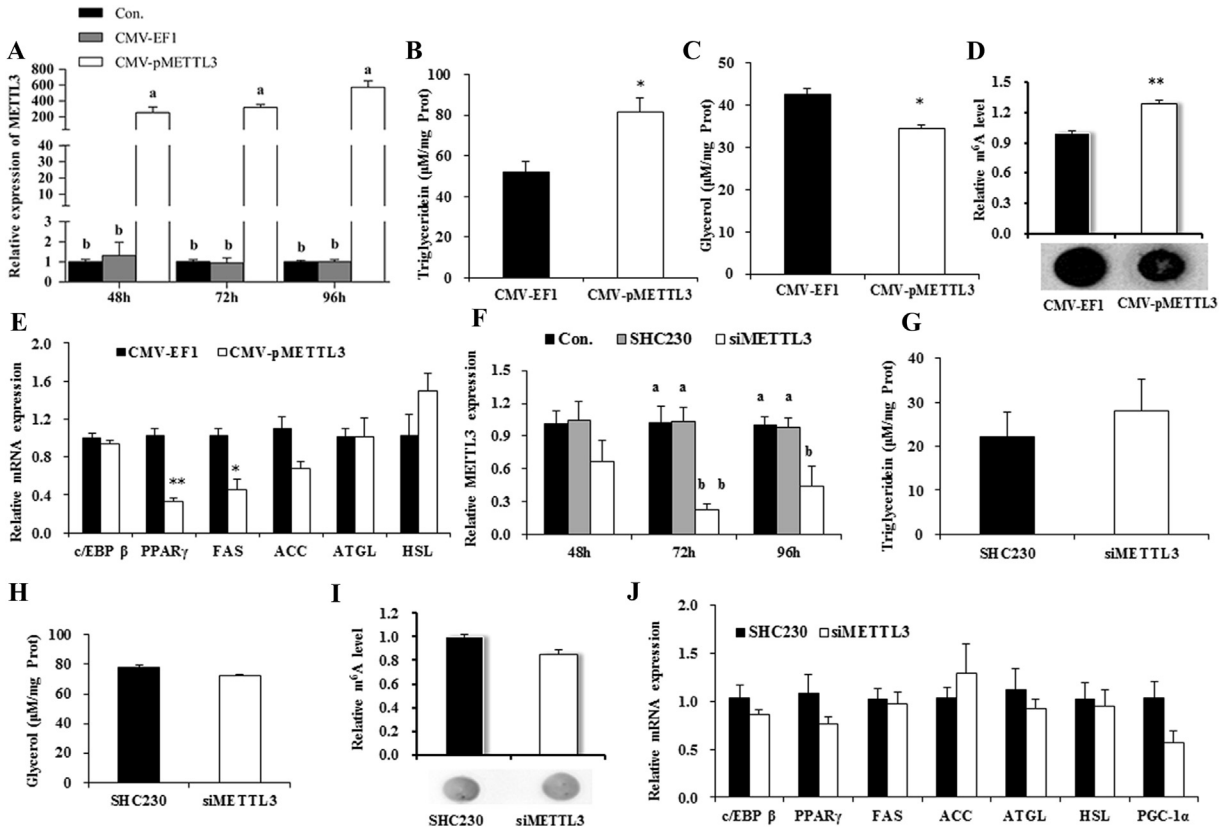

Fig. 2. Effect of METLL3 on lipid accumulation, mRNA m^{6}A level and gene expressions in porcine adipocytes. (A) Effect of CMV- METLL3 on METLL3 mRNA expression in porcine adipocytes. (B) Effect of METLL3 overexpression on triglyceride content in porcine adipocytes. (C) Effect of METLL3 overexpression on glycerol content in culture medium. (D) Effect of METLL3 overexpression on mRNA m^{6}A level in porcine adipocytes. (E) Effect of METLL3 overexpression on mRNA expression in porcine adipocytes. (F) Effect of SiMETLL3 on METLL3 mRNA expression in porcine adipocytes. (G) Effect of METLL3 knockdown on triglyceride content in porcine adipocytes. (H) Effect of METLL3 knockdown on glycerol content in culture medium. (I) Effect of METLL3 knockdown on mRNA m^{6}A level in porcine adipocytes. (J) Effect of METLL3 knockdown on mRNA expression in porcine adipocytes. METTL3, methyltransferase like 3; m^{6}A, N6-methyladenosine; c/EBPβ, CCAAT/enhancer-binding protein β; PPARγ, proliferator-activated receptor γ; ACC, Acetyl-CoA carboxylase; FAS, fatty acid synthase; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase. Data are means ± SEM; n = 3. *, P < 0.05; **, P < 0.001.

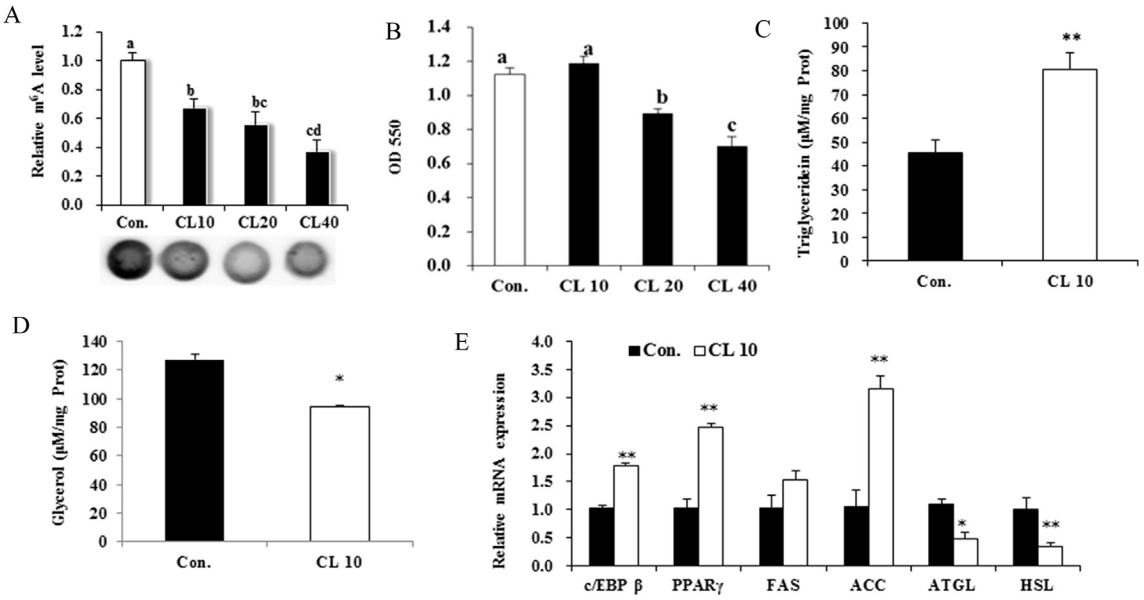

Fig. 3. Effect of cycloleucine on lipid accumulation, mRNA m^{6}A level and gene expressions in porcine adipocytes. (A) Effect of cycloleucine on mRNA m^{6}A level in porcine adipocytes. (B) Effect of cycloleucine on porcine adipocytes growth. (C) Effect of cycloleucine on triglyceride content in porcine adipocytes. (D) Effect of cycloleucine on glycerol content in culture medium. (E) Effect of cycloleucine on mRNA expression in porcine adipocytes. m^{6}A, N6-methyladenosine; c/EBPβ, CCAAT/enhancer-binding protein β; PPARγ, proliferator-activated receptor γ; ACC, Acetyl-CoA carboxylase; FAS, fatty acid synthase; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase. Data are means ± SEM; n = 3. *, $P < 0.05$; **, $P < 0.001$.

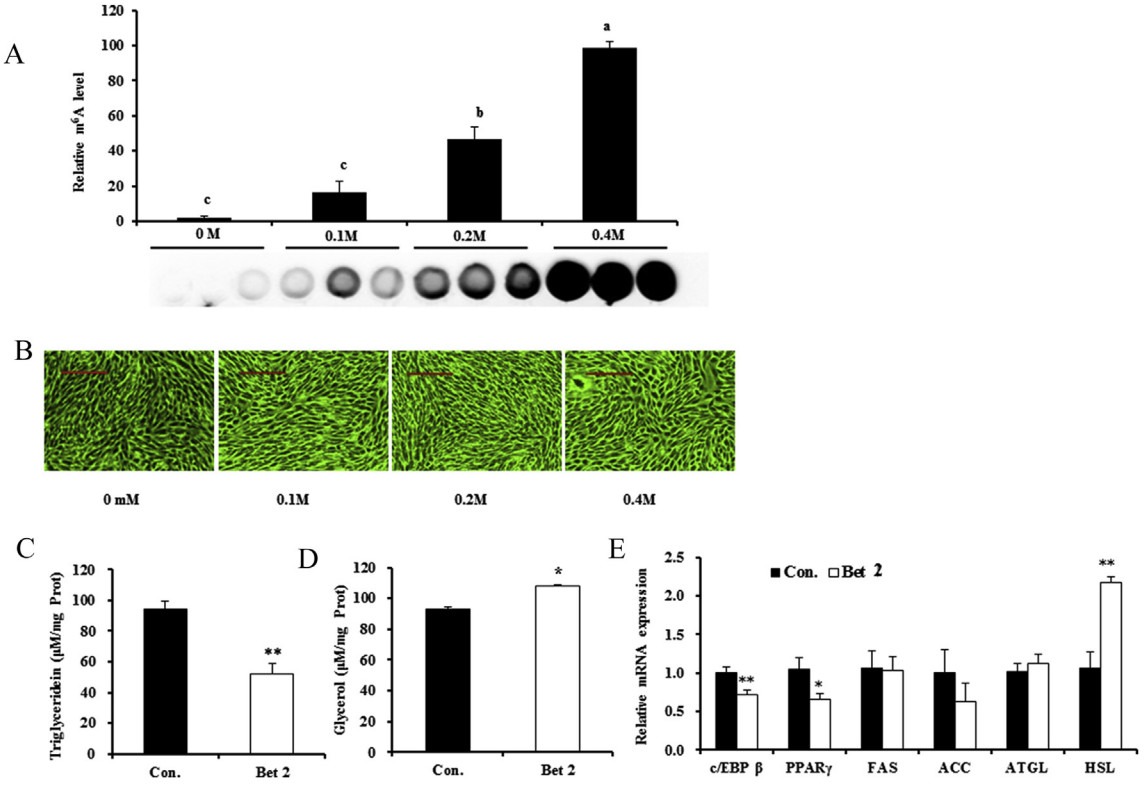

Fig. 4. Effect of betaine on lipid accumulation, mRNA m^{6}A level and gene expressions in porcine adipocytes. (A) Effect of betaine on mRNA m^{6}A level in porcine adipocytes. (B) Effect of betaine on porcine adipocytes growth. (C) Effect of betaine on triglyceride content in porcine adipocytes. (D) Effect of betaine on glycerol content in culture medium. (E) Effect of betaine on mRNA expression in porcine adipocytes. m^{6}A, N6-methyladenosine; c/EBPβ, CCAAT/enhancer-binding protein β; PPARγ, proliferator-activated receptor γ; ACC, Acetyl-CoA carboxylase; FAS, fatty acid synthase; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase. Data are means ± SEM; n = 3. *, $P < 0.05$; **, $P < 0.001$.

increased glycerol content ($P < 0.05$) (Fig. 4D) in culture medium. Bet 2 significantly decreased c/EBPβ and PPARγ, and increased HSL expression ($P < 0.05$) (Fig. 4E).

4. Discussion

Our data show that m^{6}A is directly involved in the regulation of lipid accumulation in porcine adipocytes partly by regulating related gene expression. High m^{6}A level leads to less lipid accumulation, while low m^{6}A promotes adipogenesis.

Jia provided evidence that FTO strongly prefers to demethylate N6-methyladenosine (m^{6}A) in ssRNA. By direct comparison with other substrates they conclude that m^{6}A in ssRNA is the best substrate discovered. FTO has a greater than 50-fold preference for m^{6}A over 3-methyluracil [13]. In our experiment, expected changes in levels of m^{6}A in mRNA were found when porcine adipocytes were manipulated to either overexpress FTO (which caused a decreased level of m^{6}A) or underexpress FTO (which increased the level of m^{6}A). Overexpression of FTO leads to increased food intake and a dose-dependent increase in body and fat mass in mice [14]. Loss of FTO in mice leads to increased energy expenditure and a significant reduction in adipose tissue and lean body mass [15]. Our results also suggested that FTO expression correlates positively with fat deposition in porcine adipocytes. It can be inferred the function of FTO on adipogenesis may be mediated by m^{6}A.

In addition, the level of m^{6}A can also be regulated by the N6-adenosine methyltransferase METTL3 [16]. METTL3 is one of the multicomponent N6-adenosine methyltransferase complex [17]. Overexpressed METTL3 increased m^{6}A level as expected and decreased lipid accumulation, which further confirmed the inverse effect of m^{6}A level on adipogenesis. No significant difference was observed in m^{6}A level when METTL3 was knocked down, indicating that there may exist alternative ways to m^{6}A catalytic reaction in porcine adipocytes. Liu (2013) reported another methyltransferase-like 14 (METTL14) catalyzes m^{6}A RNA methylation [8]. Together with METTL3, these two proteins form a stable heterodimer core complex of METTL3-METTL14 that functions in cellular m^{6}A installation. WTAP, a mammalian splicing factor, can interact with this complex and also affect m^{6}A methylation. Knockdown of cellular METTL3, METTL14 or WTAP shows that both METTL14 and WTAP have a larger effect on m^{6}A level than METTL3. We will further explore the effect of knockdown of METTL3, METTL14 or both of them on m^{6}A level and lipid accumulation in porcine adipocytes.

In order to reduce the FTO or METTL3 disturbance, we use chemicals to test the relationship between m^{6}A and lipid accumulation in porcine adipocytes. Cycloleucine (CL), a chemical inhibitor of methionine adenosyltransferase (MAT) [18], reduces cellular S-adenosylmethionine (SAM) level, which further affects the methylation of nuclear acid. In current study, CL is proved to be toxic to cells in high concentration and reduces m^{6}A level in a dose-depend manner. The adipogenesis promoting effect of CL verifies the results from FTO and METTL3 treatment that m^{6}A negatively regulated lipid deposition in porcine adipocytes.

Betaine, the trimethyl derivative of the amino acid glycine, is a methyl donor providing its labile methyl groups for the synthesis of several metabolically active substances such as creatine and carnitine. It is widely used as feed additive to reduce total fat and improve carcass lean gain in pigs [19,20]. Recently, the relationship between betaine and DNA methylation has been studied [21,22], no report related to betaine and mRNA m^{6}A methylation. In our study, we show that betaine can change m^{6}A level in a dose-dependent pattern. The effect that betaine reduces backfat in pig may be mediated by m^{6}A, which need to be clarified further.

m^{6}A is widely conserved among eukaryotic species that range from yeast, plants, flies to mammals, as well as among viral RNAs with a nuclear phase [7]. Although it was discovered 40 years ago [23], the development in m^{6}A study has lagged behind that of other modified bases, largely part due to the lack of available methods for detecting the presence of m^{6}A. The recent breakthroughs in the development of high-throughput assays provide tools for functional investigations of m^{6}A. Recent studies show m^{6}A may affect biological process through affecting mRNA stability and decay [24], splicing and translation [7]. In our study, genes related adipogenic differentiation and lipolysis were affected by FTO and METTL3 manipulation, and chemical treatment maybe mediated by m^{6}A. It can be deduced that m^{6}A affect gene expression maybe by affecting mRNA abundance or translation, and the effect has the specificity. The mechanism underlying m^{6}A effects need to be studied further.

In summary, mRNA m^{6}A are dynamics and can be regulated by demethylase FTO, methylase METTL3 and chemicals. m^{6}A play important role in biological process and negatively regulated adipogenesis in porcine adipocytes.

Conflict of interest

The authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.048.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.048.

References

[1] K.A. Fawcett, I. Barroso, The genetics of obesity: FTO leads the way, Trends Genet. 26 (2010) 266–274.
[2] B. Fan, Z.Q. Du, M.F. Rothschild, The fat mass and obesity-associated (FTO) gene is associated with intramuscular fat content and growth rate in the pig, Anim. Biotechnol. 20 (2009) 58–70.
[3] L. Fontanesi, E. Scotti, L. Buttazzoni, S. Dall'Olio, A. Bagnato, D.P. Lo Fiego, R. Davoli, V. Russo, Confirmed association between a single nucleotide polymorphism in the FTO gene and obesity-related traits in heavy pigs, Mol. Biol. Rep. 37 (2010) 461–466.
[4] L. Fontanesi, E. Scotti, L. Buttazzoni, R. Davoli, V. Russo, The porcine fat mass and obesity associated (FTO) gene is associated with fat deposition in Italian Duroc pigs, Anim. Genet. 40 (2009) 90–93.
[5] Y. Fu, L. Li, S. Ren, Effect of FTO expression and polymorphism on fat deposition in Suzhong pigs, Asian-Australas J. Anim. Sci. 26 (2013) 1365–1373.
[6] J.A. Bokar, Fine-tuning of RNA functions by modification and editing, in: H. Grosjean (Ed.), The Biosynthesis and Functional Roles of Methylated Nucleosides in Eukaryotic mRNA, SpringerVerlag, 2005, pp. 141–178.
[7] Y. Fu, D. Dominissini, G. Rechavi, C. He, Gene expression regulation mediated through reversible m(6)A RNA methylation, Nat. Rev. Genet. 15 (2014) 293–306.
[8] J.Z. Liu, Y.N. Yue, D.L. Han, X. Wang, Y. Fu, L. Zhang, G.F. Jia, M. Yu, Z.K. Lu, X. Deng, Q. Dai, W.Z. Chen, C. He, A METTL3-METTL14 complex mediates mammalian nuclear RNA N-6-adenosine methylation, Nat. Chem. Biol. 10 (2014) 93–95.
[9] T. Shan, Y. Ren, Y. Wang, Sirtuin 1 affects the transcriptional expression of adipose triglyceride lipase in porcine adipocytes, J. Anim. Sci. 91 (2013) 1247–1254.
[10] K.J. Shin, E.A. Wall, J.R. Zavzavadjian, L.A. Santat, J. Liu, J.I. Hwang, R. Rebres, T. Roach, W. Seaman, M.I. Simon, I.D. Fraser, A single lentiviral vector platform for microRNA-based conditional RNA interference and coordinated transgene expression, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 13759–13764.

[11] G.F. Jia, Y. Fu, X. Zhao, Q. Dai, G.Q. Zheng, Y. Yang, C.Q. Yi, T. Lindahl, T. Pan, Y.G. Yang, C. He, N6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO, Nat. Chem. Biol. 7 (2011) 885–887.
[12] X. Wang, M. Huang, Y. Wang, The effect of insulin, TNFalpha and DHA on the proliferation, differentiation and lipolysis of preadipocytes isolated from large yellow croaker (Pseudosciaena Crocea R.), PLoS One 7 (2012) e48069.
[13] G.F. Jia, Y. Fu, X. Zhao, Q. Dai, G.Q. Zheng, Y. Yang, C.Q. Yi, T. Lindahl, T. Pan, Y.G. Yang, C. He, N6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO (vol 7, pg 885, 2011), Nat. Chem. Biol. 8 (2012), 1008–1008.
[14] C. Church, L. Moir, F. McMurray, C. Girard, G.T. Banks, L. Teboul, S. Wells, J.C. Bruning, P.M. Nolan, F.M. Ashcroft, R.D. Cox, Overexpression of Fto leads to increased food intake and results in obesity, Nat. Genet. 42 (2010) 1086–U1147.
[15] J. Fischer, L. Koch, C. Emmerling, J. Vierkotten, T. Peters, J.C. Bruning, U. Ruther, Inactivation of the Fto gene protects from obesity, Nature 458 (2009) 894–898.
[16] J.A. Bokar, M.E. Shambaugh, D. Polayes, A.G. Matera, F.M. Rottman, Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N-6-adenosine)-methyltransferase, Rna-a Publ. Rna Soc. 3 (1997) 1233–1247.
[17] C.F. Hongay, T.L. Orr-Weaver, Drosophila Inducer of MEiosis 4 (IME4) is required for Notch signaling during oogenesis, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 14855–14860.
[18] D.W. Lin, B.P. Chung, P. Kaiser, S-adenosylmethionine limitation induces p38 mitogen-activated protein kinase and triggers cell cycle arrest in G1, J. Cell. Sci. 127 (2014) 50–59.
[19] J.O. Matthews, L.L. Southern, A.D. Higbie, M.A. Persica, T.D. Bidner, Effects of betaine on growth, carcass characteristics, pork quality, and plasma metabolites of finishing pigs, J. Anim. Sci. 79 (2001) 722–728.
[20] J. Sales, F. Jancik, Effects of dietary chromium supplementation on performance, carcass characteristics, and meat quality of growing-finishing swine: a meta-analysis, J. Anim. Sci. 89 (2011) 4054–4067.
[21] L. Wang, L. Chen, Y. Tan, J. Wei, Y. Chang, T. Jin, H. Zhu, Betaine supplement alleviates hepatic triglyceride accumulation of apolipoprotein E deficient mice via reducing methylation of peroxisomal proliferator-activated receptor alpha promoter, Lipids Health Dis. 12 (2013) 34.
[22] D. Cai, Y. Jia, H. Song, S. Sui, J. Lu, Z. Jiang, R. Zhao, Betaine supplementation in maternal diet modulates the epigenetic regulation of hepatic gluconeogenic genes in neonatal piglets, PLoS One 9 (2014) e105504.
[23] R. Desrosiers, K. Friderici, F. Rottman, Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 3971–3975.
[24] X. Wang, Z. Lu, A. Gomez, G.C. Hon, Y. Yue, D. Han, Y. Fu, M. Parisien, Q. Dai, G. Jia, B. Ren, T. Pan, C. He, N6-methyladenosine-dependent regulation of messenger RNA stability, Nature 505 (2014) 117–120.