Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;45:1023-1033

DOI: 10.1159/000487343
Published online: February 12, 2018

Accepted: December 14, 2017

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Original Paper

Hoxa5 Promotes Adipose Differentiation via Increasing DNA Methylation Level and Inhibiting PKA/HSL Signal Pathway in Mice

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Key Words
Hoxa5 • Adipocytes • Differentiation • Methylation • PKA/HSL pathway

Abstract:
Background/Aims: Impaired adipogenesis may be the underlying cause in the development of obesity and type II diabetes. Mechanistically, the family of Homeobox transcription factors is implicated in the regulation of adipocyte fate. Hoxa5 is highly expressed in adipocytes, and its mRNA expression is decreased during differentiation. However, the function of Hoxa5 in adipose tissue has been poorly understood. The aim of this study is to unveil the role of Hoxa5 on adipocyte differentiation and its underlying mechanisms. ***Methods:*** Quantitative real-time PCR (qPCR) and western blot were performed to determine Hoxa5 expression in primary adipocytes and in adipose tissues from mice. Lipid accumulation was evaluated by bodipy staining. Dual luciferase assay was applied to explore the transcription factor of Hoxa5 and the transcriptional target gene modulated by Hoxa5. All measurements were performed at least for three times at least. ***Results:*** A significant reduction of Hoxa5 expression was observed in adipose tissue of High Fat Diet (HFD) induced obesity mice. We determined Hoxa5 increased adipocytes differentiation and mitochondrial biogenesis in adipocytes *in vitro*. CEBPβ was determined a transcription factor of Hoxa5 and inhibited methylation level of Hoxa5 by combining on the promoter of Hoxa5. Importantly, we found Fabp4, a known positive regulator of adipocytes differentiation, was transcriptional activation by Hoxa5. In addition, Hoxa5 promotes adipocytes differentiation by inhibiting PKA/HSL pathway. ***Conclusion:*** Our study demonstrated the promoting role of Hoxa5 in adipocytes differentiation and therefore bringing a new therapeutic mean to the treatment of obesity and type II diabetes.

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Introduction

Homeobox genes, belonging to the developmental transcription factor, play a part in the determination of adipose tissue expandability and body fat distribution [1-4]. Hoxa5, one of the Hox genes, has been identified differentially expressed in adipose tissue depots [5, 6]. It is reported that the Hoxa5 expression is decreased with a high fat diet and increased

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in human adipose tissue after fat loss surgery [7, 8]. There is a strong correlation between brown adipocytes adipogenesis and Hox genes expression [9]. In addition, lipid accumulation decreased in the Hoxa5-siRNA treated 3T3-L1 adipocytes [10]. However, the regulatory mechanism of Hoxa5 on adipocytes differentiation and mitochondrial biogenesis still needs to be illustrated.

DNA methylation in promoters is closely linked to downstream gene repression. Moreover, methylation of the Hoxa5 promoter region may be responsible for silencing of gene expression [11]. Notably, there is a crosstalk between site-specific transcription factors and DNA methylation states [12, 13]. The regulation mechanism of Hoxa5 promoter activity during differentiation, especially for the transcriptional and methylation, is not clear.

In this study, we focus on the effect of Hoxa5 in the process of differentiation and mitochondrial biogenesis in mice adipocytes. We also explored the exact regulation mechanism on the promoting activity of Hoxa5. This study is aimed to elucidate the mechanism of Hoxa5 regulating adipocytes differentiation and mitochondrial biogenesis, and to open new therapeutic possibilities against obesity and type II diabetes.

Materials and Methods

Animal experiment

Six-week-old Kunming male mice were purchased from the Laboratory Animal Center of the Fourth Military Medical University (Xi'an, Shaanxi, China). The mice were randomly assigned into two groups: a high-fat diet fed group or a chow diet fed for 8 weeks. All mice handling methods and experimental protocols were followed as our previous reports [14]. Feeding procedure for mice was ATRA (50mg/kg/day) or saline was subcutaneously injected into 16-week-old mice for 5 days. Then the mice were euthanized for collection of tissues and blood. EWAT, iWAT and BAT were sampled in the epididymis, inguinal and interscapular adipose tissue, respectively.

Drug treatments

The adipocytes were treated with 1μM Cl316, 243 (Sigma, St. Louis, MO, USA) for 4h to induce lipolysis. Adipocytes were incubated with 1 μM ATRA (Sigma, St. Louis, MO, USA) for 24 h before exact cells or incubated with 1 μM Oligomycin (Sigma, St. Louis, MO, USA) for 1h. The cells were incubated with 5 μM 5-Azacytidine (Sigma, St. Louis, MO, USA) after transfection. PKA/HSL signal pathway was activated by 10 μM Forskolin (Selleck, Houston, USA) for 4h. Then adipocytes were treated with 10 μM H89 (Selleck, Houston, USA) for 2h, or 15 μM cAMPS-Rp, triethylammonium salt (R&D Systems, USA) for 2 h to block PKA/HSL signal pathway.

Immunofluorescence

The cells were incubated in 4% para formaldehyde for 20 min, and then were permeabilized by 0.1% Triton X-100 for 10 min. Non-specific binding was blocked with 5% BSA for 30 min. Cells were incubated with primary antibody against Hoxa5 (Abcam, England) at 37° C for 2 hours. Then incubated the cells at a 1:100 dilution in 2% BSA of Cy3-conjugated Donkey Anti-Rabbit IgG (Sangon Biotech, China) for 30 min. All of the washes were done in 1×PBS. An anti-fade solution containing DAPI (Solarbio, China) was used [15].

Relative mitochondrial DNA copy number assay

DNA was isolated from cells with Gene Mark Kits containing RNase A treatment and quantified by the NanoDrop Spectrophotometer. Mitochondrial DNA copy number percell was quantified as described. Real-time quantitative PCR was carried out using the real-time PCR detection system as previous described [16].

Bodipy staining and Mitochondria staining with Mitotracker deep red FM

Bodipy (Invitrogen, Carlsbad, USA) with 513 nm green florescent was diluted with DMSO to a concentration of 1 mm as work solution. When differentiating for 4 d, adipocyte lipid droplets were stained with Bodipy. The formaldehyde-fixed cells were stained with a working solution of Bodipy for 30 min at room temperature. Cells were washed with PBS for three times and image observation was taken with an inverted microscope (Nikon Instruments Europe BV, England).

To stain the mitochondria, adipocytes were plated on cover-slips. After having indicated transfection and incubation, the cells were washed with PBS and stained with 100nM MitoTracker deep red (Invitrogen, USA). Cell nucleus were counterstained by DAPI and observed by fluorescence microscope.

Luciferase report assay

Fragments containing Hoxa5 promoter sequences were sub-cloned into a pGL3-basic vector (Takara, China), respectively. HEK293T or 3T3-L1 cells were cultured in 24-well plates and co-transfected with Hoxa5 promoter plasmid and pc-CEBPβ plasmid or pcDNA3.1 plasmid. HEK293T cells also co-transfected with Fabp4 promoter plasmid and pc-Hoxa5 plasmid or pcDNA3.1 plasmid. The experimental protocols were followed as previous described [17].

Determination of DNA methylation status by bisulphite conversion

Bisulphite treatment of genomic DNA was carried out using EZ DNA Methylation-Gold™ Kit (Zymo Research) in accordance with the manufacturer's instructions. For bisulphite sequencing, PCR fragments were cloned into pGEMT-Easy vector system (Promega) and 10 clones were sequenced to determine the methylation status, the experimental procedure was as described in our previous reports in detail [18].

Cell culture

Epididymal white adipose tissues from four-week-old mice were harvested, visible fibers and blood vessels were removed and the adipose tissue was washed three times with PBS buffer containing 200U/mL penicillin (Sigma, St. Louis, USA) and 200U/mL streptomycin (Sigma, St. Louis, USA). The adipocyte culture was performed as previously described [18, 19]. Cells were differentiated after induction for 4 days.

Cells infection and transfection

The pre-adipocytes were infected with overexpression Hoxa5 adenovirus vector (pc-Hoxa5) or adenovirus interference vector of Hoxa5 (sh-Hoxa5) for 48 h at the titer of 1×10^{9} IFU/mL, and then to induce differentiation [20]. Forced expression plasmid vector of CEBPβ (pc-CEBPβ), forced expression plasmid vector of Fabp4 (pc-Fabp4) and interference vector of Fabp4 (sh-Fabp4) were kept in our lab; and the control plasmid vector was pcDNA3.1-vector. The X-treme GENE HP Reagent (Roche, Switzerland) was used in plasmids transfection. The transfection procedure was as described in our previous reports in detail [21].

qPCR and Western blot Analysis

Total RNA form adipose tissues or adipocytes were extracted with TRIpure Reagent kit (Takara, Dalian, China) and 500 ng of total RNA was reverse transcribed using the M-MLV reverse transcriptase kit (Takara, China). Primers for Hoxa5, PGC1α, PPARγ, FABP4, CEBPβ, NRf1, NRf2, Tfam, Fas, ACC, Hsl, Dnmt1, Dnmt3a, Dnmt3b and Mbd3 were synthesized by Shanghai Sangon Ltd (Shanghai, China). qPCR was performed in 25 μL reactions containing specific primers and SYBR Premix EX Taq (Takara, China). The levels of mRNAs were normalized to β-actin. The expression of genes were analyzed by the method of $2^{-\triangle\triangle Ct}$.

Antibodies against PGC1α (ab72230), CEBPβ (ab32358), FABP4 (ab92501), HSL (ab45422), PKA (ab108385), p-PKAThr197 (ab75991), FAS (ab128856), β-actin (ab8226) and GAPDH (ab9484) were from Abcam (Cambrige, England); PPARγ (Ap0686) was from Bioworld (CA, USA). The experimental procedure was as described in our previous reports in detail [22].

Statistical analysis

Statistical calculations were performed using SAS v8.0 (SAS Institute, Cary, NC). Statistical significance was determined using the one-way ANOVA test. Comparisons among individual means were made by Fisher's Least Significant Difference (LSD) post hoc test after ANOVA. Data are presented as mean ± SD; $p<0.05$ was considered to be significant.

Results

Hoxa5 expression decreased under HFD and ascended along with mitochondrial biogenesis genes by ATRA in mice adipose tissue

We found Hoxa5 expressed the highest level in mitochondrion-rich brown adipose tissue (Fig. 1A). High Fat Diet (HFD) and All-trans retinoic acid (ATRA) injection were performed

on mice. We found inguinal white tissue was smaller and darker color in the ATRA group (Fig. 1C). Increased weight of the body and adipose tissue by HFD was reversed by ATRA treatment (Fig. 1B, D). In addition, the ATRA treatment reversed the effect of HFD on serum lipids (P<0.05; for all online suppl. material, see www.karger.com/doi/10.1159/000487343 Fig. S1A). In mice inguinal white adipose tissue (iWAT), the HFD increased mRNA level of adipogenic markers PPARγ, FABP4 and Fas, while they were attenuated by ATRA. And the decreased HSL mRNA expression in HFD groups had been increased by ATRA (P<0.05; Fig. 1E). Moreover, the mRNA level of Hoxa5 and RAR α as well as mitochondrion biosynthesis related genes PGC1α and Tfam were enhanced, while Leptin expression had decreased in ATRA groups (Fig. 1F; P<0.05). Importantly, mRNA level of Hoxa5 and PGC1α in iWAT were strong positively correlated (R=0.9782; see online suppl. material, Fig. S1B).

Hoxa5 stimulates differentiation and lipid deposition in mice adipocytes

To explore the effect of Hoxa5 on adipocytes differentiation, we detected the expression of Hoxa5 in mice adipocytes in the process of differentiation. Interestingly, the Hoxa5 expression had increased before the 1st differentiated day and then decreased with the passage of differentiated time (Fig. 2A). Fig. 2D & see online suppl. material, Fig. S2A-B showed Hoxa5 mRNA and protein expression both had greatly increased by overexpression Hoxa5. Over expression of Hoxa5 increased mRNA levels of PPARγ, CEBPβ and Fabp4 on the 1st, 3rd, 5th, 7th day after being differentiated in mice adipocytes (P<0.05; Fig. 2B). And Hoxa5 increased the protein expression level of PPARγ, CEBPβ and Fabp4 (Fig.2C; P<0.05). Bodipy staining showed Hoxa5 promoted the adipocytes lipid deposition (Fig. 2E). In addition, Hoxa5 enhanced the Fabp4 and Fas mRNA level and reduced HSL mRNA level regardless of the present of lipotropic agent - Cl316, 243 treatment (see online suppl. material, Fig. S2C; P<0.05). And the effect of Hoxa5 on FABP4, FAS and HSL protein is consistent with the mRNA results (Fig. 2F; P<0.05).

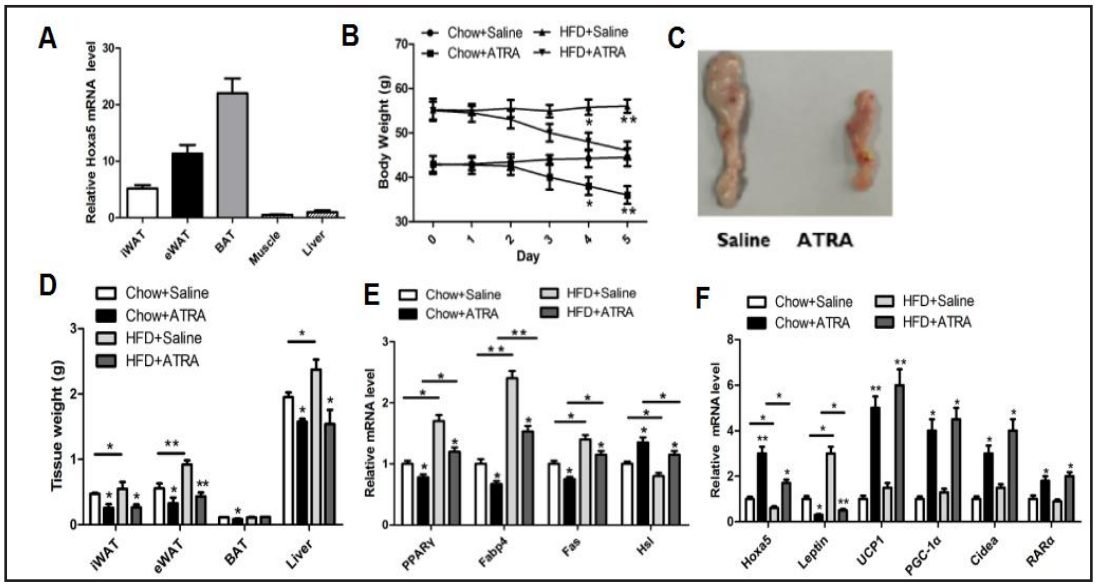

Fig. 1. Hoxa5 expression decreased under HFD and ascended along with mitochondrial biogenesis genes by ATRA in mice adipose tissue. (A) Relative Hoxa5 mRNA levels in iWAT, eWAT, BAT, muscle and liver of mice (n=6). (B)Body weight of mice fed HFD or chow diet with ATRA or saline (n=6). (C) iWAT representative picture of male mice with ATRA or saline injection (n=6). (D) Tissue weight of iWAT, eWAT, BAT and liver of mice in different groups (n=6). (E) Relative mRNA levels of PPARγ, Fabp4, Fas and Hsl in iWAT of mice in different groups (n=6). (F) Relative mRNA levels of Hoxa5, Leptin, PGC1α, Tfam and RARα in iWAT of mice in different groups (n=6). Values are means ± SD. vs. Control group, *p<0.05, ** p<0.01.

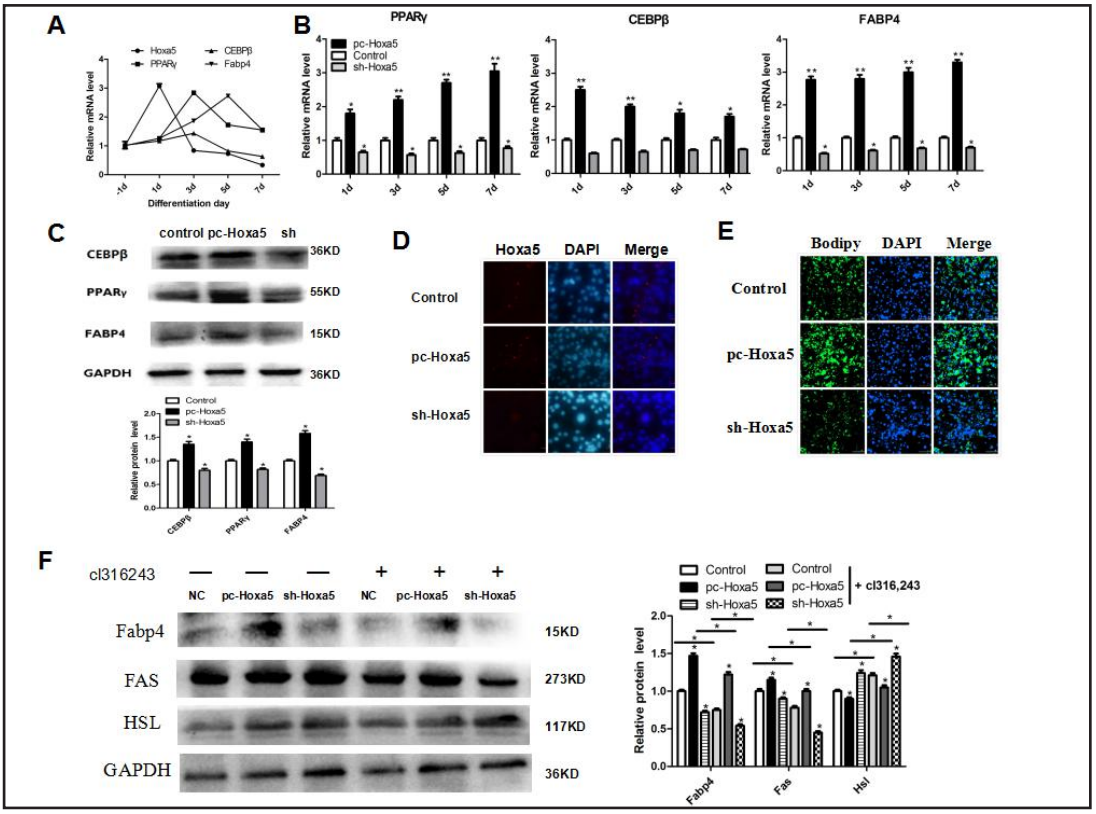

Fig. 2. Hoxa5 stimulates differentiation and lipid deposition in mice adipocytes. (A) Relative mRNA expression level of Hoxa5, PPARγ, CEBPβ and Fabp4 on the day before differentiated and the 1st, 3rd, 5th, 7th day after differentiated in mice adipocytes (n=4). (B) Relative mRNA expression level of PPARγ, CEBPβ and Fabp4 on the 1st, 3rd, 5th, 7th after differentiated in the control, pc-Hoxa5 and sh-Hoxa5 groups in mice adipocytes (n=4). (C) Representative immunoblots and densitometric quantification for CEBPβ, PPARγ and Fabp4 in the control, pc-Hoxa5 and sh-Hoxa5 groups on differentiation until 5 days. (D) Images of adipocytes stained by immunofluorescent staining in the control, pc-Hoxa5 and sh-Hoxa5 groups in mice adipocytes (n=4). (E) Images of adipocytes stained by Bodipy staining in the control, pc-Hoxa5 and sh-Hoxa5 groups in mice adipocytes on differentiation until 5 days (n=4). (F) Representative immunoblots and densitometric quantification for Fabp4, Fas and Hsl in the control, pc-Hoxa5 or sh-Hoxa5 groups with treatment of Cl316,243 for 4 h on differentiation until 5 days. Values are means ± SD. vs. Control group, *p<0.05, ** p<0.01.

Hoxa5 promotes mitochondrial biogenesis in mice adipocytes

We found Hoxa5 increased PGC-1α, Nrf-1, Nrf-2 and Tfam mRNA expression (P<0.05; Fig. 3A) and enhanced PGC-1α protein expression in adipocytes (P<0.05; Fig. 3B). ATRA treatment was performed to induce mitochondrial biogenesis. The mRNA levels of Hoxa5, PGC-1α, Nrf-1, Nrf-2 and Tfam were enhanced by ATRA (P<0.05; Fig. 3E). While, the mRNA levels of PGC-1α, Nrf-1, Nrf-2 and Tfam was reduced by oligomycin (P<0.05; see online suppl. material, Fig. S2D). And Hoxa5 increased PGC-1α, Nrf-1, Nrf-2 and Tfam expression regardless the present of ATRA or oligomycin (P<0.05; Fig. 3E & see online suppl. material, Fig. S2D). Moreover, the mtDNA level is higher in the Hoxa5 over expression group (P<0.05; Fig. 3C). Fig. 3D shows a representative picture of mitochondria staining, which revealed the content of mitochondria is higher in the pc-Hoxa5 group (P<0.05).

CEBPβ negative transcription regulation of Hoxa5 and methylation level of Hoxa5 promoter increased in differentiated adipocytes

With Genomatix software predication, a potential binding site of CEBPβ and two E-box binding sites on the Hoxa5 promoter region were discovered. And Hoxa5 mRNA level

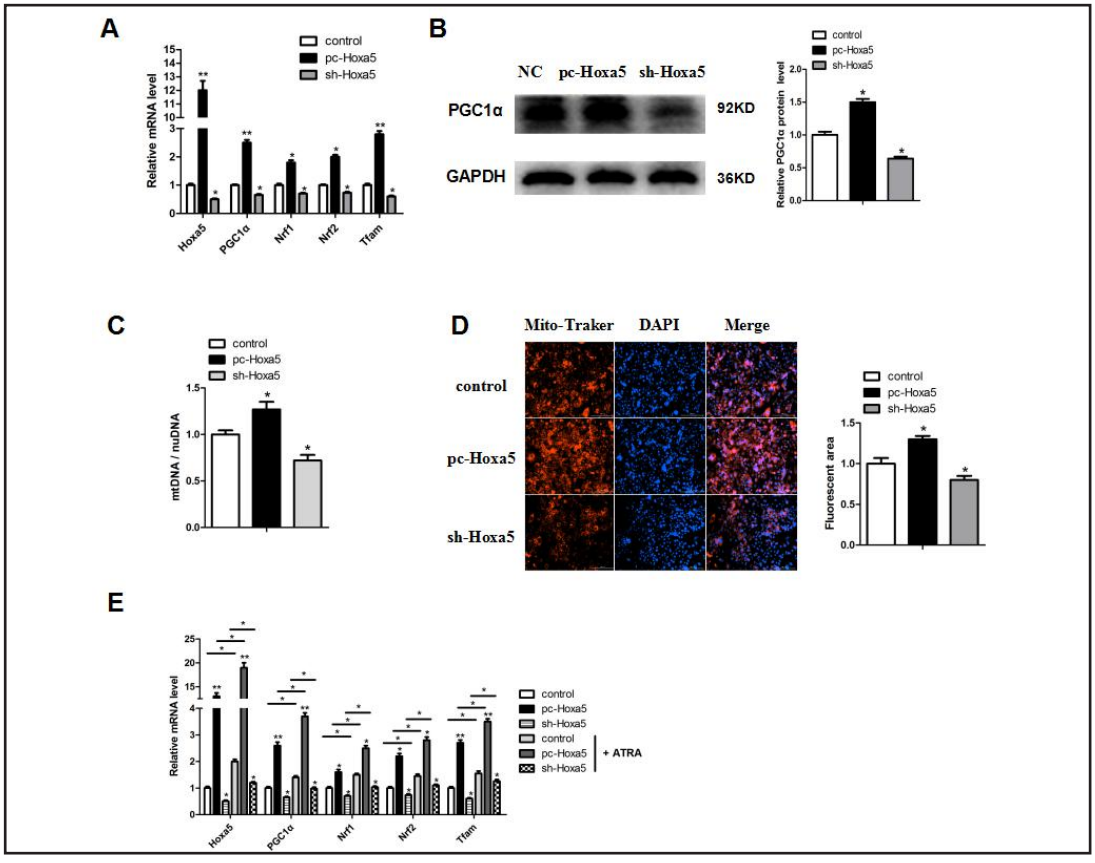

Fig. 3. Hoxa5 promotes mitochondrial biogenesis in mice adipocytes. (A) Relative mRNA expression level of Hoxa5, PGC-1α, Nrf-1, Nrf-2 and Tfam in mice adipocytes in Control group, pc-Hoxa5 group and sh-Hoxa5 group on the 4th differentiated day (n=4). (B) Representative immunoblots and densitometric quantification for PGC-1α in control group, pc-Hoxa5 group and sh-Hoxa5 group on the 4th differentiated day (n=4). (C) The ratio of mtDNA/nucDNA was assessed by qRT-PCR in mice adipocytes in Control group, pc-Hoxa5 group and sh-Hoxa5 group on the 4th differentiated day (n=4). (D) Representative picture of mitochondria staining with mito-tracker deep red FM and cell nucleus were counterstained by 4', 6-diamidino-2-phenylindole(DAPI). Cells were observed by fluorescence microscope (n=4). (E) Relative mRNA expression level of Hoxa5, PGC-1α, Nrf-1, Nrf-2 and Tfam in Control group, pc-Hoxa5 group and sh-Hoxa5 group with ATRA treatment for 24h on the 4th differentiated day (n=4). Values are means ± SD. vs. Control group, *p<0.05, ** p<0.01.

was decreased in the pc-CEBPβ group (P<0.05; Fig. 4A). Luciferase reporter assay in the transfected HEK293 cells showed the 368~382 bp upstream of Hoxa5 transcription initiation site was considered to be the binding site of CEBP β (see online suppl. material, Fig. S3A). In addition, we found the second E-box binding site (-42bp) may aggravate the transcriptional inhibition effect of CEBPβ on Hoxa5 promoter activity (Fig. 4B, see online suppl. material, Fig. S3A). We then determined the methylation level of CpG in Hoxa5 promoter in differentiated adipocytes is higher than that present in undifferentiated adipocytes (P<0.05; Fig. 4C, see online suppl. material, Fig. S3B). Moreover, mRNA levels of Dnmt1 and Dnmt3a were higher in differentiated adipocytes than that in undifferentiated adipocytes (P<0.05; Fig. 4D). The methylation inhibitor 5-azacytidine decreased Hoxa5 expression in pre-adipocytes, while increased Hoxa5 in mature adipocytes. The high-level methylation of Hoxa5 promoter repressed Hoxa5 expression (Fig. 4E).

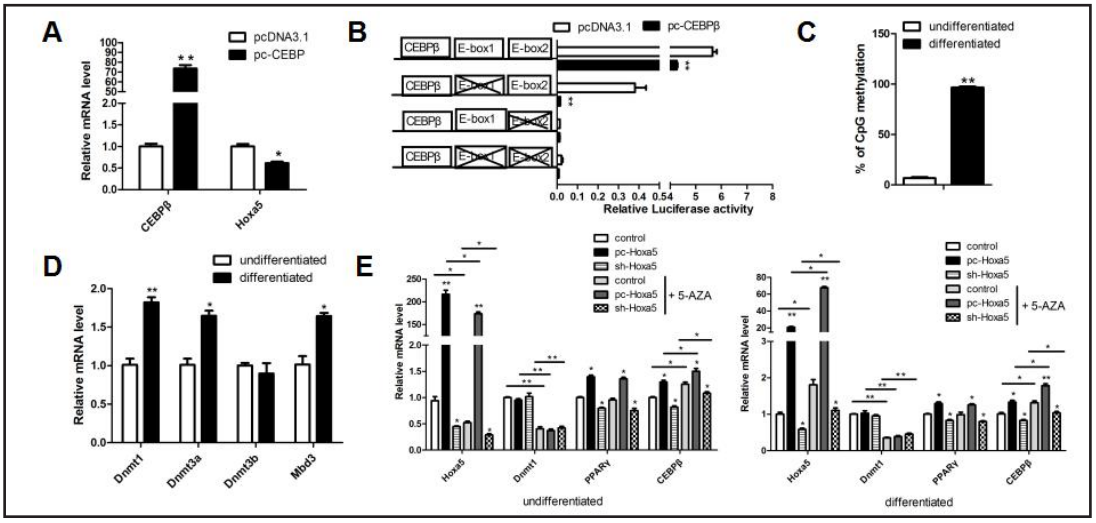

Fig. 4. CEBPβ negative transcription regulation Hoxa5 and methylation level of Hoxa5 promoter increased in differentiated adipocytes. (A) Relative mRNA expression level of CEBPβ and Hoxa5 in mice adipocytes in control group, and pc-CEBPβ group after 48h transfection (n=4). (B) Mutational fragments of Hoxa5 promoter fused to a luciferase reporter gene were co-transfected into cells together with PGL3-basic (control) (n=4). Luciferase activity was corrected for Renilla luciferase activity and normalized to control activity (n=4). (C) The methylation level of CpG in -148bp~-14bp of the Hoxa5 promoter in undifferentiated adipocytes and differentiated adipocytes (n=10). (D) Relative mRNA level of Dnmt1, Dnmt3a Dnmt3b, Mbd3 in undifferentiated adipocytes and differentiated adipocytes (n=4). (E) Relative mRNA level of Hoxa5, Dnmt1, PPARγ, CEBPβ in undifferentiated or differentiated adipocytes with pc-control, pc-Hoxa5 or sh-Hoxa5 infected for 48h under demethylation induced by 5'-Aza-deoxycytidine (n=4). Values are means ± SD. vs. Control group, *p<0.05, ** p<0.01.

Hoxa5 promoter activity had been competitively repressed by CEBPβ combining and methylation during adipocytes differentiation

We further characterized the underlying mechanisms of transcriptional inhibition and methylation on Hoxa5 promoter activity during adipocytes differentiation. Fig. 5A showed methylation-variable positions nearing CEBPβ binding site in the Hoxa5 promoter. We found methylation level had decreased in the pc-CEBPβ groups in both stages (Fig. 5B, see online suppl. material, Fig. S3C). The data indicating CEBPβ binding on Hoxa5 promoter may inhibit the methylation level of its adjacent motif. Hoxa5 expression was repressed by the pc-CEBPβ regardless of the present of 5-azacytidine treatment in both stages (Fig. 5D). Then we measured the promoter activity of Hoxa5 in 3T3-L1 cell line by luciferase reporter assay. The promoter activity of Hoxa5 was increased by pc-CEBPβ in 3T3-L1 cell line, which is consistent with the result by HEK293A cell showed, see online suppl. material, in Fig. S3A. What's more, the effect of CEBPβ transcriptional inhibition was strengthened by demethylation (Fig. 5C).

Hoxa5 promotes adipocytes differentiation by the positive transcription regulation of Fabp4 and via inhibiting PKA/HSL signaling pathway

There were three potential binding sites of Hoxa5 on the Fabp4 promoter region were discovered by software predication. The result of luciferase reporter assay showed 1466~1437bp upstream of Fabp4 transcription initiation site (Mut1) is a binding site of Hoxa5 (Fig. 6A). The co-treatment of pc-Fabp4 and pc-Hoxa5 was performed to explore the effect of Fabp4 on Hoxa5 promoting differentiation, the results showed pc-Fabp4 increased mRNA and protein level of PPARγ and CEBPβ, and pc-Hoxa5 enhanced the facilitation ($p < 0.05$; Fig. 6B, D). In addition, pc-Fabp4 increased mRNA level of Fas, ACC1 and decreased HSL mRNA level, and Hoxa5 enhanced the effect ($p < 0.05$; Fig. 6C). We further determined the PKA pathway, the cAMP/PKA signal pathway was activated by Forskolin while it can be blocked by the inhibitor H89 and cell-permeable cAMP analogue triethylammonium

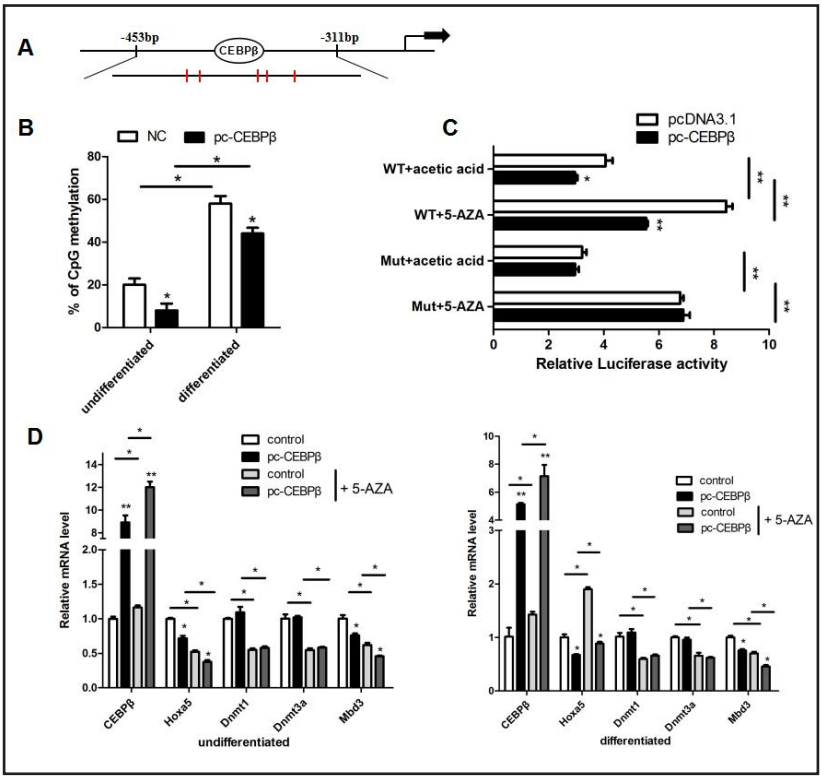

Fig. 5. Hoxa5 expression was competitively repressed by CEBPβ combining and methylation during adipocytes differentiation. (A) The methylation-variable positions nearing CEBPβ binding site in Hoxa5 promoter. (B) The methylation level of methylation-variable positions nearing CEBPβ binding site in Hoxa5 promoter in different groups (n=10). (C) Mutational fragments of Hoxa5 promoter fused to a luciferase reporter gene were co-transfected into 3T3-L1 cells together with pc-control or pc-CEBPβ under demethylation induced by 5'-Aza-deoxycytidine. (n=3). (D) Relative mRNA levels of CEBPβ, Hoxa5, Dnmt1, Dnmt3a, Mbd3 in undifferentiated or differentiated adipocytes with pc-control or pc-CEBPβ transfected for 48h under demethylation induced by 5'-Aza-deoxycytidine (n=3). Values are means ± SD. vs. Control group, *p<0.05, ** p<0.01.

salt followed our preliminary experiment. The protein level of HSL and phosphorylation of PKA were both reduced by pc-Hoxa5 ($p < 0.05$; Fig. 6E). Moreover, the PKA inhibitor H89 as well as triethylammonium salt treatment decreased PKA phosphorylation level and protein expression of HSL ($p < 0.05$; see online suppl. material, Fig. S4). In addition, Hoxa5 enhanced the protein levels of Fabp4 regardless the present of H89 or triethyla ($p < 0.05$; Fig. 6E, see online suppl. material, Fig. S4).

Discussion

Hoxa5 is one of the *Hox* genes, which are highly expressed in the adipose tissue and play an active role in regulating adipocyte functions, including differentiation and body fat distribution [23, 24]. Hoxa5 expression can be up-regulated by retinoic acid [25, 26]. Retinoic acid may reduce body fat and improve insulin sensitivity in lean and obese rodents. Importantly, it induces oxidative phosphorylation and mitochondria biogenesis in adipocytes [27-29]. The down-regulation of *Hoxa5* expression in iWAT by HFD may contribute to the adipose tissue functional changes and remodeling.

DNA methylation is reported to block the binding of some transcription factor *in vitro* [30, 31]. Intriguingly, some transcription factors such as REST and CTCF have been shown to bind methylated regions and trigger their demethylation [32]. It is reported that transcription factors binding patterns at enhancers and promoters are both guided by and actively shape the balance between active demethylation and de novo methylation [33]. As some transcription factors, such as CTCF, locally mediate low methylation level [34-36]. Here, we hypothesized that CEBPβ binding on Hoxa5 promoter inhibits methylation of a Hoxa5 promoter in adipocytes, and the result verified our assumption. The data suggested that the transcription inhibition effect of CEBPβ on Hoxa5 and the methylated modification effect competitively regulate the activity of Hoxa5 promoter.

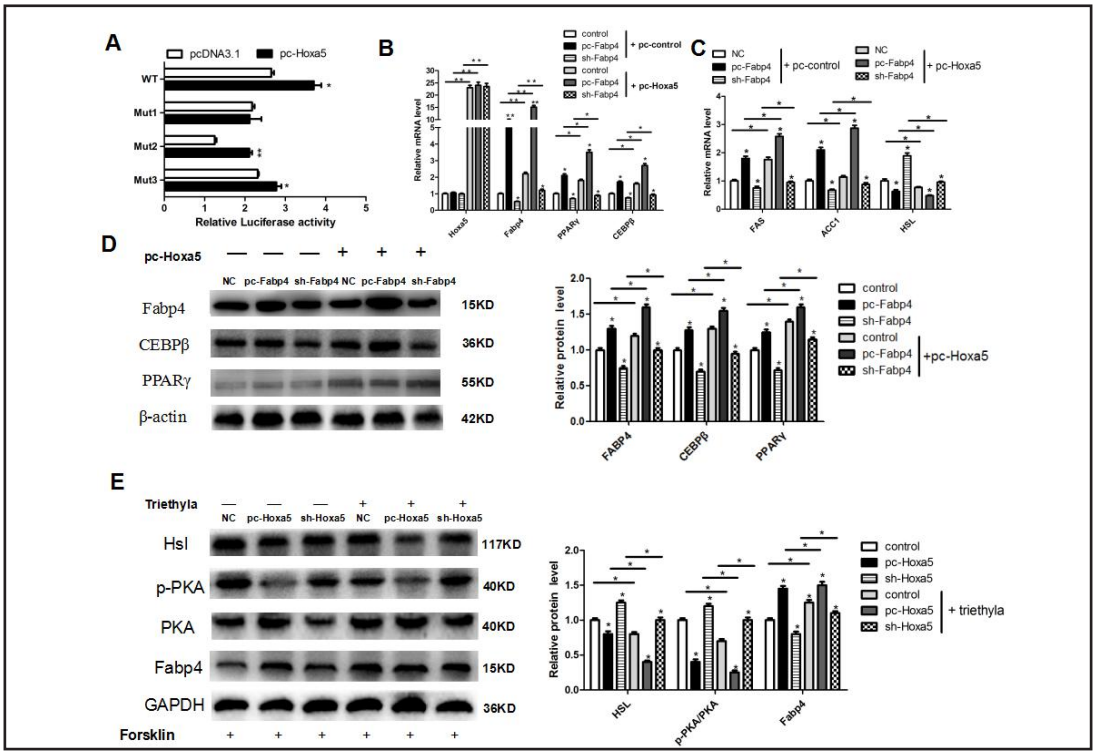

Fig. 6. Hoxa5 promotes adipocytes differentiation by positive transcription regulation of Fabp4 and via inhibiting PKA/HSL signaling pathway. (A) Mutational fragments of Fabp4 promoter fused to a luciferase reporter gene were co-transfected into cells together with pc-control or pc-Hoxa5 (n=3). Luciferase activity was corrected for Renilla luciferase activity and normalized to control activity (n=3). (B) Relative mRNA expression level of Hoxa5, Fabp4, PPARγ and CEBPβ in Control group, pc-Fabp4 group and sh-Fabp4 group with pc-Hoxa5 treatment on the 4th differentiated day (n=4). (C) Representative immunoblots and densitometric quantification for Fabp4, PPARγ and CEBPβ in Control group, pc-Hoxa5 group and sh-Hoxa5 group with pc-Hoxa5 treatment on the 4th differentiated day (n=4). (D) Relative protein expression level of Fas, ACC1 and HSL in Control group, pc-Fabp4 group and sh-Fabp4 group with pc-Hoxa5 treatment or not on the 4th differentiated day (n=4). (E) Representative immunoblots and densitometric quantification for HSL, p-PKA, total PKA, Fabp4, CEBPβ and PPARγ in Control group, pc-Hoxa5 group and sh-Hoxa5 group with cAMPS-Rp, triethylammonium salt treatment or not (n=4). Values are means ± SD. vs. Control group, *p<0.05, ** p<0.01.

FABP4 is an adipose-derived factor that integrates with components of the fasting response to maintain glucose homeostasis [37]. FABP4, identified as a cytosolic protein, is strongly up-regulated during differentiation of preadipocytes into adipocytes [21, 38]. Here, we first determined that Hoxa5 acts as a transcriptional activator of the key adipogenic factor-Fabp4. PKA promotes lipolysis by phosphorylating several proteins including lipid droplet-associated protein perilipin A, Hormone-Sensitive Lipase (HSL), and Adipose Triglyceride Lipase (ATGL) [39-41]. In this study, we found that Hoxa5 promoted adipocytes differentiation and reduced lipolysis through inhibiting the PKA/HSL signal pathway.

Conclusion

Our results demonstrated that Hoxa5 promotes adipocytes differentiation by activating the Fabp4 and inhibiting the PKA/HSL pathway (summarized, see online suppl. material, in Fig. S5). Moreover, we found CEBPβ was a novel transcriptional inhibitor of Hoxa5 and CEBPβ inhibited methylation level of Hoxa5 by combining on Hoxa5 promoters. These findings may help to uncover novel ways to intervene with the function of adipose tissue in order to promote health.

Acknowledgements

This study was financially supported by Major National Scientific Research Projects (2015CB943102), the National Nature Science Foundation of China (31572365) and the Key Sci-tech innovation team of Shaanxi province (2017KCT-24).

Disclosure Statement

The authors declare that they have no competing interest.

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