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Signal transducer and activator of transcription 5B (STAT5B) modulates adipocyte differentiation via MOF☆

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ABSTRACT

The role and mechanism of signal transducer and activator of transcription 5B (STAT5B) in adipogenesis remain unclear. In this study, our data showed that Males absent on the first (MOF) protein expression was increased during 3 T3-L1 preadipocytes differentiation accompanied with STAT5B expression increasing. Over-expression STAT5B enhanced MOF promoter trans-activation in HeLa cells. Mutagenesis assay and ChIP analysis exhibited that STAT5B was able to bind MOF promoter. Knocking-down STAT5B in 3 T3-L1 preadipocytes led to decreased expression of MOF, but resulted in increased expression of peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α (C/EBPα) and fatty acid-binding protein 4 (Fabp4), which were important factors or enzymes for adipogenesis. We also found that knocking-down MOF in 3 T3-L1 preadipocytes resulted in increased expression of PPARγ, C/EBPα and Fabp4, which was in the same trend as STAT5B knocking-down. Over-expression MOF resulted in reduced promoter trans-activation activity of C/EBPα. These results suggest that STAT5B and MOF work as negative regulators in adipogenesis, and STAT5B modulates preadipocytes differentiation partially by regulating MOF expression.

1. Introduction

The excess accumulation of fat due to the hyperplasia and hypertrophy of adipocytes will lead to obesity [1,2]. Obesity is a major risk factor for insulin resistance, type 2 diabetes mellitus and cardiovascular disease and thus has become a major health problem in modern society [3].

Growth hormone (GH) is a 22-kDa peptide that is produced by the pituitary gland and secreted into the circulation. GH plays essential roles in growth and metabolism. Deregulation of GH secretion has been implicated in the development of obesity. In obese people, GH secretion is usually reduced [4,5]. GH deficiency in GH knock-out mice causes aberrant fat accumulation and obesity [6,7].

STAT5B is an important molecule in GH signaling pathway [8]. In transgenic mice, deletion of the sequence in growth hormone receptor (GHR) gene for STAT5B activation resulted in obesity [9]. Other studies show that STAT5B activation induced by growth hormone down-regulates the expression of PPARγ, Fabp4 also known as aP2 and fatty acid synthase (Fasn) [10–13], and thus inhibits adipocyte differentiation. Similarly, drugs that activate STAT5B or over-expression of constitutively active STAT5B inhibit adipocyte differentiation [14,15]. However, some other studies indicate that STAT5B activation may be necessary for adipocyte differentiation [16,17].

MOF, known as hMOF/MYST1/KAT8 in humans, is first identified in *Drosophila*, which possesses an acetyltransferase activity [18]. In

Abbreviation: C/EBPα, CCAAT/enhancer-binding protein α; ChIP, chromatin immunoprecipitation; Fabp4, fatty acid-binding protein 4; HFD, high fat diet; MOF, Males absent on the first; PPARγ, peroxisome proliferator-activated receptor γ; STAT5B, signal transducer and activator of transcription 5B; KD, knock-down.

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Table 1
sequences of primers used for DNA molecular cloning and RT-PCR.

Target genes	Sequence
For DNA molecular cloning	
pGL3-MOF 1640 bp (+)	CGGGGTACCTGGCAGGAGCCAGTCAGTCTC
pGL3-MOF900bp (+)	CCCGAGCTCTGCTGTGCCGGAAGTGACCG
pGL3-MOF560bp (+)	CGGGGTACCACCCRGAGCGAGAGACGC
pGL3-MOF (−)	CCCGAGCTCTGCTGTGCCGGAAGTGACCG
pGL3-MOF promoter mutation (+)	GCACCATGCATGAATTTCAGGCTAGCC
pGL3-MOF promoter mutation (−)	AGCAGTATCCTGGGGATTACAGTTG
pGL3-C/EBPα2000 bp (+)	CCCCGGTACCTCACCTCAAGCACTTATCCCACTTTCTTT
pGL3-C/EBPα1500 bp (+)	CGGGGTACCCAGCAGACCTCCCTAACA
pGL3-C/EBPα800 bp (+)	CGGGGTACCCTGGAGACGCAATGAAA
pGL3-C/EBPα200 bp (+)	CGGGGTACCGTTGCGCCACGATCTCTC
pGL3-C/EBPα (−)	CCCCGAGCTCTTCGGGTCGCGAATGGCCAGGCCCGCG
STAT5B (+)	CGGGGTACCATGGCTATGTGGATACAGGC
STAT5B (−)	CCCAAGCTTTCATGACTGTGCGTGAGGG
MOF-FLAG (+)	TGCTCTAGAATGGCGGCATATGGGAGCTACAGCT GCAGTTG
MOF-FLAG (−)	CGGGGTACCTTACTTGTCATCGTCGTCCTTGTAGCC TTCTT GGAAAGCTTGACTGTTTGTG
For RT-PCR	
18 s (+)	CGCGGTTCTATTTTGTTGGT
18 s (−)	AGTCGGCATCGTTTATGGTC
C/EBPα (+)	GCGGGAACGCAACAACATC
C/EBPα (−)	GTCACTGGTCAACTCCAGCAC
PPARγ2 (+)	TGGTGACTTTATGGAGCCTAA
PPARγ2 (−)	GGCGAACAGCTGAGAGGACTCTG
Fabp4 (+)	AAGGTGAAGAGCATCATAACCCT
Fabp4 (−)	TCACGCCTTTCATAACACATTCC
MOF (+)	ACCGTGGAGATCGGAGAAAC
MOF (−)	GCCTGCTAAAGCCCACATAGTGA
STAT5B (+)	CACAGTGGATCGAAAGCCAAG
STAT5B (−)	AGCTGGGTGGCCTTAATGTTC

following studies, MOF has been demonstrated to possess multiple biological activities, including gene transcription regulation [19], chromatin structure maintenances, cell cycle control, DNA damage and repair [20]. More recently, MOF is suggested to play essential roles in embryonic development and tumorigenesis [21,22].

Previous study reports that STAT5B could bind the GAS cassette (TTCNNNGAA), and thus regulates gene expression [23]. In the promoter region of MOF gene, we found a GAS cassette-like sequence in the upstream from −431 bp to −423 bp (TTCAATGAA). We speculated that STAT5B might play a role in modulation of MOF gene expression.

In this report, we demonstrated that knock-down of STAT5B reduced MOF expression in 3 T3-L1 preadipocytes. In addition, knock-down of either STAT5B or MOF led to enhanced adipocyte differentiation. These results indicate that STAT5B plays an inhibitory role in adipogenesis, and the effect of STAT5B is at least partially mediated by MOF.

2. Materials and methods

2.1. Cell culture

Human epithelial carcinoma cells (HeLa) were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) with 10% fetal bovine serum (FBS, Hyclone), 100 U/ml penicillin and 100 mg streptomycin at 37 °C in a humidified atmosphere (95% air and 5% CO_2). For adipogenesis, 3 T3-L1 preadipocytes were grown to confluence in DMEM with 10% newborn calf serum (NCS, Gibco), after two days the culture medium was replaced with differentiation medium containing 10% FBS, 1 μM dexamethasone (Sigma), 0.5 mM 3-isobutyl-1-methylxanthie (IBMX) (Sigma), and 10 μg/ml insulin (Sigma). Two days later, the medium was changed to DMEM with 10% FBS, 10 μg/ml insulin for another 2 days. Then the cells were maintained in DMEM with 10% FBS. Oil red O straining was used to observe adipocyte differentiation. Briefly, after washed with Phosphate Buffered Saline (PBS) and fixed with 4% paraformaldehyde for 10 min, the cells were then washed twice with PBS, stained with 60% saturated Oil Red O for 10 min, and washed twice with 60% isopropanol.

2.2. DNA molecular cloning

The cDNAs of mouse STAT5B and MOF were obtained from C57BL/6 mouse liver mRNA by reverse transcription-PCR and ligated with pcDNA3.0 vector at KpnI and HindIII sites, the newly-made cDNAs were sequenced by Sangon Biotech to exclude the mutations. Full-length MOF promoter DNA fragment (2000 bp) was obtained from the genomic DNA of C57BL/6 mouse liver by PCR and was ligated with pGL3-Basic at KpnI and SacI sites; the PCR primers used to amplify MOF promoter were as follows: 5′CGGGGTACCGAGTTCTGACCTCAAGC TGC3′ and 5′CCCGAGCTCTGCTGTGCCGGAAGTGACCG3′. The MOF promoter-derived fragments, including pGL3-1640 bp, pGL3-900 bp and pGL3-560 bp were amplified by PCR with high fidelity DNA polymerase and inserted into the same vector as the full length, and a sequence substitution mutation (TTCAATGAA to *ATGC*ATGAA, −431 bp to −428 bp) of pGL3-Full-length MOF promoter was generated by using site directed mutagenesis strategy. The pGL3-basic and pRL-TK vectors were from Promega. C/EBPα luciferase reporter constructs was made in the same way as MOF promoter. The primers used are listed in Table 1.

2.3. Preparation of STAT5B or MOF KD cell lines

Oligos of shRNAs specifically targeting to mouse STAT5B and MOF were cloned into lentiviral vector pGLV-3H1. The oligo sequences for these shRNA are as follows:

STAT5B: 5-AACGTACAACAATGGATTTACTTTCAAGAGAAGTAAATCC ATTGTTGTACTTTTTC-3

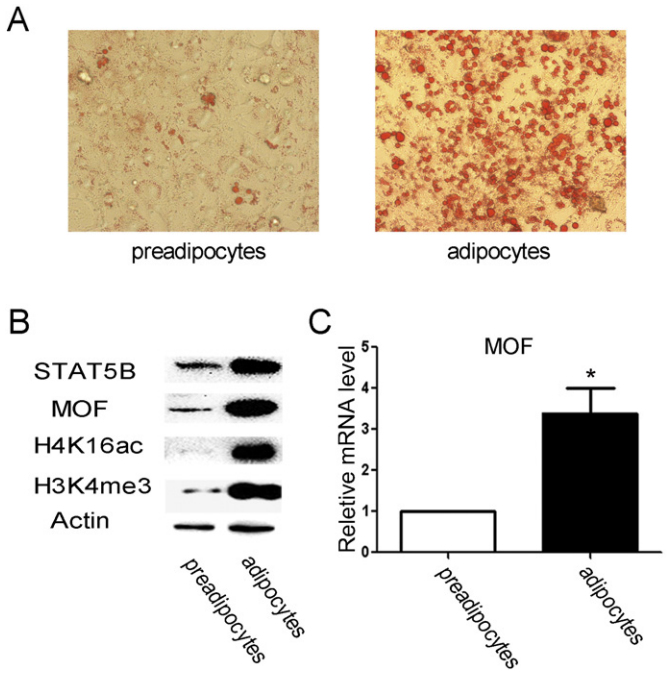

Fig. 1. The expression of STAT5B and MOF are different between 3 T3-L1 predipocytes and adipocytes. (A) 3 T3-L1 preadipocytes and adipocytes were stained with Oil-red O to verify that differentiation had been completed. (B) Comparison of STAT5B and MOF protein level between preadipocytes and differentiated adipocytes with Western blot. (C) The mRNA level of MOF between preadipocytes and adipocytes was analyzed with TR-PCR. (n = 3 for each sample, *, $p < 0.05$).

5-TCGAGAAAAAGTACAACAATGGATTTACTTCTCTTGAAAGTAAATC CATTGTTGTACGTT-3.

MOF: 5-GATCCGCCGAGAAGAATTCTATGTTTCAAGAGAACATAGAAT TCTTCTCGGCTTTTTTG-3

5-AATTCAAAAAAGCCGAGAAGAATTCTATGTTCTCTTGAAACATAGA ATTCTTCTCGGCG-3.

To generate stable cell lines, 3 T3-L1 preadipocytes were infected with lentivirus containing STAT5B or MOF shRNA or scramble shRNA to be negative control (NC). After 48 h post-infection, the cells were incubated with drug selection medium (normal medium with puromycin 2 μg/ml) for 2 weeks, clones were selected and amplified and characterized.

2.4. Animal study

Six-week old male C57BL/6 mice were purchased from the Beijing HFK Bioscience CO.LTD. Animal experiments were conducted according to the Principles of Laboratory Animal Care established by the National Institutes of Health. All experiments were approved by the Animal Care and Use Committee at Shandong University. Mice were divided into two groups, with one normal feeding group fed with a control diet containing 5% fat and the other group fed with a high-fat diet containing 20% fat. Animal body weights were measured once a week and the body weight 30% greater than the normal diet mouse was considered to be fat. The animals were sacrificed following deep anesthesia with ether. Visceral adipose tissues were collected and snap frozen in liquid nitrogen.

2.5. RNA extraction and real-time quantitative-PCR (RT-PCR) performing

Total RNA was extracted with TRIzol (9109, TaKaRa) according to manufacturer instructions from 3 T3-L1 preadipocytes and adipocytes, reverse transcription was carried out according to the manufacturer's instruction with the ReverTra Ace® qPCR RT kit (FSQ-101, TOYOBO). RT-PCR was performed by monitoring the increase in fluorescence of the SYBR green dye (UltraSYBR Mixture CW0956) using the CFX96TM Real-Time System (Bio-Rad) according to the manufacturer's instructions. 18 s rRNA was used as an internal control. The primers used are listed in Table 1.

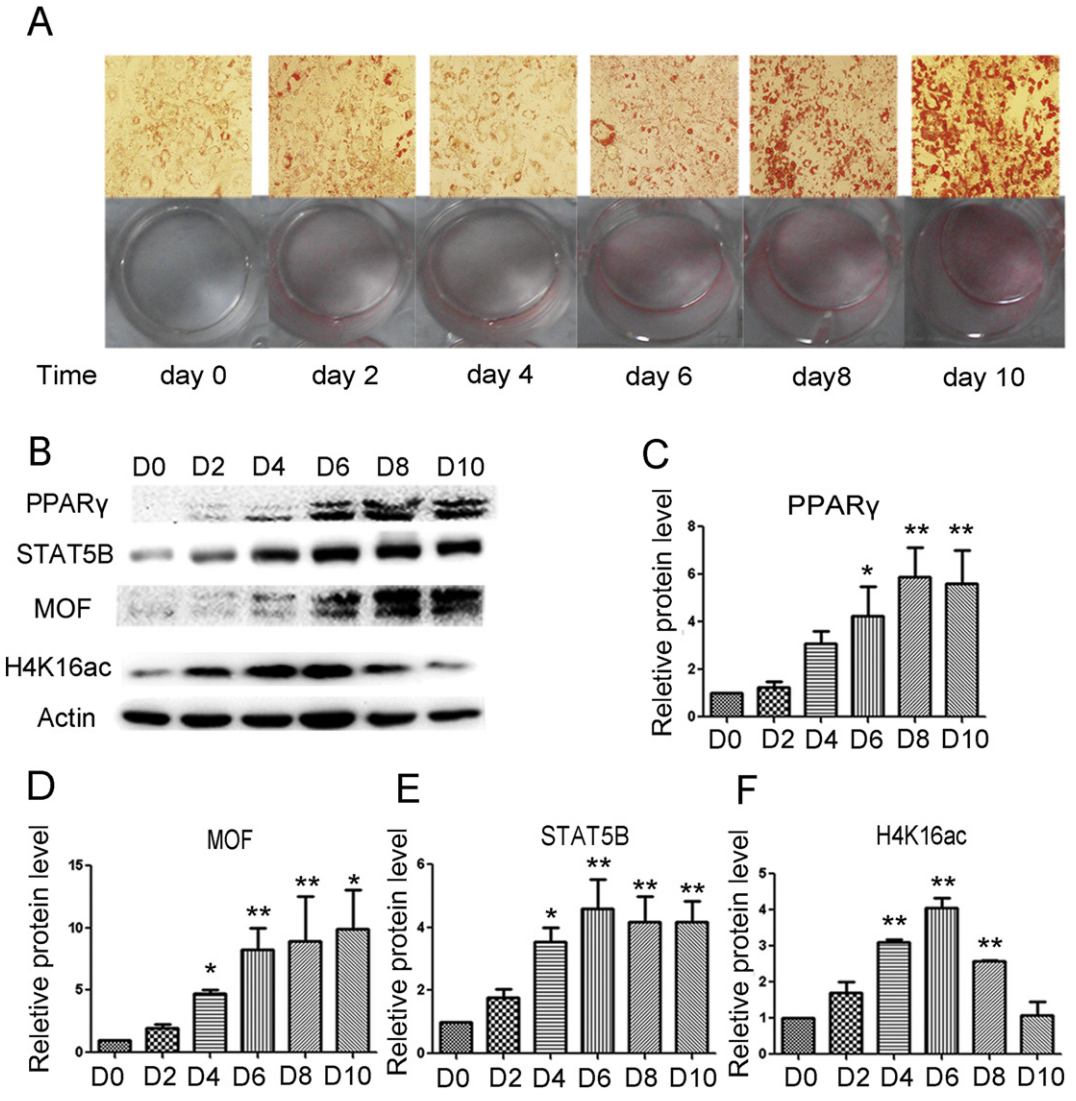

Fig. 2. MOF expression is increased in concert with STAT5B during 3 T3-L1 adipocyte differentiation. (A) 3 T3-L1 preadipocytes were induced to differentiate into mature adipocytes in the adipogenesis induction medium as described in Materials and Methods. At different induction days, cells were subjected to Oil-red O staining. (B) STAT5B and MOF protein expression profiles during 3 T3-L1 preadipocytes differentiation. The H4K16ac level was also detected during the adipogenesis process. PPARγ was used as a marker for adipogenesis and β-actin as internal loading control. (C, D, E, F) Semi-quantitative analyses of Western blotting (n = 3 for each sample, *, $p < 0.05$; **, $p < 0.01$).

2.6. Luciferase assay

Transfection was performed with lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. One day before transfection, 2×10^5 cells were seeded in one well of 24-well plate with 500 μl of growth medium without antibiotics so that cells would be 90–95% confluent at the time of transfection. Cells were co-transfected with 400 ng luciferase reporter plasmid (pGL3-Full-length MOF promoter or pGL3-MOF promoter deletion or mutation) or PGL3-basic (control), 400 ng protein expression plasmid (pcDNA-STAT5B) or 400 ng pcDNA (vector) and 40 ng pRL-TK(a Renilla luciferase plasmid). For each transfection, 2 μl lipofectamine 2000 was used. After transfection for 24 h, HeLa cells in 24-well plates were lysed for luciferase assay. Luciferase activities were determined by luciferase assay kit from Beyotime Biotechnology. Luciferase activity was normalized to Renilla luciferase activity, which was determined as described previously [24]. Transfections were performed in triplicate for each independent experiment. The luciferase assay of MOF on C/EBPα promoter was similar to what described above.

2.7. Western blotting

3 T3-L1 preadipocytes, 3 T3-L1 adipocytes or epididymal fat of normal feeding and HFD mice were lysed in RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% tritonX-100, 1% sodium deoxycholate, 0.1% SDS, Beyotime Biotechnology), supplemented with protease inhibitor cocktail (Sigma), and the protein content was measured using the BCA Protein Assay Kit (Sangon Biotech). Western blotting was carried out as previously described [25], using mouse anti-MOF antibody (1:1000), rabbit anti-STAT5B antibody (1:1000), from Santa Cruz Biotechnology; rabbit anti-PPARγ antibody (1:1000), rabbit anti-Fasn antibody(1:1000), rabbit anti-Fabp4 antibody (1:1000), rabbit anti-H4K16ac antibody (1:1000) from Abcam, mouse anti-beta-actin antibody (1:2000) and secondary antibodies conjugated to horseradish peroxidase (1:5000) from ZSGB-BIO.

2.8. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) was performed with 1.0×10^7 cells essentially as described [26]. A rabbit anti-STAT5B (Santa Cruz Biotechnology) was used to precipitate STAT5B binding DNA fragment, and PCR primers F: 5′-CCCCAAGCATCCCATAAA-3′ R: 5′CTCCTCCT GCCTTGGGTT-3′ were exploited to amplify STAT5B binding site.

2.9. Statistical analysis

All data are expressed as the mean ± SEM. Analysis of differences was performed using the two-tailed Student's t-test or with analysis of variance (ANOVA) in Graph Pad Prism 5.0. A value of $p < 0.05$ (*) was considered statistically significant, $p < 0.01$ (**) was considered very significant.

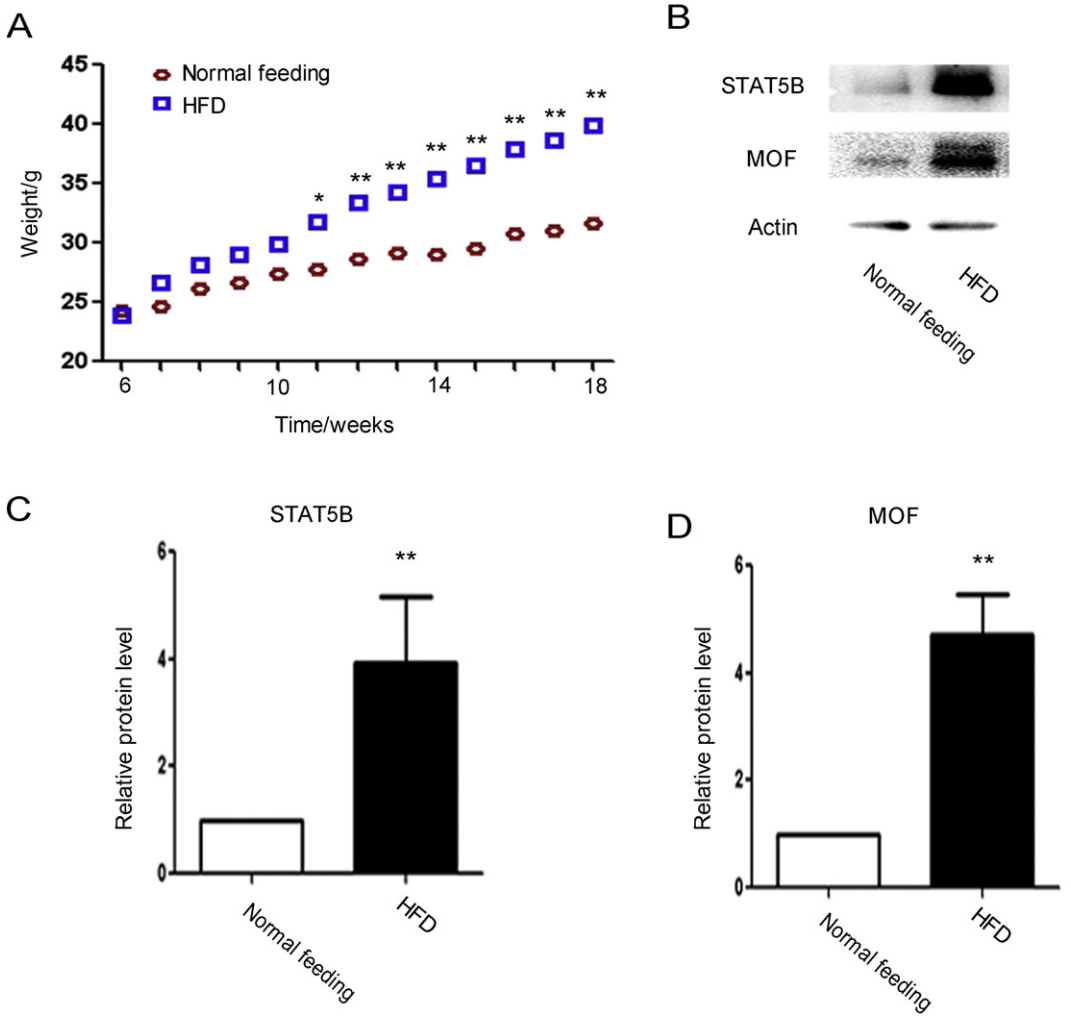

Fig. 3. STAT5B and MOF expression are increased in white adipose tissue from HFD fed obese mice. (A) C57BL/6 mice fed with normal diet or high fat diet (HFD) for 13 weeks, the body weight was measured every week. When body weight of HFD group reached 20% excess the normal control group, it was regarded that the obesity model was established. n = 6 (B) Western blot analysis the expression of STAT5B and MOF in mice WAT (Epididymal fat). (C, D) Semi-quantitative analyses of Western blotting (n = 6 for each sample, *, $p < 0.05$; **, $p < 0.01$).

3. Results

3.1. STAT5B and MOF expression are different between 3 T3-L1 predipocytes and adipocytes

We observed that STAT5B and MOF expression were different between undifferentiated and differentiated 3 T3-L1 cells (Fig. 1A). The protein level of MOF was elevated in differentiated 3 T3-L1 cell accompanied with STAT5B protein level (Fig. 1B). This suggests that during adipogenesis the expression of STAT5B was positively correlated with the expression of MOF. We also detected H4K16ac and H3K4me3 levels in these different periods of cells. As the main downstream molecule of MOF, H4K16ac was also increased, moreover, the modification of H3K4me3 which coincided with H4K16ac showed the same pattern (Fig. 1B). The mRNA level of MOF was elevated in differentiated 3 T3-L1 cells (Fig. 1C).

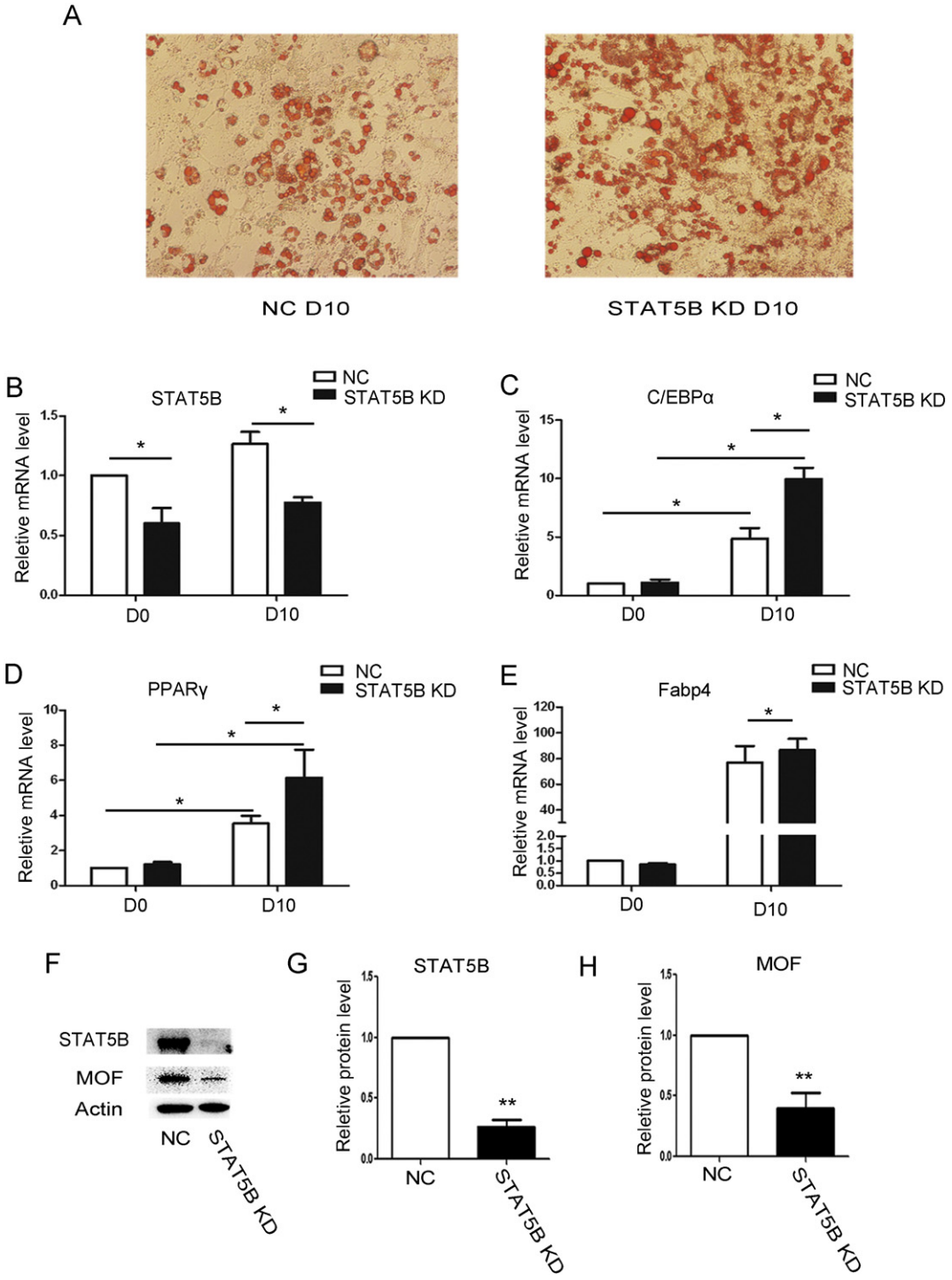

Fig. 4. STAT5B regulates genes associated with adipogenesis in 3 T3-L1 adipocytes and MOF expression is downregulated in STAT5B knocking-down preadipocytes. (A) After differentiated for ten days, NC and STAT5B KD cells were subjected to Oil-red O staining. (B, C, D, E) mRNA expression levels of STAT5B, C/EBPα, PPARγ and Fabp4 in both preadipocytes and adipocytes were analyzed with real-time PCR. The mRNA level was calculated as a ratio to 18 s rRNA. The results are described as the mean ± SEM of three independent experiments. *, $p < 0.05$; **, $p < 0.01$, vs. NC. (F) STAT5B and MOF protein expression in STAT5B knocking-down preadipocytes were analyzed with Western blot. (G, H) Semi-quantitative analyses of Western blotting ($n = 3$ for each sample, *, $p < 0.05$; **, $p < 0.01$).

3.2. MOF expression are induced during 3 T3-L1 differentiation

To identify the dynamics of MOF during adipogenesis, 3 T3-L1 preadipocytes cells were cultured and induced to differentiation. Cells were harvested every two days, 3 T3-L1 cells were fully differentiated after ten days induction (Fig. 2A). The protein level of MOF was gradually elevated in the process of adipogenesis accompanied with the increase of STAT5B protein level (Fig. 2B). PPARγ was the critical marker for adipogenesis, which was exploited to monitor the differentiation level of preadipocytes. We also examined H4K16ac which was the main substrate of MOF activation and found that H4K16ac level was also up-regulated [27].

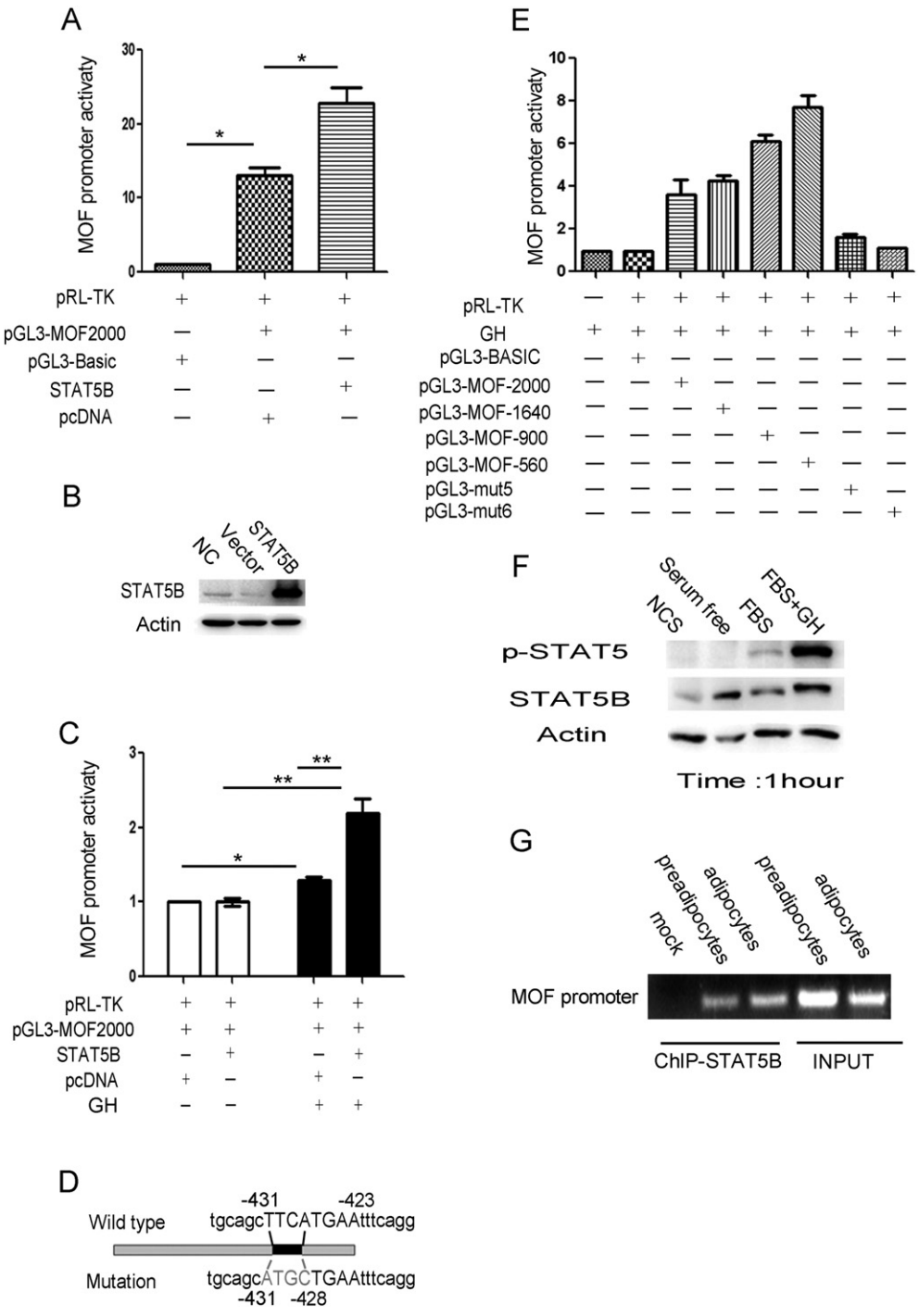

Fig. 5. STAT5B binds to MOF promoter and modulates MOF trans-activation. (A) Luciferase activity of 2000 bp MOF promoter in HeLa cells was significantly activated by STAT5B. (B) STAT5B expression in samples from A was analyzed with Western blot. (C) After starving for 3 h, HeLa cells were treated with 125 ng/ml GH in starvation medium for 6 h. Luciferase activity of 2000 bp MOF promoter was activated by GH. (D) Schematic of the mutation GAS sequence (−431 to −423) in MOF promoter. (E) GH enhanced MOF promoter trans-activation in 3 T3-L1 preadipocytes. When the STAT5B binding site in MOF promoter was mutated, STAT5B failed to enhance MOF promoter trans-activation. (F) STAT5B was activated by GH treatment. (G) ChIP assay showed that GAS region in MOF promoter was co-precipitated with anti-STAT5B antibody in 3 T3-L1 cells, rabbit IgG was used to be negative control. (n = 3 for each sample, *, $p < 0.05$; **, $p < 0.01$).

3.3. STAT5B and MOF protein levels are both increased in white adipose tissue (WAT) of HFD-fed obese mice

To determine whether STAT5B and MOF were up-regulated in vivo in adipocytes, we fed C57BL/6 mice with normal diet and HFD, After 13 weeks feeding, the body weights of obese mice increased significantly in the HFD group compared with the normal diet group (Fig. 3A). The animals were sacrificed after deep anesthesia with ether. WAT were extracted from epididymal fat and analyzed with Western blotting. We found that STAT5B and MOF were both elevated in epididymal fat of HFD group (Fig. 3B, C, D).

3.4. Knock-down of STAT5B in preadipocytes increases the expression of adipogenesis genes and decreases the expression of MOF

To further confirm the role of STAT5B in preadipocytes and adipocytes, lentivirus expressing STAT5B shRNA or scramble shRNA was used to infect 3 T3-L1 preadipocytes. After selected with puromycin, we obtained the cell lines, in which shRNA was stably expressed. We induced control (NC, stably express scramble shRNA) and STAT5B knock-down 3 T3-L1 cell to differentiation for ten days (Fig. 4A), and then detected genes that associated with adipocyte differentiation by RT-PCR. The results showed that pro-adipogenesis genes such as PPARγ, C/EBPα and Fabp4 were up-regulated in STAT5B knock-down adipocytes compared to the NC cells (Fig. 4B, C, D, and E). These data suggested that the decrease of STAT5B was favorable to the differentiation of 3 T3-L1 preadipocytes. As expected, MOF expression declined accompanied with the decrease of STAT5B in STAT5B knock-down 3 T3-L1 cells (Fig. 4F). Taken together, all these results suggest that STAT5B may act as a negative regulator in adipocyte differentiation and there may be some connection between STAT5B and MOF during adipogenesis.

3.5. STAT5B binds to MOF promoter and enhances MOF transcription

To determine whether STAT5B had the effect on MOF trans-activation, we did luciferase assay. As shown in Fig. 5A and B, over-expression STAT5B increased the luciferase activity of pGL3-MOF2000 reporter. HeLa cells were transfect with pcDNA-STAT5B, pGL3-MOF2000, then treated with GH; our data showed that GH elevated MOF promoter trans-activation (Fig. 5C) in HeLa cells. To narrow-down the STAT5B regulatory element in MOF promoter, we generated a series truncation MOF reporters (pGL3-1640 bp, pGL3-920 bp, pGL3-560 bp) and a potential STAT5B binding defective (−431 bp to −423 bp TTCAATGAA) MOF reporter (pGL3-2000 mutation) as shown in Fig. 5D, and did luciferase assays with these reporters in 3 T3-L1 preadipocytes cells. Compared with the pGL3-basic, all plasmids except pGL3-2000 mutation increased MOF promoter activity after treated with GH for 6 h (Fig. 5E). In fact, medium that contained fetal bovine serum could activate STAT5B, in Fig. 5F we showed that STAT5B activation in 3 T3-L1 cells were much higher when treated with GH in medium with fetal bovine serum. 3 T3-L1 preadipocytes and adipocytes cells

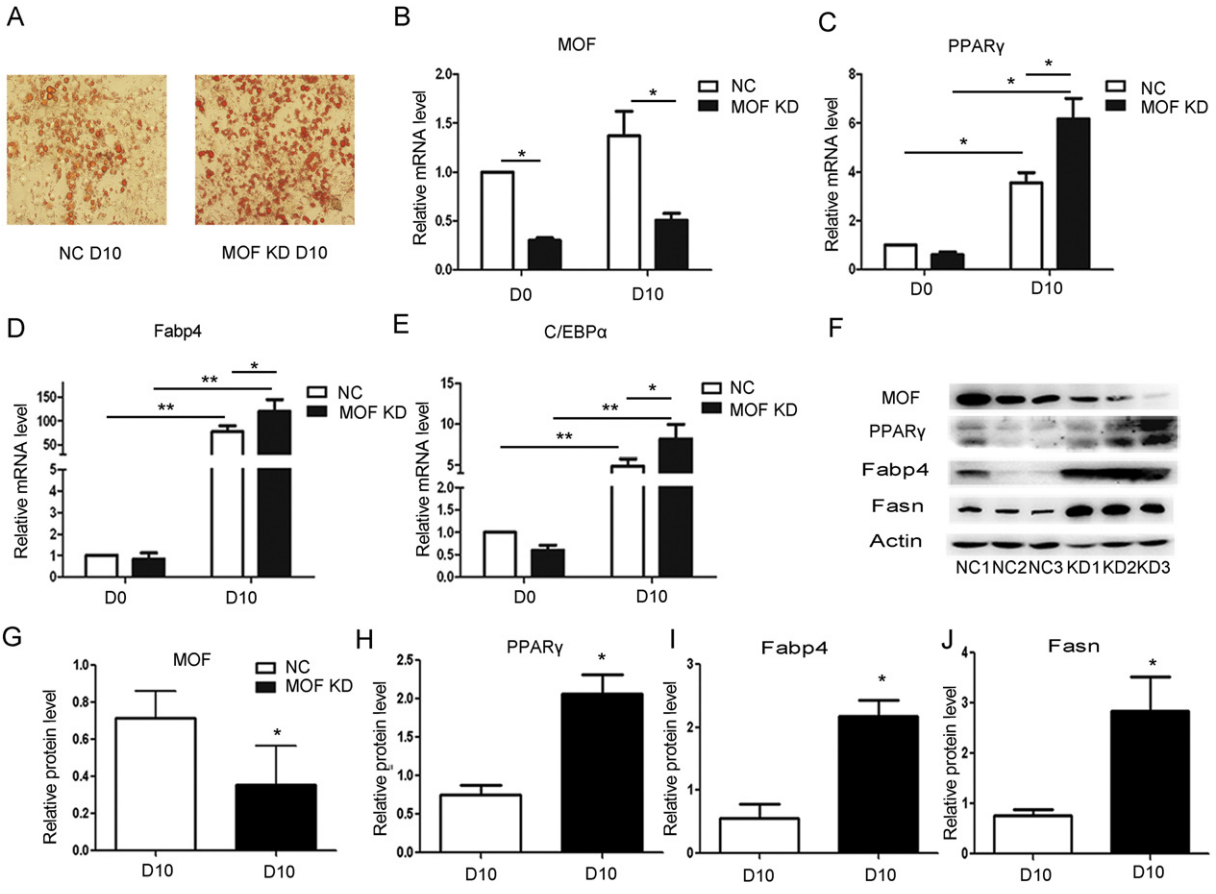

Fig. 6. MOF knocking-down effects adipocyte differentiation. (A) After differentiated for ten days, NC and MOF KD cells were subjected to Oil-red O staining. (B, C, D, E) mRNA expression levels of MOF, PPARγ, C/EBPα and Fabp4 in both preadipocytes and adipocytes were evaluated by RT-PCR using RNA extracted from NC cells and MOF knockdown cells. The mRNA level was calculated as a ratio to 18 s RNA. The results are shown as the mean ± SEM of three independent experiments. *, $p < 0.05$; **, $p < 0.01$, vs. NC. (F) Protein expression of genes associated with adipogenesis in MOF knockdown adipocytes were detected by Western blot. (G, H, I, J) Quantitative analyses of Western blotting, representative result of Western blotting. ($n = 3$ for each sample, *, $p < 0.05$; **, $p < 0.01$).

were applied to ChIP assay with a STAT5B specific antibody. Immunoprecipitated DNA was used to amplify the DNA fragment coverage of STAT5B putative fragment by PCR (Fig. 5G). Our data confirmed that STAT5B bound to MOF promoter at TTCAATGAA sequence site.

3.6. Knock-down of MOF increases the expression of adipogenesis genes

We exploited lentivirus expressing MOF shRNA and infected 3 T3-L1 preadipocytes. After selection, we obtained the MOF knocking-down cells and control cells. These cells were applied for differentiation (Fig. 6A). RT-PCR results showed the similar tendency in mRNA expression of PPARγ, C/EBPα or Fabp4 in MOF knocking-down cells compared to the NC cells during adipogenesis (Fig. 6B, C, D, and E). Western blot analysis showed that the protein expression of PPARγ, Fabp4, or Fasn was up-regulated in MOF knocking-down adipocytes (Fig. 6F). The mRNA expression of PPARγ and Fabp4 was in concert with their protein expression when MOF was knocked-down. It should be noted that there was no difference in STAT5B expression between negative control (NC) and MOF knocking-down preadipocytes, but when induced for ten days MOF knocking-down cells had a higher level of STAT5B (data not shown). All these data suggested that MOF knocking-down caused similar modulation in adipocyte differentiation as STAT5B knocking-down did, which indicated MOF might play an important role in adipocyte differentiation as a downstream molecule of STAT5B.

3.7. MOF inhibits the transcription activity of C/EBPα promoter

Luciferase assay were exploited to determine the effect of MOF on promoter transcription activity of C/EBPα or PPARγ. As shown in Fig. 7, over-expression MOF inhibited the activity of C/EBPα promoter in HeLa cells, while over-expression MOF did not change the promoter transcription activity of PPARγ (data not shown). We also generated a series truncation C/EBPα reporter (pGL3-C/EBPα1500bp, pGL3-C/EBPα800bp, and pGL3-C/EBPα200bp) and found that the inhibited effect of MOF to C/EBPα promoter was only attenuated in pGL3-C/EBPα200bp. These results implied that MOF was a negative regulator for C/EBPα promoter trans-activation and the regulated element might between −800 bp and −200 bp.

4. Discussion

Stephens first demonstrated that protein levels of STAT5A and 5B were increased during the differentiation of 3 T3-L1 preadipocytes [28,29]. Deletion of STAT5A, STAT5B or both in mice resulted in significantly reduced fat pad size [30]. These findings suggested that STAT5

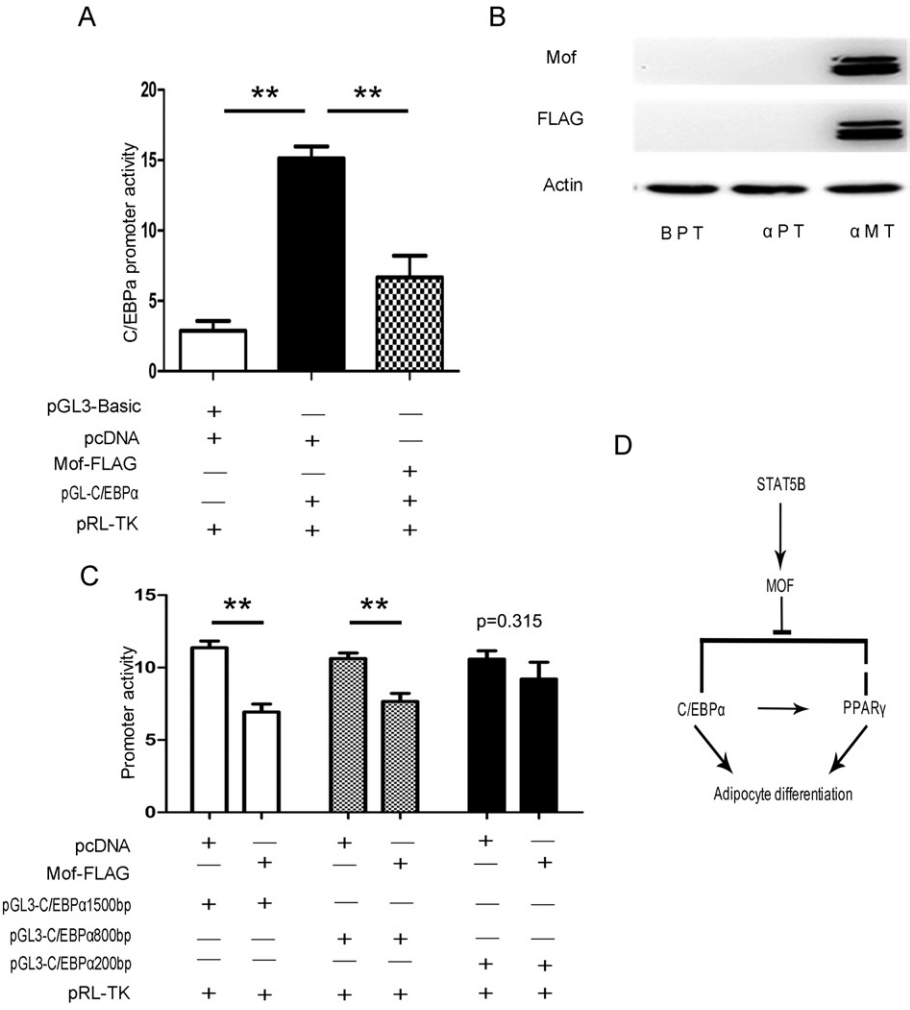

Fig. 7. MOF impacts C/EBPα promoter trans-activation. (A) Luciferase activity of 2000 bp C/EBPα promoter in HeLa cells was significantly inhibited by over expression of MOF. (B) MOF expression in samples from HeLa cells were analyzed with Western blot, BPT stands for transfectant with basic, pcDNA and pRL-TK; αPT stands for transfectant with C/EBPα promoter, pcDNA and pRL-TK; αMT stands for tansfectant with C/EBPα promoter, MOF-FLAG and pRL-TK. (C) Luciferase activity of C/EBPα promoter fragments (pGL3-C/EBPα1500 bp, pGL3-C/EBPα800 bp, and pGL3-C/EBPα200 bp) in HeLa cells were analyzed and only pGL3-C/EBPα200bp trans-activity was not inhibited by over expression of MOF. (n = 3 for each sample, *, $p < 0.05$; **, $p < 0.01$). (D) Schematic for STAT5B modulates adipocyte differentiation via MOF. STAT5B negatively modulates adipogenesis via regulating MOF expression and MOF inhibit the expression of C/EBPα and PPARγ.

proteins may play important roles in adipogenesis. Previous studies showed that ectopic expression of STAT5A rather than STAT5B induced fat cell differentiation [31,32]. In the present study, we demonstrated that STAT5B protein expression was increased during adipocyte differentiation, which is in agreement with a previous report [28]. Furthermore, knock-down of STAT5B in 3 T3-L1 preadipocytes increased the expression of PPARγ, C/EBPα and Fabp4, which are markers of adipocyte differentiation. Our findings are in consistency with a previous report showing that STAT5B over-expression down-regulated PPARγ and C/EBPα [15].

Interestingly, we found that knock-down of STAT5B resulted in decreased MOF expression. To confirm the link between STAT5B and MOF, we examined the MOF promoter sequence and found a GAS cassette in the upstream from −431 bp to −423 bp (TTCAATGAA) as previously reported [23]. Global histone H4K16 acetylation in most organisms is due to the KAT8/MOF/MYST protein [33]. There are two mammalian MOF complexes that regulate transcription activation, MSL and MSL1v1 which regulate different regions of target genes. It is important that not only histone but also non-histone can be acetylated by MOF-MSL 1v1, such as p53-k120 [34]. MOF can be autoacetylated by itself on K274, but SIRT1 deacetylate MOF and increase the recruitment of MOF on chromatin then activate gene expression [35]. It seems that acetylation of transcriptional regulator may down-regulate its ability for DNA binding.

MOF protein was increased during 3 T3-L1 adipocyte differentiation, and the expression of MOF and STAT5B proteins were much higher in white adipose tissues of HFD induced obese mice than control mice. We proposed that STAT5B might regulate MOF expression by binding to its promoter. Luciferase reporter assay showed that over-expression of STAT5B enhanced MOF promoter transcription activity, in which the STAT5B binding sequence was indispensable. Additional experiments in this study demonstrated that knock-down of MOF in 3 T3-L1 preadipocytes resulted in increased expression of PPARγ, Fabp4, Fasn and C/EBPα. These findings suggested that STAT5B may modulate adipocyte differentiation partially by regulating MOF expression. To our knowledge, this is the first report proposing MOF as a negative regulator of adipocyte differentiation.

The precise mechanism underlying the action of MOF in adipocyte differentiation was not clear. A previous study showed that over-expression of C/EBPα could regulate PPARγ trans-activation with steroid receptor coactivator-3 (SRC-3) in mouse embryonic fibroblasts (MEF) [36]. Herein, we demonstrated that over-expression of MOF inhibited C/EBPα promoter trans-activation in HeLa cells. We thus speculated that MOF might inhibit PPARγ expression via regulating C/EBPα.

5. Conclusion

In this study, we demonstrated that STAT5B is a negative regulator of adipocyte differentiation. MOF expression increases in parallel with STAT5B during adipogenesis. Knocking-down STAT5B expression in preadipocytes results in decreased expression of MOF and increased expression of adipogenesis activators or enzymes. Knocking-down MOF expression also resulted in increased expression of adipogenesis activators in preadipocytes. STAT5B over-expression enhances MOF promoter trans-activation and STAT5B is associated with MOF promoter in the STAT5B putative binding area. Over-expression MOF inhibits C/EBPα promoter trans-activation. We therefore, conclude that STAT5B negatively modulates adipogenesis via regulating MOF expression, which subsequently inhibits C/EBPα promoter trans-activation. The significance of STAT5B/MOF pathway in adipogenesis needs further investigation.

Competing interests

The authors declare there are no competing financial interests.

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