Klf7 modulates the differentiation and proliferation of chicken preadipocyte

Zhiwei Zhang1,2,3, Haixia Wang1,2,3, Yingning Sun1,2,3,4, Hui Li1,2,3*, and Ning Wang1,2,3

1Key Laboratory of Chicken Genetics and Breeding, Ministry of Agriculture, Harbin 150030, China
2Key Laboratory of Animal Genetics, Breeding and Reproduction, Education Department of Heilongjiang province, Harbin 150030, China
3College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China
4Biolog and Forestry College, Qi juhar University, Qi juhar 161006, China
*Correspondence address. Tel: +86-451-55191516; Fax: +86-451-55103336; E-mail: lihui@neau.edu.cn (H.L.)/Tel: +86-451-55191770; E-mail: ningwang2001@yahoo.com (N.W.)

Krüppel-like factor 7 (Klf7) has been extensively studied in the mammalian species, but its function in avian species is unclear. The objective of this study was to reveal the function of chicken Klf7 (Gallus gallus Klf7, gKlf7) in adipogenesis. The results of real-time reverse transcription polymerase chain reaction demonstrated that the relative mRNA level of chicken Klf7 (gKlf7/β-Actin) in the abdominal adipose tissue was significantly associated with the abdominal fat content and the age of broilers (P < 0.05), and gKlf7 was more highly expressed in preadipocytes than in mature adipocytes (P < 0.05). In addition, Oil red O staining showed that gKlf7 inhibited chicken preadipocyte differentiation, and MTT assay indicated that gKlf7 overexpression promoted preadipocyte proliferation. Additionally, luciferase assays showed that gKlf7 overexpression suppressed the chicken CCAAT/enhancer-binding protein α (C/ebpα), fatty acid synthase (Fasn), and lipoprotein lipase (Lpl) promoter activities (P < 0.05), and gKlf7 knockdown increased the chicken peroxisome proliferator-activated receptor γ (Ppary), C/ebpα and fatty acid-binding protein 4 (Fabp4) promoter activities (P < 0.05). Together, our study demonstrated that chicken Klf7 inhibits preadipocyte differentiation and promotes preadipocyte proliferation.

Keywords chicken; Krüppel-like factor 7; preadipocyte; differentiation; proliferation

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Introduction

Krüppel-like factors (KLFs) that regulate gene expression by binding to the CACCC/GC/GT boxes in target gene promoters through their highly conserved three C-terminal C_2H_2 zinc finger domains have been shown to play important roles in a variety of cellular processes during embryonic development and in the adult organism [1]. KLF7, a member of the KLF family, was firstly isolated by degenerate polymerase chain reaction (PCR) corresponding to the DNA-binding domain of erythroid KLF using cDNA prepared from human vascular endothelial cells as template [2]. KLF7 is also named as ubiquitous KLF, as it is broadly expressed at low levels in many adult human tissues [2]. The Klf7 null mice died within 2 days after birth due to widespread neurological defects resulting from incorrect development of axonal pathways, indicating that Klf7 plays an important role in nervous system development [3]. Genetic association analysis in human population indicated that KLF7 is a candidate gene for obesity and type 2 diabetes [4,5], and the functional studies in both human and mouse showed that KLF7 inhibits preadipocyte differentiation [6], along with the down-regulation of many differentiation marker genes, including peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α (C/EBPα) and fatty acid-binding protein 4 (FABP4) [6]. In addition, KLF7 also regulates the expression of adipocyto- kines in the differentiated human adipocytes [6], inhibits glucose-induced insulin secretion in pancreatic β-cells, and takes part in the regulation of type 2 diabetes [6].

Our previous study has indicated that the structure and tissue expression pattern of chicken Klf7 (Gallus gallus Klf7, gKlf7) was similar to its orthologs in mammalian species (unpublished data). We also found that a single nucleotide polymorphism in the coding region of gKlf7 was significantly associated with fatness traits in chicken (unpublished data). The objective of this study was to investigate the function of chicken Klf7 in preadipocyte differentiation and proliferation in vitro. The results provide evidence that the Klf7 is involved in chicken adipogenesis.

Materials and Methods

Experimental birds and management

All animal experiments were conducted according to the guidelines for the care and use of experimental animals
established by the Ministry of Science and Technology of the People’s Republic of China (Approval number: 2006-398), and was approved by the Laboratory Animal Management Committee of Northeast Agricultural University. In total, 115 male birds, from the 14th generation population of Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF), were used. The information of NEAUHLF has been described previously [7]. In brief, after 14 generations of divergent selection for abdominal fatness, the abdominal fat percentage at 7 weeks of age in the fat chicken line was 4.45 times as that of the lean line. All birds were kept in similar environmental conditions and had free access to feed and water.

Tissues
Several male birds (each line three to six birds) were killed at each week from 1 to 12 weeks of age, and the abdominal fat tissue was collected immediately. The collected tissues were flash-frozen and stored in liquid nitrogen until the extraction of RNA.

Chicken Klf7-overexpression and Klf7-RNA interference plasmid construction
The full-length coding sequence of gKlf7 (891 bp; GenBank accession number: JQ736790) was generated by amplification from chicken adipose tissue cDNA using the following primers: sense, 5'-CGGAATTCCGAGGATGATGCCTGCAAGTATGCAGGAATGGATGGCAGGGGATGATACG-3'; anti-sense, 5'-GTGTTACCCTCTTCTATCGATGTGCCAGTGTGATGGCAGGGGATGATATATTTTTTGAAA-3'. The amplified PCR products were separated on a 0.8% agarose gel and the desired band was purified. The purified chicken Klf7 cDNA was subcloned into pMD-18T vector (Takara, Dalian, China) and verified by direct sequencing. The full-length coding region of gKlf7 was generated by pMD-18T-gKlf7 plasmid by treating with EcoRI and KpnI, and subcloned into pCMV-myc vector (Clontech, Mountain View, USA) to obtain the Klf7-overexpression vector, pCMV-myc-gKlf7. The target sequences of the gKlf7 selected for RNA interference (RNAi) comprised nucleotides 273 to 291 relative to the translational start site, and negative control nucleotides was also designed as control group (Table 1). To construct expression vectors for each of the corresponding small interfering RNAs (siRNAs), we synthesized the DNA fragment encoding the sense and antisense sequences of the siRNA, and the short double chain sequences were obtained by the denaturation at 94°C for 7 min and annealing at the room temperature. Then the double chain sequence products were subcloned into the BamHI and Hind III sites of pGenesil-1 plasmid (Genesil, Wuhan, China), and the gKlf7-RNA interference vector and its negative control plasmid, pGenesil-1-siKlf7 and pGenesil-1-NC (NC) were constructed.

Preparation of stromal-vascular cell and fat cell fractions and cell culture
Chicken stromal-vascular and fat cells were isolated according to the following procedure. First, abdominal fat tissue (3–5 g) was collected from 12-day-old chickens, minced, and incubated with 2 mg/ml of collagenase I (Sigma, St Louis, USA) for 1 h in a shaking water bath (180 rpm, 37°C). The suspension was then passed through a 100- and 600-μm nylon cell strainer (BD Falcon, New York, USA) to remove undigested tissue. The filtrate was centrifuged for 10 min at 200 g. The top layer (fat cell fraction) and the pellet (stromal-vascular cell fraction) were collected as chicken fat and stromal-vascular cells, respectively.

The separated chicken stromal-vascular cells (chicken preadipocytes) were seeded at a density of 1 × 10⁵ cells/cm² in DMED/F12 medium (Invitrogen, New York, USA) supplemented with 10% fetal calf serum (Invitrogen) and maintained at 37°C in a humidified, 5% CO2 atmosphere. Until about 70–90% confluence (days 3–4), the cells were passaged and seeded into 6-well plates at a density of 1 × 10⁵ cells/cm². After 12 h, when the cells reached 60–80% confluent, they were transfected with the gKlf7-overexpression plasmid, gKlf7-RNA interference plasmid, as well as their control plasmids, respectively, using the FuGENE HD transfection reagent (Roche, Mannheim, Germany) according to the manufacturer’s recommendations. For differentiation assay, 160 μM oleate (Sigma) was added into the medium to induce preadipocyte differentiation at 24 h after transfection.

Oil red O staining
Oil red O staining of intracellular lipid droplets was performed in 6-well plates. At 48 h after differentiation, chicken preadipocytes were washed with phosphate

<table>
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buffered saline (PBS) and fixed in 10% formaldehyde for 10 min. After rinsing with distilled water, they were stained with 0.5% oil red O working solution, prepared by vigorously mixing three parts of a stock solution (0.5% oil red O in isopropanol; Sigma) with two parts of water for 5 min and filtering through a 0.4-μm filter. Excess staining was removed by rinsing twice with PBS. The dye was extracted by isopropanol incubation for 15 min at room temperature. Quantitative assessment was obtained by spectrophotometric analysis of absorbance of a 3-fold dilution of the extracted dye at 500 nm.

RNA isolation and reverse transcription

**quantitative PCR (RT-qPCR)**

Total RNA of tissues (each 100 mg) and cells was extracted using Trizol (Invitrogen) following the manufacturer’s protocol. RNA quality was assessed by denaturing formaldehyde agarose gel electrophoresis. Reverse transcription was performed using 1 μg of total RNA, an oligo (dT) anchor primer, and ImProm-II reverse transcriptase (Promega, Madison, USA). Reverse transcription conditions for each cDNA amplification were 25°C for 5 min, 42°C for 60 min, and 70°C for 15 min.

Real-time quantitative PCR (qPCR) was used to detect target gene expression using the SYBR Premix Ex Taq (Takara) and a 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA) with the primers shown in **Table 2**. Chicken β-Actin (Gallus gallus β-Actin, gβ-Actin) was used as an internal reference. Part (1 μl) of each reverse transcription reaction product was amplified in a 20-μl PCR system. Reaction mixtures were incubated in an ABI Prism 7500 sequence detection system (Applied Biosystems) pro grammed to conduct 1 cycle at 95°C for 30 s and 40 cycles at 95°C for 5 s and, at 60°C for 34 s. Dissociation curves were analyzed using the Dissociation Curve 1.0 software (Applied Biosystems) for each PCR reaction to detect and eliminate possible primer–dimer artifacts. All reactions were performed in triplicate. The relative amounts of the mRNAs were calculated by the comparative cycle-time method.

**Western blot assays**

Chicken preadipocytes and DF1 cells transfected with pCMV-myc-gKlf7 or pCMV-myc vector for 2 days were lysed in RIPA buffer [PBS, pH 7.4, containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium do decyl sulphate (SDS), and a protease inhibitor cocktail]. Then, the cell lysates were added into 5× denaturing loading buffer and boiled in boiling water for 5 min. Cell lysates were separated on 5–12% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After incubation with the primary antibody for myc-tag (1:200; Clontech) or chicken GAPDH (1:1000; Beyotime, Beijing, China), a secondary horseradish peroxide-conjugated antibody (1:5000; Beyotime) was added, and a BeyoECL Plus kit (Beyotime) was used for detection.

**Luciferase reporter assays**

DF-1 cells (kindly provide by the Harbin Veterinary Research Institute, Harbin, China) were grown in DMED/F12 medium (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen), and plated in 12-well dishes. In the transfection assays, a fixed amount of total DNA (1 μg) was transfected for each well, using the FuGENE HD transfection reagent (Roche), and the transfection system was shown in Supplementary Table S1. After transfection and incubation for 48 h, the cells were lysed in 250 μl of passive lysis buffer (Promega), and portions of the lysate were subjected to assays for firefly and Renilla luciferase using the Dual-Luciferase Reporter Assay System (Promega). Promoter activity of each construct was expressed as the ratio of Firefly/Renilla luciferase activity.

**MTT assays**

After the chicken preadipocytes were transfected with the gKlf7-overexpression plasmid, gKlf7-RNA interference plasmid, as well as their control plasmids, respectively, and incubation for 24 h, these cells were passaged and seeded into 96-well plates at a concentration of 5000 cells per well, respectively. At the designated time points, including 24, 48, 72, 96 and 120 h, 20 μl 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) solution (5 mg/ml; Sigma) was added in the medium, then the cells were incubated at 37°C for 4 h. After that the medium was removed, and 200 μl of DMSO (Sigma) was added per well, shaking the plate on a rocking platform at 60 rpm for

<table>
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Table 2 Primers used in the qPCR analysis
15 min so that the complete dissolving was achieved. The absorbance was recorded with enzyme-labeled instrument (BioRad, Hercules, USA) at 492 nm. All experiments were carried out in triplicates.

Statistical analysis
Data are expressed as mean ± SD. Comparison between two groups was performed by unpaired two-tailed Student’s t-test. Statistical analysis among more than two groups was performed by PROC GLM procedure followed by the Duncan's multiple test, with the following models:

\[ Y = \mu + A + L + e \]  \hspace{1cm} (1)

\[ Y = \mu + F + e \]  \hspace{1cm} (2)

Model (1) was used for tissue samples, where \( Y \) is the dependent variable (the \( gKLF7 \) expression level), \( \mu \) is the population mean, \( A \) is the fixed effect of the age factor, \( L \) is the line (broiler line selected by high and low abdominal fat content) as fixed effect and \( e \) is the random error. Model (2) was used for cell samples, where \( Y \) is the dependent variable (the \( gKLF7 \) expression level), \( \mu \) is the population mean, \( F \) is the fixed effect of the factor of differentiation time and \( e \) is the random error. Differences were considered significant at \( P < 0.05 \) unless otherwise indicated. All analyses were performed using the SAS software system (version 9.2; SAS Institute Inc., Cary, USA).

Results
Expression pattern of \( gKlf7 \) during chicken adipose tissue development
qPCR analysis showed that the \( gKlf7 \) was expressed in chicken abdominal fat tissues during adipose tissue development, and statistical analysis indicated that the relative \( gKlf7 \) mRNA level (\( gKlf7/g\beta-Actin \)) in the chicken abdominal fat tissue was significantly associated with the broiler lines (selected by high and low abdominal fat content, respectively; \( P = 0.007 \)), and the adipose tissue \( gKlf7 \) mRNA level was significantly higher in lean broiler line than in fat broiler line \( [P < 0.01; \text{Fig. 1(A)}] \). In addition, the \( gKlf7 \) mRNA level was also significantly associated with the age of broilers \( (P = 0.0007) \), and significantly changed during the chicken adipose tissue development. Its expression peaked at 1 week of age \( [P < 0.01; \text{Fig. 1(B)}] \). Additionally, the comparison of \( gKlf7 \) expression in the abdominal fat tissue between fat and lean broilers at each age showed that, at 2 and 5 weeks of age, the \( gKlf7 \) expression in lean males was significantly greater than that in fat males \( [P < 0.05; \text{Fig. 1(B)}] \).

Expression pattern of \( Klf7 \) in chicken adipocytes
qPCR analysis showed that the \( gKlf7 \) mRNA expression fluctuated during chicken preadipocyte differentiation induced by oleate. During the differentiation, \( gKlf7 \) expression decreased at early stage of differentiation and remained at low level at 24 h and 48 h after induction of differentiation. Then its expression increased and peaked at 72 h,
Figure 2 Expression of Klf7 during chicken preadipocyte differentiation

(A) Klf7 mRNA expression during chicken preadipocyte differentiation induced by oleate in vitro was detected by qPCR. Cells were harvested at designated time points. β-Actin was used as an internal control. The diagram shows the relative quantification of chicken Klf7 expression. The different lowercase letters above error bars indicate significant different Klf7 expression levels among time points (GLM followed by Duncan’s multiple test, *P < 0.05). (B) Klf7 mRNA expression in chicken preadipocytes (SV) and mature adipocytes (FC) was detected using RT-qPCR. β-Actin was used as an internal control. The diagram shows the relative quantification of chicken Klf7 expression. *P < 0.05 (Student’s t-test).

Figure 3 Effects of Klf7-knockdown and Klf7-overexpression on chicken preadipocyte differentiation

(A) Oil red O staining of chicken preadipocytes which transfected with pGenesil-1-siKlf7 (Klf7-siRNA) or pGenesil-1-NC (NC), induced into differentiation by oleate for 48 h. (B) The effect of the chicken Klf7 knockdown by RNA interference. The diagram shows the expression levels of Klf7 mRNA. (C) The lipid content of chicken preadipocytes which were transfected with pGenesil-1-siKlf7 (Klf7-siRNA) or NC, induced into differentiate by oleate for 48 h, and absorbance was measured at 500 nm. (D) Oil red O staining of chicken preadipocytes which were transfected with pCMV-myc-gKlf7 (Klf7-overexpressed) or pCMV-myc (empty vector, EV), induced into differentiate by oleate for 48 h. (E) Western blot analysis of gKlf7 overexpression in chicken preadipocytes transfected with pCMV-myc-gKlf7 (Klf7-overexpressed) or EV. (F) The lipid content of chicken preadipocytes transfected with pCMV-myc-gKlf7 (Klf7-overexpressed) or EV, induced into differentiation by oleate for 48 h. The diagram shows the lipid contents in the chicken preadipocytes by measuring the absorbance at 500 nm. *P < 0.05; **P < 0.01 (Student’s t-test).
and fell steadily afterwards \((P < 0.05; \text{Fig. 2})\). In addition, the \(g\text{Klf7}\) expression was much higher in preadipocytes (SV cell fraction) than in mature adipocytes (FC cell fraction) \((P < 0.05; \text{Fig. 2})\).

**Effect of Klf7 on chicken preadipocytes differentiation**

The \(g\text{Klf7}\) mRNA level was higher in preadipocytes than in mature adipocytes, suggesting that \(g\text{Klf7}\) may play a potentially inhibitory role in chicken adipocyte differentiation. To directly validate this hypothesis, we constructed a \(g\text{Klf7}\)-RNA interference plasmid, pGenesil-1-si\(g\text{Klf7}\), which could knockdown the mRNA expression level of \(g\text{Klf7}\) in chicken preadipocytes \([P < 0.01; \text{Fig. 3(B)}]\), and a \(g\text{Klf7}\)-overexpression plasmid, pCMV-myc-\(g\text{Klf7}\), which could express \(g\text{Klf7}\) protein in chicken preadipocytes \([\text{Fig. 3(E)}]\), and then performed the transient transfection to analyze the effect of the knockdown or overexpression of \(g\text{Klf7}\) on chicken preadipocyte differentiation. Oil red O staining demonstrated that, compared with the control groups, the \(g\text{Klf7}\)-silencing cells exhibited an increase in the intracellular lipid accumulation \([P < 0.05; \text{Fig. 3(A, C)}]\), and the \(g\text{Klf7}\)-overexpressing cells exhibited a decrease in the intracellular lipid accumulation \([P < 0.05; \text{Fig. 3(D, F)}]\).

**Effect of Klf7 on chicken preadipocytes proliferation**

The preadipocyte proliferation was also an important process of adipose tissue development. In order to investigate the role of \(g\text{Klf7}\) on the proliferation of chicken preadipocytes, the MTT assay was used to assess cell proliferation in this study. The results showed that, compared with the control group, the \(g\text{Klf7}\)-overexpressing cells exhibited an enhanced ability in cell proliferation, especially, at two time points, 48 and 120 h after transfection \([P < 0.05; \text{Fig. 4(A)}]\). However, no significant difference in cell proliferation was observed between the \(g\text{Klf7}\)-knockdowning cells and their control group \([P > 0.05; \text{Fig. 4(B)}]\).

**Effect of Klf7 on promoter activities of chicken \(Ppary\), \(C/e\beta p\alpha\), \(Fas\), \(Lpl\), and \(Fabp4\)**

\(C/e\beta p\alpha\) and \(Ppary\) are key positive regulators in chicken adipogenesis \(\text{[8]}\), and fatty acid synthase (\(FASN\)), lipoprotein lipase (\(LPL\)) and \(Fabp4\) are all important functional proteins in adipose tissue and adipocytes \(\text{[9–11]}\). To reveal the role of \(g\text{Klf7}\) on the transcription regulation of chicken \(C/e\beta p\alpha\), \(Ppary\), \(Fas\), \(Lpl\), and \(Fabp4\), the luciferase assays were conducted in this study. The results showed that the \(g\text{Klf7}\) overexpression significantly suppressed the \(Lpl\), \(C/e\beta p\alpha\), and \(Fas\) promoter activities \((P < 0.05)\), but had no significant effect on the promoter activities of \(Ppary\) and \(Fabp4\) \((P > 0.05; \text{Fig. 5})\). In contrast, the knockdown of \(g\text{Klf7}\) significantly increased \(C/e\beta p\alpha\), \(Ppary\), and \(Fabp4\) promoter activities \((P < 0.05)\), and had no significant effect on the promoter activities of \(Lpl\) and \(Fas\) \((P > 0.05; \text{Fig. 5})\).

**Discussion**

Our previous study has found that a single nucleotide polymorphism in the coding region of \(g\text{Klf7}\) was significantly associated with the chicken fatness traits (unpublished data). Here, this study showed that the relative \(g\text{Klf7}\) mRNA level \((g\text{Klf7}/g\beta-\text{Actin})\) in the chicken abdominal fat tissue was significantly associated with the divergent selection of abdominal fat content \((P < 0.01)\), and the abdominal fat \(g\text{Klf7}\) expression level was greater in the lean broilers than in the fat broilers \([P < 0.01; \text{Fig. 1(A)}]\), indicating that \(g\text{Klf7}\) might have a negative effect on the abdominal fat deposit in chicken, consistent with the previous reports in mammalian species that \(Klf7\) suppresses the differentiation...
Figure 5: Effects of Klf7 overexpression and knockdown on the promoter activities of chicken Ppary, Cebpα, Fasn, Lpl, and Fabp4. (A) Effects of Klf7 on the promoter activities of chicken Ppary, Cebpα, Fasn, Lpl, and Fabp4. Luciferase assays were conducted in DF1 cells, the promoter activities were expressed as ratios of Firefly/Renilla luciferase activity. The diagrams show the quantification of promoter activities. (B) Western blot analysis of chicken Klf7 overexpression in DF1 cells transfected with pCMV-myc-gKlf7 or EV. (C) The effect of the chicken Klf7 knockdown of by RNAi in DF1 cells. The diagram shows the expression levels of Klf7 mRNA. Klf7-overexpressed: cells transfected with pCMV-myc-gKlf7; EV: cells transfected with pCMV-myc; Klf7-siRNA: cells transfected with pGenesil-1-siKlf7; NC: cells transfected with pGenesil-1-NC. *P < 0.05; **P < 0.01 (Student’s t-test).
of preadipocytes [6,12]. There are strong correlations of abdominal fat with cardiovascular disease [13], type 2 diabetes [14], insulin resistance [15], inflammatory diseases [16], and other obesity-related diseases [17]. Thus, our results may provide a clue for understanding and curing the obesity-related diseases.

Although some correlation of Klf7 expression and preadipocyte differentiation has been revealed in the mammalian species [6,12], the Klf7 expression pattern during adipose tissue development remains unclear. This study showed that the gKlf7 mRNA level in the chicken abdominal fat tissue was significantly associated with the age of broilers (P < 0.01), indicating that the Klf7 expression is developmentally regulated in the abdominal fat tissues, suggested that Klf7 was involved in the chicken abdominal fat tissue development. In addition, our results showed that the gKlf7 expression in abdominal fat tissue peaked at 1 week of age (P < 0.01; Fig. 1(D)), the earliest development time point of this study, suggested that Klf7 might mainly function in the early stage of chicken abdominal fat tissue development. Additionally, the Klf7 expression level of lean broilers was significantly higher than that of fat broilers at 2 and 5 weeks of age (P < 0.05; Fig. 1(B)), shed a light that Klf7 plays an important role in chicken abdominal adipose development from 2 to 5 weeks of age.

Preadipocyte differentiation is an important cellular process in adipose tissue development. Previous studies in human and mouse have demonstrated that Klf7 is a negative regulator of preadipocyte differentiation [6,12]. This study also showed that gKlf7 is an inhibitor of chicken preadipocyte differentiation (Fig. 3). However, unlike other negative regulator of preadipocyte differentiation [18], whose expression was down-regulated steadily after differentiation, gKlf7 expression decreased at early stage of differentiation and then increased, and peaked at 72 h after induction of differentiation. Its mouse ortholog also has the similar expression trend during 3T3-L1 preadipocyte differentiation [6,12]. These results suggest that, like its mouse ortholog, gKlf7 might functions in preadipocytes and mature adipocytes.

The proliferation of preadipocyte is another important process of adipose tissue development. In this study, the MTT assay showed that overexpression of gKlf7 promotes the proliferation of chicken preadipocytes (Fig. 4), indicating that gKlf7 has a positive role in the chicken preadipocyte proliferation. However, consistent with the previous report in mouse embryonic stem cells [19], no significant difference in the cell proliferation was observed between the gKlf7-silencing preadipocytes and their control group (P > 0.05; Fig. 4(B)), indicating that down-regulation of gKlf7 have no obvious effect on the cell proliferation, suggesting that Klf7 have no effect on cell proliferation at high dose.

Previous study in the mouse has shown that Klf7 play important roles in the adipogenesis [6]. However, little is known about its target genes. Currently, the luciferase assays showed that gKlf7 overexpression suppressed chicken Lpl, C/ebpa, and Fasn promoter activities (P < 0.05; Fig. 5) and the knockdown of gKlf7 increased chicken C/ebpa, Ppar, and Fabp4 promoter activities (P < 0.05; Fig. 5). C/EBPα is a cell cycle regulator and a key regulator of adipocyte differentiation [20]. It promotes anti-proliferation through inhibition of cyclin-dependent kinases 2, 4, and 6 and repression of S-phase gene transcription [21]. C/ebpa also facilitates the preadipocyte differentiation by maintaining the expression of Ppar and trans-activating the expression of many adipocyte-specific genes in mature fat cells [22]. Suppression of the C/ebpa promoter by Klf7 was demonstrated by both gKlf7 overexpression and gKlf7 silencing studies, strongly indicating that Klf7 plays a negative role in the transcriptional regulation of chicken C/ebpa, and C/ebpa might be a target gene of chicken Klf7. It is possible that gKlf7 inhibits chicken preadipocyte differentiation and promotes chicken preadipocyte proliferation via regulating C/ebpa expression. The previous studies in mammalian and avian species have shown that Ppar is the dominant positive regulator in the adipocyte differentiation [23,24]. In this study, knockdown of gKlf7 promoted the expression of chicken Ppar, which is consistent with the increased differentiation in gKlf7-silencing preadipocytes. FASN, LPL, and Fabp4 are all important functional proteins in the adipose tissues and adipocytes [9–11], gKlf7 overexpression inhibited the promoter activities of chicken Fasn and Lpl, and gKlf7 knockdown increased the chicken Fabp4 promoter activity. All these data were consistent with the negative role of Klf7 played in the chicken adipogenesis, and also provided evidence that like mammalian Klf7, the chicken Klf7 might play a role in mature adipocytes.

It is important to note that the effect of Klf7 overexpression on the promoter activities of chicken Lpl, Fasn, Ppar, and Fabp4 was not exactly opposite to that of Klf7-knockdown. It is possible that the regulation of Klf7 on the promoter activities of these genes was indirect, and overexpression and knockdown of Klf7 might use different mechanisms to regulate these genes. The molecular mechanism by which gKLF7 regulates these genes, remains to be investigated.

Collectively, this study revealed that the gKlf7 mRNA level in the abdominal fat tissues was significantly associated with the divergent selection of abdominal fat content. And gKlf7 inhibited the chicken preadipocyte differentiation, and promoted the proliferation of chicken preadipocytes.

Supplementary Data

Supplementary data are available at ABBS online.
Funding

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