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The novel gene pFAM134B positively regulates fat deposition in the subcutaneous fat of *Sus scrofa*

ZhangQin Yuan, DeGuang Song, YiZhen Wang *

Institute of Feed Science, Zhejiang University, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, PR China

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ABSTRACT

In this study, we analyzed the global gene expression profiles in the subcutaneous fat (SAT) of Jinhua pigs and Landrace pigs at 90 d. Several genes were significantly highly expressed in Jinhua pigs, including genes encoding the rate limiting enzymes in the TCA cycle, fatty acid activation, fatty acid synthesis and triglyceride synthesis. We identified a novel gene tagged by the EST sequences as public No. BF702245.1, which was named porcine FAM134B (pFAM134B) and the pFAM134B mRNA levels of SAT was significantly higher in Jinhua pigs than that in Landrace pigs at 90 d ($P < 0.01$). Then the effects of pFAM134B on lipid accumulation were investigated by using RNAi and gene overexpression in the subcutaneous adipocytes. The results showed that pFAM134B played a significant positive role in regulating lipid deposition by increasing the mRNA levels of PPARγ, lipogenic genes fatty acid synthetase (FAS) and acetyl-CoA carboxylase (ACC) ($P < 0.01$) and reducing the mRNA levels of adipose triglyceride lipase (ATGL) and lipase, hormone-sensitive (HSL) ($P < 0.01$). This study implied that pFAM134B might be a positive factor in lipid deposition, providing insight into the control of fat accumulation and lipid-related disorders.

1. Introduction

Fat content is a key index to evaluate pork quality [1]. In the pig production, excessive fat deposition affects animal health, production efficiency and marketability of animal products. Therefore, reducing of the fat deposition in adipose tissues of meat-producing animals is highly desirable for both producers and consumers [2,3]. The development of adipose tissue and the accumulation of lipids is a continuous process that depends on genetic, hormonal, and dietary factors, which includes the hypertrophy of existing adipocytes and the proliferation and differentiation of new ones [4]. The previous studies have shown obvious differences in the adipose tissue deposition rate between lean and fatty pigs breeds [5]. Particularly, as the main part of fat tissues, the subcutaneous fat (SAT) content in the Chinese local pigs is higher than in other commercial pigs breeds [6–8]. Jinhua pig, a traditional slow growing breed with high fat content, shows strong capacity of adipogenesis [9,10]. In contrast, Landrace pig, a commercial breed of Danish origin selected over many generations for rapid growth and enhanced carcass yield, shows low activities adipogenesis which lead to trace amounts of fat depot [11–13]. The difference of the genotypes between the two breeds is the foundation for resulting in the difference of phenotypes of the difference of the SAT deposition. It was reported that the body fat ratio and back fat thickness of Jinhua pigs was significantly higher than that of the Landrace pigs at each developmental stage, especially 3.34 and 3.42-fold increase at 90 d, respectively [14].

The underlying mechanism of fat deposition in fatty and lean pigs could be elucidated using these two pig models. Therefore, the objective of this study is to investigate the expression of genes involved in lipogenesis, lipolysis and fatty acid transport in SAT using the Affymetrix GeneChip Porcine Genome Array to compare the expression pattern of lipid metabolism associated genes in pigs with different fat deposition capacity between fatty and lean pigs, and then search for a novel gene with the potential function involving in fat deposition.

2. Materials and methods

2.1. Experimental animals and samples

Eighteen castrated Jinhua (Jinhua II breed) and Landrace (Danish breed) pigs were raised and had ad libitum access to

Abbreviations: SAT, subcutaneous fat; pFAM134B, porcine FAM134B.

* Corresponding author at: Institute of Feed Science, Zhejiang University, 866 Yuhangtang Road, Hangzhou, Zhejiang Province 310058, PR China. Fax: +86 571 88982729.

E-mail address: yzwang321@zju.edu.cn (Y. Wang).

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Table 1
Sequences of oligonucleotide primers used in quantitative polymerase chain reaction (qPCR) assays.

Gene name	Forward Reverse	Primer sequences (5′–3′)	Amplicon length (bp)
18s rRNA	F	CCC ACG GAA TCG AGA AAG AG	122
	R	TTG ACG GAA GGG CAC CA	
pFAM134B	F	AGG GTCTCA GAT GTC AGC ATG CCT	198
	R	TGT CAG CCA CTA CCA ACC ATC CCT	
ATGL	F	TCA CCA ACA CCA GCA TCC A	95
	R	GCA CAT CTC TCG AAG CAC CA	
HSL	F	ACC CTC GGC TGT CAA CTT CTT	84
	R	TCC TCC TTG GTG CTA ATC TCG T	
PPARγ	F	CAT CCT CGC GGG AAA GG	70
	R	GGC CAT ACA CAG TGT CTC CAT GT	
FAS	F	CTA CCT TGT GGA TCA CTG CAT AGA	114
	R	GGC GTC TCC TCC AAG TTC TG	
ACC	F	GGA GAC AAA CAG GGA CCA TTA CA	144
	R	CAG GGA CTG CCG AAA CAT C	

commercial diets (nutrients levels according to the NRC) under similar conditions during the whole experimental period and were slaughtered after a 24 h fasting period at 90 d. The SAT was collected after exsanguinations and directly frozen in liquid nitrogen and stored at −80 °C until use for isolation of total RNA. All experiments described in the study were performed in full accordance with the guidelines for animal experiments released by the National Institute of Animal Health and with a permit (License No.: GB/T 14925-94). For culture of the subcutaneous adipocyte precursor cells in vitro, 3 newborn Duroc × Landrace × Yorkshire pigs were given an overdose of sodium thiopental and exsanguinated. The subcutaneous fat was removed, and preadipocytes were prepared by previously published methods [15].

2.2. RNA extraction, microarray hybridization and microarray data analysis

The RNA extraction, microarray hybridization and microarray data analysis were carried out by using the published methods previously [14].

2.3. Cloning of the pFAM134B gene

To obtain the full-length cDNA sequence of pFAM134B, the RACE technology was carried out to clone the 5′-ends of pFAM134B by using the SMARTTM RACE cDNA Amplification Kit and GeneRacer Kit (Invitrogen Biotechnology Co. Ltd., Shanghai, China). Briefly, for 5′-RACE, 5′ phosphates and the 5′ cap structure were removed from the total RNA from porcine tissues, the GeneRacer RNA Oligo sequence (5′-CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA-3′) to the 5′ end of the prepared mRNA was ligated and the 5′ RACE cDNA template was then obtained by reverse transcribing the ligated mRNA according to the manufacturer's instructions. Two steps were required to obtain the full length of pFAM134B cDNA. The first reaction of PCR was performed using a combination of sm-FAM134B-R1 (5′-GCTGTACNTGAAGATTGAAGGGATGGTTGG-3′) and 10 * UPM using the 5′ RACE cDNA template. The PCR condition was as follows: 94 °C for 2 min, 5 cycles of 94 °C for 30 s and 72 °C for 5 min, 5 cycles of 94 °C for 30 s, 70 °C for 30 s and 72 °C for 5 min, 27 cycles of 94 °C for 30 s, 68 °C for 30 s and 72 °C for 5 min. Then the product was further identified using another primer (sm-FAM134B-R2: 5′-GGGTTAATATCACCCACTGCAAGACGAGAG-3′) that is located on the downstream of sm-FAM134B-R1. The PCR condition used was: 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 68 °C for 30 s and 72 °C for 4 min, then 72 °C for 10 min. The resulting PCR product obtained from this step was isolated, cloned, and sequenced. By ligation of the overlapping sequence, full-length pFAM134BcDNA was obtained. The gene specific primer sm-FAM134B-R1 and sm-FAM134B-R2 was designed based on the pFAM134B EST (BF702245.1) available in GenBank.

2.4. qPCR

The primer sequences, melting temperatures and expected product sizes for the genes analyzed are shown in Table 1. The qPCR was performed using SYBR green I nucleic acid dye on an BIO–RAD CFX96 Real Time PCR System (BIO–RAD, Foster City, CA, USA) to quantify the target genes expression levels. Data are expressed as the ratio between expression of the target genes and that of the housekeeping gene 18s rRNA. To calculate the mRNA expression of selective genes, the ΔCt values was used for detection of their mRNA related to internal control 18s rRNA expression using the $2^{-\triangle\triangle Ct}$ method [16].

2.5. RNA interference (RNAi)

Based on our previous cloned complete sequence of pFAM134B (GenBank Accession No. JX854456), 3 potential small interference RNA (siRNA) target sites were determined using the Qiagen siRNA design program. These were confirmed with BLAST for specificity. Oligonucleotides to generate the plasmid-based siRNA were cloned into pYr-1.1-hU6-EGFP neo plasmids (Ambion), and all constructs were confirmed by sequencing. Transfection efficiency was assessed by expression of the reporter gene EGFP (green color) harbored by the plasmid and qPCR for target gene expression. The most effective target sequence (GTCACAAAGATGACAGTGA) of pFAM134B for RNAi (pFAM134B-siRNA) was identified, and the RNAi conditions were optimized. For RNA interference

Table 2
List of representative adipose metabolism-related genes differentially expressed in the SAT of Jinhua pigs and Landrace pigs at 90 d.

Gene ID	Gene name	Gene symbol	J/L Z score ($P < 0.05$)
AF052691.1	Leptin	LEP	8.03
NM_213814.1	Salivary lipocalin	SAL	3.41
NM_213938.1	3-Oxoacid CoA transferase 1	OXCT1	2.34
NM_213909.1	Glutamate-ammonia ligase	GLUL	2.89
BX667605	Phosphoglycerate mutase 2	PGAM2	2.28
NM_214246.1	Carboxylesterase	CES	2.45
NM_214351.1	Beta-1,3-N-acetylgalactosaminyltransferase 1	B3GALNT1	2.66
NM_214423.1	Cytochrome P450 3A29	CYP3A29	2.78
X93016.1	Malic enzyme 1	ME1	3.86
BX676168	Phosphoenolpyruvate carboxykinase 1 (soluble)	PCK1	−3.79
CN155220	Glucuronidase, beta	GUSB	−2.35
CN166665	Lipin1	LPIN1	−3.43
NM_214099.1	Insulin-like growth factor binding protein 5	IGFBP5	−2.53

experiments, porcine adipocytes were transfected with 3 μg/ml pFAM134B-siRNA and negative control siRNA (empty plasmid) respectively. Transfections were performed using LipofectamineTM 2000 (Invitrogen Life Technologies) according to the manufacturer's protocol. Gene expression and glycerol release were determined 48 h after transfection. Gene expression was determined by qPCR. The glycerol content in culture medium was determined using glycerol kit (Applygen Technologies Inc.). Intracellular triglyceride was assayed using triglyceride assay kit (Applygen Technologies Inc.).

2.6. RNA over expression

The EGFP fragment was collected from the pEGFP-N1 plasmid after digestion with HindIII and NotI. At the same time, the pcDNA3.1 plasmid was digested with HindIII and NotI. The EGFP was ligated to the fragment from pcDNA3.1 by using T4 DNA ligase forming pcDNA3.1-EGFP which was used for genetic transformation. The pFAM134B construct was prepared with polymerase chain reaction amplification of the pFAM134B coding sequence using the complete pFAM134B cDNA sequence in pUCm-T (Invitrogen) as a template. The primers were designed to add HindIII sites to the 5′ end and EcoRI sites to the 3′ end of the amplified product. The HindIII- and EcoRI-digested polymerase chain reaction fragment was inserted into the HindIII and EcoRI sites of the pcDNA3.1-EGFP vector. The PCR products were purified using the QIAEX II gel extraction system (Qiagen) and subcloned into pcDNA3.1-EGFP expression vector (Invitrogen) according to the manufacturer's protocol. For RNA overexpression experiments, porcine adipocytes were transfected with 3 μg/ml pcDNA3.1-pFAM134B, negative control vector (pcDNA3.1, empty plasmid).Transfections were performed using LipofectamineTM 2000 (Invitrogen Life Technologies) according to the manufacturer's protocol. Gene expression and glycerol release were determined 48 h after transfection. The glycerol content in culture medium was determined using glycerol kit (Applygen Technologies Inc. Beijing, China). Intracellular triglyceride was assayed using a triglyceride assay kit (Applygen Technologies Inc.).

2.7. Statistical analysis

All experimental data are presented as means ± SEM. Comparisons were made by unpaired, 2 tailed, Student's t tests or 1 or 2 way ANOVA, as appropriate. Significance was set at $P < 0.05$.

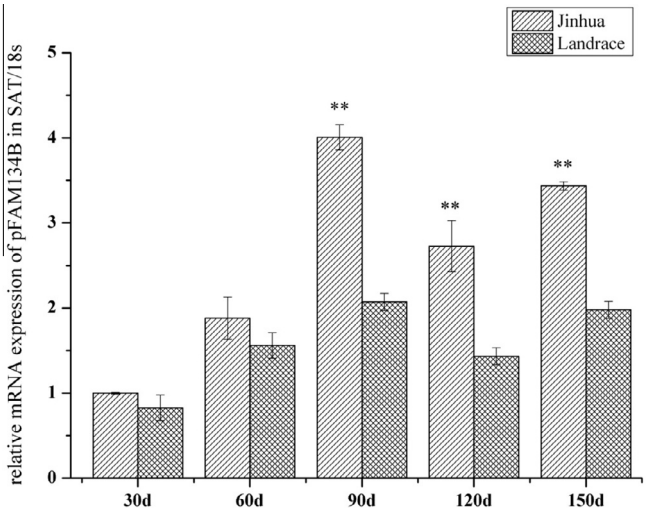

Fig. 1. Comparative analysis of pFAM134B mRNA expression in SAT between Jinhua pigs and Landrace pigs at different stages. The qPCR values are shown as expression fold changes after normalization against the control 18s rRNA. Data are presented as means ± standard error. $^{**}P < 0.01$.

3. Results and discussion

3.1. Jinhua pigs exhibited significantly increased expression of key lipogenic genes in their subcutaneous fat

Here we analyzed the differentially expressed genes of SAT in Jinhua pigs and Landrace pigs at 90 d to elucidate the relationship between the differential gene expression patterns and the phenotypic differences. A total of 458 genes were identified with at least a 2.0-fold difference ($P < 0.05$) between the two breeds. Among these, 235 genes were up-regulated in Jinhua pigs (Jinhua-up genes) (Supplementary Table 1) and 223 genes were down-regulated (Jinhua-down genes) (Supplementary Table 2). Table 2 lists representative differentially expressed genes known to be related to adipose deposition based on the OMIM database from National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/omim/) and relevant publications describing their biological functions.

Using biological function GO analysis, we detected that the significantly more highly expressed genes in Jinhua pigs include rate-limiting enzymes in glycolysis, the TCA cycle, fatty acid activation, fatty acid synthesis and desaturation, triglyceride synthesis, and cholesterol synthesis (Table 2). These genes include stearoyl-CoA desaturase, fatty acid synthase, acyl-CoA synthetase short-chain family member 2, and 1-acylglycerol-3-phosphate O-acyltransferase 1, leptin, salivary lipocalin, 3-oxoacid CoA transferase 1, glutamate-ammonia ligase (glutamine synthetase), and phosphoglycerate mutase 2, etc. Several genes encoding lipid metabolism proteins were expressed at a higher level in Landrace pigs, including CPT1A, 11-beta hydroxysteroid dehydrogenase isoform 1, ectonucleotide pyrophosphatase/phosphodiesterase 1, adipose differentiation-related protein, pyruvate dehydrogenase kinase, isozyme 4, Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3F, ATPase, Na+/K+ transporting, beta 1 polypeptide, fumarate hydratase, and lysozyme (renal amyloidosis) as well as others. These genes are known to be involved mainly in regulating fatty acid oxidation, suggesting that the SAT of Landrace pigs has more fatty acid oxidation activity than deposition activity. Our data clearly demonstrate that there are large differences between the two different breeds in lipid metabolism at the transcriptional level. Phenotypically, Landrace pigs possess a thinner subcutaneous fat layer and lower body fat ratio compared to Jinhua pigs at the same age when fed the same diet. This phenotype is consistent with the down-regulation of lipogenic genes in the Landrace pigs.

3.2. The full length cloning of pFAM134B cDNA and study on the developmental pattern and breed differences of pFAM134B expression in SAT

Through the bioinformatics analysis, the expression of the gene tagged by EST sequence with the public No. BF702245.1 was much higher than Landrace in SAT at 90 d, which is coincident with the rules that IMF of LD and SAT is increased significantly at 90 d compared with Landrace pigs [14], we presumed that it might play some functional roles in fat deposition. Full-length cDNA sequence of the candidate gene was cloned by RACE technology according to the known EST sequence (BF702245.1), and then submitted to NCBI (GenBank Accession No. JX854456). The BLAST analysis of the deduced amino acid sequences with the other species indicated that the candidate gene sharing FAM134B 84% similarity with human and rat FAM134B gene. Therefore, the gene was named as porcine FAM134B gene, abbreviated as pFAM134B for the following experiments (Supplementary Fig. 1).

FAM134B (family with sequence similarity 134, member B) encoding a newly identified Golgi protein belongs to a family of

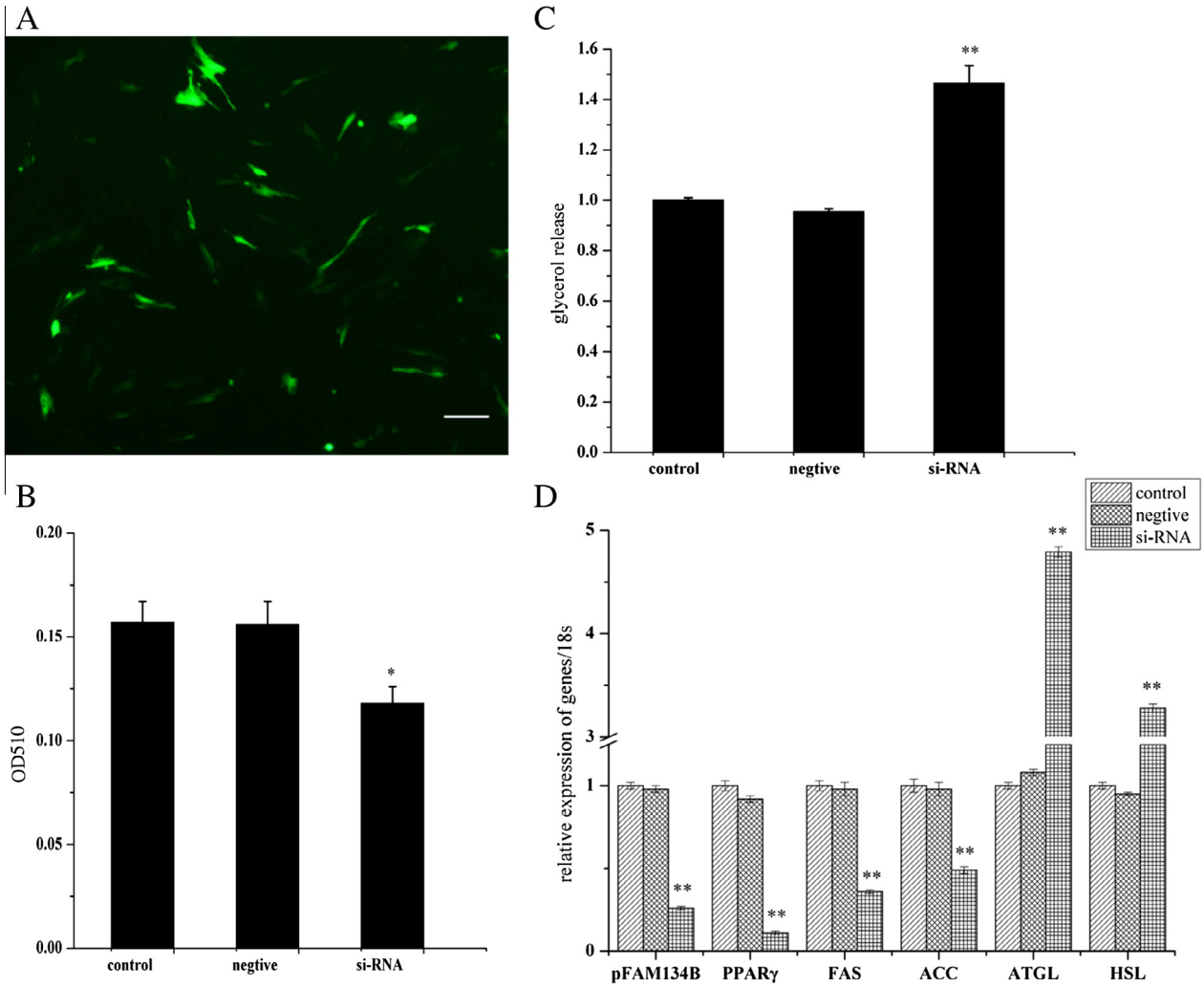

Fig. 2. Effects of pFAM134B on lipid deposition after siRNA transfection in subcutaneous adipocytes. (A) Successful transfection was assessed by expression of the reporter gene EGFP (green color) harbored by the plasmid in subcutaneous adipocytes. Scale bars: 100 μm. (B) Total triglyceride in adipocytes. (C) Relative glycerol release in the culture medium. (D) qPCR analysis of gene mRNA expression in adipocytes. siRNA: pFAM134B specific siRNA; negative: empty plasmid. The qPCR values are shown as expression fold changes after normalization against the control 18s rRNA. Data are presented as means ± standard error from three replicates. $^{*}P < 0.05$, $^{**}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

three genes, FAM134A, FAM134B and FAM134C, of unknown function. In mice, FAM134B is predominantly expressed in sensory and autonomic ganglia and is a component of the Golgi matrix [17]. Since the FAM134B protein overlaps with the cis-Golgi marker giantin and has a similar response to brefeldin A as Golgi resident matrix proteins, and the Golgi apparatus is made of stacks of flattened cisternae (from cis to trans); newly synthesised lipids and proteins enter the cis-Golgi network from the endoplasmic reticulum, are modified, and leave via the trans-Golgi network to be distributed to their final destinations, and we recently found that the pFAM134B was highly expressed in the subcutaneous fat of Jinhua pigs. Putatively, the pFAM134B may also be involved in the regulation of lipolysis. Then we further investigate the effects of pFAM134B repression and overexpression on lipid storage.

The breed difference study results showed that, in the SAT, pFAM134B gene expression in Jinhua pigs was higher than that in Landrace pigs after 90 d significantly ($P < 0.01$, Fig. 1). The genetic factors on fat deposition comprise a large number of genes with different effects. As one of these genes, pFAM134B gene mRNA expression level in SAT of Jinhua pigs was significantly higher than that of Landrace pigs. This result was highly coincident with the result of gene chips and previous studies that Jinhua pig has significantly higher subcutaneous fat, namely fat synthesis ability of Jinhua pigs is much stronger than Landrace pigs. The pFAM134B, significantly higher expressed in SAT of Jinhua pig during the key period of fat deposition, might be related to the synthesis of fat and positively regulate fat deposition. The developmental expression pattern of different growth stage study results showed that, the pFAM134B mRNA level in SAT was significantly higher at 90 d ($P < 0.01$, Fig. 1). The study of the pFAM134B expression in SAT at different growth stage including 30 d, 60 d, 90 d, 120 d and 150 d shows that the pFAM134B gene expression pattern has certain relevance with the body fat ratio. This is basically accord with the pig fat deposition law, which the body fat metabolism become more actively and deposition capacity become stronger till reaching the peak at the adult age.

3.3. Positive effects of the pFAM134B on fat deposition

After the treatment of subcutaneous adipocytes with pFAM134B-siRNA vector, there were much green fluorescent protein expressed in the adipocytes, suggested that the cells were transfected successfully (Fig. 2A). We found that the total triglyceride level in cells was significantly down-regulated ($P < 0.05$, Fig. 2B),

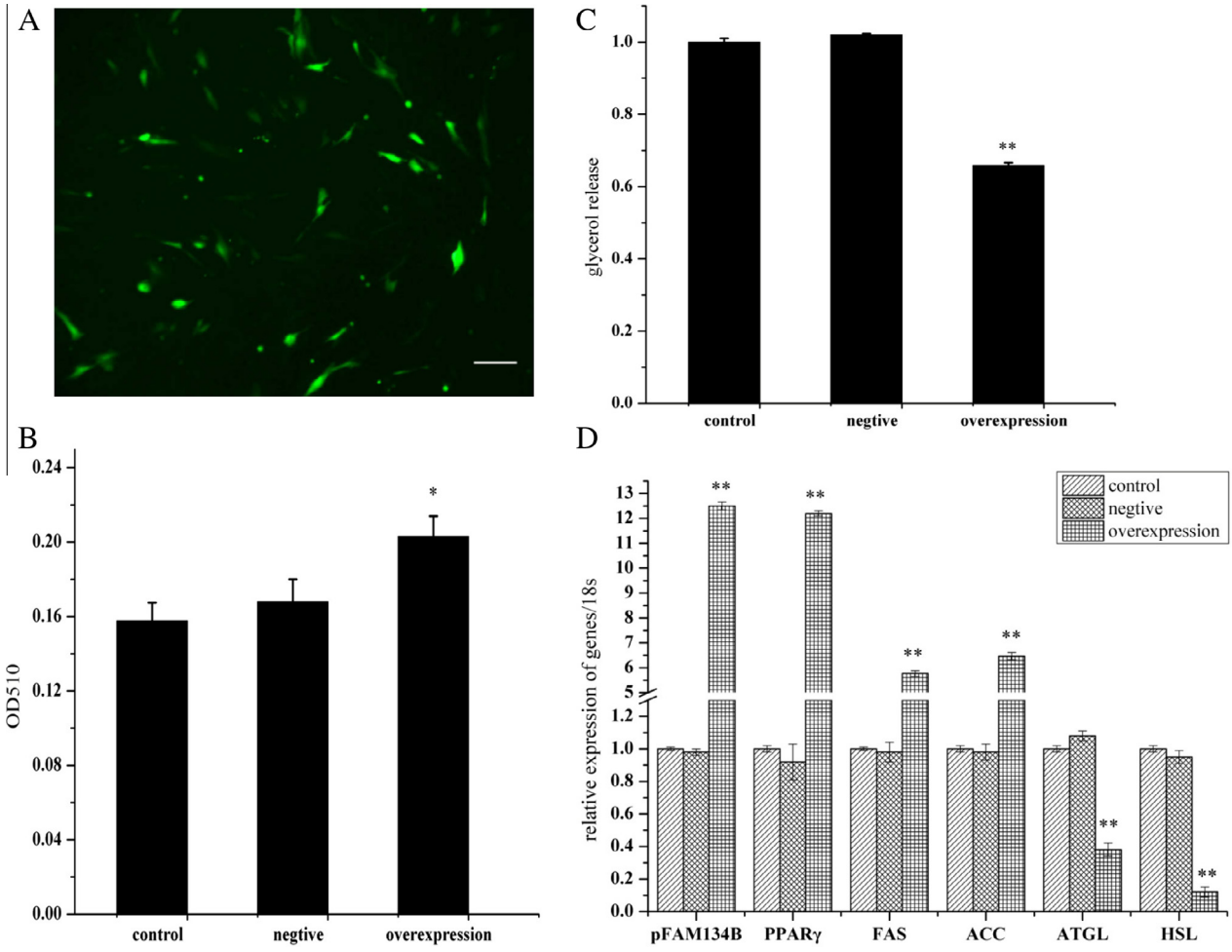

Fig. 3. Effects of pFAM134B on lipid deposition after transfection of pcDNA3.1-EGFP-pFAM134B in subcutaneous adipocytes. (A) Successful transfection was assessed by expression of the reporter gene EGFP (green color) harbored by the plasmid in subcutaneous adipocytes. Scale bars: 100 μm. (B) Total triglyceride in adipocytes. (C) Relative glycerol release in the culture medium. (D) qPCR analysis of gene mRNA expression levels in adipocytes. Overexpression: pcDNA3.1-EGFP-pFAM134B construct, negative: mock vector pcDNA3.1. The qPCR values are shown as expression fold changes after normalization against the control 18s rRNA. Data are presented as means ± standard error from three replicates. $^{*}P < 0.05$, $^{**}P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

while the glycerol release was up regulated ($P < 0.01$, Fig. 2C). Along with the decreasing pFAM134B mRNA level, the transcriptional factor PPARγ mRNA level was significantly decreased in adipocytes ($P < 0.01$, Fig. 2D). Fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) encoding two key enzymes for the synthesis of fat were significantly down-regulated ($P < 0.01$, Fig. 2D). Moreover, adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) gene being responsible for the hydrolysis of fat were significantly up-regulated ($P < 0.01$, Fig. 2D). These results suggested that the repression of pFAM134B in adipocytes led to a substantial reduction in lipid accumulation.

Meanwhile, the pFAM134B overexpression significantly increased the total triglyceride level of cells ($P < 0.05$, Fig. 3B) and the mRNA expression levels of PPARγ, FAS and ACC genes ($P < 0.01$, Fig. 3D), decreased the glycerol release in the culture medium and the mRNA expression levels of ATGL and HSL in adipocytes ($P < 0.01$, Fig. 3C and D). These results demonstrated that the pFAM134B up-regulated fat deposition in cultured adipocytes, which was consistent with the results above. Taken together, these findings underscore a critical role of pFAM134B in the subcutaneous fat deposition.

The coordinated regulation between lipogenesis and lipolysis determines fat mass. And the two opposing biochemical processes, lipogenesis and lipolysis, are controlled by different enzymes. ACC catalyzes the first step in fatty acid biosynthesis and is considered a rate-limiting enzyme for lipogenesis in pigs [18]. The fatty acid synthase (FAS) catalyses de novo lipid synthesis in the cytosol by converting acetyl-CoA to the long-chain fatty acid palmitate (C16), which is further synthesized into triacylglycerols, and stored in adipose tissue [19]. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are the major rate determining enzymes for lipolysis in adipocytes [20]. ATGL is believed to catalyze the initial step in TG hydrolysis in the adipocyte and HSL hydrolyzes diglyceride (DG) and minor amounts of TG. In this study, pFAM134B increased ACC and FAS mRNA expression, decreased ATGL and HSL mRNA expression, further reduced TG accumulation and enhanced glycerol release from porcine adipocytes, suggesting that pFAM134B plays a positive role in lipid accumulation.

Moreover, our results showed that gene expression of PPARγ was also changed by different treatment of pFAM134B in porcine adipocytes. The role of PPARγ as the master regulator of adipogenesis is supported by overwhelming evidence from both in vivo and in vitro studies. The expression of PPARγ induces the expression of adipocyte specific genes, resulting in the promotion of intracellular fat storage [21–23]. Once the activity of PPARγ is established, the adipogenic program proceeds to completion with no additional stimulation required [24,25]. Here we showed that the PPARγ

expression was markedly suppressed during adipose conversion in adipocytes transfected with pFAM134B siRNA construct. In pFAM134B's absence, the expression of PPARγ was severely impaired, suggesting its suppression by siRNA probably disrupted adipogenesis. Meanwhile, the transcript level of PPARγ was markedly increased in the pFAM134B overexpressed adipocyte. FAS, ACC, ATGL and HSL are known to be targets for transactivation by PPARγ. Taken together, these findings reveal that the mode of action of pFAM134B on lipid deposition and its related genes expression is via the regulation of PPARγ expression. Therefore, pFAM134B might play an important role in fat accumulation by promoting PPARγ expression. This study implied that pFAM134B might be a positive factor in lipid deposition, providing insight into the control of fat accumulation and lipid related disorders.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.117.

References

[1] J.D. Wood, M. Enser, A.V. Fisher, et al., Fat deposition, fatty acid composition and meat quality: a review, Meat Sci. 78 (2008) 343–358.
[2] A. Fortin, W.M. Robertson, A.K.W. Tong, The eating quality of Canadian pork and its relationship with intramuscular fat, Meat Sci. 69 (2005) 297–305.
[3] J.P. Jiang, J. Zhou, J. Chen, et al., Effect of chicken egg yolk antibody against adipose tissue plasma membranes on carcass composition and lipogenic hormones and enzymes in pigs, Livest. Sci. 107 (2007) 235–243.
[4] L. Bouraoui, J. Gutierrez, I. Navarro, Regulation of proliferation and differentiation of adipocyte precursor cells in rainbow trout (*Oncorhynchus mykiss*), J. Endocrinol. 198 (2008) 459–469.
[5] M.Z. Li, X.W. Li, L. Zhu, Differential expression analysis and regulatory network reconstruction for genes associated with muscle growth and adipose deposition in obese and lean pigs, Prog. Nat. Sci. 18 (2008) 387–399.
[6] H.K. Kinyamu, R.C. Ewan, Energy and protein metabolism of the Chinese pig, J. Anim. Sci. 72 (1994) 2068–2074.
[7] J.T. Yen, J.A. Nienaber, J. Klindt, J.D. Crouse, Effect of ractopamine on growth, carcass traits, and fasting heat production of US contemporary crossbred and Chinese Meishan pure- and crossbred pigs, J. Anim. Sci. 69 (1991) 4810–4817.
[8] L.D. Young, Comparison of Meishan, Fengjing, Minzhu and Duroc swine: effects on postweaning growth, feed efficiency, and carcass traits, J. Anim. Sci. 70 (1992) 2020–2029.
[9] Z.G. Miao, L.J. Wang, Z.R. Xu, et al., Developmental changes of carcass composition, meat quality and organs in the Jinhua pig and Landrace, Animal 3 (2009) 468–473.
[10] J. Guo, T.Z. Shan, T. Wu, et al., Comparisons of different muscle metabolic enzymes and muscle fiber types in Jinhua and Landrace pigs, J. Anim. Sci. 89 (2011) 185–191.
[11] F.W. Dai, D.Y. Feng, Q.Y. Cao, et al., Developmental differences in carcass, meat quality and muscle fibre characteristics between the Landrace and a Chinese native pig, S. Afr. J. Anim. Sci. 39 (2009) 267–273.
[12] N.D. Cameron, P.D. Warriss, S.J. Porter, M.B. Enser, Comparison of Duroc and British Landrace pigs for meat and eating quality, Meat Sci. 27 (1990) 227–247.
[13] J.D. Wood, A.J. Kempster, P.J. David, M. Bovey, Observations on carcass and meat quality in Duroc, Landrace and Duroc × Landrace pigs, British Society of Animal Production Winter Meeting, in: Scarborough Programme and summaries (Paper 98), (1987), pp. 2.
[14] T. Wu, Z.H. Zhang, Z.Q. Yuan, et al., Distinctive genes determine different intramuscular fat and muscle fiber ratios of the longissimus dorsi muscles in Jinhua and Landrace pigs, PLoS One 8 (1) (2013) e53181, http://dx.doi.org/10.1371/journal.pone.0053181.
[15] T.Z. Shan, Y.Z. Wang, Y.J. Liu, et al., Developmental expression of the lipoprotein lipase gene in porcine subcutaneous adipose tissue, J. Anim. Feed Sci. 15 (2006) 621–629.
[16] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method, Method 25 (2001) 402–408.
[17] M. Shekarabi, N. Girard, J.B. Rivière, et al., Mutations in the nervous system – specific HSN2 exon of WNK1 cause hereditary sensory neuropathy type II, J. Clin. Invest. 118 (2008) 2496–2505.
[18] R.A. Scott, S.G. Cornelius, H.J. Mersmann, Effects of age on lipogenesis and lipolysis in lean and obese swine, J. Anim. Sci. 52 (1981) 505–511.
[19] M.J. Griffin, H.S. Sul, Insulin regulation of fatty acid synthase gene transcription: roles of usf and srebp-1c, IUBMB Life 56 (10) (2004) 595–600.
[20] M. Schweiger, R. Schreiber, G. Haemmerle, et al., Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism, J. Biol. Chem. 281 (2006) 40236–40241.
[21] P. Tontonoz, E. Hu, B.M. Spiegelman, Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor, Cell 79 (1994) 1147–1156.
[22] Z. Wu, E.D. Rosen, R. Brun, et al., Cross-regulation of C/EBPα and PPARγ controls the transcriptional pathway of adipogenesis and insulin sensitivity, Mol. Cell. 3 (1999) 151–158.
[23] S.O. Freytag, D.L. Paielli, J.D. Gilbert, Ectopic expression of the CCAAT/enhancer-binding protein α promotes the adipogenic program in a variety of mouse fibroblastic cells, Genes Dev. 8 (1994) 1654–1663.
[24] E.D. Rosen, P. Sarraf, A.E. Troy, et al., PPARγ is required for the differentiation of adipose tissue in vivo and in vitro, Mol. Cell. 4 (1999) 611–617.
[25] N. Arimura, T. Horiba, M. Imagawa, et al., The peroxisome proliferator-activated receptor γ regulates expression of the perilipin gene in adipocytes, J. Biol. Chem. 279 (2004) 10070–10076.