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

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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.

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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