

## Different Transcription Profiles of *SOCS-3*, *ob* and *IGF-I* Genes and their Possible Correlations in Obese and Lean Pigs

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**Abstract** Pig breeds have significant differences in fat deposition and muscle development ability. However, the molecular mechanism behind these differences is still unknown. In this study, the expression patterns of three candidate genes, suppressor of cytokine signaling 3 (*SOCS-3*), obesity (*ob*) and insulin-like growth factor I (*IGF-I*), which are involved in adipose metabolism or muscle development, were analyzed. Total RNA was extracted from dorsal subcutaneous adipose tissue and longissimus of 8-month-old Bamei and Largewhite pigs. Semiquantitative reverse transcription-polymerase chain reaction was used to determine the expression levels of the *SOCS-3* and *ob* genes in adipose tissue, and *SOCS-3* and *IGF-I* genes in muscle tissue. The results showed that in adipose tissue the expression level of *SOCS-3* was significantly higher in Bamei (obese) pigs than that in Largewhite (lean) pigs ( $P < 0.01$ ). However, in muscle tissue it was significantly lower in Bamei than that in Largewhite pigs ( $P < 0.01$ ). Furthermore, the expression of *SOCS-3* was positively correlated to that of *ob* in adipose tissue and that of *IGF-I* in muscle tissue. These findings suggest that the difference in *SOCS-3* gene expression levels in adipose and muscle tissues, the relationship between *SOCS-3* and *ob* in adipose tissue, and that between *SOCS-3* and *IGF-I* in muscle tissue, might contribute to the different fat deposition and muscle development ability between obese and lean pigs.

**Key words** *SOCS-3*; *ob*; *IGF-I*; fat deposition; muscle development; obese pigs; lean pigs

Different pig breeds show diverse fat accumulation and muscle development ability. The Bamei pig, a domesticated eutherian mammal line, has evolved over 3000 years in China. Its distinct feature is its high body fat content. The finishing Bamei pigs have an average back-fat thickness of approximately 8 cm, and have 20–25 kg attar and suet, accounting for approximately 25% of its body weight [1]; the total fat content is more than 50%. In contrast, the Largewhite pig has a relatively low fat content of approximately 36%. Recently, molecular biology technology has revealed that some available genes correlated with fat deposition and muscle development, in particular, the obesity (*ob*) and insulin-like growth factor I (*IGF-I*) genes. Leptin, the product of the *ob* gene, is secreted by adipocytes, and is known as a regulator of food intake and

energy expenditure [2,3]. *IGF-I* is considered one of the indispensable regulators of skeletal muscle development [4]. However, the mechanism of the two genes that regulates fat deposition and muscle development remains to be clarified.

A recently described family of proteins, the suppressor of cytokine signaling (SOCS) family, acts in a negative feedback loop to regulate cytokine or growth factor signaling [5]. *SOCS-3* is one of its members. A number of studies have shown that leptin can induce the expression of *SOCS-3*, and the inducible *SOCS-3* can inversely suppress leptin signaling [6]. An increase in *SOCS-3* expression was considered a sign of leptin resistance, which was closely correlated to obesity-related diseases in human [7]. Although the mechanism responsible for the development of adipose tissue leptin resistance is unknown, a role for increased suppressor of *SOCS-3* inhibition of the leptin receptor has been shown in rodent [8,9]. Furthermore, it has not been investigated whether *SOCS-3* is attributed to

Received: October 24, 2006 Accepted: January 14, 2007

This work was supported by the grants from the National Basic Research Program of China (No. 2004CB117506) and the National Natural Science Foundation of China (No. 30471267)

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DOI: 10.1111/j.1745-7270.2007.00274.x

the development of adipose tissue. SOCS-3 can also negatively regulate insulin signaling [10]. The receptors for insulin and IGF-I share considerable structural homology, and both insulin and IGF-I act on some of the same intracellular signaling pathways [11].

SOCS-3, *ob* and IGF-I are attractive targets for explaining the genetic differences in different pig breeds. Therefore, the objective of this research is to analyze the differential expression of *SOCS-3*, *ob* and *IGF-I* genes in obese and lean pigs.

## Materials and Methods

### Animals

All procedures involving animals were licensed under the Animals Scientific Procedures Act of 1986. Nine Bamei pigs were obtained from the conservation factory of the Bamei breed in Qinghai, China. Nine Largewhite pigs were from the boar factory attached to the Northwest A&F University (Yangling, China). Pigs were fed to an age of 8 months. Food and water were available *ad libitum* with all other environmental conditions unaltered. All samples were rapidly collected by operation under anesthesia, and were frozen in liquid nitrogen.

### Total RNA extraction and reverse transcription

Total RNA was extracted from dorsal subcutaneous adipose tissue and longissimus of 8-month-old Bamei and Largewhite breeds according to the TriPure reagent kit protocol (BioTek, Beijing, China). One microgram of total RNA was reverse transcribed using the Fermentas RevertAid first-strand cDNA synthesis kit (Fermentas, Burlington, Canada) according to the manufacturer's protocol. Briefly, the RNA was incubated with reverse transcriptase (Fermentas), random hexamer primer, and dNTP mix (Hualuyuan Biotechnology, Guangzhou, China) in a 20  $\mu$ l reaction system at 42 °C for 60 min. The reaction was inactivated by incubation at 70 °C for 10 min. The samples were subsequently stored at -20 °C for later use.

### Semiquantitative PCR

Semiquantitative polymerase chain reaction (SQ PCR) was used with  $\beta$ -actin as a standard for each reaction. The following primer sequences were used: *SOCS-3* forward (F), 5'-GTGCGCCATGGTCACCCAC-3'; *SOCS-3* reverse (R), 5'-GTCCAGGAAGTCCCGAAT-3'; *ob* F, 5'-CAAGACGATTGTCACCAGG-3'; *ob* R, 5'-CAGGGTCTGGTCCATCTTG-3'; *IGF-I* F, 5'-GGAGCTGTGATCTG-

AGGA-3'; *IGF-IR*, 5'-ACAGTAACCTCGTGCAGA-3';  $\beta$ -actin F, 5'-ACTGCCGCATCCTCTTCCTC-3'; and  $\beta$ -actin R, 5'-CTCCTGCTTGCTGATCCACATC-3'. All primers were purchased from Shanghai Sangon Biotechnology (Shanghai, China). The *SOCS-3* primers produced a product with a size of 644 bp and were designed based on GenBank accession number NM053565. The *IGF-I* primers produced a product size of 222 bp and were designed based on GenBank accession number AB003362. The *ob* primers produced a product size of 133 bp and were designed based on GenBank accession number SSU59894. The  $\beta$ -actin primers produced a product with a size of 399 bp and were designed based on GenBank accession number BC063166.

One microliter of each reverse transcription reaction product was mixed with 18.05  $\mu$ l sterile deionized water, 2.5  $\mu$ l 10 $\times$ PCR buffer, 1.0  $\mu$ l of 10 mM dNTP mix, 0.5  $\mu$ l of 0.5  $\mu$ M primers (sense and antisense), 0.6 U of 500 U *Taq* DNA polymerase and 1.25  $\mu$ l of 1.25 mM MgCl<sub>2</sub> in a final volume of 25  $\mu$ l. For all PCR reactions  $\beta$ -actin amplifications were carried out in parallel under identical conditions. All of the above reagents were purchased from Fermentas. *SOCS-3* amplifications were carried out in a PTC-200 DNA engine (MJ Research, Massachusetts, USA) with an initial denaturing step of 95 °C for 10 min, followed by 28 cycles of 50 s at 95 °C, 1 min at 62.8 °C, and 1 min at 72 °C with cDNA reversed from adipose and muscle tissue. The final cycle ended with 10 min at 72 °C. For *IGF-I* amplification, an initial denaturing step was carried out at 94 °C for 5 min, followed by 28 cycles of 50 s at 95 °C, 1 min at 56 °C, and 1 min at 72 °C with cDNA reversed from muscle tissue. The final cycle ended with 10 min at 72 °C. For *ob* and  $\beta$ -actin amplifications, an initial denaturing step was carried out at 95 °C for 7 min, followed by 28 cycles of 1 min at 94 °C, 1 min at 55.6 °C, and 1 min at 72 °C with cDNA reversed from both adipose tissue and muscle tissue for  $\beta$ -actin amplification, and with adipose tissue for *ob* amplification. The final cycle ended with 10 min at 72 °C. The PCR products were then separated on a 1% agarose gel and stained with ethidium bromide. The gels were scanned, and signal quantification was carried out with Dolphin-DOC software (version 1.3.2, Taiwan, China). The signal determined for each target was subsequently normalized to the signal for the  $\beta$ -actin target.

### Statistical analysis

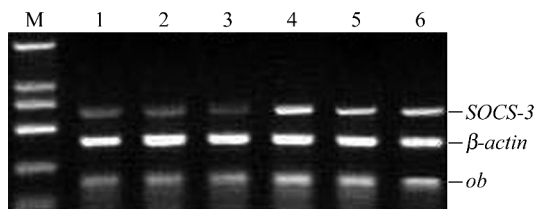
All of the data are expressed as mean $\pm$ SD. Statistical significance was determined using Student's *t* test. Correlations were analyzed using Spearman's and Pearson's

correlation tests followed by a two-tailed Student's *t*-test of significance. *P* values less than 0.01 were considered to be significantly different.

## Results

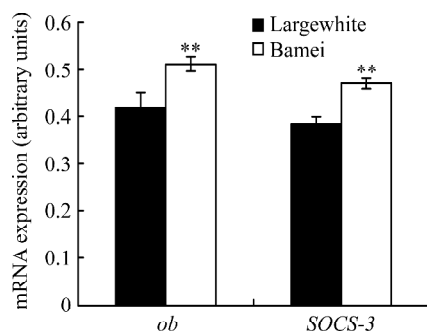
### Expression of *SOCS-3* and *ob* genes in adipose tissue

To determine whether *SOCS-3* is involved in the regulation of fat deposition, total RNA was isolated from dorsal subcutaneous adipose tissue of 8-month-old Bamei and Largewhite pigs. SQ reverse transcription (RT)-PCR analysis showed that the expression of the *SOCS-3* gene was significantly higher in Bamei pigs than in Largewhite pigs ( $P < 0.01$ ) (Figs 1, 2). It was suggested that the fat deposition was regulated by the *ob* gene, so a similar analysis was carried out for that gene. Like *SOCS-3*, the



**Fig. 1** Electrophoresis results of *SOCS-3* and *ob* mRNA in the adipose tissue of Bamei and Largewhite pigs

Total RNA was isolated from dorsal subcutaneous adipose tissue of Bamei and Largewhite pigs. Semiquantitative reverse transcription-polymerase chain reaction was carried out to detect *SOCS-3*, *ob* or  $\beta$ -actin transcripts. These results were obtained from three independent experiments. M, marker DL2000; 1–3, amplification results of Largewhite pigs; 4–6, amplification results of Bamei pigs.



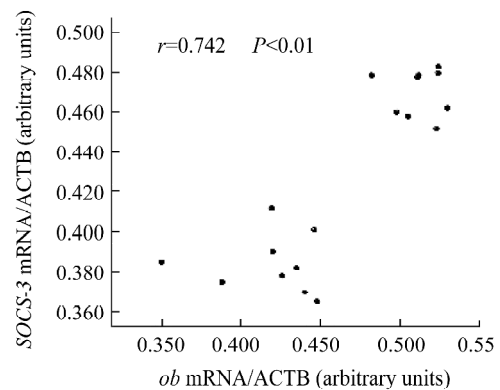
**Fig. 2** Quantification of signal from Fig. 1 and its significance test

The signal derived from Fig. 1 was normalized to the signal from the  $\beta$ -actin target, and subjected to Student's *t* test. These results were obtained from three independent experiments. \*\*A value significantly different from the Largewhite pigs ( $P < 0.01$ ).

expression of *ob* was significantly higher in Bamei pigs than in Largewhite pigs ( $P < 0.01$ ) (Figs 1, 2).

### Correlation between the expression of *SOCS-3* and *ob*

In order to further investigate the relationship of *SOCS-3* and *ob* in adipose tissue, data derived from Figs. 1 and 2 were used and subjected to Spearman's and Pearson's correlation tests. The results (Fig. 3) show that the expression of *SOCS-3* was positively correlated with that of *ob* ( $r = 0.742$ ,  $P < 0.01$ ) in adipose tissue. These findings suggest that *SOCS-3* participates in regulating the fat deposition in pigs.



**Fig. 3** Correlation between the expression of the *SOCS-3* and *ob* genes in Bamei and Largewhite pigs

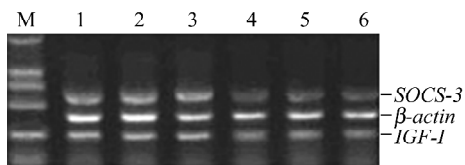
Data from Fig. 2 was derived and subjected to bivariate correlation analysis. These results were obtained from three independent experiments. *r*, coefficient of correlation.

### Expression of *SOCS-3* and *IGF-I* in muscle tissue

To examine the role of *SOCS-3* in the regulation of muscle development, total RNA was isolated from longissimus tissue of 8-month-old Bamei and Largewhite pigs. SQ RT-PCR analysis showed that, unlike in adipose tissue, the expression of *SOCS-3* mRNA in longissimus was significantly higher in Largewhite pigs than in Bamei pigs ( $P < 0.01$ ) (Figs. 4, 5). Similarly, the expression of the *IGF-I* gene in longissimus was significantly higher in Largewhite pigs than in Bamei pigs ( $P < 0.01$ ) (Figs. 4, 5), supporting the previous findings that muscle development was regulated by the *IGF-I* gene. IGF-I is a positive regulator of the development. It can affect and induce the expression of many factors. *SOCS-3* is one of them.

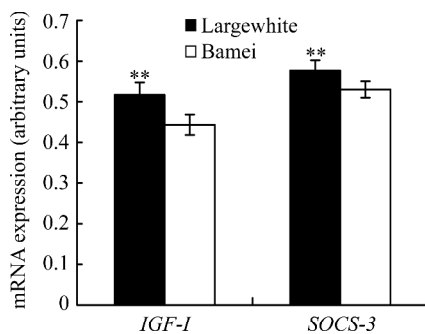
### Correlation between the expression of *SOCS-3* and *IGF-I*

In order to further investigate the relationship between



**Fig. 4** Electrophoresis results of *SOCS-3* and *IGF-I* mRNA in the skeletal muscle of Bamei and Largewhite pigs

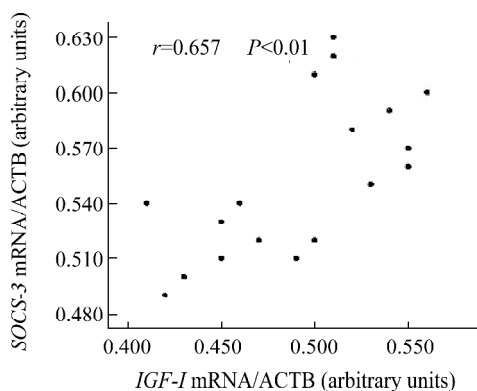
Total RNA was isolated from longissimus of Bamei and Largewhite pigs. Reverse transcription-polymerase chain reaction was carried out to detect *SOCS-3*, *IGF-I* or  $\beta$ -actin transcripts. These results were obtained from three independent experiments. M, marker DL2000; 1–3, amplification results of Largewhite pigs; 4–6, amplification results of Bamei pigs.



**Fig. 5** Quantification of signal from Fig. 4 and its significance test

The signal derived from Fig. 4 was normalized to the signal from the  $\beta$ -actin target, and subjected to Student's *t* test. These results were obtained from three independent experiments. \*\* A value significantly different from the Bamei pigs ( $P < 0.01$ ).

*SOCS-3* and *IGF-I* in skeletal muscle, data derived from Figs. 4 and 5 were used and subjected to Spearman's and Pearson's correlation test. The results (Fig. 6) indicate



**Fig. 6** Correlation between the expression of *SOCS-3* and *IGF-I* mRNA in Bamei and Largewhite pigs

Data from Fig. 5 was derived and subjected to bivariate correlation analysis. These results were obtained from three independent experiments. *r*, coefficient of correlation.

that the expression of *SOCS-3* was positively correlated with that of *IGF-I* ( $r=0.657$ ,  $P < 0.01$ ) in skeletal muscle. These findings suggest that *SOCS-3* participated in regulating the development of skeletal muscle in pigs.

## Discussion

There are several novel findings in this study of fat deposition and muscle development by investigating the expression of *SOCS-3* mRNA and its potential role in pigs. Our data indicate that high levels of *SOCS-3* mRNA expression in obese pig adipose tissue were related to the more dorsal subcutaneous adipose tissue. Increased *SOCS-3* mRNA expression might enhance skeletal muscle growth. An increase in *SOCS-3* mRNA appears to positively mediate IGF-I signaling. These data suggest for the first time a potential molecular mechanism mediated by *SOCS-3* mRNA expression, which results in increased fat deposition and skeletal muscle development in pigs. This study provides further information on the mechanisms of obesity in both pigs and humans.

Leptin is the product of the *ob* gene. It can alleviate obesity through affecting the animal's appetite and energy expenditure [3]. As leptin has the function of reducing fat deposition, it has been used to cure human obesity. However, it was found that recombinant leptin therapy failed to overcome obesity. In fact, only approximately 5% of obese people lack leptin. On the contrary, obesity is always associated with leptin resistance as evidenced by hyper-leptinemia [12].

One of the reasons for leptin resistance is an increase in the suppressor of leptin signaling [9]. *SOCS-3* is one of the negative regulators of leptin signaling, so it is the candidate for causing leptin resistance [9]. Our experiment indicates higher levels of *SOCS-3* in adipose tissue of obese pigs, which is a positive correlation with the expression of *ob*. Thus, we thought that high levels of *SOCS-3* mRNA expression in adipose tissue of obese pigs results in leptin resistance, which causes the excessive fat deposition in subcutaneous adipose tissue.

It has been recognized that IGF-I is important for the prenatal and postnatal development of skeletal muscle [13]. *In vitro* myogenesis studies have shown that IGF-I regulates myoblast proliferation and differentiation [14]. Certainly, strong evidence exists for the role of IGF-I in muscle development. Early studies showed that IGF-I stimulates myoblast proliferation and differentiation in a concentration-dependent manner [15]. There is evidence that locally produced IGF-I might be important in the

muscle regeneration process. IGF-I can promote skeletal muscle hypertrophy or regeneration under certain conditions [4]. However, the mechanism of its regulation in muscle development remains to be defined.

Recently, SOCS-3 has been implicated as having the ability to regulate insulin signaling in cultured adipocytes [16]. Because insulin and IGF-I share many signaling properties, it is possible that SOCS-3 could regulate IGF-I signaling as well. In human embryonic kidney 293 cells, Dey *et al.* [17] indeed found that SOCS-3 interacts constitutively with the IGF-I receptor *in vitro* in intact cells. Because the activation of the IGF-I receptor is critical for the induction of myoblast differentiation [18], it is possible that SOCS-3 is critical for myoblast differentiation. IGF-I is necessary for differentiation [18], and myoblast production of IGF-I increases during the differentiation process [19]. Therefore IGF-I could induce SOCS-3 expression.

In this study, it is shown that *SOCS-3* mRNA was positively detected in skeletal muscle, and the expression level of *SOCS-3* was significantly higher in Largewhite pigs than in Bamei pigs. Further correlation analysis indicated that the expression of *SOCS-3* mRNA was positively correlated with that of *IGF-I*. As we know, the muscle mass of Largewhite pigs is significantly higher than that of Bamei pigs. IGF-I positively regulates the development. Therefore, we could deduce that IGF-I induces the expression of *SOCS-3* mRNA, and the inducible *SOCS-3* promotes muscle development through positively regulating IGF-I signaling. This finding is consistent with the results obtained by Spangenburg [20]. This might be one of the reasons for the higher skeletal muscle content in lean pig breeds.

Although the original descriptions indicated that the SOCS family acted in an inhibitory fashion, SOCS proteins do not always act in a negative way. Recent evidence, together with this study, suggests that under certain conditions the SOCS family of proteins could also induce various biological mechanisms [21,22].

In conclusion, these data show for the first time that increased *SOCS-3* mRNA expression in both adipose and skeletal muscle tissue is related to excessive fat deposition and more muscle growth. Future studies should examine changes in protein content of SOCS-3 in adipose tissue and skeletal muscle and establish whether regulating the expression of SOCS-3 is effective in overcoming the shortage of Chinese domestic pig breeds. It is promising that SOCS-3 will be the new obesity therapy target. Clearly, the regulation mechanism of SOCS-3 in fat deposition and skeletal muscle development is still elusive and requires further investigation.

## Acknowledgments

We thank Dr. Bin WU and Wansheng LIU for their critical reading of the manuscript.

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Edited by  
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