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Castration-induced changes in microRNA expression profiles in subcutaneous adipose tissue of male pigs

Zhaowei Cai · Lifan Zhang · Minli Chen · Xiaoling Jiang · Ningying Xu

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Abstract MicroRNAs (miRNAs) are class of molecular regulators found to participate in numerous biological processes, such as adipogenesis and obesity in mammals. To determine the roles of miRNAs involved in castration-induced body fatness, we investigated the different miRNA expression patterns in subcutaneous adipose tissue between intact and castrated male pigs. Our results showed that castration led to decrease serum testosterone but increase serum Leptin levels ($P< 0.01$). Moreover, castration also increased adipocyte size, body fat content and backfat thickness in male pigs ($P< 0.01$). Meanwhile, miRNA expression profiles in adipose tissue were changed by castration, and 18 miRNAs were considered as the differentially expressed candidates between intact and castrated male pigs. Furthermore, functional analysis indicated that the differential expressed miRNAs and their target genes are involved in the regulation of fatty acid metabolism. In brief, our present study provides a comprehensive view on how miRNAs works in subcutaneous adipose tissue with castration. These results suggested that miRNAs might play an important role in the castration-induced fat deposition in male pigs.

Keywords Adipose tissue · Microarray · MicroRNA · Pig · Testosterone

Introduction

The metabolism of adipose tissue is known to be affected by gonadal steroid hormones such as testosterone (Andersen et al. 2010; B langer et al. 2006; Varlamov et al. 2012). For example, testosterone deficiency caused by castration increases the quantity of adipose tissue in male rat (Li and Bjorntorp 1995) and castration can also increase body fatness in male pigs (Christoffersen et al. 2010). On the other hand, obesity has usually been associated with reduced plasma testosterone levels, which play an important role in modulating of fat accumulation in both humans and animals, but unfortunately, the possible molecular mechanisms underlying this action are still not clear.

MicroRNAs (miRNAs) are endogenous small RNAs that regulate gene expression at the post-transcriptional level and have been shown to have important roles in numerous disease or physiological metabolism processes (Lhakhang and Chaudhry 2012). ManyinsTJ0-1.24619996TD(ad)15.10000038(i)13.8999

· M. Chen
Laboratory Animal Research Center,
Zhejiang Chinese Medical University, Hangzhou 310053, China

Z. Cai · X. Jiang · N. Xu (✉)
College of Animal Science, Zhejiang University,
No. 388 Yuhangtang Road, Hangzhou 310058, China
e-mail: nyxu@zju.edu.cn

L. Zhang
College of Animal Science and Technology,
Nanjing Agricultural University, Nanjing 210095, China

adipose tissue of genetically obese mice compared with lean mice (Lin et al. 2009). Another one, miR-130 also inhibits adipogenesis by targeting PPARγ mRNA, and it has been observed that adipose tissue from obese women had lower expression of miR-130 with higher expression of PPARγ mRNA than non-obese women (Lee et al. 2011).

But up to now, only a few studies have been conducted to investigate the role of miRNAs on regulation of fat deposition in domestic pigs, and most of them focused on identification of novel miRNAs in porcine adipose tissue (Li et al. 2011b;

Hybridization images were collected using a GenePix 4000B laser scanner (Molecular Devices, Sunnyvale, CA) and digitized using Array-Pro image analysis software (Media Cybernetics, Sliver, Spring, MD).

Data were analyzed by first subtracting the background and then normalizing the signals using a LOWESS filter (Locally-weighted Regression) (Bolstad et al. 2003). The ratio of the two sets of detected signals (Log2 transformed, balanced) and P-values of the *t*-test were calculated. Differentially expressed miRNAs with $P<0.01$ were selected for further analysis.

Stem-loop real-time RT- PCR

A miRNA quantification method described by Li et al. (2011a) was used, with some modification. For miRNA quantification, each RT reaction consisted of 1 μg of total RNA mixed with 2.0 μL of 5×RT buffer which included dNTPs (Takara), 1.0 μL of 10 μM stem-loop RT primer (Table 1), 0.5 μL RNase inhibitor (Takara), and 0.5 μL reverse transcriptase (Takara) in a final volume of 10 μL. The admixture was incubated at 42 °C for 60 min, 70 °C for 15 min, and then held at 4 °C. Real-time PCR was performed using SYBR Green Master Mix (Takara) on the StepOne™ Software v2.0 (Applied Biosystems) according to the manufacturer's instructions. The PCR volume was 20 μL, containing 1 μL diluted RT product, with the following cycling conditions: 95 °C for 30 s, followed by 40 cycles for 95 °C for 5 s and 60 °C for 30 s. Porcine miR-24 was used as an internal control and all PCR reactions were run in triplicate. The comparative $2^{-\Delta\Delta}$Ct method (Livak and Schmittgen 2001) was employed to determine the expression level differences.

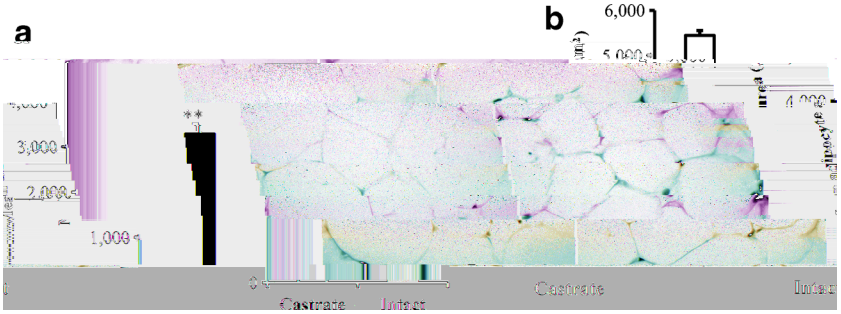

Fig. 1 Histological sections of subcutaneous adipose tissue from intact and castrated male pigs. **a** H&E staining of subcutaneous adipose tissue from intact and castrated male pigs (shown at×400 magnification); **b** The mean adipocyte size in intact and castrated male pigs. Adipocyte area was measured using Image J software from three different animals per group (60 fat cells for each individual). $^{**}P<0.01$

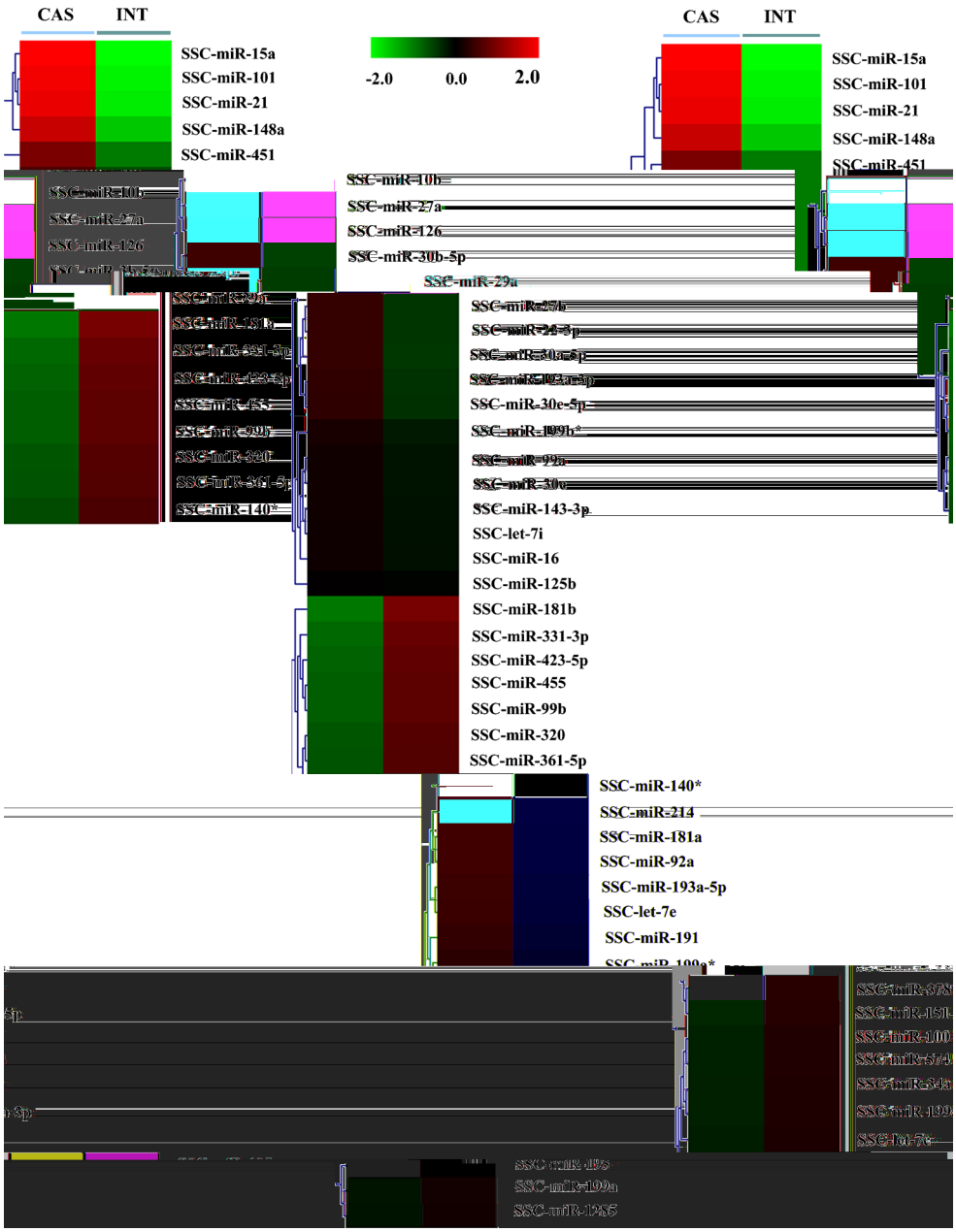

Fig. 2 Hierarchical cluster heat map for miRNAs expressed in subcutaneous adipose tissue from intact and castrated male pigs. **a** miRNAs with a signal value >500 and a *p*-value <0.01 (high signal group miRNAs); **b** miRNAs whose expression level showed a fold change ≥2 (differentially expressed miRNAs). "CAS" means castrated male pigs; "INT" means intact male pigs. The relative transcript abundance of each miRNA is color coded. The *red* or *green* color indicates relatively high or low expression, respectively

Results

Castration induces changes in serum hormones and body fatness

The effect of castration on serum hormones and body fatness traits are shown in Table 2. Castration can significantly reduce serum testosterone concentrations (*P*

activity and lipid metabolism process while the specific GO of the target genes related to the down-regulated miRNAs were involved in protein binding, Golgi apparatus, anti-apoptosis and cell development (Fig. 4 and Supplementary Table S1). To further investigate the role of miRNAs in castration-induced fat deposition, a miRNA-mRNA regulatory network was subsequently established for the lipid metabolism process (Fig. 4c). Interestingly, miR-27a, miR-15a, miR-101, miR-30b-5p, miR-148a, miR-126 and miR-21 interacted with their target genes and were involved in lipid/fatty acid metabolism, suggesting that these miRNAs and their candidate targets might play important roles in castration-induced adiposity in male pigs.

Discussion

Castrated and intact male pigs demonstrate striking difference in body fatness (Mersmann 1984; Christoffersen et al. 2010). However, the molecular mechanisms that cause such differences are still not clear. In the past few years, many researchers have shown that miRNAs are involved in adipose tissue

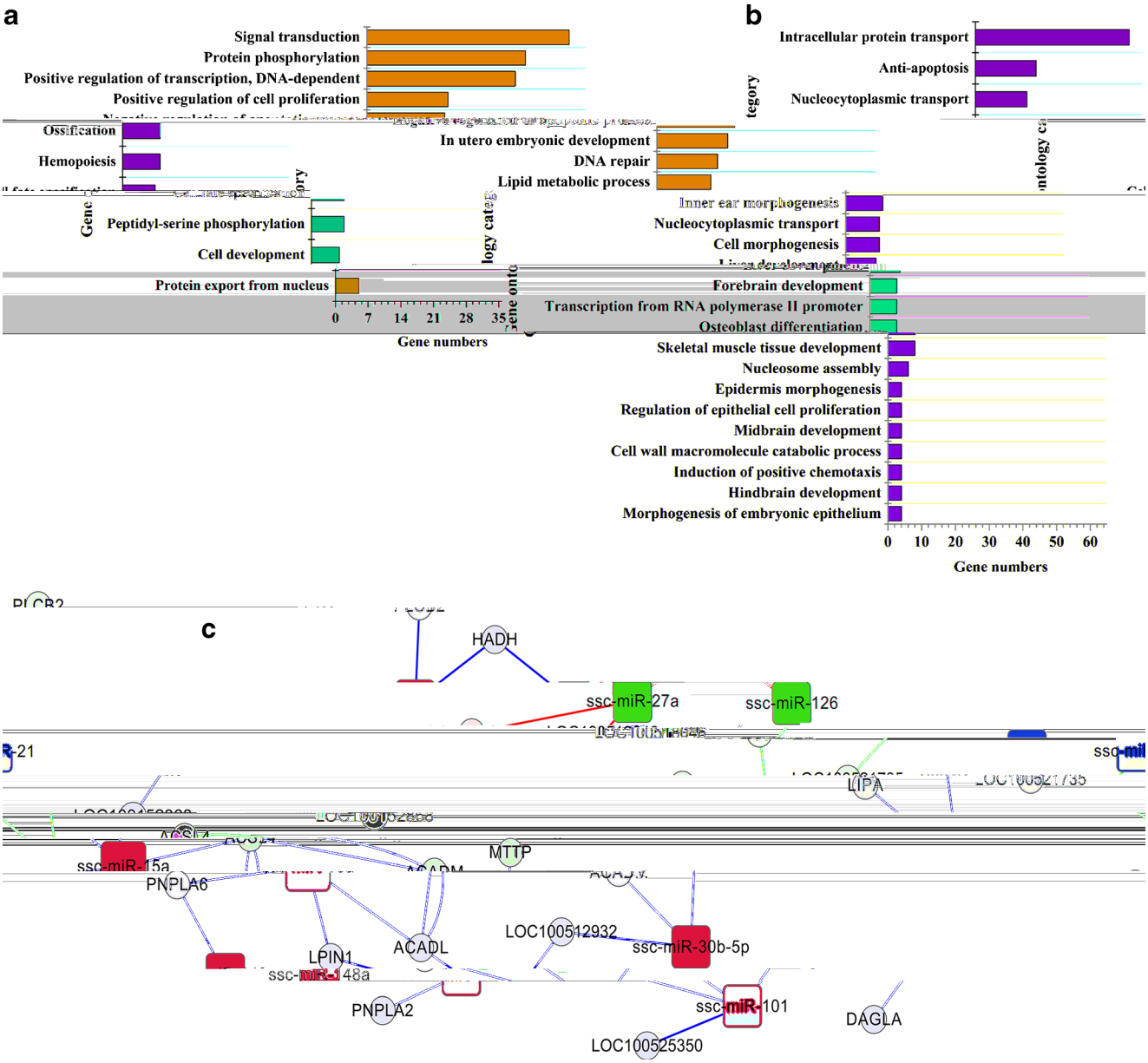

Fig. 4 Gene ontology (GO) analysis of genes targeted by differentially expressed miRNAs. **a** GOs targeted by up-expressed miRNAs. **b** GOs targeted by down-expressed miRNAs. The *vertical axis* is the GO category, and the

development and obesity (Takanabe et al. 2008; Xie et al. 2009; Gerin et al. 2010). Therefore, we used a miRNA mi-

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