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Differential expression analysis and regulatory network reconstruction for genes associated with muscle growth and adipose deposition in obese and lean pigs

Mingzhou Li [a], Xuewei Li [a,*], Li Zhu [a], Xiaokun Teng [b], Huasheng Xiao [b], Surong Shuai [a], Lei Chen [a], Qiang Li [a], Yujiao Guo [a]

[a] *Laboratory of Pork Quality, College of Animal Science and Technology, Sichuan Agricultural University, Ya'an 625014, China*
[b] *National Engineering Center for Biochip at Shanghai, Shanghai 201203, China*

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Abstract

During the growth and development of skeletal muscle cells and adipose cells, the regulatory mechanism of micro-effect polygenes determines porcine meat quality, carcass characteristics and other relative quantitative traits. Obese and lean type pig breeds show obvious differences in muscle growth and adipose deposition; however, the molecular mechanism underlying this phenotypic variation remains unknown. We used pathway-focused oligo microarray studies to examine the expression changes of 140 genes associated with muscle growth and adipose deposition in *longissimus dorsi* muscle at six growth stages (birth, 1, 2, 3, 4 and 5 months) of Landrace (a leaner, Western breed) and Taihu pigs (a fatty, indigenous, Chinese breed). Variance analysis (ANOVA) revealed that differences in the expression of 18 genes in Landrace pigs and three genes in Taihu pigs were very significant (FDR-adjusted permutation, $P < 0.01$) and differences for 22 genes in Landrace pigs and seven genes in Taihu pigs were significant (FDR-adjusted permutation, $P < 0.05$) among six growth stages. Clustering analysis revealed a high level of significance (FDR-adjusted, $P < 0.01$) for four gene expression patterns, in which genes that strongly up-regulated were mainly associated with the positive regulation of myofiber formation and fatty acid biogenesis and genes that strongly down-regulated were mainly associated with the inhibition of cell proliferation and positive regulation of fatty acid β-oxidation. Based on a dynamic Bayesian network (DBN) model, gene regulatory networks (GRNs) were reconstructed from time-series data for each pig breed. These two GRNs initially revealed the distinct differences in physiological and biochemical aspects of muscle growth and adipose deposition between the two pig breeds; from these results, some potential key genes could be identified. Quantitative real-time RT-PCR (QRT-PCR) was used to verify the microarray data for five modulated genes, and a good correlation between the two measures of expression was observed for both two pig breeds at different growth stages ($r = 0.876 \pm 0.095$). These results highlight some possible candidate genes for porcine meat quality and carcass traits and provide some data on which gene(s) should be further studied for elucidating the molecular mechanism of muscle growth and fat deposition.

Keywords: Pig; Muscle growth; Adipose deposition; Pathway-focused microarray; Differential gene expression; Gene regulatory network (GRN)

1. Introduction

The growth and development of muscle and adipose tissue are complex physiological processes involving co-expressional and co-restricted multi-genes. The pig (*Sus scrofa*) has become one of the most important farm

* Corresponding author. Tel.: +86 13608266187; fax: +86 835 2886080.
E-mail address: lixuewei9125@126.com (X. Li).

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animals. Anatomical, physiological, pathological and genomic similarities between pig and human have suggested that the pig could be considered a model species for human health issues. Therefore, the use of pigs as research animals will benefit both animal agriculture and biomedical research. The present studies mainly focus on candidate genes which influence muscle growth and adipose deposition, especially genes for pork quality. In dynamic views, the competition between growth rates of skeletal muscle cells and adipose cells *in vivo* not only influences pork quality, but also simultaneously determines porcine productive type [1].

At present, little is known about the molecular basis of pork growth; in particular, the genetic complexity underlying the phenotypic variation that is related to porcine breeding programmes and selection criteria remains only partially understood. In a postgenomic era, functional genomics, including analysis of the transcriptome and the proteome, provides new opportunities for understanding the interactions of functional genes and how these influence the production of meat [2].

Western pig breeds have been intensively selected over the past two decades for rapid, large and efficient accretion of muscle, which is believed to have led to deterioration in meat quality. Landrace, a typical lean-type western breed, is now widely used for commercial production throughout the world. While indigenous Chinese pig breeds have lower growth rates and a lower lean meat content than conventional western pig breeds, they have proved superior in terms of perceived meat quality. The Taihu variety is a typical indigenous Chinese breed of pig [3].

Here, we describe a pathway-focused analysis of gene expression changes in *longissimus dorsi* muscle in Landrace and Taihu pigs at birth, 1, 2, 3, 4 and 5 months. Our results could be beneficial to researchers attempting to generate, through genetic engineering, pigs which have both improved meat quality and lean carcasses.

2. Materials and methods

2.1. Animals and tissue collection

Landrace and Taihu pigs (12 sows and 12 boars for each breed) were used in this study. Two male and two female piglets were randomly assigned to each stage for each breed with ad libitum access to feed under same normal conditions. The piglets were weaned simultaneously at 28 ± 1 day of age. A starter diet that provided 3.40 Mcal kg^{-1} metabolizable energy (ME), 20.00% crude protein and 1.15% lysine during 1–2 months after weaning. In 2–4 months, the diet contained 3.40 Mcal kg^{-1} ME, 17.90% crude protein and 0.83% lysine. In 4–5 months, the diet contained 3.40 Mcal kg^{-1} ME, 15.00% crude protein and 1.15% lysine. The animals were reared in compliance with national regulations for the humane care and use of animals in research. The pigs were sacrificed at a commercial slaughterhouse at birth, 1, 2, 3, 4 and 5 months of age. The *longissimus dorsi* muscle near the last 3rd or 4th rib was rapidly and manually dissected from each cleaved pig. These samples (0.3–0.4 cm in thickness) were immediately submerged in RNA*later* (Qiagen, Germany) for RNA preservation, after which they were crushed to powder with liquid nitrogen, subdivided (per 80–100 mg) and stored at −70 °C until further use.

2.2. Muscle measurements

After sacrifice, all muscle tissues were fixed in 10% neutral-buffered formalin solution, embedded in paraffin using TP1020 semi-enclosed tissue processor (Leica, Germany), sliced at a thickness of 6 μm using RM2135 rotary microtome (Leica, Germany) and stained with hematoxylin and eosin (H&E). The myofiber cross-sectional area (CSA) was counted in average of 100 fibers in randomly selected fields using TE2000 fluorescence microscope (Nikon, Japan) and Image Pro-Plus 6.0 software (Media-Cybernetics, USA). The intramuscular fat (IMF) content was measured by heat extraction-oil weight method using SoxtecAvanti2055 extraction system (Foss, Denmark).

2.3. Construction of porcine pathway-focused microarray

One hundred and forty genes that were involved in biological processes of skeletal muscle growth and adipose deposition were selected with the aid of gene annotations of Gene Ontology (GO) terms and hundreds of biomedical literatures using Onto-Design [4] and GoPubMed [5] tools. The set of 140 oligonucleotides (Pig Genome Oligo Microarray Database, http://omad.operon.com/pig/query.php) represents porcine cDNAs and ESTs and was designed from TIGR TC cDNA sequences (SsGI release 12.0, http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=pig). In addition, there were six positive control genes and six negative controls including three *Arabidopsis* genes and three randomized sequences known to have minimal cross-hybridization with mammalian transcripts. All pig-specific ~70-mer oligonucleotides were designed within 1000 bp of an annotated 3′ end, the cross-oligonucleotide percentage identity <70%. No oligonucleotide has 20 contiguous bases in common with any other oligonucleotide. No oligonucleotide has repeats of >8 bases or a potential hairpin stem >9 bp.

The synthesized oligonucleotides were spotted at the National Engineering Center for Biochip at Shanghai, China. Each oligonucleotide was spotted four times and each control gene was spotted eight times on GAPS II slides (Corning, USA) using OmniGrid100 microarrayer (Gene-Machine, USA). Oligonucleotides were ultraviolet (UV) cross-linked to the slides after spotting according to the manufacturer's protocol for all slides. All information about this pathway-focused oligo microarray has been submitted to the NCBI Gene Expression Omnibus (GEO) database under the Accession No. GPL5171.