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Expression and variation of *Myf5* and *MyoD1* genes in different tissues of Wuzhishan pigs

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ABSTRACT. The myogenic regulatory factor (MRF) family includes *Myf5*, *MyoD1*, *Myf4*, and *Mfy6* genes. This experiment assessed the variation of *Myf5* and *MyoD1* genes from birth to maturity (30, 210, and 360 days) in the back muscle tissue of Wuzhishan pigs (WZSP), and the expression of *Myf5* and *MyoD1* mRNA in the heart, liver, lung, spleen, kidney, muscle, stomach, and intestine tissues were also examined. The results indicate that the expression level of mRNA for *Myf5* and *MyoD1* genes in the back muscle tissue is directly proportional to age ($P < 0.05$). Furthermore, of the eight adult pig tissue types that were tested, the expression of *Myf5* and *MyoD1* was highest in the muscle tissue.

Key words: Wuzhishan pig (WZSP); *Myf5*; *MyoD1*; Real-time fluorescence quantification PCR

INTRODUCTION

Obtaining the desired qualities and traits in meat is a complex process that is influenced by genetic and environmental factors. These factors can affect the animal before and after birth. For instance, genetic factors may influence the expression of genes that affect muscle formation during prenatal development. Following birth, the increase in muscle fiber size, muscle tissue formation, and postnatal growth are mainly controlled by myogenic regulatory factors (MRFs), including *Myf5* genes and *MyoD1* genes that belonged to the MRF family (Buckingham, 1996; Pownall et al., 2006). Funk et al. (1991) pointed out that MRFs can be divided into two categories. The first category includes the *MyoD1* and *Myf5* MRFs, which are expressed during the course of myoblast proliferation. These genes are also involved in the expression of myogenic cell lines that are associated with regulation. The second category includes the *MyoG* and *Myf6*, which are required to complete the formation of muscle fibers (Wyszyńska-Koko et al., 2006; Zhang et al., 2014). In the MRF family, *MyoD1* and *Myf5* genes have a high degree of homology, but they perform complementary roles (Tang et al., 2007). Therefore, studies of *MyoD1* and *Myf5* provide the experimental material needed to further discuss the mutual regulatory effects of these regulatory factors on meat quality.

The Wuzhishan pig (WZSP) belongs to the Suidae family (Mammalia: Artiodactyla), and the breed was included in the 2000 National Animal Genetic Resources Protection List. The breed was developed in Hainan, China, and is one of the endangered small breeds. WZSP displays several interesting features such as strong resistance, genetic stability, low fat content, a thick back, rich amino acid content, and a high metabolism (Wu et al., 2008). This experiment used real-time PCR to examine the differential expression of *Myf5* and *MyoD1* genes and the variations associated with muscle growth, height, and meat quality.

MATERIAL AND METHODS

Material

Nine WZSPs of the following ages from the farm of the CATAS were used in the present study: three at 30 days old, three at 210 days old, and three at 360 days old. We collected heart, liver, kidney, lung, spleen, muscle, stomach, and intestinal tissues from each specimen. The tissues were frozen using liquid nitrogen, and were then stored at -80°C until use.

Reagents and instruments

We utilized the following items that were purchased from Takara Biotechnology Co., Ltd., RNAiso Plus (Total RNA extraction reagent), the reverse transcription kit, and the fluorescence quantitative PCR kit (SYBR® Premix Ex Taq™). The Mastercycler ep realplex 4 was used for quantitative PCR analyses (Eppendorf).

Quantitative PCR

Total RNA extraction and reverse transcription

Total RNA was extracted from various organs according to the manufacturer protocol

(RNAiso Plus). The nucleic acid concentration and ultraviolet spectroscopy were used to determine the purity, and the RNA samples were diluted for further use.

Reverse transcription reaction mix: 20 µL 5X Prime Script™ buffer (final concentration 1X); 0.5 µL Prime Script™ RT Enzyme Mix; 0.5 µL 50 µM Oligo dT Primer (final concentration 25 pmol); 0.5 µL 100 µM random 6-mer primers (final concentration 50 pmol); 0.5 µL total RNA; a maximum 10 µL of RNase Free dH_2O.

Reverse transcription conditions: 37°C for 15 min; 85°C for 5 s. Products were stored at -20°C until use.

Primer design

Primers were designed using the Primer Premier 5.0 program (Shanghai Sangon Biological Engineering Co., Ltd.; Table 1).

Table 1. Real-time RT-PCR primer information.

Gene name	Sequences of primers (5'→3')	Products (bp)	Annealing/elongation (°C)	Accession No.
Myf5	F: AGGTGCACCACGACTAACCCCA R: TCCACCTGTTCCCTCAGCAGC	107	59.0	XM_001924362.2
MyoD1	F: GACCACTAACGCCGACCGCC R: GGCAGCCGCTGATTCGGGTT	123	60.0	NM_001002824.1
**β-actin*	F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC	114	62.0	DQ178122

*Housekeeping gene.

Real-time PCR

Real-time PCR experiments were performed on the MasterCycler RealPlex4 platform using the SYBR Green I dye method. The total 25-µL reaction mix contained the following components: 12.5 µL SYBR® Premix Ex Taq™; 1 µL primer (10 µM); 2 µL cDNA template, and 9.5 µL ddH_2O. The PCR cycling parameters included an initial preheating at 95°C for 30 s and 40 cycles of PCR denaturation at 95.0°C for 5 s, annealing at 60.5°/58.5°C for 20 s, and an extension at 72°C for 10 s.

Data and statistics

To ensure *Myf5* and *MyoD1* testing efficiency, we referred to Winer et al. (1999). First, we determined the expression level of *Myf5* and *MyoD1* in 360-day-old WZSP muscle tissues. Subsequently, the measured expression levels and $2^{-\Delta\Delta Ct}$ were used to calculate the relative mRNA expression levels of the target *Myf5* and *MyoD1* genes (Livak and Schmittgen, 2001). The housekeeping gene, β-actin, was utilized for internal calibration, and the SAS software was used to analyze the data. This methodology was applied to analyses of the relative expression of *Myf5* and *MyoD1* in WZSP muscle tissue (from birth to maturity) and the expression of *Myf5* and *MyoD1* in various WZSP tissues.

RESULTS

Analysis of relative expression *Myf5* and *MyoD1* WZSP mature back muscle tissue

The results indicate that as the age of WZSPs increases, the expression of *Myf5* and *MyoD1* mRNA in the back muscle tissue also increases (Figure 1 and Table 2).

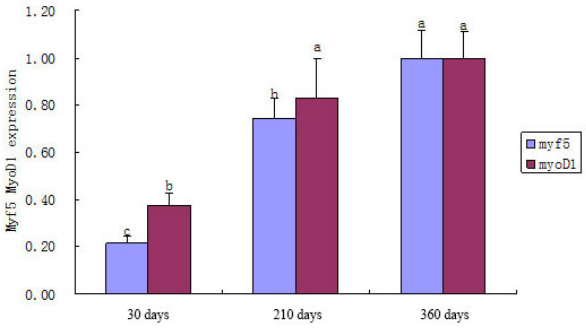

Figure 1. *Myf5* and *MyoD1* mRNA levels at different ages (from birth to maturity) in the back muscle tissue of Wuzhishan pigs. The letters refer to significant differences ($P < 0.05$).

Table 2. *Myf5* and *MyoD1* mRNA levels at different ages (from birth to maturity) in the back muscle tissue of Wuzhishan pig (the expression of *Myf5* and *MyoD1* in the 360-day-old specimens is the relative expression).

Target gene	Expression level		
	30 days	210 days	360 days
Myf5	0.21 ± 0.032^{c}	0.75 ± 0.084^{b}	1.00 ± 0.116^{a}
MyoD1	0.37 ± 0.055^{b}	0.83 ± 0.171^{a}	1.00 ± 0.110^{a}

The superscripts refer to significant differences ($P < 0.05$).

Analysis of relative expression of *Myf5* and *MyoD1* genes in various organs

The results show that *Myf5* and *MyoD1* expressed in heart, liver, lung, spleen, kidney, stomach, and intestinal tissues. However, the expression of the genes is highest in muscle tissue (Figure 2 and Table 3).

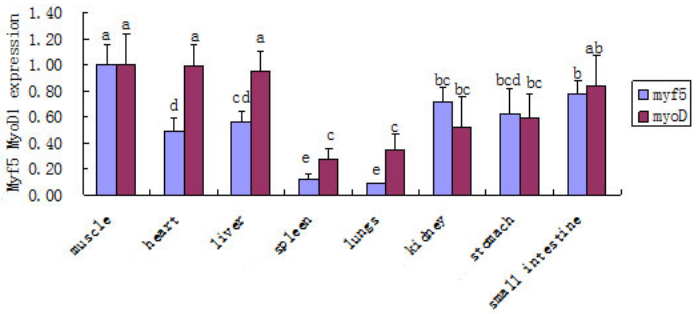

Figure 2. *Myf5* and *MyoD1* mRNA levels in eight tissues of Wuzhishan pigs. The letters refer to significant differences ($P < 0.05$).

Table 3. *Myf5* and *MyfoD1* mRNA levels in eight tissues of Wuzhishan pigs (the expression of the *Myf5* and *MyfoD1* in muscle tissues is the relative expression).

Objective gene	Expression							
	Muscle	Heart	Liver	Spleen	Lungs	Kidney	Stomach	Small intestine
Myf5	1.00 ± 0.146^{a}	$0.49 \pm 0.\ 0.099^{d}$	0.56 ± 0.081^{cd}	0.12 ± 0.032^{e}	0.09 ± 0.006^{e}	0.71 ± 0.108^{bc}	0.62 ± 0.197^{bcd}	0.77 ± 0.095^{b}
MyoD1	1.00 ± 0.236^{a}	0.98 ± 0.168^{a}	0.95 ± 0.150^{a}	0.27 ± 0.082^{c}	0.34 ± 0.124^{c}	0.52 ± 0.228^{bc}	0.59 ± 0.189^{bc}	$0.84 \pm 0.236a^{b}$

The superscripts refer to significant differences ($P < 0.05$).

DISCUSSION

Skeletal muscle is composed of fibers that begin to develop during the embryonic stage; however, an increase in the number of muscle fibers occurs 70 days after birth. During the developmental process, embryonic muscle cells are the first to be induced by *Myf5*, but *MyoD1* will compensate for any loss of muscle caused by *Myf5* deficiencies. Similarly, a lack of *MyoD1* will not drastically influence myogenic differentiation. Thus, there is a complementary, overlapping relationship between the functions of the two genes. However, when both genes are missing, the differentiation of skeletal muscle cells and myoblasts does not occur (Daumas et al., 1997). The effects of *Myf5* and *MyoD1* play key roles in muscle formation. Studies of the genes were focused on the molecular structure (Guo et al., 2009), polymorphisms (Wu et al., 2013), pathology (Blum and Dynlacht, 2013), and the mechanisms or embryonic stages that associated with the expression levels in different breeds (Sarti et al., 2014). Lefaucheur and Ecolan (2005) found that in the first 75 days following birth, the total amount of skeletal muscle fiber is far less in Meishan pigs than in Large White pigs. This difference is a direct result of the higher postnatal muscle development capacity of Large White pigs as compared to Meishan pigs. Moreover, Tang et al. (2007) found that embryonic Landrace and Tongcheng pigs vary in terms of gene expression. For instance, the skeletal muscle of Tongcheng pigs has a more complex molecular mechanism and a relatively slow growth rate (Tang et al., 2007). WZSPs also grow slowly, but it is not yet know if the low production of meat is related to expression of *Myf5* and *MyoD1*.

The expression of *Myf5* and *MyoD1* genes not only plays an important role in the regulation of muscle growth, but the genes also affect other organs (Sun et al., 2014). However, no other studies have examined the expression of the genes in different WZSP tissues. This experiment confirms that while *Myf5* and *MyoD1* expression is highest in muscle tissues, the genes are also expressed in a variety of organs, including the heart, liver, lungs, spleen, kidneys, stomach, and intestines. Therefore, the results provide further impetus to study the variation of *Myf5* and *MyoD1* expression in WZSP muscle tissues.

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