MRFs GENE EXPRESSION IN WUZHUMUQIN SHEEP DURING POSTNATAL DVELOPMENT

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Abstract –To investigate the MRFs gene expression patterns of Wuzhumuqin sheep, three major of muscles, including longissimus dorsi (LD), Biceps femoris (BF) and Triceps brachii (TB), were obtained from 18 Wuzhumuqin rams. This study was designed to detect the Wuzhumuqin sheep's Myf5, MRF4 and Myogenin genes expression patterns at six different postnatal time points by real-time PCR. The results showed that the trend of MRFs gene was highly similar in different muscle during muscle growth *(P>0.05)*. During postnatal development, Myf5 gene expression was trend to declined, while MRF4 and Myogenin gene expression values were appeared to stable in Wuzhumuqin sheep in LD muscle. In BF muscle, MRF4 and Myogenin gene expressions were trend to increased whereas Myf5 gene expression seem to more stable with aged. There was a higher level of MRF4 mRNA expression value and lower level of Myogenin mRNA expression value was obtained in TB muscle during muscle growth. In conclusion, these results suggest that the level of MRFs gene expression is trend to stable in natural grazing Wuzhumuqin sheep during postnatal development.

Key Words –Myf5 gene, MRF4 gene, Myogenin gene.

I. INTRODUCTION

The Chinese native sheep, Wuzhumuqin sheep, is a kind of Mongolian sheep. Wuzhumuqin sheep is come from Xilin-Gol grassland in Inner Mongolia. Most Chinese consumers largely prefer Wuzhumuqin sheep to the other breed, because they believe that the flavor and juiciness of Wuzhumuqin sheep is better than the others such as Xiaoweihanyang or kind of hybrid rams. Thence, Wuzhumuqin sheep has been regarded as the most expensive and high quality sheep for decades in China. However, very few scientific studies have examined the gene expression pattern of myogenic regulatory factors during muscle mass, especially in relation to the natural grazing Wuzhumuqin rams. The past decade has been reported that skeletal muscle growth has regulated by several factors including myogenic regulatory factors (MRFs), satellite cells and MSTN. MRFs is known as the vital members of the basic helix-loop-helix (bHLH) class of transcription factors, is capable of participating the muscle precursor cells proliferation, the postnatal muscular function and the muscle fiber formation, as well as regulating skeletal muscle differentiation. Myogenic regulatory factors have four specific genes, including MyoD, Myf5, MRF4 and Myogenin, that is almost exclusively expressed in skeletal-muscle lineage [1-3]. In these family groups, each gene has its special roles during muscle growth and the myogenesis stability is balanced by self-adjustment and mutual activation. As the one of family member has absent, the others compensates and performs some of the role of it [4]. Accumulating evidences suggest that non-muscle has formed in MyoD, MRF4 or Myf5 gene knockout mouse, as well as in MRF4, MyoD and Myogenin simultaneously abolished mice [5-6]. The expression level of MRFs is improved in regenerating muscle, denervated muscle, and hindlimb muscle [7]. Besides, MyoD and Myf5 over-expression can govern the embryonic myogenesis, which is highly similar to postnatal myogenesis [8]. However, MRFs gene does not always have a high value of gene expression level in muscles during muscle growth. In the soleus muscle, MRF4 gene expression has reduced and myogenin gene expression has unchanged. These studies suggest that each gene of myogenic regulatory factor has its special expression pattern in different muscle. Therefore, the objective of this study was to investigate the gene expression pattern of MRFs gene in Wuzhumuqin rams during postnatal development.

II. MATERIALS AND METHODS

Animals and muscle samples

All procedures for this study were conducted under a protocol approved by the Institutional Animal Care and Use Committee in the College of Animal Science and Technology, Inner Mongolian Agricultural University, China. Three major muscles including Longissimus dorsi (LD), Biceps femoris (BF) and Triceps brachii (TB) of eighteen Wuzhumuqin sheep were taken aged from 1 month until 18 month. Muscle samples were taken at the adjacent to the 13th thoracic vertebra of LD, central portion of BF and TB muscle, respectively. 100 mg of each muscle was taken for genetic analysis within 1 h post-mortem and frozen in isopentane chilled with liquid nitrogen. Total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After extraction, the RNA samples were used to synthesize first-strand cDNA using the Ex Script TM RT reagent Kit (Takara, Tokyo, Japan).

Primer design and Quantitative Real-time PCR

Based on the conserved regions of MRFs sequences from mice, human, cattle, goat, and sheep, four primer pairs were designed to amplify the sheep's MRFs gene family. The primers were used in real-time PCR as followed:

Myf5 (F:CACGACCAACCCTAACCAGA;
R: TGGTGATCCGATCCACTATGCT),
MRF4 (F:ATGCAGGAGTTAGGGGTGGA;
R: TGTTCCTCCGAGGAAATGCTG),
Myogenin(F:CACTCTGAGGGAGAAGCGCAG;
R: TGTGGACTGCAGGAGGCACTA)
RPL7(F:CGAAAGGCAAGGAGGAGGAAGCTTAT;
R: TGTTAATTGACGCCTTGTTGAGCT).

The real-time PCR was performed using the Cycler I Q Detection System (Bio-Rad) Real-Time PCR with the 2-ΔΔCt method and RPL7 as the housekeeping gene. PCR was carried out in a total volume of 25μL containing 2μL first-strand cDNA, 12.5μL SYBR@ Premix Ex Taq TM II, 1μL each primer, 8.5μL ddH_2O (TaKaRa, Tokyo, Japan). The amplification program included an initial denaturation step at 95°C for 30s, 40 cycles of denaturation at 95°C for 5s, and annealing at 55.7 – 63°C for 30s, extension 72°C for 1min. A melting program ranging from 55°C to 95°C with a heating rate of 0.5°C/10s was carried out to create melt curves. Reactions were performed in triplicate and a negative control was also performed in parallel.

Statistical analysis

The experimental data were analyzed by the analysis of variance procedure of statistical analysis systems (SAS, 2002), and the Duncan's multiple range test was used to determine the significant differences among means at a 5% level of significance (SAS, 2002). Real-time PCR data were presented as Mean ± SE, and each sample was triplications. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR data. Differences were considered significant when $P < 0.05$.

III. RESULTS AND DISCUSSION

Three major of myogenic regulatory factor gene expression patterns, including Myf5, MRF4 and Myogenin gene, were observed in Wuzhumuqin sheep during postnatal muscle growth (Fig.1-3). The level of MRFs gene expression values were peaked at 1 month or 18 month, as well as had similar gene expression patterns for the next stages. In LD muscle, the level of Myf5 gene expression was trend to declined, while MRF4 and Myogenin gene expression level seem to more stable ($P > 0.05$) (Fig.1). Regarding MRFs gene expression patterns, we found that the level of MRF4 gene expression were higher than Myogenin expression value in the most of times in LD muscle (Fig.1). MRF4 and Myf5 gene expression had an increasing trend until 3month in BF muscle, at the same time Myogenin gene expression was declined in BF muscle. Afterwards, all of these mRNA level appeared to satble during muscle growth ($P > 0.05$) (Fig.2). In TB muscle, MRF4 mRNA level was increased then peaked at the 18 month, whereas Myogenin mRNA level was waved during muscle mass. However, there was no significantly changes for Myf5 mRNA level in TB muscle during postnatal development ($P > 0.05$) (Fig.3). Many researchers found that MRFs was highly related to the muscle growth in the different muscle during muscle growth [9-11]. In this research, the results confirmed that myogenic regulatory factors were closely related to the muscle growth, had self- adjusment and mutual activation during poatanal muscle growth.

Fig 1. The gene expression profiles of myogenic regulatory factors (MRFs) genes in longissimus dorsi (LD) at different months (P >0.05)

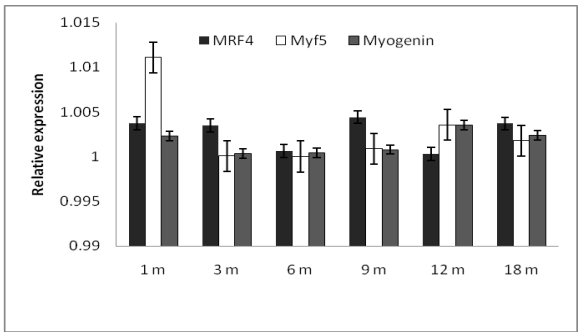


Fig 2. The gene expression profiles of myogenic regulatory factors (MRFs) genes in Biceps femoris (BF) at different months (P >0.05)

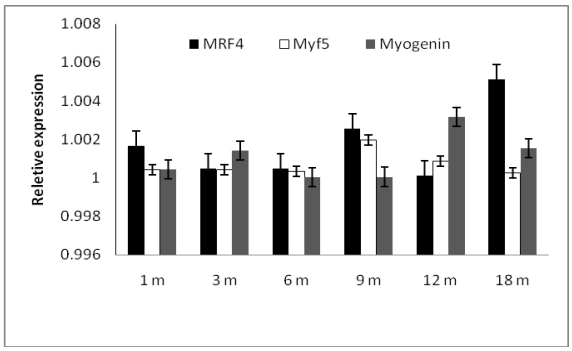
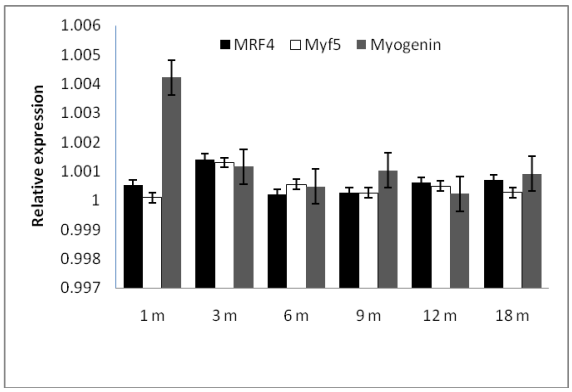


Fig 3. The gene expression profiles of myogenic regulatory factors (MRFs) genes in Triceps brachii (TB) at different months (P >0.05)



IV. CONCLUSION

The gene expression pattrns of MRFs family were highly similar and seem to be stable in different muscles. And, there was a potentially interaction among these factors during postnatal development in Wuzhumuqin sheep.

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