ANALYSIS OF GENETIC CHARACTERISTICS OF WUZHUMUQIN SHEEP MYOSTATIN GENE

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Abstract –Wuzhumuqin sheep was employed in this study. We used the PCR technique to amplified the Myostatin (MSTN) gene of wuzhumuqin sheep, and then carried out cloning and sequencing. Full-length sequence of MSTN gene was obtained by Sequence analysis using molecular biology software. The results showed that wuzhumuqin sheep MSTN gene had 3 exons and 2 introns. The size of exonⅠ, exonⅡand exonⅢwere 373, 374, 381bp, respectively. The size of intronⅠand intronⅡwere 1833, 2030bp, respectively. The Full-length sequence of CDS was 1128bp and composed of 375 amino acids. Theoretical molecular weight of encoding protein of MSTN gene was 42.8 kDa and PI was 7.01. Highest level of Leu (9.9%) was observed and followed by Lys (8.3%). The secondary structure of MSTN protein was mainly α-helix and random coil structure, which have characteristics of transmembrane protein. The transmembrane domain located in AA6-AA23 and showed secretion signal peptide structure that amino acids sequence was MQKLQICVYIYLFMLIVA. MSTN may be characterized with TGF-β family.

Key Words – Wuzhumuqin sheep, Myostatin, RT-PCR

I. INTRODUCTION

Myostatin (MSTN) gene was first identified by Mcpherron *et al.* [1] in 1997. It is a member of transforming growth factor (TGF -β) family that plays an important regulation role in muscle growth [2]. MSTN negatively control the muscle cell growth and development through inhibition of the transcriptional activity of myogenic differentiation (MyoD) members. Its expression was negatively correlated with the change of the muscle weight [1]. Scientists have been doing research on the MSTN gene in a variety of animals, such as mouse [3], cows [4], goats [5], zebrafish [6], chickens [7] and other species. Myostatin knockout mice showed a dramatic increase of skeletal muscle mass [1]. The double muscle breeds of cattle carrying the natural mutations in the myostatin gene have significantly more muscle mass than standard breeds. It makes double muscle cow meat production is 1.3 times higher than that of ordinary cow [8]. Kocamis *et al.* [9] reported that they have found detectable MSTN mRNA expression in skeletal muscle of porcine fetus in their pig MSTN study. Rodgers *et al.* [10] also reported that there are two types of MSTN in the american trout, one type was specifically expressed in the ovary, and the another type was expressed in the red muscle and brain. The research on the MSTN gene was not only limited in animals, there were some studies also pointed to human [11].

The wuzhumuqin sheep is a kind of excellent Mongolian sheep stock that formed by the long term breeding in Xilin-Gol grassland in Inner Mongolia. The wuzhumuqin sheep usually has longer body than other sheep breed that caused by the more spinal section number (usually 14 ribs in pure breed of wuzhumuqin sheep), and which is an extremely valuable genetic pool for the genetic study. Our previous study for the first time reported the role of MSTN expression changes in the growth of wuzhumuqin sheep under the natural grazing conditions. But the whole MSTN gene cloning and analysis on this breed has not been reported.

In this study, we selected the wuzhumuqin sheep under the natural grazing condition as our objective, and cloned and sequenced the MSTN gene of purebred (multiple thoracic individual) wuzhumuqin sheep, and sequence alignment with other species, meanwhile speculated the secondary and tertiary structures and some physical and chemical properties of the coding protein. The study will provide reliable information on the establishment of wuzhumuqin sheep gene library, and also provide the theoretical basis for the improvement of local sheep breed and the study on the usage of MSTN gene as a molecular breeding technology of wuzhumuqin sheep.

II. MATERIALS AND METHODS

Genomic DNA was extracted by Blood Genomic DNA Extraction Kit (TIANGEN) from wuzhumuqin sheep blood samples.

PCR reaction: 50 ul reaction system is applied in this experiment, the concentration and volume of each component are as follows: the template DNA 4 ul, forward primer (10 um) 2 ul, reverse primer (10 um) 2 ul, premix TaqTM 25 ul, ddH2O 17 ul. The PCR reaction conditions: 95 ℃ modified 5 min, 98 ℃ degeneration 10 s, 30 s annealing, 72 ℃ for extension 1 min, 30 cycle, 72 ℃ for extension 10 min, 4 ℃ heat preservation, amplification products with 1% agarose gel electrophoresis detection.

III. RESULTS AND DISCUSSION

DNAMAN V6.0 software was used for sequence splicing and obtained wuzhumuqin sheep MSTN gene sequences. Sequence analysis revealed that the wuzhumuqin sheep MSTN gene spanned 5068bp, including partial sequence region, all exons and intron sequences. Wuzhumuqin sheep MSTN gene contained three exons which size were 373bp, 374bp, 381bp and two introns which size were 1833bp, 2030bp, respectively. Exon Ⅰ contains the start codon ATG, exon Ⅲ contains a stop codon TGA. BioEdit biological analysis revealed that the full length CDS of wuzhumuqin sheep MSTN gene sequence was 1128bp and composed of 375 amino acids (Fig1) compared with other animal species gene sequence(Table1).

DNAMAN biological software was used to analysis the wuzhumuqin sheep MSTN gene coding region of whole nucleotide sequences compared with other sheep and goat. Results showed that one base substitution (C→T) was detected in 1122 site of wuzhumuqin sheep MSTN gene coding region compared with other sheep. As well as it compared with goat MSTN gene coding region, six base substitution (T→C) in 126 site, (A→G) in 189 site, (G→A) in 329 site, (C→T) in 903 site, (C→T) in 930 site, (A→C) in 1094 site, were identified in wuzhumuqin sheep MSTN gene coding region, respectively. In addition, similarities between amino acid sequence of wuzhumuqin sheep and other sheep MSTN gene showed 100%, while similarities between the goat, cattle, horse, pig, human, chimpanzee, baboon, rat, dog, turkey, chicken, rabbit and zebrafish were 99.5%, 93.3%, 94.4%, 95.5%, 94.1%, 94.4%, 94.1%, 90.9%, 93.1%, 88.0%, 87.7%, 94.7% and 66.8%, respectively (Table1).

The amino acid sequences were analyzed by ProtParam and obtained the basic parameters of MSTN precursor protein, wuzhumuqin sheep MSTN gene encoding protein formula was C1912H3018N512O561S21, theoretical molecular weight was about 42.8 kDa, PI was 7.01; the amino acids leucine occupied maximum content of 9.9%, lysine was 8.3% followed. Aqueous solution extinction coefficient at 280 nm was approximately 51 ~630. Instability coefficient of the protein was 44.87, the average hydrophilic coefficient was 0.411.

SOPMA biological analysis predicted wuzhumuqin sheep MSTN gene encoding protein secondary structure, original secondary structure of α-helical (Alpha helix), β-fold (Extended strand)occurred: number of α-helix was 94, representing 25.07% of all structures; number of β-folded was 77, representing 20.53% of all structures; especially number of random coil was 183, representing 48.80% of all structures. TMpred software analysis predicted transmembrane structure of wuzhumuqin sheep MSTN gene encoding protein, results showed that wuzhumuqin sheep MSTN gene encoding protein belongs to the transmembrane protein, AA6 - AA23 was transmembrane region (theoretical values were 1422, significantly higher than the software default 500) (Fig. 2). Gentry analysis predicted that wuzhumuqin sheep MSTN gene encoded a protein having a secretion signal peptide structure, the amino acid sequence was

MQKLQICVYIYLFMLIVA. This result was consistent with reports of Helen [12] (Fig 3).

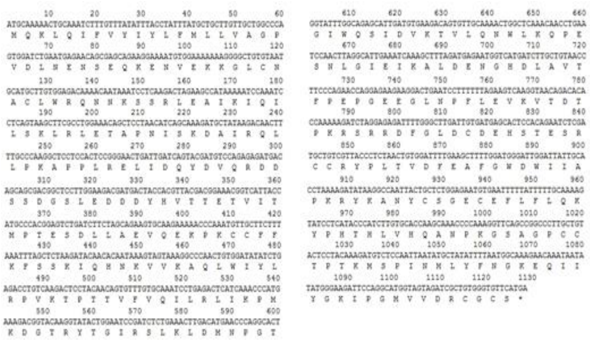

Fig. 1 CDS sequence and amino-acid sequences of MSTN

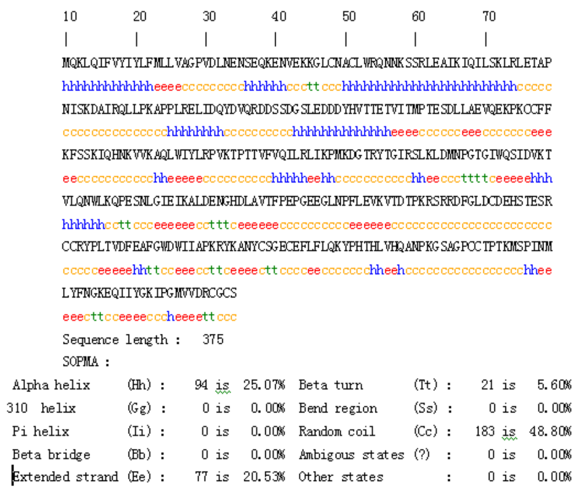

Fig. 2 Analysis of wuzhumuqin sheep MSTN gene encoding protein secondary structure

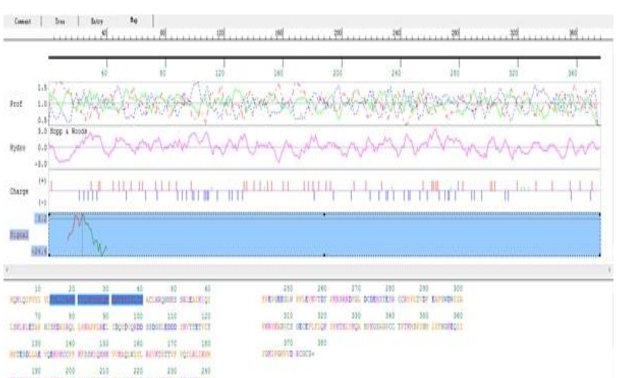

Fig. 3 The signal peptide analysis of Ujumqin sheep MSTN gene

Species	Ujumqin sheep	Sheep	Goat	Cattle	Horse	Pig	Human	Chimpanzee	Baboon	Rat	Dog	Turkey	Chicken	Rabbit	Zebrafish
Ujumqin sheep	100														
Sheep	100	100													
Goat	99.5	99.5	100												
Cattle	93.3	93.3	92.8	100											
Horse	94.4	94.4	93.9	94.9	100										
Pig	95.5	95.5	94.9	95.7	98.1	100									
Human	94.1	94.1	93.6	94.1	97.3	97.9	100								
Chimpanzee	94.4	94.4	93.9	94.4	97.6	98.1	99.7	100							
Baboon	94.1	94.1	93.6	94.1	97.3	97.9	99.5	99.7	100						
Rat	90.9	90.9	90.4	91.2	94.7	94.9	94.9	95.2	94.9	100					
Dog	93.1	93.1	92.5	93.3	96.3	96.8	95.7	96.0	95.7	93.3	100				
Turkey	88.0	88.0	87.5	88.3	91.5	91.7	92.3	92.0	91.7	89.9	89.9	100			
Chicken	87.7	87.7	87.2	88.0	90.9	91.5	92.0	91.7	91.5	89.9	89.6	99.2	100		
Rabbit	94.7	94.7	94.1	94.4	97.6	98.4	97.9	98.1	97.9	95.2	95.7	92.3	92.0	100	
Zebrafish	66.8	66.8	66.3	66.0	68.2	68.2	68.7	68.5	68.2	67.4	67.7	68.5	68.7	68.2	100

Table 1 Using biological software DNAMAN calculate amino acid sequence similarity between the species (Wuzhumuqin sheep →Ujumqin sheep).

IV. CONCLUSION

In this study, five cloned fragments were spliced and its full-length gene sequence were 5068bp. Biological analysis shows that Wuzhumuqin sheep MSTN gene has two introns and three exons. Size of Exon Ⅰ, exon Ⅱ and exon Ⅲ were 373bp, 374bp, 381bp and size of intron Ⅰ and intron Ⅱ were 1833bp, 2030bp. High similarity of MSTN gene exist in Wuzhumuqin sheep and other sheep, reached 99.9%, mutation only occured once in position 1122 (C → T), however it did not result in amino acid changes.

Further explanation revealed that wuzhumuqin sheep and other sheeps has closer kinship, although there was some differences nucleotide level, but there was no difference amino acid level, so it would not influence the encoded protein structure. Wuzhumuqin sheep and sheep MSTN gene encoding protein sequences has the same composition, so the regulation of gene expression in the way between the two species may also be the same. Meanwhile, results showed that DNA level between wuzhumuqin sheep and other species have difference because they may have different origins, evolution and extent of breeding. The reason need to further research.

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