Molecular Cloning, Characterization and Tissue Distribution of MyoGenin Gene in Goose (Anser cygnoides)

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Abstract: MyoGenin (MyoG) is a basic Helix-Loop-Helix (bHLH) transcription factor that belongs to the Muscle-specific transcription factors (MRFs) family which plays critical roles in regulating the skeletal muscle development and growth. In this study, the complete coding sequence and genomic DNA sequence of goose MyoG gene were cloned and characterized. The goose MyoG CDS was composed of 684bp that encoded a 227 amino acid protein, including a highly conserved basic helix-loop-helix domain. Multiple sequence alignments and phylogenetic analysis indicated that the deduced goose MyoG protein was conserved in vertebrates, especially in the avian species. The goose MyoG genomic DNA sequence we obtained was 3444bp and consisted of 3 exons and 2 introns. Semi-quantitative RT-PCR analysis demonstrated that the goose MyoG mRNA was specifically expressed in the breast muscle and leg muscle tissues, little or no expression was observed in heart, liver, spleen, lung, kidney, muscular stomach, brain, intestine and sebum. These data will serve as a foundation for further insight into the functions of the MyoG gene in Chinese domestic goose.

Key words: MyoGenin, transcription, kidney, characterized, stomach

INTRODUCTION

The MyoGenic Regulatory Factors (MRFs) family is a kind of MyoGenic basic Helix-Loop-Helix (bHLH) transcription factors and consists of four structurally and functionally related genes including MyoG, Myf-5, MyoD, and Myf-6. All the four genes shared homology with the region coding for bHLH domain and were involved in the muscle development from commitment and proliferation through muscle fiber formation during embryonic development to their postnatal maturation and function (Fujis-Sekara et al., 1990; Pas et al., 1999). Therefore, the MRFs family genes are considered as candidate genes for meat production traits in farm animals (Verner et al., 2007). MyoGenin, also known as MyoG or Myf4 has a central position within the MRFs gene family because it is the only factor expresses in all skeletal muscle cells and has a major influence on the number of muscle fibers during Myogenesis (Pas et al., 1999; Wright et al., 1989; Megeney et al., 1996). In mice, MyoG was essential for the development of functional skeletal muscle (Hasty et al., 1993). In chickens, MyoG gene was the major gene affecting the muscle fiber traits (Wang et al., 2007). In pigs, studies showed that the polymorphisms on MyoG gene were related to the meat production traits, including the number of muscle fibers, birth weight, growth rate and thickness of backfat (Pas et al., 1999; Tepas et al., 1996; Lin et al., 2001; Kim et al., 2009). All the above results suggest that MyoG gene is associated with the formation of muscle and plays crucial roles on the meat yield and quality.

As an important agricultural poultry, meat yield and quality traits are main economically traits in geese. However, our understanding on the biological function of goose MyoG gene has lagged behind due to the lack of complete coding sequence in GenBank database. Thus, the purpose of the present study was to clone and characterize the goose MyoG gene and analyze its tissue expression profile in different tissues of Chinese domestic goose. These data will be useful for the further studies of goose MyoG gene function.

MATERIALS AND METHODS

Animals and tissues: Three healthy female Zhedong White geese (12 weeks old) were obtained from the Institute of Zhedong-White Goose (Xiangshan) and all geese were set free in an open ground with a free
swimming pool and reared under normal management conditions. Muscle tissue from one Zhedong-White goose was used for cloning the goose MyoG cDNA sequence. A total of 11 tissues, including heart, liver, spleen, lung, kidney, breast muscle, leg muscle, brain, intestine, muscular stomach and sebum were surgically removed from each goose and used for tissue distribution analysis. All geese were slaughtered by a ventral cut of the neck blood vessels with 10 s after the end of the stun. The tissues were collected immediately, frozen in liquid nitrogen and stored at -80°C until RNA extraction. Blood samples were withdrawn from the three Zhedong-White geese, stored at -20°C until genomic DNA was extracted. All animal procedures were handled in compliance with the law of the people’s republic of china on animal protection.

Nucleic acid isolation and cDNA synthesis: Total RNA was extracted from the above 11 tissues with Trizol Reagent (Invitrogen, Carlsbad, CA, USA) using the Tissue Ruptor (TOYOCO, Japan). First-strand cDNA was synthesized using PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Japan). Genomic DNA was isolated from the collected blood samples of the above three geese using the AXYGEN DNA Isolation Kit (AXYGEN, USA). DNA concentration and quality were measured using a ND-1000 spectrophotometer (Nano Drop, USA) and the concentrations were adjusted approximately 300 ng μL and stored at -20°C for use.

Sequence cloning of the goose MyoG gene: Based on the partial mRna sequence of MyoG in goose (GI: 83265397) and the complete coding sequence in duck (GI: 29604134) and chicken (GI: 59745437), two pairs of primers (MyoG-F1/MyoG-R1 and MyoG-F2/MyoG-R2, Table 1) were designed to amplify the complete coding sequence of goose MyoG gene. The PCR condition initially started with a 94°C 3 min followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature, 30 s at 72°C and an extension step of 5 min at 72°C. PCR products were applied on 2.5% agarose gel electrophoresis and purified using the PCR purification Kit (TransGen Biotech Co., Ltd., Beijing, China). The purified PCR products were cloned into the PMD19-T vectors (TaKaRa, Japan), the positive clones were sequenced by Sangon Biotech Co., Ltd (Shanghai, China).

Based on the obtained cDNA sequence from the above RT-PCR reactions and the reference genomic DNA sequence of duck MyoG gene (NW-004676592), the genomic DNA sequence of goose MyoG gene were amplified using the two pairs of primers (MyoG-0F1/MyoGs-GR1 and MyoG-0F2/MyoG-0R2, table) from one DNA sample of Zhedong-White goose. The PCR profile was 5 min at 94°C followed by 36 cycles of 30 s at 94°C, 30 s at annealing temperature, 2-3 min at 72°C and a final extension of 10 min at 72°C. PCR products were gel purified, cloned and sequenced according the above methods.

Sequence analysis: The obtained cDNA and genomic DNA sequences were matched using DNAANAN software (Pointe-Claire, Canada). The amino acid sequence was predicted using ORF Finder (http://www.ncbi.nlm.nih.gov/projects/orf/). Sequence similarity was analyzed with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments of the amino acid sequences were performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2) and edited with BOXSHADE (http://bioweb.pasteur.fr/seqanal/interfaces/boxshade.html). ProtParam were used to predict the molecular weight and isoelectric point. Signal peptide sequence was predicted by Signal P 4.1 (http://www.cbs.dtu.dk/services/SignalP/). The transmembrane domain was predicted by TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). Phylogenetic tree was constructed using MEGA 6.0 and based on the Neighbor-Joining (NJ) method with a bootstrap of 1000 repetitions (Tamura et al., 2013).

Tissue distribution of goose MyoG gene: To measure the mRNA expression levels of goose MyoG in various tissues, semi-quantitative RT-PCR was performed with primers MyoG-RT-F/MyoG-RT-R (Table). Amplification of goose GAPDH gene was used as an internal control (GAPDH-F/GAPDH-R, Table 1). The amplified GAPDH fragment spanned intron 4 which was applied to exclude the possibility of DNA contamination during all RT-PCR reactions. The semi-quantitative RT-PCR amplifications were carried out in triplicate in a total volume of 20 μL, PCR conditions were as follows: 94°C, 4 min; 25-36 cycles with 94°C, 45 s; 65°C, 40 s; 72°C, 45 s and a final extension at 72°C for 10 min. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The PCR fragments were visualized on 2.5% agarose gels stained with ethidium bromide and visualized with ultraviolet light.

RESULTS AND DISCUSSION

Molecular cloning and characterization of goose MyoG gene: A 684 bp cDNA sequence was amplified by RT-PCR using two pairs of primers from the muscle tissue of Zhedong-White goose. The cDNA (GenBank: KF893286) comprised an ORF of 681 bp encoding a 227 amino acids
Fig. 1: Nucleotide and deduced amino acid sequences of the goose MyoG gene and multiple alignments of MyoG amino acid sequences among different species: a) The letters underlined indicate the start codon (ATG) and the stop codon (TAA) is indicated with an asterisk. The bHLH domain is indicated with dotted lines; b) Red amino acids indicate 100% conserved sequences. Less-conserved and non-conserved amino acids are shown in blue and black, respectively. Whereas “-” indicates deletion of an amino acid. The highly conserved bHLH domains are boxed.

protein with a calculated molecular mass of 25.74 kDa and an isoelectric point of 5.31 (Fig. 1a). A Gen Bank database search using BLAST revealed that the goose MyoG gene was consistent with those of other species with 97, 91, 90, 84, 81, 76, 75, 75 and 74% identity to Anas platyrhynchos (XM_013094032), Gallus gallus (FJ882411), Meleagris gallopavo (NM_001303170), Mus musculus (NM_031189), Ortygolagus cuniculus (FJ605116), Equus caballus (AB608013), Homo sapiens (NM_002479), Sus scrofa (NM_001012406), Bos taurus (NM_001111325) and Ovis aries (GU550517). Meanwhile, BLASTP analysis displayed that the predicted protein shares 98, 94, 94, 70, 71, 68, 69, 70, 71 and 70% amino acid sequence similarity with the MyoG protein sequences of the above species. The results of multiple alignments analysis showed that the deduced goose MyoG amino acid sequence was more similar to those of avian species, particularly to ducks (Fig. 1b). To elucidate the genomic organization, a total of 3444 bp in length genomic sequence (GenBank: KP895286) of goose MyoG gene was
Table 1: Primers used in this study

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<th>Primers purpose</th>
<th>Primer name</th>
<th>Primer sequence (5’-3’)</th>
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Fig. 2: Tissue distribution of the goose MyoG gene in different tissues

cloned using the primers MyoG-GF1/MyoG-GR1 and MyoG-GF2/MyoG-GR2 (Table 1). BLAST similarity analysis showed that the goose MyoG genomic sequence (GenBank: KT250042) shared high similarity with duck and chicken MyoG genomic DNA sequences. The goose MyoG genomic DNA sequence consisted of three exons of 480, 82 and 122 bp as well as two introns of 2022 and 737 bp, respectively. All exon-intron splice junction sequences conformed to the “GT-AG” rule.

Protein structure prediction and phylogenetic-tree construction of MyoG: The goose MyoG protein was predicted to be a non-secreted protein, due to it did not contain a signal peptide sequence which was consistent with that of sheep (Zhang et al., 2014). Meanwhile, it was a non-transmembrane protein, because no transmembrane helices were predicted. Moreover, the analysis from the NCBI conserved domains database showed that the goose MyoG protein contained a typical bHLH domain, which was a common feature of the bHLH transcription factors (Fig. 1a). The phylogenetic tree displayed that the MyoG proteins from eleven different species were divided into two major groups. The Anser cygnoides, Anas platyrhynchos (XP_012949486), Gallus gallus (NP_989515) and Meleagris gallopavo (NP_001290099) were grouped into a cluster, the mammalian species, including Mus musculus (NP_112466), Homo sapiens (NP_002470), Oryctolagus cuniculus (NP_001171220), Equus caballus (BAP59063), Sus scrofa (NP_001102120), Bos taurus (NP_001104795) and Ovis aries (NP_001167580) belonged to the other group. The phylogenetic analysis demonstrated that the goose MyoG exhibited a closer genetic relationship with those of avian species, particularly with the MyoG protein of duck.

Tissue distribution of goose MyoG gene: To characterize the tissue expression pattern of goose MyoG gene, semi-quantitative RT-PCR was performed with total RNA from eleven different goose tissues. The results showed that the goose MyoG mRNA was specifically expressed in the breast muscle and leg muscle tissues. Little or no expression was detected in heart, liver, spleen, lung, kidney, brain, intestine, muscular stomach and sebum tissues (Fig. 2).

Like other genes in the MRFs gene family, MyoG gene belongs to an important member of the MyoGenic bHLH transcription factors with the typical bHLH domain. In this research, molecular cloning, structural and phylogenetic analysis of goose MyoG gene were firstly performed. The cloning of goose MyoG provided a
material basis for further functional studies of goose MyoG molecules. Both of the multiple alignments and the phylogenetic analysis displayed the deduced protein of goose MyoG shared high amino acid identities with its counterparts in other species, especially with avian species. This is consistent with our previous studies on other goose genes (Wang et al., 2014; Liu et al., 2014). The close genetic relationship between goose and these avian species indicates that they may have similar biological functions. Further study should examine whether amino acid differences between the MyoG proteins of birds versus mammals contribute to functional differences.

Similar to other genes in MRFs family, the goose MyoG genomic DNA is composed of three exons and two introns which is consistent with the genome organization of MyoG gene in human, mouse, pig and fish (Soumillon et al., 1997, 1998; Tan et al., 2002). It is worth mentioning that the three exons encoded three different domains, respectively. The first exon encodes the bHLH domain, second exon encodes the transcriptional activation domain and the last exon encodes the conserved C-terminal domain (Soumillon et al., 1997; Schwarz et al., 1992). It is surprising that the genome structure of goose MyoG gene is different from that of duck, human, mouse and pig. The goose MyoG introns (2022 and 737 bp) are longer than the corresponding introns of ducks (1681 and 668 bp), humans (131 and 125 bp), mice (513 and 526 bp) and pigs (785 and 639 bp). In ducks, the expression of MyoG was detected both in the breast muscle and leg muscle at different stages of the fetal duck development (Liu et al., 2010). Our finding demonstrated that the goose MyoG only expressed in the breast muscle and leg muscle tissues. These data indicate that MyoG gene may play important roles in regulating muscle development and growth in waterfowl.

CONCLUSION

In summary, the goose MyoG gene was isolated and characterized for the first time in this study. The goose MyoG transcript was specifically expressed in the muscle tissues. All the results derived from this study established the primary foundation for further insight into the function of the MyoG gene in goose.

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REFERENCES


