연제 7

Structure and Expression of the Chicken Myostatin Gene

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ABSTRACT

A new murine TGF-β family member, myostatin(growth/differentiation factor-8), is expressed specifically in developing and adult skeletal muscle and may be a negative regulator of skeletal muscle development. This study aims at characterization and identification of genomic organization of chicken myostatin gene. In this study, we identified the genomic organization and sequence of chicken myostatin gene. Results of RT-PCR and Northern blots from various tissues showed different mRNA expression levels in developmental stages of chick embryos and demonstrated strong expression of myostatin mRNA in skeletal muscle. These facts suggest that chicken myostatin gene would play an important role not only in skeletal muscle cell but also in other tissues.

INTRODUCTION

Skeletal muscle development and regeneration are regulated positively by a variety of growth and transcription factors that affect both muscle stem cell proliferation and myotube differentiation. In particular, growth factors such as FGFs, IGFs and TGF-βs play a critical role in promoting myogenesis(Musaro et al., 1999). More recantly, a megative regulatror of skeletal muscle growth, myostatin, was described. Myostatin, also known as growth differentiation factor-8(GDF-8), is a member of TGF-β superfamily(McPherron & Lee, 1997).

Mice completely lacking myostatin sowed a profound increase in skeletal muscle growth; thus implicated in both hypertrophy and hyperplasia of muscle(McPherron et al., 1997). The two breeds of cattle with characteristics of increased muscle mass (double muscling), Belgian Blue and Piedmontese, have been found to contain mutations within the myostatin coding sequence(McPherron & Lee, 1997). Myostatin expression in porcine skeletal muscle peaked prenatally and greater expression was associated with low birth weight.

In chicken, complete cDNA sequence was reported, but neither the genomic structure nor expression patterns of the myostatin gene has been examined. Therefore, this study was carried out to determine the myostatin gene structure and to examine expression patterns in chicken.

MATERIALS & METHODS

Molecular cloning of chicken myostatin cDNA

The chicken myostatin cDNA was amplified by PCR using the forward primer (5-ATG AAAAAGCTAGCAGTCTATGTT-3) and reverse primer (5-TCATGAGCACCCGCA ACGATCTAC-3) (Genebank Accession No; AF019621). Amplified products were cloned into pUC18 vector.

Isolation and sequencing of genomic DNA

To isolate genomic clone of chicken myostatin gene, from chicken genomic library plaque hybridization was performed using radioactively labelled chicken myostatin cDNA probes.

Sequencing was carried out by using the PRISM BigDye terminator mix and the PRISM 377 ABI PRISM DNA sequencer (Perkin Elmer, Foster, CA).

RT-PCR and Northern blotting

Two sets of primers were synthesized based on the chicken cDNA sequence. Total RNA was extracted using the TRIZOL Reagent (GIBCO BRL, Gaithersburg, MD). For Northern blot analysis, the 1.1-kb, complete cDNA for the chicken myostatin was used as probes after labelling with the [α -^{32}P]dCTP using the random labeling method.

RESULTS and DISCUSSION

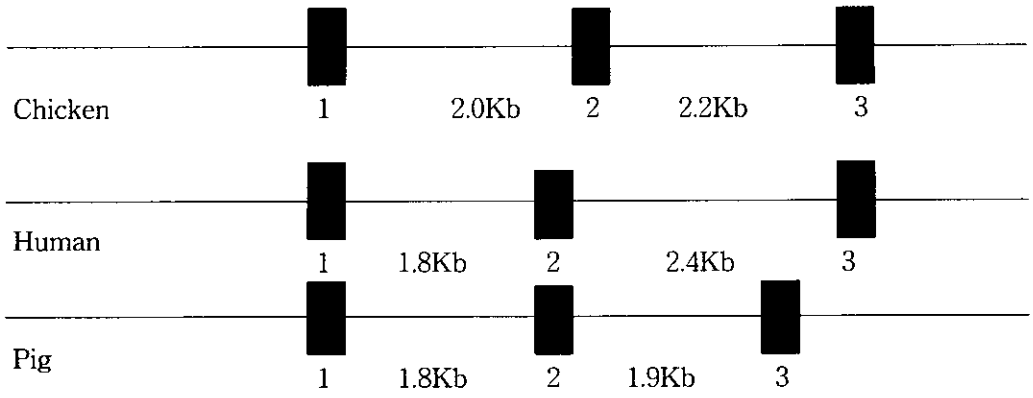

Figure 1. Genomic structures of myostatin genes in chicken, human and pig. Filled boxes indicate the relative locations of exons 1-3.

In this study, genomic organization and sequence of the chicken myostatin gene was revealed to contain three exons and two introns. Analysis of 5′ regulatory region reveals several cis-elements including GATA-1-binding sites and GRE. Northern blots and RT-PCR results of different tissues demonstrated strong expression of myostatin mRNA in the skeletal muscle and showed different mRNA expressions in developmental stages of chick embryos. Unlike in mammals, myostatin mRNA is expressed in various tissues, although the most intensive signal was shown in the skeletal muscle. Our results suggest that expression of the myostatin is developmentally regulated and its role may be not restricted to the skeletal muscle development in chicken.

(**Key Words** : GDF-8, myostatin, White Leghorn, skeletal muscle, gene expression)

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