연제 6

Characterization of chicken myostatin gene and expression in avian tissues

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Introduction

Myostatin (GDF-8) is recently identified as a member of the transforming growth factor (TGF)-beta superfamily of growth factors which are multifunctional peptides that cont-rol proliferation, differentiation and other functions in many cell types. A new murine TGF-β family member, growth/differentiation factor-8 (GDF-8), is expressed specifically in developing and adult skeletal muscle and may be a negative regulator of skeletal muscle development and growth (Mcpherron A.C. et al., 1997). The two breeds of cattle that were characterized by increased muscle mass (double muscling), Belgian Blue and Piedmontese had mutations in the myostatin coding sequence (Mcpherron A.C. et al., 1997). Myostatin expression in porcine skeletal muscle peaks prenatally and that greater expression was associated with low birth weight. Postnatally, myostatin mRNA was detected in skeletal muscle and mammary gland. Additionally, myostatin mRNA abundance was not changed by porcine growth hormone administration in growing animals (Shaoquan. et al., 1998).

In this study we identified the genomic organization and sequence of the chicken myostatin gene in the skeletal muscle. The expression level of myostatin mRNA in several organs was examined by northern analysis.

Materials and methods

Localization of Intron/Exon junctions

The chicken genomic myostatin gene was amplified by PCR using the forward primer (5'-ATG CAA AAG CTA GCA GTC TAT GTT-3') and reverse primer (5'-TCA TGA GCA CCC GCA ACG ATC TAC-3') which were

designed according to the chic-ken myostatin cDNA sequence. The conditions for PCR were 94℃ and 30sec for denaturation, 55℃, 1min for annealing and 72℃, 2min for extention, then performed by 35cyc-les. PCR products were inserted into pCR-TOPO vector(CLONETECH, TOPO-TA cloning kit), and sequenced. This sequence was confirmed by Southern blot analysis.

RT-PCR

Two sets of primer for the RT-PCR were synthesized based on the chicken cDNA seq-uence. The first set was described above. The second primer sequences used were the sense primer (5′-GTT TTG CTG GAA GGA ACT-3′) and antisense primer (5′-GCT CCA AAC ACT GAA GAA-3′). Total chicken skeletal muscle RNA was submitted to reverse transcription-PCR with the oligo dT primer and the gene specific primers.

Northern blot analysis

Total RNA was extracted from muscle, brain, liver, oviduct of White Leghor n chicken using TRIZOL regeant (GIBCO BRL). Northern blot analysis was perf ormed by using a 1128bp ^{32}P-labeled myostatin cDNA probe.

Results and discussion

The chicken myostatin gene have three exons and two introns. We confirmed the sequences of introns 1 and 2 and their junctions with the flanking exons. The 2103bp Intron 1 was located between nucleotide 373 and 374 of the cDNA sequence. Intron 2 was 2272bp long and followed nucleotide 747. We hybridized with myostatin cDNA probes for a northern blot containing total RNA isolated from several chicken organs. A single 1.1kb mRNA species was expressed in the chicken skeletal muscle, brain, but mRNA of myostatin was not expressed in liver and oviduct. These data indicate that myostatin expression is not restricted to skeletal muscle.

(Key words; TGF-beta, myostatin, GDF-8, White Leghorn, tissue specificity)

References

Mcpherron A.C. et al., Nature., 387:83-90, 1997.

Nestor F. Gonzalez-Cadavid. et al., PNAS., 95:14938-14943, 1998.

Mcpherron. A.C. and Lee.S.J, PNAS., 94:12457-12461, 1997.

Shaoquan. J, I, R.L.Losinski, et al., Am. J. Physiol., 275:1265-1273, 1998.