Altered Expression of Myostatin Gene in the Progressive Muscular Dystrophy Patients

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Abstract Progressive muscular dystrophy is a group of inherited disorders characterized by progressive skeletal muscle wasting and weakness which is not of neurogenic origin. Myostatin is a new member of the TGF-β super-family is a negative regulator of skeletal muscle growth. To investigate the possible involvement of myostatin in the development of progressive muscular dystrophy we cloned and sequenced myostatin cDNAs from the progressive muscular dystrophy patients by RT-PCR. Levels of myostatin mRNA and protein in the patients were analyzed by semi-quantitative RT-PCR and Western blot respectively. We did not find any mutations in the myostatin cDNA sequences from the progressive muscular dystrophy patients in this study. However we found that the levels of myostatin transcripts were reduced in some patients and the processing and maturation of myostatin protein were inhibited in some patients. Our data demonstrated that the pathogenesis of some types or subtypes of progressive muscular dystrophy is probably associated with the altered myostatin expression and the processing inhibition of myostatin protein.

Key words progressive muscular dystrophy myostatin protein processing

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Myostatin

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Supported by the National Natural Science Foundation of China No. 30200162 National Science Foundation for Distinguished Young Scholars No. 30025027 and Chinese National Programs for High Technology Research and Development No. 2001AA222031 2003AA221071
最近发现的骨骼肌生长发育抑制因子。为探讨基因与进行性肌营养不良病理发生的相关性,采用方法克隆了患者的基因并测序、分析肌营养不良患者是否存在基因突变;然后采用半定量方法检测患者中基因的表达水平是否发生改变,同时用方法分析了肌营养不良患者中基因蛋白的表达情况。结果发现,所研究的肌营养不良患者中没有携带基因突变,但一些患者的基因转录水平降低,部分患者蛋白加工障碍。结果提示,一些类型(亚型)的进行性肌营养不良可能与肌肉抑制素基因表达异常、蛋白加工障碍有关。

关键词:进行性肌营养不良;肌肉抑制素基因;蛋白加工

中图分类号:

文献标识码:

文章编号:
GAPDH

1.2.2 DNA Myostatin

DNA Myostatin p125\textsuperscript{5}AAACTGTAATA-ATCTTGCCATG-3\textsuperscript{5} CTGT-TCTCATTTAGATCCACTG-3\textsuperscript{5} DNA p125 p126 Myostatin

10 x PCR 2 μL 10 pmol/μL dNTPs 2 μL 5 mmol/L 5 μL DNA 50 ng/μL Taq DNA 1 μL 20 μL 94°C 5 min 94°C 30 s 55°C 30 s 72°C 1 min 30 s 72°C 10 min

0.8% BlastN

1.2.3 Myostatin cDNA PCR BlastN

1.2.4 Western blot Myostatin

50 mmol/L Tris-HClpH7.4 0.5 mmol/L DTT 150 mmol/L NaCl 50 μg/mL PMSF 10 μg/mL Leupeptin 10 μg/mL aprotinin 5 min −70°C 15 μg/mL SDS-PAGE 30 mA PVDF

10% TBS-T 1 h 1:2 000 Myostatin IgG Santa Cruz 1 h TBS-T 6 5 min HRP 1 h TBS-T 6 5 min SuperSignal Chemiluminescent Substrate 1 min

2

2.1

1 8

Fig. 1 Muscle biopsies of four muscular dystrophy patients by HE staining

2Hββ represent No. of cases N represents normal control.
2.2 Myostatin

For the study of the correlation between the muscle dystrophy gene and the progression of muscular dystrophy, this study first analyzed whether the patients involved in the experiment showed any mutations in the muscle dystrophy gene. According to the muscle dystrophy gene sequence (GenBank accession number: AF019627), primers 947 and 9476 were designed to amplify the patients’ muscle dystrophy gene using muscle biopsy tissue as template, and the PCR products were purified and sequenced. The results showed no mutations. The muscle dystrophy gene expression level analysis in normal and dystrophic muscle tissues was further conducted using semiquantitative PCR. Primers 947 and 9476 were used to amplify the muscle dystrophy gene using muscle biopsy tissue as template, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. After 30 cycles of amplification, the products were subjected to agarose gel electrophoresis. The results showed that all normal and dystrophic muscle tissues could amplify the muscle dystrophy gene, and except for case 6 (case 8), the expression level of the muscle dystrophy gene in the other cases was significantly lower than that of the normal muscle tissue. This result indicates that muscle dystrophy may be related to the processing failure of the muscle dystrophy protein.

2.4 Myostatin

For the study of the relationship between muscle dystrophy gene expression and the progression of muscular dystrophy, this study further analyzed the patients’ muscle dystrophy gene promoter region. Primers 947 and 9476 were used to amplify the gene promoter region using muscle biopsy tissue as template, and the PCR products were purified and sequenced. The results showed no mutations. It is speculated that the differences in various transcriptional regulatory factors play a key role in the downregulation of muscle dystrophy gene expression.

2.5 Myostatin

Fig. 3 Myostatin expression in skeletal muscle tissues from the patients and normal person by Western blot

1-8: No. of cases [9]: Normal control. The arrow showed three forms of myostatin.

3

Fig. 2 Expression level of Myostatin gene in skeletal muscle tissues from the patients and normal person by semi-quantitative RT-PCR method

Lanes from 1 to 8 are RT-PCR products with total RNA from muscle biopsy of eight patients as template. Lane 9 is that of the normal person. M is 100 bp DNA Ladder.
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References


