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Original article

Intron-retained transcripts of the spinal muscular atrophy genes, *SMN1* and *SMN2*

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Abstract

Background: The *SMN* genes, *SMN1* and *SMN2*, are highly homologous genes which are related to the development or clinical severity of spinal muscular atrophy. Some alternative splicing patterns of the *SMN* genes have been well documented. In 2007, an *SMN1* transcript with a full sequence of intron 3 was reported as the first intron-retained *SMN* transcript.

Methods: Intron-retained *SMN* transcripts in various cells and tissues were studied using reverse transcription (RT)-PCR. HeLa cells were used for subcellular localization of the transcripts and protein expression analysis with Western blotting.

Results: Two intron-retained *SMN* transcripts were detected, which contain full sequences of intron 2b or intron 3. These transcripts were produced from *SMN1* and *SMN2*, and ubiquitously expressed in human cells and tissues. Western blotting analysis showed no proteins derived from the intron-retained transcripts. Fractionation analysis showed that these intron-retained transcripts were localized mainly in the nucleus. Contrary to our expectation, the intron-retained transcript levels decreased during the treatment of cycloheximide, an inhibitor of nonsense-mediated decay (NMD), suggesting that they were not targets of NMD.

Conclusion: Intron 2b-retained *SMN* transcript and intron3-retained *SMN* transcript were ubiquitously expressed in human cells and tissues. The intron-retained transcripts were mainly localized in the nucleus and decreased through non-NMD pathway.

Keywords: Spinal muscular atrophy; *SMN*; Intron retention; Nonsense mediated decay

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1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disorder characterized by muscle weakness and atrophy. Genetic linkage analysis has mapped the SMA locus to chromosome 5q13 [1,2] and the survival motor neuron (*SMN*) gene has been identified as the responsible genes for SMA [3]. Two highly homologous copies of *SMN* are present in SMA locus, namely, *SMN1* and *SMN2* [3,4].

These two *SMN* genes are almost identical except for five nucleotide differences [3,5]. A single nucleotide difference in exon 7 alters the splicing pattern of both genes, resulting in exon 7 inclusion in *SMN1* transcripts and exon 7 exclusion in *SMN2* transcripts [6,7]. Inclusion or exclusion of exon 7 is not the only alternative splicing events in the *SMN* genes. Gennarelli et al. reported transcripts lacking exon 5 generated from *SMN1* and *SMN2* [8]. Singh et al. clarified the diversity of splice isoforms of *SMN* transcripts using a multiple-exon-skipping detection assay (MESDA) [9].

As for a distinct form of alternative splicing, intron retention, only one case has been reported until now for *SMN*. In 2007, Setola et al. described the first case of a transcript with intron 3 retention generated from *SMN1* (a-*SMN*) and its protein product, axonal-SMN (a-SMN) [10]. According to them, the a-SMN regulates axonogenesis of motor neurons [10]. This may be an example of "production of novel isoform with specific functions" through intron retention. Other intron-retained *SMN* transcripts, as well as a-*SMN* transcript, might have some function related to the pathogenesis of SMA.

It was commonly thought that intron retention is a form of mis-splicing, and that the intron-retained transcripts with premature termination codons (PTCs) may produce potentially harmful proteins. Hence, to prevent the intron-retained transcripts from being translated into potentially harmful products, these transcripts will be removed by nonsense-mediated decay (NMD) [11]. However, it has become increasingly known that intron retention also has several functions, such as regulation of gene expression via NMD and other mRNA decay mechanisms, production of novel isoform of RNA or protein for specific stages of development, providing signal for localization to specific cells/tissues, marking adjacent exons for splicing, etc. [12].

We confirmed the presence of two intron-retained *SMN* transcripts: one contained the full sequences of intron 2b and the other contained the full sequence of intron 3. The former has never been reported so far, and the latter was the same as the transcript which was identified by Setola et al. in 2007 [10]. To characterize the intron 2b-retained and intron 3-retained transcripts, we studied the expression levels of these transcripts in different cells and tissues as well as their intracellular localization and response to an NMD inhibitor (cycloheximide; CHX) in HeLa cells. We also examined in this study whether the plausible proteins are derived from the intron-retained transcripts in HeLa cells.

2. Materials and methods

2.1. HeLa, HEK293, HepG2 cell lines and fibroblasts

HeLa, HEK293 and HepG2 cell lines were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). These cells were confirmed to individually carry two copies of *SMN1* and two copies of *SMN2*. Control fibroblast cell line (Control fibroblasts) was established from a healthy male who carried 2 copies of *SMN1* and 2 copies of *SMN2*. SMA fibroblast cell line (SMA fibroblasts) was established from a female patient with SMA type 3, who had complete absence of *SMN1* but carried 3 copies of *SMN2*.

The HeLa, HEK293 and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM)–high glucose (Sigma-Aldrich, St. Louis, MO, USA) containing 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.25 μg/ml amphotericin B (Nacalai Tesque, Inc, Kyoto, Japan), and 8% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich) in 5% CO_2 at 37 °C. The fibroblast cell lines were maintained in DMEM–low glucose (Sigma-Aldrich) containing 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.25 μg/ml amphotericin B (Nacalai Tesque, Inc), and 10% heat-inactivated FBS (Sigma-Aldrich) in 5% CO_2 at 37 °C. Fibroblast cells were analyzed at 10–18 passages.

2.2. RNA from a human tissue panel

Intron-retained *SMN* transcripts in 20 tissues of Asian and Caucasian origins were analyzed using Human Total RNA Master Panel II (Clontech Laboratories, Inc., Mountain View, CA, USA).

2.3. cDNA synthesis, RT-PCR, electrophoresis and nucleotide sequencing

cDNA synthesis was performed at 55 °C for 30 min in a total volume of 20 μl containing 1 μg of total RNA, 60 μM of random hexamer primers, 1 mM dNTPs, 50 mM Tris/HCl, 30 mM KCl, 8 mM $MgCl_2$ pH 8.5, 20 U of protector RNase inhibitor and 10 U of Transcriptor reverse transcriptase (Roche Diagnostics GmbH, Mannheim, Germany).

An aliquot of cDNA, which was equivalent to 50 ng of total RNA extracted from the cells, was applied in a reverse-transcription PCR (RT-PCR) tube. In our RT-PCR experiments, Roche FastStart™ Taq DNA Polymerase (Roche Diagnostics) and KOD FX Neo

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