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Research Report

Identification of a new *RTN3* transcript, *RTN3-A1*, and its distribution in adult mouse brain ☆

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Abstract

The Reticulon (RTN) family of proteins is thought to play important roles in the regulation of neuronal regeneration. In this study, we have identified a novel alternative splicing isoform of the *RTN* gene family, *RTN3-A1*, which contains an additional 2.3-kb exon. The transcripts of human and mouse *RTN3-A1* (about 5.0 kb) were first discovered by database sequence mining and analysis, and verified by cloning and sequencing. Northern blot analysis of 16 human tissues with a common probe of *RTN3* transcripts and a specific probe for *RTN3-A1* demonstrated that human *RTN3-A1* is expressed mainly in brain tissues with a weak expression in the skeletal muscle. With Western blot analysis, the expected 100-kDa RTN3-A1 protein was detected in mouse brain. In situ hybridization with a mouse *RTN3-A1*-specific cRNA probe revealed that the mouse *RTN3-A1* mRNA was regionally expressed in the neurons of the cerebral cortex, hippocampus, hypothalamus, and cerebellum of the adult mouse brain. In contrast to the transcripts of *RTN1* and *RTN2*, *RTN3-A1* shares some significant similarity with *RTN4-A* in exon structure, tissue distribution, and brain expression profile. Since other reports have shown that RTN4-A inhibits neuronal outgrowth and restricts the plasticity of the central nervous system, we speculate that RTN3-A1 might play certain roles in the central nervous system.

Theme: Cellular and molecular biology
Topic: Gene structure and function: general

Keywords: Reticulon 3; Splicing isoform; In situ hybridization; Central nervous system; Nogo-A

1. Introduction

The Reticulon (RTN) family of proteins seems to be very important, since some of them have been shown to regulate the growth of certain types of cancer and to regulate neuro-outgrowth and -regeneration. Four members of the Reticulon (RTN) family have been identified in mammals: *RTN1*, *-2*, *-3*, and *-4/NOGO*. They all associate with the endoplasmic reticulum through a C-terminal reticulon-homolog domain, which consists of two large hydrophobic segments [15]. Each gene in this family has been reported to produce two or more alternative spliced forms. Except *RTN3*, each of them has been reported to have a long transcript, which is primarily expressed in the brain tissue [15].

RTN1 is the first identified mammalian *RTN* family member, known as *NSP* (Neuroendocrine-Specific Protein) gene [1,18]. Two isoforms of RTN1, RTN1-A and RTN1-B, aggregate as homo- and heteropolymers in small-cell lung carcinoma cell lines [18,20]. *RTN4* produces three transcripts (*RTN4-A*, *RTN4-B1*, and *RTN4-C*) [5,12,15]. RTN4-A has been reported as one of the few identified inhibitors of neuronal outgrowth and of regeneration of adult mammalian central nervous system [2,5,8,11,16,22], whereas RTN4-B1

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seems to regulate apoptosis in cancer cells [9,13,17,24]. Its shortest isoform, RTN4-C, can reduce the axonal regeneration rate in vivo [7].

Three transcripts of *RTN3* have been reported, but their functions remain unknown. Two of them, *RTN3-B1a* and *RTN3-B1b* (the 2.5-kb and 1.7-kb *RTN3* transcripts), have been found to share a common ORF (open reading fragment) and produce the same protein product, RTN3-B1 (originally referred to as RTN3). Both transcripts are ubiquitously expressed in almost all tissues, and their protein forms an interactive complex with RTN4-B1 in vitro [10,14,17]. The third transcript, named *RTN3-B2* in this article, consists of the full length of *RTN3-B1a* and an extra 57-bp exon [14]. In this study, we report the isolation, identification, and characterization of a novel long transcript of *RTN3*, *RTN3-A1*, in both humans and mice, which is highly expressed in brain tissues.

2. Material and methods

2.1. Sequence data mining and analysis

Using the sequence of human *RTN3-B1a* (reported *RTN3*, NM_006054), we mined expressed sequence tags (ESTs) in the GenBank database and discovered a mouse cDNA clone (CB519708). This cDNA fragment was encoded not only by the first exon of *RTN3* but also by a portion of its first intron. With its sequence, further BLAST (Basic Local Alignment Search Tool) search on the GenBank database was carried out and a series of human ESTs (ESTs:BI667331, AK127079, etc.) were revealed. These ESTs were then assembled to become a 4937-bp human *RTN3* contig, human *RTN3-A1* (AY750848). Another contig, human *RTN3-A2*, lacking a 57-bp exon of *RTN3-A1*, was also assembled (AY427821). Similarly, a 5013-bp putative mouse *RTN3* transcript, mouse *RTN3-A1* (AY750849), and a 4956-bp putative mouse, *RTN3-A2* (AY427822), were assembled too.

2.2. Tissue collection

Mouse brain, skeletal muscle, and liver tissues were collected from C57BL/6 mouse deeply anesthetized with halothane. These tissues were immersed in liquid nitrogen immediately. All procedures performed on animal during this study conformed to U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.3. Cloning and sequencing of human RTN3-A1

Human *RTN3-A1* was amplified from a Marathon-Ready™ brain cDNA Library (Clontech) with LA Taq™ DNA polymerase (TaKaRa) using the primer pair hPAF/hPFR (Table 1, Fig. 1). PCR condition was as follows: 4 min at 95 °C, 30 cycles of 25 s at 98 °C, 1 min at 60 °C, 10 min at 72 °C, followed by a final extension of 10 min at 72 °C. This 4.9-kb transcript was then subcloned into the pMD 18-T vector (TaKaRa) and was sequenced on an ABI PRISM sequencer using primer pairs hPAF/hPAR, hPBF/hPBR, hPCF/hPCR, hPDF/hPDR, hPEF/hPER, and hPFF/hPFR (Table 1, Fig. 1).

2.4. RT-PCR

Total RNA of mouse brain and skeletal muscle was isolated, respectively, from 100 mg brain tissue and 100 mg skeletal muscle tissue with TRIZOL LS Reagent (Gibco BRL). cDNA was synthesized using 10 μg of total RNA, 40 U of M-MLV reverse transcriptase (Promega), and 100 pmol of *RTN3-A1/RTN3-A2*-specific downstream primer RTPA2R (Table 1, Fig. 1) according to the manufacturer's instructions. PCRs were carried out using primer pairs RTPA1F/RTPA1R and RTPA2F/RTPA2R (Table 1, Fig. 1) on the cDNA synthesized above. These fragments were cloned and sequenced.

Table 1
The primers for cloning of *RTN* fragments

Name	Sequence	Name	Sequence
RTN3F	GTATCTCTTTTCACCCTTCTCC	RTN3R	CTGTTGCATTTCTGGTTTCCATG
RTN4F	TTCAAGTACCAGTTCGTGAGGGAGC	RTN4R	AATGATCTATCTGTGCCTGATGCCG
hPAF	GAGTCAGTCAGTCTGTCGGAGTC	hPAR	GAGTACTCAGTAGGTGGTTTCTG
hPBF	TCTTGCAGCAGGAGTTCATTGTG	hPBR	TTCTGCATATTGCCTTGCACACC
hPCF	AGGTGATTGGGCAGAAGCATCTC	hPCR	GAAGTCTGTCAGAAGTCTTGCTAG
hPDF	CTCCAGTAGCATCTCTTGACTTAG	hPDR	CCTTCTTCTGACTTCTGTACAGC
hPEF	TGGCACCACGCTGATCATGCTGC	hPER	GAGTATCAGGGGTAGCTGCTCAC
hPFF	GTGGTAGAGCCTTTACCTGTAGC	hPFR	AAGACAATCACTGACTTCCTGGG
RTPA1F	CCCTACGTCTCTCTTCACCCTTC	RTPA1R	GCAAAGAGAGCTCAATCCCGCTTG
RTPA2F	TGCGGCTCCTCGTGTGCGGCG	RTPA2R	AAATGTGTTCTGCCTTAGACTGCCC
ISHup	cagagatgcaATTAACCCTCACTAAAGGAGAATTC**ccacaggtgactggacagaagc**		
ISHdn	ccaagcttcTAATACGACTCACTATAGGG**ctgtgatgtcctctattactgtg**		

The primers for cloning of *RTN3* and *RTN4* fragments. Of primers ISHup and ISHdn, the RNA polymerase promoter sequences were shown in upper case and the mouse *RTN3-A1* sequences were shown in regular bold font.