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.
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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

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.
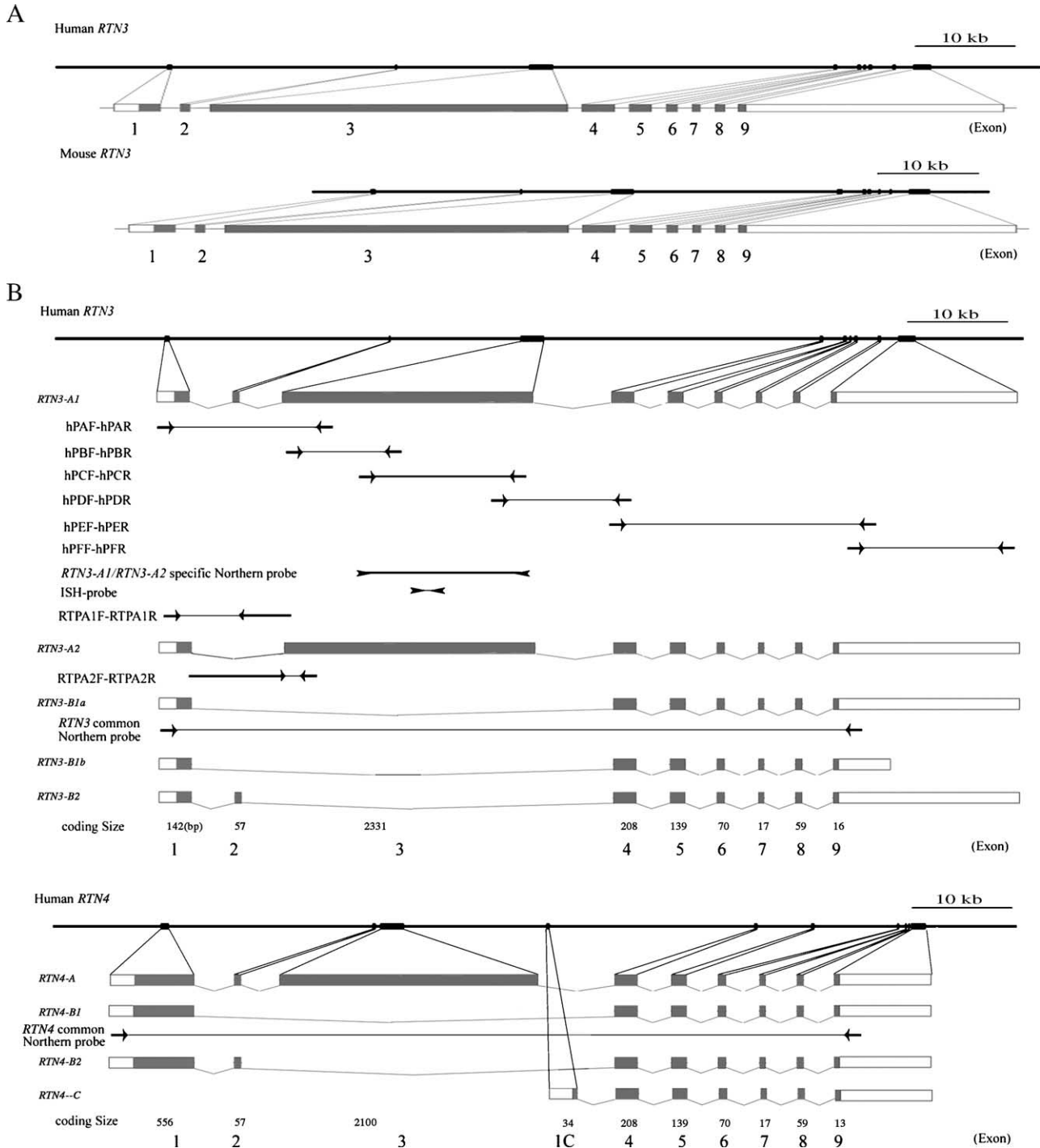
Fig. 1. Gene structure of human/mouse RTN3 and the comparison of RTN3 transcripts with RTN4 transcripts. Panel A shows the gene structure of human and mouse RTN3. Each of them has nine exons. Human RTN3 is about 78.5 kb and mouse RTN3 is about 57 kb. Panel B compares the genome structures of human RTN3 and human RTN4. The genomic structure of RTN3 is very similar to that of RTN4. Five transcripts so far have been discovered to be derived from the RTN3 gene. RTN4 has three main transcripts: RTN4-A, RTN4-B1, and RTN4-C. Panel B also shows the location of the primer pairs and probes on the corresponding regions of human RTN3. The solid boxes refer to the translated segments of each gene. The white boxes refer to the 5′ UTR (untranslated region) or 3′ UTR.

2.5. Northern blot analysis

The RTN3-B1a ORF fragment from a part of its 1st exon to 3′ terminal (778 bp, nucleotides 147–924) was amplified on a human brain cDNA library (Gibco BRL) with primer pair RTN3F/RTN3R (Table 1, Fig. 1). This RTN3-B1a fragment could recognize RTN3-A1, RTN3-A2, RTN3-B1, and RTN3-B2. The RTN4 transcript-recognizing fragment

was amplified on a human brain cDNA library (Gibco BRL) with primer pair RTN4F and RTN4R (Table 1, Fig. 1). This fragment was 970 bp from a part of the 1st exon to the 3′ terminal of *RTN4-B1* (NM_153828, nucleotides 365–1334). The human *RTN3-A1/RTN3-A2*-specific fragment was amplified from the 3rd exon of *RTN3* with primer pair hPCF/hPCR from the human *RTN3-A1* clone that we had obtained. The length of this fragment was 1321 bp. All these

fragments were cloned and sequenced. On a Northern blot membrane (Clontech) containing the mRNAs of 16 human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocyte) and under the stringent conditions described previously [23], Northern blot hybridizations were carried out separately using α-[^{32}p]-dATP-labeled (Random Primers labeling Kit,
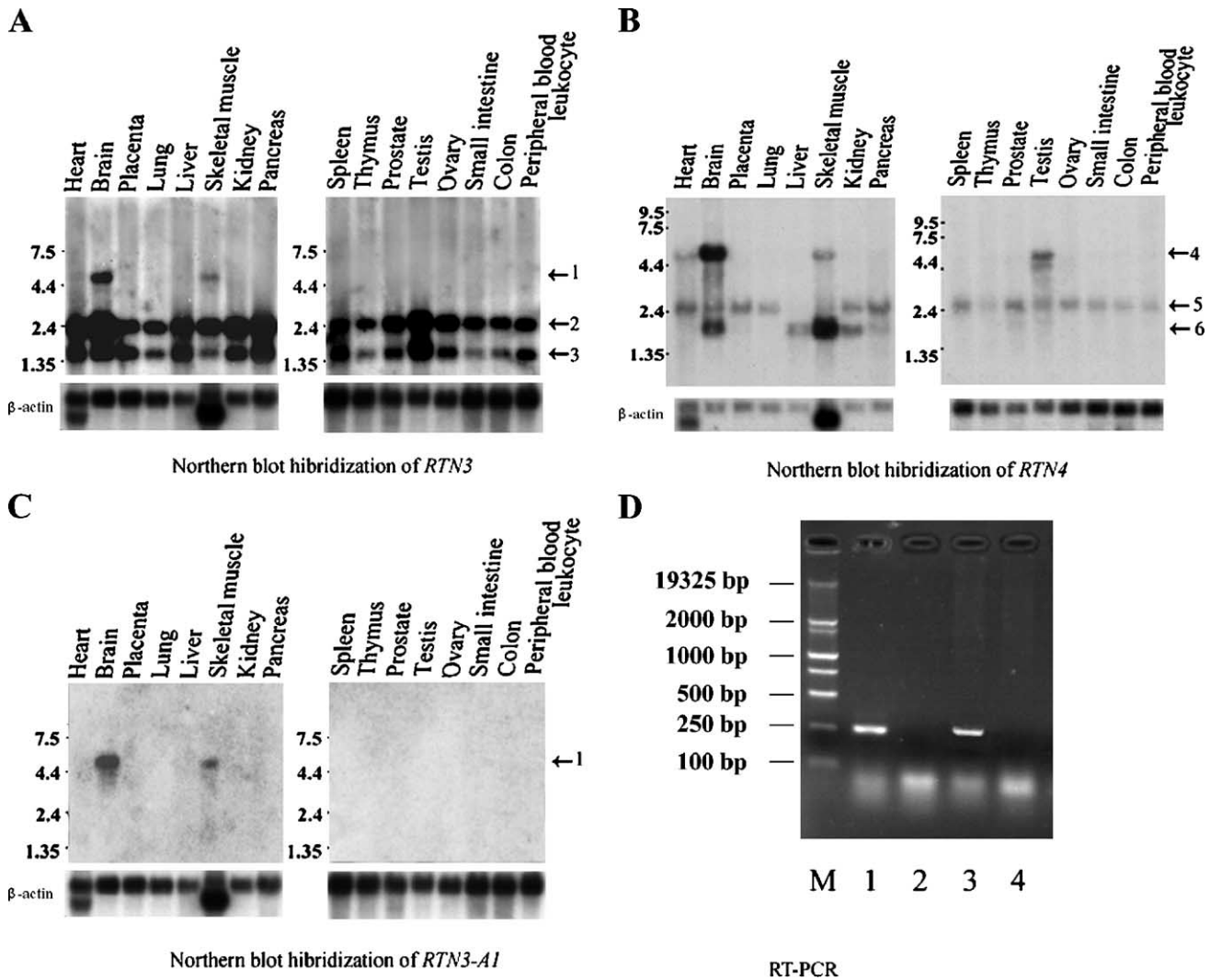

Fig. 2. Expression profile of *RTN3-A1* and the comparison of expression profile of *RTN3* transcripts with *RTN4* transcripts. Panel A illustrates three main transcripts of *RTN3*: 4.9 kb, 2.6 kb, and 1.7 kb. Panel B illustrates three main transcripts of *RTN4* in different tissues: 4.9 kb, 2.7 kb, and 1.7 kb. Panel C: Northern blot analysis with a portion of the third exon of *RTN3* as a probe, which is specific for *RTN3-A1* and *RTN3-A2*, confirms that this 4.9-kb *RTN3* transcript is *RTN3-A1*, considering that RT-PCRs excluded the expression of *RTN3-A2* in brain and skeletal muscle tissue (see below). Panel D illustrates the RT-PCR results separately using *RTN3-A1*- and *RTN3-A2*-specific primer pairs. The 267-bp *RTN3-A1*-specific fragment is detected in normal brain tissue and skeletal muscle tissue while no *RTN3-A2* expression is detected in these tissues. The *RTN3-A2*-specific primer pair RTPA2F/RTPA2R was validated (data not showed). M refers to the DNA marker ladders; lanes 1 and 2 are the PCR product from mouse brain cDNA pool; lane 3 and lane 4 are from skeletal muscle cDNA pool; lane 1 and lane 3 are with *RTN3-A1*-specific primer pair RTPA1F/RTPA1R; lanes 2 and 4 are the PCR product with *RTN3-A2*-specific primer pair RTPA2F/RTPA2R. Together with the expression profile of the 4.9-kb *RTN3* transcripts and the result of cloning and sequencing, this evidence indicates that *RTN3-A2* is not expressed in the tissues we investigated here. Therefore, the fragments shared exclusively by *RTN3-A1* and *RTN3-A2* can be regarded as *RTN3-A1*-specific, and *RTN3-A2* is not under discussion in the expression study in this article. Altogether, the 4.9-kb band represents the expression of *RTN3-A1*. *RTN3-A1* has a high expression in brain tissue with a slight expression in skeletal muscle tissue (arrow 1 in panels A and C, 4.9 kb). Besides a weak expression in testis, *RTN4-A* is strongly expressed in the brain tissue and slightly expressed in the skeletal muscle tissue (arrow 4 in panel B, 4.9 kb). *RTN3-B1a/RTN3-B1b* and *RTN4-B1* are almost expressed in all the tissues tested. Arrow 2 shows *RTN3-B1a/RTN3-B2* (2.6 kb), arrow 3 shows *RTN3-B1b* (1.7 kb), arrow 5 shows *RTN4-B1* (2.7 kb), and arrow 6 shows *RTN4-C* (1.7 kb).

Amersham) probes obtained from the fragments prepared above: the 778-bp fragment of human *RTN3-B1a*, the 970-bp fragment of human *RTN4-B1*, and the 1321-bp human *RTN3-A1/RTN3-A2*-specific fragment. β-actin probe was used as a control.

2.6. Western blot analysis

Protein extractions from mouse liver and brain were isolated with the protocol reported [25]. Proteins were separated by electrophoresis on a 10% polyacrylamide gel containing 0.1% SDS, and were transferred to a nitro-cellulose membrane (Advantech, Tokyo) for Western blot analysis. The polyclonal antibody (Santa Cruz) against the common C-terminal 19 amino acids of human and mouse RTN3 isoforms (SIVEKIQAKLPGIAKKKAE) was applied (diluted 1:300) afterward, followed by horseradish perox-idase (HRP)-conjugated donkey anti-goat IgG (SIGMA). To confirm the amount of total protein loaded in each lane, blots were hybridized afterward with a monoclonal β-actin antibody (SIGMA). Signals were visualized though auto-radiography after treatment with the ECL detection system (Amersham Pharmacia Biotech).

2.7. In situ hybridization

To prepare the probes for *RTN3-A1* in situ hybridization, T3 and T7 RNA polymerase promoter sequences were separately constructed upstream and downstream of a 282-bp mouse *RTN3-A1/RTN3-A2*-specific fragment, which was generated by PCR with primer pair ISHup/ISHdn (Table 1, Fig. 1) on T-vector carrying the mouse *RTN3-A1/RTN3-A2*-specific fragment we obtained. The resulting PCR product was cloned and its sequence was then confirmed. The mouse *RTN3-A1/RTN3-A2*-specific DIG-labeled antisense and sense cRNAs were obtained via in vitro transcription directed by the T3 and T7 promoters (Promega), respec-tively, with the DIG-labeled cytidine triphosphate (Roche) according to the in vitro transcriptional procedure [3]. Cryosections (10 μm) were prepared from adult C57BL/6 mouse brain as previously described [21]. The in situ hybridization process followed the described method [21]. Observed and evaluated with a light microscope, the purple hybridization signals were shown at the sites where the DIG-dUTP-labeled probe had bound to mouse *RTN3-A1* mRNA. As a control, adjacent sections were hybridized with the sense probe complementary to the antisense probe.

3. Results

3.1. Sequence characteristics of RTN3-A1

We cloned the 4937-bp sequence of *RTN3-A1* in human brain tissue and confirmed its sequence. *RTN3-A1* consists of all of the nine exons of *RTN3* (Fig. 1). Among these nine

exons, the characteristic 2.3-kb large exon is newly reported here. Sharing the same initiation and stop codon as human *RTN3-B1a*, human *RTN3-A1* contains a 3099-bp ORF encoding a 1032 amino acid protein with a predicted molecular weight of 112 kDa.

RTN3-A1 carries a conservative 3′ terminal region (exons 4–9) as the other family members (the similarity is about 80%) (Fig. 1). Preceding these exons, both *RTN3-A1* and *RTN4-A* have a large exon (about 2.4 kb) following a 57-bp mini exon. However, the homology of the encoded amino acid sequence between the 2.4-kb exons of *RTN3-A1* and of *RTN4-A* is very low (about 20%), which is also true for the amino acid sequence encoded by the 57-bp exon in *RTN3-A1* and *RTN4-A* (about 30%). Therefore, the sequence homology between protein RTN3-A1 and RTN4-A is limited to the RTN domain at the C-termini. This is consistent with homology comparison among the other RTN members [15].

The 57-kb mouse *RTN3* gene shares a similar exon–intron structure to the 80-kb human *RTN3* gene in genome (Fig. 1A). With the same initiation and stop codon as mouse *RTN3-B1a*, mouse *RTN3-A1* possesses a 2895-bp ORF corresponding to a 101-kDa mouse RTN3-A1. The result of Western blot analysis verified the existence of *RTN3-A1* in mouse brain (Fig. 3). The full length of human and mouse RTN3-A1 amino acid shares 62% of the sequence. However, the homology of the 3′ termini between these two proteins is much higher (93%) than that of the 5′ termini (56%). This indicates that the
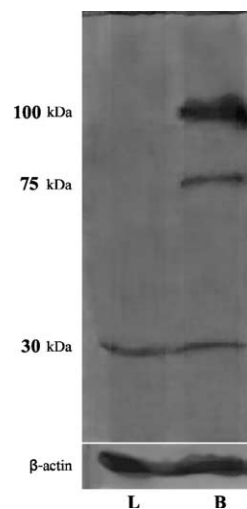

Fig. 3. Western blot analysis. With a polyclonal antibody recognizing the common 19 amino acids of the N-termini of mouse RTN3 proteins (including RTN3-A1 and RTN3-B1/RTN3-B2), a 100-kDa protein band is demonstrated in brain tissue, which corresponds to the mouse RTN3-A1 (predicted molecular weight 101 kDa). A band of about 30 kDa with a proximate molecular weight of RTN3-B1/RTN3-B2 (predicted 27 kDa) is also detected in the liver and brain. Additionally, an unidentified band with apparent relative molecular mass at 75 kDa is observed in brain tissue. Because its intensity is much less than that of RTN3-A1, this band may be the modified RTN3-A1 or another unidentified isoform of RTN members (L is for Liver and B for brain tissue).

5′ terminal of *RTN3-A1* evolutes faster than the 3′ terminal among different species.

3.2. Transcripts of RTN3 and their denomination

So far, five transcripts derived from human *RTN3* have been found: *RTN3-A1*, *RTN3-A2*, *RTN3-B1a*, *RTN3-B1b*, and *RTN3-B2*. The 4.9-kb *RTN3-A1* transcript is the new and longest one. It is named as human *RTN3-A1* according to the traditional nomenclature of the *RTN1*, *-2*, and especially the *RTN4* [15]. The 2nd new transcript *RTN3-A2* possesses all exons of *RTN3-A1* except the 57-bp one. Since *RTN3-A2* has been found only in melanoma, this transcript would be a minority. The 2.5-kb *RTN3* transcript (NM_006054) and the 1.7-kb *RTN3* transcript (BC010556) share a common ORF and may produce a 27-kDa identical product; the reason is that the same exons of gene *RTN3* are shared by these two transcripts and the 2.5-kb *RTN3* transcript has a much longer untranslated 3′ region [17]. Their common protein product is labeled RTN3-B1, and the 2.5-kb, 1.7-kb transcripts are labeled *RTN3-B1a* and *RTN3-B1b* respectively. In addition, another human *RTN3* transcript (BK001684) was submitted to GenBank [14]. This transcript consists of the full-length sequence of *RTN3-B1a* plus an extra 57-bp exon (Fig. 1), and it is renamed as *RTN3-B2* in this study. Human *RTN3* and mouse *RTN3* are alternatively spliced and expressed in a similar manner. Therefore, this nomenclature of human *RTN3* is also applied to mouse *RTN3* transcripts.

3.3. Tissue expression pattern of RTN3-A1

Northern blot analysis and RT-PCR result revealed that the 4.9-kb *RTN3-A1* was expressed mainly in brain tissue with a weak expression in skeletal muscle tissue; no *RTN3-A2* expression was detected in normal tissues studied (Fig. 2). In addition, the expression profile of protein RTN3-A1 verified the expression of its mRNA in brain tissue (Fig. 3). Furthermore, considering the established in vitro interaction of RTN3-B1 and RTN4-B1 [17], together with the similar genome structure of *RTN3* and *RTN4* (Fig. 1), we carried out a Northern blot analysis with common probes against the *RTN4* transcripts in order to have an in-parallel comparison of tissue expression profile between *RTN3* and *RTN4* transcripts. The result showed that *RTN3-A1* and *RTN4-A* did share a similar
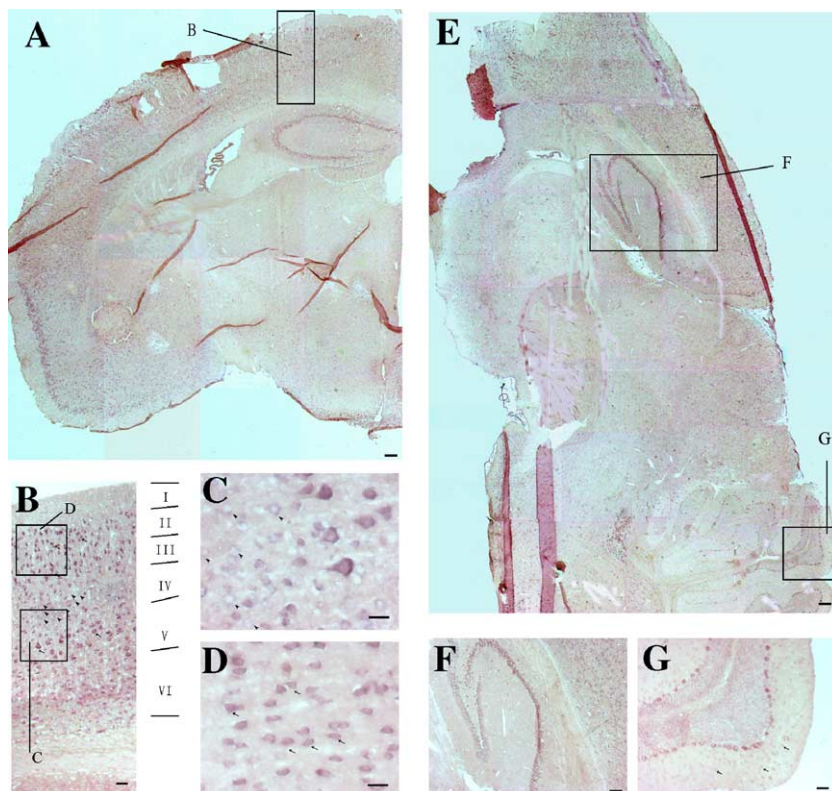

Fig. 4. Distribution of *RTN3-A1* in adult mouse brain. In situ hybridization with the *RTN3-A1*-specific probe demonstrates the expression of mouse *RTN3-A1* in the cerebral cortex, hippocampus, cerebellum, and hypothalmatic region. Panel A illustrates a coronal section and panel E illustrates a sagittal section. In the cerebral cortex, the bodies of pyramidal cells in layer V and the bodies of granule cells in layer II/III show strong signals (arrow in panel B, D). Some cells with round nucleus in layer IV are also weakly stained (arrowhead in panel B, C). These stains might indicate the expression of *RTN3-A1* in oligodendrocytes, but further investigation is needed before a clear conclusion can be drawn. On the other hand, no signal is shown in layer I (panel B). In the hippocampus, a high level of *RTN3-A1* mRNA is found in pyramid cells of regions CA1–CA4 and lower signals are detected in the granule neurons of the dentate gyrus (panel F). In the cerebellum area, the Purkinje cells are strongly stained (panel G). Some glia cells in the molecular layer also show slight signals (arrows in panel G). (Scale bar: 150 μm in panels A and E; 100 μm in panel F; 50 μm in panels B and G; 20 μm in panels C and D).

tissue expression profile (Fig. 2B). This was also true for RTN3-B1a/RTN3-B1b and RTN4-B1.

3.4. Distribution of RTN3-A1 in adult mouse brain

To further characterize the expression of RTN3-A1 in mouse brain, an in situ hybridization was accomplished. The results showed that mouse RTN3-A1 was regionally expressed in the neurons of the cerebral cortex, hippocampus, hypothalamus, and cerebellum of adult mouse brain (Fig 4). Additionally, some unidentified staining is also detected.

4. Discussion

In this report, we identified the human and mouse RTN3-A1, and redefined the early reported transcripts of the RTN3 gene [10,17] according to the nomenclature tradition of the RTN gene family. Additionally, we demonstrated the expression profile of RTN3-A1 in human tissues and its distribution in mouse brain. Moreover, we found that some characters are shared by RTN3-A1 and RTN4-A, especially the transcript distribution and exon−intron structure. This might indicate a close relationship between RTN3-A1 and RTN4-A.

RTN3 and RTN4 have a similar genome structure (Fig. 1). The human genes RTN3 and RTN4 are both about 80 kb. As for their intron−exon structure, except for exon 1C that is exclusively expressed in RTN4-C, the size of the protein-encoding region of exons in RTN3 is almost the same as that of the corresponding exon in RTN4, especially the 57-bp second exon and the 2.4-kb third exon of both RTN3-A1 and RTN4-A. By contrast, human gene RTN1 is about 270 kb

and RTN2 is only about 12 kb [19]. Except for the exons encoding the reticulon domain being the same size as the corresponding exon of different RTN members, no obvious characteristic of RTN2 or RTN1 was shared by other mammalian RTN members in the exon−intron structure (data not show).

In contrast to the expression of RTN1 and RTN2 transcripts, RTN3-A1 and RTN4-A share a similar expression profile. In human tissues, except for the low-level expression of RTN4-A in testis, both RTN4-A and RTN3-A1 are expressed mainly in brain and weakly in skeletal muscle tissue among the examined samples (Fig. 2). However, RTN1-A is expressed mainly in brain tissue and slightly in placenta, lung, pancreas, spleen, prostate, testis, and peripheral blood leukocyte. RTN1-C is expressed mainly in brain tissue and slightly in testis and small intestine. RTN2-A is expressed almost exclusively in brain tissue with slight expression in small intestine and colon tissue, and RTN2-B is expressed mainly in skeletal muscle and weakly in heart, placenta, skeletal muscle, pancreas, spleen, prostate, testis, small intestine, and colon tissues [15] (Table 2).

Additionally, the expressions of RTN3-A1 and RTN4-A are overlapped in the regions and cells of adult brain tissue [6] (Fig. 4). In the neocortex, their mRNAs were detected in layers II−VI. In the hippocampus, their expressions were found in the pyramid cell of CA1−CA4 with a weaker signal in the granule cells shown in the dentate gyrus. In the cerebellum, their obvious expressions were discovered in the Purkinje cells.

It is established that RTN3-B1 and RTN4-B interact with each other as a complex [25]. This is consistent with the parallel expression of RTN3-B1 and RTN4-B1 (Table 2). Since RTN3-A1 possesses the full-length amino acid

Table 2
Tissue expression of the main RTN transcripts

Tissue	Transcript								
	RTN3-A1	RTN3-B1	RTN4-A	RTN4-B1	RTN4-C	RTN1-A	RTN1-C	RTN2-A/B	RTN2-C
Heart		+++		+++					++
Brain	+++	+++	+++	+++	++	+++	+++	+++	
Placenta		+++		+++		+/−			+
Lung		+++		+++	+	+			
Liver		+++							
Skeletal muscle	+	+++	+	+++	+++				+++
Kidney		+++		+++					
Pancreas		+++		+++		+/−			+
Spleen		+++		+++		+			+/−
Thymus		+++		+++					
Prostate		+++		+++		+/−			++
Testis		+++	+	+++		+/−	+/−		+/−
Ovary		+++		+++					
Small intestine		+++		+++			+	+/−	+/−
Colon		+++		+++				+/−	+/−
Peripheral blood leukocyte		+++		+++		+/−			

Tissue expression of the main human RTN transcripts. "+" shows the positive signal of the expression. More "+" shows stronger expression. "−" shows slight expression which sometimes cannot detected. The data are based on our Northern blot analysis (some pictures not shown), of which the early reported transcripts are generally consistent with the previous result [1,23].

sequence of RTN3-B1 and RTN4-A possesses that of RTN4-B1 (Fig. 1), obviously, the domains that are responsible for the interaction clearly are separately covered by RTN3-A1 and RTN4-A. Together with their similar expression pattern in tissues and brain cells, RTN3-A1 might interact with RTN4-A and might be involved in the function of RTN4-A in the central nervous system.

Note, when the present manuscript was under review, different evidences with similar conclusions and an alternative nomenclature were also described on *RTN3* alternative splicing forms [4].

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