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Molecular cloning and tissue-specific expression analysis of mouse spinesin, a type II transmembrane serine protease 5☆,☆☆

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Abstract

We have previously reported novel serine proteases isolated from cDNA libraries of the human and mouse central nervous system (CNS) by PCR using degenerate oligodeoxyribonucleotide primers designed on the basis of the serine protease motifs, AAHC and DSGGP. Here we report a newly isolated serine protease from the mouse CNS. This protease is homologous (77.9% identical) to human spinesin type II transmembrane serine protease 5. Mouse spinesin (m-spinesin) is also composed of (from the N-terminus) a short cytoplasmic domain, a transmembrane domain, a stem region containing a scavenger-receptor-like domain, and a serine protease domain, as is h-spinesin. We also isolated type 1, type 2, and type 3 variant cDNAs of m-spinesin. Full-length spinesin (type 4) and type 3 contain all the domains, whereas type 1 and type 2 variants lack the cytoplasmic, transmembrane, and scavenger-receptor-like domains. Subcellular localization of the variant forms was analyzed using enhanced green fluorescent protein (EGFP) fusion proteins. EGFP-type 4 fusion protein was predominantly localized to the ER, Golgi apparatus, and plasma membrane, whereas EGFP-type 1 was localized to the cytoplasm, reflecting differential classification of m-spinesin variants into transmembrane and cytoplasmic types. We analyzed the distribution of m-spinesin variants in mouse tissues, using RT-PCR with variant-specific primer sets. Interestingly, transmembrane-type spinesin, types 3 and 4, was specifically expressed in the spinal cord, whereas cytoplasmic type, type 1, was expressed in multiple tissues, including the cerebrum and cerebellum. Therefore, m-spinesin variants may have distinct biological functions arising from organ-specific variant expression.

Keywords: Spinesin; TMPRSS5; Serine protease; Splicing variant; Spinal cord

Serine proteases have important roles in various kinds of biological processes in the central nervous system (CNS) and blood coagulation system. In the last few years, type II transmembrane serine proteases (TMPRSS) have been cloned and reported as enterokinase [1], hepsin [2], human airway trypsin-like protease (HAT) [3], corin [4,5], epithin [6], matriptase [7], TMPRSS2 [8,9], TMPRSS3 [10,11], and seprase [12], tumor-associated differentially expressed gene (TADG)-12 [13], and TAGD-15 [14]. These TMPRSS contain common structures: a short cytoplasmic domain, a transmembrane domain, a stem region, and a serine protease domain. The stem region contains various modulatory elements such as a frizzled domain, SEA domain, CUB domain, MAM domain, low-density lipoprotein (LDL)-receptor class A

☆ *Abbreviations:* TMPRSS, type II transmembrane serine proteases; EGFP, enhanced green fluorescent protein; ER, endoplasmic reticulum.

☆☆ *Data deposition.* The nucleotide sequences reported in this paper have been deposited in the DDB/GenBank/EMBL databases (Accession Nos. AB041037, AB016230, AB016229, and AB016423).

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domain, and scavenger-receptor domain [15]. The functions of these domains have not yet been clarified, although they may play critical roles in intracellular signaling and the recognition of other molecules, such as proteolytic substrates and inhibitors. It is speculated that these various domain structures facilitate the diverse biological functions of the serine proteases.

In the CNS, serine proteases such as thrombin and tissue-type plasminogen activator (tPA) play important roles in neurite outgrowth [16,17], cell migration [18,19], long-lasting potentiation [20,21], learning and memory [22–24], excitotoxic cell death [25–27], and regeneration or recovery from injury to the nervous system [28]. To elucidate the functions of serine proteases in the CNS, we have isolated novel serine proteases expressed in the brain and spinal cord, such as neurosin/PRSS9 [29–32], neuropsin [33], hippostasin/PRSS20 [34–37], motopsin/PRSS12 [38,39], and spinesin/TMPRSS5 [40]. Neurosin and hippostasin, the genes of which are found on chromosome 19ql3.3, are secreted and belong to the kallikrein-like serine protease family. We confirmed the organ-specific alternative splicing of hippostasin/KLKll [33–37]. Motopsin, the gene for which is located on chromosome 5, has a complicated structure similar to that of TMPRSS, including (from the N-terminus) a proline-rich domain, a kringle domain, three scavenger-receptor cysteine-rich domains, and a protease domain. However, motopsin has a putative signal sequence at the N-terminus with no obvious hydrophobic transmembrane domain and thus appears to be a secreted protease [37]. On the other hand, spinesin/TMPRSS5 has a type II transmembrane structure, consisting of (from the N-terminus) a short cytoplasmic domain, a transmembrane domain, a stem region containing a scavenger-receptor-like domain, and a serine protease domain [40]. In this paper, we report the isolation of mouse spinesin, a human spinesin homolog, and the characterization of its variant forms.

Materials and methods

Preparation of total or messenger RNAs from mice tissues. Mice tissues were isolated from BALB/c or BDFl mice (CLEA Japan, Tokyo, Japan) that had been perfused with 0.1 M phosphate buffer (pH 7.4). The tissues were preserved at −80 °C after freezing in liquid nitrogen. Total RNAs from various tissues were prepared with TRIzol (Invitrogen, Carlsbad, CA), followed by purification with an RNeasy Mini Kit (Qiagen GmbH, Germany). mRNAs were purified with oligo(dT)-cellulose (Amersham Biosciences, Little Chalfont, UK) from the total RNAs of various tissues.

Isolation of mouse spinesin cDNAs. To determine the nucleotide sequence of full-length mouse spinesin mRNA, we performed 3′- and 5′-rapid amplification of cDNA ends (RACE) using the Rapid Amplification of cDNA Ends Kit (Invitrogen) and the Marathon cDNA Amplification Kit (Clontech, CA), respectively. For 3′-RACE, the first-strand cDNA was synthesized from mouse brain, spinal cord, and preputial gland mRNAs using an adaptor primer, 5′-GGCCACG CGTCGACTAGTACT$_{17}$-3′. The cDNA was first amplified between forward primer 1 (5′-ATGGTGGAGAAGATCATTCCT-3′) and the adaptor primer, and then reamplified using forward primer 2 (5′-TA CAGTGCCCAGAACCATG-3′) and the adaptor primer. 5′-RACE was performed according to the manufacturer's instructions. Briefly, double-stranded (ds) cDNAs were synthesized with reverse primer 1 (5′-CTGTTGCAGAGGTGGGTGCTGA-3′), followed by ligation of the Marathon cDNA adaptor. The RACE polymerase chain reaction (PCR) was performed using reverse primer 1 and the adaptor primer 5′-CCATCCTAATACGACTCACTATAGGGC-3′. 5′- and 3′-RACE products were cloned into pGEM-T Easy vector (Promega, WI), and then sequenced using an automatic DSQ-1000 sequencer (Shimadzu, Kyoto, Japan).

Analysis of cellular localization. To investigate m-spinesin localization, the coding regions of m-spinesin type 1 and type 4 were amplified using the primer sets, 5′-GAAGATCTATGGAAGCC CAGGTAGGGC-3′/5′-TGGAATTCTGCTTCTTCGGCTAGCG-3′ and 5′-GAAGATCTATGAGTCCAACACTGGATGA-3′/5′-TGGA ATTCTGCTTCTTCGGCTAGCG-3′, respectively. PCR products were digested with *Bgl*II and *Eco*RI, and then inserted into the *Bgl*II–*Eco*RI sites of pEGFP-Cl (Clontech). The constructed plasmids were transfected into COS-1 cells with LipofectAmine (Invitrogen). The transfected cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (HyClone, UT) for three days. The enhanced green fluorescent protein (EGFP) fusion protein was visualized using an Olympus inverted fluorescence microscope (Olympus, Tokyo, Japan), and images were captured using Pro600ES (Pixera, CA).

Analysis of splicing variants in various tissues. Total RNAs from various tissues were prepared as described above. cDNAs were synthesized with ThermoScript reverse transcriptase (Invitrogen). To detect mouse spinesin splicing variants, we designed primer sets specific for each variant (Table 1). As a positive control for RNA preparation and cDNA synthesis, we performed a RT-PCR of glycerol-3-phosphate dehydrogenase (GPDH), using the primer set (5′-GGCATT GCTCTCAATGACAA-3′/5′-TGTGAGGGAGATGCTCAGTG-3′). PCR was carried out for 35–40 cycles (30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C) using r*Taq* DNA polymerase (ToYoBo, Osaka, Japan).

Results

Cloning mouse spinesin and its variants

We isolated novel serine proteases from cDNA libraries constructed from human and mouse CNS, using PCR with degenerate oligodeoxyribonucleotide primers designed on the basis of the serine protease motifs, AAHC

Table 1
PCR Primes

Name	Sequence
mBSSP2F5	5′-ATAGTTGGCGGCCAAGCTGT-3′
mBSSP2R5CGC	5′-GCGGACCTGCACAGTGTCAT-3′
Type 1 F	5′-CTTTCAGAATAAATGGAGAGG-3′
Type 1 R	5′-TAACCCACAGAAGCCCTACC-3′
Type 2 F	5′-GCTGGGCTGTTGAATCAATC-3′
Type 2 R	5′-CGTCCCAGAGATGGATGGAGA-3′
Type 3 F	5′-GCTGCTGGATCTTCAACCAC-3′
Type 3 R	5′-CCTCCATTTATTCTGAAAGATAC-3′
Type 4 F	5′-GCTGACTCATATGAGTCCAAC3′
Type 4 R	5′-CCTCCATTTATTCTGAAAGATAC-3′

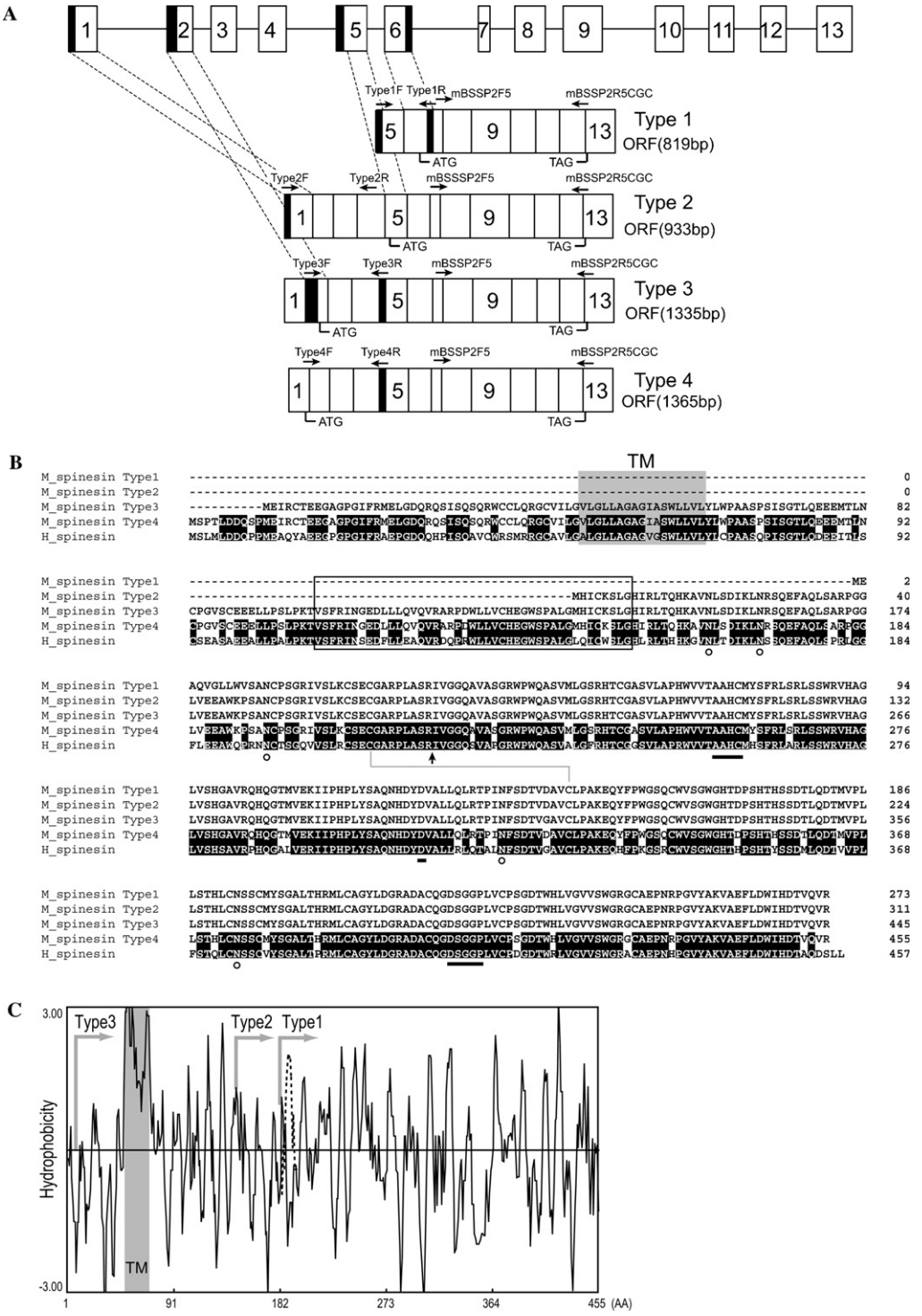

Fig. 1. The structures of m-spinesin and its variants. (A) The genomic structure and variant mRNAs of m-spinesin are schematically shown: the characterized exons of m-spinesin are indicated with boxes. The sites of insertions and deletions are shown with closed boxes. The putative open reading frames (ORF) of these variants are indicated by initial and terminal codons, and are 819, 933, 1335, and 1365 bp in length for the type 1, 2, 3, and 4 variants, respectively. Arrows indicate the sites of primer sets used for RT-PCR, as described in Table 1. (B) Amino acid sequences encoding m-spinesin variants are aligned with that of h-spinesin. The amino acid residues conserved between m-spinesin type 4 and h-spinesin are represented with white letters. The transmembrane and scavenger-receptor-like domains are indicated with a shaded box and an open box, respectively. The essential triads, the putative processing sites, and the putative N-glycosylation sites are marked with bars, arrows, and open circles, respectively. The putative disulfide bridge within the protease domain is shown with a connecting line. (C) The hydropathy plot of m-spinesin type 4 was calculated with the Kyte and Doolittle method with averaging over a window of five residues [43]. A putative transmembrane domain is shown with a shaded box. Arrows indicate the locations of the putative initiation codons of m-spinesin variants.

and DSGGP. A 459 bp DNA fragment was amplified and cloned into the pGEM-T Easy vector, followed by nucleotide sequence analysis. The nucleotide sequence revealed that this DNA fragment encodes a novel serine protease. To characterize this serine protease, we isolated the cDNA clone encoding this protein from mouse brain, spinal cord, and preputial gland using 5′- and 3′-RACE-PCR. Using primer 2 and the adaptor primer, we isolated the longest clone of the 3′-RACE products, which was 1519 bp in length. The 5′-RACE product was 1021 bp in length and overlapped the 5′ end of the 3′-RACE product, indicating that the apparent full-length cDNA of this novel serine protease is 2265 bp in length (Fig. 1A, type 4).

An open reading frame (ORF) of 1365 bp, encoding a polypeptide of 455 residues, was predicted by a Kyte–Doolittle hydropathy plot to contain a transmembrane region (Fig. 1C). A homology search indicated that this polypeptide is homologous to human spinesin (h-spinesin)/TMPRSS5, previously reported by Yamaguchi et al. [40] (Fig. 1B). h-Spinesin is predominantly expressed in the human brain and spinal cord, and is composed of (from the N-terminus) a short cytoplasmic domain, a transmembrane domain, a stem region containing a scavenger-receptor-like domain, and a serine protease domain. Full-length m-spinesin also contains these domains, the putative N-glycosylation sites Asn^{163}, Asn^{170}, Asn^{319}, and Asn^{375}, and a putative disulfide bridge linking the pro- and catalytic domains (Cys^{209} and Cys^{328}), as occurs in h-spinesin (Fig. 1C, type 4). Comparison with the mouse genomic sequence revealed that m-spinesin is composed of 13 exons and 12 introns, and that all the introns contain the canonical GT/AG dinucleotides at both ends.

We also isolated three kinds of m-spinesin variants, type 1, type 2, and type 3, by 5′-RACE-PCR (Fig. 1B). Type 3 and type 2 variants are generated, respectively, by the insertion of a 43 bp intron into exon 2 and a 10 bp deletion at exon 5, resulting in a shift in the translation reading frame (Fig. 1 A, types 2 and 3). Both type 3 and type 2 variants use an alternative acceptor site AG at intron 2 and exon 5, respectively, so these variants are generated by alternative splicing. The type 1 variant is generated by deletion of exons 1–4 and the insertion of an intron downstream from exon 6 (Fig. 1A, type 1). Although it is unclear whether translation products are synthesized from these variant mRNAs, potential initiation codons were located in their downstream regions, suggesting that these variants may encode m-spinesin isoforms that lack the N-terminal 10, 144, and 182 residues, respectively (Fig. 1C). The putative proteins encoded by the type 1 and 2 variants lack a transmembrane domain and a scavenger-receptor-like domain (Fig. 1B).

Although h-spinesin is predominantly expressed in the CNS [40], we analyzed the distribution of m-spinesin in various mouse tissues in detail using RT-PCR (Fig. 2). We designed the primers (m-2 F and m-2 R) to regions within the putative mature protease domain, which should detect all types of m-spinesin variants (Table 1). m-Spinesin was detected by RT-PCR in almost all tissues (Fig. 2). In particular, it was highly expressed in brainstem, spinal cord, and thymus (Fig. 2, lanes 4, 5, and 10).

Localization of m-spinesin EGFP-type 1 and -type 4 fusion proteins

To investigate the localization of the spinesin variant forms, we constructed expression vectors for m-spinesin EGFP-type 1 or -type 4 fusion protein. In COS-1 cells transfected with the m-spinesin EGFP-type 4 expression vector, the fluorescent signal was predominantly observed in the ER, Golgi apparatus, and cell surface (Fig. 3B), whereas the fluorescent signal of the EGFP-type 1 fusion protein was observed in the cytoplasm (Fig. 3A). These fusion proteins were predominantly detected at 57 and 77 kDa by Western blotting analysis using anti-GFP antibodies (Fig. 3C). Although m-spinesin type 3 and type 4 have no apparent signal sequence, PSORT II analysis predicted preferential ER, Golgi apparatus, and plasma membrane localization. On the other hand, types 1 and 2 were predicted to have preferential cytoplasmic localization. Our observations are consistent with the PSORT II predictions, and suggest that the differential subcellular localization of m-spinesin may be involved in the proteolysis of distinct substrates.

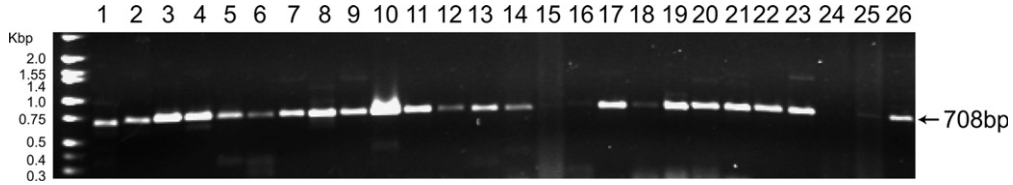

Fig. 2. Distribution analysis of m-spinesin in mouse tissues using RT-PCR. RT-PCR of total RNAs from various tissues was performed using mBSSP2F5 and mBSSP2 R5CGC primers, as described in Fig. 1 and Table 1. Lanes 1, cerebrum; 2, cerebellum; 3, brainstem; 4, spinal cord; 5, fetal brain (E20); 6, ophthalmus; 7, salivary gland; 8, heart; 9, lung; 10, thymus; 11, esophagus; 12, intestine; 13, liver; 14, spleen; 15, pancreas; 16, kidney; 17, adrenal gland; 18, large vessels; 19, skeletal muscle; 20, testis; 21, prostate; 22, ovary; 23, placenta; 24, breast; 25, preputial gland; and 26, bone marrow.

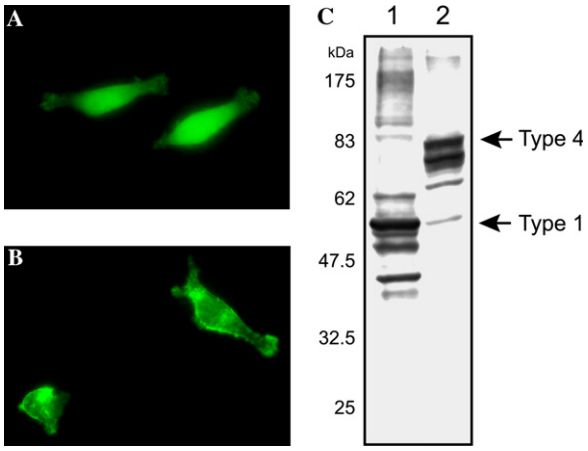

Fig. 3. Localization of m-spinesin EGFP-type 1 and -type 4 fusion proteins in COS-1 cells. COS-1 cells were transfected with expression vectors encoding EGFP-type 1 (A) or EGFP-type 4 (B) fusion proteins. After three days, the EGFP fusion proteins were visualized using a fluorescence microscope. The fluorescent signal of EGFP-type 1 fusion protein was strongly detected in the cytoplasm, whereas the fluorescent signal of EGFP-type 4 fusion protein was predominantly detected in the ER, Golgi apparatus, and plasma membrane. (C) These cells were extracted with sample buffer, followed by Western blotting analysis with anti-GFP polyclonal antibodies. Each fusion protein was predominantly detected at 57 and 77 kDa, corresponding to type 1 (lane 1) and type 4 (lane 2), respectively.

Expression of m-spinesin splicing variants in various tissues

To investigate the expression of m-spinesin variants in various tissues, we designed primer sets specific to each variant (Fig. 4A). Type 4 m-spinesin, a transmembrane type spinesin, was predominantly detected in the spinal cord and brainstem (Fig. 4B). Similarly, a band consistent with m-spinesin type 1 (270 bp) was detected in the spinal cord and brainstem (Fig. 4C), and a longer PCR product (~500 bp) was observed in several tissues, including the cerebrum, cerebellum, brainstem, lung, intestine, and prostate (Fig. 4C, asterisk). This longer product is predicted to be generated from an unidentified splicing variant of m-spinesin, because a ~500 bp PCR product is generated by the insertion of a 204 bp intron between exon 5 and exon 6. This prediction is consistent with the presence of the canonical GT/AG dinucleotides at both sites. This insertion generates a premature termination codon when generated in type 2, type 3, and type 4. On the other hand, when this insertion is generated in type 1, it is probable that the unidentified variant encodes the same polypeptide as the type 1 isoform, because this intron is inserted into the 5′-untranslated region (UTR) of m-spinesin type 1 (Fig. 4A). Interestingly, this unidentified splicing variant was not detected in the spinal cord, despite the expression of the type 1 variant in that tissue. This result suggests that the type 1 variant is generated by spinal-cord-specific splicing. We also examined the expression of m-spinesin types 2 and 3, using specific primer sets (Table 1). The PCR products corresponding to m-spinesin types 2 and 3 were weakly detected only in the spinal cord (Figs. 4C and D). These results suggest that the transmembrane-type m-spinesins, such as type 3 and type 4, are specifically expressed in the spinal cord, whereas the cytoplasmic-type m-spinesin, type 1, is expressed in multiple tissues.

Discussion

We have cloned the full-length cDNA encoding a serine protease of 455 residues from cDNA libraries constructed from mouse brain, spinal cord, and preputial gland. The amino acid sequence of this serine protease is 77.9% identical to that of h-spinesin, a type II transmembrane serine protease 5. The short cytoplasmic domain, transmembrane domain, scavenger-receptor-like domain, and serine protease domain are highly conserved between human and mouse spinesins. Cys^{209} and Cys^{328} of m-spinesin probably form a disulfide bridge linking the pro- and catalytic domains, as occurs in h-spinesin [40]. We also isolated variant cDNAs encoding at least three kinds of m-spinesin isoforms, type 1, type 2, and type 3. The type 3 variant uses an alternative acceptor site AG within intron 2, resulting in a 43 bp insertion. The type 2 variant is also spliced at an alternative acceptor site AG within exon 5, resulting in a 10 bp deletion. Although these variants, type 3 and type 2, cause a shift in the translation reading frame, a potential initiation codon for in-frame translation is located at nucleotides 116 and 516, respectively. These splicing variants potentially encode putative polypeptides of 445 or 311 residues, respectively, as the longest open reading frame. However, the PCR products corresponding to m-spinesin types 2 and 3 were weakly detected only in the spinal cord, suggesting that the products of these variants may hardly be synthesized. We also isolated a short variant, designated type 1, which was generated by the deletion of exons 1–4 and the insertion of an intron downstream from exon 6, resulting in a transcript encoding a putative polypeptide of 273 residues. As a result of further RT-PCR analysis, we also detected an alternative splicing product with an intron inserted between exon 5 and exon 6. This intron contained canonical GT/AG dinucleotides at both ends. Interestingly, this intron-inserted variant was not detected in the spinal cord, suggesting tissue-specific alternative splicing as observed for the type 3 and type 2 variants. This insertion was generated within the 5′-UTR of the type 1 variant mRNA, suggesting that this variant encodes the same polypeptide as the type 1 isoform. The shorter m-spinesins, with no transmembrane domain or scavenger-receptor-like domain (type 1 and

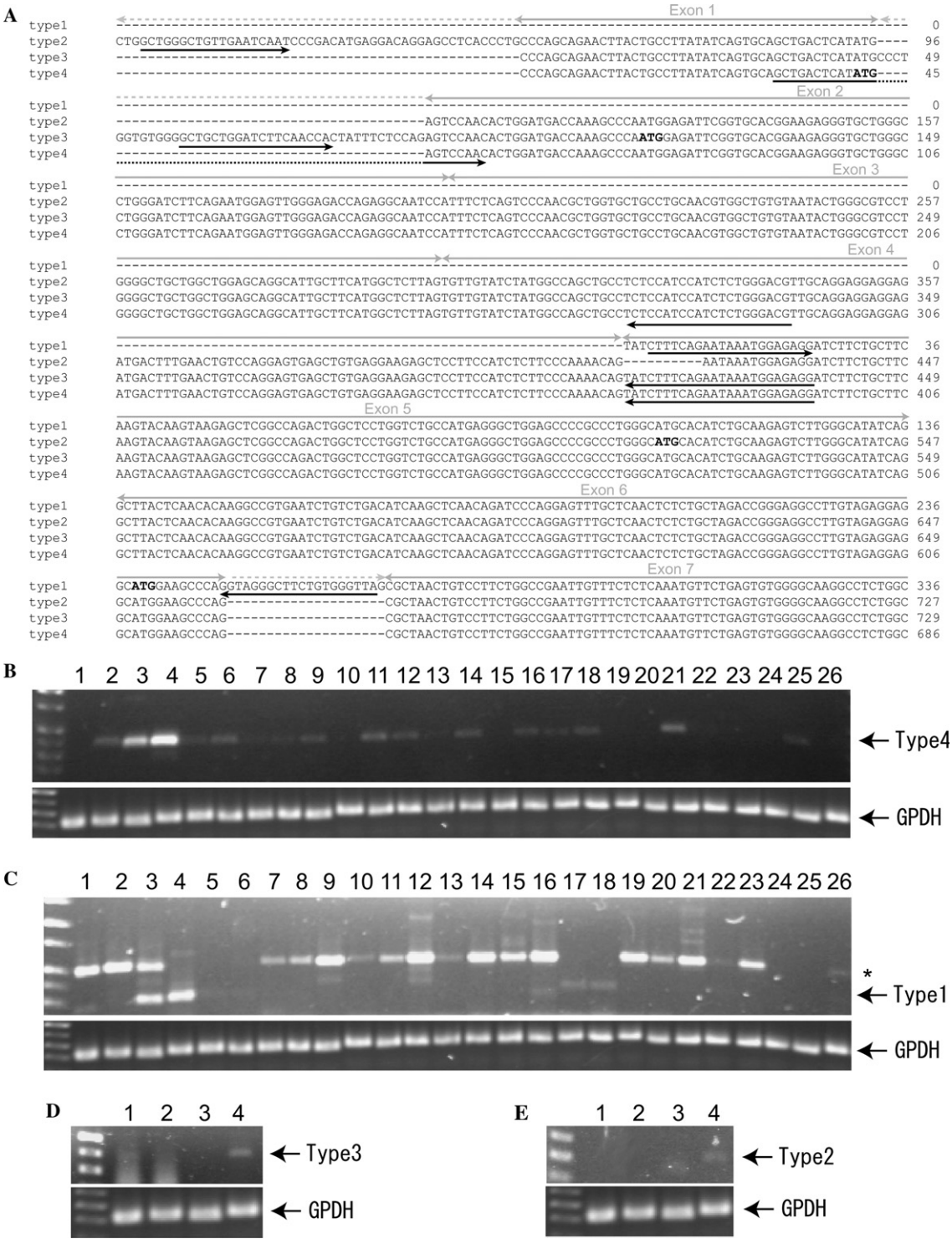

Fig. 4. Organ-specific expression of m-spinesin variants. (A) To confirm the expression of m-spinesin variants in various tissues, we designed variant-specific primer sets (closed arrows). The putative initiation codon of the m-spinesin variants is represented with bold letters. (B,C) The m-spinesin variants type 4 and type 1 were amplified from various tissue cDNAs using the primer sets described in (A). Lanes 1, cerebrum; 2, cerebellum; 3, brainstem; 4, spinal cord; 5, fetal brain (E20); 6, ophthalmus; 7, salivary gland; 8, heart; 9, lung; 10, thymus; 11, esophagus; 12, intestine; 13, liver; 14, spleen; 15, pancreas; 16, kidney; 17, adrenal gland; 18, large vessels; 19, skeletal muscle; 20, testis; 21, prostate; 22, ovary; 23, placenta; 24, breast; 25, preputial gland; and 26, bone marrow. The PCR product of m-spinesin variant type 4 or type 1 was 361 bp or 266 bp in length (closed arrows), respectively. When the type 1-specific primer set was used, a 500 bp PCR product was detected in various tissues (asterisk). This product may have been amplified from a variant with an intron inserted between exon 5 and exon 6. As a positive control for RNA preparation, glycerol-3-phosphate dehydrogenase (GPDH) was also amplified with a GPDH-specific primer set, as described in Materials and methods. Similarly, m-spinesin variant type 3 (D) and type 2 (E) were also confirmed with RT-PCR. Lanes 1, cerebrum; 2, cerebellum; 3, brainstem; and 4, spinal cord. The PCR products corresponding to m-spinesin types 2 and 3 were weakly detected only in the spinal cord (crossed arrows).

type 2), were localized to the cytoplasm, whereas the longer m-spinesins containing all the domains (type 3 and type 4) were localized to the ER, Golgi apparatus, and plasma membrane. This observation is consistent with the subcellular localizations predicted by the PSORT II algorithm. The lack of a scavenger-like domain and the differential subcellular localizations of m-spinesin should generate differential regulation of proteolysis and substrate(s) recognition. Another TMPRSS, TADG-12, also undergoes alternative splicing [13]. TADG-12 variant isoform 2 also lacks a hydrophobic putative transmembrane region, although the subcellular localization of the TADG-12 variant isoform has not been reported. Based on our observation and this report, the subcellular localizations and different domain structures of the proteases that are generated by alternative splicing or transcription may reflect differential proteolytic substrates for the proteases and may play distinct roles in cellular functions.

To confirm the organ distribution of m-spinesin variants, we analyzed their expression in mouse tissues using variant-specific primer sets (Table 1). The transmembrane-type m-spinesin, type 3 and type 4, was predominantly detected in the spinal cord and brainstem. The detection of m-spinesin type 4 in the brainstem was probably due to contamination with the spinal cord. Therefore, transmembrane-type m-spinesin is specifically expressed in the spinal cord. On the other hand, the cytoplasmic-type m-spinesin, type 1, was expressed in various organs, including the cerebrum, cerebellum, brainstem, lung, intestine, and prostate. In the thymus, m-spinesin was not amplified with primer sets directed against all variant types, although it was detected using primers directed against the protease domain, suggesting that an unidentified variant of m-spinesin is expressed in the thymus. The mechanism of spinal-cord-specific splicing has not yet been clarified. However, mRNA encoding the *N*-methyl-D-aspartate receptor R1 subunit (NMDAR1) is alternatively spliced in the rat lumbar spinal cord [41], suggesting tissue-specific alternative splicing. Furthermore, an in vitro splicing assay revealed that α-tropomyosin undergoes an alternative splicing in neuron extract [42]. These reports suggest that an organ-specific splicing mechanism is involved in m-spinesin splicing, resulting in the generation of type 2, type 3, and an unidentified variant with an intron 5 insert. On the other hand, it is unclear whether the type 1 variant is generated by alternative splicing or by distinct transcription initiation. Although we computationally predicted a transcription initiation site in the genomic sequence upstream from exon 5, we found no predicted promoter sequence. Further investigation is required to establish how the type 1 variant is generated. We have previously isolated organ- or embryonic developmental stage-specific variants of other serine proteases. Hippostasin/PRSS20 has three kinds of variants, and two of these are specifically expressed in the prostate, one with an insertion in the N-terminal region and the other with an insertion in the protease domain [36,37]. In mouse, neuropsin has adult-brain-type and embryonic-brain-type isoforms [33–35]. From this evidence, we speculate that the variation in the isoforms of the serine proteases reflects organ-specific biological functions, resulting in differential recognition of proteolytic substrates. This speculation is consistent with the organ-specific expression of the m-spinesin variant, type 4 spinesin. Type 4 spinesin may play a specific role in motoneuron function, because h-spinesin is predominantly localized at neighboring synapses of the anterior neuron [40]. In future research, we will investigate the specific function of type 4 m-spinesin in the spinal cord and the organ-specific mechanism that generates the m-spinesin variants.

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