Spinesin/TMPRSS5, a Novel Transmembrane Serine Protease, Cloned from Human Spinal Cord

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A cDNA encoding a novel serine protease, which we designated spinesin, has been cloned from human spinal cord. The longest open reading frame was 457 amino acids. A homology search revealed that the human spinesin gene was located at chromosome 11q23 and contained 13 exons, the gene structure being similar to that of TMPRSS3 whose gene is also located on 11q23. Spinesin has a simple 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. Unlike TMPRSS3, it carries no low density lipoprotein receptor domain in the stem region. The extracellular region carries five N-glycosylation sites. The sequence of the protease domain carried the essential triad His, Asp, and Ser and showed some similarity to that of TMPRSS2, hepsin, HAT, MT-SP1, TMPRSS3, and corin, sharing 45.5, 41.9, 41.3, 40.3, 39.1, and 38.5% identity, respectively. The putative mature protease domain preceded by H2D-DDDKK was produced in Escherichia coli, purified, and successfully activated by immobilized enterokinase. Its optimal pH was about 10. It cleaved synthetic substrates for trypsin, which is inhibited by p-amidinophenylmethanesulfonyl fluoride hydrochloride but not by antipain or leupeptin. Northern blot analysis against mRNA from human tissues including liver, lung, placenta, and heart demonstrated a specific expression of spinesin mRNA in the brain. Immunohistochemically, spinesin was predominantly expressed in neurons, in their axons, and at the synapses of motoneurons in the spinal cord. In addition, some oligodendrocytes were clearly stained. These results indicate that spinesin is transported to the synapses through the axons after its synthesis in the cytoplasm and may play important roles at the synapses. Further analyses are required to clarify its roles at the synapses and in oligodendrocytes.

Serine proteases have essential functions in biological processes such as the activation of complement and blood coagulation. Recently some serine proteases have been reported to contain a transmembrane domain that anchors the protease molecule to the cell membrane. During the last few years, many type II transmembrane serine proteases (TTSPs, referred to in this article as TMPRSS) from mammals have been cloned and reported, namely enterokinase (1), hepsin (2), HAT (3), corin (4, 5), MT-SP1 (epithelin) (6), matriptase (7), TMPRSS2 (epiteliadin) (8, 9), TMPRSS3 (10, 11), seprase (12), TADG12 (13), and TADG15 (14)

The common structural features of TMPRSSs are that they contain, from the N terminus, a short cytoplasmic domain, a transmembrane domain, a stem region, and a serine protease domain, the latter two being outside of the cell. The stem region varies in length and contains various modulatory domains. The length of these proteases ranges from 400 to over 1000 amino acid residues. The longest is corin at 1042 residues (4), and the shortest is hepsin at 417 residues. Hepsin has the simplest domain structure, having no unique modulatory domain in the stem region (2). On the other hand, enterokinase at a length of 1019 residues has the most complicated multiple domains in the stem region, i.e., a SEA (sea urchin sperm protein-enterokinase-gain) domain; two low density lipoprotein receptor class A domains; two CUB (Cls/Cr, urchin embryonic growth factor, and bone morphogenetic protein 1) domains; a MAP (meprin, A5 antigen, and receptor protein phosphatase μ) domain; and an SRRC (scavenger receptor cysteine-rich) domain (1).

At present, the roles of these domains have not been clarified, although the presence of a cytoplasmic domain suggests involvement in intracellular signaling. The various domains in the stem region may function in the recognition of other molecules, e.g. proteolytic substrates and inhibitors as well as other proteins and ligands, soluble or matrix-bound, on other cells, suggesting important roles for TMPRSSs in the body (for a review, see Ref. 15). Corin and matriptase process atrial natriuretic peptide (16) and hepatocyte growth factor (17), respectively. Enterokinase has long been known to have an essential role in the processing of digestive proteases (18).

We have been studying the brain-specific serine proteases and have newly cloned and characterized neurosin/PRSS9 (19), hippocastain/PRSS20 (20, 21), and motopin/PRSS12 (22, 23). Neurosin and hippocastain, whose genes are found on chromosome 19q13.3, are secreted and belong to the kallikrein-like serine protease family. Motopin, whose gene is located on...
chromosome 5, has a unique and complicated structure similar to TMPRSS5, 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 without an obvious hydrophobic transmembrane domain and thus would appear to be a secreted protease.

As part of our continuing efforts to characterize serine proteases from the CNS, we have cloned from a human spinal cord mRNA pool a TMPRSS that we designated spinesin or TMPRSS5. As far as we know, this is the first report of a TMPRSS identified in the CNS.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human tissues of the CNS for immunohistological analyses were obtained with informed consent within 12 h of death. Cerebrospinal fluids (CSFs) were obtained with informed consent from patients with non-neurological disease. Human brain mRNA, multiple tissue Northern blots, and tissue extracts were purchased from CLONTECH (Palo Alto, CA). Tissue culture media, supplements, pTrcHisB vector, and competent *Escherichia coli* DH5α cells were from Invitrogen. All other chemicals were obtained from Wako Chemicals (Osaka, Japan).

**Antibodies**—Rabbit polyclonal antibodies for Western blotting and immunohistochemical analyses were raised against two KLH-conjugated peptides, KLH-CGSRAEEALLP (anti-human spinesin A) and KLH-CAGLVSASVHRPHQG (anti-human spinesin B), and purified using protein A-Sepharose (Amersham Biosciences, Inc.). The former peptide sequence is derived from the stem region, and the latter was derived from the protease domain (see Fig. 1).

**Isolation of Human Spinesin cDNA Clones**—Poly(A)+ RNA from human CNS (CLONTECH) was reverse-transcribed by using the SuperScript PremiCipation System (Invitrogen) according to the instruction manual. PCR with a pair of degenerate primers, DP-S and DP-A (Table 1), was performed as described previously (22, 24). The PCR products were ligated into the pGEM-T Easy vector (Promega, Madison, WI), cloned, and sequenced using an automatic sequencer (DSQ-1000, Shimadzu Co., Kyoto Japan). A clone carrying a 465-bp fragment was found to have a novel serine protease-related sequence. Based on this sequence, specific primers were synthesized for the rapid amplification of cDNA ends (RACE, Table I). For 3′-RACE, human CNS poly(A)+ RNA was reverse-transcribed using oligo(dT) with an adaptor primer sequence at the 5′-end, TGGAAGAATACGCGGCCGCAGT17. The cDNA was then amplified using forward primer 1 and the adaptor primer, products of which were further amplified by nested PCR using primer 2 and the adaptor primer. 5′-RACE was performed using a Marathon cDNA amplification kit (CLONTECH) according to the instruction manual. In brief, nested PCR with AP2 and primer 3 was performed using products of PCR with primer 4 and AP1 as a template. Northern Blot Hybridization—Northern blot hybridization against human multiple tissues was carried out using a commercially available membrane (CLONTECH). The cDNA carrying the full-length human spinesin open reading frame amplified using primers 5 and 6 was labeled by the random labeling method using a [γ-32P]ATP labeling kit (Takara Shuzo Co., Ltd., Shiga, Japan). Hybridization was carried out in ExpressHyb hybridization solution (CLONTECH) at 65°C overnight, and the final wash was performed in 0.1× saline/sodium phosphate/EDTA containing 0.1% SDS at room temperature for 10 min. The radioactivity was detected using an FLA-2000 image analyzer (Fuji Photo Film Co. Ltd., Tokyo, Japan).

**Immunohistochemical Analysis**—The CNS including spinal cord from a non-neurological patient (65-year-old Japanese male) was obtained 2–12 h after death. Small blocks were dissected and fixed in 0.1 m phosphate-buffered 4% paraformaldehyde for 2 days and then stored in 0.1 m phosphate-buffered saline containing 15% sucrose and 0.1% sodium azide and kept at −70°C until use. Sections were cut on an cryostat at 20-μm thickness and washed in phosphate-buffered saline. The antibody, dilution 1:2000 with phosphate-buffered saline-Tween, was incubated with the specimens at 4°C for 4 h. After a wash with phosphate-buffered saline-Tween, the slides were incubated with alkaline phosphatase-labeled goat anti-rabbit IgG for 60 min at room temperature. After another wash, immunoreactivity was visualized with 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate. Counter staining was not performed.

**Expression and Purification of Recombinant Spinesin in a Bacterial Expression System**—To obtain an active recombinant spinesin, a cDNA fragment encoding the putative mature enzyme of spinesin (Ile128-Leu227) was amplified by PCR (forward primer, ATATGGTTTGGCGACTCTGCTGT; reverse primer, primer 6 in Fig. 1) and subcloned into pTrcHisB between the BamHI site, which had been treated with mung bean exonuclease following the instructions of the manufacturer, and the EcoRI site. The resultant vector carrying chimeric cDNA encoding H3-DDDK(223-231)-L227 was transformed into DH5α, and the recombinant protein was induced using 0.7 m isopropyl-β-D-thiogalactopyranoside. The recombinant protein in the cells from 1 ml of culture, mostly in inclusion bodies, was collected and suspended in 20 mM Tris-HCl, pH 8.0, containing 0.2 mM NaCl and 1% Triton X-100. The suspension (5 ml) was sonicated, and inclusion bodies were collected by centrifugation and resuspended. After three rounds of sonication and centrifugation, the final pellet was dissolved with 5 ml of 8 M urea in the same buffer without Triton X-100 by sonication and shaken at room temperature for 1 h. Then the solution was diluted 10 times with 20 mM Tris-HCl, pH 8.0, containing 0.2 mM NaCl under vigorous stirring and centrifuged for 30 min at 3,500 rpm to remove debris. The supernatant was tested using Boc-Gln-Ala-Arg-MCA as a substrate in either 0.1 M saline/sodium phosphate or 0.2 M NaCl containing 1% Triton X-100, pH 8.0, containing 0.2 mM NaCl. The activity of spinesin treated with 0.01 unit of recombinant enterokinase at 38°C was assayed by measuring the absorbance at 380 nm, emission at 460 nm) was measured using a plate reader (Cytofluor 2300, Millipore, Bedford, MA). The effect of pH on the activity of spinesin treated with 0.01 unit of recombinant enterokinase was tested using Boc-Gln-Ala-Arg-MCA as a substrate in either 0.1 M phosphate buffer or 0.1 M Tris-HCl buffer that contained 0.2 mM NaCl. The reaction was carried out under the conditions described above. An inhibitor profile was obtained by preincubating for 30 min at 37°C with a final concentration of 1 μM aminophenylmethanesulfonfluoride hydrochloride, 1 mM leupeptin, or 1 mM antipain. The remaining enzyme activity was expressed relative to a control value obtained by adding buffer without inhibitor.

**Zymography**—Gelatin or casein (270 μg/ml) was copolymerized in a standard 12.5% SDS-polyacrylamide gel. The active recombinant spinesin (100 ng) was electrophoresed at a constant current of 20 mA under nonreducing condition. The gel was washed with 20 mM Tris-HCl (pH 8.0), 0.2 mM NaCl containing 1% Triton X-100 at 37°C for 3 h and then incubated in 20 mM Tris-HCl, pH 8.0, containing 0.2 mM NaCl at 37°C overnight. The gel was stained with Coomassie Brilliant Blue. The samples were applied to a 12.5% polyacrylamide gel containing 0.1% SDS and electrophoresed. The separated proteins were transferred onto polyvinylidene difluoride membrane and then incubated overnight at room temperature with anti-human spinesin A or B diluted 2000-fold with 20 mM Tris-HCl, pH 7.4, containing 0.05% Tween 20 and 0.2 mM NaCl. After a wash with the same buffer, the membrane was incubated with alkaline phosphatase-labeled goat anti-rabbit IgG for 60 min at room temperature. After another wash, immuno-

**TABLE I**

| Name | Sequence |
|------|----------|
| DP-S | GTGCTCAGNCGCCGCATGTCG |
| DP-A | AGGCCGCCGCDSWRTCVCC |
| Primer 1 | ACTGCTGCACATTGTATG |
| Primer 2 | GCTCTCAACTTCTACGAC |
| Primer 3 | AGGGGGCGGCGCTATCTCC |
| Primer 4 | ACTGCTACCTTGGCA |
| Primer 5 | CTTGTACACGTCGTACGAC |
| Primer 6 | AAGGATGACGGAAGAACACCAGACCTGAG |
| Adaptor primer | TGGAGAAGATAGCGGGCGGAG |
| AP1 | CCATCTAAATTACGACTCTATAGGG |
| AP2 | ACTCATTAGGCGCTTAGGCGC |

Data from Table I.
noreactivity was visualized with 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate.

RESULTS

Nucleic Acid Sequence of Human Spinesin cDNA—PCR using degenerate primers designed from serine protease motifs, AAHC and DSGGP, amplified a 465-bp fragment from a cDNA library of human spinal cord. Further detailed study of the library was performed since the sequence analysis of the fragment showed that it encoded a novel serine protease. The longest clone of the 3'-RACE products obtained using primer 2 and adaptor primer contained 1213 bp including a poly(A) tract. The sequence of the 5'-RACE product was 1381 bp long, and its 3'-end overlapped with the 5'-end of the 3'-RACE product (Table I), enabling the determination of the apparent full-length cDNA of 2265 bp including the 5'- and 3'-untranslated region for a novel serine protease. The longest open reading frame was 1371 bp, which encoded 457 amino acids. This protein was termed spinesin for spinal cord-enriched trypsin-like protease. A homology search against the GenBank™ data base showed that the human spinesin gene spanned 18.8 kb on chromosome 11 and was composed of 13 exons and 12 introns (Fig. 1). The GT-AG rule for exon-intron boundaries was conserved except for the 5'-donor site of the eighth intron, the sequence of which was GC. The cDNA sequence contained three possible initiation codons at the 5'-end, the third codon conforming best to the Kozak consensus sequence (Fig. 1).

Structure of Human Spinesin Deduced from Nucleotide Sequence—Hydropathy plots (Fig. 2) revealed an apparent hydrophobicity of the deduced amino acid sequence of spinesin. The method of Hopp and Woods (25) was used with averaging over a window of 10 residues. Hydrophobic residues show negative values, whereas hydrophilic residues show positive values. The structure of spinesin is illustrated under the plot. TM, transmembrane domain; SR-like, scavenger receptor-like domain; ✫, cysteine residues; upside-down ⌅, N-glycosylation sites.

FIG. 1. Structures of cDNA, deduced amino acids, and gene of human spinesin. Comparison of the nucleotide sequences of human spinesin cDNA and human chromosome 11q clone DNA (GenBank™ accession no. AP002436) revealed the structure of the human spinesin gene. The 13 exons are boxed, and the intervening sequences are not shown except for the exon/intron boundaries. The exon/intron boundary consensus (GT or GC/AG) sequence and poly(A) signal are double underlined. Amino acids are numbered starting from the putative first initiating Met. A transmembrane and a scavenger receptor-like domain are indicated by white and shaded letters, respectively. The essential triad and the putative processing site are indicated by an underline and by an arrow, respectively. Putative N-glycosylation sites are circled.

FIG. 2. Hydropathy plot of the deduced amino acid sequence of spinesin. The method of Hopp and Woods (25) was used with averaging over a window of 10 residues. Hydrophobic residues show negative values, whereas hydrophilic residues show positive values. The structure of spinesin is illustrated under the plot. TM, transmembrane domain; SR-like, scavenger receptor-like domain; ✫, cysteine residues; upside-down ⌅, N-glycosylation sites.
phobic region at Ala–Leu, suggesting it to be a transmembrane portion. Both ends of the transmembrane sequence are flanked by a Cys residue that might form a disulfide bond with another Cys residue on each side of the membrane. Five putative N-glycosylation sites exist on the sequence C-terminal to the transmembrane portion, suggesting that the molecule is a type II transmembrane glycoprotein (Figs. 1 and 2). Accordingly the N-terminal cytoplasmic domain and the N-terminal sequence of the stem region between the transmembrane and scavenger receptor-like domain (see below) each carry only two Cys residues that might form a disulfide bridge on each side of the membrane, i.e. Cys41–Cys49 and Cys73–Cys93. A serine protease domain was located at Ile218–Leu457 in the C-terminal half of the molecule and contained the HDS (His, Asp, Ser) triad essential for catalytic activity of a serine protease (Figs. 1, 2, and 3). The stem region connecting the transmembrane and catalytic domains spans from Cys73 to Arg217 and carries a scavenger receptor-like domain at Val110–Gly152 that contains two cysteines that probably form a disulfide bond, Cys135–Cys148 (15). The disulfide bridge linking pro- and catalytic domains seems to be formed between Cys209 and Cys328, which are conserved among TMPRSSs (Fig. 3) (15). A homology search using the protease domain of spinesin showed that it shares 45.5, 41.9, 41.3, 40.3, 39.1, and 38.5% amino acids with human TMPRSS2, hepsin, HAT, MT-SP1, TMPRSS3, and corin, respectively (Fig. 3). Nine of 10 cysteine residues in the mature enzyme domain of spinesin were well conserved among other TMPRSSs. A putative cleavage site for processing to generate a mature form is tentatively assigned between Arg217 and Ile218, which is in the highly conserved activation motif of the serine protease (15). Of the five putative N-glycosylation sites, three are in the stem region, and two are in the mature enzyme region (Fig. 2).

Enzyme Characteristics of Recombinant Spinesin Produced in E. coli—To demonstrate that the putative serine protease domain of spinesin has enzymatic activity, a chimeric protein in which Ile218–Leu457 of spinesin was fused downstream of H6DDDK was expressed in E. coli. The products were purified from extensively washed and solubilized inclusion bodies using Talon chelate column chromatography. SDS-PAGE of the purified recombinant chimeric spinesin, H6DDDK-(I–L), was subjected to 12.5% SDS-PAGE under reducing conditions. A, left and right panels show Coomassie Brilliant Blue staining and Western blot using anti-spinesin B, respectively. Lane 1, purified inclusion body; lane 2, fraction passed through a Talon column; lanes 3 and 4, consecutive fractions of 5 mM imidazole buffer wash; lanes 5 and 6, consecutive fractions of 100 mM imidazole buffer eluate, B, enzyme fractions electrophoresed through gelatin-polyacrylamide gel as described under "Experimental Procedures." Lane a, nontreated/Talon-purified chimeric spinesin; lane b, 0.05 unit of entero kinase in 20 mM Tris-HCl, pH 8.0, containing 0.2 M NaCl; lane c, entero kinase-treated (0.05 unit) chimeric spinesin. C, Western blot analysis of brain homogenate and CSFs. Lane 1, brain extract; lanes 2–4, CSF samples from three non-CNS diseased patients.
purified protein showed a single band (Fig. 4A, left, lane 5) of 30 kDa, which was immunoreactive with the anti-human spinesin B in Western blot analysis (Fig. 4A, right, lane 5). The purified recombinant spinesin was activated by treatment with an immobilized enterokinase column to remove His<sub>6</sub>DDDDK. The activated spinesin cleaved the synthetic trypsin substrate Boc-Gln-Ala-Arg-MCA (Fig. 5A). Using this substrate, the pH optimum was estimated to be about pH 10 (Fig. 5B). p-Amidinophenylmethanesulfonyl fluoride hydrochloride inhibited spinesin activity by more than 75% at 1 μM, but antipain and leupeptin showed no inhibitory effect at 1 mM (Fig. 5C).

As is shown in Fig. 4B, the recombinant spinesin gained an ability to cleave gelatin after treatment with 0.05 unit of enterokinase, while neither purified recombinant spinesin before activation or 0.05 unit of enterokinase itself cleaved gelatin. Interestingly, casein is not cleaved by the activated recombinant spinesin (data not shown).

Localization of Spinesin in the Human CNS—First, Northern blot analysis was performed to see which human tissues produce spinesin mRNA using a commercially available RNA blot. A clear band was observed at 2.3 kbp in brain but not in kidney, liver, lung, placenta, or heart suggesting a specific expression of spinesin in the CNS (Fig. 6).

The presence of spinesin was verified by Western blot analysis of human brain homogenate, which showed a protein band at 52 kDa that was detectable with anti-human spinesin A anti-serum (Fig. 4C). The size suggests that it is a full-length spinesin with possibly five N-glycosylated sugar chains. No other distinct bands were apparent using anti-human spinesin B suggesting that the 52-kDa protein is the major molecule present in the brain (data not shown). Spinesin was also detected in the CSF. In some cases, a more rapidly migrating band at about 50 kDa seems dominant (Fig. 4C, lanes 2 and 4).

Detailed immunohistochemical analysis using anti-human spinesin A showed that at the anterior horn of the spinal cord, neuronal cells and their axons were stained (Fig. 7A). The transverse section of the spinal cord revealed many axons to be positively stained (Fig. 7B), and among the oligodendrocytes, sporadic staining of the cytoplasm and dendrites was evident (Fig. 7C). In addition, the synapses on motor neurons were also stained (Fig. 7D). Neuronal cells of the substantia nigra and oculomotor nerve were also strongly stained as well as their axons (data not shown).

DISCUSSION

We have cloned a cDNA encoding a serine protease, designated spinesin or TMPRSS5, of a tentative size of 457 amino acid residues from a human spinal cord cDNA library. It apparently belongs to the TMPRSS family having an N-terminal cytoplasmic domain, a transmembrane domain, a scavenger receptor-like domain in the stem region, and a protease domain. As far as we know, this is the first report of a TMPRSS gene family that of TMPRSS3 (11). Both are composed of 13 exons spanning the same chromosome. On the other hand, human enterokinase and TMPRSS2 are located at 21q21 and 21q22.3, respectively. Interestingly the gene structure of spinesin is highly similar to that of TMPRSS3 (11). Both are composed of 13 exons spanning 21–24 kb. The protease domain is encoded on exons 8–13, and the transmembrane domain is encoded on the exons 3 and 4. The stem region is encoded on exons 4–8. The gene structure of both human hepsin and TMPRSS2 is also similar to that of spinesin, so it seems possible that the TMPRSS gene family
Experiments were performed as described under “Experimental Procedures.” A, anterior horn of the spinal cord. Representative positive neuronal cells (arrow) and their axons (arrowhead) are indicated. a, no positive signals in a similar region immunostained using normal rabbit serum. B, transverse section of the spinal cord. Representative positive myelinated axons (arrow) and oligodendrocytes (arrowhead) are shown. C, magnified view of a transverse section of the spinal cord. The sporadic staining of cell bodies (white arrow) and dendrites of oligodendrocytes (black arrowhead) is demonstrated. D, magnified view of the anterior horn. Arrows indicate spot-like staining of synapses in contact with motoneurons.

By analogy also, we predict that the cleaved catalytic domain is linked with the C-terminal side of the stem region by a disulfide bond formed between Cys²⁰⁹ and Cys³²⁸ (Figs. 1 and 3). However, Western blot analysis using anti-spinesin A and B on the brain homogenate revealed the presence of a single major 52 kDa band that is far bigger than the predicted catalytic domain of 240 amino acid residues even if it was N-glycosylated at two sites. Whether a smaller active form of the enzyme is present at a level below the detection limit and/or uncleaved 52-kDa spinesin has enzymatic activity remains to be seen. The mechanism underlying the production of 50-kDa spinesin in the CSF and whether the enzyme has activity are also left for future studies.

Immunohistochemical analysis along with Northern blot analysis showed clearly that spinesin is located in the CNS. The neuronal cells and their axons at the anterior horn of the spinal cord were clearly immunopositive. Spinesin was stained in the substantia nigra, oculomotor nucleus, and temporal lobe (data not shown). Furthermore, spinesin was demonstrated at the synapses of the spinal cord. From these results, it seems that spinesin produced in the neuronal cytoplasm may be transported along the axons to the synapses at the anterior horn of the spinal cord. We predict that spinesin is present in the presynaptic regions.

As shown in Fig. 7, B and C, the oligodendrocytes were also stained. The transverse section of the spinal cord clearly demonstrated spinesin in both neuronal axons and oligodendrocytes. The physiological roles of spinesin in the synapses and oligodendrocytes could be different naturally, and further analysis is required to elucidate spinesin functions in different cell types. It should be noted here that, among TMPRSSs, only TMPRSS2 was reported to be present in the brain, having been detected at the mRNA level using human RNA master blot (8), but further examinations such as experiments on the cellular localization of the protein are needed.

The proteolytic activities of membrane-anchored proteins such as membrane-type metalloproteinases and ADAM (a disintegrin-like and metalloproteinase) may play roles in activating events that take place on the cell surface. These enzymes...
also may interact with extracellular matrices and proteins on adjacent cells. The enzymatic activity of a few TMPRSSs has been demonstrated. Gelatin, fibrinogen, fibronectin, and laminin are cleaved by TMPRSSs (15). Corin is a processing enzyme of proatrial natriuretic peptide (16), and matriptase processes hepatocyte growth factor as an activator (17). Activated spine-sin mainly cleaved trypsin substrates among synthetic forms and cleaved gelatin but not casein.

In summary, we have cloned spinesin/TMPRSS5, a protein that encodes 457 amino acids including a cytoplasmic domain, transmembrane domain, a scavenger receptor-like domain, and a serine protease domain, from human spinal cord mRNA. Spinesin is dominantly expressed at synapses. We predict that axonal spinesin is transported to synaptic junctions for cleavage of protein(s) in the presynaptic regions and that the spine-sin dominantly expressed in some oligodendrocytes may activate or inactivate other proteins on the cell surface. We are continuing our efforts to elucidate the biological and pathophysiological functions of spinesin including identifying physiological substrates, interacting molecules, and the exact localization of the molecule in the body including CSF.

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Spinesin/TMPRSS5, a Novel Transmembrane Serine Protease, Cloned from Human Spinal Cord
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