Identification of Strain-specific Variants of Mouse Adamts13 Gene Encoding von Willebrand Factor-cleaving Protease*

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Human ADAMTS13 was recently identified as a gene encoding von Willebrand factor-cleaving protease, hADAMTS13. Both congenital and acquired defects in this enzyme can cause thrombotic thrombocytopenic purpura. hADAMTS13 consists of 1,427 amino acid residues and is composed of multiple structural domains including thrombospondin type 1 motifs and CUB domains. To analyze the functional roles of these domains in vivo, we determined the cDNA sequence of the mouse ortholog, mADAMTS13. Unexpectedly, two forms of the mouse Adamts13 gene were isolated that differed in the insertion of an intracisternal A particle (IAP) retrotransposon including a premature stop codon. The IAP insertion was found in BALB/c, C3H/He, C57BL/6, and DBA/2 strains but not in the 129/Sv strain. The outbred ICR strain had either the IAP-free or IAP-inserted allele. The IAP-free mAdams13 encoded mADAMTS13L, a protein of 1,426 amino acid residues with the same domain organization as hADAMTS13. In contrast, IAP-inserted mAdams13 encoded a C-terminally truncated enzyme, mADAMTS13S, that is comprised of only 1,037 amino acid residues and lacking the C-terminal two thrombospondin type 1 motifs and two CUB domains. Strain specificity was also confirmed by reverse transcription-PCR and Northern blot analyses. Both recombinant mADAMTS13L and mADAMTS13S exhibited von Willebrand factor cleaving activities in vitro. The natural variation in mouse ADAMTS13 should allow for the determination of hitherto unknown functions of its C-terminal domains in vivo.

von Willebrand factor (VWF)1 is a large glycoprotein that mediates adhesion between the platelet surface and damaged subendothelium (1, 2). VWF is mainly synthesized in endothelial cells and secreted into the circulating blood as unusually large VWF (UL-VWF) multimers (1, 2). In healthy individuals, UL-VWF multimers are cleaved to smaller sizes in plasma (3). If cleavage is impaired, however, UL-VWF multimers accumulate in the plasma. Because UL-VWF multimers possess an extremely high thrombotic activity (4, 5), UL-VWF multimers in the circulation lead to platelet clumping at the sites of vascular injury. The importance of VWF proteolysis is best illustrated by the severe consequences of thrombotic thrombocytopenic purpura, a condition associated with increased levels of UL-VWF multimers (6). This disease is characterized by microangiopathic hemolytic anemia, thrombocytopenia, neurological dysfunction, renal failure, and fever (7). The mortality of affected patients may exceed 90% without treatment such as plasma exchange.

Human ADAMTS13 (hADAMTS13), an enzyme responsible for the proteolytic processing of UL-VWF multimers, was recently purified, and its partial amino acid sequence was determined (8–10). hADAMTS13 cleaves a peptidyl bond between Tyr1665 and Met1666 in the VWF A2 domain (11–13). The gene encoding hADAMTS13 was identified as a member of the “a disintegrin-like and metalloprotease with thrombospondin type 1 motif (ADAMTS)” family and designated as ADAMTS13 (8, 14, 15). ADAMTS13 contains 29 exons and spans ~37 kb on chromosome 9q34 (8, 14, 15). The mRNA is detected primarily in liver (8, 14, 15). Analysis of genomic DNA in patients with congenital thrombotic thrombocytopenic purpura revealed that mutations of ADAMTS13 could lead to an inactive enzyme (15–20). Notably, a common single nucleotide polymorphism, P475S, with ~10% heterozygosity in the Japanese population, resulted in a decrease of enzymatic activity (16).

hADAMTS13 consists of several different domains: a signal peptide, a propeptide, a repolinysin-like metalloprotease domain, a disintegrin-like domain, a thrombospondin type 1 (TSP1) motif, a cysteine-rich domain, a spacer domain, seven additional TSP1 repeats, and two CUB domains. In vitro studies using C-terminally truncated hADAMTS13 constructs revealed that the C-terminal TSP1 motifs and CUB domains were dispensable to maintain the VWF cleaving activity (21, 22). However, the biochemical and physiological roles of these domains in vivo remain to be resolved. As a first step to develop suitable animal models for following potential roles of this enzyme in vivo, we have cloned the mouse ortholog of ADAMTS13, mADAMTS13, and determined its complete genomic structure. In the present study, we report two types of the Adamts13 gene in mice caused by the strain-specific insertion of an intracisternal A-particle (IAP) retrotransposon. We further examine the VWF cleaving activity of mADAMTS13.
EXPERIMENTAL PROCEDURES

Animals—Male 129/Sv mice were purchased from Clea Japan, Inc. Male BALB/c, C3H/He, C57BL/6, DBA/2, and ICR mice were purchased from SLC Inc. Blood (~100 μl) was collected by cardiac puncture into a syringe containing 10 μl of 3.8% sodium citrate and centrifuged to obtain plasma. Spleen and liver were excised, rinsed in phosphate-buffered saline, and immediately used for DNA and RNA preparation.

DNA Sequencing—All of the sequence analyses were performed by 373A or 3700 automated DNA sequencer (Applied Biosystems) with a Big Dye Terminator Kit (Applied Biosystems).

Determination of the mADAMTS13 cDNA Sequence—Total RNA was prepared from the livers of 5C7BL/6 and 129/Sv mice with Isogen (NipponGene), and poly(A)^+ RNA was purified with an mRNA purification kit (Amersham Biosciences) according to the manufacturer's instructions. The cDNA was synthesized from the poly(A)^+ RNA with a first strand cDNA synthesis kit (Amerham Biosciences). PCR was carried out with primers designed from the genomic DNA sequence (forward sequence in 5'-untranslated region, 5'-AGGAAAGCTCCACAGGATGAAAACACTGCTGGC-3', reverse sequence within the metalloprotease domain, 5'-TCAGAGAGGTTAGATTTTCAATC-3', the primer downstream of the IAP target site, 5'-TGCCAGATGGAGAGA-3'), and the underlined sequence is the inserted C-terminal His6 tag. PCR products were cloned into pCR2.1 vector using a TA CloningTM kit (Invitrogen) and sequenced.

In addition, 3'-RACE was performed using a 3'-Full RACE Core Set (Takara), according to the manufacturer's instructions. After reverse transcription from liver poly(A)^+ RNA, PCR was performed using the Adaptor Primer provided with the kit and a gene-specific forward primer within the metalloprotease domain, 5'-TGGAGTTGTCCTGATGCAACACGCA-3'. The second PCR was performed using the first PCR products as a template with Adaptor Primer and a gene-specific intron forward primer, 5'-CATCACCCTTTTCTCATTCTAATCTGGAC-3'. The cycling parameters were as follows: 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 3 min, followed by 72°C for 7 min. PCR products were cloned into pCR2.1 vector and sequenced.

PCR Analysis of IAP Insertion in the Adams3 Gene—Genomic DNA was extracted from ear punches of 129/Sv, BALB/c, C3H/He, C57BL/6, DBA/2, and ICR mice by DNeasy Tissue Kit (Qiagen). Presence or absence of an IAP insertion in the Adams3 gene was determined by PCR with HotStarTaq DNA polymerase (Qiagen). The amplification was carried out using mixture of three primers; the intron 23 forward primer, 5'-ACCTCTCAAGGTTTGGGAGTCTA-3', the IAP-specific reverse primer, 5'-TCAGCCGCACTTTGTTGACGGGAGA-3', and the primer downstream of the IAP target site, 5'-TGGCCAGATGGCCATGATTAACTCTT-3'. PCR products were directly sequenced.

Southern blot Analysis—Genomic DNA prepared from spleens was digested with EcoRV, separated on 0.7% agarose gel, and transferred to a nylon membrane by standard capillary blotting techniques. A genomic fragment (442 bp) of the IAP insert was produced by PCR with the primer set: 5'-TAGGCGACCATGATGGTATTAG-3' and 5'-TGTCGTCTCTTCCAGAACATCTT-3' and labeled with fluorescein-d11-dUTP using Gene Images random prime labeling module (Amerham Biosciences). The blot was hybridized with the probe, and the hybridized probe was detected using the Antifluorescein-AP conjugate detection reagent (Amersham Biosciences) according to the manufacturer's instructions. Chemiluminescence was measured by an LAS-1000plus image analyzer (Fujifilm).

Preparation of Recombinant Substrate (mVWF73) for Enzymatic Assay—To examine the enzymatic activities of mADAMTS13, we prepared the recombinant substrate as described previously (24). In brief, a D1596-T1668 region of mouse VWF was amplified by RT-PCR using RNA from a C57BL/6 mouse liver. The primers, 5'-GGGATCCGCGGTGAGAAGCAG-3' and 5'-GCGGAGAAGGAGTCTGGA-3', were used for the amplification. Lowercase letters indicate added restriction enzyme sites, and the underlined sequence is the inserted C-terminal His8 tag (H). The PCR product was digested with BamHI and EcoRI and cloned into the corresponding sites of pEXK6-1 (Amerham Biosciences), a glutathione-S-transferase (GST) fusion expression vector. The resulting plasmid encoding GST-D1596-T1668 was introduced into Escherichia coli, BL21 (Stratagene), and expression was induced by the addition of isopropyl-p-thiogalactoside. The bacterial cells were collected and lysed with Celllytic B (Sigma), followed by centrifugation. The soluble fraction was subjected to a nickel-nitrilotriacetic acid spin Kit (Qiagen) and further to a MicroSpin GST purification module (Amerham Biosciences). The purified protein, designated GST-mVWF73-H, was used as substrates for enzymatic assays. The molecular mass of GST-mVWF73-H was 35.7 kDa. If mADAMTS13 cleaves the expected site, the size of the N-terminal portion including the GST tag will be 28.0 kDa.

Transient Expression of mADAMTS13—The entire open reading frame (ORF) constructs with C-terminal FLAG sequence (DYKD-DDDK) were prepared for two types of mouse ADS13M, mADAMTS13L (GenBank™ accession number AB112382) and mADAMTS13S (GenBank™ accession number AB071302), by PCR. Each PCR product was inserted into pCAG-neo mammalian expression vector (25). The resulting plasmids were transfected into HeLa cells using FuGENE 6 (Roche Applied Science) as described previously (16). Forty-eight hours after transfection, the media were collected and concentrated using Centricon YM-30 (Millipore). The cells together with extracellular matrix were lysed in SDS sample buffer (10 mM Tris-HCl, 100 mM NaCl, 1% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.05% bromophenol blue).
Tris-HCl, 2% SDS, 50 mM dithiothreitol, 2 mM EDTA, 0.02% bromphenol blue, 6% glycerol, pH 6.8).

Recombinant proteins were detected by SDS-PAGE and Western blot as described previously (16). For culture media, a rabbit anti-mADAMTS13 polyclonal antibody (described below) and an HRP-labeled goat anti-rabbit IgG antibody (Kirkegaard & Perry Laboratories) were used for detection after SDS-PAGE under nonreducing condition. For cell lysates, an anti-FLAG M2 monoclonal antibody (Sigma) and an HRP-labeled goat anti-mouse IgG antibody (Kirkegaard & Perry Laboratories) were used for detection after SDS-PAGE under reducing condition. Chemiluminescence was developed using the Western Lighting Chemiluminescence Reagent Plus (PerkinElmer Life Sciences) and detected by an LAS-1000plus image analyzer (Fujifilm).

**Preparation of Polyclonal Antibody against Mouse ADAMTS13**

-A polyclonal antiserum against mADAMTS13 was raised by DNA-based immunization protocols. Rabbits were immunized by intradermal injection with ~1 mg of mADAMTS13 expression plasmid at 25 sites on the back. Booster immunizations were carried out by the same protocol 3 weeks after the primary immunization. Serum was collected 3 weeks after the second immunization. The IgG fraction was then prepared by an affinity chromatography using a protein G column (Amersham Biosciences).

**Enzymatic Assay**—Purified GST-mVWF73-H (500 ng) was incubated with recombinant mADAMTS13L or mADAMTS13S in 40 μl of reaction buffer (5 mM Tris-HCl, 10 mM BaCl2, 0.01% Tween 20, and 1 mM p-amidinophenylmethanesulfonyl fluoride hydrochloride, pH 8.0) at 37 °C for 1 h. The reaction was stopped by adding 10 μl of SDS sample buffer (50 mM Tris-HCl, 10% SDS, 250 mM dithiothreitol, 10 mM EDTA, 0.1% bromphenol blue, 30% glycerol, pH 6.8). The samples were sub-

**Fig. 2. Genotyping of mouse Adamts13.** A, diagram of a segment of the Adamts13 gene around the IAP insertion site. The sites of primers used for the genotyping PCR are indicated by arrows. The EcoRV fragments detected in Southern blot analysis are indicated by double-headed arrows. B, PCR analysis. In mixture of three primers, F and R1 primers generate a 305-bp product specific for the IAP-free Adamts13 gene, whereas F and R2 primers generate a 230-bp product specific for the IAP-inserted Adamts13 gene. C, Southern blot analysis. Genomic DNA from each mouse strain was digested with EcoRV and hybridized with the probe that detects a 1.5-kb fragment in the IAP-free allele and a 4.3-kb fragment in the IAP-inserted allele.

**Fig. 3. RT-PCR of Adamts13 mRNA in liver.** PCR primers are shown as arrows indicating direction at their approximate locations. In combination of three primers, S and A1 primers generate a 540-bp product specific to the IAP-free transcript, whereas primers S and A2 generate a 370-bp product specific to the IAP-inserted transcript.

Tris-HCl, 2% SDS, 50 mM dithiothreitol, 2 mM EDTA, 0.02% bromphenol blue, 6% glycerol, pH 6.8).

Recombinant proteins were detected by SDS-PAGE and Western blot as described previously (16). For culture media, a rabbit anti-mADAMTS13 polyclonal antibody (described below) and an HRP-labeled goat anti-rabbit IgG antibody (Kirkegaard & Perry Laboratories) were used for detection after SDS-PAGE under nonreducing condition. For cell lysates, an anti-FLAG M2 monoclonal antibody (Sigma) and an HRP-labeled goat anti-mouse IgG antibody (Kirkegaard & Perry Laboratories) were used for detection after SDS-PAGE under reducing condition. Chemiluminescence was developed using the Western Lighting Chemiluminescence Reagent Plus (PerkinElmer Life Sciences) and detected by an LAS-1000plus image analyzer (Fujifilm).

**Preparation of Polyclonal Antibody against Mouse ADAMTS13**—A polyclonal antiserum against mADAMTS13 was raised by DNA-based immunization protocols. Rabbits were immunized by intradermal injection with ~1 mg of mADAMTS13 expression plasmid at 25 sites on the back. Booster immunizations were carried out by the same protocol 3 weeks after the primary immunization. Serum was collected 3 weeks after the second immunization. The IgG fraction was then prepared by an affinity chromatography using a protein G column (Amersham Biosciences).

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**Fig. 4. Northern blot analysis of mouse Adamts13 mRNA.** A, expression of Adamts13 mRNA in liver. Poly(A) RNA isolated from liver of indicated strains was probed with a 1.3-kb Adamts13 cDNA corresponding to exons 3–13. The approximate sizes of the IAP-free (5.0 kb) and the IAP chimeric (3.5 kb) transcripts are indicated by arrowheads. B, expression of Adamts13 mRNA in tissues from BALB/c mice. C, expression of Adamts13 mRNA in tissues from Swiss/Webster mice. The sizes of RNA markers are shown at the left.
These results implied that the Adamts13 mRNA, we performed RT-PCR and 3'-RACE using poly(A)/H11032 tissues of the BALB/c and the Swiss Webster strains. As shown RNA from the liver of a C57BL/6 mouse. Unexpectedly, the lytic activity of plasma from five mouse strains in the same way.

RESULTS

Identification of Adamts13 in the C57BL/6 Strain—To identify the orthologous mouse gene of human ADAMTS13, we performed a BLAST search in the public database, based on the human ADAMTS13 cDNA sequence (GenBank™ accession number AB069698, 4,284-nucleotide ORF) reported by Soejima et al. (8). This search led us to identify a compatible genomic sequence (GenBank™ accession number AC090008) derived from the C57BL/6 strain. This sequence was located on chromosome 2, band A3 and contained 29 conserved exons similar to human ADAMTS13. To obtain the cDNA to corresponding mRNA, we performed RT-PCR and 3'-RACE using poly(A)/H11001 RNA from various mouse strains. The obtained sequences indicated that the Adamts13 cDNA (GenBank™ accession number AB112302) contained a 4,281-nucleotide ORF similar to human ADAMTS13 (4,284-nucleotide ORF). To determine the complete genomic sequence of Adamts13 in the 129/Sv strain, we screened a 129/Sv mouse genomic library. Sequence analysis of positive phage clones confirmed the absence of IAP in the Adamts13 gene. Adamts13 in the 129/Sv strain (GenBank™ accession number AB099445) contained 29 exons like human ADAMTS13 and spanned ~30 kb.

To examine the effect of IAP on Adamts13 mRNA splicing, RT-PCR was performed using liver poly(A)/H11011 RNA from six mouse strains (Fig. 3). The exon 21/22-specific sense primer, the exon 24/25-specific antisense primer, and the pseudo-exon 24-specific antisense primer were mixed and used for the amplification. We detected the IAP chimeric transcript in four inbred strains with the IAP insertion. In contrast, the IAP-free transcript was observed in the 129/Sv strain. The heterogeneous expression of two types of transcripts was observed in samples from the ICR strain. To characterize the transcripts in more detail, Northern blot analysis of liver RNA was carried out using a 1.3-kb probe spanning exons 3–13 of Adamts13 cDNA (Fig. 4A). The RNA was prepared from the same animals as used for the Southern blot analysis. An ~3.5-kb mRNA corresponding to the size of IAP chimeric transcript was detected in the C57BL/6 and the ICR strains. The IAP-free transcript of ~5.0 kb was observed in the 129/Sv and the ICR strains.

Thus, these results clearly indicate the presence of two types of mouse Adamts13 in a strain-specific manner (Fig. 5). Adamts13 of the 129/Sv strain encodes an ADAMTS13 protein containing 1,426 amino acid residues with the same domain structure as hADAMTS13, designated mADAMTS13L. Adamts13 of the BALB/c, C3H/He, C57BL/6, and DBA/2 strains encodes the shorter ADAMTS13 protein including only 1,037 amino acid residues, designated mADAMTS13S. In this protein, the C-terminal two TSP1 and two CUB domains are replaced with the 16-amino acid sequence, ALVWEEAAPT-FAVTRWR, derived from the IAP. The outbred ICR strain carries either the IAP-free or IAP-inserted allele or both.

Expression of the Adamts13 mRNA in Mouse Tissues—To study the expression pattern of the mouse Adamts13 gene, we analyzed Northern blots containing poly(A)/H11011 RNA from various tissues of the BALB/c and the Swiss Webster strains. As shown
in Fig. 4 (B and C), Adanuts13 mRNA in both strains was exclusively observed in the liver, suggesting that mADAMTS13 is primarily synthesized in the liver, similar to hADAMTS13. A single transcript of 3.5 kb was expressed in liver of the BALB/c strain with the IAP insertion. In contrast, two transcripts of 5.0 and 3.5 kb were detected in liver of the outbred Swiss Webster strain, suggesting that this strain may carry two types of alleles, like the ICR strain. Both SwissWebster and ICR strains are derived from a colony of Swiss mice.

Comparison of the Deduced Amino Acid Sequences of mADAMTS13 and hADAMTS13—The deduced amino acid sequences of mouse and human ADAMTS13 were aligned (Fig. 6). The overall sequence identity between mADAMTS13 and hADAMTS13 was ~70%. The highest identity (>80%) was observed in the disintegrin-like domain, the TSP1-1 domain, and the Cys-rich domain. Relatively low conservation (<60%) was observed in the signal peptide, propeptide, and TSP1–4 domains.

mADAMTS13 contained eight potential N-glycosylation sites, six of which were conserved in hADAMTS13. mADAMTS13 also included several motifs characteristic for each domain of hADAMTS13, such as a furin cleavage sequence at the end of the protein.

Fig. 6. Alignment of deduced amino acid sequences of mADAMTS13 and hADAMTS13. Sequences are represented with the single-letter code, and residues that differ from mADAMTS13L are shaded. Each structural domain is underlined. The predicted furin cleavage site (RXKR) is marked with an arrow. The IAP-derived 16 amino acid residues in mADAMTS13S are boxed. Open circles indicate the potential N-glycosylation sites.
The small size difference of recombinant enzymes in medium and cell lysates was due to the difference of electrophoretic condition. The faster migrating bands seen in medium and cell lysates expressing mADAMTS13 in the cell lysate. The cell lysates including extracellular matrixes were analyzed by Western blot with an anti-FLAG antibody under reducing condition. The size of protein markers is indicated at the left. The size of protein markers is indicated at the left.

The VWF cleaving activities of recombinant proteins were measured by the degradation of the specific recombinant substrate, GST-mVWF73-H. The relative concentration of recombinant mADAMTS13 in the culture medium was determined by chemiluminescent intensities on Western blot, and equal amounts were used for the enzymatic assay. The substrate, GST-mVWF73-H, was incubated with serial dilutions of the culture medium, and the cleavage product including the N-terminal GST tag was visualized by Western blot using anti-GST (Fig. 8). When the substrate was incubated with the medium of mADAMTS13L-transfected cells, a band appeared with the expected size of the N-terminal portion (28 kDa) in a concentration-dependent manner, indicating the cleaving activity of recombinant mADAMTS13L (Fig. 8A). No degradation was observed after the incubation of GST-mVWF73-H with the medium from untransfected cells. The cleaved band was also detected after incubation with the mADAMTS13S culture medium, and the chemiluminescent intensities of the product bands were almost equal to those obtained by mADAMTS13L (Fig. 8A). We confirmed that the degradation of GST-mVWF73-H by mADAMTS13 was also time-dependent, and the rate of the product formation by mADAMTS13S was similar to that by mADAMTS13L (Fig. 8B).

The VWF cleaving Activity of Mouse Plasma—To examine the ADAMTS13 activity in plasma from various mouse strains, we collected plasma samples from five strains and carried out the enzymatic assay using GST-mVWF73-H. As shown in Fig. 9, plasma from all strains cleaved GST-mVWF73-H. Comparison of the product levels did not reveal a significant difference among strains. This suggested that the IAP insertion into the Adams13 gene does not affect the in vitro cleavage of GST-mVWF73-H by plasma.

**DISCUSSION**

In this study, we identified two isoforms of the mouse Adams13 gene that result from the strain-specific insertion of...
Fig. 9. Cleavage of GST-mVWF73-H by mouse plasma. GST-mVWF73-H was incubated with plasma samples from mice with (BALB/c, C3H/He, C57/BL6, and DBA/2) or without (129/Sv) the IAP insertion in the Adamts13 gene. The products were analyzed by Western blot using an anti-GST antibody. The results from three animals/strain are shown.

an IAP-retrotransposon. The IAP-free Adamts13 gene contained 29 exons, and the deduced protein sequence included 1,426 amino acid residues with the same domain organization as hADAMTS13. In contrast, the IAP-inserted Adamts13 gene contained only 24 exons encoding 1,037 amino acids having a truncated C terminus.

The inserted IAP is one of the endogenous transposable elements, which is closely related to retroviruses and transposed via the reverse transcription of an RNA intermediate (26, 27). The IAP element contains two long terminal repeats with the signals for the initiation/regulation of transcription and for the polyadenylation of transcripts (28). IAP insertions into introns have been shown to cause formation of chimeric transcripts (29–32), similar to our findings in the Adamts13 gene. We noted that the presence of IAP in the Adamts13 gene induces the appearance of a cryptic splicing site followed by a premature in-frame stop codon and a polyadenylation signal derived from the IAP long terminal repeat. As a result, the insertion leads to replacement of the last 405 amino acid residues corresponding to two TSP1 motifs and two CUB domains with the IAP-encoded 16 amino acid residues.

Northern blot and RT-PCR analyses confirmed that the IAP chimeric short transcript (3.5 kb) and the IAP-free long transcript (5 kb) were expressed in a strain-specific manner. Both types of transcripts were specifically expressed in the liver, consistent with expression of the human ADAMTS13 gene. It should be noted that the IAP insertion could not completely abolish the formation of mADAMTS13L mRNA. The RT-PCR products (540-bp; Fig. 3) characteristic of mADAMTS13L mRNA were also detectable in the strains with the IAP insertion when using a large amount of template (data not shown). A small amount of mADAMTS13L protein may be expressed in mice with the IAP-inserted Adamts13 gene such as BALB/c, C3H/He, C57/BL6, and DBA/2 strains. Incidentally, the RT-PCR and 3′-RACE data did not show any splicing variants that encoded mADAMTS13S-like protein in the IAP-free strains.

Recently, we developed a novel recombinant substrate, GST-VWF73-H, to measure hADAMTS13 activity (24). GST-VWF73-H is a partial region of human VWF flanked by GST and His6 tags. Because of difficulty in isolating VWF from mouse plasma, we have also prepared the recombinant substrate, GST-mVWF73-H, based on the mouse VWF cDNA sequence. Both mouse and human plasma efficiently cleaved GST-mVWF73-H and produced a fragment of the expected size. Mouse plasma also cleaved the substrate for hADAMTS13, GST-VWF73-H (data not shown).

Both recombinant mADAMTS13L and mADAMTS13S were secreted into the culture medium of HeLa cells. This result indicates that the IAP insertion does not abolish secretion of mADAMTS13 from cells. The recombinant mADAMTS13L and mADAMTS13S cleaved GST-mVWF73-H with nearly the same efficiency. Similarly, a deletion mutant of hADAMTS13 in mimercy of mADAMTS13S was also secreted efficiently from HeLa cells and cleaved GST-VWF73-H with normal activity (data not shown). In previous reports, we and others found that deletion mutants of hADAMTS13 devoid of the C-terminal TSP1 motifs and CUB domains retained VWF cleaving activity (21, 22). Therefore, our current observation on mouse and human recombinant proteins was consistent with these previous studies. Moreover, the plasma VWF cleaving activities in mice were also comparable among the strains with or without the IAP insertion in the Adamts13 gene. The C-terminal two TSP1 motifs and two CUB domains of mADAMTS13S may contribute to activity but are not essential for the VWF cleavage, at least in vitro.

The fact that several common strains of mice have a naturally truncated form of ADAMTS13 allows us to hypothesize that the truncated domains are not necessary in vivo. However, several mutations in TSP1-7, TSP1-8, CUB-1, and CUB-2 domains of hADAMTS13 were reported to associate with congenital thrombotic thrombocytopenic purpura (15, 17–20). It is still unclear whether these mutants are secreted from cells, as is the case with mADAMTS13S. To date, two mutations, R1123C and 4143insA, were characterized by expression analysis, and both impaired secretion of the enzyme from cells (19, 20). The C-terminal mutations found in thrombotic thrombocytopenic purpura patients may influence their synthesis or secretion.

Bernardo et al. (33) reported that several short peptides within the regions from TSP1-6 to the C terminus of hADAMTS13 block VWF cleavage on the endothelial cell surface under flow conditions. This finding suggests an important role for the C-terminal domains in vivo. Although our results clearly show that the mouse has managed without full-length ADAMTS13, the relative importance of ADAMTS13 for regulation of VWF activity may be different between human and mouse. A gene targeting technique of mouse Adamts13 will help to clarify the physiological contribution of ADAMTS13.

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