Matrilin-4, a new member of the matrilin family of extracellular matrix proteins

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Abstract Mouse cDNA encoding for matrilin-4 was cloned and the primary structure of this fourth member of the matrilin family was deduced from the nucleotide sequence. The protein precursor of 624 amino acids consists of a putative signal peptide, two vWFA-like domains linked by four epidermal growth factor-like modules and a potential coiled-coil α-helical oligomerization domain at the C-terminus. The predicted M_r of the mature protein is 66 442. Expression in lung, brain, sternum, kidney and heart was detected by Northern blot analysis of mouse mRNA. Additionally an alternatively spliced mRNA lacking the sequence coding for the first vWFA domain was found in 7 weeks old mice leading to a protein precursor of 434 amino acids and a predicted M_r of the mature protein of 45 468.

Key words: Matrilin; Extracellular matrix; Von Willebrand factor type A; Epidermal growth factor; Coiled-coil α-helix; Alternative splicing

1. Introduction

The matrilins are a family of extracellular matrix proteins with a multidomain structure consisting of von Willebrand factor type A-like domains, EGF-like domains and an α-helical coiled-coil domain. The matrilins accordingly belong to the von Willebrand factor A superfamily that consists of proteins with different domain organization and a variety of functions [1]. Several vWFA-like domains have been implicated in interactions with collagen [1].

To date three matrilins have been known, cartilage matrix protein (CMP) now alternatively named matrilin-1 [2] and the recently cloned matrilin-2 [2] and matrilin-3 [3,4]. Matrilin-1 is a trimer of identical ellipsoid subunits assembled via their C-terminal extension domains in a coiled-coil α-helix [5]. Aggrecan [6,7] and type II collagen-containing fibrils [8] have been proposed as binding partners for matrilin-1 and it has been suggested that matrilin-1 might play an integrating role in cartilage extracellular matrix organization as a bridging molecule between these two major constituents [7]. It has also been shown that matrilin-1 can form filamentous networks independent of collagen fibrils [9]. Matrilin-1 is expressed only in some types of the hyaline cartilage [10] whereas matrilin-2 is found in calvaria, uterus, heart, skeletal muscle, brain and skin but not in normal cartilage [2]. The expression patterns appear to be complementary and the tissue distribution of matrilin-3 shows similarities to that of matrilin-1 [3,4]. It has been proposed from the structural homologies and the complementary sites of expression, that matrilin-1 and -2 may have a similar function in different forms of extracellular matrix [2]. The primary structure of matrilin-1 has been deduced from chicken [11], man [12] and mouse [13] cDNA, that of matrilin-2 from mouse and partially from human cDNA [2] and that of matrilin-3 from mouse [3], chicken [4] and partially from human cDNA [3,4]. In all cases the sequence is highly conserved between these species, with the vWFA-like domain being most conserved and the coiled-coil the least conserved domain. The modular structure of the three is very similar with each containing one or two vWFA-like domains, varying numbers of EGF-like domains after the N-terminal first vWFA-like domain and a C-terminal oligomerization domain. Matrilin-2 possesses an additional unique sequence of 75 amino acids between the second vWFA-like domain and the oligomerization unit and it has recently been shown that synthetic peptides corresponding to the C-terminal domain of matrilin-2 assemble into a three-stranded α-helical coiled-coil [14]. Matrilin-2 and -3 have a highly positively charged stretch of amino acid residues between the signal peptide and the first vWFA-like domain. Matrilin-3 lacks the second vWFA-like domain seen in matrilin-1 and -2.

Supposing the existence of more members of this new family of extracellular matrix proteins, we looked for gene products with a similar modular structure by searching in the EST databases. Here we report on the primary structure of matrilin-4 from mouse.

2. Materials and methods

2.1. Clones, 5′ RACE and RT-PCR

The matrilin-4 mouse EST clones were obtained from IMAGE consortium [15]. 5′ RACE was performed with the Marathon cDNA Amplification kit (Clontech) following manufacturers instructions using polyA$^+$-RNA prepared with the Oligotex mRNA-kit (Quiagen) from 19 days old mouse total embryos (CD1/C57Bl/6 hybrids). We used the adaptor primer AP1 (Clontech) and the gene specific primer m48: 5′-GTTCATAGCGTACTGGATCGCC-3′ (nt 461–440 on the cDNA sequence, Fig. 1) designed from the EST sequences for the first reaction and the nested adaptor primer AP2 (Clontech) and primer m48 for a semi-nested second PCR reaction. A faint band with the highest molecular weight was eluted from the agarose gel and used as a template in a third PCR reaction with AP2 and m48. Each PCR was performed with 40 cycles under following conditions: 1 min 95°C, 1 min 63°C, 1 min 72°C. To exclude PCR artifacts after a total of 120 cycles of PCR we performed at least four independent RT-PCR reactions with mRNA prepared from kidney of adult CD1 mice to get an errorless sequence by direct sequencing of

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Abbreviations: vWFA, von Willebrand factor type A; EGF, epidermal growth factor; EST, expressed sequence tag; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription polymerase chain reaction; RACE, rapid amplification of cDNA ends

The nucleotide sequence reported in this paper has been submitted to the EMBL nucleotide sequence database with accession number AJ006140.

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the RT-PCR products. After reverse transcription with Superscript II (BRL), PCR was carried out with Amplitaq (Perkin Elmer) using primers m45, 5′-AACCATCCACCAGGAGAACAAG-3′ (nt 1275–1254) and m412, 5′-GCTCTGTAGCCGCACCTCTG-3′ (nt 9–28) for 40 cycles under following conditions: 1 min 95°C, 1 min 63°C, 1 min 72°C. The RT-PCR reactions for the alternatively spliced mRNA were performed under the same conditions.

2.2. DNA sequencing

DNA sequencing was performed on double-stranded plasmid DNA and PCR products using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit and the products were resolved on an ABI Prism 377 Automated Sequencer (Perkin-Elmer/Applied Biosystems). Plasmids were sequenced two times in both directions, RT-PCR products sequenced directly at least 4 times in both directions to exclude PCR artifacts. Nucleotide sequence analysis was performed with the programs of the GCG package [16].

2.3. Northern blot analysis

Total RNA of various tissues was extracted from 7 weeks old female CD1 mice by the guanidinium-thiocyanate method. PolyA$^+$-RNA was prepared from the total RNA using the Oligotex mRNA-kit (Quiagen). Three to six μg aliquots of polyA$^+$-RNA were electrophoresed on a 1.2% denaturing agarose-formaldehyde gel, blotted and hybridized. The conditions for the last two washing steps were: 0.1×SSC, 0.1% SDS at 65°C for 10 min each.

3. Results

3.1. Mouse matrilin-4

A search in the EST database yielded several mouse ESTs (AA003393, AA050024, AA462743, AA030414, W65581, AA016343, AA289820 and AA048871) with a sequence identity to the other matrilins of about 50% that could not be attributed to one of the known matrilins. They could be arranged in two independent sets of overlapping clones. By RT-PCR (data not shown) we could show that both sets code for the same protein. By sequencing we found that the delivered N-terminal ESTs (AA289820 and AA048871) were contaminated and did not contain the desired sequence. Sequencing of the remaining most N-terminal EST AA003393 yielded the C-terminal half of matrilin-4. By 5′ RACE we cloned the N-terminal half. The combined cDNA for mouse matrilin-4 covered 2193 bp (Fig. 1). The 5′ untranslated sequence was 89 bp long and the first ATG codon was followed by an open reading frame of 1872 bp ending with an TGA stop codon and 229 bp 3′ untranslated sequence that lacked a polyadenylation signal. The protein predicted from the open reading frame consisted of 624 amino acids preceded by a putative signal

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GGCCCGTCGCTCTGTAGCCGCACCTCTGAGCTGTCCGGCAGGCTCAGCTGCAGGCTGTGACACTGTGGTTCGTCATCCTTCCAAGCACCATGAGAGGCCC  100
                                                                                          M  R  G  P     4
CTGCTGCTGGCCACTCTCTCTGTTGCTGCTCTTCCTGCAGTCCTGGGAAACTCAGCTCCAGTCTGCAGGTCCTAGGTGCTATAATGGGCCCCTGGATTTG  200
 C  C  W  P  L  S  L  L  L  L  F  L  Q  S  W  E  T↓ Q  L  Q  S  A  G  P  R  C  Y  N  G  P  L  D  L    37
GTGTTCATGATTGATAGCTCCCGCAGCGTGCGCCCCTTCGAGTTTGAGACCATGAGGCAGTTCCTAGTGGGCCTCCTCCGCAGCCTGGACGTGGGTCTGA  300
V  F  M  I  D  S  S  R  S  V  R  P  F  E  F  E  T  M  R  Q  F  L  V  G  L  L  R  S  L  D  V  G  L  N   71
ACGCCACGCGCGTTGGGGTGATCCAGTATTCTAGTCAAGTGCAAAGCGTCTTTCCCCTGGGCGCCTTTTCGCGCCGCGAGGACATGGAACGAGCCATCCG  400
  A  T  R  V  G  V  I  Q  Y  S  S  Q  V  Q  S  V  F  P  L  G  A  F  S  R  R  E  D  M  E  R  A  I  R  104
CGCGGTGGTGCCGCTGGCGCAGGGCACCATGACCGGGCTGGCGATCCAGTACGCTATGAACGTGGCCTTCAGCGAGGCCGAAGGCGCGCGCCCATCGGAG  500
 A  V  V  P  L  A  Q  G  T  M  T  G  L  A  I  Q  Y  A  M  N  V  A  F  S  E  A  E  G  A  R  P  S  E   137
GAGCGAGTGCCGCGCGTCTTGGTCATCGTGACAGACGGGCGACCTCAAGACCGAGTGGCCGAAGTGGCCGCTCAGGCGCGCGCCCGCGGCATTGAGATCT  600
E  R  V  P  R  V  L  V  I  V  T  D  G  R  P  Q  D  R  V  A  E  V  A  A  Q  A  R  A  R  G  I  E  I  Y  171
ATGCGGTAGGGGTACAGCGAGCTGACGTGGGCTCTCTTCGCACCATGGCTTCGCCGCCGCTGGATCAGCATGTCTTCTTAGTGGAGTCCTTCGATCTCAT  700
  A  V  G  V  Q  R  A  D  V  G  S  L  R  T  M  A  S  P  P  L  D  Q  H  V  F  L  V  E  S  F  D  L  I  204
CCAGGAGTTTGGCCTGCAGTTCCAGGGCCGCCTGTGCGGGAAGGACCTGTGTGCTGAGTTGGTTCATGGCTGCCAACACCTGTGTGTCAACGCCCCAGGA  800
 Q  E  F  G  L  Q  F  Q  G  R  L  C  G  K  D  L  C  A  E  L  V  H  G  C  Q  H  L  C  V  N  A  P  G   237
ACATTCTACTGCGCCTGCAACTCTGGCTACAAGCTAGCACCAGATAACAAGAATTGTTTGGCCTTGGATCTCTGCGCTGAAGGAACCCATGGCTGTGAAC  900
T  F  Y  C  A  C  N  S  G  Y  K  L  A  P  D  N  K  N  C  L  A  L  D  L  C  A  E  G  T  H  G  C  E  H  271
ACCTCTGTGTCAACTCCGTGGACTCGTATTTCTGTCGTTGCCGAGCTGGCTTTGCGCTCCAGCAGGACCAGAGGAGCTGCAGAGCCATTGACTACTGCAG 1000
  L  C  V  N  S  V  D  S  Y  F  C  R  C  R  A  G  F  A  L  Q  Q  D  Q  R  S  C  R  A  I  D  Y  C  S  304
CTTTGGAAACCACAGCTGCCAGCATGAGTGTGTGAGCACTTTGGCGGGGCCACAGTGTCGCTGCAGAGAGGGCCACGACCTGCTACCTGATGGGAGAAGC 1100
 F  G  N  H  S  C  Q  H  E  C  V  S  T  L  A  G  P  Q  C  R  C  R  E  G  H  D  L  L  P  D  G  R  S   337
TGTCGGGTCAGGGACTTCTGCAATGGTGTGGACCATGGCTGTGAGTTCCAGTGTGTGAGTGAGGGTCTTTCCTTCCACTGCCTGTGCCCTGAGGGGAGGC 1200
C  R  V  R  D  F  C  N  G  V  D  H  G  C  E  F  Q  C  V  S  E  G  L  S  F  H  C  L  C  P  E  G  R  R  371
GACTTCAGGCTGATGGCAAGAGCTGTGACCGGTGCCGGGAGGGCCACGTGGATCTTGTTCTCCTGGTGGATGGTTCCAAGAGCGTGCGCCCACAGAACTT 1300
  L  Q  A  D  G  K  S  C  D  R  C  R  E  G  H  V  D  L  V  L  L  V  D  G  S  K  S  V  R  P  Q  N  F  404
CGAGCTGGTGAAGCGCTTCGTGAACCAGATTGTGGATTTCCTTGACGTGTCTCCCGAGGGCACACGTGTTGGGCTGGTGCAGTTCTCCAGCCGGGTGCGC 1400
 E  L  V  K  R  F  V  N  Q  I  V  D  F  L  D  V  S  P  E  G  T  R  V  G  L  V  Q  F  S  S  R  V  R   437
ACCGAGTTCCCCCTGGGCCGCTATGGCACCGCAGCTGAGGTGAAGCAGGCAGTTTTGGCCGTGGAGTACATGGAGCGCGGCACCATGACAGGGCTGGCCC 1500
T  E  F  P  L  G  R  Y  G  T  A  A  E  V  K  Q  A  V  L  A  V  E  Y  M  E  R  G  T  M  T  G  L  A  L  471
TGCGTCACATGGTGGAGCACAGCTTCTCTGAGGCGCAGGGCGCGCGGCCTCGCGACCTCAACGTGCCTCGCGTGGGCTTGGTGTTCACTGATGGCCGCTC 1600
  R  H  M  V  E  H  S  F  S  E  A  Q  G  A  R  P  R  D  L  N  V  P  R  V  G  L  V  F  T  D  G  R  S  504
TCAGGATGACATTTCAGTGTGGGCAGCTCGTGCCAAAGAGGAAGGCATCGTCATGTATGCCGTGGGCGTGGGTAAGGCTGTGGAGGAAGAGCTGCGTGAG 1700
 Q  D  D  I  S  V  W  A  A  R  A  K  E  E  G  I  V  M  Y  A  V  G  V  G  K  A  V  E  E  E  L  R  E   537
ATCGCATCGGAACCATCGGAGTTGCACGTGTCCTATTCTCCGGACTTTAGCACCATGACGCACCTGCTGGAGAATCTCAAAGGCAGCATTTGCCCAGAGG 1800
I  A  S  E  P  S  E  L  H  V  S  Y  S  P  D  F  S  T  M  T  H  L  L  E  N  L  K  G  S  I  C  P  E  E  571
AGGGCATTGGCGCGGGGACAGAGCTTCGGAGTCCCTGCGAATGCGAAAGCCTCGTGGAGTTCCAGGGCCGCACGCTGGGGGCGCTCGAGAGCCTGACGCA 1900
  G  I  G  A  G  T  E  L  R  S  P  C  E  C  E  S  L  V  E  F  Q  G  R  T  L  G  A  L  E  S  L  T  Q  604
GAACCTGGCTCGGCTGACAGAGCGCCTGGAGGAGCTGGAGAACCAGCTGGCTAGCCGAAAGTGAGGGTCGCACGCTGTCTGAGCAGCCACGCGAGACCGA 2000
 N  L  A  R  L  T  E  R  L  E  E  L  E  N  Q  L  A  S  R  K  *                                        624
GGCCGAGCCCCGCAGCCTGGACCGTCCAGGAGGGGGCGCTGGCGGGCCCCACGCGCTGCCTTCGAGCTGGCCTCACCTGGACCAGGAGCCAGATTTAGGG 2100
GATGGGGATGAGGGGATGGGGAGGTGCTGGTGGGGAAGGACTGCTACACTCGCCGCGAGCTTTGTTGGATTTCTTTTTTTGTTTTCCTTTTCT        2193
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Fig. 1. Nucleotide and deduced amino acid sequences of mouse matrilin-4. nt 1–28 is from CD1/C57BL/6J hybrids, nt 29–1281 is from CD1 and nt 1282–2193 is from C57BL/6J mice. Polymorphisms: nt 1045 is A instead of C in C57BL/6J leading to aa (319) Glu instead of Ala and nt 1126 is A instead of G in C57BL/6J leading to aa (346) Asp instead of Gly. Arrowhead, predicted propeptidase cleavage site; the potential *N*-linked glycosylation sites (amino acid residues 71–73 and 307–309) are underlined.

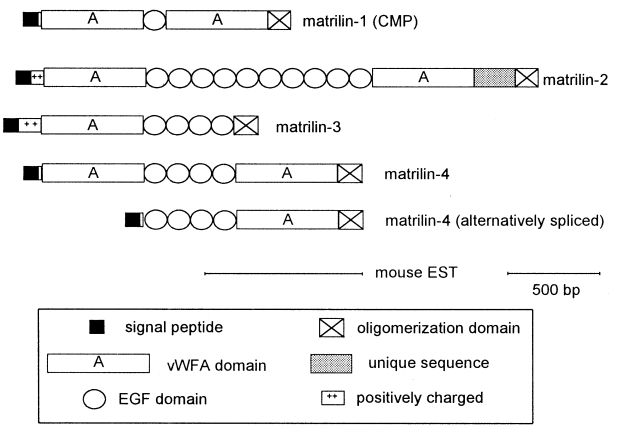

Fig. 2. Comparison of the modular structure of the matrilin family. The horizontal line denotes the coding region of the uncomplete mouse matrilin-4 cDNA clone (ID 427166) [15].

peptide of 21 amino acids as predicted by a method using neural networks [17] (Fig. 1). The mature secreted protein has a predicted M_r of 66 442 and has the characteristic domain structure of the matrilin family (Fig. 2). It contains two vWFA-like domains linked by four EGF-like domains and a putative C-terminal oligomerization domain. The identity to mouse matrilin-1, -2 and -3 is 50.6%, 52.8% and 48.5% respectively. The first vWFA-like domain (Asn^{71}-Ala-Thr) and the third EGF-like domain (Asn^{307}-His-Ser) of the mouse matrilin-4 contain a potential *N*-glycosylation site.

A sequence alignment of the different modules with their counterparts in the other matrilins of mouse shows the striking homology (Fig. 3). The sequence identity of the first vWFA-like domain of matrilin-4 to the first vWFA-like domains of matrilin-1, -2 and -3 is 49.2%, 52.4% and 51.3% respectively and for the second vWFA-like domain of matrilin-4 to the second vWFA domains of matrilin-1 and -2 is 56.0% and 58.8% respectively. The sequence identity between both vWFA domains of matrilin-4 is 41.7%. The metal ion-dependent adhesion site (MIDAS) motif [18], the two flanking cysteine residues and the six hydrophobic residues that are highly conserved in vWFA-like domains [19] are also present in matrilin-4. The structure consists of alternating amphipatic α-helices and hydrophobic β strands (Fig. 3A) and is in good agreement with the structure of the vWFA-like domain of the α subunit of the A domain of the integrins CR3 [18] and LFA-1 [20] and the A3 domain of the von Willebrand factor [21] which have been determined at high resolution. The four EGF-like domains, which are characterized by the spacing of six cysteines, have an average sequence identity with each other of 42.0%. Comparing the EGF-like domains of matrilin-4 with the EGF-like domains of the other matrilins, the identity is highest (58.5%) between the first EGF-like domain of matrilin-4 and the second EGF-like domain of matrilin-2 and lowest (29.3%) between the third EGF-like domain of matrilin-4 and fourth EGF-like domain of matrilin-3. The average sequence identity with respect to the other matrilins is 44.7%. The EGF-like domains of matrilin-4 lack the key residues involved in Ca^{2+}-binding [22] to EGF-like domains and do also not contain the extra amino acid residue found in the EGF-like domains of human and mouse matrilin-3 (Fig. 3B). The potential oligomerization domain has the lowest homology to the other matrilins (Fig. 3C). While the degree of identity is 38.2% to matrilin-1, 40.4% to matrilin-2 and only 20.7% to matrilin-3 the positions a and d of the heptad repeats are well conserved. The COILS program [23] detects 4.5 heptad repeats that are also found in matrilin-1 and -2. The pair of closely spaced cysteine residues at the beginning of the oligomerization domain is conserved in all four matrilins. In the case of matrilin-1 it was shown that these cysteines form interchain disulfide bonds [24].

3.2. Alternative splicing

By RT-PCR we found an alternatively spliced mRNA in lung, sternum, brain, kidney and heart of 7 weeks old mice lacking 190 amino acid residues coding for the first vWFA-like domain (Fig. 2). This splice variant was not detected in 19.5 days embryos or in 2, 8, and 21 days old animals (data not shown). The codons $G^{168}G^{169}T^{170}$ of aa 27 (Gly) and $G^{738}G^{739}G^{740}$ of aa 217 (Gly) (Fig. 1) are fused to a resulting new GGG codon (Gly) leading to the transition nucleotide sequence $G^{165}C^{166}A^{167}GGG^{740}A^{741}A^{742}G^{743}$ and the corresponding amino acid sequence $A^{26}GK^{218}$. The exact splice site will be determined when the genomic sequence is known. The alternative splicing leads to a mature protein product that has a predicted M_r of 45 468.

3.3. Gene expression

We studied the expression of the matrilin-4 gene in different mouse tissues by Northern hybridization with polyA$^+$-RNA (Fig. 4). The strongest signal, a 2.7 kbp band, could be detected in liver, weak signals also in brain and sternum and very weak signals in kidney and heart. Additionally a 4.0 kbp and a 2.45 kbp band was detected in lung, brain and sternum. The 2.1 kbp band present in lung and very weakly in brain was due to the differentially spliced mRNA as the signal was absent when the blot was hybridized with a probe coding for the first vWFA-like domain alone (data not shown).

4. Discussion

We have isolated and characterized mouse cDNA for matrilin-4, the fourth member of a novel family of extracellular matrix proteins, as evidenced from the striking sequence similarity of the protein to the other three members which are the nearest homologues detected in the databases. Matrilin-4 shares the modular composition of vWFA-like, EGF-like and coiled-coil domains. Although there are strong homologies between matrilin-4 and the previously known matrilins there are clear differences in the domain arrangement. The vWFA domains and the coiled-coil domain of matrilin-4 have the highest similarity to the respective domains of matrilin-2. The gene is mainly expressed in lung, where the mRNA could be derived from bronchial cartilage but the minor expression in brain, sternum, kidney and heart shows that matrilin-4, as matrilin-2, is expressed also in tissues other than cartilage. It has been proposed that matrilin-2 may have a tissue specific role similar to that of matrilin-1, which binds to collagen fibrils and proteoglycan in cartilage and possibly connects these structures [2]. Similarly, matrilin-4 may take over this role especially in lung where matrilin-2 on the basis of Northern hybridization is not expressed [2].

The conservation of the MIDAS motif [18] in the vWFA-like domain implies the dependence on divalent cations for function although it has been shown recently from crystallo-

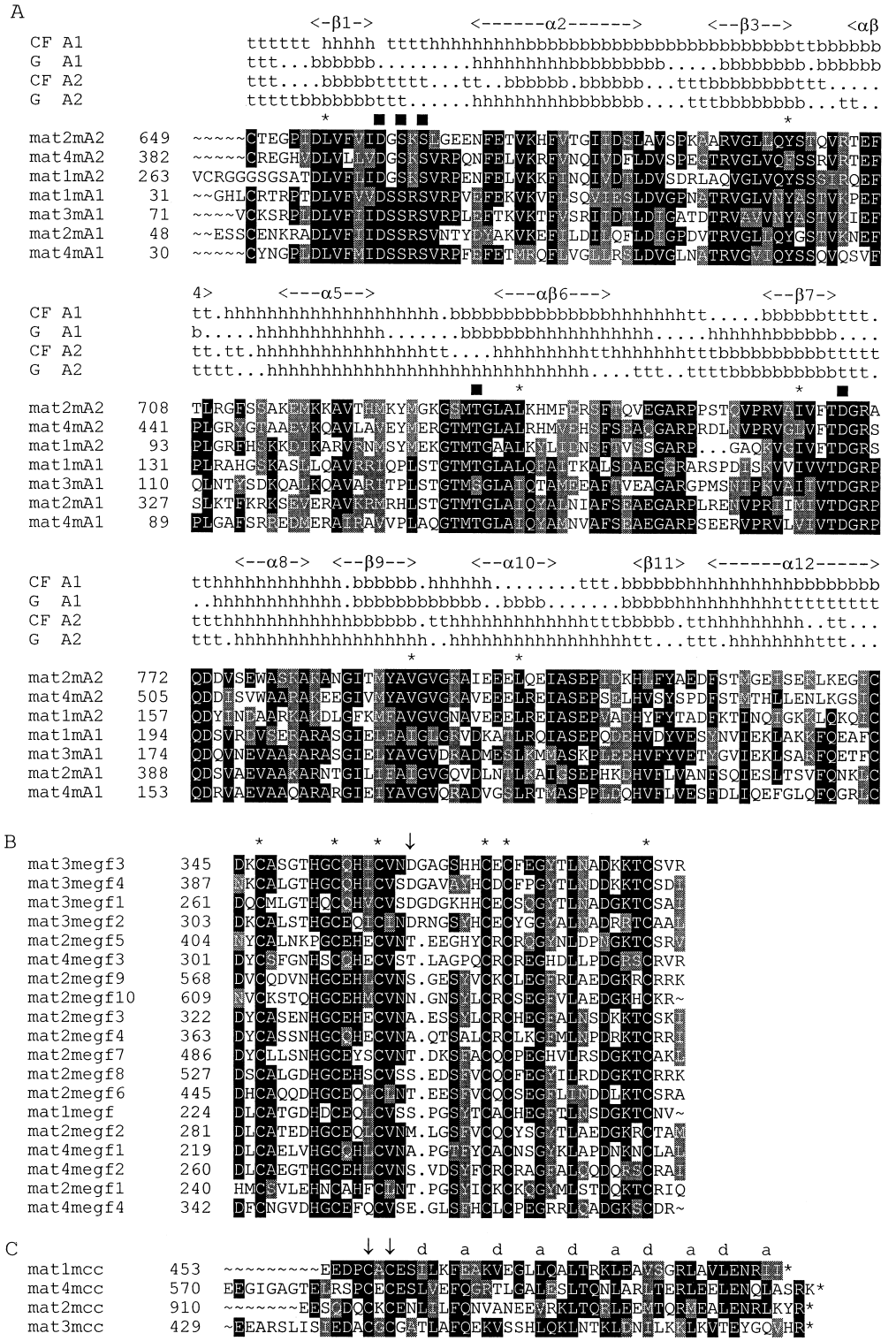

Fig. 3. Amino acid sequence alignments of the murine matrilin modules. Murine sequences were aligned by the PILEUP program of the GCG package, using the default parameters. Matrilin-1 (CMP) [13] is numbered from the first codon identified, matrilin-2 [4], matrilin-3 [3] and matrilin-4 are numbered from the first amino acid of the protein precursor. A: Sequence alignment of the vWFA-like modules. The locations of the α-helices and β-sheets determined from averaged structure predictions of 75 modules [19] are indicated by the arrow ranges β1-α12. Structure predictions by the Chou-Fasman (CF) and Garnier (G) methods for the vWFA-like modules of matrilin-4 are shown underneath (b, β-sheet; h, α-helix; t, turn). The conserved metal ion-dependent adhesion site [18] and the conserved hydrophobic moities [19] are denoted with (■) or (*) respectively. B: Sequence alignment of the EGF-like modules. The conserved positions of the cysteine residues are marked by asterisks, the additional aspartic acid residue of the matrilin-3 EGF-like repeats is marked by an arrow. C: Sequence alignment of the α-helical coiled-coil domains. The positions a and d of the heptad repeats are indicated above. The conserved cysteine residues at the N-terminal end of the coiled-coil region are marked with arrows.

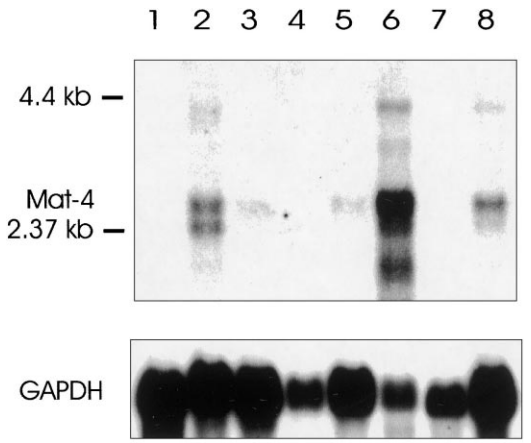

Fig. 4. Distribution of matrilin-4 mRNA in various mouse tissues. Northern hybridization of 3–6 μg polyA$^+$-RNA from 7 weeks old female CD1 mice. Lane 1 to 8: Skeletal muscle, brain, heart, liver, kidney, lung, ear and sternum, respectively. The blot was hybridized with a PCR fragment of mouse matrilin-4 cDNA (nt 8–1275) (upper part) and subsequently with a GAPDH cDNA fragment (lower part) to estimate the relative abundance of the matrilin-4 message. The exposure time for the matrilin-4 autoradiography was 12 h using a phosphor imager (Molecular Dynamics). The image is overexposed with respect to the lane for lung to show the weak signals in the other lanes. The spot in lane 4 is due to an artifact.

graphic data of the A3 domain of the von Willebrand factor that it must not necessarily have this function [21,25]. The presence of heptad repeats indicates the oligomerization of subunits via a coiled-coil α-helical structure. The number of subunits in the oligomer cannot be predicted from the sequence alone. It was recently demonstrated that the exchange of a single amino acid residue in the coiled-coil domain of matrilin-1 leads to the assembly of a tetramer instead of a trimer [26].

In 7 weeks old mice an alternatively spliced matrilin-4 mRNA was detected beneath the full length mRNA lacking the sequence coding for the first vWFA-like domain. This mRNA could not be seen in developing mice. The domain structure of the resulting shorter form of matrilin-4 is somewhat similar to the structure of the matrilin-3 in that both possess only one vWFA-like domain and differ only in the position of the EGF-like domains. As to date nothing is known about the binding partners of the different vWFA-like domains of matrilin-4, the functional consequences can not be predicted. In the case of matrilin-3 it was very recently shown that it can form heterooligomers with matrilin-1 [27].

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