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Minireview

The matrilins – adaptor proteins in the extracellular matrix

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Abstract The matrilins form a four-member family of modular, multisubunit matrix proteins, which are expressed in cartilage but also in many other forms of extracellular matrix. They participate in the formation of fibrillar or filamentous structures and are often associated with collagens. It appears that they mediate interactions between collagen-containing fibrils and other matrix constituents, such as aggrecan. This adaptor function may be modulated by physiological proteolysis that causes the loss of single subunits and thereby a decrease in binding avidity. Attempts to study matrilin function by gene inactivation in mouse have been frustrating and so far not yielded pronounced phenotypes, presumably because of the extensive redundancy within the family allowing compensation by one family member for another. However, mutations in matrilin-3 in humans cause different forms of chondrodysplasias and perhaps also hand osteoarthritis. As loss of matrilin-3 is not critical in mouse, these phenotypes are likely to be caused by dominant negative effects.

Keywords: Matrilin; Extracellular matrix; Cartilage; Collagen; Fibril; Chondrodysplasia

1. Introduction

The matrilins are a family of non-collagenous extracellular matrix proteins that form a subbranch of the superfamily of proteins containing von Willebrand factor A (VWA) domains (for review, see [1]). VWA domains are present in a large number of extracellular and intracellular proteins and often participate in protein–protein interactions leading to the formation of multiprotein complexes.

2. The family members and their structure

The matrilin family consists of four members with a closely similar domain structure (Fig. 1). Two VWA domains are connected by a varying number of epidermal growth factor (EGF)-like domains. These are followed by a C-terminal α-helical coiled-coil domain which allows the oligomerisation of the single subunits in a bouquet-like fashion. Only matrilin-3 lacks the second VWA domain and here the EGF-like domains are directly connected to the coiled-coil domain. In addition, matrilin-2 and -3 contain a stretch of amino acid residues at the N-terminus with a high frequency of positively charged side chains. Uniquely, matrilin-2 contains a module between the second VWA domain and the oligomerisation domain that has no homology to any other known protein sequence.

VWA domains consist of about 200 amino acid residues in a classical Rossman fold with a central β-sheet surrounded by α-helices. A MIDAS (metal ion dependent adhesion site) motif (DXSXSXnTXnD), which may be involved in ligand binding, is perfectly conserved in nearly all matrilin VWA domains. The VWA domains of matrilins represent an own subgroup of the VWA domains of extracellular matrix proteins (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) [2]. All matrilin VWA domains have higher identity to the members of the subgroup than to any other VWA domain, indicating that they originate from a common ancestor VWA domain [3]. Phylogenetic studies assigned the first and the second VWA domains into two different groups, of which each splits into a matrilin-1/3 and a matrilin-2/4 subbranch [3]. Among other VWA domain family members, the VWA domains of different collagens, vitrin, α1 integrin, WARP and AMACO have the highest identity (≈30%). It is probable that the VWA domains are the principal interaction modules of matrilins, since matrilin-1 and -3 splice variants exist in zebrafish that lack all EGF domains [4].

All other matrilins contain at least one EGF-like domain, the highest number being in a splice variant of zebrafish matrilin-4 where 12 EGF domains are present [4]. EGF domains contain 40–50 amino acid residues including six conserved disulphide bonds. The structure shows a two-stranded β-sheet and often EGF domains are present in multiple copies [5]. A comparison of the matrilin EGF domains with the key residues of calcium-binding motifs in calcium binding EGF domains [5,6] showed no match with the consensus sequence. Neither was a non-canonical calcium-binding site [7] detected in matrilin EGF domains. Database searches show that EGF domains of scube proteins, fibulins and fibrillins have the highest homologies to matrilin EGF domains.

The matrilins contain a C-terminal α-helical coiled-coil domain that allows oligomerisation of the subunits in a

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Abbreviations: COMP, cartilage oligomeric matrix protein; EGF, epidermal growth factor; p.c., post coitum; VWA, von Willebrand factor A

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bouquet-like fashion. Coiled-coil domains are characterised by heptad repeats (a–g) of amino acid residues that typically have non-polar amino acids at position a and d. The coiled-coil domains of matrilins contain 4.5 heptad repeats showing the least perfect match with the consensus sequence for matrilin-3. By electron microscopy of full-length proteins, it has been shown that matrilin-1 and -4 form homo-trimers [8,9], whereas matrilin-2 and -3 form homo-tetramers [10,11] (Fig. 1). The oligomerisation was also studied by using recombinantly expressed coiled-coil domains [12]. In contrast, here the coiled-coil domain of matrilin-2 formed only trimers. It could well be that the oligomerisation is influenced by the unique domain adjacent to the coiled-coil domain of matrilin-2. Hetero-oligomeric forms of matrilin-1 and -3 have been isolated from fetal human and calf cartilage [11,13,14]. There is no conclusive evidence for natural occurrence of other hetero-oligomers, but a SDS–PAGE band from an extract of newborn mouse epiphyseal cartilage was shown by peptide mass fingerprinting to contain both matrilin-1 and -4 (G. Sengle, unpublished results). The analysis of oligomerisation in mixtures of isolated coiled-coil domains showed a broad range of interactions and the only hetero-oligomers not found were those containing matrilin-2 and -3 or matrilin-3 and -4 [12].

Matrilin-1, which was first purified from bovine cartilage, is the prototype member of the family and was originally called "cartilage matrix protein" or CMP [15]. It is an abundant protein in many forms of cartilage. The single subunit has a molecular weight of 52 000 Da and contains 3.9% carbohydrate, probably in the form of N-linked glycans [15]. In electron microscopy matrilin-1 shows the typical bouquet-like structure where the single VWA domains of the subunits are not resolved, indicating an interaction between VWA1 and VWA2 (Fig. 1). The solution structure of the oligomerisation domain of matrilin-1 has been determined by heteronuclear NMR spectroscopy. As predicted, the domain folds into a parallel, disulfide-linked, three-stranded, α-helical coiled-coil, spanning five heptad repeats in the amino acid sequence [16].

Matrilin-2 is the largest matrilin with a calculated molecular weight of 104 300 Da and also carries N-linked glycans [10]. Similar to matrilin-1, in electron microscopy the two VWA domains appear to bind to each other causing the subunit to form a loop extending from the central coiled-coil (Fig. 1). Both recombinant matrilin-2 and the protein detected by immunoblot in tissue extracts display an unusual extent of structural heterogeneity, most likely due to proteolytic processing.

Matrilin-3 is the smallest family member and is mostly co-expressed with matrilin-1 [11,17]. By MALDI-TOF mass spectrometry, the molecular weight was determined to 49 300 Da. This value closely matches the calculated mass, indicating the lack of glycosylation. Due to the absence of the second VWA domain, there is no self-interaction in the subunits and in electron microscopy the tetrameric matrilin-3 appears to have more extended, flexible arms (Fig. 1).

Matrilin-4 contains in mouse four EGF domains between the two VWA domains [9,18]. The recombinant protein has a molecular weight of 72 900 Da indicating 7% posttranslational modifications. Although matrilin-4 carries two VWA domains, in electron microscopy these show no obvious interaction (Fig. 1). The images show the three C-terminal VWA domains at the center, presumably held together by the coiled-coil domain. The subunits extend from this central structure and end in globular domains representing the N-terminal VWA domain. The structure of matrilin-4 is reminiscent of that of matrilin-3, except for the latter protein forming tetramers.

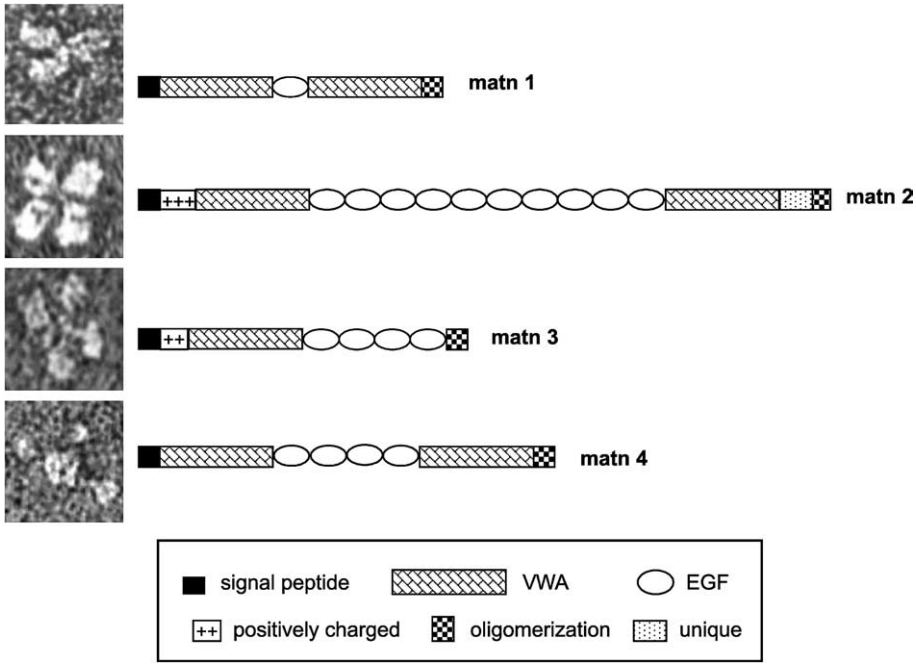

Fig. 1. Domain structure of mouse matrilins. On the left, electron microscopy after negative staining is shown. For explanation of symbols, see frame below figure. The electron micrographs were reproduced from [8–11] with permission.

3. Expression and processing

Studies on matrilins have been focussed on cartilage. All four members of the matrilin family have been shown to be present in cartilage matrix, even though it is mainly matrilin-1 and -3 that are prominent in this tissue. Further, matrilin expression is strictly regulated also within cartilage tissue, both in a temporal and in a spatial manner. Matrilin-1 can be detected by antibody labelling in myocardium at 9.5 days post coitum (p.c.) [19]. This expression is, however, transient and of unknown physiological significance. More lasting expression is seen for both matrilin-1 and -3 in the condensing mesenchyme at day 12.5 p.c. As skeletal development continues, the expression domains of matrilin-1 and -3 are similar to that of collagen II, but in late development it becomes noticeable that deposition of these matrilins at the articular surface is not as pronounced as in deeper cartilage layers [19,20]. In the mouse tibial growth plate matrilin-1 and -3 proteins are abundant in resting, proliferating and hypertrophic cartilage [20], but in situ hybridisation analysis for matrilin-1 mRNA showed a downregulation concomitant with the progress of hypertrophy at later stages of chondrogenesis [21]. As calcification occurs matrilin-1 and -3 become incorporated into the calcified cartilage that is gradually replaced by proper bone tissue. Continued expression of matrilin-1 is seen in tissues that remain cartilaginous during the whole lifespan, e.g., in costal cartilage and in the nasal septum, while matrilin-3 expression in these tissues ceases after birth [20]. It is likely that most matrilin biosynthesis occurs early in life, but that protein remains because of a slow turnover.

The expression patterns of matrilin-2 and -4 are much broader than those of matrilin-1 and -3 and, despite being present also in cartilage, those proteins are found mainly in loose connective tissue. The first matrilin-2 expression in the mouse embryo is in heart, just like for matrilin-1, but matrilin-2 is expressed later, at day 10.5 p.c., and the expression continues [20]. Later in development, it is produced by a wide variety of connective tissue cells, but also by smooth muscle cells and some epithelia [10]. Matrilin-2 protein is deposited by these cells into their pericellular matrix. In some cases, it becomes associated with basement membranes, even though it is uncertain if it is an integral basement membrane protein. In other cases, matrilin-2 is found as a component of a filamentous network of unknown overall composition [10]. In general, matrilin-2 has a complementary expression pattern to matrilin-1 and -3, even though there is some matrilin-2 present also in cartilage. Matrilin-4 in turn is the most ubiquitous of all matrilins and appears to be present, wherever another matrilin is found [9]. It can be detected by immunohistochemistry in the ectoplacental cone already at day 7.5 p.c. [9]. The expression in nervous tissue is more pronounced than for other matrilins, and indeed the brain appears to be the most abundant tissue source for matrilin-4.

The tissue distribution of all or some matrilins has also been determined in man [22], chicken [23,24] and zebrafish [4]. From the limited data available matrilin expression patterns appear highly conserved.

The genetic basis for matrilin-1 gene expression has been studied in some detail in the chicken system. A minimal promoter has been defined that functions both in chondrocytes and fibroblasts [25]. An enhancer exerts a chondrocyte-specific stimulation on the promoter activity and a silencer inhibits activity both in chondrocytes and fibroblasts. The enhancer is independent of the developmental stage of the chondrocytes, while promoter upstream control regions appear to restrict the promoter activity to certain chondrocyte developmental stages [26]. Transgenic experiments with the chicken matrilin-1 promoter in mouse have indicated that the tissue-specific control elements are divided between the promoter upstream and intronic regions in a manner similar to that of the *Col11a2* gene [27].

Matrilins, in particular matrilin-2 and -4, when isolated from tissues or cell cultures are often degraded and actually seen as a ladder of bands in SDS–PAGE representing, in addition to the full-length proteins, fragments lacking the greatest part of one or more subunits [9,10]. This processing was studied in some detail for matrilin-4, which when recombinantly expressed in human embryonic kidney-derived 293 cells is found as a mixture of monomers, dimers and trimers [9]. Analysis of fragments by MALDI-TOF mass spectrometry and Edman degradation showed that the cleavage occurs at a distinct site in a short linker region which resides between the C-terminal VWA domain and the coiled-coil. The processing results in an almost complete subunit being released from the major part of the molecule consisting of the coiled-coil together with remaining subunits. Similar linker regions occur also in the other matrilins, but it is noteworthy that in matrilin-1, which is the least sensitive to proteolysis, this linker is the shortest. At least for matrilin-4 it has been shown that fragments corresponding to those characterised for the recombinant protein occur also in tissue extracts [9]. It appears that this depolymerisation is a physiological process and it may serve the purpose of decreasing the avidity of matrilins for their ligands and thereby cause a disassembly of supramolecular structures held together by matrilins.

4. Interactions and potential functions

Matrilin-1 was first recognised as a protein tightly bound to aggrecan and copurified with aggrecan in a variety of separation methods [28]. The complexes appear to be formed by protein–protein interactions occurring along the extended chrondroitin–sulphate-attachment region of aggrecan. The bound matrilin-1 molecules with time become covalently cross-linked to the aggrecan core protein, with at least some of the crosslinks not being sensitive to reduction [29]. It is not known if this complex formation is unique for matrilin-1 and aggrecan or if also other matrilins can bind to proteoglycans, perhaps to other members of the lectican family.

An association was also found between matrilin-1 and cartilage collagen fibrils and immunolabelling at the electron microscopy level even indicated a certain periodicity in the matrilin-1 distribution [30]. Chondrocyte cell culture experiments showed matrilin-1 present in two kinds of pericellular filaments, where one type was collagen-dependent, as it required ascorbate for formation, and the other not [31]. Similar staining of filaments in the pericellular matrix of cultured cells has been seen for each of the other matrilins (Fig. 2) and, in case of matrilin-2 and -4, not only around chondrocytes, but also in cultures of other cell types that express these matrilins [9–11].

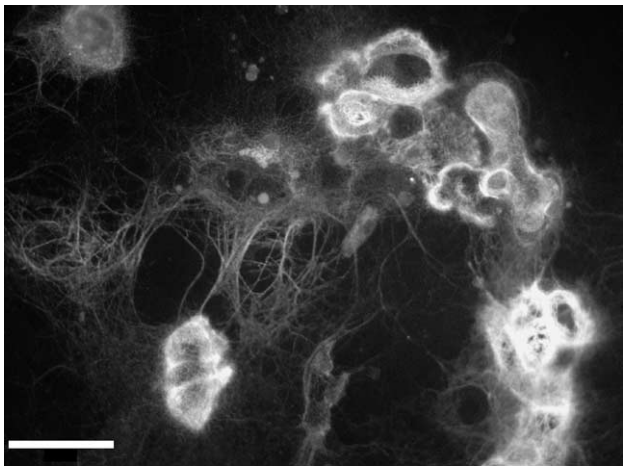

Fig. 2. Immunofluorescence microscopy of matrilin-3 in the extracellular matrix of primary mouse chondrocytes. Matrilin-3 was detected with affinity-purified primary antibodies and a Alexa-Fluor 488®-conjugated secondary antibody. The bar is 50 μm.

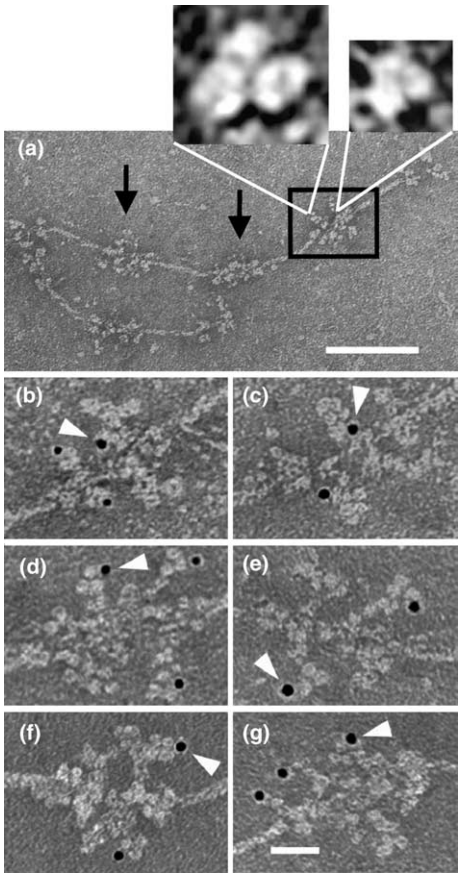

Fig. 3. Structure of intact collagen VI from the Swarm rat chondrosarcoma as seen by electron microscopy after negative staining. (a) Collagen VI microfibrils with different molecules bound close to the N-terminal parts of the collagen VI tetramers are visible (arrows). Representative particles exhibiting multidomain structures of different sizes are shown at higher magnification (insets). Using specific gold-labelled antibodies these molecules are identified as biglycan (b), decorin (c), matrilin-1 (d), matrilin-3 (e), matrilin-4 (f), and chondroadherin (g). Thus, complexes of matrilin-1, -3, or -4 and the leucine-rich repeat proteoglycans biglycan or decorin, binding close to the collagen VI N-termini, are identified. The bars represent 100 nm (a) and 20 nm ((b)–(g)). Reproduced from [34] with permission.

Experiments have been performed to identify molecules that interact with matrilins to form such matrix assemblies. In studies of matrilin-1, -3, and -4 [32] and matrilin-2 [33] a variety of collagens were found to bind to matrilins in solid phase assays. However, some non-collagenous molecules, in particular cartilage oligomeric matrix protein (COMP) and decorin, bound to matrilins with higher affinity [32] and as COMP and decorin are known to interact with collagens, it may well be that matrilins are attached to collagen fibrils as part of a "sandwich" where other components may be the direct and high-affinity collagen binders. Evidence in this direction comes from studies where the composition and assembly of molecular complexes attached to collagen VI-containing microfibrils were studied [34]. With the use of gold-labelled antibodies or matrix proteins as probes, it could be shown that decorin and biglycan attach to VWA domains in the N-terminal region of collagen VI and that matrilins were in turn bound to these small, leucine-rich repeat proteoglycans (Fig. 3). At the periphery of these assemblies, matrilins could be seen to connect the collagen VI-containing microfibrils to aggrecan core proteins or to collagen II-containing fibrils.

5. Matrilins in disease and in genetic mouse models

Mutations in matrilin-3 were found to be linked to autosomal dominant forms of multiple epiphyseal dysplasia (MED), a relatively mild and clinically variable osteochondrodysplasia, primarily characterised by delayed and irregular ossification of the epiphyses and early onset osteoarthritis [35]. The mutations mostly affect residues within the conserved β strands of the single VWA domain of matrilin-3 [36,37]. In bilateral hereditary micro-epiphyseal dysplasia (BHMED), which gives a skeletal phenotype similar to but still distinct from common MED, a site close to the β-strands in the VWA domain of matrilin-3 is affected [38]. MED is commonly caused by autosomal dominant mutations in the genes encoding COMP [39] and the α1, α2, and α3 chains of type IX collagen (*COL9A1*, *COL9A2*, and *COL9A3*) [40–42] and it is of interest that mutations in the functionally related protein matrilin-3 causes similar phenotypes. In addition, in an autosomal recessive form of another osteochondrodysplasia, spondylo-epi-metaphyseal dysplasia (SEMD), that is associated with vertebral, epiphyseal, and metaphyseal anomalies, again the matrilin-3 gene is affected [43]. The disease is caused by a change of a cysteine into a serine in the first EGF domain of matrilin-3, which could lead to a disturbance in the disulphide bond formation. In a genomic screen of the Icelandic population, a mutation in the first EGF domain of matrilin-3 was linked to the occurrence of hand osteoarthritis. Slightly more than 2% of patients with hand osteoarthritis carry the mutation [44], but it was also identified in unrelated controls [36,44]. In a recent study, the influence of those MED, SEMD and hand osteoarthritis mutations on the secretion of matrilin-3 was studied [45]. Whereas matrilin-3 carrying the hand osteoarthritis mutation could be secreted by chondrocytes at a similar rate as wildtype matrilin-3, the matrilin-3 mutants causing MED and SMED, respectively, were retained in the

endoplasmic reticulum. It is likely that this retention causes a chondrocyte dysfunction by which MED and SMED phenotypes could be explained. Similar observations were earlier made for COMP mutations leading to MED (for review see [46,47]). In contrast, the mutant matrilin-3 that has been linked to hand osteoarthritis is synthesised, processed, secreted and deposited in a way indistinguishable from the wildtype protein, suggesting, at the most, subtle effects of this mutation on the structure and function of the protein [45].

Matrilin-1, which has a similar tissue distribution as matrilin-3 and is abundant in cartilage, has been excluded as the mutant locus in several heritable chondrodysplasias [48]. In particular, MED mutations were not found in the four exons coding for the VWA domains of matrilin-1 in 43 unrelated MED patients [36]. The linkage of the matrilin-1 locus to osteoarthritis is controversially discussed. Whereas a linkage was found in the Dutch population [49], no linkage was seen among the British [50]. No genetic disorders have been found involving mutations in matrilin-2 or -4.

Relapsing polychondritis is a rare autoimmune disease of unknown origin characterised by recurrent episodes of inflammation and progressive destruction of cartilaginous tissues [51]. In such patients autoantibodies against matrilin-1 were detected [52,53]. An animal model for the disease can be induced by immunizing rats or mice with matrilin-1 [54,55]. In such experimental relapsing polychondritis, it was shown that B-cells as well as the complement factor C5 are important for the induction [55]. In addition to the humoral immune response, also macrophages and $CD4^+$ T cells are involved in the pathogenesis, whereas Il10 has the potential to suppress the disease [56].

Matrilins may potentially serve as markers for a disturbed cartilage metabolism in joint disease. It has been reported that the expression of matrilin-1 is enhanced in knee osteoarthritic cartilage and knee or hip rheumatoid arthritic cartilage [57]. In addition, elevated serum levels of matrilin-1 were detected in patients with active rheumatoid arthritis [58]. Matrilin-1 also showed an increased expression in specimens from arthritic condylar cartilage of the temporomandibular joints [59]. Similarly, the expression of matrilin-3 has been shown to be increased in knee osteoarthritic cartilage [60].

In the light of the clear role of matrilins in human disease, it was surprising that all mouse matrilin null mutants generated so far show no obvious phenotype [61–64]. In one of the matrilin-1 knockouts, an abnormal fibril organisation and a slightly increased diameter of collagen type II fibrils was detected [64], but no similar findings were made in an independently performed matrilin-1 gene inactivation [61]. Although the matrilin-4 null mutants are not yet available, the lack of obvious phenotypes in the single knockouts of matrilin-1, -2 and -3 indicates a considerable redundancy within the matrilin family.

6. Perspectives

Despite increased information on matrilin interactions the detailed in vivo functions are still not known. It is clear that matrilins serve as adaptors in the assembly of supramolecular structures in the extracellular matrix, but we do not know to what extent this role is static or dynamic in nature. Matrilins need to be studied in genetic models, but the redundancy within the family has caused problems in this regard and the three single gene inactivations performed so far for matrilin-1, -2 and -3 have not yielded any change in phenotype. These studies are at present being continued through the establishment of double knockouts and, in parallel, a knock-down approach is taken in zebrafish in the hope that compensation is less pronounced in this model organism.

In addition to participating in extracellular matrix assembly matrilins may participate in cell–matrix interactions. Matrilin-containing fibrils and filaments certainly occur close to the cell surface (Fig. 2), but despite an early report on integrin $\alpha1\beta1$ being a matrilin-1 receptor [65], the potential cellular interactions of matrilins have not been systematically studied.

Interaction studies performed with matrilins and other matrix proteins point to the oligomeric structure of the proteins being important for high-affinity binding. This has made the mapping of binding sites difficult, but indirect results point to the VWA domains being most important in mediating interactions with other matrix proteins. It would be of great interest to define binding sites on matrilin domains by mutagenesis, but such studies would become easier if the three-dimensional structure of matrilin domains could be determined. The degree of identity to VWA domains of known structure is not high enough to yield reliable structures of matrilin VWA domains by modelling approaches and it would therefore not be possible to mutate only surface-exposed residues. Experimental determination of the three-dimensional structure of at least one matrilin VWA domain would overcome this problem.

Such studies would allow not only promote the determination of matrilin structure and function, but also yield insight into how mutations in these proteins may cause inherited disease. Even though intracellular accumulation of misfolded proteins may be a major contributor to the phenotypes seen in matrilin-3 dependent osteochondrodysplasias, other mutations causing more discrete changes may cause phenotypes by other mechanisms.

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References

[1] Whittaker, C.A. and Hynes, R.O. (2002) Distribution and evolution of von Willebrand/integrin A domains: Widely dispersed domains with roles in cell adhesion and elsewhere. Mol. Biol. Cell 13, 3369–3387.

[2] Marchler-Bauer, A., Anderson, J.B., Cherukuri, P.F., DeWeese-Scott, C., Geer, L.Y., Gwadz, M., He, S., Hurwitz, D.I., Jackson, J.D., Ke, Z., Lanczycki, C.J., Liebert, C.A., Liu, C., Lu, F., Marchler, G.H., Mullokandov, M., Shoemaker, B.A., Simonyan, V., Song, J.S., Thiessen, P.A., Yamashita, R.A., Yin, J.J., Zhang, D. and Bryant, S.H. (2005) CDD: A conserved domain database for protein classification. Nucleic Acids Res. 33 (Database Issue), D192–D196.

[3] Deák, F., Wagener, R., Kiss, I. and Paulsson, M. (1999) The matrilins: A novel family of oligomeric extracellular matrix proteins. Matrix Biol. 18, 55–64.

[4] Ko, Y.P., Kobbe, B., Paulsson, M. and Wagener, R. (2005) Zebrafish (Danio rerio) matrilins: Shared and divergent

characteristics with their mammalian counterparts. Biochem. J. 386, 367–379.
[5] Rao, Z., Handford, P., Mayhew, M., Knott, V., Brownlee, G.G. and Stuart, D. (1995) The structure of a Ca^{2+}-binding epidermal growth factor-like domain: Its role in protein–protein interactions. Cell 82, 131–141.
[6] Handford, P.A., Mayhew, M., Baron, M., Winship, P.R., Campbell, I.D. and Brownlee, G.G. (1991) Key residues involved in calcium-binding motifs in EGF-like domains. Nature 351, 164–167.
[7] Malby, S., Pickering, R., Saha, S., Smallridge, R., Linse, S. and Downing, A.K. (2001) The first epidermal growth factor-like domain of the low-density lipoprotein receptor contains a noncanonical calcium binding site. Biochemistry 40, 2555–2563.
[8] Hauser, N. and Paulsson, M. (1994) Native cartilage matrix protein (CMP). A compact trimer of subunits assembled via a coiled-coil α-helix. J. Biol. Chem. 269, 25747–25753.
[9] Klatt, A.R., Nitsche, D.P., Kobbe, B., Macht, M., Paulsson, M. and Wagener, R. (2001) Molecular structure, processing, and tissue distribution of matrilin-4. J. Biol. Chem. 276, 17267–17275.
[10] Piecha, D., Muratoglu, S., Mörgelin, M., Hauser, N., Studer, D., Kiss, I., Paulsson, M. and Deák, F. (1999) Matrilin-2, a large, oligomeric matrix protein, is expressed by a great variety of cells and forms fibrillar networks. J. Biol. Chem. 274, 13353–13361.
[11] Klatt, A.R., Nitsche, D.P., Kobbe, B., Mörgelin, M., Paulsson, M. and Wagener, R. (2000) Molecular structure and tissue distribution of matrilin-3, a filament-forming extracellular matrix protein expressed during skeletal development. J. Biol. Chem. 275, 3999–4006.
[12] Frank, S., Schulthess, T., Landwehr, R., Lustig, A., Mini, T., Jeno, P., Engel, J. and Kammerer, R.A. (2002) Characterization of the matrilin coiled-coil domains reveals seven novel isoforms. J. Biol. Chem. 277, 19071–19079.
[13] Kleemann-Fischer, D., Kleemann, G.R., Engel, D., Yates 3rd, J.R., Wu, J.J. and Eyre, D.R. (2001) Molecular properties of matrilin-3 isolated from human growth cartilage. Arch. Biochem. Biophys. 387, 209–215.
[14] Wu, J.J. and Eyre, D.R. (1998) Matrilin-3 forms disulfide-linked oligomers with matrilin-1 in bovine epiphyseal cartilage. J. Biol. Chem. 273, 17433–17438.
[15] Paulsson, M. and Heinegård, D. (1981) Purification and structural characterization of a cartilage matrix protein. Biochem. J. 197, 367–375.
[16] Dames, S.A., Kammerer, R.A., Wiltscheck, R., Engel, J. and Alexandrescu, A.T. (1998) NMR structure of a parallel homotrimeric coiled-coil. Nat. Struct. Biol. 5, 687–691.
[17] Wagener, R., Kobbe, B. and Paulsson, M. (1997) Primary structure of matrilin-3, a new member of a family of extracellular matrix proteins related to cartilage matrix protein (matrilin-1) and von Willebrand factor. FEBS Lett. 413, 129–134.
[18] Wagener, R., Kobbe, B. and Paulsson, M. (1998) Matrilin-4, a new member of the matrilin family of extracellular matrix proteins. FEBS Lett. 436, 123–127.
[19] Segat, D., Frie, C., Nitsche, P.D., Klatt, A.R., Piecha, D., Korpos, E., Deák, F., Wagener, R., Paulsson, M. and Smyth, N. (2000) Expression of matrilin-1, -2 and -3 in developing mouse limbs and heart. Matrix Biol. 19, 649–655.
[20] Klatt, A.R., Paulsson, M. and Wagener, R. (2002) Expression of matrilins during maturation of mouse skeletal tissues. Matrix Biol. 21, 289–296.
[21] Aszódi, A., Hauser, N., Studer, D., Paulsson, M., Hiripi, L. and Bösze, Z. (1996) Cloning, sequencing and expression analysis of mouse cartilage matrix protein cDNA. Eur. J. Biochem. 236, 970–977.
[22] Mundlos, S. and Zabel, B. (1994) Developmental expression of human cartilage matrix protein. Dev. Dyn. 199, 241–252.
[23] Stirpe, N.S. and Goetinck, P.F. (1989) Gene regulation during cartilage differentiation: Temporal and spatial expression of link protein and cartilage matrix protein in the developing limb. Development 107, 23–33.
[24] Belluoccio, D. and Trueb, B. (1997) Matrilin-3 from chicken cartilage. FEBS Lett. 415, 212–216.
[25] Kiss, I., Bösze, Z., Szabó, P., Altanchimeg, R., Barta, E. and Deák, F. (1990) Identification of positive and negative regulatory regions controlling expression of the cartilage matrix protein gene. Mol. Cell. Biol. 10, 2432–2436.
[26] Muratoglu, S., Bachrati, C., Malpeli, M., Szabó, P., Neri, M., Dozin, B., Deák, F., Cancedda, R. and Kiss, I. (1995) Expression of the cartilage matrix protein gene at different chondrocyte developmental stages. Eur. J. Cell Biol. 68, 411–418.
[27] Karcagi, I., Rauch, T., Hiripi, L., Rentsendorj, O., Nagy, A., Bösze, Z. and Kiss, I. (2004) Functional analysis of the regulatory regions of the matrilin-1 gene in transgenic mice reveals modular arrangement of tissue-specific control elements. Matrix Biol. 22, 605–618.
[28] Paulsson, M. and Heinegård, D. (1979) Matrix proteins bound to associatively prepared proteoglycans from bovine cartilage. Biochem. J. 183, 539–545.
[29] Hauser, N., Paulsson, M., Heinegård, D. and Mörgelin, M. (1996) Interaction of cartilage matrix protein with aggrecan. Increased covalent cross-linking with tissue maturation. J. Biol. Chem. 271, 32247–32252.
[30] Winterbottom, N., Tondravi, M.M., Harrington, T.L., Klier, F.G., Vertel, B.M. and Goetinck, P.F. (1992) Cartilage matrix protein is a component of the collagen fibril of cartilage. Dev. Dyn. 193, 266–276.
[31] Chen, Q., Johnson, D.M., Haudenschild, D.R., Tondravi, M.M. and Goetinck, P.F. (1995) Cartilage matrix protein forms a type II collagen-independent filamentous network: Analysis in primary cell cultures with a retrovirus expression system. Mol. Biol. Cell 6, 1743–1753.
[32] Mann, H.H., Özbek, S., Engel, J., Paulsson, M. and Wagener, R. (2004) Interactions between the cartilage oligomeric matrix protein and matrilins. Implications for matrix assembly and the pathogenesis of chondrodysplasias. J. Biol. Chem. 279, 25294–25298.
[33] Piecha, D., Wiberg, C., Mörgelin, M., Reinhardt, D.P., Deák, F., Maurer, P. and Paulsson, M. (2002) Matrilin-2 interacts with itself and with other extracellular matrix proteins. Biochem. J. 367, 715–721.
[34] Wiberg, C., Klatt, A.R., Wagener, R., Paulsson, M., Bateman, J.F., Heinegård, D. and Mörgelin, M. (2003) Complexes of matrilin-1 and biglycan or decorin connect collagen VI microfibrils to both collagen II and aggrecan. J. Biol. Chem. 278, 37698–37704.
[35] Chapman, K.L., Mortier, G.R., Chapman, K., Loughlin, J., Grant, M.E. and Briggs, M.D. (2001) Mutations in the region encoding the von Willebrand factor A domain of matrilin-3 are associated with multiple epiphyseal dysplasia. Nat. Genet. 28, 393–396.
[36] Jackson, G.C., Barker, F.S., Jakkula, E., Czarny-Ratajczak, M., Makitie, O., Cole, W.G., Wright, M.J., Smithson, S.F., Suri, M., Rogala, P., Mortier, G.R., Baldock, C., Wallace, A., Elles, R., Ala-Kokko, L. and Briggs, M.D. (2004) Missense mutations in the β strands of the single A-domain of matrilin-3 result in multiple epiphyseal dysplasia. J. Med. Genet. 41, 52–59.
[37] Mabuchi, A., Haga, N., Maeda, K., Nakashima, E., Manabe, N., Hiraoka, H., Kitoh, H., Kosaki, R., Nishimura, G., Ohashi, H. and Ikegawa, S. (2004) Novel and recurrent mutations clustered in the von Willebrand factor A domain of MATN3 in multiple epiphyseal dysplasia. Hum. Mutat. 24, 439–440.
[38] Mostert, A.K., Dijkstra, P.F., Jansen, B.R., van Horn, J.R., de Graaf, B., Heutink, P. and Lindhout, D. (2003) Familial multiple epiphyseal dysplasia due to a matrilin-3 mutation: Further delineation of the phenotype including 40 years follow-up. Am. J. Med. Genet. A 120, 490–497.
[39] Briggs, M.D., Hoffman, S.M., King, L.M., Olsen, A.S., Mohrenweiser, H., Leroy, J., Mortier, G.R., Rimoin, D.L., Lachman, R.S., Gaines, E.S., Cekleniak, J.A., Knowlton, R.G. and Cohn, D.H. (1995) Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. Nat. Genet. 10, 330–336.
[40] Czarny-Ratajczak, M., Lohiniva, J., Rogala, P., Kozlowski, K., Perala, M., Carter, L., Spector, T.D., Kolodziej, L., Seppanen, U., Glazar, R., Krolewski, J., Latos-Bielenska, A. and Ala-Kokko, L. (2001) A mutation in COL9A1 causes multiple epiphyseal dysplasia: Further evidence for locus heterogeneity. Am. J. Hum. Genet. 69, 969–980.

[41] Muragaki, Y., Mariman, E.C., van Beersum, S.E., Perala, M., van Mourik, J.B., Warman, M.L., Olsen, B.R. and Hamel, B.C. (1996) A mutation in the gene encoding the α2 chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2). Nat. Genet. 12, 103–105.
[42] Paassilta, P., Lohiniva, J., Annunen, S., Bonaventure, J., Le Merrer, M., Pai, L. and Ala-Kokko, L. (1999) COL9A3: A third locus for multiple epiphyseal dysplasia. Am. J. Hum. Genet. 64, 1036–1044.
[43] Borochowitz, Z.U., Scheffer, D., Adir, V., Dagoneau, N., Munnich, A. and Cormier-Daire, V. (2004) Spondylo-epi-metaphyseal dysplasia (SEMD) matrilin 3 type: Homozygote matrilin 3 mutation in a novel form of SEMD. J. Med. Genet. 41, 366–372.
[44] Stefansson, S.E., Jonsson, H., Ingvarsson, T., Manolescu, I., Jonsson, H.H., Olafsdottir, G., Palsdottir, E., Stefansdottir, G., Sveinbjornsdottir, G., Frigge, M.L., Kong, A., Gulcher, J.R. and Stefansson, K. (2003) Genomewide scan for hand osteoarthritis: A novel mutation in matrilin-3. Am. J. Hum. Genet. 72, 1448–1459.
[45] Otten, C., Wagener, R., Paulsson, M. and Zaucke, F. (in press) Matrilin-3 mutations that cause chondrodysplasias interfere with protein trafficking while a mutation associated with hand osteoarthritis does not. J. Med. Genet.
[46] Posey, K.L., Hayes, E., Haynes, R. and Hecht, J.T. (2004) Role of TSP-5/COMP in pseudoachondroplasia. Int. J. Biochem. Cell Biol. 36, 1005–1012.
[47] Briggs, M.D. and Chapman, K.L. (2002) Pseudoachondroplasia and multiple epiphyseal dysplasia: Mutation review, molecular interactions, and genotype to phenotype correlations. Hum. Mutat. 19, 465–478.
[48] Loughlin, J., Irven, C. and Sykes, B. (1994) Exclusion of the cartilage link protein and the cartilage matrix protein genes as the mutant loci in several heritable chondrodysplasias. Hum. Genet. 94, 698–700.
[49] Meulenbelt, I., Bijkerk, C., de Wildt, S.C., Miedema, H.S., Valkenburg, H.A., Breedveld, F.C., Pols, H.A., Te Koppele, J.M., Sloos, V.F., Hofman, A., Slagboom, P.E. and van Duijn, C.M. (1997) Investigation of the association of the CRTM and CRTL1 genes with radiographically evident osteoarthritis in subjects from the Rotterdam study. Arthritis Rheum. 40, 1760–1765.
[50] Loughlin, J., Dowling, B., Mustafa, Z., Smith, A., Sykes, B. and Chapman, K. (2000) Analysis of the association of the matrillin-1 gene (CRTM) with osteoarthritis: Comment on the article by Meulenbelt et al. Arthritis Rheum. 43, 1423–1425.
[51] Gergely Jr., P. and Poor, G. (2004) Relapsing polychondritis. Best Pract. Res. Clin. Rheumatol. 18, 723–738.
[52] Buckner, J.H., Wu, J.J., Reife, R.A., Terato, K. and Eyre, D.R. (2000) Autoreactivity against matrilin-1 in a patient with relapsing polychondritis. Arthritis Rheum. 43, 939–943.
[53] Hansson, A.S., Heinegård, D., Piette, J.C., Burkhardt, H. and Holmdahl, R. (2001) The occurrence of autoantibodies to matrilin 1 reflects a tissue-specific response to cartilage of the respiratory tract in patients with relapsing polychondritis. Arthritis Rheum. 44, 2402–2412.
[54] Hansson, A.S., Heinegård, D. and Holmdahl, R. (1999) A new animal model for relapsing polychondritis, induced by cartilage matrix protein (matrilin-1). J. Clin. Invest. 104, 589–598.
[55] Hansson, A.S., Johannesson, M., Svensson, L., Nandakumar, K.S., Heinegård, D. and Holmdahl, R. (2004) Relapsing polychondritis, induced in mice with matrilin 1, is an antibody- and complement-dependent disease. Am. J. Pathol. 164, 959–966.
[56] Hansson, A.S., Johansson, A.C. and Holmdahl, R. (2004) Critical role of the major histocompatibility complex and IL-10 in matrilin-1-induced relapsing polychondritis in mice. Arthritis Res. Ther. 6, R484–R491.
[57] Okimura, A., Okada, Y., Makihira, S., Pan, H., Yu, L., Tanne, K., Imai, K., Yamada, H., Kawamoto, T., Noshiro, M., Yan, W. and Kato, Y. (1997) Enhancement of cartilage matrix protein synthesis in arthritic cartilage. Arthritis Rheum. 40, 1029–1036.
[58] Saxne, T. and Heinegård, D. (1989) Involvement of nonarticular cartilage, as demonstrated by release of a cartilage-specific protein, in rheumatoid arthritis. Arthritis Rheum. 32, 1080–1086.
[59] Ohno, S., Murakami, K., Tanimoto, K., Sugiyama, H., Makihira, S., Shibata, T., Yoneno, K., Kato, Y. and Tanne, K. (2003) Immunohistochemical study of matrilin-1 in arthritic articular cartilage of the mandibular condyle. J. Oral Pathol. Med. 32, 237–242.
[60] Pullig, O., Weseloh, G., Klatt, A.R., Wagener, R. and Swoboda, B. (2002) Matrilin-3 in human articular cartilage: Increased expression in osteoarthritis. Osteoarthritis Cartilage 10, 253–263.
[61] Aszódi, A., Bateman, J.F., Hirsch, E., Baranyi, M., Hunziker, E.B., Hauser, N., Bösze, Z. and Fässler, R. (1999) Normal skeletal development of mice lacking matrilin 1: Redundant function of matrilins in cartilage. Mol. Cell. Biol. 19, 7841–7845.
[62] Mátés, L., Nicolae, C., Mörgelin, M., Deák, F., Kiss, I. and Aszódi, A. (2004) Mice lacking the extracellular matrix adaptor protein matrilin-2 develop without obvious abnormalities. Matrix Biol. 23, 195–204.
[63] Ko, Y., Kobbe, B., Nicolae, C., Miosge, N., Paulsson, M., Wagener, R. and Aszódi, A. (2004) Matrilin-3 is dispensable for mouse skeletal growth and development. Mol. Cell. Biol. 24, 1691–1699.
[64] Huang, X., Birk, D.E. and Goetinck, P.F. (1999) Mice lacking matrilin-1 (cartilage matrix protein) have alterations in type II collagen fibrillogenesis and fibril organization. Dev. Dyn. 216, 434–441.
[65] Makihira, S., Yan, W., Ohno, S., Kawamoto, T., Fujimoto, K., Okimura, A., Yoshida, E., Noshiro, M., Hamada, T. and Kato, Y. (1999) Enhancement of cell adhesion and spreading by a cartilage-specific noncollagenous protein, cartilage matrix protein (CMP/Matrilin-1), via integrin α1β1. J. Biol. Chem. 274, 11417–11423.