Matrilin-2 interacts with itself and with other extracellular matrix proteins

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Matrilin-2 is a component of extracellular filamentous networks. To study the interactions by which it can be integrated into such assemblies, full-length and truncated forms of matrilin-2 were recombinantly expressed in HEK-293 cells and purified from conditioned medium. The recombinant proteins, when used in interaction assays, showed affinity to matrilin-2 itself, but also to other collagenous and non-collagenous extracellular matrix proteins. The interaction between matrilin-2 and collagen I was studied in greater detail and could be shown to occur at distinct sites on the collagen I molecule and to have a K_D of about 3×10^{-8} M. Interactions with some non-collagenous protein

ligands were even stronger, with matrilin-2 binding to fibrillin-2, fibronectin and laminin-1–nidogen-1 complexes, with K_D values in the range of 10^{-8} – 10^{-11} M. Co-localization of matrilin-2 with these ligands in the dermal-epidermal basement membrane, in the microfibrils extending from the basement membrane into the dermis, and in the dermal extracellular matrix, indicates a physiological relevance of the interactions in the assembly of supramolecular extracellular matrix structures.

Key words: collagen, fibrillin, fibronectin, laminin, skin.

INTRODUCTION

The matrilins are characterized by having subunits containing von Willebrand factor A (vWFA)-like domains, which are connected by a variable number of epidermal growth factor (EGF)-like modules, and a series of heptad repeats at their C-terminal ends (for a review see [1]). The subunits of matrilin-1, -2 and -4 contain two vWFA-like domains, while that of matrilin-3 carries only one. All matrilins can form homo-oligomers by the C-terminal heptad repeats folding into a coiled-coil α -helix and, in addition, matrilin-1 and -3 have been shown to assemble into hetero-oligomers of variable stoichiometry [2,3]. The homo-oligomer of matrilin-1 consists of three subunits [4], while for matrilin-2, -3 and -4 multiple assembly forms are detected in tissue extracts and cell culture media [3,5,6].

Matrilin-2 is the largest known matrilin with a subunit molecular mass of 106 800 Da predicted from the mouse sequence [7]. Its two vWFA-like domains are connected by 10 EGF-like modules and followed by a unique segment not found in other matrilins and a C-terminal coiled-coil region. Electron microscopy revealed a loop structure for each subunit that appears to be closed at the two vWFA-like domains and further showed that the largest assembly form is a tetramer [5]. Matrilin-2 is expressed in a variety of tissues, including both loose and dense connective tissue as well as subepithelial basement membranes in skin and the digestive tract. However, in cartilage, where matrilin-1, -3 and -4 are abundant matrix components, only a weak expression of matrilin-2, mainly restricted to the epiphyseal growth plate, has been observed [5,8,9].

Little is known about the function and ligand interactions of matrilins in tissues and most studies have focused on matrilin-1. This protein was initially isolated as a cartilage matrix protein associated with aggrecan [10] by an interaction with distinct sites

along the chondroitin sulphate-attachment region of the aggrecan core protein [11]. It was further identified as a component of filaments found in the pericellular matrix of cultured chondrocytes [12]. Two populations of matrilin-1-containing filaments have been distinguished, one containing collagen and the other not [13,14]. Similarly, matrilin-2 [5], -3 [3] and -4 [6] could be detected in filaments surrounding cultured smooth muscle cells, chondrosarcoma cells and fibroblasts, respectively. Thus, it has been suggested that the matrilins may represent adapter proteins connecting proteoglycans to the collagen network and, on the other hand, forming collagen-independent structures [1]. Interestingly, matrilin-1-deficient mice, created by gene targeting, do not show any obvious phenotypical changes [15] and only a moderately abnormal cartilage collagen fibrillogenesis, without obvious effects on skeletal development [16]. Presumably matrilin-3 and -4, which are expressed at similar locations in skeletal tissues, can compensate for the loss of matrilin-1. The present study is the first to attempt a functional characterization of matrilin-2. We have identified a set of extracellular matrix proteins that are both localized together with matrilin-2 *in vivo* and have binding affinity for this protein, and have examined some of these interactions in detail.

EXPERIMENTAL

Expression and purification of recombinant matrilin-2 variants

To allow simple identification of the recombinant proteins we use a nomenclature where mtr2 represents the full-length protein, mtr2-cc is a deletion mutant lacking the coiled-coil domain and mtr2-A1-cc contains the 10 EGF-like repeats and the C-terminal vWFA-like domain. The domain structure of the polypeptides is

Abbreviations used: EGF, epidermal growth factor; RU, resonance units; vWFA, von Willebrand factor A.

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Figure 1 Schematic drawings of the domain structure of recombinant matrilin-2 variants

sp indicates the signal peptide; + is the positively charged region; A1 and A2 are the two VWFA-like domains; un is the unique domain and cc is the coiled-coil domain.

shown in Figure 1. The full-length mtr-2 and truncated mtr2-A1-cc cDNAs (Figure 1) were obtained as described previously [5]. The cDNA encoding mtr2-cc (Figure 1) was amplified by PCR using primers which inserted an *NheI* restriction site at the 5' end and a *NotI* site 3' to the stop codon: 5'-ATTAGCTAGCTACAGTCCACATGTGCAGC-3' and 5'-TTAAGCGGCCGCTCATTACATGCCTTCTTTCAGC-3'. After digestion with *NheI* and *NotI*, a 2.5 kb cDNA fragment was inserted into the expression vector pCEP-Pu in frame with the sequence of the signal peptide of BM40 [17]. All constructs were verified by sequencing. The recombinant plasmids carrying cDNAs encoding mtr2, mtr2-cc and mtr2-A1-cc were introduced by electroporation into the human embryonic kidney cell line HEK-293-EBNA (Invitrogen). Transfected cells were selected with 1.0 µg/ml puromycin and grown to confluency. Secretion of full-length or truncated forms of recombinant matrilin-2 into the cell culture medium was confirmed by SDS/PAGE of medium samples from transfected and wild-type cells and by immunoblotting using a specific antiserum against matrilin-2 [5].

The purification of the full-length protein mtr2 and mtr2-A1-cc was done as described in detail previously [5]. The serum-free culture medium from mtr2-cc-expressing cells was diluted 3-fold with 50 mM Tris/HCl, pH 7.4, and applied to a column of DEAE-Sepharose Fast Flow (Amersham Biosciences). Bound proteins were eluted with a linear gradient of 0.05–0.5 M NaCl in the same buffer. mtr2-cc-containing fractions were diluted 3-fold with 50 mM Tris/HCl, pH 7.4, and applied to a Source 15Q FPLC column (Amersham Biosciences). The proteins were eluted with a gradient of 0.05–0.3 M NaCl in the same buffer. Final purification and concentration was achieved through binding of mtr2-cc to a heparin-Sepharose CL6B column (Amersham Biosciences) and elution with 0.3 M NaCl/50 mM Tris/HCl, pH 7.4.

Ligand-binding assays

The collagens used in ligand-binding assays were purchased from Sigma. They had been isolated after pepsin digestion from the following tissues: collagen I from calf skin, collagen II from bovine tracheal cartilage, and collagens III, IV and V from human placenta. Each collagen was dissolved in 0.1 M acetic acid, pH 2.5. Murine laminin-1–nidogen-1 complexes [18] and bovine fibronectin [19] were isolated according to established procedures. Production and purification of the recombinant 110 kDa N-terminal fragment (rF37) of human fibrillin-2 has been described in detail previously [20].

The binding affinities between matrilin-2 and other extra-

cellular matrix proteins were measured by surface plasmon resonance using the BIAcore™ 2000 (BIAcore). Immobilization of proteins to the CM5 sensor chip was achieved by the *N*-ethyl-*N*-(3-dimethylaminopropyl)-carbodi-imidehydrochloride/*N*-hydroxysuccinimide coupling procedure at a flow rate of 5 µl/min. The surface of the carboxymethylated sensor chip was activated by addition of 30 µl of 0.2 M *N*-ethyl-*N*-(3-dimethylaminopropyl)-carbodi-imidehydrochloride/0.05 M *N*-hydroxysuccinimide solution. mtr2, mtr2-cc and mtr2-A1-cc were coupled to the activated chip in 10 mM sodium acetate, pH 4.0, which resulted in 16000 resonance units (RU) for mtr2, 10000 RU for mtr2-cc and 9000 RU for mtr2-A1-cc on one chip and 3000 RU for mtr2, 9000 RU for mtr2-cc and 9300 RU for mtr2-A1-cc on a second chip. Residual activated esters were blocked with 1 M ethanolamine in 0.1 M sodium hydrogen carbonate, pH 5.3. For controls, one surface on each chip was activated and blocked under identical conditions without coupling a protein. Binding studies with different ligands were performed in 0.15 M NaCl/10 mM Tris, pH 7.4, with 0.05 % Surfactant P-20 (BIAcore) at a flow rate of 30 µl/min for 1 min. BIAevaluation software version 2.1 was used to calculate dissociation constants.

Immunohistochemistry

A polyclonal antiserum against murine mtr2-A1-cc was raised in rabbit, purified by affinity adsorption to the antigen and tested for specificity by immunoblot [5]. Mouse monoclonal antibodies were obtained directed against human collagen I (CIIC1; Sigma), collagen IV (M3F7; Developmental Studies Hybridoma Bank), human cellular fibronectin (Sigma) and human fibrillin-1 (mAb201; kind gift of Dr Lynn Y. Sakai, Shriners Hospital for Children, Portland, OR, U.S.A.). Double immunofluorescence stainings were performed on adult human and newborn rat skin cryosections. After blocking with 1 % (w/v) BSA in PBS for 1 h, the sections were incubated with combinations of the polyclonal antiserum to matrilin-2 and one of the various mouse monoclonal antibodies for 1 h, followed by Cy3-conjugated affinity pure goat anti-rabbit IgG and Cy2-conjugated affinity pure goat anti-mouse IgG (Jackson ImmunoResearch Laboratories). The staining patterns were analysed with a Zeiss Axiophot microscope equipped with a fluorescence source.

Electron microscopy

Matrilin-2 and procollagen I, isolated from calf tendon fibroblasts [21], were mixed in equimolar amounts (typical concentrations 50–100 µg/ml in Tris-buffered saline) and incubated for 1 h at 4 °C. Samples were then diluted 10 times with Tris-buffered saline, adsorbed to 400-mesh carbon-coated copper grids for 1 min, washed briefly with two drops of water and stained with two drops of 0.75 % uranyl formate. The grids were rendered hydrophilic by glow discharge at low pressure in air. Specimens were observed in a Jeol 1200 EX transmission electron microscope operated at 60 kV accelerating voltage. Images were recorded on Kodak SO-163 plates without pre-irradiation at a dose of typically 2000 electrons/nm². Evaluation of the data from electron micrographs was done as described previously [22].

RESULTS

Preparation of recombinant matrilin-2 polypeptides

We produced three recombinant polypeptides of mouse matrilin-2, which were purified from the medium of stably transfected HEK-293-EBNA cells [5]. mtr2 represents the full-length protein, mtr2-cc is a deletion mutant lacking the coiled-coil domain and

Figure 2 SDS/PAGE analysis of the purified recombinant matrilin-2 variants used in binding assays

Electrophoresis was performed in 4–15% gradient polyacrylamide gels for mtr2 and in 10% polyacrylamide gels for mtr2-cc and mtr2-A1-cc without (–SH) or with (+SH) prior reduction of samples. Proteins were detected by Coomassie Blue staining.

mtr2-A1-cc contains the 10 EGF-like repeats and the C-terminal vWFA-like domain (Figure 1). SDS/PAGE of the preparations demonstrated their purity (Figure 2). The deletion mutants mtr2-

cc and mtr2-A1-cc gave major bands before and after reduction which corresponded well with the calculated molecular masses of the constructs (mtr2-cc, ≈ 90 kDa; mtr2-A1-cc, ≈ 70 kDa). In the reduced form, the full-length mtr2 polypeptide showed the expected molecular mass (≈ 125 kDa) plus a smaller band (≈ 100 kDa), probably representing a proteolytic breakdown product. In the non-reduced state, mtr2 in addition showed oligomers of different sizes, which are likely to represent dimers, trimers and tetramers of mtr2 (Figure 2). This assignment of bands is supported by earlier electron microscopy analysis [5]. Incomplete assembly of matrilin-2 is seen in tissue extracts and cultures of various cell types and probably represents a limited physiological proteolysis [5]. A related pattern of proteolytic fragments is seen for matrilin-4 and in this case a specific cleavage site, located close to the coiled-coil domain, could be identified [6]. Two glutamic acid residues directly N-terminal of the matrilin-4 cleavage site are conserved in matrilin-2.

Matrilin-2 self-interactions

The matrilin-2-containing filaments, observed in our earlier studies [5], may be formed by self-interactions between matrilin-2 molecules, by the association of matrilin-2 with a filament-forming protein, or by a combination of both mechanisms. To assess the potential of matrilin-2 for self-interactions we prepared BIAcore CM5 sensor chips carrying covalently bound matrilin-2 and matrilin-2 fragments and used these in surface plasmon resonance measurements with the same proteins as soluble ligands. In a first experiment, a sensor chip with mtr2, mtr2-cc and mtr2-A1-cc coupled to individual surfaces at a high density was used (Figures 3A–3C). All three matrilin-2 variants, when added in solution, bound significantly only to the smallest

Figure 3 Surface plasmon resonance measurements of matrilin-2 self-interaction

(A) mtr2 (100 nM) in the fluid phase was tested for interactions with 16000 RU of mtr2 (trace a), 10000 RU of mtr2-cc (trace b) and 9000 RU of mtr2-A1-cc (trace c) coupled to the chip. The same chip was used to investigate (B) 150 nM mtr2-cc and (C) 250 nM mtr2-A1-cc. (D) mtr2 (100 nM) in the fluid phase was tested for binding to 3000 RU of mtr2 (trace a), 9000 RU of mtr2-cc (trace b) and 9300 RU of mtr2-A1-cc (trace c) coupled to a separate chip.

Figure 4 Co-distribution of matrilin-2 and its ligands in skin

Sections of adult human skin (**A**, **C**, **D**) or newborn rat skin (**B**) were subjected to double immunofluorescence with specific antibodies directed against matrilin-2 (red) or the potential ligands (green) collagen IV (**A**), collagen I (**B**), fibronectin (**C**) and fibrillin-1 (**D**). The scale bar represents 0.1 mm in **A**, **C** and **D** and 0.2 mm in **B**. bm, basement membrane; ca, capillary; d, dermis; e, epidermis; f, hair follicle; n, nerve.

fragment mtr2-A1-cc, containing a single vWFA-like domain. As previous electron microscopy has shown that the matrilin-2 subunits form loops through a contact between the two vWFA-like domains [5], it may be that the presence of a single vWFA-like domain in mtr2-A1-cc favours interactions with exogenous ligands. Alternatively, self-interactions between mtr2 or mtr2-cc molecules when coupled at high density on the chip surface may make these partially unavailable for binding. To test this hypothesis, a second sensor chip was prepared carrying mtr2 at a 5-fold lower concentration. With this sensor chip, binding was observed with mtr2 added in solution (Figure 3D). Because of complex dissociation profiles K_D values could not be calculated for the matrilin-2 self-interactions. Even so, the results clearly demonstrate a self-interaction between matrilin-2 molecules and thereby a mechanism by which matrilin-2 may form homotypic filaments.

Co-localization of matrilin-2 and its potential ligands in human skin

To determine with which other extracellular matrix proteins matrilin-2 occurs *in vivo* and which may be its physiological interaction partners, we investigated the co-localization between matrilin-2 and some potential ligands in skin sections, using

Figure 5 Surface plasmon resonance measurements of the interaction between matrilin-2 and collagen I

(**A**) Collagen I (25 nM) in the fluid phase was tested for interactions with 3000 RU of mtr2 (trace a), 9000 RU of mtr2-cc (trace b) and 9300 RU of mtr2-A1-cc (trace c) coupled to the chip. In (**B**) the same experiment was repeated in the presence of (I) 25 nM mtr2-A1-cc and (II) 2.5 nM mtr2-A1-cc as a competitor in the fluid phase.

double immunofluorescence microscopy with specific antibodies. Collagen IV was used as a marker for basement membranes. An exact co-localization of matrilin-2 and collagen IV was observed at the dermo-epidermal junction of human skin and often in structures surrounding hair follicles and blood vessels (Figure 4A). Since laminin is found in basement membranes together with collagen IV, matrilin-2 is expressed in close proximity to laminin. Co-localization of collagen I and matrilin-2 was observed in parts of the upper dermis and, most prominently, in the subcutis of rat (Figure 4B). In recent studies we found that while matrilin-2 is found both in papillary dermis and basement membranes in mouse [5], it is more restricted to the basement membrane zone in human skin [23]. Fibronectin and matrilin-2 were often found in distinct expression domains but showed a clear overlap in some parts of the epidermal-dermal junction of human skin (Figure 4C). An interesting partial co-localization was observed for matrilin-2 and fibrillin-1, a marker for microfibrillar structures also containing fibrillin-2 [24], in and below basement membranes of human skin (Figure 4D). On the

Figure 6 Negative-staining electron microscopy of complexes formed between procollagen I and matrilin-2 and distribution of binding sites for matrilin-2 along procollagen I molecules

(A) Arrowheads and asterisks indicate, respectively, matrilin-2 molecules and the C-terminal globular domain of procollagen I. Scale bar, 100 nm. (B) The relative frequency denotes the fraction of matrilin-2 molecules that are bound to procollagen I within an interval of ± 2.5 nm.

basis of these results, studies of heterotypic interactions of matrilin-2 were focused on collagen I and fibrillin.

Analysis of the interactions between matrilin-2 and collagen I by surface plasmon resonance and electron microscopy

The binding of collagen I to the matrilin-2 proteins was studied by surface plasmon resonance. When collagen I was passed over the chip at a concentration of 25 nM, binding to both the full-length mtr2 and the truncated proteins mtr2-cc and mtr2-A1-cc was observed (Figure 5A). However, only the binding of collagen I to mtr2-A1-cc showed the expected concentration dependence (results not shown) and could be analysed quantitatively. A biphasic dissociation was observed and an overall K_D of about 3×10^{-8} M was calculated. The specificity of the interactions could be confirmed by an inhibition approach. Injection of pre-mixed samples of 25 nM collagen I with 2.5 nM mtr2-A1-cc as an inhibitor resulted in a reduction of the binding, as compared with the case without inhibitor, and at an equimolar ratio of

ligand to inhibitor binding was abolished (Figure 5B). Binding of collagen II, III, IV and V to the recombinant matrilin-2 polypeptides was also demonstrated by surface plasmon resonance and in most cases the strongest signals were obtained with mtr2-A1-cc (results not shown).

The interaction of matrilin-2 with collagen I was further studied by negative-staining electron microscopy of complexes preformed in solution. Procollagen I was used since the presence of a globular domain at the C-terminus allows one to determine the polarity of the molecules. Fields of well-resolved particles showed a high proportion of mtr2 bound to the collagen molecules, both at the C-terminus and along the triple helix (Figure 6A). Occasionally, two procollagen I molecules were seen to be connected by one molecule of mtr2, indicating the multivalent binding of this protein to procollagen I. The distribution of matrilin-2 particles along the procollagen molecules was measured for 200 complexes (Figure 6B). Preferential binding was detected to sites close to the C-terminus and at a distance of 110, 150 and 270 nm from the C-terminal end of procollagen I. The results demonstrate that, in addition to being able to self-aggregate, matrilin-2 can interact with collagen I and potentially be associated with the surface of collagen I-containing fibrils in a manner analogous with that of matrilin-1 bound to cartilage collagen fibrils.

Analysis of the interactions between matrilin-2 and non-collagenous proteins by surface plasmon resonance

The localization of matrilin-2 to microfibrils and basement membranes (Figure 4) prompted us to screen for interactions with some major molecular components of those structures. A recombinant 110 kDa fragment corresponding to the N-terminal portion of the microfibrillar protein fibrillin-2 bound in a dose-dependent manner to all three matrilin-2 variants (Figure 7 and results not shown). The binding curves were fitted to a 1:1 Langmuir binding model and resulted in dissociation constants of 3×10^{-9} M for mtr2, 6×10^{-9} M for mtr2-cc and 2×10^{-9} M for mtr2-A1-cc. Fibronectin and laminin-1–nidogen-1 complexes both showed a strong dose-dependent binding to mtr2-A1-cc while no interaction was observed with the larger matrilin-2 proteins (results not shown). K_D values of 6×10^{-11} M and 3×10^{-11} M were calculated for the interactions between mtr2-A1-cc and fibronectin or laminin-1–nidogen-1, respectively. Even though we have not yet characterized these interactions in as much detail as the matrilin-2 self-interactions and those between matrilin-2 and collagen I, the results show the ability of matrilin-2 to bind with high affinity to a spectrum of extracellular matrix ligands found in different subcompartments of, for example, the skin.

DISCUSSION

The extracellular organization of matrilin-2 is largely unknown, except for its localization in pericellular networks of cultured smooth muscle cells and the tissue distribution in both stroma and basement membranes of many tissues [5,8,9,23]. This prompted us to identify extracellular matrix ligands for matrilin-2 and to determine in which supramolecular extracellular assemblies it may be a component.

Our early attempts to investigate molecular interactions of matrilin-2 with other extracellular matrix proteins were hampered by the self-aggregation properties of matrilin-2. Therefore, we first characterized the binding of matrilin-2 to itself. Studies of matrilin-2, employing electron microscopy after negative staining, have indicated that the vWFA1 and vWFA2 domains within

Figure 7 Dose-dependent binding of the 110 kDa N-terminal fragment of fibrillin-2 to full-length matrilin-2 detected by surface plasmon resonance spectroscopy

Different concentrations of the recombinant fibrillin-2 fragment rF37 were tested for binding to (A) 9300 RU of mtr2-A1-cc and (B) 16000 RU of mtr2 coupled to the chip.

a single subunit may bind to each other [5]. It is feasible that similar interactions may occur between two matrilin-2 molecules that are physically close to each other in solution or in an assembled matrix. Indeed, recombinant matrilin-1 lacking the N-terminal vWFA1 domain does not assemble into filaments, indicating a crucial role for this domain in assembly [13]. When different recombinant matrilin-2 variants, harbouring either one (mtr2-A1-cc) or two (mtr2, mtr-cc) vWFA-like domains, were coupled to a sensor chip at high densities and used as ligands for homotypic interaction studies by surface plasmon resonance, only immobilized mtr2-A1-cc bound to the larger recombinant polypeptides (Figure 3). From these results, we conclude that intramolecular interactions between vWFA1 and vWFA2, either on the same subunit or between different subunits, block available binding sites for the soluble ligands. This phenomenon was dependent on the concentration of the ligand used in the coupling procedure. When a sensor chip was prepared with a lower coupling density, full-length matrilin-2 bound to the chip surface was seen to bind to matrilin-2 added in solution, indicating that intermolecular interactions were favoured under those conditions. The results demonstrate the potential of matrilin-2 for homotypic association, which may lead to the formation of filamentous structures.

Double immunofluorescence microscopy on skin sections demonstrated a partial co-localization of matrilin-2 with collagen

I, collagen IV, fibronectin and fibrillin-1 (Figure 4). Thereby, collagen IV served as a marker for basement membranes and fibrillin-1 for microfibrils, while both collagen I and fibronectin are predominant components of the dermal extracellular matrix. All these proteins, as well as molecules that are closely co-localized with one of them, e.g. laminin and fibrillin-2, are therefore potentially relevant as physiological ligands of matrilin-2.

Since collagen I is one of the major structural components of many extracellular matrices and it is expressed in many tissues where matrilin-2 is present (see e.g. Figure 4B), we investigated the interaction between matrilin-2 and collagen I in some detail. All matrilin-2 variants bound significantly to collagen I in surface plasmon resonance interaction experiments (Figure 5). This interaction could be inhibited by mtr2-A1-cc, the smallest matrilin-2 polypeptide, demonstrating that the interaction between matrilin-2 and collagen I is specific. Analysis of preformed complexes between matrilin-2 and procollagen I by electron microscopy after negative staining demonstrated four discrete binding sites along the procollagen I molecule (Figure 6). The fact that a high proportion of all matrilin-2 particles were found associated with procollagen I further confirms the strong binding affinity between these ligands. Earlier studies of matrilin-1 have shown an association of this molecule with the cartilage collagen fibrils [12–14], made up from collagen II, XI and IX. The results indicate that collagen association is a property of both matrilin-1 and -2, which may be shared by all matrilins.

It is interesting that matrilin-2 co-localizes with microfibrils in and directly below the dermo-epidermal basement membrane (Figure 4) and that matrilin-2 strongly binds fibrillin-2 (Figure 7). Fibrillin-2, together with its close homologue fibrillin-1, is organized in 10–12 nm-diameter microfibrils which often intersect into the dermo-epidermal basement membrane [25]. The role of this anchorage and the underlying molecular interactions are not known. The interactions with components both of microfibrils and basement membranes presented here identify matrilin-2 as a candidate protein for being a molecular adapter between these structures. If indeed matrilin-2 serves this purpose, then it might be relevant also in wound healing and in some forms of inherited connective tissue disease.

In conclusion, matrilin-2 is a widespread extracellular matrix component that can interact with itself and with other both collagenous and non-collagenous matrix molecules. This interaction repertoire, together with its oligomeric structure, renders matrilin-2 a good candidate as an adapter or mediator molecule for interactions between other matrix macromolecules during the assembly of an extracellular matrix. It has the potential to serve such functions in and between basement membranes, microfibrils and in the dermal extracellular matrix.

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REFERENCES

- Deák, F., Wagener, R., Kiss, I. and Paulsson, M. (1999) The matrilins: a novel family of oligomeric extracellular matrix proteins. *Matrix Biol.* **18**, 55–66
- 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
- Klatt, A. R., Nitsche, P. D., 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

- 4 Paulsson, M. and Heinegård, D. (1981) Purification and structural characterization of a cartilage matrix protein. *Biochem. J.* **197**, 367–375
- 5 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
- 6 Klatt, A., 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
- 7 Deák, F., Piecha, D., Bachrati, C., Paulsson, M. and Kiss, I. (1997) Primary structure and expression of matrilin-2, the closest relative of cartilage matrix protein within the von Willebrand factor type A-like module superfamily. *J. Biol. Chem.* **272**, 9268–9274
- 8 Segat, D., Frie, C., Nitsche, D. P., 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**, 549–555
- 9 Klatt, A. R., Paulsson, M. and Wagener, R. (2002) Expression of matrilins during maturation of mouse skeletal tissues. *Matrix Biol.* **21**, 289–296
- 10 Paulsson, M. and Heinegård, D. (1979) Matrix proteins bound to associatively prepared proteoglycans from bovine cartilage. *Biochem. J.* **183**, 539–545
- 11 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
- 12 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
- 13 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
- 14 Chen, Q., Zhang, Y., Johnson, D. M. and Goetinck, P. F. (1999) Assembly of a novel cartilage matrix protein filamentous network: molecular basis of differential requirement of von Willebrand factor A domains. *Mol. Biol. Cell* **10**, 2149–2162
- 15 Aszodi, A., Bateman, J. F., Hirsch, E., Baranyi, M., Hunziker, E. B., Hauser, N., Böse, Z. and Fässler, R. (1999) Normal skeletal development of mice lacking matrilin 1: redundant function of matrilins in cartilage? *Mol. Biol. Cell* **19**, 7841–7845
- 16 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
- 17 Kohfeldt, E., Maurer, P., Vannahme, C. and Timpl, R. (1997) Properties of the extracellular calcium binding module of the proteoglycan testican. *FEBS Lett.* **414**, 557–561
- 18 Paulsson, M., Aumailley, M., Deutzmann, R., Timpl, R., Beck, K. and Engel, J. (1987) Laminin-nidogen complex. Extraction with chelating agents and structural characterization. *Eur. J. Biochem.* **166**, 11–19
- 19 Miekka, S. I., Ingham, K. C. and Menache, D. (1982) Rapid methods for isolation of human plasma fibronectin. *Thromb. Res. Suppl.* **27**, 1–14
- 20 Keene, D. R., Jordan, C. D., Reinhardt, D. P., Ridgway, C. C., Ono, R. N., Corson, G. M., Fairhurst, M., Sussman, M. D., Memoli, V. A. and Sakai, L. Y. (1997) Fibrillin-1 in human cartilage: developmental expression and formation of special banded fibers. *J. Histochem. Cytochem.* **45**, 1069–1082
- 21 Kadler, K. E., Hojima, Y. and Prockop, D. J. (1987) Assembly of collagen fibrils *de novo* by cleavage of the type I pC-collagen with procollagen C-proteinase. Assay of critical concentration demonstrates that collagen self-assembly is a classical example of an entropy-driven process. *J. Biol. Chem.* **262**, 15696–15701
- 22 Engel, J. and Furthmayr, H. (1987) Electron microscopy and other physical methods for the characterization of extracellular matrix components: laminin, fibronectin, collagen IV, collagen VI, and proteoglycans. *Methods Enzymol.* **145**, 3–78
- 23 Piecha, D., Hartmann, K., Kobbe, B., Haase, I., Mauch, C., Krieg, T. and Paulsson, M. (2002) Expression of matrilin-2 in human skin. *J. Invest. Dermatol.* **119**, 38–43
- 24 Zhang, H., Apfelroth, S. D., Hu, W., Davis, E. C., Sanguineti, C., Bonadio, J., Mecham, R. P. and Ramirez, F. (1994) Structure and expression of fibrillin-2, a novel microfibrillar component preferentially located in elastic matrices. *J. Cell Biol.* **124**, 855–863
- 25 Tsuji, T. (1980) Elastic fibres in the dermal papilla. Scanning and transmission electron microscopic studies. *Br. J. Dermatol.* **102**, 413–417

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