The procollagen N-proteinases ADAMTS2, 3 and 14 in pathophysiology

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Abstract

Collagen fibers are the main components of most of the extracellular matrices where they provide a structural support to cells, tissues and organs. Fibril-forming procollagens are synthetized as individual chains that associate to form homo- or hetero-trimers. They are characterized by the presence of a central triple helical domain flanked by amino and carboxy propeptides. Although there are some exceptions, these two propeptides have to be proteolytically removed to allow the almost spontaneous assembly of the trimers into collagen fibrils and fibers. While the carboxy-propeptide is mainly cleaved by proteinases from the tolloid family, the amino-propeptide is usually processed by procollagen N-proteinases: ADAMTS2, 3 and 14.

This review summarizes the current knowledge concerning this subfamily of ADAMTS enzymes and discusses their potential involvement in physiopathological processes that are not directly linked to fibrillar procollagen processing.

Introduction

Fibrillar collagens are the most abundant proteins and the main constituents of the extracellular matrix. They are the principal source of tensile strength in animal tissues and define the shape and form of tissues in which they occur. The formation of collagen fibers is a multi-step process [1]. After synthesis, modifications by specific enzymes and proper folding, fibrillar collagens are secreted as pro-molecules formed by a large central triple helical domain extended by propeptides at both extremities. These amino- and carboxy-propeptides are then cleaved, respectively by the procollagen N-proteinases (ADAMTS2, 3 and 14) and the C-proteinases (see Vadon-Le Goff et al. in this Special issue), which reduces the solubility of the collagen molecules and induces their almost spontaneous assembly into elongated and cylindrical collagen fibrils (Fig. 1A).

The first description of procollagen N-endopeptidase activity was made in the context of a study trying to determine the cause of dermatosparaxis, a rare genetic disease that appeared in Belgian cattle herds during an inbreeding program [2,3]. Affected animals were mainly characterized by extreme skin fragility. Electron microscopy and biochemical analyses showed the presence of highly abnormal collagen fibrils in the dermis of dermatosparactic calves resulting from the accumulation of type I aminoprocollagen (type I collagen still retaining its aminopropeptide). The demonstration that this longer form of collagen could be processed into normal chains of expected size by extracts of normal calf skin suggested that the procollagen molecule itself was not defective and that the enzyme responsible for its cleavage was either absent or inactive. Semi-purified preparations containing N-endopeptidase activity were obtained from chick embryo [4] and calf tendon [5] but did not allow identifying the enzyme. The determination of partial amino acid sequences from enzyme purified from calf skin [6] was a key step because it allowed designing DNA probes and primers that were used
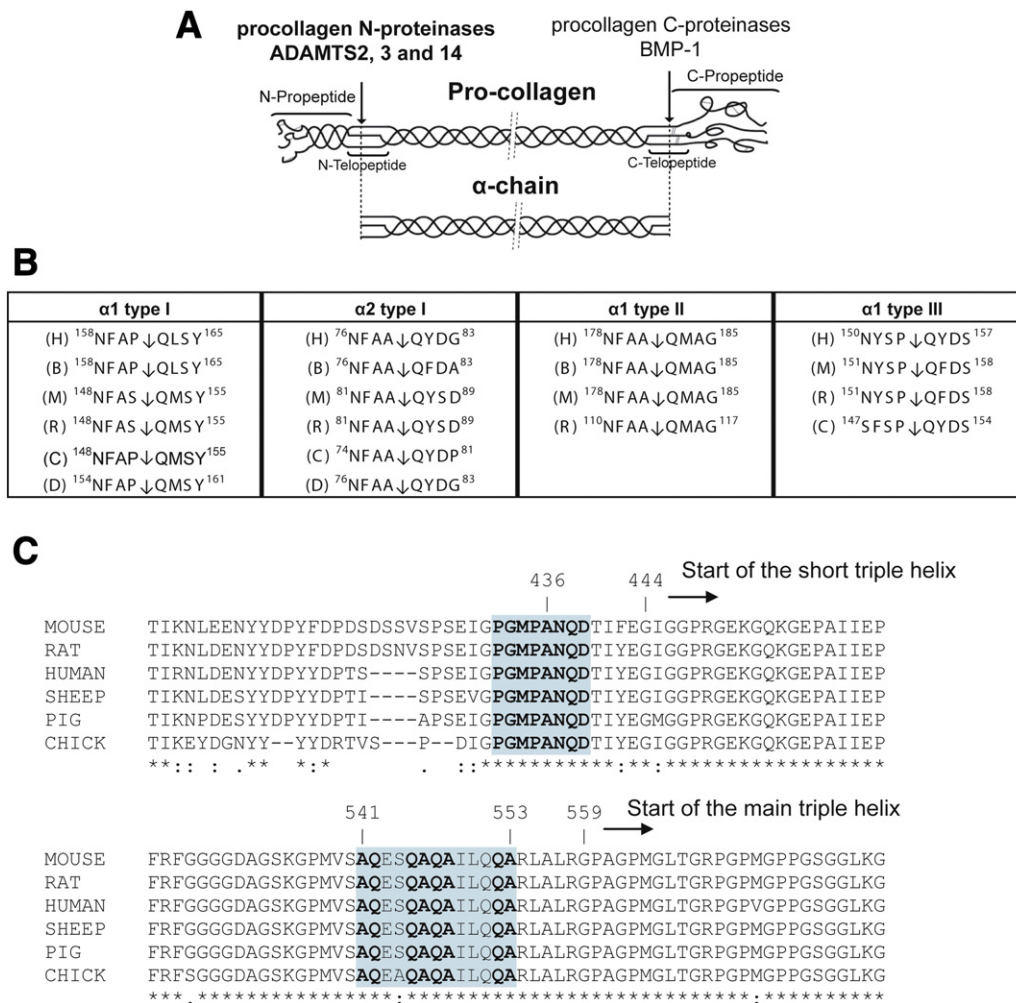
Fig. 1. Procollagen aminopeptidase cleavage sites. (A) Procollagen processing by the procollagen N-proteinases (ADAMTS2, 3 and 14) and the C-proteinases. (B) Sequence homology of ADAMTS2 cleavage sites in types I–III fibrillar collagens from different species: (H) human, (B) bovine, (M) mouse, (R) rat, (C) chicken and (D) dog. The cleavage sites are indicated by arrows. The numbering of amino acids is assigned according to the Universal Protein Resource (UniProt) database. (C) Sequence alignment (ClustalW on NPS@) of α1 type V collagen from different species. A cleavage site has been experimentally determined at position 435/436 (in human and mouse sequences) upstream of the short triple helical domain. A highly conserved sequence presenting repetitions of AQ residues potentially cleaved by ADAMTS2 is however observed between the short and the main triple helix as in types I–III collagens, which suggests that processing by procollagen N-proteinases could occur at this site even if not yet firmly demonstrated. (*) Identical, (:) strongly similar, (.) weakly similar.

to clone its cDNA and to characterize its gene, both in man and bovine [7,8]. Based on domain similarities with ADAMTS1 that was cloned almost simultaneously [9], this procollagen N-endopeptidase was named ADAMTS2.

Two other enzymes displaying procollagen processing activity were described later. The first report related to ADAMTS3 was the cloning of a human cDNA (KIAA0366) [10] displaying sequence similarities with ADAMTS2, although its procollagen N-endopeptidase activity was demonstrated later [11,12]. ADAMTS14 was cloned by three different groups [13–15]. Its distribution pattern and activity were also characterized, demonstrating a low but significant procollagen N-endopeptidase activity in vitro [15].

Amino acid sequence alignment and domain organization clearly show that these three procollagen N-propeptidases belong to the "A Disintegrin And Metalloproteinase with ThromboSpondin type I domain" proteases (ADAMTS) family (M12B ADAM branch) [16]. Beside their specific enzymatic activity, they are characterized by the presence of four thrombospondin type I domains (TSR1) and a C-terminal procollagen N-proteinase (PNP) domain

comprising a "Protease and LACunin" (PLAC) domain (for more details about the ADAMTS family, see Dubail and Apte in this Special issue).

Amino-endopeptidase function of ADAMTS2, 3 and 14

Regulation of expression and activity

The most investigated and characterized procollagen N-endopeptidase is ADAMTS2. It is mainly expressed by fibroblasts and cells of mesenchymal origin and its expression correlates with the expression of type I and type III collagens. As examples, its synthesis is increased by TGF beta in vitro [17] and in fibrotic lesions in vivo [18]. ADAMTS2 is synthesized as an inactive proenzyme which is activated by mammalian subtilisins, such as furin, which cleave between the prodomain and the metalloproteinase domain [19]. It has also been shown that an autocatalytic cleavage occurring within the carboxy-terminal end, possibly in the PNP-domain, increases its activity [19]. A similar activation process was also reported for ADAMTS1, 4, 8, 9 and 12 [20], suggesting that this step of regulation could be a common feature of the ADAMTS proteases. Based on sequence homologies and Western blot studies showing similar band patterns, ADAMTS3 and 14 are supposed to be activated in a same manner as ADAMTS2 despite the fact that it was not demonstrated.

As for the other ADAMTS, the activity of ADAMTS2, 3 and 14 requires a neutral to slightly basic pH and the presence of zinc and calcium ions [6]. To date no highly specific inhibitor of procollagen N-propeptidases has been reported. As for many other proteinases, α2-macroglobulin can physically entraps ADAMTS2 after cleavage within its bait region. TIMP3 has also been shown to inhibit the aminopropeptide cleavage of type I, II and III collagen when added in excess in the presence of heparin [21] However, TIMP3 is a wide-spectrum inhibitor with activities towards several MMPs, ADAMs and ADAMTSs [22]. Papilin has also been reported to be an inhibitor of ADAMTS2, by binding both the enzyme and the substrate [23]. The precise mechanism of inhibition has not yet been determined but could be related to the presence of similar domains (Cys-rich and several TSR1) in papilin and in the C-terminal ancillary domains of ADAMTS2. If true, it would explain why the C-terminal part of ADAMTS2, rather than its metalloproteinase domain, seems to be responsible for the recognition of its procollagen substrates [19]. It would suggest also that other proteins possessing domains similar to those found in the ancillary domains of ADAMTS2 (ADAMTSL, other proteins possessing TSR1 domains) could regulate its activity.

The activity of proteinases can be also controlled by other mechanisms such as clearing by internalization into cells or co-localization with their substrates. ADAMTS2, 3 and 14, although being secreted, are immobilized at the cell surface, or very close to it, at a location where procollagen processing is physiologically performed. The ancillary domains, especially the second TSR1, are required for efficient interactions with the cell layer compartment and with extracellular matrix components [19]. As heparin and high salt concentrations can interfere with binding, this suggests that proteoglycans are probably involved. In vitro, it has been shown that ADAMTS2 induces the apoptosis of endothelial cells by a mechanism independent of its catalytic activity but potentially related to interactions with a cell surface receptor [24]. In this model, efficient internalization process has also been observed but its role, either as a simple clearing mechanism or a specific intracellular function, is still unknown.

Further to proteolytic processing, the domain composition of ADAMTS2 can also result from an alternative splicing mechanism. Beside the classical 1211 amino acid full length enzyme, a shorter form has been described. It is formed by the first 543 amino acids of the long form (corresponding to exons 1–10) followed by 23 amino acids encoded by an alternative exon present in intron 10 of the long form. This truncated form does contain the metalloproteinase domain but does not show any significant amino-procollagen peptidase activity. Determination of its potential physiological relevance would require additional characterizations. In ADAMTS14, the nine last bases of exon 6 can be also alternatively spliced but it does not seem to modify its enzymatic activity. The existence of alternative exons 1 was also reported [15], but their biological impact was not yet determined.

Spatial distribution and substrate specificity

The preferential substrate specificity of ADAMTS2, 3 and 14 regarding the different fibrillar procollagens is dictated first by their tissue distribution. High expression of ADAMTS2 is detected in all type I collagen-rich tissues from fetal calf such as skin, bones, tendons and aorta, which supports its importance for type I collagen maturation [7]. ADAMTS3 is mainly expressed in cartilage, where it colocalizes with type II procollagen, and in the nervous system [11,25]. ADAMTS14 is usually co-expressed with ADAMTS2, although at a lower level, suggesting potential functional redundancy. These results demonstrate that a first level of specificity of these proteinases is guided by their spatial and temporal localization with their respective substrates.

As ADAMTS2, 3 and 14 were discovered because of their aminoprocollagen endopeptidase activity, it

is not surprising that most of the knowledge about their substrate specificity and requirement is directly linked to collagen. In type I and type II procollagens, the cleavage is performed between the short amino-terminal and the main central collagen domains. This specific 3D conformation seems to be important since individual procollagen chains are not cleaved by ADAMTS2. It has also been shown that mutations slightly affecting the stability of the beginning of the collagen domain decrease the rate of aminoprocollagen processing, which leads to an Osteogenesis Imperfecta/Ehlers–Danlos mixed syndrome in human [26]. Alignments of cleaved sequences in various species have allowed the identification of a preferential cleavage site consisting in a small aliphatic residue at the P1 position and a glutamine at P1' (Fig. 1B).

The existence of an enzyme specific for type III procollagen processing was initially proposed since some enzyme preparations were active on type I and not on type III procollagens, and vice versa [5,27,28]. However, it was later shown that recombinant ADAMTS2 is also the main enzyme responsible for the processing of the aminopropeptide of type III procollagen [17,19,25] and that the cleavage site is identical to that of type I and type II procollagens (Fig. 1A). These contradictory results between the first and more recent data could be explained by the use of extracts only partially purified in the initial experiments and suggest the existence of potential cofactor(s) or inhibitor(s) that would be differently co-purified and that would regulate specifically the processing of the different fibrillar procollagens.

The cleavage site for type V procollagen has been more complex to determine because this minor fibrillar collagen is hard to purify in significant amounts in conditions that both preserve its non collagenous domains and prevent contamination by type I/III collagens. Therefore, many studies have been performed using recombinant procollagen. Processing by BMP1 has been reported between the large globular N-propeptide and the variable region of α1(V) [29] and between the small and the large triple helical domain of α3(V) [30]. A cleavage of α1(V) chain by ADAMTS2 has also been demonstrated [19]. However its localization (between the variable and the short triple helical domains) and its site (P-A) are distinct from those reported for types I, II and III procollagens (Fig. 1). It does not mean however that cleavage cannot occur in the more conventional site between the short and the long triple helical domains since the consensus sequence found in the other fibrillar collagens is also present (AQESQAQ) and conserved between species (Fig. 1C). Although the physiological relevance of the processing between the variable and the short triple helix of type V procollagen has not been demonstrated yet, it does show however that ADAMTS2, and thus probably also ADAMTS3 and 14, can be active in situations more diverse than

initially thought. This hypothesis is further supported by the fact that ADAMTS2 is able to cleave itself within the C-terminal ancillary domains [19].

To date, little is reported concerning ADAMTS3 and ADAMTS14 activity. ADAMTS14 processes type I procollagen (α1 and α2 chains) and its strong sequence homology with ADAMTS2 suggests that the cleavage should occur at the same site [15]. ADAMTS3 is less expressed in human skin and skin fibroblasts than ADAMTS2, but is more abundant in cartilage. It has been demonstrated that ADAMTS3 cleaves the aminopropeptide of type II collagen in swarm rat chondrosarcoma RCS-LTC cells stably transfected with human ADAMTS3 [11]. More recently, it has been shown in co-transfection studies that ADAMTS3 promotes the release of a proteolytically-cleaved active form of VEGF-C, a process that increases VEGF-R3 signaling [31]. This work further supports the fact that the substrates repertoire of the procollagen N-proteinases ADAMTS2, 3 and 14 is wider than initially expected.

Other biological functions of ADAMTS2, 3 and 14

Beside its role during procollagen processing, the best characterized property of ADAMTS2 is its potent anti-angiogenic activity [24]. In vitro, recombinant ADAMTS2 rapidly induces the apoptosis of endothelial cells while fibroblasts or smooth muscle cells, for example, are not affected. In vivo, the formation of tumors in nude mice by HEK cells is strongly reduced when they overexpress ADAMTS2, an observation that was correlated to a reduced intratumoral vascularization but that could also involve direct anti-tumor effects (unpublished personal data). These effects do not rely on the catalytic activity of the enzyme but rather seem to be related to one of its C-terminal domains. Co-purification and co-localization studies identified nucleolin as a potential endothelial cell surface receptor for ADAMTS2, which makes sense since nucleolin is one of the receptors for endostatin [32], a potent anti-angiogenic molecule.

A puzzling observation about ADAMTS2 was the description of its overexpression by macrophages and peripheral blood monocytes stimulated by glucocorticoids [33]. As these cells have little chance to be implicated in procollagen processing, it means that other functions or substrates should be involved. In this context, it is worth mentioning that several molecules involved in innate defenses (C1q, ficolins, MBL) have triple-helical collagen-like domain. Therefore, it would be most interesting to evaluate if they are substrates (or binding partners) of ADAMTS2, which would open a so far unexpected field of research about the so-called aminoprocollagen endopeptidases.

Procollagen aminopeptidase KO mouse models

Until now, only Adamts2-deficient mice have been produced and thoroughly phenotypically characterized [34]. Heterozygous males and females display a normal phenotype. Their mating produces a normal Mendelian ratio of Adamts2-KO pups that cannot be distinguished from their heterozygous or wild-type littermates. By two weeks of age, their skin progressively becomes fragile. Similarly, while testes are normal at birth, seminiferous tubules display strong abnormalities in the adult with an absence of spermatozoids production. Female were initially reported as being "normally" fertile [34]. However, despite numerous mating between Adamts2-KO females and wild-type males, only a limited number of pups have been obtained later (personal observation). Although the reason for such reduced fertility was not thoroughly investigated, it does not seem to be caused by impaired parturition and could be related to connective tissue-related problems preventing efficient fertilization.

As for dermatosparactic calves, skin fragility is caused by excessive accumulation of aminoprocollagen I resulting in the formation of abnormal collagen fibrils (Fig. 2). The proportion of aminoprocollagen I/fully processed alpha I chains is high in the skin and low in other tissues, such as tendon, which explains the absence of obvious tendon alterations. Electron microscopy analyses confirm these functional and biochemical data since fibrils are strongly disorganized in the skin and much less altered in tendons. It was suggested that ADAMTS14, which is co-expressed with ADAMTS2 in different connective tissues, could be responsible for this partial alternative aminoprocollagen endopeptidase activity [25].

As the role of ADAMTS2 is crucial for collagen fibrils formation, it was investigated whether its inhibition/absence could be considered as a way to treat fibrosis [18]. Carbon tetrachloride injection and bile duct ligation were used to induce liver fibrosis. In Adamts2-KO mouse livers, collagen fibers were thinner and more irregular than in the control littermates, which correlated also with faster collagen degradation. Although the more obvious explanation relates to the role of ADAMTS2 for procollagen processing, potential implications at other levels of regulation could not be excluded.

The dermatosparactic type of Ehlers-Danlos syndrome and other inherited diseases

The Ehlers–Danlos Syndrome (EDS) comprises a heterogeneous group of diseases characterized by fragility of the soft connective tissues and widespread manifestations in skin, ligaments and joints, blood vessels and internal organs. The clinical spectrum varies from mild skin and joint hyperlaxity to severe physical disability and life-threatening vascular complications. The Villefranche classification [35] recognizes six subtypes, most of which are linked to mutations in one of the genes encoding fibrillar collagen proteins or enzymes involved in post-translational modification of these proteins [36].

The arthrochalasic type of EDS, caused by mutations affecting the aminoprocollagen cleavage site in alpha1 or alpha2 type I procollagen, is mainly characterized by joint hyperlaxity while skin collagen fibers are only moderately affected. Surprisingly, the absence of ADAMTS2 activity leads to a different type of disease, the dermatosparactic type of EDS (also previously known as EDS-type VIIC) [8,37]. The main clinical manifestation of this rare genetic disease is the fragility of the skin, as in Adamts2-KO mice, but joint laxity is usually only moderate, which contrasts with the hypermobility in arthrochalasic EDS. This could result from the strong variation in type I procollagen processing from one tissue to another. It could be also related to the abundance of type III procollagen in skin while it is almost absent in tendons and ligaments. Processing by ADAMTS2 of other macromolecules involved in fibril formation (FACITs, proteoglycan …) is another hypothesis that would be worth investigating. Patients have also orofacial characteristics and are at risk of rupture of internal organs due to soft tissue fragility. The formation of large hematomas after minor trauma is also reported [38] and has been attributed to the lack of collagen type III maturation [17] and/or to overall skin fragility, which does not exclude the participation of some other mechanisms related to blood coagulation. This hypothesis is in line with a recent genome-wide association study performed on a large cohort of 270 family-based trios and describing a strong association ($P = 2.9 \times 10^{-6}$) between the ADAMTS2 gene and predisposition to pediatric stroke [39].

Still in the cardiovascular field, it has also been shown that ADAMTS2 and ADAMTS3, but not ADAMTS14, are more abundant in the culprit plaques from patients presenting with acute myocardial infarction (AMI) versus stable angina. Their expression overlap the area positive for CD31 or CD68 suggesting their expression by endothelial cells or macrophages [40].

Genetic associations have been also reported for ADAMTS14. Polymorphic markers found in the Adamts14 gene seem to be linked to the predisposition to multiple sclerosis [41]. Two other studies identified the Adamts14 gene as potentially implicated in knee osteoarthritis in woman [42,43]. The molecular mechanism potentially involving ADAMTS14 in these pathologies remains unknown.
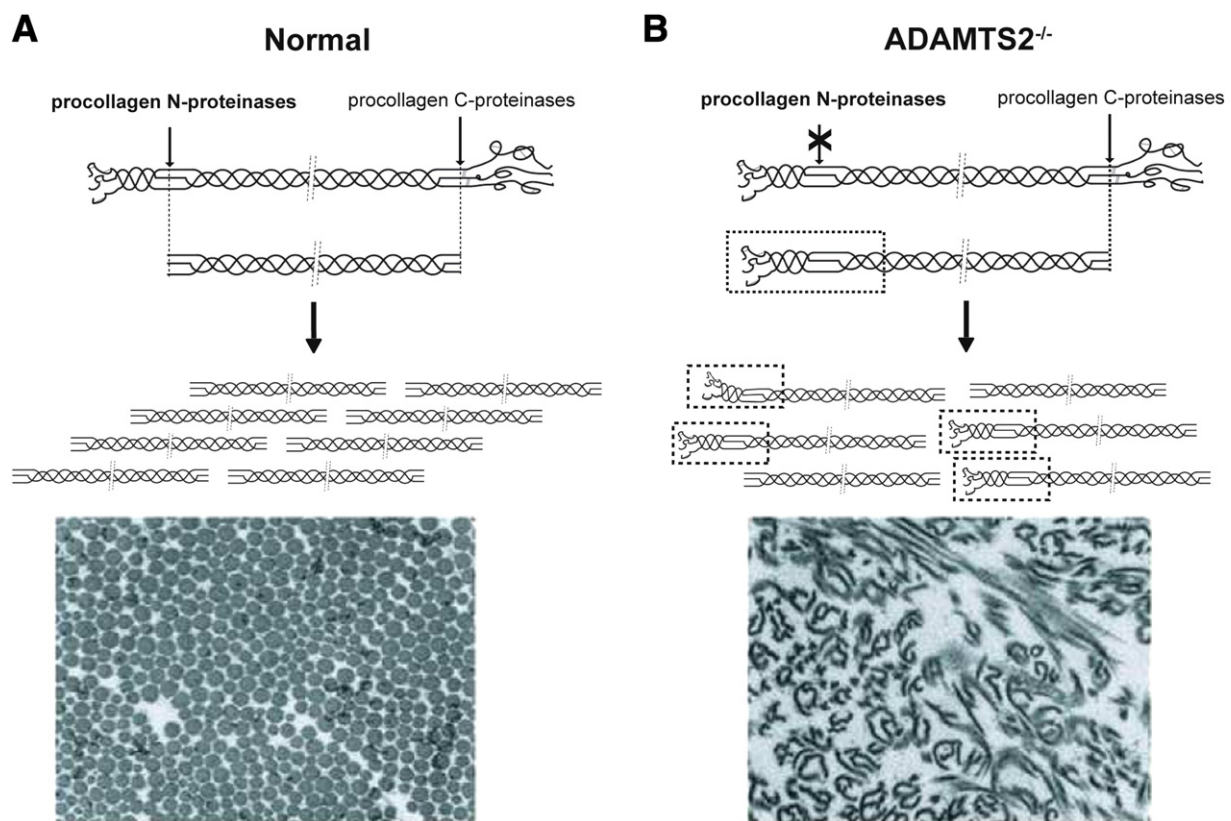
Fig. 2. ADAMTS2 is crucial for fibrillar collagen organization. (A) Collagen fibrils are formed by the progressive assembly of mature α chains after cleavage of the propeptides by procollagen N- and C-proteinases. These fibrils are elongated and cylindrical in cross section as visualized by transmission electron microscopy in a normal mouse skin. (B) In absence of ADAMTS2 activity, aminoprocollagen (collagen molecule still retaining its aminopropeptide) accumulates. This interferes with collagen assembly and leads to the formation of ribbon-like collagen structures with poor mechanical properties, which, in turn, is responsible for the skin fragility observed in patients with dermatosparactic EDS or in animals with dermatosparaxis.

Conclusions and perspectives

As dramatically seen in the dermatosparactic type of EDS, ADAMTS2 is crucial for fibrillar collagen maturation. This largely explain why most of the studies about ADAMTS2, but also about its closest relatives ADAMTS3 and ADAMTS14, have been mainly focused on collagen and extracellular matrix biology. Although procollagens are certainly key physiological targets of these enzymes, several observations suggest the existence of other functions in the fields of immunity and fertility. The observation that ADAMTS2 inhibits angiogenesis independently of its catalytic activity further suggests the existence of other regulatory mechanisms, possibly through functional interactions with cell surface receptors. Genetic linkages between the procollagen N-proteinase genes and blood related pathologies, multiple sclerosis and osteoarthritis, together with the recent demonstration that ADAMTS3 participates in the VEGF-C activation and in lymphangiogenesis, clearly show that the roles of these three enzymes are much more diverse than initially expected. The extensive characterization of the phenotype of Adamts3 and Adamts14-KO mice (ongoing research) and large scale evaluation of their substrates repertoire should shed a new light on their biology and the functions of the entire ADAMTS family.

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