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The multiple, complex roles of versican and its proteolytic turnover by ADAMTS proteases during embryogenesis☆

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Abstract

Embryonic development is an exceptionally dynamic process, requiring a provisional extracellular matrix that is amenable to rapid remodeling, and proteolytic or non-proteolytic mechanisms that can remodel the major components of this matrix. Versican is a chondroitin-sulfate proteoglycan that forms highly hydrated complexes with hyaluronan and is widely distributed in the provisional matrix of mammalian embryos. It has been extensively studied in the context of cardiovascular morphogenesis, neural crest cell migration and skeletal development. Analysis of *Vcan* transgenic mice has established the requirement for versican in cardiac development and its role in skeletogenesis. The ADAMTS family includes several versican-degrading proteases that are active during remodeling of the embryonic provisional matrix, especially during sculpting of versican-rich tissues. Versican is cleaved at specific peptide bonds by ADAMTS proteases, and the cleavage products are detectable by neo-epitope antibodies. Myocardial compaction, closure of the secondary palate (in which neural crest derived cells participate), endocardial cushion remodeling, myogenesis and interdigital web regression are developmental contexts in which ADAMTS-mediated versican proteolysis has been identified as a crucial requirement. ADAMTS proteases are expressed coordinately and function cooperatively in many of these contexts. In addition to versican clearance, ADAMTS proteases generate a bioactive versican fragment containing the N-terminal G1 domain, which we have named versikine. This review promotes the view that the embryonic extracellular matrix has evolved not only to provide a permissive environment for embryo growth and morphogenesis, but through its dissolution to influence and regulate cellular processes.

Keywords

A disintegrin-like and metalloprotease domain with thrombospondin type 1 motifs; ADAMTS; Versican; Embryogenesis; Soft tissue syndactyly; Cardiac jelly; Heart valve; Limb development; Cleft palate; Melanoblast

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1. Introduction

The vertebrate embryonic extracellular matrix (ECM) is compositionally, organizationally and mechanically distinct from the extensively specialized and mechanically robust ECMs of adult tissues. It is a provisional or temporary matrix, such as that present during the early stages of wound healing. Because of extensive and rapid cell migration, folding, branching and sculpting of tissues required for embryogenesis, a mature or adult ECM, such as one comprising a well-established collagenous structure, could be an impediment to successful development. Instead, the provisional matrix is enriched in hyaluronan, fibronectin and proteoglycans such as versican, rendering it highly hydrated and malleable. mRNA expression of versican, the focus of this review, is the highest during embryogenesis and is much reduced in most adult tissues (Naso et al., 1995), which are more cellular, contain more collagen, elastin and other ECM components, but, with the exception of the brain, relatively little versican. It could be speculated that the embryonic provisionalmatrix evolved to support morphogenesis, and consequently, required the evolution of specific mechanisms to dissolve it during tissue sculpting to achieve the mature form, or prior to replacement by specialized ECM. This model has a precedent in the requirement for tissue collagenase in rapid collagen remodeling during resorption of the vestigial tail and fin, a defining event in amphibian metamorphosis (Gross and Lapiere, 1962). By analogy, it could be argued that specific proteolytic activities are necessary for digesting the relatively collagen-poor provisional matrix during mammalian embryogenesis. This review will focus on the developmental role of versican and the mechanisms of its clearance during embryogenesis. Indeed, one purpose of this reviewis to emphasize the extraordinarily broad, yet specific role of ADAMTS proteases in versican removal during morphogenesis. Coordinated evolution of provisional matrix with specific proteolysis mechanisms that remodel it, and especially, the existence of optimized temporal and spatial relationships of the protease and substrate (using versican as a model) is a key concept underlying this review.

2. Structure and genetics of versican

Versican (synonyms: PG-M, CSPG2), was identified in the culture medium of metabolically labeled fibroblasts (Coster et al., 1979), initially characterized as a proteoglycan from chick limb buds and first cloned from a human placenta cDNA library (Kimata et al., 1986; Zimmermann and Ruoslahti, 1989). It is a chondroitin sulfate (CS) proteoglycan belonging to a family of hyaluronan (HA) binding proteins (also referred to as hyalectans or lecticans) that includes aggrecan, brevican and neurocan (Yamaguchi, 2000). Versican is found in vertebrates, but not in invertebrate model organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster* (Ismat et al., 2013).

Vcan is located on human chromosome 5q (the mouse gene, *Vcan* is on chromosome 13) and contains 15 exons (Iozzo et al., 1992). It comprises three major domains with distinct functions: an N-terminal G (globular) 1 domain, which mediates HA binding through two link protein-like modules (link modules, Fig. 1), one or both of two alternatively spliced, extended glycosaminoglycan (GAG) attachment domains (GAGα and GAGβ), and a C-terminal G3 domain (Zimmermann and Ruoslahti, 1989; Wight, 2002). The versican–HA complex may be further stabilized by link protein, but link protein appears not to be essential

for formation of the complex. Versican participates in several intermolecular interactions through its G3 domain, such as with fibrillins, fibulins, tenascin-R, fibronectin and integrin β1, as well as with cytokines, selectins, apolipoproteins and CD44 via the GAG-chains as previously reviewed (Wu et al., 2005). Four versican splice variants result from alternate splicing of exons 7 and 8, encoding the GAGα and GAGβ domains, respectively (Fig. 1). Versican V0 contains both GAG attachment exons and is the largest isoform, containing up to 23 CS chains. Versican V1 contains only exon 8 and has up to 15 CS chains, versican V2 contains only exon 7 and has up to 8 GAG attachment sites, whereas versican V3 does not contain either large exon and thus lacks CS chains (Dours-Zimmermann and Zimmermann, 1994; Zako et al., 1995). A V4 variant mRNA, predicting a truncated GAGβ domain from utilization of a cryptic splice site in exon 8, has 5 predicted CS attachment sites, and was recently identified in human breast cancer (Kischel et al., 2010). Versican isoforms V0 and V1 are expressed in the developing heart and limb, vascular smooth muscle cells, fibroblasts, and many non-neural cells, whereas versican V2 and V0 are the predominant isoforms in the nervous system (Schmalfeldt et al., 1998; Evanko et al., 1999; Lemire et al., 1999; Zimmermann and Dours-Zimmermann, 2008).

Wagner syndrome (OMIM: 143200) and erosive vitreoretinopathy are allelic autosomal dominant eye disorders caused by *Vcan* mutations. These mutations cluster around the consensus splice sites flanking exon 8, and are thought to lead to exon skipping. This removes the GAGβ domain, and reduces the proportion of V0 and V1 isoforms, in relation to V2 and V3 isoforms (Kloeckener-Gruissem et al., 2006; Mukhopadhyay et al., 2006; Brezin et al., 2011). Clinically, these disorders result in an optically empty vitreous cavity but appear to have no extra-ocular/systemic effects (Meredith et al., 2007).

3. ADAMTS proteolysis of versican

Most analyses of ECM proteolysis focus on matrix metalloproteinases (MMPs), which are thought to have central roles in ECM turnover. Although MMPs can degrade versican (Perides et al., 1995; Halpert et al., 1996; Barascuk et al., 2013), none of the observed developmental defects in MMP-deficient model systems have been attributed to defective versican proteolysis. In contrast, based on several interesting developmental outcomes of ADAMTS gene mutagenesis, much interest in recent years has focused on ADAMTS proteases. Like MMPs, ADAMTS proteases are zinc-dependent multi-domain proteinases and have an N-terminal domain organization that includes a propeptide and a zinc/calcium metalloprotease domain with a characteristic cysteine signature (Apte, 2009). A hallmark of ADAMTS proteases is their C-terminal ancillary domain, which has a characteristic domain structure containing one or more thrombospondin type 1 motifs. Of the 19 members of the ADAMTS family, 6 have been identified as proteoglycanases and are capable of cleaving versican; these include ADAMTS1, 4, 5, 9, 15, and 20 (Sandy et al., 2001; Somerville et al., 2003; Longpre et al., 2009; Dancevic et al., 2013). ADAMTS9 and ADAMTS20 are highly homologous, and ADAMTS1, -4, -5 and -15 form an evolutionarily related cluster. Investigations into the mechanism of aggrecan proteolysis by ADAMTS proteoglycanases predicted consensus cleavage sites in the versican GAGα and GAGβ domains, i.e., two sites in versican V0; Glu^{405}–Gln^{406} located in GAGα and Glu^{1428}–Ala^{1429} in GAGβ (all human sequence enumerations), and the corresponding single sites in the V1 and V2 isoforms,

Glu^{441}–Ala^{442} and Glu^{405}–Gln^{406} respectively (Sandy et al., 2001; Westling et al., 2004). These were experimentally validated both in vivo and in vitro using neoepitope antibodies to the predicted new C-terminal sequences resulting from cleavage, anti-NIVSFE^{405} (human V2 sequence enumeration) and anti-DPEAAE^{441} (human V1 sequence enumeration) (Sandy et al., 2001; Westling et al., 2004). These antibodies, particularly anti-DPEAAE^{441}, have been exceptionally useful in identifying versican processing during embryogenesis. Several other cleavage sites have been predicted using peptide fragments, such as Glu^{950}–Gly^{951} (in the V2 isoform), and Tyr^{423}–Ile^{424} (in the V1 isoform) (human sequence enumeration) but are not yet validated as being physiologically relevant (Jonsson-Rylander et al., 2005). The molecular interactions between versican and ADAMTS proteases are not fully understood, but ongoing studies suggest a major role for the CS chains of versican in binding to an ADAMTS protease (Foulcer et al., manuscript in preparation).

4. Major developmental contexts and functions for versican

4.1. Versican and cardiac development

The microenvironment for myocardial growth and trabeculation is provided by cardiac jelly, which is enriched in hyaluronan and versican, and located between the endocardium and developing myocardium (Stankunas et al., 2008; Gittenberger-de Groot et al., 2013). The cardiac developmental role of *Vcan* was first elucidated in the *Vcan*^{hdf} (*hdf*, *heart defect*)mutant, in which transgene insertion disrupted *Vcan*, resulting in loss of all versican splice variants, i.e., a null allele (Yamamura et al., 1997; Mjaatvedt et al., 1998). *Vcan* expression via a *LacZ* reporter in this transgene showed expression from E8.0 in the heart field region that generates the right ventricle and conus cordis. *Vcan*$^{hdf/hdf}$ embryos did not survive past 10.5 days of gestation (E10.5), were small, with defects of the anterior segment of the primordial heart, and the primitive right ventricle was abnormal and lacked an outflow track (Yamamura et al., 1997; Mjaatvedt et al., 1998). In addition to the myocardium, *Vcan* is expressed during formation of the endocardial cushions that are the precursors of the heart valve leaflets (Henderson and Copp, 1998). In *Vcan*$^{hdf/hdf}$ embryos, these cardiac jelly and mesenchyme-containing swellings between the endocardium and the myocardium do not form (Markwald et al., 1981; Mjaatvedt et al., 1998). Since explants of valve primordia of *Vcan*$^{hdf/hdf}$ embryos can form endothelium-transformed mesenchyme, a defect in matrix expansion of the cardiac cushions, which are enriched in versican, was implicated. Indeed, versican, HA and link protein are each expressed in the expanding cushions, suggesting they form a functional complex required for cushion expansion. Hyaluronan synthase 2 (*Has2*) null embryos have a similar cardiac phenotype as *Vcan*$^{hdf/hdf}$ embryos, suggesting that a hyaluronan–versican composite matrix is crucial during heart development in mice (Camenisch et al., 2000).

That this crucial composite ECM likely takes the form of a HA–versican complex was bolstered by a gene-targeted allele (*Vcan*$^{\Delta 3}$), from which a mutant versican with reduced HA binding was produced (Hatano et al., 2012). *Vcan*$^{\Delta 3/\Delta 3}$ mice in the congenic C57BL/6 background died around E10.5, like *Vcan*$^{hdf/hdf}$ embryos mice, with defective endocardial cushion development, whereas some *Vcan*$^{\Delta 3/\Delta 3}$ embryos survived longer in a mixed genetic background, and had a ventricular septal defect. This suggests that disruption of the HA–

versican interaction, albeit concomitant with reduction of *Vcan* expression, could lead to heart defects.

In zebrafish, versican (*Cspg2*) is expressed in the AV canal by both endocardial and myocardial cells of the valve primordia (Walsh and Stainier, 2001; Hurlstone et al., 2003). Treatment of zebrafish embryos with Amiodarone, an anti-arrhythmia drug, caused a massive ectopic upregulation of versican in the atrium, the ventricle and the AV canal and resulted in a loss of endocardial cushion formation in developing embryos that was restored by morpholino-mediated knockdown of versican (Chen et al., 2012). This suggested that tight regulation of versican expression was crucial for normal heart development. A similar outcome arose from miR-138 knockdown in zebrafish embryos (Morton et al., 2008). miR-138 is critical for the chamber-specific expression of versican, and restricts versican expression to the AV canal region via *Raldh2*-mediated retinoic acid signaling, while repressing the *Cspg2* transcript in the ventricles. The mouse versican 3′ UTR contains a miR-138 responsive binding site and *Vcan* transcript and protein levels are reduced when miR-138 is introduced in NIH 3T3 cells (Morton et al., 2008).

During in-vitro differentiation of human embryonic stem cells into cardiomyocytes, dynamic HA synthesis and versican expression were reported (Chan et al., 2010). Ventricular myocardial cells underwent terminal differentiation concurrently with versican downregulation during development, implicating versican in regulating myocardial cell proliferation and differentiation (Henderson and Copp, 1998). Collectively these studies demonstrate that versican is essential for normal heart development, regulating heart tube segmentation, endocardial cushion formation, ventricular septation, and myocardial differentiation. The literature thus clearly indicates that tight temporal and site-specific regulation of versican expression is required for normal heart development.

4.2. Versican in neural crest cell migration

Upon closure of the neural tube, streams of neural crest cells (NCCs) migrate from the ectoderm toward the peripheral target sites via specific migratory routes (Mayor and Theveneau, 2013). For example, melanoblasts take a dorso-lateral path to their eventual destination in the skin. It is believed that versican-rich areas are non-permissive tracts/barriers for NCC migration whereas fibronectin and laminin provide pro-migratory substrates for these cells (Landolt et al., 1995; Dutt et al., 2006). However, there is also evidence from chick embryos, using versican V0/V1 immobilized on transplantable micromembranes, that some migratory NCCs may be attracted toward versican-rich matrices (Perissinotto et al., 2000).

One of the three pathways of NCC migration is directly through developing sclerotomes. In chick embryos, versican V0/V1 isoforms are present at a higher density in the caudal halves of each sclerotome (Dutt et al., 2006), whereas NCC migrates through the rostral sclerotome (Serbedzija et al., 1989). A similar rostral sclerotome migratory path has been demonstrated during mouse embryogenesis (Serbedzija et al., 1990), suggesting that versican present in the caudal sclerotome may lead to preferred rostral sclerotome migration by NCCs. Direct evidence for versican as a barrier for NCC migration came from in-vitro stripe-choice assays, utilizing stripes coated with different substrates. Preferential migration of NCC

through stripes composed of fibronectin and laminin was observed in these assays and may reflect expression of pro-migratory adhesion molecules such as integrins, which are vital for NCC migration (Lallier and Bronner-Fraser, 1993; Kil et al., 1996). A recent study shows that peripheral axon guidance during chick hind limb development is also dependent on an inhibitory role by versican V0/V1 isoforms, similar to NCC migration (Dutt et al., 2011).

Additional evidence supporting versican as a negative regulator for NCC migration in mice came from the *Pax3 Splotch* mutant (*Pax3*^{Sp}). *Pax3*$^{Sp/Sp}$ embryos have upregulated *Vcan* mRNA and exhibit anomalies such as pigmentation defects, cardiac outflow tract septation defects, and absent dorsal root ganglia, all indicative of NCC compromise (Tremblay et al., 1995; Conway et al., 1997a,b; Henderson et al., 1997). *Pax3*$^{Sp/Sp}$ NCCs migrate normally when surrounded by wild type ECM (Mansouri et al., 2001), supporting the possibility that *Vcan* upregulation may in part be responsible for defective NCC migration observed in these embryos. *Pax3* and *Vcan* show mutually exclusive expression domains, but when *Pax3* is lost, the *Vcan* expression domain expands along the NCC migratory path, suggesting that *Pax3* directly or indirectly regulates *Vcan* expression (Henderson et al., 1997).

In *Xenopus laevis*, the versican V0 isoform is highly restricted to the dorsal organizer and the neural plate cells during development (Casini et al., 2008), potentially playing important developmental roles in these tissues during gastrulation and neurulation, respectively. *Vcan* expression is intimately associated with hair follicle development (Kishimoto et al., 1999) and may have a role in formation and maintenance of these and other integumentary structures, in addition to melanoblast colonization of hair follicles.

4.3. Versican in limb development

The appendicular skeleton is formed from cartilage anlagen that are preceded by mesenchymal condensations. Versican V0 and V1 are expressed in early limb bud mesenchyme, and upregulated in prechondrogenic mesenchymal condensations (Kamiya et al., 2006; Shepard et al., 2008). Chondrogenesis is severely compromised in micromass cultures established from *Vcan*$^{hdf/hdf}$ limb bud mesenchyme (Williams et al., 2005). Following chondrogenesis, versican persists in the perichondrium around the cartilage anlagen, which now strongly upregulate aggrecan expression, and in the prospective joint interzones (Shibata et al., 2001; Capehart, 2010; Nagchowdhuri et al., 2012). Mice with conditional *Vcan* deletion in limb mesenchyme developed distal limb anomalies, including tilted joints, clefts in proximal phalanges, delayed chondrocyte differentiation in distal skeletal elements and abnormal nests of hypertrophic chondrocytes (Choocheep et al., 2010). The joint interzones did not bind TGFβ in the absence of versican, suggesting that versican enabled proper localization of TGFβ in the limb mesenchyme.

5. Developmental significance of versican processing

The developmental significance of versican processing by ADAMTS proteases has been hitherto uncovered at the sites of the most dramatic sculpting of the provisional matrix, namely regression of interdigital webs, sculpting, redirection and migration of the secondary palate shelves prior to their midline fusion, resorption of cardiac jelly during myocardial compaction, and remodeling of endocardial cushions to form mature heart valve leaflets.

These findings complement the developmental contexts described above in which versican is functionally significant, and correlate with high expression levels of one or more ADAMTS proteases. These studies elucidate the developmental significance of ADAMTS proteases and illustrate how proteolysis of versican deposited in the early embryo could be a regulator of morphogenetic processes during subsequent development, upon expression of these proteases.

5.1. ADAMTS proteases are essential for versican processing in cardiac development

During myocardial growth and trabeculation, endocardial ADAMTS1 expression is repressed by a transcriptional complex, which includes *Brg1*, a chromatin-remodeling protein (Stankunas et al., 2008). Once myocardial growth is achieved, ADAMTS1 is de-repressed to stifle further growth and achieve compaction of the developing myocardium. In mice with *Brg1* inactivation, ADAMTS1 was upregulated and implicated in premature resorption of versican in the cardiac jelly, leading to an abnormally early termination of myocardial trabeculation (Stankunas et al., 2008). E13.5 *Adamts1*$^{-/-}$ embryos had greater trabeculation than wild-type littermates, further supporting a physiological role for ADAMTS1 in myocardial compaction. Subsequent work showed that in mice lacking fibulin-1, an ADAMTS cofactor, versican proteolysis was reduced, leading to increased trabecular cardiomyocyte proliferation (Lee et al., 2005; McCulloch et al., 2009b; Cooley et al., 2012).

Versican cleavage occurs sub-endocardially as the endocardial cells transform to form the cushion mesenchyme, and is co-localized with ADAMTS1 and fibulin-1 (Kern et al., 2006). Later during development, endocardial cushions are rapidly remodeled (sculpted) to achieve their mature structure, and cleaved versican is broadly distributed around cushion mesenchyme cells. Valve anomalies associated with accumulation of versican were seen in both *Adamts9*$^{+/-}$ mice and *Adamts5*$^{-/-}$ mice (Kern et al., 2007, 2010). In particular, *Adamts5*$^{-/-}$ mice have unsculpted pulmonic valves and adult *Adamts5*$^{-/-}$ mice have myxomatous mitral valves, suggestive of failed remodeling of the matured valve. Versican remodeling has also been observed as the myocardium regresses around the outflow tract and is replaced by smooth muscle cells (Kern et al., 2007). Altered TGFβ and BMP signaling have been noted in association with reduced versican processing, although the detailed mechanism underlying this effect is presently unclear (Kern et al., 2010; Dupuis et al., 2011, 2013).

5.2. Cooperation of ADAMTS9 and ADAMTS20 in closure of the secondary palate

The secondary palate separates the oral and nasal cavities and forms by the midline fusion of two palatal shelves that arise from the maxilla, migrate to the midline and fuse. *Adamts20* mutant mice (*Adamts20*$^{bt/bt}$, see below) have a low incidence of cleft palate, but *Adamts9* haploinsufficiency in *Adamts20*$^{bt/bt}$ mice resulted in fully penetrant cleft palate in mice owing to reduced shelf elevation, sculpting and growth (Enomoto et al., 2010). *Adamts20* was expressed in the palate mesenchyme, which is of NCC lineage, whereas *Adamts9* expression was seen exclusively in mesoderm-derived microvascular endothelial cells (Enomoto et al., 2010), implying coordination between these two different lineages. Reduced sculpting of the shelves and decreased growth were accompanied by accumulation

of ECM and reduced cell density, with decreased cell proliferation in palate mesenchyme of the *Adamts9*$^{+/-}$ *and Adamts20*$^{bt/bt}$ embryos (Enomoto et al., 2010). Furthermore, the palates of these embryos showed a clear reduction of processed versican as evident from reduced anti-DPEAAE staining. As in the syndactyly mutants described below, *Vcan* haploinsufficiency in the *Adamts20*$^{bt/bt}$, background also led to cleft palate, implying that versican was a necessary partner of ADAMTS proteases during palate closure, possibly by providing a bioactive fragment (Enomoto et al., 2010). However, local administration of the G1-DPEAAE^{441} N-terminal ADAMTS-generated versican fragment, named versikine, did not rescue the proliferation defect, suggesting that another mechanism or a different versican fragment may be operational in this context. Interestingly, the G3 domain of versican, which contains EGF-like repeats, was previously implicated in regulation of cell proliferation (Zhang et al., 1999).

5.3. ADAMTS proteolysis of versican in interdigital web regression

Interdigital webs are present in birds that swim, such as ducks and geese. In bats the forelimbs have webs that increase the surface area for flight. Webs do not develop as new structures in these species, but rather, represent the persistence of interdigital tissue that is formed by default during development of the distal limb (autopod). During embryogenesis, the autopod is the last limb segment to develop, and in most mammals, retains interdigital webs until the specification of the digits is completed. Once patterning is complete, the webs regress with massive apoptosis of the interdigit mesenchyme. The webs contain a provisional ECM whose fate and resorption mechanism was until recently, unknown and largely ignored. Although interdigital webs may seem trivial, the dexterity afforded by freed digits is crucial for survival in the wild, and web regression in terrestrial mammals is a key evolutionary development. Persistence of one or more interdigital webs, termed soft-tissue syndactyly, is a common birth defect in humans.

Immediately prior to apoptosis, ADAMTS1, ADAMTS5, ADAMTS9 and ADAMTS20 are upregulated in the webs (Thai and Iruela-Arispe, 2002; McCulloch et al., 2009a,b). Although single ADAMTS mutants, with the exception of *Adamts9* (Dubail et al., manuscript in revision) have a low incidence of soft-tissue syndactyly, combinatorial mutants of ADAMTS5, ADAMTS9 and ADAMTS20 showed fully penetrant soft tissue syndactyly (McCulloch et al., 2009b). Additional genetic evidence established that the ADAMTS co-factor fibulin-1 (Lee et al., 2005), and versican itself, were essential for web regression and that versikine was causally implicated in induction of apoptosis (McCulloch et al., 2009b). Specifically, beads soaked in versikine could induce apoptosis in ADAMTS-deficient interdigital tissues. Versikine activity was shown to be independent of and parallel to the established FGF–BMP pathway governing apoptosis during interdigital tissue regression, indicating that cell and ECM turnover are coordinated and interdependent during web regression.

5.4. ADAMTS proteolysis of versican in other developmental contexts

The *Adamts20* mutant *Belted* (*Adamts20*^{bt}), is a recessive allele with unpigmented regions (white spotting) in the lumbar torso of mutant mice (Rao et al., 2003). Melanoblasts, which are of NCC origin, migrate normally in this mutant, but fail to colonize the hair follicles,

where they eventually reside. Increased melanoblast apoptosis in *Adamts20*$^{Bt/Bt}$ embryos was associated with reduced versican processing (Silver et al., 2008). Interestingly, throughout melanoblast migration and colonization of hair follicles, *Adamts20* is not expressed by melanoblasts, but by adjacent dermal mesenchymal cells (Rao et al., 2003). This suggests that versican proteolysis provides environmental cues or survival signals for melanoblasts. Moreover, *Adamts20*$^{Bt/Bt}$ melanoblasts have reduced sensitivity to soluble Kit-ligand, a crucial signal for melanoblast survival, suggesting a link between versican processing and Kit-signaling that is presently unclear (Silver et al., 2008). *Adamts9* haploinsufficiency in *Adamts20*$^{Bt/Bt}$ mice increased the extent of depigmented hair follicles (Silver et al., 2008), identifying a crucial cooperative role for these highly homologous proteases in addition to web regression and palatogenesis. However, it is not known if the function of ADAMTS9 in melanoblast development is temporally and spatially identical to that of ADAMTS20.

Skeletal muscle arises fromthe fusion of myoblasts to form multinucleated myotubes, a process that is accompanied by versican processing (Stupka et al., 2013). *Adamts5* and *Adamts15* are expressed during myoblast fusion in developing embryonic muscle and differentiating C2C12 cells. *Adamts5* knockdown in vitro was shown to impair myoblast fusion, associated with expansion of a HA and versican-rich matrix, and could be rescued with catalytically active but not inactive ADAMTS5 or ADAMTS15. Instead, inactive ADAMTS5, ADAMTS15, or full-length V1 versican impaired myoblast fusion (Stupka et al., 2013). However, a myopathy has not yet been observed in ADAMTS deficient animals, suggesting that specific combinatorial mutants, such as of *Adamts5* and *Adamts15*, may be required to elucidate it.

6. Conclusions and future directions

In summary, versican is a crucial component of the provisional matrix that is required for tissue expansion and regulates morphogenetic cell movements in the embryo. Versican accumulation occurs primarily during the embryonic periods from 8 to 14 days of gestation in the mouse, and once organogenesis and morphogenesis are sufficiently advanced, versican content of tissues is drastically reduced by a combination of decreased gene expression (Naso et al., 1995) and increased proteolysis, much of it, apparently, mediated by ADAMTS proteases (this review). The literature reviewed here supports the concept that specific proteolytic mechanisms evolved to clear away versican once its embryonic functions were met. However, the specific mechanisms that evolved for embryonic versican proteolysis (i.e., ADAMTS proteases), may do more than simply clear the versican, i.e. they may provide a bioactive molecule, such as versikine (Fig. 2). Indeed, the process of versican breakdown is spatially and temporally highly regulated in developmental contexts, with versican deposition typically preceding upregulation of ADAMTS protease mRNA, which shows a high degree of temporal and spatial specificity in the various developmental contexts cited here.

The investigations to date raise several intriguing questions: Since versican is a widespread component of embryonic matrices, are there other organ sites where versicanolysis is crucial, such as the brain, or organs that arise from epithelial–mesenchyme interactions, such

as the lung and kidney? In light of several intriguing in vitro effects of versican and versican domains on cells (Yang et al., 1999; Wight, 2002; Yee et al., 2007), does the versican-rich provisional matrix promote specific cell behaviors, and are these abrogated or reversed by ADAMTS proteolysis? An unexplored and potentially important context for versican turnover is the fetal–maternal axis, including extra-embryonic tissues such as the umbilical cord, which contains a provisional matrix rich in HA and versican called Wharton's jelly. What proteolytic mechanisms remodel Wharton's jelly and enable umbilical cord growth? Since versican guides NCC migration, does versican proteolysis participate in regulating NCC dispersal to target sites, and in the brain, for establishing neural projections and networks? Are bioactive fragments other than versikine generated from versican? How does versikine regulate and interact with cells? Does it have a cellular receptor that transduces a signal, or does it act by disruption of an ECM network followed by cellular sensing of that disruption? What co-factors other than fibulin-1 regulate versican proteolysis? DoMMPs and ADAMTS proteases collaborate specifically in versican processing? Is versican proteolysis coordinated with turnover of other provisional matrix components such as fibronectin and HA, and does it influence their turnover? These intriguing questions deserve to be the focus of future investigations.

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Abbreviations

ADAMTS	A disintegrin-like and metalloprotease domain with thrombospondin type 1 motifs
ECM	extracellularmatrix
GAG	glycosaminoglycan
HA	hyaluronan
CS	chondroitin sulfate

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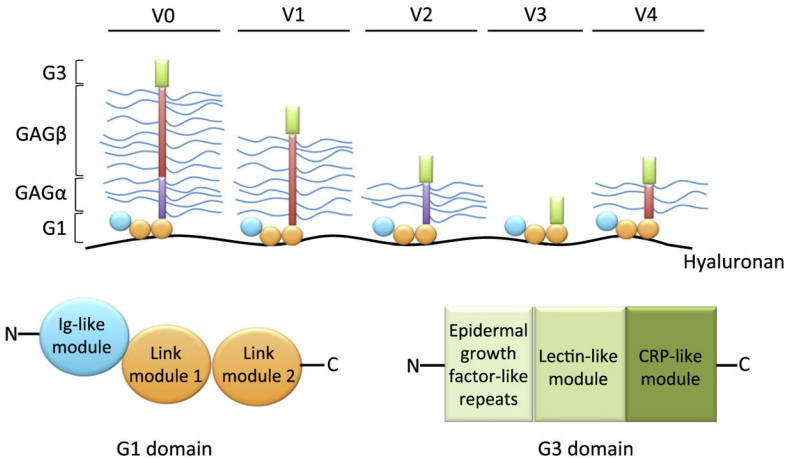

Fig. 1.

Domain structure of versican splice variants: Each versican variant shown binds to hyaluronan through the G1 domain, and has additional interactions through the G3 domain. As shown, inclusion of the alternatively spliced CS-bearing GAGα (violet) and GAGβ (red) regions defines distinct splice variants. CS-chains are shown as wavy blue lines. The cartoons at the bottom define the subdomains identified within the G1 and G3 domains.

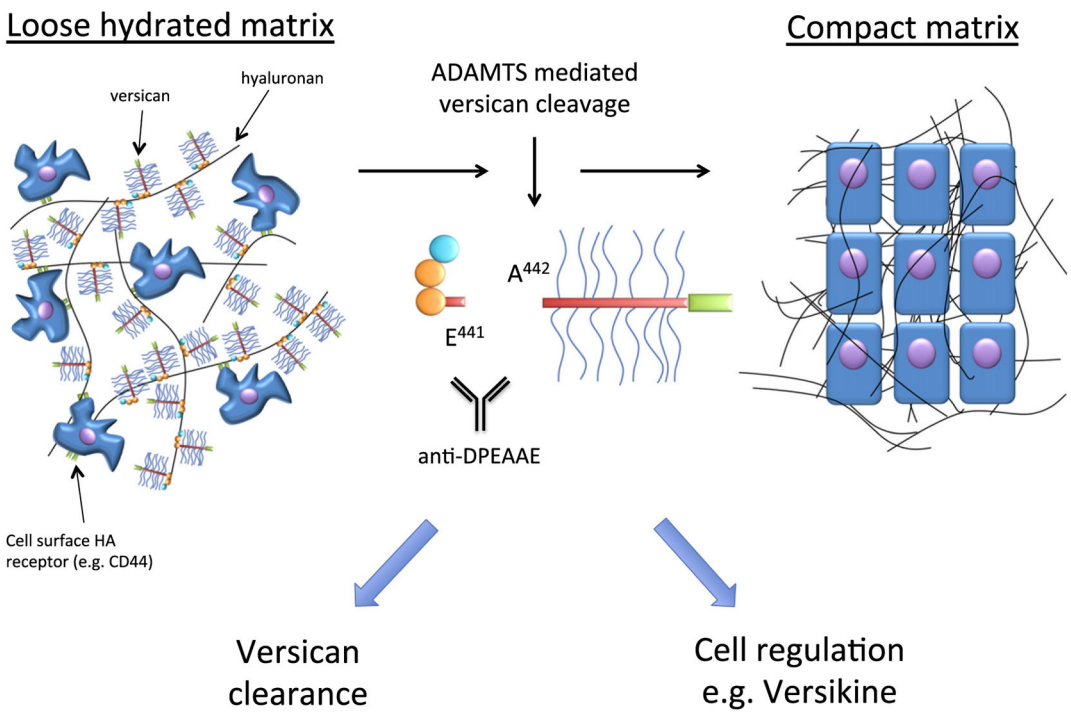

Fig. 2.

Dual function of versican and versican proteolysis in provisional ECM: During early development, the HA–versican composite forms a loose hydrated matrix (left) that is permissive form orphogenesis and amenable to rapid remodeling. Specific ADAMTS proteases cleave versican, leading to compaction of this matrix (right) concurrent with removal of other components of the provisional matrix, such as hyaluronan by other mechanisms, and elaboration of specialized matrix components (black fibers). Versican proteolysis by ADAMTS proteases occurs at a specific peptide bond (center). In addition to versican clearance and ECM remodeling occurring via the ADAMTS activity, versican fragments, such as versikine, may influence cell proliferation and apoptosis locally.