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Review

Lecticans: organizers of the brain extracellular matrix

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Abstract. Lecticans are a family of chondroitin sulfate proteoglycans, encompassing aggrecan, versican, neurocan and brevican. These proteoglycans are characterized by the presence of a hyaluronan-binding domain and a C-type lectin domain in their core proteins. Through these domains, lecticans interact with carbohydrate and protein ligands in the extracellular matrix and act as linkers of these extracellular matrix molecules. In adult brain, lecticans are thought to interact with hyaluronan and tenascin-R to form a ternary complex. We propose that the hyaluronan-lectican-tenascin-R complex constitutes the core assembly of the adult brain extracellular matrix, which is found mainly in pericellular spaces of neurons as 'perineuronal nets'.

Key words. Proteoglycan; chondroitin sulfate; extracellular matrix; nervous system; neurite outgrowth; axon regeneration.

Introduction

Proteoglycans are a group of proteins which have attached glycosaminoglycans, negatively charged polysaccharide chains composed of repeating disaccharide units [1]. Proteoglycans are major components of extracellular matrices (ECMs). Both chondroitin sulfate and heparan sulfate proteoglycans are present in various ECMs. Lecticans are a family of chondroitin sulfate proteoglycans (CSPGs) [2–4]. Four lecticans have been identified by molecular cloning, namely aggrecan, versican, neurocan and brevican. The prototype lectican is the well-known, large cartilage proteoglycan, aggrecan [5]. It was originally thought that this type of proteoglycan is expressed exclusively in cartilage. A second lectican, versican, was identified in fibroblasts [6], demonstrating that proteoglycans related to aggrecan are present in various connective tissues and are not limited to cartilage. More recently, two nervous system-specific lecticans, neurocan [7] and brevican [8], have been identified. Both aggrecan and versican were later shown to be expressed in the nervous tissues. These observations suggest that lecticans play important roles in the ECM of the nervous system. This review describes recent developments in lectican research, with an emphasis on their role in the nervous system.

Structure of the lectican core proteins

The hallmark of lecticans is the presence of globular domains at the N-terminal and C-terminal ends of their core proteins (fig. 1). These globular domains are connected by a structurally diverse central domain, which contains the attachment sites for chondroitin sulfate chains. The N-terminal globular domain, which is homologous to hyaluronan-binding proteins such as the cartilage link protein and CD44, binds hyaluronan. The C-terminal globular domain contains a C-type lectin domain flanked by EGF- and complement regulatory protein (CRP)-like domains. Based on these structural domains which potentially bind carbohydrate ligands, this family of proteoglycans has been named lecticans [3]. They are also called hyalectans (*hyalur*onan plus *lect*in) [4].

The N-terminal globular domain

The N-terminal globular domain (G1 domain) of lecticans consists of an immunoglobulin (Ig)-like loop and two link protein-like tandem repeats (these repeats are called proteoglycan tandem repeat (PTR) domains or, more broadly, 'link modules'). Aggrecan has an additional globular domain (G2 domain) that consists of only two link modules without the Ig-like loop. An ~ 130 residue nonglobular region (interglobular domain) connects the G1 and G2 domain. Versican, neurocan and brevican do not contain G2 domains.

The Ig-like loop is not highly conserved among the members of lecticans ($\sim 40\%$ identity at the amino acid level), whereas the PTR domains are more highly conserved ($\sim 60\%$). The PTR domain of lecticans is $\sim 50\%$ homologous with that of the cartilage link protein. The Ig-like loop contains two conserved cysteine residues, whereas each tandem repeat contains four conserved cysteine residues. The pattern of disulfide bonding has been determined for the G1 and G2 domains of aggrecan [9, 10], and a PTR domain is thought to form a double loop structure linked by these conserved cysteine residues.

The crystal structure of the link module has been determined with human TSG-6 (tumor necrosis factor-stimulated gene 6) [11]. TSG-6, which is upregulated in inflammation including arthritis, is a secreted 35-kDa protein containing a single link module. The link module of TSG-6 consists of two α-helices and two antiparallel β-sheets arranged around a large hydrophobic core. It is likely that the PTR domains of lecticans and the cartilage link protein have similar structures. Interestingly, the structure of the link module of TSG-6 is very similar to that of the C-type lectin domain [11]. This suggests that the N-terminal and C-terminal globular domains of lecticans have similar three-dimensional structures, and that the N-terminal globular domains of lecticans bind carbohydrates other than hyaluronan.

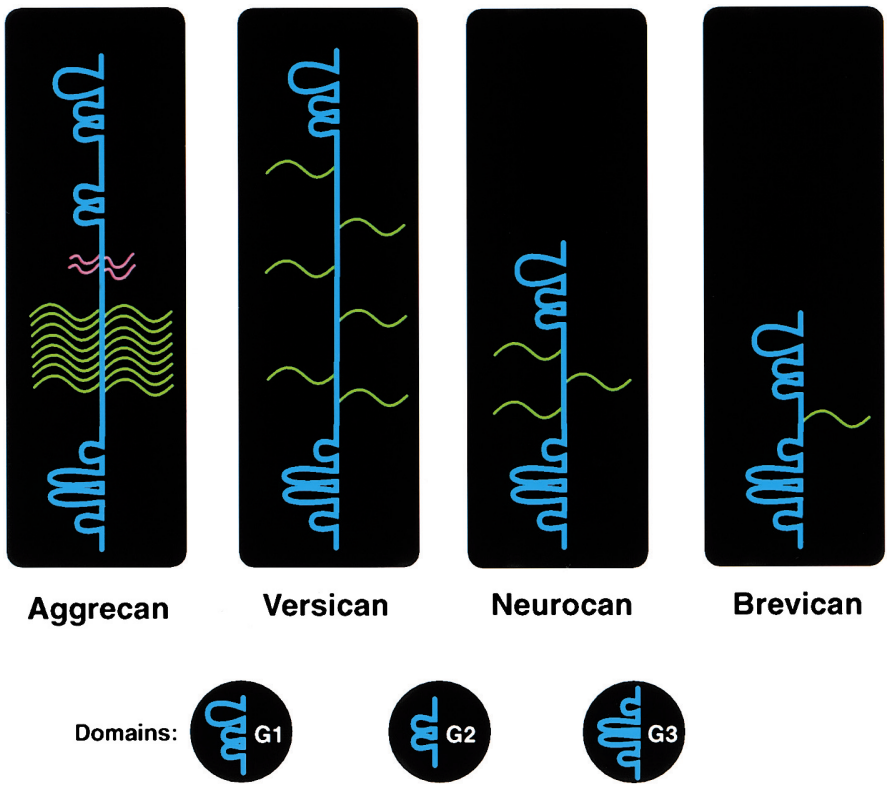

Figure 1. Domain structures of lecticans. All lecticans contain N-terminal G1 domains and C-terminal G3 domains. Only aggrecan contains the G2 domain. The G1 domain consists of an Ig-like loop and two link modules, whereas the G2 domain consists only of two link modules. The G3 domain consists of one or two EGF repeats, a C-type lectin domain and CRP-like domain. All lecticans contain chondroitin sulfate chains (*yellow*) in the central domain. Aggrecan also contains keratan sulfate chains (*pink*) in the N-terminal part of the central domain.

The central domain

The central domain of lecticans is highly diverse in terms of size and sequence. Versican has the longest central domain with about 1700 amino acid residues, whereas brevican has the shortest with about 300 residues. One versican splicing variant even lacks the entire central domain (see below). Unlike the N-terminal and C-terminal globular domains, the central domains of lecticans have no cysteine residues and are likely to have highly extended three-dimensional structures. Essentially all glycosaminoglycan attachment sites are present in the central domains. The numbers of potential glycosaminoglycan attachment sites in the central domains are $\sim$120, $\sim$20, $\sim$7 and $\sim$3, for aggrecan, versican, neurocan and brevican, respectively.

In the case of aggrecan and versican, subdomains have been identified within the central domain. Aggrecan contains a subdomain consisting of repeated hexapeptide sequences just downstream of its G2 domain [12]. This subdomain provides attachment sites for keratan sulfate chains [12–14]. Unlike human aggrecan, rat and mouse aggrecan does not contain this keratan sulfate-attachment domain. Adjacent to the keratan sulfate subdomain is the chondroitin sulfate-attachment domain. This subdomain of aggrecan contains $\sim$120 serine-glycine dipeptide sequences, which are attachment sites for chondroitin sulfate chains. The central domain of mouse versican consists of two alternatively spliced subdomains (CSα and CSβ) [15]. Both CSα and CSβ subdomains contain attachment sites for chondroitin sulfate chains. The central domains of neurocan and brevican are much shorter than those of aggrecan and versican. Unlike aggrecan and versican, no subdomains have been described in neurocan or brevican.

The C-terminal globular domain

The C-terminal globular domain (G3 domain) consists of one or two EGF repeats, a C-type lectin domain and a CRP-like domain. The combination of these three types of domains is also seen in the selectin family of adhesion molecules. The arrangement of the domains is, however, different between lecticans and selectins: in the case of selectins, the lectin domain is located at the N-terminus of the molecule, followed by the EGF repeats and CRP-like domains. Versican and neurocan have two EGF repeats, whereas brevican has one. Aggrecan has splice variants with one or two EGF repeats [16]. The EGF repeats are well conserved among lecticans ($\sim$60% identity). The C-type lectin domain is also well conserved among the members of the family ($\sim$60% identity). Homologies between these domains and other C-type lectins range from $\sim$20% for mannose-binding protein (MBP) and $\sim$25% for selectins. Compared with the EGF repeats and C-type lectin domains, the CRP-like domain is less well conserved among lecticans ($\sim$40% identity).

The C-type lectin domains of lecticans contain six cysteine residues, four of which are conserved in MBP and selectins. The pattern of disulfide bonding in this domain has been determined for aggrecan and shows that these four cysteine residues form disulfide bonds similar to other C-type lectins [9]. The crystal structure of several C-type lectin domains has been determined, including MBP [17] and E-selectin [18]. These studies show that the domain contains two α-helices connected by three antiparallel β-strands. Molecular modeling indicates that the C-type lectin domain of aggrecan has a three-dimensional structure very similar to that of MBP [19].

Carbohydrate moieties

Lecticans exist as either CSPGs or simple glycoproteins lacking glycosaminoglycan chains. Reflecting the numbers of potential glycosaminoglycan attachment sites, the numbers of chondroitin sulfate chains attached to lecticans differ greatly. Aggrecan isolated from cartilage carries approximately 100 chondroitin sulfate chains, indicating that most of the potential attachment sites are actually substituted by chondroitin sulfate chains. Neurocan from P (postnatal day) 7 rat brain contains three 22-kDa chondroitin sulfate chains, whereas neurocan from adult brain (with a truncated 150-kDa core protein) contains a single 32-kDa chondroitin sulfate chain [20]. A significant portion of brevican found in adult brain exists as simple glycoproteins lacking chondroitin sulfate chains [8, 21]. Aggrecan and neurocan carry keratan sulfate chains in addition to chondroitin sulfate chains [20, 22]. There has been no report describing the presence of heparan sulfate chains on lecticans. Some lecticans contain unusual carbohydrates other than glycosaminoglycans and common N- and O-linked carbohydrate chains. Neurocan contains carbohydrate chains carrying HNK-1-reactive 3′-sulfated terminal glucuronic acid determinants [23].

Splicing variants

A number of lectican isoforms derived from alternative splicing have been described. At least four isoforms exist for mouse versican (V0, V1, V2 and V3 isoforms), and alternative splicing of these isoforms occurs in the central domain [15, 24]. Interestingly, the V3 variant lacks the entire central domain: the G1 domain is immediately followed by the first EGF repeat of the G3 domain [24]. Thus, the V3 isoform is likely to be devoid of glycosaminoglycan chains.

Another interesting example of alternative splicing is a glycosylphosphatidylinositol (GPI)-anchored form of brevican [25]. In this isoform, the central domain is followed by a short signal sequence for the attachment of GPI anchors. This protein product is found in rat brain and is the only example of a membrane-bound lectican.

Proteolytic processing of lectican

All lecticans have been shown to undergo proteolytic cleavage of the core proteins. The proteolytically cleaved N-terminal 60-kDa fragment of versican is known as glial hyaluronate-binding protein (GHBP) [26]. Neurocan undergoes developmentally regulated proteolytic cleavage [20]. In embryonic and early postnatal brain, most of the neurocan core proteins are uncleaved 250-kDa form, whereas in adult brain a 150-kDa cleaved form predominates. The amino acid sequences at these cleavage sites in versican and neurocan are not similar to each other nor to the aggrecanase cleavage site described below.

In contrast to versican and neurocan, aggrecan and brevican undergo proteolytic cleavage at similar sites. Aggrecan has been shown to be cleaved in vitro by various metalloproteinases in the region between the G1 and G2 domains ('interglobular domain'). The metalloproteinases mediating this cleavage include matrix metalloproteinase (MMP)-1, -2, -3 and -8 [27]. Proteolytic fragments of aggrecan are found in the synovial fluid of osteoarthritis patients, and the degradation of the aggrecan core protein is thought to be involved in pathogenesis of osteoarthritis. The cleavage site in arthritis patients is, however, distinct from the cleavage sites by MMPs and other known proteinases. This sequence is distinct from those for the known proteinases including MMPs. Aggrecan in cartilage explant cultures is also cleaved at the same site. The putative proteinase responsible for this cleavage has been called aggrecanase [28–30] (fig. 2), and the identification of aggrecanase has been a subject of intense interest because of its clinical implications.

The proteolytic cleavage site in brevican shows intriguing similarity to the aggrecanase cleavage site [31] (fig. 2). The sequences surrounding the cleavage sites are similar: EGE/ARG for aggrecan and ESE/SRG for brevican. The distance from the nearest upstream cysteine residue, which represents the C-terminal end of the G1 domain, to the cleavage sites is also similar. Moreover, any substantial sequence homology between brevican and aggrecan in the regions other than globular domains is restricted to the vicinity of the cleavage site.

Aggrecanase has recently been identified as a novel ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family protein and designated ADAMTS-4/aggrecanase-1 [32]. Another novel ADAMTS molecule (ADAMTS-11/aggrecanase-2) also possesses aggrecanase activity [33]. These observations suggest the possibility that ADAMTS family molecules are also involved in the cleavage of brevican. The identification of the proteinase which cleaves brevican in the brain ('brevicanase') can have a significant impact on the study of glioma invasion, since it has been reported that expression of the N-terminal fragment of brevican renders noninvasive glioma cells highly invasive [34].

Molecular interactions of lecticans

The N-terminal globular domain

Interactions through the N-terminal globular domains have been studied extensively with regard to the interaction of aggrecan with hyaluronan and the cartilage link protein. In cartilage, aggrecan forms a ternary complex with hyaluronan and the link protein. The link protein, an $\sim$50-kDa glycoprotein consisting of an Ig-like loop and two link modules, plays a crucial role in stabilizing the complex by binding to both aggrecan and hyaluronan. The dissociation constant for the aggrecan-hyaluronan interaction is in the order of 10^{-7} M [35, 36], and that for the aggrecan-link protein interaction is 10^{-8} M [35].

The G1 domain of aggrecan contains the binding site for hyaluronan and link protein. These interactions require proper disulfide-linked folding of the domain, as reduction abolishes the binding activities. More recently, the hyaluronan-binding site within the aggrecan G1 domain was identified [36]. The G1 domain consists of three subdomains; A (the Ig-like loop), B (the first tandem repeat) and B′ (the second tandem repeat). It was shown that the binding to hyaluronan requires fragments containing both B and B′ subdomains, and that single subdomains do not have significant hyaluronan-binding activity [36]. On the other hand, there is evidence that the A subdomain is involved in binding to link protein [37, 38]. The presence of the A subdomain enhances the interaction of the B-B′ fragment with hyaluronan [36].

Other lecticans also bind hyaluronan. A 60-kDa N-terminal fragment of versican containing the G1 domain was known as a hyaluronan-binding protein (GHAP) until molecular cloning unambiguously identified GHAP as a fragment of versican [26]. Recombinant proteins containing the N-terminal versican fragment also binds hyaluronan [39]. Neurocan has also been shown to bind hyaluronan [20].

The role of the G2 domain, which is present only in aggrecan, is not known. Despite a high level of homology and conservation of crucial cysteine residues be-

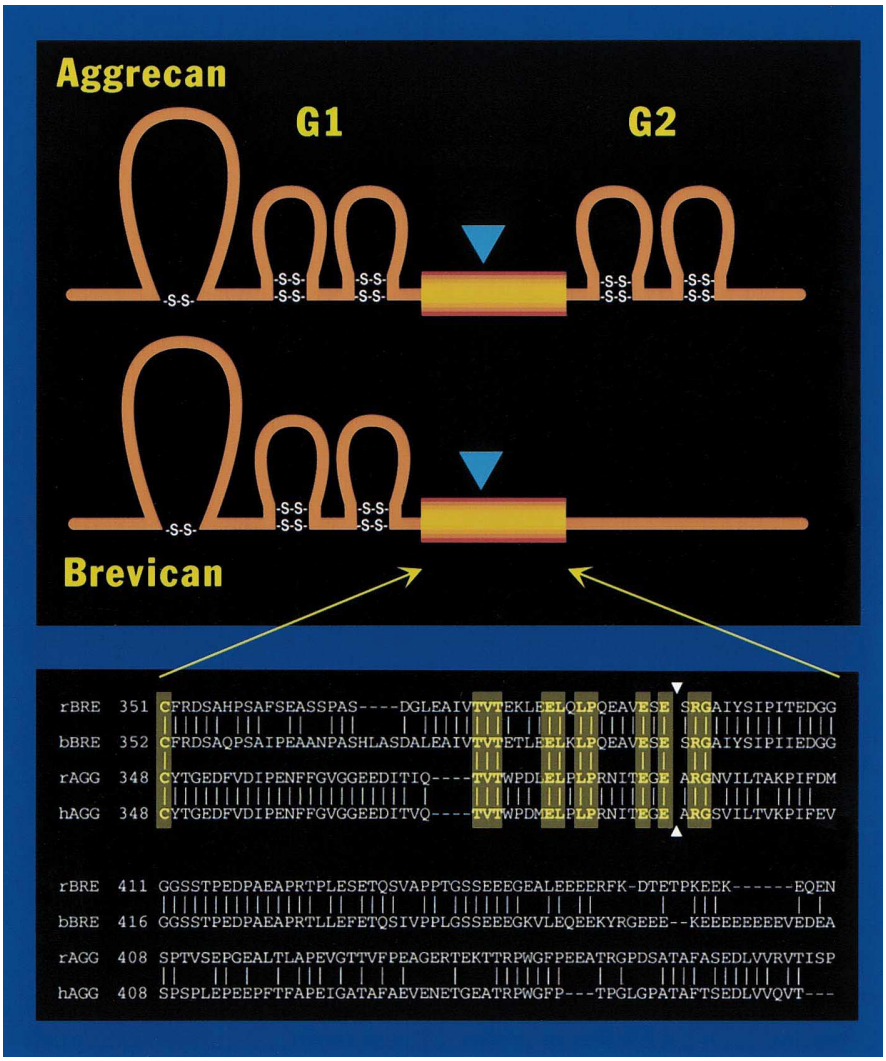

Figure 2. The aggrecanase cleavage sites in aggrecan and brevican. Upper panel: Cleavage of aggrecan by aggrecanase occurs at the EGE/ARG sequence 44 residues downstream of the G1 domain in the interglobular domain. Brevican is cleaved at the ESE/SRG sequence 44 residues downstream of the G1 domain. The triangles indicate cleavage sites. Lower panel: Comparison of amino acid sequences of aggrecan and brevican surrounding the aggrecanase cleavage site. rBRE, rat brevican; bBRE, bovine brevican; rAGG, rat aggrecan; hAGG, human aggrecan. Yellow boxes indicate residues conserved in all four sequences. Note that there are no homologies between brevican and aggrecan following the cleavage site.

tween the G1 and G2 domains, the G2 domain fragments from cartilage aggrecan or those generated as recombinant proteins do not bind hyaluronan [36].

The C-terminal globular domain (G3 domain)

Unlike the N-terminal globular domain, the physiological ligands for the C-terminal globular domains of lecticans have long been elusive. Although earlier studies showed that the C-type lectin domain of aggrecan and versican binds simple carbohydrates and glycosaminoglycans, including fucose, galactose, *N*-acetylglucosamine [40–42] and heparin/heparan sulfate [43], the physiological significance of these interactions is not understood.

A strong candidate for a physiological ligand for the C-terminal globular domain is tenascin-R. Tenascin-R is a large molecular weight ECM glycoprotein expressed predominantly in the nervous system [44, 45]. It was first demonstrated that recombinant lectin domain of versican binds tenascin-R in a calcium-dependent manner, as expected of a carbohydrate-protein interaction

mediated by a C-type lectin domain [42]. Recombinant lectin domains of other three lecticans also bind tenascin-R in a calcium-dependent manner [46]. It was initially thought that the lectin domains bind to the carbohydrate chains attached to the tenascin-R polypeptide, but this proved not to be the case. Deglycosylation studies and binding experiments with nonglycosylated recombinant proteins revealed that these interactions are mediated by protein-protein interactions between the C-type lectin domains of lecticans and tenascin-R polypeptides and do not require any carbohydrates on tenascin-R [46]. The binding site for the lectin domain is the fibronectin type III domains 3–5 of tenascin-R.

There are qualitative differences among the interactions of four lecticans with tenascin-R, and it is not clear whether all four lecticans are physiologically relevant ligands for tenascin-R. For example, the lectin domain of neurocan does not bind natural tenascin-R efficiently, though it binds to the recombinant fragment of FN3–5 of tenascin-R [46]. On the other hand, there is strong evidence that the brevican-tenascin-R interaction is physiologically relevant in the adult nervous system. First, among the four lecticans the brevican lectin domain has the highest affinity for tenascin-R: surface plasmon resonance analysis revealed that brevican lectin has at least a 10-fold higher affinity for tenascin-R than other lectican lectins [46]. Second, natural brevican coimmunoprecipitates with tenascin-R from adult rat brain extracts [46]. Finally, brevican colocalizes with tenascin-R in a number of nuclei and reticular formations in the adult central nervous system [47]. In these sites, both brevican and tenascin-R show pericellular staining around cell bodies and proximal dendrites of large neurons. This pericellular staining has been identified to represent 'perineuronal nets', specialized extracellular matrices found predominantly in adult brain (see below for detail). These observations strongly suggest that the brevican-tenascin-R interaction is physiologically significant in the adult brain, though this notion does not exclude the possibility that other lecticans also bind tenascin-R in vivo, since they have also been observed in perineuronal nets [47–49].

Taken together, these observations suggest that tenascin-R is a physiological ligand for lecticans in the nervous system. It is not known, however, whether the lectin domains of lecticans, which can bind carbohydrates in vitro [40–43], have physiological carbohydrate ligands. Since the lectin domain of versican binds heparin and heparan sulfate in vitro, it is suggested that heparan sulfate proteoglycans are physiological ligands for versican lectin domain [43]. There is, however, little evidence supporting this possibility at present. Other potential in vivo ligands are sulfated cell surface glycolipids. It has recently been shown that the lectin domains of all four lecticans bind sulfated cell surface glycolipids, namely sulfatides and HNK-1-reactive sulfoglucuronyl glycolipids (SGGLs) [50]. These interactions are divalent cation-dependent as expected of C-type lectin interactions. Sulfate residues (attached to galactose and glucuronic acid residues for sulfatides and SGGLs, respectively) are required for recognition by lectin domains. There are several pieces of evidence for the physiological significance of these interactions. Like lecticans, sulfatides and SGGLs are abundant in nervous tissues. Sulfatides are present mainly in various fiber tracts produced by oligodendrocytes ensheathing the axons [51, 52], whereas SGGLs are abundant in the embryonic cerebral cortex and adult cerebellum [53]. The interaction between the brevican lectin domain and cell surface sulfatides or SGGLs supports adhesion of cells expressing these glycolipids, suggesting that the glycolipids act as cell surface receptors for lecticans present in brain ECM [50].

Interactions involving the neurocan C-terminal globular domain have been extensively studied. It was initially shown that the 150-kDa C-terminal fragment of the neurocan core protein binds tenascin-C [54]. Rauch et al. [55] demonstrated that the G3 domain of neurocan (including the EGF repeat, the C-type lectin domain and the CRP-like domain) binds a tenascin-C fragment consisting of fibronectin type III domains 4 and 5. On the other hand, Milev et al. [56] reported that the neurocan core protein binds the fibrinogen-like domain of tenascin-C (the binding site on the neurocan core protein for this interaction has not been defined). These conflicting findings suggest that neurocan-tenascin-C interaction may involve multiple sites [55]. In addition to tenascin-C, neurocan has been shown to bind N-CAM [57, 58], Ng-CAM/L1 [58], Nr-CAM [59], contactin [59], tenascin-R [59], TAG-1/axonin [60], heparin-binding growth-associated molecule (HB-GAM) [59] and amphoterin [59]. Binding of HB-GAM and amphoterin requires chondroitin sulfate chains on neurocan [59].

Little is known about the physiological ligands for the G3 domains of lecticans in nonneural tissues. Recently, Aspberg et al. [61] reported that fibulin-1, an ECM glycoprotein expressed in various connective tissues, binds the lectin domain of aggrecan and versican. Interestingly, the lectin domains of brain-specific lecticans (neurocan and brevican) do not bind fibulin-1.

Tissue distribution of lecticans

Each lectican has a characteristic distribution pattern. Aggrecan is the most abundant proteoglycan in cartilage. Versican is the most ubiquitously expressed of the four lecticans [48, 62, 63]. Whereas aggrecan and versi-

can are expressed mainly in connective tissues, neurocan and brevican are largely restricted to neural tissues. In Northern blotting, messenger RNAs (mRNAs) of neurocan and brevican are detected only in nervous tissues [8, 25, 64, 65]. These observations do not preclude a low level of expression of these lecticans in nonneural tissues. Several ESTs (expression sequence tags) representing neurocan or brevican derived from nonneural tissues are found in the databases.

All four lecticans are expressed in the nervous system [48, 66, 67]. The four lecticans exhibit different temporal expression patterns during brain development [67]. In the rat brain, aggrecan and brevican exhibit a similar increasing expression pattern: their concentrations in the brain extracts increase steadily from E (embryonic day) 14 to P (postnatal day) 150, when they reaches plateau. In contrast, neurocan shows $\sim$5-fold increase during embryonic development, reaches a peak at P2-6, and then rapidly declines thereafter. Interestingly, splicing variants of versican show distinct expression patterns. Versican isoforms containing the CSα domain show an increasing expression pattern during the period of P10 to P100, whereas isoforms containing the CSβ domain show a pattern similar to that of neurocan. In terms of absolute quantity, brevican and the V2 form of versican are the most abundant CSPGs in the adult brain [68, 69].

In primary cultures, lecticans are expressed in both neuronal and glial cell types. Versican is expressed in cultured oligodendrocytes and Schwann cells [70, 71]. Brevican mRNA has been detected in primary cultures of cerebellar astrocytes but not in granule neurons [8]. Brevican is also expressed in oligodendrocytes [72] [T. Ogawa and Y. Yamaguchi, unpublished results]. Neurocan is produced in both neurons and astrocytes in vitro [73].

In brain tissues, brevican is detected in association with neuroglial sheaths of velate protoplasmic astrocytes in the cerebellar granular layer [69], in immature oligodendrocytes of the hippocampal fimbria [T. Ogawa and Y. Yamaguchi, unpublished results], and in perineuronal nets of large neurons [47]. Versican is also detected in perineuronal nets [47, 48]. In adult rat cerebellum, neurocan mRNA and immunoreactivity are detected in two types of cerebellar neurons, namely granule cells and Purkinje cells [74, 75].

Biological functions of lecticans

Aside from the obvious 'role' of aggrecan as the essential component of cartilage ECM, there is much circumstantial evidence that lecticans play important roles in development. Most of these functions are related to their role as modulators of cell adhesion, migration and neurite outgrowth.

Immunohistochemical studies in developing embryos reveal remarkable spatiotemporal patterns of chondroitin sulfates. The epitopes of chondroitin sulfates (and keratan sulfates in some cases) are localized in so-called barriers against cell migration and axon growth [76–78]. These barriers are present in several strategic locations in developing embryos and function as critical guidance cues for axon growth and neural crest cell migration. For example, in the developing chick somite, chondroitin sulfates are predominantly localized in regions through which neural crest cells do not migrate [76]. In chick and quail embryos, chondroitin 6-sulfate is located in axon barriers, including the posterior sclerotome, perinotochordal mesenchyme, the roof plate of the spinal cord and the early limb bud [77]. In developing retina, the regression of chondroitin sulfate expression correlates with the progress of differentiation and axon outgrowth of ganglion cells: ganglion cells extend axons in the direction of the optic fissure coincident with the disappearance of chondroitin sulfates [78, 79]. Chondroitinase treatment of cultured retinas causes disruption of this differentiation process, resulting in axons extending in all directions [78].

It has not been determined which CSPGs are responsible for such barrier effects. It is likely, however, that lecticans, the largest group of nervous system CSPGs, are involved in repulsive activity of these barriers. Each lectican acts as a repulsive substrate in in vitro assays. All four lecticans inhibit neurite outgrowth in a variety of neuronal cell populations [58, 63, 69, 79, 80]. In all of these cases, the inhibitory activities reside in the chondroitin sulfate moieties. It should be noted that chondroitin sulfate is not always inhibitory to neurite outgrowth. The chondroitin sulfate moiety of the proteoglycan known as DSD-1-PG promotes neurite outgrowth [81]. (DSD-1-PG has recently been identified as phosphacan [82].) There are, however, no reports that chondroitin sulfate moieties of lecticans promote neurite outgrowth.

While chondroitin sulfate chains have potent effects on cell adhesion and neurite outgrowth, the effects of CSPGs on these processes are not solely due to the chondroitin sulfate moieties. There is increasing evidence that core proteins of CSPGs have their own effects on neurite outgrowth. Phosphacan, which is a major nonlectican CSPG in the nervous system, promotes neurite outgrowth through the interaction of its carbonic anhydrase domain with contactin, an immunoglobulin superfamily adhesion molecule expressed by neurons [83, 84]. Aggrecan inhibits adhesion and migration of neural crest cells in vitro [85]. This activity resides in the hyaluronan-binding domain of aggrecan. As described above, the lectin domains of lecticans support adhesion of cells expressing surface sulfatides or HNK-2-reactive SGGLs [50]. Recently, we have

found that the substrate of the nonproteoglycan form of brevican promotes neurite outgrowth of primary cortical and hippocampal neurons [R. Miura and Y. Yamaguchi, unpublished results]. These observations suggest that lectican and cell surface-sulfated glycolipids comprise a novel cell recognition system. It is likely that, like brevican, the lectin domains of other lecticans promote neurite outgrowth since they bind to these glycolipids [50].

Thus lecticans can have both inhibitory and promoting effects on neurite outgrowth; the inhibitory effect in their chondroitin sulfate chains and the promoting effect on their core proteins. It is still unknown what the overall effect of such molecules on neurite outgrowth in vivo is. However, it should be noted that lecticans do not always carry chondroitin sulfate chains. For instance, brevican is a 'part-time' proteoglycan [8, 21]. The V3 isoform of versican lacks the entire central domain where glycosaminoglycan attachment sites are located [24]. These nonproteoglycan forms of lecticans could act as promoting substrates for growing neurites. Indeed, the nonproteoglycan form of brevican and HNK-1 carbohydrates colocalize in areas of prenatal hippocampus in which dendrites and axons are actively extending [R. Miura and Y. Yamaguchi, unpublished results]. These observations suggest the possibility that lecticans, particularly brevican and versican, act as bifunctional regulators of neurite outgrowth. Such a dual effect of a single CSPG suggests that the regulation of chondroitin sulfate chain synthesis could serve as a biological switch determining whether a CSPG is inhibitory or stimulatory to neurite outgrowth.

Lecticans as the organizers of the brain extracellular matrix—the HLT matrix model

With globular domains in the N- and C-termini, the structure of lecticans suggests that they act as molecular bridges linking two types of ligands. The identification of tenascin-R as a ligand of the lectin domains has made lecticans strong candidates as organizers of brain ECM.

The ECM of the adult brain is unusual in several respects. First, unlike other organs, the brain does not contain histologically well-defined stromal spaces. Before the 1970s, it was generally accepted that brain tissue is totally packed with neurons and glial cells, and therefore no appreciable amounts of 'classical' stroma or ECM could exist in the brain. This view was later amended by the discovery of significant amounts of extracellular space filled with ECM-like materials [86]. Second, most common ECM proteins, such as fibronectin and collagens, are not found in adult brain parenchyma [87–89]. In contrast, various types proteoglycans are abundantly expressed in the adult brain [2–4, 21, 90–92].

In adult brain, ECM materials are mainly present in intercellular spaces between neurons and glial cells. It is believed that the neuronal cell surface feature called perineuronal nets (PNNs) represent a form of the adult brain ECM [93–95] (fig. 3). PNNs were first described by Camillo Golgi and Santiago Ramon y Cajal in the 1890s as reticular networks observed on the surface of neuronal cell bodies and proximal dendrites. The ECM materials deposited in the space between neurons and astrocytic processes ensheathing the neurons are visualized as netlike features on the neuronal cell surface (fig. 4). Both neurons and their ensheathing glial cells contribute to the secretion and deposition of the materials forming PNN [94]. Immunohistochemical studies demonstrated that a unique set of ECM molecules are present in PNN. Such molecules include lecticans (versican [31, 47, 48], brevican [47], neurocan [96], Cat-301 antigen [97]), DSD-1-PG [98], hyaluronan [99], tenascin-C [100] and tenascin-R [47, 101]. It is intriguing to note that the two known ligands for lecticans, tenascin-R and hyaluronan, are components of PNN. This observation led us to hypothesize that hyaluronan, lecticans and tenascin-R form a ternary complex ("hyaluronan-lecticantenascin-R [HLT] complex") in PNN, with lectican linking the two ligands through the N-terminal hyaluronan-binding domain and the C-terminal lectin domain (figs 4 and 5A). Considering the multimeric nature of tenascin-R, we propose that the association of these molecules results in the formation of an organized lattice of hyaluronan in the intercellular spaces.

The HLT model predicts that the 'tightness' of the hyaluronan lattice can be altered by lecticans (fig. 5).

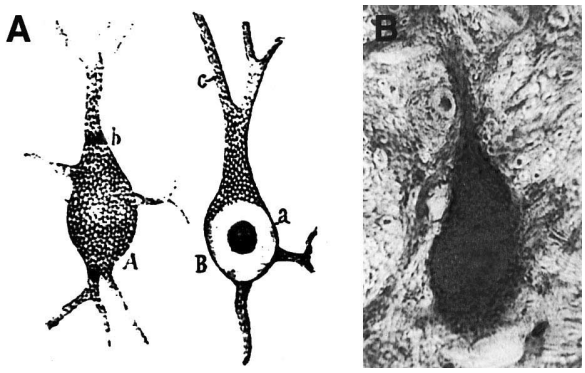

Figure 3. Perineuronal nets (PNN). (*A*) Drawing of PNN by Ramon y Cajal (1898). Modified from [94]. (*B*) Localization of brevican in PNN of a large neuron in the gigantocellular reticular nucleus in adult rat brain stem.

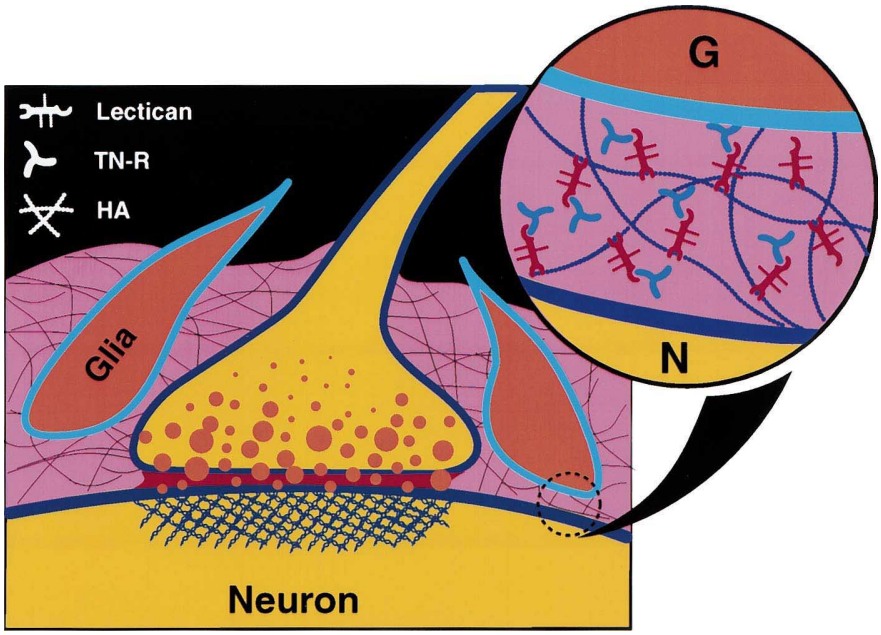

Figure 4. A schematic view of the PNN. PNN represents brain ECM deposited in the spaces between neuronal cell surface and glial sheath. We hypothesize that lecticans form complexes with hyaluronan and tenascin-R and in PNN.

For instance, a lectican with a higher affinity for tenascin-R may increase the level of cross-linking of the hyaluronan lattice (i.e. tightening of the matrix). A lectican with a shorter central domain could make the matrix tighter than one with a longer central domain and a more extended shape. On the other hand, disruption or weakening of the lectican-tenascin-R and/or lectican-hyaluronan interactions could decrease the level of cross-linking (i.e. 'loosening' of the matrix) (fig. 5B). Such disruption or weakening could be caused by the cleavage of the lectican core proteins by proteinases, expression of truncated forms of lecticans or expression of a lectican with a low affinity to their ligands. Loosening the matrix could also be caused by an increase of hyaluronan relative to lecticans in the matrix. The physical property of the HLT matrix may also be altered by the glycosaminoglycan moiety of the lectican involved in the HLT matrix. The number of attached chondroitin sulfate chains can differ greatly among lecticans, ranging from more than 100 for aggrecan to none for the nonproteoglycan form of brevican and the V3 isoform of versican. Therefore, by expressing brevican rather than aggrecan, the brain ECM would contain smaller amounts of chondroitin sulfate, while it maintains the same tripartite interaction.

This model sheds light on the function of PNN. Both lecticans and tenascin-R are repulsive molecules against neurite outgrowth. The hyaluronan matrix acts as a physical barrier preventing the direct contact of particulate materials such as cells (the exclusion of particles such as fixed red blood cells from pericellular spaces is used as an in vitro assay to detect cell surface hyaluronan [102]). Thus the HLT complex deposited on neuronal surfaces may be a highly repulsive barrier against approaching axons and dendrites. This idea is consistent with the observation that neuronal surfaces covered by PNN are devoid of synaptic contacts [103]. In fact, it has been speculated that a role of PNN is to present a nonpermissive substrate that blocks formation of new synaptic contacts [94]. Thus, the lectican-tenascin-R complex may be instrumental in inhibition of synapse formation on PNN-covered neuronal surfaces.

Our model also has implications for developmental regulation of neural plasticity. In the developing brain, a large number of synapses are initially formed. While the majority of these initial synapses are eliminated during the early postnatal period, some synapses are stabilized as mature synapses, which are functionally more efficient and less plastic. Such synaptic elimination and stabilization are believed to constitute a cellular basis for the so-called critical period of neural plasticity [104, 105]. Hockfield et al. postulated that this developmental modification of synapses is accompanied, or possibly caused, by the conversion of an embryonic

type ECM, which is fluid and occupies larger extracellular spaces, to an adult type ECM, which is less fluid and occupies smaller extracellular spaces [106]. Acidic polysaccharides, such as hyaluronan and chondroitin sulfate, have large hydrodynamic volumes and may play crucial roles in regulating the fluidity and the size of extracellular spaces. We speculate that lecticans function to mediate developmental changes from embryonic to adult-type ECM by regulating the level of cross-linking of the hyaluronan matrix and the amount of chondroitin sulfate introduced into brain ECM. In fact, Cat-301 antigen [49, 97, 106], the molecule implicated in Hockfield's hypothesis, is likely to be a lectican [107].

Another biological phenomenon in which adult brain ECM is likely to be involved is glioma invasion. Glioma cells are known for their highly invasive behavior in the brain, whereas they rarely metastasize extracranially [108, 109]. In contrast, various nonneural tumor cells, which metastasize to the brain and form metastatic brain tumors, are not usually as invasive in the brain as they are in the original lesion [110]. It is thought that the unique composition of the adult brain ECM is a factor determining differences in invasiveness of glioma and nonneural tumors [3]. There are data suggesting that the hyaluronan matrix is involved in glioma invasion: hyaluronan facilitates glioma invasion in vitro [111, 112], and the hyaluronan content of tumors correlates with their malignancy [113, 114]. These observations suggest that modifications of the HLT matrix are important in defining the invasive behavior of glioma cells. In fact, Zhang et al. [34] have reported strong evidence that the modification of the HLT matrix is indeed involved in glioma invasion: they found that the overexpression of the N-terminal fragment of brevican in glioma cells makes noninvasive glioma cells more invasive. Although this study did not provide a mechanism for this effect, we think the HLT model can be applied to these finding: the N-terminal brevican fragment expressed in transfected glioma cells presumably competes with the endogenous hyaluronan-lectican interaction in brain tissues and results in the loosening of the HLT matrix, a condition favorable for glioma cell invasion.

Although the HLT model provides bases to explain the organization and function of brain ECM, it should be emphasized that this is a simplified model and does not include other matrix molecules present in PNN. For instance, phosphacan (DSD-1-PG) has not been incorporated into the model. Also, I did not include the potential role of the lectican-cell surface glycolipid interactions in the organization of PNN, partly because there are simply not enough data to formulate a feasible working hypothesis. It is clear that the organization of the brain ECM will eventually be explained by a model including these molecules. In the meantime, the HLT model is useful to design experiments to test the validity of the hypothesis, thereby elucidating the molecular organization and biological functions of the brain ECM.

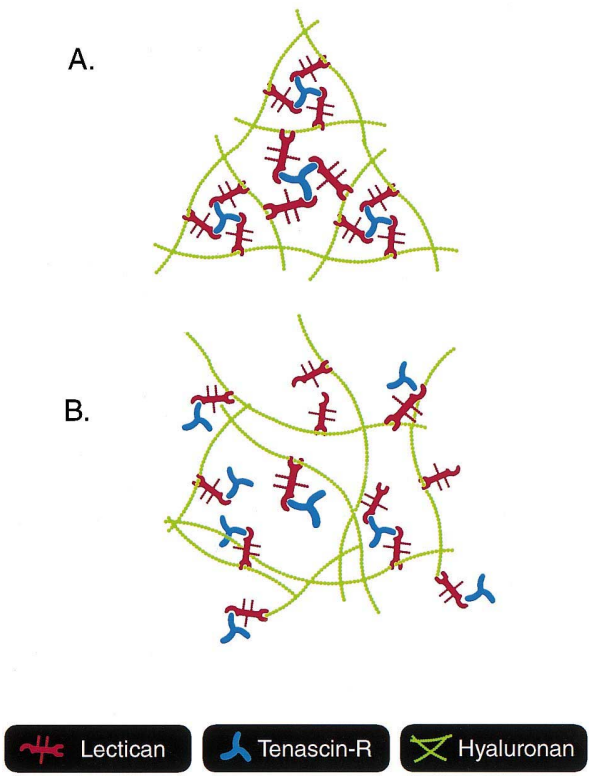

Figure 5. The HLT model of the brain ECM. We suggest that lecticans play a central role in assembling a highly ordered matrix of hyaluronan in the adult brain ECM. Lecticans bind hyaluronan and tenascin-R through their G1 and G3 domains, respectively, and form a ternary complex (*A*). To simplify the model, this figure depicts a situation in which all tenascin-R molecules are present as trimers. When the interactions of lecticans with these two ligands are disrupted or the amount of hyaluronan relative to lecticans and tenascin-R is increased, the matrix becomes 'loose'. (*B*) Such a loose matrix may provide a favorable environment for cell migration and axon growth during development and the infiltration of glioma cells.

Concluding remarks

In this article, I reviewed biochemical and biological properties of lecticans and introduced the HLT model of the brain ECMs. Identification of ligands for the N- and C-terminal globular domains of lecticans has made it clear that a major role of lecticans is to link different ECM molecules, thereby assembling highly organized hyaluronan-containing ECMs. The type and amount of lecticans incorporated into the ECM greatly impacts the physical and biological properties of the matrix. Modu-

lation of the brain ECM by lecticans would have significant effects on physiological brain functions. Finally, there are reports that abnormal PNN associates with human neurological diseases [115–117]. Elucidation of the physiological role of the HLT matrix may shed light on the involvement of the brain ECM in nonneoplastic neurological disorders.

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