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Review

Extracellular matrix components associated with remodeling processes in brain

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Abstract. In the central nervous system, various extracellular matrix components have been identified which are strongly expressed during development and in most areas of the brain down-regulated during maturation. Examples are tenascin-C, neurocan and hyaluronan. While tenascin-C is well known to be associated with morphogenic events and the active contribution of hyaluronan to various physiological processes is increasingly acknowledged, neurocan belongs to a class of molecules thought to be generally more associated with barrier functions: chondroitin sulfate proteoglycans. Consideration of these and related molecules and their processing in the context of the general organization of the brain extracellular matrix, their changes during brain maturation and their implication in different types of remodeling processes in adult brain, like normal and pathological synaptic plasticity, inflammatory and dementia-associated diseases and gliomas, may indicate that components of the extracellular matrix could provide valuable early information about the pathological state of the brain.

Key words. Extracellular matrix; hyaluronan; tenascin; neurocan; lectican; link protein; matrix metalloproteinase; cerebrospinal fluid.

Introduction

The structural plasticity of tissues is mainly evident during their development and maturation, and also during remodeling processes in adulthood. In most cases extensive remodeling of a mature tissue is a consequence of pathological and repair processes, although in certain organs, for example bone and in the mammary gland, tissue remodeling is part of their normal physiology. In the adult hippocampus, synaptic plasticity in association with memory formation can be considered a prerequisite for the normal function of this part of the brain. However, it can also be a consequence of neuronal death caused by metabolic perturbations. Various insults and diseases, some of them associated with inflammatory processes, have been shown or are likely to trigger remodeling of neural tissue.
Remodeling of adult tissues may reactivate molecular mechanisms utilized during their initial development. In such situations, one would expect the respective development-associated molecules to be up-regulated or reexpressed. In the central nervous system, various extracellular matrix components have been identified which are strongly expressed during development, and in most areas of the brain, down-regulated during maturation. Examples of such components are tenascin-C, an oligomeric glycoprotein [1], neurocan, a chondroitin sulfate proteoglycan [2] and also the polysaccharide hyaluronan [3].
Tenascin-C is known to be associated with a large variety of morphogenic events in different tissues, and its expression increases whenever tissue regeneration or remodeling occur, as in healing wounds and during oncogenesis [4]. The analysis of various human brain tumors indicates a negative correlation between tenascin-C expression and the survival time of patients, and several groups have started treating patients with multiforme glioblastomas using radi-

olabeled anti-tenascin-C antibodies [5]. An association of hyaluronan with tumors has also been observed [6]. Having for some time been mainly considered as a passive space-filling material rendering the tissue more permissive to cellular migrations and complex remodeling events, the active contribution of hyaluronan to a variety of physiological processes, like the development of an immune response, is increasingly acknowledged [7]. Neurocan can interact with both tenascin-C and hyaluronan, and unless it is specifically proteolytically processed by MMP-2, it can connect them. As a chondroitin sulfate proteoglycan, neurocan belongs to a class of molecules thought to be generally associated with barrier functions and thus, inhibitory for tissue plasticity [8–10]. Neurocan is, for example, one of the first molecules up-regulated after spinal cord injury [11], and treatment of lesion sites with chondroitinase ABC, an enzyme digesting the glycosaminoglycan side chains of chondroitin sulfate proteoglycans, enhances axonal growth and functional recovery after such injury [12].

This review will look at these and related molecules and their processing in the context of the general organization of the brain extracellular matrix and changes during brain maturation and in different types of remodeling processes in adult brain, such as normal and pathological synaptic plasticity, inflammatory and dementia-associated diseases, gliomas and angiogenesis. In addition, perineuronal nets, structures composed of extracellular matrix molecules and observed to be inhibitory for tissue plasticity, will be discussed. Work on the human brain and its most accessible component, cerebrospinal fluid, may well indicate that components of the extracellular matrix could give valuable early information about the pathological state of the brain.

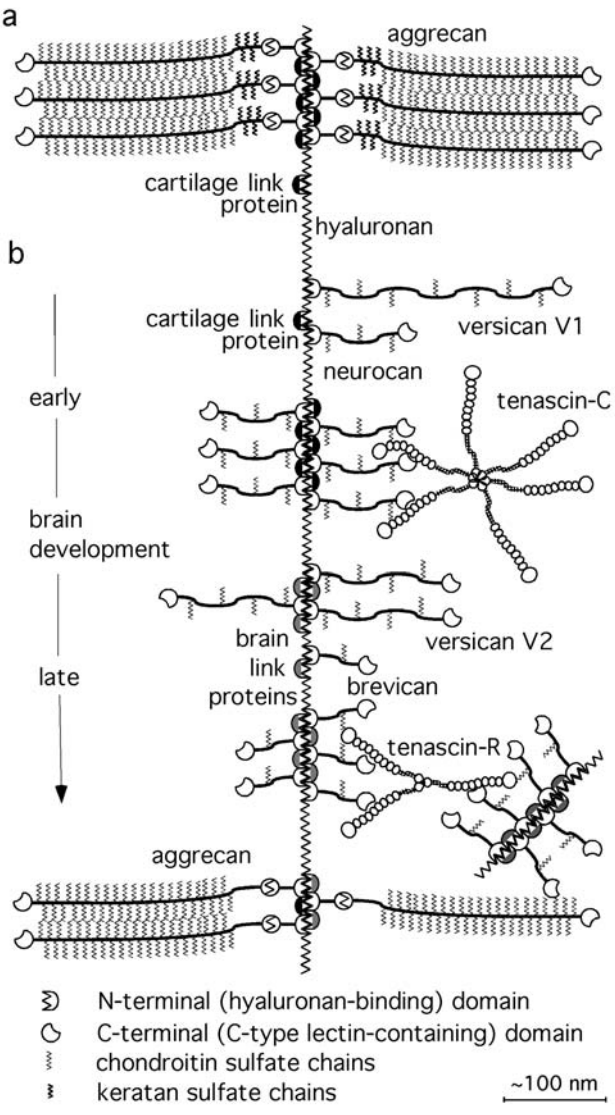

Figure 1. Schematic presentation of the molecules of the lectican family, and their interaction with hyaluronan and tenascins. Proteoglycans and tenascins are presented according to rotary shadowing electron micrographs. The proteoglycans are characterized by two (in aggrecan, three) domains with a globular shape connected by filamentous extended regions, which are highly glycosylated (mucin-like) and provide the attachment sites for the glycosaminoglycan chains. The depicted sizes of brevican and versican variants reflect the number of amino acids within their central region in comparison to neurocan, which extends over 60–90 nm [160]. Tenascins are characterized by their oligomeric structure [35, 161]. (*a*) Schematic presentation of cartilage-hyaluronan-aggrecan aggregates, consisting of aggrecan, hyaluronan and cartilage link protein. (*b*) Developmental changes in the expression of lecticans, tenascins and link proteins in brain. Characteristic molecules of the early developing brain are versican V1, neurocan, tenascin-C and cartilage link protein. Characteristic molecules of the mature brain are versican V2, brevican, aggrecan, tenascin-R and brain link proteins. Due to their polyvalency, tenascins are able to interconnect and cross-link lectican-hyaluronan aggregates. Note that in cartilage but not in brain, aggrecan is additionally modified with keratan sulfate chains [162].

The brain extracellular matrix

In the central nervous system, the extracellular space represents a developmentally decreasing compartment. Its volume fraction of the brain condenses from about 40% at early developmental stages to about 20% in adulthood [13]. This space is occupied by a quite unique extracellular matrix [14], whose structural organization is mainly based on hyaluronan, a linear polysaccharide. Hyaluronan can aggregate chondroitin sulfate proteoglycans to lampbrush-like superstructures [15] (fig. 1). The prototype of such aggregates was identified in cartilage, where it is composed of hyaluronan, the chondroitin sulfate proteoglycan aggrecan and link protein. Cartilage link protein is a small glycoprotein able to bind to both other components, hyaluronan and aggrecan [16]. Due to the negatively charged hyaluronan polymer and the dense substitution of aggrecan with strongly negatively charged chondroitin sulfate chains, these aggregates have a strong tendency to become hydrated and to ex-

pand in volume in aqueous solution. In cartilage, the expansion of the volume is restrained by a rigid network of fibrillar collagens. These counteracting biophysical forces are responsible for the special elastic properties of cartilage [17].

Although the relative volume occupied by the extracellular matrix in cartilage is much larger than in brain, the basic organization of brain extracellular matrix is likely to follow the same principles [18, 19]. However, in contrast to aggrecan, other hyaluronan-binding proteoglycans present in the extracellular matrix of the brain are less densely modified with chondroitin sulfate chains [20, 21]. This reduces their capacity to become hydrated, and consequently the tendency of the aggregates to expand. Considering, in addition, that any expansion of the brain tissue would be restrained by the skull, a counteracting network of collagen fibrils would not be necessary and, indeed, does not exist in brain.

The three hyaluronan-binding proteoglycans homologous to aggrecan, which could be involved in the generation of aggregates in the brain, are versican, neurocan and brevican [20] (fig. 1). Together, the four molecules represent the lectican family of chondroitin sulfate proteoglycans. Except for distinct splice variants, like the versican V3 form and a GPI-linked brevican variant, all four lecticans have a similar shape with terminal globular domains separated by an elongated, highly glycosylated central region. More recently, three additional link proteins have been identified, two of them specifically in the brain [22–24]. The modular composition of the four link proteins is identical, and identical to the hyaluronan-binding N-terminal globular domains of the four lecticans. They consist of two hyaluronan-binding link modules and one module of the immunoglobulin type.

The C-terminal globular ends of the lecticans have been shown to bind to glycolipids and to various glycoproteins [20, 25]. Several of these glycoproteins have an oligomeric molecular organization and, thus, a polyvalent character. Observations by rotary shadowing electron microscopy show that fibulin-2, a dimeric glycoprotein, can interact with the C-terminal end of versican, thereby cross-linking two versican molecules [26]. Oligomeric glycoproteins of the tenascin family (fig. 1) appear to be especially suitable for cross-linking hyaluronan aggregates in the central nervous system. Interactions of the C-terminal domains of lecticans with tenascin-R and tenascin-C have been analyzed and found to be highly promiscuous, but not completely redundant; tenascin-C, for example, can interact avidly with the C-terminal domain of neurocan, but barely with that of brevican, while tenascin-R interacts most strongly with the C-terminal domain of brevican [27–29]. Interactions of a recently identified additional member of this family, tenascin-N [30], with particular lecticans appear likely, but have not yet been reported.

During maturation of the central nervous system, characteristic changes in the composition of the molecules of the extracellular matrix have been observed [22, 31, 32]. Many molecules, however, appear to be replaced by members of the same protein family, permitting the maintenance of the overall structural organization. Typical molecules in the developing rodent brain are neurocan, the versican V1 splice variant, tenascin-C and cartilage link protein, while their homologues brevican, the somewhat shorter versican V2 splice variant, and tenascin-R are major components of the adult rodent brain (fig. 1) [33–35]. Also predominantly expressed in the mature nervous system are aggrecan, tenascin-N and brain link proteins [22, 24, 30, 31, 36]. Estimating the quantitative contribution of these latter components is difficult, because results of preparative purifications of these molecules from adult brain tissue have not yet been presented.

On the basis of their abundance, close temporal expression and avid physical interaction, brevican and tenascin-R can be considered as a representative pair of mature brain matrix molecules. Both molecules are only expressed in the central nervous system. Similarly, neurocan and tenascin-C can be considered as representatives of juvenile brain matrix, both proteins decreasing significantly in brain after the first postnatal week [37, 38]. During the same period, an increasing fraction of neurocan is proteolytically processed in a manner which separates the hyaluronan-binding from the tenascin-binding part of the molecule. The extent to which this cleavage compromises the overall biological activity of neurocan is uncertain, since the generated fragments are, for example, still able to interact with neural cell adhesion molecules and inhibit homophilic interactions between them [2]. Neurocan and tenascin-C have also been observed outside the central nervous system. While tenascin-C has been found to be associated with a large variety of remodeling events in different tissues [4], neurocan expression outside the nervous system appears to be rather restricted, to activated T lymphocytes, lymph nodes and the developing avian heart [23, 39, 40]. However, activated T lymphocytes can enter almost any part of the body. Thus, in principle, neurocan could participate in inflammation-associated remodeling processes in any tissue infiltrated by these cells.

Evidence for an association of typical juvenile matrix components, especially neurocan and tenascin-C, with remodeling processes in adult brain

Normal brain-function-related structural plasticity

Synaptic plasticity reflects activity-dependent changes in the efficacy of synaptic transmission. An activity-dependent enhancement of the synaptic transmission, termed long-term potentiation (LTP), which can be experimen-

tally assessed in hippocampal slice cultures, is commonly considered to function as a cellular mechanism for learning and memory [41]. The persistent lack of direct proof may reflect the difficulty to extrapolate from massive experimentally induced activation to the sparse activity found in vivo which may, in addition, create radically different spatial patterns of postsynaptic activation [42]. LTP is differentiated, based on the temporal characteristics, into a transient enhancement or early phase-LTP (E-LTP), lasting less than 1–2 h and a longer-lasting late phase, L-LTP, lasting more than 1–2 h. L-LTP, but not E-LTP, is associated with synthesis of new proteins and recruitment of new sites of synaptic transmission [43]. Thus, L-LTP represents a synaptic remodeling process. The involvement of extracellular matrix molecules in this normal brain-function-related remodeling event has been recently comprehensively reviewed [44].

An analysis of hippocampal slices of mice deficient in the chondroitin sulfate proteoglycan neurocan showed a normal initial E-LTP, while L-LTP was impaired [45]. Interestingly, pretreatment of hippocampal slices from wild-type mice with chondroitinase ABC that completely removed chondroitin sulfate from the extracellular matrix compromised E-LTP, mediating a twofold reduction in potentiation [46]. Thus, in the context of synaptic plasticity, the absence or reduction of chondroitin sulfate appears to be inhibitory. Chondroitin sulfate chains present in inter- or perisynaptic spaces might sequester basic proteins like pleiothrophin, which has been shown to bind to the chondroitin sulfate chains of neurocan and to interfere with hippocampal LTP [47]. In tenascin-C knockout mice also, where only measurements of E-LTP were reported, these were found to be reduced [48]. In addition, induction of LTP in vivo was found to be associated with an increase in hippocampal tenascin-C expression [49]. Apart from the hippocampus, tenascin-C has been reported to be present in another region exhibiting structural plasticity in adult brain, the neurohypophysis [50]. Here, remodeling of the glial coverage of neuronal elements occurs in a secretory-activity-dependent fashion.

These observations in rodent brains confirm a role of tenascin-C, but also indicate an involvement of neurocan in normal brain-function-related structural plasticity. Consistently, in human brain, moderate to strong tenascin-C immunopositivity was observed in the CA-1 to CA-2 sectors and perforant path of the temporal lobe, whereas only low to moderate immunopositivity was evident in white matter, extending weakly into the first layer of the gray matter. No immunopositivity was noticed in other areas [5].

Pathological synaptic and structural plasticity

Hippocampal remodeling processes are, apart from their important role in normal brain function, observed in response to pathological and repair processes such as pathological loss of hippocampal pyramidal neurons. Such a loss of neurons can be induced by toxic or pharmacological insults, or may be a consequence of a pathologically increased excitability of those neurons, which is observed in cases of epilepsy.

Long-term ethanol intake (20% ethanol in drinking water) in rats led after 30 weeks to a notable loss of hippocampal pyramidal cells, for example, a decrease of 19% and 18% in the CA1 and CA3 subdivisions, respectively. Surprisingly, despite this neuronal loss, the total number of synapses between mossy fibers and the CA3 pyramids was unaffected. This observation suggests that in a synaptic-remodeling process, new signal-transmitting contacts were formed between the surviving neurons [51]. Synaptic reorganization with functional connectivity in the hippocampal CA1 field was observed electrophysiologically in isolated CA1 areas after neuronal loss due to kainate-treatment-induced epilepsy [52]. Kainate-induced epileptic seizures have been shown to increase the deposition of neurocan in the hippocampal CA1 field [53]. Newly deposited neurocan was found to be present in its complete, proteolytically unprocessed form. Kainate treatment also increased the hippocampal deposition of tenascin-C [54]. After a different kind of metabolic insult, focal cerebral ischemia, tenascin was found to be among the early up-regulated genes in the periinfarctional area [55] (table 1).

Table 1. Alterations in the expression/deposition of juvenile matrix molecules and matrix metalloproteinases related to brain pathology.

Injury/ disease	Matrix molecule	Matrix metalloproteinase
Epileptic seizures	tenascin-C up [54] neurocan up [53]	MMP-9 up [85] ADAMTS-1/4 up [86]
MS, acute (EAE) center edge	tenascin-C down [65] neurocan down [64] neurocan up [64]	MMP-9 up [98]
MS, chronic	tenascin-C up [65] neurocan down [64]	MMP-2 up [98]
Alzheimer's disease	(activated astrocytes)*	ADAMTS-4 up [90]
Glioma	hyaluronan up [101] tenascin-C up [77] (TGF-beta up)‡	MMP-2 up [93]
Ischemic injury, stroke	tenascin-C mRNA up [55]	MMP-9 up [97]
Denervation-induced axonal sprouting	tenascin-C up [57] neurocan up [56]	
Physical injury	tenascin-C up [11] neurocan up [11]	

* Activated astrocytes have been shown to express neurocan [59] and tenascin-C [57].

‡ TGF-beta has been shown to induce neurocan [59] and tenascin-C [76] expression.

Expression of neurocan and tenascin-C by reactive astrocytes was observed in association with axonal sprouting in the molecular layer of the dentate gyrus. This remodeling process in adult brain was induced by denervation of the respective layer via a lesion of the entorhinal cortex [56, 57]. Increased expression of both neurocan and tenascin-C could also be observed after direct mechanical insults, like cortical stab wounds [53, 58–60], transection of the postcommissural fornix [61; C. Stichel and U. Rauch, unpublished observation] and spinal cord injury [11]. Unfortunately, these observations, mostly derived from independent investigations, do not distinguish whether the induction of these two molecules occurred in a coordinated or merely parallel fashion.

Inflammation and dementia-associated diseases

Multiple sclerosis (MS) is a progressive inflammatory disease driven by a proinflammatory T cell response against myelin components [62]. In the initial stage of the disease, the inflammation occurs episodically and eventually leaves residual neurological damage by remodeling myelinated axonal tracts into glial scar tissue, which is inhospitable to axonal regrowth and regeneration [63]. Thus, two consecutive changes occur in the tissue: first, an active inflammatory lesion, followed by a chronic inactive lesion. Experimentally, T cell infiltration into the central nervous system leading to demyelination can be induced by immunization with a myelin-derived protein or peptide. Such animal models of MS, termed experimental autoimmune encephalomyelitis (EAE), are used to study the onset and initial events of the disease.

In MS-affected human brains, prominent neurocan deposition was observed at the edge of active lesions, while less neurocan than in adjacent unaffected white matter was apparent in the center of active lesions and in chronic inactive lesions [64]. In contrast, there was a striking loss of tenascin-C in acute lesions up to the edge and even extending 1–2 mm further into adjacent macroscopically normal-appearing white matter, while in chronic lesions, tenascin-C staining was at levels equal to or greater than those seen in adjacent white matter [65]. Thus, during MS-associated remodeling processes, the deposition of neurocan and tenascin-C is affected, but not in parallel as in many other instances. Neurocan deposition was increased in regions where active remodeling of the matrix was ongoing, while tenascin-C became a more static structural component of the resulting resident gliotic scar tissue.

No indication of a significant alteration in neurocan or tenascin-C expression was evident in a study in which cDNA libraries, either derived from human MS plaques or from control areas, were compared by a large-scale sequencing approach [66]. However, the deposition of extracellular matrix molecules does not necessarily correlate with the abundance of their mRNA. In brevican knockout mice, for example, an increased deposition of neurocan without noticeable differences in neurocan mRNA levels was observed [67]. For other extracellular matrix molecules, the large-scale sequencing approach revealed only a significant up-regulation of osteopontin and amyloid beta precursor-like protein 1 (APLP-1), a transmembrane molecule with an extensive extracellular domain. Elevation of osteopontin, a phosphorylated acidic glycoprotein of about 40 kDa, is also seen in EAE. Osteopontin has been implicated in the regulation of inflammation, tissue remodeling and cell survival in various other conditions [68]. After focal stroke, for example, osteopontin mRNA induction and protein deposition was observed in the matrix of ischemic brain [69]. Although osteopontin is not likely to represent a major structural extracellular matrix component, it could influence the structure and organization of the hyaluronan-linked extracellular matrix via an integrin-aided activation of CD44, a cellular hyaluronan receptor [70]. Interestingly, for not fully clarified reasons, antibodies to CD44 are able to prevent the development of EAE [71].

Alzheimer's disease, the prototype of dementia, is also associated with an inflammatory cytopathology thought to represent an innate immune response to the accumulation of extracellular deposits of amyloid. The amyloid plaques consist mainly of a 40- to 42-amino-acid peptide, A-beta, derived from a transmembrane protein, amyloid beta precursor protein (APP). Interestingly, APP is a homologue of APLP-1, which is up-regulated in MS. Typical for an innate immune response, the A-beta plaque areas show little or no lymphocytes or monocytes, but contain activated microglia cells and astrocytes [62]. The presence of juvenile brain ECM molecules in such plaque areas has not been established. However, their involvement could be assumed, since in other instances (see above) reactive astrocytes have been shown to express neurocan and tenascin-C [56, 57, 59].

The extracellular portion of the APP molecule itself might be considered as a juvenile matrix constituent. It can be shed from the cell surface and is highly expressed during early developmental stages [72]. APP shows increased expression levels during reactive processes. Attenuation of gliosis, which could be achieved by an inhibition of costimulatory interactions of the immunoregulatory molecule CD40, led to a marked decrease in beta-amyloid pathology [73]. This decrease was characterized by a reduced amyloidogenic processing of APP and increased circulating levels of beta-amyloid peptides. These observations could indicate that the ability of A-beta peptides to aggregate, a crucial event in Alzheimer pathogenesis, might depend on the composition of the local extracellular matrix, which, in turn, is determined by the secretion products of the associated gliotic cells.

Gliomas and angiogenesis

Malignant glioma cells and sprouting endothelial cells attracted by tumor-derived factors are among the limited types of cells which can migrate within adult brain tissue. Although the mechanisms glioma cells have acquired to mediate their migration might differ from case to case, an important factor in their strategies is assumed to be the remodeling of the extracellular matrix [74].

Malignant gliomas are often associated with an increased expression of transforming growth factor-beta (TGF-beta), considered as a mechanism to escape immune surveillance [75], but also shown to enhance expression of neurocan and tenascin-C [59, 76]. Glioma-associated perivascular and cyst wall depositions of tenascin-C appear to correlate with glioma malignancy [5, 77–79], and are likely to interfere with the maintenance of the blood-brain barrier [80]. No association of neurocan with brain tumors or tumor-induced angiogenesis has yet been reported. However, perivascular neurocan deposits have been found in association with aberrant vessels in dystrophic rat retinas [81], and vessels associated with active MS lesions in humans [64].

Other extracellular matrix components involved in remodeling processes

Proteases

Tenascin-C has been implicated in the induction and activation of matrix metalloproteinases (MMPs) [4], which, like other types of extracellular proteases, can participate in matrix remodeling processes [82].

On the involvement of proteases in LTP, attention has focused on two serine-type proteolytic systems, the plasminogen activator/plasmin system and neuropsin, and their suggested substrates, laminin and fibronectin, respectively [83]. While the activity of both systems appears to support the manifestation of L-LTP, a loss of the plasminogen activator/plasmin system was observed to protect against excitotoxin-induced epileptic seizures, while a loss of neuropsin renders mice more susceptible to excitotoxic insults. The neuroprotective effect of plasminogen activator or plasminogen deficiency was attributed to the maintenance of interstitial depositions of laminin, which has recently been specified as laminin-10 [84].

The observation of an excitotoxin-protective effect in the absence of just the plasminogen activator/plasmin system is surprising, since metalloproteinases were found to be up-regulated by excitotoxic insults, in particular MMP-9, ADAMTS1 and ADAMTS4 [85, 86]. ADAMTS1 and ADAMTS4 are proteases involved in the proteolytic processing of aggrecan, brevican and the versican V2 variant, prominent constituents of the adult brain extracellular matrix [87, 88]. These proteases recognize a defined cleavage site in their central region, creating essentially an N-terminal hyaluronan-binding glycoprotein fragment and a C-terminal proteoglycan fragment (fig. 2). Interestingly, a similar motif is also present and recognized by these proteases in the versican V1 variant [89] (fig. 2). The observation that ADAMTS-4 is transcriptionally induced in beta-amyloid-treated rat astrocytes indicates that the proteolytic degradation of aggrecan, brevican and versican might also be involved in matrix-remodeling processes in Alzheimer's disease [90].

Production and proteolytic processing of brevican by ADAMTS-4 have been associated with increased infiltrative activity of gliomas [87]. Interestingly, rather than the secretion of the entire molecule, the secretion of just the hyaluronan-binding region of brevican increases the infiltrative capacity of glioma cells [91]. Other proteinases implicated in glioma progression and related cross-activation events are MMP-2, membrane type 1 matrix metalloproteinase (MT1-MMP) and the plasminogen activator/plasmin system [92, 93]. Neurocan, whose proteolytic processing increases during rat development [37], is a designated substrate of MMP-2, whose activity in adult rat brain is moderate, but constitutive [94]. MMP-2 has been identified as the neurocan-processing enzyme by a comparison of the amino acid sequence surrounding the processing site in rat brain with an MMP cleavage site database (fig. 2) [95, 96]. Consistently, in Western blots of human glioma tissue, neurocan fragments could be identified with an antiserum raised against the neoepitope which is generated by this processing event [E. Englund and U. Rauch, unpublished observation]. However, since neurocan is constitutively processed in rat brain, and no healthy human control tissue was analyzed, the pathobiological significance of this observation is not clear. In the kainate-treated rat hippocampus, neurocan was up-regulated and found to be

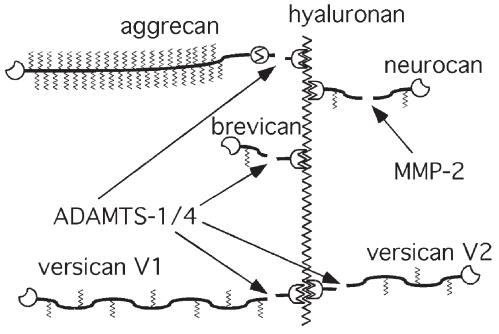

Figure 2. Proteolytic cleavage of lecticans by metalloproteinases. Aggrecan, brevican and the versican V1 and versican V2 splice variant are cleaved by ADAMTS-1 or ADAMTS-4. This cleavage generates hyaluronan-binding fragments not modified with glycosaminoglycan chains. Neurocan is cleaved by MMP-2 in a more central position and both generated fragments are modified with glycosaminoglycan chains. Note that the sequences of the entire extended regions of the versican V1 and V2 variants are different and, therefore, also the sites recognized by the protease.

deposited in its complete, proteolytically unprocessed form [53]. This impairment of an immediate proteolytic processing of newly deposited neurocan might be related to the lack of concomitant up-regulation of MMP-2 by this treatment, in marked contrast to the kainite-induced upregulation of hippocampal MMP-9 [85]. Similarly, in the hippocampus, a strong immediate up-regulation of MMP-9 was observed after transient global cerebral ischemia, while a significant up-regulation of MMP-2 was noticed after a delay of 3 days [97].

MMP-9 is further prominently up-regulated in acute MS lesions, whereas MMP-2 is more likely to participate in the remodeling of the extracellular matrix in chronic disease states [98]. Therefore, one can speculate whether the prominent deposition of neurocan but lack of tenascin-C at the edges of active MS lesions might be related to differences in their susceptibility to MMP-9 cleavage. MMP-9 is also up-regulated in EAE and other inflammatory neurological diseases [92]. Other metalloproteinases found to be up-regulated in inflamed human central nervous system tissue include ADAM-10 and ADAM-17 [99]. During EAE, in addition to the increase in metalloproteinases, a coordinated induction of the plasminogen activator system, with upregulation of tissue and urokinase-type plasminogen activator (tPA and uPA), PA inhibitor and uPA receptor was observed [100].

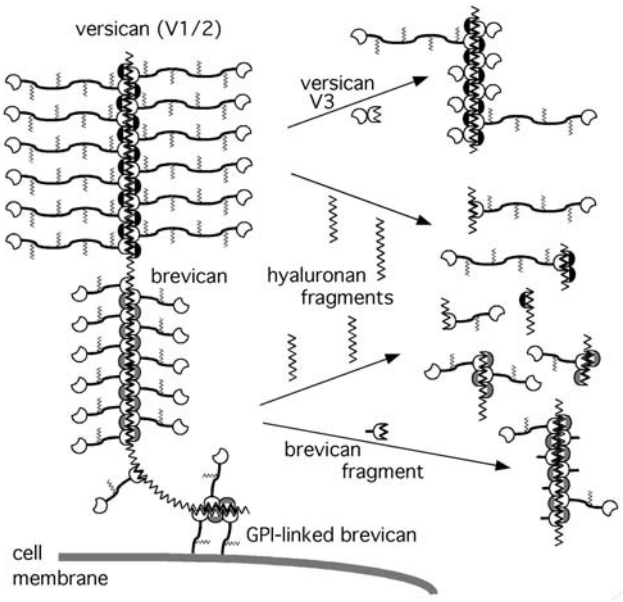

Figure 3. Potential effects of hyaluronan fragments, the versican V3 splice variant or N-terminal fragments of lecticans on hyaluronan aggregates. The versican V3 variant has no extended central region and is not modified with chondroitin sulfate chains. This molecule could compete with versican proteoglycans for link protein supported hyaluronan docking sites. As a consequence, the volume taken up by hyaluronan proteoglycan aggregates could be considerably reduced. Proteoglycans (versican V1 and V2 variants), which are not able to participate in aggregate formation, either by being competed out by versican V3 or by binding to small hyaluronan fragments, are prone to endocytosis and degradation. While the versican V3 variant could be involved in the modulation of aggregates in a variety of tissues, in brain, the hyaluronan-binding domain of brevican could compete with full-size brevican molecules for docking sites in hyaluronan aggregates. Moreover, in brain, hyaluronan-binding domains could also interfere with interactions of the GPI-linked brevican variant and hyaluronan, thereby weakening or breaking the binding of hyaluronan to the cell surface.

Hyaluronan, hyaluronidase and hyaluronan-binding molecules

In normal adult rodent brain, 85% of the hyaluronan is insoluble in water, while only 10% of the hyaluronan in brains of 1-week-old rats is not water extractable [32]. This indicates that in adult brain hyaluronan is mostly present as a central filament of aggregates, associated with hyaluronan-binding proteins and proteoglycans (fig. 1). Increased hyaluronan deposition has been implicated in glioma progression [101, 102], although an increased production of hyaluronan alone does not seem to be sufficient, unless it is associated with concomitant hyaluronidase expression [103]. Increased expression of hyaluronan is likely to change the ratio between the number of hyaluronan-binding proteoglycans and link proteins on the one hand, and the number of potential docking places for these proteins along the hyaluronan molecules on the other. This would render hyaluronan less substituted with those proteins and a better target for hyaluronidase digestion. Voluminous hyaluronan proteoglycan aggregates could be efficiently converted to smaller aggregates or just single proteoglycans with a small hyaluronan fragment bound to them (fig. 3). This could make extracellular matrix constituents more easily accessible to the endocytotic degradation pathway.

An effect similar to a combination of increased hyaluronan and hyaluronidase secretion could be expected from an application of short hyaluronan fragments. Such fragments have been shown to enhance angiogenesis [104]. A crucial prerequisite for angiogenesis is the migration of endothelial cells. Migrating endothelial cells have recently been reported to express the versican V3 variant, a versican glycoprotein variant lacking the glycosaminoglycan-substituted domain of the molecule [105] (fig. 3). These molecules might compete with proteoglycans for hyaluronan docking sites, converting voluminous proteoglycan aggregates into thin protein filaments (fig. 3). Experimental evidence that such a conversion is indeed occurring has been obtained with smooth muscle cells overexpressing the versican V3 variant [106]. These cells display increased adhesiveness in conjunction with a decreased distance between the membrane of the cell and the culture dish. Increased adhesiveness was also observed for HEK 293 cells expressing only the N-terminal, hyaluronan-binding domain of neurocan [107]. By analogy, the same effect could be caused by the hyaluronan-binding N-ter-

minal brevican fragment. The secretion of this molecule has been shown to enhance the infiltrative capacity of gliomas much more efficiently than the secretion of the complete brevican proteoglycan [91] (fig. 3). In the mature brain, such competition could be particularly effective, because brevican can also be expressed as a GPI-linked variant and bind hyaluronan to the surface of cells directly [108] (fig. 3). An increased turnover of brevican has been suggested as the basis for glioma progression in a tissue disruption model [74]. Interestingly, these views are contradicted by observations made in an artificial environment, matrigel, a mixture of basement membrane components. In this environment, the overexpression of the hyaluronan-binding domain of brevican inhibits glioma cell invasion [109]. As noted in this study, malignant forms of glioblastoma rarely metastasize to distant sites. Such a process would require the transition through basement membranes and other different environments. Thus, properties required for glioma cells to migrate might be most efficient in brain tissue.

The studies presented above indicate that the expression of hyaluronan-binding molecules with the ability to compete with full-length proteoglycans for docking sites on hyaluronan molecules is likely to change the biophysical properties of the matrix in general and enhance the migratory ability of cells especially in brain. However, the effect of the individual hyaluronan-binding domains of the lecticans could still have a tissue-specific component, since the four link proteins with distinct tissue expression patterns which have been identified [23] might have different preferences in supporting the hyaluronan-binding ability of the four lectican proteoglycans. Cartilage link protein, the best-characterized of the four link proteins, does evidently support the hyaluronan-binding ability of aggrecan [15, 16, 110]. Consistently, aggrecan is strongly decreased in cartilage of cartilage link protein knockout mice [111]. Cartilage link protein also supports the hyaluronan binding ability of neurocan [112]. Brain link protein 1 colocalizes with versican at the nodes of Ranvier in white-matter tracts [113]. Moreover, in the brains of brevican knockout mice, a decrease in brain link protein 2 was observed [24], despite an increased deposition of neurocan which was also noticed in the brains of brevican knockout mice [67]. This points to a specific interdependence of brain link protein 2 and brevican. Thus, interference of the N-terminal brevican fragment with the formation of hyaluronan-proteoglycan aggregates might be most effective in a tissue where brain link protein 2 is also expressed. However, the specificity of support of each link protein for the binding of the individual lecticans to hyaluronan appears to be more complex than a simple one-to-one relationship and remains to be further elucidated.

The above points are not likely to explain adequately the entire influence of hyaluronan on cell motility. Cellular

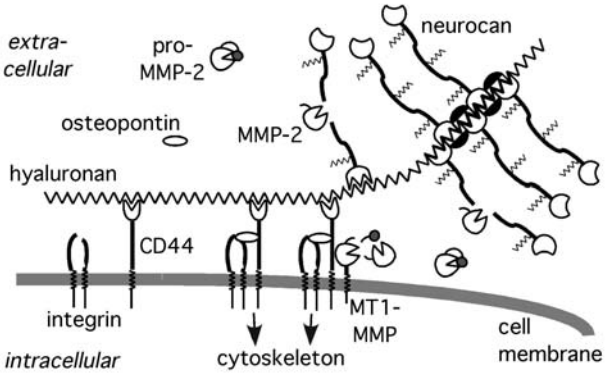

Figure 4. Possible scenario of hyaluronan-associated interactions involved in remodeling processes. Osteopontin binds to alphaV beta3 integrin and to CD44, thereby increasing the concentration of hyaluronan-binding CD44 molecules on the cell surface. Both integrin and CD44 can interact with the cytoskeleton and, thereby, be directed to particular regions of the cell, like the migratory front. MT1-MMP can bind to CD44 and, thereby, be localized to the same region. CD44 can bind hyaluronan and eventually position MT1-MMP in the vicinity of extracellular neurocan-hyaluronan aggregates. There, MT1-MMP activates pro-MMP-2 into the proteolytic active form of MMP-2.

receptors, like CD44, LYVE-1, RHAMM and Toll-like receptor 4 have the potential to sense hyaluronan or derived oligosaccharides and, in addition, to interact with the cytoskeleton or activate cells via signaling cascades [114–116]. Those interactions can be further modulated by third components, for example osteopontin [70]. Interestingly, in cultured cells, CD44 can bind to MT1-MMP, a membrane protease with the potential to activate MMP-2, and to mediate the transport of MT1-MMP to the migration front [117]. Taken together, these observations could imply a scenario where osteopontin-activated CD44 directs MMP-2 activating MT1-MMP close to hyaluronan-bound neurocan molecules, thereby eventually triggering their proteolytic processing (fig. 4). How the interaction of hyaluronan with soluble binding proteins and proteoglycans influences the interaction with cellular receptors and consecutive cellular signaling cascades, extracellular proteolytic activity and the general behavior of cells in a three-dimensional environment is a challenging question.

Brain structures inhibitory for remodeling processes

Hyaluronan and all lecticans, or their respective N-terminal part, have been identified as components of perineuronal nets [36, 118–122]. Tenascin-R also appears to be an important structural component, since these structures are less developed in tenascin-R knockout mice [121, 123]. In mature brain perineuronal nets, which are complexes of extracellular matrix molecules interposed between a meshwork of glial processes, surround neuronal

cell bodies and proximal dendrites in a net-like structure that interdigitates with synaptic contacts [124]. These structures have been implicated in the stabilization of synapses and maintenance of cellular relationships in the adult brain, while impeding novel formation of synaptic contacts [124]. Some protective role of perineuronal-net-associated proteoglycans has been suggested, since a negative correlation with the susceptibility of neurons to Alzheimer's disease, indicated by the presence of neurofibrillary tangles, has been noticed [10, 125]. The cortical distribution of neurofibrillary tangles in Alzheimer's diseased brains matches the pattern of neurons that retain their capacity for plastic remodeling [126]. Thus, perineuronal nets appear to be inhibitory for plastic remodeling. The probability of this conclusion has recently been directly demonstrated by a reactivation of ocular dominance plasticity in the adult visual cortex of rats by treatment of inhibitory perineuronal nets with chondroitinase ABC [127, 128]. During physiological remodeling processes, mechanisms like those discussed in the previous sections, proteolysis and 'hyaluronolysis,' could impair the structural integrity and interfere with the plasticity-inhibitory activity of perineuronal nets. In Alzheimer's disease, aggrecan, brevican and versican might be prone to proteolytic processing by ADAMTS-4 (fig. 2), which is transcriptionally induced in beta-amyloid-treated rat astrocytes [90]. A destruction of perineuronal nets or down-regulation of their elementary components might, in addition, occur in parallel with or precede the up-regulation of more juvenile matrix components. Whether the assembly of perineuronal nets in general represents mainly an active process coordinated by local cells or, rather, the lack or down-regulation of mechanisms which are inhibitory for their deposition, appears to be an interesting question for further investigations.

An inverse relationship to the distribution of neurofibrillary tangles in Alzheimer's diseased cortex has also been observed for areas of myelogenesis [129], indicating that white-matter tracts are also inhibitory for plastic remodeling. Interestingly, the content of hyaluronan in white-matter tracts was found to be higher than in gray matter [118]. Typical hyaluronan locations in white matter are the nodes of Ranvier, where versican and brain link protein 1 have also been observed [113, 118]. Proteoglycan-hyaluronan aggregates may prevent axons from sprouting collaterals at these locations. Brevican is also present in white-matter tracts [130], while neurocan staining disappears from unmyelinated cerebellar fiber tracts after myelination [37]. In marked contrast to its association with perineuronal nets, no association with white-matter tracts has been reported for aggrecan [36]. Differences in the composition of plasticity-inhibitory hyaluronan aggregates between gray and white matter, as well as spatial and biochemical extracellular matrix heterogeneities within the gray matter, are likely to reflect differences in tissue architecture and to contribute to the functional specialization of those brain regions. Distinct remodeling strategies might be required for their modification or penetration by migrating cells.

Matrix components in human brain and cerebrospinal fluid

Unfortunately, at least for neuroscientists, the composition of the extracellular matrix in normal human brain remains poorly characterized. Histochemical analysis of extracellular matrix molecules has mainly focused on glycosaminoglycan structures, like those of hyaluronan and chondroitin sulfate [118, 125, 131–133]. The superior histochemical availability of these structures might be related to a longer resistance to degradation in post-mortem tissue. The protein component of proteoglycans endocytosed by human fibroblasts, for example, is degraded 10–15 times more rapidly than the glycosaminoglycan moiety [134]. Assuming that the results of a recent study with antibodies against protein epitopes do not merely reflect spatial differences in degradation, the temporal lobe seems to have the highest tenascin-C levels in the mature human brain [5], possibly reflecting a more juvenile matrix composition, which is more permissive for remodeling events than other parts. In patients with mesial temporal lobe epilepsy, markedly increased chondroitin sulfate levels and increased hyaluronan levels have been observed in the dissected tissue [135, 136]. Changes in the composition of the extracellular matrix might precede or even be causally involved in cellular degeneration and death. Therefore, extracellular matrix molecules could represent important early markers of disease.

The least invasive way to obtain significant, although limited information about the status of extracellular matrix in human brain is the analysis of cerebrospinal fluid (CSF). In principle, extracellular matrix molecules should be able to reach the CSF by passive diffusion through the extracellular space, unless they are sequestered by immobile ligands or trapped by dense molecular networks, like basement membranes. Interestingly, in the periventricular zone, basement membranes appear truncated, terminating in expansions, named fractones [137]. This may help even large extracellular matrix components, like reelin, tenascin-C and proteoglycans to gain access to the ventricle. The levels of reelin, or rather major fragments of reelin, in CSF were found to be altered in patients with frontotemporal dementia and Alzheimer's disease [138], although the observation of reelin in CSF appears surprising, since in mammals, no reelin-positive cells were detected in the vicinity of the telencephalic ventricular zone [139]. Less surprising is the observation of proteolytic fragments of reelin, since several proteases,

such as MMP-2, MMP-9, [98, 140–142] and ADAM-17 [99] have been identified in CSF. Their activity might be balanced by protease inhibitors, such as TIMP-1 and TIMP-2, which were observed in CSF as well [98, 140–142]. Other extracellular matrix molecules identified in CSF by immuno- or affinity-chemical detection methods are soluble uPA receptor [143], glycosaminoglycans [136, 144], tenascin-C [145], TGF-beta 1 [146], cartilage glycoprotein-39 [147], the entire soluble ectodomain or the beta-amyloid peptide of APP [148] and major proteolytic fragments of neurocan [A. Grubb and U. Rauch, unpublished observation], while only small proteolytic peptides derived from testican and osteopontin were found [149].

The concentration of many of these substances has been correlated to certain pathological incidences, like epilepsy (chondroitin sulfate, hyaluronan), multiple sclerosis (MMP-9, ADAM-17), meningitis (suPA, hyaluronan, MMP-9, cartilage glycoprotein-39), encephalitis (hyaluronan), cerebral infarction (hyaluronan), hydrocephalus (TGF-beta, hyaluronan), neurodegenerating diseases (reelin, A-beta peptide, TIMP-1) or tumors (suPA, tenascin-C). Thus, various pathological insults and associated remodeling events are reflected by changes in the extracellular matrix components in CSF and could provide valuable information about the pathological state of the brain. The analysis of CSF proteins has therefore been considered as a promising topic in proteomic research [150]. Interestingly, a high-resolution separation of human CSF and subsequent identification of about 500 spots revealed only one of the molecules mentioned above, cartilage glycoprotein-39 [151]. This observation might indicate some limitations of the proteomic approach with respect to the identification of structural extracellular matrix compounds, which are often considerably post-translationally modified, although this is not impossible, as demonstrated by the identification of fibulin-1 [152].

Concluding remarks

There is ample evidence that brain remodeling processes are characterized by an up-regulation of extracellular matrix components, which are otherwise mainly expressed during early develomental stages. Interestingly, one of them, neurocan, belongs to a class of molecules, chondroitin sulfate proteoglycans, generally thought to be inhibitory for tissue plasticity. Treatment of inhibitory sites with chondroitinase ABC enhances axonal growth and functional recovery after spinal cord injury [12] and restores ocular dominance plasticity in the adult visual cortex of rats [127]. However, although present and useful as a marker, neurocan does not seem to be essential for the generation of plasticity-inhibitory structures like perineuronal nets, since those are also evident in neurocan knockout mice [153]. Moreover, neurocan appears to enhance synaptic plasticity. Thus, the functional relevance of the presence of neurocan in plasticity-inhibitory structures is not clear and remains to be investigated. In the absence of neurocan, the gross anatomical development of mouse brains is normal [45], although on closer inspection, alterations in the spatial arrangement of hippocampal pyramidal cells can be noticed [U. Rauch, unpublished observation].

Careful inspection and elaborated test paradigms were necessary to detect alterations in tenascin-C knockout mice [48, 154–156]. While no splice variants of neurocan have been described, the structural and functional properties of tenascin-C can change due to multiple alternatively spliced exons [157]. Apart from the obvious steric effect, increasingly convincing evidence for other particular functions of variable regions, like interactions with other molecules [158], and neurite-outgrowth-promoting activities [159] are emerging. This might render even minor proteolytic fragments of this apparently primarily structural matrix molecule biologically active and might draw more attention to the identification of the particular tenascin-C splice variant.

In summary, the evidence compiled in this review suggests that like tenascin-C, neurocan, although a chondroitin sulfate proteoglycan, might, rather, be considered as a component of an extracellular matrix permissive for remodeling events. In perspective, neurocan might be useful as a marker or target molecule for diseases involving tissue remodeling of the central nervous system.

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