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## ADAMTS expression and function in central nervous system injury and disorders

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### Abstract

The components of the adult extracellular matrix in the central nervous system form a lattice-like structure that is deposited as perineuronal nets, around axon initial segments and as synapse-associated matrix. An abundant component of this matrix is the lecticans, chondroitin sulfate-bearing proteoglycans that are the major substrate for several members of the ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) family. Since lecticans are key regulators of neural plasticity, ADAMTS cleavage of lecticans would likely also contribute to neuroplasticity. Indeed, many studies have examined the neuroplastic contribution of the ADAMTSs to damage and recovery after injury and in central nervous system disease. Much of this data supports a role for the ADAMTSs in recovery and repair following spinal cord injury by stimulating axonal outgrowth after degradation of a glial scar and improving synaptic plasticity following seizure-induced neural damage in the brain. The action of the ADAMTSs in chronic diseases of the central nervous system appears to be more complex and less well-defined. Increasing evidence indicates that lecticans participate in synaptic plasticity in neurodegenerative disease states. It will be interesting to examine how ADAMTS expression and action would affect the progression of these diseases.

### Keywords

Brevican; Chondroitin sulfate; Spinal cord injury; Seizure; Neuroplasticity; Glial scar; Proteolytic fragment

### Extracellular matrix in the developing and adult CNS

The extracellular matrix (ECM) in the central nervous system (CNS) shares similarities to other tissues, although there are several aspects of nervous system matrix that make it unique [1]. In the CNS there is little collagen or other fibril forming molecules. The extracellular space in the parenchyma is limited to 20% of the total CNS volume [2] and is filled with a lattice-forming structure composed of hyaluronan, glycoproteins including tenascin C/R, and

a small family of chondroitin sulfate (CS)-bearing proteoglycans (PGs) termed lecticans [3–5]. The lecticans include CNS-specific brevican and neurocan [6], aggrecan (also highly abundant in cartilage), and versican (found in nearly all tissues including blood vessels and basement membranes) [4,7]. The amino terminus of lecticans binds hyaluronan and the carboxy terminus binds tenascin.

The make-up of the matrix changes significantly during CNS development [8,9] and is quite varied in different regions of the adult CNS. During development, the ECM directly influences neuronal and glial migration and differentiation, neurogenesis, and axonal outgrowth and guidance that are vital for synaptogenesis [10–12]. Compared to the ECM during early development and later developmental “critical periods”, adult tECM is located just adjacent to the active zone of many synapses and directly affects synaptic plasticity and stability [3,6]. Additionally in the adult CNS, the ECM forms perineuronal nets (PNNs) around selected neuronal soma where it can influence synapses on the membrane of the cell body [13]. After injury to the CNS, glial scars form that contains robust amounts of ECM molecules, especially CS-bearing PGs, secreted mainly by activated glia. Although these scars are thought to assist in the isolation of the injured region of the brain, the scar inhibits repair mechanisms [11]. In fact, be it synaptic or neurite plasticity, the role of CS-bearing PGs appears to be negative in every case, but little is known about how the presence and turnover of matrix is regulated. However, it is known that extracellular proteases act selectively on lecticans, other CS-bearing PGs, other matrix proteins, and a small number of non-matrix protein substrates. These proteinase families are called a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) and matrix metalloproteinases (MMPs) [5,14]; tissue plasminogen activator is also capable of cleaving lecticans. Changes in deposits of matrix protein in the CNS involve the action of these proteases. The remainder of this chapter will describe what is known about the expression and activity of ADAMTS glutamyl-endopeptidases (proteases that cleave on the carboxy-end of glutamate or aspartate residues), in the CNS, how they influence the functional plasticity of brain ECM in CNS injury and disease, and the prospects for future discovery.

## Detection of changes in ADAMTS activity

One consistent and evident feature of Western blots conducted for identification of the lectican proteins, brevican, aggrecan, versican and neurocan, is the presence of low molecular weight fragments detected by antibodies originally raised to bind epitopes on the full length proteins. These fragments were initially observed for aggrecan and led to the notion that specific proteinases could cleave the full length protein [15]. The cleavage sites for each lectican in the intraglobular domain are conserved across species and are located near the junction between the N-terminal G1 domain and the central, CS-bearing domain. MMPs and ADAMTSs cleave specific but different sites in this region. Originally, a protein termed glial hyaluronan binding protein was thought to be an independent protein, but was found to be an ADAMTS-derived N-terminal fragment of versican V2 [16].

The C-terminal peptide sequences of the N-terminal fragments produced by lectican proteolysis were used to raise “neoepitope” antibodies that specifically recognize only the cleaved fragment, and not the intact proteoglycan [17,18]. These neoepitope antibodies may

be used in immunohistochemistry and Western blots to determine when there is an increase in the lectican fragment (and potentially a decrease in full length lectican) that would signify increased activity of the ADAMTSs or MMPs in a particular region of the brain after injury, in a brain disorder, or after treatment with an agent that may affect the activity of these proteinases. For example, a Western blot using an antibody against the brevican N-terminal region shows a typical ~55 kDa fragment (Fig. 1c). Brevican may be cleaved by either ADAMTSs or MMPs at sites that are proximate to one another. The N-terminal fragments produced by ADAMTS or MMP cleavage of brevican are 55 kDa and 53 kDa, respectively (Fig. 1D, E), and thus an increase in either the 55 kDa or 53 kDa fragment may signify increased ADAMTS or MMP activity, respectively. Since ADAMTSs require proteolytic activation to generate a functional protease, or may be inhibited by endogenous TIMP3, detecting an altered quantity of ADAMTSs alone does not signify a change in protease activity. However, changes in the levels of the ADAMTS-specific lectican fragment indicate that the activity of the proteinase has changed. The major ADAMTSs expressed in the brain which have the ability to cleave lecticans are ADAMT1, ADAMTS4, ADAMTS5, ADAMTS8, and ADAMTS9. It should also be noted that the lecticans are not the only substrates for these ADAMTSs, as they can cleave phosphacan [19] and reelin [20,21].

### ADAMTS in the CNS: regional and cellular expression in the CNS

ADAMTSs are expressed throughout the CNS, including the spinal cord, brain stem, hippocampus, striatum and cortex, as detected by direct immunohistochemistry, Western blot, RT-PCR, and by using ADAMTS-specific neopeptide antibodies to detect changes in fragments of various lectican substrates [21–29]. ADAMTS4 appears to be the most highly expressed ADAMTS in the brain of the adult under basal conditions [23], although ADAMTS1 was found to be expressed in the mouse and rat brain during development [30,31], in the mouse motor neurons after hypoglossal nerve injury [32] and in the frontal cortex of human Down's syndrome, Alzheimer's and Pick's disease tissue [25]. In the frontal cortex of mice, transcripts for the ADAMTS proteoglycanases were compared at postnatal day 8, day 28 and in the adult. ADAMTS1 and ADAMTS4 had higher transcript levels at all developmental periods compared to ADAMTS5, ADAMTS9 and ADAMTS15. Most interestingly, ADAMTS4 levels were quite low at P8, increased markedly at P28 and decreased to between these two levels in the adult [29]. ADAMTS4 is found in astrocytes and microglia in spinal cord *in vivo* and it increased in experimental autoimmune encephalomyelitis [27]. Neuronal expression of ADAMTS4 mRNA was observed in normal rat hippocampus—dentate gyrus granule cells and CA1 and CA3 neurons—and cortical neurons; its expression markedly increased in these regions following kainic acid-induced seizures [23]. In addition, ADAMTS1 mRNA markedly increased in the dentate gyrus and CA1 and CA3 regions of the hippocampus following kainic acid-induced seizures. This expression was closely associated with an increase in the ADAMTS-specific N-terminal cleavage fragment of brevican [23]. *In vivo* data supports that most ADAMTSs are produced by astrocytes, especially after injury, although ADAMTSs are also produced by neurons and microglia [33]. *In vitro*, many of the proteoglycan-cleaving ADAMTSs are produced by astrocytes and neurons [33]. In cultured astrocytes ADAMTS4 is expressed basally and inhibited in response to treatment with transforming growth factor  $\beta$  [34] and its production

is stimulated by  $\beta$ -amyloid [35]. Further, tumor necrosis factor  $\alpha$  induced the production of ADAMTS1 and ADAMTS4 in astrocytes [36]. Overall, most data indicates that ADAMTSs increase their expression in response to CNS disease, CNS disorders or after CNS injury. In these studies, there is either speculation about how the ADAMTSs might influence CNS repair or some studies produced data to support the functional role of the ADAMTSs.

## The functional role of ADAMTSs in the CNS

The specific role(s) ADAMTSs play in physiological and pathological nervous system functions remains under investigation. Studies have predominantly focused on ADAMTS family members that can cleave lecticans. There is substantive data that demonstrates that ADAMTS cleavage of brevican may facilitate the invasiveness of the glial tumors. ADAMTS4 mRNA is expressed by glioma cell lines with invasive properties and early data indicated that cleavage of brevican by ADAMTS4 is closely associated with invasiveness of these brain tumors [22,37,38]. Interestingly, only ADAMTS5 mRNA was upregulated in human glioma tissue. Although increased transcript alone does not indicate increased proteolytic activity, ADAMTS5 may also be involved in glioma invasiveness [39]. Compounds that could inhibit ADAMTS cleavage of brevican could potentially serve to limit glioma migration and aid in surgical removal. However, in other pathological conditions, increasing ADAMTS activity could prove beneficial.

Several studies indicate that the ADAMTSs are involved in recovery from spinal cord injury (SCI). SCI leads to a glial scar that is rich in growth inhibitory molecules, including lecticans, which diminishes functional recovery. Following SCI, ADAMTS1, 5, and 9 (but not ADAMTS4) mRNA was upregulated and this change was accompanied by increased cleavage of aggrecan, brevican, and versican [40]. Although ADAMTS4 mRNA was not altered in this report, another study demonstrated both increased ADAMTS4 protein and aggrecan cleavage following SCI [19]. Additionally, ADAMTS13 mRNA, a family member that cleaves von Willebrand Factor (vWF) but not lecticans, was increased in astrocytes and microglia following SCI. While vWF is systemically important for regulating blood coagulation, upregulation at the injury site was hypothesized to regulate secondary inflammation that would result from blood brain barrier breakdown. Given the prominent lectican component of the glial scar, recent studies examined the ability of ADAMTS4 to aid in recovery from SCI. When ADAMTS4 was infused into the rat spinal cord following SCI, it promoted functional recovery comparable to that observed with chondroitinase ABC, a bacterial enzyme that cleaves chondroitin sulfate from CS-bearing PGs [19]. Recently, tPA was shown to cleave pro-ADAMTS4 into its active form. Infusion of either tPA or active ADAMTS4 at the lesion after SCI promoted axonal sprouting and functional recovery [33]. Overall, ADAMTS4 may be a potentially promising therapeutic candidate that could increase plasticity and functional recovery after SCI, especially when delivered soon after the injury.

In studies both within and outside the CNS, ADAMTSs have been identified as having inflammatory and anti-angiogenic actions [33], including their necessity for macrophage invasion *in vitro* [41] and monocyte/macrophage differentiation and activation [42]. In addition, because several ADAMTSs can cleave versican deposited in the vasculature, it is

possible that the ADAMTSs, especially ADAMTS4, may contribute to the opening of the blood brain barrier after spinal cord injury or ischemic stroke. Although ADAMTS1 was the first ADAMTS to be cloned and identified with both inflammatory and anti-angiogenic properties [43], other ADAMTSs also show these actions [33]. Both the inflammatory and anti-angiogenic properties of the ADAMTSs have the potential to contribute to repair following ischemic stroke, other brain injury and certainly in a number of different neurodegenerative disease states. In fact, the ADAMTSs have a great deal of speculative potential toward their role in neural and synaptic plasticity, and potential for repair mechanisms that involves turnover of the ECM proteoglycans [5].

Indeed, increasing evidence suggests that ADAMTSs may play a role in neuroplasticity. After kainic acid-induced seizure, there was an association between increased ADAMTS-derived proteolytic brevicin fragment and a loss of synaptic density (as detected by reduced synaptophysin immunoreactivity) in dentate gyrus outer molecular layer. ADAMTS cleavage of brevicin occurred early with elevated brevicin fragment by 8 h of post-seizure. This finding suggested that the disrupted brevicin–tenascin–hyaluronan complex reduced the stability of synapses and may have led to synapse loss [23]. Localized inhibition of ADAMTS activity using a small molecule inhibitor [44] or localized treatment with siRNA that would block ADAMTS expression to determine if these treatments would reverse the loss of synaptic density might directly verify and support this finding. On the other hand, when ADAMTS activity was examined for longer periods after localized entorhinal cortex lesion, data indicated that ADAMTSs may exert a positive action in stimulating neuritic sprouting, synaptic remodeling and synaptogenesis in the outer molecular layer of the dentate gyrus; this role would make ADAMTSs crucial for recovery and repair after neuronal and/or synaptic loss [24]. Additional evidence for the potential role of ADAMTSs in neuroplasticity comes from *in vitro* work. When compared to several MMPs that cleave CS-bearing PGs, ADAMTS4 not only increased neurite outgrowth on an inhibitory CS-bearing PG or astrocyte-derived matrix, but also did not process laminin or induce neurotoxicity [45]. Cortical neurons transfected with either ADAMTS4 cDNA or a mutant ADAMTS4 cDNA that produced an inactive proteinase or direct treatment with recombinant ADAMTS4 all exhibited increased neurite outgrowth. The recombinant form of ADAMTS4 stimulated neurite extension in a dose-dependent manner. Thus, it was speculated that the thrombospondin repeats in ADAMTS4 may have induced neurite extension since expressing the inactive form of the protease was just as effective as the active form in stimulating neurite outgrowth [46]. In this study, ADAMTS-induced neuritic growth was shown to be dependent on extracellular signal-regulated kinase 1/2 (ERK-1/2) signaling [46]. This data suggests that ADAMTSs may increase plasticity even in the absence of lectican cleavage [46].

ADAMTS1, although not widely studied in the CNS, appears to play a role in developmental synaptogenesis. In one month old and adult ADAMTS1 knockout mice, several synaptic markers, including PSD-95, synaptophysin and SNAP-25 showed reduced levels in the frontal cortex extracts compared to wild type mice [29,47] and this was associated with an increase in neurocan during early postnatal development. Interestingly, this effect was sexually dimorphic as only female ADAMTS1 knockout mice exhibited these changes. It is unclear, however, whether these changes in synaptic density in female ADAMTS1

knockouts were related to the changes in matrix proteins since the alterations in the neurocan were observed about a week after birth [29]. ADAMTS1 is located on chromosome 21, and thus in Down syndrome or trisomy 21, it would be expected to be upregulated. In the Ts65Dn mouse model of Down syndrome, there was altered synaptic density in the stratum oriens of the hippocampus that was unexpectedly associated with increased deposition of versican V2 in the same region. It is possible that the overexpression of versican was associated with altered inhibitory neurotransmission in this region [48]. Taken together, the majority of data suggests that the ADAMTSs are involved in the activation of plasticity mechanisms, possibly on neurites and synapses, a role that is not surprising since the CS-bearing PGs can inhibit both neurite outgrowth and synaptic plasticity.

Future investigation into the role of ADAMTSs in nervous system disorders may involve synaptic plasticity. There is abundant evidence that lecticans and other CS-bearing proteoglycans have a marked influence on a host of nervous system disorders and diseases [13]. For example, recent data demonstrated a defect in ocular dominance plasticity in two different amyloid precursor protein overexpressing Alzheimer disease model mice at one month of age that was associated with increased ECM deposition [49]. Also, elevated ECM proteins, including brevican, were found at an early age in Alzheimer model mice associated with a loss of contextual fear memory and an LTP defect. Treatment with chondroitinase ABC restored contextual memory as well as LTP [50]. In such models it would be quite interesting to determine whether increased expression of ADAMTSs can produce the same effects as treatment with chondroitinase ABC. This prospect would be especially important since the ADAMTSs are naturally produced proteins where chondroitinase ABC is a bacterial enzyme. This has been performed in spinal cord injury and overexpression of the ADAMTSs was as effective as chondroitinase ABC in producing behavioral recovery [19].

The role of the ADAMTSs in the CNS continues to evolve. More and more evidence indicates that they play an important role in neuroplasticity as well as nervous system dysfunctions. It is hopeful and possible that ADAMTS family members may be utilized to develop therapies for CNS injury and neurodegenerative and neurological disorders.

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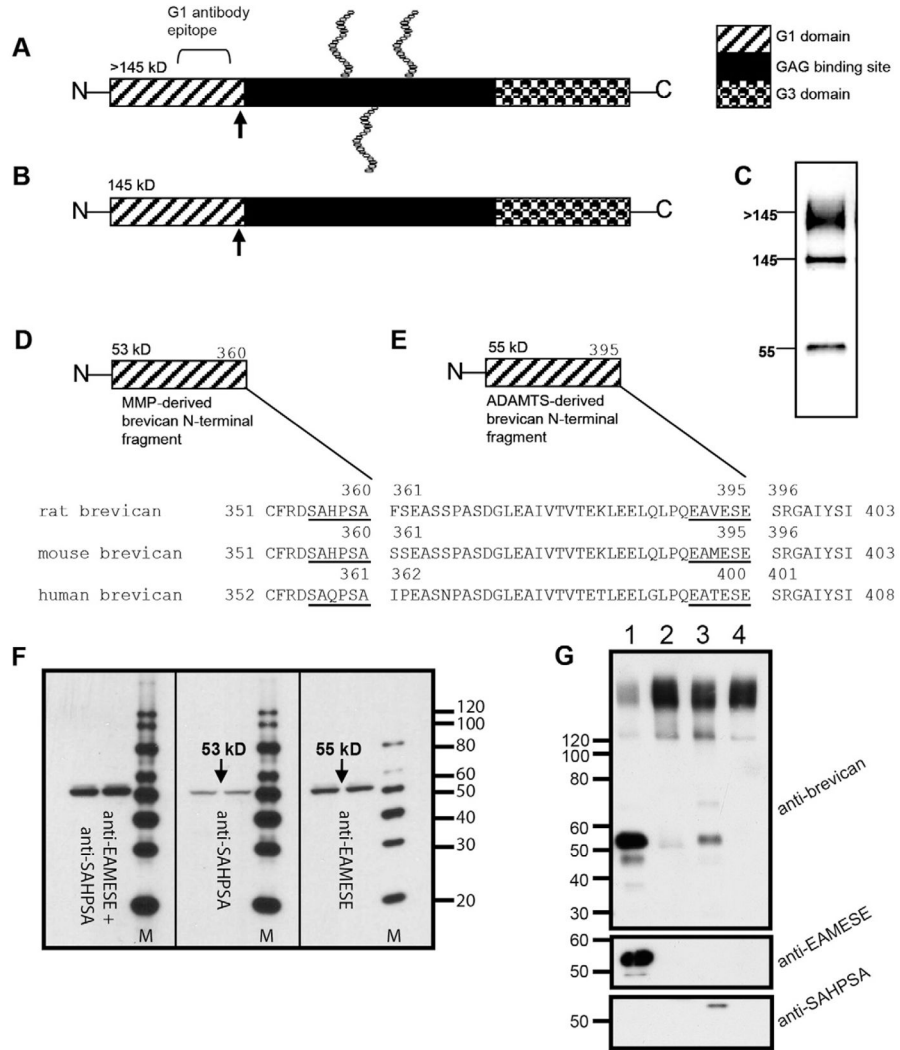
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**Fig. 1.** Detection of brevicin isoforms and proteolytic degradation by endogenous proteases at specific cleavage sites. Brevican is secreted as a >145 kDa protein bearing 1–3 CS chains (A). Brevican is also secreted as the holoprotein without CS chains at 145 kDa (B). When probed on Western blot with an N-terminal antibody (BD Biosciences, San Jose, CA) three immunoreactive bands appear: a >145 kDa smear (glycosylated brevican), the 145 kDa core protein, and a ~55 kDa proteolytic fragment. Arrows in (A) and (B) indicate proteolytic cleavage sites. Fragments of brevican are generated by endogenous proteases, the MMPs (D) and ADAMTSs (E). Each has a distinct, specific cleavage site sequence on the brevican protein. Shown here are the specific cleavage sequences for the MMPs and ADAMTSs in mouse, rat and human brevican (based on data from [51]). The MMP cleavage-site is 35 amino acid residues upstream from the ADAMTS-specific site (D and E). Distinct “neoepitope” antibodies recognize the MMP- and ADAMTS-derived cleavage fragments of brevican in mouse brain extracts subjected to Western blot on 4–20% gradient SDS-PAGE gels (F). Anti-SAHPSA recognizes the 53 kDa, MMP-derived fragment of brevican (F; middle panel) whereas anti-EAMESE detects the 55 kDa, ADAMTS-derived form (F; right

panel). Mixing the two antibodies detects a “thicker” band in this region (F; left panel). “M” indicates molecular weight markers in (F). Antibodies recognize distinct products after proteolytic cleavage with hrADAMTS4 or hrMMP-2 (G). Proteoglycan purified from the mouse brain was incubated with 50 nM hrADAMTS-4 (G, lane 1), 50 nM hrADAMTS4 + 5 mM EDTA (G, lane 2), 50 nM hrMMP-2 (G, lane 3) or 50 nM hrMMP-2 + 5 mM EDTA (G, lane 4) and immunoblotted for brevican. Note that the ADAMTS-derived brevican fragment was selectively recognized by anti-EAMESE and the brevican MMP product was recognized by anti-SAHPSA (Figure used with permission from Journal of Neurochemistry [52]).

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