Brevican and phosphacan expression and localization following transient middle cerebral artery occlusion in the rat

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Abstract

The ECM (extracellular matrix) is a complex molecular framework that provides physical support to cells and tissues, while also providing signals for cell growth, migration, differentiation and survival. The ECM of the CNS (central nervous system) is unusual in that it is rich in CSPGs (chondroitin sulfate proteoglycans), hyaluronan and tenascins. The CSPGs are widely expressed throughout the developing and adult CNS and have a role in guiding or limiting neurite outgrowth and cell migration. Alterations in the synthesis or breakdown of the ECM may contribute to disease processes. Here, we examine changes in the brain-specific CSPGs, brevican and phosphacan, following transient middle cerebral artery occlusion, a model of stroke in the rat. We have investigated their expression at various time points as well as their spatial relationship with ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs 4). The co-localization of ADAMTS or its activity may indicate a functional role for this matrix–protease pair in degeneration/regeneration processes that occur in stroke.

Introduction

Focal brain ischaemia induces a complex cascade of events leading to ECM (extracellular matrix) remodelling, gliosis and neovascularization. Changes in the levels of CSPGs (chondroitin sulfate proteoglycans) are relevant to neuronal survival and regeneration after an ischaemic event. The CSPGs present in the CNS (central nervous system) include neurocan, phosphacan and brevican. Brevican is a nervous system-specific CSPG and is most abundant in the adult brain. Phosphacan represents the extracellular domain of the transmembrane RPTPβ (receptor protein tyrosine phosphatase β), which is known to inhibit neurite outgrowth.

The ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzymes include some that preferentially cleave the aggregating CSPGs, aggrecan, versican and brevican at specific Glu-Xaa bonds but may have wider substrate specificities.

ECM changes in brain injury

Few previous studies have examined cell-type-specific expression of ECM proteins in the injured CNS. In a rat model of spinal cord injury, Jones et al. [1] have shown that 24 h after injury, brevican immunolabelling was moderately up-regulated and continued to increase 2 weeks after injury. Phosphacan immunolabelling however decreased immediately after injury but later recovered and peaked at 2 months. Elevated MMP (matrix metalloproteinase) expression is observed in various CNS pathologies including stroke [2,3], suggesting that MMPs participate directly in the pathological processes or are induced in response to injury for reorganization purposes. In animal models of stroke, metalloprotease inhibitors have previously demonstrated success in reduction of CNS damage.

ADAMTS proteoglycansases in the CNS

ADAMTS-1, -4, -5, -8, -9 and -15 demonstrate proteoglycanase activity, suggesting that they play a role in CNS turnover [4]. They are sequestered in the ECM via interactions with sulfated glycosaminoglycans [5]. ADAMTS expression has been detected in the CNS [6] and is known to be altered in disease states [7,8].

ADAMTS are activated intracellularly and secreted as active enzymes, and proteolytic processing alters both their localization and activity. The processed forms have the ability to cleave different ECM components.

Our recent research has focused on changes in the expression of brevican and phosphacan in CNS tissue of rats following MCAO (transient middle cerebral artery occlusion) as a model of stroke.

Changes in CSPG turnover following CNS injury

CNS trauma and stroke lead to a reactive gliosis characterized by increased GFAP (glial fibrillary acidic protein) expression
and hypertrophy of astrocytes [9], which is accompanied by alterations to many brain ECM components. We have previously reported that ADAMTS-1 and -4 mRNA and protein were all constitutively expressed in normal and stroke tissue and that their expression was up-regulated following occlusion [8]. ADAMTS-1 and -4 degrade CSPGs and an increase in degradation of ECM components may aid recovery by removal of the CSPGs that are inhibitory to neurite outgrowth [10]. Conversely, increased ADAMTS-mediated CSPG degradation may potentiate brain injury by disruption of the blood–brain barrier, enabling infiltration of inflammatory cells.

Our more recent observations have also demonstrated the constitutive expression of brevican and phosphacan at both the mRNA and protein levels. Brevican and phosphacan mRNA levels are unchanged in tMCAO tissue compared with sham operated at 24 h and 5 days post-tMCAO in the ipsilateral and contralateral hemispheres of experimental animals, although there appeared to be a reduction in brevican mRNA at 6 h post-tMCAO. Brevican protein levels are decreased in a small number of animals post-tMCAO, but overall no significant changes were observed in the protein levels of brevican or phosphacan.

Immunohistochemical analysis of rat tMCAO tissue sections revealed that brevican immunoreactivity is associated with vWF (von Willebrand factor)-positive endothelial cells and GFAP-positive astrocytes, consistent with previous studies [11]. Phosphacan staining had a diffuse punctate staining pattern, with some apparently associated with GFAP-positive astrocytes. Brevican (Figure 1) and phosphacan were co-localized with ADAMTS-4 immunoreactivity on astrocytes. A decrease in phosphacan immunostaining is observed following tMCAO, accompanied by an increase in ADAMTS-4 expression. This suggests that the decrease in phosphacan immediately post-hypoxia could be due to increased levels of proteolytic enzymes, namely ADAMTS-4, rather than a decrease in expression levels.

Jones et al. [1] reported a decrease in phosphacan immunolabelling in tissue near the region of spinal cord injury until day 3, followed by a significant rise up to 8 weeks after injury, whereas brevican was reported as being up-regulated after 24 h and continued to increase up to 8 weeks post-injury. Phosphacan was localized to astrocytes in the Purkinje cell layer and extending across the molecular layer. Our observations showed some phosphacan immunoreactivity to be localized to astrocytes. The localization of the CSPGs within the brain appears to be varied. Vitellaro-Zuccarello et al. [12] examined the regional distribution of several CSPGs and reported that they displayed distinct patterns of laminar distribution and association with specific neuronal subsets. Although there are no consistent differences in brevican protein levels between the sham and stroke brains, there were, however, localized dense areas of brevican immunostaining observed in the ipsilateral hemisphere, 5 days post-occlusion. This was not seen throughout the whole tissue and only in small areas. This would suggest that the changes observed in CSPG expression are confined to specific areas within the affected hemisphere and are masked when protein is extracted from the whole hemisphere and compared with contralateral hemisphere. Laser capture microdissection would therefore be an alternative approach to isolate the specific areas to quantify alterations in protein and mRNA expression. ADAMTS-4 immunoreactivity was also greatly increased in the areas of increased brevican staining. Therefore ADAMTS-4 may exhibit different specificities for the different CSPGs. An alternative explanation could be that the antibody used for

Figure 1 | Confocal scanning laser microscopy of immunohistochemistry on 5 day post-tMCAO tissue from the ipsilateral hemisphere
(A) ADAMTS-4, (B) brevican, and (C) co-localization. True co-localized pixels are represented in white in the composite image.
the detection of brevican also detected the cleavage products of the proteolysis of brevican by ADAMTS-4. Yuan et al. [13] showed an increase in ADAMTSs-mediated brevican proteolysis in the outer molecular layer of the dentate gyrus in a kainate-induced CNS lesion. Carulli et al. [14] reported expression of brevican within the molecular layer and granule cell layer, but different cell types and different levels of expression between the layers, in the adult rat brain.

In the glial scar formed after CNS injury, various components of the ECM, including the CSPGs, are increased, which in turn prevent axonal regeneration [10]. Davies et al. [15] showed that suppression of CSPGs markedly promoted axonal extension from transplanted adult sensory neurons to the marginal area after spinal cord injury. It is thus possible that regulation of the expression of CSPGs will be an important measure to control plasticity and reconstruction of the neural network.

Summary
The identification of the cell types responsible for producing putatively inhibitory ECM molecules after CNS injury is important for designing strategies to neutralize their effects. It remains to be determined whether the increase in ADAMTS expression is beneficial or detrimental to the healing process via effects on CSPG levels. Further studies will use cleavage-specific antibodies to detect the different forms of the breakdown products and to give us a clearer picture of any proteolysis that is occurring. As this becomes more clearly defined, new therapeutic targets may emerge.

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References
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