Chondroitin sulphate proteoglycans in the central nervous system: changes and synthesis after injury

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Abstract
Chondroitin sulphate proteoglycans (CSPGs) are up-regulated in the central nervous system after injury, specifically around the lesion site where the glial scar forms. This structure contains astrocytes, oligodendrocyte precursor cells, microglia and meningeal cells, and forms an inhibitory substrate for axon re-growth. CSPGs have been shown to be closely involved in this neuronal growth inhibition, specifically through their sugar chains. These chains are composed of repeats of the same disaccharide unit carrying sulphate groups in different positions. The sulphation pattern directly influences the CSPG binding properties and function; the specific sulphation pattern required for the inhibitory activity of these molecules on axon growth is unknown at present. The expression of the chondroitin sulphotransferases, which sulphate the disaccharide residues of CSPGs and thus are responsible for the structural diversity of the chondroitin sulphate sugar chains, is regulated differently in central nervous system during development and after injury, suggesting the implication of a specific sulphation pattern in the inhibitory activity of CSPGs.

Introduction
After an injury to the central nervous system (CNS), neurons are not able to regenerate their axons, and as a consequence most of them die by necrosis or through an apoptotic process. The inability of axons to re-grow is a characteristic of the mammalian CNS that was acquired late during evolution. The majority of neurons in the peripheral nervous system (PNS) and the lower-vertebrate CNS can survive a lesion and regenerate their processes, restoring the functionality of the lesioned tissue [1]. This observation suggests that CNS neurons might not have lost their regenerative capacity completely; indeed, many types of CNS neurons are able to elongate their axons through fragments of peripheral nerve grafts if they are injured [2].

The molecular mechanisms involved in the regulation of axon growth are still not clear. It is known that the extrinsic environment can promote or inhibit the elongation of neurites. In the PNS, as well as in the olfactory tract (an area of the adult CNS where axons can extend), specific molecules in the extracellular matrix (ECM) and in the surrounding glia, such as laminin and heparan sulphate proteoglycans, are able to promote axon growth [3]. However, these molecules fail to enhance axon elongation in the injured CNS, suggesting the presence of an interfering inhibitory system between these molecules and the signalling mechanisms promoting axon growth [4]. Indeed, over the past few years several molecules with a repulsive effect on growing axons have been identified both in the myelin surrounding the CNS axons and in the glial scar that forms after a CNS injury. The outer myelin membrane contains non-permissive molecules, such as Nogo and myelin-associated glycoprotein. The disruption of myelin due to the injury could expose these normally unexposed inhibitory proteins to the regenerating axons [1]. Moreover, the glial scar that forms after a CNS injury behaves as a barrier for growing axons [5]. It has been demonstrated by Davies et al. [6] that even PNS neurons, which can grow in normal CNS white matter tracts, cannot cross the scar substrate. This strong inhibitory effect is mediated by two types of ECM molecules, tenascins and chondroitin sulphate proteoglycans (CSPGs) [5].

CSPGs and the inhibition of axon re-growth
CSPGs consist of a protein core and long, unbranched polysaccharides [glycosaminoglycans (GAGs)] comprising chondroitin sulphate (CS) disaccharide unit repeats. The core proteins known to bear mainly CS GAGs are hyalectans (brevican, neurocan, versican, aggrecan), NG2, phosphacan, appican, decorin, biglycan and neuroglycan C [7]. In addition, six different CS disaccharide units can form the GAGs. Each of these carries one or two sulphate groups in different positions depending on the presence and the activity of different chondroitin sulphotransferases (CSSTs), the enzymes responsible for the sulphation of GAGs, in the Golgi apparatus. Along the sugar chains, the presence of different disaccharide units results in the formation of specific
structural motifs known as CS-A, CS-C, CS-D and CS-E. The sulphation pattern of each of these units influences the binding properties of the GAGs and thus the overall function of the CSPGs [8].

The inhibitory activity of CSPGs on axon re-growth is mediated mainly by the GAGs and by their structural motifs. Treatment with chondroitinase ABC, an enzyme able to cleave the sugar chains without altering the core protein structure, promotes axon re-growth both in vitro and in vivo [9–12]. Different astrocytic cell lines, i.e. permissive A7 and non-permissive Neu7, have been analysed for CSPG expression. In Neu7 cells, NG2 was highly expressed, and treatment with chondroitinase improved the growth of axons on these non-permissive cells. A7 astrocytes did not show high levels of NG2 expression [9].

In lesions of the nigrostriatal tract, treatment with chondroitinase ABC promoted axon re-growth [10], and its injection into the site of injury also promoted functional recovery of rats with spinal cord damage [11]. Moreover, on a nitrocellulose membrane with adhesive glial scar tissue, axon growth was promoted by treatment with the same enzyme, and this positive effect was laminin dependent [12]. The ECM of the glial scar contains high levels of CS GAGs. Davies et al. [13] have shown that neurons cannot cross the glial scar, in which CS levels are increased. The monoclonal antibody used for these studies, CS-56, preferentially binds CS-D, and thus it is not clear whether other types of CS around the injured area in the CNS are also up-regulated and involved in the inhibition of axon elongation.

Controversially, some studies also describe how certain CS GAGs are able to promote axon growth in some developmental models. CS-E has a growth-promoting effect on CNS embryonic axons in the mouse [8]. However, other studies have described how neurites tend to grow in CS GAG-free areas during early development [3,7]. CSPGs are highly expressed in embryonic brain, and have a primary role in axonal guidance and path finding during the development of the CNS in different organisms [3,7]. The proportions of the different CS subtypes seem to be critical for the neuro-regulatory activity of CS GAGs, and they are finely regulated during the development of both chicken and mouse brain [14,15]. The expression of CSSTs, the enzymes responsible for the structural diversity of CS GAGs, is highly regulated during brain development both in chicken and in mouse [14,15]. Interestingly their expression also changes after PNS injury [16]. Our recent data show that CSST transcription also changes in the CNS after injury. Specifically, the CSSTs involved in the synthesis of CS-C and CS-D are up-regulated in the area where the glial scar forms. Their expression is also very high in Neu7 non-permissive cells, in astrocytes after treatment with brain-injury-related cytokines and in oligodendrocyte precursor cells (OPCs).

CSPG core proteins are also up-regulated in the glial scar and during development [5,7]; however, their structural role in the inhibition of axon growth is still controversial. In rat cortex, neurocan expression increases in the glial scar, particularly after injury [17]. Even though OPCs have been shown to produce neurocan in vitro, the source of this CSPG in the scar seems to be reactive astrocytes, as shown by in situ hybridization experiments [18]. Moreover, various factors (transforming growth factors α and β and epidermal growth factor) produced in response to injury can increase neurocan expression in cultured astrocytes [17]. Versican has also been shown to be up-regulated in injured tissue, probably by OPCs [19]. The proliferation of this cell type around the injured area also causes an increase in the level of NG2 immunoreactivity, particularly 7 days after the lesion [5]. Other CSPGs, such as brevican, decorin and biglycan, have also been shown to be up-regulated after injury [5].

Despite all of these findings, it is still not clear which core protein and which CS subtypes are involved in the inhibition of neurite elongation in vivo both during development and after injury, and through which ligands and molecular signaling pathways CSPGs communicate with neurons. For this reason, the role of CSPGs in impeding axon regeneration is to some extent still controversial, and further studies need to be performed in order to develop useful treatments and to overcome the inhibitory activity of these molecules on axon growth.

References

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