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Glial cell extracellular matrix: boundaries for axon growth in development and regeneration

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Abstract. Astrocytes and other glia in the central nervous system are now thought to produce molecules that negatively modulate axon growth, thereby influencing axon pathfinding in both development and regeneration. The relevant evidence for glial cell boundaries and the inhibitory molecules present in these extracellular matrix structures is discussed in this minireview.

Key words: Astrocyte – Glia – Proteoglycan – Injury – Necrosis – Cavitation

Introduction

In an effort to understand axon pathfinding during the development and regeneration of the central nervous system (CNS), numerous studies have examined the abilities of various substrates to support the elongation of neuronal processes. Glial cells of the CNS have long been recognized as important contributors to the CNS as a structural framework for neurons, and although structural considerations are certainly important for axon growth, the cell-surface and extracellular matrix molecules produced by glial cells have recently been demonstrated to play critical roles in the abilities of these cells to support neurons and their processes. Since the mid-1980s, a number of molecules that are produced by astrocytes or their precursors and that encourage axon growth have been discovered (Liesi and Silver 1988; Tomaselli et al. 1988; Smith et al. 1990; Serafini et al. 1996). Even adult astrocytes can produce axon-supportive molecules such as laminin (Liesi et al. 1984; Bernstein et al. 1985; McKeon et al. 1991, 1995; Frisen et al. 1995). As discussed in this minireview, increasing evidence suggests that another important role of astrocytes and other glia in the CNS is the production of molecules that negatively modulate axon growth, thereby enabling glia to influence axon pathfinding in both development and regeneration.

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Glial cell boundaries in development

Glial cells of the astrocyte lineage play an integral role during development of the nervous system as a substrate for neuronal migration and axon elongation in vivo (Silver et al. 1993; Rakic 1995), and neonatal astrocytes have been shown to be a supportive substrate for axon growth in vitro (Fallon 1985; Rudge et al. 1989; Ard et al. 1991; Bahr et al. 1995). Glial cells are recognized not only for their axon supportive properties, but also for establishing functional boundaries important for guiding developing neuronal connections. Within the developing nervous system, such boundaries that are implicated in glial guidance of axons are present in the diencephalic/telencephalic junction (Silver 1984), the optic chiasm (Navascues et al. 1987; Silver et al. 1987; Godement et al. 1990; Sretavan 1990; Cole and McCabe 1991), the optic tectum (Snow et al. 1990b; Jhaveri 1993), anterior commissure (Cummings et al. 1997), somatosensory barrel fields of the cortex (Cooper and Steindler 1986), the midbrain (Garcia-Abreu et al. 1995), the roof plate of the spinal cord and tectum (Wilkinson et al. 1987; Joosten and Gribnau 1989; McMahon and Moon 1989), the developing floor plate (Dodd and Jessell 1988; Bovolenta and Dodd 1990; Kuwada et al. 1990; Placzek et al. 1990; Serafini et al. 1996), and extracellular channels of the retina and optic nerve (Silver and Robb 1979; Krayanek and Goldberg 1981; Silver 1984). These glial structures are also found at the midline of the developing forebrain, in the cerebral commissures, partitioning the diencephalon, and bordering the corpus callosum, internal capsule, and anterior commissure (Silver et al. 1982, 1993). They seem to play an integral role in axon guidance during embryogenesis by providing structural

and/or molecular cues that repel growing axons or redirect them toward other pathways.

Extracellular matrix molecules associated with boundaries in development

Various studies have recently been conducted to investigate the molecular basis of the glial boundaries found in the development of the nervous system. Many strategies have examined molecules that are present in such boundary regions but that are not present in other areas of the developing embryo; these approaches have implicated several molecules that are located in the previously described axon inhibitory regions and that may functionally serve as repulsors to growing neuronal processes. Tenascin is one such molecule that is associated with glial cell boundaries in development. It is found associated with developing axons in the spinal cord (Pindzola et al. 1993), optic nerve (Bartsch et al. 1994), optic tectum (Perez and Halfter 1993), olfactory bulb (Gonzalez et al. 1993; Gonzalez and Silver 1994), and barrel fields of the somatosensory cortex (Cooper and Steindler 1989; Mitrovic et al. 1994). Various members of the proteoglycan family have also been shown to be associated with similar glial boundaries in the development of the roof plate and midline dorsal tectum (Snow et al. 1990b; Katoh-Semba et al. 1995), roof plate and midline rhombencephalon and mesencephalon (Cole and McCabe 1991; McCabe and Cole 1992), dorsal root entry zone and dorsal columns in the spinal cord (Pindzola et al. 1993), olfactory bulb (Gonzalez et al. 1993; Gonzalez and Silver 1994), cortical barrels, thalamic nuclei, geniculate nucleus, and cerebellum (Cooper and Steindler 1986; Steindler et al. 1988, 1990; Geisert and Bidanset 1993; Robson and Geisert 1994; Seo and Geisert 1995; Watanabe et al. 1995), posterior sclerotome (Oakley and Tosney 1991; Landolt et al. 1995), optic nerve (Perez and Halfter 1993), and retina (Brittis et al. 1992; Brittis and Silver 1994; Brittis et al. 1995). The spacial and temporal expression of these molecules suggests a contribution to the molecular environment of glial boundaries and may serve to direct the growth of axons to other regions during development.

Boundary molecules directly modulate axon growth

These families of boundary molecules have been directly demonstrated as having the ability to modulate the growth of axons as purified substrates or as part of the extracellular matrix produced by CNS astrocytes. Experiments in vitro have demonstrated that certain forms of tenascin can be inhibitory for axon growth, whereas others can also modulate neurite extension in a positive manner (Grierson et al. 1990; Perez and Halfter 1993; Taylor et al. 1993; Faissner et al. 1994; Chiquet-Ehrismann et al. 1995). Proteoglycan molecules of various types also can inhibit neurite outgrowth as a result of their glycosaminoglycan (GAG) sugar chains (Snow et al. 1990a; Cole and McCabe 1991; Fichard et al. 1991; Snow et al. 1991; Bovolenta et al. 1993; Canning et al. 1993, 1996; Katoh-Semba et al. 1995) and sometimes as a function of their core proteins (Oohira et al. 1991; Geisert and Bidanset 1993; Dou and Levine 1994). It has also been suggested that, in certain situations, proteoglycans may positively influence neurons and/or axon growth (Iijima et al. 1991; Maeda et al. 1995; Challacombe and Elam 1997; Kappler et al. 1997). In addition to possible direct effects of the proteoglycan core molecule or GAG chains, a recent report highlights the differences between the inhibition of thalamic neurons in the developing cortical plate versus the stimulation of these neurons in the subplate region and suggests that proteoglycans in vivo may modulate such positive or negative axon growth via interactions with other molecules that bind to the GAG epitopes (Emerling and Lander 1996). Interactions such as those between proteoglycan molecules and identified growth promoting substances have been previously described and are discussed elsewhere (Ruoslahti and Yamaguchi 1991; Grumet et al. 1993; Friedlander et al. 1994; Milev et al. 1994; Burg et al. 1995).

Formation of cellular and molecular boundaries following CNS injury

Unlike the robust regenerative response of the peripheral nervous system (PNS) to injury (for a review, see Guth 1956), trauma to the adult mammalian CNS leads to permanent disability with little or no functional regeneration of injured axons (Ramón y Cajal 1928). Classic descriptions of injury to the CNS suggested for many years that regenerative failure was attributable primarily to a structural barrier to axon growth, viz., a glial scar composed of astrocytes and connective tissue (Windle and Chambers 1950; Windle et al. 1952; Clemente and Windle 1954). Recent studies indicate that the astrocytic scar that often forms following injury does not prevent axon growth simply by a mechanical mechanism (Reier et al. 1983; Guth et al. 1986; Davies et al. 1996); this has led investigators to search for both cellular and molecular explanations for the ways in which glial cells may contribute to the lack of regeneration of axons injured in the CNS (for a review, see Fitch and Silver 1997b).

Both tenascin and certain proteoglycans are upregulated following injury to the CNS. Tenascin levels increase following trauma to the brain (Laywell and Steindler 1991; McKeon et al. 1991, 1995; Laywell et al. 1992; Brodkey et al. 1995; Lips et al. 1995) and the spinal cord (Pindzola et al. 1993; Zhang et al. 1995). Similarly, chondroitin sulfate proteoglycans persist in the extracellular matrix of the CNS following injury, e.g., in the spinal cord following dorsal root injury (Pindzola et al. 1993), in the brain following stab wounds (Levine 1994; Fitch and Silver 1997a), in the fornix following transection (Lips et al. 1995), and in the spinal cord following penetrating crush injury (Fitch and Silver 1997a). Post-injury responses thus include the production of the same types of boundary molecules that have been described as having axon inhibitory functions during the

development of the nervous system. Extracellular matrix molecules produced in response to injury on an implanted piece of nitrocellulose in vivo provide a substrate that is inhibitory to axon regeneration in vitro, demonstrating that these kinds of molecules produced after injury may indeed functionally inhibit regeneration (McKeon et al. 1991, 1995). In addition, the spacial and temporal appearance of these putative inhibitory molecules implicates them in the failure of adult axons to regenerate successfully after trauma. Adult reactive astrocytes in the vicinity of CNS lesions are poorly supportive substrates for axon growth (Rudge et al. 1989; Smith et al. 1990; Geisert and Stewart 1991; Bahr et al. 1995; Le Roux and Reh 1996) and this seems to be due, at least in part, to these types of boundary molecules that are produced by astrocytes and are triggered to become reactive in specific ways (Grierson et al. 1990; McKeon et al. 1991, 1995; Ard et al. 1993; Canning et al. 1993, 1996; Dou and Levine 1994; Hoke et al. 1994; Smith-Thomas et al. 1994; Chiquet-Ehrismann et al. 1995).

What triggers the upregulation of boundary molecules after CNS injury?

Questions remain concerning the molecular triggers that are responsible for the production of inhibitory extracellular matrix molecules. One series of studies has demonstrated that β-amyloid protein is a trigger for the increased production of inhibitory proteoglycans by astrocytes (Canning et al. 1993, 1996; Hoke et al. 1994); this may explain the presence of these molecules around the plaques found in Alzheimer's disease (DeWitt et al. 1993). Our recent experiments in vivo demonstrate that increases in chondroitin sulfate proteoglycans after CNS injury are associated with the breakdown of the blood brain barrier and infiltrating macrophages at the lesion site, suggesting that serum factors or inflammatory cytokines play a role in the molecular cascade leading to extracellular matrix production in the immediate vicinity of the developing glial scar (Fitch and Silver 1997a). As a number of therapeutic strategies designed to modify the immune response have shown promise (Bracken et al. 1990; Guth et al. 1994a, b; Zhang et al. 1997), it is possible that such benefits may result from limiting inflammation-induced increases in putative inhibitory molecules after trauma. In addition, strategies designed to prevent widespread breakdown of the blood brain barrier after injury could lead to clinically promising therapies by similarly reducing a potential trigger for the production of molecules that may inhibit regeneration.

What are the functions of boundary molecules following trauma?

Proteoglycans may influence the normal environment of the adult CNS to favor the inhibition of axon growth in an attempt to maintain normal synaptic connections (Kalb and Hockfield 1990). Therefore, the upregulation of inhibitory molecules after injury may be one mechanism that the adult nervous system uses to prevent aberrant growth of axons and the formation of inappropriate connections. The previously described interactions of proteoglycans with growth factors (Ruoslahti and Yamaguchi 1991; Grumet et al. 1993; Burg et al. 1995; Emerling and Lander 1996) may serve to hinder long distance axon growth by binding and functionally removing important growth signals from the injury site, or they may act as a "trophic oasis" past which axons are unwilling to regenerate.

Chondroitin sulfate proteoglycans have been described at the interface between developing cavities and the surrounding CNS parenchyma and produce what is effectively a molecular boundary between the destruction of tissue via progressive necrosis and the surrounding viable tissue (Fitch and Silver 1997a). This response of the mature CNS to injury may represent a "walling off" of injured CNS tissue as a protective response for the healthy tissue surrounding the wound epicenter. Since proteoglycans have been demonstrated to inhibit the phagocytosis and destruction of β-amyloid protein by macrophages (Shaffer et al. 1995), an attractive hypothesis is that proteoglycans have a protective function to prevent secondary damage within the CNS after trauma, thus limiting the devastating process of progressive necrosis. The inhibitory properties of these molecules on axonal growth may simply be an unfortunate side-effect of a normal wound-healing response of CNS tissue. Strategies to modulate the production of these molecules may be one way of approaching the enhancement of the regenerative response by adult neurons in future interdisciplinary approaches to the therapy of CNS injury.

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