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Minireview

Building bridges with astrocytes for spinal cord repair

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Abstract

Simultaneous suppression of glial scarring and a general enhancement of axonal outgrowth has now been accomplished in an adult rat model of spinal cord transection. Transplantation of a novel astrocyte cell type derived from glial-restricted precursors *in vitro* raise the eventual possibility of cellular therapy for spinal cord injury.

The functional regeneration of neuronal processes in the injured nervous system poses a formidable challenge. During development, axons - the long processes of neurons - grow long distances through complex terrains in stereotypical patterns to connect with the appropriate targets and facilitate effective communication. Much energy has been expended by researchers in investigating how axonal growth and connectivity are guided and regulated. While a complete understanding is still a long way off, it is clear that these are complex processes, involving multiple molecular cues that occur in stereotyped sequences. Many of the cues mediating axonal growth and guidance are lost in the adult central nervous system (CNS) and these processes are further disrupted by injury, resulting in disoriented axons. The injury itself releases inhibitors of axon growth from white matter (bundled tracts of axons) [1,2] and local endogenous glial cells, the supporting non-neuronal cells of the nervous system, respond to the insult with increased production of a variety of growth inhibitors [3,4]. In addition to these environmental changes, there are intrinsic differences in the growth responses of immature and adult axons - adult axons grow less strongly. In consequence, it seems that the key to functional regeneration in the injured adult spinal cord is the simultaneous modification of multiple inhibitory cues - a demanding task that requires a particularly special type of glial cell. In a recent paper in *Journal of Biology*, Davies *et al.* [5] describe the identification of such cells and their transplantation to promote a remarkable regeneration of adult axons after spinal cord transection in the rat.

The cells utilized by Davies *et al.* [5] are termed GRP-derived astrocytes. This unusual name derives from the origins of the cells and reflects recent advances in our understanding of the cellular development of the CNS. Classical morphological studies identified the major epochs of neural development, in which neurons arise before glial cells [6]. Evidence that all major cell types might be derived from multipotent stem cells emerged from *in vitro* assays in which 'neurosphere'-producing cells were isolated, passaged and shown to generate neurons and the glial cell types astrocytes and oligodendrocytes [7]. These observations prompted an intensive search to define intermediate cell types between a multipotent stem cell and the fully differentiated cellular products. Using a series of *in vitro* approaches, Davies *et al.* [5] identified precursor cells derived from multipotent stem

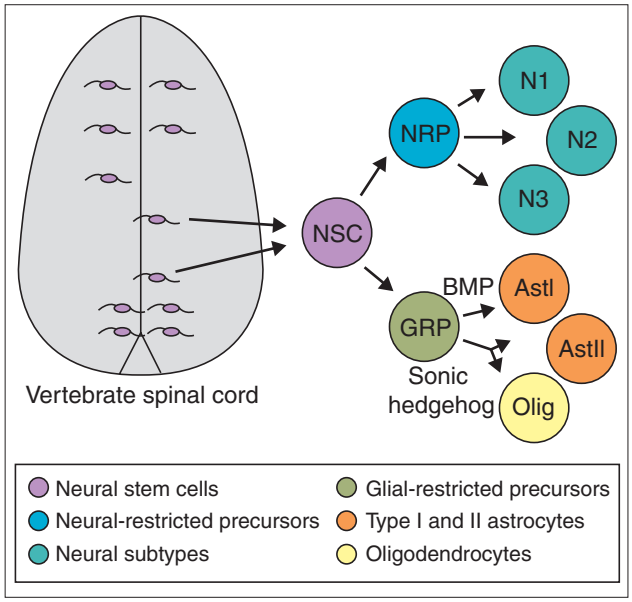

Figure 1
A model for the sequential generation of distinct cell types in the vertebrate CNS. Neural stem cells (NSCs) from the rat embryonic brain give rise to progenitors that are restricted to neuronal or glial fates. *In vitro* treatment of glial-derived precursors (GRPs) with members of the bone morphogenetic protein (BMP) family of secreted signaling molecules drives their differentiation into a distinct subtype of astrocyte (type I astrocyte, AstI) that promotes repair when transplanted to the injured adult spinal cord. In contrast, treatment of GRPs with the secreted protein Sonic hedgehog (Shh), a member of a different family of signaling molecules, causes their differentiation into type II astrocytes (AstII) and oligodendrocytes.

cells that appeared to be restricted to generating either neurons (neuron-restricted precursors, NRPs) or glial cells (glial-restricted precursors (GRPs) that give rise to astrocytes and oligodendrocytes) (Figure 1). Treatment of GRPs with a particular cocktail of growth factors and cytokines results in a population of cells, the GRP-derived astrocytes, that express canonical characteristics of astrocytes such as expression of the intermediate filament protein GFAP (glial fibrillary acidic protein). These were then used in the transplant studies. Remarkably, the GRP-derived astrocytes are far more effective at promoting axonal regeneration than are their less committed ancestor cells. Recent studies from other laboratories have used similar approaches to examine the ability of transplanted neural stem cells [8] or NRPs and GRPs [9] to promote spinal cord repair. Those analyses demonstrated the survival, migration and integration of the transplanted cells into the host tissue, but the characterization of axonal regrowth by Davies *et al.* [5] reveals that these precursor cell types have a very limited capacity to support axonal regeneration.

The striking axonal regeneration seen by the authors following GRP-derived astrocyte transplantation raises the critical issue of what is special about these cells. It seems likely that one of the main keys to enhanced axonal regeneration is modulation of the endogenous host cells' response to injury rather than the provision of specific molecular promoters of axonal elongation by the transplanted cells. For example, the regeneration-promoting abilities of GRP-derived astrocytes are not restricted to particular populations of neurons. Davies *et al.* [5] severed the rubrospinal tract - a population of axons that runs from the brain and is involved in relaying information that controls muscle function. Animals with injuries to the rubrospinal tract had lost the ability to coordinate their fore- and hindlimbs precisely. After transplantation of GRP-derived astrocytes to the site of the lesion, the team observed increased regrowth of the rubrospinal tract axons into the injury site compared with untreated animals, and enhanced recovery of locomotor function.

In other experiments the authors found that GRP-derived astrocytes enhanced the growth of axons from transplants of sensory dorsal-root ganglion neurons through a lesion. These axons, which normally derive from neurons located outside the spinal cord, are likely to use distinct molecular cues for outgrowth compared with the rubrospinal tract axons, and it is unlikely that both sets of cues are expressed at the same time by the GRP-derived astrocytes. Rather, the GRP-derived astrocytes appear to modulate shared responses of adult CNS cells to injury, and provide an environment that recapitulates essential properties of the developing CNS.

Two aspects seem particularly important. The first is the suppression of glial scarring that normally accompanies injury to the CNS. Glial scars result in excessive growth in size of astrocytes (hypertrophy) and upregulation of the production of various proteoglycans that inhibit axonal growth. The onset of the scarring response is significantly delayed by transplanting GRP-derived astrocytes, which perhaps allows a window of opportunity for regenerating axons to traverse the lesion site. Second, transplantation of GRP-derived astrocytes imposes a striking linear orientation on host glial cells such that they provide a more uniform environment through which the regenerating axons grow more easily.

Davies *et al.* [5] provide two main insights that will be important in approaches to promoting spinal cord repair by cell transplantation. First, the selection of the appropriate cell type is critical for regeneration. Cells that are too immature or uncommitted are relatively ineffective, presumably because their fate can be dictated by signals at the injury site. Cells

that are too mature, as in the host, are relatively ineffective, presumably because they are programmed to form glial scars. Thus, the level of commitment or cell differentiation is key. Second, creation of a 'regeneration-permissive' environment is not neuron-specific. The commonalities of axon outgrowth seem sufficient for different types of neurons to be able to benefit from the same treatment. These observations suggest that axon regeneration is perhaps fundamentally different from initial axonal pathfinding during development, which appears to be exquisitely neuron-specific [10].

This study also raises a number of interesting questions on the *in vivo* correlates of GRP-derived astrocytes. For example, do they represent a distinct cell population in the intact CNS or are they simply a product of *in vitro* 'cell engineering'? The origin and lineages of glial cells in the CNS has been extensively studied, particularly in the spinal cord. Although several models have been proposed linking neurons and oligodendrocytes in a common lineage [11,12], more recent studies suggest that this is unlikely [13,14]. Earlier studies had linked astrocytes and oligodendrocytes in a common lineage [15], and previous work [16] from two of the authors of Davies *et al.* [5] demonstrated a more primitive glial precursor that generates different types of astrocytes and oligodendrocytes. The molecular cues used by Davies *et al.* [5] to generate GRP-derived astrocytes are operative in the intact CNS but are likely to be used in concert with multiple other signals to specify other cell types, including neurons [17-20]. The origins of astrocytes *in vivo* remain unclear. Indeed, in other regions of the CNS astrocytes have been proposed to represent stem cells on the basis of the expression of GFAP, and clonal studies *in vitro* suggest a significant diversity among spinal cord astrocytes [21]. The precise assessment of lineage associations between cells of the CNS and identification of intermediate cell types require novel approaches and the generation of new molecular markers.

Ultimately, it will be essential to unravel the cellular and molecular bases of the phenomena described by Davies *et al*. [5]. What is it about GRP-derived astrocytes that facilitates their orientation and what are the molecular mechanisms by which they promote axonal growth? These questions will not be answered easily. The model of cell transplantation into the injured spinal cord is extremely complex. Multiple cell interactions are occurring simultaneously and early interactions are likely to establish cascades of subsequent events. Such complexity limits the use of modern DNA array-based discovery approaches, and insights are more likely to come from cell-based strategies. The hope is that identification of critical upstream steps in the regulation of the host glial response to CNS injury that are modulated by GRP-derived astrocytes might lead to the identification of key regulators that could be targets for pharmacological therapeutics.

Regardless of whether there is a precise *in vivo* counterpart of the GRP-derived astrocytes and of the molecular mechanisms by which these cells promote axon elongation, the studies by Davies *et al.* [5] reveal both the importance of cellular maturity in promoting axonal regeneration and provide a source of cells for effective therapeutic approaches aimed at adult spinal cord regeneration.

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