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Review

Regeneration-based therapies for spinal cord injuries

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Abstract

Although it has been long believed that the damaged central nervous system does not regenerate upon injury, there is an emerging hope for regeneration-based therapy of the damaged central nervous system (CNS) due to the progress of developmental biology and regenerative medicine including stem cell biology. In this review, we have summarized recent studies aimed at the development of regeneration-based therapeutic approaches for spinal cord injuries, including therapy with anti-inflammatory cytokines, transplantation of neural stem/precursor cells and induction of axonal regeneration.

Keywords: Interleukin-6 (IL-6); Neural stem cells; Reactive astrocytes; Semaphorin 3A; Spinal cord injury (SCI); Stat3

Contents

1. Why does the adult mammalian spinal cord lack regenerative capacities?

Traumatic spinal cord injury (SCI) affects many people, including young people, and can result in severe damage, leading to loss of motor and sensory function caudal to the level of injury, by severing descending and ascending fiber tracts (Ogawa et al., 2002; Okano et al., 2006). However, the effects of current conventional treatments are modest at best and consequently there is a great need for novel "regenerative" treatment strategies that could significantly protect and/or restore functions following SCI (Hofsteller, 2005). In order to develop such "regenerative" treatment strategies, it is obviously important to elucidate the underlying mechanism as to why the adult mammalian spinal cord has extremely low regenerative capacities. Many lines of evidence have indicated that apparent lack of regenerative

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Table 1
Inhibitory factors for axonal regenerations and their blocking reagents

Inhibitory factors	Established molecules	Blocking reagents
Myelin-derived proteins	MAG, Nogo-A, OMgp	Nogo-66 receptor antagonist peptide (a)
Glial scar	CSPGs	C-ABC (b) Rho-kinase inhibitor (c)
Extracellular matrix-derived factors	Semaphorin3A	SM-216289 (d)

References: (a) GrandPre et al., 2002; (b) Bradbury et al., 2002; (c) Monnier et al., 2003; (d) Kaneko et al., 2006.

capacities of the adult mammalian spinal cord, could result from (i) the inhibitory character of CNS myelin and injury-induced glial scars for axonal regeneration (Table 1), (ii) the apparent inability of endogenous adult Neural Stem Cells (NSCs) in the spinal cord to induce *de novo* neurogenesis upon injury, and (iii) the lack of sufficient trophic support mechanisms (reviewed by Okano et al., 2003, 2006).

2. Stem cells-based transplantation studies on SCI

Experimental therapeutic methods have been previously reported on the transplantation of peripheral nerves (Richardson et al., 1980) and fetal spinal cord (Bregman, 1987) for spinal cord injuries. These studies indicated that the introduction of an appropriate environment into the injured site within the spinal cord can cause injured axons to regenerate. Practically, however, the above-mentioned protocols are almost impossible to apply for human SCI patients. For example, therapeutic approaches involving the transplantation of fetal CNS tissues came up against the double problem of obtaining a large enough amount of the donor tissues and also the ethical controversy surrounding the use of human fetal tissues.

To overcome such practical and ethical issues, transplantation therapies have been developed for SCI animal models including primates using *in vitro* expanded neural stem/precursor cells (NSPCs), including a mixed cell population of NSCs and neural precursor cells (Ogawa et al., 2002; Iwanami et al., 2005a,b; Cummings et al., 2005; Okada et al., 2005), embryonic stem (ES) cells-derived neural precursor cells (McDonald et al., 1999), or human ES cells-derived oligodendrocyte progenitor cells (OPCs). We found that appropriate time window for the *in vitro* expanded fetal NSPC transplantation is crucial to obtain the maximum therapeutic effects (Ogawa et al., 2002; Okano, 2002a,b, in press; Okano et al., 2003, 2006).

Several possible explanations for functional improvement through these transplantation studies may be advanced as follows: (1) Neurons derived from the grafted cells ''relayed'' signals from the disrupted fibers in the host, including local circuit interneurons or ascending fibers that existed in the dorsal column (Bregman et al., 1993); (2) oligodendrocytes derived from grafted cells might have remyelinated fibers that had been demyelinated as a result of injury and restored the salutatory conduction along the neuronal axons of long projection neuron (Cummings et al., 2005; Keirstead et al., 2005); (3) astrocytes-derived from donor neural progenitor cells might have played active roles in the generation of neuronal cells (Ogawa et al., 2002), axonal regeneration of host neuronal axons (Garcia-Abreu et al., 2000), enhancement of axonal extension of donor-derived neurons, synapse formation, and/or physiological maturation of neuronal cells (Blondel et al., 2000); (4) trophic effects (indicating that functional improvement may not be dependent on the transplanted human fetal NSPCs, which had been expanded *in vitro*, becoming functional neurons and making the right connections, but rather on the secretion of trophic factors from the transplanted cells) might also be effective for the survival and differentiation of host cells in the injured spinal cord, leading to functional recovery. The observed functional recovery might not result from a single mechanism. On the other hand, Cummings et al. (2005) showed that engraftment of *in vitro* expanded human fetal NSPCs into the immunodeficient mouse SCI model was associated with locomotor recovery. Interestingly, observed functional recovery was abolished by selective ablation of engrafted cells by diphtheria toxin, which is selective for human cells rather than rodent cells. Thus, the survival of engrafted human fetal NSPCs, which had been expanded *in vitro*, and their progenies in the host was shown to play a role in the maintenance of improved performance. These findings indicate that differentiation of engrafted human fetal NSCs to myelinating oligodendrocytes and neurons with synaptic connections to host neurons rater than the trophic effects could be mechanisms for sustained locomotor recovery in this model.

Ogawa et al. (2002) showed that a narrow therapeutic time window can allow successful transplantation. This brief window of opportunity might arise because the microenvironment in the host spinal cord changes rapidly after the injury (Okano, 2002a,b; Okano et al., 2003). Recent reports have shown that transient severe inflammation occurs around the injured site during the acute phase, which immediately follows the injury. During this time the levels of many inflammatory cytokines that have neurotoxic or astrocyte-inducing effects, such as IL-1, IL-6, and TNFα, increase and then decline sharply within 24 h (Nakamura et al., 2003), indicating that the microenvironment of the acute phase is not suitable for survival of grafted cells. In fact, the transplantation of *in vitro* expanded fetal NSPCs results in mitogenic neurogenesis when the transplantation into the injured adult rat spinal cord is carried out 9 days after injury, but not when the transplantation is done within a few days of the injury (Ogawa et al., 2002). The chronic phase of spinal cord injury is not likely to be appropriate for therapeutic transplantation due to the formation of enlarged cysts and the development of glial scarring, which might inhibit axonal regeneration (reviewed by Okano, 2002a,b) (Fig. 1).

3. Supporting treatments with NSPCs-transplantation

To achieve greater effects of fetal or adult NSPCs-mediated cell therapy on various types of CNS damage including SCI,