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Reconstructing neural circuits using transplanted neural stem cells in the injured spinal cord

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Traumatic spinal cord injury is one of the most common causes of disability in young adults. Restoring independent ambulation in such patients is considered one of the biggest challenges in regenerative medicine because repair of spinal cord injury involves the complex processes of axonal regeneration, remyelination, and formation of new synaptic connections. In this issue of the *JCI*, Abematsu et al. report their attempts to rise to this challenge, showing in a mouse model of severe spinal cord injury that spinal neuronal circuits can be restored by neural stem cell transplantation, leading to impressive functional recovery in the hind limbs.

Traumatic brain and spinal cord injury (SCI) is one of the most common causes of disability in young adults and poses a huge social and economical burden (1). The impact of nonpenetrating, blunt (contusive) trauma to the spinal cord causes abrupt discontinuation of axonal projections. There is also release of neurotoxic compounds and inflammatory mediators that add to neuronal and oligodendroglial cell death during the first hours and days after injury. This is followed by secondary processes of loss of myelin, degeneration of axons, and formation of a glial scar that inhibits spontaneous regeneration. Currently, there is no proven reparative treatment for SCI. Restoring independent ambulation in paraplegic trauma victims is considered one of the biggest challenges in regenerative medicine. This would require the development of approaches to achieve effective transmission of electrical impulses through the lesion. To this end, it is necessary to induce

robust regeneration of severed axons, their covering with myelin sheaths — a process termed remyelination — and formation of new synaptic connections. With this notion in mind, studies of neural stem cell transplantation have been performed in experimental models of SCI during the last decade and have demonstrated increased growth of severed host axons and improved remyelination (2). In this issue of the *JCI*, Abematsu et al. (3) show that transplanting neural stem cells epigenetically directed to differentiate into neurons can promote the reconstruction of spinal neuronal circuits, leading to impressive functional recovery in mouse hind limbs following SCI.

## Impediments and therapeutic targets in spinal cord repair

Restorative therapy in SCI should be directed against several targets. During the immediate hours and days after injury, the priority is to minimize inflammatory and neurotoxic damage. In the following days and weeks, the focus should be on halting the secondary processes of demyelination, axonal degeneration, and scar formation. However, meaningful recovery after SCI is

ultimately an issue of axonal regeneration. There are several axonal projections passing through the spinal cord. The corticospinal tract consists of fibers that originate in cortical neurons and send axons extending through the brain and spinal cord (reaching over one meter long) to innervate spinal lower motor neurons. These, in turn, transmit the message for muscles to contract. Other descending tracts that are involved in execution of motor functions, such as the proprio-, rubro-, reticulo-, and raphespinal tracts, are multisynaptic and therefore consist of neurons with shorter axons. Direct growth of corticospinal fibers through the lesion to reinnervate distal segments that are caudal (below) to the site of injury is very limited, since corticospinal axons are the neuronal population with the least regenerative capacity. The multisynaptic spinal tracts are considered to have better inherent regenerative capacity than corticospinal tracts (4). Plastic mechanisms that have been shown to contribute to functional recovery after SCI include local sprouting of fibers to innervate undamaged collateral fibers, spinal interneurons, and multisynaptic tracts.

Restrictions to axonal regeneration in the CNS include both extrinsic and intrinsic factors (5). There are several known myelin- and extracellular matrix–derived molecules that are inhibitory to axonal regeneration. Removal of myelin (6), neutralization of myelin-derived inhibitory molecules such as Nogo (7), and neutralization of the extracellular matrix component chondroitin sulfate proteoglycan (8) all result in improved axonal growth.
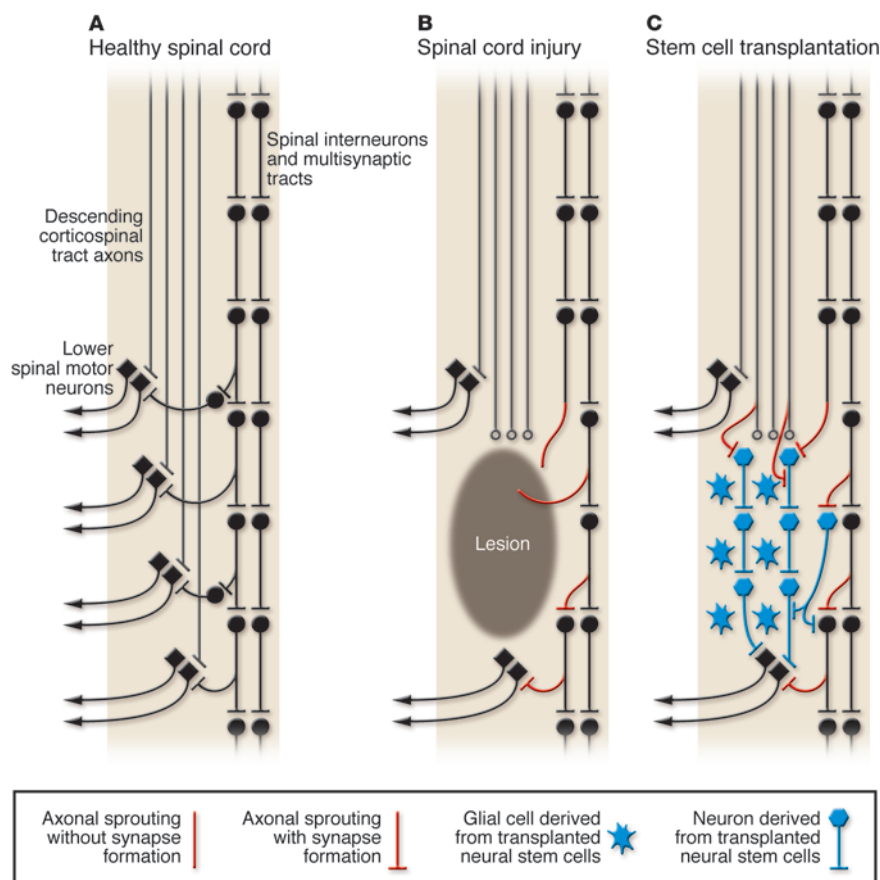
Figure 1
Regeneration of the injured spinal cord by neural stem cell transplantation. Simplified illustration of longitudinal sections of the spinal cord. Shown are motor projections that control voluntary muscle activity and their response to injury and to stem cell transplantation. (**A**) Descending corticospinal tract axons innervate directly the lower spinal motor neurons (diamonds), which exit the spinal cord in a segmental manner to innervate voluntary muscles. Motor activity is also modulated and controlled by spinal interneurons and multisynaptic tracts (circles). (**B**) After severe SCI with discontinuation of most spinal projections, there is a nonpermissive environment (shadowed area) for repair processes. Axonal sprouting from surviving spinal neurons (in red) is mostly inefficient, and very few form new synapses with spinal neurons that reside below the injured area. (**C**) Neural stem cells transplanted into the injured spinal cord differentiate into glia (blue stars) and neurons (blue hexagons). The glial cells create a permissive environment for regeneration, resulting in increased sprouting of surviving nerve fibers. The transplanted neurons form multiple synaptic connections with surviving neurons and with spinal segments below the lesion, resulting in substantially improved transmission of information through the lesion.

These strategies provide only limited benefit, and as such it has become clear that targeting a single molecule will not be sufficient for meaningful repair. A successful strategy must address the range of injurious and inhibitory mechanisms. This is a core rationale for the use of cell therapy for spinal cord regeneration.

Stem cell transplantation for spinal cord regeneration

In view of the limited capacity of the CNS to repair itself, the science of stem cell transplantation therapy emerged initially with the intention of replacing damaged cells. Until now, this approach seemed feasible for diseases that affect a specific type of neural cell and pathway, such as replacing dopaminergic neurons in Parkinson disease or myelinating the brain in disorders of myelin. However, reconstitution of more complex CNS neuronal circuits by cell transplantation, such as in victims of stroke or trauma, appeared to be a much more difficult task. The last decade of research has highlighted several additional mechanisms by which transplanted cells may enhance recovery. Various types

of precursor cells (including neural stem cells, embryonic stem cell–derived neural progenitors, and bone marrow stromal stem cells) demonstrate powerful immunomodulatory and neurotrophic properties (reviewed in ref. 9) that reduce the acute deleterious inflammatory process and induce a permissive environment for axonal regeneration after SCI (10). Numerous studies have shown that these precursor cells exhibit their therapeutic properties by multiple mechanisms, for example, production of a myriad of neurotrophic growth factors that enhance axonal regeneration (11); induction of matrix metalloproteinases that degrade the extracellular matrix and cell surface molecules that impede axonal regeneration, thus enabling axons to extend through the glial scar (12); induction of angiogenesis in the lesioned tissue, which provides trophic support and enables tissue repair (13); provision of proper realignment and guidance to enable axonal regeneration along long fiber tracts (14); and increase of remyelination in the lesion by both endogenous and graft-derived myelin forming–cells to enhance action potential conduction and

limit secondary axonal degeneration (15). To this end, combination therapy with chondroitinase to destroy the inhibitory extracellular matrix component chondroitin sulfate proteoglycan followed by infusion of neurotrophic growth factors and neural stem cells has been shown to act synergistically in the repair of chronically injured rat spinal cord (16). In all these strategies, cell therapy was aimed primarily at supporting endogenous repair processes to improve motor function of experimental animals. This improvement was achieved by the generation of graft-derived glia and other supporting cells that promoted endogenous axonal regeneration and by the generation of graft-derived remyelinating cells that ensheathed the demyelinated endogenous axons.

Structural remodeling of injured spinal cord by transplanted stem cells

Can cell therapy regenerate the spinal cord directly by integrating into spinal cord neuronal circuits? Central to this question is the delineation of the mechanisms of axonal regeneration in the spinal cord. Replacing corticospinal fibers with transplanted

neurons that stretch from the motor cortex to distal spinal segments is still far from practical. However, graft-derived neurons may participate directly in the structural remodeling of remaining neuronal systems. To that end, the study of Abematsu and colleagues (3) shows that in mice, transplanted neurons can indeed integrate in this way and serve as a new intraspinal relay to reinstate neural communication between the segments above and below the lesion. To overcome the strong bias of neural stem cells for acquiring a glial fate, the researchers epigenetically directed the differentiation of neural stem cells into neurons using the inhibitory effect of valproic acid on histone-deacetylase activity. They then showed that transplantation of these cells led to very good functional recovery in mice following SCI, to the extent that the mice were able to bear their own weight and walk efficiently. Importantly, this effect was dependent on neuronal differentiation of the grafted neural stem cells. Furthermore, no recovery of injured corticospinal tracts was observed; rather, generation of graft-derived multiple synapse–forming neurons was observed. Evidence that the transplanted neurons were directly responsible for recovery was provided by the demonstration that when transgenic neural stem cells expressing the diphtheria toxin receptor were transplanted, their removal by injection of diphtheria toxin reversed the beneficial effects of transplantation. These results provide convincing evidence of direct reconstruction of neural circuits in the spinal cord by neurons derived from transplanted stem cells (Figure 1).

Implications from experimental animals to human patients
Are the findings by Abematsu et al. relevant to human patients? From the basic biological mechanistic point of view, thus far, studies on the therapeutic effects of rodent-derived cell grafts have proven to be predictive of the behavior of their human counterparts. This introduces expectations that their findings will be replicated with human neural stem cells (17, 18). A more difficult question is to what extent the animal model represents the human disease. Obviously, this depends on which patients will be selected for initiating clinical trials. The American Spinal Injury Association (ASIA) has classified 4 levels of trauma severity. The most severe forms are in patients with complete loss of function below the lesion site (ASIA-A)

or with partial sensory sparing (ASIA-B). Interestingly, even in the severe cases of injury by contusive trauma, there is often some spared tissue with continuity of the spinal cord, which is a necessary substrate for regeneration. These factors and the gravity of the condition of these patients (without any therapeutic alternative) render them good candidates for stem cell therapy. Importantly, the occurrence of some spontaneous functional improvement in the majority of human patients, even those with severe injury (19), suggests a similar (albeit very limited) type of spontaneous plasticity of spared projections as seen in rodents. In their study, Abematsu et al. (3) used a model of severe SCI, mimicking the type of injuries that are likely to serve as candidates for regenerative cell therapy. However, the main shortcoming of the mouse model of SCI lies in differences in the anatomic-physiologic organization of motor systems between rodents and primates. Gait and most other voluntary limb motor functions in humans are much more dependent on the corticospinal tract than in rodents. It therefore remains to be seen whether the formation of relays by transplanted neurons can indeed lead to functional recovery of the human spinal cord. Perhaps an answer to this question will be found in studying whether transplanted neurons connect preferentially to certain tracts. Moreover, can these preferences be manipulated by partially mimicking normal development in order to maximize the efficiency of regeneration (20)? Furthermore, can the multiple therapeutic effects of neural stem cell transplantation via direct neuronal regeneration and their proregenerative properties be combined to achieve the maximal synergistic therapeutic benefit? The experimental tools described so clearly by Abematsu et al. (3) will continue to be of use in efforts to answer these questions and meet these challenges.

Acknowledgments

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