

● PERSPECTIVE

Dynamic expression of ATF3 as a novel tool to study activation and migration of endogenous spinal stem cells and their role in neural repair

One of the major problems of modern neurobiology is how to replace dead or damaged neurons in the human brain or spinal cord after injury or as a consequence of neurodegenerative diseases. In fact, because adult mammalian neurons are post-mitotic cells that cannot divide to replace dead cells, loss due to lesion or disease is permanent. Furthermore, surviving neurons have modest capacity to regenerate their damaged axons and re-establish functional connections. Thus, a gradual neurodegenerative scenario with certain similarities in stroke, brain or spinal cord injuries and neurological diseases like Alzheimer's disease is produced. These conditions represent the major disease burden of the modern world in terms of mortality, disability, productivity loss and health-care costs (World Health Organization, 2008). While much effort has been directed to understand the molecular and cellular mechanisms involved in the pathology of these diseases to set new effective treatments, many neuroprotective and regenerative approaches, although showing positive results in preclinical studies, have so far failed to provide strong benefit to patients.

Spinal cord injury (SCI) of traumatic and non-traumatic origin (cancer, osteoporosis, spinal stenosis, infection, vascular disorders, *etc.*) produces lifelong, devastating consequences for patients who now number 40 million worldwide, with growing incidence every year (van den Berg et al., 2010): thus, reversal of paralysis and recovery of sensory dysfunction are critically important challenges. Despite improvements in the early management of SCI, there are no licensed treatments to significantly ameliorate neurological outcome. Recent therapeutic strategies for SCI arising from animal studies have mostly focused on stem cells, which might provide trophic and immunomodulatory factors to the injured spinal cord tissue, and may, thus, enhance axonal growth and contrast neuroinflammation, with the possibility to replace dead neurons. Common problems inherent to stem cell transplant are the risk of immune rejection and the need for an external source of cells (embryonal), with related ethical concerns. Clinical studies in which SCI patients received different types of stem cells have recently been reported but, unfortunately, there is no fully documented functional benefit for the majority of the patients (Neirinckx et al., 2014). This realization begs the question why the spinal cord, that intrinsically contains stem cells even in the adult (Johansson et al., 1999), cannot repair the initial damage. If endogenous neuronal stem/progenitor cells (SPCs) could be exploited for cell-based regenerative treatment of SCI, the risk of immune rejection and the need of the heterologous cells would be avoided.

Hope that the manipulation of endogenous spinal neural stem cells could represent one valid alternative to stem cell transplantation comes from the fact that the embryonal mammalian central nervous system (CNS) can readily recover after injury. This ability to regenerate injured spinal tissue, with complete recovery of locomotor function, unfortunately ceases soon after birth. The exception is marsupials that are born very immature and their capacity to regenerate spinal cord after injury is extended to the neonatal age. For example, the South American opossum can completely regenerate spinal cord tissue after injury up to 17 days postnatally, but then this process is abruptly stopped (Mladinic et al., 2009). Although the intrinsic

and extrinsic factors that allow neuronal tissue recovery after injury in newborn opossums are not fully understood, the process is likely to be related to the abundance of neuronal endogenous SPCs in the developing spinal tissue.

Can the endogenous stem cell potential be activated in the adult human CNS to contribute to functional recovery of the brain or spinal cord tissue after injury? The stem cell potential in the adult mammalian CNS is, however, limited: in the brain it resides in the subventricular zone of the lateral ventricles of the forebrain, and in the subgranular zone of the dentate gyrus of the hippocampus. Additional, recently discovered potential reservoirs of progenitor cells have been also found in the walls of the microvasculature (perivascular niches, harboring pericytes that express mesenchymal cell markers) and in the meninges. In the caudal nervous system, the neural stem cell potential resides mainly within the population of ependymal cells lining the central canal of the spinal cord (Johansson et al., 1999). In mammals, including humans, proliferation of ependymal cells and their progeny is a frequent event during embryonic and early postnatal development, but then it significantly declines. The neural stem cells present in the adult spinal cord are recruited and proliferate after spinal cord injury (Yamamoto et al., 2001), producing scar-forming astrocytes and myelinating oligodendrocytes (Meletis et al., 2008). Recent reports indicate an important role of spinal endogenous stem cells in restricting the tissue damage and neural loss after injury through the formation of a glial scar and exerting a neurotrophic effect for survival of neurons adjacent to the lesion (Sabelstrom et al., 2013). Nevertheless, this activity is insufficient to reverse the functional deficit in patients with SCI. Hence, controlled activation, migration and differentiation of SPCs could be critically important for neuronal regeneration after SCI. This goal, however, needs full understanding of the canonical signaling pathways and genes regulating SPCs quiescence and their fate in

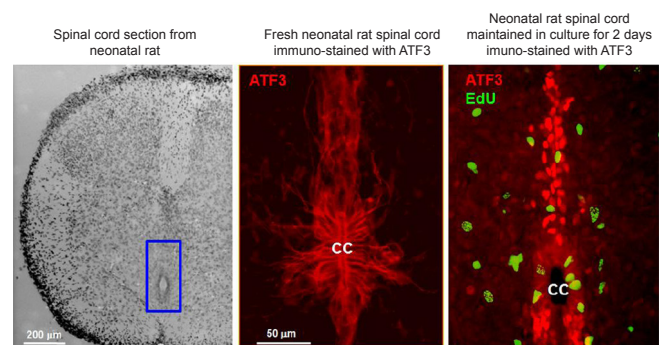


Figure 1 Activation and migration of ATF3-positive spinal stem cells. Left panel: 30 µm thick section of the neonatal rat spinal cord stained with the nuclear dye (4',6-diamidino-2-phenylindole (DAPI) to visualize nuclei of spinal cells (black negative image of bright fluorescence signal). The central spinal region containing the ependymal zone is marked with the blue rectangle. This region is shown at higher magnification in the middle and right panels.

Middle panel: the region around the central canal of the freshly fixed spinal cord (CC) labelled with a fluorescent antibody to visualize the fibrillary staining of the activating transcription factor 3 (ATF3; red).

Right panel: the region around the central canal of the spinal cord (CC) from a spinal cord maintained in culture for two days, to allow the activation of spinal ependymal stem cells. Markers are a fluorescent antibody to visualize ATF3 (red) or to observe the incorporation of (5-ethynyl-2'-deoxyuridine (EdU)) into proliferating cells (green). ATF3 is expressed in the nuclei of the activated spinal cord stem/progenitor cells that migrate from the ependymal zone around the spinal cord central canal versus dorsal and ventral funiculi, in a cell formation called funicular migratory stream (FMS). Proliferating cells that are also ATF3 positive are shown in yellow (modified from Mladinic et al., 2014).

normal and pathological conditions. Moreover, studying SPCs is especially difficult because of their heterogeneity as well as lack of specific expressional markers, because the ones currently used significantly overlap with those of mature astrocytes. Thus, the availability of new tools to monitor activation, migration and differentiation of spinal SPCs and their molecular switches is a major objective. These approaches may be complementary to understand if the same molecular and cellular events taking place during normal brain development are involved in post-injury adaptive plasticity potentially leading to spontaneous recovery, and if axonal sprouting following CNS injury can be further enhanced by intensive neurorehabilitation procedures.

One important contribution to this field may come from *in vitro* spinal cord injury models, in which the basic molecular and cellular mechanisms responsible for death, survival and regeneration of neurons after injury can be more easily investigated than *in vivo* and useful correlations between damage and loss of locomotor network function can be obtained. In the course of studies aimed at revealing the molecular mechanisms underlying SCI pathophysiology, spinal ependymal stem cells (identified as positive to markers such as nestin, vimentin and SOX2) were discovered to be intensely immunostained for the Activating transcription factor 3 (ATF3) in the neonatal and adult spinal cord (Mladinic et al., 2014). ATF3 belongs to the mammalian ATF/cAMP responsive element-binding (CREB) protein family of the basic leucine zipper (bZIP) transcription factors and is thought to control cell cycle and cell death machinery. It has been reported that the ATF3 has a role in survival and regeneration of peripheral axons and that it acts as regulator of neuronal survival against excitotoxic and ischemic brain damage (Zhang et al., 2011). However, the expression of ATF3 had not been previously reported in any type of stem or progenitor cells and its role in development of the intact central nervous system remains to be clarified.

The expression of ATF3 by rat ependymal SPCs is dynamically regulated: when the ependymal stem cells become activated *in vitro*, the ATF3 immunostaining changes from cytoplasmic to nuclear, which provides the unique possibility to detect and quantify activated cells and to follow up their migration and fate (Figure 1). The activation of SPCs is observed in association with their migration from the ependymal region surrounding a central canal toward the ventral and dorsal funiculi (Mladinic et al., 2014; Figure 1), a phenomenon reminiscent of the rostral migratory stream of brain subventricular stem cells. Although future studies have to decipher the role of ATF3 in SPC activation and migration, employing ATF3 as a readout tool to distinguish between quiescent and migrating rat spinal endogenous SPCs can help characterize the intracellular and the extracellular factors that control their activation and fate after injury.

Despite recent progress in understanding certain molecular and cellular mechanisms responsible for maintenance and mobilization of stem cells as well as neuronal regeneration, a number of fundamental questions remain: is the extracellular matrix of the SPC niche important for the activation of ependymal stem cells and what are the factors contained in blood or cerebrospinal fluid that can influence stem cell quiescence? What are the main molecular and cellular differences that determine whether the spinal tissue can (young mammals) or cannot (adult) regenerate after injury? What genes are differentially expressed in quiescent and migrating ependyma-derived stem cells? How can differentiation of the activated SPCs into neurons or glia be monitored? Using ATF3 as a tool to identify the activated, migrating spinal SPCs, may help answer such questions. Thereafter, it should be possible to address therapeutically related issues regarding the fate and the potential integration of SPCs into existing circuits of the spinal cord.

Because brain or spinal cord tissue reacts to injury in an

archetypal fashion, data arising from SPC studies might expand our general knowledge about neuronal stem cell maintenance and mobilization, and might be exploited for developing new therapeutic strategies to treat human patients with different neurodegenerative diseases. Recently, certain drugs and biomaterial scaffolds in combination with growth factors have been shown to enhance endogenous neurogenesis after stroke and to be promising for promoting structural and functional restoration of stroke-damaged neuronal tissue. Whether these data are applicable to SCI and have an impact on SPC activity remains to be elucidated.

Many questions are still open, but new discoveries regarding molecular and cellular events underlying development, regeneration and stem cell maintenance are bringing us closer to solving the major problem of effective medical treatment for CNS injuries and diseases.

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Accepted: 2015-03-31

doi:10.4103/1673-5374.156961 <http://www.nrronline.org/>
Mladinic M, Nistri A (2015) Dynamic expression of ATF3 as a novel tool to study activation and migration of endogenous spinal stem cells and their role in neural repair. *Neural Regen Res* 10(5):713-714.

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