

The Differential Intracellular Expression of the Novel Marker ATF-3 Characterizes the Quiescent or Activated State of Endogenous Spinal Stem Cells: A Tool to Study up Neurorepair?

Miranda Mladinic^{1,2*} and Andrea Nistri²

¹Department of Biotechnology, University of Rijeka, Rijeka, Croatia

²Neuroscience Department, International School for Advanced Studies (SISSA), Trieste, Italy

*Corresponding author: Miranda Mladinic, Associate Professor, Department of Biotechnology, University of Rijeka, Rijeka, Croatia, Tel: 385 51 584567; E-mail: mirandamp@uniri.hr

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Editorial

Worldwide, spinal cord injury (SCI) remains a major cause of disability with serious consequences in terms of personal and social costs [1]. Thus, important issues are how to protect the spinal cord to limit its initial damage, how to repair a lesion, and how to facilitate recovery by exploiting surviving tissue. These needs are currently unmet because our knowledge of the detailed structure of the neuronal networks responsible for human locomotion is scanty and our control over the mechanisms involved in neuronal death and regeneration is very limited. The molecular mechanisms underlying neuronal death after SCI are incompletely understood so that specific strategies for neuroprotection remain preliminary [2-4]. While many neuroprotective molecules have been reported to be experimentally effective for neuronal survival after SCI, very few have reached the clinical testing stage and none of them has provided efficacious treatment for SCI patients [5]. The reasons for such a clinical failure are complex and may include the diversity of protocols used to induce injury in animal models and the difficulty of detailed animal tissue analysis beyond a single time point so that a relatively narrow window of pathophysiology may be explored [6,7]. In clinical settings, the large majority of SCI cases are managed at late stages after the patient's conditions have been stabilized following the primary lesion. Hence, damage repair rather than neuroprotection becomes a crucial goal.

Exogenous stem cells for SCI therapy

In the mammalian central nervous system, lesioned neurons often do not regrow axons and, even if they might be induced to sprout fibers, they fail to re-innervate strategic network targets. Furthermore, because surviving cells do not replace or substitute dead postmitotic neurons, a neurodegenerative scenario is progressively established.

Within this framework of SCI studies, recent therapeutic strategies have mostly focused on repair mechanisms using stem cells [8,9]. One approach is to transplant embryonic or adult stem/progenitor cells (with or without prior manipulation *ex vivo*) into the site of spinal cord damage [10-13]. These studies have shown that stem cells might provide trophic and immunomodulatory factors to the injured spinal cord tissue, and may, thus, enhance axonal growth, remyelination of spared axons (by newly formed oligodendrocytes) and contrast neuroinflammation [1]. Even though generation of new neurons has been reported [14,15], the idea that these cells will replace dead neurons and integrate within neuronal circuits remains, however, a conjecture only [1]. The common problems inherent to transplantation-based strategies, namely the risk of immune rejection and the need for an external source of cells with related ethical

concerns, were recently addressed by generating autologous pluripotent stem cells from the skin fibroblasts manipulated with viral vectors [16]. These preliminary results need careful longitudinal studies to validate their safety especially in terms of cancerogenic potential.

There is, at present, an intense debate on the challenges to successful translation of promising cellular therapies and approaches from the experimental preclinical studies to clinically efficacious treatment for patients [17]. Details of the clinical studies in which SCI patients received different types of stem cell have recently been described [18,19]. Unfortunately, there is no fully documented functional benefit for the majority of the SCI patients recruited in such trials.

In particular, the first clinical trial was run by Geron USA with transplantation of human embryonic-derived oligodendrocyte progenitor cells. This study was approved by the FDA against a background of controversy regarding the ethical and safety concerns (formation of teratoma). Nevertheless, while the safety of the procedure was shown, no neurological recovery of the patients was obtained so that the trial was abruptly stopped [18,19]. Transplanting olfactory ensheathing glial cells, which offer the possibility of autologous transplantation, has confirmed the safety of this procedure in clinical trials without, however, providing documented motor improvement [19,20]. Likewise, even though transplantation of bone marrow derived mesenchymal stem cells has shown promising results in preclinical studies on animals (rats, pigs, non-human primates) with improved locomotor performance, this procedure has failed to show functional benefit to SCI patients in several small cohort studies [19]. Despite the fact that Schwann cells have long been investigated in preclinical transplantation research, very few clinical trials with SCI patients have been performed: one small trial completed after autologous transplantation, has shown the safety of the procedure, with no data on functional outcome [19,21].

The positive results obtained in animal studies using neural stem cells isolated from embryonic or adult spinal cord and brain, have encouraged translational studies using human central nervous system stem cells (HuCNS-SC). While this work started in 2011 in Switzerland and was sponsored by StemCells Inc, its results have not yet been published [19]. New hopes for transplantation strategies come from induced pluripotent stem cells that share many characteristics with embryonic stem cells, thus, allowing autologous transplantation to circumvent ethical concerns. Future studies are needed for their safety (teratoma induction?) and usefulness to SCI patients. Even though more pre-clinical studies with other types of

stem cell, like for example umbilical cord and adipose derived mesenchymal stem cells are required, two clinical trials have been already concluded or are in progress in Korea, with no data about the outcome [19].

Endogenous spinal stem cells

Activating the endogenous stem cells normally present in the mammalian spinal cord could represent a valid alternative to stem cell transplantation strategy after injury, since this is a non-invasive method that does not require immune suppression [22]. Indeed, in lower vertebrates, which can completely and spontaneously recover after SCI, endogenous spinal stem cells play a major role in the spinal cord regeneration process [23]. The mammalian spinal cord niches containing stem and progenitor cells capable of adult neurogenesis have been less investigated than those in the subventricular zone of the lateral ventricles of the forebrain, and in the subgranular zone of the dentate gyrus of the hippocampus [24]. The neural stem cells potential in the adult mammalian caudal nervous system resides mainly within the population of ependymal cells lining the spinal central canal [25]. Here, several cell types reside with different morphology, location, function and specific markers (ependymocytes, tanycytes, cerebral-fluid contacting neurons, etc.), plus a pool of stem and progenitor cells readily activated and recruited after experimental spinal damage [23]. Even though their sustained adult neurogenesis was not observed [23,26], the neural stem cells present in the adult spinal cord are recruited and proliferate after SCI [27] to produce scar-forming astrocytes and myelinating oligodendrocytes [22]. They can also be pharmacologically (with growth factors) and genetically manipulated to stimulate neurogenesis and oligodendrogenesis [28]. Recent reports have indicated an important role of spinal endogenous stem cells in restricting the tissue damage and neural loss after injury through the formation of glial scar and exerting the neurotrophic effect required for survival of neurons adjacent to the lesion [29].

Although spinal cord endogenous stem/progenitor cells represent a potential source for future SCI treatments, there is the immediate need to fully identify them prior to any manipulation for their recruitment after injury. Spinal stem/progenitor cells are especially difficult to identify due to their heterogeneity as well as lack of specific expressional markers, since the ones currently in use significantly overlap with those of mature astrocytes [30]. In addition, there is no specific marker to discriminate between quiescent and activated ependymal spinal cells, nor any one capable of monitoring their migration and differentiation after injury. Furthermore, the signalling pathways and genes that control the fate of the spinal cord stem/progenitor cells in normal and pathological situations, are largely unknown [23]. In fact, the transcription factors controlling spinal cord stem cells should be extensively studied because to date they have been investigated with *in vitro* primary cultures only [27] and their applicability to the *in vivo* tissue remains to be tested.

One important contribution to this field may come from *in vitro* spinal cord injury models, in which the basic molecular mechanisms involved in the death, survival and regeneration of neurons after SCI can be investigated at preselected time points and useful correlations between damage and loss of locomotor network function can be obtained [3,31].

In the course of the studies trying to reveal basic molecular mechanisms involved in the pathophysiology of the SCI using the *in vitro* spinal cord preparation, activation of spinal stem/progenitor cells

(positive for stem/progenitor cells markers such as nestin, vimentin or the SOX2 transcription factor) is observed in association with their migration from the ependymal region surrounding a central canal toward the ventral and dorsal funiculi. This pattern is largely reminiscent of the rostral migratory stream of brain subventricular stem cells [32] (**Figure 1**).

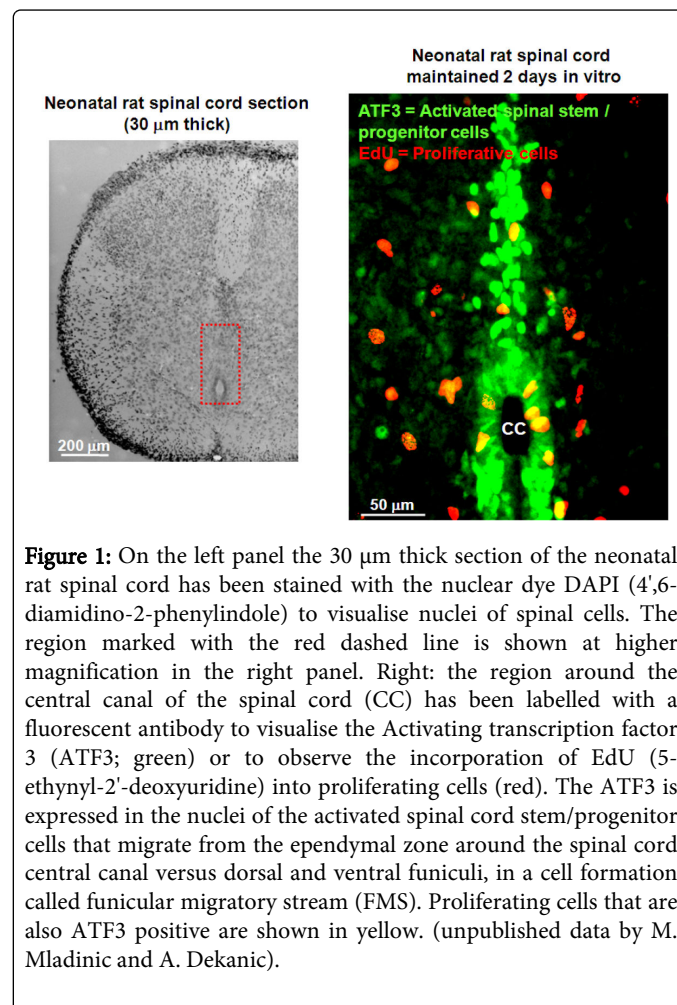


Figure 1: On the left panel the 30 µm thick section of the neonatal rat spinal cord has been stained with the nuclear dye DAPI (4',6-diamidino-2-phenylindole) to visualise nuclei of spinal cells. The region marked with the red dashed line is shown at higher magnification in the right panel. Right: the region around the central canal of the spinal cord (CC) has been labelled with a fluorescent antibody to visualise the Activating transcription factor 3 (ATF3; green) or to observe the incorporation of EdU (5-ethynyl-2'-deoxyuridine) into proliferating cells (red). The 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. (unpublished data by M. Mladinic and A. Dekanic).

Both quiescent and migrating spinal stem/progenitor cells express the Activating transcription factor 3 (ATF3), which currently has no clear role in neuronal development of the intact nervous system, and its expression has not been previously reported in any type of stem/progenitor cell. ATF3 belongs to the mammalian ATF/cAMP responsive element-binding (CREB) protein family of the Basic Leucine Zipper (bZIP) transcription factors [33] and is thought to be a stress inducible gene and an adaptive response gene, which, when activated by various stimuli, can control cell cycle and cell death machinery [34]. Although ATF3 expression is normally very low in central nervous system, it is markedly upregulated in response to injury and closely linked to survival and regeneration of peripheral axons [34]. ATF3 is supposed to have a role in neurite growth and regeneration [35] and it has been identified as regulator of neuronal survival against excitotoxic and ischemic brain damage [36,37].

It is particularly interesting that ATF3 expression in spinal ependymal stem/progenitor cells is dynamic, as this protein is localized to the cytoplasm of such cells when they are quiescent, and it

is detected in the nucleus when cells became activated [32] (Figure 1). This discovery opens the new possibility to track down migrating spinal stem/progenitor cells after injury and to identify the molecules that control their activation (for example miRNA differentially expressed in quiescent versus migrating ependyma-derived cells). Furthermore, the nuclear ATF3 expression in migrating spinal stem/progenitor cells gives the possibility to monitor their translocation and differentiation (versus neuronal or glial cell lineages) after injury.

Although future studies have to decipher the role of ATF3 in spinal stem/progenitor cell maintenance and mobilization, the identification of ATF3 as a unique marker to distinguish between quiescent and migrating spinal endogenous stem/progenitor cells can help characterizing those factors that control their activation and fate after injury, and might become a reporter molecule in studies of drug testing for SCI outcome.

Conclusion

The former disappointing results from clinical studies exploring neurorepair strategies to restore neuronal connectivity in the networks that underlie standing and walking, should stimulate novel approaches aimed at exploiting the endogenous rewiring potential of the ependymal stem/progenitor cells. These goals demand precise basic knowledge of their niche topography, their transcriptional potential, metabolism, and physiology. Thus, in the future, full genomic and functional characterization of these cells should provide new opportunities to control their migration toward the lesion sites and their neuronal differentiation.

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