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PERSPECTIVE

Endogenous neural progenitor cells in the repair of the injured spinal cord

Stem cell treatments, and in particular, stem cell transplants have been identified as potential therapeutic strategies for a range of neurodegenerative and acquired conditions of the central nervous system (CNS). Stem cell transplants are seen as a way of replacing lost neurons, or providing a cellular environment that is more permissible for axon and cell regeneration. An alternate strategy to transplantation of exogenous stem cells is the recruitment and manipulation of endogenous neural progenitor cells (NPC). NPCs exist in the spinal cord and have been shown to proliferate, migrate and differentiate in response to injury (Mothe and Tator, 2005; Meletis et al., 2008; Hamilton et al., 2009). NPCs may have a wider role than previously thought and it has been reported that NPCs also engage in 'cross talk' with immune cells and may be able to modulate the inflammatory response following injury. Our research has focussed on determining the exact timing and location of NPC activation after injury. It is clear that there will be a critical window of opportunity to implement a treatment that harnesses the NPC response to spinal cord injury (SCI), and that this must occur before the full cascade of inflammation has been initiated.

Traumatic SCI causes the destruction of neuronal and glial cells leading to the disruption of sensory and/or motor functions. To date, there is no known cure for SCI. There is evidence that primitive vertebrates such as the teleost fish (Zupanc and Sîrbulescu, 2012) and lizards (Fisher et al., 2012) are able to regenerate spinal tissue after trauma resulting in extensive axonal regrowth and functional recovery. In these animals it is the recruitment of NPCs that plays a major role in the regeneration. NPCs are the precursor to all of the neural cells - neurons, astrocytes and oligodendrocytes. Following injury, including axonomy, in the lower vertebrates several genes and structural proteins such as Sox1 and fibroblast growth factor-2 are upregulated (Ferretti et al., 2001) which drives the regeneration of neuronal cells and eventually leads to an elongation and regrowth of the spinal cord.

Vertebrate mammals have lost this capacity to regrow nervous tissue following injury even though NPCs are found lining of the central canal of the spinal cord. During non-pathological conditions, they remain in a quiescent state in the ependymal layer and demonstrate minimal proliferation activity to maintain the homeostasis of neural cells in the spinal cord (Hamilton et al., 2009). In rodent models NPCs are observed to increase proliferation and differentiation significantly and then migrate to the lesion area following a SCI (Mothe and Tator, 2005; Meletis et al., 2008). However, in rodent models, migrating NPCs are more likely to differentiate into astrocytes forming the glial scar (Mothe and Tator, 2005). While differentiation into all three lineages can occur *in vitro* under the correct growth conditions, no spontaneous differentiation of NPCs into neurons *in vivo* has been documented. One study by Guo et al. (2014) induced the transcription factor Sox11 *via* a lentiviral vector in a mouse SCI model, and demonstrated that it is possible to differentiate NPCs into neurons *in vivo*. In ependymal cells, the activation of Sox11 is associated with an upregulation of Nestin, a type VI intermediate filament protein found predominately in NPCs in the developing or injured spinal cord (Tzeng, 2002). Consequently these cells have the potential to be manipulated *in situ*, offering a novel approach to restore the injured spinal cord and much effort is made to identify the response of NPCs to traumatic SCI in relation to location, time and inflammation.

Activation of endogenous neural progenitor cells throughout the neuroaxis: Following injury to the CNS, we have shown that there is an increase in Nestin reactivity beyond the lesion site in humans (Cawsey et al., 2015). In our rat model of T_{10} contusion injury an increased expression of Nestin was also observed both caudally and rostrally to the injury site (**Figure 1A**, **B**). This suggests a whole spinal cord response to a discrete lesion localized to the T_{10} region of the spinal cord. The response may be occurring due to chemical signals, such as inflammatory cytokines, travelling through the cerebrospinal fluid (CSF) and interacting with the quiescent endogenous NPCs located beyond the lesion site. Exactly why NPCs are reacting distal to the injury site is unknown at this stage, although it is possible that the ependymal cells have a surveillance role involved in 'sampling' the CSF in the same way in which tanycytes lining the third ventricle are thought to communicate with the hypothalamus. Our studies have used the immunohistochemical expression (**Figure 1C–E**) of Nestin as a marker of endogenous NPCs. Following SCI, nestin positive ependymal cells have a very similar morphology to that described for tanycytes, with long basal processes, and interactions with blood vessels, however, whether they have similar functions in regenerating the CNS during an injury is difficult to determine since endogenous NPCs are not a homogenous cell population.

Modulation of immune responses by neural progenitor cells: While the majority of studies have classically targeted endogenous NPCs as a structural or cell replacement treatment after SCI, another significant role for NPCs in the post-injury microenvironment is emerging. That is the role of immune responses within the CNS following trauma. In recent years, a high degree of interaction and 'cross-talk' has been highlighted between the nervous and immune systems (Cusimano et al., 2012). The term 'therapeutic plasticity' was coined to describe NPCs emerging abilities to modulate their surrounding environment, as well as respond to its cues, and instruct other innate and immune cells after trauma (Cusimano et al., 2012). The beneficial effects of NPCs as immune modulators include secreting a variety of neurotrophic molecules, responding to the cues from the microenvironment, as well as instructing the innate immune cells *via* cellular junction coupling between NPCs and active phagocytes including macrophages and microglia. A recent study highlighted the ability of transplanted NPCs to survive undifferentiated at the peri-lesion area, in order to modulate the expression of inflammatory gene transcripts (Cusimano et al., 2012). As a consequence, the number of pro-inflammatory macrophages was reduced at the lesion area at 7 days post-injury, with the ultimate results of limited secondary injury and improved healing. There is also mounting evidence to suggest that NPCs in the CNS can switch to a 'chaperone' phenotype to assist in tissue repair *via* inherent regulatory and protective activities (Hauwel et al., 2005). This is likely mediated by growth and trophic factors such as brain-derived neurotrophic factor (BDNF), members of the nerve growth factor (NGF) family and the prevalent cytokines in the microenvironment. This releasing and receiving of effector molecules place greater emphasis on the synergy between NPCs and immune cells. From this, the endogenous NPCs may also be manipulated to engage in modulation of immune responses as a reparative strategy for SCI.

Window of opportunity to manipulate endogenous neural progenitor cells: There will be a valuable window of opportunity, within a relatively short time following injury, to manipulate endogenous NPCs as a novel approach for SCI repair. We have shown that endogenous NPCs at the central canal are activated within 1 week, and most likely within 24 hours, after SCI in a rat model, while the astrogliosis (GFAP reactivity) starts forming at the lesion area from 1 week (Mao et al., 2016). These activated NPCs are thought to induce astrogliogenesis spontaneously, contributing to the formation of astroglial scar at the lesion area. Therefore, therapeutic interventions should be conducted to promote neurogenesis, or reduce inflammation, prior to the astroglial differentiation in order to build up functional nerve tissue instead of astroglial scar. It is postulated that a reduction in astrogliosis with a simultaneous increased survival of neurons is beneficial for spinal cord repair and functional recovery. In addition, early interventions to manipulate endogenous NPCs

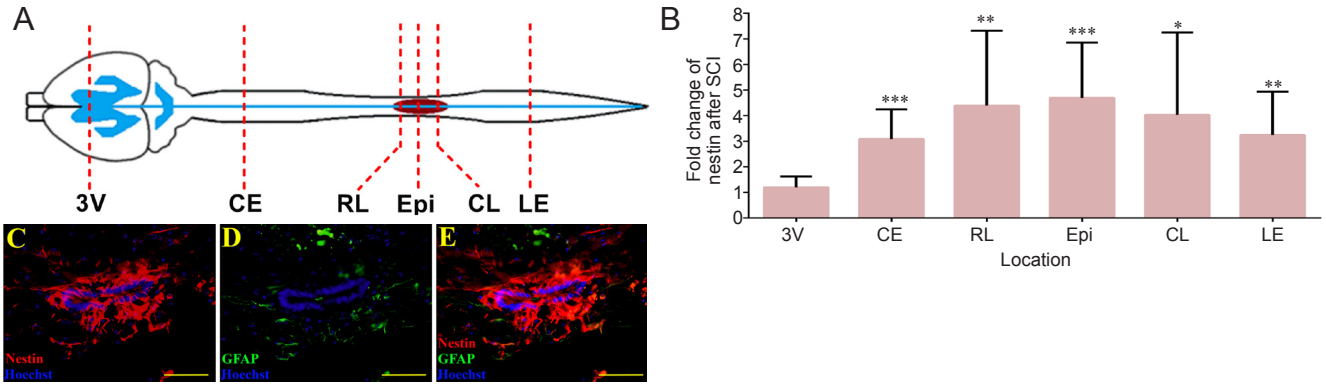

Figure 1 Nestin expression throughout the neuroaxis following a contusion injury at T_{10} in a rat spinal cord.
(A) A schematic of the central nervous system with a spinal cord injury (SCI; red). The blue structure represents the neuroaxis consisting of the ventricular systems of the brain and the central canal of the spinal cord. Nestin immunoreactivity was measured at the median eminence of the third ventricle (3V), cervical enlargement (CE), 2.25 mm rostral to the epicentre of lesion (RL), the T_{10} epicentre of lesion (Epi), 2.25 mm caudal to the epicentre of lesion (CL) and lumbar enlargement (LE). (B) Fold change of Nestin immunoreactivity following spinal cord injury throughout the neuroaxis compared to the sham. (C) Fluorescent immunohistochemistry demonstrated the Nestin positive cells (red) at the ependymal area of the spinal cord while the nuclei were counterstained by Hoechst (blue) at 2.25 mm rostral to the epicentre of the lesion. (D) Fluorescent immunohistochemistry demonstrated the glial fibrillary acidic protein positive cells (green) at the ependymal area of the spinal cord while the nuclei were counterstained by Hoechst (blue) at 2.25 mm rostral to the epicentre of lesion. (E) The merged image of Nestin (red) and glial fibrillary acidic protein (green) staining at the ependymal layer on rat spinal cord transverse sections at 2.25 mm rostral to the epicentre of lesion. Nestin positive cells at the central canal did not express glial fibrillary acidic protein. Scale bars: 25 μm. *t*-test, injury *vs.* sham, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

may be able to circumvent the deleterious chronic inflammatory responses occurring. The initial inflammatory response at the acute phase of SCI is required, however the persistent pro-inflammatory response is ultimately detrimental. We have shown a robust pro-inflammatory response within the first week following a SCI involving neutrophils, peaking at 24 hours. This is followed by a significant influx of macrophages, predominantly M1 like, peaking at 1 week. It is the continuation of these responses that becomes detrimental to recovery and an early modulation by NPCs will assist in alleviating this as well as promoting neuronal survival.

Summary and conclusions: There is rising evidence indicating that manipulation of the endogenous NPCs could be an innovative platform for the treatment of acute SCI. The use of endogenous NPCs has the advantages of avoiding risky surgical procedures, bypassing invasive delivery systems, eliminating tumorigenesis and reducing a rejective immune response over transplantation of stem/progenitor cells. It also negates ethical concerns regarding the use of embryonic stem cells or stem cells from alternate sources that might delay the application of cell grafts for SCI patients.

However, our research, and that of others, highlights the need for further investigation regarding spinal cord NPCs. What is their normal role in the spinal cord? How can a treatment be targeted at the local injury site only, not the entire CNS? What are the characteristics of the endogenous NPCs in the injured spinal cord, especially in relation to inflammatory responses? What are the sub-populations of cells in the ependymal layer of the central canal and what is their contribution to injury response? Further research needs be conducted to develop a viable strategy to manipulate the endogenous NPCs into neuronal termination to repair the injured spinal cord, and to modulate the inflammatory response to reduce ongoing tissue damage.

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