Chapter 11

Neural Stem/Progenitor Cells for Spinal Cord Regeneration

Ryan Salewski, Hamideh Emrani and
Michael G. Fehlings

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55054

1. Introduction

Central nervous system (CNS) repair and regeneration following traumatic injury or disease pathology is a widely studied and widely debated field. This book chapter will outline the pathology of spinal cord injury (SCI) with particular focus on how it lends itself to cell-based intervention. Next, we will outline the different populations of cells proposed for SCI treatment, including neural stem/progenitor cells (NSPC), and methods of generating clinically relevant NSPCs from adult tissue, embryonic stem (ES) cells and induce pluripotent stem (iPS) sources. Lastly, we will examine the use of NSPCs in SCI models with a specific focus on how the environment affects the transplanted population and how the transplanted cells modulate the spinal cord niche.

2. Pathology of spinal cord injury

Spinal cord injury (SCI) is a devastating event that significantly affects the morbidity and quality of life in adults (Average age of patients at the time of injury is 38.0 years old). [1] The prevalence of traumatic SCI worldwide is approximately 750 per million with an increasing annual incidence. [2] In order to be able to develop effective treatments for SCI, it is necessary to have a detailed understanding of the pathophysiological events that happen during SCI in the body and how they interact with each other to cause the functional deficits seen in patients. These events have complicated roles post-SCI. For example, the inflammatory response and reactive astrogliosis that are seen post-SCI have damaging *and* neuroprotective and –restorative effects. [3]

The pathophysiology of SCI is biphasic; it consists of a "primary" and a "secondary" stage. The primary injury is the result of an initial mechanical insult, which is followed by a series of cellular and molecular events that, in turn, lead to further destruction of the spinal cord tissue. It is well known that the mechanical injury seldom results in complete transection of the spinal cord. Most fracture dislocations or burst fractures of the spine result in disc displacement into and laceration or compression of the cord resulting in blood vessel, axonal and cellular membrane disruption.

Decreased blood supply to the tissue and cells of the spinal cord results in nutrient and oxygen deficiency in the injured tissue, which in turn is followed by a cascade of biochemical or "secondary" events that eventually lead to further tissue necrosis. [3-5] Thus, the primary insult precedes sequential pathological changes such as further vascular dysfunction and hemorrhage, edema, ischemia, excitotoxicity, electrolyte shifts, free radical production, inflammation, axonal and neuronal necrosis, demyelination, cyst formation and infarction. [5] The events will be discussed in further detail in following sections and are summarized in Figure 1.

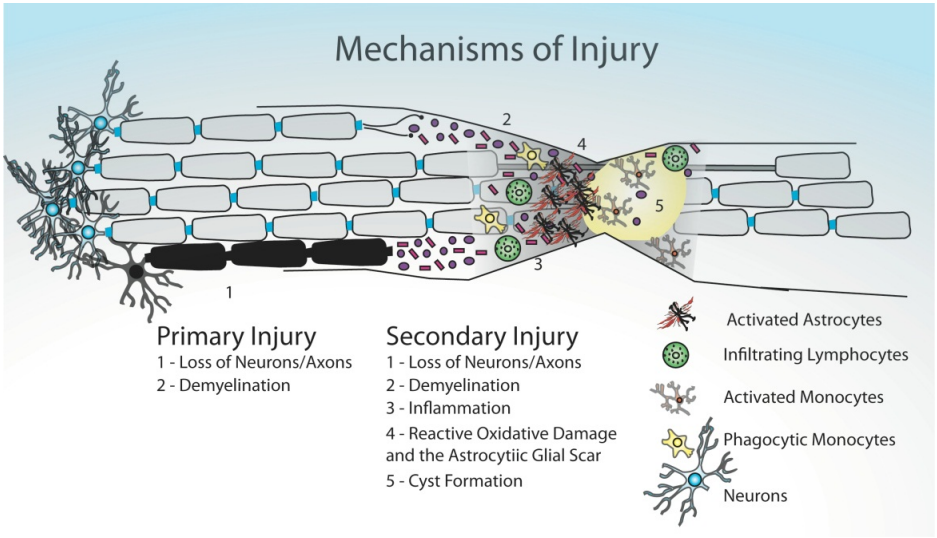

Figure 1. Mechanisms of Injury in SCI. SCI pathology results from several mechanisms occurring both concurrently and in sequence. Primary mechanical insult results in initial loss of axons (1) and demyelination (2). Subsequent secondary injury is characterized by further neuronal/axonal cell death (1) and myelin degradation (2), usually due to secondary inflammation from infiltrating lymphocytes and monocytes (3) and also reactive oxygen species secreted by activated astrocytes composing the glial scar (4). Both the glial scar (4) and post-traumatic cyst or syrinx formation (5) pro- vide physical impediments to regeneration, and cyst formation can further damage neurons by exerting physical pressure on their damaged axons. * from *Ruff and Fehlings. (2010). Panminerva Med 52:125-147, with permission*

SCI is a complex set of damaging events that occur at the cellular level and it can be divided into four main stages: the immediate, acute, intermediate, and chronic phase of SCI.[3] The

first two hours post-SCI is the immediate stage during which the body is going through processes as a result of the primary injury itself. Severing of axons, neural and glial death, and spinal shock, all instantly result in loss of function below and at the level of the injury. The spinal cord becomes swollen which is accompanied by hemorrhage into the grey matter, cellular necrosis, vascular disruption and ischemia. Vascular disruption leads to further hemorrhage into the white matter and, as a result of the combined effect of edema and hemorrhage, many segments of the spinal cord, rostral and caudal to the site of injury, become ischemic. [6] Even though gross histopathological changes may not be visible at this stage, many pathophysiological events have already started. For example, activation of microglial cells and an instantaneous increase in pro-inflammatory cytokines TNFand IL and excitotoxic levels of extracellular glutamate can be detected within minutes of an injury. [3, 7, 8]

2.1. Stages SCI progression

2.1.1. Acute stage

During the acute stage of the SCI, the events of the secondary injury prevail. This is subdivided into early acute (2-48 hours) and subacute (2 days-2weeks). Vascular disruption, hemorrhage, and the resulting ischemia are central constituents of the early acute stage [5, 7], which result in the loss of normal autoregulatory mechanisms. The loss of ionic homeostasis immediately following SCI and excitotoxicity are closely related processes that each significantly contribute to the propagation of cellular injury after SCI. The ionic deregulation plays an important role in increasing necrosis and cell death following SCI. For instance, irregularity of Ca2+ concentration initiates activation of calpains and caspase cascades which in turn cause further cellular apoptosis. [9] Additionally, activated microglia express FAS ligand receptors and signaling through the p75 neurotrophin receptors that initiate the caspase and calpain cascade leading to proteolysis and DNA cleavage by effector caspases and trigger even further apoptotic cell death. [10] As a result of these events, a large number of cells, including oligodendrocytes, die. In addition to death of the neural cells, loss of calcium, sodium and potassium ionic channel homeostasis, and demyelination disrupt signal transduction in the spinal cord and result in sensorimotor inactivity and paralysis. [3-5]

The extracellular levels of glutamate rise rapidly due to direct injury to cells and failure of energy-dependent transporters, notably the Na+K+ adenosine triphosphatase membrane transporter that normally functions to regulate extracellular concentrations of ions, glutamate, and other molecules. [11] Excessive activation of glutamate receptors leads to an increase in the influx of Na+ and Ca2+ through the NMDA and alpha-amino-3-hydroxy-5-methyl-isoxazoleproprionate/kainate receptors and excitotoxicity. [12] This causes further neural and glial death.

Reactive oxygen (O2) species (ROS) is at detectable levels 12 hours after SCI and remains elevated for almost one week, returning to preinjury levels 4-5 weeks post-injury. The increased amount of ROS leads to increased membrane lipid oxidation and an increase in cellular lysis, organelle damage and Ca2+ ion concentration irregularities. [13, 14] It has been

shown that Peroxynitrite, which is generated through reaction of ROS and nitric acid, is directly involved in the induction of neural apoptosis in rat SCI. [13]

The brain blood barrier (BBB) is a highly selective endothelial filter for transport of compounds in and out of the central nervous system. This becomes highly permeable as a result of the primary mechanical insult to the cord and presence of inflammatory mediators. It has been shown that peak BBB permeability occurs at 24 hours following injury in the rat and returns control levels by 2 weeks. [15] Two inflammatory mediators – TNF and IL-1 - are known to increase vascular permeability. In addition, other compounds released by glial cells or invading immune cells which are believed to play a role in increasing BBB permeability include ROS, nitric oxide, histamine, matrix metalloproteinases, and elastase. [3, 13] Additionally, disruption of the vasculature following SCI results in the breakage of the BBB, leading to an inflammatory response and infiltration of astrocytes, microglia, T cells, neutrophils, and invading monocytes. Invasion of macrophages and neutrophils to the site of injury increases cellular disruption and causes further inflammatory-triggered necrosis. Inflammation then results in activation of microglia which, together with leukocytes, cause malfunction of oxidative metabolism in demyelinated axons and proliferation of astrocytes as well as increased expression of glial fibrillary acid protein (GFAP) and the formation of a glial or astrocytic scar. [2-4] Further to this, a multitude of noncellular mediators, including TNF, interferons, and ILs play important roles.

The inflammatory response in SCI is a highly complex event with a dual nature. Some aspects of the inflammatory response further advance the secondary injury phase, while others are beneficial in removing cellular debris and starting the regenerative process [16]. For instance, TNF plays an important role in cellular death post-SCI and its inhibition has been shown to result in functional neurological recovery after SCI. However, it has also been shown to have a neuroprotective role in *in vitro* and murine models of SCI. [17, 18]

2.1.2. Subacute phase

The subacute phase lasts from 2 days to 2 weeks after the initial injury. This is the stage where it is hoped that therapeutic strategies currently in development, including cellular treatments and strategies, will be helpful. For instance, we have shown in our lab that transplantation of adult murine neural precursor cells at 2 weeks post-injury promotes remyelination and functional recovery. Such effects were not seen when the cells were transplanted at later time points or during the chronic stage of the injury. [19] Keirstead et al, have also reported the failure of transplanted human ESC-derived oligodendrocyte progenitor cells to survive, migrate and promote functional recovery when they were injected to the site of injury either at the time of injury or after the subacute phase. [20]

During the initial hours to days following SCI, astrocytes undergo cytotoxic edema and necrotic cellular death; however, a second delayed astrocytic response happens during the subacute phase. During this stage, there is a significant increase in the astrocytic intermediate filament GFAP as a result of the proliferative and hypertrophic activity of astrocytes. These reactive astrocytes form the astrocytic (gliotic) scar through their interweaving cytoplasmic processes, which becomes a physical and chemical barrier to axonal regeneration. It is

important to note that in humans, there is significantly less astroglial scarring observed than is observed in rodent SCI models. [21] Other than scar formation, astrocytes also promote the reestablishment of ionic homeostasis and the integrity of the BBB, which is beneficial in decreasing edema and immune cell infiltration. [22]

During the subacute stage of the injury, there is a significantly higher phagocytic response observed in the CNS compared to the PNS. While this could partly account for the lack of regenerative ability seen in the CNS, the phagocytic response is believed to be efficient in cell debris removal and, to some extent, in promotion of axonal growth inside the lesion. [13]

2.1.3. Intermediate phase

The intermediate phase starts at two weeks and continues to 6 months post-injury. Main characteristics of this stage are continuous maturation of the gliotic scar and the start of axonal regeneration. Regenerative axonal sprouting has been observed in corticospinal tract axons in rat models of contusive SCI from 3 weeks to 3 months following injury, and reticulospinal fibers have been observed from 3 to 8 months post injury. [23] It is encouraging to observe that regenerative potential still exists in adult spinal cord, however, these regenerations have not been able to produce significant functional recovery in severe SCI.

2.1.4. Chronic phase

The chronic phase starts about 6 months after the initial SCI and lasts throughout the lifetime of the SCI patient. Further gliotic scar formation, development of cysts and syrinxes within the lesion, and continuous Wallerian degeneration of severed and injured axons are the main characteristics of this stage. [3, 23-25] Two to three years post-injury, the lesion has usually stabilized and is characterized by formation of a cyst cavity and mayelomalacia, which is the final stage of necrotic death. [26] Treatment strategies at this stage focus on rehabilitative approaches to promote plasticity, regeneration and remyelination of injured axons. Unfortunately, to date there has not been any report of the application of stem cells at this stage resulting in any significant functional improvement.

2.2. Cell-based approaches in SCI

As explained in the previous section, in the pathophysiology of SCI, some processes can be considered to be double-edged swords with both positive and negative effects and interations., These are not yet completely understood. One example of such an interaction is the astrocytic response which, on one hand results in the development of astrocytic scar limiting axonal regeneration and functional recovery, and on the other results in reestablishment of the BBB, ionic homeostasis, and decreased immune cell infiltration. What makes the pathophysiology of SCI and the translation of treatment strategies into the clinic even more complicated is its variance and uniqueness among different patients with different cases of injury. Experimental SCI models, which are mostly rodent models, consist of a homogeneous population, in contrast to the vastly heterogeneous human SCI population.

Two main aims of cell-based treatment for SCI are replacing lost or injured cells such as oligodendrocytes and neurons, or providing the cells with a microenvironment that supports or enhances the neuroprotective and regenerative ability of cells within the lesion. Stem and progenitor cells, and Schwann cells are examples of cells that are being used in studies to replace lost cells. Additionally, many studies have shown that these cells have the ability to remyelinate axons in the injury site while also providing them with supportive growth and neuroprotective factors. [1, 19, 27-29]

3. Cell populations for SCI treatment

Even though modern surgical and medical treatment approaches have dramatically decreased mortality rate due to SCI, current clinical techniques provide only modest efficacy in improving outcomes for patients, especially during the chronic stages of the disease. Thus, there is a need for new techniques that could improve recovery for SCI patients. Cell replacement strategies use stem cells because of their long-term proliferative abilities and the trophic support they provide to the regenerating tissue. In the following sections we will briefly review different types of stem cells that are being investigated as cell sources in therapeutic approaches for SCI.

3.1 Cell types used in SCI therapy

3.1.1. Mesenchymal stem cells

Multipotent mesenchymal stem cells (MSCs) are non-hematopoietic multipotential progenitor cells that were initially characterized by Caplan as cells with self-renewing capacity that can give rise to skeleton and other lineages of the mesenchyme through the mesengenic process [30, 31]. However, with the exception of a few stem cells, which are progenitors with "clonal self-renewal and multilineage potential", MSCs represent a non-homogenous population of cells and not all of the cells have the same potential for differentiation. Therefore, the International Society for Cellular Therapy (ISCT), suggests usage of the term, "Multipotent mesenchymal stromal cells", for the plastic adherent population of cells isolated from stromal tissue of bone. [32] Additionally, in 2006 Dominici et al., from the International Society for Cellular Therapy, defined a minimum set of criteria for MSCs some of which include lacking hematopoietic cell surface markers such as CD34 (endothelial marker) and CD45 (pan-leukocyte marker), and containing cell surface markers such as CD105 (endoglin), CD90 (Thy-1), and CD73 (ecto 5′ nucleotidase) and to be able to generate at least three lineages of the mesenchyme [33]. MSCs are primarily isolated from bone in adults but they also reside in adipose tissue, cartilage, synovium, periosteum, muscles and fetal tissue such as placenta and umbilical cord.

There are many studies on the application of MSCs to SCI models with variable reports on the ability of the cells to survive, integrate, and differentiate into neuronal lineages. [34] MSCs from both rats and humans have been shown to be capable of generating neural morphology and expressing neural and astrocytic markers *in vitro*. However, they have similar differentiation profiles only when transplanted into an embryonic or developing CNS. When trans-

planted into adult rat CNS, neither rat nor human MSCs were able to differentiate into neuronal cells. Mostly, they generated perivascular macrophages in the rat brain. [35-37]

There are other studies in which transplanted MSCs were tracked in rat or mouse injury models through GFP or electromagnetic tagging coupled with immunochemistry and MR imaging. Even though both types of studies report functional improvements, only a fraction of cells are reported to show expression of neuronal or astrocytic markers and only a very small number of cells are reported as having differentiated into neurons. [37-39] There are differing reports on the extent of MSC transplantation benefits in functional recovery. MSCs have shown to be effective at 4 weeks up to 1 year after the injury [41], depending on the timing of transplantation from the time of injury [40] to 1 week [37, 38, 41], or 3 months post injury [42]. Other studies show lack of any functional benefit observable through the Basso Beattie Bresnahan (BBB) open field locomotor scoring system. [43] However, all of the mentioned studies report on the ability of MSC transplants to preserve the injured tissue, both gray and white matter, and to provide micro environmental cues supportive of axonal growth, rather than replacing lost cells. [42, 43] Given the inconsistency seen in the results of transplanting MSCs into SCI models in rats, mice and primates, it is necessary to have a better understanding of the type of cells in the MSC population that are being transplanted[44] and investigate all the possible mechanisms behind the functional recovery observed in these models.

3.1.2. Schwann cells

Schwann cells are the myelinating cells in the peripheral nervous system. Since the first transplantation of SCs into SCI by Duncan et al. in 1981 [34], these cells have been used in many different SCI models and have been shown to be able to remyelinate axons and provide a permissive environment for them to regenerate and grow. Binge et al. [45] showed that SCs also have a small but significant effect on increasing functional recovery. However, their remyelinating and regenerating ability does not go beyond the growth-permissive surface, which explains why there is only a slight improvement in functional recovery. [3, 44-46] Additionally, it has been shown that axons in the corticospinal tract (CST) remain unaffected by SC transplants. [34] Also, they have been shown to generate a more active astrocytic response compared to other cell types such as NPCs which results in less efficient integration of these cells into the injured spinal cord. [34] As a result, the clinical application of SCs alone for SCI treatment appears extremely limited.

To overcome this limitation, many combinatorial strategies have evolved which associate SCs with growth factors or bioengineering scaffolds and other cells types to enhance recovery. [4, 47-50] Additionally, new sources for SCs are being investigated such as skin derived precursor cells from the dermis of the skin [51-54] and MSCs. [55-58] However, it is yet to be proven whether these cells hold any advantage in treating SCI compared with SCs that are derived from the PNS.

3.1.3. Olfactory ensheathing cells

OECs are glial cells derived from the olfactory bulb or lamina propria of the olfactory mucosa and are termed olfactory nerve Schwann cells due to morphological similarities to SCs. Having a lifelong capacity to proliferate and being able to facilitate passage of new axons from

regenerating receptor neurons in the PNS in the olfactory mucosa to the target neurons in the CNS in the olfactory bulb glomeruli, has made them an excellent candidate for cell treatment strategies in SCI. [58, 59] They may be specifically advantageous when co-transplanted with SCs, since they can overcome SCs' limitations in passing the transplant graft and entering the injured CNS environment to produce functional synapses. [3, 60, 61]

OECs create a permissive environment for axonal growth and regeneration by interacting with the astrocytes in the glial scar and promoting angiogenesis. [62] This neuroprotective effect has led to their use in numerous clinical trials outside North America. However, they all report mixed results with none showing a significant benefit. [3, 27] Additionally, there are numerous axonal regeneration claims seen in OEC literature, which have not been confirmed by other studies and there are inconsistent reports on their regenerative capacity *in vivo*. The reasons for such discrepancies are not completely understood but could be attributed to variability in cell sources, cultures, and injury models studied. [63, 64] Thus, there is a need for further studies on the biology of OECs and more refined criteria set for isolation of these cells in order for their translation into the clinic to be feasible. [34]

3.1.4. Neural stem/progenitor cells

Neural Stem/Progenitor Cells (NSPCs) are multipotent stem cells that self-renew and differentiate into lineage-specific neural precursor cells, which can give rise to neurons, astrocytes, and oligodendrocytes through asymmetric cell division. [30] Indeed, neural precursor cells and oligodendroglial precursor cells have been shown to replace damaged cells, secrete trophic factors, regulate gliosis and scar formation, reduce cystic cavity size and axonal destruction, as well as to remyelinate axons.[3, 27, 65] However, the scarcity of adult NSPCs limits the clinical translation of transplanting these cells in injured tissue. Therefore, alternative routes to derive NSPCs have been studied.

4. Generation of NSPC from various sources

4.1. Adult tissue derived NSPCs

NSPCs can be derived from various regions along the neuroaxis during embryonic development and in adult life [66, 67]. These cells retain their mulipotentiality and can generate neural cells in culture. NPSCs have been isolated from the subependymal zone of the adult mammalian brain and from ependymal and non-ependymal regions of the adult mammalian spinal cord. [68-70] Single adult NPSCs can be isolated *in vitro* in the presence of growth factors (epithelial growth factor, EGF; fibroblast growth factor, FGF) that enable the proliferation and formation of clonally-derived free-floating colonies. The differentiation and survival of cellular subpopulations can be promoted *in vitro* by exposure to bone morphogenetic proteins (BMPs) to produce astrocytes [71, 72], insulin-like growth factor (IGF)-I, interleukin-1 (IL-1), Neuregulin-1 (Nrg-1) to generate oligodendrocyte [73, 74], and neurogenin-2 to produce neurons [75, 76].

For experimental purposes neurospheres can be generated from the germinal zone of the adult mouse brain, according to well-established techniques [77, 78]. Briefly, the subventricular zone

(SVZ) of the forebrain of mice can be dissected and transferred to a low calcium aCSF solution. Cells are plated on uncoated tissue culture flasks in serum free medium containing FGF2 and EGF for 7 days. The neurospheres are passaged weekly by mechanical dissociation in the same medium. This method of generating NSPCs has obvious clinical limitations as it requires brain tissue to generate the renewable cell population.

4.2. Embryonic stem cell (ESC) derived-NSPCs

The first isolation of a pluripotent population of cells from the mouse embryo over 30 years ago revolutionized the emerging field of regenerative medicine. [79, 80] Once a similar population of cells were identified and isolated from a human source, [81] the possibilities and potential for clinical application for the derivatives of these cells became limitless. Neurons and glial cells were among the first differentiated cells to be generated from these pluripotent cells. Although there are some differences between human and murine ES cells, such as LIF responsiveness *in vitro* [82], many of the neural differentiation protocols established for mouse ES cells have been adapted for use with human ES cells. There are multiple strategies that are implemented to generate neural cells from ES cells with varying degrees of cell homogeneity, differentiation potential, efficiency and time requirements. In general, two strategies exist to direct ES cells to NSPC: the first uses embryoid body (EB) formation to mimic the physiological neurodevelopment of the embryo and the second involves removal of all cues that would direct the cells to a non-ectoderm lineage by limiting cell-cell interactions with low cell density culture and environmental signals, with serum-free media.

4.2.1. NSPC generation with EB intermediate

Using EB intermediates to generate NSPCs and their differentiated products mimics the physiological environment that produces neuroectoderm. [83, 84] In brief, the ES cells are transferred from their expansion conditions on feeder cells to a suspension culture and allowed to form aggregates. Within a few days, the EBs formed resemble an anterior pre-streak stage embryo with an epiblast-like core. [85] At this stage the cells are still able to form cells from all three germ layers. Next conditions are such that they drive the neuroectoderm lineage while inhibiting the endoderm and mesoderm fates concentrating the NSPC population and thus yield cells that can differentiate to neurons and glial cells.

The use of retinoic acid (RA) with the EB based neuralization protocols can vastly improve the neural lineage cells produced. [86, 87] Many studies have shown that RA plays a key role during neurogenesis, both *in vitro* conditions as well as physiologically. RA treatment directs cell towards those of the posterior CNS. [88] The general culturing process involves EB formation, as described above, for 4 days, followed by RA treatment (4-/4+) and then 1 week of adherent conditions. A high proportion of the differentiated cells have neural properties, with positive identification for both glutamatergic and motor neurons. [88, 89]

Other neuralization protocols that use an EB intermediate, such as culturing with carcinoma or stromal cell-line conditioned media or via selection in defined media conditions, can be used to generate NSPCs with relatively high efficiency. [90, 91] However, a key limitation with NSPCs generated from EB intermediates exists; despite success with these approaches, concerns related to the restricted potential of NSPCs remain. The involvement of EB formation

creates a risk for the persistence of non-neural cells in the final population. This has been associated with teratoma formation and increase tumorigenicity. The persistence of non-neural cell can be traced back to the non-specific differentiation pattern associated with the initial aggregation of EB.

4.2.2. NSPC generation from default pathway

In order to circumvent EB intermediates, and its limitations, during neuralization of pluripotent cells, our lab has opted to use the default pathways to direct ES cells to a neural lineage. ([92]; Figure 2) This pathway relies on the fact that, in the absence of extrinsic signaling to form non-ectoderm lineage, the cells will adopt a neuroectoderm fate. [93, 94] The default mechanism is based on studies that showed the inhibition of BMP signaling by protein inhibitors or by gene expression manipulation leads to neural lineage commitment. A small percentage (0.2%) of single ESCs cultured under serum-free, low cell density condition proliferate in the presence of LIF to form floating neurospheres of cells that express the neuroepithelial markers Nestin and Sox1, but downregulate the ES cell markers Oct4 and SSEA1. [93, 94] Moreover, a small proportion of cells derived from primary neurospheres can generate secondary colonies when subcloned, which are independent of LIF, but are dependent on FGF2. These cells have been termed definitive (dNSPC). [94]

In order to assess the clinical potential of the NSPCs generated by this method, with specific focus on SCI, our lab has extensively characterized the ES-derived dNSPCs *in vitro*. [92] In brief, ES cells were directed to primitive and definitive NSPC fates. Their mRNA profile was evaluated using RT-PCR and *in vitro* differentiation patterns were quantified and compared to aNSPCs populations isolated from the SVZ. The ES-dNSPC populations were similar to aNPCs analyzed at the mRNA level with a significant decrease in pluripotency (*Nanog, Oct4*) and stemness (*Tdgf1, Dnmt3b, Gaf3*) markers with increased transcription of neural-specific markers (*Pax6, Nestin, Olig2, Synaphysin*). The pNSPC retained many of the pluripotency and stem markers associated with undifferentiated ES cells reflecting the importance of driving the NSPC to a definitive state. To assess the *in vitro* differentiation pattern of the ES-derived NSPCs and aNSPC controls, the cells were cultured on matrigel for one week with 1% FBS media. The cells were immunolabelled for NSPCs (Nestin), neurons (BetaIII Tubulin), astrocytes (GFAP), and oligodendrocytes (Olig2, PDGFRa, CNPase). Both ES-dNSPCs and aNSPCs yielded primarily differentiated neural cells with the majority being astrocytes (~65%). The largest proportion of cells from the ES-pNSPCs were undefined or retained the NSPC marker Nestin. Although astrocytic differentiation may not be the desired cell, this differentiation into astrocytic cells demonstrates that the *in vitro* environment is very different from the *in vivo* niche.

The potential of these cells to survive, integrate, and differentiate *in vivo* is critical to evaluating their role in regenerative medicine applications. Since remyelination following SCI is a likely potential mechanism of recovery, our lab uses a dysmyelinated *Shiverer* mouse to assess the *in vivo* potential of our ES-dNSPC. *Shiverer* mice lack compact myelin basic protein (MBP) and therefore are an ideal candidate to evaluate the myelination ability of these cells. [95] In general, the Shiverer mouse, under isoflurane anaesthesia, received a T6-T7 laminectomy followed by 4 intraspinal injections of ES-dNSPC (100,000 in 2ul of media). The mice were immune suppressed with continued cyclosporine A treatment. Six weeks following transplantation the

animals were perfused and spinal tissue was fixed with 4% PFA. Cryosections of tissue were immunohistochemically labeled for neural cell markers. The ES-dNSPCs were able to survive and integrate into the host tissue in a similar pattern to that previously described with aNPCs. The transplanted cells preferentially migrated to the white matter tracts and differentiated to oligodendrocytes with multiple processes that expressed MBP. The quantification of the differentiation pattern showed that the transplanted cells become primary mature APC+ oligodendrocytes with limited astroglial and neuronal fates. The variability between *in vivo* and *in vitro* differentiation potential can be attributed to the instructive nature of the spinal cord niche. The *Shiverer* spinal cord has been shown to have greater amounts of NG2-expressing oligodendrocyte progenitors. [96] The environment that leads to the excess of these progenitors is also acting on the transplanted cells. This instructive/permissive niche could be a result of signaling molecules such as GRO-1 [97] and neuregulin (NGR) [98], both of which are released by astrocytes and neurons.

ES cells are the most defined source of readily available pluripotent cells that can be used in cell-based treatment for SCI. Although, they have some safety and ethical concerns, this population will remain extremely relevant in the future of spinal cord regeneration.

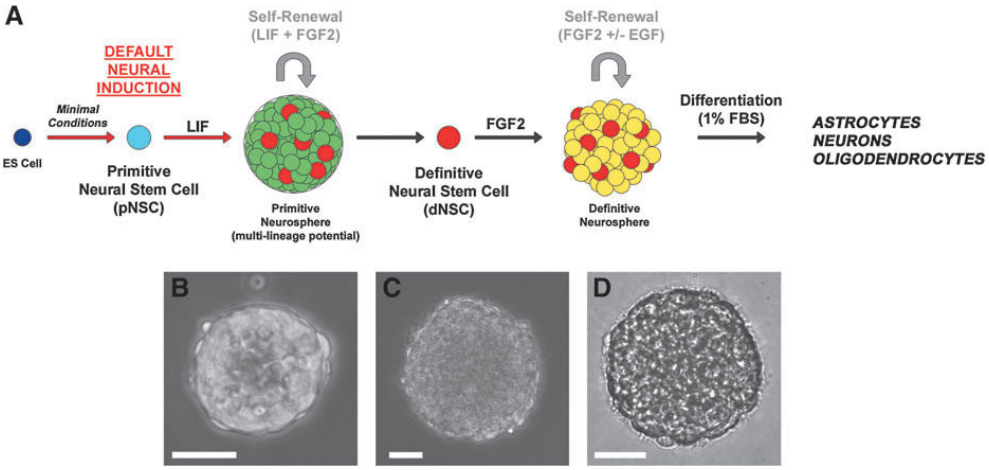

Figure 2. Default neural differentiation of embryonic stem cells. (A) Schematic representation of neural induction of ESCs through the default pathway: Individual ESCs when cultured at low density in minimal serum-free media containing LIF acquire a neural identity through a default mechanism. These neural stem cells colonies are termed pNSPCs, are LIF- dependent, and divide to form clonally-produced floating neurosphere colonies. pNSPC-derived neurospheres can be dissociated into single cells and passaged indefinitely in serum-free media containing LIF or can be passaged into serum-free media containing FGF2 to produce a distinct population of FGF2-dependent cells termed dNSPCs that also divide to form clonally-produced neurospheres. These spheres can also be passaged indefinitely and when differentiated produce all three cell types of the neural lineage. (B) Phase-contrast image of a neurosphere colony generated by a pNSPC. (C) Phase-contrast image of a neurosphere colony generated by a dNSPC. (D) Phase-contrast image of a neurosphere generated by adult neural stem cells derived from the subependymal layer of the adult mouse forebrain. For B–D, bar equals 50 mm. ESCs, embryonic stem cells; LIF, leukemia inhibitory factor; dNSPC, definitve neural stem cell; pNSPC, primitive neural stem cell; FGF2, basic fibroblast growth factor; Olig2, oligodendrocyte transcription factor 2. *from *Rowland et al., 2011, Stem cell and Develop. 20(11); 1829-1845, with permission.*

4.3. Induced pluripotent stem cells (IPSC) derived NSPCs

Given the aforementioned concerns with ES cells combined with the immunogenicity that arises from allograft transplantation, the search for a patient-specific and accessible cell source has been a principle endeavor of regenerative medicine. Historically, many techniques and strategies have been developed to accomplish this aim, most notably somatic cell nuclear transfers (SCNT). SCNT is the process by which the nucleus of the somatic cell being reprogrammed is transferred to an enucleated ooctye [99]. This technique became famous in the late 1990s when Dr. Ian Wilmut cloned the first mammal, a sheep named "Dolly". [100] Although there is no obvious mechanism that would preclude SCNT from reprogramming human cells, this technique has yet to be successfully applied in human cells. Furthermore, the requirement for donor ooctyes, combined with an inefficient and technically difficult processes makes SCNT unlikely to be a viable option for clinical application even in the most ideal circumstances.

The discovery of IPS cell technology has made a significant stride towards realizing the promise of patient-specific regenerative medicine. IPS cells are somatic cells that have been reprogrammed to ectopically express certain transcription factors that induce an ES cell-like state, in terms of their differentiation potential and response to *in vitro* culture conditions. [28] This technology allows for a constant and relatively easy method of generating cells for autologous transplantation from readily available cell sources such as skin cells. In 2006, Takahashi and Yamanaka used retrovirus transfected with 24 transcription factors in mouse embryonic fibroblasts (MEF). [101] Through careful elimination they were able to reduce the required genes to four: OCT4, Sox2, KLF4 and c-myc. The expression of these four genes was sufficient to revert the MEFs to an undifferentiated, pluripotent state that was verified using teratoma formation and contribution to a chimeric mouse. Since the initial characterization of the iPS cells there has been a tremendous amount of research further expand and refine the technology. The Yamanaka factors have been used to reprogrammed various cells from tissues from a variety of species including mice [101], rats [102], rhesus monkeys [103], and humans [104-107].

The first generation of iPS cell technology is not without its shortcomings and clinical obstacles. The process of reprogramming can be slow and inefficient and includes oncogenic potential of the factors themselves, insertion mutatgenesis, and the risks associated with the use of viral vectors. All of this can contribute to limited clinical translational potential. Fortunately, substantial research has rapidly developed iPS techniques that are viral vector and mutation free. For example, piggyBac transposition is a viral-free system that can be used to deliver the reprogramming factors in both human and mice fibroblasts [108, 109]. A single transposon containing all four iPS transcription factors is introduced to the cells and reprogrammed colonies are selected. IPS cell lines with a single insertion site are identified and transposon is seamlessly excised yielding stable, reprogrammed cell lines that are do not contain any exogenous DNA or have any insertion mutation. Other viral-free and mutation-free methods exist including using lentiviruses [110, 111],an episomal system [112], and the use of recombinant proteins [113], to generate more clinically relevant iPS cells.

Even with the advancement in iPS cell generation, intrinsic differences between ES and iPS cells exist. IPS and ES are often described as "indistinguishable", however, key differences have been identified. The initial characterization of the iPS cells from Takahashi and Yamanaka noted variation in global gene expression as well as differences in epigenetic characters,

specifically histone methylation.[101] Epigenetic memory of iPS cells can influence the differentiation potential and the differentiation of the iPS cells, favouring the identity of the tissue of origin. [114] The impact of epigenetic variability still remains an area of debate and methods to optimize iPS cells are underway.

4.3.1. Neuralization of IPS cells

The conceptual outline of how iPS cells may be used in the treatment of SCI is described in figure 3. [28] Many of the *in vitro* neuralization protocols outlined above for generating NSPCs and terminally differentiated neural cells, neurons, astroglia and oligodendrocyte, from ES cells can be applied to iPS cells. iPS cell have been used in models of Parkinson's [110, 115], spinal muscular atrophy (SMA) [116], amyotrophic lateral sclerosis (ALS) [117], and SCI [118]. Although there have been positive results using iPS-derived neural cells for cell-based treatment, the variation in iPS cells has resulted in variability in the safety and neuralization of these cells. A comprehensive evaluation of NSPCs from mouse iPS cell lines generated from various tissue sources and reprogramming methods has shown differences in tumorigenesis between iPS cell lines compared to NSPCs from ES cell lines. [119]

4.3.2. Improving the IPS neuralization using NOTCH pathway agonism

The variation in neuralization potential between iPS cell lines and their ES cell counterparts represents an area of regenerative medicine that, if not addressed, could be an obstacle to clinical translation. Our lab specifically examined the difference in neuralization potential of multiple iPS cells lines. [120] We used iPS cells generated using piggyBac transposon technology to eliminate variation that could be attributed to the resurgence of the silenced transgenes or due to insertion mutations. [108, 109] Furthermore, the default pathway of neuralization was utilized to avoid issues associated with the EB formation as an intermediate step in generating NSPCs from a pluripotent cell source. Even with the additional precautions to help generate safe and clinically-relevant cells, there was retention of plurioptency markers as well as persistence of non-ectoderm lineage markers. In order for the potential for iPS technology to be realized, methods to effectively and consistently generate definitive cells must be developed.

We examined the role of BMP antagonism during the initial neuralization of the pluripotent IPS cells. We identited this as a possible area where IPS cell neuralization could be improved. Noggin, a BMP antagonist, has previously been shown to increase the number of neurospheres generated using the default pathway and ESCs.[94] BMPs are involved in directing pluripotent cells to an endoderm lineage fate. Although the inclusion of Noggin during the *in vitro* culture of primary primitive NSPCs did increase the number of neurospheres, it did not affect the character of the NSPCs as determined by a battery of gene markers analyzed by RT-PCR. Furthermore, there was no difference on the subsequent mRNA profile between definitive NSPCs with or without Noggin treatment. These data led us to conclusion that the variability in the PB-IPS cells' response to the default pathway is not a result of poor initial neuralization but likely due to incomplete transition from primitive NSPC to definitive NSPCs state.

The NOTCH pathway has been shown to be involved in many aspects of neurodevelopment and its role persists in the adult CNS. [121, 122] Disruption in NOTCH signaling leads to a

reduction in NSPCs while conversely the induction of this pathway promotes NSPCs *in vivo*. [123, 124] The NSPC niche of the SVZ expresses receptors and ligands of the NOTCH pathway. [125] Delta-like ligands (DLL) or Jagged are the principle ligands of the NOTCH pathway, and interact with the membrane-bound NOTCH receptor. The NOTCH intracellular domain (NICD) is cleaved by gamme-sectrase. NICD is translocated to the nucleus to facilitate the transcription of targets such as Hair and enhancer of split, HES genes. [126] Furthermore, NOTCH appears to play a critical role in transition from primitive to definitive neural state. Primitive NSPCs were readily generated in LIF-dependent culture conditions from ES cells as well as NSPCs isolated from E7.5 embyros from NOTCH-deficient sources. However, passage of these cells to a definitive state was disrupted indicating a crucial role for NOTCH. [124, 127] Using these data we hypothesized that agonizing the NOTCH pathway during the neuralization of PB-iPSs would improve the neural character of the definitive NSPCs generated and thus, improve the clinical relevance and translation potential of the cells.

We demonstrated that the addition of recombinant mouse DLL4 to the definitive culture conditions of the default pathway of neuralization improved the generation of definitive NSPCs compare to those cells grown in parallel using standard default conditions. [120] DLL4 was selected to this pathway since DLL4 is most avid ligand for the NOTCH1 receptor.[128] The definitive neurospheres treated with DLL4 produced a greater number of spheres that retained a free-floating phenotype while untreated spheres showed extensive adhesion and signs of differentiation. Also, the mRNA profile of the DLL4 treated dNSPC showed a reduction in pluripotency markers (*Lin28, Nanog, Oct3/4)* as well as reduction or elimination of endodermal markers (*Gata6, Afp*) compared to control cells. This pattern was confirmed at the protein level with immuncytochemistry. The dNSPCs were also cultured in chamberslides matrigel in SFM containing 1% FBS to induce the differentiation of the cells. The differentiation profile of the DLL4-treated definitive NSPCs reflected the ES-derived dNSPCs as well as aNSPCs. Primarily differentiated neural cells were identified following 1 week of differentiation with mostly GFAP+ astrocytes in the iPS-dNSPC(+DLL4) group compared to non-treated iPS-dNSPCs that were primarily positive for the undifferentiated NSPC marker Nestin or were not labeled by any of the neural markers used. Lastly, the neurons, oligodendrocytes and astrocytes from DLL4-treated dNSPCs were shown to be electrophysiologically functional.

In addition to the NOTCH pathway, Sonic Hedgehog (SHH) and WNT signaling are both known to play roles in neurodevelopment. These pathways have been shown to have independent and interconnected mechanisms of action and there is evidence of considerable crosstalk with NOTCH signaling. SHH expression can be up-regulated in a time dependent profile with the Jagged1 in NSPCs *in vitro*. [125] WNT signaling has been shown to influence the transition of primitive NSPCs to definitive state through the manipulation of *Hes* expression. [129, 130]

iPS cell technology combined with the default pathway of neuralization has tremendous potential to revolutionize the treatment of SCI. Patients could someday use their own skin cells to regenerate and repair their injury (Figure 3).

http://dx.doi.org/10.5772/55054

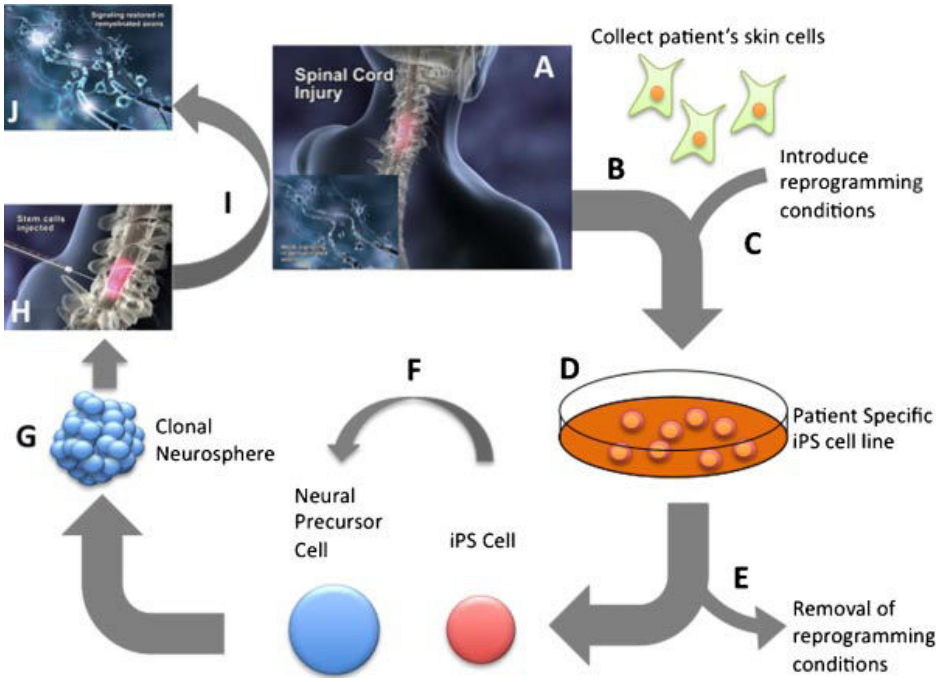

Figure 3. A general schematic representation of the generation of iPS cells, the promotion of neural precursor cells, and their use in spinal cord remyelination. A: Following a spinal cord injury, demyelination occurs resulting in vulnerable axons and impaired CNS function. B: The patient's fibroblasts, or skin cells, are harvested. C: Reprogramming conditions/factors are introduced to induce self-renewal and pluripotency properties. D: The cells are now iPS cells. E: When full and independent reprogramming is achieved, reprogramming condition/factors are removed. F: Neuralization of the iPS cell to NPCs under minimal conditions. G: Growth factors (i.e., FGF) further differentiate the cells to become definitive neurospheres. H: iPS cell-derived neural stem/precursor cells can be injected into the injured spinal cord. I: Once transplanted, these cells can differentiate CNS cells such as myelinating oligodendrocytes. J: iPS-NPC-mediated remyelination, or by some unidentified neuroprotective effect, can result in functional recovery following spinal cord injury. * from *Salewski et al., 2010, J. Cell Physiol. 222; 515-521, with permission*

5. Transplantation of neural stem/progenitor cells into the injured spinal cord

5.1. Model of SCI and NSPC transplantation

The *in vivo* regenerative and neuroprotective effect of the cell-based treatments must be evaluated in preclinical animal models. The rodent clip compression model of SCI developed in our lab mimics injuries observed in human SCI, in terms of primary and secondary injury processes and, in particular, with regards to lesion and cavity formation. This creates a situation where we can optimize the cell culture and transplantation paradigm while extensively

characterizing and evaluating the safety and efficiency of our NSPCs in a clinically relevant small animal model prior to their implementation into larger animal models or a clinical trial.

In general, our SCI model involves injured rats that receive a clip compression injury of spinal cord either at the cervical or thoracic level. [19, 65] The model characteristics, histopathology, assessment of axonal integrity, molecular examination of axonal structure, and behavioural assessment have been extensively characterized. [131-134] This model of moderately severe SCI, results in a central cavitation and loss of 80% of axons in the spinal cord white matter, demyelination of the surviving axons in the residual subpial rim and spastic paraparesis. Following acute (2 week) or chronic (6 weeks) SCI, we have administered four intraspinal injections of aNSPCs in growth media to the rats at locations 2mm caudal and 2mm rostral to the injury site. To enhance the survival of the transplanted cells, growth factors [PDGF-AA bFGF and EGF] in a solution containing aCSF, BSA (100 μg/ml) and gentamycine (50 μg/ml) has been infused intrathecally to the area of transplantation for 7 days using a microcatheter connected to an osmotic minipump. A battery of behavioural tests, histological outcomes and electrophysiological measures are used to determine the therapeutic effect aNPC transplantation.

5.2. Effect of transplant on subacute and chronic injury

In our study of subacute intervention following SCI, transplantation was delayed to two weeks following injury. [19] The animals were examined using a number of measures of locomotor function, including the BBB scale, ladder walk analysis and gait/coordination assessment, for eight weeks post-SCI. At the eight week endpoint of the experiment, the animals were euthanized and their tissue collected for histological and electron microscopy.

The transplanted NSPCs had substantial survival (~40%) in the spinal cord when the growth factor mini osmotic pump was used and these cells demonstrated multipotency. In contrast to their *in vitro* differentiation profile, the transplanted cells mostly became oligodendrocytes in the injured spinal cord. In fact, the cells preferentially migrated to the white matter tract and showed extensive myelination. ([19]; Figure 4) The role of myelination will be fully addressed when we discuss potential mechanisms of neuroprotection/neurorepair. Behavioral and functional benefits from aNSPC engraftment were observed. There was significant locomotor recovery compared to the injured control groups. The BBB is a 21 point scale that is used to evaluate hindlimb locomotion in an open field. [43] Immediately following the SCI, all rats were fully paraplegic and exhibited no hindlimb function. The control rats were able to recover to a BBB score of 8, denoting plantar placement of the paw without weight support. The experimental group that received the aNSPC treatment was able to reach a score of 10 on the BBB. Although a 2-point increase may seem negligible, the BBB is a non-linear scale and these animals show occasional weight supported plantar stepping which is a substantial functional improvement. Furthermore, the animals that received the transplanted cells show improvement on other independent behavioral tests including grid walk and footprint tests. Our results show that the improved recovery of locomotor function in the transplanted groups was a result of the effects of the aNPCs since there was no significant difference in the walking ability of the plain injured or sham controls in all behavioral tests used.

http://dx.doi.org/10.5772/55054

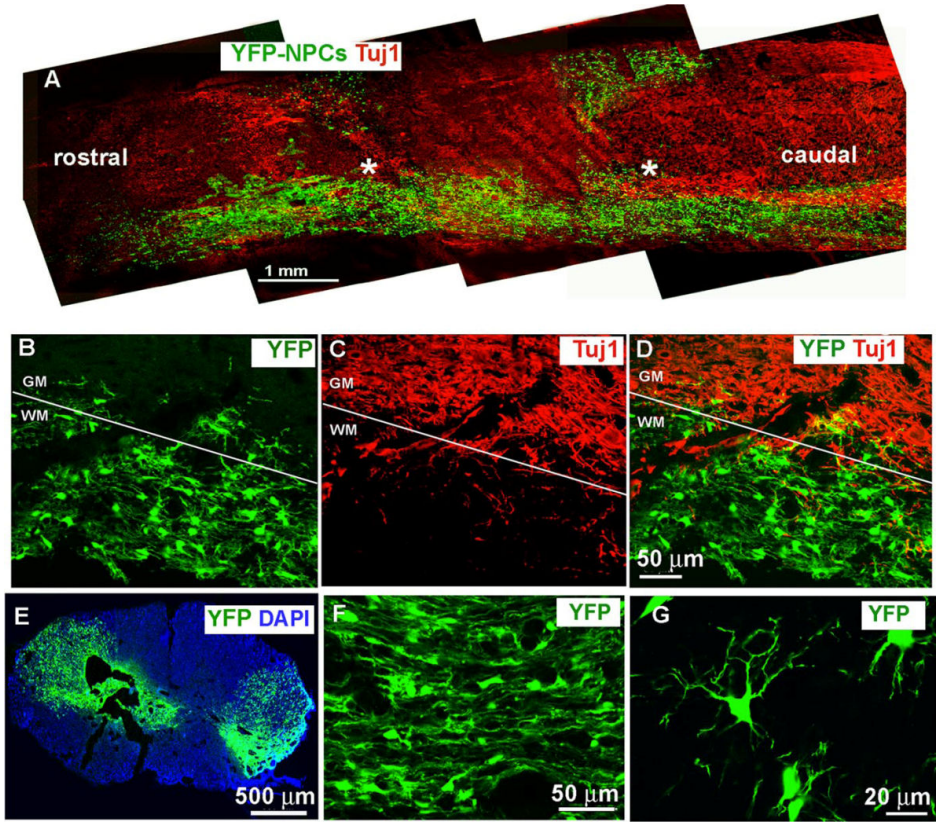

Figure 4. YFP-NPCs in the spinal cord of a subacutely injured rat 8 weeks after transplantation. A, A confocal image from a longitudinal section of an injured spinal cord taken from the dorsal spinal cord of a transplanted rat above the central cavity. A low-magnified image shows the extent of YFP-NPC survival within the injured spinal cord 8 weeks after transplantation. Grafted YFP-NPCs (green) were dispersed along the rostrocaudal axis of the spinal cord 5 mm away from the implantation sites (*). YFP-NPCs also migrated to the contralateral site of the spinal cord to a lesser extent. Double labeling with the neuronal marker betaIII tubulin (Tuj1) showed that YFP-NPCs reside predominantly in the white-matter area (A–D). Our histological data showed no signs of tumor formation in the spinal cord. E, Confocal image of a transverse section of the spinal cord from a transplanted rat (8 weeks after transplantation) showing the distribution of YFP-NPCs in the lateral columns. F, G, YFP cells mainly showed multipolar morphology and extended numerous branches in the white-matter tissue along the length of axons. WM, White matter; GM, gray matter. * from *Karimi-Abdolrezaee et al, 2006, J Neurosci 26:3377-3389, with permission*

The successful implementation of the NSPC therapy in the subacute model is a key step forward in spinal cord regenerative medicine. It has a relevant therapeutic window, although narrow, and requires intervention shortly after the initial injury. The chronic injured population represents a large section of the SCI community and currently many of the proposed treatments cannot be used to repair their injuries. The chronic injured environment is characterized by many additional challenges including cell loss, a cystic cavity and the inhibitory influences of the glial scar. [65]

We noted that when aNSPCs were transplanted at 6 weeks post-SCI, the chronically injured spinal cord was not an hospitable environment. To address this, we examined methods to modify the SCI environment to create a permissive environment. Our data suggested that expression of chondroitin sulfate proteoglycans (CSPG) reduces the survival and thus, the therapeutic potential of our aNPC treatment. [65] Chondroitinase ABC (ChABC) was administered to the chronic SCI environment to breakdown CSPGs that were upregulated as a result of the injury. We then analyzed the synergistic effect of the transplanted aNSPCs with ChABC and growth factor pump on the repair and plasticity of the chronically injured spinal cord. Survival, integration and migration of the transplanted cells in the chronic SCI with ChABC was extensive compare to treatment without ChABC and growth factors where survival was low and the transplanted cells remained at the injection site. ([65]; Figure 5) The differentiation potential of the aNSPCs was similar to that observed in the subacute injury/transplanted paradigm with the majority of cells displaying oligodendrocyte markers. There was also improvement observed at the functional locomotor level. The animals that received the combinatorial treatment of ChABC followed by aNSPCs and GF pump had a significant improvement on the BBB scale as well as with ladder-walk analysis. These behavioral improvements were also linked to neuroanatomical changes associated with the aNPC therapy. This is the first successful application of an aNSPC-based treatment for chronic SCI and represents a critical step forward improving the lives of patient currently living with SCI.

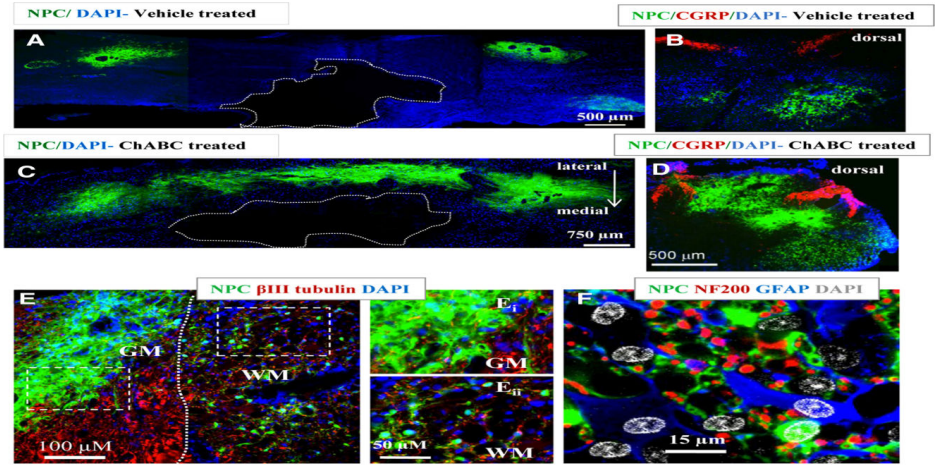

Figure 5. ChABC treatment greatly optimizes NPC transplantation in the chronically injured spinal cord. A–D, Confocal images of longitudinal and cross sections of chronically injured spinal cord transplanted with NPCs at 9 weeks after transplantation show a significant increase in the number of surviving NPCs (green) in ChABC-treated spinal cord (C,D) compared to the vehicle-treated spinal cords (A, B). D, The majority of NPCs integrated within white matter areas including dorsal, lateral, and ventral columns. Quantification of transplant volume revealed a 6.2-fold increase in ChABC-treated rats (n6, 3.70.79mm3) compared to vehicle-treated rats (n6, 0.60.24mm3) (p0.01, Student's t test). NPC cell survival analysis also showed a 5.7-fold increase in number of surviving NPCs (YFP/DAPI-positive cells) in ChABC-treated rats (28.256.77%) relative to the vehicle-treated ones (4.91.96%) (p0.01, Student's t test). ChABC treatment resulted in a significant increase in rostral and caudal migration of NPCs from the injection sites (4.20.50mm)

compared to vehicle treatment (1.40.4mm)(p0.001, Student's t test). We also found a 4.6-fold increase in the total length of rostrocaudal distribution of NPCs in ChABC-treated rats (13.02mmin the best case, average 8.321.5 mm) compared to vehicle-treated rats (2.24mminthe best case, average 1.820.5mm)(p0.005, Student's t test). * *from Karimi-Abdolrezaee et al, 2010, J Neurosci 30:1657-1676., with permission*

5.3. Possible mechanism of neurorepair/neuroprotection

Although our lab and others have clearly shown that cell-based therapies for SCI can be beneficial, the mechanisms by which the benefits are achieved still remains an area of debate. Trophic factor support [40, 135-137] and remyelination [19, 27, 65] as well as other mechanisms including plasticity and axon integrity have been suggested as possible mechanisms of recovery with cell-based treatments for SCI.

5.3.1. Trophic factor support

Trophin delivery as a potential mechanism of benefit in cell-based treatments, such as the use of bone marrow stromal cells (BMSC) following trauma, was first proposed in response to evidence that there was a degree of neuroprotection despite a lack of survival of transplanted cells. [40, 135-137] Trophic factors have been shown to have apoptotic effects[138], enhance axonal regrowth[139], promote endogenous remyelination[140, 141], and neuronal plasticity[142, 143]. BMSCs as well as aNSPCs have been shown by our lab and others to produces these trophic factors *in vitro*, however little evidence to show their involvement *in vivo* has been shown. [144]

To study trophin production and involvement *in vivo*, we examine both brain- and spinal-derived NPCs, compared them to BMSCs, and transplanted them into injured rat spinal cords. [145] Candidate trophic factor (NGF, BDNF, NT-3, NT-4/5, GDNF, CNTF, PDGF-A, EGF, bFGF, LIF, IGF-1, GGF2, TGF-b1, VEGF-A) expression was examined in spinal tissue homogenates as well as specifically in the transplanted cell population by FAC sorting for the eGFP labeled cells. The neurotrophin levels were increased following SCI and the transplantation of aNSPC with GF pump further elevated specific trophic factors, in particular, GDNF, LIF and bFGF. Furthermore, the analysis of the FAC sorted transplanted population of aNSPC showed increased neurotrophin production, specifically CNTF, EGF and bFGF. The study was the first to shown that both changes in the host tissue as well as the transplanted aNSPCs can influence trophic factor levels *in vivo*. Although further research is required to determine if trophic factor support is the sole mechanism that conveys neuro-protection or neuro-repair, our research does shows that it is a likely contributing factor to the functional improvements observed.

5.3.2. Remyelination

Myelin is a critical component of the CNS and is required for its proper functioning. Myelin is needed for the precise molecular organization of the axon, particularly with regards to nodal architecture. The arrangement of Na+ channels, K+ channels and contactin-associated protein at and around the nodes of Ranvier is closely linked to myelin, and a disruption in myelination results in loss of nodal organization. Furthermore, following SCI there is extensive loss of white matter and often otherwise functional axons are bare and rendered useless. In this situation,

restoring the myelination of these axons can have large effects on the overall outcome and locomotor function since relatively few spared axons can have a tremendous effect on improving neural outcomes.

These data suggest that the remyelination could be the mechanism by which aNSPC transplantation is able to repair the injured spinal cord. Following our subacute intervention as well as chronic injury with combinatorial therapy, we observed migration of engrafted cells to white matter tracts, mature oligodendrocytic differentiation and expression of myelin basic protein (MBP) by these cells associated with axons. ([19]; Figure 6) Furthermore, the exogenous myelination was confirmed by electron microscopy to form multilayer compact myelin around axons and to restore the nodal architecture as shown by immune labeling for Kv1.2 and panKa.

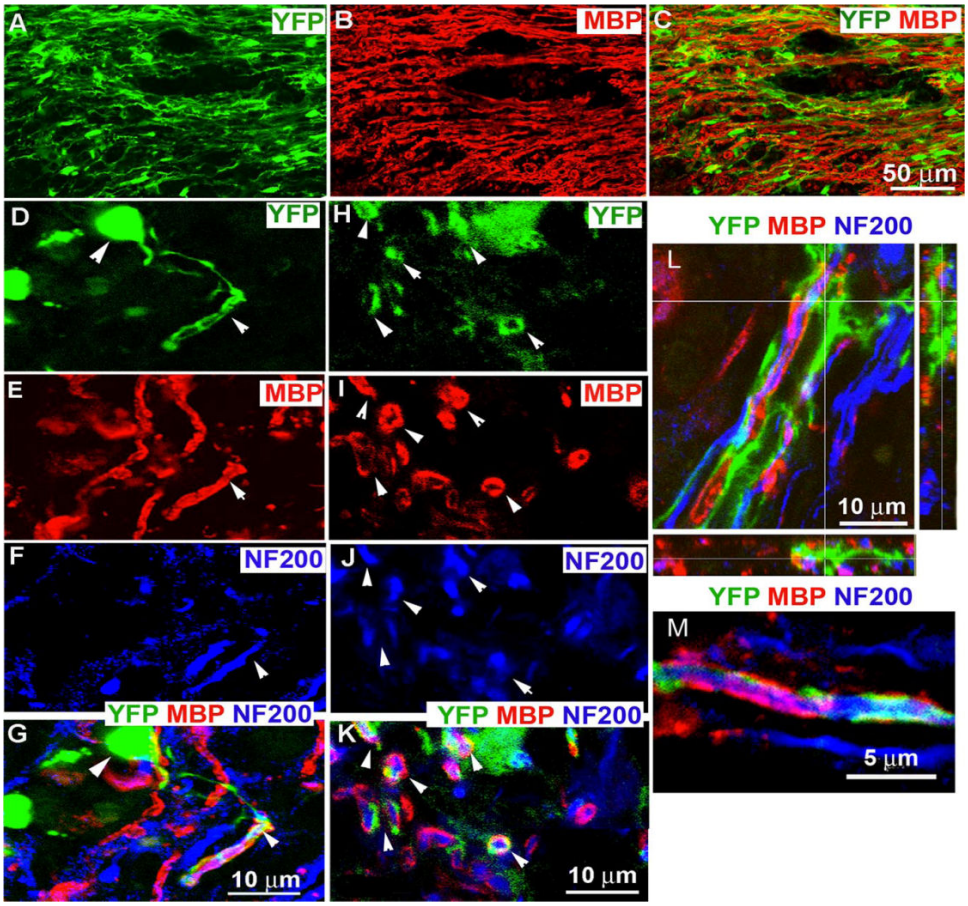

Figure 6. Exogenous remyelination following subscute SCI with aNSPC transplantation: YFP-NPC-derived oligodendrocytes generate MBP and ensheath the injured axons of the spinal cord. A–C, Confocal images of longitudinal sections of an injured spinal cord 8 weeks after transplantation. The area grafted with YFP-NPCs (green) displays a

robust expression of MBP (red) in the white matter of an injured spinal cord. Cell bodies of donor cells are surrounded with MBP. Triple-labeling experiments on longitudinal (D–G) and cross (H–K) sections of spinal cord white matter showed that MBP-expressing YFP-NPCs ensheathed the injured axons (identified by NF200; blue). These images (D–G) clearly show the oligodendrocyte morphology of one grafted YFP cell (arrowheads) that extends its processes and expresses MBP around an injured axon and the close proximity of these cells with newly myelinated axons. L, M, Images taken by deconvolution confocal microscopy show a higher-magnification image confirming axonal ensheathment of MBP-expressing YFP-NPCs around the injured axons. *from *Karimi-Abdolrezaee et al, 2006, J Neurosci 26:3377-3389, with permission*

Although exogenous myelination has been proposed as a mechanism for aNSPC-mediated recovery, potential endogenous myelination cannot be ruled out. There is still extensive research required to delineate the precise role of remyelination and specifically the role of remyelination induced by transplanted cells.

5.3.3. Other mechanisms

In addition to the roles of trophic support and myelination, other mechanisms have been proposed. It is possible that effects on axon integrity and neuronal plasticity play key roles in SCI recovery. We noted positive effects in these areas following our combinatorial strategy of ChABC and aNSPC in the chronically injured spinal cord.[65] Cortiospinal tract (CST) labeling using PKC-g and anterograde tracing showed evidence of enhanced axonal integrity and collateral spouting. This phenomena was seen only at the local level with long-distance CST regeneration beyond the lesion not observed. Promotion of plasticity of serontonergic (5HT) fibers was observed with ChABC and aNSPC therapy as noted by a significant increase in its immunointensity rostral to the injury epicenter. The 5HT positive fibers were observed in areas of aNSPC engraftment. Many mechanisms are likely working in concert to elicit the behavioral and functional outcomes observed by our lab and others after application of aNSPC therapy.

6. Conclusions

Spinal cord injury pathology creates a situation where cell-based interventions are attractive as potential treatments to promote repair and regeneration. There are a variety of cell sources available for this aim, however, in our research experience, NSPCs are the most likely to provide lasting and appropriate neurological recovery. Both adult brain and spinal tissue are sources of NSPCs for transplantation along with the pluripotent cells (ESC and iPSC) when differentiated *in vitro* to a restricted NSPC identity. Once transplanted into the injured spinal cord, the spinal niche directs the NSPCs to terminally differentiated neural cell types, both neuronal and glial. Our work suggests remyelination via oligodendrocytes is the principal mechanism of recovery although trophic support as well as neuronal lineages that are created are other possible mechanisms by which NSPC treatment is beneficial in SCI. Our results with chronic SCI, which is characterized by cavitation and glial scarring, has shown that stem cell therapy alone may be of limited benefit. Thus, combinatorial stem cell approaches with bioengineered strategies, such as use of chondroitinase, will be a key area of future research in the field of neuro-regenerative medicine.

Author details

Ryan Salewski[1], Hamideh Emrani[1] and Michael G. Fehlings[2]

1 Toronto Western Research Institute, University Health Network, Toronto Western Hospital, Canada

2 Division of Neurosurgery and Spinal Program University of Toronto; Krembil Neuroscience Centre, UHN, Toronto Western Hospital, Canada

References

[1] Eftekharpour E, S Karimi-Abdolrezaee and MG Fehlings. (2008). Current status of experimental cell replacement approaches to spinal cord injury. Neurosurg Focus 24:E19.

[2] Wyndaele M and JJ Wyndaele. (2006). Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey? Spinal Cord 44:523-529.

[3] Rowland JW, GW Hawryluk, B Kwon and MG Fehlings. (2008). Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. Neurosurg Focus 25:E2.

[4] Ruff CA and MG Fehlings. (2010). Neural stem cells in regenerative medicine: bridging the gap. Panminerva Med 52:125-147.

[5] Tator CH and MG Fehlings. (1991). Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 75:15-26.

[6] Kakulas BA. (2004). Neuropathology: the foundation for new treatments in spinal cord injury. Spinal Cord 42:549-563.

[7] Tator CH and I Koyanagi. (1997). Vascular mechanisms in the pathophysiology of human spinal cord injury. J Neurosurg 86:483-492.

[8] Pineau I and S Lacroix. (2007). Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500:267-285.

[9] Schanne FA, AB Kane, EE Young and JL Farber. (1979). Calcium dependence of toxic cell death: a final common pathway. Science 206:700-702.

[10] Nagata S and P Golstein. (1995). The Fas death factor. Science 267:1449-1456.

[11] Lipton SA and PA Rosenberg. (1994). Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 330:613-622.

[12] Park E, AA Velumian and MG Fehlings. (2004). The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma 21:754-774.

[13] Donnelly DJ and PG Popovich. (2008). Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp Neurol 209:378-388.

[14] Xiong Y, AG Rabchevsky and ED Hall. (2007). Role of peroxynitrite in secondary oxidative damage after spinal cord injury. J Neurochem 100:639-649.

[15] Noble LJ and JR Wrathall. (1989). Distribution and time course of protein extravasation in the rat spinal cord after contusive injury. Brain Res 482:57-66.

[16] Fleming JC, MD Norenberg, DA Ramsay, GA Dekaban, AE Marcillo, AD Saenz, M Pasquale-Styles, WD Dietrich and LC Weaver. (2006). The cellular inflammatory response in human spinal cords after injury. Brain 129:3249-3269.

[17] Cheng B, S Christakos and MP Mattson. (1994). Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. Neuron 12:139-153.

[18] Kim GM, J Xu, SK Song, P Yan, G Ku, XM Xu and CY Hsu. (2001). Tumor necrosis factor receptor deletion reduces nuclear factor-kappaB activation, cellular inhibitor of apoptosis protein 2 expression, and functional recovery after traumatic spinal cord injury. J Neurosci 21:6617-6625.

[19] Karimi-Abdolrezaee S, E Eftekharpour, J Wang, CM Morshead and MG Fehlings. (2006). Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci 26:3377-3389.

[20] Keirstead HS, G Nistor, G Bernal, M Totoiu, F Cloutier, K Sharp and O Steward. (2005). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 25:4694-4705.

[21] Hagg T and M Oudega. (2006). Degenerative and spontaneous regenerative processes after spinal cord injury. J Neurotrauma 23:264-280.

[22] Herrmann JE, T Imura, B Song, J Qi, Y Ao, TK Nguyen, RA Korsak, K Takeda, S Akira and MV Sofroniew. (2008). STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. J Neurosci 28:7231-7243.

[23] Hill CE, MS Beattie and JC Bresnahan. (2001). Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. Exp Neurol 171:153-169.

[24] Ehlers MD. (2004). Deconstructing the axon: Wallerian degeneration and the ubiquitin-proteasome system. Trends Neurosci 27:3-6.

[25] Coleman MP and VH Perry. (2002). Axon pathology in neurological disease: a neglected therapeutic target. Trends Neurosci 25:532-537.

[26] McDonald JW, D Becker, CL Sadowsky, JA Jane, Sr., TE Conturo and LM Schultz. (2002). Late recovery following spinal cord injury. Case report and review of the literature. J Neurosurg 97:252-265.

[27] Eftekharpour E, S Karimi-Abdolrezaee, J Wang, H El Beheiry, C Morshead and MG Fehlings. (2007). Myelination of congenitally dysmyelinated spinal cord axons by adult neural precursor cells results in formation of nodes of Ranvier and improved axonal conduction. J Neurosci 27:3416-3428.

[28] Salewski RP, E Eftekharpour and MG Fehlings. (2010). Are induced pluripotent stem cells the future of cell-based regenerative therapies for spinal cord injury? J Cell Physiol 222:515-521.

[29] Hawryluk GW, J Rowland, BK Kwon and MG Fehlings. (2008). Protection and repair of the injured spinal cord: a review of completed, ongoing, and planned clinical trials for acute spinal cord injury. Neurosurg Focus 25:E14.

[30] Caplan AI. (1991). Mesenchymal stem cells. J Orthop Res 9:641-650.

[31] Caplan AI. (1994). The mesengenic process. Clin Plast Surg 21:429-435.

[32] Horwitz EM, K Le Blanc, M Dominici, I Mueller, I Slaper-Cortenbach, FC Marini, RJ Deans, DS Krause and A Keating. (2005). Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 7:393-395.

[33] Dominici M, K Le Blanc, I Mueller, I Slaper-Cortenbach, F Marini, D Krause, R Deans, A Keating, D Prockop and E Horwitz. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315-317.

[34] Tetzlaff W, EB Okon, S Karimi-Abdolrezaee, CE Hill, JS Sparling, JR Plemel, WT Plunet, EC Tsai, D Baptiste, LJ Smithson, MD Kawaja, MG Fehlings and BK Kwon. (2011). A systematic review of cellular transplantation therapies for spinal cord injury. J Neurotrauma 28:1611-1682.

[35] Vallieres L and PE Sawchenko. (2003). Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. J Neurosci 23:5197-5207.

[36] Massengale M, AJ Wagers, H Vogel and IL Weissman. (2005). Hematopoietic cells maintain hematopoietic fates upon entering the brain. J Exp Med 201:1579-1589.

[37] Parr AM, CH Tator and A Keating. (2007). Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 40:609-619.

[38] Lee J, S Kuroda, H Shichinohe, J Ikeda, T Seki, K Hida, M Tada, K Sawada and Y Iwasaki. (2003). Migration and differentiation of nuclear fluorescence-labeled bone marrow stromal cells after transplantation into cerebral infarct and spinal cord injury in mice. Neuropathology 23:169-180.

[39] Koda M, S Okada, T Nakayama, S Koshizuka, T Kamada, Y Nishio, Y Someya, K Yoshinaga, A Okawa, H Moriya and M Yamazaki. (2005). Hematopoietic stem cell and marrow stromal cell for spinal cord injury in mice. Neuroreport 16:1763-1767.

[40] Wu S, Y Suzuki, Y Ejiri, T Noda, H Bai, M Kitada, K Kataoka, M Ohta, H Chou and C Ide. (2003). Bone marrow stromal cells enhance differentiation of cocultured neurosphere cells and promote regeneration of injured spinal cord. J Neurosci Res 72:343-351.

[41] Hofstetter CP, EJ Schwarz, D Hess, J Widenfalk, A El Manira, DJ Prockop and L Olson. (2002). Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc Natl Acad Sci U S A 99:2199-2204.

[42] Zurita M and J Vaquero. (2006). Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation. Neurosci Lett 402:51-56.

[43] Basso DM, MS Beattie and JC Bresnahan. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12:1-21.

[44] Pinzon A, B Calancie, M Oudega and BR Noga. (2001). Conduction of impulses by axons regenerated in a Schwann cell graft in the transected adult rat thoracic spinal cord. J Neurosci Res 64:533-541.

[45] Xu XM, V Guenard, N Kleitman and MB Bunge. (1995). Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. J Comp Neurol 351:145-160.

[46] Pearse DD, AR Sanchez, FC Pereira, CM Andrade, R Puzis, Y Pressman, K Golden, BM Kitay, B Blits, PM Wood and MB Bunge. (2007). Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: Survival, migration, axon association, and functional recovery. Glia 55:976-1000.

[47] Tom VJ and JD Houle. (2008). Intraspinal microinjection of chondroitinase ABC following injury promotes axonal regeneration out of a peripheral nerve graft bridge. Exp Neurol 211:315-319.

[48] Houle JD, VJ Tom, D Mayes, G Wagoner, N Phillips and J Silver. (2006). Combining an autologous peripheral nervous system "bridge" and matrix modification by chon-

droitinase allows robust, functional regeneration beyond a hemisection lesion of the adult rat spinal cord. J Neurosci 26:7405-7415.

[49] Fouad K, L Schnell, MB Bunge, ME Schwab, T Liebscher and DD Pearse. (2005). Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. J Neurosci 25:1169-1178.

[50] Lavdas AA, J Chen, F Papastefanaki, S Chen, M Schachner, R Matsas and D Thomaidou. (2010). Schwann cells engineered to express the cell adhesion molecule L1 accelerate myelination and motor recovery after spinal cord injury. Exp Neurol 221:206-216.

[51] Toma JG, M Akhavan, KJ Fernandes, F Barnabe-Heider, A Sadikot, DR Kaplan and FD Miller. (2001). Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 3:778-784.

[52] Toma JG, IA McKenzie, D Bagli and FD Miller. (2005). Isolation and characterization of multipotent skin-derived precursors from human skin. Stem Cells 23:727-737.

[53] Biernaskie J, JS Sparling, J Liu, CP Shannon, JR Plemel, Y Xie, FD Miller and W Tetzlaff. (2007). Skin-derived precursors generate myelinating Schwann cells that promote remyelination and functional recovery after contusion spinal cord injury. J Neurosci 27:9545-9559.

[54] McKenzie IA, J Biernaskie, JG Toma, R Midha and FD Miller. (2006). Skin-derived precursors generate myelinating Schwann cells for the injured and dysmyelinated nervous system. J Neurosci 26:6651-6660.

[55] Kamada T, M Koda, M Dezawa, K Yoshinaga, M Hashimoto, S Koshizuka, Y Nishio, H Moriya and M Yamazaki. (2005). Transplantation of bone marrow stromal cell-derived Schwann cells promotes axonal regeneration and functional recovery after complete transection of adult rat spinal cord. J Neuropathol Exp Neurol 64:37-45.

[56] Wakao S, T Hayashi, M Kitada, M Kohama, D Matsue, N Teramoto, T Ose, Y Itokazu, K Koshino, H Watabe, H Iida, T Takamoto, Y Tabata and M Dezawa. (2010). Long-term observation of auto-cell transplantation in non-human primate reveals safety and efficiency of bone marrow stromal cell-derived Schwann cells in peripheral nerve regeneration. Exp Neurol 223:537-547.

[57] Park HW, MJ Lim, H Jung, SP Lee, KS Paik and MS Chang. (2010). Human mesenchymal stem cell-derived Schwann cell-like cells exhibit neurotrophic effects, via distinct growth factor production, in a model of spinal cord injury. Glia 58:1118-1132.

[58] Ruff CA, JT Wilcox and MG Fehlings. (2011). Cell-based transplantation strategies to promote plasticity following spinal cord injury. Exp Neurol 235:78-90.

[59] Doucette R. (1991). PNS-CNS transitional zone of the first cranial nerve. J Comp Neurol 312:451-466.

[60] Ramon-Cueto A, MI Cordero, FF Santos-Benito and J Avila. (2000). Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. Neuron 25:425-435.

[61] Ramon-Cueto A, GW Plant, J Avila and MB Bunge. (1998). Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. J Neurosci 18:3803-3815.

[62] Richter MW and AJ Roskams. (2008). Olfactory ensheathing cell transplantation following spinal cord injury: hype or hope? Exp Neurol 209:353-367.

[63] Au E, MW Richter, AJ Vincent, W Tetzlaff, R Aebersold, EH Sage and AJ Roskams. (2007). SPARC from olfactory ensheathing cells stimulates Schwann cells to promote neurite outgrowth and enhances spinal cord repair. J Neurosci 27:7208-7221.

[64] Richter MW, PA Fletcher, J Liu, W Tetzlaff and AJ Roskams. (2005). Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential axon sprouting in the lesioned spinal cord. J Neurosci 25:10700-10711.

[65] Karimi-Abdolrezaee S, E Eftekharpour, J Wang, D Schut and MG Fehlings. (2010). Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. J Neurosci 30:1657-1676.

[66] Morshead CM, BA Reynolds, CG Craig, MW McBurney, WA Staines, D Morassutti, S Weiss and D van der Kooy. (1994). Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron 13:1071-1082.

[67] Weiss S, C Dunne, J Hewson, C Wohl, M Wheatley, AC Peterson and BA Reynolds. (1996). Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci 16:7599-7609.

[68] Weiss S, BA Reynolds, AL Vescovi, C Morshead, CG Craig and D van der Kooy. (1996). Is there a neural stem cell in the mammalian forebrain? Trends Neurosci 19:387-393.

[69] Martens DJ, V Tropepe and D van Der Kooy. (2000). Separate proliferation kinetics of fibroblast growth factor-responsive and epidermal growth factor-responsive neural stem cells within the embryonic forebrain germinal zone. J Neurosci 20:1085-1095.

[70] Yamamoto S, N Yamamoto, T Kitamura, K Nakamura and M Nakafuku. (2001). Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. Exp Neurol 172:115-127.

[71] Gross RE, MF Mehler, PC Mabie, Z Zang, L Santschi and JA Kessler. (1996). Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron 17:595-606.

[72] Weissman IL, DJ Anderson and F Gage. (2001). Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol 17:387-403.

[73] Calaora V, B Rogister, K Bismuth, K Murray, H Brandt, P Leprince, M Marchionni and M Dubois-Dalcq. (2001). Neuregulin signaling regulates neural precursor growth and the generation of oligodendrocytes in vitro. J Neurosci 21:4740-4751.

[74] Hsieh J, JB Aimone, BK Kaspar, T Kuwabara, K Nakashima and FH Gage. (2004). IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes. J Cell Biol 164:111-122.

[75] Falk A, N Holmstrom, M Carlen, R Cassidy, C Lundberg and J Frisen. (2002). Gene delivery to adult neural stem cells. Exp Cell Res 279:34-39.

[76] Hofstetter CP, NA Holmstrom, JA Lilja, P Schweinhardt, J Hao, C Spenger, Z Wiesenfeld-Hallin, SN Kurpad, J Frisen and L Olson. (2005). Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat Neurosci 8:346-353.

[77] Chiasson BJ, V Tropepe, CM Morshead and D van der Kooy. (1999). Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics. J Neurosci 19:4462-4471.

[78] Craig CG, R D'sa, CM Morshead, A Roach and D van der Kooy. (1999). Migrational analysis of the constitutively proliferating subependyma population in adult mouse forebrain. Neuroscience 93:1197-1206.

[79] Evans MJ and MH Kaufman. (1981). Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154-156.

[80] Martin GR. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A 78:7634-7638.

[81] Thomson JA, J Itskovitz-Eldor, SS Shapiro, MA Waknitz, JJ Swiergiel, VS Marshall and JM Jones. (1998). Embryonic stem cell lines derived from human blastocysts. Science 282:1145-1147.

[82] Brons IG, LE Smithers, MW Trotter, P Rugg-Gunn, B Sun, SM Chuva de Sousa Lopes, SK Howlett, A Clarkson, L Ahrlund-Richter, RA Pedersen and L Vallier. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature 448:191-195.

[83] Pierce GB and FJ Dixon, Jr. (1959). Testicular teratomas. I. Demonstration of teratogenesis by metamorphosis of multipotential cells. Cancer 12:573-583.

[84] Martin GR, LM Wiley and I Damjanov. (1977). The development of cystic embryoid bodies in vitro from clonal teratocarcinoma stem cells. Dev Biol 61:230-244.

[85] Keller G. (2005). Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes Dev 19:1129-1155.

[86] McDonald JW, XZ Liu, Y Qu, S Liu, SK Mickey, D Turetsky, DI Gottlieb and DW Choi. (1999). Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 5:1410-1412.

[87] Bain G, D Kitchens, M Yao, JE Huettner and DI Gottlieb. (1995). Embryonic stem cells express neuronal properties in vitro. Dev Biol 168:342-357.

[88] Li XJ, ZW Du, ED Zarnowska, M Pankratz, LO Hansen, RA Pearce and SC Zhang. (2005). Specification of motoneurons from human embryonic stem cells. Nat Biotechnol 23:215-221.

[89] Bibel M, J Richter, K Schrenk, KL Tucker, V Staiger, M Korte, M Goetz and YA Barde. (2004). Differentiation of mouse embryonic stem cells into a defined neuronal lineage. Nat Neurosci 7:1003-1009.

[90] Rathjen J, JA Lake, MD Bettess, JM Washington, G Chapman and PD Rathjen. (1999). Formation of a primitive ectoderm like cell population, EPL cells, from ES cells in response to biologically derived factors. J Cell Sci 112 (Pt 5):601-612.

[91] Lee H, GA Shamy, Y Elkabetz, CM Schofield, NL Harrsion, G Panagiotakos, ND Socci, V Tabar and L Studer. (2007). Directed differentiation and transplantation of human embryonic stem cell-derived motoneurons. Stem Cells 25:1931-1939.

[92] Rowland JW, JJ Lee, RP Salewski, E Eftekharpour, D van der Kooy and MG Fehlings. (2011). Generation of neural stem cells from embryonic stem cells using the default mechanism: in vitro and in vivo characterization. Stem Cells Dev 20:1829-1845.

[93] Tropepe V, S Hitoshi, C Sirard, TW Mak, J Rossant and D van der Kooy. (2001). Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism. Neuron 30:65-78.

[94] Smukler SR, SB Runciman, S Xu and D van der Kooy. (2006). Embryonic stem cells assume a primitive neural stem cell fate in the absence of extrinsic influences. J Cell Biol 172:79-90.

[95] Chernoff GF. (1981). Shiverer: an autosomal recessive mutant mouse with myelin deficiency. J Hered 72:128.

[96] Bu J, A Banki, Q Wu and A Nishiyama. (2004). Increased NG2(+) glial cell proliferation and oligodendrocyte generation in the hypomyelinating mutant shiverer. Glia 48:51-63.

[97] Wu Q, RH Miller, RM Ransohoff, S Robinson, J Bu and A Nishiyama. (2000). Elevated levels of the chemokine GRO-1 correlate with elevated oligodendrocyte progenitor proliferation in the jimpy mutant. J Neurosci 20:2609-2617.

[98] Canoll PD, JM Musacchio, R Hardy, R Reynolds, MA Marchionni and JL Salzer. (1996). GGF/neuregulin is a neuronal signal that promotes the proliferation and survival and inhibits the differentiation of oligodendrocyte progenitors. Neuron 17:229-243.

[99] Hochedlinger K and R Jaenisch. (2006). Nuclear reprogramming and pluripotency. Nature 441:1061-1067.

[100] Campbell KH, J McWhir, WA Ritchie and I Wilmut. (1996). Sheep cloned by nuclear transfer from a cultured cell line. Nature 380:64-66.

[101] Takahashi K and S Yamanaka. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663-676.

[102] Liu H, F Zhu, J Yong, P Zhang, P Hou, H Li, W Jiang, J Cai, M Liu, K Cui, X Qu, T Xiang, D Lu, X Chi, G Gao, W Ji, M Ding and H Deng. (2008). Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. Cell Stem Cell 3:587-590.

[103] Liao J, C Cui, S Chen, J Ren, J Chen, Y Gao, H Li, N Jia, L Cheng, H Xiao and L Xiao. (2009). Generation of induced pluripotent stem cell lines from adult rat cells. Cell Stem Cell 4:11-15.

[104] Takahashi K, K Tanabe, M Ohnuki, M Narita, T Ichisaka, K Tomoda and S Yamanaka. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861-872.

[105] Yu J, MA Vodyanik, K Smuga-Otto, J Antosiewicz-Bourget, JL Frane, S Tian, J Nie, GA Jonsdottir, V Ruotti, R Stewart, Slukvin, II and JA Thomson. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917-1920.

[106] Lowry WE, L Richter, R Yachechko, AD Pyle, J Tchieu, R Sridharan, AT Clark and K Plath. (2008). Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci U S A 105:2883-2888.

[107] Park IH, R Zhao, JA West, A Yabuuchi, H Huo, TA Ince, PH Lerou, MW Lensch and GQ Daley. (2008). Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451:141-146.

[108] Kaji K, K Norrby, A Paca, M Mileikovsky, P Mohseni and K Woltjen. (2009). Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458:771-775.

[109] Woltjen K, IP Michael, P Mohseni, R Desai, M Mileikovsky, R Hamalainen, R Cowling, W Wang, P Liu, M Gertsenstein, K Kaji, HK Sung and A Nagy. (2009). piggyBac

transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458:766-770.

[110] Soldner F, D Hockemeyer, C Beard, Q Gao, GW Bell, EG Cook, G Hargus, A Blak, O Cooper, M Mitalipova, O Isacson and R Jaenisch. (2009). Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136:964-977.

[111] Sommer CA, M Stadtfeld, GJ Murphy, K Hochedlinger, DN Kotton and G Mostoslavsky. (2009). Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. Stem Cells 27:543-549.

[112] Yu J, K Hu, K Smuga-Otto, S Tian, R Stewart, Slukvin, II and JA Thomson. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. Science 324:797-801.

[113] Zhou H, S Wu, JY Joo, S Zhu, DW Han, T Lin, S Trauger, G Bien, S Yao, Y Zhu, G Siuzdak, HR Scholer, L Duan and S Ding. (2009). Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4:381-384.

[114] Kim K, A Doi, B Wen, K Ng, R Zhao, P Cahan, J Kim, MJ Aryee, H Ji, LI Ehrlich, A Yabuuchi, A Takeuchi, KC Cunniff, H Hongguang, S McKinney-Freeman, O Naveiras, TJ Yoon, RA Irizarry, N Jung, J Seita, J Hanna, P Murakami, R Jaenisch, R Weissleder, SH Orkin, IL Weissman, AP Feinberg and GQ Daley. (2010). Epigenetic memory in induced pluripotent stem cells. Nature 467:285-290.

[115] Wernig M, JP Zhao, J Pruszak, E Hedlund, D Fu, F Soldner, V Broccoli, M Constantine-Paton, O Isacson and R Jaenisch. (2008). Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A 105:5856-5861.

[116] Ebert AD, J Yu, FF Rose, Jr., VB Mattis, CL Lorson, JA Thomson and CN Svendsen. (2009). Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457:277-280.

[117] Dimos JT, KT Rodolfa, KK Niakan, LM Weisenthal, H Mitsumoto, W Chung, GF Croft, G Saphier, R Leibel, R Goland, H Wichterle, CE Henderson and K Eggan. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321:1218-1221.

[118] Tsuji O, K Miura, Y Okada, K Fujiyoshi, M Mukaino, N Nagoshi, K Kitamura, G Kumagai, M Nishino, S Tomisato, H Higashi, T Nagai, H Katoh, K Kohda, Y Matsuzaki, M Yuzaki, E Ikeda, Y Toyama, M Nakamura, S Yamanaka and H Okano. (2010). Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci U S A 107:12704-12709.

[119] Miura K, Y Okada, T Aoi, A Okada, K Takahashi, K Okita, M Nakagawa, M Koyanagi, K Tanabe, M Ohnuki, D Ogawa, E Ikeda, H Okano and S Yamanaka. (2009). Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol 27:743-745.

[120] Salewski RP, J Buttigieg, RA Mitchell, D van der Kooy, A Nagy and MG Fehlings. (2012). The Generation of Definitive Neural Stem Cells from PiggyBac Transposon-Induced Pluripotent Stem Cells Can Be Enhanced by Induction of the NOTCH Signaling Pathway. Stem Cells Dev.

[121] Yoon K and N Gaiano. (2005). Notch signaling in the mammalian central nervous system: insights from mouse mutants. Nat Neurosci 8:709-715.

[122] Louvi A and S Artavanis-Tsakonas. (2006). Notch signalling in vertebrate neural development. Nat Rev Neurosci 7:93-102.

[123] Nakamura Y, S Sakakibara, T Miyata, M Ogawa, T Shimazaki, S Weiss, R Kageyama and H Okano. (2000). The bHLH gene hes1 as a repressor of the neuronal commitment of CNS stem cells. J Neurosci 20:283-293.

[124] Hitoshi S, T Alexson, V Tropepe, D Donoviel, AJ Elia, JS Nye, RA Conlon, TW Mak, A Bernstein and D van der Kooy. (2002). Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev 16:846-858.

[125] Androutsellis-Theotokis A, RR Leker, F Soldner, DJ Hoeppner, R Ravin, SW Poser, MA Rueger, SK Bae, R Kittappa and RD McKay. (2006). Notch signalling regulates stem cell numbers in vitro and in vivo. Nature 442:823-826.

[126] Ables JL, JJ Breunig, AJ Eisch and P Rakic. (2011). Not(ch) just development: Notch signalling in the adult brain. Nat Rev Neurosci 12:269-283.

[127] Hitoshi S, RM Seaberg, C Koscik, T Alexson, S Kusunoki, I Kanazawa, S Tsuji and D van der Kooy. (2004). Primitive neural stem cells from the mammalian epiblast differentiate to definitive neural stem cells under the control of Notch signaling. Genes Dev 18:1806-1811.

[128] D'Souza B, L Meloty-Kapella and G Weinmaster. (2010). Canonical and non-canonical Notch ligands. Curr Top Dev Biol 92:73-129.

[129] Dang LT and V Tropepe. (2010). FGF dependent regulation of Zfhx1b gene expression promotes the formation of definitive neural stem cells in the mouse anterior neurectoderm. Neural Dev 5.

[130] Hirsch C, LM Campano, S Wohrle and A Hecht. (2007). Canonical Wnt signaling transiently stimulates proliferation and enhances neurogenesis in neonatal neural progenitor cultures. Exp Cell Res 313:572-587.

[131] Fehlings MG and CH Tator. (1995). The relationships among the severity of spinal cord injury, residual neurological function, axon counts, and counts of retrogradely labeled neurons after experimental spinal cord injury. Exp Neurol 132:220-228.

[132] Fehlings MG, CH Tator and RD Linden. (1989). The relationships among the severity of spinal cord injury, motor and somatosensory evoked potentials and spinal cord blood flow. Electroencephalogr Clin Neurophysiol 74:241-259.

[133] Nashmi R and MG Fehlings. (2001). Mechanisms of axonal dysfunction after spinal cord injury: with an emphasis on the role of voltage-gated potassium channels. Brain Res Brain Res Rev 38:165-191.

[134] Nashmi R, OT Jones and MG Fehlings. (2000). Abnormal axonal physiology is associated with altered expression and distribution of Kv1.1 and Kv1.2 K+ channels after chronic spinal cord injury. Eur J Neurosci 12:491-506.

[135] Enzmann GU, RL Benton, JF Talbott, Q Cao and SR Whittemore. (2006). Functional considerations of stem cell transplantation therapy for spinal cord repair. J Neurotrauma 23:479-495.

[136] Koshizuka S, S Okada, A Okawa, M Koda, M Murasawa, M Hashimoto, T Kamada, K Yoshinaga, M Murakami, H Moriya and M Yamazaki. (2004). Transplanted hematopoietic stem cells from bone marrow differentiate into neural lineage cells and promote functional recovery after spinal cord injury in mice. J Neuropathol Exp Neurol 63:64-72.

[137] Ohta M, Y Suzuki, T Noda, Y Ejiri, M Dezawa, K Kataoka, H Chou, N Ishikawa, N Matsumoto, Y Iwashita, E Mizuta, S Kuno and C Ide. (2004). Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. Exp Neurol 187:266-278.

[138] Koda M, M Murakami, H Ino, K Yoshinaga, O Ikeda, M Hashimoto, M Yamazaki, C Nakayama and H Moriya. (2002). Brain-derived neurotrophic factor suppresses delayed apoptosis of oligodendrocytes after spinal cord injury in rats. J Neurotrauma 19:777-785.

[139] Neuhuber B, B Timothy Himes, JS Shumsky, G Gallo and I Fischer. (2005). Axon growth and recovery of function supported by human bone marrow stromal cells in the injured spinal cord exhibit donor variations. Brain Res 1035:73-85.

[140] Tolwani RJ, JM Cosgaya, S Varma, R Jacob, LE Kuo and EM Shooter. (2004). BDNF overexpression produces a long-term increase in myelin formation in the peripheral nervous system. J Neurosci Res 77:662-669.

[141] Hempstead BL and JL Salzer. (2002). Neurobiology. A glial spin on neurotrophins. Science 298:1184-1186.

[142] Nakamura M, H Okano, Y Toyama, HN Dai, TP Finn and BS Bregman. (2005). Transplantation of embryonic spinal cord-derived neurospheres support growth of supra-

spinal projections and functional recovery after spinal cord injury in the neonatal rat. J Neurosci Res 81:457-468.

[143] Chen Q, L Zhou and HD Shine. (2006). Expression of neurotrophin-3 promotes axonal plasticity in the acute but not chronic injured spinal cord. J Neurotrauma 23:1254-1260.

[144] Hawryluk GW, AJ Mothe, M Chamankhah, J Wang, C Tator and MG Fehlings. (2010). In vitro characterization of trophic factor expression in neural precursor cells. Stem Cells Dev 21:432-447.

[145] Hawryluk GW, A Mothe, J Wang, S Wang, C Tator and MG Fehlings. (2012). An in vivo characterization of trophic factor production following neural precursor cell or bone marrow stromal cell transplantation for spinal cord injury. Stem Cells Dev 21:2222-2238.