Review

Stem Cell-Based Therapy for Spinal Cord Injury

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Stem cells (SCs) represent a new therapeutic approach for spinal cord injury (SCI) by enabling improved sensory and motor functions in animal models. The main goal of SC-based therapy for SCI is the replacement of neurons and glial cells that undergo cell death soon after injury. Stem cells are able to promote remyelination via oligodendroglia cell replacement to produce trophic factors enhancing neurite outgrowth, axonal elongation, and fiber density and to activate resident or transplanted progenitor cells across the lesion cavity. While several SC transplantation strategies have shown promising yet partial efficacy, mechanistic proof is generally lacking and is arguably the largest impediment toward faster progress and clinical application. The main challenge ahead is to spur on cooperation between clinicians, researchers, and patients in order to define and optimize the mechanisms of SC function and to establish the ideal source/s of SCs that produce efficient and also safe therapeutic approaches.

Key words: Stem cells (SCs); Spinal cord injury (SCI); Differentiation; Remyelination; Inflammation

INTRODUCTION

Spinal cord injury (SCI) is a devastating disorder with frustrating implications both for the individual and the society. Spinal cord injury has a profound effect on a patient's physical and psychosocial well-being because it often results in permanent loss of bodily functions affecting limb movement, somatosensation, reproductive organs, bladder, and bowel. With respect to the costs of health care and living expenses, SCI poses a substantial burden on the healthcare system: the average lifetime cost of treating an individual with SCI is up to $2 million with $7 billion spent annually for caring for the SCI patients in the US alone (70).

By definition, SCI can be traumatic and nontraumatic, depending on the cause of injury (Table 1). Epidemiological data show that the incidence of traumatic SCI in the US ranges from 27 to 83 per million while in Europe it is approximately 10–30 new cases per million (41,117). The prevalence of nontraumatic spinal cord lesions is unknown due to the absence of state and federal registries. Spinal cord injury results in the marked loss of neurons and other cell types at injury site immediately after injury which is exacerbated with time. The pathophysiology of SCI can be divided clinically into two phases: primary injury phase and secondary injury phase (Fig. 1 and Table 2).

Primary Injury Phase

Primary injury is due to the direct compression and contusion of the spinal cord by fractured and displaced bone fragments and disc material as a result of fracture–dislocation or burst fracture of the spine (92). The nerve cells are damaged, axons are disrupted, and neural cell membranes are ruptured. Injury of blood vessels is followed by microhemorrhages in the central gray matter

Table 1. The Main Causes of Spinal Cord Lesions

The main causes of traumatic SCI are:

1. motor vehicle accidents
2. violence
3. falls
4. recreational activities

The main causes of nontraumatic SCI are:

1. genetic and metabolic diseases of CNS
2. degenerative disorders of CNS
3. primary and metastatic (intramedullary and extramedullary) tumors
4. congenital and developmental diseases of CNS
5. infectious (viral, bacterial, fungal, parasitic) and inflammatory diseases
6. toxins
7. ischemic diseases of CNS

CNS, central nervous system.

that spreads out radially and axially, leading to spinal cord swelling and secondary ischemia (70). Ischemia, altered ion balance, and toxins (24) released from disrupted neural membranes trigger a secondary injury cascade that further exacerbates SCI.

Secondary Injury Phase

The secondary injury phase is described as a complex damage that occurs at the cellular level as a result of an interrelated series of pathophysiological processes including electrolyte imbalance, ischemia, excitotoxicity, oxidative stress, inflammation, and massive cell death due to immune response to the injury (92). Secondary injury starts with depolarization and voltage-dependent opening of sodium, potassium, and calcium ion channels. Overload of calcium ions initiates mitochondrial dysfunction and the activation of cytoplasmic nitric oxide synthase and phospholipase A2, which leads to microvascular damage and consequential ischemia (35). Damaged cells, axons,
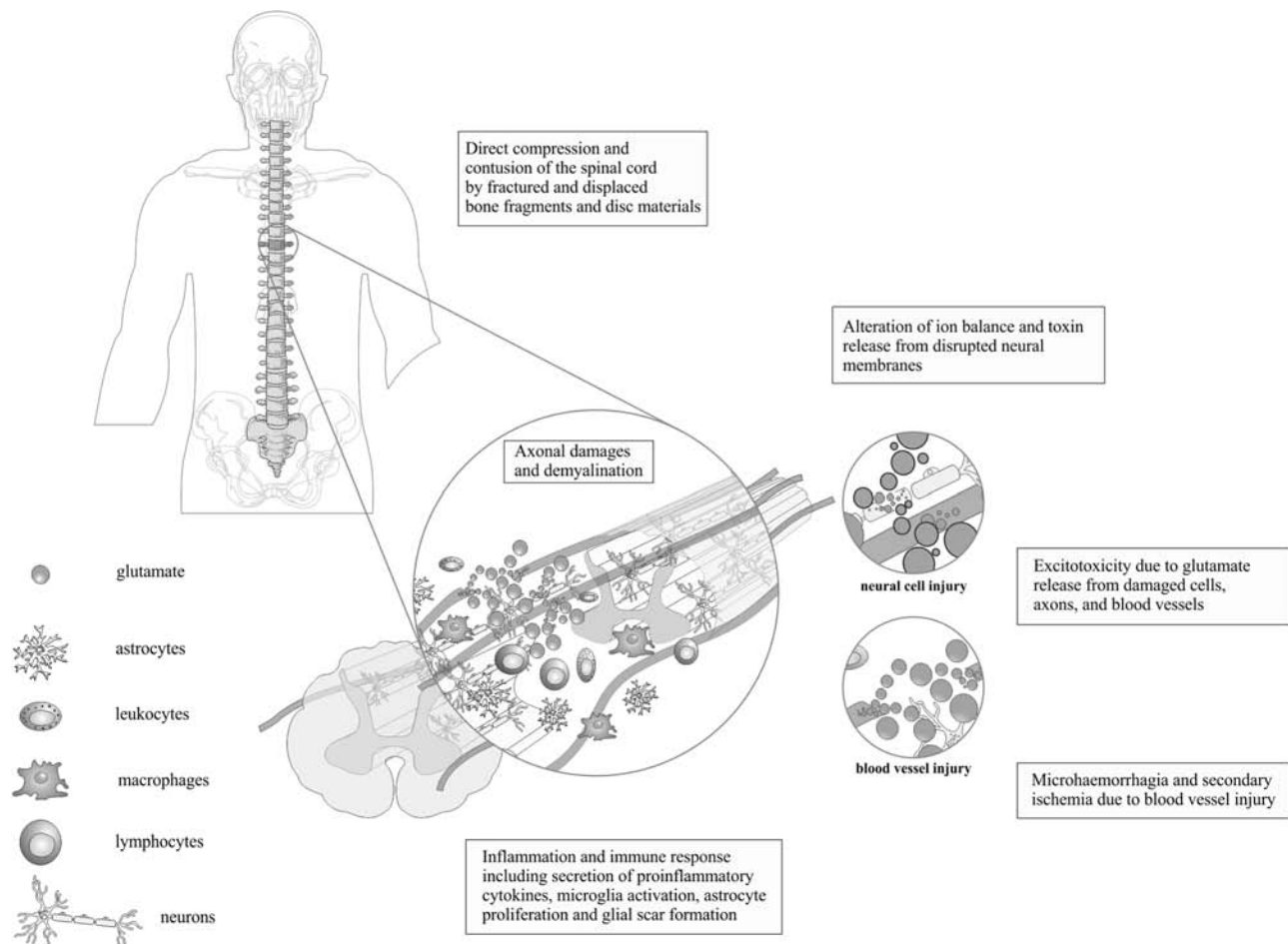
Figure 1. The mechanisms involved in spinal cord injury. Spinal cord injury can be sustained through different mechanisms, with the following common main abnormalities leading to tissue damage: (i) destruction from direct trauma; (ii) compression by bone fragments, hematoma, or disk material; and (iii) ischemia from damage or impingement of the spinal blood vessels.

Table 2. A Summary of Pathophysiological Events Following SCI

Primary injury phase

1. Direct compression and contusion of the spinal cord by fractured and displaced bone fragments and disc materials
2. Neural cell injury and disruption of axons
3. Alteration of ion balance and toxin release from disrupted neural membranes
4. Microhemorrhagia and secondary ischemia due to blood vessel injury

Secondary injury phase

1. Excitotoxicity due to glutamate release from damaged cells, axons, and blood vessels
2. Electrolyte imbalance, increased production of free radicals and oxidative stress
3. Inflammation due to the loss of blood–brain barrier and leukocyte migration to the injury site
4. Immune response including secretion of proinflammatory cytokines, microglia activation, astrocyte proliferation and glial scar formation
5. Fas receptor activation and caspase cascade initiation
6. Apoptosis and necrosis of neural cells
7. Axonal damages and demyelination

and blood vessels release toxic chemicals including glutamate that attack intact neighboring cells in a highly disruptive process known as excitotoxicity (70). Glutamate, normally secreted in minimal amounts at the end tips of many axons, binds to receptors on target neurons and stimulates them to conduct impulses. After SCI, glutamate is massively released from injured spinal neurons, axons, and astrocytes, overexciting neighboring neurons and triggering the production of free radicals. During the progression of SCI, the level of free radicals constantly increases at the lesion site leading to the reduction in membrane permeability. Cascades of radical-mediated peroxidation affect cell membranes through oxidation of lipid bilayer membranes and disrupt the electron transport chain portion of the metabolic process (31). The loss of ionic homeostasis accompanied by excitotoxicity and oxidative stress leads to massive cell death in SCI. Many functional neurons and glia including oligodendrocytes (the nervous system's myelin-producing cells) die by apoptosis and necrosis (7). Although the precise mechanism of oligodendrocyte apoptosis is not clearly known, oligodendrocytes can undergo excitotoxic cell death through their glutamate receptors or through Fas receptor/Fas ligand interaction (5,12). This is achieved by activation of Fas receptors (located on the surface of oligodendrocytes) and by Fas ligands that are expressed by activated microglia (12) triggering caspase-mediated apoptotic cell death of oligodendrocytes. The loss of

oligodendrocytes effectively initiates axon demyelination and blocks transmission of action potentials.

The blood–brain barrier normally works as a highly selective filter that prevents influx of mononuclear cells (MNCs) from the blood into the central nervous system (CNS). However, SCI results in increased permeability of the blood–brain barrier, allowing massive MNC infiltration in the medullar tissue that triggers an inflammatory response. At the injury site, levels of proinflammatory cytokines, particularly interleukin 1α and -β, and tumor necrosis factor-α (86) are increased, leading to the activation of microglia cells (51). In turn, activated microglia cells increase the expression of monocyte chemoattractant protein-1 and chemokines that direct leukocytes to the site of injury (2), promoting inflammation. Furthermore, as previously described, activated microglia cells express Fas ligands that interact with Fas receptors on neurons and oligodendrocytes, initiating apoptotic cell death. In SCI cases, microglia cells activate T-cells that pass through the blood–brain barrier and infiltrate damaged spinal cord. Both T lymphocytes and glial cells are able to protect neurons from secondary degeneration either by releasing neurotrophins (T-cells) or by forming scars (glial cells) that isolate neural tissue from inflammatory cells and decrease neuroinflammation (33,73). Several hours after SCI, astrocytes in the lesion site proliferate, join together tightly, and form astrocytic (glial) scars that are beneficial for the reestablishment of physical and chemical integrity of the spinal cord but are also responsible for prevention and complications of neuroregeneration (33).

The commonly affected areas of SCI are the cervical and lumbar spine. Damage to upper motoneurons results in hyperreflexia, hypertonia, and muscle weaknesses, while the insults to lower motoneurons cause hyporeflexia, hypotonia, and muscle atrophy (69,114). After SCI, the entire nervous system should be carefully examined because posttraumatic multilevel cord injury is not rare and SCI could be accompanied by serious brain injury (19). The severity of SCI is determined by neurological assessment often using the five-level (A–E) American Spinal Injury Association (ASIA) impairment scale (70). Although various bodily functions are affected after SCI, the absence of bowel and bladder control, limitations in hand use, and difficulty in breathing are critical (70); therefore, the primary goal of restorative therapy for SCI should be improvement of these functions. This could be achieved by preventing progressive cell death through replacement of damaged cells, by repairing the myelin sheath, and by reconnecting injured nerve fibers with their original targets that all lead to partial or complete rehabilitation of nerve and muscle function. Preclinical and clinical studies have shown that SCs might play an important role in many of these processes. Therefore, we review here the potential of human

Table 3. Therapeutic Potential of Stem Cells for Treatment of Spinal Cord Injury

Stem cells that have the ability to differentiate toward defined neural cell types in vitro:

  hESCs and iPSCs: neurons, glial cells and motoneurons.
  MSCs: NSCs, neurons, astrocytes, oligodendrocytes and Schwann cells.
  NSCs: neurons, oligodendrocytes and astrocytes.

Main mechanisms responsible for functional recovery of stem cell-treated animal models of spinal cord injury:

  hESCs: differentiation into neuronal and glial cells, modulation of local immune response, neuronal protection and activation of endogenous neurogenesis.
  MSCs: providing trophic support to damaged neurons by secreting angiogenic and neurotrophic factors and modulation of local immune response.
  NSCs: differentiation into oligodendrocytes and astrocytes.

Limitations for stem cell-based therapy in humans:

  hESCs and iPSCs: potential for tumor formation.
  MSCs: the proof of functional neurons derived from transplanted MSCs has not been provided yet.
  NSCs: graft rejection, lack of neurotrophic factors, decrease of differentiation potential after several passages, formation of glial scars.
  hESCs, human embryonic stem cells; MSCs, mesenchymal stem cells; NSCs, neural stem cells; iPSCs, induced pluripotent stem cells.

embryonic SCs (hESCs), adult SCs (ASCs) including mesenchymal SCs (MSCs), neural SCs (NSCs), and a new source of reprogrammed somatic cells: induced pluripotent SCs (iPSCs) as a potential therapeutic agent in the treatment of SCI (Table 3).

CURRENT STEM CELL-BASED THERAPIES FOR SPINAL CORD INJURY

Human Embryonic Stem Cells

Human embryonic stem cells are pluripotent cells derived from the inner cell mass of the early blastocyst with the ability to proliferate for a long period under in vitro conditions and with a potential for differentiation into a broad range of cell types including specific cells of neuronal or glial fates (26). In view of this, hESCs are a promising source for generation of differentiated oligodendrocytes and motoneurons (63,78), as a potential novel approach to treat SCI. Clinical applications of hESCs critically depend on their ability to differentiate toward defined and purified neural cell types in vitro. Recently, considerable progress has been achieved. Several studies, including our own (4,25,26,32,63,78), have focused on the improvement of the methods for predifferentiation of hESCs into neural or neuronal precursors prior to

cell transplantation in animal models of SCI. As a result improved protocols for relatively efficient generation and propagation of motoneuron progenitors (MPs) and oligodendrocyte progenitors (OPCs) via targeted differentiation of hESCs have been developed (25,78). Motoneuron progenitors derived from hESCs have the ability to mature and develop fundamental functions of normal motoneurons in vitro including directional growth of long axons (25). Transplantation of hESC-derived OPCs can efficiently recover locomotor function in contusion and transection animal models of SCI (25,78). Furthermore, in contusion models of SCI, surviving axons persist in the subpial rim of white matter. Most importantly, transplanted hESC-derived OPCs survive, integrate, differentiate, and remyelinate damaged tissue, resulting in a significant improvement of locomotor function of rats with spinal cord contusions (78).

For a long time, it was believed that, after complete spinal transaction, there were no spared host axons or spontaneous regeneration (41,70). However, we recently demonstrated (25) that OPCs and MPs derived from hESCs, when transplanted into the rat spinal cord immediately after injury, have the ability to migrate and engraft for at least 4 months. The main mechanism responsible for locomotor recovery of these animals is the ability of hESC-derived OPCs and MPs to differentiate into neuronal and glial cells after transplantation. However, it seems that the regenerative mechanism of hESC therapy for SCI does not depend exclusively on the differentiation potential of transplanted cells. Immunomodulatory characteristics of transplanted hESC-derived OPCs could also be responsible for the significant recovery of animals after SCI. Within the lesion, SCs and hESC-derived OPCs are able to generate a paracrine/trophic environment and modulate the local immune response promoting neuronal protection and activation of endogenous neurogenesis (50,74,87).

Despite promising results obtained in preclinical studies, there are several concerns regarding the safety of transplantation of hESCs in humans, including the formation of teratoma (64). The possible reason for this problem could be the usage of different cell lines, various differentiation protocols, and transplantation of heterogeneous cell populations. Therefore, prolonged differentiation of hESCs (8), inhibition of signaling pathways activating cell proliferation (13,64), and pure cell population eliminates the incidence of tumor formation (8). As a consequence, a range of clinical trials involving administration of hESCs or hESC-derived OPCs for SCI treatment has already taken place including the one from Geron, which attempted to discern the safety of stem cell therapy in SCI on humans by using hESC-derived OPCs (known as "GRNOPC1") in order to remyelinate axons within the injured spinal cord (for more information,

please see http://www.geron.com/GRNOPC1Trial). Food and Drug Administration (FDA) regulatory authority halted the Geron trial after preclinical studies showed that some of SCI animals treated with Geron's cell line developed small spinal cysts at the treatment site. The FDA requested further characterization of differentiated cells and more preclinical trials with GRNOPC1 cells in animal models. The company subsequently reported to have identified batches of GRNOPC1 cells that did not cause cyst formation in animal models. Following these results, the company received regulatory approval to proceed with clinical trials, and the first patient with SCI received GRNOPC1 cells in October 2010. All patients enrolled in the Geron trial received GRNOPC1 cells within acute phase (7–14 days after SCI). This strategy was based on the studies conducted in animal models that showed that transplantation of cells in chronic phase results in insignificant remyelination and poor locomotor improvement (50). Patients with thoracic SCI are more often enrolled in Phase 1 clinical trials for cell transplantation than people with other SCI types since the cell loss in these patients may not be life threatening as opposed to cervical injury. Regarding this matter, all patients enrolled in the Geron trial had a thoracic ASIA grade A SCI with neurological levels of T3 to T10. Unfortunately, in November 2011, due to financial reasons, the company announced the end of the medical research using hESCs for treatment of SCI and stated that the data obtained from enrolled Phase 1 patients will be available in 2013.

Adult Stem Cells

Adult SCs (ASCs) have been identified in many organs and tissues including bone marrow, brain, spinal cord, skin, skeletal muscle, and bone (88). Depending on the tissue of origin, ASCs exhibit the potency to differentiate into multiple or specific cell types, and their main role is believed to be protective (88).

Although numerous preclinical studies have been conducted, there is still no unique consensus about which types of ASCs are most effective for the treatment of SCI. Transplantation of MSCs, olfactory ensheathing cells (OECs), and NSCs showed similar effects in the experimental models of SCI (38,67). All these cells are able to incorporate into damaged spinal cord and promote regeneration of damaged axons, leading to partial neurological improvements (38).

Significantly higher bar grip power and spontaneous motor activity were noticed in marmosets with contused spinal cords after transplantation of in vitro-expanded human NSCs (43), while enhanced regenerative sprouting of the rubrospinal tract, better locomotion, and hind limb function were seen in rats with SCI after implantation of OECs overexpressing the trophic factor neurotrophin-3 (94). Although the precise mechanisms by which SCs

function in SCI are still unknown, it seems that different SCs showed similar beneficial effect by using different mechanisms: NSCs mainly differentiate into neurons, astrocytes, and oligodendrocytes in spinal cord lesions, while MSCs principally act as neuroprotectors by secreting various angiogenic and neurotrophic factors providing trophic support to damage neurons (Fig. 2) (53,95).

Mesenchymal Stem Cells

Due to their immunomodulatory ability and capacity for self-renewal and differentiation into tissues of mesodermal origin, MSCs are ASCs that are most often used in preclinical and clinical studies for the treatment of various diseases including SCI. Although many studies (82,88,96,119) optimistically reported that MSCs could be transdifferentiated or converted into NSCs, neurons, astrocytes, oligodendrocytes, and Schwann cells (37,49,113), definitive proof of functional neurons derived from transplanted MSCs has not been provided. There is a general agreement in the literature that the benefits of MSC therapy in SCI are a result of indirect environmental modification rather than direct translineage conversion to functional oligodendrocytes or neurons (93). In SCI treatment, MSCs are thought to act as neuroprotectors by secreting various angiogenic and neurotrophic factors such as brain-derived neurotrophic factor, nerve growth factor, vascular endothelial growth factor, and hepatocyte growth factor (53,95), providing trophic support to damaged neurons and resulting in clinical improvement in patients with SCI.

The most widely applied SC-based therapy for SCI treatment in humans is the transplantation of bone marrow (BM)-derived MSCs (BMMSCs) (93). Mesenchymal SCs can be safely and easily obtained from human BM, and autologous transplantation of BMMSCs is a minimally invasive procedure that could be used successfully as novel cell-based transplant strategy for SCI therapy (93). During the last 5 years, several clinical trials, using transplantation of autologous BMMSCs for treatment of patients with SCI, were performed in Brazil (10,17), India (57,80), Argentina (75), Czech Republic (100), Russia (14), Turkey (21), and South Korea (118). Autologous BMMSCs were transplanted around the spinal cord lesion in 35 patients with complete SCI (patients were in acute, subacute, and chronic stages of the disease) (118). The main side effect was neuropathic pain observed in 7.7% of MSC-treated patients (118). During the 10-month follow-up period, 30.4% of patients who received MSCs in acute and subacute stages showed significant improvements in the ASIA scale (118). Sykova et al. (100) suggested that MSCs should be transplanted during a so-called therapeutic window of 3–4 weeks following SCI, which is, according to their results, critical for the success of MSC-based therapy for SCI (100). Intravenous or intraarterial
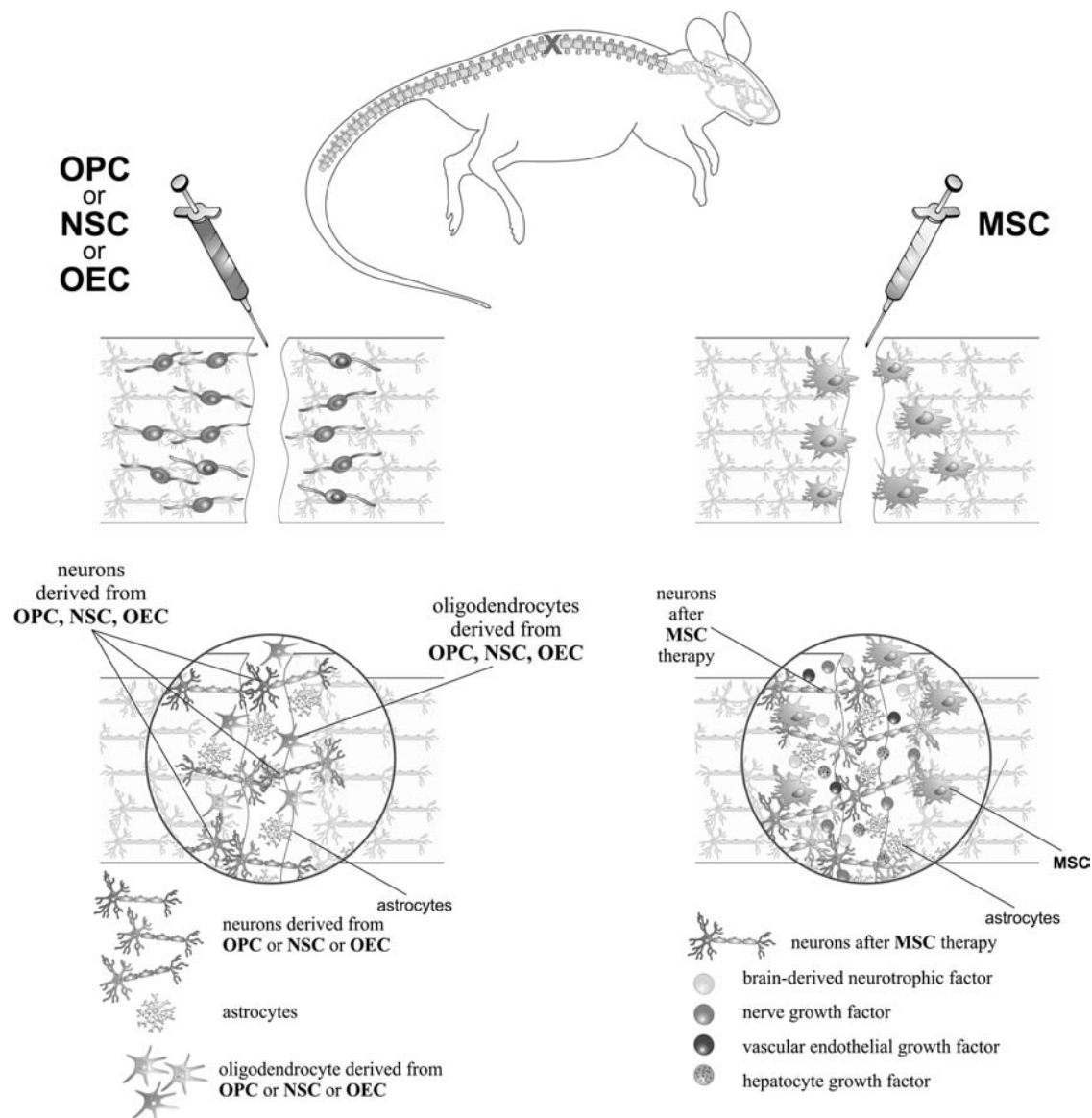
Figure 2. The main mechanisms responsible for functional recovery of stem cell-treated animal models with spinal cord injury (SCI). Transplantation of NSCs, OPCs, and OECs efficiently recovered locomotor function in both contusion and transection animal models of SCI, while MSCs act as neuroprotectors and promote early functional recovery after SCI by secreting various angiogenic and neurotrophic factors. MSC, mesenchymal stem cell; NSC, neural stem cell; OEC, olfactory ensheathing cell; OPC, oligodendrocyte progenitor.

injection of autologous BMMSCs resulted in sensory and motor improvements in five of eight patients with complete SCI in the subacute stage of disease, while significant recovery of locomotor functions was observed in only 1 of 12 chronic patients with SCI (100). Another large clinical trial performed in India (57), consisting of 297 patients with chronic SCI, showed that intrathecal transplantation of autologous BMMSCs leads to clinical improvements in patients with chronic SCI. However, caution should be exerted when results from this study are interpreted. Patients are followed up for only 3 months,

and detailed information about the period from injury to MSC transplantation was not obtained (57). Other studies have also shown improvements in MSC-treated patients that are in the chronic stage of SCI (10,17,21). Neurological improvements were seen in 66.7% (14) and 100% (10), respectively, of patients with chronic SCI enrolled in clinical trials performed in Russia and Brazil. In these studies, cells were transplanted either directly in the lesion or intravenously and intrathecally simultaneously. In addition, after a 30-month follow-up trial, Cristante and coworkers (17) showed that intraarterial

delivery of BMMSCs resulted in electrophysiological improvement in chronic SCI patients with paraplegia or tetraplegia.

Although results from these clinical studies are promising, all these trials showed no universal consistency in terms of donor age/sex and cell passaging/culture conditions. As the mechanism by which MSCs function remains unknown and considering the inconsistency associated with cell sourcing and conditions employed, further studies must be done in order to isolate and characterize more homogeneous BMMSC populations to obtain reliable, repeatable results.

Olfactory Ensheathing Cells

OECs are specialized glial cells that are found in the nerve fiber layer of the olfactory bulb and in the nasal olfactory mucosa (102). Based on their unique property of bridging the peripheral and CNS and providing a channel for peripheral axonal growth into the CNS (89), several preclinical studies investigated therapeutic potential of OECs in the treatment of incomplete and complete SCI and reported neural tissue sparing, axon remyelination, and significant improvements in motor performance after OEC transplantation (28,30,42,56,66,76,90,91). The improvements depended on the SCI model, the duration of the study, the source, the age, and the methods used to prepare and transplant the OECs (29). Cells derived from the olfactory bulbs of adult rodents were the most commonly used in the SCI studies (105). The most promising OECs for SCI therapy were p75-nerve growth factor receptor (NGFR)-positive OECs (94). These cells were able to promote axon regeneration, intermingle with astrocytes, and migrate well within the reactive astrocytic environment after injury (58,59). Severe contusions of the spinal cord in a rat model were efficiently treated after cotransplantation of BMSCs and human p75-positive OECs harvested from the outer layers of the olfactory bulbs from human fetuses (22). Although there was no functional improvement of thoracic SCI after injection of OECs alone, the transplantation of both OECs and Schwann cells promoted behavioral benefits in contused animals (84). In the experimental models of complete thoracic transaction, transplantation of OECs resulted in the regeneration of corticospinal axons and led to improvements of motor behavior 3 and 7 months after injury (56,90). In addition, studies that investigated therapeutic potential of adult bulb-derived OECs transplanted in animals with partial spinal cord lesion showed that OECs were able to improve directed forepaw reaching after dorsal column transection, as well as electrolytic lesions of the dorsal columns (65,77). These improvements are likely to be due to corticospinal axon regeneration and/or the enhancement of plasticity and neural cell sparing in the host spinal cord.

In rats with spinal cord unilateral cervical (C4) corticospinal transection, functional improvements and behavioral benefits were noticed after transplantation of adult bulb-derived OECs that were genetically engineered to express the trophic factor neurotrophin-3 (94). These OECs were far more effective in promoting long-distance maintenance/regeneration of lesioned corticospinal axons due to the enhanced plasticity and neuroprotection (94). Data from experimental studies suggest that facilitation of axonal regrowth, remyelination, and neuroprotection are the main mechanisms responsible for the therapeutic effects of OECs (89,102,105). However, data from clinical studies that used OECs for the treatment of SCI patients were not encouraging (89). Feron and colleagues (27) reported neither adverse effects nor neurological improvement in patients with complete thoracic SCI 1 year after transplantation of autologous OECs. Lima and colleagues (68) transplanted minced olfactory mucosa tissue in the cavity of the cervical and thoracic SCI. According to their results, olfactory mucosa autograft transplantation into the human injured spinal cord is feasible and relatively safe. However, the treatment was not significantly efficient. The problem is that they used tissue that contains OECs but also many other cell types.

Taken together, these data suggest that there is obviously a significant difference between promising results obtained from preclinical studies versus discouraging data from clinical trials. The reason for this discrepancy is not fully understood, but variability of the cell sources and culture conditions could be important contributing factors (105), suggesting that protocols for OEC isolation and growth need improvement.

Adult and Fetal-Derived Neural Stem Cells

Adult neural SCs are a subpopulation of ASCs naturally present in the adult brain and spinal cord. In the brain, multipotent NSCs reside in the periventricular subependymal layer and the subgranular zone of the dentate gyrus, while in the spinal cord, they can be found in the ependymal regions lining the central canal (36,71,74,106,116). Astrocyte and oligodendrocyte progenitors reside throughout the neural axis compiling 3–5% of all glial cells (106,116). A remarkable and underappreciated mechanism of self-repair is the proliferation of NSCs and glial progenitor cells following SCI. This robust response leads to significant replacement of glial elements and correlates to the period of early functional recovery seen in incomplete lesions (116). Newly generated progenitors, including multipotent ependymal and glial progenitor cells, home to the lesion site where they differentiate into both astrocytic-like and oligodendroglial lineage cells, indicating their regenerative potential (36,71,74,120). Early after injury, some progenitor populations participate in scar formation as noted in SCI and demyelinating lesions (106). However, the majority

of the replaced glia are oligodendrocytes, and recent data demonstrate that these cells afford significant myelin restoration of spared axons (61).

Although NSCs have the potential to differentiate into neurons, oligodendrocytes, and astrocytes in vitro (40), it is believed that, following SCI, endogenous NSCs differentiate mostly into oligodendrocytes and astrocytes (6). Despite significant endogenous cellular replacement that is mediated by NSCs, injury repair is incomplete. Of particular note is the lack of repair at the lesion epicenter, which remains the domain of the immune system (1). Despite a significant homing effect of NSCs to the lesion epicenter, there are clearly factors (such as netrin) that prevent progenitor cells from regenerating the lesion core as occurs in regenerative species such as urodeles and fish (85). Indeed, understanding how to boost endogenous cellular replacement, particularly in the lesion core, is an underdeveloped research area that has big clinical potential.

Many investigators have achieved successful but partial functional improvement by supplementation of endogenous NSC activity with transplanted NSCs. Our group has recently reported a functional motor recovery after transplantation of OPCs differentiated from ependymal stem progenitor cells that were derived from the adult rat spinal cord suffering a traumatic lesion (74). Oligodendrocytes are able to remyelinate the axons in white matter, and astrocytes may secrete many neurotrophic factors supporting axonal regeneration and cell survival (16,20,52). Although several studies confirmed that transplantation of adult spinal cord-derived NSCs promotes early functional recovery after SCI (43,83), there are very few reports (8,39,48) that describe the precise mechanism of action and factors responsible for integration of transplanted NSCs in injured spinal cord. It seems that the source of transplanted NSCs and methods used for their isolation prior to implantation are critical for a successful NSC survival and integration after transplantation in damaged spinal cord (39,48). Additionally, time of transplantation, the method of immunosuppression used, and type of injury (contusion vs. transection) affect the mechanism(s) of recovery (26,93). Several shortcomings limit their clinical use including formation of glial scars and the lack of neurotrophic factors after NSC transplantation. Since most sources of NSCs are exogenous allograft or xenograft, their transplantation may cause graft rejection (41). In addition, the differentiation potential of NSCs decreases after several passages under in vitro conditions (3,115). Despite these challenges, a part of the scientific community believes that NSCs represent an ideal candidate for cell-based treatment of SCI due to functional improvement noticed after their transplantation (18,39,48,74), low rates of tumorigenesis (52), and the opportunity for autologous transplantation (41).

In addition to adult-derived NSCs, neural and glial precursors derived from postnatal and fetal nervous system also show therapeutic promise. Investigators have used neural and glial-restricted precursors that produce functional improvement when injected early after injury (11). Multipotent populations of NSCs have been generated from human fetal tissue transplanted into immune-compromised mice (39). Unlike adult NSCs, fetal-derived NSCs generate neurons in addition to glia in the injured spinal cord. The almost singular proof that human fetal NSCs transplanted at subchronic periods generate functional improvements in mice has generated enough excitement to proceed to a clinical trial. Swissmedic, the Swiss regulatory agency for therapeutic products, has authorized a Phase I/II clinical trial in which SCI patients will receive human purified NSCs at 3 and 12 months postinjury. This NSC-based therapy for SCI is being developed by Anderson and Cummings of UC Irvine's Sue and Bill Gross Stem Cell Research Center in collaboration with researchers at StemCells, Inc., and is expected to begin in 2011 at the Zurich's University Hospital, Balgrist (for more information, please see http://www.today.uci.edu/news/2010/12/nr_swisstrial_101207.php).

Looking to the Future: Induced Pluripotent Stem Cells

A scientific breakthrough achieved by Takahashi and Yamanaka in 2006 (101) demonstrated that it is possible to reprogram somatic cells back to the pluripotency stage by transducing a few key transcription factors creating iPSCs. Upregulation of "Yamanaka factors": sex-determining region Y box-containing gene 2 (*SOX2*), octamer-binding transcription factor 3/4 (*OCT4*), tumor suppressor Krüppel-like factor 4 (*KLF4*), and protooncogene *c-MYC* allow somatic cells from mice and humans to be reprogrammed back into pluripotent cells (60). These cells have normal karyotype, express telomerase activity, exhibit morphology and markers of hESCs, and maintain the developmental potential to differentiate into all cell types including neurons, glia, NSCs, and motoneurons (23,98,112). Thus, the use of iPSC technology could be an alternative therapeutic approach to nonautologous transplantation of SC-based therapy for SCI. Derived from the patient's own somatic cells without the usage of early embryos, iPSCs represent an ethically acceptable cell source as opposed to hESCs. In addition, use of autologous iPSCs may mitigate the need for immune suppression, although this requires careful study. Despite their promise, iPSCs are still not approved for clinical trials due to concerns surrounding cell senescence and long-term tumorigenicity (72). This is due to the FDA and European EMA requiring iPSCs to be produced without the use of integrating vectors. To date, iPSCs have not been put through the extensive preclinical safety required

by regulatory agencies, and some data indicate there may be an increased tumor risk (72,111). Most iPSCs have been generated with integrating vectors, which may not be silenced efficiently or could disrupt endogenous genes (111). Nevertheless, the field of iPSCs is moving forward at an amazing rate, and preclinical efficacy in a rodent model of SCI has been established (109). New iPSC generation methods do not require the use of oncogenes or integrating vectors to generate iPSCs. In combination with a new technology termed "directed differentiation," many of the reprogramming issues are being solved and will alleviate safety concerns (109). It is also important to note that the scrutiny placed on the safety of iPSC-derived transplants must also be leveled on all other competing technologies that require expansion in vitro. For example, all individual ESC lines, as well as postnatal and adult NSC lines, must go through rigorous in vivo testing for tumorigenicity. These guidelines are continuously being altered and leave no short path to clinic. All clinical trials approved by regulatory agencies must be from cells derived from a mother line or stock of SCs that has gone through extensive and expensive safety testing. This is in part the reason many companies have vetted and remained steadfast toward a particular line of SCs for therapeutic trial. It is a financial and time-consuming hurdle to generate or even modify a protocol for the derivation of a therapeutic cell line that limits movement of SCs to clinic. In this regard, the use of patient specific iPSCs is at a significant disadvantage. Nevertheless, research is accelerating toward the development of optimized growth and differentiation protocols and reliable safety assays to bring the therapeutic potential of iPSCs to life.

Timing and Targets for Stem Cell Therapy

Current and planned SC-based clinical trials in SCI are targeting the acutely injured. This is necessitated by the relative lack of evidence for therapeutic value of SCs in chronic rodent injuries. The acutely injured patient is not the ideal population to evaluate recovery of function since it is impossible to discern how much natural recovery will ensue. Combined with the huge variability in the types and levels with which humans exhibit spinal trauma, patient selection, and evaluation is a challenge and one that needs to be carefully considered. The basis for the return of function in rodents is theorized to be remyelination of spared axons (97). However, the initial patients are likely to have little or no spared axons, making remyelination unlikely to have efficacious results. Nevertheless, these trials are very important as they will establish safety and lead the way for future work if safety is confirmed. In addition, there is a plethora of data indicating that placement of stem or progenitor cells within an acute period (0–14 days after injury) have beneficial results (93). This

remarkable body of literature argues strongly for an acute clinical trial despite the challenges of patient selection and inherent variability in recovery, which will decrease the power of analysis. It may also be a difficult debate to decide which cells should be modeled first. Data exist for BM, ESCs, hematopoietic cells, adult NSCs, fetal precursor cells, and postnatal progenitors having benefit in the acute setting. With this wide of a cell repertoire but a dearth of mechanistic understanding, it is tempting to posit that most immature or stem-like cells provide benefit in the acute setting by a general trophic mechanism. Until further challenged, we might consider that cell type is less important than the factors they produce or the indirect effects they promulgate. Indeed, future work might mitigate the need for cells at all and allow clinicians to administer SC factors in lieu of transplantation.

In contrast to the widely published evidence for partial repair by transplanting SCs in the acute setting, the chronic injury has been less tractable. However, there is renewed hope for the chronically injured population with limited but rigorous new data showing rodent regeneration in the subchronic time period (4–6 weeks following injury) (93). Nevertheless, the targets for the chronically injured are only slightly better defined than for the acute lesion. The most common stated goal of chronic transplantation is remyelination of spared axons, but the dogmatic assumption of chronic demyelination has been recently challenged and needs to be rigorously studied in both animal models and patients (61). Similar to the findings in peripheral nerve, it appears that demyelinated, spared axons may only persist for a short time after injury. Combined with evidence of rare or limited chronic demyelination in human samples, we must reconsider targets and mechanisms in order to accelerate progress (54). It may also be prudent to adjust future clinical trials by enrolling a defined population where physiological evidence of chronic demyelination of spared axons is unequivocal (9). It is likely that we will have to accept that mechanisms of trauma, vascular and inflammatory insults, and finally recovery could be more complex, subtle, and multimodal. The research community must dig deeper into the new studies also showing extensive spontaneous remyelination and functional return in the chronically injured and be open to novel regenerative mechanisms. For example, it may be that the accumulation of scar or inhibitory factors in the chronic injury requires digestion or blockade in order to unmask myelination targets (85,93) or make neuritis responsive to plasticity signals. The formation of relay neurons, nonmyelinating glia, or supportive astrocytes may be the key to SC-based mechanisms that deliver regenerative function. None of these mechanisms are exclusive nor have they likely been optimized by existing SC transplantation approaches.

In the past few years, considerable progress has been done combining SC-based treatment, bioengineered tissue scaffolds, and peripheral nerve grafts designed to provide mechanical support for axonal regrowth and to serve as a local delivery system for growth factors or as an SC carrier (15,110). A variety of biomaterials, both synthetic (polylactic acid, polyglycolic acid) and natural (hyaluronic acid, alginate, collagen, agarose, chitosan, matrigel, and methylcellulose hydrogel), have been modified to fabricate tissue scaffolds (99). Some success for the formation of neural tissue has been achieved using synthetic hydrogels in combination with NSCs, (79,103), while transplantation of OECs on hydrogel scaffolds resulted with regeneration of dorsal root axons and led to increased axonal growth across transected rat spinal cords (28). The enhanced regeneration of neurons and functional recovery of rats with SCI was seen after use of scaffolds that released brain-derived neurotrophic factor (BDNF) and after transplantation of BDNF-producing fibroblasts delivered within alginate matrices (44,107). In humans, axonal regeneration beyond the lesion site has been noticed in patients who received agarose scaffolds seeded with autologous bone marrow stromal cells expressing trophic factor neurotrophin-3 (34).

Finally, it is important, with respect to identifying therapeutic targets, that we consider the models upon which clinical trials are being based. In this regard, studies looking at the counterindications of cell transplantation, aside from the obvious caveat of tumor formation, are just becoming widespread. For example, most studies have not adequately addressed the potential emergence of pain syndromes despite literature indicating this is possible (20). Any therapy where the goal is to increase plasticity may result in unwanted pain fiber growth, directly or indirectly. Another modeling concern is that existing rodent models typically focus on the recovery of locomotion. While rodents are quadrupeds, humans utilize inherently more complex bipedal locomotion. Locomotion in a quadruped can be facilitated by limited regeneration (less than 3% of a tract), whereas human control of locomotion may require significantly more (104). It will be important to not exceed the public's expectations by assuming that limited repair of quadrupedal locomotion will equate to the recovery of walking in humans. Many laboratories have added forelimb functional testing, which may be a step in the direction of clinical relevance. This is due to the fact that the wiring and utilization of the rodent forepaw is likely more similar to humans than locomotion. The majority of human SC transplants have been modeled in immune-compromised rodents (18). While these results allow us to test the cellular basis of recovery without xenograft rejection, they leave in question how the intact immune system will augment or interfere with SC function clinically. Finally,

if current and near-future clinical trials fail to show any efficacy it may signal that the complexity of the human nervous system and immune system has been underestimated. This may argue that larger model species, such as dogs or monkeys, are important intermediaries before the next leap to humans (9,45).

Autologous or Allogenic Stem Cell Therapy for Spinal Cord Injury?

Among all human stem cells, MSCs are the best candidates for allogenic stem cell therapy. The culture-expanded human MSCs express only major histocompatibility complex (MHC) class I molecules, do not express MHC class II and costimulator molecules (62), cannot be antigen-presenting cells, and are invisible to the host's immune system after transplantation (55,108). Both autologous and allogenic MSC transplantation showed therapeutic effect in the experimental model of SCI (46). From a therapeutic point of view, autologous MSCs exhibited more beneficial therapeutic potential than allogenic MSCs, but both autologous and allogenic MSCs managed to rehabilitate locomotor and nociceptive function in dogs with SCI (46). In addition, 5 weeks after injury, the size of the spinal cord lesions was approximately the same among autologous and allogeneic MSC-treated groups but significantly reduced when compared with untreated animals.

Clinical studies using allogenic stem cells are rare because of the concerns about safety. The human leukocyte antigen (HLA) compatibility must be first considered in all allogenic clinical trials because the transplantation of HLA-mismatched SCs is unsafe. Kang and colleagues reported a case study of allogenic HLA-matched human umbilical cord blood-derived stem cell (hUCBSC) transplantation into the injured spinal cord site of a 37-year-old female patient with SCI (47). The hUCBSCs managed to improve sensory perception and movement in the patient's hips and thighs within 41 days of cell transplantation. Multiple radiological analyses suggested that hUCBSC therapy led to a regeneration of the spinal cord at the injured site and at some of the cauda equina below it (47). Despite promising results obtained from this study, it is noteworthy to mention that hUCBSCs are known to be more immune naive than any other adult cells (81). Therefore, safety issues such as immune reaction in HLA-mismatched allogeneic transplantation must be considered in all future allogeneic clinical trials.

CONCLUSIONS

The main goal of SC-based therapy for SCI is the regeneration and replacement of neurons and glial cells that undergo cell death soon after injury. Stem cells represent the newest and the most successful therapeutic approach for SCI, enabling improved and efficient sensory and motor functions in animal models. Stem cells are able to

promote remyelination via oligodendroglial cell replacement; produce trophic factors enhancing neurite outgrowth, axonal elongation, and fiber density; and activate resident or transplanted progenitor cells across the lesion cavity. Despite this, we have yet to validate specific mechanisms for SC transplantation. Published data supporting acute transplantation outweigh chronic intervention 100 to 1, but this is fortunately changing. Many investigators push hard to establish cell type-specific mechanisms of recovery, while others have begun to ask if trophic factors or immune modulation is in itself the mechanistic basis of acute SC therapy. Numerous studies suggest that SCs are able to enhance recovery following SCI, but no single SC type, when transplanted, seems sufficient to support a robust regenerative response that will lead to complete recovery of SCI. Successful SC-based therapy for SCI requires better understanding of SC differentiation pathways and SC survival upon transplantation. Protocols for differentiation of hESCs, MSCs, OECs, NSCs, and iPSCs into pure population of functional neural cells need improvement. Further reproducible studies using animal especially primate models should be done in order to investigate the precise mechanisms of reconstructing pathways and synapses, integration, survival, and action of transplanted SCs in injured spinal cord. Therefore, the first long-term results of the trials focused on impact of stem cell-based therapies for SCI are eagerly awaited. Until then, the ideal source of SCs for efficient and safe cell-based therapy for SCI remains a challenging issue that requires more investigation and continuous cooperation between clinicians, researchers, and patients.

KEY MESSAGES

- The main goal of SC-based therapy for SCI is regeneration and replacement of neurons and glial cells that undergo cell death soon after injury.
- Transplantation of hESC-derived OPCs and MPs efficiently recovered locomotor function both in contusion and transection animal models of SCI.
- The most widely applied SC-based therapy for SCI treatment in humans is transplantation of bone marrow-derived MSCs that are thought to act as a neuroprotectors by secreting various angiogenic and neurotrophic factors. However, the proof of functional neurons derived from transplanted MSCs has not been provided yet.
- Several preclinical studies showed that transplantation of adult spinal cord-derived NSCs promote early functional recovery after SCI through differentiation into oligodendrocytes.
- Reprogramming of somatic cells back to the pluripotency stage by transducing a few key transcription factors results in the creation of iPSCs and

represents a novel patient specific therapeutic approach for SCI treatment.
- iPSCs are still not allowed in initial clinical trials due to concerns about their tumorigenicity.
- The ideal source of SCs for efficient and safe cell-based therapy for SCI remains a challenging issue that requires more investigation and continuous cooperation between clinicians, researchers, and patients that should result with new SC-based strategies for the safe and successful treatment of SCI.

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