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Abstract

A large body of work has been published on transplantation of a wide range of neural stem and progenitor cell types derived from the developing and adult CNS, as well as from pluripotent embryonic stem cells, in models of traumatic spinal cord injury (SCI). However, many of these cell-based approaches present practical issues for clinical translation such as ethical cell derivation, generation of potentially large numbers of homogenously prepared cells, and immune rejection. With the advent of induced Pluripotent Stem (iPS) cell technology, many of these issues may potentially be overcome. To date, a number of studies have demonstrated integration, differentiation into mature CNS lineages, migration and long-term safety of iPS cell transplants in a variety of SCI models, as well as therapeutic benefits in some cases. Given the clinical potential of this advance in stem cell biology, we present a concise review of studies published to date involving iPS cell transplantation in animal models of SCI.

Introduction

Traumatic spinal cord injury (SCI) and its motor, sensory and autonomic consequences have a devastating impact on patient quality of life [1]. In the United States alone, there are around 276,000 individuals currently living with SCI (with even higher published estimates) and approximately 12,500 new cases per year [2]. Major causes of SCI include vehicular accidents, falls, sports injuries and violence [1]. SCI represents a heterogeneous set of conditions resulting from differences in the location, type and severity of trauma, as well as on the consequent types and degree of functional impairment. As the central nervous system (CNS) has limited potential to spontaneously regenerate, a first line treatment for SCI patients often involves interventions such as surgical stabilization and decompression and high dose methylprednisolone, followed by long-term approaches such as physical rehabilitation and pharmacological treatments for problems like chronic neuropathic pain

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[3]. Although used, controversies on the efficacy of therapies such as methylprednisolone and decompression remain [4].

To overcome the non-regenerative state of the CNS, cell transplantation provides a potentially powerful approach to repair and/or replace damaged elements of the injured spinal cord. A number of these transplant-based interventions using cell types derived from the developing and adult CNS, as well as from pluripotent embryonic (ES) stem cells, have demonstrated therapeutic efficacy in various animal models of SCI [5]. Despite success with many of these cell types, practical issues including ethical derivation, necessity for long-term immunosuppression of the patient recipient, and isolation and expansion of large numbers of cells in a uniform manner are impediments to clinical translation. With the advent of induced Pluripotent Stem (iPS) cell technology [6], many of these issues may potentially be overcome. Given the clinical relevance of this advance in stem cell biology, we will review studies published to date involving iPS cell transplantation in animal models of traumatic SCI.

Spinal cord injury pathophysiology

SCI progression generally consists of three major temporal phases [7]. The primary injury is characterized by direct tissue trauma, resulting in early loss of various CNS cell types, axotomy of passing axonal fibers, and blood vessel and blood brain barrier disruption [8, 9]. The initial trauma sets into course a sequence of secondary pathological events that occur over the hours, days and even weeks following injury, causing significant additional degeneration and consequent functional loss [7]. A large number of underlying cellular mechanisms are responsible for secondary injury processes, including excitotoxicity, immune cell activation, and oxidative damage [10]. In the chronic stages following SCI, little-to-no long-term recovery occurs due to issues such as minimal axonal growth/regeneration, modest functional remyelination, and lack of a robust response by endogenous neural stem and progenitor cells [11–16].

Cell transplantation as a therapy for SCI

Cell transplantation provides a therapeutic tool to target a number of these SCI pathological processes. Transplants can (1) replace damaged and loss CNS cell types (2), provide neurotrophic support and modulate the host immune response to minimize secondary injury, (3) enhance axonal plasticity by reducing the growth inhibitory environment of the injured spinal cord and by providing a cellular substrate for axonal extension in the lesion site, amongst a number of other potential benefits [17, 18]. To date, a variety of cell types have been tested in models of SCI to varying degrees of success. These include neural cells types such as peripheral nerve grafts, Schwann cells [19–21], olfactory ensheathing glia [22–25], dissociated fetal tissue, multipotent neural stem cells (NSCs), lineage-restricted neural progenitor cells (NPCs), and mature CNS cells. In addition, non-neural cell classes have also been tested, including genetically-modified fibroblasts, bone marrow stromal cells and activated macrophages. NSCs and NPCs are particularly promising sources for SCI given that they can actually replace mature CNS cell types, as well as contribute to other beneficial processes such as immune modulation.

Background on iPS cell technology

Nearly 10 years ago, the laboratory of Shinya Yamanaka developed a method for the *in vitro* conversion of adult rodent somatic cells into pluripotent ES cell-like cells (termed “induced Pluripotent Stem cells” or “iPS cells”) via retroviral introduction of several pluripotency related genes - Oct3/4, Sox2, c-Myc and Klf48 [6]. This work was then extended to the generation of human iPS cells using a similar combination of pluripotency factors [26]. NSCs and NPCs have traditionally been obtained from fetal or adult nervous system tissue or from pluripotent ES cells, all of which are associated with ethical concerns and practical issues of standardization and generation of adequate numbers of cells for transplantation in potentially large numbers of patients. iPS cell technology allows for homogeneous derivation of cell types in large quantities for applications such as therapeutic transplantation, potentially in an autologous fashion from the eventual patient recipient [27–31].

However, iPS cell use of is not devoid of risks. One of the main concerns regarding this technology is the potential for uncontrolled proliferation and even tumor formation given their pluripotent state (ES cells present a similar concern) [32, 33]. Also, the use of viral vectors that can integrate randomly into host genome could disrupt important regulators of cell division such as tumor suppressors or could result in the activation of oncogenes [6, 34, 35]. In addition, iPS cell generation involves the introduction of factors that regulate the cell’s proliferative state, and so it is imperative when using this method that there is a tight screening of oncogenic capacity prior to transplantation [36]. Alternative strategies are being developed to minimize this risk. *PiggyBac* transposition [37, 38], episomal vectors [39–44], microRNA [45, 46], and delivery of recombinant reprogramming proteins [47–49] are examples of these new methods. More recently, small-molecule compounds like CHIR99021 (glycogen synthase kinase 3 inhibitor), RepSox (TGF-β inhibitor), DZNep (s-adenosylhomocysteine hydrolase inhibitor), valproic acid (histone deacetylase inhibitor), tranylcypromine (lysine-specific demethylase 1 inhibitor), forskolin (adenylate cyclase activator) and TTNPB (retinoic acid receptor ligand) have been used to generate iPS cells [50]. More extensive discussion of these issues are available [44]. These new methods can reduce the risk of malignant transformation of transplanted cells and can serve as alternatives to “classic” viral transduction. Also, emerging iPS cell technologies that require less time to induce pluripotency (and subsequently to differentiate the iPS cells into mature cell types of interest) would provide much needed methodological improvements for achieving autologous transplantation at earlier stages of disease in conditions such as SCI.

Overview of iPS cell transplantation in SCI models

Despite the promise of this approach, the iPS cell transplantation field is in its infancy with respect to evaluating long-term *in vivo* integration and therapeutic usefulness in relevant SCI models. A number of studies have reported significant therapeutic benefit when NSCs/NPCs derived from either mouse [33] or human [36, 51, 52] iPS cells were transplanted into contusion or cavity-type models of rodent SCI, as well as in non-human primate models [53]. Given the xenografting paradigm, most studies of iPS cell transplantation in SCI animal models have employed either immunosuppressed rodents or animals with a

genetically-compromised immune response. In many cases, cells were delivered in a multipotent NSC-like state and resulted in mixed differentiation into glial phenotypes, including astrocytes, and various neuronal subtypes. Across all of these published studies, the phenotypic state of cells (derived *in vitro* from iPS cells prior to injection) at the time of transplantation into injured spinal cord ranged from undifferentiated neurospheres [53], NSCs [52], mixed cell lineages to even astrocytes only [54]. While these studies were able to achieve some functional benefit, future work may require more phenotypically targeted strategies, each of which depends on the nature of the SCI pathology (e.g. type of injury, anatomical locations affected, etc.) and the specific cell lineages being targeted for replacement. Nevertheless, these studies have collectively demonstrated therapeuitc properties of iPS cell-derived transplants in the injured spinal cord environment, including synaptic integration into endogenous neuronal circuitry [36, 51].

Therapeutic effects of iPS cell transplantation in SCI

Following transplantation of human iPS cell-derived neurospheres into an immunodeficient mouse model of SCI, Nori and colleagues demonstrated functional efficacy that was accompanied by a variety of histopathological improvements, including decreased neuronal death and demyelination and increased angiogenesis and axonal growth [36]. Transplanted cells survived for prolonged lengths of time and differentiated into various mature CNS lineages. Transplants also secreted a variety of neurotrophic factors, including NT3, NT4, CNTF, VEGF and PECAM, which may explain, for example, the increased angiogenesis and axonal growth observed in both this study and by Kobayashi et al. in the marmoset contusion SCI model [53]. Using a model of thoracic contusion SCI in rats, Romanyuk and colleagues showed that transplantation of iPS cell-derived NPCs one week post-SCI promoted tissue sparing and improvement in a number of functional motor tests, even though only approximately 10% of transplanted cells survived in injured spinal cord [52]. Similar to the neurotrophin production noted in the Nori study, the authors observed increased levels of human-specific neurotrophic factors, possibly underlying the observed therapeutic benefit. Together, these findings point to the multi-faceted potential of transplanted iPS cells in SCI; not only are they capable of replacing lost cell types, but they are also able to modulate the environment of the injured host spinal cord.

In the majority of studies involving iPS cell transplantation in SCI models, significant graft survival, integration and, often times, migration from injection sites were observed. Using a thoracic spinal contusion model in Nonobese diabetic-severe combined immunodeficiency (NOD-SCID) mice, Fujimoto and colleagues showed that transplantation of human iPS-derived neuroepithelial-like NSCs promotes functional motor recovery via integration of transplant-derived neurons and connection with host neuronal circuitry [51]. Along these lines, Lu and colleagues showed results indicating that even iPS cells derived from older subjects can be used successfully for SCI treatment. After a C5 hemisection, human iPS cell-derived NSCs from an 86 year-old were transplanted into adult immunodeficient rats. At 3 months follow-up, transplanted cells survived and the cells that differentiated into neurons showed long-distance axonal outgrowth and made extensive synaptic connections with host neurons, even outside of the lesion area [55]. Tang and colleagues transplanted human iPS cell-derived NSCs labelled *in vitro* with superparamagnetic iron oxide particles

into both a rat model of traumatic brain injury and a monkey model of SCI at 1 week post-injury. Using recurrent *in vivo* magnetic resonance imaging (MRI) tracking until 30 days post-injury, the authors showed that transplant-derived cells could progressively migrate from injection sites, which was accompanied by significant motor function recovery [56]. Despite the graft integration shown in these studies, it has yet to be mechanistically determined how these cells are promoting therapeutic effects. It may, for example, be that long-term graft integration and cell replacement of mature CNS lineages are not even necessary if transplanted cells can exert benefit via transient processes such as neuroprotection. This issue, however, is not unique to transplantation of iPS cells, but it relevant to NSC/NPC transplantation in general in SCI and other CNS diseases.

Studies to date using iPS cell transplantation in SCI models have not focused on astrocyte replacement. To begin to utilize astrocyte replacement in a mechanistically-targeted fashion based on their crucial functions in the intact nervous system, we transplanted human iPS cell-derived astrocytes (hiPSAs) as a strategy for restoring extracellular glutamate homeostasis in the injured spinal cord. Astrocytes are responsible for the vast majority of glutamate uptake throughout the CNS via expression of the plasma membrane transporter, glutamate transporter 1 (GLT1), thereby playing a central role in maintaining normal synaptic communication and preventing glutamate-mediated excitotoxicity. Following SCI, astrocyte GLT1 expression and function are severely compromised, which contributes to excitotoxicity-induced cell death during the delayed secondary injury phase. We derived pluripotent iPS cells from non-diseased human donors, subsequently generated glial progenitors and then differentiated these cells into hiPSAs prior to transplantation. In a unilateral cervical contusion model of rat SCI, we injected hiPSAs engineered to overexpress GLT1 into the cervical ventral horn as a therapeutic strategy for reconstituting GLT1 function, preventing excitotoxicity, and consequently protecting respiratory phrenic motor neurons and preserving diaphragm function. Transplants survived for long periods of time in the injured cervical spinal cord, did not form tumors or show uncontrolled proliferation, differentiated into only GFAP-positive astrocytes, and did not localize to ectopic locations or differentiate into unexpected lineages. GLT1 overexpressing hiPSAs (engineered using an AAV8- GLT1 vector *in vitro* prior to injection) expressed persistently high levels of GLT1 protein following transplantation, and overexpression also enhanced GLT1-mediated glutamate uptake compared to control cells. Furthermore, these overexpressing hiPSA transplants promoted significant survival of phrenic motor neurons, preservation of diaphragm neuromuscular junction innervation, and protection of diaphragmatic respiratory function. Our findings demonstrate the therapeutic value of targeting mature astrocyte properties using iPS cell transplantation in the injured spinal cord. Given the long list of important astrocyte functions, this strategy represents just one example of using this astrocyte-targeted approach in SCI.

Collectively, these studies indicate the therapeutic utility of iPS cell-derived transplantation for SCI potentially via properties such as differentiation into mature CNS cell types, neuronal integration into host circuitry, production of neurotrophic factors, and distribution from injection sites to areas of pathology.

Lack of therapeutic effects / negative effects of iPS cell transplantation in SCI

Not all studies with iPS cell transplantation reported beneficial outcomes in SCI models. Pomeshchik and colleagues did not observe improved function when human iPS cell-derived NPCs were transplanted 7 days after contusion SCI [57]. However, the authors also did not find long-term survival of grafted cells in these mice receiving a Tacrolimus immune suppression regimen. Contrary to this report, a number of studies employing human iPS cell-derived transplants have noted significant survival and differentiation into mature CNS cell types following injection into the adult spinal cord of immunosuppressed rodents [58, 59]. In our study with hiPSAs described above, we also observed robust and persistent transplant integration in the contused spinal cord using a modified immune suppression protocol consisting of both Tacrolimus and Rapamycin in mice or cyclosporine only in rats. Transplant rejection due to immunesuppression regimen problems may not be relevant to autologous delivery; nevertheless, it is unlikely that autologous transplantation will be used in all cases because of issues such as minimal time available between trauma and transplantation (as discussed below). It will therefore be crucial to address this important issue in animal models prior to clinical translation.

A study from the Horner group [60] reported lack of therapeutic improvement with transplantation of human iPS cell-derived NPCs in a SCI model, despite significant graft integration. However, cells were delivered at a chronic time point following injury, which may represent an environment less amenable to transplant-induced plasticity than delivery at very early stages post-trauma. As for any cell-based intervention, the timing of iPS cell transplantation will have important therapeutic consequences. The temporal delivery paradigm will depend on both the disease mechanism(s) being targeting (e.g. early neuroprotection, delayed regeneration, etc.) and practical issues encountered in the clinical setting (e.g. need for patient stabilization prior to invasive interventions).

A recent study from the Steward lab reported that transplantation of a mixed population of rodent-derived glial and neuronal progenitors (which were not derived from iPS cells) into transection SCI resulted in ectopic engraftment of large numbers of cells at locations such as the central canal and pial surface of the spinal cord and the 4th ventricle [61], providing a note of caution when using transplantation of any class of NSC/NPC in SCI. This issue is particularly relevant to strategies employing cells derived from pluripotent sources such as ES and iPS cells given the possibility of incomplete and/or inefficient differentiation. For example, Tsuji and colleagues generated lines of both “safe” and “unsafe” neurospheres from mouse iPS cells [33]. In a mouse model of contusion SCI, the “safe” cells successfully survived, differentiated along mature CNS lineages and promoted functional recovery, serotonergic axon growth and remyelination without tumor formation. On the contrary, the “unsafe” neurospheres produced teratomas and associated functional impairment in the same SCI model. Unlike the Steward and Tsuji papers, the other iPS cell transplantation studies we describe in this review did not systematically assess distribution of transplant-derived cells throughout the neuraxis. In future experiments, it will be important for the field to assess cell fate at long-term time points post-transplantation, as well as possible ectopic

localization away from injections sites, to establish the safety of tested cells before proceeding to the clinic.

In all of these studies using iPS cells in SCI, transplanted cells differentiated into one or more mature CNS cell types (i.e. neurons, astrocytes and oligodendrocytes) in the injured spinal cord, although smaller percentages of undifferentiated nestin+ neural precursors were also seen even out to relatively late time points post-transplantation [33, 36, 53]. Along these lines, we noted the presence of a small residual population of proliferating graft-derived cells out to four weeks post-transplantation (the latest time point examined) when we injected hiPSAs into our SCI model. Even though we and others [36] did not observe overt tumor formation or extensive migration away from injection sites beyond only a few spinal segments, all of these studies collectively demonstrate the importance of efficient *in vitro* pre-differentiation prior to transplantation, which may require a combination of both positive selection of differentiated cell types and negative selection for residual pluripotent stem cells (and possibly even less immature NSCs and NPCs). Even with the use of this *in vitro* pre-selection, it will still be necessary to comprehensively conduct longer term follow-up (preferably out to a year or more post-transplantation) of the safety of these cells in SCI animal models.

Mechanical allodynia (a form of neuropathic pain) was observed when iPS cell-derived astrocytes were transplanted into a contusion SCI model [54]. In addition to this work, other studies have similarly reported sensory hypersensitivity in SCI models accompanying transplantation of progenitor-derived astrocytes [62, 63], possibly due to increased neuronal plasticity induced by transplantation of immature astrocyte populations [64]. However, in a large body of work, we and others [60, 65, 66] have not found such increased sensitivity, including following hiPSA transplantation [60]. The discrepancy amongst these studies may be due to heterogeneity in the subtypes of astrocytes being injected [62, 67]. Nevertheless, this suggests caution with respect to potentially inducing unexpected functional outcomes after transplantation that can be very debilitating to patients, though this particular example is not specific to iPS cells.

A comprehensive summary of all these relevant papers using iPS cells in SCI animal models is presented in Table 1.

Transplantation of iPS cells in other spinal cord disease models

The application of iPS cells for treatment of spinal cord diseases is not exclusive to SCI. For example, Simone and colleagues transplanted iPS cell-derived NSCs into a rat model of Spinal muscular atrophy with respiratory distress type 1 (SMARD1). The authors reported a number of results similar to those already described in SCI models such as survival and differentiation of transplanted cells. They also found phenotypic improvement due to motor neuron protection, suggesting trophic influences of the transplanted cells. In support of this mechanism, through *in vitro* co-culturing studies with motor neurons generated from human SMARD1-iPS cells, they reported that iPS cell-derived NSCs increased motor neuron axonal length via neurotrophic factor production [68].

Clinical implications of iPS cells for therapeutic transplantation in SCI

A number of practical issues will need to be addressed before moving iPS cell transplantation to the clinic in SCI and other nervous system diseases. Protocols for *in vitro* generation of iPS cells will need to be optimized (and possibly standardized), including the preferred somatic cell types used, the specific pluripotency factors and vectors used for reprogramming (discussed above), and the differentiation procedures used to efficiently generate cell types of interest from iPS cells without leaving residual undifferentiated cells. Importantly, these considerations will play a major role in preventing uncontrolled proliferation after transplantation.

Specifically with respect to targeting relative early events following SCI (e.g. secondary degeneration), autologous derivation of cells may not be relevant given the extended time needed (at least based on current induction technologies) to generate iPS cells and subsequently differentiate them prior to delivery. Instead, cells to be used for transplantation could be obtained from banks of immune/HLA-matched cells [69]. Given the need to extensively test iPS cell lines prior to transplantation into a patient, as well as the time that will be required for generating cells for each individual patient, this approach may actually be practically preferable to autologous derivation in some cases [70]. However, with such as allogeneic approach, the choice of appropriate immune suppression regimens will become a key factor, as will consideration of toxicity associated with long-term administration of such drugs. Nevertheless, autologous transplantation of iPS cells does seem practically plausible for transplanting cells into the chronic SCI condition (to target, for example, remyelination and axon regeneration) given the extended time frame from trauma to cell delivery.

As human stem cell lines have shown donor variability in SCI models [71], future studies will also need to investigate *in vivo* properties and therapeutic efficacy of human iPS cells derived from multiple donors in an attempt to move this approach towards clinical translation, particularly if banks of cells will be used to provide transplants for large numbers of patients. We may find, for example, that various donor lines differ in their therapeutic properties (e.g. levels and types of neurotrophins produced) and/or differentiation potential and consequently that certain lines are more suited to particular disease conditions depending on the mechanistic biological needs of a given disease or even disease sub-type.

With a number of studies having already demonstrated safety and therapeutic efficacy of iPS cell transplantation in animal models of SCI, clinical trials are beginning to move forward. For example, Okano and colleagues are currently preparing a clinical trial for SCI following sub-acute transplantation of iPS-derived NSCs/NPCs [72].

Conclusions

iPS cell technology provides a novel and clinically-relevant source of cells for CNS transplantation given that these cells avoid ethical issues associated with ES cell derivation and can be autologously derived from the patient in some cases, thereby avoiding toxic immune suppression. The iPS cell transplantation field is rapidly progressing, particularly

with respect both to testing therapeutic potential in SCI models and overcoming many of the practical hurdles associated with clinical translation. While a collective body of work has demonstrated that transplantation of CNS cells types derived from iPS cells into various models of SCI can promote histopathological and function recovery, many of these studies also provide important cautionary notes, mainly regarding the safety of the cell lines and the risk of tumor formation, which can be extremely detrimental to patients. Once practical issues associated with iPS cell transplantation are resolved and protocols for ensuring transplant safety are established, individualized therapies using iPS cells will be within closer reach for a wide range of CNS disease conditions, including SCI.

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List of abbreviations

CNS	central nervous system
ES	embryonic stem cells
GLT1	glutamate transporter 1
hiPSA	human iPS cell-derived astrocyte
iPS	induced Pluripotent Stem cells
MRI	magnetic resonance imaging
NOD-SCID	nonobese diabetic-severe combined immunodeficiency
NPC	neural progenitor cells
NSC	neural stem cells
SCI	spinal cord injury
SMARD1	Spinal muscular atrophy with respiratory distress type 1

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Table 1

Published studies with iPS cell transplantation in SCI animal models (presented in chronological order of publication).

Reference	SCI species	SCI model	Immunology	iPS cell transplant			Results
				Donor	Time of transplant post-SCI	Transplanted cell type	
Tsuji et al., 2010 [33]	Mouse	Thoracic contusion	NOD-SCID mouse	Mouse	9 days	Neurospheres	Survival: 42 days post-injury Differentiation: Hu+ neurons, GFAP+ astrocytes, FSGT+ oligodendrocytes Migration: 4mm rostral and caudal Function: motor recovery Histology: increased myelination; increased serotoninergic distal fibers; expression of NT-3 and BDNF; no tumorigenesis
Hayashi et al., 2011 [54]	Rat	Thoracic contusion	Cyclosporine immune suppression	Mouse	3 days or 7 days	Astrocytes	Survival: 8 weeks post-injury Differentiation: GFAP-astrocytes Migration: None Function: no motor recovery; hyperalgesia Histology: axonal regrowth; tissue sparing; no differences from controls: no tumorigenesis
Nori et al., 2011 [36]	Mouse	Thoracic contusion	NOD-SCID mouse	Human	9 days	Neurospheres	Survival: 112 days post injury Differentiation: NeuN+ and βIIItubulin+ neurons; GFAP+ astrocytes; APC+ oligodendrocytes Migration: yes Function: motor and electrophysiological recovery Histology: tissue sparing; axonal regrowth; decreased demyelination; expression of NGF, BDNF and HGF; no tumorigenesis
Fujimoto et al., 2012 [51]	Mouse	Thoracic contusion	NOD-SCID mouse	Human	7 days	Neuroepithelial-like stem cells (NES)	Survival: 8 weeks post-injury Differentiation: Tuj1+ neurons; GFAP+ astrocytes; MBP+ and APC+ oligodendrocytes Migration: yes Function: motor recovery Histology: no CST neurons in the lesion; neuronal recovery by bridging
Kobayashi et al., 2012 [53]	Marmoset	Cervical contusion	Cyclosporine immune suppression	Human	9 days	Neurospheres	Survival: 112 days post-injury Differentiation: NeuN+ neurons, GFAP+ astrocytes; Olig1+ oligodendrocytes Migration: yes Function: motor recovery

Reference	SCI species	SCI model	Immunology	iPS cell transplant			Results
				Donor	Time of transplant post-SCI	Transplanted cell type	
							Histology: axonal regrowth; prevention of demyelination; expr Histology: axonal regrowth; prevention of demyelination; expr Histology: axonal regrowth; prevention of demyelination; e Histology: axonal regrowth; prevention of demyel
Nutt et al., 2013 [60]	Rat	Cervical contusion	Cyclosporine immune suppression	Human	4 weeks	Caudalized NPCs	Survival: 12 weeks post-injury Differentiation: Tuj1+ neurons; GFAP+ astrocytes Function: no motor recovery; allodynia Histology: lesion with cavitation, myelin loss and gliosis; bridging with host neurons; no tumorigenesis
Lu et al., 2014 [55]	Rat	Cervical hemi-section	Athymic nude rat	Human	2 weeks	NSCs	Survival: 3 months Differentiation: NeuN+ neurons; GFAP+ astrocytes Migration: cell distribution over 3 segments; axon extension across large extenxt of neuraxis Function: motor recovery Histology: synaptic connection to host neurons; presence of collagen rifts between rostral and caudal; no tumorigenesis
Haidet-Phillips et al., 2014 [58]	Rat	No injury	Cyclosporine immune suppression	Human	Not applicable	Astrocyte progenitors	Survival: 12 weeks Differentiation: GFAP+ astrocytes Migration: within 1mm Histology: no tumorigenesis
Sareen et al., 2014 [59]	Rat	No injury	Athymic nude rat	Human	Not applicable	NPCs	Survival: 3 weeks Migration: not assessed Histology: no tumorigenesis
Romanyuk et al., 2014 [52]	Rat	Thoracic contusion	Cyclosporine, Azathioprine and Methylprednisolone immune suppression	Human	1 week	NPCs	Survival: 17 weeks Differentiation: neurons and astrocytes Migration: yes Function: motor recovery; no allodynia Histology: white matter sparing; expression of NGF, FGF8 and GDNF; no tumorigenesis
Pomeshchik et al., 2014 [57]	Mouse	Thoracic contusion	Tacrolimus immune suppression	Human	7 days	NPCs	Survival: low Differentiation: no Migration: yes Function: no motor recovery Histology: no differences in lesion size; accumulation of activated microglia surrounding grafts; no tumorigenesis