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Stem cells and spinal cord injury

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Abstract

Spinal cord injury (SCI) is a devastating disorder with detrimental implications for both, the individual and for society. Spinal cord injury has a profound effect on patient's physical and psychosocial wellbeing because it often results in permanent loss of bodily functions affecting limb movement, somatosensation, reproductive organs, bladder and bowel. There are many strategies to cure SCI including transplantation of stem cells (SC). 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 and represents the newest and the most successful therapeutic approach for SCI enabling improved and efficient sensor and motor functions in animal models. These cells are able to promote remyelination via oligodendroglial cell replacement, produce trophic factors enhancing neurite outgrowth, axonal elongation and fibre density and activate resident or transplanted progenitor cells across the lesion cavity. Despite this there is a need to validate specific mechanisms for different SC sources and SC transplantation.

Keywords: spinal cord injury, stem cells transplantation

1. Introduction

Spinal cord injury (SCI) is an important cause of neurologic disability after trauma and although prevention programs have been initiated, there is no evidence that the incidence is declining [Tator and Fehlings, 1991]. Furthermore, no satisfactory treatment is currently available [Fehlings and Tator, 1991], [Su et al. 2010]. According to the SCI information network, approximately 300000 people suffer from SCI in the US and nearly 11000 new cases are reported annually. The estimated cost of these patients is US$ 8 billion annually, with individual costs of up to 1.35 million over the course of their life. Importantly, the average age of an SCI patient is 30 years, indicating that it incapacitates individuals in the prime of their life. The high incidence in young age groups is attributable to the large number of cases of road traffic accidents and sports injuries in this group. However, no cure is currently available for these individuals. The lack of mobility and increased dependence of SCI patients causes increasing psychological stress along with secondary complications such as urinary tract infection and pressure sores, requiring constant hospitalization further burdening the health care system. However, an overall understanding of the basic pathophysiology underlying the disease is essential.

Spinal cord injury may result in severe sensory and motor deficits at and below the level of injury due to extensive neuronal loss, acute axonal damage, demyelination and scar formation [McDonald and Sadowsky, 2002]. The severity of SCI ranges from incomplete myelopathy/paraparesis to complete paraplegia. The actual damage is exacerbated by the immune response to spinal trauma. The post-traumatic microenvironment has been shown to cause apoptosis and damage to surrounding functional neurons. Any potential method for functional restoration must incorporate neuroprotective strategies along with the re-establishment of ascending/descending tracts and local synapses. Due to the limited capacity of axonal regeneration and the presence of local inhibitory factors, recovery is minimal.

Currently, the pathophysiology of SCI is established in 2 stages: i) primary and ii) secondary lesions. Laceration, contusion, compression, and concussion represent primary lesion, due to the physical and mechanical trauma to the spinal cord mainly causing structural disturbance [Fehlings and Tator, 1999], [McDonald and Sadowsky, 2002], [Rowland et al. 2008]. In the majority of cases of human SCI, the mechanism of the primary lesion is acute compression or laceration of the spinal cord due to displacement of bone or disc into the canal during fracture dislocation or burst fracture of the spine. Residual neural connections usually persist after this stage indicating potential for recovery, despite that functional loss may be complete [McDonald and Sadowsky, 2002], [Rowland et al., 2008]. In the following stage, secondary lesion mainly causes functional disturbance, in the presence of ischemia and microvascular damage [Hall and Springer, 2004], [Dougherty and Hochman, 2008] glutamatergic excitotoxity, oxidative stress, and inflammation [Genovese and Cuzzocrea, 2008]. It is not so clear when this stage begins and finishes. Most of the cell death in consequence of SCI is due to secondary lesion and begins centrally and affects the cell body first [Hall and Springer, 2004], [Genovese and Cuzzocrea, 2008], [Rowland et al. 2008]. Another important reason is swelling and hemorrhage into the cord leads to vascular disturbance, with emphasis on vascular mechanisms, causing depression in oxygen and nutrients support [Genovese and Cuzzocrea, 2008]. Considering all these mechanisms involved in SCI as consequences there are different strategies to help patients with SCI.

2. Strategies to cure SCI

Currently, there is a multiplicity of interventions to promote recovery from an SCI: i) treatments immediately following the trauma (treating inflammation and limiting initial degeneration) and long-term procedures (stimulating axonal growth, promoting new growth through substrate or guidance molecules, ii) blocking molecules that inhibit regeneration, iii) building bridges to span the lesion cavity and iv) supplying new cells to replace lost ones including SC transplantation [Moalem et al. 2000], [Gris et al. 2007], [Genovese and Cuzzocrea, 2008].

3. Therapeutic strategies using stem cells

There are different types and sources of stem cells including human embryonic stem cells, fetal stem cells, adult stem cells (hematopoietic, mesenchymal) and tissue specific stem cells, for instance neural stem cells.

4. Human embryonic stem cells

Human embryonic stem cells (hESC) are pluripotent cells derived from the inner cell mass of the early blastocyst with 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 [Nistor et al. 2005], [Lee et al. 2007], [Baizabal and Covarrubias, 2009], [Erceg et al. 2009], [Gil et al. 2009], [Erceg et al. 2010]. In view of this hESC are a promising source of differentiated oligodendrocytes and motoneurons [Lee et al. 2007], [Erceg et al. 2009] as a potential novel approach to treat SCI. Clinical applications of hESC 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 [Nistor et al. 2005], [Lee et al. 2007], [Erceg et al. 2009], [Baizabal and Covarrubias, 2009], [Erceg et al. 2010] improved the methods for differentiation of hESC 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 (MP) and oligodendrocyte progenitors (OPC) via targeted differentiation of hESC have been developed [Erceg et al. 2010]. Motoneuron progenitors derived from hESC have the ability to mature and develop fundamental functions of normal motoneurons *in vitro* including directional growth of long axons [Keirstead et al. 2005]. Transplantation of hESC-derived OPC can efficiently recover locomotor function in contusion and transection animal models of SCI [Keirstead et al. 2005], [Erceg et al. 2009], [Erceg et al. 2010].

Despite promising results obtained in preclinical studies, there are several concerns regarding the safety of transplantation of hESC in humans, including the formation of teratomas [Li et al. 2008]. The possible reason for this problem could be the usage of different cell lines, various differentiation protocols and most important, transplantation of heterogeneous cell populations.

5. Umbilical cord blood stem cells

It is not completely clear whether human umbilical cord blood stem cells (UCBSC) have potential to differentiate into neural cells under both conditions, *in vivo* and *in vitro*. However it is known that this source of stem cells downregulates apoptotic genes, Fas and tissue plasminogen activator and blocks activation of caspases 3 and 8. In cases of SCI, UCBSC could control apoptosis, demyelination, and scar formation [Sobani et al. 2010]. Umbilical cord blood is a potential vast source of primitive hematopoietic stem and progenitor cells available for clinical application [Butler and Menitove, 2011]. However, the functionality and maturity of differentiated cells need to proven in more details using electrophysiology and animal disease models and other more competent *in vitro* and *in vivo* assays.

6. Bone marrow stromal cells

Adult bone marrow (BM) is a source of stem cells with power to differentiate in osteocytes, chondrocytes, myocytes, hepatocytes, epithelial linings, glia, neurons, and Schwan cells. In an average BM harvest, only 0.125% of the cells are in fact bone marrow stromal cells (BMSC), and an age-dependent inverse correlation with number of cells isolated in the first passage has also been demonstrated. However, it has also been noted that sufficient BMSC can be successfully cultured for an auto transplant from SCI patients (Kamada et al. 2005). BMSC delivery routes include intravenous, intra-arterial, intrathecal, and lumbar puncture with

evidences that these cells migrate mainly to the injury site. It was evaluated whether transplantation of Schwann cells derived from BMSC would promote axonal regeneration and functional recovery in completely transected SC in adult rats. BMSC were induced to differentiate into Schwann cells *in vitro*. A 4 mm segment of rat spinal cord was removed completely at the T7 level. An ultrafiltration membrane tube, filled with a mixture of matrigel (MG) and BMSC or MG alone, was grafted into the gap. In the MG and BMSC group, the number of neurofilament and tyrosine hydroxylase-immunoreactive nerve fibers was significantly higher compared with the MG alone group, although 5-hydroxytryptamine or calcitonin gene-related peptide-immunoreactive fibers were rarely detectable in both groups. In the MG and BMSC group, significant recovery of the hind limb function was recognized, which was abolished by transection of the graft 6 weeks after transplantation. These results demonstrated that transplantation of BMSC promoted axonal regeneration of injured SC, resulting in recovery of hind limb function in rats [Kamada et al. 2005].

7. Olfactory ensheathing cells

Olfactory bulb-derived (central) ensheathing cell (OB) transplants have shown significant promise in rat models of SCI, as well as the use of lamina propria-derived (peripheral) olfactory ensheathing cells (LP) in both experimental and clinical trials [Richter et al. 2005]. Both cell types exhibit morphologic and antigenic similarities *in vitro*, and, after transplantation, OB and LP attenuate lesion and cavity formation and promote angiogenesis, endogenous Schwann cell infiltration, and axonal sprouting. However, an increased mitotic rate and migratory ability of LP *in vitro* was observed *in vivo* due to their migration within the SC, reduced cavity formation and lesion size, and stimulated outgrowth of axonal subpopulation when compared with OB [Rizek and Kawaia, 2006]. For otherwise, there is an important aspect concerning remyelination, that is, olfactory ensheathing cells do not myelinate axons in their native environment [Yamamoto et al. 2009]. Therefore, it is not clear how the myelination happens. According to the literature, it would be preferable to obtain reparative cells from an olfactory mucosal biopsy via intranasal endoscopy rather than requiring the more invasive intracranial approach to remove an olfactory bulb [Yamamoto et al. 2009]. But, when compared with previous findings with bulbar cells, the mucosal cell cultures contained only 5% of olfactory ensheathing cells and a conversely much larger proportion of fibroblasts. These cell preparations showed minimal migratory ability and failed to form complete bridges across the lesion [Yamamoto et al. 2009].

There are a great number of primitive stem cells in the OB with power for regeneration as mentioned above. Therefore, olfactory ensheathing cells can be considered as a promising source to promote neuroregeneration in cases of SCI. Another advantage is that they can be used as autografts. Nevertheless, several preclinical and clinical trials with high number of patients using olfactory ensheathing cells must be carried out to consider this possibility as effective for SCI.

8. Neural stem progenitor cells

Neural stem progenitor cells (NSPC) may be an important potential graft material for cell therapeutics after SCI. The use of NSPC-enriched population (including ependymal cells) derived from human fetal spinal cord (embryonic week 8 to 9) and expanded *in vitro* by neurosphere formation seems to be a feasible alternative [Nakamura et al. 2005]. According to this experience, it was seen that NSPC labeled with BrdU or culture medium were transplanted

into the adult primate marmoset spinal cord, after contusion injury at C5 level. Grafted NSPC survived and migrated up to 7 mm far from the lesion epicenter. Besides, double-staining with TuJ1 for neuron, GFAP for astrocyte, or CNPase for oligodendrocyte and BrdU revealed that grafted NSPC differentiated into neurons and oligodendrocytes eight weeks after transplantation. Furthermore, behavioral assessment of forelimb muscle strength using a bar grip test and amount of spontaneous motor activity using infrared rays monitoring revealed that the grafted NSPC significantly increased both of them compared with those of control group [Nakamura et al. 2005], [Parr et al. 2008].

The effect of SC-derived NSPC after delayed transplantation into the injured adult rat SC with or without earlier transplantation of BMSC was evaluated using either BMSC or culture medium for transplantation. NSPC or culture medium were transplanted 9 days after injury (for more details see Parr et al. 2008). However, transplantation of the BMSC resulted in a trend toward improved survival of the NSPC, but there was no increase in locomotor function. Transplantation of adult rat NSPC produced significant early functional improvement after SCI, suggesting an early neuroprotective action associated with oligodendrocytes survival and axonal ensheathment by transplanted NSPC [Parr et al. 2008].

9. Conclusion

Stem cell transplantation as a strategy for spinal cord regeneration is at the forefront now. The animal studies and *in vitro* studies provide a solid platform to proceed to well-designed human studies on stem cell transplantation for SCI. More than two decades ago, optimism prevailed with the discovery of molecules in mature CNS that inhibited neurite growth. Unfortunately, the problem is multi-factorial and the answer too lies in a multi-factorial approach. It will require choosing the right source and type of SC, priming them appropriately and transplanting them with right conditions and factors to persuade them into doing the functional job. However, the key strategy in developing the therapeutic basis of SC transplantation for spinal cord regeneration is to completely remove the pseudo-science and opportunism. All the trials should be based on stringent ethical, scientific and clinical criteria and efforts at the global level.

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Извод

Матичне ћелије и повреда кичмене мождине

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Резиме

Повреда кичмене мождине (СЦИ) је разорни поремећај са штетним последицама како за појединца тако и за друштво. Повреда кичмене мождине има далекосежан утицај на физичко и физиолошко стање пацијента, јер често доводи до трајног губитка телесних функција које утичу на покретање екстремитета, соматску осетљивост, репородуктивне органе, бешику и црева. Постоје бројне стратегије за лечење СЦИ, укључујући трансплантацију матичних челија (СЦ). Основни циљ терапије на бази СЦ за СЦИ је обнављање и замена неурона и глија ћелија које претрпе изумирање ћелија убрзо после повреде и она представља најновији и најуспешнији терапеутски приступ код СЦИ који омогућава побољшане и ефикасне сензорске и моторне функције на анималним моделима. Ове ћелије могу да поспеше ремијелинацију путем замене олигодендроглијских ћелија, да произведу трофичке факторе који побуђују раст неурита, аксоналну елонгацију и густину влакана и активирају резидентне или пресађене прогениторске ћелије преко шупљине лезије. Упркос томе, постоји потреба за валидацијом специфичних механизама за различите изворе СЦ и СЦ трансплантацију.

Кључне речи: повреда кичмене мождине, трансплантација матичних ћелија

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