

STEM CELLS: NEW HOPE FOR SPINAL CORD INJURY

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MATIČNE ČELIJE: NOVA NADA ZA POVREDE KIČMENE MOŽDINE

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ABSTRACT

Stem cell therapy offers several attractive strategies for spinal cord repair. The regenerative potential of pluripotent stem cells was confirmed in an animal model of Spinal Cord Injury (SCI); nevertheless, optimized growth and differentiation protocols along with reliable safety assays should be established prior to the clinical application of hESCs and iPSCs. The therapeutic effects of mesenchymal stem cells (MSCs) in SCI result from neurotrophin secretion, angiogenesis, and anti-inflammatory actions. Several preclinical SCI studies have reported that the occurrence of axonal extension, remyelination and neuroprotection occur after the transplantation of olfactory ensheathing cells (OECs). The transplantation of neural stem cells NSCs (NSCs) promotes partial functional improvement after SCI because of their potential to differentiate into neurons, oligodendrocytes, and astrocytes. The ideal source of stem cells for safe and efficient cell-based therapy for SCI remains a challenging issue that requires further investigation.

SAŽETAK

Terapija matičnim ćelijama pruža nekoliko atraktivnih mogućnosti za lečenje povreda kičmene moždine. Regenerativni potencijal pluripotentnih matičnih ćelija je potvrđen u animalnim modelima povreda kičmene moždine, međutim, protokoli za kultivaciju i diferencijaciju ovih ćelija kao i testovi za potvrdu njihove bezbednosti tek moraju biti ustanovljeni kako bi se hESCs i iPSCs primenile u kliničkoj praksi. Terapijski efekat MSCs u povredi kičmene moždine se zasniva na sposobnosti ovih ćelija da sekretuju neurotrofne i antiinflamatorne faktore, kao i da promovišu angiogenezu. U nekoliko predkliničkih studija su pokazani rast aksona, remijelinizacija i neuroprotektivno delovanje OECs. Transplantacija NSCs doprinosi funkcionalnom oporavku nakon povreda kičmene moždine diferencijacijom NSCs u neurone, oligodendrocite i astrocite. Otkrivanje idealnog izvora matičnih ćelija za efikasnu i bezbednu terapiju povreda kičmene moždine i još uvek je izazov i zahteva dalja istraživanja.



INTRODUCTION

Spinal cord injury (SCI) is a devastating condition with permanent lifelong consequences (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 (1). SCI usually results in sudden and long-lasting locomotor and sensory neuron degeneration below the injury (2).

The pathophysiological processes that underlie SCI comprise the primary and secondary phases of injury. During the primary phase, because of the direct mechanical trauma of the spinal cord by fractured and displaced bone fragments and disc material, there is massive axonal damage as well as neuronal and glial cell losses (3, 4, 5). During the secondary phase of injury, further tissue damage occurs mostly from ischemia, electrolyte imbalance, inflammatory response, oxidative stress and excitotoxicity (3). Despite

major advances in the medical and surgical care of SCI patients, there are currently no effective therapies for the treatment of traumatic SCI in humans (2). Stem cell therapy offers several attractive strategies for spinal cord repair. Stem cells may play an important role in the replacement of damaged neuronal and glial cells, axonal regeneration and remyelination, the restoration of neuronal circuitry, and the production of neurotrophic factors, anti-inflammatory cytokines, and other molecules that promote tissue repair and neovascularization.

In this review, we will evaluate the therapeutic role of human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), and olfactory ensheathing cells (OECs) for treating SCI, and we will cover some of the clinical trials that aim to translate laboratory stem cell research into clinical practice.



Table 1. Therapeutic potential of stem cells for treatment of spinal cord injury

Stem cell source	Advantages	Disadvantages
hESCs	differentiation into neurons and glia, modulation of local immune response, activation of endogenous neurogenesis.	ethical issues, immune rejection, potential for tumor formation.
iPSCs	differentiation into neurons, glia, and neural progenitor cells.	potential for tumor formation.
MSCs	providing trophic support to damaged neurons by secreting angiogenic and neurotrophic factors, modulation of local immune response.	no universal consistency in cell sourcing, and the optimal administration method.
NSCs	differentiation into oligodendrocytes and astrocytes.	immune rejection, formation of glial scars.

hESCs, human embryonic stem cells; iPSCs, induced pluripotent stem cells; MSCs, mesenchymal stem cells; NSCs, neural stem cells.

Human Embryonic Stem Cells

hESCs are derived from the inner cell mass of human blastocysts; they have the ability to proliferate by maintaining both their pluripotency and their ability to differentiate into nearly all cell types, including neuronal and glial cells (6). Improved protocols have been developed to differentiate hESCs into motoneuron progenitors (MPs) and oligodendrocyte progenitors (OPCs) (7, 8). The transplantation of hESC-derived MPs and OPCs can efficiently recover locomotor function in both contusion and transection animal models of SCI (7, 8). The regenerative mechanism of hESC therapy for SCI depends on the potential of hESC-derived OPCs and MPs to differentiate into neuronal and glial cells and the immunomodulatory characteristics of transplanted hESC-derived OPCs (7, 1).

Based on promising preclinical data from hESC-derived OPC transplants in rodent SCI models, the Food and Drug Administration (FDA) approved the first hESC clinical trial in 2009. The Geron Company attempted to test the safety of hESC-derived OPCs in human SCIs. Two million hESC-derived OPC cells (GRNOPC1) within the acute phase were

transplanted directly into the spinal cord of four ASIA A patients with complete thoracic SCI. In 2011, Geron discontinued this trial for financial reasons. The preliminary results indicated that GRNOPC1s do not cause any harm, but the debate about the efficacy of these cells still continues.

Concerns about the transplantation of hESC-derived neural cells to treating SCI are related to the ethical issues of cell derivation, the immune rejection of transplanted cells, the use of differentiation protocols that still involve mediums, growth factors, and supplements of animal origin, and the possibility of teratoma formation from incomplete or aberrant differentiation resulting in the formation of non-neural cells (6, 9).

Induced Pluripotent Stem Cells

iPSCs were originally generated by the ectopic expression of four transcription factors called, namely Oct4, Sox2, Klf4, and c-Myc in fibroblast cells by Takahashi and Yamanaka in 2006 (10). iPSCs show morphological, transcriptional, epigenetic, and phenotypic similarity to hESCs and can differentiate towards any cell in the human body including neurons, glia, neural progenitor cells (NPCs), and motoneurons (11).

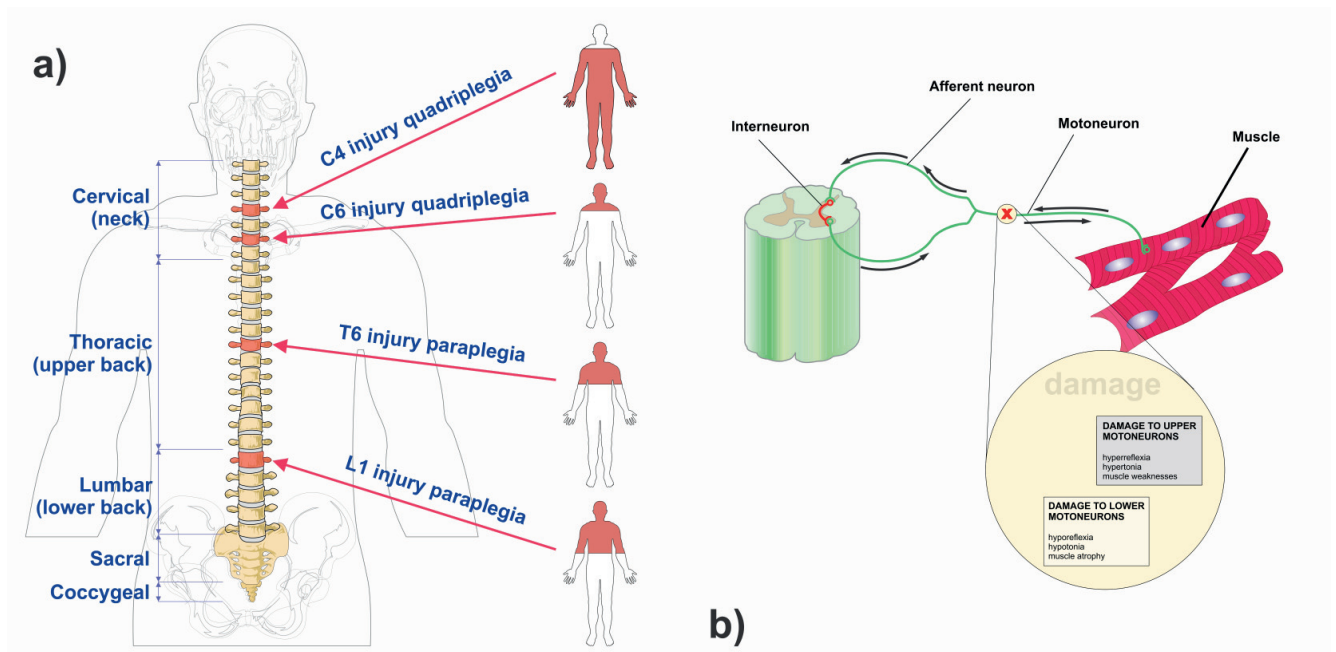


Figure 1: Spinal cord injury: a) clinical signs and b) site of injury

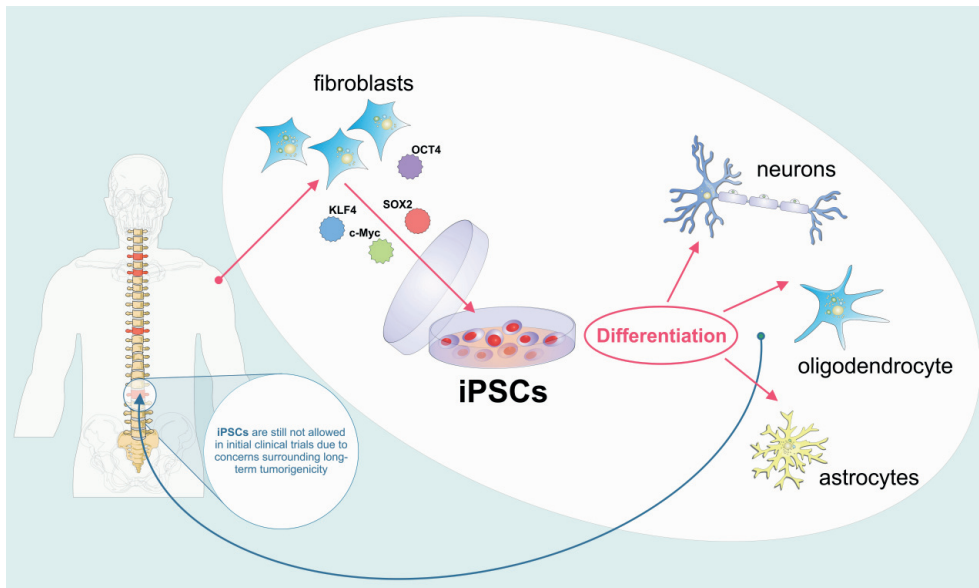


Figure 2: Method for derivation of iPSCs from adult somatic cell by introducing OCT4, SOX2, KLF4 and c-Myc. iPSCs can differentiate toward neurons, oligodendrocytes and astrocytes.

Patient-specific iPSCs derived from somatic cells through the ectopic expression of a defined set of factors do not present ethical and immunological concerns (11). The primary concern about the use of these cells in clinical trials was with the reprogramming technology that involved viral vectors and their tumourigenicity (2). Some of the reprogramming issues are solved by the deriving iPSCs by through nonviral methods such as mRNA or chemicals and small molecules (12, 13). The regenerative potential of iPSCs was confirmed in a rodent model of SCI (14, 15); nevertheless, optimized growth and differentiation protocols and reliable safety assays should be established prior to the clinical application of iPSCs.

Mesenchymal Stem Cells

MSCs are adult, self-renewable, multipotent cells that can be found in almost all postnatal tissues (16). In addition to their stem/progenitor properties, MSCs have been shown to possess broad immunomodulatory abilities (16). The therapeutic effects of MSCs in SCI result from neurotrophin secretion, angiogenesis, and antiinflammatory actions, rather than direct translineage conversion to functional oligodendrocytes or neurons (17, 18, 19, 20, 21). Engrafted MSCs act as neuroprotectors by secreting brain-derived neurotrophic factor (BDNF), glia cell line-derived neurotrophic factor (GDNF),

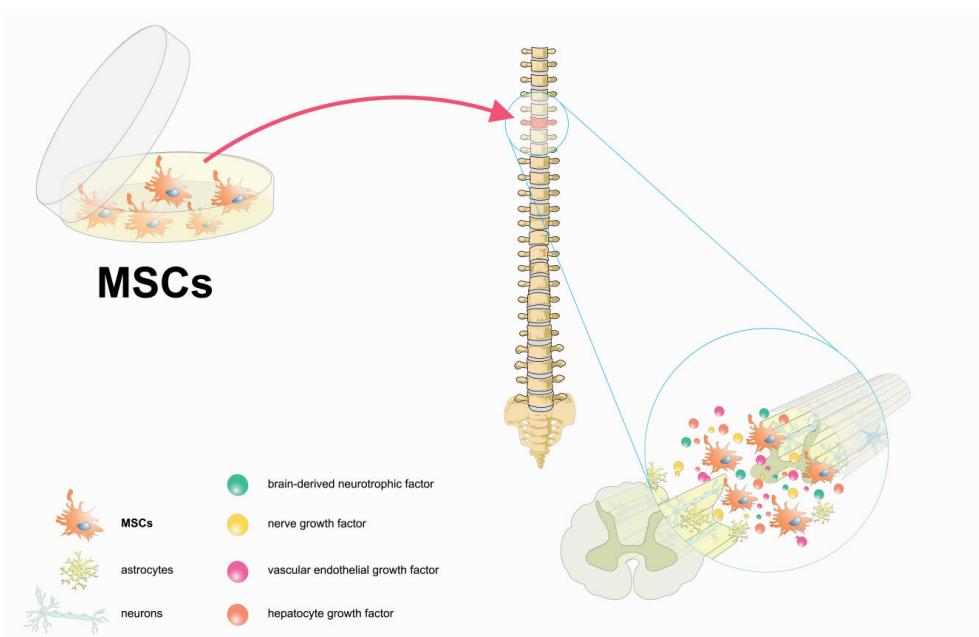


Figure 3: Transplanted MSCs act as neuroprotectors in spinal cord injuries by producing growth factors and anti-inflammatory cytokines.



nerve growth factor (NGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (22, 23).

The number of clinical trials that employ MSCs for SCI treatment is increasing, indicating that despite several questions that still need to be addressed at pre-clinical levels, MSCs are considered potentially beneficial for translational studies (24). The pathological processes that occur at the lesion site in SCIs evolve over time, from the acute to subacute to chronic phases; therefore, transplantation at different times post-lesion may have varied effects (25). Sykova et al. suggested that performing MSC transplantation within a therapeutic window of 3-4 weeks following SCI is critical for the success of MSC-based therapy (26). Yoon et al. studied the effects of autologous bone marrow MSC transplantation in combination with the administration of granulocyte-macrophage colony-stimulating factor to 35 patients with complete SCIs at acute, subacute and chronic stages (27). No serious complications were reported, and 30.4% of patients who received MSCs at acute and subacute stages showed significant improvements in their ASIA scale position (27). Few clinical studies have shown neurological improvements in MSC-treated patients who during the chronic stage of SCI, when the glial scar is already present (28, 29, 30, 31, 32). In these studies, MSCs were transplanted directly into the lesion intrathecally, intravenously and intrathecally (simultaneously) at once, and intraarterially.

Although the clinical study results are promising, there are important issues that should be addressed to achieve successful MSC-based therapy, that is, the universal consistency in cell sourcing and culture conditions, the ideal cell quantity and the optimal administration method (24, 1).

Olfactory Ensheathing Cells

OECs are a unique population of macroglia found in the lamina propria of olfactory mucosa, around the olfactory nerve fascicles and in the two outer layers of the olfactory bulb. OECs have the dual nature of astroglial cells and Schwann cells (33). Several preclinical SCI studies have re-

ported the occurrence of axonal extension, remyelination and neuroprotection after OEC transplantation (34, 35, 36, 37, 38). OECs migrate to injured sites and secrete a large number of factors that are necessary for the growth, development, differentiation, and maturation of different types of neurons and reduce astrocyte activity and glial scar formation (39, 40). Spinal cord regeneration and functional recovery depend on the nature and source of OECs, the injury model, the graft cell preparation, the time of transplantation, and the transplantation procedures (25).

Feron et al. performed a single-blind phase I clinical trial in which three patients with SCI (chronic injuries) received autologous OECs. The feasibility of the procedure and the safety of these cells were reported, but there was no evidence of clinical efficacy (41, 42). Lima et al. reported that the transplantation of minced olfactory mucosa in patients with chronic SCI was not significantly efficient (44). By contrast, recent clinical studies suggested that there was a neurological improvement in SCI patients after OEC transplantation (44, 45).

Neural Stem Cells

NSCs are multipotent cells with the potential to differentiate into neurons, oligodendrocytes, and astrocytes, and they can be efficiently propagated *in vitro* (46).

NSCs can be found in the periventricular subependymal layer, in the subgranular zone of the dentate gyrus, and in the ependymal regions lining the central canal (47). The activation of resident ependymal stem cells following SCI is not sufficient to promote recovery because the cells differentiate mostly into astrocytes and oligodendrocytes (48, 49). Several preclinical studies confirmed that the transplantation of NSCs promotes a partial functional improvement after SCI (50, 51). The transplantation of OPCs that had differentiated from ependymal stem cells efficiently recovered the locomotor function of an SCI animal model (47). The source of NSCs, the methods of cell isolation and preparation, the time of transplantation, the chosen

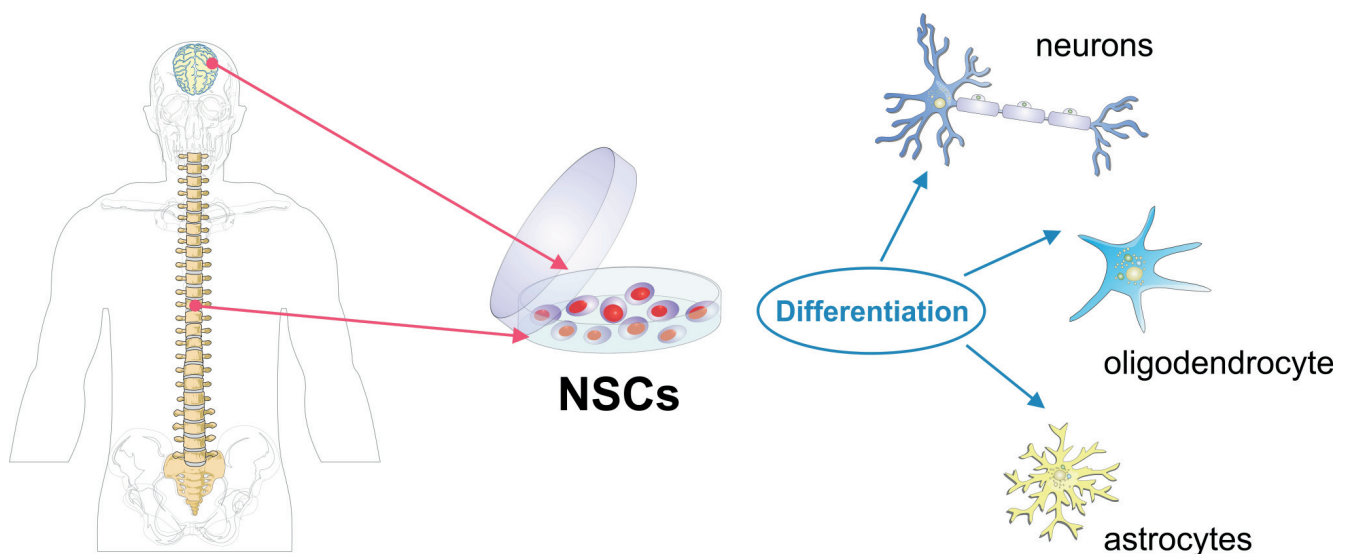


Figure 4: Potential uses of NSCs which were isolated from the adult brain and spinal cord as a source of neurons, oligodendrocytes, and astrocytes.



immunosuppression, and the type of injury (contusion vs. transection) are important issues for achieving successful NSC-based therapy after SCI (49). Human NSCs have been isolated from foetal brains, and spinal cords have been isolated from aborted fetuses. Unlike adult NSCs, foetal-derived NSCs generate neurons in addition to glia in the injured spinal cord (1).

Currently, two human trials involving allogeneic NSCs for SCI are ongoing. The primary objectives of these studies are to determine the long term safety and preliminary efficacy of NSC transplantation in subjects with thoracic spinal cord trauma.

CONCLUSIONS

Numerous preclinical studies suggest that stem cells are able to enhance recovery following SCI. However, the ideal source of stem cells for the efficient and safe cell-based therapy of SCI remains a challenging issue that requires further investigation and continuous cooperation between clinicians, researchers, and patients.

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