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REVIEW ARTICLE

Stem Cell–Based Therapies for Spinal Cord Injury

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Abstract

Summary: Spinal cord injury (SCI) results in loss of nervous tissue and consequently loss of motor and sensory function. There is no treatment available that restores the injury-induced loss of function to a degree that an independent life can be guaranteed. Transplantation of stem cells or progenitors may support spinal cord repair. Stem cells are characterized by self-renewal and their ability to become any cell in an organism. Promising results have been obtained in experimental models of SCI. Stem cells can be directed to differentiate into neurons or glia in vitro, which can be used for replacement of neural cells lost after SCI. Neuroprotective and axon regeneration-promoting effects have also been credited to transplanted stem cells. There are still issues related to stem cell transplantation that need to be resolved, including ethical concerns. This paper reviews the current status of stem cell application for spinal cord repair.

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Key Words: Spinal cord injuries; Stem cells; Totipotency; Pluripotency; Cell transplantation; Neuroprotection; Axon regeneration; Gene therapy; Ethics

INTRODUCTION

Stem cells proliferate, migrate, and differentiate to form organisms during embryogenesis. During adulthood, stem cells are present within tissues/organs including the central nervous system (1–5), where they may differentiate into neurons (6). Since the identification and characterization of stem cells, a great deal of interest has been given to their potential for treatment of spinal cord injury (SCI), traumatic brain injury, and degenerative brain diseases (7–12). Considering their characteristic abilities to self-renew and differentiate into any cell type in the body, the therapeutic promise of stem cells is justified. Before effective therapies can be developed, several issues need to be addressed and resolved. These issues range from increasing our basic knowledge about the stem cell's biology to prevailing over moral concerns fueled by religious and/or political ideas.

STEM CELL DEFINITIONS

A stem cell is defined by its ability of self-renewal and its totipotency. Self-renewal is characterized by the ability to undergo an asymmetric division in which one of the resulting cells remains a "stem cell," without signs of aging, and the other (daughter) cell becomes restricted to one of the germ layers. A stem cell may become quiescent and at later stages re-enter the cycle of cell division (13,14) (Figure 1).

A true stem cell is a totipotent cell; it can become any cell type present in an organism. Many consider the zygote to be the only true totipotent (stem) cell because it is able to differentiate into either a placenta cell or an embryonic cell. Others define the cells of the inner cell mass within the blastocyst as embryonic stem cells (ESCs). These cells are pluripotent because they can not become a placenta cell (Table 1). Besides ESCs, undifferentiated cells can be found among differentiated cells of a specific tissue after birth. These cells are known as adult stem cells, although a better term would be "somatic stem cell" because they are also present in children and umbilical cords. There is ample evidence that adult stem cells are not restricted to a particular germ layer and can transdifferentiate (15–19). An

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Table 1. Terms Most Frequently Used in Stem Cell Biology

Totipotent cell	Differentiates into any cell type without exception. Also "stem cell"
Pluripotent cell	Differentiates into any cell type present within a germ layer
Multipotent cell	Differentiates into cells of a particular cell lineage (in a germ layer)
Unipotent cell	Differentiates into only 1 type of cell and differs from non–stem cell because it is able to self-renew
Self-renewal	Asymmetrical division producing 1 identical cell and 1 "daughter" cell that enters the determination phases
Zygote	Fusion of spermatozoid and egg cell; develops into blastocyst
Inner cell mass	Clump of cells within blastocyst; the original (totipotent) embryonic stem (ES) cells
Embryonic stem cell	Undifferentiated cell present in the inner mass cells of a blastocyst
Adult stem cell	Undifferentiated cell in differentiated tissue; better term is "somatic stem cell" because this type can also be found in children
De-differentiation	Reversion of partially or terminally differentiated cell to an earlier developmental stage in its own lineage
Transdifferentiation	Change of cell's fate, ie, cell enters a lineage that was not the original destination

important advantage of adult stem cells over ESCs is that they can be harvested without destruction of an embryo. As a result, adult stem cells have gained ample interest for their application in a variety of disorders (see below).

Differentiation

The pluripotent stem cell differentiates into a multipotent cell of the 3 germ layers. These 3 layers are the ectodermal layer (from which skin and neural tissue originate), the mesodermal layer (connective tissue, muscle, bone, and blood cells), and the endodermal layer (gastrointestinal tract and internal glandular organs) (Figure 2).

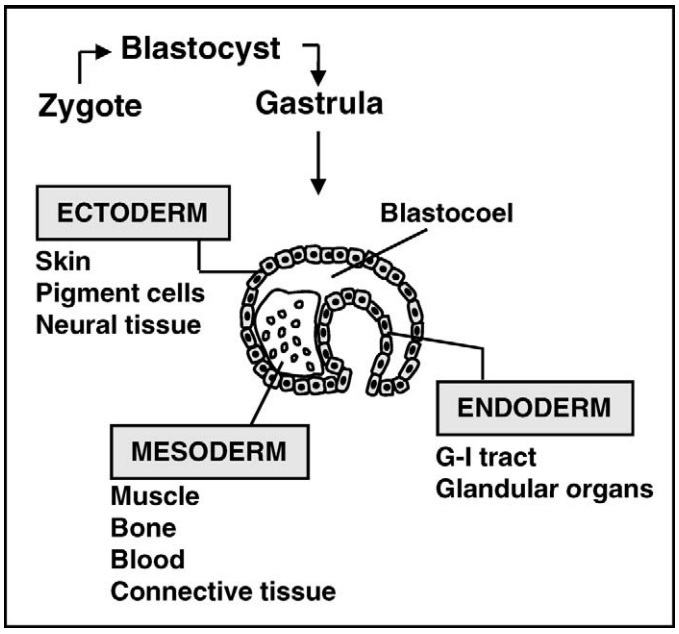

Figure 1. *All tissues in an organism originate from the 3 germ layers: the ectoderm layer, endoderm layer, and the mesoderm layer. Neural cells that form the central and peripheral nervous system derive from the ectoderm.*

The multipotent cell differentiates into a unipotent cell of a particular cell lineage within its own germ layer (Figure 2). The unipotent cell is capable of becoming a cell type within that particular cell lineage (Figure 2). At the successive phases of differentiation (or determination), the resulting progeny are known as progenitor cells; "stem cell-like" cells capable of self-renewal. Within the central nervous system, unipotent neural progenitors become the neurons and glial cells present in brain and spinal cord (Figure 2).

Transdifferentiation

In classic embryology, the totipotent stem cell becomes unipotent through successive phases of fate restriction. The steps in this process were thought to be irreversible. However, recently it was shown in vitro that the fate of multipotent cells can be changed to another germ layer (15–19). This process is known as transdifferentiation. The unlimited potential of transdifferentiation prompted many investigators to obtain cells that normally derive from stem cells that are more difficult to harvest from stem cells that are easier to harvest. For instance, it is less complicated to harvest stem cells from skin (20,21) or bone marrow (22,23) than from the brain (24,25). Thus, it would be more efficient to obtain neural cells from skin- or bone marrow–derived stem cells through transdifferentiation.

Transdifferentiation has often been shown using nonspecific markers and ignoring possible artifacts caused by culturing methods (26,27). Therefore, the existence of transdifferentiation is still debated (27,28). It

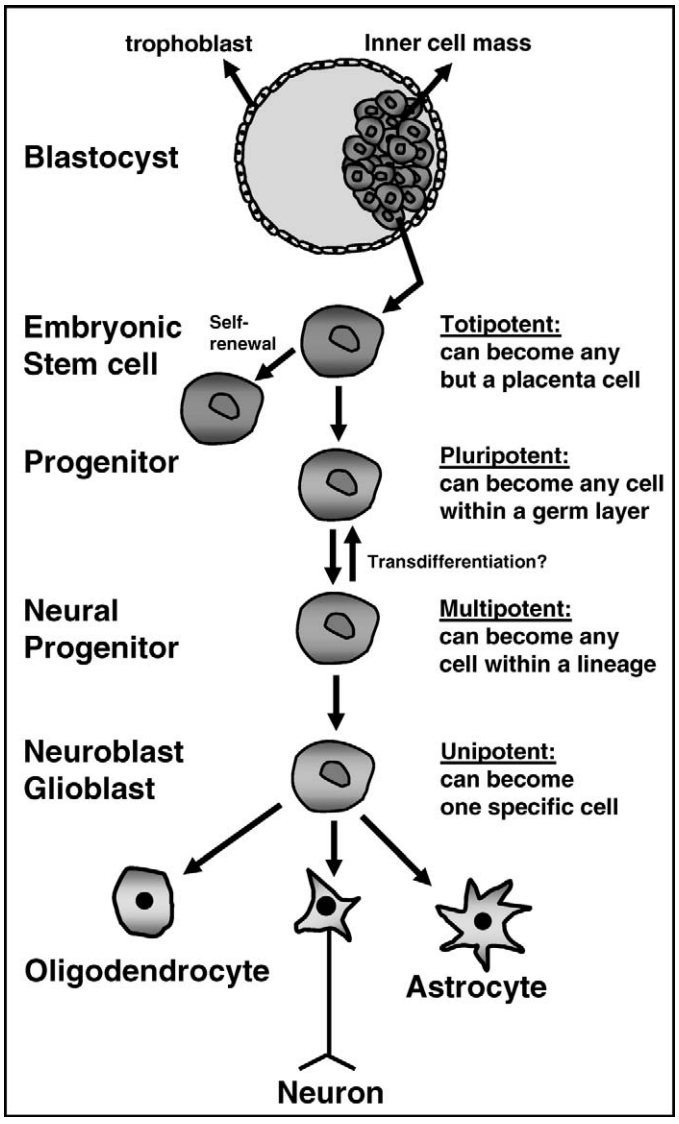

Figure 2. *From embryonic stem cell to differentiated neural cell. Embryonic stem cells from the inner cell mass of the blastocyst are pluripotent and undergo phases of differentiation that change them into unipotent cells. This depicts the generation of neural cells; oligodendrocytes, neurons, and astrocytes.*

should be kept in mind that forced differentiation into a cell from a lineage within an unnatural germ layer could result in abnormal phenotypes that, after grafting, could induce carcinogenesis (29).

POTENTIAL FOR SPINAL CORD REPAIR

After SCI, endogenous regenerative events occur, indicating that the spinal cord attempts to repair itself. Schwann cells, the myelinating and regeneration-promoting cell in the peripheral nervous system, migrate from spinal roots into the damaged tissue and myelinate spinal cord axons (30,31). The expression of regeneration-associated genes is increased in damaged neurons (32,33). There is a surge in proliferation of local adult stem cells and progenitor cells (34–36). However, axonal growth is thwarted by growth inhibitors present on oligodendrocyte myelin debris and on cells that form scar tissue (37–39). Also, the newborn stem cells and progenitor cells do not integrate functionally into the injured spinal cord tissue. Thus, the endogenous regenerative events that occur after injury fail to repair the spinal cord.

Improved functional outcome after SCI may be elicited by neuroprotective approaches that limit secondary tissue loss and thus the loss of function. Alternatively, functional recovery could be elicited by axon growth-promoting approaches that result in restoration of damaged and/or formation of new axon circuits that could become involved in function. There is little doubt that stem cells and neural progenitor cells could become invaluable components of repair strategies for the spinal cord. They can become neural cells that may support anatomical/functional recovery. Alternatively, they may secrete growth factors that could support neuroprotection and/or axon regeneration (Figure 3). The potential of stem cells or progenitor cells to support spinal cord repair has been studied extensively (40–42). Their shortcomings for repair are also understood (43,44). Over the last decade, stem cells have often been studied without implementing explicit criteria that would define the used cells as such. Consequently, the therapeutic potential of true stem/progenitor cells is still unknown. Other matters related to the use of stem/progenitor cells for SCI also need to be resolved before effective therapies can be developed. How can the cells be best obtained? Do they need to be differentiated in vitro before transplantation? How can survival of grafted stem/progenitor cells be improved and uncontrolled division and differentiation be prevented (45)? How can functional integration of the transplanted cells be improved?

Cell Replacement in the Injured Spinal Cord

Considering the ability of stem cells to become any cell type, their potential use for cell replacement strategies is common sense. With the appropriate combination of (growth) factors (induction cocktail), ESCs can be used to obtain neurons and glial cells (46,47). ES-derived neurons can survive and integrate after injection into the injured rat spinal cord (48). It was shown that transplanted mouse ESCs myelinate axons in the myelin-deficient *shiverer* rat spinal cord (49). Also, mouse ESCs grafted into the injured (normal) rat spinal cord result in improved functional recovery (50). Importantly, ESCs were found to survive well within the injured spinal cord, suggesting that long-term treatments could be achieved using this approach (51).

Human ESC can be directed toward multipotent neural precursors (52), motor neurons (53,54), and oligodendrocyte progenitor cells (55). The latter were found to differentiate into mature oligodendrocytes in vitro and in vivo (56). Moreover, these cells are able to

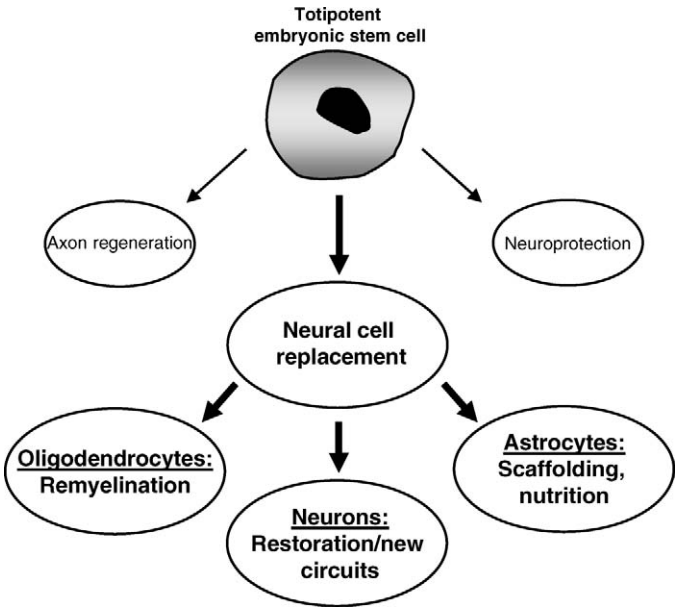

Figure 3. *Potential effects of stem cells on spinal cord repair. Although transplanted stem cells could elicit axon regeneration and/or neuroprotection through secretion of growth factors, the most logical contribution to repair could come from their ability to replace lost neural cells. This could result in remyelination of demyelinated axons if they become oligodendrocytes, restoration of (new) circuits if they become neurons, and providing scaffolding and nutrition of the injured area if they become astrocytes. Generally, the last is not preferred because astrocytes express a number of axon growth inhibitory molecules that could prevent axon regeneration and thus limit the overall restoration.*

myelinate axons after transplantation into the spinal cord of myelin-deficient *shiverer* mice and adult rats (55).

Neural progenitor cells (ie, multipotent cells from which the cells of the central nervous system arise) often aggregate into neurospheres. Cao et al (57) showed that neural progenitor cells transplanted into the injured rat spinal cord favored differentiation into astrocytes. These results indicated the need for differentiation protocols before grafting (58). Fetal neural precursor cells genetically modified to express noggin, an antagonist of bone morphogenetic protein, differentiate preferably into neurons and oligodendrocytes (59). Transplantation of these cells into the injured mouse spinal cord resulted in improved functional outcome (59). However, this result could not be shown by others using the same approach (60).

Human neural progenitor cells can be harvested from blastocyst-stage embryos and manipulated to generate functional neurons and glia (61). When human neural progenitor cells were grafted into the injured rat spinal cord, some of them were found to differentiate into oligodendrocytes (62,63). Moreover, this finding was accompanied by improved functional outcome (62,63).

Mesenchymal stem cells from bone marrow may also have therapeutic promise for SCI (64,65). Although still debated (66), these particular adult stem cells have been shown to differentiate into bone, fat, tendon, and cartilage cells (67). It has been published that these cells can also transdifferentiate in vitro into liver (68), skeletal (69,70), and cardiac muscle (71,72) cells and into central nervous system cells (68,70,73–77). This makes mesenchymal bone marrow stromal stem cells interesting for strategies for repair of the injured spinal cord. Many medical fields are exploring mesenchymal stem cells, for instance, for repair of the heart after myocardial infarction (78,79), osteogenesis imperfecta in orthopedics (80,81), organogenesis in internal medicine (82,83), intervertebral disk disease in neurosurgery (84–87), and stroke/neurodegenerative diseases in neurology (88–90).

Neuroprotection

A neuroprotective strategy implemented soon after SCI would be the first line of defense against injury-induced tissue loss and could contribute to an improved neurological outcome. It has been shown that neural progenitor cells can protect against excitotoxicity (91,92). Also, neural progenitor cells secrete a variety of molecules that could protect neural cells from death mechanisms other than excitotoxicity (91,92). Thus, transplantation of these cells into the injured spinal cord could in fact exert neuroprotective effects. Bone marrow stromal cells have also been shown to elicit neuroprotective effects because grafting into the injured adult rat spinal cord resulted in tissue sparing (93,94). This may have resulted from the secretion of a number of growth factors (95–98).

Axon Regeneration

Promoting axon growth in the injured spinal cord could contribute to restoring function. The ability of neural progenitor cells to secrete a variety of neurotrophic factors indicates that they could promote growth of damaged axons (91,92). Adult neural progenitor cells were found to provide a permissive guiding substrate for corticospinal axon regeneration after spinal cord injury (99). The stem cell–like olfactory ensheathing cells assist axon regeneration in the injured spinal cord in a different manner. These cells are capable of preventing axons from recognizing growth inhibitory molecules thereby allowing them to elongate into otherwise inhibitory terrain (100,101).

CLINICAL APPLICATION TO SCI

The translation of approaches developed in the laboratory involving stem cells into the clinic is in progress. The use of stem cells harvested from tissue from an adult has facilitated the use of stem cells in the clinic because it has practically dismissed the moral objections surrounding the use of stem cells derived from an embryo. Nevertheless, for reasons described below, the use of

ESCs is often preferred over that of adult stem cells. Use of human ESCs for spinal cord repair in the United States has been proposed by Geron, a California-based biotechnology company. Application of adult human stem cells for treatment of SCI is in progress in many countries around the world (102). For instance, autologous bone marrow–derived stem cells have been transplanted in the injured spinal cords of 25 patients in Guayaquil, Ecuador, a trial that is supported by a California-based biotechnology company, PrimeCell Therapeutics. Encouraging results have been reported such as improved walking and sensory perception. It has been suggested that surmounting the ethical hurdles (see below) could benefit the clinical application of ESCs (103).

EMBRYONIC VS ADULT STEM CELLS

ESCs can develop into more than 200 different cell types present in the human body (104) and under the appropriate circumstances into an entire organism (105). Human ESCs have been isolated from blastocyst-stage embryos (106). They have also been created using somatic cell nuclear transfer (107,108) or parthenogenetic activation of eggs (109,110). Isolated ESCs do not undergo senescence and retain high telomerase activity and normal cell cycle signaling, which explains their rapid proliferation in culture (111,112). These plastic characteristics make the ESC suitable for central nervous system repair strategies. However, transplantation of ESC can result in teratomas because of uncontrollable cell proliferation (113–115). Also, ESCs in culture may undergo genomic and epigenetic changes that could lead to transformation, although this can be prevented using proper culture techniques (116). Transplanted ESC are prone to be rejected after injection into adult tissue, and long-term treatment with immunosuppressive drugs may be required to prevent this loss (114). These findings have to some extent tempered the enthusiasm for application of ESC in repair strategies for the central nervous system, despite the fact that ESC possess by far the greatest potential and could be applied in a broad selection of reparative cell therapies.

An alternative for ESC are stem cells obtained from tissue after birth. For instance, neural progenitor cells have been harvested from adult brain (117,118) and spinal cord (119). However, adult stem cells are less plastic than ESCs and divide less frequently in culture (120). Also, their differentiation potential may decrease in time (121). This makes them a possible but somewhat limited alternative for ESCs. On the other hand, they offer the advantage that they can be transplanted without genetic modifications or pretreatments. Immune rejection would not be an issue with adult stem cells when the cells are isolated from the patient (autografting) (122). Also, adult stem cells show a high degree of genomic stability during culture (123,124) and usually do not result in tumor formation (124). Finally, there is much less moral concern surrounding the use of adult stem cells because they can be harvested from the patient. These latter features support the use of adult stem cells over ESCs for strategies aimed at repairing the central nervous system. This is certainly true if strategies can be developed that circumvent the potential drawbacks of using adult stem cells such as the lower plastic ability and lower rate of proliferation in vitro compared with ESCs.

ETHICAL AND SOCIAL CONCERNS

One of the issues that surround the use of ESCs is the time point at which an embryo is considered to be a person (125–127). According to the Roman Catholic Church and other religious institutions, an embryo "must be treated from conception as a living person" (128). This implies that a blastocyst cannot be used to harvest cells. Others consider an embryo to be a person only after the 20th week of gestation (125,126), implying that ESCs can be harvested from blastocysts. Also, in that case, ESCs could be harvested from embryos that were generated but not selected for in vitro fertilization. These would otherwise be discarded.

Discussions on what constitutes "life" and when does "life" start are often intense because they are driven by moral concerns fueled by religious and political ideas. These issues need to be addressed with respect to all opponents. Rules regarding the harvest and use of stem cells can only be set after full agreement by all groups within a society.

Ethical issues that surround the use of adult stem cells mostly involve their possible misuse (129). For instance, oocytes can be derived from stem cells of male origin, which allows the production of a child from one or two male biological parents (130–132). The potential biological problems and psychological effects on the child are unknown. It would also be possible that the offspring develops defects because of acquisition of pairs of (recessive) genes (130–132).

Therapeutic cloning and genetic manipulation are other issues that surround the use of stem cells. Cloning of cells, genetically matched for the host, could in theory be beneficial for organ transplantation because it may solve issues such as organ shortage and rejection. Genetic manipulation could convert ESCs into gametes, which would allow germ line gene therapy (GLGT) (131).

INDUCED PLURIPOTENT STEM CELLS

It is now possible to obtain pluripotent cells by reprogramming differentiated cells, such as fibroblasts, through the introduction of 4 transcription factors, OCT3/4 (octamer-4), SOX2 (sex-determining region Y-box2), KLF4 (Kruppel-like factor), and MYC (induced pluripotent stem (iPS) cells (133,134). This new technology was first described by Takahashi and Yamanaka (135) for mouse fibroblasts and has now been applied for other mouse cells (136) and for human somatic cells (137). Of the 4 transcription factors, MYC and KLF4 can be substituted by others (138,139). The underlying mech-

anisms for this typically straightforward and robust reprogramming procedure are still unknown and intensely debated. At present, it is still unclear in how closely iPS cells resemble conventional ESCs and whether application of iPS cells would result in similar functional results as can be obtained with ESCs. Comparative gene expression profiles of human ESCs and human iPS cells is now ongoing (137,140). Several hurdles need to be overcome before iPS cell technology can produce cells for clinical use (133), such as the use of retroviral vectors to introduce the transcription factors and the need for selection markers to identify the reprogrammed cells, as well as the use of the oncogene MYC and the integration of retroviral vectors into the genome. These needs are required for proper reprogramming, but they modify the cell genetically and modified cells face regulatory obstacles for therapeutic applications. Nevertheless, it is evident that iPS cell technology is promising and has opened exciting avenues for the clinical application of pluripotent cells without the ethical obstacles that go along with the use of ESCs.

CONCLUSIONS

Stem cells hold promise for spinal cord repair, but their true potential has not yet clearly been shown. At this time, stem cell–based therapies are at an early stage, and the associated risks are still unclear. When a patient has a disabling or life-threatening disease, a case might be made for surmounting the existing ethical and social barriers to enable treatment. Changing ethical barriers will not be accomplished overnight. Most likely, stem cell science will advance faster than the debate on ethical issues. Therefore, to enable future use of stem cells for therapeutic purposes, discussions on all related issues and especially the moral aspects need to be held today. As with any medical intervention, the questions to be asked are whether this approach is the most likely one to achieve success and whether the risks justify the benefits.

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