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Concise Review: Human Pluripotent Stem Cells in the Treatment of Spinal Cord Injury

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ABSTRACT

Spinal cord injury (SCI) results in neural loss and consequently motor and sensory impairment below the injury. There are currently no effective therapies for the treatment of traumatic SCI in humans. Different kinds of cells including embryonic, fetal, and adult stem cells have been transplanted into animal models of SCI resulting in sensorimotor benefits. Transplantation of human embryonic stem cell (hESC)- or induced pluripotent stem cell (hiPSC)-derived neural cells is nowadays a promising therapy for SCI. This review updates the recent progress in preclinical studies and discusses the advantages and flaws of various neural cell types derived from hESCs and hiPSCs. Before introducing the stem cell replacement strategies in clinical practice, this complex field needs to advance significantly in understanding the lesion itself, the animal model adequacy, and improve cell replacement source. This knowledge will contribute to the successful translation from animals to humans and lead to established guidelines for rigorous safety screening in order to be implemented in clinical practice. STEM CELLS *2012;30:1787–1792*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

In the last decade, many reports have demonstrated significant recovery after early medical treatment of spinal cord injury (SCI), although there is still no effective cure. SCI usually results in long-lasting locomotor and sensory neuron degeneration below the injury. Cell transplantation is considered as a promising approach to replace damaged cells and promote neuroprotective and neuroregenerative repair. Human pluripotent stem cells, including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), hold great potential as a source for cell replacement therapies in humans. Self-renewal and multilineage differentiation toward virtually any cell type of human body are two unique properties that make these cells the most promising sources for tissue regeneration. After encouraging preclinical studies using hESC derivates, clinical trials have been initiated focusing on safety and efficacy.

BRIEF SUMMARY OF PATHOLOGICAL EVENTS AFTER SCI

The pathological events following SCI have been thoroughly documented in our last review [1] and can be summarized in two complex phases [2]. In acute phase, there is a massive axonal loss occurring within days following SCI [3] as well as neuronal and glial cells loss [4] in the lesion epicenter creating a fluid-filled cyst. In addition, massive death of oligodendrocytes results in the inability of spared neurons to regenerate their axons [5]. In the secondary injury phase, further tissue damage occurs mostly due to massive production of free radicals, excessive release of excitatory neurotransmitters, and inflammatory response. The massive cell death, provoked by apoptosis and necrosis, affects all functional neurons and glial cell population, including oligodendrocytes [6] in this secondary phase. One of the important events that contribute to pathophysiological state after SCI is astrogliosis. Astrocytes

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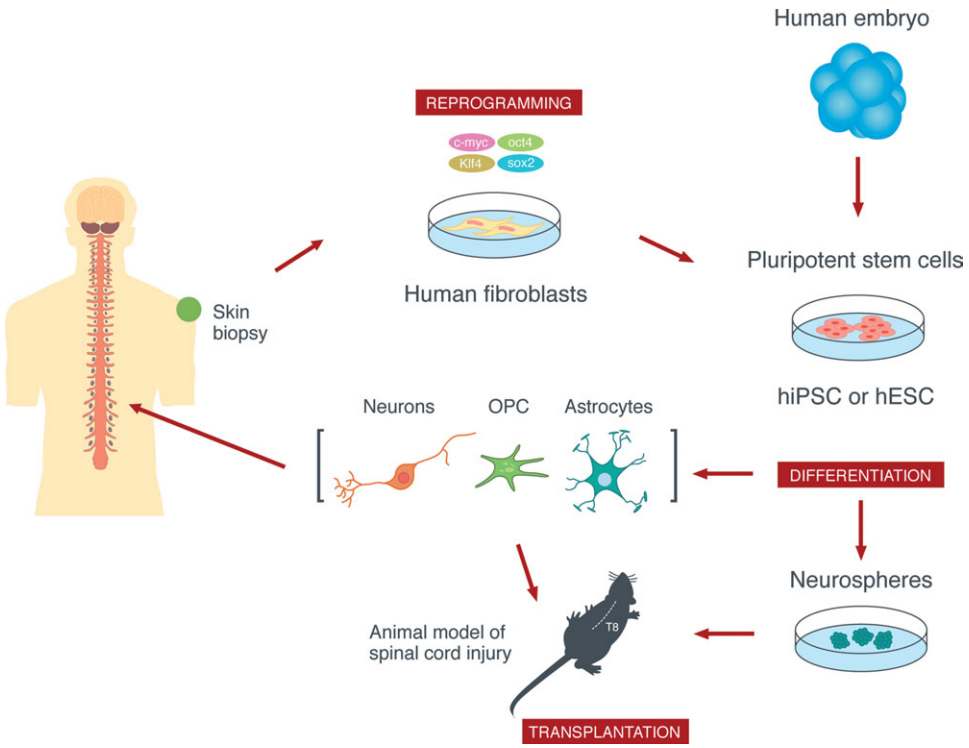

Figure 1. Sources of stem cells used in preclinical studies and clinical treatment. hiPSCs can be generated by reprogramming patient's fibroblasts and hESCs can be differentiated toward neural progenitors: OPC, MP and astrocytes, or neurospheres and transplanted to animal models or humans. Abbreviations: hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; MP, motoneuron progenitors; OPCs, oligodendrocytes progenitor cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in the lesion site proliferate and increase the expression of glial fibrillary acid protein [7]. These reactive astrocytes, due to their large-cell bodies and processes, join together tightly and form glial scars. The scar formation is tightly correlated with inflammatory process caused by microglial cell uptake and secretion of chondroitin sulfate proteoglycans and other proteins that are known to inhibit axonal growth such as slit proteins [8] or ephrin-B2 [9]. The inhibitory molecules secreted by glial scar-forming cells prevent functional recovery of the CNS contributing to locomotor impairment [10].

In summary, the spinal cord lesion induces massive cell loss, with oligodendrocytes and neural cell death, leading to neurological dysfunction. For these reasons, in addition to axonal regeneration and neural protection strategies, many experimental studies suggest that transplantation of undamaged cells into the site of injury may eventually be an effective therapy to overcome the lost locomotor function. By cell therapy, scientists and clinicians hope to restore spinal cord function by creating an environment that promotes remyelination using oligodendrocytes, axon elongation, and formation of circuits with new neurons. During the last two decades, the search for new cell sources has been revolutionized by the discovery of hESCs and hiPSCs, encouraging the development of stem cell-based reparative approaches for many disorders, including SCI [11].

hESCs

hESCs are pluripotent cells and can be derived from the inner cell mass of the early blastocyst. These cells are characterized by 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 [12–14]. In the context of cell therapy for SCI, oligodendrocytes and neurons are of particular interest. Oligodendrocytes play a crucial role in CNS providing myelin sheaths around axons enabling fast propagation of nerve impulses. SCI-induced massive cell death and loss of oligodendrocytes results in demyelination of spared axons leading to locomotor impairment. Delivery of early neural or oligodendrocyte progenitor cells (OPCs) as a source for remyelination processes including migration and mature differentiation of these cells could be a promising strategy for spinal cord repair [13]. In view of this, hESCs have been described as a promising source of differentiated oligodendrocytes and motoneurons [12–14]

Clinical application of hESCs critically depends on their ability to differentiate toward defined and pure neural cell types in vitro. In other words, generation of OPCs without traces of hESCs, is fundamental in case of SCI. Several studies, including our own [11-13, 15], have focused on the improvement of the existing protocols for differentiation of hESCs toward neural precursors prior to cell transplantation in animal models of SCI (Fig. 1). Keirstead et al. [13, 16] were the first to describe an efficient protocol for production of OPCs from hESC. Differentiation of hESCs based on specific coatings and media supplementation by triiodothyronine and morphogens such as epidermal growth factor in a timely fashion yields OPCs at more than 90% [17]. The injection of these cells into the contusion rat model, in acute phase of SCI, led to remyelination and partial restoration of locomotor function. The same effects were observed when SCI was performed in the cervical part [18]. In the contusion model used by these authors, even after severe contusive SCI, surviving

axons persist in the subpial rim of white matter and the restoration of the oligodendrocyte population by replacement therapy has been considered as an attractive strategy to promote remyelination [13, 16, 19, 20]. Although several groups [21, 22], including our own [11], validated this protocol and showed significant locomotor improvement in different rodent models, our results revealed different aspects and mechanisms behind the regenerative potential of OPC when transplanted in complete transection rat model of SCI either alone or in combination with motoneuron progenitors (MPs) [11]. We studied the completely transected spinal cords with no evidence of spared host axons, and found that transplanted OPCs differentiated toward neuronal cells, as confirmed by the presence of human specific $NF70^+$ neurofilaments in the lesion site. This is in contrast to previous claims that hESCs differentiate exclusively toward a pure population of OPCs [13, 16]. We hypothesized that the potential of OPCs to rescue locomotor activity is due to the presence of heterogeneous cell types or multiple character of transplanted progenitors. By analyzing the transcription phenotype of obtained OPCs at day 42, we observed strong expression of *OLIG2* [23–25], a developmental marker of MPs during the neurogenic phase. In rodents, OLIG2-expressing spinal progenitors from the MP domain are a source of both motoneurons and OPCs [26, 27]. It has been proposed that transplanted OPCs may undergo their last division in order to adapt to a new environment and respond appropriately to environmental cues [28]. The study of Shihabuddin et al. [29] describes that the in vitro-expanded neural progenitors from adult spinal cord (non-neurogenic zone) respond to physiological cues in vivo, by site-specific differentiation, generating proper neurons and/or glial cells depending on the transplantation site. Although we can confirm the commitment of generated OPCs to oligodendroglial fate [13, 16], neuronal cells were also observed in vitro, with 20% of differentiated cells being $TUJ1^+$. We believe that the presence of neuronal progenitors within transplanted OPCs caused significant locomotor recovery of animals. OPCs were also described to generate a paracrine/trophic environment and positively modulate the local immune response as well as promote neuronal protection and activation of endogenous neurogenesis [19, 30] suggesting that regenerative mechanisms do not depend exclusively on a specific cell lineage. It is possible that the fate of OPCs is different when transplanted in contused spinal cords showing a tendency to differentiate toward oligodendrocytes due to the existence of spared host axons promoting remyelination. On the other hand, a more complex environment in complete transection model demands sophisticated regenerative mechanisms to bridge the gap produced by SCI requiring neurons besides the oligodendrocytes.

Despite the critics regarding the lack of reproducibility of the preclinical results in independent laboratories and failure to test large animals, as a scientific requirement prior to its translation to humans [20, 31-33], the first clinical trial was initiated by Geron company using the protocol of Keirstead and collaborators. The trial attempted to test the safety of stem cell therapy in four SCI (ASIA grade-A SCI with neurological level of T3 to T10) patients by using hESC-derived OPCs (known as "GRNOPC1") in order to remyelinate axons within the injured spinal cord. The patients enrolled in the trial received 2 million GRNOPC1 cells within acute phase (7–14 days after SCI) as opposed to chronic phase that is expected to result in insignificant remyelination and poor locomotor improvement, as shown in animal models [13]. Another bias regarding patient selection is that thoracic SCI are more often enrolled in phase I clinical trials for cell transplantation than patients with other SCI types since the cell loss in these patients may not be life threatening as opposed to cervical injury. Immediately after first Geron announcements, this clinical trial suffered criticisms and raised ethical concerns regarding the design and selection of patients for this study [34]. Besides the general concerns related with any hESC-derived cell therapy, Bretzner et al. (2011) [34] point out great discrepancies between the target population of the patients enrolled in the trial, having subacute complete SCI, and animal models used in preclinical studies, contused rats with incomplete injury. The authors also state that these patients may be especially susceptible to "therapeutic misconception," being highly motivated to be cured rather than fully understanding the potential risks and goals of this trial [34], as this trial aims to elucidate the safety concerns, and not to establish stem cell therapy. Furthermore, alternative target populations such as chronic complete SCI patients are suggested, in which assessment of safety is more appropriate as these patients are fully aware of clinical trial purpose and outcomes. Subacute incomplete SCI patients are also candidates since most of the preclinical studies were performed in animal contusion model with incomplete lesion [34]. As a response of Bretzner's critiques, authors strongly defend clinical protocol and patients selected for the study [35]. The two opposing views were reconciliated in the work of Solbakk and Zoloth [36], pointing out that unresolvable ethical and epistemological challenges underlie any type of translational research in human beings [36]. To summarize, these contrasting views demonstrate the lack of well-established standards in translating preclinical data into humans.

A year after initiating the trial, investigators reported "no serious adverse events" and that the four treated patients entered a period of long-term follow-up [http://ir.geron.com/phoenix.zhtml?c = 67323&p = irol-newsArticle&ID = 1635760&highlight =]. According to Geron, the procedure of cell transplantation occurred without surgical complications or adverse events and neurological changes, neither evidence of cavitation nor immune response to injected cells probably due to the administration of low dose of immunosuppressive drug. Unfortunately, in November 2011, the trial was discontinued [http://ir.geron.com/phoenix.zhtml?c = 67323&p = irol-newsArticle&ID = 1635764&highlight]. Geron justified its decision on grounds of "capital scarcity and uncertain economic conditions" disappointing many patients with SCI worldwide. After a huge investment in stem cell therapy and first U.S. Food and Drug Administration approval to test the safety of the stem cell-based product, this decision called into question the effectiveness of the trial among the scientific and patient community. Nevertheless, the scientists are not discouraged by this trial's halting, and due to promising preclinical data and positive preliminary report from another hESC-based trial [37], they believe in the future of hESC-based therapy.

During the decades the spinal cord repair strategies targeted mostly axons and oligodendrocytes while neural population of gray matter (motoneurons and interneurons) was put aside until hESCs were shown to be an efficient source of these cells, capable to restore intraspinal circuitry, and improve functional motor neurons in animals [38, 39]. Various authors have provided more or less efficient protocols for generation of spinal motoneurons from hESCs by applying different cell culture conditions [40–45]. Retinoic acid (RA) is used to instruct embryoid bodies (EBs) toward neuroepithelial cells at the primitive stage to adopt the spinal cord neuronal fate followed by sonic hedgehog treatment to convert them to spinal motoneurons. The low yield of generated MPs [44] and heterogeneity of derived cell population are some of the main obstacles for their clinical use. Singh Roy and colleagues [44] show that the yield can be improved by

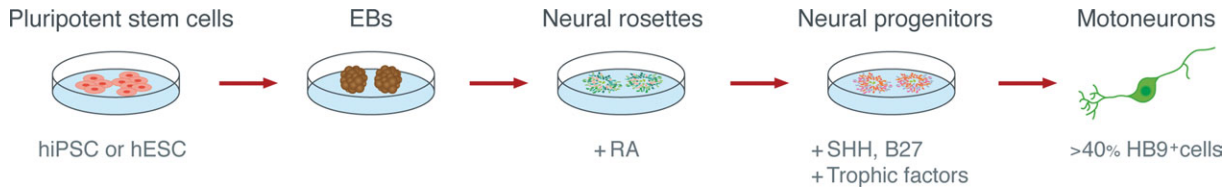

Figure 2. A scheme of the most efficient protocol for motoneuron differentiation from hESC and ihPSC (based on Hu and Zhang [46]). The pluripotent stem cells form EBs in low attachment plate. In adherent conditions, neuroepithelial structures called rosettes are formed. The addition of RA, SHH, and trophic factors results in the motoneuron phenotype (>40% $HB9^+$ cells). Abbreviations: EBs, embryoid bodies; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; RA, retinoic Acid; SHH, sonic hedgehog.

transfecting hESCs with HB9 (motoneuron-specific marker) enhancer coupled with green fluorescent protein for efficient cell sorting of generated motoneurons. Generated motoneurons were functionally active by electrophysiological recording only if cocultured with myocytes. Directed generation and isolation to purity of specific motoneuron phenotypes from hESCs has still to be accomplished. The largest yield ever achieved of generated motoneurons is approximately 50% of total differentiated cells [46] (Fig. 2). In the first preclinical study using MPs, we modified the protocol of Li et al. [47] to assess the regenerative potential of these cells in rat model of completely transected spinal cord [11]. In this article, we showed that MPs were able to mature and develop fundamental functions of normal motoneurons in vitro (expressing OLIG2, ISL1, and HOXC5), including directional growth of long axons, confirming the results from the previous study [47]. The locomotor improvement, shown by Basso, Beattie and Bresnahan (BBB) score of MP treated rats, was higher than in the controls, and immunohistochemistry analysis confirmed that hESC-derived MPs survive, migrate, and engraft for at least 120 days in the lesion site [11]. These data suggest that the application of in vitro preconditioning may allow efficient generation of new neurons in non-neurogenic regions as is the SCI site. Interestingly, the immunohistochemistry analysis showed clear evidence that these progenitors have the capacity to finally differentiate to both mature oligodendrocytes and neurons in the lesion site. Using this strategy, we did not observe any formation of anatomically, physiologically, and functionally active motor units between transplanted axons and host muscles, but the fact that these cells innervate the lesion site filling the gap between the rostral and caudal stumps as well as significantly improved locomotor function of lesioned rats suggests that hESC-derived MP have regenerative potential [11].

Transplantation of hESC-derived neural progenitor cells with scaffolds made of three-dimensional biomaterials such as laminin, fibronectin, or collagen [48, 49] could present an advantage because they provide an adhesive support and may serve to deliver growth factors. This strategy has been used in a recent study where hESC-derived neural progenitors were transplanted into a rat model of SCI using collagen scaffolds [50].

iPSCs

The use of hESCs remains controversial facing major obstacles such as ethical issues, low engraftment rates, immune rejection, and tumorigenicity, which have impeded efforts toward clinical translation. The solution to the ethical concerns was offered by the discovery of hiPSCs, which can be derived from adult somatic cells by ectopic expression of a defined set of factors [51] (Fig. 1). Human iPSCs are derived from individual patients, making it possible to develop customized stem cell therapies, generate disease-specific stem cell lines, and even perform gene correction [52]. hiPSCs are capable of differentiating toward all cell types, including neurons, glia, neural progenitor cells (NPCs), and motoneurons for different purposes [53–56]. The recently developed disease-specific hiPSCs from the patients with Rett syndrome [57], spinal muscular atrophy [58], Huntington disease [59], Friedrich ataxia [60], Parkinson disease [61], or amyotrophic lateral sclerosis [53], as human cell models will help understand many neurodegenerative and neurodevelopmental disorders providing unique in vitro model of human disease and development.

Initially, the main concern for using these cells in clinics was reprogramming technology that involved viral vectors. The development of nonviral approach such as mRNA [62] or chemicals and small molecules [63] makes this cell source very attractive. The initial enthusiasm about the expected hiPSCs' immunotolerance in donors was put in question by work of Zhao et al. [64]. The authors showed that some mouse iPSCs (miPSCs) induced immune response, influenced by reprogramming method, in syngenic mice. The immature miPSCs, used in this study, are not to be applied for transplantation, and further investigations have to answer whether tumor antigens continue to be expressed in differentiated cells [65]. The distinguishable features of hiPSCs are different gene expression, histone methylation as well as the epigenetic memory from the source tissue [66, 67]. hiPSCs are thought to be more tumorigenic than hESCs due to genetic and epigenetic aberrations [68]. To address this issue, Tsuji et al. [69] proposed that each iPSC line has to be pre-evaluated to assess the teratoma formation after cell transplantation in animal models as different iPSC lines vary in differentiation capacity and teratoma formation. This study shows the functional recovery in contused rats grafted with miPSC-derived neurospheres by three possible mechanisms: remyelination by miPSC-derived oligodendrocytes, axonal regrowth, and trophic support. In another study, the regenerative potential of pre-evaluated hiPSCs was confirmed in the same model [70]. Transplanted cells survived, migrated, and differentiated toward all neural cells (astrocytes, neurons, and oligodendrocytes) [70], thus validating hiPSCs as neural cells source in a preclinical study and representing an important step toward clinical practice. Recently, Fujimoto et al. [71] showed that hiPSC-derived neuroepithelial-like cells could be an efficient cell source for treatment of SCI opening the way to another clinical study.

Conclusions

Since 1998, when first hESC line was generated, there is a latent skepticism about their use in clinical practice. The first human clinical trial involving hESC-derived GRNOPC1 injected in patients showed that these cells do not cause any harm, but the debate about these cells is still alive. The

investigators and bioethicians express several concerns regarding the safety of hESC transplantation in SCI in humans: (a) the absence of replication of preclinical results in larger animals (primates), evolutionally and anatomically closer to the humans [32, 33], (b) the possibility of teratoma formation following hESC-derived neural cell engraftment due to the hypothetical presence of undifferentiated hESCs, which could be harmful in humans [72, 73], (c) presence of undifferentiated hESCs as well as other type of differentiated cells could trigger neurological disfunctions in humans causing aberrant axonal sprouting [34], (d) direct competition of transplanted cells with endogenous progenitor-derived cells [34] questioning the contribution of spontaneous differentiation [34], (e) the use of differentiation protocols that still involve mediums, growth factors, and supplements of animal origin [15], (f) immune rejection of transplanted cell, and (g) ethical concern. Extensive research efforts are needed in order to resolve these complex issues and develop a safe cell therapy practice.

The use of hiPSCs and their derivates in a future treatment of SCI circumvents the ethical concerns but shares the same safety concerns as hESCs. hiPSCs face another challenge regarding their use in clinical practice such as reprogramming mechanism that requires additional basic investigation. A novel strategy to generate differentiated cells was offered by Vierbuchen et al. [74], who combined three well-known transcription factors: Ascl1, Brn2, and Mytl1 (or Zic1), in order to directly convert mouse embryonic and postnatal fibroblasts into functional neurons called induced neuronal (iN) cells, without passing through pluripotent stem cell stage. Although this study remains to be profounded in many aspects such as stability of iN cells, viability, gene expression, or in vivo survival [75], it represents an interesting approach for the treatment of SCI. In situ targeted reprogramming of non-neural cells of injured spinal cord into desired neural cells would mark a new era in cell-based therapy to treat the lesion.

Recent improvements in stem cell therapy are encouraging, however, the effect of the grafted cells on local tissue and endogenous neural stem cells and the mechanism of functional recovery are poorly understood. While survival and differentiation of the transplanted cells are well-demonstrated events, remyelination and glial cell replacement [13] still lack direct evidence as regenerative mechanisms. Rigorous mechanistic studies need to be performed in order to elucidate signaling pathways ongoing in the lesion in addition to those activated by transplanted cells. Any improvement in the basic knowledge of the ongoing events will lead to increased reproducibility and safety of cell therapy in humans. Based on the above observations, the ideal source of stem cells for efficient and safe cell replacement remains a challenge requiring further investigation.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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