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Concise Review: Laying the Groundwork for a First-In-Human Study of an Induced Pluripotent Stem Cell-Based Intervention for Spinal Cord Injury

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ABSTRACT

There have been numerous attempts to develop stem cell transplantation approaches to promote the regeneration of spinal cord injury (SCI). Our multicenter team is currently planning to launch a first-in-human clinical study of an induced pluripotent stem cell (iPSC)-based cell transplant intervention for subacute SCI. This trial was conducted as class I regenerative medicine protocol as provided for under Japan's Act on the Safety of Regenerative Medicine, using neural stem/progenitor cells derived from a clinical-grade, integration-free human "iPSC stock" generated by the Kyoto University Center for iPS Cell Research and Application. In the present article, we describe how we are preparing to initiate this clinical study, including addressing the issues of safety and tumorigenesis as well as practical problems that must be overcome to enable the development of therapeutic interventions for patients with chronic SCI. STEM CELLS 2019;37:6–13

SIGNIFICANCE STATEMENT

There is an increasing interest in the regenerative medicine using induced pluripotent stem cells (iPSCs). This article reviews the process in preparation for the first human trial of iPSC-based cell therapy for spinal cord injury, including addressing the issues of safety and tumorigenesis. Practical problems, which must be overcome to enable the development of therapeutic interventions for patients with chronic spinal cord injury, are described.

INTRODUCTION

Spinal cord injury (SCI) results in severe neurological dysfunction, including motor, sensory, and autonomic paralysis. The annual incidence of SCI in Japan is approximately 5,000, and the prevalence of chronic SCI is approximately 150,000 [1]. This is consistent with a report that the annual incidence of SCI worldwide varies from 8.0 to 246.0 cases per million [2]. In current practice, treatments for acute SCI are limited to stabilization of the injured spine and decompression of the injured spinal cord in the acute stage, followed by rehabilitation to restore physical function and activity to the extent possible. No therapeutic approach has been established for regenerating injured spinal cord. However, the most recent guidelines suggested that blood pressure augmentation and high-dose steroids within 8 hours of injury would be treatment options for SCI [3].

In recent years, there have been many attempts to develop cell transplantation or drug therapies to promote regeneration of the damaged spinal cord. There are two major principal currents of research in regenerative medicine for SCI. The first involves stem cell-based interventions using neural stem/progenitor cells (NS/PCs) derived from fetal tissues [4], embryonic stem cells (ESCs) [5], Schwann cells [6], mesenchymal stem cells [7], olfactory ensheathing cells [8], or induced pluripotent stem cells (iPSCs) [9–11]. Recently, direct reprogramming techniques have also attracted significant attention as a source of NS/PCs [12–14]. The second focus is on the use of neurotrophic factors, such as hepatocyte growth factor [15], IL-10 [16], granulocyte colony stimulating factor [17], and reagents against axon growth inhibitors typified by Semaphorin 3A [18], Nogo [19], Rho [20], and chondroitin

sulfate proteoglycans [21]. More effective treatments for SCI may be achievable through a combinatorial application involving both cells and neurotrophic factors.

Although the majority of SCI patients are in the chronic phase, most therapeutically focused studies to date have focused on the acute or subacute phase. Several recent reports showed that stem cell transplantation alone does not yield positive effects in chronic SCI models [22, 23], an issue that must be addressed in future studies. The establishment of effective treatments for chronic SCI patients with decreased therapeutic reactivity thus remains an urgent issue [24].

We have been conducting preclinical research to establish an iPSC-based approach to spinal regenerative medicine. The reason we chose to use iPSC is largely influenced by the current Japanese regulatory framework as well as a number of ethical considerations. Clinical trials using cells from other sources for treatment of SCI have already been started in the U.S., E.U., Pakistan, People's Republic of China, Korea, and elsewhere [25–30]. In these clinical trials, the safety and effectiveness of cells such as fetal-derived NS/PCs, ESC-derived NS/PCs, autologous mesenchymal stromal cells, umbilical cord blood mononuclear cells are being examined. However, clinical trials using iPSCs targeting SCI have not yet been conducted at this point.

In this review, we outline the preparations for first-in-human trials of iPSCs-based cell transplant interventions for subacute SCI under the new Japanese legal system for regenerative medicine and prospects for the development of new interventions for chronic SCI.

iPSCs-Based Cell Therapy and Japan's New Law Systems on Regenerative Medicine

In 2006, it was discovered that mature somatic cells can be reprogrammed to a pluripotent state by gene transfer, generating iPSCs [31]. These cells exhibit pluripotent stemness similar to that of ESCs, and were first established by introducing four reprogramming factors (Sox2, Oct3/4, c-Myc, and Klf4) into somatic cells. Another attractive feature of iPSCs is that they can be derived from a patient's own somatic cells; a clinical research study of autologous iPSC-derived retinal pigment epithelium (RPE) sheet transplantation for exudative age-related macular degeneration patient was initiated in September 2014 by Masayo Takahashi's group in Kobe, Japan, representing the first clinical study involving an iPSC-derived product. The engrafted iPSC-RPE sheet showed no sign of immune rejection for the 1-year monitoring period following transplantation [32].

However, the costs of quality testing and safety concerns for autologous cell transplants have led to increased interest in allogeneic strategies. In an effort to mitigate these practical problems, Japan has launched a program to develop an "iPSC stock" derived from "human leukocyte antigen (HLA) super-donors," who are homozygous at the three major HLA gene loci, to maintain a pool of safe iPSC clones corresponding to various HLA types. This program is performed mainly by Center for iPS Cell Research and Application (CiRA) at Kyoto University, which contributed greatly to the development of iPSC technology. For example, frequencies of

HLA-A*24:02; HLA-B*52:01; HLA-DRB1*15:02 haplotypes in the Japanese population are reported to be the most frequent (8.5%), and iPSCs established from the individuals who are homozygous for this haplotype are expected to be immunologically matched to approximately 17% of the Japanese population. It has been estimated that an iPSC bank comprised of ∼140 unique HLA-homozygous donors would cover ∼90% of the Japanese population [33]. Globally, an iPSC bank comprising 100 iPSC lines representing the most frequent HLA types from each major ethnic background would cover 78% of U.S. residents of European heritage, 63% of Asian, 52% of Hispanic, and 45% of African American [34]. Masayo Taka shashi's group recently transplanted an allogeneic cell suspension of iPSC-RPE derived from a super-donor carrying the most common HLA haplotype in Japan, which matched the recipient's HLA type, following in vivo animal studies in which macaque monkey iPSC-RPE cells derived from Major Histocompatibility Complex (MHC) homozygote iPSC lines were successfully transplanted into the eyes of MHC-matched heterozygote donors without immunosuppression [35].

Other research groups plan to use allogenic and HLA-unmatched iPSCs as donors of transplantation. The Australian company Cynata Therapeutics plans to study potential clinical uses of iPSC-derived mesenchymal stromal cells (MSCs) generated from healthy donors as immune suppressor cells in graft-versus-host disease patients in the UK and other countries (http://cynata.com/). Curtis et al. transplanted unmatched fetus-derived NS/PCs into chronic SCI patients (*n* = 4) and reported that no rejection reaction was observed, even after the termination of a temporary immunosuppressant [25]. Recently, the Japanese government health ministry approved a plan proposed by Yoshiki Sawa's group at Osaka University group to begin a pilot study of iPSC-derived cardiomyocyte cell sheet transplantation in heart failure patients; this study will use allogenic iPSCs from the CiRA cell bank. Our own group is currently proposing the first human trial of allogenic iPSC-based cell transplantation for subacute SCI, also using CiRA-derived iPSC. If approved, this study was conducted under the terms set forth in the Act on the Safety of Regenerative Medicine (ASRM).

In 2014, Japan introduced two legal reforms; the ASRM and a set of amendments to the Pharmaceuticals, Medical Devices and Other Therapeutic Products Act (PMD Act). The PMD Act governs the review and approval of "regenerative medical products" intended for commercial distribution, although acknowledging the heterogeneity of cells used in medical products [36]. Notably, the PMD Act introduced a new pathway for conditional and time-limited approval of regenerative medical products.

In contrast, the ASRM governs the development and use of regenerative medicine in both noncommercialized academic clinical studies and private medical practices operating outside the national health insurance system. It adopts a risk-based approach to strengthen safety oversight [37]. The Act classifies regenerative medicine in three categories: class I (high risk); class II (medium risk); and class III (low risk) [36, 37]). iPSC-based cell transplantation falls in the high-risk group (class I), along with approaches using ESCs, transgenic or genetically modified cells, xenogeneic cells, and allogeneic cells [36]. Under ASRM, any medical institution that plans to conduct a

clinical study of or offer iPSC-based cell therapies must undergo review by a Certified Special Committee for Regenerative Medicine (CSCRM) as class I regenerative medicine techniques [37].

PREPARING THE FIRST HUMAN TRIAL OF IPSCS-BASED CELL THERAPY FOR SCI OF SUBACUTE PHASE

To date our group has focused on applications of NS/PCs and reported positive therapeutic effects from the use of rodent (rat/mouse) fetus-derived NS/PCs for mouse/rat injured spinal cord (contusion injury model) at the subacute phase [38, 39]. We additionally transplanted human fetal-derived NS/PCs into nonhuman primate (common marmoset) injured spinal cord, and observed positive effects on motor function recovery [4]. Likewise, significant motor function recovery was reported for NS/PCs derived from mouse ESCs in a mouse SCI model at the subacute phase [40]. However, Japanese governmental guidelines over stem cell research and development that were in effect from 2006 to 2014 prevented us from initiating clinical research using fetus- or ESC-derived NS/PCs, as such studies would involve harvesting cells from aborted fetuses or surplus embryos from in vitro fertilization attempts. iPSCs, which were established in 2006 [31], have made it possible to avoid some of the ethical and regulatory issues surrounding the use of ESC- or fetus-derived cells.

Together with the invention of the iPSC-technology, the above-mentioned restricted situation led us to start the research into clinical application of iPSC-derived NS/PCs (iPSC-NS/PCs)-transplantation for SCI in collaboration with Kyoto University since 2006. As a first step, we developed a method for preparing NS/PCs from mouse [41] and human iPSCs [10], and determined that transplantation of mouse iPSC-NS/PCs into a mouse SCI model and of human iPSC-NS/PCs into an immune-deficient mouse SCI model at the subacute phase promotes motor function recovery and improves motor evoked potential [9, 10]. Moreover, we transplanted human iPSC-NS/PCs into a common marmoset SCI model [4] and found that human iPSC-NC/PCs are able to differentiate into neural trilineage cells (neurons: 52%; astrocytes: 31%; and oligodendrocytes: 27%), form synaptic connections with host neurons, reduce post-SCI demyelination (preservation of 1.5–2 times larger myelinated areas), and consequently promote better motor function recovery [11]. Several mechanisms may support this functional recovery after stem cell-derived NS/PCs transplantation, including (a) creating a permissive substrate for axonal growth; (b) providing cells that remyelinate spared but demyelinated axons; (c) supplying trophic support reducing the damage and rescuing neurons and oligodendrocytes; and (d) enhancing axonal plasticity and replacing lost neurons to reconstruct local circuitry [42–44]. These iPSC-NS/PCs transplantation experiments were conducted in models at the subacute phase of SCI, not at the chronic phase (Fig. 1).

At the same time, we discovered that there are "safe" and "dangerous" human iPSC clones, and that transplantation of "dangerous" human iPSC-clone-derived NS/PCs resulted in neural tumor-like proliferation of transplants by 103 days postinjury (dpi) [47]. The proliferated tissue was substantially different from teratoma (a form of benign tumor that results

from contamination of undifferentiated human iPSCs), and consisted of immature neural cells exhibiting differentiation-resistance. The following analysis revealed that such "dangerous human iPSC-NSPCs" can result from genetic instabilities, such as transgene activation [47], epigenetic events [48], or other genetic modifications such as abnormal karyotype or copy number variations (CNVs) [49] that occur or increase over the course of the culture process. Pre-evaluation of iPSCs and iPSC-NSPCs before transplantation is thus essential for the detection of "dangerous" iPSC-NS/PCs, as is the establishment of fail-safe systems after human iPSC-NS/PC transplantation.

There are five key issues in improving the safety of human iPSC-NS/PCs: (a) preventing the use of genetically unstable human iPSCs; (b) preventing contamination by undifferentiated pluripotent cells in iPSC-NS/PCs; (c) preventing the transformation of progenitor cells into "dangerous iPSC-NS/PCs;" (d) minimizing the risk of proliferation of differentiation-resistant "dangerous iPSC-NS/PCs" in vivo; and (5) removal/ablation of cells after the transplant. In (a)–(c), quality monitoring of iPSC clones and derivative iPSC-NS/PCs in vitro is indispensable. in vitro quality checks of iPSCs require evaluation of the completeness of reprograming status (surface markers, capacity of cellular differentiation, and so on) and genetic stability (karyotypes, CNVs, and so on).

Additionally, to prepare clinical grade human iPSC-NS/PCs, it is important to establish a procedure for obtaining NS/PCs from feeder-free human iPSCs using a xeno-free medium [50]. The evaluation of each iPSC clone-derived NS/PCs in vitro must include confirmation of completeness of the cellular induction process, by using the target NS/PC markers (e.g., SOX1 and PSA-NCAM) and pluripotency markers for the original iPSCs (e.g., OCT3/4 and TRA1-60), preferably by more than two methods, such as fluorescence activated cell sorting (using surface markers), sequencing of RNAs, and immunocytochemistry of intracellular phenotypic markers. The function of induced cells (cellular shape, cell cycle status, proliferation speed, survival rate, and so on), genomic information (karyotype, gene sequences, CNVs, and so on), and the cellular terminal differentiation capacity should also be evaluated. Additionally, since DNA methylation status is known to change over the culture period, the number of days required for the culture process and the DNA methylation status of the final iPSC-NS/PCs should also be monitored [48].

To minimize the risk of proliferation of differentiation-resistant "dangerous iPSC-NSPCs" in vivo (the fourth issue mentioned above), we focused on gamma secretase inhibitor (GSI), which inhibits Notch signaling, which is crucial for maintaining the undifferentiated state. We found that pretreatment of iPSC-NS/PCs with GSI before transplantation promoted neuronal differentiation and prevented tumor formation, even for "dangerous" clone-derived NS/PCs [51, 52].

As the last issue to enhance cell safety, the simplest approach for removal of cells is surgical resection of the transplant. However, in cases in which the transplanted cells invade or metastasize to other sites, the effectiveness of surgical approaches may be limited. Thus, other approaches, including the use of immune-rejection [53], or the use of a "suicide transgene" [54] may be needed for human clinical studies in the future. However, the introduction of a suicide gene system in the clinical setting would involve heightened oversight, since
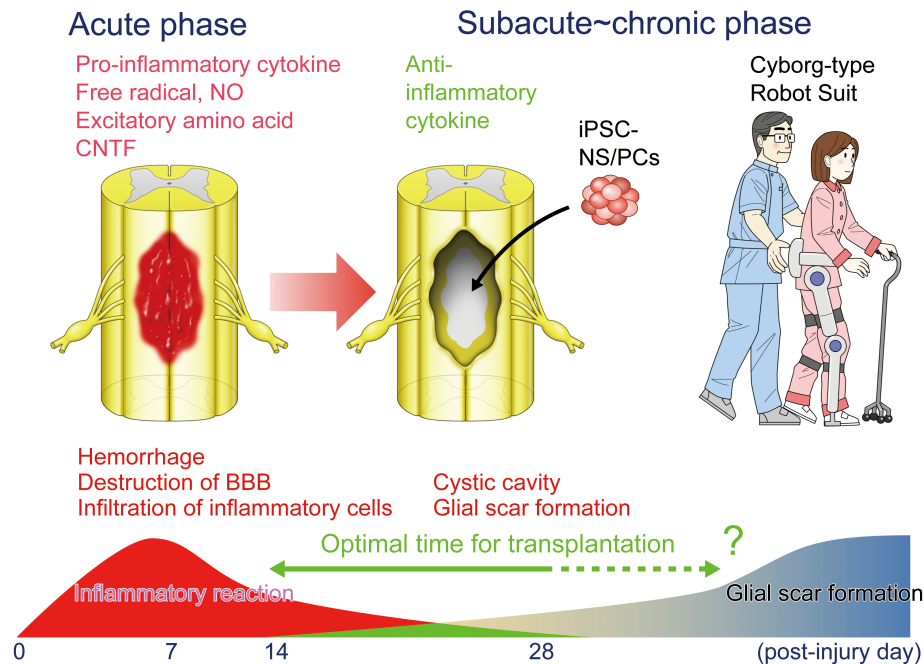
Figure 1. Therapeutic strategy for spinal cord injury (SCI) based on changes in the microenvironment. The microenvironment within SCI changes over the time course following the primary mechanical trauma resulting in the SCI (modified from [45]). Analysis of this time course suggests that 14–28 days postinjury, that is, the subacute phase, is the most appropriate for stem cell transplantation in human patients. It has been reported that neural stem/progenitor cell (NS/PC) transplantation alone is not sufficient to induce significant functional recovery in chronic SCI. However, given the synergistic effects of rehabilitation and NS/PC transplantation in chronic SCI model mice [46], a combination intervention involving transplantation of induced pluripotent stem cell-NS/PCs and use of a robotic device are being planned for future clinical studies.

this would require viral (AAV or lentivirus) transduction of the gene into the graft.

In vivo analyses are also essential in preclinical safety testing of iPSC-based cell interventions for SCI. For this, we adopted two methods: transplanting the cells into intact striata of immunodeficient mice, or transplanting the cells into the injured spinal cords of immunodeficient mice [49]. By comparing histology 12–26 weeks after the injection of iPSC-NSPCs, we confirmed that both methods produce equivalent results. Use of intact striata is technically easier, and the mice survive longer, which makes it easier to study long-term safety. On the other hand, the use of injured mice has the advantage of being more similar to the state of human patients. However, the injured spinal cord in mouse is quite different in terms of graft volume, that is, the estimated maximum cell number that can be transplanted into the injured cord was less than that of the brain (striata). Moreover, in both model cases, it is extremely difficult to obtain longer survival (over 1 year) for immune-deficient mice. The method will thus need to continue to be optimized in the future.

Although recently the generation time of autologous human iPSC-NS/PCs has been drastically reduced in the research setting [55, 56], the establishment of autologous human iPSC-NS/PCs takes at least 6 months, and several additional months are needed for safety testing. If quality testing were to be performed for all SCI patient-derived autologous iPSCs, the costs would be extremely high. Thus the issues of time and expense represent significant obstacles to autologous transplantation of iPSC-NS/PCs to SCI patients in the subacute phase [57]. Given these issues, in our planned first-in-human

clinical study, we opted to use clinical-grade allogenic iPSC clones produced by CiRA, which have already passed through quality and safety tests. Based on our series of investigations on the safety issues, we developed the draft of standard operating procedures to prepare iPSC-NS/PCs for use in the proposed clinical study, in which cells were transplanted to SCI patients (Fig. 2). These plans are currently under evaluation as a class I regenerative medicine protocol by a CSCRM, pursuant to the ASRM.

We believe that our proposed clinical trial program must meet higher safety standards than interventions using cells from other sources, due to the gene transfer process used in generating iPSCs and the long culture period for differentiation. However, even allowing for these heightened safety considerations, the use of iPSC-derived cells remains attractive for several reasons, including their pluripotency, lower ethical tensions, clearer regulatory provisions, and in the case of autologous iPSCs, the reduced risk of immune rejection [58]. Indeed, iPSCs are used in spinal cord regeneration studies worldwide. Lu et al. reported that iPSC-derived NS/PCs exhibit remarkable axonal outgrowth in the injured spinal cord, even when the cells are derived from an elderly person [59]. Ruzicka et al. reported the superiority of the therapeutic effects of iPSC derived neural progenitors compared with bone marrow-derived MSCs and fetus-derived neural progenitors in a rat SCI model [60]. Strnadel et al. reported that transient immunosuppression was sufficient following transplantation of allogenic iPSC-NS/PC in a porcine model [61]. Fujimoto et al. generated human iPSC-derived long-term self-renewing neuroepithelial-like stem cells, and reported the effectiveness of these cells in
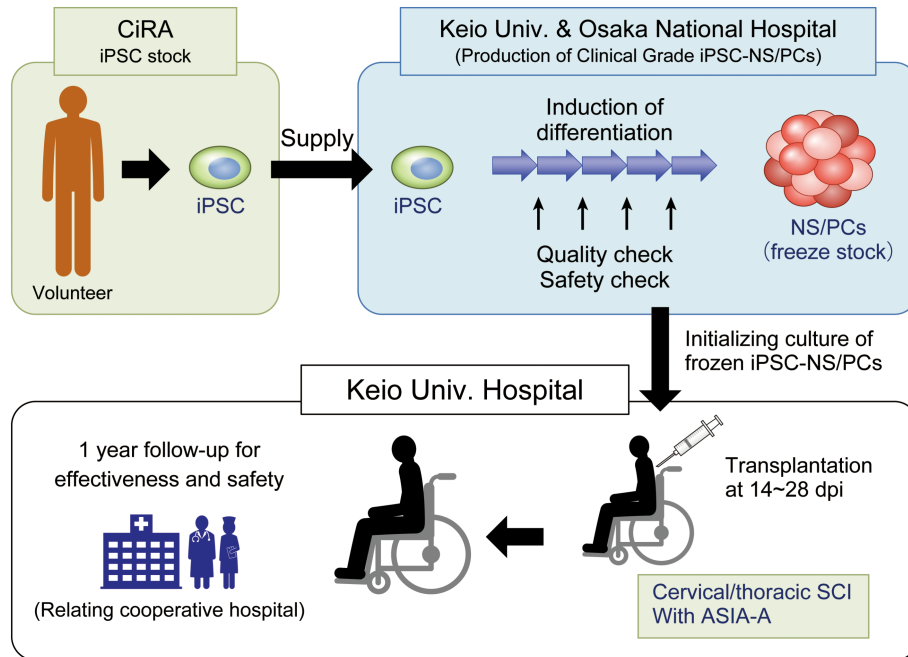
Figure 2. Overview of induced pluripotent stem cell-neural stem/progenitor cell (iPSCs-NS/PCs) "first-in-man" clinical trial. The iPSCs-NS/PCs "first-in-man" clinical trial for SCI patients at subacute phase are being planned using the clinical grade iPSCs-NS/PCs generated at Keio University School of Medicine and Osaka National Hospital, National Hospital Organization from human "iPSC stock" prepared in CiRA [37, 57]. The clinical grade iPSC-NS/PCs were kept at frozen state until a few days before the surgery. iPSCs-NS/PCs were transplanted into injured spinal cords of the patients of subacute phase (14–28 days postinjury). After the transplantation, patients were followed-up in terms of safety.

a subacute contusive SCI model in immunodeficient mice [62]. All these reports support the utility of iPSCs as a cell source for studies of cell transplantation in SCI.

CELL TRANSPLANTATION THERAPY AND REHABILITATION FOR CHRONIC SCI

The development of an effective therapy for chronic SCI is a key goal in SCI research. Many groups have conducted studies aimed at uses of cell transplantation, including iPSCs-derived NS/PCs for chronic SCI. Recently, the first human trial for chronic SCI (n = 4) using fetal NS/PCs was reported [25], which primarily focused on transplantation safety. However, previous studies have indicated that functional recovery by cell transplantation alone is not clear for SCI at chronic stage. For example, Kumamaru et al. used whole transcriptome analyses to show that, although grafted NS/PCs produce many regenerative/neurotrophic molecules and the chronically injured spinal cord exhibits the potential to permit NS/PCs to differentiate into neurons and oligodendrocytes, NS/PCs did not improve locomotor function in the chronic stage. In this regard, the authors highlight the importance of environmental modulation [22]. Consistent to Kumamaru's report, our group also conducted similar experiments and reported that there was no functional recovery in the chronic phase (42 dpi), whereas there was no significant difference in the transplants' viability or differentiation rate between the subacute (9 dpi) and chronic phase (42 dpi) [23]. We speculated that within the damaged spinal cord microenvironment, the glial scar formation and inflammatory phenotype may be the most efficient targets to

modify to achieve functional recovery by NS/PC transplantation at the chronic phase.

To gain more insight into chronic contuse SCI, we referred to analyses of transplantation of Neurotrophin-3 (NT-3)-expressing rat fetus-derived NS/PCs into the contusive injured spinal cord of rat at the chronic phase. Although there was no improvement in motor function in transplantation of normal NS/PCs, NT-3-expressing NS/PCs enhanced motor function recovery and remyelination in a rat thoracic SCI model, even at the chronic stage (42 dpi; Basso, Beattie and Bresnahan scale (BBB) subscore at 84 dpi showed significant recovery) [63]. It has been reported that NT-3 expression is significantly increased in the injured spinal cord (rat hemisection model) at early-chronic phase (28 dpi) following walking training [64]. We suggested that NS/PC-transplantation combined with walking training might produce synergistic effects on motor function recovery in chronic SCI. However, there are very few reports on the combination of NS/PCs-transplantation and rehabilitation. There have, however, been several reports on combination use of rehabilitation and cell transplantation other than NS/PCs. In 2011, Takeoka et al. reported the effects of combination of rehabilitation with olfactory ensheathing glia (OEG) grafts. They transplanted OEGs immediately after transection injury of rat thoracic spinal cord and started treadmill bipedal exercise with partial weight support, and found that this promoted a fourfold increase in regenerating axons within the caudal stump of transected spinal cords, and consequently improved hindlimb function and electrophysiological states [65]. Sun et al. transplanted both OEGs and Schwann cells into a moderate contusion-injured rat spinal cord at 14 dpi, and

reported that, in combination with treadmill training, this contributed to enhanced motor function recovery (BBB score: 13) compared with the controls (BBB score: 10) [66]. In a study of combined NS/PCs-transplantation and rehabilitation, Hwang et al. transplanted NS/PCs into contused rat spinal cord at 7 days after injury, followed by treadmill training with partial weight support for 8 weeks. The combination approach promoted motor function recovery (BBB score: 16) compared with the control (BBB score: 9), enhanced tissue protection, and preserved more myelinated area. The grafted NS/PCs differentiated into more neurons and oligodendrocytes (30% βIII tubulin$^+$ neurons and 25% CC1$^+$ oligodendrocytes) compared with the control group (20% neurons and oligodendrocytes), and the number of Nestin$^+$ undifferentiated cells decreased (control: 25% and treadmill: 15%) at 9 weeks postinjury, which the authors attributed to the reduction of stress caused by active oxygen or active nitrogen through IGF-1 signaling [67].

Thus, our understanding of the synergistic effects of cell transplantation combined with rehabilitation at the chronic phase continues to expand. However, the pathology of chronic SCI remains to be elucidated. We analyzed the mechanisms underlying spasticity, which is a major problem in chronic SCI patients, and found that in a contuse SCI rat model, the expression of BDNF was increased at the lumbar enlargement of spinal cord after treadmill training, resulting in increased expression of KCC2 and suppression of spasticity [67]. We also studied the combination of treadmill training and transplantation of mouse fetus-NS/PCs for mice thoracic cord contusion injury from 7 weeks after injury (chronic stage), and found improved hind limb motor function in the combined group (NS/PCs + treadmill). This superior functional recovery, even at the chronic stage, was enabled by the combined effects of the improvement of injured spinal cord electrical conduction, newly developed fibers and synapses within the central pattern generator at the lumbar enlargement, and appropriate motor control [46]. In a study of treadmill training in chronic SCI, we confirmed improvement of hind limb motor function by concurrent use of chondroitinase ABC (C-ABC) via intrathecal continuous administration (BBB score: 6 at 14 weeks postinjury, compared with the control BBB score: 3) for severe rat thoracic chronic contusion injury model. The mechanism of functional recovery in this system was attributed to an increase in serotonergic nerve fibers at the lesion site [68].

CONCLUSION

In this review, we described the preparation for first-in-human transplantation of iPSC-NS/PCs for subacute-complete SCI patients. After confirmed safety and further efficacy of this treatment, there would still be a long way to put this technology into practical use in general. Meanwhile, there are various situations such as incomplete injury or transection injury in the state of spinal cord injury, and some additional approach seems to be necessary to treat them. Further development of future treatment is expected.

FUTURE PROSPECTS AND ASSIGNMENTS

In this review, we have outlined studies on the pathology and treatment of subacute to chronic SCI. Overcoming chronic SCI remains the most formidable outstanding challenge for SCI researchers. Several important findings on stem cell transplantation and medication approaches for chronic SCI have been reported in recent years, but none promises to fully restore function. The trinity of stem cell transplantation, medication, and rehabilitation thus appears to be the way forward. We need to aim at the most effective reconstruction of organized neural circuits by iPSCs-based cell therapy after chemical suppression of axon growth inhibitory factors at the lesion site, although pursuing further rehabilitation, including future applications such as robotic engineering, functional electrical stimulation, and their combination.

AUTHOR CONTRIBUTIONS

O.T., K.S., H.O.: manuscript writing; M.N., H.O.: administrative support; O.T., N.N., J.K., R.Y., K.S., T.I., T.O., M.I., T.S., K.F., S.T., N.N., M.S., K.F., K.Y., and S.Y.: played major roles in the designing and conducting a wide range of studies needed to justify a first-in-human of iPSCs-based cell therapy for SCI. All of the authors have approved of the final manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

H.O. receives financial compensation as a founding scientist of SanBio Co., Ltd. and K-Pharma, Inc. M.N. is a founding scientist of K-Pharma, Inc. R.Y. and M.I. declared leadership position with Sumitomo Dainippon Pharma Co., Ltd. S.Y. declared consulting, research funding from iPS Academia Japan. The other authors indicated no potential conflicts of interest.

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