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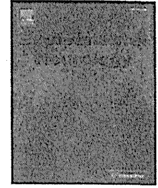

Review

Regeneration of the damaged central nervous system through reprogramming technology: Basic concepts and potential application for cell replacement therapy

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ABSTRACT

Neural stem cell (NSC) transplantation provides a new approach for the repair of damage to the central nervous system (CNS), including that resulting from cerebral infarction and spinal cord injury (SCI). In the past, there were no reputable means of converting non-neural somatic cells into neural cells. This status was overturned by the establishment of induced pluripotent stem (iPS) cells, which have pluripotency akin to that of embryonic stem (ES) cells and can differentiate into most cells of the three germ layers. If differentiated somatic cells could be reprogrammed into iPS cells, and if these iPS cells could be induced to differentiate once again, it would be theoretically possible to obtain a large number of neural cells. However, this is not yet feasible due to the limitations of existing stem cell technology. Induction of neural cells from iPS cells is currently hindered by two distinct problems: 1) the preparation of specific types of targeted neural cells requires extensive cell culture, and 2) tumors are likely to form due to the presence of residual undifferentiated cells following transplantation of the induced cells. By contrast, direct induction methods permit the generation of target cells from somatic cells without the transitional iPS cell stage. This review outlines the present-day status of research surrounding the direct induction of NSCs from somatic cells, as well as the perspectives for the future clinical application of this technique for cell replacement therapy following CNS injury.

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Introduction

It is well-known that the mature human central nervous system (CNS) shows little potential for regeneration (CNS). In contrast to individuals with bone fractures, where the pre-fracture state is often restored by appropriate treatment, patients rarely achieve a full recovery after CNS injury resulting from trauma or neurodegeneration. In fact, patients with spinal cord injury (SCI), cerebral infarction or neurodegenerative diseases are likely to suffer from the pathological sequelae for the rest of their lives.

Cell replacement therapy involves the transplantation of neural stem cells (NSCs) and is a promising regenerative strategy for the repair of CNS damage. A number of studies in mice, rats and other animal models report that NSC-transplantation can result in the recovery of function from neurological disorders that are conventionally difficult to treat,

Abbreviations: cAMP, cyclic AMP; C/EBP, CCAAT-enhancer-binding protein; CNS, central nervous system; EB, embryoid body; EGF, epidermal growth factor; ES cell, embryonic stem cell; FGF-2, fibroblast growth factor-2; GSK, glycogen synthase kinase; iNSC, induced neural stem cell; iPS cell, induced pluripotent stem cell; LIF, leukemia inhibitory factor; NSC, neural stem cell; SCI, spinal cord injury; TGF, transforming growth factor.

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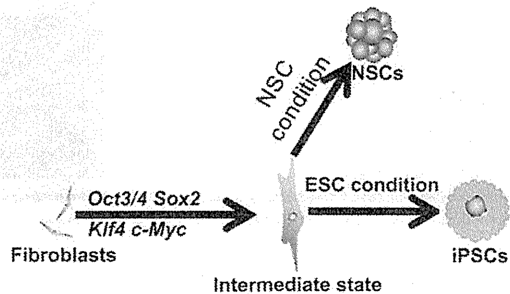

Fig. 1. Schematic diagram showing direct vs. indirect induction of NSCs. The direct conversion of fibroblasts into NSCs requires a three-fold shorter period of culture to obtain mature NSCs with gliogenic competency relative to the indirect induction of NSCs from iPS cells. These NSCs can be maintained by passage for more than 1 year.

such as SCI (Ogawa et al., 2002), cerebral infarction (Oki et al., 2012), amyotrophic lateral sclerosis (Boulis et al., 2011) and Alzheimer's disease (Blurton-Jones et al., 2009). The clinical application of transplanted fetal brain-derived or embryonic stem (ES) cell-derived NSCs in humans engenders problems such as immunological rejection and ethical considerations; however, induced pluripotent stem (iPS) cell technology is expected to overcome these complications (Takahashi et al., 2007). This technology allows the preparation of NSCs from iPS cells derived from the patient's own somatic cells for subsequent autografting. In addition to iPS technology, there is an increasing interest in the direct induction of NSCs from somatic cells for potential autografting therapy following SCI (Fig. 1). Recent findings and perspectives for the use of cell replacement therapy to repair CNS damage, with a particular emphasis on the spinal cord, will be discussed in this review article.

iPS cell technology and cell replacement therapy for SCI

iPS cell technology is anticipated to allow the acquisition of cells from a mature somatic origin with pluripotency similar to ES cells. Multiple systems for inducing ES cell-derived populations associated with each germ layer have already been established, and the use of these systems is expected to similarly enable the induction of NSCs from iPS cells (Okada et al., 2008; Reynolds and Weiss, 1992). Thus, cell replacement therapy with autografting is likely to be feasible in the future. Many neurological diseases develop rapidly in elderly humans, and human iPS cells can even be obtained from the somatic cells of humans aged over 100 years (Yagi et al., 2012). Therefore, cell replacement therapy is also theoretically applicable to the treatment of patients of advanced age.

Despite this positive outlook, at least three problems need to be overcome before the autografting of NSCs established from human iPS cells can be realized. The first problem pertains to the time required to prepare the target cells (Okada et al., 2008; Takahashi and Yamanaka, 2006; Tsuji et al., 2010). Establishment of iPS cells from human cells is more time-consuming than their establishment from mouse cells (Takahashi et al., 2007), and the subsequent induction steps involve complex and rather prolonged incubation periods (Koch et al., 2009; Nori et al., 2011; Yagi et al., 2011). Studies using animal models of SCI show that NSC-transplantation during the subacute phase of injury (within 14 days of injury in rodents) is necessary for optimal functional recovery (Fig. 2) in view of the changes in the microenvironment of the injured spinal cord (Iwanami et al., 2005; Ogawa et al., 2002; Okada et al., 2006). With the currently established incubation techniques, however, it is difficult to establish iPS cells from somatic cells during this relatively short time frame for use in cell replacement therapy.

The second problem relates to the fact that NSCs must differentiate into neurons and glia to enable sufficient functional recovery following their transplantation into SCI patients (Kumagai et al., 2009; Nori et al., 2011; Tsuji et al., 2010). This is probably because the following processes all play a significant role in functional recovery: 1) incorporation of neurons derived from the transplanted cells into the host's neural circuit; 2) re-myelination by oligodendrocytes derived from the transplanted cells; and 3) trophic effects of astrocytes derived from the transplanted cells (Kumagai et al., 2009; Nori et al., 2011; Tsuji et al., 2010).

Regarding the differentiation potential of NSCs, NSCs induced from mouse pluripotent stem cells (ES and iPS cells) via embryoid body (EB) formation can differentiate specifically into neurons as long as they remain in the primary neurosphere state without undergoing passaging. If these cells undergo passaging and enter the secondary neurosphere state, they can differentiate not only into neurons, but also into glia (Miura et al., 2009; Naka et al., 2008; Okada et al., 2008). When transplanted into animal models of SCI, NSCs in the secondary neurosphere state exert useful therapeutic effects, while NSCs in the primary neurosphere state do not (Kumagai et al., 2009; Tsuji et al., 2010).

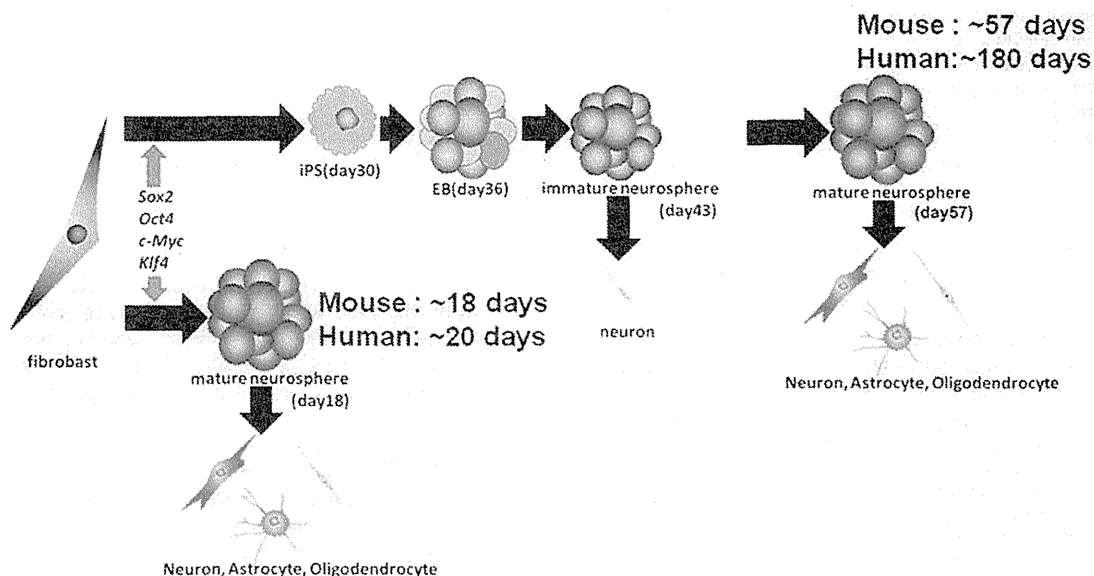

Fig. 2. Schematic diagram of autograft transplantation of induced NSCs. For the optimal treatment of SCI with cell replacement therapy, NSC transplantation must be performed in the subacute phase of injury. The rapid induction of mature NSCs by direct conversion may thus provide the best source of cells for this treatment.

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Furthermore, induction of NSCs that have the potential to differentiate into glia from established iPS cells via EB formation takes about 60 days in mice and 180 days in humans after gene transfer into somatic cells (e.g. skin cells). Therefore, if iPS cells are only generated after the onset of SCI, the patient will have already proceeded to the chronic stage of the disease before the cells become available for transplantation (i.e. to a stage where cell transplantation is no longer expected to be effective).

The third problem pertains to the risk of teratoma formation from residual undifferentiated cells among the NSCs used for transplantation (Miura et al., 2009). Humans have a long lifespan, and it is essential that tumorigenesis related to cell transplantation does not occur for at least 10 years after grafting. It is, therefore, imperative to establish NSCs that are free from undifferentiated cells for use in cell replacement therapy.

To overcome these problems, it seems indispensable to adopt a strategy of allogeneic transplantation, which involves the establishment of a bank of iPS cells and a bank of NSCs derived from these iPS cells (Okano et al., in press). If autologous transplantation is to be adopted, a technique must be developed that will allow the rapid induction of NSCs with the potential to differentiate into both neurons and glia from somatic cells (skin, blood, etc.). Such direct induction is expected to serve as a breakthrough in resolving the above difficulties regarding cell replacement therapy.

Concept of direct induction

"Direct induction" is a collective term used to refer to methods of induction aimed at establishing target cells from differentiated somatic cells, without the intermediary stage of iPS cells. The first report on direct induction was published in 1987 (Davis et al., 1987), much earlier than the initial description of iPS cells. In this report and a follow-up study, Weintraub and colleagues demonstrated that transduction with the *MyoD* gene resulted in the alteration of fibroblasts into myoblasts (Davis et al., 1987; Weintraub et al., 1989). Next, conversion of B cells into macrophages by the forced expression of CCAAT-enhancer-binding protein (C/EBP) α or C/EBPβ was reported in 2004 (Xie et al., 2004). However, all of these reports pertained to direct conversion (conversion through differentiation) among cells belonging to the mesoderm layer, and none dealt with direct conversion among cells of different germ layers. However, in 2006, Yamanaka and colleagues succeeded in establishing pluripotent stem cells (i.e., iPS cells) from adult mouse fibroblasts (Takahashi and Yamanaka, 2006), triggering remarkable changes in the research field of reprogramming and transdifferentiation.

As a first step, the forced expression of 24 genes known to be predominantly expressed in ES cells was performed in fibroblasts, resulting in the successful establishment of iPS cells with pluripotency akin to that of ES cells. The same investigators attempted to limit the number of genes needed for induced pluripotency among these 24 genes, ultimately demonstrating the indispensability of four genes, *Oct4*, *Sox2*, *Klf4* and *c-Myc*. Using the same approach, multiple groups have succeeded in establishing ectodermal NSCs from mesodermal fibroblasts. The techniques employed by these groups can be roughly divided into two types: 1) forced expression of genes required in the target cells; and 2) forced expression of reprogramming factors (used at the time of iPS cell establishment) to yield partially pluripotent cells, followed by the incubation of these cells in appropriate culture media to obtain the desired target lineage. Both techniques enable acquisition

Table 1
List of currently reported NS/PCs directly induced from mouse somatic cells.

Ref.	Cell source	Transgenes	Gene expression control	Culture period	Colony-forming efficiency (%)	Passage	Growth factor	Cells generated
Kim[a]	MEF (primary) Adult mouse fibroblast	*Oct4* *Sox2* *Klf4* *c-Myc*	Trans-gene expression was restricted to six days using the tet-ON system.	13 days	0.69	<5	EGF FGF2 FGF4	Neuron Astrocyte
Matsui[b]	Adult mouse fibroblast MEF	*Oct4* *Sox2* *Klf4* *c-Myc*	NP	18 days	0.03	>40	LIF EGF FGF2	Neuron Astrocyte Oligodendrocyte
Lujan[c]	MEF	*Brn2* *FoxG1* *Sox2*	Trans-gene-expression was terminated at the beginning of differentiation.	24 days	Unknown	>20	EGF FGF2	Neuron Astrocyte Oligodendrocyte
Sheng[d]	Mouse Sertoli cell	*Ascl1* *Ngn2* *Hes1* *Id1* *Pax6* *Brn2* *Sox2* *Klf4* *c-Myc*	NP	30 days	0.002	>25	EGF FGF2	Neuron Astrocyte Oligodendrocyte
Thier[e]	MEF Adult mouse fibroblast	*Oct4* *Sox2* *Klf4* *c-Myc*	The expression of Oct4 was restricted to five days by direct delivery of the OCT4 protein.	18 days	0.008	>50	EGF FGF2	Neuron Astrocyte Oligodendrocyte
Han[f]	MEF	*E47/Tcf3* *Brn4* *Sox2* *Klf4* *c-Myc*	NP	28–35 days	<0.01	>130	EGF FGF2	Neuron Astrocyte Oligodendrocyte
Ring[g]	MEF	*Sox2*	NP	>40 days	0.96	>40	EGF FGF2	Neuron Astrocyte Oligodendrocyte

[a] Kim et al. (2011).
[b] Matsui et al. (2012).
[c] Lujan et al. (2012).
[d] Sheng et al. (2012).
[e] Thier et al. (2012).
[f] Han et al. (2012).
[g] Ring et al. (2012).

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of the desired target cells without the intermediary stage of iPS cells. The elimination of the iPS cell stage is expected to reduce the risk of teratoma formation from residual undifferentiated cells after induction of differentiation, thereby improving the safety of cell replacement therapy. Furthermore, these techniques are expected to shorten the time required to obtain the target cells to a duration comparable with that of iPS cell-mediated induction. To date, at least 7 cases of direct induction of NSCs have been reported (Han et al., 2012; Kim et al., 2011; Lujan et al., 2012; Matsui et al., 2012; Ring et al., 2012; Sheng et al., 2012; Thier et al., 2012) (Table 1). In the forthcoming sections, the characteristics of the NSCs established using these techniques will be outlined, accompanied by a discussion of the roadmap to clinical application of directly induced NSCs and the issues that must be resolved prior to their use.

Direct induction of NSCs

Direct induction of neuronal cells was first reported in 2010 by Wernig and colleagues (Vierbuchen et al., 2010). These investigators listed up to 19 genes expressed in neuronal cells and their precursor cells as candidate genes for NSC induction and also identified the indispensable genes among these 19 genes, akin to the approach of Takahashi and Yamanaka (2006). Transduction with three of the 19 genes (*Brn2*, *Myt1l* and *Ascl1*) enabled the efficient induction of neural cells, which were termed "induced neurons" (iNs) (Vierbuchen et al., 2010). Subsequently, a series of reports has been published regarding the direct induction of neural cells from human fibroblasts (Pang et al., 2011), neuronal cells from the fibroblasts of patients with familial Alzheimer's disease (Qiang et al., 2011), dopaminergic neurons (Pfisterer et al., 2011) and spinal cord motor neurons (Son et al., 2011) from somatic cells, and so on. At present, efforts are underway to establish a technique for the generation of specific subtypes of neural cells by direct induction, in addition to simply creating a general population of neural cells. The outcomes of this research are likely to have a major impact on the advancement of relevant pathophysiological analyses and pharmaceutical research related to neurological disorders.

However, these iNs are unlikely to be an appropriate cellular resource for the promotion of functional recovery via cell replacement therapy following CNS injury. To achieve functional recovery in the damaged CNS, the presence of diffusible factors secreted from glial cells (astrocytes) also plays an important role, in addition to the repair of neural circuitry via transplanted iNs. Furthermore, because the majority of grafted cells are lost without undergoing engraftment (Yasuda et al., 2011), transplantation of a large number of cells with proliferative activity seems essential for a successful outcome. Our experiments demonstrated that functional recovery in animal models of SCI requires the transplantation of numerous mature NSCs with the potential to differentiate into astrocytes (Kumagai et al., 2009; Nori et al., 2011; Ogawa et al., 2002). In general, most studies concerning cell transplantation into animal models of neurological disease have shown that grafted astrogenic NSCs afford a high therapeutic efficacy (Blurton-Jones et al., 2009; Boulis et al., 2011; Ogawa et al., 2002; Oki et al., 2012; Parish et al., 2008). These studies support the hypothesis that the most advantageous results can be expected from cell replacement therapy using NSCs with self-renewal capacity and the potential to differentiate into all cell types of the nervous system.

The establishment of NSCs by direct induction was first reported by Kim et al. (2011). Prior to this, Takahashi and Yamanaka (2006) demonstrated that transduction of somatic cells with four genes (*Oct4*, *Sox2*, *Klf4* and *c-Myc*) enabled their reprogramming into an undifferentiated state resembling the ES cell phenotype. Kim and colleagues adapted this process to transduce mouse fibroblasts with the above-mentioned genes, cells were then incubated and reprogramming was completed using conditions optimized for NSCs. In this way, directly reprogrammed NSCs were effectively generated. Unlike iNs, the neuronal progenitor cells established by this method had the potential to divide, albeit to a limited extent, and could be passaged for several generations. However, despite having the capability to differentiate into both neuronal and glial cells, they failed to differentiate into oligodendrocytes.

Our own group (Matsui et al., 2012), as well as the groups of Lujan et al. (2012) and Sheng et al. (2012) (discussed below), simultaneously established induced NSCs (iNSCs) with a stable self-renewal capacity and the potential to differentiate into all three types of nervous system cells (neurons, astrocytes and oligodendrocytes). However, the three studies employed almost entirely different methods. Like Kim et al. (2011), our group transduced mouse fibroblasts with *Oct4*, *Sox2*, *Klf4* and *c-Myc*, harvested the fibroblasts before their conversion into iPS cells, and cultured them in suspension in serum-free medium supplemented with leukemia inhibitory factor (LIF) and fibroblast growth factor-2 (FGF-2) (Fig. 3). In this way, iNSCs were established in a relatively short period of time (18 days after gene transduction, including 4 days of adherent culture and 14 days of suspension culture). The resultant cells were mature NSCs capable of differentiating into neurons, astrocytes and oligodendrocytes when incubated with the appropriate growth factors *in vitro* or when transplanted into the living body. Moreover, the NSCs could be maintained in culture for one year or longer. We have since confirmed that these iNSCs retain the capability to differentiate into nervous system cells even after prolonged maintenance in the undifferentiated state.

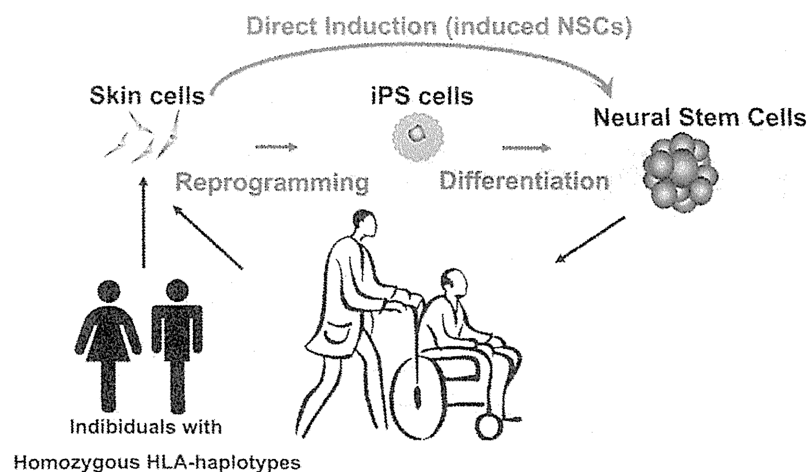

Fig. 3. Partially reprogrammed fibroblasts can form both neurospheres and iPS cell colonies. Fibroblasts differentiate into NSCs following suspension culture in serum-free medium containing LIF and FGF-2 upon transduction with four reprogramming factors (Oct4, Sox2, Klf4 and c-Myc). By contrast, fibroblasts are reprogrammed to the pluripotent state and give rise to colonies of iPS cells after adherent culture on feeder cells and in the presence of LIF (i.e., under ES cell culture conditions).

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We additionally demonstrated that iNSCs could be obtained without the intermediary stage of *Nanog*-GFP-positive iPS cells, if LIF was temporarily omitted, and epidermal growth factor (EGF) was instead added during the period of suspension culture. These results were attained using iNSCs derived from fibroblasts isolated from *Nanog*-GFP mice (Matsui et al., 2012). The iNSCs established by this method were almost entirely free from undifferentiated multipotent cells. Therefore, these iNSCs are a promising source of cells for transplantation therapy, provided that their safety is endorsed.

Lujan et al. (2012) employed a technique resembling that used for the generation of iN cells and iPS cells to transduce mouse fibroblasts with 11 transcription factors known to be expressed specifically in NSCs. These investigators generated iNSCs with self-renewal capacity and the potential to differentiate into all three types of nervous system cells. Later, Lujan et al. (2012) attempted to limit the number of factors needed to establish pluripotency by using an approach similar to that adopted by Takahashi and Yamanaka (2006) and demonstrated that transduction of only two factors (*FoxG1* and *Brn2*) enabled the direct conversion of fibroblasts into NSCs.

In contrast to the research performed by our group and that of Lujan et al., in which fibroblasts were used to establish iNSCs, Sheng et al. (2012) generated iNSCs via transduction of Sertoli cells (collected from the genital glands of mouse fetuses) with nine genes (*Pax6*, *Ngn2*, *Hes1*, *Id1*, *Ascl1*, *Brn2*, *c-Myc*, *Klf4* and *Sox2*). Although this group attempted to reduce the number of transduced genes, eight of the nine genes, excluding *Sox2*, were found to be indispensable. This finding is especially noteworthy in view of the fact that most of the successful demonstrations of iNSC generation from fibroblasts have employed Sox2. The requirement for a large number of reprogramming genes in Sertoli cells but not fibroblasts may be attributable to the difference in the gene expression profile between the two cell types.

A number of studies were subsequently published concerning the direct induction of NSCs. For example, Thier et al. (2012) reported the direct induction of NSCs from mouse fibroblasts using four factors (Oct4, Sox2, Klf4 and c-Myc), which were identical to those employed by Kim et al. and our group. However, the Thier et al. study differed in that a retroviral vector was employed for transduction with *Klf4*, *Sox2* and *c-Myc*, but not *Oct4*. Instead, the encoded Oct4 protein was directly introduced into fibroblasts, circumventing its continued gene expression and the reprogramming of fibroblasts into an iPS cell-like state. Furthermore, Han et al. (2012) transduced cells with *Brn4* and *E47/Tcf3* in addition to *Sox2*, *Klf4* and *c-Myc*, resulting in iNSCs with the ability to differentiate into all three types of nervous system cells and the capacity for long-term maintenance. Hence, these iNSCs were similar to those established by our group (Matsui et al., 2012) and that of Thier et al. (2012), whereas the iNSCs established by Kim et al. (2011) (i.e. using a tet-ON system and limiting the expression of Klf4, Sox2 and c-Myc to the first six days) lacked both self-renewal properties and the potential to differentiate into oligodendrocytes.

By contrast, the iNSCs established by our group (characterized by continued expression, to some extent, of Oct 4, Klf4, Sox2 and c-Myc, despite silencing) (Matsui et al., 2012) and the iNSCs established by Thier et al. (2012) (characterized by continued expression of Klf4, Sox2 and c-Myc, but not Oct4) exhibited a self-renewal capacity, as well as the potential to differentiate into oligodendrocytes. Hence, Klf4, Sox2 and/or c-Myc might be needed for the long-term maintenance of NSCs.

The current status of the direct induction of NSCs using mouse cells has been outlined above. Several groups are now attempting to create NSCs from human cells using the same techniques. For example, our group adapted a technique similar to that used to generate mouse iNSCs from human fibroblasts. As a result, NSCs capable of differentiating into βIII tubulin- and GFAP-positive cells were obtained following a short period of culture (about 20 days), despite the low efficiency of transduction (Matsui et al., 2012). In addition, Ring et al. (2012) attempted to establish mouse iNSCs through forced expression of Sox2, Bmi-1, TLX, Hes1 and Oct1, which are known to be expressed in NSCs, and eventually demonstrated that transduction with Sox2 alone enabled direct conversion of mouse MEFs into iNSCs. Surprisingly, this technique was also applicable to human fetal fibroblasts, culminating in the generation of human iNSCs with self-renewal capacity and the ability to differentiate into neurons, astrocytes and oligodendrocytes for the first time (Ring et al., 2012). This was also the first report demonstrating direct conversion between germ layers using only one factor.

The investigation by Ring and colleagues also demonstrated that iNSC colonies could not be obtained by adherent culture on a plastic dish with gelatin, but required culture on glass coated with poly-o-lysine and laminin. It has been previously shown that human NSCs express laminin receptors, and that laminin stimulates the proliferation of human and mouse NSCs (Flanagan et al., 2006). These findings indicate that optimization of extracellular matrix-derived signals, in addition to the provision of suitable growth factors, is critical for obtaining the desired target cells through direct conversion. Since the generation of human iNSCs in this manner did not involve oncogenic factors (e.g., Oct4 and/or c-Myc), and the expression of Sox2 (introduced via a retroviral vector) was gradually decreased due to silencing, Ring et al. (2012) made a convincing argument that these iNSCs might represent a useful source of biological material for cell transplantation therapy. Nonetheless, a limitation of this study is that long-term incubation (for 60 days) was needed to obtain sufficient numbers of iNSCs for autografting. Furthermore, mouse/human fibroblasts of fetal origin were employed, rather than cells of mature mouse/human origin. Considering that many patients who require NSC autografts are adults, the generation of NSCs of adult origin would be beneficial for clinical application.

Open issues and perspectives for the future

As illustrated above, new findings related to stem cell transplantation have been published frequently since Kim et al. first reported the direct induction of NSCs in 2011 (Fig. 1). The preceding sections discussed the usefulness of NSCs established from fibroblasts. However, a number of issues must be resolved before these cells can be applied clinically. In this section, we discuss the problems that require resolution prior to the successful realization of cell transplantation therapy using NSCs, as well as the possibility of pharmacological approaches to achieve the same end. As already described, a large number of cells are needed for NSC transplantation therapy (Nori et al., 2011; Ogawa et al., 2002; Tsuji et al., 2010). The induction period of the cells that will be grafted needs to be shortened, so that transplantation can be performed before the disease has progressed to a stage that is untreatable using this approach. This is true not only for the treatment of SCI, but also for the treatment of other neurological disorders.

Experiments on NSC induction using ES cells have been repeatedly carried out, starting before NSCs were generated by direct induction. These investigations have identified many low molecular weight compounds that can potentially improve the efficiency of NSC generation and control the phenotype of the established NSCs. These compounds may also be applicable to the creation of NSCs by direct induction. For example, cyclic AMP (cAMP) reportedly suppresses apoptosis of NSCs and can be employed for the induction of NSCs (Smukler et al., 2006). This compound has been successfully used by our group for the direct induction of NSCs; notably, the addition of cAMP to the culture medium markedly increased the efficiency of NSC induction (Matsui et al., 2012).

The transforming growth factor (TGF)-β/SMAD pathway also promotes the maintenance of human ES cells through its actions on the SMAD protein (Li et al., 2011a). When a compound inhibiting this pathway was added to the culture medium, the differentiation of human ES cells into NSCs was accelerated. A similar effect was reported for

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glycogen synthase kinase (GSK)-3β inhibitors and notch signal inhibitors. Indeed, NSCs were recently generated from human ES cells in only 7 days by the simultaneous inhibition of TGF-β, GSK-3β and Notch-signaling (Li et al., 2011a). This result pertains only to NSC induction using ES cells, and it is anticipated that even with this technology, incubation for 2 months or more will be required to establish NSCs from human somatic cells via iPS cells. However, if these findings were applied to modify the current direct induction technique for the establishment of NSCs without mediating the iPS cells, there may be potential for further shortening of the incubation period. Advances in this technology are expected in the near future.

Clearly, one of the most important issues related to the clinical application of NSCs is the integration of exogenous transgenes into the NSC genome. To date, several reports have been published concerning methods for the efficient induction of NSCs, either indirectly (via mediating the iPS cells) or directly (from the somatic cells). Without exception, all of these methods require transduction of exogenous genes, such as *Klf4*, *Oct4*, *Sox2*, *c-Myc* and *FoxG1*; however, numerous reports suggest that these genes are associated with tumorigenesis. Thus, establishment of NSCs by forced gene expression involves the risk of teratomas, gliomas, etc. (Moon et al., 2011; Schmidt et al., 1988; Seoane et al., 2004; Wang et al., 2009). Retroviral vectors and lentiviral vectors are primarily used for the transduction of exogenous genes, but there are also significant problems associated with the use of the viral vectors themselves. For instance, the number of gene copies inserted, the chromosomal site of integration, and so on, are not constant, resulting in numerous random effects. If iNSCs can be established without using exogenous transgenes (e.g. by the addition of low molecular weight compounds), it will be possible to realize safer, more efficient and better-standardized incubation systems that may prove advantageous for clinical application.

As far as the establishment of human and mouse iPS cells is concerned, the required number of exogenous genes has been successfully reduced to one (*Oct4*) following the addition of low molecular weight compounds to the culture medium (Li et al., 2011b; Zhu et al., 2010). These findings have increased confidence that suitable small compounds will be discovered to mediate the direct induction of NSCs. At present, many millions of small chemical compounds are accessible at research institutions and pharmaceutical companies around the world. If large-scale screening is carried out with these chemical compounds, remarkable advances may be achieved in the field of NSC technology.

Despite the hope for early clinical application of directly induced NSCs, to date, no published reports have evaluated the therapeutic efficacy of these cells. However, a report has recently been published regarding the efficacy of directly induced myocardial cells for the treatment of myocardial infarction (Qian et al., 2012). Undoubtedly, similar reports regarding the efficacy of directly induced NSCs for the treatment of neurological disorders will soon appear and they are eagerly anticipated.

Author contributions

T.M., W.A., M.N. and H.O. designed the research. T.M. and H.O. wrote the manuscript.

Conflict of interest

Authors have no conflicts of interest related to this study.

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2 iPS細胞を用いた脊髄損傷治療の開発

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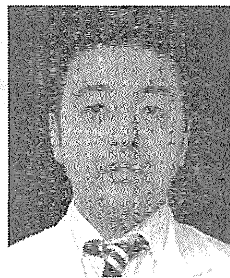

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Key words :iPS cell, neural stem/progenitor cell, safety issue

Abstract
近年，動物実験レベルで脊髄損傷に対する細胞移植療法の報告が数多くなされている。これまで我々は神経幹細胞に注目し，胎児（胎仔）及び，ES細胞由来神経幹細胞の脊髄損傷に対する有効性を報告してきたが，倫理的，免疫学的問題から我が国での臨床応用は困難な状況である。そこで注目を集めたのがiPS細胞である。当研究室でも安全性の確認されたマウス及びヒトiPS細胞由来神経幹細胞をマウス並びにサル脊髄損傷モデルへ移植し，その有効性を報告した。しかしその一方で，造腫瘍性の高いiPS細胞由来神経幹細胞は移植後に腫瘍を形成することも明らかになった。iPS細胞臨床応用にあたり十分な安全性の評価が極めて重要である。

はじめに

中枢神経系である脊髄に損傷が起こると，損傷部以下の知覚・運動・自律神経系の麻痺を呈する。集学的医療の進歩により脊髄損傷患者の平均余命は健常人と同等になってきているが，損傷された脊髄自体を直接治療する方法が無いのが現状である。しかし，幹細胞研究の急速な進歩により，動物実験レベルでは細胞移植をはじめ損傷脊髄の修復を促す治療法が多数報告されるようになってきている。基礎研究で得られた結果を臨床の現場で応用可能となれば，脊髄損傷に対する新たな治療法を確立することが可能となると言えよう。

近年，中枢神経系の再生医療の戦略として神経幹/前駆細胞（Neural Stem/Progenitor Cells: NS/PCs），胚性幹（Embryonic Stem: ES）細胞，人工多能性幹（induced Pluripotent Stem: iPS）細胞などを用いた細胞移植療法に世界的な注目が集まっている。特に，iPS細胞を用いた細胞移植療法の研究は急速に進んでおり，iPS細胞から種々の細胞への分化誘導法の開発や，試験管内での疾病状態の再現，疾患モデル動物への移植に関する研究が相次いで報告されている。

本稿では，我々がこれまで行ってきたNS/PCs・ES細胞を用いた細胞移植研究に触れながら，現在再生医療分野で最も注目されているiPS細胞を用いた脊髄損傷治療の現状と今後の展望について概説したい。

1．神経幹/前駆細胞の培養法

我々は，移植に使用するNS/PCsの選択的培養法としてReinoldsらが1992年に報告したニューロスフェア法[1]を使用している。この方法

Development of iPS cell transplantation therapy for spinal cord injury: Osahiko Tsuji[1,2], Satoshi Nori[2,3], Masaya Nakamura[2], 1) Department of Orthopaedic Surgery, Saitama Social Insurance Hospital, Urawa Ward, Saitama Prefecture, 330-0074, Japan. 2) Department of Orthopaedic Surgery, Keio University School of Medicine, Shinjuku Ward, Tokyo, 160-8582, Japan. 3) Department of Orthopedic Surgery, Tokyo Dental College Ichikawa General Hospital, Ichikawa, Chiba Prefecture, 272-8513, Japan.

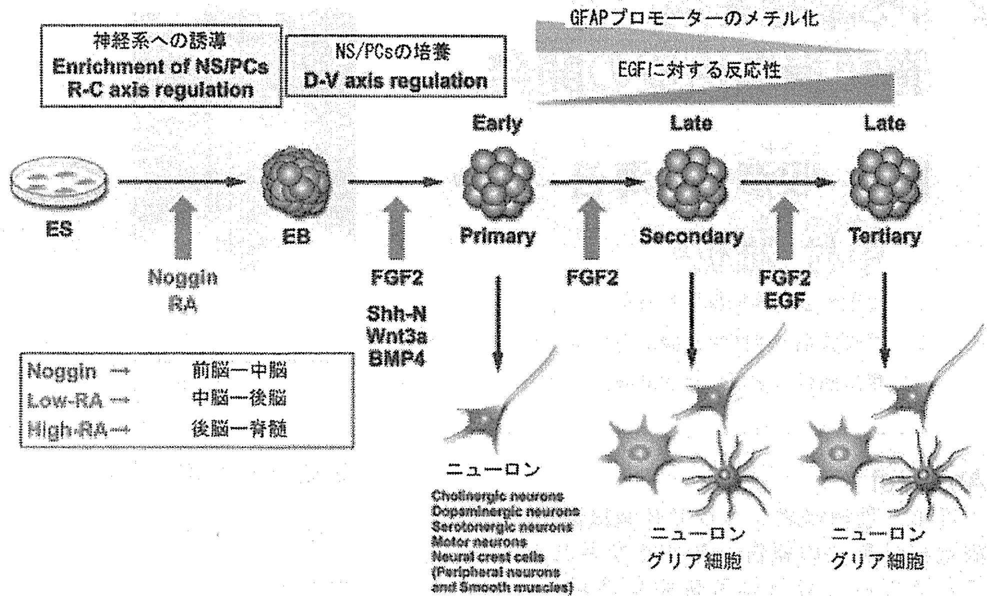

図1　マウスES細胞由来ニューロスフェア培養法による神経発生の再現（文献9を改変）

ES細胞から胚葉体（Embryoid Body：EB）を形成したのち神経系に誘導をかけてニューロスフェアを形成した[9]。このニューロスフェアを継代していくことで，生体内における神経発生を*in vitro*で再現することが可能であった。

は，胎生期の中枢神経系より採取した神経幹細胞を含む細胞群を分離した後に，非接着性培養皿で特定の栄養因子の存在する無血清培地により培養する方法で，神経幹細胞が選択的に浮遊した状態で増殖しニューロスフェアと呼ばれる細胞塊を形成する。

このニューロスフェアは，継代を繰り返すことができ（自己複製能），神経系3系統細胞である，ニューロン，アストロサイト，オリゴデンドロサイトへの分化能（多分化能）を有している。

2．神経幹細胞を用いた脊髄損傷治療

これまでに我々は，このニューロスフェア法により得られたラットNS/PCsをラット損傷脊髄に，さらにヒトNS/PCsをサル損傷脊髄へと移植し，運動機能の有意な改善が得られることを報告し[1,8]，胎児由来NS/PCs移植をヒト脊髄損傷治療に臨床応用するべく研究を進めてきた。しかし，NS/PCsを得るためには，中絶胎児からの細胞採取が必要であり，倫理的観点から我が国での臨床応用は困難なありほぼ不可能と言わざるを得ない。

3．ES細胞を用いた脊髄損傷治療

ヒトES細胞は，さまざまな細胞移植療法の重要な細胞源になり得るものとして注目されている。我々は，ES細胞からNS/PCsへの誘導法を構築した[9]。ES細胞から胚様体（Embryoid Body: EB）を形成し，その後神経系への誘導をかけニューロスフェアを形成する誘導法である（図1）。

このマウスES細胞由来NS/PCsの治療効果を検証するため，マウス脊髄損傷モデルに移植を行った。組織学的評価で軸索再生，再髄鞘化，血管新生を認め，下肢運動機能の改善を認めた[5]。しかし，ES細胞の作製にはヒト余剰胚を用いるため，胎児由来NS/PCs移植のケースと同様に倫理的問題から我が国での臨床応用は困難な状況である。

4．iPS細胞を用いた脊髄損傷治療

iPS細胞は，ES細胞と同等の増殖能・分化能

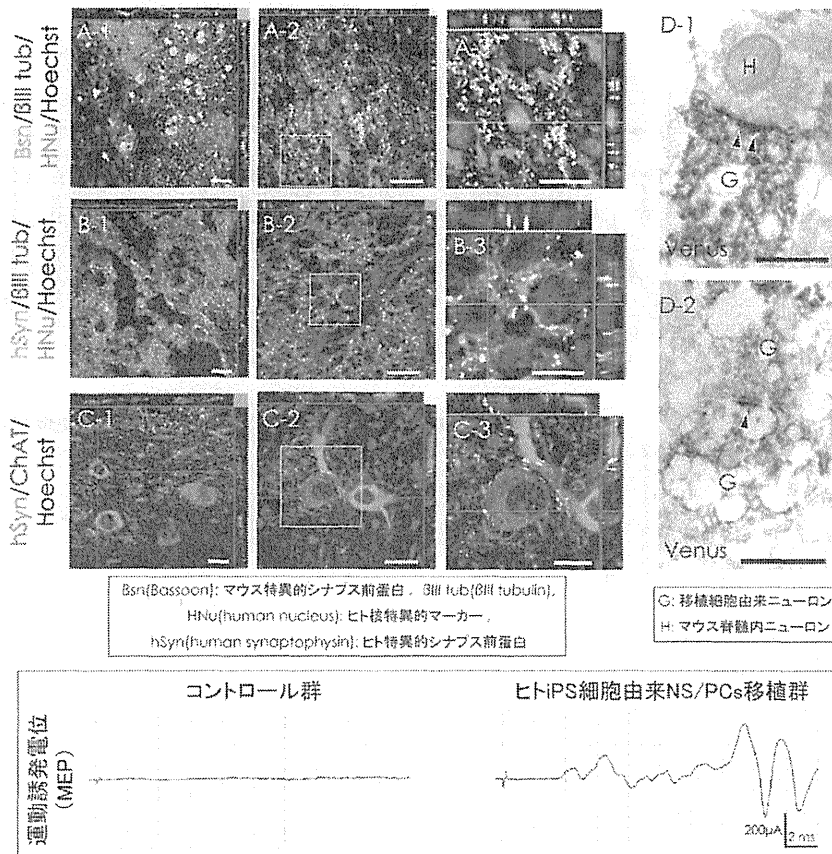

図2　ヒトiPS細胞由来ニューロンとマウス脊髄内ニューロンとのシナプス形成（文献7を改変）
免疫組織学的解析より，ヒトiPS細胞由来ニューロンはマウス特異的シナプス前蛋白とシナプスを形成（A）し，マウス脊髄内ニューロンはヒト特異的シナプス前蛋白とシナプスを形成（B，C）していた。電子顕微鏡による解析でもヒトiPS細胞由来ニューロンとマウス脊髄内ニューロンとのシナプス形成が確認された（D）。運動誘発電位（MEP）を計測したところ，201B7由来NS/PCs移植群でMEPの波形が検出されたのに対し，コントロール群では波形が検出されなかった[7]。

をもつ人工多能性幹細胞であり，線維芽細胞等の体細胞（somatic cell）に各種の初期化遺伝子を導入することで作製される。京都大学の山中伸弥教授らは，皮膚線維芽細胞からiPS細胞を樹立することに成功し，前述した幹細胞を用いる際の倫理的問題に解決の糸口を与え大きな注目を集め，2012年にノーベル医学生理学賞を受賞している[12,13]。

①マウスiPS細胞由来NS/PCsを用いた
脊髄損傷治療

われわれは，マウスES細胞のNS/PCsへの誘導培養法をマウスiPS細胞に応用しマウス成体皮膚線維芽細胞由来のiPS細胞からニューロスフェアを作製した。このマウスiPS細胞由来NS/PCsのクローンごとの安全性を，免疫不全マウス（NOD/scidマウス）の大脳（線条体）に移植し長期観察することで確認し，その上でマウス脊髄損傷モデルに移植した。その結果，“危険な”クローン由来のNS/PCs移植群は脊髄内で奇形腫を形成した。一方，“安全な”クローン由来NS/PCs移植群では腫瘍形成を認めず，下肢運動機能評価で有意な回復を確認した。このことより，臨床応用可能な成体組織由来のiPS細胞は，厳密にその安全性を検討すれば，脊髄損傷治療への有用な細胞源となることが示唆された[14]。

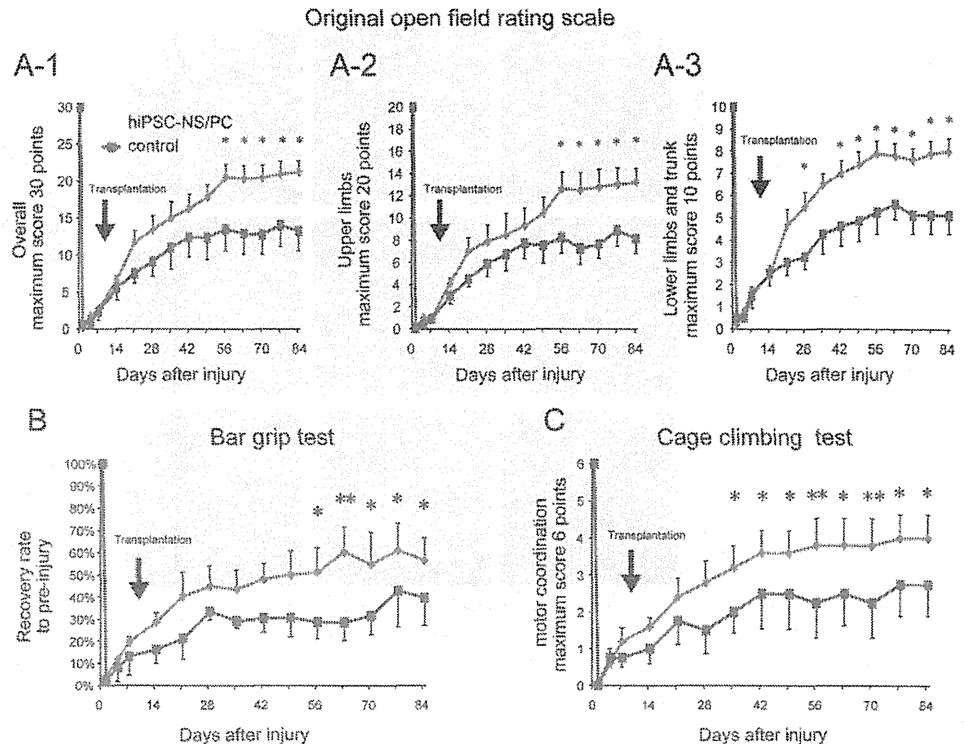

図3　コモンマーモセット脊髄損傷モデルに対するヒトiPS細胞由来NS/PCs移植後の運動機能評価（文献4を改変）

コモンマーモセット脊髄損傷後の運動機能評価法であるOriginal open field rating scale（A），Bar grip test（B），Cage climbing test（C）においてヒトiPS細胞由来NS/PCs移植群はコントロール群に比べて有意に運動機能の改善を認めた[4]。

②ヒトiPS細胞由来NS/PCsを用いた脊髄損傷治療

次にわれわれはヒトiPS細胞を用いた移植実験を行った。成人顔面皮膚由来の線維芽細胞に*Oct3/4*, *Sox2*, *Klf4*, *c-Myc*を導入して作製し，脳への移植実験で安全性を確認した201B7というヒトiPS細胞株[12]を用いた。この201B7をNS/PCsに分化誘導しニューロスフェアを作製した後，201B7由来NS/PCsの治療効果を確認するため，免疫不全マウス脊髄損傷モデルに移植を行った。移植細胞はマウス脊髄内で生着し，ニューロン，アストロサイト，オリゴデンドロサイトへの分化を認めた。移植細胞の約50％がニューロンへと分化しており，免疫組織学的解析および電子顕微鏡による解析から移植細胞由来ニューロンとマウス損傷脊髄内ニューロンがシナプスを形成していることを確認した（図2）。運動誘発電位（Motor Evoked Potential: MEP）を計測したところ，201B7由来NS/PCs移植群のみでMEPの波形を検出できた（図2）。これらの結果より，201B7由来ニューロンがマウス脊髄内で介在ニューロンとして機能して神経伝導に寄与している可能性が示唆された。各種運動機能評価では，移植群はコントロール群に比して有意な下肢運動機能の改善が認められた。さらに，安全性を確認するため，脊髄損傷後112日（4ヶ月）まで経過観察したが，腫瘍形成は認められなかった[7]。

さらにわれわれは，ヒトiPS細胞の臨床応用に向けた最終のステップとして，いわゆる前臨床研究に着手しており，前述した安全なiPS細胞クローン由来NS/PCsをサル（コモンマーモセット）脊髄損傷モデルに移植し組織学的評価で軸索再生，再髄鞘化，血管新生を認め，良好な運動機能の改善（図3）を確認している[4]。サル損傷脊髄内においても，免疫抑制剤投与下にヒトiPS細胞クローン由来NS/PCsは腫瘍形成することなく生着し，ニューロン，アストロサイト，オリゴデンドロサイトへと分化していることが確認された[4]。

5．今後の課題と展望

iPS細胞は脊髄損傷に対する細胞移植療法の有力な細胞供給源となる可能性を秘めている。これまでの我々の研究で用いてきたiPS細胞は，樹立時に初期化遺伝子を導入する際にレトロウイルスベクターを用いていた。レトロウイルスは遺伝子のプロモーター付近に組み込まれ，近傍の内在性遺伝子の発現状態を変化させることにより腫瘍化をもたらす危険性がある。この問題を解決するため，プラスミドベクターを用いた方法[10]，トランスポゾンの一種であるPiggy Bacを用いた手法[16]，染色体外に挿入しいずれ消失するエピソーマルベクターの導入[17]，プラスミドベクターよりも長期間の発現が可能であるミニサイクルベクター[2]，薬剤により導入遺伝子を置き換える方法18)などが相次いで報告されている。また，2011年にはiPS細胞の作製効率のみならず安全性も飛躍的に向上させるGlis1遺伝子が発見されており，iPS細胞は実用化に向けて着々とその安全面において改良が重ねられてきている[6]。

また，神経細胞への分化誘導法に目を向けると，線維芽細胞からiPS細胞へのリプログラミングを介さずに直接神経細胞に誘導したinduced Neuronal（iN）細胞も報告されてきている[3,15]。今後，このiN細胞の分化能，自己複製能，移植細胞としての安全性について詳細に評価する必要がある。

脊髄損傷に対する細胞移植治療の臨床応用にはまだまだ多くの課題があり，乗り越えるべき障壁は決して低くはない。しかしながら，一つ一つ研究を積み重ねていくことで，近い将来脊髄損傷の麻痺に苦しむ多くの患者さんに希望の光を投げかけることが出来るものと確信している。

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シンポジウム

1．再生医学・再生医療の最前線
4）iPS細胞を用いた脊髄再生医療の展望—基礎から臨床へ—

中村　雅也[1)]　岡野　栄之[2)]　戸山　芳昭[1)]

Key words：neural stem/progenitor cells，cell transplantation，spinal cord injury

はじめに

我が国では年間約5千人以上の新規脊髄損傷患者が発生しており，慢性期脊髄損傷患者は延べ20万人以上に達している．集学的医療の進歩により脊髄損傷患者の平均余命は健常人と変わらなくなってきているが，医療の発達した現代でも損傷された脊髄そのものを治療する方法はいまだ確立されていないため，運動・感覚・排泄などの機能障害が長期間にわたり患者を苦しめている．しかし近年の神経科学の進歩に伴い，脊髄損傷に対する細胞移植療法をはじめとする様々な治療法に関する前臨床研究が報告されるようになってきた．これらの報告により，損傷した脊髄でも微小環境が整えば再生することが示され'中枢神経系は一度損傷を受けると再生しない'という通説はもはや過去のものとなりつつある．

本シンポジウムでは，我々がこれまで行ってきた細胞移植研究，特に人工多能性幹細胞（induced pluripotent stem cell：以下iPS細胞）に関する基礎研究の現状に触れながら，脊髄再生医療における細胞移植療法の現状と今後の展望について概説する．

1．脊髄損傷に対する細胞移植療法

我々は胎児由来神経幹/前駆細胞(neural stem/progenitor cells：NS/PCs)に着目し，ラット胎仔由来NS/PCsをラット損傷脊髄に，さらにヒト胎児由来NS/PCsをサル（コモンマーモセット）損傷脊髄へと移植し，運動機能評価および組織学的評価において良好な結果を報告してきた[1,2]．海外に目を向けると，2011年に米国のStem Cell社による胎児由来NS/PCsを用いた慢性期胸髄損傷患者に対する第I/II相治験が，スイスのチューリヒ大学バルグリスト病院で開始されたことで，世界的に大きな注目を集めている．

しかしNS/PCsを得るためには中絶胎児からの

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110th Scientific Meeting of the Japanese Society of Internal Medicine：Symposium：I. Frontier of the Regenerative Medicine；4）Perspective on regenerative medicine for spinal cord injury using iPS cell—From bench to bedside—.

Masaya Nakamura[1)], Hideyuki Okano[2)] and Yoshiaki Toyama[1)]：[1)]Department of Orthopaedic Surgery, Keio University School of Medicine, Japan and [2)]Department of Physiology, Keio University School of Medicine, Japan.

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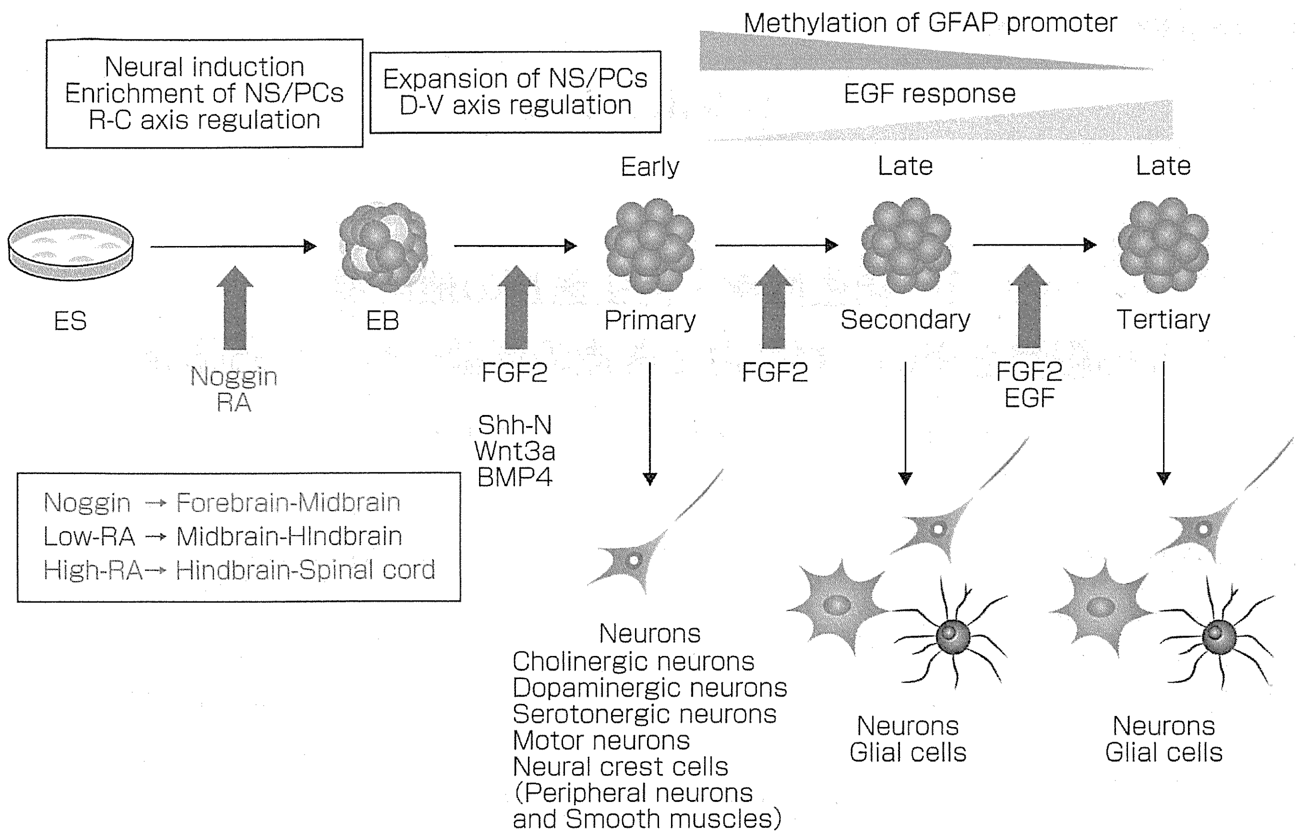

図1．マウスES細胞由来ニューロスフェア培養法による神経発生システム（文献7より改変）
ES細胞から胚葉体（embryoid body：EB）を形成したのち神経系に誘導し，ニューロスフェアを形成した．ニューロスフェアを継代することで，生体内における神経発生を*in vitro*で再現することが可能となった．

細胞採取が必須となるため，倫理的観点から我が国における臨床応用の実現は現時点では不可能と言わざるを得ない．また胎児由来NS/PCsを移植治療に用いる際，他家移植であるため免疫拒絶反応が起こる可能性がある．中枢神経においても他の臓器と同様に，MHC（主要組織適合遺伝子複合体）の違いによる免疫拒絶により組織の生着を妨げる[3,4]という報告もされており，細胞移植治療における大きな問題の一つとなっている．

一方，1981年にマウス胚盤胞期の受精卵から樹立されたES細胞は多分化能と自己複製能を持ち，万能細胞と言われている．ES細胞が1998年にヒト胚から作製されて以来，さまざまな細胞移植療法の重要な細胞源になり得るものとして注目されてきた．1999年にMcDonaldらがES細胞からNS/PCsを誘導し，ラット損傷脊髄へ移植し，良好な機能回復を報告した[5]．さらに2005年にはKeirsteadらがヒトES細胞からオリゴデンドロサイト前駆細胞への分化誘導に成功し，ラット損傷脊髄への移植を行い，脱髄した軸索の再髄鞘化と下肢運動機能の回復を報告した[6]．我々もES細胞からNS/PCsを誘導するシステムを構築し（図1）[7]，マウスES細胞由来NS/PCsをマウス損傷脊髄に移植し，組織学的評価で，軸索の成長，再髄鞘化および血管新生を認め，良好な下肢運動機能の改善を認めた[8]．しかしヒトへの臨床応用を考えた場合，ES細胞の樹立には不妊治療における余剰胚を用いるため，胎児由来NS/PCsと同様に倫理的な問題及び，免疫拒絶の問題は避けられない．

2. iPS細胞を用いた脊髄損傷治療

前述した幹細胞を用いる際の諸問題に解決の糸口を与えたのが，山中伸弥教授らにより樹立されたiPS細胞である[9,10]．線維芽細胞に数種類の初期化遺伝子を導入することで体細胞をリプログラミングし作製されたiPS細胞は，ES細胞と同等の増殖能・分化能をもつ人工多能性幹細胞である．iPS細胞は患者自身の体細胞から樹立することが可能であり，先に述べた倫理的問題や免疫拒絶の問題を解決する技術として期待されている．

1）マウスiPS細胞由来NS/PCsを用いた脊髄損傷治療

われわれは2006年の山中教授らによるiPS細胞の発表以降，慶應義塾大学と京都大学で，脊髄損傷に対するiPS細胞由来NS/PCs移植の臨床応用に向けた共同研究を開始した．まず，前述したマウスES細胞の神経幹細胞への誘導培養法をマウスiPS細胞に応用し，マウスiPS細胞由来NS/PCを作製した．さらにその安全性を確認するために，免疫不全（nonobese diabetic-severe combined immunodeficient：NOD-scid）マウスの大脳の線条体に移植した．その結果，成体組織由来（tail tip fibrobrast：TTF）iPS細胞を分化誘導させた際に残存する未分化細胞の比率が0.01％より高いと腫瘍形成が起こりやすいことが判明した[11]．この結果を踏まえて，当研究室の辻らはマウスTTF由来のiPS細胞を用いてマウス損傷脊髄への移植実験を行った．TTF由来iPS細胞のうち，安全性が確認できた335D1と腫瘍形成が認められた“危険な”256H13と256H18の3つのクローンをそれぞれ神経分化誘導し，マウス脊髄損傷モデルに移植を行った．その結果，“危険な”クローン由来のNS/PCs移植群は脊髄内で巨大な奇形腫を形成し，下肢運動機能の低下が認められた．一方で“安全な”335D1由来NS/PCs移植群では腫瘍形成を認めず，対照群と比較して下肢運動機能の有意な回復がみられた（図2）．このことより，臨床応用が可能な成体組織由来iPS細胞は，厳密にその安全性を検討すれば，脊髄損傷治療への有用な細胞源となることが示唆された[12]．

2）ヒトiPS細胞由来NS/PCsを用いた脊髄損傷治療

臨床応用へ向けた次の一歩として，当研究室の海苔らはヒトiPS細胞を用いた脊髄損傷治療への有効性の検討を行った．成人顔面皮膚にレトロウイルスを用いて*Oct3/4*，*Sox2*，*Klf4*，*c-Myc*を導入して作製し，安全性を確認した201B7細胞株からマウスiPS細胞と同様の方法でNS/PCsへ分化誘導した．201B7由来NS/PCsの治療効果を確認するため，NOD-scidマウスの損傷脊髄に移植した．なお，移植細胞を標識するためにレンチウイルスを用いて蛍光蛋白質Venus（改変型yellow fluorescent protein：YFP）をNS/PCsに遺伝子導入した．移植細胞はマウス脊髄内で生着し，NeuNおよびβIII tubulin陽性のニューロン，GFAP陽性のアストロサイト，APC陽性のオリゴデンドロサイトへの分化を認めた．移植細胞の約50％がβIII tubulin陽性のニューロンへと分化しており，そのうちの約70％がGAD67陽性のGABA作動性ニューロンへと分化していた．さらに，免疫組織学的解析および電子顕微鏡による解析から201B7由来ニューロンとマウス損傷脊髄内ニューロンがシナプスを形成していることが明らかになった（図3）．運動誘発電位（motor evoked potential：MEP）を計測したところ，201B7由来NS/PCs移植群ではMEPの波形が検出されたのに対し，PBSを注入した対照群ではMEPの波形は検出されなかった（図4）．このことより，201B7由来NS/PCがマウス脊髄内で介在ニューロンとして機能して神経回路の再構築を行っている可能性が示唆された．さらに移植細胞由来アストロサイトによる神経栄養因子の自己分泌，傍分泌作用により血管新生，神経再生，組織保護作用も認められた．これらの結

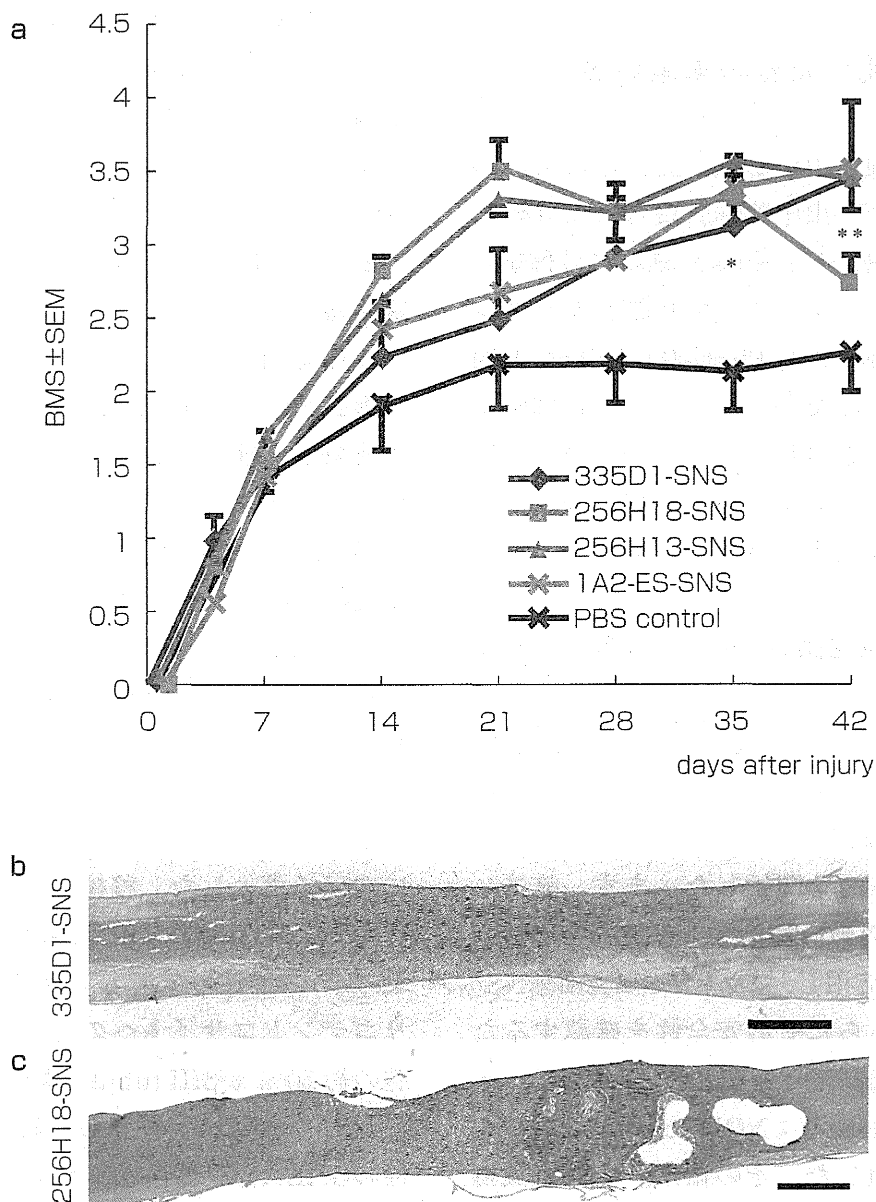

図 2. マウス脊髄損傷モデルに対する成体マウスiPS細胞由来NS/PCs移植後の組織像と下肢運動機能評価（文献 12 より改変）

"安全な" 335D1 由来NS/PCs移植後には腫瘍形成は認められず，下肢運動機能の改善が認められた．これに対して，"危険な" 256H18 および 256H13 由来NS/PCs移植後に奇形腫の形成を認め，一度回復した下肢運動機能が脊髄損傷後 6 週目で低下した．

果としてBMS, Rotarodトレッドミルテスト, foot printによる歩行解析において良好な下肢運動機能の改善が認められた（図 4）．さらに，長期にわたる 201B7 由来NS/PCs移植の安全性を確認するため脊髄損傷後 112 日まで経過を観察したところ，細胞移植群では良好な下肢運動機能の回復が継続し，腫瘍形成も認められなかった[13]．

3. 今後の課題と展望

以上のように，iPS細胞は脊髄損傷に対する細胞移植療法の有力な細胞供給源となる可能性を秘めている．2011 年に京都大学の山中教授らにより発表された*Glis1* 遺伝子は，iPS細胞の作製効

Bsn（Bassoon）：マウス特異的シナプス前蛋白, βⅢ tub（βⅢ tubulin）, HNu（human nucleus）：ヒト核特異的マーカー, hSyn（human synaptophysin）：ヒト特異的シナプス前蛋白

G：移植細胞由来ニューロン
H：マウス脊髄内ニューロン

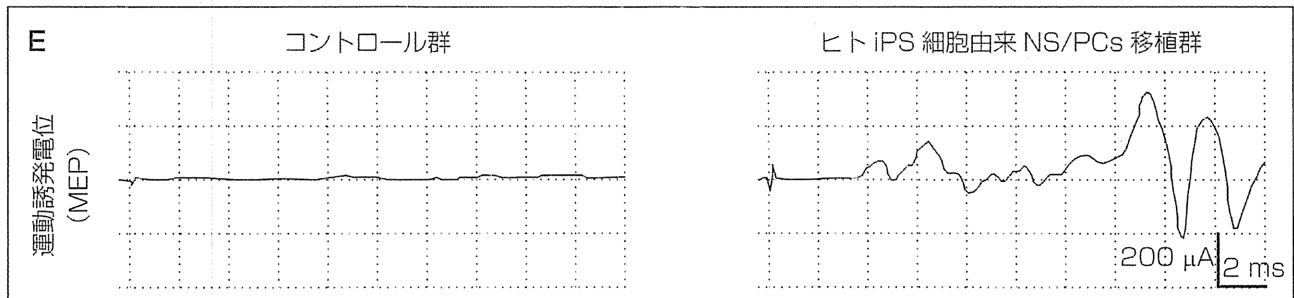

図3. ヒトiPS細胞由来ニューロンとマウス脊髄内ニューロンのシナプス形成（文献13より改変）
免疫組織学的解析より，ヒトiPS細胞由来ニューロンはマウス脊髄内ニューロンとシナプスを形成していた（A～D）．電子顕微鏡による解析でもヒトiPS細胞由来ニューロンとマウス脊髄内ニューロンとのシナプス形成が確認された（D）．運動誘発電位（MEP）を計測したところ，ヒトiPS細胞由来NS/PCs移植群でMEPの波形が検出されたのに対し，コントロール群では波形が検出されなかった（E）．

率のみならず安全性も飛躍的に向上させることが可能である．この発見はiPS細胞の実用化を予感させる内容であり，世界中の幹細胞研究者を興奮させた[14)]．これまでわれわれが報告してきた移植研究にはiPS細胞樹立時における初期化遺伝子の導入に，レトロウイルスを用いてきた．レトロウイルスは遺伝子のプロモーター付近に組み込まれることが多く，近傍の内在性遺伝子の発現状態を変化させ腫瘍化をもたらす危険性がある．この問題を解決するため，ゲノム挿入の

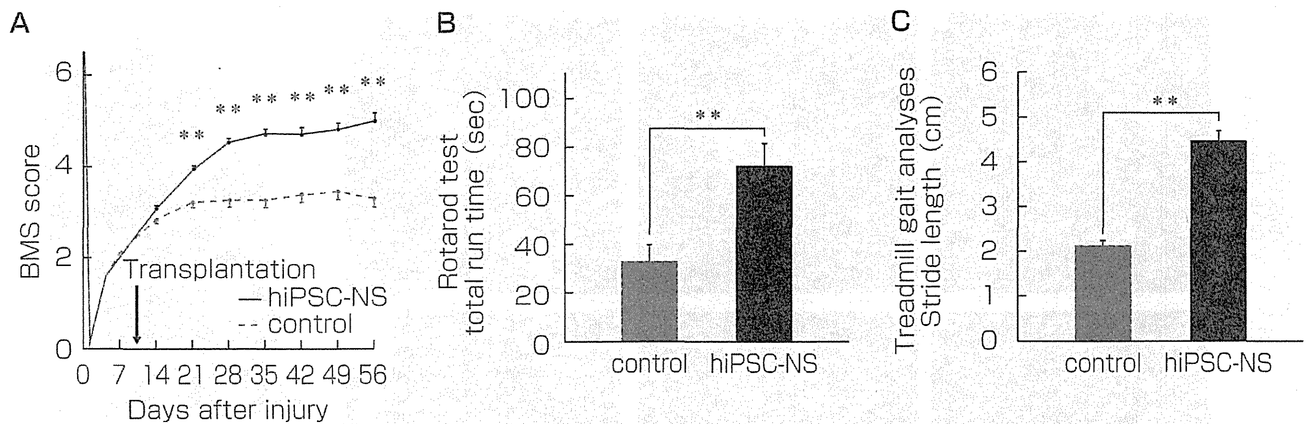

図4. マウス脊髄損傷モデルに対するヒトiPS細胞由来NS/PCs移植後の後肢運動機能評価（文献13より改変）
マウス脊髄損傷後の下肢運動機能評価法であるBMS（A），Rota-rod treadmill test（B），foot printによる歩行解析（C）においてヒトiPS細胞由来NS/PCs移植群はコントロール群に比べて有意に下肢運動機能の改善を認めた．

起こらないプラスミドベクターを用いた方法[15]，染色体外に挿入しいずれ消失するエピソーマルベクターの導入[16]，プラスミドベクターよりも長期間の発現が可能であるミニサイクルベクター[17]，薬剤による導入遺伝子の一部置き換えの成功[18]などが相次いで報告されている．今後，これらの方法で作製されたintegration free iPS細胞を用いて，1）移植前の安全なiPS細胞クローンの選別法，2）最終産物であるiPS-NSCの安全性基準の確立，3）GMP準拠した臨床に使用できるiPS-NSCの製造を行っていく予定である．1），2）に関しては既に基礎研究からその方向性は定まりつつある．3）に関しては産業化を見据えて企業を巻き込んだ研究体制の構築が求められている．これらの研究成果を結集して，平成28年までには脊髄損傷に対するiPS由来NS/PCを用いた臨床研究の開始を目指したいと考えている．

著者のCOI（conflicts of interest）開示：本論文発表内容に関連して特に申告なし

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