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Stem Cell Transplantation Combined with Niche Modification: A Novel Strategy for Treatment of Neurodegeneration

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Introduction

Stem cells are self-renewable and multipotent, and exist in various tissues including fetal brains. There is a growing knowledge of how stem cells differentiate into mature cells and how the niche, a microenvironment surrounding stem cells, regulates their proliferation and differentiation. Consequently, establishment of a cell replacement therapy to treat many neurological disorders that are presently incurable is strongly anticipated.

In this report, we present a survey of experimental trials of treatment using neural stem cells in animal models of various neurological disorders such as Parkinson's disease (PD), multiple sclerosis, stroke, spinal cord injury, and perinatal hypoxia-ischemia. In some of these trials, neural stem cells were transplanted in combination with modulation of the extracellular matrix of host tissues. Although many problems remain to be solved in advance of clinical use, stem cell transplantation combined with niche modulation could be a novel strategy for treatment of various neurological disorders.

Keywords: neural stem cells; transplantation; niche.

1. Features of neural stem cells and their niches

Stem cells are defined by their self-renewal and multipotent properties. In other words, they have the potential to replicate themselves and to differentiate into various types of cells. Stem cells are derived from various tissues including blastocysts (embryonic stem cells), bone marrow (bone marrow stromal cells), fetal brain (neural stem cells), and umbilical cord (umbilical cord blood cells)[1]. Recently, pluripotent cells with features similar to those of stem cells were induced from differentiated cells such as skin fibroblasts [2].

Neural stem cells (NSCs) exist in germinal zones (ventricular zone, VZ; and subventricular zone, SVZ) of the developing central nervous system (CNS). In the adult CNS, NSCs are also present in SVZ and can proliferate. In the developing CNS, most NSCs migrate from VZ to pia surfaces and differentiate into neurons and glial cells. The proliferation and differentiation of NSCs are regulated by both intrinsic factors, such as transcription factors, and extrinsic factors, such as growth factors including fibroblast growth factors (FGFs) and epidermal growth factors (EGFs)[3]. NSCs proliferate actively in culture and form cell aggregates called neurospheres in the presence of these growth factors.

Stem cell populations are established in the 'niches', which are specific anatomic locations that support stem cell reproduction or self-renewal [4]. The niches control the stem cell number and prevent stem cell depletion and/or dysplastic cell growth. Niche elements consist of niche cells and extracellular matrix. In mouse bone marrow, osteoblasts are prominent niche cells and osteopontin is the matrix for haematopoietic stem cells [5,6]. In the mouse brain, endothelial cells were shown to regulate the NSC number, and are therefore believed to be niche cells [7]. Tenascin C, a glycoprotein that exists in SVZ of the developing CNS and controls NSC proliferation, is believed to be an extracellular matrix component of the NSC niche [8]. Chondroitin sulfate proteoglycans (CSPGs) are the major constituent of the extracellular matrix of various regions of the CNS including VZ of the fetal rat telencephalon, and chondroitin sulfates (CS) isolated from the developing telencephalon promote FGF2-mediated proliferation of NSCs [9]. Thus, CS polysaccharides participate in the regulation of NSC functions as a niche matrix constituent.

2. Experimental trials of cell transplantation therapy in animal models of various neurological disorders

Most neurological disorders are incurable, and their causes are yet unknown. Based on accumulating

knowledge on NSC proliferation and differentiation, establishment of a cell replacement therapy for these neurological disorders is currently anticipated. Therefore, many experimental trials employing cell transplantation therapy are on-going in animal models of various neurological disorders.

Parkinson's disease (PD) is a common chronic neurodegenerative disorder characterized by tremor, rigidity, and hypokinesia. The pathological hallmark is a gradual loss of nigrostriatal dopamine (DA)-containing neurons. Clinical trials involving transplantation of human fetal mesencephalic tissue in PD patients have demonstrated that grafted DA neurons can reinnervate the denervated striatum, release DA, and become functionally integrated into neural circuits of the host [10, 11, 12]. However, functional outcomes after transplantation have been variable, and limited tissue availability has hindered further developments. Cell replacement therapy using embryonic stem (ES) cells provides a promising alternative, but the risk of teratoma formation and poor survival of grafted human ES-cell-derived DA cells in animal models have prevented their use in clinical trials [13]. Currently, a cell replacement therapy using a method for generating a large number of DA neurons from stem cells is under investigation. Transplantation of bone marrow stromal cells (MSCs), which had been transfected with the Notch intracellular domain in the presence of glial-cell-line-derived neurotrophic factor, brought about improvement in a PD mouse model [14]. Ventral midbrain (VM) neurospheres, pretreated with sonic hedgehog and FGF8 and transfected with Wnt5a, generated 10-fold more DA neurons than conventional VM-neurospheres [15]. Transplantation of these cells into PD mice resulted in significant functional recovery [15].

Multiple sclerosis (MS) is caused by inflammation-induced destruction of the myelin sheath that surrounds axons. Myelin-producing oligodendrocyte progenitor cells (OPCs) are abundant in the adult human brain, and are also present in chronic demyelinated MS lesions. Thus, it is important to find a way to enhance remyelination in MS patients. Recently, it was found that astrocyte-derived hyaluronan had accumulated in demyelinated lesions of MS patients and prevented the maturation of endogenous OPCs [16]. To compensate for this demyelination, remyelinating cell transplantation therapy was attempted in a chronic MS mouse model. Although the injected NSCs migrated to inflammatory demyelinating lesions and induced recovery, grafted NSCs remained undifferentiated and suppressed the proinflammatory mechanism [17, 18].

Focal brain ischemia results from blockage of the blood supply. Degeneration of brain tissue following a stroke leads to functional impairment with a limited capacity for self-repair. After the insults, patients must endure a diminished quality of life that persists throughout their lifetimes and would therefore desire greater functional recovery. Based on the observation that new neurons are generated in specific regions of the adult brain, a trial to activate endogenous neural progenitors and regenerate hippocampal neurons after ischemic brain injury has been proposed [19]. Another trial using MSCs was conducted in an ischemic rat model. Surprisingly, intravenous administration of MSCs was shown to lead to significant functional recovery after cerebral ischemia [20]. In another interesting study, treatment of stroke with MSCs was found to enhance angiogenesis in the host brain via increases in levels of endogenous vascular endothelial growth factor (VEGF) and its receptor [21]. In addition, intravenous delivery of the $CD34^+$ subpopulation of human umbilical cord blood cells enhanced angiogenesis, neurogenesis, and morphological and functional recovery in an animal model of stroke [22]. This recovery was observed even though the number of grafted cells entering the brain was not significant.

Spinal cord injuries interrupt ascending and descending axonal pathways, and lead to a loss of movement, sensation, and autonomic control below the site of injury. Until recently, CNS neurons have been thought to degenerate after injury. Many trials of treatment of spinal cord injuries have been conducted in rodent models. One of these involved enzymatic removal of CS by injection of chondroitinase ABC (ChABC) into the lesion site, and the CS removal resulted in promotion of axon regeneration following spinal cord injury [23]. CS is the major component of glial scars which are believed to prevent axonal regeneration [24]. Injection of MSCs into the cerebrospinal fluid also promoted functional recovery from spinal cord injury in a rat model [25]. Moreover, injection of NSCs combined with ChABC promoted functional recovery [26].

Neonatal hypoxic-ischemic encephalopathy (HIE) usually occurs perinatally and results in long-term neurological disabilities in children. Although various neuroprotective strategies have been studied, options for the management of HI brain injury are currently very limited. Transplantation of bone-marrow-derived multipotent adult progenitor cells into the hippocampus of HIE model rats ameliorated motor deficits associated with HI injury [27]. In addition, ES-cell-derived cell transplantation resulted in improvement in learning ability and memory in an HIE mouse model [28]. Recently, Sato et al. (2008) showed that transplantation of NSCs, combined with ChABC, into cerebroventricles reduced the infarct size in an HIE rat model [29]. CSPGs exist in an NSC environment in vivo, indicating that CSPGs are a niche matrix component [9]. Some CSPGs support survival of neurons at the lesion site in conjunction with various glycosaminoglycan-binding neurotrophic factors, such as FGFs and pleiotrophin [24]. It is conceivable that ChABC treatment

changes the microenvironment of grafted NSCs and can increase neurotrophic factor release accessibility (Figure 1).

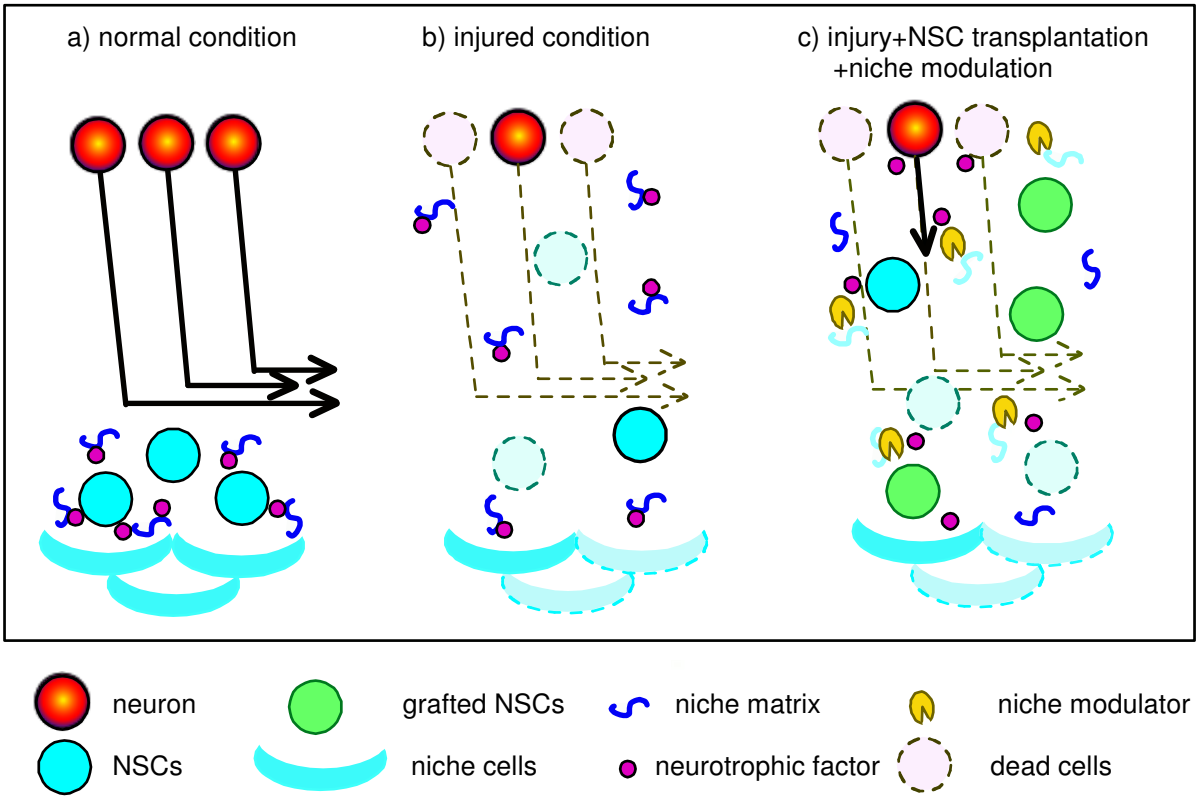

Figure 1. A possible role of niche modulation in stem cell transplantation. a) In the normal CNS, neurons have elongated axons and neural stem cells (NSCs) are stable inside their niche. Activities of neurotrophic factors that are synthesized by NSCs and niche cells are suppressed by their binding to niche matrix molecules such as chondroitin sulfate. b) When CNS tissue is injured, many neurons and axons degenerate, and endogenous NSCs may lose their niches. Neurotrophic factors may commonly be combined with niche matrix molecules, resulting in continued suppression of their neurotrophic effects. c) When NSCs are transplanted together with niche modulators, modification of niches can occur. The modulation may help neurotrophic factors become soluble and facilitate access to NSCs and injured neurons. Consequently, the neurotrophic and/or neuroprotective effects of NSCs could be enhanced.

Considering the results of the various experimental trials described above, transplanted cells do not always replace degenerated neural cells. Rather, they often accumulate around injured regions where they secrete neurotrophic factors and induce angiogenesis, neurogenesis, and/or neuroprotection. Therefore, niche modulation such as that resulting from ChABC treatment would enhance stem cell transplantation therapy. A more precise understanding of the roles of niche components in regulation of stem cell functions would advance developments in stem cell therapy.

3. Problems to be solved in advance of clinical use

Although the experimental approaches described above resulted in significant functional improvements in each animal model, most cannot be applied to clinical trials at the present time. Several problems must be overcome prior to their clinical use. First, control of graft-versus host disease (GVHD) remains a concern. Most experiments using heterografts or allografts require immunosuppressants. However, induced pluripotent stem (iPS) cells [2] can help solve this problem. Since iPS cells are established from cells of individual patients, transplantation of these cells would be considered a self-graft. Second, we must regulate the proliferation of stem cells. Since stem cells have strong growth potential, they readily form tumors. ES cells, especially, are reported to sometimes induce teratoma in grafted animals. Third, we need to have a precise understanding of the regulatory mechanisms involved in differentiation of stem cells. For example, expansion of proper neuronal subpopulations such as that of DA neurons is critical for clinical treatment of neurodegenerative disorders such as PD. Similarly, expansion of OPCs is crucial for MS therapy.

From another viewpoint, there is a possibility that NSC transplantation itself induces some unexpected side effects, such as the development of neuropsychiatric deseases, etc. Since we currently lack reliable ways to examine neuropsychiatric

disorders in rodent models, it is not known whether NSC transplantation may cause these disorders, even after an interval of several years. We need to exclude this possibility through careful study of primate models before we can apply this approach to human clinical trials.

NSC transplantation remains a long-sought goal of patients, clinicians and medical co-workers who must deal with incurable neurological disorders. Therefore, we should persevere in our step-by-step search for optimal ways to use stem cell transplantation therapy.

4. Conclusions

There have been an increasing number of reports on stem cell transplantation therapy in animal models of various neurological disorders. Results of many of these trials showed that the host animals achieved functional recovery without long-term survival of the grafted NSCs. Rather, the transplanted cells tended to accumulate around the injured regions soon after transplantation, and seemed to secrete neurotrophic factors and to induce angiogenesis, neurogenesis, and/or neuroprotection. We should aim for greater understanding of the regulatory mechanisms underlying stem cell proliferation and differentiation including the role of the niche in stem cell physiology. We would then be able to employ this knowledge in stem cell treatment of various human neurodegenerative diseases.

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