STRATEGIES TOWARD CNS-REGENERATION USING INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem (iPS) cells are pluripotent stem cells directly reprogrammed from cultured mouse fibroblast by introducing *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. Cells obtained using this technology, which allows the ethical issues and immunological rejection associated with embryonic stem (ES) cells to be avoided, might be a clinically useful source for cell replacement therapies. Here we demonstrate that murine iPS cells formed neurospheres that produced electrophysiologically functional neurons, astrocytes, and oligodendrocytes. Secondary neurospheres (SNSs) generated from various mouse iPS cell showed their neural differentiation capacity and teratoma formation after transplantation into the brain of immunodeficient NOD/SCID mice. We found that origin (source of somatic cells) of the iPS cells are the crucial determinant for the potential tumorigenicity of iPS-derived neural stem/progenitor cells and that their tumorigenicity results from the persistent presence of undifferentiated cells within the SNSs. Furthermore, transplantation of non-tumorigenic *Nanog*-iPS-derived SNSs into mouse spinal cord injury (SCI) model promoted locomotor function recovery. Surprisingly, SNSs derived from c-Myc minus iPS cells generated without drug selection showed robust tumorigenesis, in spite of their potential to contribute adult chimeric mice without tumor formation..

Keywords: neural stem/precursor cells, induced pluripotent stem (iPS) cells, regeneration, spinal cord injury

1. Introduction

It had been long believed that adult mammalian central nervous system (CNS) do not regenerate upon their injury. However, we wanted to challenge this dogma taking advantage of stem cell technology. The major strategies for the regeneration of the damage CNS would include, i) Transplantation of stem/ progenitor cells, and ii) Activation of endogenous stem cells and self-repair mechanisms[1-4]. We have investigated transplantation therapy for spinal cord injury (SCI) model for more than ten years using cell sources including fetal CNS-derived neural stem/progenitor cells (NS/PCs) [5-8], ES-derived NS/PCs, induced pluripotent stem (iPS) cells-derived NS/PCs and neural crest stem cells [9, 10]. Here, I will mainly talk about cell therapy for SCI model using iPS cells-derived NS/PCs.

2.1. *Cell Therapy for SCI Model Using iPS Cells-Derived NS/PCs*

The iPS cells are pluripotent stem cells directly reprogrammed from cultured mouse fibroblasts by the introduction of Oct3/4, Sox2, c-Myc, and Klf4 [11]. Various types of

induced pluripotent stem (iPS) cells, each exhibiting different biological properties, have been established by different methods [12]. Their properties, including their differentiation potentials and tumorigenic activities (teratoma-forming potentials) in different contexts, should be investigated in detail from the aspects of safety and efficacy of cell transplantation therapies.

2.2. *Evaluation of the Safety of NS/PCs Derived from iPS Cells*

First, we evaluated the safety of mouse-derived induced pluripotent stem (iPS) cells through secondary neurosphere (SNS) formation, according to the method for neural differentiation of mouse ES cells we reported [13, 14]. We generated SNSs from various mouse iPS cell clones and examined their neural differentiation capacity and teratoma-forming potential after transplantation into the brains of immunodeficient NOD/SCID mice. In collaboration with Prof. Shinya Yamanaka's laboratory, we used 36 iPS cell clones, which differ in (i) their origin (source somatic cells), (ii) presence or absence of c-Myc retroviral transduction, and (iii) presence or absence of drug selection for *Nanog* expression. While the NS/PCs generated from the various mouse iPS cells showed similar neural differentiation capacity, they showed enormously different teratoma-forming potential after transplantation into the brain (striatum) of NOD/SCID mice. Accordingly, we came to the conclusion that the origin of the iPS cells are a crucial determinant of the tumorigenic potential of iPS-derived NS/PCs and that their tumorigenic potential is co-related to the persistent presence of undifferentiated cells within the iPS-cell-derived NS/PCs[15].

2.3. *Transplantation of NS/PCs Derived from Pre-Evaluated Safe iPS Cells*

Next, we also demonstrated that transplantation of pre-evaluated "safe" iPS-cell-derived NS/PCs into a mouse model of spinal cord injury (SCI) resulted in graft-derived neurogenesis and myelination, various non-cell autonomous trophic effects against the host spinal cords, and long-lasting recovery of locomotor function, without tumor formation. On the other hand, transplantation of NS/PCs (SNSs), derived from pre-evaluated 'unsafe' iPS cells, judged from the persistent presence of undifferentiated cells within the iPS-derived neurpsheres and/or robust teratoma formation potential upon transplantation into NOD/SCID mice brains, resulted in a certain degree of functional recovery for a short period. However, it was eventually followed by teratoma formation within the spinal cord and deterioration of locomotor function, possibly due to the tumor mass effects.

Thus, properly pre-evaluated iPS clone-derived cells must be a promising cell source for future transplantation therapy. Our present study is the first demonstration of the therapeutic potential of iPS-derived NS/PCs for the repair of SCI. Taken together, prior to the start of clinical trials of human CNS disorders using iPS cells, it is essential to pre-evaluate each iPS cell clone carefully, and to conduct preclinical transplantation tests using appropriate primate models under stringent clinical settings. I would also like to

describe about the results of our recent experiments involving transplantation of human iPS-derived NS/PCs into a mouse model of SCI.

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