Review: Direct conversion in Neuro-regenerative Medicine

Myung Rae Park[1], Danbi Song[1], Jihye Park[1], Kang Seok Lee[1], Suk Hwan Joung[1], Jeong Beom Kim[1*]

[1]Max Planck Partner Group-Molecular Biomedicine Laboratory, School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, South Korea

Abstract. Recent advances in direct conversion technology are of great significance in neuro-regenerative medicine. Conversion of somatic cells to a desired cell type is next generation reprogramming technology for clinical applications. The novel strategy of direct conversion of somatic cells into expandable multipotent neural cells (induced multipotent neural cells, iMNCs) and specific neurons (induced neurons, iNs) opens a new window of opportunities for cell therapy, disease specific modeling, and drug discovery for brain diseases.

Keywords: Direct conversion, multipotent neural cells, somatic cells, neurodegenerative disorders

1 Introduction

Regenerative medicine opens new windows for effective and safe therapies for incurable and debilitating disorders. Stem cells, which can be isolated from human tissues, are considered as an essential resource in regenerative medicine. There are several types of stem cells such as embryonic stem cells (ESCs), adult stem cells, and induced pluripotent stem cells (iPSCs) depending on their developmental sources [1]. The discovery of iPSCs in 2006 not only offered an opportunity for a patient-specific cell-based therapy but also provided disease-specific modeling, which can be used as a valuable platform for drug screening. Furthermore, the derivations of iPSCs from adult cells avoided the ethical debate concerning the derivation of ESCs from human embryos [2]. However, many practical obstacles still remain to bring stem cell technology from bench to bed. One of the limitations is difficulty in inducing differentiation of pluripotent stem cells into a desired cell type. It takes significant time and efforts to reprogram somatic cells to pluripotent cells and later induce them into desired cells. Another limitation is that there still is a high possibility that remaining undifferentiated cells may form a teratoma during differentiation within the human body. To date, there are many reviews on induced conversion of specialized cells into other cell types by using lineage specific transcription factors. In the past few years, many research groups have focused on direct conversion between somatic cells, within or outside their original lineages [3]. Neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS) and Multiple Sclerosis (MS) occur as the result of neurodegenerative processes

that lead to the functional loss of the nervous system. In this review, we will discuss recent studies in which various neurodegenerative disorders have been modeled via the direct conversion of somatic cells to neural lineages.

2 Induced neurons (iNs) for neurodegenerative diseases

2.1 Dopaminergic neurons

Dopaminergic (DA) neurons which are mostly found in the substantia nigra pars compacta (SNpc), a region of midbrain, mediate motor control, reward, and arousal. The loss of dopaminergic neurons contributes to the motor symptoms of PD such as resting rigidity, tremors, and postural instability. For clinical approach, transplantation of directly converted dopaminergic neurons is a prospective way that can replace the degenerated dopaminergic neurons in PD patients.

Several groups have reported on the direct conversion of induced dopaminergic (iDA) neurons from the mouse [4, 5] and human fibroblasts [4, 6]. These groups assessed the functionality of iDA neurons by transplantation of the neurons into PD animal models [4-6]. Liu et al. converted human fibroblasts into iDA neurons by five transcription factors (*Ngn2*, *Sox2*, *Ascl1*, *Nurr1*, and *Pitx3*). They grafted the iDA neurons into a PD rat model [6]. The symptoms of PD were alleviated and it showed more stabilized rotational behaviors at 8 weeks post injection. Caiazzo et al. identified three transcription factors (*Ascl1*, *Lmxl1a*, and *Nurr1*) that converted functional iDA neurons from mouse embryonic fibroblasts and fetal and adult human fibroblasts [4]. They grafted the iDA neurons, which were generated by these three factors, into the newborn mouse brains. Two and six weeks after the transplantation, they found that iDA neurons integrated into the host tissue efficiently. Kim et al. transplanted iDA neurons, which were converted by six transcription factors (*Pitx3*, *Ascl1*, *Lmx1a*, *Nurr1*, *Foxa2*, and *EN1*) from mouse tail tip fibroblasts , into 6-hydroxydopamine (6-OHDA) lesioned mice [5]. They confirmed the survival of the majority of transplanted iDA neurons and the expression of DA neuron markers *in vivo*. From the work of these groups, it was demonstrated that direct conversion of mouse and human fibroblast into iDA neurons has potential for clinical applications.

2.2 Motor neurons

Motor neurons (MNs) are involved in actuating muscular movement by receiving information from the central nervous system (CNS) and controlling musculature. Amyotrophic lateral sclerosis (ALS), also called Lou Gehrig disease, is one of the prototypical motor neuron diseases caused by the global degeneration of MNs in the brain and spinal cord. Neuromuscular dysfunction symptoms of ALS lead to progressive physical paralysis and death.
For the treatment of ALS, there have been multiple approaches for the generation of

motor neurons (MNs). Son and his coworkers directly converted the mouse and human fibroblasts into functional spinal motor neurons [7]. By forced expression of seven transcription factors (*Ascl1, Brn2, Myt1l, Lhx3, Hb9, Isl1*, and *Ngn2*), iMNs were obtained from the mouse fibroblasts within 1~2 weeks. They prepared fibroblasts from the tails of adult *Hb9::GFP* mice and monitored the efficiency of iMN induction by GFP intensity signals, which was measured to be between 5% and 10%. In the case of human iMNs, the same group also trans-differentiated human embryonic fibroblasts by a similar set of factors (*Ascl1, Brn2, Myt1l, Lhx3, Hb9, Isl1*, and *Ngn2*) with one additional factor *NEUROD1* [7]. The efficiency was relatively low (0.05%) and the process was further protracted (4~5 weeks).

3   Induced Multipotent neural cells (iMNCs)

As shown in previous studies, iNs, which are fully differentiated neurons, could be applied to treat a variety of neurodegenerative disorders. However, there are several limitations in this type of cells: iNs are not expandable since they are in fully differentiated state. Moreover, they can be applied to a specific neurodegenerative disorder. On the other hand, induced multipotent neural cells (iMNCs) such as induced oligodendrocyte precursor cells (iOPCs) or induced neural stem cells (iNSCs) are self-renewable and multipotent cells that can differentiate into certain neural cell types constituting the central and peripheral nervous system.

3.1 Induced oligodendrocyte precursor cells (iOPCs)

Oligodendrocyte precursor cells (OPCs) can differentiate into neuroglial cells including oligodendrocytes which produce the myelin sheath to insulate axons in the CNS. Multiple sclerosis (MS) is a chronic demyelinating disease in which the membranous sheath of nerve cells in the brain and spinal cord are damaged. In this respect, directly converted iOPCs can be considered as an appropriate treatment for MS.

Recently, two research groups have reported that direct conversion of mouse fibroblast to iOPCs. Najm F.J et al. showed that the delivery of three transcription factors (*Sox10, Olig2,* and *Nkx 6.2*) converted the mouse fibroblasts to iOPCs [8]. This group performed the direct conversion of iOPCs from mouse fibroblasts by infecting eight transcription factors (*ST18, Gm98, Myt1, Nkx2.2, Nkx6.2, Olig1, Olig2* and *Sox10*). After screening of the eight selected transcription factors, three of them (*Sox10, Olig2* and *Nkx 6.2*) turned out to be sufficient to generate iOPCs [8]. Another group, Yang, N et al., showed that a different combination of three transcription factors (*Sox10, Olig2,* and *Zfp536*) converted mouse fibroblast to iOPCs [9]. To verify the functionality of iOPCs, they were transplanted into disease model mice. 12 weeks after injection, it was demonstrated that iOPCs can differentiate into myelinogenic oligodendrocytes expressing oligodendrocyte markers as well as dense myelin sheaths [9].

3.2 Induced neural stem cells (iNSCs)

Induced neural stem cells (iNSCs) which are directly converted from somatic cells are expected to have great potential for clinical applications to a wide range of neurodegenerative disorders including PD, ALS, MS, AD, brain injury and spinal cord injury. Many research groups have generated iNSCs from the mouse and human fibroblasts. Thier et al. generated iNSCs transdifferentiated from MEFs by using the Yamanaka factors (*c-Myc, Klf4, Sox2,* and *Oct4*) and strictly regulating *Oct4* expression for the first five days [10]. Vierbuchen, T. and his colleagues converted MEFs into iNSCs using five factors (*c-Myc, Klf4, Sox2, E47,* and *Brn4*) [11]. Ring, K.L et al. showed the same results not only in the mouse but also in the human fibroblasts using a single factor (*SOX2*) with high efficiency on coverslips coated with

NSC-permissive substrates poly-L-ornithine and laminin [12]. Han et al. reported that the combination of *Brn4, Klf4, c-Myc, Sox2* and *E47* converted mouse fibroblasts to iNSCs as evidenced by the presence of typical NSC markers (*Sox2, Olig10* and *Mash/Ascl1*). These iNSCs can easily differentiate into functional neurons or neuroglial cells [13]. This raises hope for clinical applications in neurodegenerative disorders.

4   Conclusions

Direct conversion technology offers a new strategy for effective and safe treatment for neurodegenerative diseases. This technology might be the platform for approaches to treat incurable neural disorders. In contrast to iPS technology, somatic cell direct conversion is rapid and safe. Compared to iNs studies the conversion of somatic cells into multipotent stem cells holds enormous potential for a cell-based therapy, disease modeling, and drug screening. iMNCs are promising for cell therapies because they can be expandable *in vitro* culture system and be differentiated into a desire cell fate. In addition, they are valuable resources for understanding the mechanism of neurodegenerative diseases. Future studies will unveil the mechanism of direct conversion to increase the efficiency in generating iMNCs.

Reference

1. Connick, P., R. Patani, and S. Chandran, Stem cells as a resource for regenerative neurology. Pract Neurol, 2011. **11**(1): p. 29-36.
2. Takahashi, K., et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell, 2007. **131**(5): p. 861-72.
3. Graf, T., Historical origins of transdifferentiation and reprogramming. Cell Stem Cell, 2011. **9**(6): p. 504-16.
4. Caiazzo, M., et al., Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature, 2011. **476**(7359): p. 224-7.
5. Kim, J., et al., Functional integration of dopaminergic neurons directly converted from mouse fibroblasts. Cell Stem Cell, 2011. **9**(5): p. 413-9.

6. Liu, X., et al., Direct reprogramming of human fibroblasts into dopaminergic neuron-like cells. Cell Res, 2012. **22**(2): p. 321-32.
7. Son, E.Y., et al., Conversion of mouse and human fibroblasts into functional spinal motor neurons. Cell Stem Cell, 2011. **9**(3): p. 205-18.
8. Najm, F.J., et al., Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. Nat Biotechnol, 2013. **31**(5): p. 426-33.
9. Yang, N., et al., Generation of oligodendroglial cells by direct lineage conversion. Nat Biotechnol, 2013. **31**(5): p. 434-9.
10. Thier, M., et al., Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell, 2012. **10**(4): p. 473-9.
11. Vierbuchen, T., et al., Direct conversion of fibroblasts to functional neurons by defined factors. Nature, 2010. **463**(7284): p. 1035-41.
12. Ring, K.L., et al., Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. Cell Stem Cell, 2012. **11**(1): p. 100-9.
13. Han, Dong W., et al., Direct Reprogramming of Fibroblasts into Neural Stem Cells by Defined Factors. Cell Stem Cell, 2012. **10**(4): p. 465-472.