Recent Progress in Cell Reprogramming Technology for Cell Transplantation Therapy

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Abstract

The discovery of induced pluripotent stem (iPS) cells opened the gate for reprogramming technology with which we can change the cell fate through overexpression of master transcriptional factors. Now we can prepare various kinds of neuronal cells directly induced from somatic cells. It has been reported that overexpression of a neuron-specific transcriptional factors might change the cell fate of endogenous astroglia to neuronal cells in vivo. In addition, some research groups demonstrated that chemical compound can induce chemical-induced neuronal cells, without transcriptional factors overexpression. In this review, we briefly review recent progress in the induced neuronal (iN) cells, and discuss the possibility of application for cell transplantation therapy.

Key words: induced pluripotent stem cells, induced neuronal cells, in vivo direct reprogramming, chemical-induced neuronal cells, stroke

Introduction

Stroke is the second leading cause of death in the world, and results in a drastic reduction in the quality of life. However, effective therapeutic method is now very limited, especially in the chronic phase of a stroke, therefore a novel therapeutic strategy for the chronic phase of a stroke is now required. Recently, the discovery of induced pluripotent stem (iPS) cells opened the gate for stroke regenerative therapy, because iPS cell can produce patient-derived neurons. In addition, recently direct reprogramming methods has been established. Both induced neuronal stem (iNS) cells and induced neuronal (iN) cells, can be directly produced from somatic cells.

In this review, we briefly review recent progress in the iN cells, and discuss the possibility of application for cell transplantation therapy of post-stroke patients.

I. iPS cells technology

In 2006, Prof. Yamanaka firstly established murine iPS cells by overexpressing four transcriptional factors (Oct3/4, Sox2, c-Myc, and Klf4) in mouse fibroblasts. Of note, they found that these key transcription factors (TFs) from 20 candidates were strongly expressed in embryonic stem (ES) cells.1) iPS cells can retain high replication competence and pluripotency, and can differentiate into various kinds of cells. The iPS cells characteristics were very similar to ES cells, indicating that overexpression of key TFs can change cell fate. Since iPS cells can be induced from a patient’s skin fibroblasts, there are no immunoreactive and/or ethical issues, which are found in ES cells. Therefore, iPS cells are believed to be a promising cell resource for cell transplantation/replacement therapy. Several scientific papers have demonstrated that human iPS cells-derived neuronal stem cells/neuronal progenitors, when transplanted into the stroke murine model brain, showed a therapeutic effect such as the recovery of motor function. For example, Oki et al. produced long-term self-renewing neuroepithelial-like stem cells from adult human fibroblast-derived iPS cells, and transplanted them into the stroke mouse model. They found that motor function had already recovered at the time point of first week after transplantation. Functional recovery was observed soon after cell transplantation, then the observed therapeutic effect was regarded to be derived from neurotrophic factors released from transplanted cells.2) It is well known that only the replacement of injured neuron cannot contribute for stroke recovery. Transplantation of exogenous
cells including mesenchymal stem cells, which is also promising cell resource, is also currently being investigated for stroke and other neurological disorders.\textsuperscript{3,4}

II. Discovery of iN cells

Some Japanese research groups have started or planned to conduct clinical transplantation therapy trials using iPS cells for age-related macular degeneration, spinal cord injury, and Parkinson disease.\textsuperscript{5} However, iPS cells might form tumors, especially in pathological conditions such as post-stroke.\textsuperscript{6} In addition, it is likely to be difficult to monitor tumor formation for more than 2 years, because animal model cannot survive longer period. Therefore, a new technology and strategy supplying neuronal cells to damaged brains, is required. Research findings of iPS cells suggested that overexpression of ES cell-specific TFs could convert fibroblasts to ES cell-like iPS cells. From this finding, many researchers have overexpressed neuron-specific TFs in skin/lung fibroblasts, in order to convert these fibroblasts into neuronal cells. In 2010, Wernig et al. firstly established murine iN cells by overexpressing three neuron-specific TFs (Ascl1, Brn2, and Myt1l) in mouse fibroblasts. They found that these iN cells showed a glutamatergic neuronal phenotype with action potential, as recorded by electric patch-clump analysis.\textsuperscript{7} Until now various kinds of iN cells, such as dopaminergic neurons and motor neurons, have been reported (Table 1).\textsuperscript{8–23} Interestingly, Ascl1 appears to be a key factor in the induction of iN cells, and the specific combination of Ascl1 plus other factors can convert somatic cells to specific neuronal cells. In terms of cell transplantation therapy, it has already been reported that induced dopaminergic neurons transplantation increased the level of striatal dopamine, showing a therapeutic effect in 6-hydroxydopamine (6-OHDA)-treated rats.\textsuperscript{8} Compared with iPS cells, iN cells are regarded as safer, and easier to induce within a relatively short time frame. But the iN cell conversion process stops cell cycle making it difficult to prepare sufficient quantities of iN cells for cell transplantation therapy. iNS cells were developed to overcome this problem. In 2012, Han et al. found that a combination of TFs (Sox2, Brn4, Klf4, c-Myc) that successfully induced mouse fibroblasts

| Target cells       | Original cells                  | Combination of transcriptional factors for reprogramming | Reference                  |
|--------------------|---------------------------------|----------------------------------------------------------|-----------------------------|
| Glutamatergic neurons | mice fibroblasts, mice hepatocytes | Ascl1, Brn2, Myt1                                        | Vierbuchen et al., 2010\textsuperscript{7} |
|                    | human fibroblasts               | Ascl1, Brn2, Myt1, NeuroD1                                | Marro et al., 2011\textsuperscript{10} |
|                    | astroglia in stab-injured cortex | NeuroD1                                                  | Pang et al., 2011\textsuperscript{11} |
|                    | human fibroblasts               | Brn2, Myt1, miR-124                                      | Qiang et al., 2011\textsuperscript{12} |
|                    | astroglia in stab-injured cortex | NeuroD1                                                  | Yoo et al., 2011\textsuperscript{13} |
|                    | mouse fibroblasts               | CHIR99021, Forskolin, I-BET151, ISX9                     | Ambasudhan et al., 2011\textsuperscript{14} |
|                    | human fibroblasts               | CHIR99021, Forskolin, VPA, Repsox, SP600125, GO6983, Y-27632 | Guo et al., 2014\textsuperscript{15} |
| Dopaminergic neurons | mice/human fibroblasts          | Ascl1, Lmx1a, Nurr1                                       | Hu et al., 2015\textsuperscript{15} |
|                    | mouse fibroblasts               | Ascl1, Lmx1a, Nurr1, Pitx3, Foxa2, En1                   | Li et al., 2015\textsuperscript{15} |
|                    | human fibroblasts               | Ascl1, Brn2, Myt1, Lmx1a, FoxA2                          | Caiazzo et al., 2011\textsuperscript{16} |
| Motor neurons      | mouse/human fibroblasts         | Ascl1, Brn2, Myt1, NeuroD1, Lhx3, Hb9, Isl1, Ngn2       | Pfisterer et al., 2011\textsuperscript{17} |
| Neural stem cells  | mouse fibroblasts               | Sox2, Brn2, FoxG1                                        | Son et al., 2011\textsuperscript{18} |
|                    | mouse fibroblasts               | Sox2, Brn4/Pou3f4, Klf4, c-Myc, E47/Tcf3                 | Lujan et al., 2011\textsuperscript{19} |
|                    | mouse/human fibroblasts         | Sox2                                                     | Han et al., 2012\textsuperscript{20} |
|                    |                                  |                                                          | Ring et al., 2012\textsuperscript{21} |

Modified from Yamashita et al., 2014\textsuperscript{23}
directly to iNS cells.\textsuperscript{9} Han et al. evaluated the therapeutic effect of cell transplantation using iNS cells in the spinal cord injury rat model. They also found that engrafted iNS cells could differentiate into neuronal lineages with synapses, enhancing the recovery of locomotor function.\textsuperscript{24} Therefore, iNS cells can be regarded as a promising cell resource candidate for cell transplantation/replacement therapy (Fig. 1).

III. Development of direct reprogramming technology

Recently, a lot of novel findings in the field of iN cells are reported every year. In particular, \textit{in vivo} direct conversion technology and chemical-induced neuronal (CiN) cells are attracting the most attention. In a clinical setting, the culture medium, including calf/bovine serum, can be problematic as they may be infectious materials against the human body. Thus, if endogenous non-neuronal cells such as astroglia can be converted to required neurons called as “\textit{in vivo} direct conversion,” it could be a simple and straightforward way of supplying required new neuronal cells to the injured brain. Until now, astroglia as well as pericytes have been reported to be directly reprogrammed into neuronal cells in cell culture systems.\textsuperscript{25,26} In 2013, Torper et al. showed that endogenous mouse astroglia could be converted into NeuN-positive neuronal cells \textit{in vivo}.\textsuperscript{27} In 2014, Guo et al. reported that reactive glial cells in the cortex of the stab-injured mice model could be directly reprogrammed into functional neurons \textit{in vivo} by overexpressing a single neural TF, NeuroD1.\textsuperscript{15} These findings suggested that \textit{in vivo} direct reprogramming technology is a hopeful method supplying required neurons for the human central nervous system.

In 2015, two different research teams published that CiN cells could be established using a cocktail of chemical compounds including forskolin (a cyclic adenosine monophosphate agonist), and CHIR99021 (a glycogen synthase kinase 3 beta inhibitor) (Fig. 1).\textsuperscript{21,22} In this method, mouse/human skin fibroblasts were successfully converted to neuronal cells without overexpressing TFs, indicating that the chemical cocktail can replace previously reported reprogramming TFs, leading to easier and more stable reprogramming methods that supply neuronal cells.

Conclusion

This review briefly highlights recent progress in the development of direct reprogramming technology for cell transplantation therapy. Especially \textit{in vivo} direct reprogramming technology may be a simple and hopeful method as new cell replacement therapy, because cell preparation and transplantation are not required. Clinical trials using iPS cells are ongoing, but it is important to combine these technologies or to choose appropriate strategies depending on the target disease.

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Conflicts of Interest Disclosure

The authors declare no conflicts of interest.

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