Neural Progenitors by Direct Reprogramming: Strategies for the Treatment of Parkinson's and Alzheimer's Diseases

Changhai Tian1,2 and Jialin C. Zheng1,2,3,*

1Center for Translational Neurodegeneration and Regenerative Therapy, Shanghai Tenth People's Hospital affiliated to Tongji University School of Medicine, Shanghai, China
2Department of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, Nebraska
3Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska

Age-related neurodegenerative diseases, such as Parkinson's (PD) and Alzheimer's (AD) disease, are seriously threatening the health of Chinese citizens, with PD and AD patient populations increasing at a rate of 100,000 and 300,000 per year, respectively (1, 2). Although there is no cure for AD or PD, a variety of medications can provide relief from some of the symptoms. Recently, the development of direct reprogramming technologies has shed light on promising strategies for stem cell-based therapies for AD and PD. This review will focus on neural progenitor cells and induced neural progenitor cells studied in our laboratory, and discuss their potential applications in AD and PD treatment.

Neural Progenitors from Fetal Tissues

Multipotent neural progenitor cells (NPCs) exist in the mammalian developing and adult nervous system, and are capable of migrating toward the specific sites and giving rise to the main components of the nervous system. Transplantation of NPCs is a promising therapy for various neurodegenerative diseases and brain injuries (3–5). It has been reported that the transplantation of NPCs into the brain contributes to the improvement of cognitive impairment in animal models of PD and AD. This occurs through cell replacement, the release of specific neurotransmitters, and the production of neurotrophic factors that protect injured neurons and promote neuronal growth (6–10). The transplantation of NPCs derived from fetal brain raises serious ethical concerns, particularly in the acquisition of the fetal tissue, but the understanding gained from these in vitro experiments will be useful for the future application of NPCs obtained from non-fetal sources. Our laboratory has been working on human NPCs for many years and has revealed that C-X-C motif chemokine 12 (CXCL12, also known as stromal cell-derived factor 1) plays an important role not only in increasing cell proliferation through the Akt/Foxo3a signaling pathway, but also in protecting against NPC apoptosis through chemokine receptor CXCR7- and CXCR4-mediated endocytotic signaling pathways (11, 12). These data provide insight into the

*Corresponding Author: jzheng@unmc.edu.
essential role for CXCL12 in neurogenesis and also suggests a novel role for CXCR7 in NPC survival contributing to neurogenesis, as well as potentially offering some theoretical guidance for NPC-based therapy.

**Neural Progenitor Cells Generated by Direct Reprogramming**

Selective degeneration of functional neurons is associated with the pathogenesis of neurodegenerative disorders, such as degeneration of midbrain dopaminergic neurons in PD (13) and forebrain cholinergic neurons in AD (14). How to achieve sufficient cell replacement to halt PD or AD progression, or possibly even provide a cure, is the main challenge. The discovery of induced pluripotent stem (iPS) cells has facilitated the derivation of stem cells from adult somatic cells for the personalized treatment of PD and AD without depending on fetal tissues. However, ethical and safety concerns still exist. Recently, neuronal subtypes including dopaminergic and cholinergic neurons have been generated successfully through direct reprogramming of somatic cells by expression of developmental genes (15–17). The low yield of neurons from this method has, however, limited its broad application in cell transplantation. In addition to NPCs obtained from iPS cell differentiation (Figure 1, path A) and neuronal subtypes induced by direct reprogramming (Figure 1, path E), the direct reprogramming of NPCs from differentiated, non-neuronal somatic cells (Figure 1, paths B and C) (18–21) will not only provide an alternative to fetal tissue or pluripotent cells as precursors, but also furnish a potentially unlimited source of neurons. Our laboratory was among the first to successfully convert fibroblasts into induced NPCs (iNPCs) by ectopic expression of transcription factors. iNPCs share many characteristics with primary NPCs and are able to differentiate into neurons (Figure 2). Recently, the direct reprogramming of iNPCs has also been achieved by forced expression of single factor (22) or by 3D sphere culture of fibroblasts on low attachment surfaces (23). These findings suggest that neural progenitor cell fate can be reprogrammed by modifying intrinsic and extrinsic cues.

Stem cell-based therapy for AD and PD is essentially based on the regeneration of different neuronal subtypes, such as dopaminergic neurons in PD and forebrain cholinergic neurons in AD. Recently, we have been working on the direct reprogramming of somatic cells into region-specific iNPCs (Figure 1, path D) as well as subtype-specific iNPCs (Figure 1, path F) by overexpression of defined growth factors. Both of these pathways show promise for AD and PD therapies. Direct in vivo conversion of somatic cells, such as fibroblasts and astrocytes, into functional neurons has also been achieved (24), providing proof of principle that it may be feasible to convert somatic cells—such as activated astrocytes—into region-or subtype-specific iNPCs in the brains of AD and PD patients. In the future, the further development of this technology will undoubtedly provide promising strategies for the effective treatment of AD and PD.

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References

Figure 1. Strategies to Generate iNPCs from Somatic Cells

iNPCs can be induced by conceptually different mechanisms. (A) Ectopic expression of Yamanaka factors converts somatic cells to induced pluripotent (iPS) cells that can then be differentiated into primitive iNPCs, and subsequently into different subtypes of neurons based on differentiation conditions. (B) Ectopic expression of Yamanaka factors converts somatic cells to primitive iNPCs through an intermediate unstable pluripotent stage. (C) Direct reprogramming of primitive iNPCs by expression of lineage-specific transcription factors. (D) Direct reprogramming of region-specific iNPCs by expression of lineage-specific transcription factors and location-specific determinants. (E) Generation of neuronal subtypes through direct conversion of cells in vitro and in vivo. (F) Generation of neuronal progenitor subtypes through direct conversion of somatic cells using sets of defined transcription factor.
Figure 2. Direct Conversion of Fibroblasts into NPCs by a Novel Combination of Transcription Factors

(A) Schematic showing direct reprogramming of fibroblasts derived from E/Nestin:EGFP transgenic mice into NPCs using defined transcription factors including Brn2, Sox2, Bmi1, TLX and c-Myc. (B) Differentiation of iNPCs into neurons (panel a; MAP-2, red), astrocytes (panel b; GFAP, red), oligodendrocytes (panel c; O4, red). Panel d shows synapse formation between neurons (β-III Tubulin, green; Synaptophysin, red), with magnification of white box in d shown in panel e. Nuclei are stained with DAPI (blue). EGFP, enhanced green fluorescent protein; MAP-2, microtubule-associated protein 2; GFAP, glial fibrillary acidic protein; O4, marker for oligodendrocytes.