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Induced neural stem cells: Methods of reprogramming and potential therapeutic applications

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ABSTRACT

Developmental studies and experimental data have enabled us to assert that the terminal cell differentiation state is reversible, and that altering the balance of specific transcription factors could be a powerful strategy for inducing pluripotency. Due to the risks related to using induced pluripotent cells in clinical applications, biologists are now striving to develop methods to induce a committed differentiated cell type by direct conversion of another cell line. Several reprogramming factors have been discovered, and some cellular phenotypes have been obtained by novel transdifferentiation processes. It has been recently demonstrated that induced neural stem cells (iNSCs) can be obtained from rodent and human somatic cells, like fibroblasts, through the forced expression of defined transcription factors. To date, two different approaches have been successfully used to obtain iNSCs: a direct method and an indirect method that involves an intermediate destabilized state. The possibility to induce characterized iNSCs from human cells, *e.g.* fibroblasts, has opened new horizons for research in human disease modelling and cellular therapeutic applications in the neurological field.

This review focuses on reported reprogramming techniques and innovative techniques that can be further explored in this area, as well as on the criteria for the phenotypic characterization of iNSCs and their use in developing novel therapeutic strategies for neurological diseases.

Contents

Abbreviations: BrdU, bromodeoxyuridine; bHLH, basic helix–loop–helix; CNS, central nervous system; CPPs, cell-penetrating peptides; DCX, doublecortin; EGF, epidermal growth factor; (E)GFP, (enhanced) green fluorescent protein; ESCs, embryonic stem cells; FGF, fibroblast growth factor; GFAP, glial fibrillary acidic protein; hiNSCs, human induced neural stem cells; iMN, induced motoneurons; iNPCs, induced neural precursor cells; iN, induced neuronal cells; iNSCs, induced neural stem cells; iPSCs, induced pluripotent stem cells; LIF, leukemia inhibitory factor; MAP2, microtubule-associated protein 2; MEFs, mouse embryonic fibroblasts; mRNA, messenger RNA; miRNAs, microRNAs; NSCs, neural stem cells; PLP, oligodendroglial proteolipid protein; PTDs, protein transduction domains; RiPSCs, RNA-induced pluripotent stem cells; Sox2, Sry-related high mobility group box transcription; TH, tyrosine-hydroxylase; Tuj1, III-β tubulin.

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1. Introduction

It has been demonstrated that pluripotency can be restored to adult somatic cells through ectopic co-expression of defined transcription factors, thus proving that the fate of somatic cells is not immutable and paving the way for modelling human diseases and personalized cell therapies (Maherali et al., 2007; Okita et al., 2007; Takahashi et al., 2007; Takahashi and Yamanaka, 2006; Wernig et al., 2007). Since the seminal study of Takahashi and Yamanaka (2006), substantial advancements have been made to improve both the efficiency and safety of the reprogramming steps. Disease- and patient-specific induced pluripotent stem cell (iPSC) lines have been established for several disorders, including major neurodegenerative diseases, such as Parkinson's disease (Park et al., 2008; Soldner et al., 2011), amyotrophic lateral sclerosis (Dimos et al., 2008), spinal muscular atrophy (Ebert et al., 2009), Alzheimer's disease (Israel et al., 2012), Huntington's disease (Park et al., 2008), and schizophrenia (Brennand et al., 2011). However, the generation of patient-specific iPSCs is still a technical and time-demanding procedure and problems, such as epigenetic changes during the reprogramming process, must be addressed before iPSC technology can be routinely used.

Moreover, both undifferentiated iPSCs and the cells derived from them harbour potential tumourigenic risks (Fong et al., 2010; Miura et al., 2009; Yamanaka, 2009); this limits their direct use in cell transplantation applications and makes their differentiation into the desired cell type highly necessary.

The limitations of iPSC technology have prompted investigation into the possibility of directly reprogramming adult cells to become the desired lineage phenotype, bypassing the step of a pluripotent state. There is evidence that forced expression of determined transcription factors can convert one differentiated cell type into another one. In a first set of experiments, fibroblasts were directly reprogrammed into induced neuronal (iN) cells by inducing the expression of neuronal lineage-specific transcription factors; Vierbuchen et al. (2010) demonstrated that the combined expression of only three factors—Ascl1, Brn2 (alternatively known as Pou3f2), and Myt1l—was sufficient to rapidly and efficiently obtain functional neurons from mouse embryonic and postnatal fibroblasts. The iN cells showed neuronal properties, electrophysiological activity, and the capacity to form synapses, and they did not maintain the morphological and molecular features of the initial donor cells (Vierbuchen et al., 2010). These findings clearly showed that the over-expression of few "master" factors is sufficient to drive a relatively rapid, direct specific lineage change in cells derived from different embryonic layers. This represents an important goal because previous works reported direct reprogramming of cells into different cell lines deriving from the same germinal layer, like the differentiation of adult pancreatic exocrine cells into beta-cells (Zhou et al., 2008). Generation of iN cells from non-neural lineages could represent the starting point for using these cells in regenerative medicine, and could also be useful for improving our knowledge about neural development and neurological disease pathogenesis.

Additional studies have demonstrated that expression of subtype-specific regulator factors in mouse and human fibroblasts results in the establishment of specific neuronal subtypes, like dopaminergic neurons (Caiazzo et al., 2011; Pfisterer et al., 2011; Liu et al., 2012) and motoneurons (Son et al., 2011). Furthermore, fibroblasts have now also been directly converted into other cell types, including cardiomyocytes (Efe et al., 2011; Ieda et al., 2010), blood cell progenitors (Szabo et al., 2010), hepatocytes (Huang et al., 2011; Sekiya and Suzuki, 2011), retinal pigment epithelium-like cells (Zhang et al., 2013), myocytes (Bichsel et al., 2013), embryonic Sertoli-like cells (Buganim et al., 2012), and adipocytes (Zhu et al., 2012).

However, because differentiated cells are post-mitotic, not dividing cells, it is a challenge to generate sufficient amounts of cells for further basic and clinical applications. To overcome these obstacles, recent experimental works have focused on the direct differentiation of fibroblasts into stem cells without going through an iPS state. For example Han et al. (2011) demonstrated the possibility to direct reprogram fibroblasts into epiblast stem cells using the same Yamanaka's four factors (Oct4, Sox2, Klf4 and c-Myc), but overcoming iPS stage under appropriate culture conditions. As regarding our interest, several papers showed direct conversion of fibroblasts into induced neural stem cells (iNSCs), which are characterized by the ability to self-renew and differentiate into neurons and glia, providing a potentially unlimited source of neuro-ectodermal cells.

In this review, we discuss recent advancements in the direct reprogramming of fibroblasts into multipotent neural stem/progenitor cells (Fig. 1), and suggest criteria to successfully identify reprogrammed cells and perspectives for clinical applications.

2. Conversion of fibroblasts into iNSCs: how to achieve this goal?

The establishment of iNSCs from fibroblasts has become a hot topic among stem cell scientists. Two different reprogramming

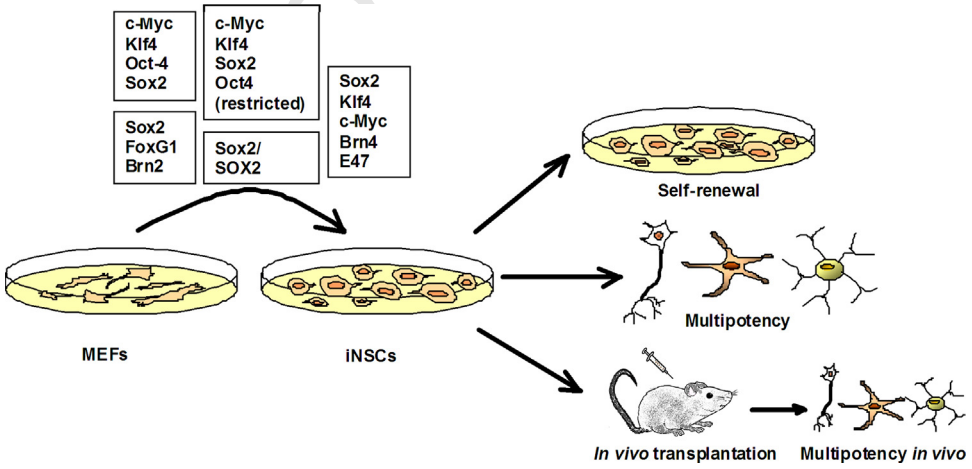

Fig. 1. Direct reprogramming of fibroblasts into iNSCs, and their features. Culturing fibroblasts with reprogramming factors (c-Myc, Klf4, Oct4, Sox2, FoxG1, Brn2, Brn4, and E47) in different combinations, as described in the text, produces iNSCs with self-renewal potentiality. iNSCs can differentiate into neurons, astrocytes, and oligodendrocytes *in vitro* and *in vivo* after transplantation.

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