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Lineage reprogramming
A shortcut to generating functional neurons from fibroblasts

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A series of recent publications have shown that human fibroblasts can be directly converted to functional neurons using defined combinations of transcription factors.[1-3] The resulting neurons are called induced neurons (iN). When compared with induced pluripotent stem (iPS) cell technology, where somatic cells can be reprogrammed to neurons via a pluripotent stem cell state, lineage reprogramming offers an appealing short cut to generate functional neurons from fibroblasts that has several advantages: the process is quicker, less laborious and does not involve a stem cell intermediate.[4] Thus, the generation of iNs opens up new and exciting possibilities for generating patient- and/or disease-specific neurons for disease modeling and brain repair.

Generation of dopamine (DA) neurons using direct conversion is of particular interest due to their involvement in Parkinson disease (PD). By tailoring the cocktail of conversion factors to include DA neuron fate determinants, iN cells expressing genes and proteins characteristic of dopamine neurons can be formed from mouse and human fibroblasts.[1,3] Dopaminergic iN cells can be obtained from fibroblasts using different sets of transcription factors (Ascl1, Brn2, Myt1l, FoxA2 and Lmx1a[3] or Ascl1, Nurr1 and Lmx1a[1]). Both transcription factor cocktails give rise to DA-iNs displaying functional properties of DA neurons in vitro, as demonstrated by whole-cell patch clamp recordings showing spontaneous action potentials, including pacemaker-like activity and potassium-evoked DA release[1,3,5] (summarized in **Fig. 1A**).

Encouraging results shows that mouse iN cells survive transplantation into neonatal animals.[1] The in vitro data for the human iN (hiNs) cells are also promising and point toward functioning DA neurons being formed via direct conversion of fibroblasts. However, it is now necessary to test their ability to survive and integrate in the adult brain and to confirm their capacity to restore functionality in animal models of PD (**Fig. 1B**). Human cells mature slowly after grafting, and a major obstacle facing these kinds of studies is the severe immune reaction induced by human cells transplanted into rodents. The immune reactions induced by such discordant xenografts are difficult to suppress long-term by standard immunosuppressive treatments without severe health problems of the treated host.[6] The use of newborn or genetically modified immune-compromised animals can at least partly override these difficulties, but the use of such animals may preclude some necessary functionality tests. Nevertheless, there are a number of ways through which functional integration of DA neurons after transplantation can and should be assessed.

First, the long-term survival and phenotype of the grafted hiNs should be confirmed on a histological level. This analysis should include analysis of graft content using antibodies specific for human cells in combination with markers specific for neurons, fibroblasts and glia as well as markers to exclude proliferating cells in the graft. The dopaminergic properties of the neurons should be assessed after grafting by expression of the necessary proteins for DA handling, such as TH, VMAT2, DAT. Furthermore, innervation of target structures should be studied, and the mesencephalic identity of the DA neurons should be confirmed using an exhaustive list of markers, including Pitx3, Nurr1, En1, Lmx1a/b and FoxA2.

Second, electrophysiological recordings and voltametric/amperometric techniques can be used to assess DA release and functional integration with the host neurons.[7] Using these methods, it is possible to witness live stimulation and/or DA release events. Specific pharmacological manipulation to determine active and functional dopamine autoreceptors (D_2) and transporters (VMAT2/DAT) or optogentic inhibition/stimulation of pre- and post-synaptic contacts can be included to refine the analysis.[8] Such analytical techniques will provide valuable and necessary high-resolution information on the synaptic activity and neurochemical behavior of transplanted iN cells.

Lastly, functional restoration of motor behaviors is primarily dependent on functional innervation of the grafted cells, and disease-related functionality is typically established using behavioral tests that model the motor symptomology of PD.[9] Therefore, combinations of different spontaneous and drug-induced behavioral tests that model various aspect of PD should be used to determine if grafted DA-iNs restore motor deficits in rodent models of the disease.[10]

We foresee that there will be a major focus in the field to develop new and safe methods for generating human iN cells that will make them suitable for clinical use, paralleling the developments of the iPS field. A major advantage of iNs over iPS cells is that they are, at least in theory,

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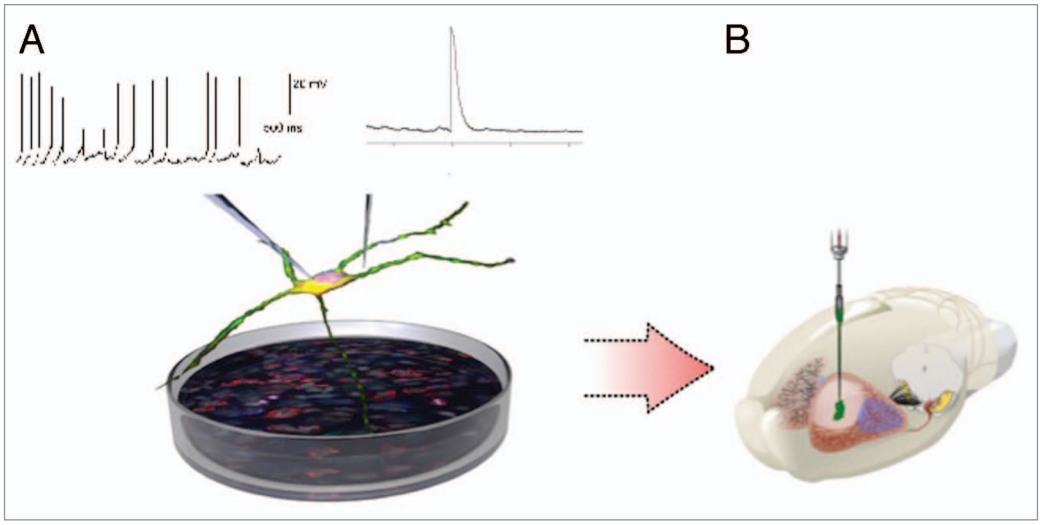

Figure 1. Dopaminergic iN cells can be generated via direct conversion of fibroblasts using defined sets of transcription factors.[1,3] This results in iNs with functional properties of DA neurons in vitro, as demonstrated by whole-cell patch clamp recordings (left graph in A) and potassium-evoked DA release (right graph in A). The functionality of human DA-iN cells remains to be determined after grafting in animal models of PD (B).

much less likely to generate tumors associated with grafting of pluripotent stem cells.[11] If the human DA-iN cells holds up to their promise and prove to be safe, survive long-term and restore function in the diseased brain, they will be a prime candidate cell source when developing future strategies for regenerative medicine.

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