Potential of dopamine neurons derived from human induced pluripotent stem cells reprogrammed by mRNA for Parkinson's disease

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Résumé

Parkinson's disease (PD) is a neurodegenerative disorder associated with a progressive loss of dopaminergic (DA) neurons in the substantia nigra *pars compact* (SNpc), leading to a loss of dopamine in the target brain region, the striatum. Cell replacement therapy in PD aim to restore the DA neurotransmission by transplanting new midbrain dopamine neurons precursors. Human induced pluripotent stem cells (hiPSC) is one of the most promising source of cells for autologous transplantation as these cells are theoretically able to differentiate into any cell type of any organ. Besides, they are an ethical and unlimited source of transplantable cells and potentially reduces the risk of transmissible infections and immune reactions following cellular therapy
Here, we used adult human dermal fibroblasts to reprogram to a pluripotent state, using following mRNAs reprogramming factors OCT4, SOX2, KLF4, c-MYC, NANOG and LIN28. The mRNA technology permits the generation of hiPSC under non-hypoxic, feeder-free conditions and eliminates the risk of genomic integration. After characterization of the pluripotency of hiPSC, we induced the *in vitro* differentiation into dopaminergic neurons using a three-step system. During differentiation, the cells were first induced into midbrain-specified floor plate progenitor cells. After expansion, the cells were differentiated into mature dopaminergic neurons.

After 25 days of differentiation, the cells expressed DA precursor markers Nurr1 (38,7 ± 9%) and FoxA2 (71 ± 6,1%). After 51 days of differentiation, 43,6 ± 13% of the cells are NeuN+. Among these neurons, around 70% are tyrosine hydroxylase + (TH). We grafted hiPSC derived DA precursors (day 25 of differentiation) into the 6-OHDA lesioned SN in a NOD SCID mice model of PD. Eight months after transplantation, 46% of grafted cells are NeuN+ and 35,5% of neurons express DA marker TH. Among DA neurons, 46,3% are GIRK2+, a marker of SNpc DA neurons. Furthermore, iPSC-derived dopamine neurons can innervate the correct target structure, the dorsal striatum. Large numbers of hNCAM+ axons extending rostrally along the nigrostriatal pathway and the medial forebrain bundle toward target area the dorsal striatum. In the dorsal striatum, graft-derived hNCAM+ fibers co-expressed TH. Interestingly, grafted animals show functional recovery in the apomorphine-induced rotation

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test, 6 months after transplantation. Moreover, the observed functional recovery is correlated with the number of TH neurons present in the graft (Spearman r = 0,8214, p< 0,02).
We have shown the possibility to obtain DA neurons of SNpc subtype from mRNA-reprogrammed hiPSC compatible with future clinical applications. More importantly, hiPSC-derived neurons grafted into the lesioned SN express midbrain specific DA markers and allow the reconstruction of degenerated nigrostriatal pathway in an animal model of PD.

Mots-Clés: Parkinson, hiPSC, Mouse model, Dopaminergic differentiation, Reprogrammation, ARNm, Transplantation, Graft, Dopaminergic neurons