DOPAMINE NEURONS DERIVED FROM HUMAN iPSCs SURVIVE AND REVERSE MOTOR ASYMMETRY IN ANIMAL MODELS OF PARKINSON’S DISEASE

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Introduction

Recent studies have indicated that human embryonic stem cells and induced pluripotent stem cells (iPSCs) differentiated into midbrain dopamine (iPSC-mDA) neurons provide functional benefit in animal models of Parkinson’s disease (PD) (Krich et al., 2011; Morisawa et al., 2011; Kralisch et al., 2014; Ballew et al., 2015). Cryopreservation of post-mitotic iPSC-mDA neurons represents a significant initial advancement for clinical translation of pluripotent stem cell technologies as they are readily and reproducibly thawed, allowing for rapid access to large numbers of highly pure, autologous cells. In the present study, we examined the engraftment potential of iPSC-mDA neurons after transplantation into the rodent brain. iPSC-mDA neurons were derived from epigenetically reprogrammed human blood samples and cryopreserved in large master cell banks. After thawing, iPSC-mDA neurons retained high viability and maintained gene and protein expression patterns consistent with the midbrain lineage (Kiri et al., 2011). Patch clamp recordings revealed normal electrophysiological characteristics with firing of evoked spontaneous action potentials, post synaptic currents, as well as functional ion channels with characteristics similar to native neurons. In addition, biochemical analysis of iPSC-mDA neurons indicated production of dopamine. Here we demonstrate reversal of motor asymmetry as measured by d-amphetamine- and apomorphine-induced rotations. Immunohistochemical analysis revealed robust long term graft survival and extensive reinnervation of the host striatum.

Methods

iPSC-mDA neurons were cryopreserved on day 18 of differentiation then thawed and plated before M1 isolation. Real-time quantitative PCR was performed using Taqman Gene Expression Assays. ANOVA for FoxA2/Tyr expression was applied to cryopreserved iPSC-mDA neurons at day 3 post-thaw. Immunocytochemistry for FoxA2/mAb and brightfield images for morphology were taken at day 7 post-thaw. Western blots were performed at 8-weeks post-thaw. Dopamine secretion by Cell Dopa Neurospheres stimulated by HHI or HHS with potassium chloride (KCl) was measured by ELISA at 14-days post-thaw. Single- and whole-cell patch clamp recordings were taken at day 21 post-thaw. Female Sprague Dawley rats received medial forebrain bundle injection (MFB) injections of 6-OHDA. Rats with confirmed behavioral losses were selected for transplantation (20-30 mg, 22-23 weeks of age) and immunomunoprecipitated daily with Cypate (100g/kg, 2% P.A.) beginning two days prior to injection. P1 (150kU/mg) iPSC-mDA were injected unilaterally into the striatum. AP=0.55mm, ML=3.21mm, DL=-5.0mm from bregma. 

Figure 1: iPSC-mDA Gene Expression Profile

Figure 2: Viability and Purity of iPSC-mDA Neurons

Figure 3: Biochemical Analysis of iPSC-mDA Neurons

Figure 4: DA Release and Electrophysiological Activity of iPSC-mDA Neurons

Figure 5: Striatal Graft Survival and Safety After 2 Weeks in vivo

Figure 6: Maintenance of Midbrain Dopaminergic Phenotype in Grafts in Vivo

Figure 7: Functional Analysis of Cryopreserved iPSC-mDA Neurons in Parkinsonian Rats

Figure 8: Three Month iPSC-mDA Graft Survival in MPTP-Treated Macaques

Conclusions

1. The three process yields high purity and viability of midbrain dopamine neurons with functional ion channels and physiologically relevant responses to drug inhibition in vivo.
2. iPSC-mDA maintain midbrain dopaminergic phenotype in vitro and following transplantation into the rat striatum.
3. Robust graft survival and fiber outgrowth found in both the rat and MPTP monkey brain.
4. Re-innervation of the host rat striatum induced full recovery in d-amphetamine-induced rotations.

Future Directions

2. Demonstrate long-term safety and functional efficacy with GMP iPSC-mDA neurons in pre-IND studies.