CHAPTER

Characterization and criteria of embryonic stem and induced pluripotent stem cells for a dopamine replacement therapy

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Abstract

Human pluripotent stem cells provide new choices for sources of A9-type dopaminergic (DA) neurons in clinical trials of neural transplantation for patients with Parkinson's disease (PD). For example, "self" and HLA-matched A9 DA neurons may improve the patient-to-patient variability observed in previous clinical trials using fetal DA neurons and obviate the need for long-term immunosuppression in the patient. Normal chromosomal structure and minimal somatic mutations in pluripotent stem cells are necessary criteria for assuring the safe and reproducible transplantation of differentiated DA neurons into patients with PD in clinical trials. However, with these new choices of cell source, the application of pluripotency assays as criteria to ensure pluripotent stem cell quality becomes less relevant. New more relevant standards of quality control, assurance, and function are required. We suggest that quality assurance measures for pluripotent stem cells need to focus upon readouts for authentic midbrain DA neurons, their integration and growth using *in vivo* assays, and their long-term functional stability.

Keywords

allogeneic transplantation, cell therapy, embryonic stem, induced pluripotent stem, isogenic transplantation, Parkinson's disease, quality control, transplantation

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1 INTRODUCTION

Stem cells are characterized by their capacity for self-renewal and differentiation. By definition, pluripotent stem cells can differentiate into any somatic lineage, as most stringently shown by tetraploid embryo complementation in the mouse. Practical and ethical concerns limit such stringent assays for examining the pluripotency of human cells. Instead, the formation of well-differentiated teratomas in immunodeficient mice is the current "gold standard" (Brivanlou et al., 2003). Such analysis is frequently complemented by immunocytochemistry and quantitative reverse transcription polymerase chain reaction for markers of pluripotency and all three germ layers after embryoid body differentiation, bisulfite sequencing to determine changes in the methylation of pluripotency-associated promoters and karyotype analysis by Giemsa staining of isolated metaphase chromosomes (G-banding) (Soldner et al., 2009). However, as the field moves toward using human pluripotent stem cells to provide cell types to treat diseases, the application of pluripotency assays as criteria to ensure cell quality becomes less relevant. In this chapter, we propose that the pluripotent characterization of cell sources for therapeutically relevant cell types is not central to future quality assurance efforts for cell therapy in Parkinson's disease (PD). Instead, we propose that quality assurance measures for pluripotent stem cells need to focus upon readouts for (1) effective and high yield differentiation of dopaminergic (DA) neurons that (2) do not proliferate in *in vivo* assays, (3) and are functionally stable.

2 CHARACTERIZING HUMAN PLURIPOTENT STEM CELL QUALITY AND SAFETY FOR CELL THERAPY IN PD

Clinical trials for PD have transplanted cell preparations dissected from the human fetal ventral midbrain (Fig. 1; Björklund et al., 1980; Brundin et al., 1988; Lindvall et al., 1988; Ma et al., 2010; Mendez et al., 2005; Piccini et al., 1999; Politis et al., 2010; Widner et al., 1992). This population of cells contains nigral (A9) and ventral tegmental area (A10) types of DA neurons, other types of non-DA neurons including serotonergic neurons, and glia (Mendez et al., 2005). Human pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, can provide an enriched population of therapeutically relevant A9 DA neurons needed for treating PD patients (Fig. 1; Bjorklund et al., 2002; Cooper et al., 2010; Deacon et al., 1998; Dinsmore et al., 1996; Hedlund et al., 2008; Kim et al., 2002; Kriks et al., 2011; Lee et al., 2000; Wernig et al., 2008). However, the clinical laboratory providing the differentiated neurons needs to be confident that the quality of the ES or iPS cells is sufficient for differentiation into A9 DA neurons that are safe and function over the long term after transplantation. Unlike other medical conditions, cell therapy for PD does not require an acute time frame to prepare cells. Each patient will most likely receive immature A9 DA neurons from a single differentiation preparation independent of pluripotent cell source. While iPS cells are clearly not ES cells (Bock et al.,

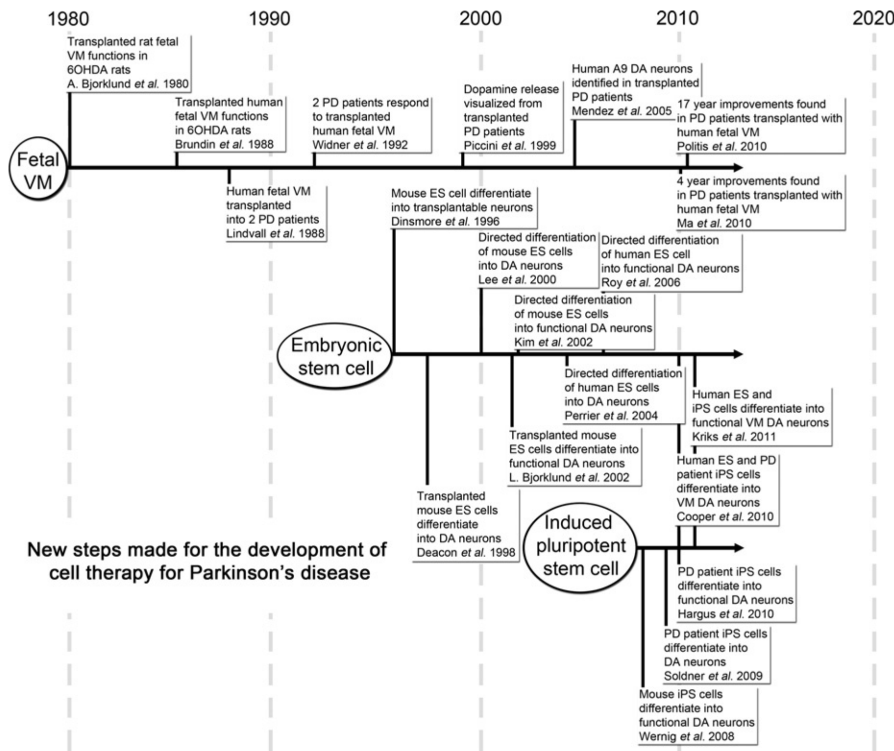

FIGURE 1

Outline of new steps made for the development of cell therapy for PD. Timeline of the scientific literature describing the critical steps in the progress of cell therapy for PD using immature neurons derived from fetuses, embryonic stem cells, and induced pluripotent stem cells.

2011; Chin et al., 2009; Doi et al., 2009; Soldner et al., 2009; Stadtfeld et al., 2010), our recent experimental studies have shown that neurons differentiated from sporadic PD patient-specific iPS cells are indistinguishable from neurons differentiated from other human pluripotent stem cell lines, independent of residual transgene expression and reprogramming methodology (Hargus et al., 2010; Soldner et al., 2009). However, the clinical application of human pluripotent stem cells to provide DA neurons requires higher standards of quality control and assurance. In particular, chromosomal disruption and somatic mutations have been demonstrated in many human pluripotent stem cell lines. The long-term significance of such genetic disruption in the starting pluripotent stem cell population is difficult to determine experimentally but may compromise transplanted DA neuron function and safety. Therefore, the quality of the pluripotent stem cells for each patient needs to be examined using additional assays.

3 THE RELEVANCE OF PLURIPOTENT STEM CELL-DERIVED DA NEURONS FOR CELL THERAPY IN PD

ES and iPS cells can provide a broad spectrum of histocompatibility for cell therapy, but the central nervous system is immunoprivileged. Currently, the extent of immunological compatibility required between grafted neurons and the patient for optimal treatment is an unresolved question. Clearly, human fetal A9 DA neurons that have not been specifically matched for histocompatibility with the patient can survive and function for at least 17 years after transplantation. Importantly, though, isogenic A9 DA neurons may improve the patient-to-patient variability observed in previous clinical trials using fetal DA neurons and obviate the need for long-term immunosuppression in the patient (Lindvall et al., 1989; Mendez et al., 2008; Politis et al., 2010).

4 EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS

Human ES cells are the most familiar form of pluripotent stem cells that can provide neurons for cell therapy. Banks of ES cells characterized for human leukocyte antigen (HLA) exist and are proposed to match neurons for the majority of recipients (Nakajima et al., 2007; Taylor et al., 2005). Banking efforts rely on the maintenance of high-quality ES cells over a prolonged period of time in culture. While DNA methylation patterns vary with time, human ES cells can acquire karyotypic changes (Amps et al., 2011). Therefore, careful analysis of human ES cell karyotype is required before transplanting differentiated neurons.

The frequency of quality control analysis for the pluripotent cells depends upon the cell type used. Recent studies have used nonintegrating vectors, mRNA, and recombinant proteins to generate iPS cells (Kaji et al., 2009; Okita et al., 2008; Warren et al., 2010; Woltjen et al., 2009; Zhou et al., 2009). Rarely, recombinant DNA from "nonintegrating" vectors can integrate and preventative research is needed. In contrast, mRNA and recombinant protein techniques are technically demanding but are associated with little risk of disrupting the host cell's genome.

Integration-free iPS cells represent a customized source of DA neurons that would require less frequent characterization, as the iPS cells would not need to be kept for many passages. In contrast, banked sources of ES cells would need regular characterization to ensure karyotypic stability without genetic or epigenetic changes that would compromise neither function nor safety. Whether ES or iPS cells are used, the karyotypic stability and genetic changes will need to be assessed periodically. However, the likelihood of a pluripotent stem cell acquiring a dysfunctional phenotype increases with longer periods in culture. We propose that clinical grade pluripotent stem cells should be characterized before differentiation and transplantation into each patient. Therefore, relatively high-throughput assays to ensure pluripotent stem cell quality need to be developed and standardized.

5 PRIORITIZING ASSAYS TO MONITOR PLURIPOTENT STEM CELL QUALITY

A stringent panel of assays has been developed by research scientists to show that putative human ES/iPS cells are pluripotent (Maherali and Hochedlinger, 2008). These assays include teratoma formation, embryoid body formation, changes in DNA methylation patterns, and expression of pluripotent markers. Such assays have been widely adopted in the field of disease modeling using iPS cells to minimize the clone-to-clone variation that confounds analysis of cellular phenotypes. However, for cell therapy, it remains unclear whether clinical grade pluripotent stem cells need to exhibit properties consistent with pluripotency to be useful for producing therapeutically relevant cell types for transplantation into patients. Furthermore, the methylation pattern of pluripotent stem cells fluctuates and the significance of data from bisulfite sequencing remains unclear (Amps et al., 2011).

We propose that the critical criteria for assuring pluripotent stem cell quality for cell therapy are

1. normal chromosomal structure,
2. minimal *de novo* mutations associated with neural cell transformation or DA neuron function,
3. high yield of therapeutically relevant A9 DA neurons.

These criteria are not meant to replace the existing good laboratory practices of maintaining cultures of high-quality human pluripotent stem cells. For example, passaging pluripotent stem cell colonies by manual picking is a labor intensive approach to maintaining cultures and observing potentially problematic changes in growth rate that are associated with karyotypic changes (Amps et al., 2011). Higher throughput approaches to passaging human pluripotent stem cells using enzymatic digestion and small molecules to improve cell viability during passaging can apply selection pressures that compromise genetic stability.

6 EXAMINING CHROMOSOMAL DISRUPTION IN PLURIPOTENT STEM CELLS

Cultures of both ES and iPS cells acquire chromosomal disruption during time in culture (Amps et al., 2011; Mayshar et al., 2010). Such chromosomal differences can confer a growth advantage leading to positive selection during reprogramming or time in culture. In the case of iPS cells, the chromosomal differences may arise in the parental cell or be acquired at early or late passages. While good laboratory practice dictates that pluripotent stem cells should be differentiated at the lowest possible passage numbers, routine karyotypic analysis by G-banding is needed to ensure pluripotent stem cell quality.

7 DETERMINING GENETIC MUTATIONS IN PLURIPOTENT STEM CELLS THAT COMPROMISE SAFETY AND FUNCTION OF A9 DA NEURONS

Our goal is to differentiate reproducibly safe and functional A9 DA neurons from human pluripotent stem cells. Previous concerns regarding uncontrolled graft growth are likely to be due to the neurodevelopmental potential of contaminating cortical progenitor cells within inappropriately differentiated cultures (Roy et al., 2006). Improved differentiation protocols yield more A9 DA neurons and fewer highly proliferative cells (Kirkeby et al., 2012; Kriks et al., 2011). As an extra layer of safety, neuronal purification strategies can eliminate the risk of uncontrolled cell growth (Hargus et al., 2010; Hedlund et al., 2008; Pruszak et al., 2007, 2009; Wernig et al., 2008). More importantly, pluripotent stem cells can acquire genetic mutations in culture. Recent studies have shown that iPS cells contain mutations that were present in the parental cell population before reprogramming or acquired during reprogramming and subsequent propagation (Gore et al., 2011). Therefore, genetic mutations may be selected by the culture process required for cell therapy and as such need to be analyzed for the presence of mutations associated with neuroblast transformation (Huse and Holland, 2010). Furthermore, haploinsufficiency of genes that regulate A9 DA neuron differentiation can compromise neuronal function in the adult brain (Kittappa et al., 2007; Sgado et al., 2006). Therefore, the screen for genetic changes in pluripotent stem cells also needs to analyze the integrity of genes that regulate the function and survival of A9 DA neurons, such as FOXA2, NURR1, TH, and PITX3. Good laboratory practice requires the use of low-passage cultures to minimize the risk of acquiring the mutations.

8 YIELD OF DIFFERENTIATED A9 DA NEURONS TO CONFIRM PLURIPOTENT STEM CELL QUALITY

PD patients will need a specific number of functionally integrated A9 DA neurons to improve their symptoms. Human pluripotent stem cells can provide unlimited numbers of A9 DA neurons. Indeed, standardized differentiation protocols yield consistent numbers of DA neurons across high-quality human pluripotent stem cell lines (Hargus et al., 2010; Soldner et al., 2009). In contrast, low yields of therapeutically relevant cell types can be used to identify human pluripotent stem cell lines with abnormal DNA methylation and gene expression profiles (Bock et al., 2011; Boulting et al., 2011).

Differentiation protocols will need to be standardized for PD cell therapy applications. Three major differentiation strategies have been developed: coculture-based differentiation, embryoid body-based differentiation, and dual SMAD pathway inhibition (Chambers et al., 2009; Kawasaki et al., 2000; Zhang et al., 2001). In the first approach, coculture with mesenchymal stromal cells such as MS5 cells, PA6 cells

(Barberi et al., 2003; Brederlau et al., 2006; Perrier et al., 2004), or with midbrain astrocytes (Roy et al., 2006), followed by treatment of the cultures with recombinant fibroblast growth factor 8 (FGF8) and sonic hedgehog (SHH) proteins, yielded many DA neurons. In the second approach, ES/iPS cells are neuralized as embryoid bodies in serum-free culture conditions (Zhang et al., 2001) and then further patterned toward expandable DA precursors using FGF8 and SHH which results in DA neuron formation with an efficiency comparable to that using the coculture methods (Yang et al., 2008). In both these protocols, the cells differentiate via a Pax6-expressing neural precursor cell type. When analyzing the regionalization of the DA neurons obtained via Pax6-expressing neural progenitor cells, these DA neurons were found to be mainly of a diencephalic neuronal subtype (Cooper et al., 2010). As a result, these non-A9 DA neurons survived transplantation but provided modest behavioral improvements when grafted in animal models of PD (Chiba et al., 2008; Roy et al., 2006; Yang et al., 2008).

During embryonic development, mouse and human A9 and A10 DA neurons differentiate from the most ventral (floor plate) cells of the neural tube (Bonilla et al., 2008; Ferri et al., 2007; Hebsgaard et al., 2009; Ono et al., 2007). Recent pluripotent stem cell differentiation protocols have used this developmental patterning via a midbrain-regionalized floor plate neural progenitor cell stage to differentiate authentic A9 and A10 DA neurons (Cooper et al., 2010; Kirkeby et al., 2012; Kriks et al., 2011). The latter two studies based their protocol on dual inhibition of SMAD signaling and report efficient dopamine release *in vitro* (Kriks et al., 2011) and *in vivo* after transplantation (Kirkeby et al., 2012) by a large fraction of human A9 DA neurons. More significantly, however, both studies reported exceptional functional integration leading to improved motor function without uncontrolled cell proliferation after grafting into animal models of PD (Kirkeby et al., 2012; Kriks et al., 2011).

We propose that a standardized human pluripotent stem cell differentiation protocol targeting early differentiation of floor plate neural progenitor cells is a necessary pretransplantation check. The yield of A9 DA neurons needs to be examined to ensure appropriate dosing and also provides a measure of pluripotent stem cell quality.

9 CONCLUSION

In summary, recent progress in our understanding of human pluripotent stem cells has provided new choices for pluripotent stem cell sources of A9 DA neurons to replace the use of fetal cells harvested from elective abortions for clinical trials in PD patients. Current experiments examining the function of pluripotent stem cell-derived A9 DA neurons and the significance of “self” and HLA-matched neurons will highlight the direction for pluripotent stem cell quality assurance in the clinical setting.

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References

Amps, K., Andrews, P.W., Anyfantis, G., Armstrong, L., Avery, S., Baharvand, H., Baker, J., Baker, D., Munoz, M.B., Beil, S., Benvenisty, N., Ben-Yosef, D., Biancotti, J.C., Bosman, A., Brena, R.M., Brison, D., Caisander, G., Camarasa, M.V., Chen, J., Chiao, E., Choi, Y.M., Choo, A.B., Collins, D., Colman, A., Crook, J.M., Daley, G.Q., Dalton, A., De Sousa, P.A., Denning, C., Downie, J., Dvorak, P., Montgomery, K.D., Feki, A., Ford, A., Fox, V., Fraga, A.M., Frumkin, T., Ge, L., Gokhale, P.J., Golan-Lev, T., Gourabi, H., Gropp, M., Lu, G., Hampl, A., Harron, K., Healy, L., Herath, W., Holm, F., Hovatta, O., Hyllner, J., Inamdar, M.S., Irwanto, A.K., Ishii, T., Jaconi, M., Jin, Y., Kimber, S., Kiselev, S., Knowles, B.B., Kopper, O., Kukharenko, V., Kuliev, A., Lagarkova, M.A., Laird, P.W., Lako, M., Laslett, A.L., Lavon, N., Lee, D.R., Lee, J.E., Li, C., Lim, L.S., Ludwig, T.E., Ma, Y., Maltby, E., Mateizel, I., Mayshar, Y., Mileikovsky, M., Minger, S.L., Miyazaki, T., Moon, S.Y., Moore, H., Mummery, C., Nagy, A., Nakatsuji, N., Narwani, K., Oh, S.K., Oh, S.K., Olson, C., Otonkoski, T., Pan, F., Park, I.H., Pells, S., Pera, M.F., Pereira, L.V., Qi, O., Raj, G.S., Reubinoff, B., Robins, A., Robson, P., Rossant, J., Salekdeh, G.H., Schulz, T.C., Sermon, K., Sheik Mohamed, J., Shen, H., Sherrer, E., Sidhu, K., Sivarajah, S., Skottman, H., Spits, C., Stacey, G.N., Strehl, R., Strelchenko, N., Suemori, H., Sun, B., Suuronen, R., Takahashi, K., Tuuri, T., Venu, P., Verlinsky, Y., Ward-van Oostwaard, D., Weisenberger, D.J., Wu, Y., Yamanaka, S., Young, L., Zhou, Q., 2011. Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat. Biotechnol. 29, 1132–1144.

Barberi, T., Klivenyi, P., Calingasan, N.Y., Lee, H., Kawamata, H., Loonam, K., Perrier, A.L., Bruses, J., Rubio, M.E., Topf, N., Tabar, V., Harrison, N.L., Beal, M.F., Moore, M.A., Studer, L., 2003. Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. Nat. Biotechnol. 21, 1200–1207.

Björklund, A., Schmidt, R.H., Stenevi, U., 1980. Functional reinnervation of the neostriatum in the adult rat by use of intraparenchymal grafting of dissociated cell suspensions from the substantia nigra. Cell Tissue Res. 212, 39–45.

Bjorklund, L.M., Sanchez-Pernaute, R., Chung, S., Andersson, T., Chen, I.Y.C., McNaught, K.S.P., Brownell, A.L., Jenkins, B.G., Wahlestedt, C., Kim, K.S., Isacson, O., 2002. Embryonic stem cells develop into functional dopaminergic neurons after transplantation into a Parkinson rat model. Proc. Natl. Acad. Sci. USA 99, 2344–2349.

Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boulting, G., Smith, Z.D., Ziller, M., Croft, G.F., Amoroso, M.W., Oakley, D.H., Gnirke, A., Eggan, K., Meissner, A., 2011. Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell 144, 439–452.

Bonilla, S., Hall, A.C., Pinto, L., Attardo, A., Gotz, M., Huttner, W.B., Arenas, E., 2008. Identification of midbrain floor plate radial glia-like cells as dopaminergic progenitors. Glia 56, 809–820.

Boulting, G.L., Kiskinis, E., Croft, G.F., Amoroso, M.W., Oakley, D.H., Wainger, B.J., Williams, D.J., Kahler, D.J., Yamaki, M., Davidow, L., Rodolfa, C.T., Dimos, J.T., Mikkilineni, S., MacDermott, A.B., Woolf, C.J., Henderson, C.E., Wichterle, H., Eggan, K., 2011. A functionally characterized test set of human induced pluripotent stem cells. Nat. Biotechnol. 29, 279–286.

Brederlau, A., Correia, A.S., Anisimov, S.V., Elmi, M., Paul, G., Roybon, L., Morizane, A., Bergquist, F., Riebe, I., Nannmark, U., Carta, M., Hanse, E., Takahashi, J., Sasai, Y., Funa, K., Brundin, P., Eriksson, P.S., Li, J.Y., 2006. Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of in vitro differentiation on graft survival and teratoma formation. Stem Cells 24, 1433–1440.

Brivanlou, A.H., Gage, F.H., Jaenisch, R., Jessell, T., Melton, D., Rossant, J., 2003. Stem cells. Setting standards for human embryonic stem cells. Science 300, 913–916.

Brundin, P., Strecker, R.E., Widner, H., Clark, D.J., Nilsson, O.G., Åstedt, B., Lindvall, O., Björklund, A., 1988. Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: immunological aspects, spontaneuous and drug-induced bahaviour, and dopamine release. Exp. Brain Res. 70, 192–208.

Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., Studer, L., 2009. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat. Biotechnol. 27, 275–280.

Chiba, S., Lee, Y.M., Zhou, W., Freed, C.R., 2008. Noggin enhances dopamine neuron production from human embryonic stem cells and improves behavioral outcome after transplantation into Parkinsonian rats. Stem Cells 26, 2810–2820.

Chin, M.H., Mason, M.J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J., Khvorostov, I., Ott, V., Grunstein, M., Lavon, N., Benvenisty, N., Croce, C.M., Clark, A.T., Baxter, T., Pyle, A.D., Teitell, M.A., Pelegrini, M., Plath, K., Lowry, W.E., 2009. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 5, 111–123.

Cooper, O., Hargus, G., Deleidi, M., Blak, A., Osborn, T., Marlow, E., Lee, K., Levy, A., Perez-Torres, E., Yow, A., Isacson, O., 2010. Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain dopaminergic neurons requires a high activity form of SHH, FGF8a and specific regionalization by retinoic acid. Mol. Cell. Neurosci. 45, 258–266.

Deacon, T., Dinsmore, J., Costantini, L., Ratliff, J., Isacson, O., 1998. Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. Exp. Neurol. 149, 28–41.

Dinsmore, J., Ratliff, J., Deacon, T., Pakzaban, P., Jacoby, D., Galpern, W., Isacson, O., 1996. Embryonic stem cells differentiated in vitro as a novel source of cells for transplantation. Cell Transplant. 5, 131–143.

Doi, A., Park, I.H., Wen, B., Murakami, P., Aryee, M.J., Irizarry, R., Herb, B., Ladd-Acosta, C., Rho, J., Loewer, S., Miller, J., Schlaeger, T., Daley, G.Q., Feinberg, A.P., 2009. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. Nat. Genet. 41, 1350–1353.

Ferri, A.L., Lin, W., Mavromatakis, Y.E., Wang, J.C., Sasaki, H., Whitsett, J.A., Ang, S.L., 2007. Foxa1 and Foxa2 regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. Development 134, 2761–2769.

Gore, A., Li, Z., Fung, H.L., Young, J.E., Agarwal, S., Antosiewicz-Bourget, J., Canto, I., Giorgetti, A., Israel, M.A., Kiskinis, E., Lee, J.H., Loh, Y.H., Manos, P.D., Montserrat, N., Panopoulos, A.D., Ruiz, S., Wilbert, M.L., Yu, J., Kirkness, E.F., Izpisua Belmonte, J.C., Rossi, D.J., Thomson, J.A., Eggan, K., Daley, G.Q., Goldstein, L.S., Zhang, K., 2011. Somatic coding mutations in human induced pluripotent stem cells. Nature 471, 63–67.

Hargus, G., Cooper, O., Deleidi, M., Levy, A., Lee, K., Marlow, E., Yow, A., Soldner, F., Hockemeyer, D., Hallett, P.J., Osborn, T., Jaenisch, R., Isacson, O., 2010. Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proc. Natl. Acad. Sci. USA 107, 15921–15926.

Hebsgaard, J.B., Nelander, J., Sabelstrom, H., Jonsson, M.E., Stott, S., Parmar, M., 2009. Dopamine neuron precursors within the developing human mesencephalon show radial glial characteristics. Glia 57, 1648–1658.

Hedlund, E., Pruszak, J., Lardaro, T., Ludwig, W., Vinuela, A., Kim, K.S., Isacson, O., 2008. Embryonic stem cell-derived Pitx3-enhanced green fluorescent protein midbrain dopamine neurons survive enrichment by fluorescence-activated cell sorting and function in an animal model of Parkinson's disease. Stem Cells 26, 1526–1536.

Huse, J.T., Holland, E.C., 2010. Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma. Nat. Rev. Cancer 10, 319–331.

Kaji, K., Norrby, K., Paca, A., Mileikovsky, M., Mohseni, P., Woltjen, K., 2009. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458, 771–775.

Kawasaki, H., Mizuseki, K., Nishikawa, S., Kaneko, S., Kuwana, Y., Nakanishi, S., Nishikawa, S.-I., Sasai, Y., 2000. Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. Neuron 28, 31–40.

Kim, J.-H., Auerbach, J.M., Rodriguez-Gomez, J.A., Velasco, I., Gavin, D., Lumelsky, N., Lee, S.-H., Nguyen, J., Sanchez-Pernaute, R., Bankiewicz, K., Mckay, R., 2002. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature 418, 50–56.

Kirkeby, A., Grealish, S., Wolf, D., Nelander, J., Wood, J., Lundblad, M., Lindvall, O., Parmar, M., 2012. Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. Cell Rep. 1 (6), 703–714.

Kittappa, R., Chang, W.W., Awatramani, R.B., Mckay, R.D., 2007. The foxa2 gene controls the birth and spontaneous degeneration of dopamine neurons in old age. PLoS Biol. 5, e325.

Kriks, S., Shim, J.W., Piao, J., Ganat, Y.M., Wakeman, D.R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A., Yang, L., Beal, M.F., Surmeier, D.J., Kordower, J.H., Tabar, V., Studer, L., 2011. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature 480, 547–551.

Lee, S.H., Lumelsky, N., Studer, L., Auerbach, J.M., Mckay, R.D., 2000. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. Nat. Biotechnol. 18, 675–679.

Lindvall, O., Rehncrona, S., Gustavi, B., Brundin, P., Åstedt, B., Widner, H., Lindholm, T., Björklund, A., Leenders, K., Rothwell, J., Frackowiak, R., Marsden, C.D., Johnels, B., Steg, G., Freedman, R., Hoffer, B.J., Seiger, Å., Strömberg, I., Bygdeman, M., Olson, L., 1988. Fetal dopamine-rich mesencephalic grafts in Parkinson's disease. Lancet 2, 1483–1484.

Lindvall, O., Rehncrona, S., Brundin, P., Gustavii, B., Åstedt, B., Widner, H., Lindholm, T., Björklund, A., Leenders, K.L., Rothwell, J.C., Frackowiak, R., Marsden, D., Johnels, B., Steg, G., Freedman, R., Hoffer, B.J., Seiger, Å., Bygdeman, M., Strömberg, I., Olson, L., 1989. Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. Arch. Neurol. 46, 615–631.

Ma, Y., Tang, C., Chaly, T., Greene, P., Breeze, R., Fahn, S., Freed, C., Dhawan, V., Eidelberg, D., 2010. Dopamine cell implantation in Parkinson's disease: long-term clinical and (18)F-FDOPA PET outcomes. J. Nucl. Med. 51, 7–15.

Maherali, N., Hochedlinger, K., 2008. Guidelines and techniques for the generation of induced pluripotent stem cells. Cell Stem Cell 3, 595–605.

Mayshar, Y., Ben-David, U., Lavon, N., Biancotti, J.C., Yakir, B., Clark, A.T., Plath, K., Lowry, W.E., Benvenisty, N., 2010. Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. Cell Stem Cell 7, 521–531.

Mendez, I., Sanchez-Pernaute, R., Cooper, O., Vinuela, A., Ferrari, D., Bjorklund, L., Dagher, A., Isacson, O., 2005. Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease. Brain 128, 1498–1510.

Mendez, I., Vinuela, A., Astradsson, A., Mukhida, K., Hallett, P., Robertson, H., Tierney, T., Holness, R., Dagher, A., Trojanowski, J.Q., Isacson, O., 2008. Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years. Nat. Med. 14, 507–509.

Nakajima, F., Tokunaga, K., Nakatsuji, N., 2007. Human leukocyte antigen matching estimations in a hypothetical bank of human embryonic stem cell lines in the Japanese population for use in cell transplantation therapy. Stem Cells 25, 983–985.

Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., Yamanaka, S., 2008. Generation of mouse induced pluripotent stem cells without viral vectors. Science 322, 949–953.

Ono, Y., Nakatani, T., Sakamoto, Y., Mizuhara, E., Minaki, Y., Kumai, M., Hamaguchi, A., Nishimura, M., Inoue, Y., Hayashi, H., Takahashi, J., Imai, T., 2007. Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. Development 134, 3213–3225.

Perrier, A.L., Tabar, V., Barberi, T., Rubio, M.E., Bruses, J., Topf, N., Harrison, N.L., Studer, L., 2004. Derivation of midbrain dopamine neurons from human embryonic stem cells. Proc. Natl. Acad. Sci. USA 101, 12543–12548.

Piccini, P., Brooks, D.J., Björklund, A., Gunn, R.N., Grasby, P.M., Rimoldi, O., Brundin, P., Hagell, P., Rehncrona, S., Widner, H., Lindvall, O., 1999. Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. Nat. Neurosci. 2, 1137–1140.

Politis, M., Wu, K., Loane, C., Quinn, N.P., Brooks, D.J., Rehncrona, S., Björklund, A., Lindvall, O., Piccini, P., 2010. Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. Sci. Transl. Med. 2, 38ra46.

Pruszak, J., Sonntag, K.C., Aung, M.H., Sanchez-Pernaute, R., Isacson, O., 2007. Markers and methods for cell sorting of human embryonic stem cell-derived neural cell populations. Stem Cells 25, 2257–2268.

Pruszak, J., Ludwig, W., Blak, A., Alavian, K., Isacson, O., 2009. CD15, CD24, and CD29 define a surface biomarker code for neural lineage differentiation of stem cells. Stem Cells 27, 2928–2940.

Roy, N.S., Cleren, C., Singh, S.K., Yang, L., Beal, M.F., Goldman, S.A., 2006. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat. Med. 12, 1259–1268.

Sgado, P., Alberi, L., Gherbassi, D., Galasso, S.L., Ramakers, G.M., Alavian, K.N., Smidt, M.P., Dyck, R.H., Simon, H.H., 2006. Slow progressive degeneration of nigral dopaminergic neurons in postnatal Engrailed mutant mice. Proc. Natl. Acad. Sci. USA 103, 15242–15247.

Soldner, F., Hockemeyer, D., Beard, C., Gao, Q., Bell, G.W., Cook, E.G., Hargus, G., Blak, A., Cooper, O., Mitalipova, M., Isacson, O., Jaenisch, R., 2009. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136, 964–977.

Stadtfeld, M., Apostolou, E., Akutsu, H., Fukuda, A., Follett, P., Natesan, S., Kono, T., Shioda, T., Hochedlinger, K., 2010. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. Nature 465, 175–181.

Taylor, C.J., Bolton, E.M., Pocock, S., Sharples, L.D., Pedersen, R.A., Bradley, J.A., 2005. Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. Lancet 366, 2019–2025.

Warren, L., Manos, P.D., Ahfeldt, T., Loh, Y.H., Li, H., Lau, F., Ebina, W., Mandal, P.K., Smith, Z.D., Meissner, A., Daley, G.Q., Brack, A.S., Collins, J.J., Cowan, C., Schlaeger, T.M., Rossi, D.J., 2010. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7, 618–630.

Wernig, M., Zhao, J.P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., Broccoli, V., Constantine-Paton, M., Isacson, O., Jaenisch, R., 2008. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc. Natl. Acad. Sci. USA 105, 5856–5861.

Widner, H., Tetrud, J., Rehncrona, S., Snow, B., Brundin, P., Gustavii, B., 1992. Bilateral fetal mesencephalic grafting in two patients with severe parkinsonism induced by MPTP. N. Engl. J. Med. 327, 1556–1563.

Woltjen, K., Michael, I.P., Mohseni, P., Desai, R., Mileikovsky, M., Hamalainen, R., Cowling, R., Wang, W., Liu, P., Gertsenstein, M., Kaji, K., Sung, H.K., Nagy, A., 2009. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458, 766–770.

Yang, D., Zhang, Z.J., Oldenburg, M., Ayala, M., Zhang, S.C., 2008. Human embryonic stem cell-derived dopaminergic neurons reverse functional deficit in parkinsonian rats. Stem Cells 26, 55–63.

Zhang, S., Wernig, M., Duncan, I.D., Brüstle, O., Thomson, J.A., 2001. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat. Biotechnol. 19, 1129–1133.

Zhou, H., Wu, S., Joo, J.Y., Zhu, S., Han, D.W., Lin, T., Trauger, S., Bien, G., Yao, S., Zhu, Y., Siuzdak, G., Scholer, H.R., Duan, L., Ding, S., 2009. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4, 381–384.