



**HAL**  
open science

## Can cellular models revolutionize drug discovery in Parkinson's disease?

Birgitt Schuele, Renee A. Reijo Pera, J. William Langston

► **To cite this version:**

Birgitt Schuele, Renee A. Reijo Pera, J. William Langston. Can cellular models revolutionize drug discovery in Parkinson's disease?. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2009, 1792 (11), pp.1043. 10.1016/j.bbadis.2009.08.014 . hal-00562930

**HAL Id: hal-00562930**

**<https://hal.science/hal-00562930>**

Submitted on 4 Feb 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Can cellular models revolutionize drug discovery in Parkinson's disease?

Birgitt Schuele, Renee A. Reijo Pera, J. William Langston

PII: S0925-4439(09)00197-5  
DOI: doi:[10.1016/j.bbadis.2009.08.014](https://doi.org/10.1016/j.bbadis.2009.08.014)  
Reference: BBADIS 63001

To appear in: *BBA - Molecular Basis of Disease*

Received date: 8 May 2009  
Revised date: 21 August 2009  
Accepted date: 26 August 2009



Please cite this article as: Birgitt Schuele, Renee A. Reijo Pera, J. William Langston, Can cellular models revolutionize drug discovery in Parkinson's disease?, *BBA - Molecular Basis of Disease* (2009), doi:[10.1016/j.bbadis.2009.08.014](https://doi.org/10.1016/j.bbadis.2009.08.014)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Can cellular models revolutionize drug discovery in Parkinson's disease?**

Birgitt Schuele, MD<sup>1</sup>

Renee A. Reijo Pera, PhD<sup>2</sup>

J. William Langston, MD<sup>1</sup>

<sup>1</sup>The Parkinson's Institute, Sunnyvale, CA, 94085-2935

<sup>2</sup>Center for Human Embryonic Stem Cell Research and Education; Institute for Stem Cell Biology & Regenerative Medicine; Department of Obstetrics and Gynecology, Palo Alto, CA 94304-5542

**Key words:** Parkinson's disease, neurodegeneration, iPSC, nuclear reprogramming, cellular disease models

Corresponding author:

J. William Langston, MD

The Parkinson's Institute

675 Almanor Ave, Sunnyvale, CA 94085-2936, USA

Phone: 408-542-5633, Fax: 408-734-8544

[jwlangston@thepi.org](mailto:jwlangston@thepi.org)

## Abstract

The study of mechanisms that underlie Parkinson's disease (PD), as well as translational drug development, has been hindered by the lack of appropriate models. Both cell culture systems and animal models have limitations, and to date none faithfully recapitulate all of the clinical and pathological phenotypes of the disease. In this review we examine the various cell culture model systems of PD, with a focus on different stem cell models that can be used for investigating disease mechanisms as well as drug discovery for PD. We conclude with a discussion of recent discoveries in the field of stem cell biology that have led to the ability to reprogram somatic cells to a pluripotent state via the use of a combination of genetic factors; these reprogrammed cells are termed "induced pluripotent stem cells" (iPSCs). This groundbreaking technique allows for the derivation of patient-specific cell lines from individuals with sporadic forms of PD and also those with known disease-causing mutations. Such cell lines have the potential to serve as a human cellular model of neurodegeneration and PD when differentiated into dopaminergic neurons. The hope is that these iPSC-derived dopaminergic neurons can be used to replicate the key molecular aspects of neural degeneration associated with PD. If so, this approach could lead to transformative new tools for the study of disease mechanisms. In addition, such cell lines can be potentially used for high-throughput drug screening. While not the focus of this review, ultimately it is envisioned that techniques for reprogramming of somatic cells may be optimized to a point sufficient to provide potential new avenues for stem cell-based restorative therapies.

## Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases of aging. Approx. 1-2% of the population over 65 years is affected by this disorder, and it is estimated that the number of prevalent cases of PD will double by the year 2030 [1]. Thus, there is an urgent need to better understand this neurodegenerative disease and to develop new therapies, particularly those aimed at disease modification and even prevention.

Classical clinical features of PD include rest tremor, bradykinesia (slowness of movement), rigidity and loss of postural stability. Importantly, it is now clear that a wide variety of other clinical manifestations accompany the disease, many of which are non-motor, ranging from autonomic dysfunction to dementia [2]. Classic neuropathologic features include the loss of dopaminergic neurons in the substantia nigra and the presence of neuronal intracytoplasmic and intra-axonal inclusions known as Lewy bodies and Lewy neurites respectively. New immunohistochemical staining techniques have shown that the neuritic pathology is widespread throughout the central and peripheral nervous system, thus explaining many of the non-dopaminergic features of the disease [3, 4].

The mechanisms that underlie neuronal dysfunction and eventual cell death in PD remain unknown although theories abound, ranging from oxidative stress and inflammation to abnormal protein folding and "toxic" aggregation. However, one of the major barriers to research on disease mechanisms has been the inaccessibility of targeted living neuronal populations from patients with the disease and consequent reliance on postmortem samples. Unfortunately, primary

cells from postmortem brain samples have short life-spans *in vitro*; although some postmortem and biopsy tissue preparations from rodent and human brain can provide stem/progenitor cell populations that have the potential for drug screening or other therapeutic applications [5-8]. Nevertheless, PD itself effectively ablates the very cell type of interest – the midbrain dopamine neuron. Thus, to date, it has been difficult to perform meaningful experiments on disease-affected human neural tissues. To overcome this barrier, early studies relied on embryonic midbrain cultures from rat to model the dopaminergic system. In these cultures, however, only a minor subset of neurons (1-2%) were dopaminergic and showed immunoreactivity for tyrosine hydroxylase (TH); none-the-less investigators put these cultures to use as an initial system to study toxic disease mechanisms [9-12]. However, over the years, efforts to develop dopaminergic cell lines have expanded dramatically, largely driven by the interest in obtaining cells that could be used for grafting into the adult human brain to treat the symptoms of PD. Importantly, specific markers have been established to characterize dopaminergic neurons, including TH and aromatic L-amino acid decarboxylase (AADC), both of which are involved in the synthesis of dopamine (DA). The dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2) are indispensable for DA neurotransmission and are also excellent markers for DA neurons. Transcription factors that indicate midbrain specificity include nuclear receptor related 1 (Nurr1) and paired-like homeodomain transcription factor 3 (Pitx3). Morphologically midbrain dopaminergic neurons exhibit long branched neurites with varicosities. Their terminals release DA after potassium exposure

which can be detected by high-performance liquid chromatography (HPLC). Electrophysiologic properties of dopaminergic neurons include spontaneous firing rate of ~ 4.5 Hz, prominent anomalous rectifier currents when hyperpolarized and irregular but unique spike waveforms of action potentials [13-15].

Finally, it is important to note that various groups of DA neurons show differences in the susceptibility to the neurodegenerative process that underlies PD. Specifically DA neurons of the A9 region of the substantia nigra are the most susceptible cell type in PD, compared to DA neurons from the A10 region [16-18]. Interestingly, the expression of the transcription factor Pitx3 may coax the cells towards a specific A9 DA neuronal phenotype [19], whereas Nurr1 in general promotes the differentiation to a dopaminergic phenotype [20].

### **Animal models of PD and neurodegeneration**

One of the most sought after approaches to study disease mechanisms in PD has been to develop animal models of the disorder. Both neurotoxicant and transgenic models of PD have been generated and characterized with the aim of achieving this goal [21, 22]. The main neurotoxicants that have been used to induce models of PD include 6-hydroxydopamine (6-OH), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and various pesticides including paraquat (PQ) and rotenone. All of these have been shown to induce nigrostriatal cell death in rodent and non-human primates (for review [22]). Each of these models has their strengths and weaknesses, but none fully model the disease.

The discovery of disease-causing genes and susceptibility factors for PD [23, 24] led to great hope that animal models based on these discoveries would

lead to ideal models for the disease, and in fact numerous transgenic and knock-out mice models aimed at modeling PD have been engineered over the last decade. However, aside from a limited number of alpha-synuclein mouse models, most of these transgenic lines only show subtle physiologic, neurochemical, or pathological manifestations that could be said to mirror certain aspects of PD [25-27]. Because of this, a novel line of research which is now emerging is the combination of genetic models with the administration of toxicants (model fusion) to accelerate and amplify behavioral and pathological features of neurodegeneration of PD and to study gene-environment interactions [28]. Of course, one limitation to any complex animal model, particularly vertebrate models, is that they are usually not amenable to high-throughput screening for drug discovery.

For all of these reasons, in this review we will focus on the current and evolving state of cell lines that can be used both for research on PD disease mechanisms and drug discovery. As dopaminergic nigrostriatal degeneration is one of the hallmarks of PD, we will center this review on those cell models that can be best used to study this feature of the disease. There are some very exciting new advances in the development of cellular models that have the potential to revolutionize the research landscape in PD.

### **Non-patient specific human cell lines**

Although tissue culture models of disease mechanisms have their experimental limitations, they have some significant advantages over animal models of disease, particularly in that they can be human genome-based and allow



for the direct investigation of pathophysiological characteristics in a far less time and less labor intensive manner. Not only can experiments be more rapidly performed, but, as noted above, techniques can even be developed for high-throughput screening of therapeutic compounds. To study the function of proteins that may be responsible for neurodegeneration, for example, tissue culture systems are easily amenable for gene manipulation such as over-expression of proteins of interest or specific knockdown of certain proteins to study their functional consequences. In this article we will first discuss those cellular models that are not based on stem cell biology. We will then review various stem cell models and conclude with an overview about the emerging techniques and the use of induced pluripotent stem cells (iPSCs).

***Human neuroblastoma cell lines:*** One of the most widely used cell lines for modeling certain aspects of neurodegeneration and neurotoxicity in relation to PD is the human neuroblastoma cell line SH-SY5Y line (ATCC# CRL-2266). These cells, which were originally derived in 1970 from a metastatic neuroblastoma, have moderate levels of dopamine beta hydroxylase activity. For many researchers, this cell-line has represented a favored starting point when a tissue culture model system is desired. Currently, a variety of other neuroblastoma cell lines are available as well, including the SK-N-MC (ATCC# HTB-10), SK-N-SH (ATCC# HTB-11), and SK-N-BE (ATCC# CRL-2271) cell lines. All these lines can be differentiated with retinoic acid (RA) or the phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), to neuron-like cells with respect to morphological and biochemical criteria [29, 30]. Phenotypic differences in

differentiated SH-SY5Y cells are seen depending on the differentiation conditions. For example, when RA (10  $\mu$ M for 3 days) treatment is followed by TPA (150 nM for 3 days) administering for a 6-day period, these cells express dopaminergic markers such as TH, dopamine D2 and D3 receptors, DAT, and VMAT2. On the other hand, a differentiation protocol using only RA shows only minimal expression of the above mentioned markers, but high expression of VMAT2 [31]. Several pathways have been studied to characterize the effects of RA-induced neuronal differentiation. RA activates the PI3K/Akt signaling pathway [32] as well as the ataxia telangiectasia mutated (ATM) kinase, which leads to a ATM dependent cAMP response element binding (CREB) protein phosphorylation. When these pathways are blocked by inhibitors or RNAi, the cells do not differentiate [33].

One interesting observation regarding the SH-SY5Y cell line is that there are differences in susceptibility between RA-differentiated and undifferentiated SH-SY5Y cells when exposed to neurotoxicants. For example, when cells were exposed to 6-OHDA (25 $\mu$ M) or MPP<sup>+</sup> (1mM) for 24 hrs, there were marked differences in cell viability, toxicity, response to oxidative stress and apoptosis, with the undifferentiated cells proving to be more susceptible to neurotoxins than the RA-differentiated cells. These results suggest that undifferentiated SH-SY5Y cells might be a better cellular model for studying neurotoxicity [34].

While the SH-SY5Y human neuroblastoma line has been a very useful model for PD research, there are certain shortcomings of this model, including the obvious fact that they are not authentic DA neurons. Furthermore, depending on

the experimental questions, differentiation regimes with varying concentrations of RA and/or other agents as well as the passage number of the cells, all need to be controlled. Finally, it also may be important to compare differentiated and undifferentiated cells in assays and experiments side by side.

***Embryonic carcinoma cell line:*** Another cell line that provides a somewhat different approach for PD research is the NTera-2/NT2 line (ATCC# CRL-1973). This is a malignant human pluripotent embryonic carcinoma cell line that shares many characteristics of human embryonic stem cells (hESCs), but also has characteristics of neuronal progenitor cells [35]. Three subclones of this cell line exist, but the subclone NT2/D1 is the main one that has been used for neuronal differentiation with RA. In fact, this line has been made commercially available as hNT (or LBS) neurons [36]. While these cells hold promise as experimental models, they have been disappointing in neurotransplantation studies; for example, when hNT neurons were grafted into animal models of PD they failed to induce any major behavioral benefit or substantial graft survival [37-40] and clinical trials showed overall no significant clinical benefit in patients with stroke [41].

Nevertheless, *in vitro* the NTera-2 line expresses markers of hESCs and can be differentiated into DA neurons using a culture differentiation regime and co-culture of PA-6 cells for several weeks (for a more detailed discussion on neuronal differentiation see the section in this article entitled ‘Human embryonic stem cells, alternative stem cells and directed differentiation into DA neurons’). This protocol results in a very high percentage (>80%) of TH-positive cells; these

cells express an impressive variety of dopaminergic markers including AADC, DAT, Nurr1, neurotrophic tyrosine kinase receptor, type 2 (TrkB), neurotrophic tyrosine kinase receptor, type 3 (TrkC), and GDNF family receptor alpha 1 (GFRA1). Furthermore, these cells show functional properties of dopaminergic cell lines in that they can respond to neurotransmitters, and exhibit electrophysiological excitability [42]. This model has primarily been used to understand neuronal differentiation, but it also has the potential to serve as a promising system to model dopaminergic dysfunction and degeneration.

### **First generation models of patient-specific cell lines for the study of neurodegeneration in PD**

Patient-specific cell lines are those that not only can be used to model human disease, but that are also derived directly from tissues of patients with a specific disorder.

**Cybrid Cell lines:** Cybrids are hybrid cell lines resulting from cytoplasmic fusion of cells that lack mitochondrial DNA (mtDNA) fused with platelet mtDNA from PD patients. Since mitochondrial function has been shown to be impaired in PD, specifically Complex I activity, a cybrid model of sporadic PD represents one of the first patient-specific attempts to model aspects of PD at the cellular level [43]. The first group to study cybrid cell lines in this manner compared cybrid cultures from PD patients and healthy controls. They found that Complex I (Vmax) in the cybrid cells from the patient group was 20% less than in those cultures from controls, whereas Complex IV activity was similar between

patients and controls [44]. These results have been confirmed in other independent studies using different host cell lines [45-47]. PD cybrids have also been shown to exhibit enhanced susceptibility to hydrogen peroxide and 1-methyl-4-phenylpyridinium (MPP+) [44, 48, 49], as well as an increase in reactive oxygen species (ROS) production and antioxidant enzyme activities when compared to controls. Remarkably, these PD cybrids have been reported to spontaneously develop fibrillar and vesicular inclusions within 3-4 months in culture and exhibit at least some of the biochemical features of Lewy bodies such as staining for eosin, alpha-synuclein, and ubiquitin [50-52]. In PD cybrids, a large number of mitochondria have been found to be enlarged, swollen or pale with only a few cristae [51].

Taken together, this model promises to provide interesting clues to the mitochondrial deficits in PD and how they relate to the neurodegenerative process that underlies the disease [53]. Importantly, it is one of the few cell lines to actually exhibit some aspects of an actual parkinsonian phenotype at the cellular level, and it is surprising it has not enjoyed more widespread use for the study of disease mechanisms and drug discovery. One reason may be that there are technical challenges in developing these cell lines.

***Primary human fibroblasts from patients with PD:*** Derivation of primary human fibroblasts to study disease mechanisms is another potentially appealing approach to developing patient-specific cell lines to model PD, but this approach is limited by the fact that fibroblasts in long term culture become senescent; they may also transform and/or undergo clonal selection. Furthermore,

for the study of neurodegenerative disease, there is always the question of whether or not results obtained in fibroblasts mirror the pathophysiological changes in human brain, particularly dopaminergic neurons. Perhaps because of this concern, publications about patient-specific fibroblasts are sparse. Nevertheless, a few reports show that under careful experimental conditions, useful data can be obtained from these cells in specific mutation carriers of PD associated genes [54, 55], suggesting that this line of research has promise for cellular modeling of PD. For example, gene expression profiles in fibroblasts (i) from patients with PTEN-induced putative kinase 1 (PINK1) mutations that are known to cause parkinsonism in humans and (ii) fibroblasts in which PINK1 was down-regulated by RNAi have been used to understand the function of this protein. Intriguingly, the alpha-synuclein gene (SNCA) was found to be upregulated in these model systems and expression of genes that code for synaptic proteins were increased [54]. In another study, mitochondrial respiratory function was assessed in a PINK1-deficient fibroblast line from a patient carrying a homozygous W437X nonsense mutation. Interestingly, the authors found a lower respiratory activity, enhanced ROS production, and a significant decrease in cytochrome C when the PINK1-deficient cells were compared to controls [55].

Obviously, a very exciting long-term outcome of this research utilizing peripheral tissue would be the discovery of gene or protein expression patterns that differ between patients, including sporadic cases, and healthy matched controls, and thus provide insights into disease mechanisms. Furthermore, if this

is proved to be the case, these cells could serve as an easily accessible tissue for biomarker discovery that could facilitate the diagnosis of sporadic PD.

***Human embryonic mesencephalic cells immortalized using ectopic myc expression:*** Another alternative developed towards the goal of obtaining faithful cellular model for PD is the Lund human mesencephalic (LUHMES) cell line, which is a clone of the v-myc-overexpressing tetracycline-dependent human mesencephalic cell line MESC2.10 [56]. Tetracycline down-regulates v-myc expression in this line and the cells can then be differentiated within 3-4 days into a dopaminergic phenotype when cultured in dibutyryl cAMP (cyclic adenosine monophosphate) and glial cell-derived neurotrophic factor (GDNF). These cells express TH and DAT [57], however, they cells do not maintain their phenotype after transplantation into the 6-OHDA rat model of PD [58]. Thus, while they could serve as a cellular dopaminergic model *in vitro*, they are probably not be amenable for neurotransplantation in PD [59].

Another immortalized human embryonic mesencephalic cell line has recently developed from human fetal ventral mesencephalon stem cells transduced with v-myc (ReNcell VM NSCs) [60]. This cell line can also be differentiated to exhibit a dopaminergic phenotype with expression of TH; however, further characterization of protein expression and neurophysiologic properties will be necessary to determine if these cells fulfill specific criteria of dopaminergic neurons [61]. Interestingly, this cell line was recently used to study the function of the PINK1 gene [62] as well. In this study, the cell line was transfected with siRNA targeting PINK1. Subsequently, age-dependent

neurodegeneration was observed with reduced long-term viability of these human neurons; this effect appeared to be mediated through a mitochondrial apoptosis pathway [62]. Thus, although there are limitations, these novel immortalized mesencephalic cell lines may well provide a promising model for studying disease mechanisms related to dopaminergic neurons, though additional studies are needed to validate these models.

**Human embryonic stem cells, alternative stem cells and directed differentiation into dopaminergic neurons**



A large body of literature is accumulating on the use of hESCs as a model for development of diverse cell types, including dopaminergic neurons. From a historical perspective, the major impetus for this research has been to engineer cells which could be used for basic studies of neural development and for cell replacement therapy. To date, however, the value of the system for the study of disease pathways in PD and for drug discovery has been less well appreciated. On the other hand, the great interest in developing hESCs for neurotransplantation to treat PD has led to impressive progress in developing techniques to transform pluripotent cells into dopaminergic neurons, the loss of which represents a central feature of PD. In general, culture conditions for directed differentiation of embryonic stem cells (ESCs) into dopaminergic neurons have been developed to mimic the microenvironment present in the developing organism using specific culture conditions or genetic manipulation [19, 20, 63-68]. Recently, a series of elegant studies have unraveled molecular codes for the generation of midbrain DA neurons; these require the temporal and spatial expression of specific neurotrophic and signaling factors to orchestrate the correct developmental stages of differentiation [69-71]. Although TH-positive cells can be generated under specific culture conditions, the majority of cells produced with these techniques have different phenotypes because the cells are not synchronized; thus cell states range from undifferentiated cells to various stages of neuroprogenitor cells. A challenge to this approach is that analysis of cell populations is of limited value if the cells are largely heterogeneous; methods for single cell analysis or for directing coordinated differentiation are thus needed.

By co-culturing ESCs with mouse PA-6 cells, Kawasaki and colleagues first reported a stromal cell-derived inducing activity (SDIA) of unknown signaling factors that promoted a neuronal dopaminergic phenotype in mouse and non-human primate ESCs [65, 72]. Others have subsequently achieved dopaminergic differentiation in hESCs using different variations of these techniques [73-76]. Modifications now also include the use of other cell types that show SDIA such as MS5 stromal cells or Sertoli cell co-culture [75, 77]. Protocols have also been developed to avoid co-culture using feeder-free medium for directed neuro-differentiation into a dopaminergic phenotype [78]. Neuronal differentiation in these protocols is achieved by sequential addition of different concentrations of recombinant growth and signaling factors to the culture media such as sonic hedge hog (SHH), fibroblast growth factor 8 (FGF8), brain-derived neurotrophic factor (BDNF), GDNF, or ascorbic acid. For further detailed review on culture regimes for directed differentiation of ESCs, refer to reference [59]. In summary, there is a rich literature on the process of differentiating ESCs into a dopaminergic phenotype, and this body of work will be hugely beneficial in the development of cellular models of PD in the future.

***Human embryonic and adult neural stem cells:*** Neural stem cells (NSCs) that can be isolated from adult and fetal brain and have the inherent ability to expand and to further differentiate in all neuronal and glial lineages in vitro. As a result, they have been of great interest as a source for cell replacement therapy for disease of the nervous system, including PD. For example, mesencephalic precursors from rat E12 have been shown to successfully expand

and differentiate into dopaminergic neurons (18.4% TH positive neurons) and show functional recovery in the 6-OHDA rat model [79]. Interestingly, when Studer and collaborators used the same tissue preparation under similar conditions, but at a lower oxygen concentration of 3% cell proliferation was promoted, apoptosis was reduced, and the number of dopaminergic neurons increased to 56% compared to 18% under environmental oxygen conditions of 20% [80]. This same group also showed successful expansion and differentiation of human mesencephalic and cortical precursors into DA neurons with a percentage of 21% and 7.5%, respectively [81]. However, when in vitro studies performed using this cell type as a primary source several observations were made. First, the developmental stage (fetal or postnatal), location of tissue collection in the brain, and culture conditions of the cells (monolayer vs. neurospheres) greatly affect the efficiency of generating specific DA neurons from neural precursor cells (NPCs) [82] [as reviewed in [83]]. On the other hand, others have found only limited ability to expand NPCs and to differentiate them in vitro into DA neurons [84].

While there are a number of publications on the rodent mesencephalic precursor cell lines using various induction methods [79, 85, 86], there are surprisingly few such reports utilizing human tissue. Liste and co-workers induced human forebrain NSC by overexpression of Basal cell lymphoma-extra large protein (Bcl-XL) under low oxygen conditions; these cells expressed TH, Nurr-1, and showed DA release [87, 88]. A recent study focused on amplification of human NSCs in suspension spinner flask bioreactors for transplantation

purposes from different brain regions [89, 90]. Taken together, these examples show that fetal and adult NSCs can be differentiated in vitro into dopaminergic neurons. However, more studies are needed to optimize culture conditions and differentiation approaches.

**Human mesenchymal stem cells:** Mesenchymal stem cells (MSCs) can be easily obtained from i.e. bone marrow or umbilical cord tissue [91]. Importantly, they are multipotent when expanded in culture and have low tumorigenicity [92]. Several groups have described the isolation and derivation of mesenchymal cells from olfactory mucosa and conjunctiva [93, 94]. Interestingly, the generation of putative dopaminergic cells has been described using different induction methods with a success rate for TH positive neurons between 12.7-67% [95-99]. For example, Dezawa and colleagues have reported achieving a frequency of 41% of potential TH positive neurons by transfecting MSCs from bone marrow with Notch intracellular domain and an exposure regime of forskolin, basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), and GDNF. However the frequency of TH-positive cells was only 3.9% when GDNF was omitted in the culture medium [95]. A three-step induction protocol with BDNF, nerve growth factor (NGF), neurotrophin-3 (NT-3), SHH, fibroblast growth factor 8 (FGF8), and RA generated 62.1%±5.2 TH positive cells from a subpopulation of MSCs, termed marrow isolated adult multilineage inducible (MIAMI) cells obtained from donors between 3–72 years old [98].

Several groups have also demonstrated stem cell potency of stromal cells isolated from human umbilical cord mesenchymal tissue (Wharton's jelly).

Putative dopaminergic neurons have also been derived from cells isolated from human umbilical cord using neuronal conditioned media, SHH and FGF8, however the frequency of TH positive neurons was about 12.7% [97]. Recently it has been report that, when a protocol for umbilical cord cells using a cocktail of SHH and fibroblast growth factors was used, 67% of cells showed expression of TH within 12 days in culture [100, 101]. Finally, Fallahi-Sichani and colleagues have investigated the differentiation of umbilical cord stem cells, into neuronal cells in medium under serum-free conditions. The derived neuronal cells expressed genes associated with development and/or survival of midbrain DA neurons such as engrailed homeobox 1 (En1), engrailed homeobox 2 (En2), Nurr1, Pitx3, paired box gene 2 (Pax2), wingless type MMTV integration site family, Member 1 (Wnt1) and wingless type MMTV integration site family, Member 3a (Wnt3a) [102].

With respect to patient-specific stem cells, Zhang and colleagues have characterized 18 bone-marrow derived MSCs from patients with PD and compared them to MSCs from normal adult bone marrow. The authors report that PD-derived MSCs are phenotypically indistinguishable from normal MSCs and exhibit the same differentiation potential. Furthermore, PD-derived MSCs differentiate into neuronal phenotype under a 2-wk culture protocol with GDNF and showed a TH expression in 30% of the cells [103]. Taken together these results are encouraging regarding feasibility of this approach, but many questions remain to be addressed regarding the authenticity of neuronal cells differentiated from cell types whose potency is generally limited to mesodermal derivatives.

## **Derivation of human induced pluripotent cell lines by nuclear reprogramming**

Recently, a major scientific accomplishment was achieved with the demonstration of nuclear reprogramming of adult human somatic cells into cells that are pluripotent and capable of differentiating to all three germ layers. Nuclear reprogramming is defined by the erasure of the established programs of a differentiated somatic cell to a pluripotent cell type; in the case of production of iPSCs, reprogramming is accomplished via introduction of a set of defined molecular factors, thus changing and modifying the epigenetic landmarks and the expression of transcription factors within the cell.

The field of human iPSC biology was heralded in late 2007 with the report of Takahashi and collaborators that iPSCs had been generated from adult human skin fibroblasts with a combination of four factors: Oct3/4, Sox2, Klf4, and c-Myc using a retroviral system [104]. The resulting iPSCs were characterized and shown to express markers of hESCs and to differentiate to all three germ cell layers *in vitro* and *in vivo* [104]. Concurrently, Yu et al. also demonstrated successful reprogramming of adult dermal cells with a similar set of transcription factors OCT4, SOX2, NANOG, and LIN28 with a lentiviral vector system [105]. Since then, a number of other groups have successfully generated iPSC lines by introducing two to six factors into human somatic cells with the outcome of generating cells that resemble hESCs [106-108][104-107]. Other researchers have also focused on developing strategies to increase efficiency of reprogramming by addition of small molecules, and to obtain reprogramming without integration of

transgenes in both mouse and human somatic cells by using non-integrating adenoviruses, transient transfection, a *piggyBac* transposon system, Cre/LoxP-excisable vectors, oriP/EBNA1-based episomal expression vectors, and direct protein transduction [109-120].

Although further advances in iPSC technology will be necessary before they can be used for cellular replacement therapy, work in this area is likely to advance quickly. For example, Wernig and colleagues have shown that mouse iPSC derived dopaminergic neurons can integrate in host brain that results in behavioral improvement in the 6-OHDA rat model [123].

There is currently great excitement regarding the immediate potential of nuclear reprogramming as a way to develop new models for a wide range of diseases, including PD. Indeed, one group has succeeded in generating multiple iPSC lines from patients with different disorders that were either complex or inherited, including one patient with sporadic PD [121]. Another group has successfully derived iPSC lines from patients with another neurodegenerative disorder, amyotrophic lateral sclerosis, and differentiated these cells into functional motor neurons [122]. More recently, five somatic cell lines from patients with idiopathic PD have also been reprogrammed using doxycycline inducible lentiviruses and in one sporadic PD cell line a loxP-excisable system was used to produce viral-free iPSC lines. These lines may potentially allow for optimal comparisons between cell lines from unaffected and PD patients; importantly, all PD iPSC lines were able to form TH-positive neurons [119].

However, no overt differences were noted in the lines derived from PD patients relative to controls under naïve conditions [119].

While all of the patient work cited above started with skin fibroblasts, other somatic cells are also amenable to reprogramming. For example, it has been reported that hepatocytes, keratinocytes, and blood progenitor cells can be reprogrammed at a much higher efficiency than dermal fibroblasts [124-126].

### **Changing the course of Parkinson's disease research: A vision for the future**

The main potential advantage of iPSCs as a cellular model for Parkinson's research is that it provides for the first time the opportunity to derive dopaminergic neurons from adult humans who are affected with idiopathic PD. However, since the cause or trigger for the disease in the vast majority of "sporadic" cases is unknown, we believe that a more promising source of tissue would be cells of patients carrying pathogenic mutations that cause PD since these individuals have parkinsonism of a known cause, and the cells will carry that cause with them in their DNA. Such an approach is now possible, since at least five monogenic forms of PD have been described over the last decade; indeed these monogenetic forms of parkinsonism have proved tremendous insight into pathways that also might be important for sporadic PD [23]. The two autosomal-dominant forms are represented by the SNCA gene and the Leucine-rich repeat kinase 2 (LRRK2) gene. Mutations in both genes can recapitulate many if not all of the characteristics of the clinical and pathologic features of sporadic PD [127-130]. Three recessive forms of PD (Parkin, PINK1, and oncogene DJ-1) typically



present with an earlier onset of disease and exhibit some atypical features of PD [131]. Thus, these genetic forms present a unique opportunity to enhance the chances of obtaining a phenotype that resembles PD. In this regard, patients with disease-causing mutations would appear to be the most promising because the clinical and pathological phenotype can so closely resemble sporadic PD, thus giving hope that any findings can be generalized to the vast majority of patients with non-genetic forms of PD.

The vision for this novel cellular model of PD is that it could overcome a major bottleneck for Parkinson's disease, which is the lack of a predictive preclinical model that can be used for disease research and drug discovery. A key goal that is essential for this model to be deemed successful is the development of a neurodegenerative phenotype that replicates characteristics of one or more features that are known to occur in brains of patients with PD. For this reason, if these cells do not exhibit a spontaneous pathological phenotype that is different from normal control cell lines, strategies that involve stressing these cells may be necessary to see if they are more susceptible to degeneration, and/or more prone to developing PD pathology such as alpha-synuclein aggregation. Pathological signatures may be more easily detected if cells are challenged with suspected environmental, metabolic or neural activity-based stressors.

As tantalizing as the promise of iPSCs may be, there are still challenges to be overcome. First, as in hESCs, iPSC differentiation needs to reach a point where efficient differentiation of midbrain TH neurons can be accomplished on a large scale. Second, iPSCs like hESCs are genetically-unstable and cells should

be used at low passages with frequent karyotype assessment. Finally, an understanding of the generation of iPSCs will be important, because confounding factors may increase survivability due to the acquisition of an embryonic state by an undefined process which might lead to a lack of a cellular phenotype. In spite of these limitations, there has been rapid progress in the generation of iPSCs, with continual improvements, and it currently appears that iPSCs have the potential to be a true “game changer.” However, here we are not referring to their use for neurotransplantation to treat the disease, an approach that is likely to require many more years to come to fruition, at least for PD, but rather to their use as tools for studying disease mechanisms and/or for drug screening that could halt or even reverse the clinical symptoms of PD. In our opinion, the possibility of translation of discoveries born out of iPSC biology will likely come first from their use to replicate authentic PD in a culture dish. If this can be achieved, it would likely have a huge impact on the development of disease modifying agents. In our opinion, this approach has a greater likelihood to benefit patients in a shorter time-frame than the long sought “cure of PD” that has been hoped for from cell replacement therapy (Figure 1).

## **Conclusion**

Virtually all of the cellular models described in this review have made valuable contributions to our understanding of PD or are likely to do so in the future, and without doubt many have served the scientific community well over the years (Figure 2). Each has its uses and limitations as summarized above. A number, such as patient-specific fibroblasts, immortalized neuroprogenitor cells

and stem cell lines appear to have a great deal to offer, but so far have not been utilized to their fullest as potential disease models for mechanistic research and drug screening.

However, each falls short of the vision of providing dopaminergic cell lines with ‘authentic’ PD, rather than representing “best guess” models that attempt to simulate the disease. We believe that the creation of iPSC lines from patients with PD, particularly of a known genetic cause, have the potential to do this. If these cells do in fact develop a disease phenotype, it could revolutionize research in PD. Indeed, it is not unreasonable to hope that the nuclear reprogramming of patient-specific tissue could form the foundation of multiple new lines of research that will give us novel insights about causes of PD by providing (1) *in vitro* models to understand mechanisms of PD and neurodegeneration, (2) *in vivo* models of neural degeneration in rodent or non-human primate brain, (3) cellular models for high-throughput screening of drugs/agents, and in the long-term (4) generation of DA neurons that are suitable for cell replacement therapies.

### **Acknowledgments**

The work was supported by the Blume Family foundation and The Parkinson Alliance. We thank Deepika Tewari and Lori Sterling for critical reading of the manuscript.

**Figure 1. Simplified scheme for utility of induced pluripotent stem cells and applications in Parkinson's Disease.** The scheme depicts the derivation of iPSCs, their further neuronal differentiation and the various avenues of downstream applications of this novel cellular model.

**Figure 2. Overview of human cellular models for Parkinson's disease.**

Cellular models in this review have been categorized into three groups: neuroblastoma cell lines, immortalized mesencephalic lines, and stem cells, for which examples are listed.

## References

- [1] E.R. Dorsey, R. Constantinescu, J.P. Thompson, K.M. Biglan, R.G. Holloway, K. Kieburtz, F.J. Marshall, B.M. Ravina, G. Schifitto, A. Siderowf and C.M. Tanner, Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030, *Neurology* 68 (2007) 384-6.
- [2] J.W. Langston, The Parkinson's complex: parkinsonism is just the tip of the iceberg, *Ann Neurol* 59 (2006) 591-6.
- [3] W. Poewe, Non-motor symptoms in Parkinson's disease, *Eur J Neurol* 15 Suppl 1 (2008) 14-20.
- [4] C.H. Hawkes, K. Del Tredici and H. Braak, Parkinson's disease: a dual-hit hypothesis, *Neuropathol Appl Neurobiol* 33 (2007) 599-614.
- [5] E.D. Laywell, V.G. Kukekov and D.A. Steindler, Multipotent neurospheres can be derived from forebrain subependymal zone and spinal cord of adult mice after protracted postmortem intervals, *Exp Neurol* 156 (1999) 430-3.
- [6] V.G. Kukekov, E.D. Laywell, O. Suslov, K. Davies, B. Scheffler, L.B. Thomas, T.F. O'Brien, M. Kusakabe and D.A. Steindler, Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain, *Exp Neurol* 156 (1999) 333-44.
- [7] T.D. Palmer, P.H. Schwartz, P. Taupin, B. Kaspar, S.A. Stein and F.H. Gage, Cell culture. Progenitor cells from human brain after death, *Nature* 411 (2001) 42-3.
- [8] N.M. Walton, B.M. Sutter, H.X. Chen, L.J. Chang, S.N. Roper, B. Scheffler and D.A. Steindler, Derivation and large-scale expansion of multipotent astroglial neural progenitors from adult human brain, *Development* 133 (2006) 3671-81.
- [9] D. Otto and K. Unsicker, FGF-2-mediated protection of cultured mesencephalic dopaminergic neurons against MPTP and MPP+: specificity and impact of culture conditions, non-dopaminergic neurons, and astroglial cells, *J Neurosci Res* 34 (1993) 382-93.
- [10] C. Mytilineou, G. Cohen and R.E. Heikkila, 1-Methyl-4-phenylpyridine (MPP+) is toxic to mesencephalic dopamine neurons in culture, *Neurosci Lett* 57 (1985) 19-24.
- [11] T. Fath, Y.D. Ke, P. Gunning, J. Gotz and L.M. Ittner, Primary support cultures of hippocampal and substantia nigra neurons, *Nat Protoc* 4 (2009) 78-85.
- [12] J.R. Sanchez-Ramos, P. Michel, W.J. Weiner and F. Hefti, Selective destruction of cultured dopaminergic neurons from fetal rat mesencephalon by 1-methyl-4-phenylpyridinium: cytochemical and morphological evidence, *J Neurochem* 50 (1988) 1934-44.
- [13] A.L. Perrier and L. Studer, Making and repairing the mammalian brain--in vitro production of dopaminergic neurons, *Semin Cell Dev Biol* 14 (2003) 181-9.

- [14] B.S. Bunney, L.A. Chiodo and A.A. Grace, Midbrain dopamine system electrophysiological functioning: a review and new hypothesis, *Synapse* 9 (1991) 79-94.
- [15] J.D. Elsworth and R.H. Roth, Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease, *Exp Neurol* 144 (1997) 4-9.
- [16] I. Mendez, R. Sanchez-Pernaute, O. Cooper, A. Vinuela, D. Ferrari, L. Bjorklund, A. Dagher and O. Isacson, Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease, *Brain* 128 (2005) 1498-510.
- [17] D.C. German, K.F. Manaye, P.K. Sonsalla and B.A. Brooks, Midbrain dopaminergic cell loss in Parkinson's disease and MPTP-induced parkinsonism: sparing of calbindin-D28k-containing cells, *Ann N Y Acad Sci* 648 (1992) 42-62.
- [18] E. Lynd-Balta and S.N. Haber, The organization of midbrain projections to the striatum in the primate: sensorimotor-related striatum versus ventral striatum, *Neuroscience* 59 (1994) 625-40.
- [19] S. Chung, E. Hedlund, M. Hwang, D.W. Kim, B.S. Shin, D.Y. Hwang, U. Jung Kang, O. Isacson and K.S. Kim, The homeodomain transcription factor Pitx3 facilitates differentiation of mouse embryonic stem cells into AHD2-expressing dopaminergic neurons, *Mol Cell Neurosci* 28 (2005) 241-52.
- [20] S. Chung, K.C. Sonntag, T. Andersson, L.M. Bjorklund, J.J. Park, D.W. Kim, U.J. Kang, O. Isacson and K.S. Kim, Genetic engineering of mouse embryonic stem cells by Nurr1 enhances differentiation and maturation into dopaminergic neurons, *Eur J Neurosci* 16 (2002) 1829-38.
- [21] D.J. Moore and T.M. Dawson, Value of genetic models in understanding the cause and mechanisms of Parkinson's disease, *Curr Neurol Neurosci Rep* 8 (2008) 288-96.
- [22] D.A. Di Monte, The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins?, *Lancet Neurol* 2 (2003) 531-8.
- [23] C. Klein and M.G. Schlossmacher, Parkinson disease, 10 years after its genetic revolution. Multiple clues to a complex disorder, *Neurology* (2007).
- [24] J.M. Bras and A. Singleton, Genetic susceptibility in Parkinson's disease, *Biochim Biophys Acta* (2008).
- [25] Y. Matsuoka, M. Vila, S. Lincoln, A. McCormack, M. Picciano, J. LaFrancois, X. Yu, D. Dickson, W.J. Langston, E. McGowan, M. Farrer, J. Hardy, K. Duff, S. Przedborski and D.A. Di Monte, Lack of nigral pathology in transgenic mice expressing human alpha-synuclein driven by the tyrosine hydroxylase promoter, *Neurobiol Dis* 8 (2001) 535-9.
- [26] L. Chen, B. Cagniard, T. Mathews, S. Jones, H.C. Koh, Y. Ding, P.M. Carvey, Z. Ling, U.J. Kang and X. Zhuang, Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice, *J Biol Chem* 280 (2005) 21418-26.

- [27] J.J. Palacino, D. Sagi, M.S. Goldberg, S. Krauss, C. Motz, M. Wacker, J. Klose and J. Shen, Mitochondrial dysfunction and oxidative damage in parkin-deficient mice, *J Biol Chem* 279 (2004) 18614-22.
- [28] A.B. Manning-Bog and J.W. Langston, Model fusion, the next phase in developing animal models for Parkinson's disease, *Neurotox Res* 11 (2007) 219-40.
- [29] S. Pahlman, J.C. Hoehner, E. Nanberg, F. Hedborg, S. Fagerstrom, C. Gestblom, I. Johansson, U. Larsson, E. Lavenius, E. Ortoft and et al., Differentiation and survival influences of growth factors in human neuroblastoma, *Eur J Cancer* 31A (1995) 453-8.
- [30] S. Pahlman, S. Mamaeva, G. Meyerson, M.E. Mattsson, C. Bjelfman, E. Ortoft and U. Hammerling, Human neuroblastoma cells in culture: a model for neuronal cell differentiation and function, *Acta Physiol Scand Suppl* 592 (1990) 25-37.
- [31] S.P. Presgraves, T. Ahmed, S. Borwege and J.N. Joyce, Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists, *Neurotox Res* 5 (2004) 579-98.
- [32] G. Lopez-Carballo, L. Moreno, S. Masia, P. Perez and D. Baretino, Activation of the phosphatidylinositol 3-kinase/Akt signaling pathway by retinoic acid is required for neural differentiation of SH-SY5Y human neuroblastoma cells, *J Biol Chem* 277 (2002) 25297-304.
- [33] N.D. Fernandes, Y. Sun and B.D. Price, Activation of the kinase activity of ATM by retinoic acid is required for CREB-dependent differentiation of neuroblastoma cells, *J Biol Chem* 282 (2007) 16577-84.
- [34] Y.T. Cheung, W.K. Lau, M.S. Yu, C.S. Lai, S.C. Yeung, K.F. So and R.C. Chang, Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as in vitro model in neurotoxicity research, *Neurotoxicology* 30 (2009) 127-35.
- [35] S.J. Pleasure and V.M. Lee, Ntera 2 cells: a human cell line which displays characteristics expected of a human committed neuronal progenitor cell, *J Neurosci Res* 35 (1993) 585-602.
- [36] M.B. Newman, I. Misiuta, A.E. Willing, T. Zigova, R.C. Karl, C.V. Borlongan and P.R. Sanberg, Tumorigenicity issues of embryonic carcinoma-derived stem cells: relevance to surgical trials using NT2 and hNT neural cells, *Stem Cells Dev* 14 (2005) 29-43.
- [37] J.D. Newcomb, M. Janowski, T. Zigova, S. Saporta, M.P. McGrogan, P.R. Sanberg and A.E. Willing, A comparison of dopaminergic cells from the human Ntera2/D1 cell line transplanted into the hemiparkinsonian rat, *Life Sci* 81 (2007) 441-8.
- [38] A.E. Willing, J.J. Sudberry, A.I. Othberg, S. Saporta, S.G. Poulos, D.F. Cameron, T.B. Freeman and P.R. Sanberg, Sertoli cells decrease microglial response and increase engraftment of human hNT neurons in the hemiparkinsonian rat striatum, *Brain Res Bull* 48 (1999) 441-4.
- [39] A.E. Willing, T. Zigova, M. Milliken, S. Poulos, S. Saporta, M. McGrogan, G. Snable and P.R. Sanberg, Lithium exposure enhances

- survival of NT2N cells (hNT neurons) in the hemiparkinsonian rat, *Eur J Neurosci* 16 (2002) 2271-8.
- [40] K.A. Baker, M. Hong, D. Sadi and I. Mendez, Intrastratial and intranigral grafting of hNT neurons in the 6-OHDA rat model of Parkinson's disease, *Exp Neurol* 162 (2000) 350-60.
- [41] D. Kondziolka, L. Wechsler, J. Gebel, S. DeCesare, E. Elder and C.C. Meltzer, Neuronal transplantation for motor stroke: from the laboratory to the clinic, *Phys Med Rehabil Clin N Am* 14 (2003) S153-60, xi.
- [42] C.M. Schwartz, C.E. Spivak, S.C. Baker, T.K. McDaniel, J.F. Loring, C. Nguyen, F.J. Chrest, R. Wersto, E. Arenas, X. Zeng, W.J. Freed and M.S. Rao, NTera2: a model system to study dopaminergic differentiation of human embryonic stem cells, *Stem Cells Dev* 14 (2005) 517-34.
- [43] S.S. Ghosh, R.H. Swerdlow, S.W. Miller, B. Sheeman, W.D. Parker, Jr. and R.E. Davis, Use of cytoplasmic hybrid cell lines for elucidating the role of mitochondrial dysfunction in Alzheimer's disease and Parkinson's disease, *Ann N Y Acad Sci* 893 (1999) 176-91.
- [44] R.H. Swerdlow, J.K. Parks, S.W. Miller, J.B. Tuttle, P.A. Trimmer, J.P. Sheehan, J.P. Bennett, Jr., R.E. Davis and W.D. Parker, Jr., Origin and functional consequences of the complex I defect in Parkinson's disease, *Ann Neurol* 40 (1996) 663-71.
- [45] M. Gu, J.M. Cooper, J.W. Taanman and A.H. Schapira, Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease, *Ann Neurol* 44 (1998) 177-86.
- [46] C.W. Shults and S.W. Miller, Reduced complex I activity in parkinsonian cybrids, *Movement disorders supplement* 13 (1998) 217.
- [47] M.K. Borland, K.P. Mohanakumar, J.D. Rubinstein, P.M. Keeney, J. Xie, R. Capaldi, L.D. Dunham, P.A. Trimmer and J.P. Bennett, Jr., Relationships among molecular genetic and respiratory properties of Parkinson's disease cybrid cells show similarities to Parkinson's brain tissues, *Biochim Biophys Acta* 1792 (2009) 68-74.
- [48] A.R. Esteves, A.F. Domingues, I.L. Ferreira, C. Janeiro, R.H. Swerdlow, C.R. Oliveira and S.M. Cardoso, Mitochondrial function in Parkinson's disease cybrids containing an nt2 neuron-like nuclear background, *Mitochondrion* 8 (2008) 219-28.
- [49] I.G. Onyango, J.B. Tuttle and J.P. Bennett, Jr., Brain-derived growth factor and glial cell line-derived growth factor use distinct intracellular signaling pathways to protect PD cybrids from H<sub>2</sub>O<sub>2</sub>-induced neuronal death, *Neurobiol Dis* 20 (2005) 141-54.
- [50] P.A. Trimmer, M.K. Borland, P.M. Keeney, J.P. Bennett, Jr. and W.D. Parker, Jr., Parkinson's disease transgenic mitochondrial cybrids generate Lewy inclusion bodies, *J Neurochem* 88 (2004) 800-12.
- [51] P.A. Trimmer, R.H. Swerdlow, J.K. Parks, P. Keeney, J.P. Bennett, Jr., S.W. Miller, R.E. Davis and W.D. Parker, Jr., Abnormal mitochondrial morphology in sporadic Parkinson's and Alzheimer's disease cybrid cell lines, *Exp Neurol* 162 (2000) 37-50.



- [52] R.H. Swerdlow, J.K. Parks, J.N. Davis, 2nd, D.S. Cassarino, P.A. Trimmer, L.J. Currie, J. Dougherty, W.S. Bridges, J.P. Bennett, Jr., G.F. Wooten and W.D. Parker, Matrilinial inheritance of complex I dysfunction in a multigenerational Parkinson's disease family, *Ann Neurol* 44 (1998) 873-81.
- [53] R.H. Swerdlow, Mitochondria in cybrids containing mtDNA from persons with mitochondriopathies, *J Neurosci Res* 85 (2007) 3416-28.
- [54] H.H. Hoepken, S. Gispert, M. Azizov, M. Klinkenberg, F. Ricciardi, A. Kurz, B. Morales-Gordo, M. Bonin, O. Riess, T. Gasser, D. Kogel, H. Steinmetz and G. Auburger, Parkinson patient fibroblasts show increased alpha-synuclein expression, *Exp Neurol* 212 (2008) 307-13.
- [55] C. Piccoli, A. Sardanelli, R. Scrima, M. Ripoli, G. Quarato, A. D'Aprile, F. Bellomo, S. Scacco, G. De Michele, A. Filla, A. Iuso, D. Boffoli, N. Capitanio and S. Papa, Mitochondrial Respiratory Dysfunction in Familial Parkinsonism Associated with PINK1 Mutation, *Neurochem Res* (2008).
- [56] J. Lotharius, S. Barg, P. Wiekop, C. Lundberg, H.K. Raymon and P. Brundin, Effect of mutant alpha-synuclein on dopamine homeostasis in a new human mesencephalic cell line, *J Biol Chem* 277 (2002) 38884-94.
- [57] J. Lotharius, J. Falsig, J. van Beek, S. Payne, R. Dringen, P. Brundin and M. Leist, Progressive degeneration of human mesencephalic neuron-derived cells triggered by dopamine-dependent oxidative stress is dependent on the mixed-lineage kinase pathway, *J Neurosci* 25 (2005) 6329-42.
- [58] G. Paul, N.S. Christophersen, H. Raymon, C. Kiaer, R. Smith and P. Brundin, Tyrosine hydroxylase expression is unstable in a human immortalized mesencephalic cell line--studies in vitro and after intracerebral grafting in vivo, *Mol Cell Neurosci* 34 (2007) 390-9.
- [59] P.H. Schwartz, D.J. Brick, A.E. Stover, J.F. Loring and F.J. Muller, Differentiation of neural lineage cells from human pluripotent stem cells, *Methods* 45 (2008) 142-58.
- [60] R. Donato, E.A. Miljan, S.J. Hines, S. Aouabdi, K. Pollock, S. Patel, F.A. Edwards and J.D. Sinden, Differential development of neuronal physiological responsiveness in two human neural stem cell lines, *BMC Neurosci* 8 (2007) 36.
- [61] R. Hoffrogge, S. Mikkat, C. Scharf, S. Beyer, H. Christoph, J. Pahnke, E. Mix, M. Berth, A. Uhrmacher, I.Z. Zubrzycki, E. Miljan, U. Volker and A. Rolfs, 2-DE proteome analysis of a proliferating and differentiating human neuronal stem cell line (ReNcell VM), *Proteomics* 6 (2006) 1833-47.
- [62] A. Wood-Kaczmar, S. Gandhi, Z. Yao, A.S. Abramov, E.A. Miljan, G. Keen, L. Stanyer, I. Hargreaves, K. Klupsch, E. Deas, J. Downward, L. Mansfield, P. Jat, J. Taylor, S. Heales, M.R. Duchen, D. Latchman, S.J. Tabrizi and N.W. Wood, PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons, *PLoS ONE* 3 (2008) e2455.

- [63] T. Deacon, J. Dinsmore, L.C. Costantini, J. Ratliff and O. Isacson, Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation, *Exp Neurol* 149 (1998) 28-41.
- [64] S.H. Lee, N. Lumelsky, L. Studer, J.M. Auerbach and R.D. McKay, Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells, *Nat Biotechnol* 18 (2000) 675-9.
- [65] H. Kawasaki, K. Mizuseki, S. Nishikawa, S. Kaneko, Y. Kuwana, S. Nakanishi, S.I. Nishikawa and Y. Sasai, Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity, *Neuron* 28 (2000) 31-40.
- [66] L.M. Bjorklund, R. Sanchez-Pernaute, S. Chung, T. Andersson, I.Y. Chen, K.S. McNaught, A.L. Brownell, B.G. Jenkins, C. Wahlestedt, K.S. Kim and O. Isacson, Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model, *Proc Natl Acad Sci U S A* 99 (2002) 2344-9.
- [67] N.S. Roy, C. Cleren, S.K. Singh, L. Yang, M.F. Beal and S.A. Goldman, Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes, *Nat Med* 12 (2006) 1259-68.
- [68] J.H. Kim, J.M. Auerbach, J.A. Rodriguez-Gomez, I. Velasco, D. Gavin, N. Lumelsky, S.H. Lee, J. Nguyen, R. Sanchez-Pernaute, K. Bankiewicz and R. McKay, Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease, *Nature* 418 (2002) 50-6.
- [69] J.P. Burbach and M.P. Smidt, Molecular programming of stem cells into mesodiencephalic dopaminergic neurons, *Trends Neurosci* 29 (2006) 601-3.
- [70] M.P. Smidt and J.P. Burbach, How to make a mesodiencephalic dopaminergic neuron, *Nat Rev Neurosci* 8 (2007) 21-32.
- [71] D.M. Van den Heuvel and R.J. Pasterkamp, Getting connected in the dopamine system, *Prog Neurobiol* 85 (2008) 75-93.
- [72] H. Kawasaki, H. Suemori, K. Mizuseki, K. Watanabe, F. Urano, H. Ichinose, M. Haruta, M. Takahashi, K. Yoshikawa, S. Nishikawa, N. Nakatsuji and Y. Sasai, Generation of dopaminergic neurons and pigmented epithelia from primate ES cells by stromal cell-derived inducing activity, *Proc Natl Acad Sci U S A* 99 (2002) 1580-5.
- [73] X. Zeng, J. Cai, J. Chen, Y. Luo, Z.B. You, E. Fötter, Y. Wang, B. Harvey, T. Miura, C. Backman, G.J. Chen, M.S. Rao and W.J. Freed, Dopaminergic differentiation of human embryonic stem cells, *Stem Cells* 22 (2004) 925-40.
- [74] K.A. Buytaert-Hoefen, E. Alvarez and C.R. Freed, Generation of tyrosine hydroxylase positive neurons from human embryonic stem cells after coculture with cellular substrates and exposure to GDNF, *Stem Cells* 22 (2004) 669-74.
- [75] A.L. Perrier, V. Tabar, T. Barberi, M.E. Rubio, J. Bruses, N. Topf, N.L. Harrison and L. Studer, Derivation of midbrain dopamine neurons from

- human embryonic stem cells, *Proc Natl Acad Sci U S A* 101 (2004) 12543-8.
- [76] T. Vazin, J. Chen, C.T. Lee, R. Amable and W.J. Freed, Assessment of stromal-derived inducing activity in the generation of dopaminergic neurons from human embryonic stem cells, *Stem Cells* 26 (2008) 1517-25.
- [77] F. Yue, L. Cui, K. Johkura, N. Ogiwara and K. Sasaki, Induction of midbrain dopaminergic neurons from primate embryonic stem cells by coculture with sertoli cells, *Stem Cells* 24 (2006) 1695-706.
- [78] M.S. Cho, Y.E. Lee, J.Y. Kim, S. Chung, Y.H. Cho, D.S. Kim, S.M. Kang, H. Lee, M.H. Kim, J.H. Kim, J.W. Leem, S.K. Oh, Y.M. Choi, D.Y. Hwang, J.W. Chang and D.W. Kim, Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells, *Proc Natl Acad Sci U S A* 105 (2008) 3392-7.
- [79] L. Studer, V. Tabar and R.D. McKay, Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats, *Nat Neurosci* 1 (1998) 290-5.
- [80] L. Studer, M. Csete, S.H. Lee, N. Kabbani, J. Walikonis, B. Wold and R. McKay, Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen, *J Neurosci* 20 (2000) 7377-83.
- [81] R. Sanchez-Pernaute, L. Studer, K.S. Bankiewicz, E.O. Major and R.D. McKay, In vitro generation and transplantation of precursor-derived human dopamine neurons, *J Neurosci Res* 65 (2001) 284-8.
- [82] D.M. Panchision and R.D. McKay, The control of neural stem cells by morphogenic signals, *Curr Opin Genet Dev* 12 (2002) 478-87.
- [83] B.J. Snyder and C.W. Olanow, Stem cell treatment for Parkinson's disease: an update for 2005, *Curr Opin Neurol* 18 (2005) 376-85.
- [84] S. Chung, B.S. Shin, M. Hwang, T. Lardaro, U.J. Kang, O. Isacson and K.S. Kim, Neural precursors derived from embryonic stem cells, but not those from fetal ventral mesencephalon, maintain the potential to differentiate into dopaminergic neurons after expansion in vitro, *Stem Cells* 24 (2006) 1583-93.
- [85] A. Hermann, M. Maisel, F. Wegner, S. Liebau, D.W. Kim, M. Gerlach, J. Schwarz, K.S. Kim and A. Storch, Multipotent neural stem cells from the adult tegmentum with dopaminergic potential develop essential properties of functional neurons, *Stem Cells* 24 (2006) 949-64.
- [86] E. Roussa, M. Wiehle, N. Dunker, S. Becker-Katins, O. Oehlke and K. Krieglstein, Transforming growth factor beta is required for differentiation of mouse mesencephalic progenitors into dopaminergic neurons in vitro and in vivo: ectopic induction in dorsal mesencephalon, *Stem Cells* 24 (2006) 2120-9.
- [87] I. Liste, E. Garcia-Garcia and A. Martinez-Serrano, The generation of dopaminergic neurons by human neural stem cells is enhanced by Bcl-XL, both in vitro and in vivo, *J Neurosci* 24 (2004) 10786-95.

- [88] I. Liste, E. Garcia-Garcia, C. Bueno and A. Martinez-Serrano, Bcl-XL modulates the differentiation of immortalized human neural stem cells, *Cell Death Differ* 14 (2007) 1880-92.
- [89] B.A. Baghbaderani, K. Mukhida, A. Sen, M. Hong, I. Mendez and L.A. Behie, Expansion of Human Neural Precursor Cells in Large-Scale Bioreactors for the Treatment of Neurodegenerative Disorders, *Biotechnol Prog* (2008).
- [90] K. Mukhida, B.A. Baghbaderani, M. Hong, M. Lewington, T. Phillips, M. McLeod, A. Sen, L.A. Behie and I. Mendez, Survival, differentiation, and migration of bioreactor-expanded human neural precursor cells in a model of Parkinson disease in rats, *Neurosurg Focus* 24 (2008) E8.
- [91] S. Greschat, J. Schira, P. Kury, C. Rosenbaum, M.A. de Souza Silva, G. Kogler, P. Wernet and H.W. Muller, Unrestricted somatic stem cells from human umbilical cord blood can be differentiated into neurons with a dopaminergic phenotype, *Stem Cells Dev* 17 (2008) 221-32.
- [92] P. Bianco, P.G. Robey and P.J. Simmons, Mesenchymal stem cells: revisiting history, concepts, and assays, *Cell Stem Cell* 2 (2008) 313-9.
- [93] S. Nadri, M. Soleimani, J. Kiani, A. Atashi and R. Izadpanah, Multipotent mesenchymal stem cells from adult human eye conjunctiva stromal cells, *Differentiation* 76 (2008) 223-31.
- [94] S. Nadri, M. Soleimani, Z. Mobarra and S. Amini, Expression of dopamine-associated genes on conjunctiva stromal-derived human mesenchymal stem cells, *Biochem Biophys Res Commun* 377 (2008) 423-8.
- [95] M. Dezawa, H. Kanno, M. Hoshino, H. Cho, N. Matsumoto, Y. Itokazu, N. Tajima, H. Yamada, H. Sawada, H. Ishikawa, T. Mimura, M. Kitada, Y. Suzuki and C. Ide, Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation, *J Clin Invest* 113 (2004) 1701-10.
- [96] L. Guo, F. Yin, H.Q. Meng, L. Ling, T.N. Hu-He, P. Li, C.X. Zhang, S. Yu, D.S. Duan and H.X. Fan, Differentiation of mesenchymal stem cells into dopaminergic neuron-like cells in vitro, *Biomed Environ Sci* 18 (2005) 36-42.
- [97] Y.S. Fu, Y.C. Cheng, M.Y. Lin, H. Cheng, P.M. Chu, S.C. Chou, Y.H. Shih, M.H. Ko and M.S. Sung, Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: potential therapeutic application for Parkinsonism, *Stem Cells* 24 (2006) 115-24.
- [98] V.M. Tatard, G. D'Ippolito, S. Diabira, A. Valeyev, J. Hackman, M. McCarthy, T. Bouckennooghe, P. Menei, C.N. Montero-Menei and P.C. Schiller, Neurotrophin-directed differentiation of human adult marrow stromal cells to dopaminergic-like neurons, *Bone* 40 (2007) 360-73.
- [99] S. Suon, M. Yang and L. Iacovitti, Adult human bone marrow stromal spheres express neuronal traits in vitro and in a rat model of Parkinson's disease, *Brain Res* 1106 (2006) 46-51.

- [100] K.A. Trzaska, E.V. Kuzhikandathil and P. Rameshwar, Specification of a dopaminergic phenotype from adult human mesenchymal stem cells, *Stem Cells* 25 (2007) 2797-808.
- [101] K.A. Trzaska and P. Rameshwar, Current advances in the treatment of Parkinson's disease with stem cells, *Curr Neurovasc Res* 4 (2007) 99-109.
- [102] M. Fallahi-Sichani, M. Soleimani, S.M. Najafi, J. Kiani, E. Arefian and A. Atashi, In vitro differentiation of cord blood unrestricted somatic stem cells expressing dopamine-associated genes into neuron-like cells, *Cell Biol Int* 31 (2007) 299-303.
- [103] Z. Zhang, X. Wang and S. Wang, Isolation and characterization of mesenchymal stem cells derived from bone marrow of patients with Parkinson's disease, *In Vitro Cell Dev Biol Anim* 44 (2008) 169-77.
- [104] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda and S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, *Cell* 131 (2007) 861-72.
- [105] J. Yu, M.A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J.L. Frane, S. Tian, J. Nie, G.A. Jonsdottir, V. Ruotti, R. Stewart, Slukvin, II and J.A. Thomson, Induced pluripotent stem cell lines derived from human somatic cells, *Science* 318 (2007) 1917-20.
- [106] I.H. Park, R. Zhao, J.A. West, A. Yabuuchi, H. Huo, T.A. Ince, P.H. Lerou, M.W. Lensch and G.Q. Daley, Reprogramming of human somatic cells to pluripotency with defined factors, *Nature* 451 (2008) 141-6.
- [107] M. Nakagawa, M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa and S. Yamanaka, Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts, *Nat Biotechnol* 26 (2008) 101-6.
- [108] W.E. Lowry, L. Richter, R. Yachechko, A.D. Pyle, J. Tchieu, R. Sridharan, A.T. Clark and K. Plath, Generation of human induced pluripotent stem cells from dermal fibroblasts, *Proc Natl Acad Sci U S A* 105 (2008) 2883-8.
- [109] Y. Zhao, X. Yin, H. Qin, F. Zhu, H. Liu, W. Yang, Q. Zhang, C. Xiang, P. Hou, Z. Song, Y. Liu, J. Yong, P. Zhang, J. Cai, M. Liu, H. Li, Y. Li, X. Qu, K. Cui, W. Zhang, T. Xiang, Y. Wu, Y. Zhao, C. Liu, C. Yu, K. Yuan, J. Lou, M. Ding and H. Deng, Two supporting factors greatly improve the efficiency of human iPSC generation, *Cell Stem Cell* 3 (2008) 475-9.
- [110] D. Hockemeyer, F. Soldner, E.G. Cook, Q. Gao, M. Mitalipova and R. Jaenisch, A drug-inducible system for direct reprogramming of human somatic cells to pluripotency, *Cell Stem Cell* 3 (2008) 346-53.
- [111] D. Huangfu, R. Maehr, W. Guo, A. Eijkelenboom, M. Snitow, A.E. Chen and D.A. Melton, Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds, *Nat Biotechnol* 26 (2008) 795-7.
- [112] T.S. Mikkelsen, J. Hanna, X. Zhang, M. Ku, M. Wernig, P. Schorderet, B.E. Bernstein, R. Jaenisch, E.S. Lander and A. Meissner, Dissecting direct reprogramming through integrative genomic analysis, *Nature* 454 (2008) 49-55.

- [113] M. Stadtfeld, M. Nagaya, J. Utikal, G. Weir and K. Hochedlinger, Induced Pluripotent Stem Cells Generated Without Viral Integration, *Science* (2008).
- [114] K. Okita, M. Nakagawa, H. Hyenjong, T. Ichisaka and S. Yamanaka, Generation of Mouse Induced Pluripotent Stem Cells Without Viral Vectors, *Science* (2008).
- [115] B.W. Carey, S. Markoulaki, J. Hanna, K. Saha, Q. Gao, M. Mitalipova and R. Jaenisch, Reprogramming of murine and human somatic cells using a single polycistronic vector, *Proc Natl Acad Sci U S A* (2008).
- [116] S. Eminli, J.S. Utikal, K. Arnold, R. Jaenisch and K. Hochedlinger, Reprogramming of Neural Progenitor Cells into iPS Cells in the Absence of Exogenous Sox2 Expression, *Stem Cells* (2008).
- [117] K. Kaji, K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni and K. Woltjen, Virus-free induction of pluripotency and subsequent excision of reprogramming factors, *Nature* (2009).
- [118] K. Woltjen, I.P. Michael, P. Mohseni, R. Desai, M. Mileikovsky, R. Hamalainen, R. Cowling, W. Wang, P. Liu, M. Gertsenstein, K. Kaji, H.K. Sung and A. Nagy, piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells, *Nature* (2009).
- [119] F. Soldner, D. Hockemeyer, C. Beard, Q. Gao, G.W. Bell, E.G. Cook, G. Hargus, A. Blak, O. Cooper, M. Mitalipova, O. Isacson and R. Jaenisch, Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors, *Cell* 136 (2009) 964-77.
- [120] H. Zhou, S. Wu, J.Y. Joo, S. Zhu, D.W. Han, T. Lin, S. Trauger, G. Bien, S. Yao, Y. Zhu, G. Siuzdak, H.R. Schöler, L. Duan and S. Ding, Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins, *Cell Stem Cell In Press*, Corrected Proof.
- [121] I.H. Park, N. Arora, H. Huo, N. Maherali, T. Ahfeldt, A. Shimamura, M.W. Lensch, C. Cowan, K. Hochedlinger and G.Q. Daley, Disease-specific induced pluripotent stem cells, *Cell* 134 (2008) 877-86.
- [122] J.T. Dimos, K.T. Rodolfa, K.K. Niakan, L.M. Weisenthal, H. Mitsumoto, W. Chung, G.F. Croft, G. Saphier, R. Leibel, R. Goland, H. Wichterle, C.E. Henderson and K. Eggan, Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons, *Science* 321 (2008) 1218-21.
- [123] M. Wernig, J.P. Zhao, J. Pruszak, E. Hedlund, D. Fu, F. Soldner, V. Broccoli, M. Constantine-Paton, O. Isacson and R. Jaenisch, Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease, *Proc Natl Acad Sci U S A* 105 (2008) 5856-61.
- [124] T. Aoi, K. Yae, M. Nakagawa, T. Ichisaka, K. Okita, K. Takahashi, T. Chiba and S. Yamanaka, Generation of Pluripotent Stem Cells from Adult Mouse Liver and Stomach Cells, *Science* (2008).
- [125] T. Aasen, A. Raya, M.J. Barrero, E. Garreta, A. Consiglio, F. Gonzalez, R. Vassena, J. Bilic, V. Pekarik, G. Tiscornia, M. Edel, S. Boue and J.C.

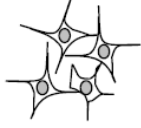
- Belmonte, Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes, *Nat Biotechnol* (2008).
- [126] Y.H. Loh, S. Agarwal, I.H. Park, A. Urbach, H. Huo, G.C. Heffner, K. Kim, J.D. Miller, K. Ng and G.Q. Daley, Generation of induced pluripotent stem cells from human blood, *Blood* (2009).
- [127] W.P. Gilks, P.M. Abou-Sleiman, S. Gandhi, S. Jain, A. Singleton, A.J. Lees, K. Shaw, K.P. Bhatia, V. Bonifati, N.P. Quinn, J. Lynch, D.G. Healy, J.L. Holton, T. Revesz and N.W. Wood, A common LRRK2 mutation in idiopathic Parkinson's disease, *Lancet* 365 (2005) 415-6.
- [128] W.C. Nichols, N. Pankratz, D. Hernandez, C. Paisan-Ruiz, S. Jain, C.A. Halter, V.E. Michaels, T. Reed, A. Rudolph, C.W. Shults, A. Singleton and T. Foroud, Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease, *Lancet* 365 (2005) 410-2.
- [129] A. Di Fonzo, C.F. Rohe, J. Ferreira, H.F. Chien, L. Vacca, F. Stocchi, L. Guedes, E. Fabrizio, M. Manfredi, N. Vanacore, S. Goldwurm, G. Breedveld, C. Sampaio, G. Meco, E. Barbosa, B.A. Oostra and V. Bonifati, A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease, *Lancet* 365 (2005) 412-5.
- [130] J. Kachergus, I.F. Mata, M. Hulihan, J.P. Taylor, S. Lincoln, J. Aasly, J.M. Gibson, O.A. Ross, T. Lynch, J. Wiley, H. Payami, J. Nutt, D.M. Maraganore, K. Czyzewski, M. Styczynska, Z.K. Wszolek, M.J. Farrer and M. Toft, Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations, *Am J Hum Genet* 76 (2005) 672-80.
- [131] M.J. Farrer, Genetics of Parkinson disease: paradigm shifts and future prospects, *Nat Rev Genet* 7 (2006) 306-18.

<b>Cell line/source</b>	<b>Examples</b>	<b>Characteristics</b>	<b>References</b>
<b>Non-patient specific human cell lines</b>			
<i>Human neuroblastoma cell lines</i>	SH-SY5Y, SK-N-MC, SK-N-BE	Expression of TH, dopamine D2 and D3 receptors, DAT, and VMAT2 upon differentiation with RA and TPA (ref.31)	29-34
<i>Embryonic carcinoma cell line</i>	Ntera-2/NT2 line, hNT (or LBS)	Under co-culture with PA6 cells expression of dopaminergic markers AADC, DAT, Nurr1, TrkB, TrkC, and GFRA1	42
<i>Immortalized Human embryonic mesencephalic cells</i>	LUHMES, ReNcell VM NSCs	LUHMES: expression of TH and DAT after in vitro differentiation, do not maintain phenotype <i>in vivo</i>	56-62
<b>First generation models of patient-specific cell lines</b>			
<i>Cybrid Cell lines</i>		Reduced complex I activity, enhanced susceptibility for H <sub>2</sub> O <sub>2</sub> and MPP <sup>+</sup> , fibrillar and vesicular inclusions after prolonged culture conditions	43-49
<i>Primary human fibroblasts</i>	PINK1-deficient fibroblasts	Upregulation of alpha-synuclein and synaptic protein genes, lower respiratory activity, increased ROS production, and decrease in cytochrome C	54, 55
<b>Stem Cell models</b>			
<i>Human embryonic stem cells</i>		Upon optimized differentiation, expression of most midbrain DA markers, DA release and electrophysiological characteristics in a subset of cells	65, 72-78
<i>Human embryonic and adult neural stem cells</i>		Expression of TH, Nurr-1, DA release	79-84, 87-90
<i>Human mesenchymal stem</i>		Overall TH expression in 12.7-67% of cells,	91-103



<i>cells</i>	expression of En1, En2, Nurr1, Pitx3, Pax2, Wnt1 and Wnt3a (ref. 102)	
<i>Human induced pluripotent stem cells</i>	TH expression in iPSCs from patients with idiopathic PD	119

Human neuroblastoma  
/carcinoma cells



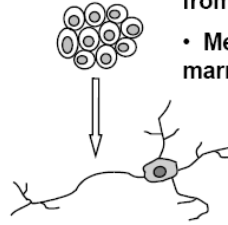
- SH-SY5Y
- SK-N-BE
- SK-N-MC
- SK-N-SH
- MN9D
- NTera-2

Immortalized human  
mesencephalic lines



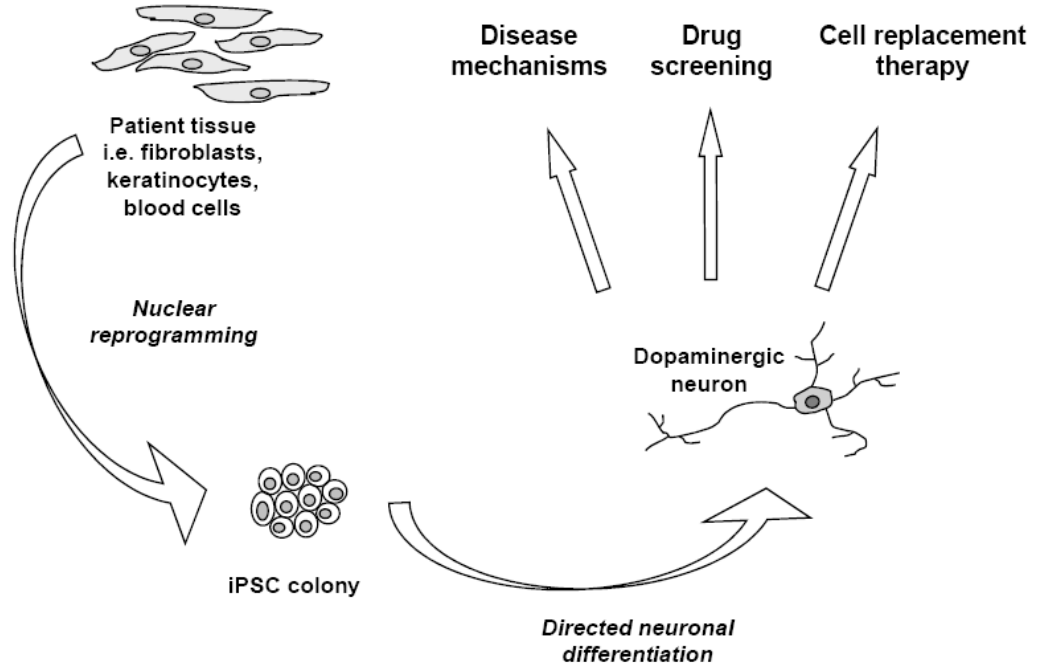
- LUHMES
- NGC-407
- ReNcell  
VM NSC

Stem cells



- Embryonic
- Neural: embryonic, adult  
from various regions
- Mesenchymal: bone  
marrow, umbilical

ACCEPTED MANUSCRIPT



ACCEPTTEL