Review Article

Dopamine-synthesizing neurons: An overview of their development and application for cell therapy

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Abstract
Cell-gene therapy is a dynamic constituent of novel medical biotechnology. Neurodegenerative disorders in which damage to or demise of specific brain cell types plays central role, are clear examples of disease candidate for cell replacement therapy. Dopaminergic (DAergic) neurons biosynthesize dopamine, a vital neurotransmitter in the central nervous system. Due to the involvement of dopamine in a number of critical physiological functions in human and other mammals, disturbed dopamine neurotransmission resulting from DAergic neuron death or damage causes a few known disorders most prominently Parkinson's disease (PD). DAergic cell replacement therapies proposed as promising approaches for PD treatment have prompted scientists to thoroughly investigate the embryonic development of DAergic neurons and their function in ordinary life. This review summarizes past and current findings in DAergic neuron development and survival. It also briefly looks at the future prospect of DAergic neuron generation *in vitro* aiming at clinical applications *in vivo*.
Keywrods**:** dopamine; dopaminergic; Parkinson disease; cell therapy

INTRODUCTION

Cell-gene therapy requires thorough understanding of biology of disease candidates and, in the first place, the normal physiology of the affected cells/tissues. In line with this notion, cell replacement strategies may, in some diseases, need imitation of developmental steps physiologically occurring *in vivo* in order to generate fresh batches of functional cells for transplantation (Wijeyekoon and Barker 2009; Korecka *et al*., 2008).

Dopamine is an important catecholamine neurotransmitter found in both vertebrates and invertebrates (Dunnett *et al*., 2005). Dopamine is synthesized by DAergic neurons (Chinta and Andersen 2005) and functions via its specific receptors found in most brain cells (Vallone *et al*., 2000; Missale *et al*., 1998). Deregulated DAergic system can cause a wide range of neurological conditions some of which have devastating impact on human life. Abnormal dopamine receptor signaling is the main cause for attention-deficit hyperactivity disorder (Heijtz *et al*., 2007), Schizophrenia (Takahashi 2006; Reynolds 1996), genetic hypertension (Banday and Lokhandwala 2008) and addiction (Volkow *et al*., 2007).

A number of important psychiatric and movement disorders most notably Parkinson's disease (PD) in the human are caused by the demise or dysfunction of DAergic neurons in the CNS (Hirsch *et al*., 1997; German *et al*., 1996). This highlights the importance of DAergic neurons for health and explains why these cell types are the subject of extensive investigation. Although the main cause of DAergic neuron death in PD and related disorders remains unknown, oxidative stress that is accelerated as age increases is amongst intrinsic contributors to DAergic cell death in these aging disorders. Neuroprotective strategies are actively explored and applied; however, substitution of dead or dying neurons would conceivably be more effective in the long-term prospect of treatment. Such strategies clearly demand a wide range of investigation on the

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source of new cells, safety and efficacy of cell transplantation, and functionality of replacing neurons upon their integration into the neural network within the patient's brain. To initiate a search for a viable external source for DAergic neuron generation, endogenous steps of DAergic neuron development at embryonic stages have to be taken to account. Numerous studies have clarified these steps at cellular and molecular levels although not the role of all elements involved in the process is fully understood yet. This review compiles data available from studies on the cell/ molecular events leading to DAergic neuron generation *in vivo* and presents a glimpse of how to apply this knowledge for PD cell replacement therapy.

Dopamine: Dopamine is one of the most intensively-studied neurotransmitters due to its critical roles in a diverse range of physiological activities. These activities are mostly related to behavior and cognition including voluntary movement, motivation and reward, inhibition of prolactin production, sleep, mood, attention, and learning (see Dunnet *et al*., 2005 for a comprehensive review).

DAergic neurons are the main source of dopamine biosynthesis and release. The neurons express a series of enzymes specifically involved in biosynthesis of the neurotransmitter (Nagatsu 1991). Tyrosine hydroxylase (TH) promotes hydroxylation of the amino acid L-tyrosine to dopamine precursor dihydroxyphenylalanine (L-DOPA) (Nagatsu *et al*., 1964). TH is found in the cytosol of all cells containing catecholamines and, therefore, widely considered for DAergic neuron characterization. Aromatic L-amino acid decarboxylase (AADC) converts L-DOPA to dopamine. Downstream catecholamines, namely norepinephrine and epinephrine, are catalysed by dopamine β-carboxylase and phenylethanolamine, respectively (Nagatsu 1991).

Dopamine exerts its functions via interactions with its receptors. These G protein-coupled receptor subtypes are divided into two main groups: D_1-like subtypes of D_1 and D_5, and D_2-like subtypes of D_2, D_3 and D_4 (Callier *et al*., 2003; reviewed in Missale *et al*., 1998). Dopamine receptors are widely expressed in the CNS, reflecting their involvement in various brain activities. In the periphery, dopamine receptors are present more prominently in kidney, vasculature, and pituitary, where they affect mainly sodium homeostasis, vascular tone, and hormone secretion (Missale *et al*., 1998). The structural and functional aspects of dopamine receptors have been extensively covered elsewhere and will not be discussed further in this review.

DAergic neurons: classification and specific characteristics: A traditional definition of DAergic neurons that is still applied to these cells was introduced by Dahlstrom and Fuxe in 1964 when they first observed them (Dahlstrom and Fuxe 1964). DAergic neuron subdivisions they defined were based on three different criteria: (1) cytoarchitechtonic features observed by non-specific staining such as the Nissl staining, (2) the DAergic phenotype of neurons and (3) the organization of midbrain DAergic neurons in dorsal and ventral tiers.

It is now well known that DAergic neurons are mainly located in the diencephalon, mesencephalon and the olfactory bulb (Bjorklund and Dunnett 2007). Approximately 90% of total brain DAergic neurons reside in the ventral part of mesencephalon (Chinta and Andersen 2005). The mammalian mesencephalic DAergic (mes-DAergic) neurons are located in three distinct brain subregions: substantia nigra pars compacta (SNpc), ventral tegmental area (VTA), and retrorubral field (RRF). The historic classification by Dahlstrom and Fuxe has designated these neurons as A8 (RRE), A9 (SNpc) and A10 (VTA) (Dahlstrom and Fuxe 1964).

Mammalian species contain different numbers of

Table 1. DAergic neurons in mammalian species. See text for more details.

Species	DAergic neuron numbers	References
Mice	3000	German *et al* 1983
Rats	45000	German and Manaye 1993
Macaca	16500	German *et al* 1983
Monkeys	590000	Pakkenberg *et al* 1991; German *et al* 1983
Humans		

DAergic neurons in their mesencephalon (Table 1). For example mice and rats possess up to 30000 and 45000, respectively, whereas the number stands, respectively, at 165000 and 590000 in macaca monkeys and humans (German and Manaye 1993; Pakkenberg *et al*., 1991; German *et al*., 1983).

The best known DAergic subdivision is the nigrostriatal (SN-ST) system which originates in the the SNpc and projects its fibers into the dorsal striatum that includes caudate and putamen (Fig. 1) (Smith and Kieval 2000). The SN-ST system is well known for its crucial role in controlling voluntary motor movement. The mesolimbic DAergic system located more medial to the SN-ST system arises from VTA DAergic neurons and sends its projections mainly into the nucleus accombens, olfactory tubercle as well as the septum, amygdale and hippocampus (Bjurklund and Dunnett 2007). These projections collectively form the mesolibmic DAergic system. Finally, the mesocortical DAergic system is constituted by DAergic neurons in the medial VTA that project to the prefrontal, cingulated and the perirhinal cortex (Bjorklund and Dunnett 2007). The mesocortical and mesolimbic DAergic systems are closely located and overlapped jointly forming the mesocorticolimbic system (Wise 2004). VTA DAergic neurons that collectively constitute the mesocorticolimbic system are involved in emotion-based behavior including motivation and reward (Phillips *et al*., 2008).

DAergic neurons: embryonic development

Early determinants: DAergic neurons develop during embryonic life in a staged fashion via distinct events mediated by certain molecules. The neurons develop from a single cell group that originates at the mesencephalic-diencephalic junction during embryonic development and projects to different forebrain targets. Several factors have been identified that determine the fate of midbrain DAergic neurons in the embryonic brain (Fig. 2). Among these factors are two secreted signaling proteins that function as early determinants to specify the identity of early proliferating DAergic progenitors: sonic hedgehog (SHH) and fibroblast growth factor 8 (FGF8) (Ye *et al*., 1998). SHH is derived from the floor plate in the ventral midline and plays essential role in the specification of different populations by controlling various transcription regulators (Placzek and Briscoe 2005; Ho and Scott 2002). FGF8 is released by the midbrain-hindbrain border (MHB) called isthmus or mid-hindbrain organizer (MHO) (Rhinn and Brand 2001). The floor plate exists throughout the entire length of the neural tube, but the MHO is a centralized organization playing a key role in controlling the size and relative location of mes-DAergic neurons (Prakash and Wurst 2006a).

Both SHH and FGF8 are necessary to induce DAergic progenitors (Ye *et al*., 1998). This has been demonstrated by the observation that SHH induces ectopic DAergic neurons in areas of the brain express-

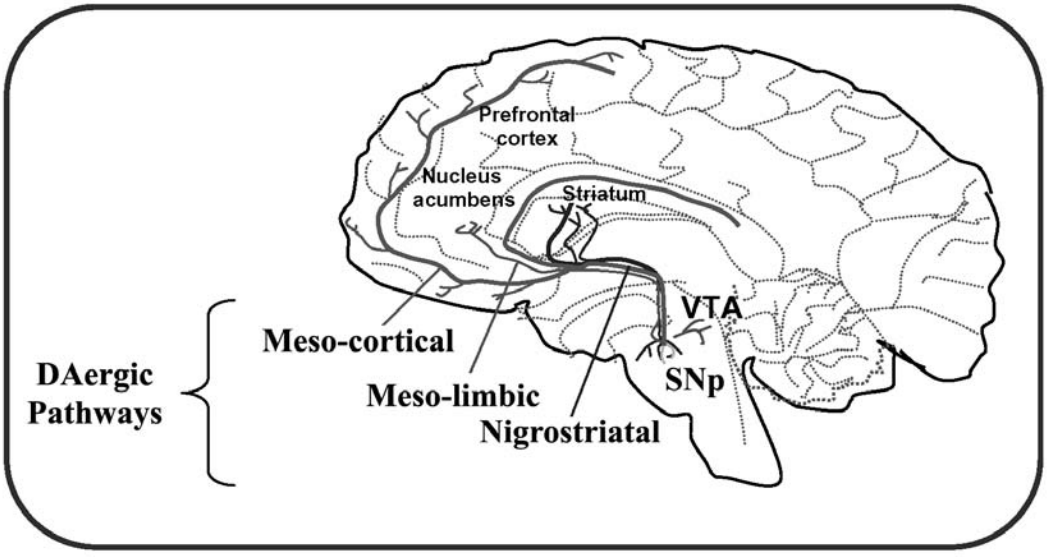

Figure 1. Dopamine projections to the forebrain: As described in the text, projections of A10 neurons extend from the ventral tegmental area (VTA) to the nucleus accombens, and profrental cerebral cortex, of A9 neurons from SNpc to dorsal striatum (caudate nucleus and putamen).

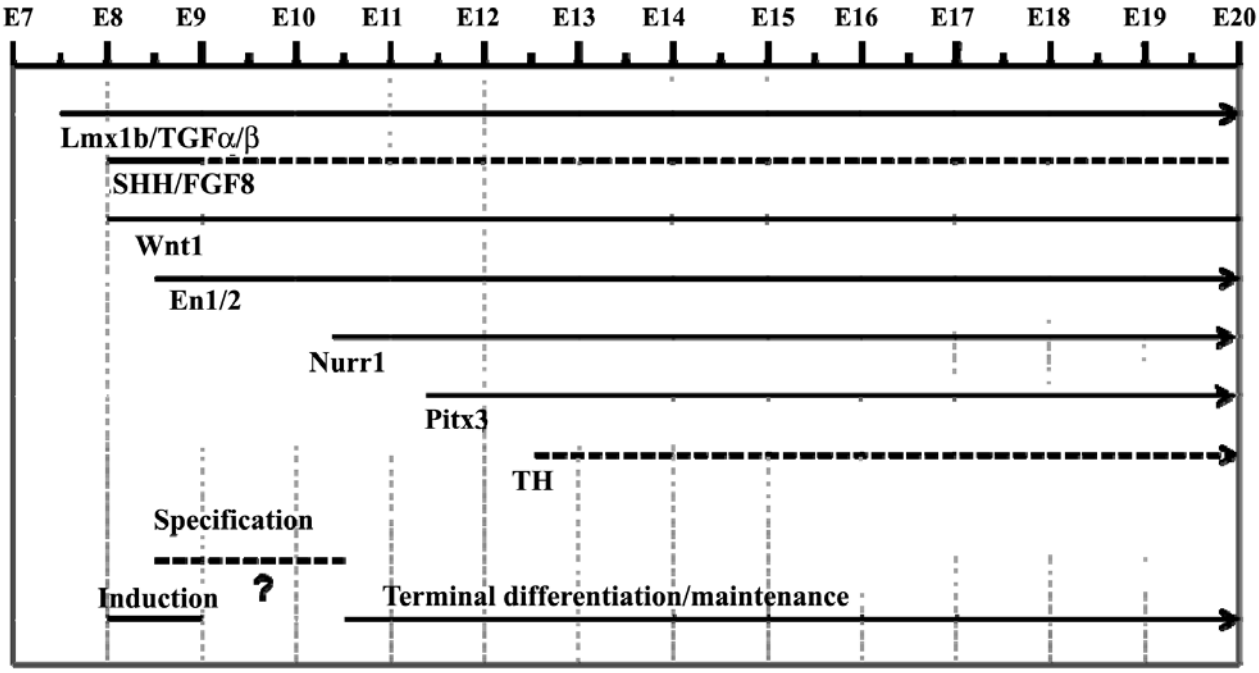

Figure 2. Chronological development of murine DAergic neurons at embryonic stages: Inducing factors that have implications on this process are shown. The top scale represents embryonic days from a day before the start of neural development. At embryonic day 8 neurolation begins shortly after gastrulation while majority of mes-DAergic neurons are generated at E13 soon after onset of TH expression. The bars below the scale indicate the onset of expression of inducing factors (transcription factors or other proteins) according to the time-scale at the top, and the time interval during which the corresponding protein is assumed to play its role in mes-DAergic development. The time interval during which the presence of the corresponding molecule is necessary for the normal development of mes-DAergic neurons is indicated by solid bars. Arrows indicate that the corresponding protein is expressed beyond mid-gestation into later fetal and postnatal stages and may also be needed at these later stages. Wherever a direct involvement of the corresponding molecule has yet to be established or its absence has no effect on normal DAergic development is indicated by dotted bars. The bars at the bottom of the Figure indicate the time intervals for induction of mes-DAergic progenitors, specification of the mes-DAergic cell fate in precursors, and terminal differentiation/maintenance of postmitotic mes-DAergic neurons in the mouse ventral midbrain. It is still not quite clear what processes and factors link early inductive signals to the molecular network controlling the differentiation and maintenance of mes-DAergic neurons in the mammalian brain. The question mark points to this uncertainty. Abbreviations: Tgf, transforming growth factor; SHH, Sonic hedgehog; FGF, fibroblast growth factor; En, engrailed; TH, tyrosine hydroxylase. Adapted from Alavian *et al*., 2008; Prakash and Wurst 2006b.

ing FGF8 (Hyness *et al*., 1997) and that DAergic neuron numbers are reduced in SHH-null mutant animals (Blaess *et al*., 2006). However, it is important to understand how SHH and FGF8 as two symmetric signals could provide sufficient information for conferring DAergic phenotype exclusively to cells that are destined to ventral midbrain. An asymmetric signal called *Wnt1* which appears at the same time of FGF8 expression and is expressed only at caudal midbrain is thought to act as mediator (Prakash *et al*., 2006a). Panhuysen *et al*., have shown that Wnt1 is important for development of mid-/hindbrain region (Panhuysen *et al*., 2004). Further studies using Wnt1-mutant forebrain cultures showed that Wnt1 is essential for DAergic neuron induction by FGF8 (Joksimovic *et al*., 2009; Castelo-Branco *et al*., 2003) and so it might act as asymetrical signal for specification of mes-DAergic progenitors (Prakash *et al*., 2006a).

LIM homeodomain family members: Lmx1a is one of the transcription factors involved in DAergic neuron development whose expression relies on SHH. Lmx1a is expressed in the ventral midbrain in a spatio-temporal pattern correlating with the emergence of DAergic neurons in the mesencephalon (Andersson *et al*., 2006). Unilateral transfection of retroviral expression vectors harboring Lmx1a-encoding cDNA into the midbrain chick embryos by in ovo electroporation ectopically induced DAergic neurons in the ventral midbrain as indicated by the induction of Nurr1, Lmx1b, and TH (Brisco *et al*., 2000; Andersson *et al*., 2006). Lmx1a seems to exert its effects through the induction of another homeobox gene Msx1 (Andersson *et al*., 2006).

Lmx1b: is another member of LIM family implicated in mes-DAergic neuron development in mouse brain

(Smidt *et al*., 2000). Lmx1b appears at E7.5 in mice (Asbreuk *et al*., 2002). Lmx1-b$^{-/-}$ mice lack signals necessary for differentiation and survival of DAergic neurons in VTA (Smidt *et al*., 2000). These findings led to the conclusion that Lmx1b is required for induction of another downstream factor Pitx3 (Smidt *et al*., 2000). Other reports indicate that Lmx1b can also induce ectopic expression of Wnt1 (Matsunaga *et al*., 2002; Adams *et al*., 2000). Since Pitx3 fails to show up in the absence of Wnt1 (Prakash *et al*., 2006a), Lmx1b is believed to induce Pitx3 expression via Wnt1 as a mediator.

Engrailed genes: Engrailed (EN)-1 and EN-2 regulate α-synuclein a protein involved in familial forms of PD (Simon 2001). A single allele of EN-1 on EN-2$^{-/-}$ mice produces a wild type-like SN and VMT. A single allele of EN-2 on EN-1$^{-/-}$, on the other hand, results in the survival of only a small proportion of DAergic neurons (Simon 2001). These finding show that EN-1 and EN-2 are differentially expressed in the brain and that the expression of the two genes continues through adulthood indicating their role in DAergic neuron survival though not in their specification. This notion is confirmed by a recent study that shows mice heterologous for both EN-1 and EN-2 (EN-1$^{+/}$-EN-2$^{+/-}$) have significantly reduced levels of striatal dopamine (Sgado *et al*., 2009).

Nurr1: is a nuclear transcription factor expressed by over 95% of DAergic neurons in the SN and VTA (Bäckman *et al*., 1999). In the mouse, Nurr1 appears at embryonic day 10.5 (Saucedo-Cardnas *et al*., 1998) which is a late stage compared to the emerging time of the signals mentioned earlier.

The fact that Nurr1 expression is maximized during embryonic development and remains high throughout lifespan (Le *et al*., 1999; Zetterstrom *et al*., 1997) reflects its important role in DAergic neuron development and survival. Various studies implicate Nurr1 in the regulation of dopamine neurotransmission. Dopamine synthesizing enzymes TH and AADC are both expressed under Nurr1 regulation (Hermanson *et al*., 2003; Smits *et al*., 2003; Saucedo-Cardinas *et al*., 1998; Zetterstrom *et al*., 1997). TH expression in DAergic neurons appears a day after Nurr1 (see below). Studies on TH promoter sequences have identified a number of Nurr1-binding motifs (Chu *et al*., 2006; Kim *et al*., 2003; Iwawaki *et al*., 2000; Sakurada *et al*., 1999). Deletion of dopamine receptor D_2 results in Nurr1 gene overexpression in mice suggesting a role for Nurr1 in autoreceptor function in DAergic neurons (Tseng *et al*., 2000). Finally, expression of factors involved in dopamine storage or reuptake such as dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) has been shown to be regulated by Nurr1 (Hermanson *et al*., 2003; Smits *et al*., 2003; Saccheti *et al*., 2001, 1999). Nurr1-mediated regulation of DAT and VMAT2 as well as its other contributions discussed below indicate that beside its role in embryonic stages, Nurr1 also functions as survival factor in DAergic neurons in adult brain.

Growth factors: *TGF-α and-β*: Transforming growth factor-α has been implicated in DAergic neuron development and later in their survival. Almost half the number of DAergic neurons was reduced in the SNpc of TGFα-null mice but not in their VTA (Blum 1998). A similar role has been discovered for TGF-*β* in chicken embryos (Farkas *et al*., 2003). Application of TGF-neutralizing antibodies in these studies revealed that TGFs are amongst early determinants of DAergic phenotype (Farkas *et al*., 2003; Blum 1998).

GDNF: glial cell-derived neurotrophic factor (GDNF) is known primarily as survival factor for DAergic neurons and its neuroprotective effects have been studied by us (Sandhu, Gardaneh *et al*., 2009) and several other groups (Akerud *et al*., 2001; Kordower *et al*., 2000; Rosenblad *et al*., 1998; Gash *et al*., 1996; Tomac *et al*., 1995). In addition, other reports indicate GDNF role in development of DAergic phenotype (Hellmich 1996; Wood *et al*., 2005). GDNF injection to the midbrain results in increased TH expression in DAergic neurons (Buytaert-Hoefen *et al*., 2004) whereas reduced TH levels occur in GDNF$^{+/-}$ mice (Boger *et al*., 2006). Human embryonic stem cells cultured with GDNF show increased potential to differentiate to TH neurons (Buytaert-Hoefen *et al*., 2004).

Recent studies point to Nurr1 interactions with GDNF and its receptor Ret. Nurr1 presence is critical for expression of Ret in DA neurons and brainstem (Wallen *et al*., 2001). GDNF itself promotes differentiation and survival of DA neurons as shown in rats and primates (Ai *et al*., 2003). In these experiments, GDNF injection into the putamen increased expression of Nurr1 (and Pitx3) suggesting that Nurr1 may influence GDNF function. Nurr1 not only enhances Ret expression but also may induce expression of GDNF itself (Sonntag *et al*., 2004; Backman *et al*., 2003). A more recent study indicates enhanced GDNF expres-

sion occurs following Nurr1 up-regulation in teratocarcinoma NT2 cells regarded as a DAergic precursor line (Hara *et al*., 2007). We are currently examining possible synergistic effects co-overexpression of Nurr1 and GDNF may have on DAergic differentiation of NT2 and other potential precursors.

Pitx3: is a homeobox gene and another player in the development and function of DAergic neurons. Indeed Pitx3 is expressed exclusively in DAergic neurons (van den Munckhof *et al*., 2003) under control of Lmx1b mentioned above. Much of our knowledge about Pitx3 comes from studies on aphakia mice that are mutant animals lacking Pitx3. In these mice the mesencephalic dopamine system develops abnormal anatomical structures leading to loss of DAergic neurons in SN and their fibers constituting the SN-ST pathway (Hwang *et al*., 2003; van den Munckhof *et al*., 2003). In fact, in aphakia embryos at day E12.5 when TH expression is initiated, DAergic neurons are absent from SNpc (Smidt *et al*., 2004a). Since Pitx3 expression is restricted to the developing ventral midbrain, the molecule probably has no significant role in the proliferation of DAergic neuron precursors or their migration. This suggests that Pitx3 may be needed for the terminal stages of differentiation or for survival of DAergic neurons. NT2 cells express Nurr1 but not TH. Our preliminary observation in this cell line showed that ectopic Pitx3 expression induces TH expression to make the cells truly dopaminergic (Gardaneh and Sandhu; unpublished observation). In line with this observation, Pitx3 has been suggested to provide vitalizing signals for the precursors entering into primordial SNpc (Smidt *et al*., 2004a). It also acts in specification of DAergic neurons strictly in SNpc compared to more general effect of Nurr1 on all midbrain DA neurons (Smidt *et al*., 2004b).

Pitx3: appears at E11.5 a day after Nurr1 and a day before TH (Korotkova *et al*., 2005). Studies on Nurr1-null mice show high-level expression of Pitx3 after E11.5 indicating that independent pathways induce Nurr1 and Pitx3. However, these mice gradually lose Pitx3 expression and show increased apoptosis in VTA neurons. This suggests Nurr1 might be important for restoring Pitx3 at the late stages of DAergic neuron development (Saucedo-Cardenas *et al*., 1998).

It is not only Nurr1 but also Pitx3 that induces VMAT2 and DAT expression (Hwang *et al*., 2009). Studies indicate further cooperative activities between Nurr1 and Pitx3 in DAergic neuron development and transmission since VMAT2 and DAT are involved in both activities (Miller *et al*., 1999). It is only in the presence of Nurr1 and Pitx3 that DAergic neurotransmission and neuron survival is fully restored. Lack of either transcription factor reduces induction levels of VMAT2 and DAT expression (Hwang *et al*., 2009) indicating a synergistic interaction between the two.

TH: is the first and rate-limiting enzyme in biosynthesis of dopamine and, as mentioned above, appears at E12.5 in mouse embryo. Expression of TH is considered a firm indicator of catecholaminergic neurons due to the initiation of the catecholamine biosynthesis pathway. Indeed TH is widely used as a clinical marker in cells that originate from neural crest and form childhood cancer neuroblastoma spanning its applications from DAergic neuron survival in PD therapy to diagnosis of minimal residual disease in NB patients (Ootsuka *et al*., 2008; Träger *et al*., 2008; eg Ito *et al*., 2004). Due to the specific pattern of TH expression in catecholaminergic neurons, we and others have shown TH promoter sequences are capable of directing DAergic-specific expression both in DAergic neurons for CNS applications (Gardaneh and O'Malley 2004) and in NB cell lines for applications in the periphery (Gardaneh *et al*., 2000). TH specificity was shown capable of promoting DAergic neuron protection by directing BCl_2 expression both constitutively and inducibly (Gardaneh *et al*., 2004). As discussed below, TH specificity has recently been applied for selecting DAergic neurons among mixed populations *in vitro* (Hedlund *et al*., 2007).

TH is not involved in DAergic neuron development but confers a DAergic phenotype to these neurons. Without TH, there will be no synthesis of dopamine and downstream catecholamines. TH mutations cause tissue-specific decrease in dopamine in nigrostriatal regions (Zafeiriou *et al*., 2009 and references therein). Mutations in TH gene have been detected in inherited forms of dystonia a disorder with symptoms similar to PD (Nagatsu and Ichinose 1999; Bautigam 1998). Degeneration of DAergic neurons in situations like PD would inevitably cause reduction or loss of TH mRNA expression (Nagatsu and Ichinose 1999).

DAergic neuron generation: an *in vitro* opportunity for cell-replacement therapy: Current treatments for disorders arising from DAergic neuron degeneration are mostly symptomatic. PD patients, in particular, are subject of administration of L-DOPA or dopamine receptor agonists and stereotaxic surgery mostly deep

Table 2. Stem and iPS cell sources for DAergic neuron generation *in vitro*.

Genes	Factors	% TH+ cells in dish	% TH+ cells survived *in vivo*	Rotational behavior test	Reference
		Human embryonic stem cells			
-	pA6 feeder	7%	Not reported	Not tested	Zeng 2004
-	pA6 feeder	8%	<1%	No recovery seen	Brederlau 2004
-	MS5, MS5-Wnt, S2 FD,	24%	Not reported	Not tested	
Sox1	FGF8, SHH, AA, cAMP,	30%	Not reported	Not tested	Perrier 2004
-	BDNF, GDNF, TGF?3	40%	21%	Improved rotational behavior	Yan 2005
-	FGF8, SHH, AA, BDNF,	86%	2.7%	Improved rotational behavior	Roy 2006
-	GDNF	90%	<1%	Improved rotational behavior	Cho 2008
-	Immortalized astrocyte FD,	27%	1%	Improved rotational behavior	Song 2008
-	SHH FGF8, ITSF, BDNF,	27%	1%	Improved rotational behavior	Yang 2008
-	GDNF	78%	Not tested	Not tested	Yang 2008
-	FGF8, SHH, AA	<30%	Not tested	Not tested	Vazin 2009
	SHH, FGF8, GDNF, TGF?3, ITS, cAMP, FGF				Shimada 2009
	FGF8, SHH, AA, cAMP, TGF?3, BDNF, GDNF,				
	FGF8, SHH, AA, cAMP, BDNF, GDNF, TGF?3				
	pA6 CM elements: SPIE				
	pA6 feeder FGF-2 + FGF-20				
		Mouse embryonic stem cells			
Nurr1	SHH, FGF8, ITSN	78%	<5%	Improved rotational behavior	Kim 2002
Nurr1	FGF8, SHH, AA	18%	Not tested	Not tested	Chung 2002
-	pA6 FD, FGF2	10%	4%	Not tested	Morizane 2006
Nurr1	pA6 FD, FGF8, SHH, AA	56%	Unclear	Test outcome unclear	
-	BMSC feeder, SHH, FGF8	32%	Unclear	Test outcome unclear.	Kim 2006 Shintani 2008
		Monkey embryonic stem cells			
-	pA6 FD	<9%	8%	Not tested	Kawazaki 2000
-	FGF-2, FGF-20	24%	27%	Improved movement	Takagi 2005
		Neural Stem Cells			
Nurr1	FGF8, SHH	94%	Not tested	Not tested	Kim 2003
Nurr1	FGF-20	-	Few	Not clear	Grothe 2004
Nurr1, Mash1	BDNF, NT-3	24%	8%	Improved behavior	Shim 2007
-					
		Induced Pluripotent Stem Cells			
-	-	5%	Unclear	Test outcome unclear	Wernig 2008
OCT4, SOX2, NANO, LIN28	ITSF, cAMP, FGF-2	6.5%	Few	No recovery seen	Cai 2009

brain stimulation (Rascol *et al*., 2002; Fahn 2006; Kondo 2002; Montgomery 1999; Frost and Osborn 2009). In recent years, cell replacement/regenerative strategies consisting in the replenishment of dying

DAergic neurons by healthy substitutes have drawn much attention. Cell sources considered potential candidates to undergo differentiation and form DAergic neurons can be endogenous and exogenous (Table 2): endogenous candidates include VM primary cultures that contain DAergic precursors, neural stem cells from subventricular zone, SN and or ST (Madhavan *et al*., 2009; Mohapel *et al*., 2005; Cooper and Isacson 2004; Storch *et al*., 2004; Fallon *et al*., 2000). Exogenous sources, on the other hand, are embryonic and mesenchymal stem cells from bone marrow, amniotic fluid, sertoli cells, retinal pigmentous epithelia (RPE), and adipocyte (Glavaski-Joksimovic *et al*., 2009; Levy *et al*., 2008; McLaughlin *et al*., 2006; Subramanian *et al*., 2002). These cell sources have been subjected to treatment with several of inducing factors discussed above either by adding these factors to cell medium or by ectopic expression of their coding sequences within the cells to direct them toward DAergic fate. Table 2 lists reports on stem cell differentiation to DA neurons and their trial in animal models of PD.

In addition, several helper cell types have been identified that possess inducing potential. These include fibroblasts, pA6 stromal cell line, sertoli cells, RPE etc. (Falk *et al*., 2009; Correia *et al*., 2008; Shamekh *et al*., 2008; Vazin *et al*., 2008; Morizane *et al*., 2006; Yue *et al*., 2006). They appear to secrete to their medium specific growth factors capable of inducing DAergic phenotype when added to the target cells (ibids).

These study cases have been complied based on their original cells sources and the year they were published. Columns labeled Genes and Factors represent inducing genes ectopically expressed in target cells and inducing/growth factors plus feeder cells/agents (shown as FD) added to their medium, respectively. References are shown by only first author and published year for space saving. Abbreviations: AA, ascorbic acid, BDNF; brain-derived neurotrophic factor; cAMP, cyclic AMP, FGF-2, fibroblast growth factor-2; FGF8, fibroblast growth factor-8, FGF-20; fibroblast growth factor-20; GDNF, glial cell line-derived neurotrophic factor; iPS, induced pluripotent; ITS, insulin-transferrin, selenium; ITSF, insulin-transferrin, selenium fibronectin; NT-3, neurotrophin-3; SHH, sonic hedgehog; TGFβ3, tumour growth factor beta 3; TH, tyrosine hydroxylase.

PD cell therapy: outstanding issues

Cell sources: In general, human embryonic stem cells amongst various candidates currently represent the most promising cell source for human cell therapy. They can produce amounts of medical grade, standardized and normally differentiable target cells for clinical use. However, their heterogeneity leading to tumor formation and production of undesired cell types must be overcome (Carson 2006). The crude mix of stem cells at various developmental stages and a number of cell lineages underline the necessity of cell selection approaches to eliminate unwanted cells and make target cells homogeneous. This homogeneity is important for PD cell therapy because serotonin cells mixed in the grafted sources appear to cause side effects (see below). Stem cells genetically transformed to express fluorescent markers under differentiation-specific promoters such as TH-GFP and Pitx3-GFP constructs (Hedlund *et al*., 2008; Hedlund *et al*., 2007) or use of DAergic surface antigens (Pruszak *et al*., 2007) could help select purely DAergic neurons.

Immunological problems: Histocompatibility remains a significant barrier to stem cell applications in clinic. Stem cell xenografts are likely to induce the recipient's immune system leading to graft rejection (Lui *et al*., 2009). In recent years powerful technologies have emerged to overcome immunogenicity of stem cell graftment. These technologies mainly include generation of 'self cells' using pluripotent cell lines, parthenogenesis, nuclear transplantation and induction of pluripotency in somatic cells. Derivation of human multipotent adult progenitors has not been definitive (Aranguren et a 2007), but alternative approaches have been reported to increase tolerance and minimize chance of rejection upon changes in defined genetic loci within a panel of stem cells (Robertson *et al*., 2007). Embryonic stem cells have been produced from diploid blastocyst stage human embryos that were developed from parthenogenetically activated oocytes (Revazova *et al*., 2007). The immunogenicity of these cells is unclear but NK cells may respond against cell lines that obtain a set of MHC-1 from only one parent. This prompted the same investigators to develop HLA homozygous parthenogenetic human stem cell lines applicable to clinical cell therapy (Revazova *et al*., 2008).

Isolation of embryonic stem cells through nuclear transfer (ntES cells) has been proven successful in many species (reviewed in Gurdon *et al*., 2003) but not in human. Even if this obstacle is eradicated, the potential immune responses could be directed towards mitochondrial histocompatibility antigens. In an ele-

gant study, Tabar *et al*., isolated fibroblasts from PD-induced mice and, by injecting their nuclei into oocytes, derived murine embryos (Tabar *et al*., 2008). They used the blastocysts from these embryos to isolate ES cells that were differentiated into DAergic neurons and injected back to the original fibroblast donor mice. The ntES self cells survived well post-transplantation and led to functional recovery. This 'therapeutic cloning' approach may help reduce immune rejection. Finally, self cells can be produced by reprogramming somatic cells using defined factors that induce pluripotency (Takahashi and Yamanaka 2006). This technology has proven useful in rat models of PD (Wernig *et al*., 2008), and more recently using human-derived cell sources (Soldner *et al*., 2009). Although iPS cells immunologically would be fully histocmpatible with the donor, until practical steps of transplantation and functional assays were taken, it may still be too soon to make a conclusion given the novelty of this technology.

Transplantation: DAergic neuron sources have to meet certain criteria before being applied for transplantation in clinic (Hagell *et al*., 2001): (1) they must have shown to alleviate parkinsonian symptoms in animal models, (2) over 100,000 of them as grafted genuine DAergic neurons have to survive in the long term, 3) they have to able to improve reconstitution of neural circuits and integrate into host striatum. Therefore, successful cell therapy aiming at neuro-regeneration and neurorepair for neurodegenerative disorders such as PD owes to the elimination of technical problems surrounding cell transplantation to brain tissue and integration to the host's neural network. PD patients have been subjected to tissue transplantation with a diverse allograft, xenograft and autograft sources. Allografts (that involve both the donor and recipient of the same species) consisting of cells from sources such as RPE of retina and fetal VM have been applied to transplantation in PD patients (Subramanian *et al*., 2002; Doudet *et al*., 2004). Xenografts (that involve the donor and recipient from different species) have been prepared and clinically applied mainly from VM tissue (Genezyme Press Release 2001; Schumacher *et al*., 2000). Autografts can be prepared from endogenously self-renewing cell sources mentioned earlier in this review. In one case, autologous tansplantaion of endogenous adult neural precursors led to a significant recovery within a 12-month time window studied (Sayles *et al*., 2004). However, the long-term outcome of this trial has not been reported.

Graft survival: Despite the ongoing attempts outlined above, the long-term outcome of DAergic neuron transplantation is still unclear. This is due, at large, to the technical hurdles associated with transplantation procedures, the source of grafted tissues and methods applied to their storage, preparation and handling. Survival of grafted cells can be improved by pre-incubation/hibernation of the cells prior to transplantation with growth factors, such as GDNF and apoptosis inhibitors (Apostolides *et al*., 1998; Mendez *et al*., 2000; Cichetti *et al*., 2002). VM cell suspension pre-incubated with Wnt1/WNt5a augments DAergic neuron number by increasing proliferation and terminal differentiation of DAergic precursors to mature DAergic neurons (Joksimovic *et al*., 2009; Castelo-Branco *et al*., 2003). For optimal recovery, specific regions of the ST need to be re-innervated (Mandel *et al*., 1990; Dunnett *et al*., 1983) and synaptic connections re-established. To achieve this goal, grafted DAergic neurons have to be distributed throughout the ST so that optimized axonal re-innervation can take place.

Heterogenous cell populations used as graft sources raise concern about graft survival and proper function. Graft-induced dyskinesia (GID) observed in several cases of transplantation was initially attributed to excessive release of DAergic from transplants enriched with DAergic neurons (Freed *et al*., 2001; Olanow *et al*., 2003). But further experiments using 6-OHDA-lesioned rat models showed that grafts containing a small proportion of DAergic compared to serotonin neurons develop a progressive worsening of L-DOPA-induced dyskinesia (LIP).

Serotonin neurons can store and release dopamine but cannot regulate dopamine release in a normal way since they don't have DAT and D_2 receptors. Also in the absence of extensive striatal dopamine innervations as occurs in advanced stages of PD, grafted serotonin neurons would aggravate LIP. Impurities like serotonin neurons are blamed for post-injection observations including formation of lewy bodies and over-expression of α-synuclein that reportedly contribute to the poor outcome of treatments (Kordower *et al*., 2008; Li *et al*., 2008). These unwanted changes in the grafted neurons are suspected cause for reduced number of surviving grafted TH^+ neurons as well as for GID (Hagell *et al*., 2002), that together with patients' age and severity of their illness contribute to unexpectedly poor improvement of their behavioral motor conditions.

Novel technologies including graft enrichment (for example using specific promoter-reporter gene constructs mentioned earlier) and iPS-based cell sources for differentiation hold promise to improve the survival and functional integration of donor DAergic neurons in hosts' nervous system. It would be interesting, for example, to see the outcome of self-cell transplantation that, as outlined above, is a newly-emerging but vibrant area of replacement therapy including PD cell therapy.

Efficacy: Will cell replacement strategies alone be capable of halting disease progression and persistence? On top of the outstanding issues outlined above, initiators of DAergic neuron death that lead to PD symptoms remain unidentified. Despite studies on the application of early indicators as biomarkers, development of biomarker-based assays for PD are still in their infancy (Eller and Williams 2009; reviewed by Antoniades and Barker 2008). Furthermore, possible sources of death signals including reactive oxygen species causing oxidative damage will persist as age grows. In this case, examination of patients who had received fetal tissue grafts revealed that some of the surviving neurons exhibit pathological changes linked to PD (Kordower *et al*., 2008; Li *et al*., 2008). These problems highlight the necessity of pursuing more comprehensive approaches than cell replacement alone.

Supportive strategies including neuroprotection combined with cell replacement is likely to enhance the efficiency of cell therapy. One such neuroprotective approach is to potentiate intrinsic properties of supporting cell sources such as astroglia as occurs *in vivo*. We and others have previously shown that GDNF-overexpressing astrocytes protect DAergic neurons against parkinsonian toxicity (Sandhu, Gardaneh *et al*., 2009). Direct intrastriatal administration of GDNF has shown effective in improving daily living and dopamine metabolism of patients (Slevin *et al*., 2006; 2005; Gill *et al*., 2003). Direct relationships between Nurr1 and neuroprotecting molecules have been established as in the case of VIP a protecting neuropeptide (Luo *et al*., 2007). Similarly, other reports indicate convergence between DAergic fate determinant Nurr1 and neurotrophic factor GDNF and its receptor Ret at some point in their signaling pathways (Li *et al*., 2009; Hara *et al*., 2007). Both Nurr1 and GDNF have shown to be life-span supporting elements of DAergic neurons (Pascual *et al*., 2008; Perlmann and Wallén-Mackenzie 2004). We are currently examining if and how GDNF-secreting astrocytes assist neurogenesis when co-cultured with stem cell-derived DAergic neurons ectopically expressing Nurr1. Another avenue that we are actively pursuing to improve DAergic neuron protection is to equip stem cell sources with an anti-oxidant defense mechanism that will involve glutathione peroxidation mediated by glutathione peroxidase-1 (GPX-1). Finally, we have shown that GDNF and glutathione can synergistically protect DAergic neurons (Sandhu, Gardaneh *et al*., 2009). The author hypothesizes that stem cell-derived GPX-1-expressing DAergic neurons co-cultured with GDNF-secreting astrocytes will enjoy better protection against toxicity (Gardaneh 2009). We are examining this hypothesis too. Such combination therapies would likely prove more effective when dealing with multifactorial complex disorders like PD where several endogenous and environmental toxins are suspected causes.

Summary and future perspective: DAergic neurons are developed *in vivo* in a step-wise fashion that involves specific molecular determinants in each step. Extensive studies have been carried out to dissect these molecular events; however, not all contributing determinants have been identified or fully resolved for their specific function. Despite dark spots that await clarification in DAergic developmental pathways, we can now rely on the main known players to produce these neurons from potential cell sources *in vitro*.

Disorders stemming from DAergic neuron degeneration would eventually require the lost cells to be compensated by their freshly prepared functional counterparts. Intrinsic and extrinsic cell sources have been identified and shown capable of becoming DAergic. To apply these sources to clinic on a routine basis, numerous problems need to be addressed. For graft cell survival and long-term function, a full set of inducing transcription/ growth factors may need to be expressed in precursors. The target cells would require purification and enrichment to maximize safety and efficacy *in vivo*. Integration to neural circuitry necessitates grafted cells to be readily traced, and, marking live cells for functional analysis is a technical challenge. Novel technologies such as iPS and ntES cell generation need to be routinely developed and fully examined for safe, non-immunogenic and non-tumorigenic source of graft supply for transplantation purposes.

It is inferable from available data that, due to complexity of PD nature, cell replacement therapy relying

only on DAergic neuron preparation and transplantation may not be a viable option in the long term. This prompts the idea of combination therapy where neurorepair and neuroprotection will be able to cooperatively restore lost cells, factors and functions and guarantee DAergic system integrity for the rest of life. This goal can only be achieved upon clarification of the entire DAergic neuron pathways and resolution of all problems that surround cell sources, transplantation procedures and immunological issues.

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References

Ai Y, Markesbery W, Zhang Z, Grondin R, Elseberry D, Gerhardt GA, Gash DM (2003). Intraputamenal infusion of GDNF in aged rhesus monkeys: distribution and dopaminergic effects. *J Comp Neurol*. 461: 250-261.

Akerud P, Canals JM, Snyder EY, Arenas E (2001). Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. *J Neurosci*. 21: 8108-8118.

Alavian KN, Scholz C, Simon HH (2008). Transcriptional regulation of mesencephalic dopaminergic neurons: the full circle of life and death. *Mov Disord*. 23: 19-28. Review.

Andersson E, Tryggvason U, Deng Q, Friling S, Alekseenko Z, Robert B, Perlmann T, Ericson J (2006). Identification of intrinsic determinants of midbrain dopamine neurons. *Cell* 124: 393-405.

Antoniades CA, Barker RA (2008). The search for biomarkers in Parkinson's disease: a critical review. *Expert Rev Neurother*. 8: 1841-52.

Apostolides C, Sanford E, Hong M, Mendez I (1998). Glial cell line-derived neurotrophic factor improves intrastriatal graft survival of stored dopaminergic cells. *Neuroscience* 83: 363-372.

Aranguren XL, Luttun A, Clavel C, Moreno C, Abizanda G, Barajas MA, Pelacho B, Uriz M, Araña M, Echavarri A, Soriano M, Andreu EJ, Merino J, Garcia-Verdugo JM, Verfaillie CM, Prósper F (2007). *In vitro* and *in vivo* arterial differentiation of human multipotent adult progenitor cells. *Blood* 109: 2634-42.

Asbreuk CHJ, Vogelaar CF, Hellemons A, Smidt MP, Burbach JPH (2002). CNS expression pattern of *Lmx1b* and coexpression with *Ptx* genes suggest functional cooperativity in the development of forebrain motor control systems. *Mol Cell Neurosci*. 21: 410-420.

Bäckman C, Perlmann T, Wallén A, Hoffer BJ, Morales M (1999). A selective group of dopaminergic neurons express Nurr1 in the adult mouse brain. *Brain Res*. 851: 125-32.

Backman C, Zhang Y, Hoffer BJ, Tomac AC (2003). Short interfering RNAs (siRNAs) for reducing dopaminergic phenotypic markers. *J Neurosci Methods*. 131: 51-56.

Banday AA, Lokhandwala MF (2008). Dopamine receptors and hypertension. *Curr Hypertens Rep*. 10: 268-75. Review.

Bjorklund A, Dunnett SB (2007). Dopamine neuron systems in the brain: an update. *Trends Neurosci*. 30: 194-202.

Blaess S, Corrales JD, Joyner AL (2006). Sonic hedgehog regulates Gli activator and repressor functions with spatial and temporal precision in the mid/hindbrain region. *Development* 133: 1799-1809.

Blum M (1998). A null mutation in TGF-α leads to a reduction in midbrain dopaminergic neurons in substantia nigra. *Nat Neurosci*. 1: 374-77.

Boger HA, Middaugh LD, Huang P, Zaman V, Smith AC, Hoffer BJ, Tomac AC, Granholm AC (2006). A partial GDNF depletion leads to earlier age related deterioration of motor function and tyrosine hydroxylase expression in the substantia nigra *Exp Neurol*. 202: 336-347.

Brederlau A, Correia AS, Anisimov SV, 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 PS, Li JY (2006). Transplantation of human embryonic stem cellderived 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.

Briscoe J, Pierani A, Jessell TM, Ericson J (2000). A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* 101: 435-445.

Buytaert-Hoefen KA, Alvarez E, Freed CR (2004). Generation of tyrosine hydroxylase positive neurons from human embryonic stem cells after coculture with cellular substrates and exposure to GDNF. *Stem Cells* 22: 669-674.

Cai J, Yang M, Poremsky E, Kidd S, Schneider JS, Iacovitti L (2009). Dopaminergic Neurons Derived from Human Induced Pluripotent Stem Cells Survive and Integrate into 6-OHDA Lesioned Rats. *Stem Cells Dev*. 2009 Oct 13 [Epub ahead of print]

Callier S, Snapyan M, Le Crom S, Prou D, Vincent JD, Vernier P (2003). Evolution and cell biology of dopamine receptors in vertebrates. *Biol Cell*. 95: 489-502. Review.

Carson CT, Aigner S, Gage FH (2006). Stem cells: the good, bad, and barely in control. *Nat Med*. 12: 1237-8.

Castelo-Branco G, Wagner J, Rodriguez FJ, Kele J, Sousa K, Rawal N, Pasolli HA, Fuchs E, Kitajewski J, Arenas E (2003). Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a. *Proc Natl Acad Sci USA*. 100: 12747-12752.

Chinta SJ, Andersen JK (2005). Dopaminergic neurons. *Int J Biochem Cell Biol*. 37: 942-946. Review.

Cho MS, Lee YE, Kim JY, Chung S, Cho YH, Kim DS, Kang SM, Lee H, Kim MH, Kim JH, Leem JW, Oh SK, Choi YM, Hwang DY, Chang JW, Kim DW (2008). Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci USA*. 105: 3392-3397.

Chu Y, Le W, Kompoliti K, Jankovic J, Mufson EJ, Kordower JH (2006). Nurr1 in Parkinson's disease and related disorders. *J Comp Neurol*. 494: 495-514.

Chung S, Sonntag KC, Andersson T, Bjorklund LM, Park JJ, Kim

DW, Kang UJ, Isacson O, Kim KS (2002). Genetic engineering of mouse embryonic stem cells by Nurr1 enhances differentiation and maturation into dopaminergic neurons. *Eur J Neurosci*. 16: 1829-1838.

Cooper O, Isacson O (2004). Intrastriatal transforming growth factor alpha delivery to a model of Parkinson's disease induces proliferation and migration of endogenous adult neural progenitor cells without differentiation into dopaminergic neurons. *J Neurosci*. 24: 8924-8931.

Correia AS, Anisimov SV, Li JY, Brundin P (2008). Growth factors and feeder cells promote differentiation of human embryonic stem cells into dopaminergic neurons: a novel role for fibroblast growth factor-20. *Front Neurosci*. 2: 26-34.

Dahlstrom A, Fuxe K (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand*. 62: 1-55.

Doudet DJ, Cornfeldt ML, Honey CR, Schweikert AW, Allen RC (2004). PET imaging of implanted human retinal pigment epithelial cells in the MPTP-induced primate model of Parkinson's disease, *Exp Neurol*. 189: 361-368.

Dunnett SB, Björklund A, Schmidt RH, Stenevi U, Iversen SD (1983). Intracerebral grafting of neuronal cell suspensions. IV. Behavioural recovery in rats with unilateral 6-OHDA lesions following implantation of nigral cell suspensions in different forebrain sites. *Acta Physiol Scand Suppl*. 522: 29-37.

Dunnett SB, Bentivoglio M, Bjurklund A, Hokfelt T (2005). Eds in "Handbook of Chemical Neuroanatomy" (A. Bjurklund, T. Hokfelt, eds), V.21: Dopamine, Chapters 5-8, Elsevier, Amsterdam.

Eller M, Williams DR (2009). Biological fluid biomarkers in neurodegenerative parkinsonism. *Nat Rev Neurol* 5: 561-70.

Fahn S (2006). Levodopa in the treatment of Parkinson's disease. *J Neural Transm Suppl*. 71: 1-15. Review.

Falk T, Zhang S, Sherman SJ (2009). Pigment epithelium derived factor (PEDF) is neuroprotective in two *in vitro* models of Parkinson's disease. *Neurosci Lett*. 458: 49-52.

Fallon J, Reid S, Kinyamu R, Opole I, Opole R, Baratta J, Korc M, Endo TL, Duong A, Nguyen G, Karkehabadhi M, Twardzik D, Patel S, Loughlin S (2000). *In vivo* induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *Proc Natl Acad Sci USA*. 97: 14686-14691.

Farkas LM, Dunker N, Roussa E, Unsicker K, Krieglstein K (2003). transforming growth factor-βs are essential for the development of midbrain dopaminergic neurons *in vitro* and *in vivo*. *J Neurosci*. 23: 5178-5186.

Freed CR, Greene PE, Breeze RE Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S (2001). Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med*. 344: 710-9.

Frost EA, Osborn I (2009). Deep brain stimulation-surgery for movement disorders and Parkinson's disease. *Int Anesthesiol Clin*. 47: 57-68. Review.

Gardaneh M (2009). Cell-gene replacement therapy for Parkinson's disease. *Proc Int Stem Cell Conf*, September 21-22, University of Newcastle, Newcastle upon Tyne, UK

Gardaneh M, Gilbert J, Haber M, Norris MD, Cohn SL, Schmidt ML, Marshall GM (2000). Synergy between 5' and 3' flanking regions of the human tyrosine hydroxylase gene ensures specific, high-level expression in neuroblastoma cells. *Neurosci Lett*. 292: 147-150.

Gardaneh M, O'Malley KL (2004). Rat tyrosine hydroxylase promoter directs tetracycline-inducible foreign gene expression in dopaminergic cell types. *Brain Res Mol Brain Res*. 126: 173-180.

Gash, DM, Zhang Z, Ovadia A, Cass WA, Yi A, Simmerman L, Russell D, Martin D, Lapchak PA, Collins F (1996) Functional recovery in parkinsonian monkeys treated with GDNF. *Nature* 380: 252-255.

Genzyme Press Release (2001). Porcine Xenografts-http://www.genzyme.com/ corp/media/Genl%20PR%20-20041901.asp; http://www.genvec.com/go.cfm?do=Press.View&prid=141.

German DC, Nelson EL, Liang CL, Speciale SG, Sinton CM, Sonsalla PK (1996). The neurotoxin MPTP causes degeneration of specific nucleus A8, A9 and A10 dopaminergic neurons in the mouse. *Neurodegeneration* 5: 299-312.

German DC, Schlusselberg DS, Woodward DJ (1983). Three-dimensional computer reconstruction of midbrain dopaminergic neuronal populations: from mouse to man. *J Neural Transm*. 57: 243-254.

German, DC, KF Manaye (1993). Midbrain dopaminergic neurons (nuclei A8, A9, and A10): Three-dimensional reconstruction in the rat. *J Comp Neurol*. 331: 297-309.

Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, Brooks DJ, Svendsen CN, Heywood P (2003). Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med*. 9: 589-95.

Glavaski-Joksimovic A, Virag T, Chang QA, West NC, Mangatu TA, McGrogan MP, Dugich-Djordjevic M, Bohn MC (2009). Reversal of dopaminergic degeneration in a parkinsonian rat following micrografting of human bone marrow-derived neural progenitors. *Cell Transplant*. Sep 28._[Epub ahead of print].

Grothe C, Timmer M, Scholz T, Winkler C, Nikkhah G, Claus P, Itoh N, Arenas E (2004). Fibroblast growth factor-20 promotes the differentiation of Nurr1-overexpressing neural stem cells into tyrosine hydroxylase-positive neurons. *Neurobiol Dis*. 17: 163-170.

Gurdon JB, Byrne JA (2003). The first half-century of nuclear transplantation. *Proc Natl Acad Sci USA*. 100: 8048-8052.

Hagell P, Brundin P (2001). Cell survival and clinical outcome following intrastriatal transplantation in Parkinson disease. *J Neuropathol Exp Neurol*. 60: 741-752.

Hagell P, Piccini P, Björklund A, Brundin P, Rehncrona S, Widner H, Crabb L, Pavese N, Oertel WH, Quinn N, Brooks DJ, Lindvall O (2002). Dyskinesias following neural transplantation in Parkinson's disease. *Nat Neurosci*. 5: 627-628.

Hara K, Matsukawa N, Yasuhara T, Xu L, Yu G, Maki M, Kawase T, Hess DC, Kim SU, Borlongan CV (2007). Transplantation of post-mitotic human neuroteratocarcinoma-overexpressing Nurr1 cells provides therapeutic benefits in experimental stroke: *in vitro* evidence of expedited neuronal differentiation and GDNF secretion. *J Neurosci Res*. 85: 1240-1251.

Hedlund E, Pruszak J, Ferree A, Viñuela A, Hong S, Isacson O, Kim KS (2007). Selection of embryonic stem cell-derived enhanced green fluorescent protein-positive dopamine neurons using the tyrosine hydroxylase promoter is confounded by reporter gene expression in immature cell populations. *Stem Cells* 25: 1126-1135.

Hedlund E, Pruszak J, Lardaro T, Ludwig W, Viñuela A, Kim KS, 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.

Heijtz RD, Kolb B, Forssberg H (2007). Motor inhibitory role of dopamine D1 receptors: implications for ADHD. *Physiol Behav*. 92: 155-160.

Hellmich HL, Kos L, Cho ES, Mahon KA, Zimmer A (1996). Embryonic expression of glial cell-line derived neurotrophic factor (GDNF) suggests multiple developmental roles in neural differentiation and epithelial-mesenchymal interactions. *Mech Dev*. 54: 95-105.

Hermanson E, Joseph B, Castro D, Lindqvist E, Aarnisalo P, Wallén A, Benoit G, Hengerer B, Olson L, Perlmann T (2003). Nurr1 regulates dopamine synthesis and storage in MN9D dopamine cells. *Exp Cell Res*. 288: 324-334.

Hirsch EC, Faucheux B, Damier P, Mouatt-Prigent A, Agid Y (1997). Neuronal vulnerability in Parkinson's disease. *J Neural Transm Suppl*. 50: 79-88. Review.

Ho KS, Scott MP (2002). Sonic hedgehog in the nervous system/functions, modifications and mechanisms. *Curr Opin Neurobiol*. 12: 57-63.

Hwang DY, Ardayfio P, Kang UJ, Semina EV, Kim KS (2003). Selective loss of dopaminergic neurons in the substantia nigra of Pitx3-deficient aphakia mice. *Brain Res Mol Brain Res*. 114: 123-31.

Hwang DY, Hong S, Jeong JW, Choi S, Kim H, Kim J, Kim KS (2009). VMAT2 and DAT are molecular targets of Pitx3 in the ventral midbrain dopamine neurons. *J Neurochem* Sep 24. [Epub ahead of print].

Hynes M, Stone DM, Dowd M, Pitts-Meek S, Goddard A, Gurney A, Rosenthal A (1997). Control of cell pattern in the neural tube by the zinc finger transcription factor and oncogene Gli-1. *Neuron* 19: 15-26.

Ito R, Asami S, Kagawa S, Motohashi S, Shichino H, Chin M, Yoshida Y, Nemoto N, Mugishima H, Suzuki T (2004). Usefulness of tyrosine hydroxylase mRNA for diagnosis and detection of minimal residual disease in neuroblastoma. *Biol Pharm Bull*. 27: 315-318.

Iwawaki T, Kohno K, Kobayashi K (2000). Identification of a potential Nurr1 response element that activates the tyrosine hydroxylase gene promoter in cultured cells. *Biochem Biophys Res Commun*. 274: 590-595.

Joksimovic M, Yun BA, Kittappa R Anderegg AM, Chang WW, Taketo MM, McKay RD, Awatramani RB (2009). Wnt antagonism of Shh facilitates midbrain floor plate neurogenesis. *Nat Neurosci*. 12: 125-131.

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

Kim DW, Chung S, Hwang M, Ferree A, Tsai HC, Park JJ, Chung S, Nam TS, Kang UJ, Isacson O, Kim KS (2006). Stromal cell-derived inducing activity, Nurr1, and signaling molecules synergistically induce dopaminergic neurons from mouse embryonic stem cells. *Stem Cells* 24: 557-567.

Kim JH, Auerbach JM, Rodríguez-Gómez JA, Velasco I, Gavin D, Lumelsky N, Lee SH, Nguyen J, Sánchez-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.

Kim KS, Kim CH, Hwang DY, Seo H, Chung S, Hong SJ, Lim JK, Anderson T, Isacson O (2003). Orphan nuclear receptor Nurr1 directly transactivates the promoter activity of the tyrosine hydroxylase gene in a cell-specific manner. *J Neurochem*. 85: 622-634.

Kim TE, Lee HS, Lee YB, Hong SH, Lee YS, Ichinose H, Kim SU, Lee MA (2003). Sonic hedgehog and FGF8 collaborate to induce dopaminergic phenotypes in the Nurr1-overexpressing neural stem cell. *Biochem Biophys Res Commun*. 305: 1040-1048.

Kondo T (2002). Initial therapy for Parkinson's disease: levodopa vs. dopamine receptor agonists. *J Neurol*. 249: 25-29. Review.

Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW (2008). Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med*. 14: 504-506.

Kordower JH, Emborg ME, Bloch J, Ma SY, Chu Y, Leventhal L, McBride J, Chen EY, Palfi S, Roitberg BZ, Brown WD, Holden JE, Pyzalski R, Taylor MD, Carvey P, Ling Z, Trono D, Hantraye P, Deglon N, Aebischer P (2000). Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 290: 767-773.

Korecka JA, Verhaagen J, Hol EM (2008). Cell-replacement and gene-therapy strategies for Parkinson's and Alzheimer's disease. *Regen Med*. 2: 425-46. Review.

Korotkova TM, Ponomarenko AA, Haas HL, Sergeeva OA (2005). Differential expression of the homeobox gene Pitx3 midbrain in dopaminergic neurons. *Eur J Neurosci*. 22: 1287-1293.

Le WD, Conneely OM, Zou L, He Y, Saucedo-Cardenas O, Jankovic J, Mosier DR, Appel SH (1999). Selective agenesis of mesencephalic dopaminergic neurons in Nurr1-deficient mice. *Exp Neurol*. 159: 451-458.

Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD (2000). Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat Biotechnol*. 18: 675-679.

Levy YS, Bahat-Stroomza M, Barzilay R, Burshtein A, Bulvik S, Barhum Y, Panet H, Melamed E, Offen D (2008). Regenerative effect of neural-induced human mesenchymal stromal cells in rat models of Parkinson's disease. *Cytotherapy*. 10: 340-352.

Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehncrona S, Björklund A, Widner H, Revesz T, Lindvall O, Brundin P (2008). Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med*. 14: 501-503.

Li L, Su Y, Zhao C, Xu Q (2009). Role of Nurr1 and Ret in induc-

ing rat embryonic neural precursors to dopaminergic neurons. *Neurol Res*. 31: 534-540.

Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F (1993). GDNF: a glial cell-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 260: 1130-1132.

Lui KO, Waldmann H, Fairchild PJ (2009). Embryonic stem cells overcoming the immunological barriers to cell replacement therapy. *Curr Stem Cell Res Ther*. 4: 70-80.

Luo Y, Henricksen LA, Giuliano RE, Prifti L, Callahan LM, Federoff HJ (2007). VIP is a transcriptional target of Nurr1 in dopaminergic cells. *Exp Neurol*. 203: 221-232.

Madhavan L, Daley BF, Paumier KL, Collier TJ (2009). Transplantation of subventricular zone neural precursors induces an endogenous precursor cell response in a rat model of Parkinson's disease. *J Comp Neurol*. 515: 102-115.

Mandel RJ, Brundin P, Bjorklund A (1990). The importance of graft placement and task complexity for transplant-induced recovery of simple and complex sensorimotor deficits in dopamine denervated rats. *Eur J Neurosci*. 2: 888-894.

McLaughlin D, Tsirimonaki E, Vallianatos G, Sakellaridis N, Chatzistamatiou T, Stavropoulos-Gioka C, Tsezou A, Messinis I, Mangoura D (2006). Stable expression of a neuronal dopaminergic progenitor phenotype in cell lines derived from human amniotic fluid cells. *J Neurosci Res*. 83: 1190-1200.

Mendez I, Hong M, Smith S, Dagher A, Desrosiers J (2000). Neural transplantation cannula and microinjector system: experimental and clinical experience. Technical note. *J Neurosurg*. 92: 493-499.

Miller GW, Gainetdinov RR, Levey AI, Caron MG (1999). Dopamine transporters and neuronal injury. *Trends Pharmacol Sci*. 20: 424-429.

Missale C, Nash SR, Robinson SW, Mohamed J, Caron MG (1998). Dopamine Receptors: From Structure to Function. *Physiol Rev*. 7: 189-225.

Mohapel P, Frielingsdorf H, Haggblad J, Zachrisson O, Brundin P (2005). Platelet-derived growth factor (PDGF-BB) and brain-derived neurotrophic factor (BDNF) induce striatal neurogenesis in adult rats with 6-hydroxydopamine lesions. *Neuroscience* 132: 767-776.

Montgomery EB (1999). Deep brain stimulation reduces symptoms of Parkinson disease. *Cleve Clin J Med*. 66: 9-11.

Morizane A, Takahashi J, Shinoyama M, Ideguchi M, Takagi Y, Fukuda H, Koyanagi M, Sasai Y, Hashimoto N (2006). Generation of graftable dopaminergic neuron progenitors from mouse ES cells by a combination of coculture and neurosphere methods. *J Neurosci Res*. 83: 1015-1027.

Nagatsu T (1991) Genes for human catecholamine-synthesizing enzymes. *Neurosci Res*. 12: 315-345.

Nagatsu T, Ichinose H (1999). Regulation of pteridine-requiring enzymes by the cofactor tetrahydrobiopterin. *Mol Neurobiol*. 19: 79-96. Review.

Nagatsu T, Levit M, Udenfriend S (1964). Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. *J. Biol. Chem*. 239: 2910-2917.

Nelson EL, Liang CL, Sinton CM, German DC (1996). Midbrain dopaminergic neurons in the mouse/computer-assisted mapping. *J Comp Neurol*. 369: 361-371.

Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, Shannon KM, Nauert GM, Perl DP (2003). A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol*. 54: 403-414.

Ootsuka S, Asami S, Sasaki T, Yoshida Y, Nemoto N, Shichino H, Chin M, Mugishima H, Suzuki T (2008). Useful markers for detecting minimal residual disease in cases of neuroblastoma. *Biol Pharm Bull*. 31: 1071-1074.

Pakkenberg B, Moller A, Gundersen HJ, Mouritzen Dam A, Pakkenberg H (1991). The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. *J Neurol Neurosurg Psychiatry*. 54: 30-33.

Panhuysen M, Vogt Weisenhorn DM, Blanquet V, Brodski C, Heinzmann U, Beisker W, Wurst W (2004). Effects of Wnt1 signaling on proliferation in the developing mid-/hindbrain region. *Mol Cell Neurosci*. 26: 101-11.

Pascual A, Hidalgo-Figueroa M, Piruat JI, Pintado CO, Gómez-Díaz R, López-Barneo J (2008). Absolute requirement of GDNF for adult catecholaminergic neuron survival. *Nat Neurosci*. 11: 755-761.

Perlmann T, Wallén-Mackenzie A (2004). Nurr1, an orphan nuclear receptor with essential functions in developing dopamine cells. *Cell Tissue Res*. 318: 45-52.

Perrier AL, Tabar V, Barberi T, Rubio ME, Bruses J, Topf N, Harrison NL, Studer L (2004). Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci USA*. 34: 12543-8.

Phillips AG, Vacca G, Ahn S (2008). A top-down perspective on dopamine, motivation and memory. *Pharmacol Biochem Behav*. 90: 236-249.

Placzek M, Briscoe J (2005). The floor plate: multiple cells, multiple signals. *Nat Rev Neurosci*. 6: 230-240.

Prakash N, Brodski C, Naserke T, Puelles E, Gogoi R, Hall A, Panhuysen M, Echevarria D, Sussel L,Weisenhorn DMV, Martinez S, Arenas E, Simeone A, Wurst W (2006a). A Wnt1-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors *in vivo*. *Development* 133: 89-98.

Prakash N, Wurst W (2006b). Development of dopaminergic neurons in the mammalian brain. *Cell Mol Life Sci* 63:187-206.

Pruszak J, Sonntag KC, Aung MH, 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.

Rascol O, Goetz C, Koller W, Poewe W, Sampaio C (2002). Treatment interventions for Parkinson's disease: an evidence based assessment. *Lancet* 359: 1589-1598.

Revazova ES, Turovets NA, Kochetkova OD, Agapova LS, Sebastian JL, Pryzhkova MV, Smolnikova VI, Kuzmichev LN, Janus JD (2008). HLA homozygous stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells*. 10: 11-24.

Revazova ES, Turovets NA, Kochetkova OD, Kindarova LB, Kuzmichev LN, Janus JD, Pryzhkova MV (2007). Patient-specific stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells*. 9: 432-449.

Reynolds GP (1996). Dopamine receptors and schizophrenia.

Biochem Soc Trans. 24: 202-205. Review.

Rhinn M, Brand M (2001). The midbrain- hindbrain boundary organizer. *Curr Opin Neurobiol*. 11: 34-42.

Robertson NJ, Brook FA, Gardner RL, Cobbold SP, Waldmann H, Fairchild PJ (2007). Embryonic stem cell-derived tissues are immunogenic but their inherent immune privilege promotes the induction of tolerance. *Proc Natl Acad Sci USA*. 104: 20920-20925.

Rosenblad C, Martinez-Serrano A, Bjorklund A (1998). Intrastriatal glial cell line-derived neurotrophic factor promotes sprouting of spared nigrostriatal dopaminergic afferents and induces recovery of function in a rat model of Parkinson's disease. *Neuroscience* 82: 129-137.

Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA (2006). Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat Med*. 12: 1259-1268.

Sacchetti P, Brownschidle LA, Granneman JG, Bannon MJ (1999). Characterization of the 5'-flanking region of the human dopamine transporter gene. *Brain Res Mol Brain Res*. 74: 167-174.

Sakurada K, Ohshima-Sakurada M, Palmer TD, Gage FH (1999). Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. *Development* 126: 4017-4026.

Sandhu JK, Gardaneh M, Iwasiow R, Lanthier P, Gangaraju S, Ribecco-Lutkiewicz M, Tremblay R, Kiuchi K, Sikorska M (2009). Astrocyte-secreted GDNF and glutathione antioxidant system protect neurons against 6OHDA cytotoxicity. *Neurobiol Dis*. 33: 405-414.

Saucedo-Cardenas O, Quintana-Hau JD, Le WD, Smidt MP, Cox JJ, De Mayo F, Burbach JP, Conneely OM (1998). Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. *Proc Natl Acad Sci USA*. 95: 4013-4018.

Sayles M, Jain M, Barker RA (2004). The cellular repair of the brain in Parkinson's disease-past, present and future. *Transpl Immunol*. 12: 321-342.

Schulz TC, Noggle SA, Palmarini GM, Weiler DA, Lyons IG, Pensa KA, Meedeniya AC, Davidson BP, Lambert NA, Condie BG (2004). Differentiation of human embryonic stem cells to dopaminergic neurons in serum-free suspension culture. *Stem Cells*. 22: 1218-1238.

Schumacher JM, Ellias SA, Palmer EP, Kott HS, Dinsmore J, Dempsey PK, Fischman AJ, Thomas C, Feldman RG, Kassissieh S, Raineri R, Manhart C, Penney D, Fink JS, Isacson O (2000). Transplantation of embryonic porcine mesencephalic tissue in patients with PD. *Neurology* 54: 1042-1050.

Shamekh R, Saporta S, Cameron DF, Willing AE, Sanberg CD, Johe K, Sanberg PR (2008). Effects of Sertoli cell-conditioned medium on ventral midbrain neural stem cells: a preliminary report. *Neurotox Res*. 13: 241-246.

Shim JW, Park CH, Bae YC, Bae JY, Chung S, Chang MY, Koh HC, Lee HS, Hwang SJ, Lee KH, Lee YS, Choi CY, Lee SH (2007). Generation of functional dopamine neurons from neural precursor cells isolated from the subventricular zone and white matter of the adult rat brain using Nurr1 overexpression. *Stem Cells* 25: 1252-1262.

Shimada H, Yoshimura N, Tsuji A, Kunisada T (2009). Differentiation of dopaminergic neurons from human embryonic stem cells: modulation of differentiation by FGF-20. *J Biosci Bioeng*. 107: 447-254.

Shintani A, Nakao N, Kakishita K, Itakura T. (2008) Generation of dopamine neurons from embryonic stem cells in the presence of the neuralizing activity of bone marrow stromal cells derived from adult mice. *J Neurosci Res*. 86: 2829-38.

Simon HH, Saueressig H, Wurst W, Goulding MD, O'Leary DD (2001). Fate of midbrain dopaminergic neurons controlled by the engrailed genes. *J Neurosci*. 21: 3126-3134.

Slevin JT, Gash DM, Smith CD, Gerhardt GA, Kryscio R, Chebrolu H, Walton A, Wagner R, Young AB (2006). Unilateral intraputaminal glial cell line-derived neurotrophic factor in patients with Parkinson disease: response to 1 year each of treatment and withdrawal. *Neurosurg Focus*. 20: E1.

Slevin JT, Gerhardt GA, Smith CD, Gash DM, Kryscio R, Young B (2005). Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputaminal infusion of glial cell line-derived neurotrophic factor. *J Neurosurg*. 102: 216-222.

Smidt MP, Asbreuk CH, Cox JJ, Chen H, Johnson RL & Burbach JP (2000). A second independent pathway for development of mesencephalic dopaminergic neurons requires Lmx1b. *Nat Neurosci*. 3: 337-341.

Smidt MP, Smits SM, Bouwmeester H, Hamers FP, Linden AJ vander, Hellemons AJ, Graw J, Burbach JP (2004a). Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene Pitx3. *Development* 131: 1145-1155.

Smidt MP, Smits SM, Burbach JP (2004b). Homeobox gene Pitx3 and its role in the development of dopamine neurons of the substantia nigra. *Cell Tissue Res*. 318: 35-43.

Smith Y, Kieval JZ (2000). Anatomy of the dopamine system in the basal ganglia. *Trends Neurosci* 23 (Suppl): S28-S33

Smits SM, Ponnio T, Conneely OM, Burbach JP, Smidt MP (2003). Involvement of Nurr1 in specifying the neurotransmitter identity of ventral midbrain dopaminergic neurons. *Eur J Neurosci*. 18: 1731-1738.

Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, 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.

Sonntag KC, Pruszak J, Yoshizaki T, van Arensbergen J, Sanchez-Pernaute R, Isacson O (2007). Enhanced yield of neuroepithelial precursors and midbrain-like dopaminergic neurons from human embryonic stem cells using the bone morphogenic protein antagonist noggin. *Stem Cells* 25: 411-418.

Sonntag KC, Simantov R, Kim KS, Isacson O (2004). Temporally induced Nurr1 can induce a non-neuronal dopaminergic cell type in embryonic stem cell differentiation. *Eur J Neurosci*. 19: 1141-1152.

Storch A, Sabolek M, Milosevic J, Schwarz SC, Schwarz J (2004). Midbrain-derived neural stem cells: from basic science to therapeutic approaches. *Cell Tissue Res*. 318: 15-22.

Subramanian T, Marchionini D, Potter EM, Cornfeldt ML (2002). Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavioral deficits without provoking a host immune response. *Cell Transplant*. 11: 207-214.

Takagi Y, Takahashi J, Saiki H, Morizane A, Hayashi T, Kishi Y, Fukuda H, Okamoto Y, Koyanagi M, Ideguchi M, Hayashi H, Imazato T, Kawasaki H, Suemori H, Omachi S, Iida H, Itoh N, Nakatsuji N, Sasai Y, Hashimoto N (2005). Dopaminergic neurons generated from monkey embryonic stem cells function in a Parkinson primate model. *J Clin Invest*. 115: 102-109.

Takahashi H, Higuchi M, Suhara T (2006). The role of extrastriatal dopamine D2 receptors in schizophrenia. *Biol Psychiatry*. 59: 919-928. Review.

Takahashi K, Yamanaka S (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663-676.

Tomac, A, Lindqvist E, Lin LF, Ogren SO, Young D, Hoffer BJ, Olson L (1995). Protection and repair of the nigrostriatal dopaminergic system by GDNF *in vivo*. *Nature* 373: 335-339.

Träger C, Vernby A, Kullman A, Ora I, Kogner P, Kågedal B (2008). mRNAs of tyrosine hydroxylase and dopa decarboxylase but not of GD2 synthase are specific for neuroblastoma minimal disease and predicts outcome for children with high-risk disease when measured at diagnosis. *Int J Cancer*. 123: 2849-55.

Tseng KY, Roubert C, Do L, Rubinstein M, Kelly MA, Grandy DK, Low MJ, Gershanik OS, Murer MG, Giros B, Raisman-Vozari R (2000). Selective increase of Nurr1 mRNA expression in mesencephalic dopaminergic neurons of D2 dopamine receptor-deficient mice. *Brain Res Mol Brain Res*. 80: 1-6.

Vallone D, Picetti R, Borrelli E. (2000) Structure and function of dopamine receptors. *Neurosci Biobehav Rev*. 24: 125-132. Review.

Van den Munckhof P, Luk KC, Ste-Marie L, Montgomery J, Blanchet PJ, Sadikot AF, Drouin J (2003). Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. *Development* 130: 2535-2542.

Vazin T, Becker KG, Chen J, Spivak CE, Lupica CR, Zhang Y, Worden L, Freed WJ (2009). A novel combination of factors, termed SPIE, which promotes dopaminergic neuron differentiation from human embryonic stem cells. *PLoS One*. 4: e6606.

Vazin T, Chen J, Lee CT, Amable R, Freed WJ (2008). Assessment of stromal-derived inducing activity in the generation of dopaminergic neurons from human embryonic stem cells. *Stem Cells* 26: 1517-25.

Volkow ND, Fowler JS, Wang GJ, Swanson JM, Telang F (2007). Dopamine in drug abuse and addiction: results of imaging studies and treatment implications. *Arch Neurol*. 64: 1575-1579. Review.

Wallen AA, Castro DS, Zetterstrom RH, Karlen M, Olson L, Ericson J, Perlmann T (2001). Orphan nuclear receptor Nurr1 is essential for Ret expression in midbrain dopamine neurons and in the brain stem. *Mol Cell Neurosci*. 18: 649-663.

Wernig M, Zhao JP, 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.

Wijeyekoon R, Barker RA (2009). Cell replacement therapy for Parkinson's disease. *Biochim Biophys Acta* 1792: 688-702.

Wise RA (2004). Dopamine, learning and motivation. *Nat Rev Neurosci*. 5: 483-494.

Wood TK, McDermott KW, Sullivan AM (2005). Differential effects of growth/ differentiation factor 5 and glial cell line-derived neurotrophic factor on dopaminergic neurons and astroglia in cultures of embryonic rat midbrain. *J Neurosci Res*. 80: 759-66.

Yan Y, Yang D, Zarnowska ED, Du Z, Werbel B, Valliere C, Pearce RA, Thomson JA, Zhang SC (2005). Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. *Stem Cells* 23: 781-790.

Yang D, Zhang ZJ, Oldenburg M, Ayala M, Zhang SC (2008). Human embryonic stem cell derived dopaminergic neurons reverse functional deficit in parkinsonian rats. *Stem Cells*. 26: 55-63.

Ye W, Shimamura K, Rubenstein JL, Hynes MA, Rosenthal A (1998). FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* 93: 755-766.

Yue F, Cui L, Johkura K, Ogiwara N, Sasaki K (2006). Induction of midbrain dopaminergic neurons from primate embryonic stem cells by coculture with sertoli cells. *Stem Cells* 24: 1695-1706.

Zafeiriou DI, Willemsen MA, Verbeek MM, Vargiami E, Ververi A, Wevers R (2009). Tyrosine hydroxylase deficiency with severe clinical course. *Mol Genet Metab*. 97: 18-20.

Zeng X, Cai J, Chen J, Luo Y, You ZB, Fotter E, Wang Y, Harvey B, Miura T, Backman C, Chen GJ, Rao MS, Freed WJ (2004). Dopaminergic differentiation of human embryonic stem cells. *Stem Cells* 22: 925-940.