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Review

Stem cells may reshape the prospect of Parkinson's disease therapy

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Abstract

The concept of cell replacement to compensate for cell loss and restore functionality has entered several disease entities including neurodegenerative disorders. Recent clinical studies have shown that transplantation of fetal dopaminergic (DA) cells into the brain of Parkinson's disease (PD) patients can reduce disease-associated motor deficits. However, the use of fetal tissue is associated with practical and ethical problems including low efficiency, variability in the clinical outcome and controversy regarding the use of fetuses as donor. An alternative cell resource could be embryonic stem (ES) cells, which can be cultivated in unlimited amounts and which have the potential to differentiate into mature DA cells. Several differentiation protocols have been developed, and some progress has been made in understanding the mechanisms underlying DA specification in ES cell development, but the "holy grail" in this paradigm, which is the production of sufficient amounts of the "right" therapeutic DA cell, has not yet been accomplished. To achieve this goal, several criteria on the transplanted DA cells need to be fulfilled, mainly addressing cell survival, accurate integration in the brain circuitry, normal function, no tumor formation, and no immunogenicity. Here, we summarize the current state of ES cell-derived DA neurogenesis and discuss the aspects involved in generating an optimal cell source for cell replacement in PD.
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1. Introduction: stem cells as a new resource for cell replacement therapies

Ongoing degeneration and death of dopaminergic (DA) neurons in the brain is the hallmark of Parkinson's disease (PD), a sickness inflicting multiple physiological and behavioral abnormalities. Chemical replacement therapy was developed and widely used as an effective way to restrain some of the behavioral symptoms of the disease, but this approach is not without faults, as motor dysfunctions and dyskinesia develop over time [56,107]. Other approaches to contain the symptoms and/or the neurodegenerative process have been therefore verified, including treatment with dopamine agonists, antioxidative agents, growth factors, deep brain stimulation, and others [9,31,32,42,118]. During the last decade or so, effort has been made in a number of laboratories, including this one, to also analyze the option of cell replacement therapy by grafting into the striatum cells that can produce dopamine, the deficient neurotransmitter. To this end, the efficiency of fetal human brain cells, retina, adrenal, and xenotransplants [7,14,91,98,119] was tested, some of which with considerable success [73]. Although very promising, this line of research is still very challenging in that it relies on the development of a convenient, plentiful, safe, and ethically acceptable source of cells; unfortunately some of these criteria are not met by the cell sources listed above. Studies conducted during the last few years, and discussed below in detail, provide encouraging indications that stem cells (SC) might fulfill all the essential attributes for a successful cell replacement therapy in PD. Needless to say, however, that much research is still necessary in order to accomplish this aspiration [78,131].

2. Using naïve embryonic stem (ES) cells for production of DA neurons

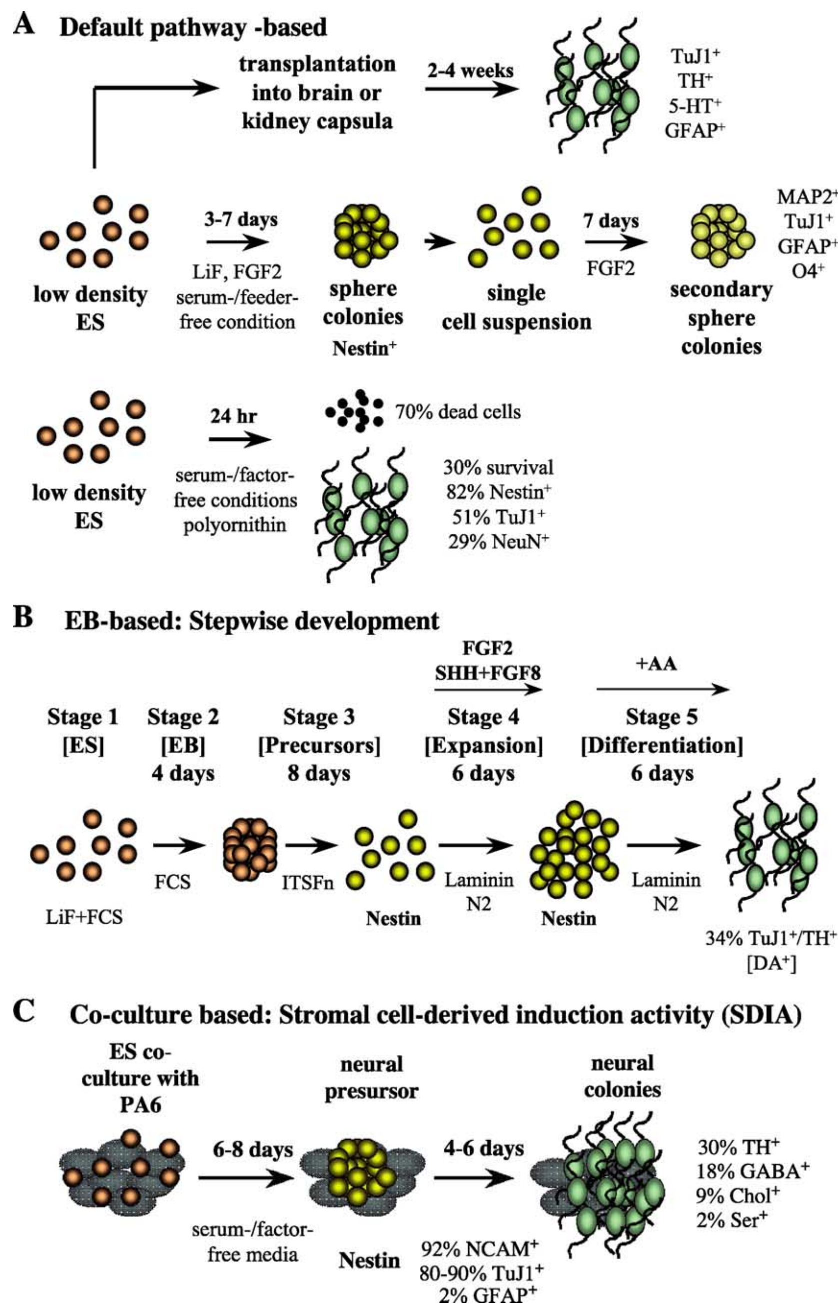
2.1. Spontaneous differentiation of ES cells to DA neurons in vivo

Several features of PD increase its attractiveness for SC therapy. First, unlike some other neurodegenerative diseases, PD symptoms are identified with the defined loss of a specific cell type, the DA neuron [56]. Second, many of the PD symptoms result from a rather localized cell loss in the midbrain, although it is clear today that some late stage symptoms are attributed to non-DA systems [17,126]. Third, over the last decade, an extensive knowledge has been accumulated from 'cell-based therapies' in PD patients transplanted with fetal or other cells. Fourth, several experimental models of PD are available today, which enhances the research effort in this field.

Multipotent cells of different origins have been used in experimental PD models, including embryonic stem (ES) cells, neural stem/progenitor cells, and immortalized cell lines. Early studies by Deacon et al. verified both neurogenesis and differentiation of mouse naïve D3 ES and transgenic E14TG2 cells in mouse and rat host brain [29] (see Fig. 1A). Transplantation of a small number of ES cells

in the brain resulted in a spontaneous production of neurons, and about 3/4 of grafts that survived contained DA neurons (tyrosine hydroxylase-positive, TH^+). Further, a PD model of 6-OHDA-lesioned rats was used in this work to analyze whether the DA neurons developing from the grafted ES cells could grow into the host striatum and thus may replace the deficient cells. Indeed, it was confirmed that the DA neurons sent axons into the lesioned striatum, preferentially to the gray matter [29]. Additional studies from this laboratory have shown that such grafted ES cells generate functional DA neurons that restore motor behavior in the rat PD experimental model [13,59].

Based on studies that identified the role of various growth factors in regulating development of dopaminergic and serotonergic neurons during embryogenesis [54,152] and their own in vitro experiments [97], McKay et al. developed an in vitro protocol for an efficient generation and differentiation of DA neurons from mouse ES cells [79] (see Fig. 1B). Cells prepared according to this protocol were functional and effective in an animal model of PD [65]. Additional efforts to generate enriched populations of DA neurons used a stromal cell-derived inducing activity (SDIA) [6,61] (see Fig. 1C). These and other studies have established the notion that naïve mouse ES cells are indeed



suitable for production of large quantities of DA neurons, which are also functional in experimental PD models.

Unraveling the mechanisms underlying these encouraging findings in rodents will contribute to adapting the method to human ES cells for successful transplantation in PD patients. Preliminary studies toward this end indicate an interesting set of differences between rodent and human ES cells [43]. Overall, effective transplantation depends on development and specification of DA neurons, a multistep process that has two main components: (a) neurogenesis and (b) expression of the DA phenotype. Some of the theoretical considerations underlying these two facets of DA development will be addressed sequentially in the following section.

2.2. The road from a totipotent ES cell to DA neurons

Early developmental studies showed that signaling factors produced by and released from a few well-defined organizing centers in the embryo take part in neural specification, otherwise the progenitor ectodermal cells adopt epithelial lineage [116]. Additional experiments established that ectoderm development, followed by neural versus epithelial specification, relies on a complex temporal and spatial regulation of several protein factors such as transforming growth factor beta (TGF-β), bone morphogenic factors (BMPs), and their protein antagonists, fibroblast growth factors (FGFs) and their receptors, sonic hedgehog (SHH) or other members of this family, and Wnts and their signaling pathway counterparts [148]. However, much less is known about the growth and morphogenic proteins that control neurogenesis and neuronal specification of ES cells, particularly when transplanted into the adult brain.

By definition, ES cells are totipotent and therefore can develop to all three cell lineages: ectoderm, mesoderm, and endoderm. When grafted into the brain for cell replacement therapy, it is critical that the ES cells will acquire the ectoderm lineage from which neurons develop. Multiple evidence indicate that neurogenesis and expression of the DA phenotype from SC is under the control of both intrinsic mechanisms as well as the host milieu, and neither one alone is sufficient to achieve a successful and functional graft. Moreover, the life history of the grafted cells (prior to transplantation) can influence the final fate of the cells in the brain, as shown in early experiments, which studied the effect of pretreatment of naïve or transgenic mouse ES cell lines with retinoic acid [29]. Yang et al., on the other hand, studied the effect of the confluence at which cells were maintained and the number of passages in vitro prior to the grafting [151]. We now know that in vitro treatments, which influence the differentiation stage of the grafted cells, are critical to their fate in the brain; this will be addressed below in detail. The recent findings that proliferation and differentiation of neuronal progenitor cells in the subventricular zone of the adult mammalian forebrain are regulated by intrinsic and extrinsic factors such as BMPs [27] suggest that ES cell neurogenesis and subtype specification may also obey rules comparable to the mechanisms in the developing brain. Lin and Rosenthal proposed that the same extrinsic and internal factors controlling DA neuron differentiation during embryogenesis are also useful to generate this cell type in vitro [82].

As to the significance of the host brain, several factors are important, including the age of the host [137], the brain region transplanted [29,123], and whether the brain is damaged or not [140]. From yet unknown reasons, apparently involving an immune induction, transplanted cells survive better in 6-OHDA-lesioned rats than in intact brain [36,140]. Whether this is relevant to PD patients would be worth analyzing. Increased recruitment of neuronal stem cells to the brain-injured site was also observed after inducing a focal stroke in the rat [160]. A very recent cDNA microarray analysis of an injured mouse brain revealed a new protein factor that induces migration of neuronal/progenitor cells to the site of injury [141]. As to the site of trans-

Fig. 1. Schematic summaries of the main ES cell differentiation paradigms for (DA) neurogenesis. (A) Summary of two differentiation protocols based on the default pathway of neural induction. Low-density ES cells were transplanted into the brain or under the kidney capsule and developed mainly into neural cells including TH^+ and $5\text{-}HT^+$ neurons ($Tuj1^+$) and astrocytes ($GFAP^+$) [13,30]. Similar observations were made by Tropepe et al. in vitro by plating low-density ES cells in the presence of LiF and FGF2 and serum-/feeder-free conditions [143]. After 3 days in culture, the ES cells developed into neural sphere colonies composed of $Nestin^+$ neural precursors. These colony-forming neural stem cells had the capacity of self-renewal and multipotency and could be plated as single cells and/or sphere colonies on polyornithine substrate. The cells could then be differentiated into neural cell types including astrocytes ($GFAP^+$), oligodendrocytes ($O4^+$), and neurons ($MAP2^+$, $Tuj1^+$). One of the features of the serum-/factor-free conditions was a relatively high percentage of dead cells (70%) after 24 h in culture. However, from the 30% surviving cells, 82% were $Nestin^+$, and 51% had a neuronal ($Tuj1^+$) phenotype. (B) ES cell-derived neurogenesis based on a stepwise cell development using the five-stage differentiation protocol [79]. This protocol was developed with the intention to recapitulate features of "normal" ES cell development by initially allowing the formation of cells from all germlayers in embryoid bodies (EB) in the presence of media containing fetal calf serum (FCS). After 4 days, the EBs were plated in defined serum-free culture conditions using insulin, transferrin, selenin, and fibronectin to select the $Nestin^+$ neural precursor population. These cells were then further propagated on polyornithine/laminin coated dishes in N2 media and FGF2. In a final step, the FGF2 was removed, and cells differentiated into neural cells for 6 to 12 days. The outcome of this protocol was a high percentage of neurons with a ventral–midbrain phenotype. To produce TH^+ neurons, the culture conditions were modified by adding the signaling factors SHH and FGF8 at stage 4 and ascorbic acid (AA) at stage 5 of the cultures, which increased the amount of functional TH^+ DA neurons to 34%. (C) Neural induction by stromal cell-derived inducing activity (SDIA). In 2000, Kawasaki et al. published an ES cell differentiation protocol that was based on the inductive property of cocultured PA6 stromal cells, which were derived from skull bone marrow [61]. ES cells grown in the presence of PA6 cells in serum- and factor-free conditions developed into $Nestin^+$ precursors, which were to 92% positive for NCAM. 80–90% of these cells became $Tuj1^+$, whereas only 2% differentiated into $GFAP^+$ astrocytes indicating strong neuronal induction of this protocol. Further analyses revealed that already after 6–8 days, the $Nestin^+$ precursor population up-regulated Tuj1 and TH expression. After 12 days in culture, the majority (30%) of the $Tuj1^+$ neurons had a TH^+ phenotype, whereas 18%, 9%, and 2% were gabaergic (GABA), cholinergic (Chol), and serotonergic (Ser), respectively.

plantation, studies with embryonic brain cells have shown that grafting into homotypic rather than heterotypic brain regions influence the grafting efficiency; it is expected that this problem will be less prominent when using ES cells.

Why would the totipotent ES cells transplanted in an adult (and foreign) brain develop and differentiate to neurons in the first place? This notion relies on early developmental models, in which ectodermal progenitor cells acquire the neural fate spontaneously, in the absence of factors derived from organizing centers, provided that the cells are placed in a low-density environment (see below). Under these conditions, therefore, the neural commitment may be controlled intrinsically by default. Additional studies led to the development of a more unifying model in which the neural default process is combined with the activity of BMP antagonists such as noggin and chordin [96,148]. Several studies with ES cells are in agreement with the default mechanism. First, when mouse ES cells were transplanted at low cell density, they developed putative DA (and serotonergic) neurons [29]. Using ES cells cultured in defined medium, Tropepe et al. showed that, at low cell density, the cells readily acquire a neural fate [143]. More recent studies from our laboratory with either naïve or knockout mouse ES cells transplanted at different grafting densities into the mouse or rat striatum are in agreement with the notion that a low rather than a high cell density is preferred for neural development [13,132]. The significance of cell densities on survival of DA neurons was also analyzed in vitro and raises the possible involvement of other factors such as extracellular matrix and cell adhesion molecules [89].

Many studies with cultured ES cells indicate that withdrawal of mitogens (such as LiF) is a prerequisite for neuronal differentiation; how does this work in vivo, and what controls ES cell proliferation versus differentiation in the grafted tissue? Aiming to address this point, it was essential to determine whether transplanted ES cells differentiate into DA neurons immediately after transplantation (without proliferation), or if they first multiply for a certain time and then differentiate. Time course analysis of ES cells transplanted at low density (2000 cells) indicate that 7 and 14 days after transplantation, 63% and 40% of the cells still express the specific ES cell marker SSEA1 [132], suggesting that many cells maintain their capacity to proliferate in vivo for at least 2 weeks. This issue should be further elucidated in the aged brain, particularly relevant to PD, as it was observed that the survival of transplanted mesencephalic cells in the aged brain is only 1/4 of the survival in the young brain [137].

The second element of neuronal specification is the acquisition of a specific neurotransmitter identity. Obtaining the DA phenotype depends on the expression of enzymes participating in DA synthesis, such as aromatic L-amino acid decarboxylase (AADC) and tyrosine hydroxylase (TH), and proteins required for DA release and uptake, such as vesicular monoamine transporter (VMAT2) and dopamine transporter (DAT). We know to day that TH expression is controlled by the transcription factors Pitx3 [21] and Nurr1 [68,115], and by cAMP [81]. Similarly, DAT expression is regulated by cAMP [114], protein kinases A [83], and Nurr1 [114]. Enhancing the expression of these genes will conceivably contribute to increase the number of DA neurons suitable for transplantation.

It is assumed, although not fully confirmed, that, at the first stage, cells have to be specified to neurons. Then, through activation of certain transcription factors and genes, some of them listed above, the cells acquire the DA phenotype. Interestingly, recent findings from this laboratory have challenged this dogma, providing evidence that, under certain experimental conditions, and possibly in vivo, the DA phenotype can also develop in nonneuronal cells [133].

3. Directed neurogenesis and differentiation of ES cells to DA neurons in vitro

3.1. Neural induction and neurogenesis in monoculture

The challenges in ES cell differentiation lie in specifically directing their development into mature cell types that potentially can be used for therapeutic application. This can be achieved chemically by applying specific in vitro culture conditions and the use of factors that are important in cell phenotype specification. For neurogenesis, and especially the generation of DA neurons, several protocols have been developed based on different aspects of ES cell differentiation (Fig. 1), which will be discussed in the following.

As mentioned above, the current understanding of neural cell development includes a default pathway of neural induction, which has been proposed in early in vivo [29] and in vitro ES cell differentiation studies [143] (Fig. 1A). Based on this model, in vitro differentiation protocols have been developed, which aimed to induce the development of neural cell populations in monocultures [153]. In these experiments, the use of different culture conditions allowed for directing neural precursors into glial cells and different neuronal subtypes, including TH^+ neurons, when the signaling factors FGF8 and SHH were present. It is of interest to note that addition of FGF was a critical requirement for neural induction, demonstrating that FGF signaling might play an important role in neural specification of mammalian ES cells [153]. An important parameter in these protocols was the immediate direction of ES cells into the neuroectoderm lineage without the formation of embryoid bodies (EB), which represent the earliest structures of germlayer development. In our studies, and similar to the results from the monocultures, transplanted ES cells spontaneously acquired a neuroectodermal faith in vivo [13,29]. However, the ES cells also developed into teratomas, suggesting that at least in vivo multiple factors seem to be involved in the default pathway of neural induction. Thus, it is unclear whether an intrinsic cellular default is sufficient to direct the ES cell into the neural

lineage. To further delineate some of the mechanisms involved in this pathway, we investigated whether deletion of key factors in TGF-β signaling might abrogate multiple germ layer formation. This involved *Smad4* and *Cripto*, which encode key components of the TGF-β signaling pathway that regulates multiple aspects of embryogenesis, including mesodermal and epidermal cell development [3,11,33,69,92,96,106,128]. Our studies demonstrated that loss of function of *Smad4* or *Cripto* was not sufficient to result in a default pathway of neural induction in in vitro and in vivo differentiation paradigms, suggesting that other factors like cell–cell contact and cellular and graft/host environment are also involved in the neural specification of differentiating ES cells [132].

3.2. Neurogenesis following the steps of embryogenesis

A different approach to direct ES cell differentiation into neural lineages was developed by Lee et al. using a five-step differentiation protocol [79] (Fig. 1B). This protocol was designed to follow the "normal" development of ES cells in embryogenesis in vitro and select the Nestin$^+$ neural precursor (NP) population to further differentiate into neurons and astrocytes. Thus, ES cells were allowed to form EBs for 4 days prior to selection of NPs in defined media conditions. After 8 days, these precursors were plated on polyornithine/fibronectin-coated dishes and were expanded in the presence of bFGF. In a final step, the cells were differentiated into neural phenotypes revealing greater than 80% neurons from which are about 5% TH$^+$. Modification of this protocol by adding the signaling factors SHH and FGF8 during expansion of NP could boost the fraction of TH$^+$ to about 20%. This protocol has been adapted and modified by several groups, including ours [24,25,64,124,132,133], and has been applied to generate transplantable DA cells used in animal models of PD [65]. Although a hallmark of ES cell differentiation into neurons and especially into the DA phenotype, the five-stage protocol is also associated with problems. For example, the development of mesodermal and endodermal cellular phenotypes cannot entirely be prevented, as demonstrated by Myosin$^+$ and Cytokeratin$^+$ cells, which could be detected after full differentiation [132]. In addition, transplanted NP cells in the brain could also give rise to teratomas, which was attributed to a small percentage of SSEA$^+$ immature ES cells that persisted during in vitro differentiation [132]. Thus, without further modification, this protocol has been shown to be suboptimal for the generation of a safe DA cell population as a source for transplantation in PD.

3.3. Neurogenesis directed by feeder cells

In 2000, Kawasaki et al. published an in vitro differentiation protocol, which they named stromal cell-derived inducing activity (SDIA) [61] (Fig. 1C). In this study, ES cells were cocultured with PA6 cells, a stromal cell line that was derived from skull bone marrow. The investigators found that the ES cells spontaneously differentiated into neurons during 14 days of culture, and about 30% of them were dopaminergic. In search for possible mechanisms, they discovered that BMP signaling inhibited neurogenesis, suggesting that the ES cells followed a default pathway of neural induction. ES cells were also transplanted into the striatum of 6-OHDA-treated mice after 12 days of in vitro differentiation. Although no functional data were reported, the neurons survived and integrated into the brain circuitry. Despite its high efficiency for the production of neurons, the function of the stromal cell line in this differentiation paradigm remained unclear. Thus, cell–cell contact and/or the release of factors might have played a role in neural induction. The SDIA protocol has been used by other investigators [64,94,125] and has also been successfully adapted to differentiate primate ES cells [62,100,125]. A second coculture protocol using the MS5 stromal cell line has been developed in Studer's group [6]. The emphasis of these studies was on the fine-tuning of the SDIA-based differentiation paradigm to specifically develop neural subtypes. Using different induction molecules, high percentages of individual neuronal subtypes such as dopaminergic, serotonergic, GABA-ergic, glutamatergic as well as astrocytes and oligodendrocytes could be achieved. A modification of this protocol has recently been adapted to primate and human ES cell differentiations, demonstrating the production of DA neurons with a midbrain-type identity [105]. In this protocol, MS5 cells have been genetically modified to express the induction signal Wnt-1, which directs midbrain-neuron specification in brain development. Coculture of ES cells with Wnt-1 expressing MS5 cells could direct their differentiation into a ventral–midbrain phenotype, and in combination with the use of SHH and FGF8, a high percentage (60–80%) of DA neurons could be generated.

3.4. Species differences

It should be mentioned that the initially described mammalian ES cell differentiation protocols are based on the mouse system and have only recently been adapted to differentiate other ES cells including primate and human [62,100,105,125]. Despite the differences between mouse and primate/human ES cells [43], these studies demonstrated that most of the fundamental mechanisms of ES cell-derived neurogenesis seem to overlap. Thus, it appears that primate and human ES cells might also follow a default pathway of neural induction, as has been suggested by the successful generation of neurons, including dopaminergic, using the SDIA-based differentiation protocols [62,105]. In addition, most of the induction and signaling factors used in the mouse differentiation protocols, such as trophic factors, SHH, FGF8, and others, have been shown to also function in primate/human ES cell differentiation [105]. Moreover,

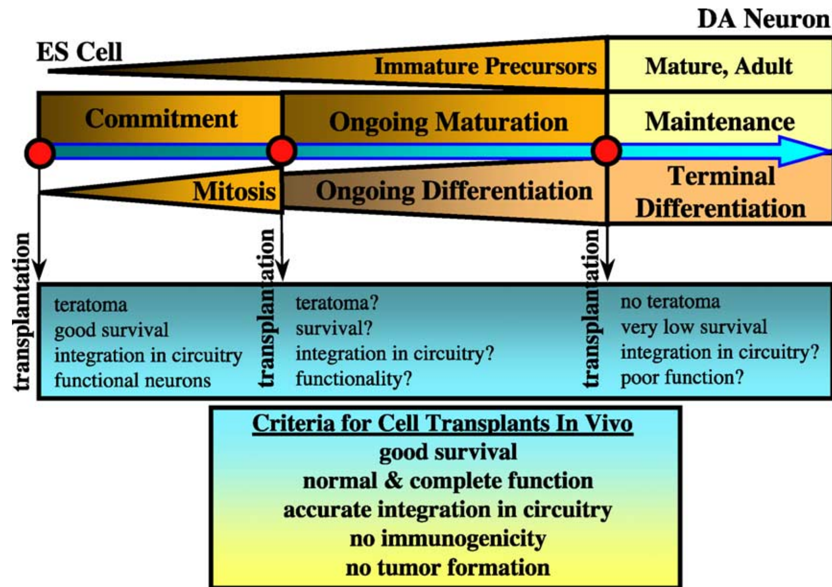


Fig. 2. ES cell-derived DA neurogenesis and its implication for transplantation. In vitro and in vivo differentiated ES cells differentiate into immature neural precursors, which develop into mature adult DA neurons. During cell development, three major phases take place: commitment into the neuroectodermal cell lineage, maturation into the DA-specific neuronal phenotype, and maintenance of cell function. In these phases, the developed cells are either mitotic, e.g., ES and lineage-committed immature precursors, or terminally differentiated immature or mature DA neurons. For transplantation purposes, it is critical to determine the optimal state of the transplanted cells. For example, it has been shown that transplantation of ES cells in the brain showed good survival and differentiation into functional DA neurons with integration in the brain circuitry but partially developed into teratomas [13]. Some of these observations have also been made by transplantation of immature precursors (unpublished observations). In contrast, transplantation of fully differentiated mature DA neurons did not reveal teratoma formation, however, poor survival and suboptimal function in the brain [85]. These results demonstrate that certain criteria for a DA graft need to be fulfilled in order to achieve optimal results in future ES cell-based transplantation paradigms for PD, which can be summarized as follows: good survival, proper function, and integration into the brain circuitry with no immunogenicity and tumor formation.

higher species ES cells can develop into EBs, which give rise to neurospheres and neural precursors [19,76,159]. These precursors can further be differentiated into glial cells and neurons [19,159]. However, there are likewise profound differences in the cell development of different species ES cells. For example, similar to species differences in the duration of gestation and embryogenesis, it appears that the time line of in vitro ES cell development is also different. In the SDIA protocols, mouse ES cell-derived neural precursors (rosettes) develop around 12 days in culture [6], in

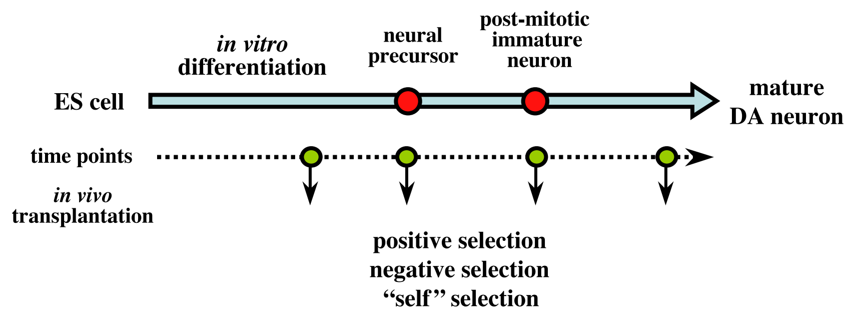


Fig. 3. Tracking the optimal DA cell for transplantation. Schematic representation of ES cell differentiation into neural precursors and mature DA neurons (see Fig. 1 for more details). Current ES cell differentiation protocols produce heterogeneous neural and nonneural cell populations that are suboptimal for transplantation in PD. Although attempts have been undertaken to enrich the fraction of DA neurons derived from ES cells, transplants still consist of contaminating other cells, which interfere in optimal function of the DA grafts. Thus, there is a need for new strategies to isolate a homogeneous DA cell population, which fulfills the criteria of an optimal transplantable cell source (see Fig. 1). Three strategies can be envisioned to achieve this goal. (1) Positive selection, e.g., the selection of the transplantable cells using marker molecules, which identify their “correct” phenotype at different time points during differentiation. For example, ES cells can be genetically engineered to express marker genes such as GFP that are linked to specific promoters, which are active in DA-specific cells like the Pitx3 [162] or TH promoter [154]. Once the promoters are active, cells can be sorted and enriched by Fluorescence Activated Cell Sorting (FACS) or Magnetic Cell Sorting (MACS). Alternatively, surface molecules can be identified that allow for direct selection of cellular subtypes by FACS, MACS, or immunopanning. (2) Negative selection, e.g., is the elimination of unwanted cells to enrich for the transplantable DA cell source. This strategy could involve a specific cell killing mechanism, which is not active in the DA-specific population and/or the use of non-DA-specific surface molecules to sort out the unwanted cells by FACS, MACS or immunopanning. (3) “Self” selection, e.g., the directed differentiation of ES cells into a transplantable homogeneous DA cell population. Several attempts to achieve this goal have been made and are discussed in this review. These include the use of chemically defined culture conditions, cocultures, signaling molecules, gene-engineering of ES cells, and the use of gene-modified stem cell lines.

contrast to primate and human ES cells, which show the typical rosette formations around day 21 and 28, respectively [105]. As discussed below, one of the crucial parameters for developing an optimal in vitro differentiation protocol for the directed differentiation of DA cells is the adjustment of the individual steps in the culture conditions to the sequential events and the intrinsic time clock of the ES cell during its development. Thus, with the higher species ES cells, a more complex and a longer timeline of cell development has to be taken into account, and adjusting the current knowledge of cell differentiation will be the challenge for future studies.

4. Currently available molecular approaches to enrich DA neurons production

4.1. Genes controlling DA neuron specification

Unraveling the molecular pathways and external factors that modulate development of DA cells should contribute to the efforts to obtain homogeneous and large quantities of cells. Two complementary pathways define the development of mesencephalic DA neurons; one involves Pitx3 and Lmx1, and the other Nurr1. Whereas Nurr1 is the key regulator of TH expression and DA phenotype [117], Lmx1 and Pitx3 are essential for the survival of mesencephalic DA cells [130].

Several external morphogenic factors play a key role in DA neuron specification during development [54]. Briefly, SHH is the main specifying protein at the dorsoventral axis of the embryo, whereas FGF8 has a major role on the anterior–posterior axis [152]. Indeed, these two proteins are now routinely used to enhance DA neuron specification in ES cell cultures (as discussed above). The usefulness of these factors in vivo, in supplement with the grafted ES cells, was not sufficiently analyzed as yet.

In the mammals, midbrain DA neurons are located in close proximity to serotonergic neurons. CNS developmental studies have indicated that these two aminergic neurons share a similar developmental profile, with SHH and FGF8 playing a common role on the two neuronal subtypes, whereas FGF4 is the specifying inducer of 5-HT neurons [6,54,152]. In cultured embryonic rat mesencephalic cells, elimination of 5-HT neurons, e.g., with anti-FGF4 or a specific serotonergic toxin, increased dramatically the expression of the DA phenotype [112]. Analysis of this approach with ES cells is certainly worthwhile, aiming to enrich the population of DA neurons.

4.2. Tracking DA neurons

Although DA neurons can be generated in current ES cell differentiation protocols, their in vivo survival and function is suboptimal. So far, the “holy grail” in this paradigm, which is the production of sufficient amounts of the “right” therapeutic DA cell, has not yet been accomplished. To eventually translate the ES cell-based transplantation approach into clinical application for PD, several requirements on the transplantable cell source have to be fulfilled: good survival in the brain, the right midbrain DA phenotype, integration and function in the midbrain circuitry, and no immunogenicity (Fig. 2). There are different ways and approaches to achieve this goal, which can be summarized into two lines of thinking. One line proposes that the transplantable cell source consists of proliferative immature neural precursors that are committed to develop entirely into functional DA neurons in the brain. The other line requires an optimal differentiation protocol that produces pure and sufficient amounts of transplantable midbrain DA neurons that survive and function properly after transplantation. In both paradigms, the emphasis lies in the generation of a homogeneous cell population from the desired cell type, which still remains challenging due to the inability of current protocols to force the development of the totipotent ES cell exclusively into the neuroectoderm–midbrain–DA lineage. A solution could be the selection of the desired cells by tracking their phenotype based on unique features that separates them from other cell lineages and subtypes (Fig. 3). For example, taking advantage of specific endogenous promoter activities that drive a marker gene expression, such as green fluorescent protein (GFP), only in the cell population that is targeted to allow their subsequent selection for further applications.

One attempt to track DA neuronal cells is targeting the TH gene, which is the earliest expressed at E11.5 in mouse development and thus can be useful to detect an early DA neural precursor population. Two approaches are currently under way: one is based on the introduction of a transcription cassette into the ES cells or neural precursors consisting of the TH promoter, which drives the GFP reporter gene [154]. The other approach takes advantage of the endogenous TH promoter and aims to insert the GFP gene into the TH locus by homologous recombination. In both strategies, it is expected that the DA-specific precursor cells up-regulate GFP gene expression during in vitro ES cell differentiation once the TH promoter is activated. These cells can then be sorted by fluorescence-activated cell sorting (FACS) and further propagated and differentiated and/or directly transplanted. Using the first approach, Yoshika et al. have shown that GFP expression indeed correlated with TH promoter activation, revealing 44% TH^{+} and GFP^{+} neurons after 12 days of differentiation in PA6 coculture experiments [154]. When transplanted in a 6-OHDA rodent model of PD, the sorted cells differentiated into mature neurons and improved amphetamine-induced rotational asymmetry. However, the efficiency of the cell sort was very low (2.7% life cells after FACS), and the transplanted cells were less effective when compared with sorted ventral mesencephalon cells from TH-GFP transgenic mice.

Using a similar approach to monitor gene expression in midbrain DA neurons, Zhao et al. have recently

reported a model system that allows for the selection of these neurons by Pitx3-directed GFP expression [162]. Since Pitx3 is a critical transcription factor in the development and maintenance of the midbrain DA neurons, it was chosen to insert the GFP gene in its locus. During embryogenesis, transgenic Pitx3-GFP$^+$ mice demonstrated reporter gene expression in all regions, which previously have been reported by other groups including the midbrain, the lens, head mesenchyme, muscles in the cranial facial region, and tongue [120,121,129]. Focusing on the ventral midbrain, GFP expression was found in the majority of TH$^+$ neurons in both the substantia nigra and ventral tegmental area. To further investigate the usefulness of the Pitx3–GFP model system for tracking DA neurons, GFP$^+$ cells were FACS sorted from E12-16 embryos, and ES cells were in vitro differentiated using the PA6 SDIA protocol. FACS sorting revealed the selection of 0.6–4% GFP$^+$ DA neurons from dissected midbrain regions. The in vitro differentiation of ES cells showed that 91% of TH$^+$ neurons were also GFP positive, and 35% of the neurons expressed DAT. However, from the total TH$^+$ and DAT$^+$ neuronal population, only 14% and 17% coexpressed GFP, respectively, indicating that the majority of the DA neurons developed in this protocol did not exhibit a mesencephalic identity. Nevertheless, these are encouraging reports to demonstrate the feasibility of targeting gene loci with reporter genes in order to select and enrich cell populations that can be used for further processing and/or transplantation in PD.

4.3. Constitutive versus regulated expression of transgenes for DA phenotypes

ES cells are accessible to genetic engineering, which can facilitate their differentiation into specific cell types. The introduction and expression of foreign genetic material can be achieved by transfection with expression plasmids or plasmids mediating homologous recombination, and virus-mediated gene transfer. So far, most of the expression systems in ES cells have been focused on constitutive gene expression based on using potent promoters that have been successfully applied in other cell systems. This includes cellular promoters such as the phosphoglycerine kinase (PGK), the elongation factor alpha 1 (EFα1), or the tyrosine kinase (TK) promoter, viral promoters from cytomegalovirus (CMV), or phage SV40, and synthetic promoters consisting of multiple elements from different species like the chicken beta actin promoter (CBA) fused to a CMV promoter enhancer segment. Most of these promoters are active in immature ES cells, although with different transcriptional activities. During differentiation, their activities can change due to the development of multilineage phenotypes. In ES cell-derived neurogenesis, it has been demonstrated that the EFα1, the CBA, and the CMV promoters are functional and good candidates to drive transgene expression in neural precursors as well as mature neural cells [1,25,146,158].

To increase the efficiency of DA-neuronal differentiation, ES cells have been genetically engineered to express key factors important for the specification of the midbrain-DA phenotype. In these approaches, investigators have focused on Nurr1, which has been implicated to play a key role in the development of midbrain DA neurons (see above). Two studies have been reported using stable Nurr1-overexpressing ES cell lines and demonstrated a significant increase in the fraction of functional midbrain-like DA neurons from 15–25% in the wild type ES to 60–80% in the recombinant cell lines under inductive differentiation conditions with SHH and FGF8 [25,65]. In addition, transplantation of such DA neurons could improve the rotation behavior in a 6-OHDA rodent animal model of PD [65]. The fraction of transplantable DA neurons could further be enhanced to 90% by using a combination of SDIA, signal induction, and Nurr1-overexpression, indicating that these factors can act synergistically [64]. It should be noted that similar induction of the DA phenotype could be achieved when Nurr1 was overexpressed in E14 cortical neural precursors in vitro using plasmid transfection or transduction assays with retroviruses [67]. However, in this case, the DA-induced neurons were not functional when transplanted into 6-OHDA-treated rats. Another study has recently shown that overexpression of Pitx3 in ES cells can induce genes that are associated with the A9 DA neuronal phenotype (Chung et al., unpublished). In this study, Pitx3 did not increase the numbers of DA neurons but could influence their development to a DA-specific neuronal subtype (A9), which preferentially is lost in PD (see below). These data demonstrated that transcription factors with distinct functions in in vivo midbrain development could also act in similar fashion in in vitro ES cell differentiation. More recently, Shim et al. reported that stably overexpressing the antiapoptotic protein Bcl-XL during ES cell differentiation enhanced survival of DA neurons and had a supportive effect on neurite outgrowth [124]. Taken together, these results demonstrate that gene-engineering mouse ES cells with factors important in midbrain DA specification and/or DA cell survival have a high potential to improve the cell source in transplantation paradigms in rodent animal models of PD. However, potential application of ES cell transplantation in clinical cell replacement therapies might require the adaptation of gene-engineering technologies to human ES cells, especially since current differentiation protocols are insufficient to generate sufficient amounts of functional and surviving cells for transplantation. Several attempts have been undertaken to modify gene expression in human ES cells. So far, it is possible to transfect these cells and target specific genomic regions through homologues recombination such as the hypoxanthine phosphoribosyltransferase-1 (HPRT-1) and the octamer-binding transcription factor 4 (Oct) [164]. This technology has shown that the Neomycin drug-resistant gene (*neo*) or the green fluorescent protein (GFP) could effectively be expressed from these loci.

In addition, human ES cells can be transduced with lentiviral vectors to stably introduce marker genes such as GFP or for the expression of drug-resistant proteins [86]. The results from these studies indicate that modification of the genome is feasible in human ES cells and could potentially be applied for the generation of DA cells as source for transplantation paradigms in PD.

4.4. Regulated gene expression in ES cells

Constitutive gene expression in ES cells is relatively easy to achieve due to the availability of potent promoter systems and/or the use of loci that allow gene expression from endogenous promoters such as the ROSA26 locus on mouse chromosome 6 [156]. However, these technologies are also afflicted with problems, since manipulation of the genome of ES cells can interfere in endogenous gene expression and change their ability to "normally" differentiate. In addition, some of the most widely used promoters vary in their transcriptional activity depending on the differentiation state of the ES cells like the CMV promoter, which has been shown to be weak in immature ES cells [24]. But more importantly, constitutive gene expression does not allow for adapting to the complex and dynamical events of transient gene expression patterns during ES cell differentiation. For example, related to the development of specific cellular phenotypes, a cascade of different genes is differentially expressed in a timely and quantitative manner. Thus, constitutive expression of transgenes can alter and/or be detrimental in normal cell development. To more physiologically adjust to the dynamics of gene expression in ES cell differentiation, it will be necessary to control the expression of the foreign genetic material. This can be achieved by inducible gene expression. One of the most effective and widely used system is the tetra- or doxycycline-dependent Tet-system [44,45]. This system contains two expression units for a transactivator (TA) binding to doxycycline and a Tet-response element (TRE) promoter, which is activated by the doxycycline–TA complex and drives the expression of the desired genetic material. Two systems have been developed using the TA for "turning off" [44] and a reverse transactivator (rtTA) for "turning on" gene expression [46]. The rtTA has recently been inserted into the mouse ES cell line J1, using a gene trap system [150], which specifically targets the ROSA26 locus that ubiquitously expresses inserted foreign genetic material from its own endogenous promoter [5,23,95,109,134,156]. This cell line has been successfully used to regulate expression of the X-chromosome inactivating gene *Xist* in ES cell differentiation [150]. More recently, we have generated and in vitro differentiated regulatory Nurr1-expressing J1–rtTA cell lines to investigate the temporal role of Nurr1 expression in DA specification [133]. Results from this study showed that induction of Nurr1 at late stages of ES cell development produced functional DA cell populations. However, these DA cells were nonneuronal, demonstrating that Nurr1 could function independently from the neural/neuronal context. Further investigation demonstrated that the ROSA26-modified J1–rtTA ES cells revealed reduced neurogenesis when compared to naïve J1 ES cells. Moreover, regulatory GFP-expressing J1–rtTA cell clones were not able to express the GFP gene in mature neurons. Although, transgene expression could be regulated at all stages of ES cell differentiation and Nurr1 could induce a DA phenotype, these results also demonstrated that combined genetic engineering using the ROSA26 locus and pTRE-containing plasmids altered the ability of the ES cells to follow "normal" neurogenesis and inducibly express transgenes in the neural cell lineage.

5. Choosing the right cell for transplantation

5.1. Naïve versus transgenic SC

The choice of whether to start with naïve or transgenic ES cells for differentiation and transplantation will depend on the outcome of protocols that produce sufficient amounts of functional DA cells and the safety of gene-engineering. As discussed in this review, many factors influence this paradigm. The advantages of using naïve ES cells lie in a reduced risk of cell transformation, since their genome is unmanipulated, while genetically modified ES cells could potentially aberrate and develop into unwanted cell types or tumor formations. In contrast to naïve ES cells, however, it has been demonstrated that gene-engineering ES cells can improve their directed development into the desired cellular phenotypes such as DA neurons [25,65]. In general, modulating the genome is a powerful tool to manipulate the outcome of ES cells in many applications including in vitro and in vivo cell differentiation and animal transgenesis. An important issue for the use of gene-modified ES cells in transplantation paradigms is their safety. This will greatly depend on the applied method and their outcome, such as the use of viral vectors or recombinant plasmids, random integration or gene targeting, and constitutive or regulated gene expression.

5.2. Precursor cells versus mature DA neurons

An important issue in current cell replacement paradigms for PD is the determination of the kind of cell that should be transplanted (Fig. 2), for example, an immature but fully committed neural precursor that differentiates in vivo into the proper DA neuron or an in vitro differentiated mature cell that possesses all the requirements of a functional DA neuron. So far, neither cell type has been shown to be optimal due to afflicted problems such as pluripotency, noncommitment, and proliferative potential of precursors or little survival, insufficient amounts, and poor function of mature DA

neurons [85]. The totipotency of the ES cell provides both the driving force and the limitation to develop into a single lineage. This excludes the use of immature ES cells as transplantation source, since the risk of teratoma development is too high. Committed neural precursors have a high potential to adapt to the host brain environment; however, they have also been shown to retain their ability to proliferate. Finally, transplanted mature DA neurons could function in vivo but needed to be grafted in high numbers to compensate for massive cell death.

5.3. DA neuronal subtypes

In PD, about 95% of the cases are sporadic with unknown etiology, and 5% are familial caused by monogenic mutations in molecules such as α-synuclein [108], ubiquitin carboxyl-terminal hydrolase-1 [80], parkin [72], DJ-1 [15], and PINKI [144] or triplication of the wild type α-synuclein gene [127]. In all cases of PD and independent of specific etiologies, there is a selective loss of the ventral midbrain neuronal subtypes in PD. The A9 DA neurons, which are located in the substantia nigra, are preferentially affected in the disease [90,102], whereas the A10 neurons in the medial and ventral tegmentum are relatively spared [41,60]. These observations indicate that a cell source that can produce A9 dopaminergic neurons will be preferential for grafting in PD patients [55,59].

6. Additional aspects of ES cell transplantation

6.1. Preventing cell death after transplantation

A major impediment in cell grafting is the high percentage of dying cells after in vivo transplantation, up to 90% in some studies [20,37]. Time course experiments pointed toward a rate of cell death, primarily within the first 24 h after transplantation [136,137]. The transplanted cells may die either by necrosis, initiated during cell preparation prior to the grafting, or due to apoptotic processes taking place in vivo after transplantation [37]. Oxidative stress, hypoxia, endogenous toxic compounds, proteolytic activity, undesired immune reaction, or withdrawal from trophic factors were suggested as possible reasons for cell death. Thus, efforts to increase cell survival were focused on improving the protocol of cell preparation [20,135], immune suppressing agents [28], protease inhibitors [53], growth factors [49], and others [34,89,157]. A more recent study showed that overexpression of the antiapoptotic mitochondrial protein Bcl-XL enhances differentiation and expression of the DA phenotype in vitro [124]. Furthermore, these cells were less sensitive to MPTP and showed improved DA functions after grafting in an experimental PD model. These findings are in agreement with an earlier work, indicating that transgeneic mouse cells overexpressing human Bcl-2 exhibit enhanced axonal growth of dopaminergic neurons in the transplanted rat striatum [51]. It also appears that the proapoptotic mitochondrial proteins Bax and Mak control proliferation of stem cell progenitors in the adult brain [84], and knockout of these genes provide protection against certain apoptotic stimuli [84,145]. It is therefore worthwhile to further study whether in addition to the conventional protective measures, transgenic cells overexpressing anti-apoptotic genes, or nonexpressing proapoptotic genes may be useful to overcome the undesired cell death in the currently used grafting protocols.

6.2. How to improve connectivity of transplanted cells to the host milieu

A crucial element in successful grafting is to ensure that once the cells acquired the desired phenotype, they will make the proper interneuronal connections with the host brain. At the moment, however, understanding the mechanisms that control neuronal connectivity is still in its infancy. Few studies analyzed the role of EphB1 and ephrin-B2, a receptor and protein of the large Eph family that are involved in providing guiding cues for axonal growth [50,99]. Both in vitro and in vivo experiments indicated that these molecules guide DA neurons of the substantia nigra or the VTA to send axons to the striatum or certain cortical regions, respectively [48,155]. A better understanding of the mode of action of these molecules, the cells that produce them, and the methods to regulate their expression at the temporal and spatial level will certainly contribute to harnessing these and similar guiding molecules, such as semaphorins [52], to improve neuronal connectivity as well as functional cross-talk between the transplanted cells and the host.

6.3. Maintaining the self-renewal capacity of cells as a practical transplantation source

Differentiation of ES cells is unwanted if a homogeneous stock of pluripotent proliferating cells is required for a continuous supply. Several genes that maintain proliferation of stem cells of various sources have been identified, including HOX11 [63], Pax [22], Akt [88], SOX10 [66], TLX [122], telomerase [113], BMP4 [110], and others. Developing a simple protocol to maintain self-renewal capacity of the ES cells in a regulated form that can be terminated when differentiation is desired will certainly improve the prospect of successful grafting in PD patients.

6.4. Using ES cells to deliver trophic factors (GDNF)

One of the theories addressing the etiology of PD suggest that the excessive death of DA neurons results from deficiency of certain growth factors such as glial cell-line-derived neurotrophic factor (GDNF). Effort has been made, therefore, to develop different techniques of GDNF delivery into the brain, and several reports showed decreased cell

death and improved motor behaviors in PD models [2,10,70,71,74,101,103,111]. Recent studies have shown that one can use ES cells or progenitors to prepare transgenic cell lines that express the GDNF protein [101]. Thus, regulated expression of GDNF, designed with the appropriate expression vector such as the Tet system, could provide an opportunity to verify whether changing the amount of GDNF released at a given time point or shutting down its expression when the cells reach full maturity may provide functional advantage.

7. Efforts to develop a universal protocol for SC transplantation in PD

7.1. The issue of SC differentiation

In vitro differentiation of ES cells, and especially directed differentiation using selective pressure during culture, is highly artificial and does not reflect normal cell development. In gestation and embryogenesis, ES cells and their progeny undergo a biological developmental program that is chronologically determined by intrinsic cellular events and the environment that they create. Since the in vitro conditions are limited, it is therefore crucial to monitor the outcome of cell development in order to determine whether the culture conditions are able to produce cell types that consist of the right and functional DA neuronal phenotype. Some of the events and factors involved in neuroectoderm formation, neural lineage specification, and DA neuronal specification have been described and used to generate DA neurons. In the mouse model, the generated DA cells could decrease PD symptoms after transplantation, indicating their potential to function properly. However, the results of these studies vary and are highly dependent on model systems, differentiation protocols, and the type of stem cells used. Recently, some of these techniques have been translated to primate and human ES cell differentiations [104,105]. However, due to their higher complexity and longer differentiation time, the success of these experiments has been limited. Although neurons with some DA characteristics could be generated, their survival after grafting in animal models of PD has been poor, and it needs to be determined if they will function as normal mesencephalic DA neurons [85]. So, what is the best way to produce functional DA cells for transplantation in PD? Probably a combination of different techniques and protocols, which adjust to the intrinsic programming and can provide the exterior milieu necessary for successfully directing ES cells into the DA neuronal lineage.

7.2. The issue of cell quality and quantities

As discussed in detail above, several protocols are currently in use to generate a transplantable DA cell source. Although DA neurons can be produced, there is a need to improve their quality (and quantity) for in vivo application. Thus, several requirements need to be fulfilled when addressing cell survival, midbrain DA specification, integration in brain circuitry, and proper function in vivo (Fig. 2). In addition, a sufficient amount of cells need to be transplanted, which greatly depend on the type and the quality of the grafted cell. In the best-case scenario, this cell either is a fully committed neural precursor that in vivo matures into functional DA neurons or an already differentiated fully functional DA neuron. Either cell type should be robust, well surviving, or properly integrating in the brain circuitry without causing an immunereactive response. So far, with the current knowledge and ES cell differentiation techniques, this cell has not been found and produced. So, what would be the most optimal differentiation protocol? Most likely, a combination of multiple techniques, which reflects the physiologic timing and pattern of ES cell development and which can enrich a cell population for further application. This could include the use of inductive signaling by stromal cells such as Wnt-1 overexpressing MS5 cells that induce neural crest and midbrain-like neural precursors [105], DA signaling factors such as SHH and FGF8, and/or trophic factors for cell proliferation and survival such as GDNF, BDNF, and others. In addition, gene-engineering the ES cells with factors important in midbrain DA specification like Nurr1, Pitx3, or lmx1b could improve their potential to differentiate into the “right” DA phenotype. Finally, it will be necessary to select and enrich a homogenous neuronal cell population for therapeutic application, which can be achieved by cell sorting based on surface markers and/or targeting specific promoter regions with reporter genes, as demonstrated for the TH, Pitx3, and Sox1 loci (Fig. 3).

7.3. Site of transplantation

The neuronal default theory and some grafting experiments of ES cells in the brain and kidney capsule [29] suggest that site of transplantation has limited influence on the fate of the grafted cell in vivo. Yet, studies using normal embryonic cell suspension or tissue explants and diverse methodologies have indicated that an adult host brain is able to send signals (directional cues) that determine neuronal specification or is able to guide the growth of extended neuronal processes, often toward the ‘correct’ brain region [12,35,57,59,149]. Moreover, xenograft studies with animals larger then rodents showed that these signaling clues are active for long distances and for an extended period of time [58,87]. It will be very useful to determine whether ephrins, other signaling proteins, or neurotrophic factors such as BDNF and GDNF that increase the survival of DA neurons may serve as axonal guiding signals after transplantation of ES cells.

7.4. Host immunology

One limiting factor in cell replacement paradigms in the CNS can be graft rejection by the host immune system.

Although the brain is considered to be an immune-privileged side [38,75,138,163], graft rejections have been observed [142]. While in allogeneic transplantation to the brain (e.g., mouse to mouse) >90% of the grafted cells survive, about 90% of the cell grafts are rejected in the xenogeneic transplantation models (e.g., mouse, primate, or human to rat). Graft rejection is mediated by both humoral and cellular immune responses [147], and in xenotransplantation, the humoral immune mechanism is based on the presence of xenogeneic natural antibodies (anti-aGal) and complement activation [4]. Although these mechanisms mostly contribute to graft rejection in the periphery, they also might play a role in transplantation of cells into the immune-privileged CNS, especially when the blood–brain barrier is compromised during implantation allowing the entry of immune-reactive cells and antibodies [8,16,26,39,77,139,147]. Graft rejection can be partially overcome by systemic application of the immune-suppressive drug cyclosporine A (CsA), which interferes with T cell function in abrogating neural rejection. However, in some animal models using murine neuroepithelial stem cells as transplants in the rat brain, no effect of CsA was observed [93]. In clinical transplantation of allogeneic VM cells for PD, CsA has been used, but there are no systematic data for its efficiency. Whether ES cell-derived DA cells survive in the brain of patients without the use of immunosuppressive treatment will be interesting to see once this paradigm has reached clinical application.

8. Future Aspects: where do we go?

8.1. Pros and cons of transplantation

As demonstrated above, a number of recent studies in experimental animals provided encouraging data concerning the usefulness of ES cells in correcting DA neuron deficiency, the prime feature of a PD brain. It is therefore expected that adapting the techniques developed for rodent ES to human ES cells, e.g., the efficient differentiation to DA neurons and the successful grafting into the adult brain, will establish a reasonable starting point for clinical studies in PD patients. However, as the architecture of the human striatum is far more complex than that of the rat or the mouse [47], and DA neurons in the human brain are associated with a whole range of complex behaviors, there is a sizeable gap in our understanding of the entire repertoire of components necessary to reestablish fully 'normal' nigrostriatal and other DA pathways, functional and free of unwanted side effects. Efforts must be made, therefore, to unravel in the future the molecular and developmental cues that instruct the implanted stem cells, once acquired the desired phenotype, to reconstruct accurate interneuronal synaptic and/or nonsynaptic contacts. Another important issue that should be resolved without any compromise is that the transplanted cells should not be competent to proliferate and to form any tumor or teratoma. A valuable 'safety net' would be to insert in all the grafted cells a suicidal transgene that may be 'switched on', should it become imperative to destroy the transplanted cells.

8.2. Exploiting endogenous neural stem/progenitor cells for neuroregeneration

An alternative methodology for cell transplantation, although exploratory at this point, is to harness the brain endogenous resource of stem cells to replace missing or dysfunctional neurons. It is now evident that the adult and even the aged brain maintain a certain pool of neural stem or progenitor cells. It is yet ambiguous whether these cells, apparently small in number, have any significant role in regeneration and/or neuronal plasticity in the undamaged brain, but recent reports demonstrated that, under certain experimental conditions, such stem/progenitors can migrate from their site of birth, e.g., the ependymal or subventricular zones, to other parts of the brain [18,160]. It would be useful to recruit such endogenous pluripotent cells to brain regions that are deficient of a specified neuron subtype, such as DA neurons in the PD brain. Using the brain's own stem/progenitor cells will obviously avoid a number of difficulties that encounter any transplantation protocol, particularly of foreign cells. However, attracting the cells to the correct brain region is certainly insufficient, as methods have to be developed to render the cells to differentiate in vivo to the desired neuronal subtype and to recapitulate both the interneuronal connectivity and the various functions of that brain region [40,161]. Needless to say that currently, we are only at the beginning of this research path.

8.3. Stem cell medicine to PD

The medical future of stem cells as a reliable and efficient therapeutic tool for PD as well other diseases may depend on the development of the know-how for isolation, proliferation, storage, and an easy access of a collection of highly homogeneous cell populations, equipped to take part in a defined tissue target, e.g., brain subregion, and possibly to repair a particular malfunction. A large pool of such specialized cells suitable for a whole variety of cell replacement therapies may assign to a stem cell as a new important role as a pharmacological tool.

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