Stem cell therapy for Parkinson's disease

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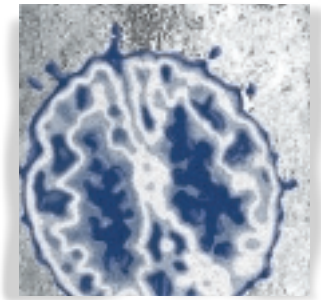

Transplantation of human fetal dopamine (DA) neurons to patients with Parkinson's disease (PD) has given proof of the principle that new neurons can survive for at least a decade, and then functionally integrate and provide significant symptomatic relief. Unfortunately, the ethical, technical, and practical limitations of using fetal DA neurons as the source for cell transplantation in PD, in combination with the development of unwanted grafting-related side effects, have put a halt to the spread of this treatment into clinical practice. Hopefully, recent advances in the fields of stem cell biology and adult neurogenesis research will lead to new exciting ways to better understand and control the biological parameters necessary for achieving safe and successful neuronal replacement in PD patients.

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Frustration over the fact that pharmacological treatments for Parkinson's disease (PD) can only provide the patient with symptomatic relief for a limited amount of time (5-15 years) has stimulated clinicians and basic scientists to seek for alternative treatment methods. Since the major contributing cause of PD has been found to be the loss or dysfunction of dopamine (DA)–producing neurons in the nigrostriatal pathway, an obvious treatment alternative would be to try to replace or protect the damaged DA neurons. This might be achieved by transplanting new DA-producing cells and/or by providing the endogenous remaining DA neurons with protective agents such as neurotrophic growth factors.

On the basis of positive results from numerous studies using animal models for PD, the first clinical transplantation studies for PD started in the mid-1980s and involved autologous transplantation of catecholamine-producing adrenal medulla cells.[1,2] Previous basic animal research involving cell implantation had convincingly shown encouraging functional effects of intrastriatal grafts of DA-producing cells[3-5] and these effects have since been confirmed in a range of animal behavioral tests.[6,7] It was shown that the observed behavioral effects are dependent on the survival of DA-producing neurons within the striatum, since the removal of transplanted tissue[8] or an immune rejection of transplanted neurons[9] reverses the transplant-induced behavioral recovery in animal studies. In addition, intrastriatal grafting in non-dopaminergic tissues produces no behavioral effects.[10,11]

The results of the first clinical trials using adrenal medulla graft proved to be quite disappointing because of the absence of any objective reductions in PD signs, which was believed to be partly due to very poor graft survival. The scientific community, however, responded quickly to this disappointment by adopting the scientifically more sound approach of transplanting PD patients with DA neurons, which were obtained from aborted fetuses.[12,13] These transplantation efforts have since con-

Selected abbreviations and acronyms

DA	*dopamine*
EG	*embryonic germ (cell)*
ES	*embryonic stem (cell)*
FGF	*fibroblast growth factor*
GDNF	*glial cell line–derived neurotrophic factor*
LIF	*leukemia inhibitory factor*
NPC	*neural progenitor cell*
PD	*Parkinson's disease*
RA	*retinoic acid*
SHH	*sonic hedgehog*
SNc	*substantia nigra compacta*

tinued as small open-label trials. The results from four centers in Sweden, France, USA, and Canada, including 26 patients, have recently been reviewed by Björklund et al,[14] and the results of these trials have been reported in numerous publications.[12,13,15-30] These open-label studies have shown that human fetal DA neurons can survive in the recipient brain for more than 10 years without being affected by ongoing disease processes. The neurons show adequate release of DA into the host[18] and, most importantly, they gradually provide substantial clinical improvement with up to 50% to 60% reductions in the Unified Parkinson's Disease Rating Scale (UPDRS). Moreover, the clinical improvements strongly correlate with recovery of movement-related activation of the host premotor and supplementary motor cortex.[14]

Most of the early transplantation efforts for PD were carried out as open-label trials. These trials gave similar results and suggested the potential benefits of cell transplantation, but concerns were raised about their validity because of the relative limited number of patients, the variable inclusion criteria, and the lack of adequate control groups. In 1992, to circumvent these issues, the National Institutes of Health (NIH) agreed to sponsor two larger controlled clinical trials. These were designed as double-blind clinical trials and even included highly controversial sham surgeries as placebo controls. The results of the first trial were published in 2001[31] and the results of the second trial have recently been reported.[32] To transplantation enthusiasts, the results were rather disappointing—even troubling. The first study showed no overall improvement on a subjective global rating scale; however, some reductions in UPDRS score were found in patients who had responded well to L-dopa treatment prior to surgery.[14,31,33] The most troubling result was that 15% of the grafted patients showed severe dyskinesias as a side effect of treatment. The second study also failed to show any significant improvements after grafting and, in this study, more than 50% of the patients developed dyskinesias.[32] In spite of the disappointing and troubling results of these recent NIH trials, most of the scientists involved seem to agree that more basic research and clinical trials are needed to be fully able to evaluate the benefits from this highly novel and still experimental treatment. A more detailed discussion of these issues can be found in Björklund et al.[14]

One issue that becomes very clear from the discussion about cell transplantation for PD is that the current method of using fetal DA neurons has major technical and practical limitations, including the limited and ethically controversial availability of human fetal DA neurons, and the potential immunological and virological complications of using nonhuman species as fetal cell sources. Therefore, most of the scientific community agree that this approach now requires a better source of transplantable DA neurons if cell therapy is ever to become a realistic and accessible treatment modality for PD. This review will focus on the various types of stem or progenitor cells currently under investigation as potential sources for cell replacement in PD. For additional reading on this subject, I would like to refer the reader to the two excellent review articles by Arenas[34] and Isacson.[35]

ES cells

Embryonic stem (ES) cells, which were first isolated from mouse blastocysts in 1981,[36,37] have been shown to proliferate indefinitely in vitro in an undifferentiated state, and to differentiate into various lineages in response to different cell culture conditions. Current extensive knowledge of cell biology, genetic manipulation, and in vitro culture methods make mouse ES cells an optimal system for potential development of unlimited transplantable cell source with reproducible genetic modification and cell biological methods.[38] It has been known for several years that mouse blastocyst–derived cell lines could differentiate into teratomas containing cells of neuroectodermal lineage after transplantation of undifferentiated cells into syngeneic mice.[39] Using retinoic acid (RA) treatment, Bain et al described the first in vitro protocol for efficient generation of neurons from ES cells.[40] However, the Bain protocol was not suitable to generate DA neurons, most probably due to the fact that RA primes the neural cells towards more "dorsal" phenotypes. Recently, Barberi et al described several protocols

for the generation of several kinds of neurons from mouse ES cells.[41] Interestingly, some reports suggest that neural differentiation from ES cells may even be a "default" option occurring unless other cell fates are actively induced.[42,43] This review will focus on the successful derivation of DA neurons from ES cells.

In vivo differentiation of DA neurons from ES cells

The first demonstration of ES cell–derived DA cells after transplantation came from Deacon et al,[44] when they showed that ES cells could spontaneously differentiate into DA neurons when grafted to either the brain or the kidney capsule. In this study, high numbers of cells (>50 000) were used and the grafts often became very large teratoma-like grafts that outgrew the target area, thus making any functional effects impossible to study. On the basis of the encouraging findings of DA cells in these large grafts, the protocol used by Deacon et al was primarily modified by decreasing the number of cells grafted. This led to smaller primarily neural grafts with numerous DA neurons, which showed beneficial functional integration in a rat model of PD.[45] Importantly, this study also highlighted the dangers of using dividing, undifferentiated ES cells for grafting, since about a quarter of the grafts still developed into teratomas, even when as few as 1000 ES cells were grafted.

In vitro differentiation of DA neurons from ES cells

Mouse ES cells

The in vitro derivation of DA neurons from mouse ES cells was first described by McKay and colleagues at the NIH.[46] They used a five-step protocol in which approximately 30% (percentage of DA neurons/total neurons) DA differentiation was obtained using treatment with fibroblast growth factor 2 (FGF2), sonic hedgehog (SHH), FGF8, and ascorbic acid. This method for derivation of DA neurons was then further refined to about 80% DA differentiation through transgenic expression of Nurr-1 in combination with FGF2, SHH, FGF8, and ascorbic acid treatment.[47] Using a similar Nurr-1 transgenic approach, the McKay group later showed functional effects of such in vitro ES cell–derived DA neurons in a rat model of PD.[48] By differentiating the ES cells from DA neurons pretransplantation, these authors claimed that they could avoid the teratoma issue seen using undifferentiated ES cells.[45] Unfortunately, teratomas can still develop even when cells are predifferentiated in vitro,[49] probably due to contamination of remaining undifferentiated ES cells within the cultures. Other protocols for the in vitro derivation of DA neurons from ES cells have been established. Kawasaki et al showed that yet unknown soluble factors (named stromal cell–derived inducing activity [SDIA]) from the PA6 stromal cell line could facilitate DA differentiation in approximately 30% to 35% of the neurons derived from ES cells; unfortunately, these DA neurons survived very poorly after grafting into the brain.[50] Barbieri et al used MS5 stromal feeder cells in combination with SHH, FGF8, ascorbic acid, and brain-derived neurotrophic factor (BDNF) treatment to obtain approximately 50% DA differentiation from normal mouse ES cells[41]; similar results have also been obtained from nuclear transfer–derived ES cells.[41,51] Furthermore, Ying et al described "significant" DA differentiation using monolayer ES cell cultures in combination with SHH, FGF8, and FGF2 treatment.[52] Thus, many recent reports have now made it clear that efficient generation (30%-80%) of DA neurons can be achieved from mouse ES cells and that such cells can survive, integrate, and show functional effects in rodent models of PD.[45,48]

Primate (nonhuman and human) ES cells

On the basis of the encouraging results from mouse ES cells and Thomson's successful generation of nonhuman primate[53,54] and human ES cell lines,[55] several labs started to investigate the possibilities of making DA neurons from primate ES cells. Kawasaki et al created DA neurons from nonhuman primate ES cells using PA6 cells and SDIA,[56] and Vrana et al showed DA differentiation from nonhuman primate parthenogenetic stem cells (Cyno-1 cells).[57] The in vitro derivation of a smaller number of DA neurons from human ES cells was described by three different groups in 2001.[58-60] We are now eagerly awaiting the first convincing demonstration of human ES cell–derived functional DA neurons in rodent or primate models of PD.

EG cells

Embryonic germ (EG) cell lines are pluripotent, self-renewing stem cells with many similarities to ES cells. The establishment of mouse EG cell lines was first described by Matsui et al[61] when they showed that the

addition of basic FGF (bFGF) to primordial germ cell (PGC) cultures in the presence of membrane-associated steel factor (SF) and leukemia inhibitory factor (LIF) enhances the growth of PGC beyond that occurring normally. In 1998, Shamblott et al created human EG cell lines after culturing gonadal ridges and mesenteries containing primordial germ cells derived from 5- to 9-week postfertilization embryos.[62] Although it has been shown that EG cells can differentiate into neurons in vivo,[63] no studies on DA differentiation have been presented so far.

Unspecified NPCs

Neural progenitor cells (NPCs) are multipotent, self-renewing cells that can differentiate into neurons, astrocytes, and oligodendrocytes. NPCs can be derived from several regions of the fetal or the adult brain[64,65] and are usually propagated as free floating clumps of cells, so-called "neurospheres" in which cells are kept dividing through stimulation via epidermal growth factor (EGF) and/or FGF2. A smaller proportion of the NPCs have been shown to differentiate into DA neurons (defined by their expression of tyrosine hydroxylase [TH], which is the rate-limiting enzyme in the DA synthetic pathway) when replated on extracellular matrix protein–coated dishes and stimulated to differentiation via conditioned media[66] or through stimulation with growth factors, such as interleukins (ILs) and glial cell line–derived neurotrophic factor (GDNF).[67] Unfortunately, although human NPCs can survive transplantation, they show no significant behavioral effects in a rat model of PD.[68]

Genetically modified NPCs and neural cell lines

Using an immortalized cerebellar neuronal cell line (C17.2), Yang et al showed that such cells could spontaneously achieve some DA features after being grafted into the DA-depleted rat striatum[69]; however, others have shown that most C17.2 cells remain undifferentiated after transplantation and many downregulate TH expression, suggesting that positive functional effects are primarily due to other mechanisms.[70] Previously, using the same C17.2 cell line in combination with transgenic overexpression of Nurr-1, a transcription factor known to be of importance for the normal development of nigral DA neurons,[71] Wagner et al had shown that such C17.2 cells could start to express TH when stimulated by conditioned media from midbrain type 1 astrocytes.[72] Another cell line that has been used in animal models for PD is the human embryonic carcinoma–derived NTN2/hNT cell line.[73] These cells differentiate into neurons upon treatment with RA and can display DA properties in vitro,[74,75] as well as in vivo, after grafting[76,77]; however, survival after grafting is usually poor and grafted animals display no significant behavioral recovery.[77]

Growth factor–producing nondopaminergic stem cells

One additional option for stem cell treatment of PD is to use stem cells as biological "pumps" for growth factors or other protective agents. Stem cells can quite easily be genetically modified to produce high amounts of such agents and could then be grafted to either the putamen or the substantia nigra compacta (SNc), where they could help by protecting the remaining endogenous DA neurons. It has been shown that stem cells producing glial cell line–derived neurotrophic factor (GDNF) can increase the survival of cocultured DA neurons[78] or cotransplanted DA neurons.[79] In addition, C17.2 cells producing GDNF[80] or the GDNF family member persephin[81] can protect the remaining DA neurons in a mouse model of PD. Since chronic injections of GDNF have shown positive effects on parkinsonian symptoms in a small clinical trial,[82] the delivery of GDNF using stem cells could become an interesting treatment alternative for PD.

Fetal midbrain dopaminergic progenitors

A possible way to compensate for the limitations in obtaining fetal DA neurons for grafting is to try to expand the numbers of fetal DA neurons via in vitro expansion of mesencephalic precursor cells. Studer et al showed that treatment of primary cultures of fetal DA neurons with FGF2 resulted in a 30-fold increase in the number of DA neurons in the cultures, and such neurons could reduce rotational asymmetry after grafting in a rat model of PD.[83] In another study, Studer et al showed that the expansion of mesencephalic precursor cells could be further increased by culturing the neurons in low (3%) oxygen concentration[84] or by adding ascorbic acid to the cultures.[85] Using a similar approach, the same group later described the expansion and differentiation of human mesencephalic precursor cells into DA neurons that survived

grafting to the rat brain.[86] One problem with this method is that the expanded mesencephalic precursor cells show such poor survival after grafting that most of the benefits of the expansion step are lost.[87] Another disadvantage is that the mesencephalic precursor cells seems to lose their ability to become DA neurons after prolonged expansion for more than 2 to 3 weeks. A different research group led by Carvey have used cytokines, such as IL-1, IL-11, LIF, and GDNF, to increase DA differentiation from rat[88-90] or human[91] mesencephalic precursor cells. Other protocols for expansion and DA differentiation of human fetal mesencephalic progenitors have also been described,[67,92] but no significant functional effects have been yet shown for such human DA neurons.

Adult neural stem cells

For many people, the use of any kind of embryonic cells is highly controversial and therefore the use of stem cells derived from adult individuals has become an attractive option. The traditional view of the nervous system used to be that no new neurons were born in adults. This concept was first challenged by Altman,[93] and later it was shown that several regions of the adult nervous system could give rise to new neurons, astrocytes, and oligodendrocytes in vitro.[65,94-96] In vivo, however, neurogenesis has so far been considered to be restricted to the subventricular zone and its projection through the rostral migratory stream to the olfactory bulb and to dentate gyrus of the hippocampus.[97] One interesting possibility for the treatment of PD would be the occurrence of endogenous neural stem cells residing within the vicinity of the adult SNc or the striatal target area, which could be stimulated to repair some of the damaged nigrostriatal circuit found in PD. With this aim, Fallon et al described how infusion of transforming growth factor α (TGFα) into the striatal parenchyma resulted in an "in vivo induction of massive proliferation, directed migration, and differentiation of neural cells" from the subventricular zone, with positive functional effects in a rat model of PD.[98] This potentially very interesting observation now awaits confirmation by other independent research groups. Moreover, it was recently suggested that there is a turnover of DA neurons in the SNc of the adult mouse and that this turnover increases when the DA neurons are toxically injured by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment.[99] Other workers have failed to find such a turnover normally ongoing in the adult SNc, but it has been shown that adult neural stem cells isolated from the SNc region have the potential to differentiate into neurons when grafted into neurogenic regions such as the hippocampus.[100] Additional research will have to show whether or not similar results can be found in humans.

Conclusion

The optimal source for transplantation is one of cells that can be efficiently and reproducibly produced at a reasonable cost in combination with showing predictable therapeutic efficacy after grafting. The cells should require minimal genetic manipulation or modification by signaling molecules in culture media to properly differentiate into the required cell types. Furthermore, they should be nonproliferative after grafting, free of infectious elements, and immunologically compatible with the host.

As reviewed in this article, research to develop transplant procedures is trying to fulfill these criteria. In contrast to the limitations of fetal cell sources, and to the cellular-molecular complexities of diverse NPCs, advances in the biology of blastula-derived ES cells suggest that this source may have important advantages over the others. However, to keep the expectations of stem cells from becoming unrealistic, it should probably be emphasized to clinicians and to patients and their families not to expect the clinical outcome using stem cell–derived DA neurons to be fundamentally "better" than what has already been achieved in the best cases using fetal DA neurons. After all, stem cells are basically just a way of obtaining a more practical and reliable source of the same type of neuron that has already been tried in clinics.

However, one potentially important benefit of using stem cell–derived DA neurons, which is separate from all logistical advantages, is that this is a much purer source of DA neurons than the currently used fetal midbrain preparations, where only about 10% of the neurons are actually DA neurons, the other 90% being primarily GABAergic (GABA, γ-aminobutyric acid). Although fetal midbrain cell preparations have been used extensively, very little is known to what extent such GABAergic cells might actually be counteracting some of the positive effects generated by the DA neurons. In addition, the 10% DA neurons will consist of both SNc (A9) and ventral tegmental midbrain (VTA, A10) DA neurons. There is selective degeneration of A9 neurons and a relative sparing of A10 neurons in PD.[101-104] These two subpopulations

of DA neurons within the SNc serve different functions and project to different brain areas (even within the SNc through dendritic release). The midline-positioned A10 DA neurons[105] project primarily to limbic and cortical regions,[106] while the neighboring A9 DA neurons (which dysfunction in PD) innervate putamen motor areas.[107] Thus, the differences between DA A10 and A9[108,109] are significant, and it might be possible to increase the functional effects of DA neuronal transplants by increasing the proportion of A9 neurons compared with A10 neurons.[110-112]

Another limiting aspect of cell therapy for PD is the fact that, in most studies, cells have been placed in the ectopic target area and not in the SNc where the actual degeneration takes place. Such an ectopic placement is necessary due to the very limited success of getting DA neurons grafted on the SNc to exhibit long-distance growth and show reestablishment of the nigrostriatal pathway. The use of stem cells for generating DA neurons for transplantation could allow for genetic or epigenetic manipulations that facilitate target finding and long-distance growth. Another option that is currently under investigation is grafting to multiple target areas within the basal ganglia circuit.[113] Thus, besides finding the optimal cell source, there are several other areas such as patient selection, study design, transplantation techniques, target selection, and combination therapies, where considerable improvements can be made[14,35,111] before making the final judgment of whether cell transplantation is a useful treatment for PD. ❑

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Terapia de células madre para la enfermedad de Parkinson

El transplante de neuronas dopaminérgicas (DA) fetales humanas a pacientes con enfermedad de Parkinson (EP) ha dado validez al principio según el cual las nuevas neuronas pueden sobrevivir por al menos una década, y después integrarse funcionalmente y aportar un alivio sintomático significativo. Desafortunadamente, las limitaciones éticas, técnicas y prácticas de utilizar neuronas DA fetales como la fuente del transplante celular en la EP, asociadas al desarrollo de efectos secundarios indeseables relacionados con el transplante, ha puesto freno a la extensión de este tratamiento a la práctica clínica. Se espera que los recientes avances en los campos de la biología de células madre y en la investigación de la neurogénesis en el adulto conducirán a nuevas y desafiantes vías para una mejor comprensión y control de los parámetros biológicos necesarios para realizar un reemplazo neuronal seguro y exitoso en pacientes con EP.

Thérapie à base de cellules souches dans la maladie de Parkinson

La transplantation de neurones dopaminergiques (DA) humains de fœtus à des patients atteints par la maladie de Parkinson (MP), a apporté la démonstration du principe selon lequel de nouveaux neurones peuvent survivre pendant au moins une décennie, et ensuite s'intégrer fonctionnellement et apporter un soulagement symptomatique significatif. Malheureusement, les restrictions éthiques, techniques et pratiques à l'utilisation des neurones DA de fœtus pour la transplantation cellulaire dans la MP, associées au développement d'effets secondaires indésirables liés aux greffes, ont mis un frein à l'extension de ce traitement en pratique clinique. Heureusement, des avancées récentes dans les domaines de la biologie des cellules souches et de la recherche sur la neurogenèse de l'adulte conduiront à explorer de nouvelles voies passionnantes pour mieux comprendre et contrôler les paramètres biologiques nécessaires pour réaliser un remplacement réussi et sans risque des neurones chez les patients atteints de la MP.

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