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Stem cells for cell therapy in Parkinson's disease

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Abstract

Clinical studies with intrastriatal transplants of human embryonic mesencephalic tissue have provided proof-of-principle for the cell replacement strategy in Parkinson's disease (PD) patients. The grafted dopaminergic neurons can reinnervate the denervated striatum, restore regulated dopamine (DA) release and movement-related frontal cortical activation, and give rise to significant symptomatic relief. However, there are several problems linked to the use of primary embryonic tissue: (i) lack of sufficient amounts of tissue for transplantation in a large number of patients; (ii) variability of functional outcome, with some patients showing major improvement and others modest if any clinical benefit; (iii) occurrence of troublesome dyskinesias in a significant proportion of patients after transplantation. Stem cells could be useful to generate large numbers of DA neurons in standardized and quality-controlled preparations. So far, neurons with at least some dopaminergic characteristics have been generated from stem cells. However, in most cases their survival after grafting in animal PD models has been poor and it is also unclear if they function as normal mesencephalic DA neurons. For the development of a clinically useful cell therapy in PD, it is also necessary to define better criteria for patient selection and how graft placement should be optimized in each patient. Several scientific issues need to be addressed before stem cell-based therapies can be tested in PD patients.

Keywords: Parkinson's disease; Transplantation; Stem cells; Striatum; Dopamine

1. Introduction

The cell replacement strategy in Parkinson's disease (PD) has been based on the idea that restoration of dopamine (DA) neurotransmission in the striatum by neural grafts, even if the disease is chronic and affects also other neuronal systems and brain regions, could lead to substantial and long-lasting functional recovery. Extensive studies in animal models have supported this strategy by showing that DA neurons in intrastriatal grafts of embryonic mesencephalic tissue display many of the morphological and functional characteristics of intrinsic DA neurons: they reinnervate the denervated striatum and form synaptic contacts with host neurons, are spontaneously active and release DA, and receive afferent inputs from the host [1]. The reinnervation by the grafts is accompanied by significant amelioration of several but not all aspects of the DA deficiency syndrome in rodents and monkeys [1,2].

Based upon these findings, clinical trials with transplantation of human embryonic mesencephalic tissue to the striatum in PD were initiated in 1987 and about 350 patients have been operated thereafter (for review of the clinical literature, see [3]). At that time, it was unknown whether neuronal replacement could at all work in the diseased human brain. Much of the scientific efforts during the past 15 years have therefore had as their main objective to provide proof-of-principle that (i) the grafted DA neurons can survive and form connections in the PD patient's brain; (ii) the patient's brain can integrate and use the grafted neurons; and (iii) the grafts can induce a measurable clinical improvement.

The aims of this article are two-fold: first, to summarize the clinical experiences with transplantation of human embryonic mesencephalic tissue, providing strong evidence that neuronal replacement can work in patients with PD, but also that the use of such tissue is associated with problems. Second, to describe current research strategies for generating DA neurons from stem cells, and to discuss the possible role of this technology for the further development of a cell replacement therapy in PD.

2. Can neural grafts survive in the Parkinson patient's brain?

Survival of grafted primary mesencephalic DA neurons, obtained from 6- to 9-week-old aborted human embryos,

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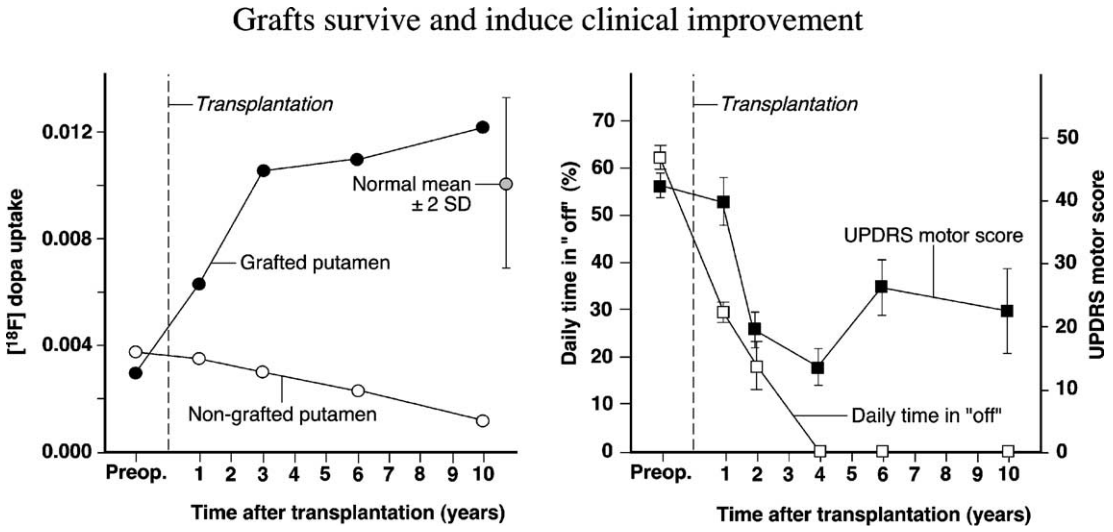

Fig. 1. Neural grafts can survive and induce clinical improvement for more than a decade in patients with PD. (Left) Fluorodopa uptake in the grafted (filled circles) and non-grafted (open circles) putamen preoperatively and at various time points after transplantation of human embryonic mesencephalic tissue unilaterally into the right putamen. Comparative data (mean ± 2 S.D.) are given for a group of 16 healthy volunteers (shaded circle with error bars). Dashed vertical line indicates time of transplantation. (Right) Percentage of the day spent in the "off"-phase (open squares) and UPDRS motor score (filled squares) in the practically defined "off" phase in the same patient. Data are mean ± 95% confidence interval. Dashed vertical line indicates time of transplantation. Data from Piccini et al. [7].

has been demonstrated in the striatum of PD patients as increased [^{18}F] fluorodopa uptake, using positron emission tomography (PET) [3] (Fig. 1), and in histopathological studies [4–6]. The grafted neurons extend their neurites up to 7 mm within the putamen, and afferent and efferent synaptic connections are established between graft and host neurons. Long-term survival of the dopaminergic grafts is possible at least up to 10 years post-transplantation despite an ongoing disease process, leading to degeneration of the patient's own DA neurons [3,7] (Fig. 1). Immunological rejection of the grafts has not been reported in any PD patient, even several years after withdrawal of immunosuppression.

3. Can neural grafts induce functional recovery?

Several research groups have demonstrated therapeutic improvement associated with graft survival [3]. In the most successful cases, patients have been able to withdraw L-dopa treatment during several years after transplantation [3] (Fig. 1). In three open label trials, patients were grafted bilaterally with tissue from about three to five donors into each putamen. According to the Unified Parkinson's Disease Rating Scale (UPDRS) [8] motor score during practically defined "off" (i.e. in the morning, at least 12 h after the last dose of antiparkinsonian medication), the overall symptomatic relief at 10–24 months postoperatively was between 30 and 40%. Even if the patients showed increased fluorodopa uptake in the putamen, indicating graft survival, the uptake after transplantation was still only about 50% of the normal mean. This probably explains the incomplete functional recovery and indicates that there is room for considerable improvement.

In the first double-blind, sham surgery-controlled study [9], there was only a modest clinical response with 18% reduction of UPDRS motor score in "off" at 12 months after bilateral putaminal grafts, but no improvement in the sham-operated group. In patients younger than 60 years, the improvement of UPDRS was 34%. These data provide the first direct evidence of a specific graft-induced improvement. However, in two patients who died after grafting, the number of dopaminergic neurons in each putamen was only between 7000 and 40,000 [9], whereas in two patients in one of the open label trials, the dopaminergic cell counts ranged from 80,000 to 135,000 [4–6]. The low cell number is probably explained by the fact that less tissue was implanted (from two donors into each putamen), and that it was stored in cell culture for up to 4 weeks before surgery. In agreement, the postoperative clinical improvement was smaller as compared to what was reported in the other patient series. These findings provide further support for the notion that the number of viable DA neurons after grafting is a factor of major importance for the magnitude of symptomatic relief.

4. Do neural grafts cause dyskinesias in patients?

Severe dyskinesias during "off" phases were observed by Freed et al. [9] in 15% of their patients after neural transplantation. Hagell et al. [10] also found that dyskinesias

(predominantly hyperkinetic, choreiform movements) increased during "off" phases postoperatively, but only in few patients constituted a clinical therapeutic problem. The severity of dyskinesias was not related to the magnitude of graft-derived dopaminergic reinnervation or symptomatic relief [10]. Thus, these data do not provide any evidence of dopaminergic overgrowth originating in the graft and resulting in a relative DA excess, as proposed by Freed et al. [9], or that effective DA neuron replacement and major recovery of motor function are coupled to the development of severe dyskinesias. The occurrence of severe dyskinesias is not a characteristic feature of DA neuron replacement per se, and therefore should not stop the further development of a cell therapy for PD. However, the underlying mechanisms must be understood so that "off"-phase dyskinesias following neural transplantation can be avoided.

5. What are the mechanisms of graft action?

5.1. Grafts restore regulated DA release in the patient's striatum

Dopamine release was assessed at 10 years postoperatively in a patient who improved markedly after transplantation and could stop L-dopa medication [7]. Binding of [^{11}C] raclopride was quantified using PET to measure DA D2 receptor occupancy by endogenous DA. Both basal and amphetamine-induced DA release as well as fluorodopa uptake was normal in the grafted putamen, which probably underlies the patient's major clinical recovery (Fig. 2).

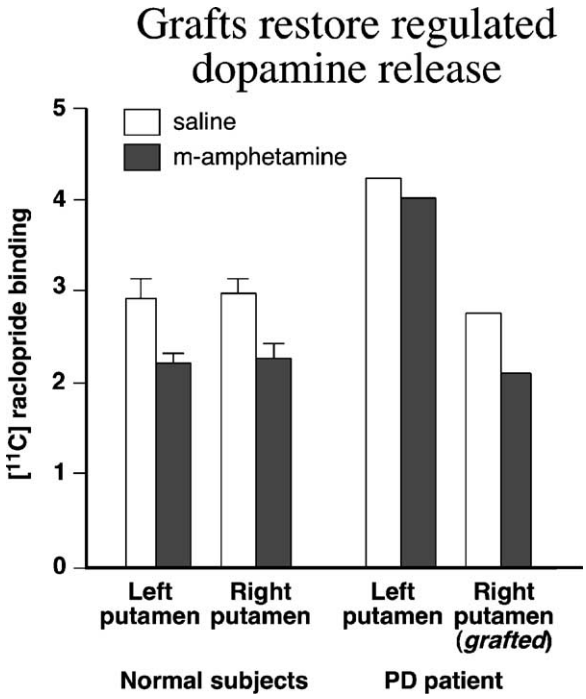

Fig. 2. Neural grafts can restore DA release in the striatum to normal levels. Basal and drug-induced DA release as assessed using [^{11}C] raclopride PET to measure DA D2 receptor occupancy by the endogenous transmitter. In the baseline condition (saline infusion; open bars), [^{11}C] raclopride binding is increased in the non-grafted putamen in the patient, while it is normal on the grafted side (right putamen). After *m*-amphetamine administration (filled bars), the binding reduction in the grafted putamen is similar to that seen in the putamen of normal subjects, whereas it is negligible in the non-grafted putamen. Same patient as in Fig. 1. Data from Piccini et al. [7].

5.2. Grafts restore movement-related frontal cortical activation

The supplementary motor area (SMA) and the dorso-lateral prefrontal cortex (DLPFC) are important in the preparation and selection of voluntary movements, their function is influenced by the basal ganglia-thalamo-cortical neural circuitries, and their impaired activation is believed to underlie parkinsonian akinesia. Four patients who were grafted bilaterally showed only a small activation of the SMA and no significant activation of the DLPFC preoperatively, as determined using regional cerebral blood flow measurements with PET [11] (Fig. 3). No improvements in activation were observed at 6.5 months after grafting, while at 18.3 months there was increased activation of both SMA and DLPFC. The time course of clinical improvement paralleled that of the increase of cortical activation (Fig. 3). In contrast, striatal fluorodopa uptake was significantly elevated already at 6.5 months with no further change thereafter (Fig. 3). Taken together, these findings indicate that successful grafts in patients with PD, by improving striatal dopaminergic neurotransmission, can restore movement-related cortical activation, which probably is necessary to induce substantial clinical improvement.

6. What is needed for successful cell therapy in Parkinson's disease?

For the further development of a cell replacement therapy for PD, it is of fundamental importance to ask which factors determine the magnitude of symptomatic relief in patients after transplantation. Based on the results from clinical trials as well as from studies in animal models, a set of requirements can be identified which probably have to be fulfilled by the grafts in order to induce marked and clinically valuable improvement: (1) the grafted cells have to express the complete machinery for DA synthesis and release, and possess the morphological and electrophysiological properties of fully mature mesencephalic DA neurons. It should be pointed out, though, that the importance of non-dopaminergic cells in the graft for the outcome after transplantation remains to be explored. These cells, which constitute about 90% of all cells in the embryonic mesencephalic grafts tested so far, could contribute to the functional recovery but may also, hypothetically, induce or contribute to adverse effects such as dyskinesias; (2) about 100,000 or more grafted DA neurons should survive long-term in each putamen; (3) the grafted DA neurons should re-establish a dense, functional,

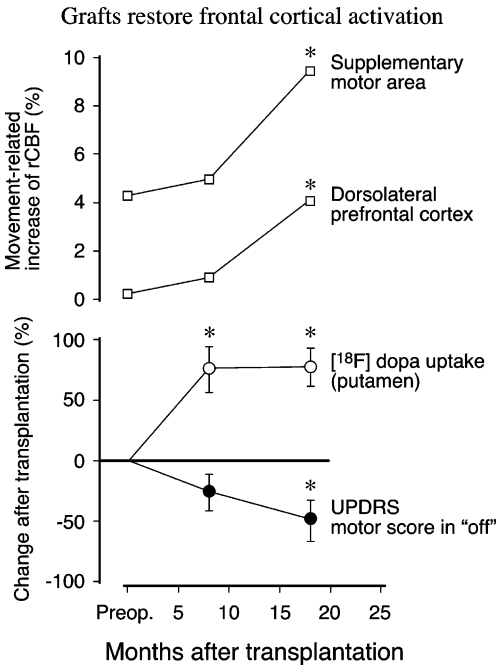

Fig. 3. Neural grafts can restore movement-related frontal cortical activation and become functionally integrated into the PD patient's brain. Movement-related increases of regional cerebral blood flow compared to resting condition in the supplementary motor area and dorsolateral prefrontal cortex (upper panel) and changes of fluorodopa uptake in the putamen and UPDRS motor score in the "off" phase (lower panel), preoperatively and at 6.5 and 18.3 months after bilateral implantation of human embryonic mesencephalic tissue into the putamen and caudate nucleus in four PD patients. Putaminal fluorodopa uptake is significantly elevated already at 6.5 months after transplantation with no further changes thereafter. In contrast, the symptomatic relief is only partial at 6.5 months and substantial clinical improvement, as measured by the UPDRS motor score, does not occur until the second postoperative year. The gradual and delayed symptomatic relief is paralleled by the recovery of movement-related cortical activation. Data are mean ± S.D. (∗) $P < 0.001$, compared to preoperatively, *t*-test. Putaminal [^{18}C] dopa uptake (open circles); UPDRS motor score in "off" (filled circles). Modified from Piccini et al. [11].

DA-releasing terminal network in large parts of the striatum; (4) the grafts have to become functionally integrated into host basal ganglia-thalamo-cortical neural circuitries. In the ideal scenario, the grafted neurons are able to reconstruct the nigrostriatal pathway and establish the appropriate afferent and efferent connections; (5) when tested preclinically in animal models of PD, the cells must be functional not only in tests of drug-induced behavior, but also in tests of spontaneous motor behavior (akinesia and limb-use tests). Deficits in these tests resemble the symptoms in PD patients.

However, a successful cell therapy for PD will also necessitate improved criteria and standards for patient selection and assessment as well as better knowledge about optimum graft placement and dosage. The distribution, degree and rate of degeneration of dopaminergic and non-dopaminergic neurons in the patient's brain will certainly influence to what extent a dopaminergic graft can restore normal function. If there is extensive dopaminergic denervation outside the grafted area, or if other systems are severely affected, the degree of symptomatic relief is likely to be only modest. In contrast, a patient with a more restricted and selective dopaminergic denervation in the striatum, which has been successfully reinnervated by the graft, probably shows substantial functional recovery. Furthermore, a rapid degeneration of the patient's own neuronal systems within and outside the transplanted areas may lead to that the postoperative improvements induced by the grafts are only transient. Detailed preoperative imaging techniques, e.g. high-resolution fluorodopa-PET, will be invaluable tools to design the optimum transplantation procedure for each individual patient.

7. Can dopamine neurons be generated from stem cells?

There are two principally different ways of using stem cells for grafting in PD: first, that the cells are predifferentiated in vitro to dopaminergic neurons prior to transplantation. Thus, stem cells could become an almost unlimited source for the generation of DA neurons. The cell preparations could be standardized and quality-controlled with respect to viability and purity. The second alternative is that the stem cells or progenitor cells differentiate in vivo to dopaminergic neurons after implantation into the striatum or substantia nigra. These neurons may integrate better as compared to primary embryonic DA neurons and, in the ideal scenario, reconstruct the nigrostriatal pathway. However, whether this will be possible is at present unknown. It will require that the mechanisms to instruct the immature stem or progenitor cells to differentiate into the missing DA neurons operate also in the PD patient's brain.

Hypothetically, DA neurons could be made from stem cells of four different sources: embryonic stem cells from the fertilized egg, neural stem cells from the embryonic or adult brain, or from stem cells in other tissues (Fig. 4). But the crucial question is of course if the generated neurons will become functional DA neurons, fulfilling the criteria described above. Another still unresolved issue is whether non-dopaminergic neurons and glial cells, normally present in the mesencephalic grafts used so far in PD patients, are important for the differentiation and function of the DA neurons. If this is the case, an enriched population of predifferentiated DA neurons may not be the optimal preparation.

The possibility to generate DA neurons has been explored using several approaches and stem cells from different sources (Fig. 4).

7.1. Embryonic stem cells (Fig. 4a)

McKay and co-workers generated DA neurons in high yield from mouse embryonic stem cells in vitro [12]. The undifferentiated stem cells were expanded and CNS stem cells selected and expanded in the presence of fibroblast growth factor 2 (FGF-2). The cells were then differentiated

to tyrosine hydroxylase (TH)-positive neurons by removal of the mitogen. Transplantation of these cells was not performed. An important step forward in the generation of DA neurons from embryonic stem cells was recently reported by the same group [13]. They overexpressed the transcription factor, Nurr-1, in mouse embryonic stem cells which were then differentiated to DA neurons in culture. The overexpression with Nurr-1 dramatically increased the yield of cells with a dopaminergic phenotype, i.e. expressing dopaminergic markers, exhibiting DA release in vitro, and electrophysiological properties of DA neurons. When the cells were grafted to the rat striatum, they survived, extended processes and improved deficits resembling PD symptoms. However, we do not yet know if the cells give rise to a functional reinnervation of the striatum, and their efficacy compared to primary embryonic DA neurons. Finally, what is the long-term safety? Is this type of genetic modification of the embryonic stem cells acceptable in a clinical setting?

Using a different approach, Sasai and co-workers co-cultured mouse embryonic stem cells with various cell lines and discovered that a bone-marrow-derived stromal cell line was a potent inducer of neuronal differentiation [15] (also see [14]). After co-culture, almost all cultures contained differentiated neurons and there was a significant yield of TH-positive neurons. These cells produced DA and showed substantial short-term survival (at 2 weeks) after transplantation to the mouse striatum. Also when using primate embryonic stem cells, the stromal cell-derived inducing activity was able to generate neurons expressing several markers of mesencephalic DA neurons [16].

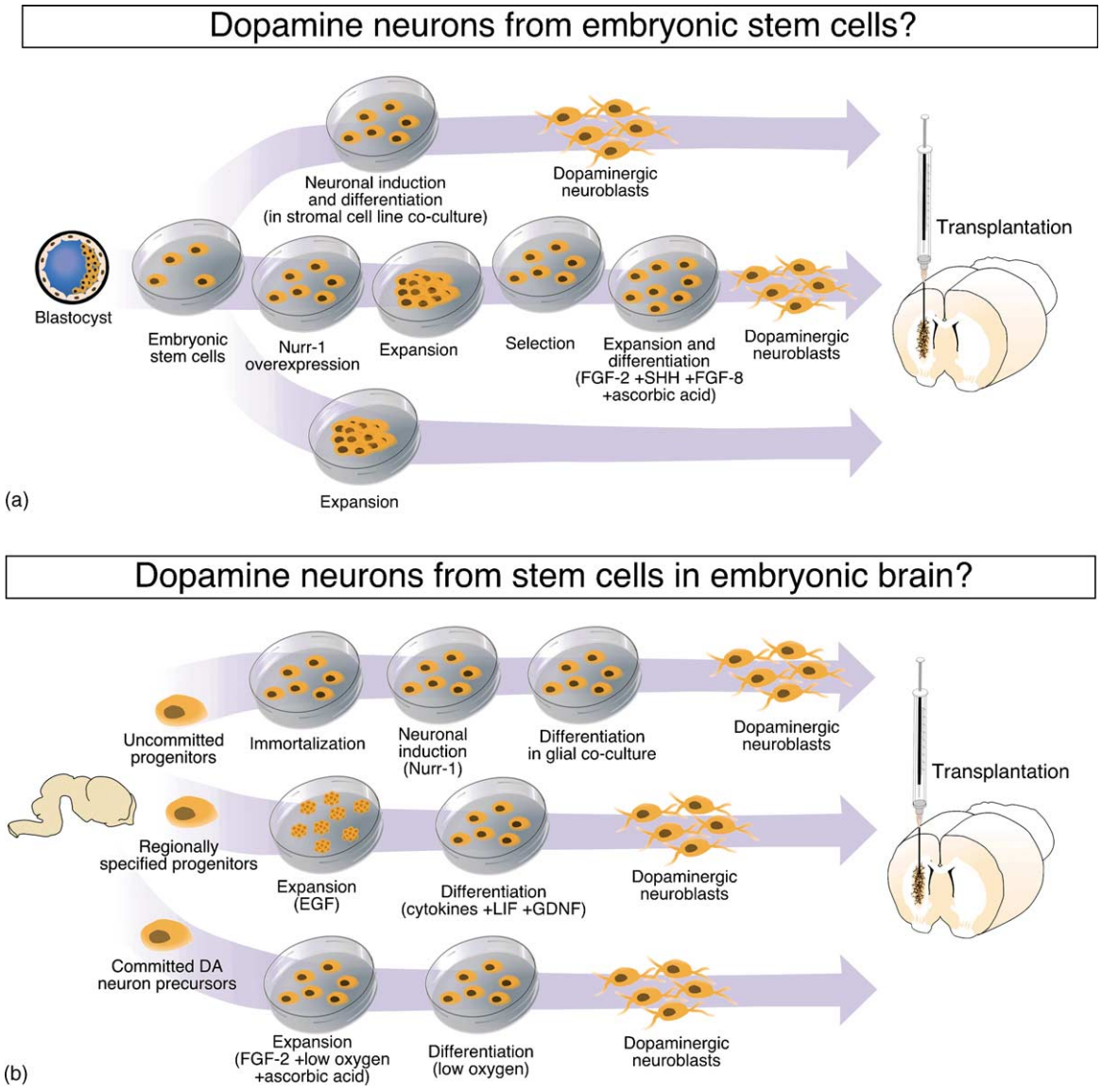

Fig. 4. Schematic illustration of different strategies to generate DA neurons for cell replacement in PD, starting from embryonic stem cells (a), from uncommitted or regionally specified progenitors, or committed DA neuron precursors in the embryonic brain (b), from stem cells in the adult brain (including neurogenesis in vivo) (c), and from stem cells in other tissues, such as bone marrow (d). For further comments, see text.

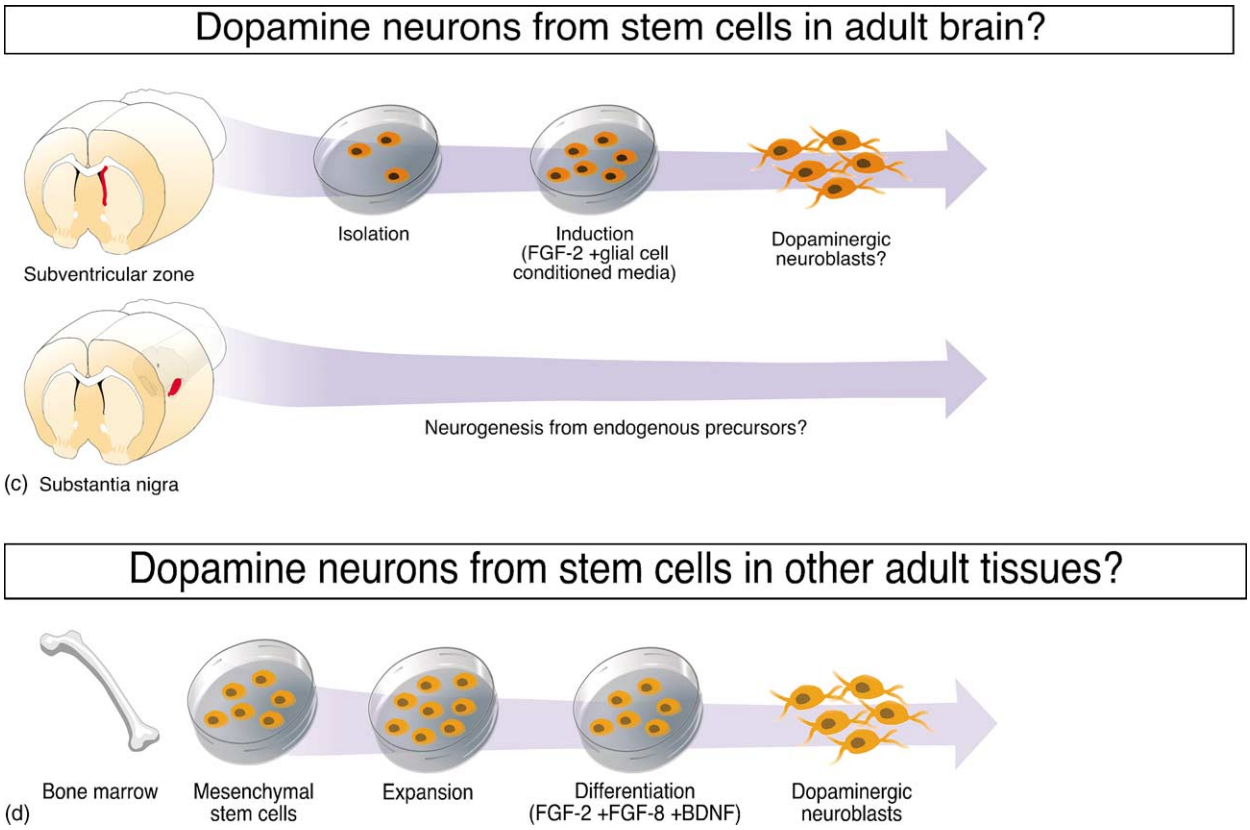

Fig. 4. (*Continued*).

These cells also released DA and had survived and extended neurites into the mouse striatum at 2 weeks after transplantation.

Is it then possible that grafted, undifferentiated embryonic stem cells can give rise to dopaminergic neurons in vivo? Some support for this strategy was given by the recent report [17] that undifferentiated mouse embryonic stem cells, implanted in low numbers into the DA denervated rat striatum, proliferated and a proportion of them differentiated into cells expressing several markers of mesencephalic dopaminergic neurons. The grafts ameliorated drug-induced rotational asymmetry but their capacity to reinnervate the striatum and release DA, as well as to improve behavioral deficits resembling the symptoms in PD, are unclear. Furthermore, about 20% of the grafted rats developed tumors which is unacceptable in a clinical perspective.

7.2. Neural stem cells in embryonic brain (Fig. 4b)

In a first approach, Studer et al. [18] expanded committed mesencephalic DA neuron precursors from rat embryos in culture. Upon removal of the mitogen, FGF-2, part of the cells differentiated into TH-positive, presumed dopaminergic neurons. The expanded cells survived transplantation to the rat striatum but the survival of the grafted TH-positive cells was poor. In recent studies, McKay and co-workers have reported that the presence of ascorbic acid promotes dopaminergic differentiation when the mesencephalic precursors are proliferated or passaged for extended periods in vitro [19]. Also, that when the predifferentiation of the precursors was carried out in cultures with low oxygen, both proliferation and dopaminergic differentiation were enhanced [20]. It is not yet known, though, whether ascorbic acid and low oxygen will increase the yield of surviving dopaminergic neurons after transplantation in vivo.

In the second approach, Carvey and co-workers [21–23] expanded mesencephalic progenitors from rat embryos under epidermal growth factor (EGF) stimulation in neurosphere cultures. The cells could subsequently be differentiated into a dopaminergic phenotype in response to signals provided by a combination of soluble (cytokines, glial cell line-derived neurotrophic factor (GDNF), and striatal conditioned medium) and insoluble (mesencephalic membrane fragments) molecules. The generated cells survived transplantation to the striatum and ameliorated rotational asymmetry in hemiparkinsonian rats. However, the survival was clearly lower as compared to that in grafts of primary embryonic mesencephalic DA neurons. The same in vitro approach combined with low oxygen has also been used to generate cells expressing dopaminergic markers and releasing DA from human embryonic mesencephalic precursors [24].

In the third approach, Wagner et al. [25] induced a dopaminergic phenotype in an immortalized multipotent

mouse neural stem cell line by overexpression of Nurr-1, in combination with as yet unidentified factors derived from type 1 astrocytes of ventral mesencephalic origin. Nurr-1 is a transcription factor which is likely to play a critical role in development of mesencephalic DA neurons. Most of the Nurr-1 transduced cells expressed the TH enzyme as well as two other markers of mesencephalic DA neurons. The engineered neurons survived transplantation to the mouse striatum but the yield was very low.

7.3. Neural stem cells in adult brain (Fig. 4c)

The finding that also the adult human brain contains neural stem cells has raised the possibility that the patient's own neural stem cells could be used to generate DA neurons. In one hypothetical approach, the cells would then be taken out, predifferentiated in vitro and reimplanted. One major advantage could be the lack of any immune reaction. However, several problems with this approach might be envisaged: first, it will probably involve extra surgery in an already diseased brain. Second, it is not known if these human cells can be expanded in sufficient numbers and if they can be differentiated into specific neurons such as DA neurons. Finally, in a patient with a chronic neurodegenerative disorder, these cells may be functionally impaired due to age, disease process or long-term drug treatment.

So far, cells with the characteristics of mesencephalic DA neurons have not been generated from stem cells in the adult animal or human brain. Daadi and Weiss [26] produced a low number of TH-expressing cells from the adult mouse SVZ in vitro by exposure to FGF-2 and glial cell conditioned media. Other properties of these cells are unknown.

Recent studies in animal models of stroke have suggested that neuronal replacement after injury in the adult brain may not require transplantation but can occur also by recruitment of endogenous precursors [27,28]. Obviously, such self-repair mechanisms, if they at all operate in the adult substantia nigra, are insufficient and need to be made much more effective. It still remains to be demonstrated, though, that neurogenesis occurs in the substantia nigra. Lie et al. [29] have shown that the adult substantia nigra contains a population of progenitor cells, which only gives rise to glial cells in situ and not to neurons. However, when exposed to the appropriate environmental signals, such as when grafted into the neurogenic area in the dentate gyrus, these cells can give rise to neurons. The major challenge will be to identify those signals which can drive the substantia nigra cells down a dopaminergic neuronal lineage.

7.4. Stem cells in other adult tissues (Fig. 4d)

From a clinical perspective, it would be ideal if stem cells could be harvested, e.g. from the patient's own bone marrow and be used to generate neurons for transplantation. However, the evidence for transdifferentiation of stem cells obtained from other adult organs into dopaminergic neurons is scarce. Although stem cells from the dermis have been shown to generate cells expressing neuron-specific markers, they did not differentiate into TH-positive, presumed dopaminergic neurons [30]. Li et al. [31] implanted bone marrow stromal cells into the DA-denervated mouse striatum and observed scattered TH-immunoreactive cells. Whether these cells exhibited other characteristics of dopaminergic neurons, including DA production, was completely unclear. Finally, Jiang et al. [32] recently described that mesenchymal stem cells from the adult bone marrow, cultured sequentially with FGF-2, FGF-8, and BDNF, generated a high proportion of cells expressing markers of dopaminergic neurons (dopamine decarboxylase- and TH-immunoreactivity). The cells also became polarized with Tau and MAP-2 expression in axonal and somatodendritic compartments, respectively. A particularly useful approach would be if the bone marrow stem cells could be administered systemically and find their way to the damaged CNS region, where they would adopt the phenotype of the missing neuron. However, in the experiments of Jiang et al. [32], intravenous infusion of the mesenchymal stem cells did not give rise to any engraftment in the brain.

To conclude, these data are promising and support the notion that it will become possible to generate DA neurons from stem cells for transplantation purposes, but there are still several unresolved issues: one problem is that the survival of these predifferentiated DA neurons after transplantation in animal models, if it has been tested, has been poor in most cases. Virtually nothing is known about long-term survival. It is also unclear if these cells display the functional characteristics of fully mature mesencephalic DA neurons after grafting. Also, relatively little is known about human cells since most studies have used cells of rodent origin.

A major challenge will be to determine the ideal composition of a graft for maximum symptomatic relief in PD. More specifically, should we aim to implant a pure population of DA neurons or should the graft also contain other defined neuron types and glial cells? The protocols for the generation of DA neurons described above all give rise to a mixed population of cells, which may not induce maximum efficacy but instead lead to adverse reactions. In any case, it would be favorable to develop procedures making it possible to select the mesencephalic DA precursor cells. Such selection could be based on surface markers, which is a common and effective strategy in the hematopoietic system. So far, FACS sorting of DA precursors has had limited success due to the difficulty in finding surface antigens for this phenotype. However, Okano et al. [34] have developed a different approach and used transgenic mice and rats expressing GFP under control of either the nestin or TH promoter (see also [33]). In both cases, fluorescent cells could be sorted by FACS, transplanted to the PD model in rats and a substantial portion survived and exerted functional effects. It remains to be shown whether similar methods can be used for isolating DA neurons from human cells.

8. Conclusions

The most important scientific conclusion from the clinical trials with transplantation of human embryonic mesencephalic tissue is that cell replacement can work in the diseased brain of PD patients. However, it is important to underscore that a clinically useful cell therapy for PD does not exist today. There are several problems linked to the use of embryonic mesencephalic tissue in current procedures, such as poor availability, variability of functional outcome, and occurrence of dyskinesias. Dopamine neurons generated from stem cells seem to be the most promising alternative for grafting in PD. However, we need to learn much more about the mechanisms of DA cell differentiation, regeneration, and functional recovery. Currently, we do not even know the best stem cell source for generating new DA neurons. Thus, the development of stem cell-based therapies for PD is still in a very early phase and it is crucial that scientists and clinicians progress with great care.

Acknowledgements

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