Transplantation of Human Retinal Pigment Epithelium Cells in the Treatment for Parkinson Disease

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SUMMARY

Background and purpose: To assess the clinical effect of transplantation of human retinal pigment epithelial (hRPE) cells into the unilateral postcommissural putamen for treatment for Parkinson disease (PD). **Methods and results:** Cells from postmortem human eye tissue (10–20 weeks of gestation) were cultured *in vitro*. Cells from -generation passage were implanted in PD postcommissural putamen with stereotactic operation in 12 patients with PD. All patients tolerated surgery well, and no major adverse events occurred. Eleven patients showed improvement in the primary outcome measure at 3 months post-treatment, particularly the Unified Parkinson's Disease Rating Scale-M score in the off state. Response reached a peak at 12 months and declined during the next 24 months. At the 36-month endpoint, there were eight patients who felt better than at baseline. Positron emission tomography (PET) showed a trend with increased dopamine (DA) release during the first 6 months. **Conclusion:** Human retinal pigment epithelial cells have the characteristics of neural progenitor cells and can be induced to differentiate into DA neurons. The results of this clinical trial suggest that the treatment of transplanted hRPE cells could improve symptoms of PD. These cells might serve as a useful source of DA neurons for neural graft in the treatment for PD.

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Introduction

Parkinson disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and reduction in striatal dopamine (DA). Current treatment for PD is primarily based on a DA replacement strategy using the DA precursor levodopa. Levodopa dramatically improves morbidity and mortality in PD, but long-term treatment is complicated by involuntary movements (dyskinesia), neuropsychiatric side effects, and so on. In addition, disease progression is associated with the development

of clinical features that do not respond to levodopa. This has led to a search for alternative treatments that can halt disease progression and/or restore the normal functions from PD.

Recent studies demonstrated that transplantation of dopaminergic neurons (DA neurons) derived from human fetuses into patients with PD can replace endogenous degenerating DA neurons and ameliorate the clinical symptoms of PD [1–3]. However, tissue availability and ethical concerns regarding the use of tissue derived from aborted human fetuses have limited clinical application in patients with PD.

There have been numerous attempts to find alternative tissues to overcome these problems. Human retinal pigment epithelial (hRPE) cells represent an alternative to human fetal tissue transplants in patients with PD [4]. These cells constitute a monolayer in the inner layer of the neural retina that produces L-dopa as a precursor to the formation of their characteristic eumelanin pigment. The cells are readily isolated from eyes obtained from eye banks and are cultured [5]. Thus, they appear to be a suitable cell type to serve as a durable source of -dopa for implant into the DA-denervated striatum.

Several recent studies have reported the successful reversal of parkinsonian deficits in rats, monkeys, and patients with PD after implantation of hRPE cells [6–8]. This improvement is thought to be due to a DA mechanism, namely the delivery of L-dopa by hRPE cells. Based on these very promising preclinical data, 12 patients with idiopathic PD were enrolling into a pilot clinical trial of intrastriatal transplantation of hRPE cells. Here, we present results from nine patients who received transplantation with the hRPE cells isolated from human aborted fetal eyes at the minimal 32 months after transplantation.

Patients and Methods

Patient Selection

Twelve patients, aged 52–88 years (median age, 66 years), were eligible to participate in the study. The patients demonstrated bilateral but asymmetric PD clinical features. All the patients have had idiopathic PD for more than 5 years (mean, 6.4 years) with at least two of the four main signs: bradykinesia, rigidity, tremor at rest, and balance problems in the off state. The baseline Hoehn and Yahr score and Unified Parkinson's Disease Rating Scale (UPDRS) score are listed in Table 1. All the patients were levodopa responders, with an improvement of at least 25 percent of the total UPDRS score after a first morning dose of levodopa. Their clinical response to drug therapy was optimized for the doses were not changed for at least 3 months before the surgery.

Criteria for exclusion from the study included secondary Parkinsonism or Parkinson's plus syndromes, hallucinations or

delusions during levodopa therapy, epilepsy, previous brain surgery, severe cognitive impairment or depression, cerebrovascular disease, evidence on MRI of another neurologic disorder, and a medical contraindication to surgery. There were no surgical contraindications in this group of patients by routine preoperative examinations. All patients acknowledged the details about the clinical study and signed the agreement for acceptance of the operation.

The protocol and a consent form describing the risks and potential benefits of the study were approved by the Institutional Review Boards of the Navy General Hospital and a Performance and Safety Monitoring Board appointed by the National Institutes of Health. Cultures of hRPE cells for transplantation were tested negative for human herpesvirus, cytomegalovirus, fungi, and bacteria.

Evaluation of the Patients

Clinical evaluations were conducted according to the guidelines described in the Core Assessment Program for Intracerebral Transplantation (CAPIT-PD), which included assessments that used the UPDRS, the Schwab and England Scale, and Hoehn and Yahr (H & Y) staging [9,10]. They were performed at baseline and at 3, 6, 12, 24, and 36 months after transplantation. All evaluations were performed by a separate evaluator who played no other role in the study. The test included assessments during "off" and "on" periods. The state of the patient was defined as "off" when testing was conducted before the patient had a first morning dose of levodopa and at least 12 h after the administration of levodopa the previous day. The "on" scores referred to the best test scores recorded during the day while the patient was taking medication. Because the doses of levodopa and other antiparkinsonian drugs are individualized for each patient, change in drug therapy can confound the interpretation of surgical outcomes. We sought to refrain from changing patients' preoperative drug schedules if possible. Brain MRI was performed preoperatively and postoperatively within 1 week and 12 and 24 months after surgery. The change in the striatal DA was assessed preoperatively and postoperatively at day 5 and at 6, 12, 24, and 32 months by positron emission tomography (PET) scans using ^{11}C - β -CFT.

Table 1 Baseline patient characteristics

No.	Age	Sex	Length of follow-up (mo)	Total UPDRS	M.B.M	ADL	MOTOR	Hoehn–Yahr	Schwab–England	PDQ-39	Madopar mg
1	69	M	36	53/94	3/3	13/25	37/66	2.5/4	65/30	67	750
2	65	F	36	126/143	6/6	39/47	81/90	4.5/5	38/20	102	750
3	73	F	36	65/98	4/6	21/36	40/56	4/4.5	40/20	80	750
4	88	F	12	72/138	5/5	19/40	48/93	3.8/5	50/30	92	750
5	56	F	12	68/78	9/10	17/24	42/44	3/3.3	49/42	74	375
6	76	M	36	48/78	2/2	14/22	33/54	2/3.8	70/50	62	1000
7	52	M	36	41/66	6/6	10/20	25/40	1.5/3	70/50	76	500
8	61	M	36	65/98	3/3	10/19	26/41	2.8/4	60/45	58	750
9	81	F	12	103/129	4/4	35/45	64/80	4.1/5	50/20	108	750
10	56	F	36	28/49	4/4	9/15	15/30	1/1.5	85/70	48	750
11	50	M	36	38/57	3/3	10/21	25/33	1.5/2.5	82/68	36	375
12	68	F	36	71/96	7/8	24/32	40/56	4.5/5	50/30	106	500

UPDRS, Unified Parkinson's Disease Rating Scale.

Donor Tissue and hRPE Cells

Human retinal pigment epithelial cells were provided by the Center for Stem Cell Research, Peking University. hRPE cells in primary culture were isolated from human aborted fetal eyes. The aborted fetal samples (10–20 weeks) were obtained from maternity hospital with mother's consent. All mothers had no history of genetic disease and infectious disease. Permission to use human tissue was granted by the Ethical Committee of Peking University Health Science Center. The RPE cell layer was dissected from the retina, directly put into sterile phosphate-buffered saline (PBS), and cut into small pieces of 1 mm³. The cell suspension was then triturated with glass pipettes gently, passed through a 70- μ m strainer, and maintained in Dulbecco modified Eagle's medium/nutrient mixture F12 (DMEM/F12; Gibco BRL, Rockville, MD) supplemented with 10% fetal bovine serum (FBS; Hyclone, Hyclone Laboratories, Logan, UT, USA), EGF (20 ng/mL; Gibco BRL), bFGF (20 ng/mL; Gibco BRL) and LIF (10 ng/mL; Chemicon, Chemicon International, Inc., Temecula, CA, USA), respectively. After 7–10 days, a few small adherent clones were appeared [11]. These clones were isolated and cultured to obtain RPE-derived neural progenitor cells (RPE-derived NPCs). Medium was replaced every 3–5 days. Passage was carried out when cells grow among 95% confluent. Cells were trypsinized with 0.25% trypsin (Gibco BRL) and were passaged at 1:3 ratios. Cell phenotypes were analyzed by FACScalibur flow cytometry (Becton Dickinson, Becton-Dickinson Biosciences, San Jose, CA, USA). Cell cycle was analyzed in a FACScalibur flow cytometry using the MODFIT software (Verity Software House, Topsham, ME, USA). Cells from passage 5–10 were used for the following experiments. Cells induced with PACAP and dbCAMP-RA-bFGF cocktail were harvested at 2 days. Determination of DA in cells suspension was measured by HPLC-electrochemical detection.

Phenotype Analysis Using FACS

Cells were trypsinized into single-cell suspension and stained with phycoerythrin (PE)-labeled antibodies anti-CD34, anti-CD44, anti-CD90, anti-CD117, or anti-CD10 or fluorescein (FITC)-labeled antibodies anti-CD14, anti-CD45, anti-CD71, anti-CD147, or anti-HLA-DR (Becton Dickinson). Mouse IgG1-PE, IgG2a-PE, IgG1-FITC, IgG2a-FITC, IgG2b-FITC (Becton Dickinson), and IgM-FITC (Southern biotech Associates, Inc., Birmingham, AL, USA) were used as isotype controls. Stained cells were analyzed by FACScalibur flow cytometry (Becton Dickinson).

RT-PCR

Total RNA was isolated with TRIzol reagent and converted to cDNA using oligo (dT)12–18 primer (Gibco BRL) with MMLV reverse transcriptase. PCR was performed in a reaction mixture containing 1 μ L of cDNA in a total volume of 25 μ L with Taq DNA polymerase (MBI). PCR amplification was carried out for 40 cycles. PCR products were run on 2% agarose gels and stained with ethidium bromide. The identities of all PCR products were confirmed by direct sequencing. Primer sets and PCR annealing temperatures are shown in Table 1.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde in PBS (0.1 M, pH 7.4), and then permeated using PBS containing 0.5% Triton X-100 or without Triton X-100 for membrane antigen staining. Fixed cells were incubated with PBS containing normal sheep serum at room temperature for 1 h and then incubated with primary antibodies for 1 h at room temperature and with secondary antibodies for 40 min. The primary antibodies include mouse anti-nestin (Neomarker, Fremont, CA, USA), ki67 (Chemicon), β -tubulin III (Chemicon), TH (Sigma, Sigma-Aldrich, St. Louis, MO, USA), oligophrenin-4 (O4) (Santa Cruz, SANTA CRUZ BIOTECHNOLOGY, INC., Santa Cruz, CA, USA), rabbit anti-glial fibrillary acidic protein (GFAP; Neomarker), PAX6 (Chemicon), TH (Chemicon), D2 receptor (Chemicon), and DAT (Chemicon). The FITC-labeled goat anti-mouse IgG or TRITC-labeled goat anti-rabbit IgG secondary antibodies were from Santa Cruz. Photographs were taken using an Olympus Immunofluorescent Microscope (Model BX51TR; Olympus Co. Ltd., Tokyo, Japan) and Apogee Instruments Microscopy Fluorescence System (Model KX85; Apogee Instruments, Inc., Auburn, CA, USA).

Method of Transplantation

At the time of surgical procedure, patients were placed in a Fisher stereotactic frame after receiving local anesthesia, and the putamen was visualized on high-field-strength MRI (1.5T) using a fast spin-echo sequence. Axial images were obtained using contiguous 3-mm sections extending from below the putamen to above the caudate in a plane. Target sites for implantation were located in the posterior putamen [12–15].

Then, patients were transferred to the operating room. After local anesthesia, a burr hole was made in the calvarium, 5 mm anterior to the coronal suture and 25 mm from midline. A total of 1×10^6 hRPE cells in 210 μ L were deposited unilaterally in the posterior putamen in one trace, which was from the ventral to dorsal of the posterior putamen. The implant cells were placed in a stainless steel cannula with outer diameter of 1.2 mm and tapering to 0.9 mm. Firstly, 70 μ L cell suspension was injected near the bottom of posterior putamen, then the needle was retracted 5 mm, and another 70 μ L cell suspension was deposited. Finally, the last 70 μ L cell suspension was injected into the location 10 mm above the first injection site near the top of the putamen [7,16]. Cells were delivered at a rate of approximately 4 μ L/min with an interval of 5 min between each deposit. After the final injection, a 5-min equilibration was made to allow the injected cell suspensions to stabilize and avoid graft withdrawal along the needle tract as the needle is removed from the brain. The injection needle withdrawal was gradual and slow with frequent brief stops to prevent any vacuum effect. Patients' ability to speak and to move their arms and legs was tested after injection. At the end of the procedure, the incision was closed and a sterile dressing was applied.

After operation, the patients were sent to the neurosurgical intensive care unit for overnight observation and then transferred to a conventional ward next morning. In general, patients were discharged from hospital within 7 days, after brain MRI and PET determined with no significant abnormalities discovered. A broad-spectrum antibiotic was administered after the surgical procedure,

and no immunosuppressive drugs were administered in this group of patients.

Safety

Serious adverse events were defined as illnesses and incidents that necessitated hospitalization or operation-caused death. Those events would be reported to the monitoring board immediately. Asymptomatic hemorrhage along a needle track during surgery was considered to be an adverse event but not a serious adverse event.

Statistical Analysis

Comparisons between the resulting baseline evaluation and the postoperative evaluation were made with repeated-measures analysis of variance. All tests were performed at the 5% significance level. Region of interest (ROI) was used to assess the data of PET, as well as an automated voxel-based statistical parametric mapping (SPM) analysis. Multivariate comparisons were made using SPSS13.0 (SPSS Inc., Chicago, IL, USA) statistical package.

Results

The characteristics of 12 patients who are enrolled in the study are shown in Table 1. Data were available for 12 patients at 3, 6, and 12 months and for nine patients at 24 and 36 months after transplantation. Three patients were excluded from our statistical analysis because of femoral neck fracture (patient 4 at 13 months) and missed follow-up (patients 5 and 9 at 24 months).

Human PE-Derived NPCs *In Vitro* Culture and Differentiation

Primary hRPE cells are small round cells morphologically, and some of the cells showed visible pigment in cytoplasm (Figure 1A). After culturing for passage, RPE cells start to differentiate into NPC-like cells with spindle-like appearance (Figure 1B). Treatment with PACAP and dbCAMP-RA-bFGF cocktail in condition medium induced RPE-derived NPCs to transform into polygonal and elongated shapes. Some of the differentiated cells were neurite-like outgrowth (Figure 1C,D).

The RPE-derived NPCs proliferated fast and have a relatively higher S phase ratio ($13.04 \pm 0.92\%$) (Figure 1G). RT-PCR results showed that the RPE-derived NPCs expressed neural stem/progenitor cell marker molecules nestin, BMI-1, sox2, and nucleostemin. The catalytic subunits of human telomerase (hTERT) and Pax6 were also expressed (Figure 1H). Surface markers have been examined by flow cytometry (FACS). The RPE-derived NPCs expressed CD147, CD90, CD10, and CD44 but did not express CD34, CD45, CD14, HLA-DR, CD38, CD117, and CD104. CD71 showed a weakly positive rate of 11.33% (Figure 2A). Immunofluorescence analysis (Figure 3) confirmed that the RPE-derived NPCs expressed nestin (Figure 3A), Pax6 (Figure 3B), and Ki67 (Figure 3C) proteins.

Cells induced with PACAP and dbCAMP-RA-bFGF cocktail were harvested at 4 days. Early neuron marker β -tubulin III (Figure 3D,E) and the oligodendrocyte marker O4 (Figure 3F,G) were

detected either by RT-PCR (Figure 1E) or by immunofluorescence analysis. These experiments suggested that human fetal RPE-derived NPCs could differentiate into neural cells at appropriate induced conditions. After 7 days of culture, the fetal RPE-derived NPCs were found to be differentiated into DA neurons in DA neuron-inducing condition medium (Figure 3H,I) and to produce DA (Figure 2B).

Safety

The neurosurgical procedure was well tolerated, and all patients were discharged from the hospital within 7 days after treatment. No serious adverse events, which are associated with cell implantation or surgical procedure, were developed. There were no obvious inflammatory responses observed after the operation. No bleeding and edema were found in brain MRI examination 3 days postoperatively. One patient withdrew from the study because of femoral neck fracture and unable to travel at 13 months (seems no relation with operation). No side effects were observed at 24 and 36 months. No mass effect and abnormal growth at the transplant sites was detected by MRI scans in 12 patients 1 year after transplantation and in nine patients 2 years after transplantation, respectively.

Clinical Assessment

All patients underwent CAPIT-PD evaluations before transplantation. Prior to the transplantation, symptoms of PD were gradually aggravated and therapeutic effects of L-DOPA were failed in these patients. Baseline demographics are presented in Table 1. Studies demonstrated that the symptoms of PD for all the patients were improved (most on the contralateral to the implanted striatum) up to 3 months after transplantation except in patient 4. The improvement reached a peak at 12 months and then started to decline during the next 24 months. After follow-up for 36 months, eight patients still felt better subjectively than their status prior to transplantation. In comparison with the baseline, the UPDRS off-state total scores were statistically significantly improved at 3 months (averaged 33%, $P = 0.000$), 6 months (averaged 38%, $P = 0.0000$), 12 months (averaged 47%, $P = 0.000$), 24 months (averaged 37%, $P = 0.000$), and 36 months (averaged 23%, $P = 0.000$) after transplantation. Three, 6, 12, and 24 months postoperatively, the mean UPDRS on-state total scores were improved by 25% ($P = 0.015$), 34% ($P = 0.006$), 41% ($P = 0.005$), and 38% ($P = 0.008$), respectively, in comparison with preoperative baseline. Although the UPDRS on-state total score was higher 36 months postoperatively (averaged 26%, $P = 0.055$), the difference was statistically insignificant, in comparison with preoperative difference. The UPDRS-M score seems to play the most important contribution to the changes in UPDRS total score (Table 2).

The daily levodopa doses were reduced from 1000 to 750 mg for patient 6 and from 750 to 500 mg for patient 10 at 6 months post-transplantation. However, they got back in rhythm of pre-transplantation after 24 months post-transplantation. The levodopa doses were persistent in other patients. Moderate to obvious reduction (30–50%) in the off time were observed in nine patients. No obvious dyskinesia was seen in nine patients at

Figure 1 Phase-contrast photograph of RPE-derived neural progenitor cells (NPCs) (400 × magnification, **A–D**). Primary RPE cells are small and round in shape. Some cells have pigment granules (**A**). RPE-derived NPCs at the fifth passage (**B**). Morphology of induced RPE-derived NPCs with dbcAMP–RA–bFGF cocktail for 1 day (**C**). RPE-derived NPCs treated with PACAP for 3 days and then treated with dbcAMP–RA–bFGF cocktail for 1 day (**D**). RT-PCR results of the differentiated ability of human fetal RPE-derived NPCs (**E–F**). Cells were preinduced with PACAP (50 nM) for 3 days and then with dbcAMP–RA–bFGF (dbcAMP 1 mM, RA, 0.5 μ M, and bFGF 100 ng/mL) cocktail for 1 day (**E**). Results of dopaminergic (DA) neuron markers' expression when RPE-derived NPCs induced with DA neuron-inducing condition medium for 7 days (**F**). Characteristics of RPE-derived NPCs analyzed by flow cytometry, RT-PCR, and G-banding analysis (**G–I**). Cell cycle of RPE-derived NPCs analyzed by flow cytometry (**G**). Identification of RPE-derived NPCs by RT-PCR (**H**). Karyotypes analyzed by G-banding. Data show the normal human diploid karyotype of chromosomes (**I**).

Figure 2 Surface marker expression of RPE-derived neural progenitor cells analyzed by flow cytometry (**A**). Determination of dopamine (DA) in cell suspension with HPLC–electrochemical detection (**B**).

Figure 3 Analysis of marker expression of RPE-derived neural progenitor cells (NPCs) by immunofluorescence staining. RPE-derived NPCs express nestin (A), Pax6 (B), and Ki67 (C) before induction. The following markers' expression in RPE-derived NPCs has been compared before and after different induced conditions: the neuron marker β -tubulin III before induction (D) and after induction (E); the oligodendrocyte marker O4 before induction (F) and after induction (G); the dopaminergic (DA) neuron-specific markers; TH before induction (H) and after induction (I); D2 receptor before induction (J) and after induction (K). (L) shows β -tubulin III expression of RPE-derived NPCs that differentiate into DA neurons. (M) shows TH expression of RPE-derived NPCs that differentiate into DA neurons. (N) is the double staining.

36 months post-transplantation. There were no significant changes in laboratory examinations as well as in neuropsychological assessments. The three patients who are excluded from statistical analysis were in a different condition. Significant change was not observed in patient 5 until we missed her. Patients 4 and 9 had been improved gradually during the first 12 months.

PET Scan

All patients underwent PET scan at day 7 before the operation and day 5 postoperatively, seven patients (patients 1, 2, 4, 5, 7, 8, and 10) at 6 months, two patients (patients 6 and 8) at 12 months, and one patient (patient 9) at 24 months.

Using ^{11}C - β -CFT as the labeling marker, PET scan was performed in the patients. Prior to transplantation, ^{11}C - β -CFT PET scans show that uptake of DAT in the head of caudate nucleus and putamen, especially in the postcommissural putamen, was significantly decreased. Quantitative analysis and comparison of the scans before implantation and 5 day after implantation revealed that the uptake of DAT in the side of implantation had no significant changes.

Mainly because these data, obtained at 6, 12, and 24 months after transplantation, were inconsistent, statistical parametric mapping (SPM) and visualized PET image analysis were used to assess the data of PET instead of ROI method. SPM is used for examining differences in brain activity recorded during functional neuroimaging experiments using neuroimaging technologies such

Table 2 Unified Parkinson's disease rating scale (UPDRS) scores in patients before and after cell implantation

	Baseline	3 months	6 months	12 months	24 months	36 months
UPDRS						
On	58.8 ± 27.7	43.9 ± 23.9 (25)	38.9 ± 20.8 (34)	34.4 ± 18.8 (41)	36.6 ± 19.9 (38)	43.8 ± 23.3 (26)
Off	82.9 ± 28.7	55.3 ± 29.7 (33)	51.1 ± 28.3 (38)	43.7 ± 25.9 (47)	52.0 ± 28.6 (37)	64.0 ± 32.1 (23)
Mentation, Behavior and Mood						
On	4.22 ± 1.71	3.55 ± 1.81	3.55 ± 1.81	3.77 ± 1.99	4.11 ± 2.03	4.44 ± 2.13
Off	4.55 ± 1.50	3.89 ± 1.76	3.89 ± 1.76	4.11 ± 1.90	4.55 ± 2.19	5.00 ± 2.34
ADL						
On	17.3 ± 9.51	12.8 ± 8.89	10.4 ± 7.21	9.11 ± 6.53	10.1 ± 7.59	11.4 ± 8.66
Off	26.3 ± 10.1	17.6 ± 11.9	15.4 ± 9.53	12.7 ± 8.50	15.3 ± 9.16	18.4 ± 9.11
Motor						
On	37.4 ± 17.4	28.1 ± 15.5	24.9 ± 12.4	21.6 ± 10.9	22.8 ± 11.2	27.9 ± 13.0
Off	51.8 ± 18.7	35.6 ± 20.2	31.9 ± 17.5	27.2 ± 16.4	32.2 ± 18.1	40.8 ± 21.4

Figure 4 Change in ^{11}C - β -CFT uptake in the striatum of patients with Parkinson disease before and after transplantation, as shown in fluorodopa positron emission tomography (PET) scans. Preoperative transaxial views are represented (A); 6-month postoperative transaxial views show that ^{11}C - β -CFT uptake on the side of implantation was increased when compared to that of baseline (B); 12-month postoperative transaxial views show that ^{11}C - β -CFT uptake on the side of implantation was increased when compared to that of 1 month; statistical parametric mapping analysis locating graph (D–F); Transaxial, coronal, and sagittal ^{11}C - β -CFT PET scans views are represented 6 months after transplantation and baseline. ^{11}C - β -CFT uptake on the side of implantation was increased when compared to that of baseline (G–I); Transaxial, coronal, and sagittal ^{11}C - β -CFT PET scans views are represented 12 months after transplantation and baseline. ^{11}C - β -CFT uptake on the side of implantation was increased when compared to that of 6 month (J–L, arrows show the side of implantation).

as PET. Of seven patients studied, six demonstrated an increasing radionuclide DAT uptake in the putamen of the implant sides 6 months after transplantation, in comparison with the same sites before implant, as well as the nongraft sides both before transplantation and 6 months after transplantation by SPM analysis, respectively (Figure 4). Patient 8 underwent PET scan at

12 months post-transplantation, and no obvious changes in their implant sides were found, in comparison with their PET scans at 6 months post-transplantation. One patient (P9) underwent PET scan at 24 months after transplantation, whose visualized PET image analysis showed that the putaminal fluorodopa uptake was greater in nongraft side than in the graft side (Figure 5).

Figure 5 Change in ^{11}C - β -CFT uptake in the striatum of patients with Parkinson disease before and after 24 month transplantation, as shown in fluorodopa positron emission tomography (PET) scans. Preoperative transaxial views are represented (A); 24 months postoperative transaxial views show that ^{11}C - β -CFT uptake on the side of implantation was increased when compared to that of baseline. At 24-month endpoint, the change from baseline in putaminal fluorodopa uptake was greater in nongraft side than in graft side in patient 9 (B), arrow shows the side of implantation).

Discussion

Human retinal pigment epithelial cells are found in the inner layer of the neural retina located between the photoreceptors and the choriocapillaris. They are the epithelial cells that form tight junctions and are considered to play an important role in maintaining the blood-retinal barrier. These cells can produce L-dopa in an intermediate step during the synthesis of their characteristic eumelanin pigment through the activity of a TH analog, tyrosinase. Interestingly, although L-dopa is reoxidized quickly in adult functioning, embryonic hrPE cells contain more stable concentrations of L-dopa [17–19]. hrPE cells can be isolated from eyes obtained from eye banks and are easily cultured. Thus, the cells appear to be a suitable cell type to serve as a durable source of L-dopa for implant into DA-denervated striatum.

Our previous study demonstrated that the NPCs derived from hrPE of 10–20 weeks of gestation could proliferate in culture for several passages and differentiate into the cells with neural phenotypes. Moreover, these fetal hrPE-derived NPCs also had the potential to differentiate into DA-specific neurons. In an *in vivo* study, we also demonstrated that the hrPE cells, which are implanted into rat striatum, could survive and express molecular markers (β -tubulin III, tyrosine hydroxylase, and GFAP) in the engraftment location without inducing immune rejection and unfavorable immune reaction. After the transplantation of hrPE cells, rotational behaviors were significantly improved in the PD rat model [20]. Based on these favorable findings regarding the behavior of hrPE cells both *in vitro* and in the animal model, we designed and conducted the clinical trial to transplant hrPE cells in patients with PD in order to treat PD.

The behavioral and motor improvements in rats, monkeys, and humans implanted with hrPE-GM have been attributed to the ability of the hrPE cells to synthesize L-dopa through the tyrosinase or tyrosine hydroxylase enzymes. Current hypothesis is that the hrPE-GM implant may work as an *in situ* “L-dopa pump” [2,21,22]. In our previous study, we provided the first evidence in support to the hypothesis that the beneficial effects of hrPE are not only from *in situ* “L-dopa pump” but also from the DA neurons derived from hrPE [20]. Using ^{11}C - β -CFT assay, we found the

accumulation of radioactive tracers to be more in the implanted putamen than in the contralateral putamen after transplantation. These results indicated that hrPE-derived NPCs injected could transform to DA neurons and secrete DA. Unfortunately, we could not use ROI method to assess the data of PET due to the inconsistency of the data.

Nevertheless, our study suggests that CFT may be a good method for *in vivo* monitoring of survival and differentiation of the implanted cells in transplantation of clinical hrPE-derived NPCs. When attempts were made to use PET for monitoring stem cell differentiation, the critical problem faced was how to choose the radioactive tracers: the markers should reflect the biological changes at different times after transplantation and should also be readily available. *In vitro* experiments proved that RPE cells expressed the D2 receptor but not the DAT, but they expressed both the D2 receptor and DAT once differentiated into DA neurons. Thus, we selected ^{11}C - β -CFT as the PET radioactive tracers for DAT in our study. Ideally, PET should give information on functional neuron transformation from stem cell differentiation *in vivo*. In the animal study of our research group, PET studies showed increased accumulation of ^{11}C -raclopride and decreased accumulation of ^{11}C - β -CFT in the injured side of striatum in both groups. ^{11}C -raclopride decreased along with a concomitant increase in ^{11}C - β -CFT after transplantation in the treated group. The changes shown by the PET studies paralleled the behavioral states and confocal microscopy observations in the treated animals [20].

The results of our study suggest that transplantation of hrPE-derived NPCs can improve symptoms and signs in patients with PD. In our study, all patients tolerated the surgical procedure well, with no major adverse events. No bleeding and edema were found in brain MRI examination after transplantation. No mass effect or abnormal growth at the transplant sites was seen on MRI scans performed 12 and 24 months after surgery. PET also showed a trend with increased DA release during the first 6 months. All the clinical results showed that the transplantation of hrPE-derived NPCs is a safe surgical procedure. Our clinical trial was an open-label study, which may be influenced by placebo response or physician bias. Therefore, a randomized, double-blind clinical trial

with a sham surgery control group will be important for our further study.

Whether immunosuppressant agents could be used in the conditioning treatment in the transplantation of allogeneic hRPE cells in patients with PD is still controversial [23,24]. Recent studies have shown that hRPE, when passively attached to gelatin microcarriers, could attain prolonged survival in the absence of immunosuppressive therapy [11,25]. In our studies, no obvious immune reactions occurred both in allogeneic hRPE cell transplantation and in animal neural stem cell transplantation, although no immunosuppressant agents were administered as conditioning treatment. However, accumulating evidence indicated that the transplantation procedure could induce damages to local blood–brain barrier, temporality edema of brain tissue, small blood vessel injury, and other local tissue inflammatory responses. Therefore, combining transplantation procedure with immunosuppressant agents as a conditioning treatment may be beneficial to overcome the possible immune reaction as well as immune rejection problems in future hRPE transplantation in patients with PD although hRPE displayed a low immunogenicity in this group of patients.

Conclusions

In summary, 12 patients with PD were treated by transplantation of hRPE-derived NPCs for phase I/II clinical trial. The results of transplantation suggest that this transplantation procedure was safe and well tolerable to the patients. No major adverse events occurred in this group of patients. The patients who received transplantation of hRPE-derived NPCs demonstrated improvement in their symptoms and signs of PD. This study suggested that hRPE-derived NPCs might serve as a useful source of DA neurons for neural graft in the treatment for PD.

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Conflict of Interest

The authors declare no conflict of interest.

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