# Photoreceptor Protection by Iris Pigment Epithelial Transplantation Transduced with AAV-Mediated Brain-Derived Neurotrophic Factor Gene

*Masayoshi Hojo,*[1] *Toshiaki Abe,*[2] *Eriko Sugano,*[1] *Yuki Yoshioka,*[1] *Yoko Saigo,*[1] *Hiroshi Tomita,*[1] *Ryosuke Wakusawa,*[1] *and Makoto Tamai*[1]

**Purpose.** To determine whether subretinal transplantation of iris pigment epithelial (IPE) cells transduced with the adeno-associated virus (AAV2)–mediated brain-derived neurotrophic factor (BDNF) gene can protect photoreceptors against phototoxicity.

**Methods.** The BDNF gene was inserted into AAV2 (AAV2-BDNF), and the recombinant AAV2 was transduced into rat IPE (AAV2-BDNF-IPE) cells at various multiplicities of infection (MOI). The concentrations of AAV capsids and BDNF were determined by enzyme-linked immunosorbent assay (ELISA). The AAV2-BDNF-IPE cells were transplanted into the subretinal space of rats, and the rats were placed under constant light on days 1 and 90 after the transplantation. The thickness of the outer nuclear layer was measured in histologic sections and compared to that of control sections. The expression of β-galactosidase (LacZ) in the subretinal space was confirmed by LacZ staining after AAV2-LacZ-IPE transplantation. BDNF gene expression after transplantation was confirmed by real-time polymerase chain reaction (PCR).

**Results.** Transduction efficiency increased with successive days in culture and increased with higher MOI in vitro. The expression of the BDNF gene in the subretinal space was higher in AAV-BDNF-IPE than with AAV2-LacZ-IPE or with IPE-only transplantation. LacZ expression was observed in the subretinal space 7 and 90 days after transplantation. A statistically significant photoreceptor protection was observed on days 1 and 90 in eyes receiving the AAV2-BDNF-IPE transplant, in both the superior transplant site and the inferior hemispheres which did not receive the transplant.

**Conclusions.** Transplantation of AAV2-BDNF-IPE cells may be an alternative method of delivering neurotrophic factors to the lesion. (*Invest Ophthalmol Vis Sci.* 2004;45:3721–3726) DOI: 10.1167/iovs.04-0059

Mutations of genes that are expressed in photoreceptor cells lead to degeneration of the photoreceptors. The genes can be associated with light-dependent or -independent pathways.[1] Most of these degenerations share a common final pathway: the apoptotic death of the photoreceptor cells.[1–3]

Intravitreal injections of neurotrophic factors can protect photoreceptor cells from apoptosis in animals with genetically programed retinal degeneration[4–6] and in animals exposed to constant light (phototoxicity).[7,8] Transplantation of cells genetically modified by neurotrophic factor genes can also protect photoreceptor cells from toxicity.[8,9] Brain-derived neurotrophic factor (BDNF) has been reported to be one of the neurotrophic factors that protects photoreceptors against phototoxicity.[8,10–12]

Gene therapy is used to treat a broad variety of diseases, and many approaches for delivering the targeted genes to the appropriate sites have been attempted. Recombinant viral vectors have been the most extensively studied, and the vectors can transport a gene to replace a defective gene,[13–15] to suppress the expression of a mutant gene,[16] or to deliver a protective gene to delay degeneration.[17,18]

Adenoassociated viruses (AAVs) are members of the Parvoviridae family, and the AAV serotype 2 (AAV2) is one of the vectors most extensively studied and developed for clinical use.[19,20] AAV2 transduction in animal models has progressed from rodents to nonhuman primates and is now being used in humans in phase I safety trials.[21,22]

Autologous iris pigment epithelial (IPE) cells are promising for transplantation into the eye because they will not be rejected.[23,24] To have neurotrophic factors expressed over a long period, we recombined the AAV2 with the BDNF gene (AAV2-BDNF), transduced the AAV2-BDNF into rat IPE (AAV2-BDNF-IPE) cells, and transplanted the AAV2-BDNF-IPE cells into the subretinal space of rats. To assess the efficacy of these procedures, the rats were exposed to phototoxic levels of light, and the retinas were examined histologically to determine whether the photoreceptors were protected by the presence of AAV2-BDNF-IPE cells.

## Materials and Methods

### Construction and Amplification of Vectors

The human BDNF gene was amplified by primers, 5′-CAGGTGAGAA-GAGTGATGACC-3′ and 5′-CATAAATCCACTATCTTCCCC-3′, from the human brain cDNA library (BD- Clontech, Palo Alto, CA). The amplified products of polymerase chain reaction (PCR) were subcloned into the T-vector (Stratagene, La Jolla, CA), and the sequences were confirmed.[25]

The cDNA was subcloned into the *Cla*I/*Eco*RI site of the AAV2 vector (AAV Helper-Free System; Stratagene) that lacked the replication (*rep*) gene, the capsid (*cap*) gene, and the flanking AAV2 inverted terminal repeats (ITR). A plasmid helper vector (pHelper) with the E2A, E4, and VA RNA genes was supplied by Stratagene. The *rep* and *cap* genes were also supplied as plasmid pAAV-RC vectors by Stratagene.

The constructed virus vectors and helper plasmids were cotransfected into human embryonic kidney (HEK293) cells expressing the E1A and E1B adenovirus genes, and the recombinant AAV2-BDNF gene

From the [1]Department of Ophthalmology and Visual Science and the [2]Division of Clinical Cell Therapy, School of Medicine, Tohoku University, Miyagi, Japan.

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Corresponding author: Toshiaki Abe, Department of Ophthalmology, Tohoku University, School of Medicine, 1-1 Seiryoumachi Aobaku Sendai, Miyagi, 980-8574 Japan; toshi@oph.med.tohoku.ac.jp.

(AAV2-BDNF) was collected by freeze–thaw cycles. Then the crude AAV2-BDNF was purified by a modification of the method of Auricchio et al.[26] Crude lysates were incubated with 1 mg each of DNase1 and RNaseA, followed by centrifugation at 4°C. The supernatant was incubated with 0.5% deoxycholic acid (Sigma-Aldrich, St. Louis, MO) for 30 minutes at 37°C. The crude viral particles were passed sequentially through a 5-$\mu$m and then an 0.8-$\mu$m pore filter (Millipore, Bedford, MA), and then applied to a heparin column equilibrated with 25 mL of PBS-MK buffer (1× phosphate-buffered saline [PBS], 1 mM $MgCl_2$, and 2.5 mM KCl) according to the manufacturer's instructions (Sigma-Aldrich). The crude viral particles were applied to a column, and the matrix was washed with 25 mL of PBS-MK buffer, followed by elution with 20 mL of PBS-MK buffer containing 1 M NaCl.[27] To adjust the NaCl concentration to physiological levels, 10× PBS was added, mixed, centrifuged, and then the virus was concentrated.

The $\beta$-galactosidase gene (LacZ; Stratagene) was inserted into the *Not*I site of the AAV2 vector. Recombinant AAV2 with the LacZ gene (AAV2-LacZ) and the AAV2 vector alone were also prepared by the same procedures.

## ELISA of AAV Capsid and BDNF Protein

To determine the virus titer, AAV2-specific capsids were assayed using an rAAV stock solution and enzyme-linked immunosorbent assay (ELISA; Progen Biotechnik, Heidelberg, Germany)[28] and the results expressed as capsids per milliliter. The BDNF proteins expressed by the transduced IPE cells were also measured (Promega Co., Madison, WI) at 14 days after the transduction of AAV2-BDNF. Approximately 20 mL of supernatant was condensed to 0.5 mL and used for determining the BDNF level. The color of the reaction products was measured with a microplate reader (MAXline; Molecular Devices Corp., Sunnyvale, CA). The total protein concentration was determined by a protein assay kit (Pierce, Rockford, IL).

## Transduction Efficiency

To determine the infectious titer, infectious center assays[29] were performed with HT1080 cells, a highly permissive cell line, according to the manufacturer's instructions (AAV Helper-Free System; Stratagene). In brief, $5 \times 10^4$ HT1080 cells were plated in 24-well tissue culture plates and cultured overnight. After cultivation, Dulbecco's modified Eagle's medium (DMEM) supplemented with 240 mM hydroxyurea and 6 mM sodium butyrate (AAV Permissive Medium; Stratagene) was added to each well and incubated for 6 hours. After washing, 200 $\mu$L of serially diluted AAV-LacZ was added to each well and further incubated for 72 hours. The expression of LacZ in the transduced HT1080 cells was determined according to the manufacturer's instructions ($\beta$-Gal Staining Kit; Invitrogen, San Diego, CA). Positive (blue) cells were counted in each well (DMIRE2; Leica Microsystems Imaging Solutions, Ltd., Cambridge, UK), and the expression level was recorded as the number of infected viral particles (stained cells) per milliliter. All counts were performed in triplicate. The efficiency was also determined in rat IPE cells by the same method, except that the incubation time was extended up to 14 days.

## Cell Culture and Transduction of Recombinant AAV2

IPE cells from Long-Evans rats were prepared as reported,[30] and cells at passages 1 and 3 were used. The 293T cells, obtained from the American Type Culture Collection (ATCC; Manassas, VA), were maintained in 5% $CO_2$ at 37°C in DMEM/F-12 nutrient mixture (DMEM/F-12) with 10% fetal bovine serum (Sigma-Aldrich).

Subconfluent IPE cells were transduced at various multiplicities of infection (MOI) for each type of recombinant AAV2. Seven to 10 days after transfection, the cells were collected by trypsinization and centrifugation and used for transplantation. Before the transplantation, the cells were washed three times, and later the supernatants were collected and genomic DNA was extracted from each wash (DNA Extraction Kit; Stratagene).

PCR was performed to detect the presence of the AAV genome in the supernatant by using the two sets of primers at the ITR and CMV promoter regions of the AAV vector. The primers were 5′-AGAGAGGGAGTGGCCAACTC-3′ and 5′-GTACTGGGCATAATGCCAGG-3′ for the ITR region and 5′-TGGAGTTCCGCGTTACATAAC-3′ and 5′-CCGCATCACCATGGTAATAG-3′ for the CMV promoter region, and were amplified 357 and 323 bp, respectively. PCR was performed in a 50-$\mu$L reaction mixture: 94°C for 1 minute for denaturation, 58°C for 2 minutes for annealing, and 72°C for 2 minutes for polymerization.[31]

## Extraction of mRNA, cDNA Generation, Reverse Transcription–Polymerase Chain Reaction, and Real-Time PCR

mRNA was extracted from each type of cell with oligo dT cellulose, and cDNAs were generated according to the manufacturer's instructions (Pharmacia Biotech Inc., Uppsala, Sweden). In addition, about one third of the retina, including the RPE and choroid, was dissected 7 and 90 days after transplantation, and mRNAs were extracted and converted to cDNA. PCR was performed in 50 $\mu$L of reaction mixture: 94°C for 1 minute for denaturation, 57°C for 2 minutes for annealing of BDNF, and 60°C for $\beta$-actin and finally, 72°C for 2 minutes for polymerization.

Real-time PCR was also performed according to the manufacturer's instructions at the selected days after AAV2 transduction.[32] The PCR products were quantified (SYBR Green I dye; BMA, Rockland, ME) and a fluorescein-detectable specific thermal cycler (Smart Cycler; TaKaRa, Kyoto, Japan). The optimal conditions for BDNF and $\beta$-actin were: 3.5 mM $MgCl_2$, 94°C for 30 seconds for 1 cycle and 94°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds, 86°C for 10 seconds for 35 cycles (for $\beta$-actin); 4.0 mM $MgCl_2$, 94°C for 30 seconds for 1 cycle and 94°C for 15 seconds, 57°C for 30 seconds, 72°C for 30 seconds, 84°C for 10 seconds for 35 cycles (for human BDNF).

The primers for the BDNF gene amplified 358 bp and $\beta$-actin 313 bp. The sequences were; 5′-AACATCCGAGGACAAGGTGG-3′ and 5′-ATACTGTCACACACGCTCAG-3′ for the human BDNF gene and 5′-CTACAATGAGCTGCGTGTGG-3′ and 5′-CGGTGAGGATCTTCATGAGG-3′ for $\beta$-actin.

## Cell Viability Test

Cell viability assays were performed with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) (CellTiter 96 Aqueous One Solution Cell Proliferation Assay; Promega) after transfection.[33] For this, 40 $\mu$L of one reagent was added to the medium (200 $\mu$L), followed for 1 hour at 37°C in humidified 5% $CO_2$. The absorbance at 490 nm was measured by spectrophotometry (Spectra Max Gemini Uvmax; Molecular Devices, Sunnyvale, CA).

## Transplantation

All procedures were preformed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Host rats were anesthetized with pentobarbital sodium (60 mg/kg body weight) and atropine sulfate (0.4 mg/kg). Recombinant AAV2-BDNF-IPE cells were injected into the superior subretinal space of the right eyes of Sprague-Dawley male rats with a 30-gauge needle on a Hamilton syringe (Hamilton, Reno, NV), and the left eyes were sham injected for control experiments.[34] The rats were 3 months old at the time of transplantation. A total of 104 rats were used for the transplantation studies with the same batch of recombinant AAVs and approximately the same passage of IPE cells for each set of experiments.

The retinas were examined by indirect ophthalmoscopy after the injection, and nine rats with eyes showing massive subretinal hemorrhage, vitreous hemorrhage, or large retinal detachments were discarded. At least five rats were used for each cell transplantation. Eight rats were used for the histologic examination for LacZ staining (described later) at 7 and 90 days after AAV-LacZ-IPE transplantation. Twenty-nine rats were also used for the BDNF gene expression after the selected times after cell transplantation.

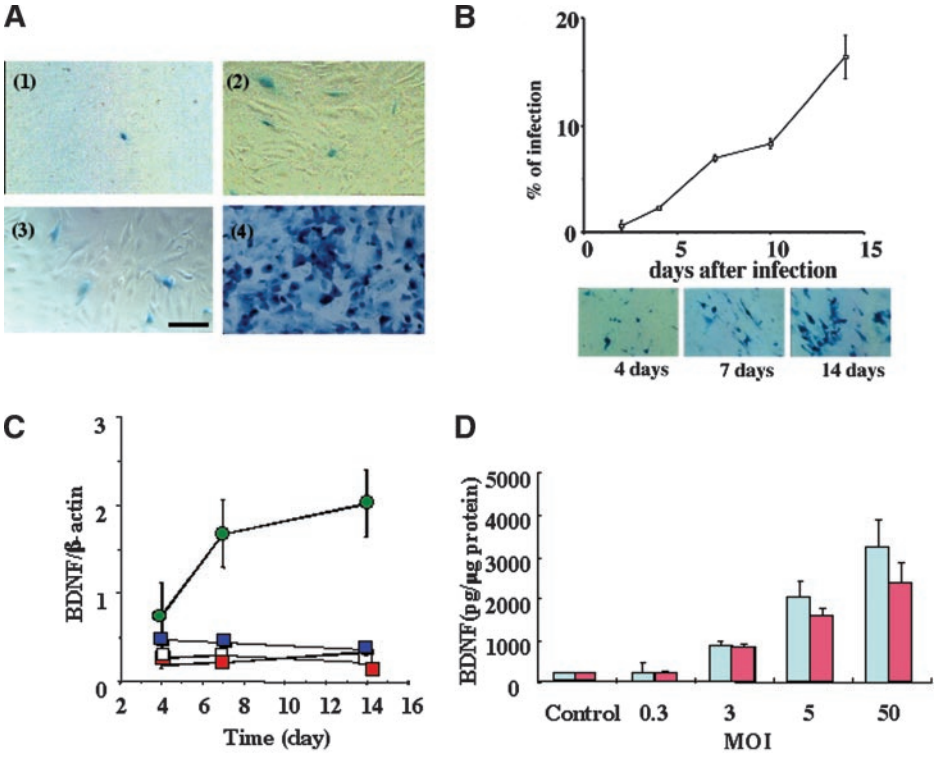


**FIGURE 1.** (**A**) Transduction efficiency. Rat IPE cells ($5 \times 10^4$) were exposed to different concentrations of AAV-LacZ, and the transfection efficiency was calculated by β-galactosidase assay. (1), (2), and (3) show MOIs of 0.3, 3, and 5 on rat IPE cells, respectively, and (4) shows the effect on HT1080 cells, the positive control. Bar, 50 μm. (**B**) The transduction efficiency increased with successive days in culture and reached approximately 15% at 4 days after transduction (MOI = 5). β-Galactosidase expression was confirmed by LacZ staining at 4, 7, and 14 days after transduction of AAV-LacZ on rat IPE cells. Error bars, SD. (**C**) Results of real-time PCR of BDNF gene. The expression of BDNF gene in AAV-BDNF-IPE is compared to that of β-actin. The level of expression increases with successive cultures (MOI = 5; *green circles*). *Blue squares*: BDNF gene expression in AAV-LacZ-IPE, *red squares*: AAV vector-transduced IPE; *open squares*: IPE only. Error bar, SD. (**D**) Results of ELISA on BDNF. Control is uninfected control rat IPE cells ($n$ = 4, passages 2–4) Error bar, SD. *Blue*: results from supernatant; *red*: cell pellets.

The needle was passed through the sclera, and $4 \times 10^4/2$ μL cells were injected. For control, the same volume of Hank's balanced salt solution (HBSS) was injected.

The rats were placed in standard cyclic room light (light-dark, 14:10 hours), and on days 1 and 90 after the transplantation, the rats were exposed to constant illuminance of 2000 to 2500 lux for 1 week. After the light exposure, the rats were killed by carbon dioxide and the eyes enucleated and processed for histologic study.[8]

### Quantification of Photoreceptor Rescue

The stained retinal sections were photographed, and the images were read into a computer system.[8] The thickness of the outer nuclear layer (ONL) was measured in five serial sections at 600, 700, 800, 1200, 1300, 1400, 2100, 2200, and 2300 μm from the optic nerve head to the ora serrata through the region of the transplantation. The thickness of the ONL in the inferior half through a nontransplant site of the same sections was also measured at 9 points for a total of 18 sites in each section.

### β-Galactosidase Activity in Histologic Sections

Seven and 90 days after AAV2-LacZ-IPE transplantation ($n$ = 4 each), the eyes were enucleated, and fixed immediately in 4% paraformaldehyde in PBS (pH 7.5) overnight at 4°C. The specimens were cryoprotected[35] and sectioned at 5 μm (Cryofilm Transfer Kit; Finetec, Tokyo, Japan) on a cryostat (LEICA CM3050; Finetec) at −16°C. The sections were stained with a β-Gal staining kit (Invitrogen) and photographed (Leica DMIRE2; Leica Microsystems Imaging Solutions Ltd.).

### Statistical Analysis

The Fisher's protected least significant difference (PLSD) test was used to determine whether differences in the ONL thickness and the BDNF gene expression were significant. $P < 0.05$ was considered to be statistically significant.

## RESULTS

### AAV Transfected IPE Cells

Our simple and efficient column chromatographic procedures purified the AAV vector to $1 \times 10^{12}$ capsids/mL (by ELISA). This corresponds to a $1.5 \times 10^8$ infectious titer using HT1080 cells. Although we used different MOIs, the transduction efficiency was very low 4 days after transduction (Fig. 1A). However, when we used an MOI of 5, the transduction efficiency of rat IPE cells increased with increasing days of culture, and it was found to be approximately 15% by infectious center assay on day 14 after transduction (Fig. 1B).

The expression of the BDNF gene after AAV-BDNF transduction also increased with successive days in culture by real-time PCR (Fig. 1C). This was not observed in the cultures with AAV-LacZ or AAV vector-transduced IPE, and only IPE culture (control).

The expression of BDNF by cultured rat AAV2-BDNF-IPE cells was 2020 pg BDNF/μg protein in the supernatant and 1740 pg BDNF/μg protein in the cell pellets of AAV-BDNF-IPE (MOI of 5), whereas uninfected IPE cells (for control) expressed 120 pg of BDNF/μg protein approximately 14 days after transduction (Fig. 1D).

PCR analysis detected no AAV DNA in the supernatant after washing the cells to be transplanted two times (data not shown).

### Cell Viability Assay

MTS assay was performed on 1, 3, 5, and 7 days after transduction on AAV-BDNF with a MOI of 3, 5, and 50. Statistical analysis showed that the cell viability of AAV transduced IPE cells was not significantly different from that of nontransduced IPE cells (data not shown).

### BDNF Gene Expression

The expression of the BDNF gene was examined by real-time PCR at the site of transplantation 7 and 90 days after transplantation. To determine the level of BDNF gene expression from transplanted cells, the primer to amplify the BDNF gene was developed from the human BDNF gene specific sequences. A higher expression of the BDNF gene was observed in transplants of AAV-BDNF-IPE than in IPE alone or AAV-LacZ-IPE at both 7 (Fig. 2A) and 90 (Fig. 2B) days after transplantation.

### Effect of IPE Transplantation

One day after transplantation, some rats showed mild subretinal hemorrhages (Fig. 3A), but histologic sections showed transplanted cells expressing LacZ in the subretinal space (Fig. 3B). One day after transplantation, the ONL was significantly thicker (asterisks) at several areas in sections from rats receiving AAV2-BDNF-IPE (diamonds) than those receiving AAV2-LacZ-IPE (squares), IPE only (triangles), and HBSS (circles) (Fig. 3C). The differences were statistically significant ($P < 0.05$) at *(AAV2-BDNF-IPE against AAV2-LacZ-IPE), †(AAV2-BDNF-IPE against IPE), and ‡(AAV2-BDNF-IPE against HBSS). Histologic examinations showed no inflammatory reaction at the transplant site (Fig. 3D).

LacZ expression was also detected in the subretinal space of the AAV2-LacZ-IPE cells, even 90 days after transplantation (Fig. 4A). LacZ expression was not observed other than in the areas receiving the transplant. Histologic examination showed no inflammatory reaction at the transplant site (Fig. 4B).

Examination of the retinas of rats kept under normal light cycling for 90 days and then exposed to constant light for 7 days showed that the ONL was significantly thicker in eyes receiving transplants of AAV2-BDNF-IPE cells than those receiving transplants of AAV-LacZ-IPE, IPE only, or HBSS injections (Fig. 4C). The regions showing statistically significant changes are indicated by *(AAV2-BDNF-IPE against AAV2-LacZ-IPE), †(AAV2-BDNF-IPE against IPE only), and ‡(AAV2-BDNF-IPE against HBSS). The AAV2-BDNF-IPE transplantation clearly rescued the photoreceptors from constant light damage at both 1 and 90 days after the transplantation.

## Discussion

Examination of eyes with AMD that have received transplants of autologous IPE cells show no clinical signs of rejection and no obvious side effects more than 4 years after transplantation.[23,24] In monkeys, autologous IPE cells transplanted into the subretinal space remained in that area for at least 6 months.[36] These observations suggested that the autologous IPE cells will remain at the site of transplantation and not be rejected. Inducing these cells to express neurotrophic factors would be a useful method to deliver neurotrophic factors to the lesion for long periods.

Although there is no known pathogenic effect of AAV in humans, the genome of AAV is incorporated into chromosomes at a region that is actively transcribing genes.[37,38] This may then induce small to large deletions of the chromosome.[37,38]

If the incorporation of the target gene leads to abnormal changes of the transduced IPE, this may be detected ex vivo by the examination of cell proliferation, cell activity, or cell morphology. In our AAV-BDND-IPE cells, no cell damage was detected by cell viability tests, and the morphology of the cells was unchanged.

We transduced the AAV-BDNF while the IPE cells were in culture. Genetically modified RPE cells have been reported to retain morphologic and biochemical characteristics of RPE cells.[39] In addition, IPE cells may also retain many functions similar to those of RPE cells in vivo.[40,41] This will permit the transplantation of autologous IPE cells in the subretinal region.[23,36] However, the cultured IPE cells may differ in several

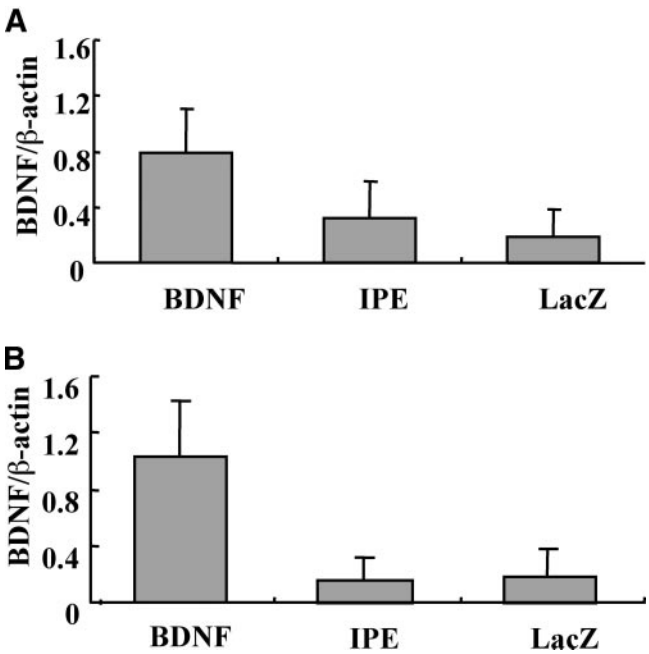


**Figure 2.** BDNF gene expression after transplantation. BDNF gene expression after subretinal transplantation was determined by real-time PCR after 7 (**A**) and 90 (**B**) days of transplantation. Higher expression of BDNF gene are found after AAV-BDNF-IPE transplantation (shown as BDNF) than after IPE only (IPE) or AAV-LacZ-IPE (LacZ). Error bars, ±SD.

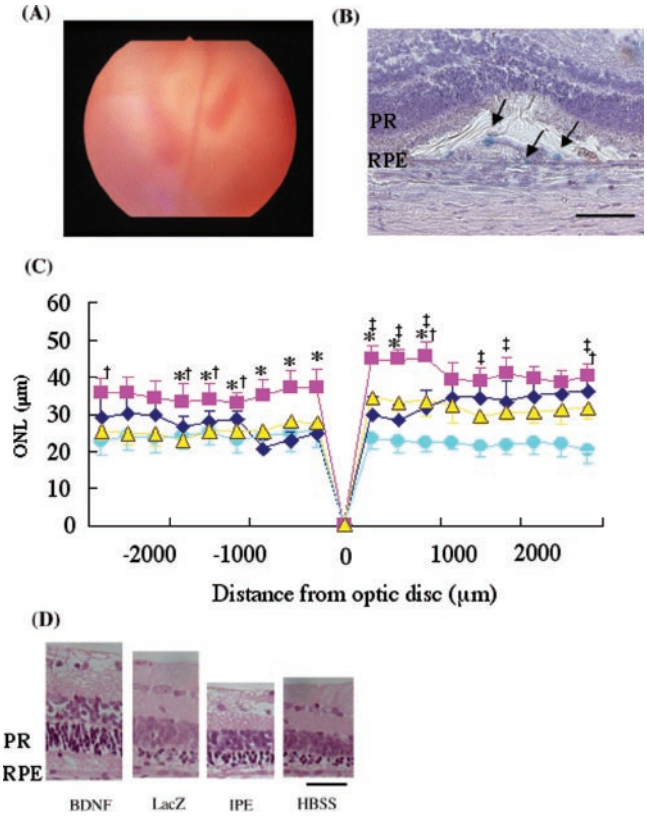


**Figure 3.** (**A**) Fundus photograph 1 day after transplantation. Mild hemorrhage appeared in the transplantation area. (**B**) Cross-section of the transplant site. Transplanted cells expressing LacZ (*arrows*) were present in the subretinal space. PR, photoreceptor; RPE, retinal pigment epithelium. Bar, 50 μm. (**C**) Effect of different types of recombinant AAV 1 day after transplantation. Significance differences (*) were observed with AAV2-BDNF-IPE (*diamonds*, $n = 6$) when compared with those of AAV2-LacZ-IPE (*squares*, $n = 5$), IPE only (*triangles*, $n = 5$), and HBSS injection (*circles*, $n = 5$). (**D**) Histologic section at 1500 μm superior to the edge of the optic disc in the transplant area. The ONLs after transplantation of AAV2-LacZ-IPE (LacZ), IPE only (IPE), and HBSS injection were thinner than that after AAV2-BDNF-IPE (BDNF). Bar, 50 μm.

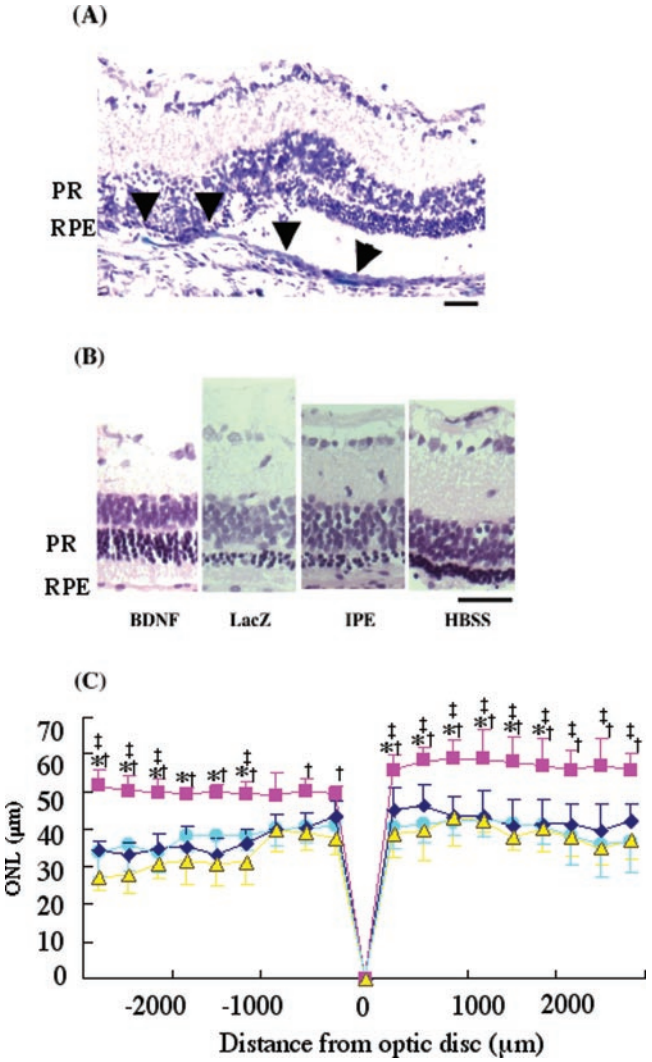


**FIGURE 4.** (**A**) β-Galactosidase activity adjacent to the transplant site. *Arrowheads*: positive staining of LacZ (*blue*). PR, photoreceptor; RPE, retinal pigment epithelium. (**B**) Histologic section at 1500 μm superior to the edge of the optic disc in the transplant area. (**C**) Effect of different types of recombinant AAV 90 days after transplantation. Statistical significance (*) was observed with AAV2-BDNF-IPE (*filled squares*, $n = 5$) and not with AAV2-LacZ-IPE (*open squares*, $n = 5$), IPE only (*filled triangles*, $n = 5$), and HBSS injection (*filled circles*, $n = 5$).

ways from those of native RPE cells that could affect the photoreceptor cell function if transplanted subretinally.

Although AAV2 has been a suitable gene vector for many different organs,[19,20,22] several studies have shown a limited efficiency of transduction that depended on the cell type.[42–45] Rat IPE cells transduced with AAV-LacZ showed an increased transduction efficiency with successive days in culture. In addition, the increased MOI also increased the transduction efficiency in our experimental conditions. Similar results were also observed with AAV-BDNF transduction. Because there is a limit in the period of cells in culture, we cannot determine the real transduction efficiency of AAV-LacZ on rat IPE. Although the transduction efficiency of AAV on rat IPE may be low during the early days of transduction, the target gene expression may be long lasting.

The transduction of AAV into B cells of chronic lymphocytic leukemia was also reported to have low transgene expression (reported to be <3%), and many methods have been used to try to improve the efficiency of AAV transduction, such as the use of the T-cell–activating molecule, CD40 ligand (CD40L).[46] The transduction process of AAV has not been fully determined, although the pathway is complex with cell surface binding, endocytosis, transport in the cytoplasm, and the nuclear events that result in the conversion of the single-stranded rAAV genome. Although the efficiency of our recombinant AAV transduction on rat IPE cells was not so high, molecular biological analysis at each step during the transduction of AAV into IPE cells may determine how the transfection efficiency can be improved.

Our results demonstrated a clear protection of AAV-BDNF-IPE transplantation against phototoxicity. The protective effect was found not only at the transplant site but extended into the inferior hemisphere that did not receive transplanted cells. These results confirmed our previous findings.[8]

We also showed that the transplanted AAV2-BDNF-IPE cells continued to be effective in protecting photoreceptor cells for at least 90 days after transplantation. In addition, no sign of inflammation was detected. Statistical analysis of the 18 points of the retina revealed that the significance after 90 days of transplantation was better than that at 1 day (*, †, and ‡, $P < 0.05$). These results suggest that the rescue effect of AAV2-BDNF-IPE may be more apparent in later days. Bennett et al.[47] reported that recombinant AAV expresses the target gene more slowly than the adenovirus-mediated gene, and the targeted gene was expressed longer. Our results agree with their findings.

The function of BDNF in protecting the retina against degeneration was suspected to be mediated by retinal glial cell (Müller cells) by expressing neurotrophic factors.[48] Together with the conglomerate morphology but not a cell sheet of AAV-BDNF-IPE in the subretinal space, our methods for protecting photoreceptor cells may be affected by more devastating retinal conditions.

The expression of the BDNF receptor and intricate intracellular signaling in the retina should be elucidated. Work examining the local reaction of the retina against continued expression of the BDNF gene as well as determining the best MOI for AAV-BDNF is ongoing in our laboratory.

It is important to point out that we did not observe any inflammation at the transplanted region histologically. The subretinal injection of adenovirus or AAV has shown a deviant immune response to viral proteins,[49] In one study, uveitis developed in 75% of eyes directly injected with recombinant AAV carrying the RPE65 gene.[50] Transduction of the target gene in the transplanted cells may be one of the methods to prevent intraocular inflammation.

In conclusion, our results demonstrated that the transplantation of IPE cells transduced with recombinant AAV2-mediated genes may serve as an alternative method of delivering the targeted gene to the lesion.

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