Human Retinal Progenitor Cell Transplantation Preserves Vision*

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Background: Human retinal progenitor cells (hRPCs) are expandable in vitro and represent a possible therapy for retinal degenerative diseases.

Results: In a rat model of retinal degeneration, transplantation of hRPCs preserved photoreceptors and visual function.

Conclusion: Subretinal injection of hRPCs rescues photoreceptors without causing adverse effects.

Significance: This study provides proof of concept for hRPC transplantation and paves the way for further studies and human trials.

Cell transplantation is a potential therapeutic strategy for retinal degenerative diseases involving the loss of photoreceptors. However, it faces challenges to clinical translation due to safety concerns and a limited supply of cells. Human retinal progenitor cells (hRPCs) from fetal neural retina are expandable in vitro and maintain an undifferentiated state. This study aimed to investigate the therapeutic potential of hRPCs transplanted into a Royal College of Surgeons (RCS) rat model of retinal degeneration. At 12 weeks, optokinetic response showed that hRPC-grafted eyes had significantly superior visual acuity compared with vehicle-treated eyes. Histological evaluation of outer nuclear layer (ONL) characteristics such as ONL thickness, spread distance, and cell count demonstrated a significantly greater preservation of the ONL in hRPC-treated eyes compared with both vehicle-treated and control eyes. The transplanted hRPCs arrested visual decline over time in the RCS rat and rescued retinal morphology, demonstrating their potential as a therapy for retinal diseases. We suggest that the preservation of visual acuity was likely achieved through host photoreceptor rescue. We found that hRPC transplantation into the subretinal space of RCS rats was well tolerated, with no adverse effects such as tumor formation noted at 12 weeks after treatment.

Retinal degenerative diseases, such as age-related macular degeneration and retinitis pigmentosa, result in death of photoreceptors and are the leading cause of blindness in the developed world (1). These conditions affect millions of people, but there are currently few effective treatments that slow or prevent disease progression (2). Cell transplantation is a potential therapy that aims to rescue or replace dying photoreceptors in the retina. Initiation of treatment relatively early in the disease process may preserve retinal structure and function through secretion of protective trophic factors by transplanted cells.

Previous studies have shown that stem cells, including embryonic or fetal retinal progenitors, neurospheres, neural stem cells, and induced pluripotent stem cells, were able to differentiate into specific retinal cell types such as photoreceptors in vitro and in vivo (3–5). Furthermore, several groups have transplanted retinal progenitor cells (RPCs)3 into animal models of retinal degenerative diseases. RPCs could migrate into the retina and differentiate into photoreceptor cells. Donor tissue came from various sources, such as rat retinal progenitor sheets (6), human embryonic stem cell-derived photoreceptors (7), and mouse rod precursors (8). Human retinal progenitor cells (hRPCs) also had the ability to migrate into degenerated retina

3 The abbreviations used are: RPC, retinal progenitor cell; AUC, area under the curve; BDNF, brain-derived neurotrophic factor; c/d, cycles/degree; cd, candela; ERG, electroretinography; hRPC, human RPC; NAC, N-acetylcysteine; OKR, optokinetic response; ONL, outer nuclear layer; Pn, postnatal day; qPCR, quantitative PCR; RCS, Royal College of Surgeons; RPE, retinal pigment epithelium.
and differentiate into mature retinal cell types (9). There have been several reports of significant visual improvement after mouse-to-mouse photoreceptor precursor transplantation (10, 11). A critical question that remains is whether transplantation of hRPCs can actually result in long term visual improvement.

Another major factor that currently limits the successful clinical application of cell transplantation is the challenge of expanding a cell line into a large enough number of cells, while keeping the cells in an undifferentiated state. Recently, Baranov et al. established an hRPC cell line derived from human fetal neural retina that can expand through multiple passages while maintaining an undifferentiated state (12). These properties make this hRPC line a useful resource for studying cell transplantation as a treatment for retinal degeneration.

The Royal College of Surgeons (RCS) rat is a widely used animal model of inherited retinal degeneration. These rats experience progressive vision loss that can be measured over time. The genetic mutation in the Mertk gene results in defective function of cells in the retinal pigment epithelium (RPE), including the failure to phagocytose rod outer segments (13). This defect causes RPE and photoreceptor degeneration and visual impairment, which can be evaluated by visual behavioral responses and electroretinography (ERG). Morphological changes in photoreceptor outer segments appear as early as postnatal day (P)16, and only scattered photoreceptor cells (cones) remain by P105. In this study, we investigated subretinal transplantation of hRPCs into RCS rats and showed that hRPCs can preserve visual function and retinal morphology.

EXPERIMENTAL PROCEDURES

hRPC Isolation and Expansion—All work with human material was performed with approval of the institutional review board of Harvard Medical School. hRPCs were isolated from human fetal neural retina at 16 weeks gestational age as described previously (9). Whole neuroretina was separated from the RPE layer, minced, and digested with collagenase I (Sigma-Aldrich). Cells and cell clusters were plated onto FN-coated flasks (Nunclon Delta) in Defined Trypsin Inhibitor (Invitrogen). At each passage, cell count was performed using a hemacytometer, and cells were plated onto a fibronectin-coated surface at a density of 20,000 cells/cm² in the same medium. All further described work was performed with a GMP-expanded hRPC cell line (GS086) at passage 9.

Immunocytochemistry—For the immunocytochemical analysis, 4000 cells were plated in each well of 16-well fibronectin-coated chamber glass slides (Nunc). After a 24-h incubation under appropriate conditions, cells were washed in PBS, fixed (cold, freshly prepared 4% paraformaldehyde), permeabilized (0.2% Triton X-100 in 5% BSA), blocked, and stained with primary antibodies at 4 °C overnight and with secondary antibodies (1:100, goat Cy3-conjugated anti-rabbit or anti-mouse; Jackson ImmunoResearch) at room temperature for 1 h. The exposure time for image recording was based on isotype control staining. Primary antibodies and their dilutions are listed in Table 1.

Flow Cytometry—For flow cytometry, hRPCs were collected and fixed in Perm/Fix buffer (BD Biosciences) at 4 °C for 20 min. The cells were washed in wash buffer (BD Biosciences) and incubated in block buffer (Pharmingen staining buffer with 2% goat serum) at room temperature for 30 min. After blocking, cells were stained with conjugated primary antibodies (SOX2-APC, CD38-PE, CD73-PE, CD133-PE, PAX6-PE, SOX2-APC IgG2a 1:20 R&D Systems) and fixed in Perm/Fix buffer (BD Biosciences) at 4 °C for 20 min. The cells were washed in wash buffer (BD Biosciences) and fixed in Perm/Fix buffer (BD Biosciences) at 4 °C for 20 min. The cells were washed in wash buffer (BD Biosciences) and incubated in block buffer (Pharmingen staining buffer with 2% goat serum) at room temperature for 30 min. After blocking, cells were stained with conjugated primary antibodies (SOX2-APC, CD38-PE, CD73-PE, CD133-PE, CD133-PE, PAX6-PE, HLA-A,B,C-PE, CD133-PE, A2B5-PE) for 1 h at room temperature. After the final wash, light scatter and fluorescence signals from each cell were measured using the Beckman Epics XL flow cytometer (10,000 events were recorded). The results were analyzed using FlowJo software (Tree Star). The ratio of positive cells within the gated population was estimated based on comparison with species-specific isotype control. Primary antibodies and their dilutions are listed in Table 2.

Table 1: Primary antibodies used for immunocytochemistry

| Antibody      | Working dilution | Source          |
|---------------|------------------|-----------------|
| β3-Tubulin (m) | 1:200            | Sigma           |
| CD44 (rab)    | 1:200            | Abcam           |
| CRX (rab)     | 1:50             | Santa Cruz      |
| Ki67 (rab)    | 1:100            | Chemicon        |
| Nestin (m)    | 1:200            | BD Biosciences  |
| NeuroD1 (rab) | 1:100            | Chemicon        |
| NRL (m)       | 1:50             | Santa Cruz      |
| OTX2 (rab)    | 1:100            | Abcam           |
| PAX6 (m)      | 1:50             | DSBB            |
| Recoverin (rab) | 1:1000          | Chemicon        |
| SSEA4 (m)     | 1:200            | Chemicon        |
| Vimentin (m)  | 1:200            | Sigma           |

Table 2: Primary antibodies used for flow cytometry

| Antibody      | Isotype | Working dilution | Source          |
|---------------|---------|------------------|-----------------|
| A2B5-PE      | IgM     | 1:20             | Millipore       |
| CD38-PE      | IgG2b   | 1:20             | Millipore       |
| CD73-PE      | IgG1    | 1:20             | Millipore       |
| CD133-PE     | IgG1    | 1:20             | Millipore       |
| HLA-A,B,C    | IgG1    | 1:20             | BD Biosciences  |
| PAX6-PE      | IgG2a   | 1:20             | BD Biosciences  |
| SOX2-APC     | IgG2a   | 1:20             | R&D Systems     |
| Control      | IgM     | 1:20             | Millipore       |
| Control      | IgG1    | 1:20             | BD Biosciences  |
| Control      | IgG2a   | 1:20             | BD Biosciences  |
| Control      | IgG2b   | 1:20             | BD Biosciences  |

Subretinal Transplantation—All procedures were conducted with the approval and under the supervision of the Institutional Animal Care Committee at the University of California San Diego and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
**Table 3**

Primary antibodies used for immunohistochemistry

| Antibody       | Working dilution | Source      |
|----------------|------------------|-------------|
| RPE65 (m)      | 1:300            | Millipore   |
| Bestrophin (m) | 1:100            | Millipore   |
| Recoverin (rab)| 1:1000           | Chemicon    |
| Opsin (g)      | 1:200            | Santa Cruz  |
| Rhodopsin (m)  | 1:250            | Santa Cruz  |
| PAX6 (rab)     | 1:50             | DSHB        |
| PKCz (rab)     | 1:1000           | Santa Cruz  |
| Human nuclear antigen (m) | 1:200 | Millipore |
| Calbindin (rab)| 1:1000           | Swant       |
| Brn3 (m)       | 1:200            | Millipore   |
| CD11b (m)      | 1:1000           | Millipore   |
| Iba1 (g)       | 1:1000           | Abcam       |
| Nestin (m)     | 1:200            | BD Biosciences |

**ERG Recording**—To monitor the efficacy of hRPCs in preserving vision, ERG studies were performed at 12 weeks after treatment before the animals were sacrificed for histology. The dark-adapted ERG response was recorded as described previously (17). In brief, rats were dark-adapted for 14 h prior to the commencement of each ERG recording session. They were deeply anesthetized as described for the surgical procedure above. Eyes were treated with 1% topical tropicamide to facilitate pupillary dilation. Each rat was tested in a fixed state and maneuvered into position for examination within a Ganzfeld bowl (Diagnosys LLC). One active lens electrode was placed on each cornea, with a subcutaneously placed ground needle electrode positioned in the tail and the reference electrodes placed subcutaneously in the head region approximately between the two eyes. Light stimulations were delivered with a xenon lamp at 0.01 and 0.3 cd/s/m² in a Ganzfeld bowl. For the photopic ERG measurement, rats were adapted at a background light of 10 cd/s/m², and light stimulation was set at 30 cd/s/m². The recordings were processed using software supplied by Diagnosys. The a- and b-wave amplitudes were measured from the minimum trough to the maximum peak following light stimulation.

**Histological Analysis**—Following ERG recordings, rats were sacrificed, and retinal cross-sections were prepared for histological evaluation of transplant survival and preservation of the ONL. Rats were euthanized with CO₂, and eyeballs were dissected out and fixed in 4% paraformaldehyde. Eyes were infiltrated with sucrose and embedded in optimal cutting temperature compound (OCT). Horizontal frozen sections (10 μm) were cut on a cryostat. Retinal sections underwent immunohistochemistry and were examined by regular and confocal microscope. Immunostaining for human-specific markers (mouse anti-TRA-1-85; Millipore) was performed. Primary antibodies and their dilutions are listed in Table 3. The secondary antibody was conjugated to Cy3 (Jackson ImmunoResearch).

**Statistical Analysis**—Differences among the experimental groups were analyzed with a t test. The Pearson correlation coefficient was used to calculate the correlation between the b-wave response and ONL cell count or ONL spread distance.

**RESULTS**

**Characterization of hRPCs in Cell Culture**—The hRPC cell line GS086 expressed markers specific for neural progenitors, including the intermediate filament nestin, SSEA4, and the microtubule subunit β3-tubulin, as well as the glial marker...
vimentin (18) (Fig. 1A). They also expressed eye development transcription factors PAX6 and NeuroD1 (19, 20) (Fig. 1A). When hRPCs were induced to differentiate by plating them on Matrigel-coated plates and treating them with Dkk1, IGF-1, and Noggin for 3 weeks, the differentiated cells showed expression of photoreceptor-specific transcription factors CRX, OTX2, and NRL (21) and the photoreceptor-specific protein recoverin (22) (Fig. 1A). Quantitative flow cytometry analysis further characterized the expression levels of various markers (Fig. 1B). Together, these markers demonstrated the neural progenitor cell phenotype of hRPCs used for transplantation.

The induction of hRPCs was further confirmed by gene expression analysis of typical early eye field transcription factor genes including PAX6, LHX2, and SIX6 (Fig. 1, C and D). Com-

![Image of Figure 1: Characterization of hRPCs.](image)
pared with human embryonic stem cells (ESCs), we found a 
>10–150-fold increase in expression of these retinal progeni-
tor markers in hRPCs. The expression level of these genes was 
maintained through multiple passages in culture. In contrast, 
the expression of pluripotency markers including NANOG and 
OCT4 was significantly decreased. We also assayed gene 
expression changes of growth factors such as brain-derived 
neurotrophic factor (BDNF) and fibroblast growth factor 2 
(FGFR2) and found that they were expressed at increased levels 
hRPCs compared with adult retina (Fig. 1E). Other growth 
factors such as TGFβ2, which promotes mitotic quiescence and 
acts as a cytostatic factor on adult neurogenesis in the 
brain, were expressed at decreased levels.

**Subretinal Transplantation of hRPCs Preserved Vision in RCS 
Rats**—RCS rats received unilateral injections (OD) of hRPCs 
group A) or vehicle (group B) at P21. The contralateral eyes 
(OS) were untreated and served as an internal control for each 
animal. Also, a group of RCS rats (group C) did not receive 
any injections and served as baseline controls. All animals 
were immunosuppressed with cyclosporine A throughout 
the experimental period. OKR studies were conducted at 5 
and 12 weeks after transplantation to examine spatial visual 
acuity. At the end of the study, none of the hRPC-trans-
planted retinas showed evidence of uncontrolled cell prolif-
eration and tumor formation.

In hRPC-grafted rats, treated eyes had preservation of visual 
acuity at 5 weeks following transplantation (P56) compared 
with the untreated contralateral eyes (OD versus OS: 0.46 ± 
0.04 versus 0.38 ± 0.05 c/d, p = 0.029). Similarly, vehicle-
treated eyes had preserved visual acuity at 5 weeks after injec-
tion compared with the untreated contralateral eyes (OD versus 
OS: 0.44 ± 0.03 versus 0.33 ± 0.03 c/d, p = 0.017). In contrast, 
at P56, the mean visual acuity in untreated control RCS rats 
group C) had deteriorated to 0.34 ± 0.04 c/d in the right eyes 
and 0.30 ± 0.03 c/d in the left eyes (p > 0.05) (Fig. 2). There was 
no significant difference in visual acuity of untreated eyes in 
groups A and B compared with that of eyes in group C.

At 12 weeks after injection (P105), there was no preservation 
of visual acuity in vehicle-treated eyes (OD versus OS: 0.29 ± 
0.02 versus 0.26 ± 0.02 c/d, p > 0.05), whereas a sustained 
protective effect on visual acuity was seen in hRPC-grafted eyes 
(OD versus OS: 0.47 ± 0.06 versus 0.22 ± 0.05 c/d, p = 0.008) 
(Fig. 3). The best visual acuity achieved in hRPC-treated eyes 
was 0.5 c/d. Furthermore, at P105, hRPC-treated eyes had a 
mean visual acuity that was significantly better than that of 
vehicle-treated eyes (0.47 ± 0.06 versus 0.29 ± 0.02, p = 0.012). 
There was no difference in the visual acuity of left and right 
eyes in group C (OD versus OS: 0.25 ± 0.026 versus 0.26 ± 0.031 c/d, 
p > 0.05).

**Subretinal Transplantation of hRPCs Improved ERG in RCS 
Rats**—Bright-flash ERG responses were tested at 12 weeks 
after transplantation (P105) to measure the electrical activity of the 
outer (a-wave) and inner (b-wave) retina. Previous studies of 
untreated retinal dystrophic eyes have shown that the ERG 
a-wave in RCS rats is typically lost by P60 whereas the b-wave 
can no longer be elicited by P90 (17). This allows graft-related 
effects to be recognized. By P105, 2 of 12 hRPC-grafted animals 
achieved significant b-waves over background performance 
compared with untreated and vehicle-injected animals, both of 
which had a low b-wave response.

**Transplanted hRPCs Survived, Integrated, and Preserved 
Photoreceptors**—DAPI nuclear staining confirmed the pres-
ence of well organized photoreceptor layers with inner and 
outer segments in the graft-protected area, while distant from 
the graft, sparsely distributed photoreceptors remained (Fig. 4, 
A and B). In contrast, vehicle-treated eyes had only one to three 
layers or sparsely distributed photoreceptor cell bodies in the 
ONL (Fig. 4, C and D), appearing similar to the retinas of age-
matched untreated dystrophic rats. As a comparison, the thickness of the ONL was observed to have 10–12 cells in nondystrophic rats (Fig. 4E). Statistical analysis of ONL measurement data demonstrated that hRPC-grafted retinas had significant preservation of the ONL compared with vehicle-treated controls at P105. Furthermore, cryosection slides of the retinas at P105 showed that the subretinally transplanted hRPCs occasionally migrated throughout the neural retinal layers, including the ONL (Fig. 5, A and D).

To compare the degree of ONL preservation between experimental groups, we measured the thickness of the ONL based on the areas under the curve (AUC) extending 50 μm on either side of the injection site. In hRPC-grafted eyes, the AUC was 1789 ± 182 μm², which was significantly larger than that of both vehicle-treated eyes (1377 ± 153 μm², p = 0.019) and untreated controls (1252 ± 156 μm², p = 0.007) (Fig. 6A). Furthermore, when cells within the AUC were counted, hRPC-grafted eyes had an average of 101 ± 11 preserved cells compared with an average of 73 ± 12 preserved cells in vehicle-treated eyes (p = 0.0203) and 62 ± 12 preserved cells in untreated eyes (p = 0.010) (Fig. 6B). hRPC transplantation resulted in an average spread distance of 1676 ± 198 μm, in which there was an average preserved cell count of 794 ± 123 in the entire ONL. Vehicle-treated eyes had a statistically lower ONL cell count of 481 ± 103 (p = 0.008) within a spread distance of 1222 ± 218 μm (p = 0.019) (Fig. 7). The ratios of the mean cell count and spread distance of hRPC to vehicle were 1.65 and 1.37, respectively. Correlation coefficient analysis showed that the b-wave response correlated well with the ONL cell count (Pearson’s r = 0.8763) and ONL spread distance (Pearson’s r = 0.8905) (Fig. 8).

DISCUSSION

Consistent with a previous report (23), we showed that our RPCs possess high expression levels of several key eye-field transcription factors (PAX6, SIX6, and LHX2) and RPC mark-
**FIGURE 5.** Confocal microscopic images of retinal sections of hRPC-transplanted eyes at P105 after transplantation. A, retinal sections were double stained with human nuclear marker (red) and recoverin (green), which showed no colocalization. Donor cells were distributed throughout multiple neural retinal layers. Grafted areas had several layers of ONL. Scale bars, 100 μm. B, retinal sections stained with antibody bestrophin showed that host RPE cells were positively stained (white arrows), whereas donor cells were negative for bestrophine (brown arrows). C, retina sections were stained with human nuclear marker (red) and nestin (green). D–F are high magnification images of A–C, respectively.

**FIGURE 6.** Measurement of ONL thickness. A, hRPC-transplanted eyes had the highest preserved ONL thickness (AUC) of 1789 ± 182 μm². The AUC value was significantly lower in vehicle-treated eyes (1377 ± 153 μm², p = 0.019) and in untreated RCS rats (1252 ± 156 μm², p = 0.007). There was no significant difference between vehicle-treated eyes and untreated eyes. B, the total numbers of cells in the ONL in the hRPC-treated group, vehicle-treated group, and control group were 101 ± 11, 73 ± 12, and 62 ± 12, respectively. hRPC-grafted eyes had a significantly higher cell count than vehicle-treated eyes (p = 0.0203) and the control group (p = 0.010). There was no significant difference between the vehicle group and control group. *, p < 0.05; **, p < 0.01. Error bars, S.E.
ers (CRX, NRL, and OTX2) compared with the ESCs from which they were derived (Fig. 1C). This is similar to what Lamba et al. observed using the H1 human ES cell line (23) and human induced pluripotent stem cells (24). Thus, the data together show that pluripotent stem cells can be directed to a retinal fate and become RPCs. We also analyzed the expression of the photoreceptor markers recoverin, opsin1, s-opsin, and rhodopsin. The expression levels of these markers decreased in the hRPCs, indicating that they were still retinal progenitor cells. Along with other data, we further confirmed that this cell line is suitable for stem cell therapy.

Engraftment of stem cells into the degenerative retina has demonstrated neuroprotective potential in animal models (25, 26). In this study, we examined whether subretinal transplantation of an hRPC cell line established from human fetal neural retina preserved vision in RCS rats. The transplantation took place at P21, the time of initiation of photoreceptor degeneration. Functional studies using OKR and histological studies showed that subretinal transplantation of hRPCs preserved photoreceptors and visual function in RCS rats.

Compared with vehicle-treated eyes, subretinal grafts of hRPCs resulted in significantly greater ONL thickness, higher numbers of preserved ONL cells, and larger spread distance of ONL cells (Figs. 6 and 7). The anatomic integrity of the ONL in hRPC-treated eyes was most preserved in the immediate vicinity of the graft site. The protective effect decreased as distance from the graft site increased. Moreover, ONL survival extended beyond the boundaries of donor cell distribution, suggesting that the survival of photoreceptors is not achieved through cell replacement but rather through rescue of host photoreceptors.

Visual function could be maintained through replacement of degenerated photoreceptor cells with new healthy cells that integrate into the host retina (27), but we found little evidence to support this claim. At P2 after subretinal injection, the hRPCs formed lumps of cells in the subretinal space, while at P105, the donor cells were found in only several layers in the subretinal space (Fig. 4, E and F). After transplantation, hRPCs were distributed throughout multiple neural retinal layers (Fig. 5, A and D). To determine whether these donor cells differentiated into mature photoreceptor cells in the retina, double labeling of grafted cells with photoreceptor markers including recoverin, opsin, rhodopsin, and human-specific anti-nuclei antibody was performed. The migrated hRPCs failed to express any cone or rod markers. In addition, the migrated hRPCs failed to express other retinal neuronal markers including PKCα, calbindin, and Brn3, indicating that the grafted cells did not differentiate into bipolar cells or retinal ganglion cells. Furthermore, there was no positive staining with RPE markers (RPE65 and bestrophin) (Fig. 5, B and E), microglia markers (Iba1 and CD11b), or Muller glia markers (GFAP). In addition, hRPCs distributed in the subretinal space or the inner retina continued to express the progenitor cell marker nestin (Fig. 5, C and F), but not PAX6 or SOX2.

Because there is significant replacement of photoreceptors, we asked whether trophic factors play a role in rescuing endogenous photoreceptors. We showed that BDNF and FGF2 were expressed at increased levels in hRPCs compared with adult retina. However, growth factors such as TGFβ2, which promotes mitotic quiescence and acts as a cytostatic factor on adult neurogenesis in the brain, were expressed at decreased levels. Our results are consistent with previous studies that indicate transplantation of Schwann cells (28) and human umbilical cord cells (25) that secrete BDNF and injection of FGF2 (29) rescue photoreceptor function, reinforcing the idea that transplanted cells can rescue host photoreceptors through the deliv-
ery of neurotrophic agents (30) and supporting the assumption that stem cells exert their effort by modifying host responses. Several experimental approaches have shown that growth factor injections into the eye can slow retinal degeneration in various rodent models (29, 31–33). However, because injected growth factors tend to have short lived effects and multiple injections are undesirable, other methods such as cell-based sustained delivery may be a rational approach for the long term maintenance of photoreceptors. Unexpectedly, significant b-waves were recordable in only two hRPC-grafted rats, whereas the majority of hRPC-grafted animals with preserved photoreceptors showed limited b-wave responses. By comparing histological data with ERG, we found that the rats with robust b-waves had a wide ONL spread distance and a greater number of preserved ONL cells. One explanation may have to do with the intrinsic properties of ERG itself. ERG gives an overall assessment of the visual response of the retina, but it is not sensitive enough in most cases to detect visual function elicited from a relatively small part of the retina (17). Because rescued photoreceptors are limited to the transplant area (2–3 mm² after full development), which corresponds to ~10–20% of the retina (10), conventional ERG may not be capable of identifying visual responses in hRPC-treated rats. Despite a flat ERG at 3 months in most of the experimental and control animals, it was possible to measure visual acuity using other methods. OKR is accepted as the most widely used test of visual function for mice and rats, due to its greater reliability (34). hRPC-treated eyes performed significantly better than untreated controls at 12 weeks. The best visual acuity was 0.5 c/d, compared with a visual acuity of 0.6 c/d in normal nontrophic rats (15). It is of interest that there was a temporary maintenance of photoreceptors. It was possible to measure visual acuity in Crx-deficient mice. Cell Stem Cell 4, 73–79. hRPCs preserve vision. Nature 444, 203–207. hRPCs Preserve Vision

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