ACS chemical biology

Letters

pubs.acs.org/acschemicalbiology

Small Molecule Mediated Proliferation of Primary Retinal Pigment Epithelial Cells

Jonathan G. Swoboda,[†,⊥] Jimmy Elliott,[‡,⊥] Vishal Deshmukh,[†] Lorenzo de Lichtervelde,[†] Weijun Shen,[§] Matthew S. Tremblay,[§] Eric C. Peters,[‡] Charles Y. Cho,[‡] Bin Lu,[∥] Sergej Girman,[∥] Shaomei Wang,*[,∥] and Peter G. Schultz*[,†]

[†]Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

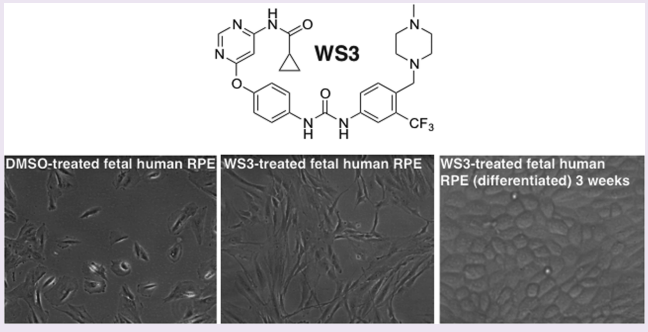
[‡]Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, California 92121, United States

[§]California Institute for Biomedical Research, 11119 North Torrey Pines Road, La Jolla, California 92037, United States

[∥]Regenerative Medicine Institute, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, California 90048, United States

Supporting Information

ABSTRACT: Retinal pigment epithelial (RPE) cells form a monolayer adjacent to the retina and play a critical role in the visual light cycle. Degeneration of RPE cells results in retinal disorders such as age-related macular degeneration. Cell transplant strategies have potential therapeutic value for such disorders; however, risks associated with an inadequate supply of donor cells limit their therapeutic success. The identification of factors that proliferate RPE cells *ex vivo* could provide a renewable source of cells for transplantation. Here, we report that a small molecule (WS3) can reversibly proliferate primary RPE cells isolated from fetal and adult human donors. Following withdrawal of WS3, RPE cells differentiate into a functional monolayer, as exhibited by their expression of mature RPE genes and phagocytosis of photoreceptor outer segments. Furthermore, chemically expanded RPE cells preserve vision when transplanted into dystrophic Royal College of Surgeons (RCS) rats, a well-established model of retinal degeneration.



Retinal pigment epithelial (RPE) cells form a tightly woven monolayer of cells positioned between the neural retina and Bruch's membrane.[1,2] RPE cells serve many functions including maintenance of the blood−retinal barrier, homeostasis of ions/nutrients, and contribution to the visual light cycle.[2] Loss of functional RPE cells leads to degenerative retinal disorders such as age-related macular degeneration (AMD).[3] Cell-based therapies using various stem cell origins (*e.g.*, embryonic and induced pluripotent) may preserve or ultimately restore vision.[4−7] Indeed, a recent clinical study on the first use of RPE cells derived from human embryonic stem cells suggests that transplantation of such cells could be an effective treatment.[8] However, controlled differentiation protocols have not generated a pure RPE population, thereby increasing the risk of tumorigenesis from the remaining undifferentiated cell population.[9] Alternatively, freshly isolated RPE cells from either adult or fetal sources could potentially restore vision.[4,10−12] Unfortunately, primary RPE cells proliferate for a finite number of passages in culture and eventually enter senescence.[11,13,14] To overcome this, we demonstrate that a previously identified proliferative molecule for β-cells [15] (WS3; Figure 1A) can reversibly induce proliferation *in vitro* of primary fetal and adult human RPE cells. We also show that an expanded RPE population remains functional *in vitro* (upon differentiation) and *in vivo*, following subretinal transplantation into the dystrophic Royal College of Surgeons (RCS) rat.[16]

Previous screening efforts identified a diarylurea class of compounds capable of proliferating primary rodent and human β-cells.[15] We tested the ability of these compounds to induce proliferation in other primary cell types that could provide potential clinical utility from *ex vivo* expansion for transplantation. We demonstrate that WS3, the most potent diarylurea analogue, proliferates primary RPE cells (rodent, human fetal, and human adult). Briefly, cryopreserved primary human fetal RPE cells at passage 1 (ScienCell Research Laboratories) were cultured until the cell number leveled off at passage 6 due to senescence, as determined by viable cell counts. Cells were plated in 384-well plates and grown for 3 days in the presence of varying concentrations of WS3 (1.0 μM to 1.0 nM) or DMSO (0.2% v/v) for an EdU proliferation assay. EdU-positive cells were quantified using high-content imaging (ImageXpress Ultra Confocal High Content Screening

Received: March 11, 2013
Accepted: April 26, 2013
Published: April 26, 2013

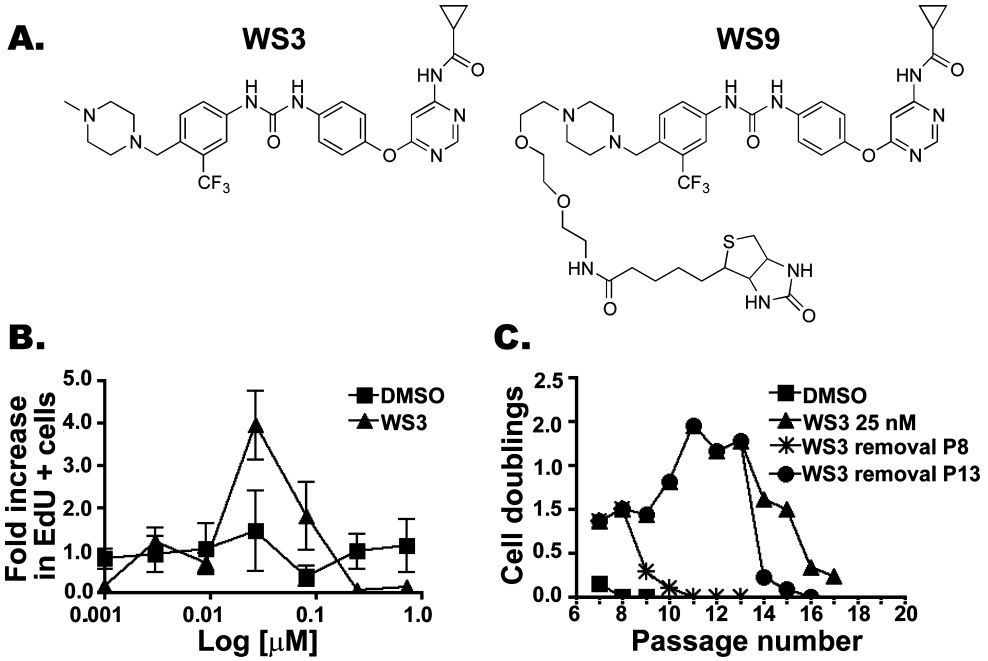


Figure 1. Proliferation of fetal human RPE cells grown in the presence of WS3. (A) Chemical structures of WS3 and WS9. Synthetic schemes have been previously published.[15] (B) WS3 shows a dose-dependent increase in EdU incorporation at passage 6 (mean ± SD, n = 4). (C) Fetal human RPE cells grown in the presence of 25 nM WS3 demonstrate that WS3-induced proliferation increases cell number and is reversible.

System). In non-proliferating RPE cells at passage 6, 25 nM WS3 induced a dose-dependent incorporation of Edu, reaching its maximum with a 4-fold increase over the DMSO control (Figure 1B). Both adult rat and adult human RPE cells responded similarly (Supplementary Figure 1). Analyses using Molecular Probes' Celltrace CFSE Cell Proliferation Kit provided additional evidence of increased cell division of fetal RPE cells in the presence of 25 nM WS3 (Supplementary Figure 2). Primary RPE cells need to be treated with WS3 prior to entering senescence (around passage 6) for the most effective expansion. However, initial expansion (passage 1−4) in the presence of WS3 does not increase the total number of cell doublings. This result suggests that the compound prevents cells from exiting the cell cycle. Since the vast majority of cells are actively dividing at early passages, addition of WS3 does not have much effect on proliferation and therefore does not need to be present during initial expansion.

For chemically induced expansion of RPE cells to be a viable strategy for cell therapy, WS3 should reversibly induce cell proliferation over multiple passages without genetic alteration. To this end, we assayed serial passages of human fetal RPE cells expanded in the presence of 25 nM WS3 and quantified the doubling rate at each passage. Passage 6 cells were plated at 50% confluence and treated with 25 nM WS3 or DMSO (0.03% v/v). The cells were split every three days, viable cell numbers were determined, and cells were replated at the starting density. DMSO-treated cells immediately ceased dividing and eventually dropped to below 0.1 doublings per passage due to cell loss (Figure 1C). WS3-treated cells continued to double through passage 15 with maximal doubling (1.95) at passage 11. Following passages 14−16, cells entered senescence but did not appear to undergo cell death. Furthermore, these cells looked similar to those untreated at passage 6. Importantly, we found that WS3-treated cells ceased dividing and cell number remained constant upon compound removal at passage 8 or 13. Given the doubling rates observed through passage 16 with WS3, a biopsy sample of 1000 RPE cells[17,18] could theoretically provide 1×10^7 viable RPE cells for differentiation and/or transplantation (Supplementary Figure 3).

Since *ex vivo* expansion is often associated with a high incidence of karyotype aberrations,[19] providing a selective growth advantage to mutated cells, we analyzed WS3-expanded RPE cells for possible chromosomal rearrangements. Fetal RPE cells were grown to passage 6, treated with 25 nM WS3 until passage 10, and then standard G-band karyotyping was performed. None of the 19 cytogenic analyses on metaphase cells revealed chromosomal abnormalities, suggesting that long-term culture of RPE cells in the presence of WS3 does not induce genetic alterations or tumorigenic events (Supplementary Figure 4).

At confluence *in vitro*, RPE cells can terminally differentiate over several weeks into a functional monolayer.[10,20] During this process, cells become pigmented, adopt a typical RPE cobblestone morphology, and are able to phagocytose photoreceptor outer segments (POS).[2] To confirm that WS3-expanded RPE cells can differentiate *in vitro*, passage 10 human fetal RPE cells were plated at high confluence and cultured in the absence of WS3 for 5 weeks until terminal differentiation. During this period, RPE cells adopted a cobblestone appearance and became pigmented (Figure 2A,B).[4] Cellular staining indicated tight junctions as seen by positive staining for ZO-1 (Figure 2C,E,F). Expression of CRALBP and RPE65 further confirmed the formation of a mature RPE monolayer (Figure 2D,F).[21] Lastly, differentiated RPE cells possessed phagocytosis activity, as demonstrated by the internalization of POS (Figure 2E). Undifferentiated cells did not express RPE cell markers or phagocytose POS (Supplementary Figure 5).

Similarly, WS3-expanded adult human RPE cells could also differentiate after compound withdrawal and acquire the pigmented, cobblestone phenotype of mature RPE cells (data not shown). In addition, gene expression analyses of differ-

dx.doi.org/10.1021/cb4001712 | *ACS Chem. Biol.* 2013, 8, 1407−1411

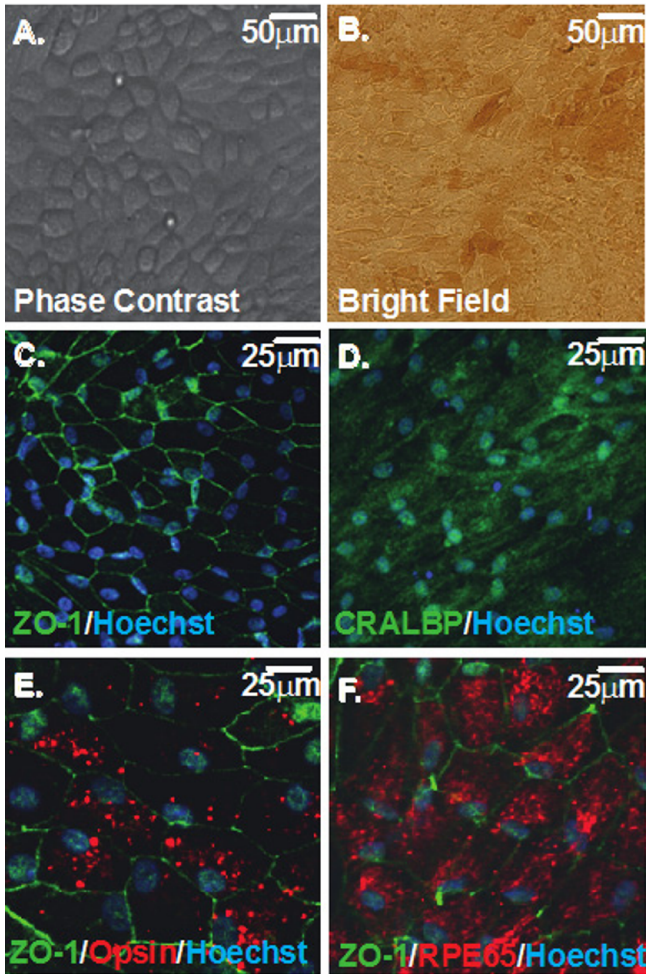


Figure 2. (A,B) WS3-expanded fetal human RPE cells grown in the presence of WS3 and differentiated for 5 weeks. (C–F) Staining of differentiated fetal RPE cells show the formation of tight junctions, expression of mature RPE markers, and function *in vitro* as determined by phagocytosis of internalized opsin.

entiated adult RPE cells via reverse transcriptase polymerase chain reaction (RT-PCR) confirmed the expression of known markers of mature RPE cells including *RPE65*, *RAX*, *MITF*, *BEST*, *PEDF*, and *PMEL* (Supplementary Figure 6).[21,22] Overall, these data show that WS3-proliferated fetal and adult human RPE cells retain their ability to differentiate into functional RPE monolayers *in vitro* upon WS3 removal. However, WS3-expanded RPE cells do not appear to differentiate as well as stem cell-derived RPE cells *in vitro*. Previous studies have shown that stem cell-derived RPE cells also differentiate better.[23,24]

To determine if *ex vivo* WS3-expanded RPE cells remain functional *in vivo*, they were transplanted into RCS rats.[16,25] RCS rats harbor an inactivating mutation in the MERTK gene, resulting in RPE cells that are incapable of POS phagocytosis.[26] Visual acuity, as tested by an optokinetic response (OKR), decreases with age, from 0.52 cycles/degree (c/d) at postnatal (P) day 30 to 0.30 c/d at P90 (as compared with 0.55–0.60 c/d in wild-type rat).[27,28] Another test for functional photoreceptors is luminance threshold (LT) recording from the superior colliculus (SC). In the RCS rat, LT elevates from 1.2 log units at P30 to 2.8 log units at P90 (as compared to less than 0.4 log units in wild-type rats). As this assay measures functional sensitivity across the visual field, it provides a topographic indication of the magnitude and the area of photoreceptor rescue across the whole retina.[29,30]

In order to assess preservation of visual acuity and LT, RCS rats transplanted with WS3-expanded rat RPE cells were analyzed at P90. OKR analysis revealed a significant difference between eyes injected with RPE cells and control eyes (p-value < 0.001, Student's t test) (Figure 3A). An average of 0.49 ± 0.02 c/d was recorded in RPE injected eyes (n = 16), whereas 0.34 ± 0.02 c/d in BSS injected (n = 8) and 0.31 ± 0.04 c/d in untreated controls (n = 8) were recorded (Figure 3A). LT recordings correlated well with visual acuity and revealed that RPE grafted eyes produced thresholds less than 1.7 log units over 35% of the SC area. In contrast, controls produced thresholds less than 1.7 log units over 2% of the SC area (Figure 3B). Thus, RPE grafted eyes have significantly lower thresholds than control eyes, indicating greater photoreceptor preservation.

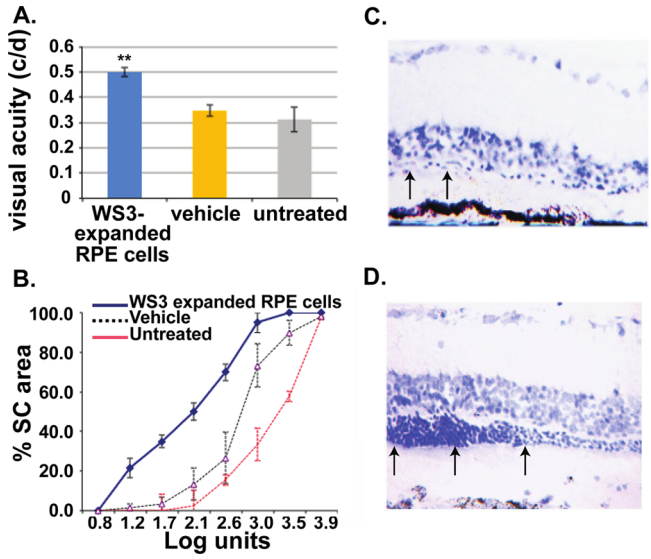


Figure 3. Restoration of a functional RPE monolayer in RCS rats at P90 following transplantation of rat RPE cells expanded with WS3. (A) Visual acuity (**p-value < 0.001, Student's t test). (B) Luminescence threshold. (C,D) Histology of rat eyes transplanted either with untreated controls (C) or WS3-expanded RPE cells (D). Arrows indicate residual photoreceptor layer. These data show that WS3-expanded RPE cells are capable of preserving photoreceptors in RCS rats.

To further support retinal lamination and photoreceptor preservation, histological examination indicated that RPE treated eyes had 4–6 layers of photoreceptors compared with a single layer in controls at P90 (Figure 3C vs D). Visual function was comparable to previously published data using human embryonic stem (hES) cell-derived RPE cells in the same animal model.[5] Human ES-RPE cells survived for longer and yielded better photoreceptor rescue, suggesting that further studies are required to improve survival and engraftment of WS3-expanded RPE cells. The number of cells injected, differentiation stage, and creation of new supports for fully differentiated RPE monolayers could improve graft integration. Long-term studies will be required to compare the effectiveness of freshly isolated, WS3-expanded, and hES-derived RPE cells in the RCS model.

dx.doi.org/10.1021/cb4001712 | *ACS Chem. Biol.* 2013, 8, 1407–1411

We next performed target identification experiments to determine if WS3 acts upon the same target as previously identified in β-cells. Again, Erb3 binding protein 1 (Ebp1) was found to be the target of WS3 in RPE cells on the basis of an affinity-based strategy using a biotin-conjugated analogue of WS3 (WS9; Figure 1A). Targeted Ebp1-knockdown and transcriptional analyses of Ebp1 targets following WS3 treatment confirmed a role for WS3 in RPE cell expansion (Supplementary Figure S7A–E), consistent with previous studies performed in β-cells.[15] However, β-cell target identification also detected IKKε as a target of WS3 based on affinity chromatography and kinase profiling (at 500 nM WS3). This target was not identified in RPE cells, and further kinase profiling must be done at a lower concentration to eliminate the possibility of alternate kinase targets. WS3 appears to act through Ebp1 as a non-cell specific proliferative molecule, and it may be useful for the *ex vivo* expansion of other primary cell types for transplantation. Additional studies are ongoing to further characterize the mechanism of action for WS3 and to identify other potential targets. Further optimization of WS3 or other such molecules may ultimately allow the expansion of small RPE biopsies to provide an adequate number of cells for autologous treatment of AMD and other visual degenerative disorders without the risks of teratoma formation associated with stem cell-derived RPE cells.

METHODS

Cell Culture. RPE cells were grown in EpiCM (ScienCell Research Laboratories) containing 2% Fetal Bovine Serum and growth supplements. Fetal RPE cells were purchased at passage one (ScienCell Research Laboratories). Both adult rat and adult human RPE cells were prepared as described in the Supporting Information. All cells were grown at 37 °C, 5% CO_2.

RPE Proliferation Assays and Growth Curves. Human RPE cells were grown to passage 6, or otherwise indicated, in EpiCM. Cells were plated in tissue-culture treated 384-well plates at a density of 1500 cells per 50 μL. Compound was immediately added to the cells in a volume of 100 nL of DMSO in a 3-fold dilution series and incubated for three days. On day three, cells were pulsed with 5 μM 5-ethynyl-2′-deoxyuridine (EdU; Invitrogen Life Technology) for 2 h, fixed with 4% paraformaldehyde, and then washed three times with Phosphate Buffered Saline (PBS). The azide/alkyne cycloaddition reaction was performed per the manufacturer's protocol (Invitrogen Life Technology, Click-iT EdU Alexa Fluor 488 Imaging Kit). Cells were then stained with 2 μg mL^{-1} of Hoechst dye in PBS. Plates were imaged on an ImageXpress Ultra Confocal High Content Screening System (Molecular Devices). Built-in algorithms for multiwavelength cell scoring were used to count total nuclei and EdU positive cells.

Growth curves were obtained by growing RPE cells at a density of 500,000 viable cells per 10 mL in a T75 flask either with 25 nM WS3 or an equivalent volume of DMSO (<1% v/v). Cells were passaged twice a week, and cell number was determined either by hemacytometry or ViCell automated cell counter (Beckman Coulter).

Immunofluorescence. Cells in 96- or 384-well plates were washed with PBS, fixed with 4% paraformaldehyde, rinsed, permeabilized with 0.1% Triton X-100, and blocked with 1% bovine serum albumin in PBS containing 0.05% Tween 20. Antibodies used for immunostaining: Anti-Opsin (Abcam catalog #ab3267) Anti-ZO1, (Invitrogen Life Technology catalog #40-2200), Anti-RPE65 (Millipore catalog #MAB5428), Anti-CRALBP (Santa Cruz biotechnology Inc. catalog #sc-59487)

RPE Transplantation Experiment in RCS Rats. All animals were housed and handled with the authorization and the supervision of the Institutional Animal Care Committee. Experiments were carried out in accordance with the National Institutes of Health guidelines regarding the care and use of animals for experimental procedures and ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The RCS rats at P22 received subretinal injection of adult rat undifferentiated RPE cells (passage 9; grown in 25 nM WS3 for 7 passages; n = 16); control rats received Balanced Salt Solution (BSS) (n = 8); and untreated rats (n = 8) using a published protocol.[5] Adult rat RPE cells were used in all transplant experiments to avoid rejection. Additionally, all rats were maintained on cyclosporine A (CyA group) (Novartis) in the drinking water (210 mg L^{-1}) from one day before transplantation until sacrifice. A RPE cell suspension containing approximately 3 × 10^4 cells in 2 μL of BSS was injected into the subretinal space through a small scleral incision from a fine glass pipet (internal diameter, 50–150 μm) attached by tubing to a 25 μL syringe (Hamilton). The cornea was punctured to reduce intraocular pressure and to limit the efflux of cells. A sham-surgery group was treated similarly, except only BSS was injected. Immediately after injection, the fundus was examined to assess retinal damage or vascular distress. Animals exhibiting such problems were removed from the study and not included in the animal counts described herein. Pigmented RCS rats received daily intraperitoneal injections of dexamethasone (1.6 mg kg^{-1}) for two weeks following transplantation to control acute inflammatory responses.

Measurement of Visual Function Following RPE Transplantation. Animals were tested for spatial visual acuity at P90 using an optometry testing apparatus (CerebraMechanics), as previously described.[31] Luminance threshold (LT) provided parallel data to the visual acuity. LT was evaluated by recording single and multiunit activity close to the surface of the superior colliculus (SC) from P90–P95 using previously described procedures.[29] Data are expressed as a graph of percentage of SC area with a LT below defined levels and as raw results. Statistical analyses were performed using GraphPad Prism. All variables were expressed as mean ± standard error of the mean. Differences between groups were compared by either Student's two tailed unpaired t test or analysis of variance. Newman–Keuls procedure was used for multiple comparison analysis. Differences were considered to be significant at $P < 0.05$.

ASSOCIATED CONTENT

Supporting Information

Additional evidence for *in vitro* proliferation and further characterization of differentiated RPE cells can be found in this section. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*(S.W.) E-mail: shaomei.wang@cshs.org. Phone: (301) 248-7970. Fax: (310) 248-8066. (P.G.S.) E-mail: schultz@scripps.edu. Phone: (858) 784-9300. Fax: (858) 784-9440.

Author Contributions

⊥These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Juvenile Diabetes Research Foundation (to J.G.S.); Hear See Hope Foundation (to B.L.); Lincy Foundation, Foundation for Fighting Blindness, Regenerative Medicine Institute at CSMC and National Institutes of Health R01EY020488-01 (to S.W.); and funds from the Skaggs Institute for Chemical Biology (to P.G.S.). This is manuscript number 21945 of The Scripps Research Institute.

REFERENCES

(1) Sparrow, J. R., Hicks, D., and Hamel, C. P. (2010) The retinal pigment epithelium in health and disease. *Curr. Mol. Med. 10*, 802–823.

(2) Strauss, O. (2005) The retinal pigment epithelium in visual function. *Physiol. Rev. 85*, 845−881.
(3) Kinnunen, K., Petrovski, G., Moe, M. C., Berta, A., and Kaarniranta, K. (2012) Molecular mechanisms of retinal pigment epithelium damage and development of age-related macular degeneration. *Acta Ophthalmol. 90*, 299−309.
(4) Lee, E., and MacLaren, R. E. (2011) Sources of retinal pigment epithelium (RPE) for replacement therapy. *Brit. J. Ophthalmol. 95*, 445−449.
(5) Lu, B., Malcuit, C., Wang, S., Girman, S., Francis, P., Lemieux, L., Lanza, R., and Lund, R. (2009) Long-term safety and function of RPE from human embryonic stem cells in preclinical models of macular degeneration. *Stem Cells 27*, 2126−2135.
(6) Lund, R. D., Adamson, P., Sauve, Y., Keegan, D. J., Girman, S. V., Wang, S., Winton, H., Kanuga, N., Kwan, A. S., Beauchene, L., Zerbib, A., Hetherington, L., Couraud, P. O., Coffey, P., and Greenwood, J. (2001) Subretinal transplantation of genetically modified human cell lines attenuates loss of visual function in dystrophic rats. *Proc. Natl. Acad. Sci. U.S.A. 98*, 9942−9947.
(7) Rowland, T. J., Buchholz, D. E., and Clegg, D. O. (2012) Pluripotent human stem cells for the treatment of retinal disease. *J. Cell. Physiol. 227*, 457−466.
(8) Schwartz, S. D., Hubschman, J. P., Heilwell, G., Franco-Cardenas, V., Pan, C. K., Ostrick, R. M., Mickunas, E., Gay, R., Klimanskaya, I., and Lanza, R. (2012) Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet 379*, 713−720.
(9) Arnhold, S., Klein, H., Semkova, I., Addicks, K., and Schraermeyer, U. (2004) Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. *Invest. Ophthalmol. Visual Sci. 45*, 4251−4255.
(10) Aronson, J. F. (1983) Human retinal pigment cell culture. *In Vitro 19*, 642−650.
(11) Kokkinopoulos, I., Shahabi, G., Colman, A., and Jeffery, G. (2011) Mature peripheral RPE cells have an intrinsic capacity to proliferate; a potential regulatory mechanism for age-related cell loss. *PloS One 6*, e18921.
(12) MacLaren, R. E., Uppal, G. S., Balaggan, K. S., Tufail, A., Munro, P. M., Milliken, A. B., Ali, R. R., Rubin, G. S., Aylward, G. W., and da Cruz, L. (2007) Autologous transplantation of the retinal pigment epithelium and choroid in the treatment of neovascular age-related macular degeneration. *Ophthalmology 114*, 561−570.
(13) Tamiya, S., Liu, L., and Kaplan, H. J. (2010) Epithelial-mesenchymal transition and proliferation of retinal pigment epithelial cells initiated upon loss of cell-cell contact. *Invest. Ophthalmol. Visual Sci. 51*, 2755−2763.
(14) Uebersax, E. D., Grindstaff, R. D., and Defoe, D. M. (2000) Survival of the retinal pigment epithelium in vitro: comparison of freshly isolated and subcultured cells. *Exp. Eye Res. 70*, 381−390.
(15) Shen, W., Tremblay, M. S., Deshmukh, V. A., Wang, W., Filippi, C. M., Harb, G., Zhang, Y. Q., Kamireddy, A., Baaten, J. E., Jin, Q., Wu, T., Swoboda, J. G., Cho, C. Y., Li, J., Laffitte, B. A., McNamara, P., Glynne, R., Wu, X., Herman, A. E., and Schultz, P. G. (2013) Small-molecule inducer of beta cell proliferation identified by high-throughput screening. *J. Am. Chem. Soc. 135*, 1669−1672.
(16) LaVail, M. M. (2001) Legacy of the RCS rat: impact of a seminal study on retinal cell biology and retinal degenerative diseases. *Prog. Brain Res. 131*, 617−627.
(17) Ishida, M., Lui, G. M., Yamani, A., Sugino, I. K., and Zarbin, M. A. (1998) Culture of human retinal pigment epithelial cells from peripheral scleral flap biopsies. *Curr. Eye Res. 17*, 392−402.
(18) Kim, K. S., Tezel, T. H., and Del Priore, L. V. (1998) Minimum number of adult human retinal pigment epithelial cells required to establish a confluent monolayer in vitro. *Curr. Eye Res. 17*, 962−969.
(19) Corselli, M., Parodi, A., Mogni, M., Sessarego, N., Kunkl, A., Dagna-Bricarelli, F., Ibatici, A., Pozzi, S., Bacigalupo, A., Frassoni, F., and Piaggio, G. (2008) Clinical scale ex vivo expansion of cord blood-derived outgrowth endothelial progenitor cells is associated with high incidence of karyotype aberrations. *Exp. Hematol. 36*, 340−349.
(20) Song, M. K., and Lui, G. M. (1990) Propagation of fetal human RPE cells: preservation of original culture morphology after serial passage. *J. Cell. Physiol. 143*, 196−203.
(21) Vaajasaari, H., Ilmarinen, T., Juuti-Uusitalo, K., Rajala, K., Onnela, N., Narkilahti, S., Suuronen, R., Hyttinen, J., Uusitalo, H., and Skottman, H. (2011) Toward the defined and xeno-free differentiation of functional human pluripotent stem cell-derived retinal pigment epithelial cells. *Mol. Vision 17*, 558−575.
(22) Strunnikova, N. V., Maminishkis, A., Barb, J. J., Wang, F., Zhi, C., Sergeev, Y., Chen, W., Edwards, A. O., Stambolian, D., Abecasis, G., Swaroop, A., Munson, P. J., and Miller, S. S. (2010) Transcriptome analysis and molecular signature of human retinal pigment epithelium. *Hum. Mol. Genet. 19*, 2468−2486.
(23) Ahmado, A., Carr, A. J., Vugler, A. A., Semo, M., Gias, C., Lawrence, J. M., Chen, L. L., Chen, F. K., Turowski, P., da Cruz, L., and Coffey, P. J. (2011) Induction of differentiation by pyruvate and DMEM in the human retinal pigment epithelium cell line ARPE-19. *Invest. Ophthalmol. Visual Sci. 52*, 7148−7159.
(24) Buchholz, D. E., Hikita, S. T., Rowland, T. J., Friedrich, A. M., Hinman, C. R., Johnson, L. V., and Clegg, D. O. (2009) Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. *Stem Cells 27*, 2427−2434.
(25) Baehr, W., and Frederick, J. M. (2009) Naturally occurring animal models with outer retina phenotypes. *Vision Res. 49*, 2636−2652.
(26) D'Cruz, P. M., Yasumura, D., Weir, J., Matthes, M. T., Abderrahim, H., LaVail, M. M., and Vollrath, D. (2000) Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. *Hum. Mol. Genet. 9*, 645−651.
(27) Lund, R. D., Wang, S., Klimanskaya, I., Holmes, T., Ramos-Kelsey, R., Lu, B., Girman, S., Bischoff, N., Sauve, Y., and Lanza, R. (2006) Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats. *Cloning Stem Cells 8*, 189−199.
(28) Wang, S., Lu, B., Girman, S., Duan, J., McFarland, T., Zhang, Q. S., Grompe, M., Adamus, G., Appukuttan, B., and Lund, R. (2010) Non-invasive stem cell therapy in a rat model for retinal degeneration and vascular pathology. *PloS One 5*, e9200.
(29) Girman, S. V., Wang, S., and Lund, R. D. (2005) Time course of deterioration of rod and cone function in RCS rat and the effects of subretinal cell grafting: a light- and dark-adaptation study. *Vision Res. 45*, 343−354.
(30) Wang, S., Lu, B., Girman, S., Holmes, T., Bischoff, N., and Lund, R. D. (2008) Morphological and functional rescue in RCS rats after RPE cell line transplantation at a later stage of degeneration. *Invest. Ophthalmol. Visual Sci. 49*, 416−421.
(31) Prusky, G. T., Alam, N. M., Beekman, S., and Douglas, R. M. (2004) Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system. *Invest. Ophthalmol. Visual Sci. 45*, 4611−4616.