GMP-compliant Human RPE Cells Derived from Embryonic Stem Cell Lines Rescue Visual Function In a Rat Model for Photoreceptor Degeneration

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Abstract

Certain retinal diseases are characterized by degeneration of the retinal pigment epithelium (RPE) which in turn results in photoreceptor loss. Examples include Stargardt macular dystrophy in humans and the genetically-determined dystrophy in the Royal College of Surgeons (RCS) rat. Such a process may also play a role in age related macular degeneration, which is responsible for compromised vision in more than 9 million people in the US alone. We investigated whether a GMP human embryonic stem cell-derived RPE (hES-RPE) cell line could rescue visual function in the dystrophic RCS rat.

GMP-compliant hES-RPE cells (developed by Advanced Cell Technology) were injected into the subretinal space of 22 day-old (P22) RCS rats that were maintained on oral cyclosporine immunosuppression, post-operatively. Functional efficacy was tested by threshold optomotor acuity and luminance thresholds recorded from the superior colliculus. All treated eyes were compared with sham-injected and untreated eyes. Histological examination was performed after these functional assessments.

Optomotor thresholds (0.48 c/d) measured at P60 were significantly ($p<0.001$) better than shams (0.27 c/d). In some instances, treated eyes showed near normal thresholds (0.536 c/d). At P90, the optomotor acuity was still maintained over 0.5 c/d in the best performers, while sham and untreated animals gave a figure of 0.16 c/d, consistent with substantial visual impairment. Superior colliculus recordings at P98 also showed much lower luminance threshold responses in cell-injected eyes with some individual recordings within the normal range (0.3 log units). Histological studies showed donor cells formed a semi-continuous, pigmented cell layer and integrated into host RPE layer. These cells expressed typical RPE cell markers such as RPE65 and bestrophin. Photoreceptor formed a layer 5-6 cells thick in rescued area compared with a single layer in sham and untreated controls at P90.

Our results show that a well-characterized GMP compliant embryonic stem cell-derived RPE cells can survive after transplantation to the subretinal space of RCS rats, integrate into host RPE layer without migration into the retina and continue to express at least some of the molecules characteristic of RPE. The cells are effective in rescuing photoreceptors from degeneration and result in significant rescue of visual function over sham-operated animals for the long term.

Introduction

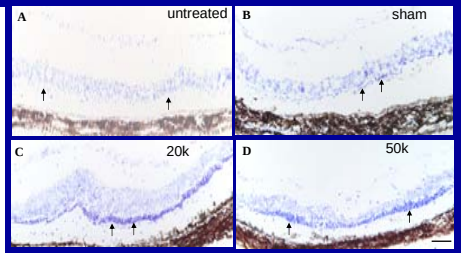
Age-related macular degeneration (ARMD) affects more than 30 million people worldwide and is the leading cause of blindness in patients over 60 in the United States, for which no suitable treatment exists. An animal model, the Royal College of Surgeons (RCS) rat provides an opportunity to explore potential therapeutic approaches that may eventually be applied in the clinic. In this animal model, a mutation in the MERTK gene affects the ability of RPE cells to phagocytose photoreceptor outer segments and leads to loss of photoreceptors between 21 and 100 days of age. Previous work has shown that freshly harvested RPE can be effective in rescuing photoreceptors in the RCS rat. A major issue in the cell-based therapy is that of providing a cell source that is readily available, safe, and ethically acceptable and which can be developed commercially in large-scale production. With this goal in mind, we investigated whether an human embryonic stem cell-derived RPE (hES-RPE) cell line could rescue visual function in the dystrophic RCS rat.

Material and Methods

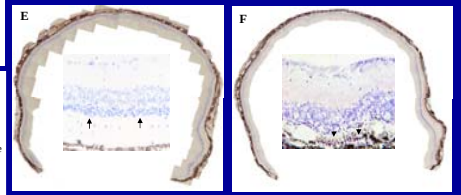
GMP-compliant hES-RPE cells from Advanced Cell Technology were injected into the subretinal space of 22 day-old (P22) RCS rats. Three cell lines designated low, medium and high pigment were given in five different dosage groups: $5x10^3$/eye (5K), $2x10^4$/eye (20k), $5x10^4$/eye (50K), $7.5x10^4$/eye (75K) and $1x10^5$/eye (100K).

1. 5K low pigmented group (n=8);
   5K medium pigmented group (n=6);
   5K high pigmented group (n=7);
2. 20K low pigmented group (n=7);
   20K medium pigmented group (n=8);
   20K high pigmented group (n=6);
3. 50K low pigmented group (n=8);
   50K medium pigmented group (n=8);
   50K high pigmented group (n=9);
4. 75K medium pigmented group (n=8);
5. 100K medium pigmented group (n=8);

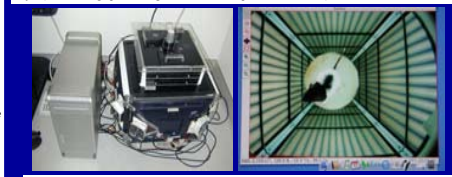
With each group of animals, 3-4 eyes received injections of sham alone. The unoperated eyes provided further baseline data. All animals were maintained on oral cyclosporine A (CyA) administered in the drinking water (210mg/l) until they were sacrificed. In addition, an intraperitoneal injection of dexamethasone was given for two weeks 2.5mg/kg/day) after surgery. Optomotor test was conducted at P60, P90, P120, P150 and P180. Tectal recordings were made at about P98 and P187. At the end of the testing, animals were sacrificed and eyes fixed with 2% paraformaldehyde. Frozen sections were cut and processed for histology and immuno-histochemistry.



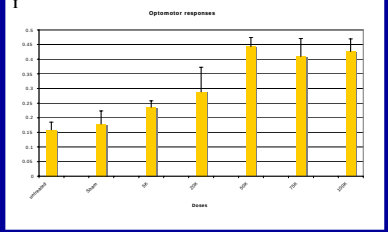
A-D: RCS retina sections at P90 stained with cresyl violet: C&D showed substantial photoreceptor rescue with hES-RPE injection, compared with untreated retina at same age (A). Sham injection (B) produced localized photoreceptor rescue (arrows). Scale bar equals 40μm.



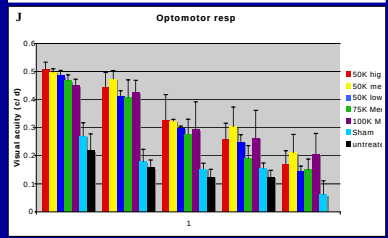
E-F: low power images of hES-RPE injected retina stained with cresyl violet (E) and human nuclear marker-1281 (Chemicon) (F): there was extensive photoreceptor rescue (arrows in high power image inside E); Human nuclear marker staining revealed that hES-RPE cells well integrated into host RPE layer (arrows in high power image inside F indicate positive donor cells).



Optomotry testing apparatus: this comprise a rotating cylinder displaying a vertical sine wave grating presented in virtual three-dimensional (3-D) space on four computer monitors arranged in a square. Rats staining unrestrained on a platform in the center of the square tracked the grating with reflexive head movements. The spatial frequency of the grating was clamped at the viewing position by repeatedly recentering the cylinder on the head of the test subject. Acuity was quantified by Increasing the spatial frequency of the grating until an optomotor response could no longer be elicited (Prusky et al . Vision Res. 2000).



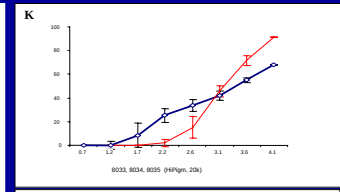
The visual acuity as measured by the optomotor system shows that animals treated with the 5K, 20K, 50K, 75K and 100K cells performed significantly better than those with the sham injection and untreated controls ($p<0.05$) at P90 days.

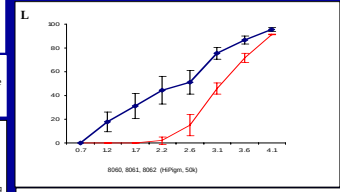


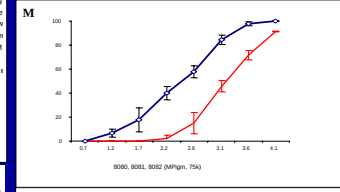
The visual acuity as measured by the optomotor test shows that animals treated with 50K, 75K and 100K cell lines performed significantly better than those with the sham injection and untreated controls ($p<0.05$) at P60 , P90, P120, P150 and P180 days, respectively.

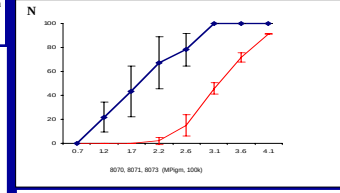
Table 1. Visual acuity of the RCS rats with hES-RPE cell line treatment

Age	Number of Eyes and with pigment	Visual acuity (c/d)		Sham		untreated	
P60	5K High	0.508	± 0.02 5	0.2 7	± 0.048	0 .22	± 0.05 5
	5K Medium	0.499	± 0.03 1				
	5K Low	0.485	± 0.01 9				
	75 K Medium	0.469	± 0.019				
	100 K Medium	0.451	± 0.022				
P90	5K High	0.444	± 0.05 2	0 .179	± 0.04 4	0 .159	± 0. 0.26
	5K Medium	0. 474	± 0.03 0				
	5K Low	0.411	± 0.020				
	75 K Medium	0.469	± 0.061				
	100 K Medium	0.426	± 0.043				
P120	5K High	0.327	± 0.09 0	0.15 3	± 0.020	0.12 4	± 0 .028
	5K Medium	0.321	± 0.06 6				
	5K Low	0.299	± 0.006				
	75 K Medium	0. 278	± 0.003				
	100 K Medium	0.295	± 0.008				
P150	5K High	0.258	± 0.05 9	0.11 4	± 0.038	0 .073	± 0 .040
	5K Medium	0.304	± 0.069				
	5K Low	0.249	± 0.02 6				
	75 K Medium	0.191	± 0.045				
	100 K Medium	0.264	± 0.09 0				
P180	5K High	0.173	± 0.04 7	0.06 2	± 0.050	0	
	5K Medium	0.213	± 0.065				
	5K Low	0.145	± 0.018				
	75 K Medium	0.153	± 0.037				
	100 K Medium	0.207	± 0.073				









Luminance threshold responses recorded across the superior colliculus, each curve (average ± SEM) shows the percent of retinal area (Y-axis) where the visual threshold is less than the corresponding value at X-axis (log units, relative to background illumination 0.02 cd/m^2). The curves in K showed that 28% of the area of the SC in animals with 20K cell line treatment gave thresholds of 2.2 log units, 45% with 50K (L), 40% with 75K (M) and 70% with 100K (N) against shams in which approximately 3% gave thresholds of 2.2 log units at P94.

O

ACT (MPigm, 100k)
Age: P98
Right eye: cell graft — Left eye: untreated
Thresholds (Log units)

	1.9	1.7	2.0		4.0	4.0	3.9	4.1
1.4	1.2	1.0	1.6		3.9	3.5	3.7	3.8
1.6	1.1	0.8	0.9		3.6	2.3	3.2	3.4
1.3	0.9	0.7	1.0		3.0	3.1	2.9	3.3

P

ACT (MPigm, 100k)
Age: P187
Right eye: cell graft — Left eye: untreated
Thresholds (Log units)

	2.6	2.4	2.5	NR	NR	NR	NR
2.1	1.9	2.1	1.6	NR	NR	NR	NR
2.4	1.6	1.4	1.9	4.1	4.4	4.6	NR
2.1	1.3	1.0	1.2	4.6	4.7	4.8	NR

Visual field is preserved in eyes receiving 100K (hES-RPE) grafts. Luminance threshold responses were recorded at P98 (O) and P187 (P) from multiple points within the superior colliculus (SC). This method quantifies functional sensitivity to light across the visual field of the eye. The topographical map depicts the luminance threshold responses (measured in log units relative to background illumination of 0.02 cd/m^2) at 15 points and 16 points both in left and right sides within SC. O. there were all points of luminance threshold responses in treated side below 2.3 log units. For comparison, the luminance threshold responses in SC in the untreated side were all above 2.3 log units at P98 of RCS rats. P. there were still 11 points of luminance threshold responses in the treated side below 2.3 log units, however, in the untreated side, 6 points were above 4.1 log units and 8 points nonresponses at P187.

Results:

1. Subretinal injection of GMP hES-RPE cell line delayed photoreceptor degeneration. There was extensive photoreceptor rescue: 5-6 cells deep in the outer nuclear layer (ONL). For comparison, the ONL was reduced to one cell deep at P90 in sham-injected RCS rats. The donor cells are able to integrate into host RPE cell layer.
2. The OptoMotor test showed a dose dependent response: 50K, 75K and 100K grafted animals performed significantly better than that of 5K, 20K, shams and untreateds at P90; also, the visual acuity of RCS rats with 50K, 75K and 100K treatments showed significant improvement ($p< 0.05$) over the sham and untreated controls, respectively at P60, P90, P120, P150 and P180 (table 1).
3. Superior colliculus recording showed that hES-RPE grafted animals had lower visual thresholds compared with sham injected and untreated groups until P187.

Conclusion:

1. This study showed that the GMP-compliant human ES-derived cell line injected into subretinal space of RCS rat can preserve photoreceptors and some visual functions in a dose-dependent manner. The mechanism of both morphological and visual functional rescue may be associated with phagocytosing photoreceptor outer segments or factor-releasing mechanisms.
2. hES-RPE may provide an effective donor cell source to rescue photoreceptors in conditions such as AMD where RPE function is compromised.

Acknowledgements

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