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Epiretinal transplantation of human bone marrow mesenchymal stem cells rescues retinal and vision function in a rat model of retinal degeneration☆

Adi Tzameret [a], Ifat Sher [a], Michael Belkin [a], Avraham J. Treves [b], Amilia Meir [b], Arnon Nagler [c], Hani Levkovitch-Verbin [d], Ygal Rotenstreich [a,1], Arieh S. Solomon [a,*,1]

[a] Goldschleger Eye Research Institute, Sackler Faculty of Medicine, Tel Aviv University, Sheba Medical Center, Tel-Hashomer, Israel
[b] Center for Stem Cells and Regenerative Medicine, Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel
[c] Hematology Division, Sheba Medical Center, Tel-Hashomer, Israel
[d] Rothberg Ophthalmic Molecular Biology Laboratory, Goldschleger Eye Research Institute, Sackler Faculty of Medicine, Tel Aviv University, Sheba Medical Center, Tel-Hashomer, Israel

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ABSTRACT

Vision incapacitation and blindness associated with incurable retinal degeneration affect millions of people worldwide. In this study, 0.25×10^6 human bone marrow stem cells (hBM-MSCs) were transplanted epiretinally in the right eye of Royal College Surgeons (RCS) rats at the age of 28 days.
Epiretinally transplanted cells were identified as a thin layer of cells along vitreous cavity, in close proximity to the retina or attached to the lens capsule, up to 6 weeks following transplantation.
Epiretinal transplantation delayed photoreceptor degeneration and rescued retinal function up to 20 weeks following cell transplantation. Visual functions remained close to normal levels in epiretinal transplantation rats. No inflammation or any other adverse effects were observed in transplanted eyes.
Our findings suggest that transplantation of hBM-MSCs as a thin epiretinal layer is effective for treatment of retinal degeneration in RCS rats, and that transplanting the cells in close proximity to the retina enhances hBM-MSC therapeutic effect compared with intravitreal injection.

1. Introduction

Retinal and macular degenerative diseases affect millions of people worldwide. Similar to other neurodegenerative diseases, there are no effective treatments that can stop retinal degeneration or restore degenerative retina. Recent advances in stem cell technology led to development of novel cell-based therapies, some are already in phase I/II clinical trials (Mu et al., 2014; Ng et al., 2014; Ramsden et al., 2013).

Studies from our group and others suggest that human bone marrow-derived mesenchymal stem cells (hBM-MSC) may be a promising source for retinal cell-based therapy. Transplanting these cells in Royal College of Surgeons (RCS) rats, a widely used model of dry age-related macular degeneration (AMD) and retinal degeneration, delayed retinal degeneration and rescued retinal and visual functions (Arnhold et al., 2007; Inoue et al., 2007; Lu et al., 2010; Tzameret et al., 2014). Accumulating evidence support the notion that hBM-MSCs act in a paracrine manner, as the cells secrete trophic and survival growth factors and cytokines, as well as protective and reparative micro- and nano-lipid microvesicles (Baglio et al., 2012; Yu et al., 2014). This hypothesis is supported by recent studies demonstrating that conditioned media collected from cultures of human adult adipose mesenchymal stem cells inhibited photoreceptor degeneration and retinal dysfunction in a mouse model of light-induced retinal damage (Sugitani et al., 2013; Tsuruma et al., 2014). Moreover, hBM-MSCs display immunosuppressive capacities (Caplan, 2009; Corcione et al., 2006; Oh et al., 2008; Sheng et al., 2008) and are potentially less immunogenic than other sources of allogenic stem cells, as studies in animals demonstrated no difference in graft survival regardless of immunosuppression (Lu et al., 2010). Furthermore, these cells offer the unique possibility of autologous transplantation that is likely to be even less immunogenic (Caplan, 2009).

Currently, one of the major challenges in stem cell-based therapy is how to safely deliver effective doses of cells to the target posterior eye

Abbreviations: AMD, age-related macular degeneration; ERG, electroretinogram; hBM-MSCs, human bone marrow mesenchymal stem cells; ONL, outer nuclear layer; PFA, paraformaldehyde; RCS, royal college surgeons; RP, retinitis pigmentosa; RPE, retinal pigment epithelium.

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* Corresponding author at: Goldschleger Eye Research Institute, Sheba Medical Center, 52621, Tel-Hashomer, Israel.
E-mail address: asolomon@post.tau.ac.il (A.S. Solomon).

[1] Equal contribution.

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tissues (retina, retinal pigment epithelium (RPE) and choroid), due to the unique anatomy and physiology of the eye. The current subretinal injection method involves three port pars plana, vitrectomy and insertion of a needle that penetrates the retina and, in doing so, detaches the photoreceptor cell layer from the RPE forming subretinal 'blebs' (Lu et al., 2009). Limited volumes can be injected and therapeutic effect is restricted to areas proximal to point of injection (Inoue et al., 2007; Lu et al., 2009; Schwartz et al., 2012). Moreover, the subretinal surgery raises a significant safety issue, as the retinal architecture across the entire retina in AMD and retinitis pigmentosa (RP) patients is fragile and the surgery can induce mechanical damage, reactive gliosis, and loss of function (Lin et al., 2009; Maguire et al., 2008; Nork et al., 2012; Wright et al., 2010). These procedural effects were documented in a recent gene therapy trial in which patients receiving a subretinal injection under the foveal region lost retinal thickness and visual acuity (Jacobson et al., 2012), suggesting that subretinal surgery presents a significant risk in treating the fovea, the target tissue in AMD. Attempts to use scaffolds to facilitate subretinal cell transplantation as a thin layer resulted in traumatic subretinal insertion and additional inflammation due to scaffold degradation (Liu et al., 2014).

We have recently developed a new cell delivery system that enabled the transplantation of hBM-MSCs as a thin layer across the extravascular spaces of the choroid in RCS rats. The graft covered most of the area of the back of the eye via a single injection with no retinal detachment or choroidal hemorrhages. Cell transplantation delayed photoreceptor degeneration throughout the whole retina and rescued retinal function for up to 5 months in RCS rats (Tzameret et al., 2014). By contrast, when hBM-MSCs were injected intravitreally, they formed a large cell clamp in the vitreous cavity and retinal function was rescued for a shorter duration, up to 12 weeks following transplantation (Tzameret et al., 2014). These findings suggested that the delivery method significantly affects therapeutic potential of transplanted cells, and that graft location, distance from the retina and graft surface area may be critical parameters for achieving effective treatment.

In the present study we examined another delivery method for hBM-MSCs to the posterior eye: transplanting the cells in the epiretina. Transplantation of hBM-MSCs using this method resulted in photoreceptor rescue across most of the retina and significantly enhanced retinal function for up to 5 months following cell transplantation.

2. Methods

2.1. Animals

The pigmented RCS strain was used in this study (D'Cruz et al., 2000; Edwards and Szamier, 1977). Rats were born and bred in the Sheba Medical Center animal facility under dim cyclic light (12 h at <5 lx, 12 h in the dark). All animal procedures and experiments were conducted with approval and under the supervision of the Institutional Animal Care Committee at the Sheba Medical Center, Tel-Hashomer, and conformed to recommendations of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Cell preparation

Primary human bone marrow mesenchymal stromal cell cultures were derived from three healthy volunteers (ages 35–50 years). Bone marrows were collected in the operating room under sterile conditions. The research was approved by the institutional review board at the Sheba Medical Center, Tel Hashomer. Bone marrow mononuclear cells were separated by Ficoll gradient (1.077 g/dl) according to the manufacturer instructions and were seeded in tissue culture flasks with culture media containing low-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 15% FCS, 100 U/ml penicillin, 100 ug/ml streptomycin and 2 mM L-glutamine. Tissue culture media was changed after 48 h and then twice a week until 70–80% confluence was reached, within 8–10 days. Cells were monitored for viability by trypan blue staining before transplantation. For immunophenotype analysis, cells were labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE) or allophycocyanin (APC) — conjugated monoclonal antibodies in 100 μl phosphate buffer for 15 min at room temperature or 30 min at 4 °C (Tzameret et al., 2014). Antibodies used were anti-CD14, CD34, CD45, CD90 and CD105 (from eBioscience) and anti-CD73 (from R&D Systems). Cultured cells were extensively washed and resuspended in PBS with 0.5% FBS, passed through a 70 μm filter and analyzed for surface marker profile by flow cytometry (FACSCalibur-Becton, Dickinson). Proliferation Index was tested within the first 72 h after seeding by XTT reagent according to the manufacturer instructions (Biological industries - Beit Haemek, Israel). For Colony Forming Unit-Fibroblasts (CFU-F) assay, the number of colonies produced in each passage per 100 cells was determined. Cells were seeded in 24 wells plate and incubated in a 5% CO_2 95% room air humidified incubator. Colonies were formed, analyzed and counted within 7–14 days after first seeding. Cells were washed to remove non-adherent colonies. Colonies were fixed with 1 ml methanol, stained with Giemsa stain, and manually counted. Cells were routinely tested for Mycoplasma contamination using EZ-PCR Mycoplasma Test Kit (Biological industries - Beit Haemek, Israel).

2.3. Epiretinal cell transplantation

Human BM-MSCs were transplanted in the epiretina of RCS rats ($n = 22$) at 28 days postnatal (designated week 0, W0), an age preceding major onset of retinal degeneration. Cells were prelabeled with the lipophilic membrane stain 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), according to manufacturer instructions (Invitrogen). Rats were under xylazine (10 mg/kg) and ketamine (75 mg/kg) intraperitoneal anesthesia. Cell transplantation was performed under a surgical microscope (Leica Wild M690; Wild Herring, Herring, Switzerland).

0.25×10^6 cells in 5 μl were transplanted epiretinally with a disposable insulin syringe of 0.3 ml volume, 0.30 mm (30G) × 8 mm (BD Micro-Fine Plus, Becton Dickinson and Company, USA). Pupils were dilated using Tropicamide 0.5% and Phenylephrine HCl diluted to 2%.

The syringe needle was introduced with its opening facing the retina and close to it. The introduction of the needle at this side created separation of the vitreous from the retina. Thus, the transplanted cells were applied directly on the surface of the retina (Supplementary Fig. 1A–E).

2.4. Electroretinogram (ERG) recording

For dark-adapted ERG, rats were kept in total darkness for 12 h prior to testing. Animals were anesthetized with intraperitoneal injection of 75 mg/kg Ketamine and 10 mg/kg Xylazine, pupils were dilated with topical 1% tropicamide and the corneas kept moist with 2.5% hydroxypropyl methylcellulose. ERGs were recorded from both eyes simultaneously using golden wire loops on the corneas. A chloride silver reference electrode was placed subcutaneously near the temporal canthus. The ground electrode was placed on the tail. For dark-adapted ERG, responses were averaged with stimulus intervals of 1 to 30 s depending on the stimulus light intensity. For light-adapted ERG, the animals were light-adapted for 10 min prior to testing and responses were averaged with stimulus intervals of 1 s.

2.5. Histology analysis

Rats were euthanized with CO_2 and the eyes were removed, fixed in formalin and embedded in paraffin. Retinal cross sections (4 μm) were cut along the vertical meridian of the eye through the optic nerve and were stained with hematoxylin and eosin.